RESEARCH PUBLICATIONS 2021 - 2022

II

NATIONAL COLLEGE (AUTONOMOUS)

COLLEGE WITH POTENTIAL FOR EXCELLENCE
NATIONALLY RE-ACCREDITED AT 'A+' GRADE BY NAAC
TIRUCHIRAPALLI-620 001

www.nct.ac.in 0431-2482995 principal@nct.ac.in

FOR PRVIVATE CIRCULATION ONLY

Contents

S.No	Department	Page No.
1	Business Administration	1
2	Biotechnology	21
3	Botany	183
4	Chemistry	427
5	Commerce	487
6	Computer Science	529
7	English	567
8	Geology	610
9	Library science	675
10	Mathematics	695
11	Physical Education	750
12	Physics	763
13	Tamil	784
14	Zoology	1083

IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

A STUDY ON THE IMPACT OF EMOTIONAL INTELLIGENCE ON EMPLOYEE WORK ENGAGEMENT BEHAVIOR KHIVRAJ MOTORS, CHENNAI.

Dr. Chandra, M. Assistant Professor, Department of Business Administration, National College(Autonomous), Trichy-1

Dr. Sivasakthi K. Assistant Professor, MBA Department, J.J. College of Engg., &Technology,Trichy-9

Abstract

Although EI has become a popular tool in organizations there is still a need for increased empirical research on the construct (Salovey, Woolery, & Mayer, 2005). This study contributes to the literature by providing more information about Emotional intelligence which may alleviate Work Engagement Behavior. It does this by building on the small existing pool of knowledge in order to extend the research on EI. The expected outcome of this study was an increased understanding of how EI impacts on Work Engagement Behavior. Emotional intelligence was measured using the 33-item Schutte Self-Report Inventory (SSRI) developed by Schutte and colleague. Engagement was measured using the shortened version of the Utrecht Work Engagement Scale (UWES) (Schaufeli et al., 2006). The scale consists of 9 items and was designed to erasure the three components of engagement: vigor, absorption, and dedication. The samples of 100 employees who are Khivraj Motors, Chennai. chosen for the study. Self Administered questionnaire distributed and information collected. Research design descriptive type with non probability purposive sampling technique was used for the study. The data were analyzed using SPSS (statistical package for social science).The statistical tools Correlation, Chi - Square Test, One – Way ANOVA, Regression Analysis were employed for the research study. From these Research Findings, managerial as well as theoretical implications have been discussed in this study.

Keywords: *goal congruence, work engagement, emotional intelligence, organizational deviance behavior*

Introduction

Emotional intelligence refers to the ability to perceive, control, and evaluate emotions. Emotional Intelligence plays an important role in helping the managers and employees to cope with this dynamic change in the business environment. Some researchers suggest that emotional intelligence can be learned and strengthened, while other claim it is an inborn characteristic. Employees need to enhance their emotional intelligence skills, apart from technical skills, which in turn will enhance their productivity on the job. Management of emotional intelligence by the team members will help in developing interpersonal skills of the team members.

Organization's to be successful, need to develop employee's emotional intelligence skills to work effectively in the organization and additionally Modern organizations need energetic and dedicated employees: people who are engaged with their work. These organizations expect proactivity, initiative and responsibility for personal development from their employees. Motivating people to engage in their work is a classic problem in organizations, complicated by the existence of multiple roles, because the attitudes, behaviors, and emotions associated with one role may spill over to another. People do not always check their problems or triumphs at the door when walking into the office or coming home from work.

Moreover, as careers have become more complex, people are increasingly faced with actively engaging in multiple roles. Within the context of the organization, people often must engage in multiple roles to fulfill job expectations. Hence this study tries to explore the relationship between Emotional Intelligence construct and work engagement behavior which really implicate the managerial and organization performance.

When emotions are experienced, such as satisfaction, anxiety or frustration, the human brain is programmed to respond to the threat and an emotional response is triggered. However, acting on that first impulse can lead to things being said or done that are later regretted. Emotional intelligence means that whilst you acknowledge that emotion, you then take a step back from that situation and let rational thought influence your actions.

Definition:

Emotional intelligence refers to emotional awareness and emotional management skills which provide the ability to balance emotion and reason so as to maximize long term happiness.

Models of EI

Currently, there are three main models of EI:

- Ability EI model
- Mixed models of EI
- Trait EI model

Different models of EI have led to the development of various instruments for the assessment of the construct. While some of these measures may overlap, most researchers agree that they tap different constructs.

Ability model

Salovey and Mayer's conception of EI strives to define EI within the confines of the standard criteria for a new intelligence Following their continuing research, their initial definition of EI was revised to "The ability to perceive emotion, integrate emotion to facilitate thought, understand emotions and to regulate emotions to promote personal growth."The ability-based model views emotions as useful sources of information that help one to make sense of and navigate the social environment.

The model proposes that individuals vary in their ability to process information of an emotional nature and in their ability to relate emotional processing to a wider cognition. This ability is seen to manifest itself in certain adaptive behaviors. The model claims that EI includes four types of abilities:

1. Perceiving emotions – the ability to detect and decipher emotions in faces, pictures, voices, and cultural artifacts—including the ability to identify one's own emotions. Perceiving emotions represents a basic aspect of emotional intelligence, as it makes all other processing of emotional information possible.
2. Using emotions – the ability to harness emotions to facilitate various cognitive activities, such as thinking and problem solving. The emotionally intelligent person can capitalize fully upon his or her changing moods in order to best fit the task at hand.
3. Understanding emotions – the ability to comprehend emotion language and to appreciate complicated relationships among emotions. For example, understanding emotions encompasses the ability to be sensitive to slight variations between emotions, and the ability to recognize and describe how emotions evolve over time.
4. Managing emotions – the ability to regulate emotions in both ourselves and in others. Therefore, the emotionally intelligent person can harness emotions, even negative ones, and manage them to achieve intended goals.

The ability EI model has been criticized in the research for lacking face and predictive validity in the workplace.

Mixed models

The model introduced by Daniel Goleman focuses on EI as a wide array of competencies and skills that drive leadership performance. Goleman's model outlines four main EI constructs:

1. Self-awareness – the ability to read one's emotions and recognize their impact while using gut feelings to guide decisions.
2. Self-management – involves controlling one's emotions and impulses and adapting to changing circumstances.
3. Social awareness – the ability to sense, understand, and react to others' emotions while comprehending social awareness.
4. Relationship management – the ability to inspire, influence, and develop others while managing conflict.

Goleman includes a set of emotional competencies within each construct of EI. Emotional competencies are not innate talents, but rather learned capabilities that must be worked on and can be

developed to achieve outstanding performance. Goleman posits that individuals are born with a general emotional intelligence that determines their potential for learning emotional competencies.

Bar-On model of emotional-social intelligence (ESI)

Bar-On defines emotional intelligence as being concerned with effectively understanding oneself and others, relating well to people, and adapting to and coping with the immediate surroundings to be more successful in dealing with environmental demands. Bar-On posits that EI develops over time and that it can be improved through training, programming, and therapy.

Bar-On hypothesizes that those individuals with higher than average EQs are in general more successful in meeting environmental demands and pressures. He also notes that a deficiency in EI can mean a lack of success and the existence of emotional problems. Problems in coping with one's environment are thought, by Bar-On, to be especially common among those individuals lacking in the subscales of reality testing, problem solving, stress tolerance, and impulse control.

In general, Bar-On considers emotional intelligence and cognitive intelligence to contribute equally to a person's general intelligence, which then offers an indication of one's potential to succeed in life. However, doubts have been expressed about this model in the research literature (in particular about the validity of self-report as an index of emotional intelligence) and in scientific settings it is being replaced by the trait emotional intelligence (trait EI) model discussed below.

Trait EI model

Petrides and colleagues proposed a conceptual distinction between the ability based model and a trait based model of EI. Trait EI is "a constellation of emotional self-perceptions located at the lower levels of personality". In lay terms, trait EI refers to an individual's self-perceptions of their emotional abilities.

This definition of EI encompasses behavioral dispositions and self perceived abilities and is measured by self report, as opposed to the ability based model which refers to actual abilities, which have proven highly resistant to scientific measurement. Trait EI should be investigated within a personality framework. An alternative label for the same construct is trait emotional self-efficacy.

The trait EI model is general and subsumes the Goleman and Bar-On models discussed above. The conceptualization of EI as a personality trait leads to a construct that lies outside the taxonomy of human cognitive ability. This is an important distinction in as much as it bears directly on the operationalization of the construct and the theories and hypotheses that are formulated about it.

Mayer & Salovey's Four Branches of Emotional Intelligence:

There are four branches of EI. They are,

Perceiving Emotion - The capacity to accurately perceive emotions in the faces or voices of others. It provides the starting point for more advanced understanding of emotions.
Using Emotion to Facilitate Thought - The capacity to utilize emotion in guiding cognitive processes.

A good system of emotional input helps in the contemplation of importantmatters. **Understanding Emotions** - Emotions convey information, therefore understanding emotional messages and the actions associated with them are central to this skill.

Managing Emotions - Emotions can be managed. Once the information behind the emotion is gathered and understood, it can be regulated and managed.

NEED FOR EI:

While the definitions of EI vary in scope and depth, many believe that it is at least as important as traditional IQ which is why many companies now use EI testing to hire new staff. Here are some ways in which a high EI quotient is beneficial:

- Helps you relate well and cooperate with others in the workplace (understanding other people's emotions and motives)
- Helps you achieve your goals (cooperation with others, emotion management, maturity)
- Maintain satisfying personal relationships (by managing and balancing your emotions)
- Live a more balanced lifestyle (personal and emotion management)
- Respond to others with empathy and compassion
- Allows you to be more playful and creative (in touch with your emotions)
- Resolve conflict more easily

Two aspects of EI:

This is the essential premise of EQ: to be successful requires the effective awareness, control and management of one's own emotions, and those of other people. EQ embraces two aspects of intelligence:

- Understanding yourself, your goals, intentions, responses, behaviour and all.
- Understanding others, and their feelings.

Five domains of emotional intelligence

Goleman identified the five 'domains' of EQ as:

1. Knowing your emotions.
2. Managing your own emotions.
3. Motivating yourself.
4. Recognising and understanding other people's emotions.
5. Managing relationships, ie., managing the emotions of others.

Advantages:

* lead to improved relationships with colleagues
* help to keep yourself under control
* help to lower stress levels
* help keep you motivated
* enable you to communicate well and influence others without conflict
* enhance your standing in the eyes of your colleagues

COMPANY PROFILE

The House of Khivraj is well known in the Automobile and Real Estate Sectors in chennai. Khivraj Group has spread its activities for Four decades in diversified fields like

- Automobile Sector
- Real Estates
- Renewable Energy Sector (Green Field Operations)
- Infrastructural activities
- IT Park

The flag ship company of the Group is " KHIVRAJ MOTORS LTD ". This Company was dealing in the vehicles manufactured by Hindustan Motors Ltd for 35 years. This company was also dealing in Two and Three Wheelers of Bajaj Auto Ltd for Four decades. Now, the company has taken Dealership for Maruti Vehicles. The company has established two Car Show-rooms.Three state-of-the-art workshops with all modern hi-tech, automated facilities are offered by the company for Cars. These workshops are ideally located in centre places. The Company is proud to have an independent Body shop with modern gadgets.

The Company has diverted the Dealership for Bajaj Auto Ltd's products to its sister concerns to have clear focus in its operations. These concerns have modern show rooms and operate speciality ideal workshops for Two and Three Wheelers. The automobile turn over of the group has crossed billion rupees.The Company has promoted many Real Estate projects in CHENNAI. The founder of the Company is considered as a doyen in Real Estate, finance and automobile business. Many land mark buildings bear the proud name of "KHIVRAJ" in CHENNAI.

The Company has diverted its Real Estate business to one of its subsidiaries viz."KHIVRAJ ESTATES LTD" . This company has launched a residential township project in a plot of 2.5 acres. The Company is building around 100,000 sq.ft in this township.

The Company has erected several Wind Electric Generators to fulfill its social obligations for environment and green power. The Company's investment in this sector has crossed Rs.100 millions.

A separate infrastructure company is promoted for promoting further Wind Electric Generators and other infrastructure activities.The management of the Company vests with Shri AJIT KUMAR CHORDIA who is a young dynamic technocrat and administrator with admirable skills in different spheres and Shri BHARAT KUMAR CHORDIA a young administrator who is known for his accumen in several fields and managerial capacity. They are guided by the Company Chairman Shri

NAVARATANMULL CHORDIA who is groomed by the Founder of the Company Late Shri KHIVRAJ CHORDIA. The group companies are well managed by dedicated professionals. The flag ship company has been granted ISO 9002 Certificate for its Sales, Services and Spares operations. The group is well known for its financial strength.

The Company is proud to be associated with M/s Ashok Leyland Ltd., M.N.Dastur & Co. Ltd, Future Software Ltd, SIFY Ltd., Industrial Development Bank of India, Kotak Mahindra Ltd, ICICI, Citi Bank, Shelters, Citi Corp, and many Government Departments and undertakings besides many others in its multi-faceted activities.Now, the Khivraj Group is venturing into "Used Cars Trade" by tying up with Maruti Udyog Ltd for True Value Outlet wherein we would be dealing in buying and selling of Used Maruti Cars. The Company is poised to grow in leaps and bounds under its dynamic and able management.

RESEARCH METHODOLOGY

This chapter focuses on the ways in which the present study was carried out. This chapter covers various aspects of this research investigation like scope of the study, objectives of the study, research design, sampling technique, tools of data collection, tools used for data analysis, limitations of the study and chapterization.

3.1 Scope of the study:

The scope of the study is to understand the employees emotions in the workplace which helps to achieve the goals by managing and balancing the emotions. This study helps the management to resolve conflict more easily and suggest some ways to improve Emotional Intelligence.

3.2 Objectives of the study:

The objectives of the study are listed below,

a) To study the demographic profile of the Employees.
b) To understand the linear association between the Emotional Intelligence and Work Engagement behavior.
c) To identify the dominant variables of Emotional Intelligence which influence the Work Engagement behavior.
d) To find out the underlying dimensions of Emotional Intelligence construct.

3.3 Hypothesis:

The hypothetical statement formulated for this study is listed below:

1) To find the linear association between the Overall Emotional Intelligence behavior and Overall Work Engagement behavior.
2) To find the association between Work Experience and changing emotions
3) To find the association between Work Experience and speaking about personal problems to others.

3.4 Research Design:

The main aim of the study is to assess the Emotional Intelligence of the employees. This study aims at describing the profile of the employees, variables that influence the work engagement behavior and the emotional intelligence of the employees. The present study focuses attention on describing the above mentioned subjects. Hence the research design is descriptive in nature.

Sampling:

Since a sample population is selected which is readily available and convenient, convenience sampling technique was adopted in this study. Employees who were considered as samples were asked to fill the questionnaire. Out of 250 employees 100 were taken as sample size.

Sampling is that part of statistical practice concerned with the selection of a subset of individuals from within a population to yield some knowledge about the whole population, especially for the purposes of making predictions based on statistical inference.

Convenience sampling (sometimes known as grab or opportunity sampling) is a type of nonprobability sampling which involves the sample being drawn from that part of the population which is close to hand. That is, a sample population selected because it is readily available and convenient. It may be through meeting the person or including a person in the sample when one meets them or chosen by finding them through technological means such as the internet or through phone. This type of sampling is most useful for pilot testing.

Tools for data collection:

Both field and secondary data is collected for this study. The field data was collected from the respondents using the questionnaire which consists of various questions regarding demographic profile, emotional intelligence and work engagement behavior. Secondary data was collected from journals, previous researchers etc.

Tools used for data analysis:

Appropriate statistical technologies are used to supplement the analysis and data interpretation. Statistical techniques like chi square, Anova, factor analysis are applied with the help of SPSS. (Statistical Package for Social Studies).

3.5 Limitations of the study:

Since the study is not limited to executive levels the respond varies widely. The measurement is perceived, rather than actual, abilities. And they are susceptible to mood and social desirability biases. This study is limited only to emotional intelligence and work engagement behavior, other dimensions like family and work can also be studied.

4.1 DATA ANALYSIS AND INTERPRETATION

4.1 Correlations

Hypothesis:

H0: There is no significant linear association between the Overall Emotional Intelligence behavior and Overall Work Engagement behavior.

H1: There is significant linear association between the Overall Emotional Intelligence behavior and Overall Work Engagement behavior.

Table 4.2.1

Correlation

		OverAll Emotional Intelligence	OverAll Work Engagement
OverAllEmotionalIntelligence	Pearson Correlation	1	.620(**)
	Sig. (2-tailed)		.000
	N	100	100
OverAllWorkEngagement	Pearson Correlation	.620(**)	1
	Sig. (2-tailed)	.000	
	N	100	100

** Correlation is significant at the 0.01 level (2-tailed).

The above correlations table displays Pearson correlation coefficients, significance values, and the number of cases with non-missing values. Pearson correlation coefficients assume the data are normally distributed. The Pearson correlation coefficient is a measure of linear association between two variables.

The values of the correlation coefficient range from -1 to 1. The sign of the correlation coefficient indicates the direction of the relationship (positive or negative).

The absolute value of the correlation coefficient indicates the strength, with larger absolute values indicating stronger relationships.

The correlation coefficients on the main diagonal are always 1.0, because each variable has a perfect positive; linear relationship with itself. Correlations above the main diagonal are a mirror image of those below. The abosulte value (.620) of the correlation coefficient indicates positive linear relationship between Overall Emotional Intelligence and Overall work Engagement.

RELIABILITY STATISTICS:

Table 4.2.2

Reliability

Cronbach"s Alpha	N of items
.803	33

The alpha values were calculated to assess the internal consistency reliabilities of the Emotional Intelligence construct and Work Engagement Behavior. The results indicate a very good acceptable value of .803. Since, Item-to-total correlations exceeded 0.50 respectively by a wide margin.

4.3 CHI SQUARE TEST:

H0: There is no significant association between Work Experience and changing emotions.

H1: There is significant association between Work Experience and changing emotions.

Table 4.3.1

Chi square

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	48.181(a)	25	.004
Likelihood Ratio	43.226	25	.013
Linear-by-Linear Association	3.792	1	.051
N of Valid Cases	100		

a 28 cells (77.8%) have expected count less than 5. The minimum expected count is .16.

A low significance value of .004 which is typically below 0.05 indicates that, **there is significant association** between employee work experience and changing emotions.

Chi-Square Tests:

H0: There is no significant association between Work Experience and speaking about personal problems to others.

H1: There is significant association between Work Experience and speaking about personal problems to others.

Table 4.3.2

Chi square

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	48.568(a)	25	.003
Likelihood Ratio	35.439	25	.081
Linear-by-Linear Association	2.524	1	.112
N of Valid Cases	100		

a 31 cells (86.1%) have expected count less than 5. The minimum expected count is .12.

A low significance value of .003 which is typically below 0.05 indicates that, **there is significant association** between employee work experience and changing emotions.

4.4FACTOR ANALYSIS

Table 4.4.1
KMO and Bartlett's Test

Kaiser-Meyer-Olkin Measure of Sampling Adequacy.		.569
Bartlett's Test of Sphericity	Approx. Chi-Square	607.996
	Df	276
	Sig.	.000

The Kaiser-Meyer-Olkin Measures of sampling adequacy of 0.569 shows that the variables and the sample size of 100 were viable and feasible to run a factor analysis.

Table 4.4.2
Total variance explained

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	3.918	16.324	16.324	3.918	16.324	16.324	2.781	11.588	11.588
2	2.391	9.961	26.285	2.391	9.961	26.285	2.271	9.464	21.051
3	2.024	8.435	34.720	2.024	8.435	34.720	1.919	7.996	29.047
4	1.689	7.039	41.759	1.689	7.039	41.759	1.845	7.687	36.734
5	1.413	5.888	47.647	1.413	5.888	47.647	1.805	7.519	44.253
6	1.366	5.692	53.340	1.366	5.692	53.340	1.669	6.952	51.206
7	1.212	5.051	58.390	1.212	5.051	58.390	1.564	6.518	57.724
8	1.046	4.360	62.751	1.046	4.360	62.751	1.206	5.026	62.751
9	.972	4.050	66.800						
10	.924	3.852	70.652						
11	.819	3.412	74.064						
12	.757	3.156	77.220						
13	.722	3.010	80.230						
14	.715	2.979	83.208						
15	.623	2.597	85.806						
16	.603	2.512	88.318						
17	.525	2.189	90.507						
18	.481	2.002	92.509						
19	.430	1.791	94.300						
20	.347	1.447	95.747						
21	.342	1.426	97.174						
22	.295	1.231	98.404						
23	.205	.855	99.260						
24	.178	.740	100.000						

	Component							
	1	2	3	4	5	6	7	8
I know when to speak about my personal problems to others		.724						
When I am faced with obstacles, I remember times I faced similar obstacles and overcame them		.798						
I expect that I will do well on most things I try								
Some of the major events of my life have led me to re-evaluate what is important and not important		.742						
Emotions are one of the things that make my life worth living	.703							
I am aware of my emotions as I experience them	.629							
I expect good things to happen								
I like to share my emotions with others								.754
When I experience a positive emotion, I know how to make it last								
I arrange events others enjoy						.767		
I seek out activities that make me happy						.736		
I present myself in a way that makes a good impression on others					.580			
When I am in a positive mood, solving problems is easy for me					.532			
By looking at their facial expressions, I recognize the emotions people are experiencing								
I know why my emotions change	.713							
When I am in a positive mood, I am able to come up with new ideas	.636							
I have control over my emotions			.643					
I motivate myself by imagining a good outcome to tasks I take on			.680					
I compliment others when they have done something well							.801	

When another person tells me about an important event in his or her life, I almost feel as though I have experienced this event myself							.549	
I know what other people are feeling just by looking at them				.790				
I help other people feel better when they are down					.732			
I use good moods to help myself keep trying in the face of obstacles					.543			
I can tell how people are feeling by listening to the tone of their voice				.657				

Table 4.4.3
Rotated Component Matrix (a)

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.
a Rotation converged in 11 iterations.

FACTOR 1: Emotions are one of the things that make my life worth living, I am aware of my emotions as I experience them, I know why my emotions change, when I am in positive mood, I am able to come up with new ideas.

FACTOR 2: I know when to speak about my personal problems to others, when I am faced with obstacles, I remember times I faced similar obstacles and overcome them, Some of the major events of my life have led me to re-evaluate what is important and not important.

FACTOR 3: I have control over my emotions, I motivate myself by imagining a good outcome of task I take on.

FACTOR 4: I know what other people are feeling just by looking at them, I can tell how people are feeling by listening to the tone of their voice.

FACTOR 5: I present myself in a way that makes good impression on others, when I am in positive mood, solving problem is easy for me, I help other people feel better when they are down, I use good moods to help myself keep trying in the face of obstacles.

FACTOR 6: I arrange events that others enjoy, I seek out activities that make me happy.

FACTOR 7: I complement others when they have done something well, when another person tells me about an important event in his or her life, I almost feel as though I have Experienced this event myself.

FACTOR 8: I like to share my emotions with others.

4.5 REGRESSION ANALYSIS:

Table 4.5.1
Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.490(a)	.240	.233	.47784
2	.601(b)	.362	.348	.44027
3	.649(c)	.421	.403	.42146
4	.699(d)	.489	.468	.39793
5	.729(e)	.531	.506	.38325
6	.721(f)	.520	.499	.38592
7	.742(g)	.550	.526	.37555

The above Regression Model Summary table displays R, R Square, Adjusted R Square, and the standard error. R is the multiple correlation coefficient, is the correlation between the observed and predicted values of the dependent variables. In the model 7 the R value of .742 indicates the moderate relationship. R squared value of .550 indicates the proportion of variation in the dependent variable explained by the regression model. The R square moderate values indicate that the model fit the data satisfactorily. Adjusted R squared value of .526 attempts to correct R squared to more closely reflect the goodness of fit of the model in the population.

Table 4.5.2
ANOVA(h)

Model		Sum of squares	Df	Mean square	F	Sig.
7	Regression	16.195	5	3.239	22.965	.000(g)
	Residual	13.258	94	.141		
	Total	29.452	99			

Table 4.5.3

Coefficients

Model		Unstandardized Coefficients		Standardized Coefficients	T	Sig.
		B	Std. Error	Beta		
7	Constant	2.888	.300		9.618	.000
	I know when to speak about my personal problems to others	.155	.027	.409	5.744	.000
	I use good moods to help myself keep trying in the face of obstacles	.119	.028	.303	4.287	.000
	By looking at their facial expressions, I recognize the emotions people are experiencing	.103	.026	.284	3.944	.000
	When I am in a positive mood, I am able to come up with new ideas	.109	.029	.264	3.704	.000
	I know what other people are feeling just by looking at them	.061	.024	.176	2.514	.014

Dependent Variable: OverAllWorkEngagement

The Unstandardized coefficients are the coefficients of the estimated regression model. The t statistics can help to determine the relative importance of each variable in the model.

Multiple R = .742, F – Value = 22.965, d.f (5.93), P – Value < 0.01, R^2 Value = .550

$Y = 2.888 + .155X_1 + .119\ X_2 + .103\ X_3 + .109X_4 + .061X_5$

The above equation shows the impact of the variables of Emotional Intelligence aspects such as, I know when to speak about my personal problems to others, I use good moods to help myself keep trying in the face of obstacles,

By looking at their facial expressions, I recognize the emotions people are experiencing, When I am in a positive mood, I am able to come up with new ideas, I know what other people are feeling just by looking at them. On the Work Engagement Behavior of the employees.

On an average if the variable namely I know when to speak about my personal problems to others, there will be .155 units increase in the Overall Work Engagement Behavior when other variables are kept constant. Moreover the result of the t test confirms that the calculated partial regression coefficient, such as (.155), (.119), (.103), (.109), and (.069) are highly significant at 1 percent level and 5 percent level. Similarly the multiple R of 0.742 shows there exist a relationship of 74.2 percent between the variables of Emotional Intelligence and Overall Work Engagement. The R square value of 0.550 exhibits that, the variables of Emotional Intelligence explained a variation of 55.0 percent on the Overall Work Engagement. Finally the result of f – test signifies that the explained variation by the above said variables in the Emotional Intelligence Constant was highly significant at 1 percent level.

From the above analysis it is concluded that the variables of Emotional Intelligence namely I know when to speak about my personal problems to others, I use good moods to help myself keep trying in the face of obstacles, By looking at their facial expressions, I recognize the emotions people are experiencing, When I am in a positive mood, I am able to come up with new ideas, I know what other people are feeling just by looking at them are the dominant variables which impact on the employee Work Engagement behavior.

FINDINGS, SUGGESTIONS AND CONCLUSION

5.1 Findings:

1) There is significant association between Work Experience and changing emotions.
2) There is significant association between Work Experience and speaking about personal problems to others.
3) Through this research model & hypothesis, the study through correlation analysis proves the alternative hypothesis and rejects the null hypothesis thereby there is a significant linear association between the Overall Emotional Intelligence and Overall Work Engagement behavior.
4) There are 8 dimensions of Emotional Intelligence, where the organization have to concentrate.

5.2 SUGGESTIONS:

1) Managers need to identify those variables which influence Work Engagement behavior apart from the existing Emotional Intelligence construct variables used for this study.
2) As a managerial implication Work Experience play a crucial role in enhancing the Emotional Intelligence behavior. So future managers need to associate the variables changing emotions and speaking about personal problems to others with Work Experience while they deal with Emotional Intelligence behavior.
3) Managers can focus on the dominant variables of Emotional Intelligence construct namely, "I know when to speak about my personal problems to others, I use good moods to help myself keep trying in the face of obstacles, By looking at their facial expressions, I recognize the emotions people are experiencing, When I am in a positive mood, I am able to come up with new ideas, I know what other people are feeling just by looking at them", to enhance Work Engagement behavior.
4) The factor analysis of the study extracts eight dimensions out of 24 variables in the research reviewed Emotional Intelligence construct. So managers need to focus on these eight Emotional Intelligence dimensions when they explore Emotional Intelligence study.

5.3 CONCLUSION:

It has been found that there is a association between emotional intelligence and work engagement behavior. Hence it is very important for the organization to concentrate on Emotional Intelligence to enhance the work engagement behavior. The variables of emotional intelligence which influence the work engagement behavior should be taken into account for better performance of the employees.

References:

- *Avey, J. B., Palanski, M. E., & Walumbwa, F. O. (2011). When leadership goes unnoticed. The moderating role of follower self- esteem on the relationship between ethical leadership and follower behavior. Journal of Business Ethics, 98(4), 573–582.*
- *Bakker, A. B., & Demerouti, E. (2008). Towards a model of work engagement. Career Development International, 13(3), 209–223.*
- *Bakker, A. B., Albrecht, S. L., & Leiter, M. P. (2011). Key questions regarding work engagement. European Journal of Work and Organizational Psychology, 20(1), 4–28.*
- *Balducci, C., Schaufeli, W. B., & Fraccaroli, F. (2011). The job demands-resources model and counterproductive work behavior: The role of job-related affect. European Journal of Work and Organizational Psychology, 20(4), 467–496.*
- *Bennett, R. J., & Robinson, S. L. (2000). Development of a measure of workplace deviance. Journal of Applied Psychology, 85(3), 349–360.*
- *Biron, M. (2010). Negative reciprocity and the association between perceived organizational ethical values and organizational deviance. Human Relations, 63(6), 875–897.*
- *Brackett, M. A., Lopes, P. N., Ivcevic, Z., Mayer, J. D., & Salovey, P. (2004). Integrating emotion and cognition: The role of emotional intelligence. In D. Dai & R. J. Sternberg (Eds.), Motivation, emotion, and cognition: Integrating perspectives on intellectual functioning (pp. 175–194). Mahwah: Lawrence Erlbaum.*
- *Carmeli, A. (2003). The relationship between emotional intelligence and work attitudes, behavior and outcomes: An examination among senior managers. Journal of Managerial Psychology, 18(7/8), 788–813.*

- *Christian, M. S., Garza, A. S., & Slaughter, J. E. (2011). Work engagement: A quantitative review and test of its relations with task and contextual performance. Personnel Psychology, 64, 89–136.*
- *Cropanzano, R., & Mitchell, M. S. (2005). Social exchange theory: An interdisciplinary review. Journal of Management, 31, 874–900.*
- *Day, A. L., & Carroll, S. A. (2004). Using an ability-based measure of emotional intelligence to predict individual performance, group performance, and group citizenship behaviors. Personality and Individual Differences, 36, 1443–1458.*
- *De Clercq, D., Thongpapanl, N., & Dimov, D. (2011). A closer look at cross-functional collaboration and product innovativeness: Contingency effects of structural and relational context. Journal of Product Innovation Management, 28(5), 680–699.*
- *Devonish, D., & Greenidge, D. (2010). The effect of organizational justice on contextual performance, counterproductive work behaviors, and task performance: Investigating the moderating role of ability-based emotional intelligence. International Journal of Selection and Assessment, 18, 75–86.*
- *Edwards, J. R., & Cable, D. A. (2009). The value of value congruence. Journal of Applied Psychology, 94(3), 654–677.*
- *Eisenhardt, K. M. (1989). Agency theory: An assessment and review. Academy of Management Review, 14, 57–74.*
- *Fornell, C. R. and Larcker, F. F. (1981). Structural Equation Models with Unobservable Variables and Measurement Error. Journal of Marketing Research, 18, 39-51.*
- *Gooty, J., Gavin, M., & Ashkanasy, N. (2009). Emotions research in OB: The 19 challenges that lie ahead. Journal of Organizational Behavior, 30(6), 833–838.*
- *Hair, J. E., Anderson, R. E., Tatham, R. L., & Black, W. C.(1998). Multivariate Data Analysis. NJ: Prentice-Hall, Upper Saddle River.*
- *Hakanen, J. J., Bakker, A. B., & Schaufeli, W. B. (2006). Burnout and work engagement among teachers. Journal of School Psychology, 43, 495–513.*
- *Halbesleben, J. R. B., Harvey, J., & Bolino, M. C. (2009). Too engaged? A conservation of resources view of the relationship between work engagement and work interference with family. Journal of Applied Psychology, 94(6), 1452–1465.*
- *Hastings, S. E., & Finegan, J. E. (2011). The role of ethical ideology in reactions to injustice. Journal of Business Ethics, 100, 689–703.*
- *Hair, J., Black, W., Babin, B., and Anderson, R.(2010). Multivariate data analysis (7th ed.). Prentice-Hall, Inc. Upper Saddle River, NJ, USA.*
- *Hayes, A. F. (2013). Introduction to Mediation, Moderation, and Conditional Process Analysis: A Regression-Based Approach. New York, NY: Guilford Press.*
- *Hayes, A. F. (2015). An Index and Test of Linear Moderated Mediation. Multivariate Behavioral Research, 50, 1–22.*
- *Hulland, J. S. (1999). Use of Partial Least Squares (PLS) in Strategic Management Research: A Review of Four Recent Studies. Strategic Management Journal, 20(4), 195–204.*
- *Jelinek, R., & Ahearne, M. (2010). Be careful what you look for: The effect of trait competitiveness and long hours on salesperson deviance and whether meaningfulness of work matters. Journal of Marketing Theory and Practice, 18(4), 303–321.*
- *Jöreskog, K. G. and Söbom, D. (1993). LISREL 8: Structural Equation Modeling with the SIMPLIS Command Language. Chicago: Scientific Software International. Kahn, W. A. (1990). Psychological conditions of personal engagement and disengagement at work. Academy of Management Journal, 33, 692–724.*
- *Kristof-Brown, A. L., & Stevens, C. K. (2001). Goal congruence in project teams: Does the fit between members' personal mastery and performance goals matter? Journal of Applied Psychology, 86, 1083–1095.*
- *Kristof-Brown, A. L., Zimmerman, R. D., & Johnson, E. C. (2005). Consequences of individuals' fit at work: A meta-analysis of person–job, person–organization, person–group, and person– supervisor fit. Personnel Psychology, 58, 281–342.*
- *Lian, H., Ferris, D. L., & Brown, D. J. (2012). Does taking the good with the bad make things worse? How abusive supervision and leader–member exchange interact to impact need satisfaction and organizational deviance. Organizational Behavior and Human Decision Processes, 117, 41–52.*
- *Lindebaum, D., & Jourdan, P. J. (2012). Positive emotions, negative emotions, or utility of discrete emotions. Journal of Organizational Behavior, 33(7), 1027–1030.*
- *Lyons, B. J., & Scott, B. A. (2012). Integrating social exchange and affective explanations for the receipt of help and harm: A social network approach. Organizational Behavior and Human Decision Processes, 117, 66–79.*
- *Matthews, G., Zeidner, M., & Roberts, R. (2004). Emotional intelligence: Science and myth. Cambridge: MIT Press.*
- *Mitchell, M. S., & Ambrose, M. L. (2007). Abusive supervision and workplace deviance and the moderating effects of negative reciprocity beliefs. Journal of Applied Psychology, 92(4), 1159–1168.*

- *Murray, J. Y., Kotabe, M., and Zhou, J. N. (2005). Strategic Alliance-Based Sourcing and Market Performance: Evidence from Foreign Firms Operating in China. Journal of International Business Studies, 36(2), 187–208.*
- *Podsakoff, P. M., MacKenzie, S. B., Lee, J. Y., & Podsakoff, N. P. (2003). Common Method Biases in Behavioral Research: A Critical Review of the Literature and Recommended Remedies. Journal of Applied Psychology, 88(5), 879–903.*
- *Rich, B. L., LePine, J. A., & Crawford, E. R. (2010). Job engagement: Antecedents and effects on job performance. Academy of Management Journal, 53(3), 617–635.*
- *Robinson, S. L., & Bennett, R. J. (1995). A typology of deviant workplace behaviors: A multidimensional scaling study. Academy of Management Journal, 38, 555– 572.*
- *Rotundo, M., & Sackett, P. R. (2002). The relative importance of task, citizenship, and counterproductive performance to global ratings of job performance: A policycapturing approach. Journal of Applied Psychology, 87, 66–80.*
- *Salovey, P., & Grewal, D. (2005). The science of emotional intelligence. Current Directions in Psychological Science, 14, 281–285.*
- *Schaufeli, W. B., & Bakker, A. B. (2004). Job demands, job resources, and their relationship with burnout and engagement: A multi-sample study. Journal of Organizational Behavior, 25, 293–315.*
- *Schaufeli, W. B., Bakker, A. B., & Salanova, M. (2006). The measurement of work engagement with a brief questionnaire: A cross-national study. Educational and Psychological Measurement, 66, 701–716.*
- *Schaufeli, W. B., Salanova, M., Gonzalez-Roma, V., & Bakker, A. B. (2002). The measurement of engagement and burnout: A two- sample confirmatory factor analytic approach. Journal of Happiness Studies, 3, 71–92.*
- *Sonnentag, S. (2003). Recovery, work engagement, and proactive behavior: A new look at the interface between nonwork and work. Journal of Applied Psychology, 88(3), 518–528.*
- *Thau, S., & Mitchell, M. S. (2010). Self-gain or self-regulation impairment? Tests of competing explanations of the supervisor abuse and employee deviance relationship through perceptions of distributive justice. Journal of Applied Psychology, 95, 1009–1031. Van Vianen, A. E. M. (2000). Person–organization fit: The match between newcomers' and recruiters' preferences for organizational cultures. Personnel Psychology, 53, 113–149. Vancouver, J. B., & Schmitt, N. W. (1991). An exploratory examination of person organization fit: Organizational goal congruence. Personnel Psychology, 44, 333–352. Vancouver, J. B., Millsap, R. E., & Peters, P. A. (1994). Multilevel analysis of organizational goal congruence. Journal of Applied Psychology, 79, 666–679.*
- *Witt, L. A. (1998). Enhancing goal congruence: A solution to organizational politics. Journal of Applied Psychology, 83, 666–674.*
- *Wong, C. S., & Law, K. S. (2002). The effects of leader and follower emotional intelligence on performance and attitude: An exploratory study. The Leadership Quarterly, 13, 243–274.*
- *Yang, J., & Diefendorff, J. (2009). The relations of daily counter- productive workplace behavior with emotions, situational antecedents, and personality moderators: A Diary Study in Hong Kong. Personnel Psychology, 62, 259–295.*
- *Zeidner, M., Matthews, G., & Roberts, R. D. (2004). Emotional intelligence in the workplace: A critical review. Applied Psychology: An International Review, 53(4), 371–399.*
- *Zeidner, M., Matthews, G., & Roberts, R. D. (2006). Emotional intelligence, coping with stress, and adaptation. In J. Ciarrochi, J. P. Forgas, & J. D. Mayer (Eds.),*
  Emotional intelligence in everyday life (pp. 100–125). New York: Psychology Press.

- *Zoghbi-Manrique-de-Lara, P. (2010). Do unfair procedures predict employees' ethical behavior by deactivating formal regulations? Journal of Business Ethics, 94(3), 4*

Received: 24 July 2020 | Revised: 7 September 2020 | Accepted: 12 September 2020

DOI: 10.1111/and.13862

Check for updates

ORIGINAL ARTICLE

First International Journal of Andrology ANDROLOGIA WILEY

Role of aposomes and epididymosomes in sperm quality control: A light and transmission electron microscopic study in an experimental rat model

Kunnathodi Faisal[1] | Mohammad Abdulkader Akbarsha[1,2]

[1]Department of Animal Science, Bharathidasan University, Tiruchirappalli, India

[2]Department of Biotechnology, National College (Autonomous), Tiruchirappalli, India

Correspondence
Mohammad Abdulkader Akbarsha, Research Coordinator & Department of Biotechnology, National College (Autonomous), Tiruchirappalli, 620 001, India.
Email: akbarbdu@gmail.com

Present address
Kunnathodi Faisal, Scientific Research Center, Prince Sultan Military Medical City, Riyadh, Kingdom of Saudi Arabia

Funding information
Department of Science and Technology, New Delhi, Government of India, Grant/Award Number: SR/SO/AS-59/2004

Abstract

The epididymis responds to adverse conditions of misshapen spermatozoa resulting from pathological changes or toxic insults by secretion of a dense matrix that segregates the latter for complete disintegration and dissolution. The objective of this study was to find the source of this matrix and the role-player of disintegration and dissolution of misshapen spermatozoa. We chose Wistar strain male rat model to tackle this issue, and the rats were administered with aflatoxin B1 for 55 days so as to increase the incidence of misshapen spermatozoa. At the end of the treatment, different segments of epididymis were processed for microscopic observations. We found that parallel with abundant misshapen spermatozoa in the epididymis the principal cells of the initial segment secrete enormous membrane-bound apical blebs called aposomes, which contain epididymosomes. The aposomes were found to coalesce so as for the content to merge and form a dense matrix that entangles the misshapen spermatozoa and segregates them from viable spermatozoa. The epididymosomes associate with the misshapen spermatozoa, and the latter is processed to disintegration and total dissolution. Therefore, we assign the role of segregation of misshapen spermatozoa from viable ones to the dense matrix of aposomes and their disintegration and dissolution to the epididymosomes.

KEYWORDS

apocrine secretion, aposome, dense matrix, epididymosome

1 | INTRODUCTION

Though apparently simple, the mammalian epididymis, formed of a single long convoluted and contorted ductus epididymidis, is in reality an amazingly complex organ (Cornwall, 2009). This complexity is contributed by the heterogeneity of epithelial cell types, consisting of principal, clear, narrow, apical, basal and halo cells (de Souza et al., 2018), and also its antero-posterior differentiation into anatomical [head (caput), body (corpus) and tail (cauda)], and histological and functional segments viz. initial segment, intermediate zone [not reported in human], caput, corpus and cauda (Robaire & Hermo, 1988). Adding to the complexity, the individual cell types perform separate and integrated functions (Cornwall, 2009). The major role attributed to the epididymal epithelium is secretion of several proteins, in a generally segment-specific manner, which contribute greatly to post-testicular physiological maturation of spermatozoa into motility and fertilising ability (Dacheux & Dacheux, 2002). There are other roles such as receptor-mediated and fluid-phase endocytosis of proteins and solutes, maintenance of the continually changing microenvironment (Hinton & Palladino, 1995), preventing reactive oxygen damage to the spermatozoa (Cornwall, 2009), detoxification of harmful substances, immune surveillance of sperm antigens and sperm transport from caput to cauda and then to the vas deferens during ejaculation

 © 2020 Wiley-VCH GmbH

(Harper, 1994). The cauda epididymidis further provides for the storage of spermatozoa until ejaculation (Akbarsha et al., 2015; Jainudeen & Hafez, 2000).

Under normal physiological conditions, there are many spermatozoa that are deformed/defective/dead due to disrupted spermatogenesis and post-testicular sperm processing mechanisms (Menkveld, 2010). The incidence of misshapen spermatozoa increases several folds in the context of pathological manifestations and/or toxic insults (Nixon et al., 2019), including in the human at times of infertility/subfertility (unpublished observation). In this context, the epididymis, in its versatility, is endowed with a sperm quality control mechanism by the way of secretion of a dense material that segregates the sperm cargo for complete disintegration and liquefaction, so as to safeguard the normal/viable spermatozoa (Faisal & Akbarsha, 2008), perhaps from the hostile environment of reactive oxygen species (Sullivan et al., 2005). Our aim was to find the source of the dense material and the critical role-player in the disintegration and liquefaction of the misshapen spermatozoa, for which we used an experimental animal model, the rat, induced into plenty of misshapen spermatozoa by treatment with aflatoxin B1 (Faisal & Akbarsha, 2008; Faisal et al., 2008), although untreated animals would also have a few-to-many misshapen spermatozoa (Cooper & Hamilton, 1977).

The epididymal epithelium, especially in the caput region, besides the classical exocrine secretory process, contributes to apocrine secretion as well (Hermo & Jacks, 2002). This secretory pathway involves formation of apical cytoplasmic blebs that detach and arrive at the luminal compartment. The apical blebs, the aposomes, disintegrate and liberate their content which includes small membrane-bound vesicles, the epididymosomes (Hermo & Jacks, 2002; Sullivan, 2015; Sullivan & Saez, 2013). Epididymosomes have a roughly spherical shape and a bi-layer membrane, and they are heterogeneous in size and content. Their size varies from 50 to 500 nm in diameter and are rich in cholesterol:lipid ratio (Frenette et al., 2003), and show complex electrophoretic patterns of associated proteins (Nixon et al., 2019; Sullivan et al., 2005). Epididymosomes are enriched in sphingomyelin and polyunsaturated membranous fatty acids, especially in arachidonic acids, in mice (Rejraji et al., 2006). Epididymosomes have been described in an increasing number of mammals, including human (Frenette et al., 2005), mouse (Rejraji et al., 2002), horse (Arienti et al., 1998), sheep (Gatti et al., 2005), chimpanzee (Frohlich & Young, 1996), hamster (Yanagimachi et al., 1985), bull (Frenette et al., 2002), cat (Rowlison et al., 2018) and rat (Belleannée, 2015). The functional maturation of the spermatozoa is accomplished *via* extensive crosstalk between the spermatozoa and the epididymal luminal content, and distinctly occurs in the complete absence of de novo gene transcription or protein translation (de Souza et al., 2018; Zhou et al., 2018). Therefore, it is ideal to implicate epididymosomes as the complex trafficking machinery of enzymes, structural proteins, immunological proteins, chaperones, cytokines and small noncoding RNAs (Reilly et al., 2016; Sharma et al., 2016; Sullivan & Mieusset, 2016; Zhou et al., 2017). Proteins of epididymosomes are selectively transferred to spermatozoa (Frenette et al., 2002; Frenette & Sullivan, 2001). Some of these proteins are GPI-anchored (Frenette & Sullivan, 2001) and have been shown to be essential for the acquisition of sperm-fertilising ability (Sullivan et al., 2005; Yanagimachi et al., 1985), whereas others have been suggested to modulate the motility of maturing spermatozoa (Frenette et al., 2005, 2006) or protection from oxidative stress (Frenette et al., 2005, 2006).

We serendipitously found lead for the dense material in which misshapen spermatozoa are embedded to contain epididymosomes. This study traces adopting light and transmission electron microscopy (TEM), epididymal epithelial aposomes to contribute to the dense material and the epididymosomes present in the aposomes to contribute to the disintegration/dissolution of the misshapen spermatozoa.

2 | MATERIALS AND METHODS

The methodology in respect of the animal study was essentially the same as described previously (Agnes & Akbarsha, 2003; Faisal & Akbarsha, 2008; Faisal et al., 2008). Briefly, ninety-day-old Wistar strain male rats, raised from a stock obtained from Indian Institute of Science, Bangalore, Karnataka, India, were used and fed on aflatoxin-free pellet feed (Sai Durga Feeds & Foods) and water ad libitum. Aflatoxin B1 (AFB1) was obtained from Sigma Chemical Company. The toxin was dissolved in a minimum quantity of ethanol and diluted in olive oil. The preparation was administered to 10 rats in the experimental group, through *intramuscular* route, at a daily dose of 20 μg/kg body weight (Agnes & Akbarsha, 2001, 2003; Faisal et al., 2008) for 55 days, the duration of one spermatogenic cycle (Clermont, 1962). This treatment is known to affect spermatogenesis and viability of epididymal spermatozoa and thus increase the load of misshapen spermatozoa (Faisal & Akbarsha, 2008; Faisal et al., 2008). Control rats of equal number received the vehicle. At the end of the experiment, control and treated rats were transcardially perfused with Karnovsky fluid under pentothal sodium anaesthesia and dissected to remove the epididymides.

Thin slices of different segments of the epididymis (initial segment, intermediate zone, caput, corpus and cauda) were fixed in 2.5% glutaraldehyde in cacodylate buffer and post-fixed in 1% osmium tetroxide. After thorough wash in the buffer, the tissue slices were dehydrated in a graded series of ethanol, cleared in propylene oxide and embedded in thin viscosity resin (Araldite CY212, SPI-Chem™). Semithin sections (1 μm thick) obtained in a Leica ultramicrotome were stained with Toluidine Blue-O (TBO; pH 4.4) for observation in a Carl Zeiss research microscope. Photomicrographs were obtained in a CCD camera (Sony) connected to a computer loaded with AxioVision (Carl Zeiss) software. To measure the size of the aposomes and epididymosomes, 100 randomly selected aposomes/epididymosomes, circular in profile, from multiple sections, were measured using AxioVision software and represented as mean ± *SD*. The areas of interest were chosen from the semithin sections and

subjected to ultrathin sectioning in the same microtome. The sections were stained with 0.1% lead citrate and 6% uranyl acetate. The sections were analysed in a transmission electron microscope (Philips 201C), and images of interest were photographed. The animal experiment was approved by the Institutional Animal Ethics Committee of Bharathidasan University.

3 | RESULTS

While exploring meticulously through the tissue sections, we found principal cells of initial segment and intermediate zone of epididymis to associate with roughly elongated to spherical electron-lucent bodies of size 3.9 ± 2 μm (Mean ± *SD*; $n = 100$), less profuse in the control rats (Figure 1a,b) compared to treated rats (Figure 1c–f), and plenty of such bodies were also seen in the lumen such as to almost fill the latter. These are known in the literature as aposomes (Rejraji et al., 2006). The aposomes present in the lumen of the epididymis and were found loosely distributed; spermatozoa were found to lie scattered amongst the aposomes (Figure 1a,f). The aposomes provided evidence of merger or coalition (Figure 1d,e) to form into conglomerates (Figure 1f). Thus, the aposomes tended to lose their identity and form the dense matrix. The spermatozoa and cell debris were seen entangled in the dense matrix (Figure 1f). The darkly stained narrow cells were protruding deep into the lumen, and some of these protrusions were appearing as pinching off such as to lie free among the luminal aposomes (Figure 1c–e).

Transmission electron microscopic observation confirmed the spherical membrane-bound aposomes in the epididymal lumen, and in most cases, spermatozoa were found scattered among the aposomes (Figure 2a). Looking into the aposomes, at high magnifications, it was seen that they contained homogeneous spherical bodies of approximately 130 nm size (Figure 2a–d). These are the epididymosomes known in the literature (Rejraji et al., 2006). The densities of epididymosomes in the aposomes were reflecting different grades, compact (Figure 2b), moderate (Figure 2c) and scattered (Figure 2d). Incidentally, an evidence that the aposomes are produced as blebs of epididymal epithelial cells could be seen in the aposomes containing cell inclusions such as mitochondria (Figure 2b–d).

The spermatozoa embedded in the dense matrix had undergone disintegration and/or dissolution (Figures 3a,b, 4a–d and 5) to different grades. There were also normal and intact spermatozoa outside the boundary of the dense matrix (Figures 3a,b and 4a–c). The sperm chromatin and outer dense fibrils are apparently tough to

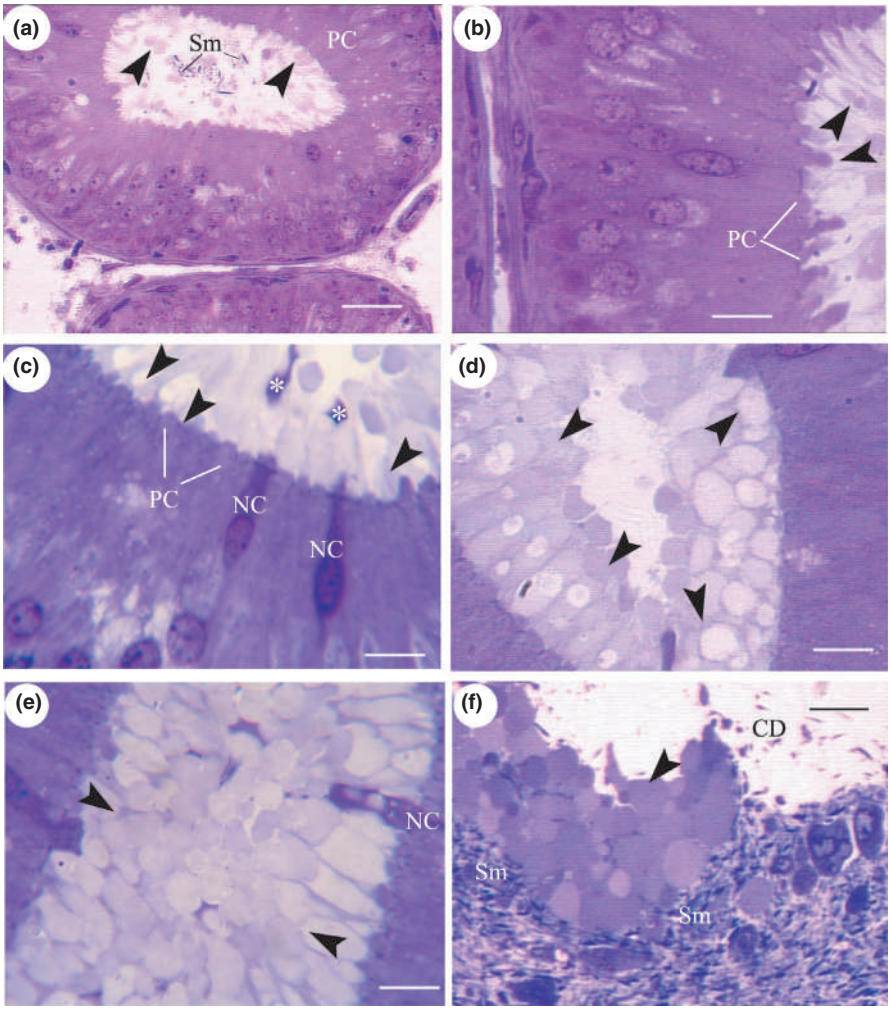


FIGURE 1 TBO-stained semithin sections of initial segment of rat epididymis. (a, b) Control rat. Principal cells (PC) secrete fewer aposomes (arrowhead); the lumen, clearly visible, contains the discharged aposomes (arrowhead) and spermatozoa (Sm). (c–f) AFB1-treated rat. (c–e) Principal cells (PC) show evidence of secretion of aposomes profusely (lightly stained; arrowheads) into the lumen, such as to almost obliterate the latter; narrow cells (NC), with apical protrusions, and their portions lying in the lumen (asterisks), are seen in (c). (f) The aposomes appear to associate among themselves by merger and form into conglomerates (arrowhead). The spermatozoa (Sm) and cell debris (CD) can be seen entangled in the dense matrix. Scale bar (a): 2.2 μm; (b–f): 1.1 μm (TBO: Toluidine Blue-O; AFB1: aflatoxin B1)

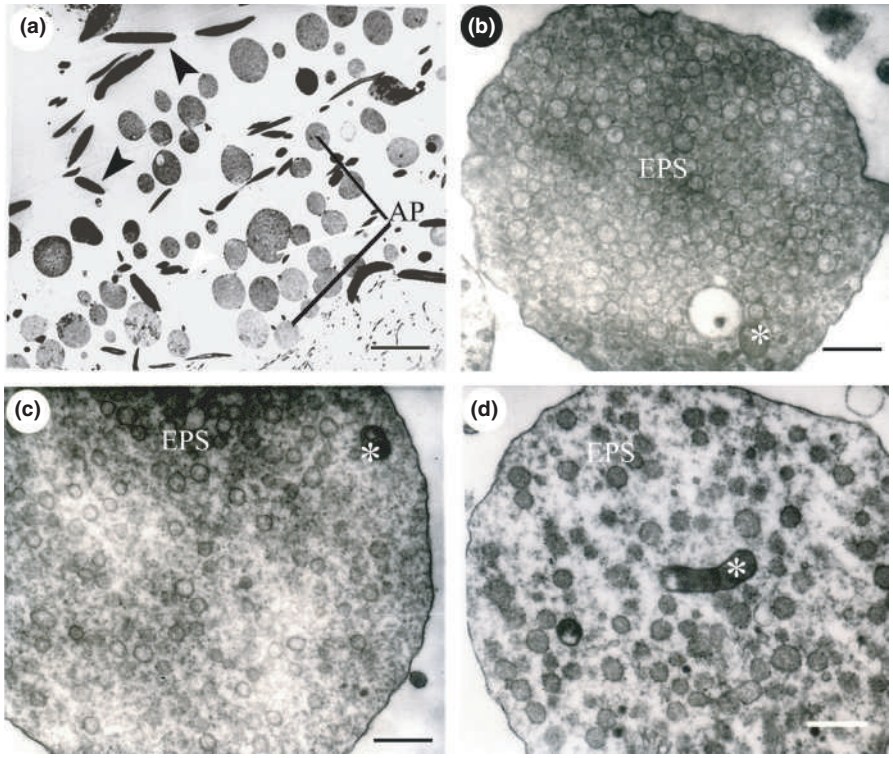


FIGURE 2 AFB1-treated rat. TEM of initial segment of epididymis. (a) Aposomes (AP) are abundantly present in the epididymal lumen, and spermatozoa (arrowheads) are scattered among them. (b–d) The aposomes contain homogenous spherical bodies, epididymosomes (EPS), which contain cell inclusions such as mitochondria (asterisks); the densities of epididymosomes in the aposomes reflect different grades, compact (b), moderate (c) and scattered (d). Scale bar (a): 6.2 μm; (b, c): 260 nm; (d): 210 nm (TEM: transmission electron micrographs)

disintegrate/dissolve (Figures 3a,b and 4a,b). But, the spermatozoa gave impression that they ultimately undergo total dissolution such as to be present as a heterogeneous material in clear vacuole-like spaces (Figure 4c,d) or as electron-dense/electron-lucent densities comparable in appearance to lipofuscin inclusions (Figure 5).

Epididymosomes were found in the dense matrix that had entangled the spermatozoa that indicate dissolution/disintegration and were seen even to be in direct contact with the spermatozoa/sperm cargo (Figure 5).

4 | DISCUSSION

Epididymosomes are known in the literature and have been reported in a variety of mammalian species, including man, as mentioned elsewhere. However, this is the first time a newer role to epididymosomes in epididymal sperm quality control is attributed, apart from their known role in protein and other content transfer to sperm surface towards its post-testicular physiological maturation (Nixon et al., 2019). We have produced evidence for the profuse discharge of epididymosomes in large membrane-bound vesicles when the lumen abounds with misshapen spermatozoa as in the context of aflatoxin toxicity, more profuse than in the control rats, and this coincides with the abundance of matrix-entangled spermatozoa in the epididymal lumen.

Yanagimachi et al. (1985) were the first to describe adopting electron microscopy, membranous vesicles and the epididymosomes, in the hamster epididymal lumen, that interact with the sperm surface, and these authors suggested that the said vesicles are involved in sperm plasma membrane cholesterol transport. The epididymal principal cells occasionally produce material in a granular form and release them by lysis of their apical membrane at the instances of toxic infliction (Agnes & Akbarsha, 2001; Akbarsha et al., 2015). These are adaptations involving structural changes mostly associated with specific functional attributes.

Rejraji et al. (2006) analysed apocrine secretion activity in the five segments of mouse epididymal epithelium and observed the formation of vesicular structures, affirming an earlier observation (Hermo & Jacks, 2002). The latter authors observed the principal cells to emit apocrine protrusions along the entire epididymal tract, but very prominently in caput epididymidis, as in the present study. In agreement with these observations, the proximal epididymis (i.e. segments I–III of the caput) was very active in apocrine secretions. However, contrary to what was reported initially by these authors, it was later found that the corpus and cauda epididymides were only very poorly active (Rejraji et al., 2006). Free vesicles resulting from blebs emitted by principal cells were also observed in the epididymal lumen. The content of the smaller vesicles was observed to contain uniformly distributed electron-dense granular material, whereas the larger vesicles looked rather empty. Even though a cytoplasmic continuity between these vesicles and epithelial cell cytosol was noticed during the apocrine secretion, the content of both these entities greatly differed from each other. A few of the vesicles had granular material aggregated in the vicinity of their membrane. Thus, their ultrastructure was almost the same as that observed for human prostasomes or prostasome-like vesicles in rat epididymis, stallion semen or bovine seminal vesicles (Arienti et al., 1998; Minelli et al., 1998). We presume that these variations in the ultrastructure, including those observed in our study, are in accordance with the heterogeneity of epididymosomes in terms of their encapsulation and interaction, discussed elsewhere.

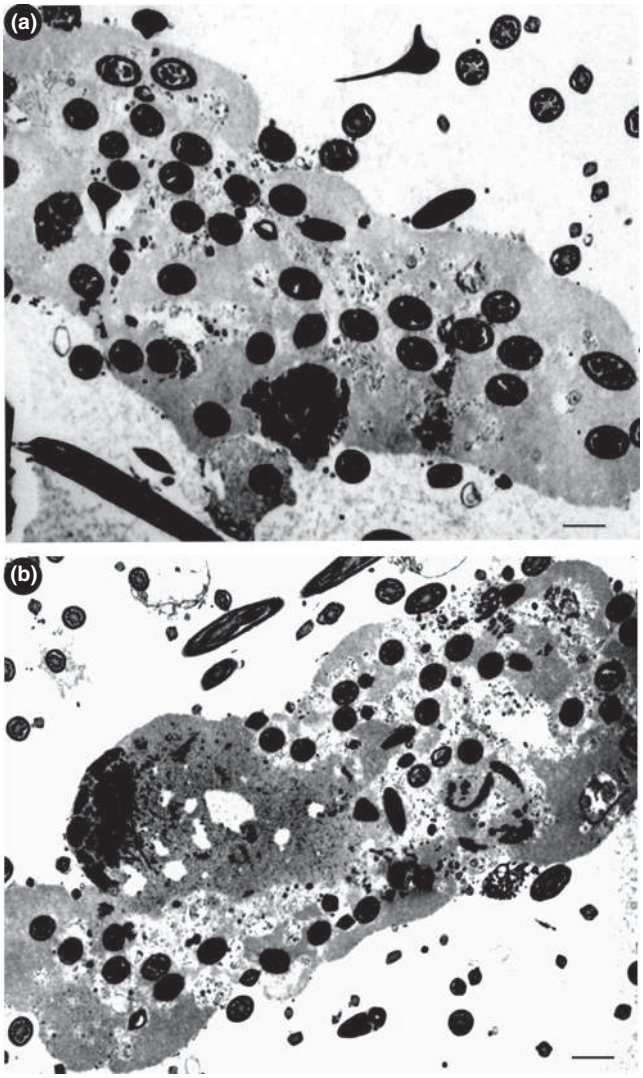


FIGURE 3 AFB1-treated rat. (a–b) TEM of dense matrix present in the caput epididymidal lumen and the spermatozoa embedded in it can be seen to undergo disintegration/dissolution to different extents. Normal spermatozoa can be seen outside the boundary of the dense matrix. Scale bar (a): 1.5 μm; (b): 2.1 μm (TEM: transmission electron micrographs)

In the bovine, two heterogeneous populations of epididymosomes have been characterised, each possessing the capacity to differentiate their investment between live and dead spermatozoa (Baskaran et al., 2020; Frenette et al., 2010). One such population is defined by their smaller diameter (~10–100 nm) and an abundance of CD9 protein (Grigor'eva et al., 2017). This population of epididymosomes exhibits preferential interaction with live spermatozoa, thus implicating them in sperm maturation/storage (Caballero et al., 2013). The other epididymosome population lacks CD9, but is loaded with epididymal sperm-binding protein 1 (ELSPBP1) and has a proclivity to interact with dead or dying spermatozoa through ELSPBP1/BLVRA (biliverdin reductase) complex (Akbarsha et al., 2015; Baskaran et al., 2020; de Souza et al., 2018; Sullivan, 2015). It is assumed that this difference in the markers may also correspond to the difference in granularity observed in the present study. The acquisition of ELSPBP1/BLVRA complex by the maturating spermatozoa from epididymosomes enables tagging of spermatozoa that must be eventually expelled, while protecting the live spermatozoa from the detrimental effects of dead/dying spermatozoa (Sullivan, 2015). Therefore, the role of epididymosomes in sperm quality control is almost well established and perhaps it is wielded via ELSBP1-rich population of epididymosomes and through the dense matrix-associated dissolution and disintegration. Nevertheless, ELSPBP1 is not the first protein known to be associated with the misshapen spermatozoa.

Proteomic analysis of human epididymosomes identified a spectrum of 146 large molecular weight proteins of varied functions including wads of proteins involved in the elimination of defective spermatozoa (Thimon et al., 2008). Amongst the various identified epididymosomal proteins, those involved in the elimination of defective spermatozoa include programmed cell death 6 (PDCD6) (Thimon et al., 2008), encoded by apoptosis-linked gene 2 (ALG/2), which is concerned with apoptosis (Vito et al., 1996). Yet another protein, Annexin V, present in the epididymosomes (Thimon et al., 2008) is also concerned with apoptosis (Gorczyca et al., 1998) and for segregation of functional spermatozoa from defective ones (Hoogendijk et al., 2009).

Ubiquitin is also associated with the epididymosomes in humans and bovine, and binds preferentially to the surface of defective spermatozoa. This has been reported in men, bulls, rhesus monkeys and mice (Sutovsky et al., 2001). The percentage of defective spermatozoa decreases as the spermatozoa descend through the epididymal passage, because of the disposal of such spermatozoa along with the ubiquitinated cytoplasmic droplets by the epididymal epithelium (Faisal & Akbarsha, 2016; Roussel et al., 1967). Thus, among its many functions, ubiquitin plays a role in extra-lysosomal protein degradation also (Robaire & Hermo, 1988). Glutathione peroxidase type 5 (GPX5) is an enzyme secreted in association with epididymosomes, and it protects the spermatozoa from oxidative stress and preserves DNA integrity during epididymal transit (Rejraji et al., 2002). This incarnates a pivotal role for epididymosomes in recognition and elimination of defective spermatozoa. Thus, in the light of earlier studies and based on the protein content of epididymosomes, there exists an alternative pathway for the elimination of misshapen spermatozoa modulated via epididymosomal interaction, that is in the dense matrix of the epididymal lumen.

Cooper and Hamilton (1977) were the first to describe and discuss in detail a 'flocculent material' or 'dense matrix' that entangles disintegrating/dissolving spermatozoa in untreated rat, mouse, hamster and guinea pig, supported by exquisite electron micrographs, and drawing strength from several earlier publications. As in the present study, these authors also noted that when the degenerating/disintegrating spermatozoa were entangled in the dense material they were in different grades of dissolution, and the intact spermatozoa were secluded from the dense material by a clear halo. It is comprehensible from this paper that sperm disintegration/dissolution and a dense matrix in which such spermatozoa are embedded would occur in normal nonseasonal males, apart from seasonal breeders

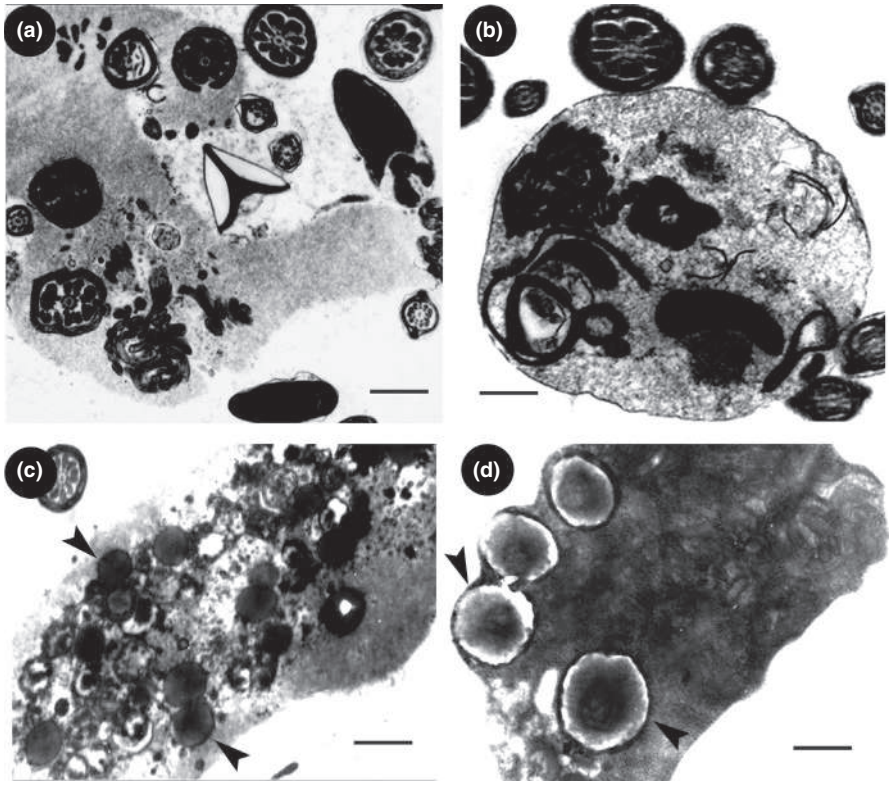


FIGURE 4 AFB1-treated rat epididymis. TEM of spermatozoa embedded in the dense matrix. The spermatozoa are in different extents of disintegration/dissolution. The liquefied material has a heterogeneous appearance (arrowheads). (a, b): caput; (c): corpus; (d): cauda. Scale bar: (a, c): 1.2 μm; (b): 2.0 μm; (d): 2.4 μm (TEM: transmission electron micrographs)

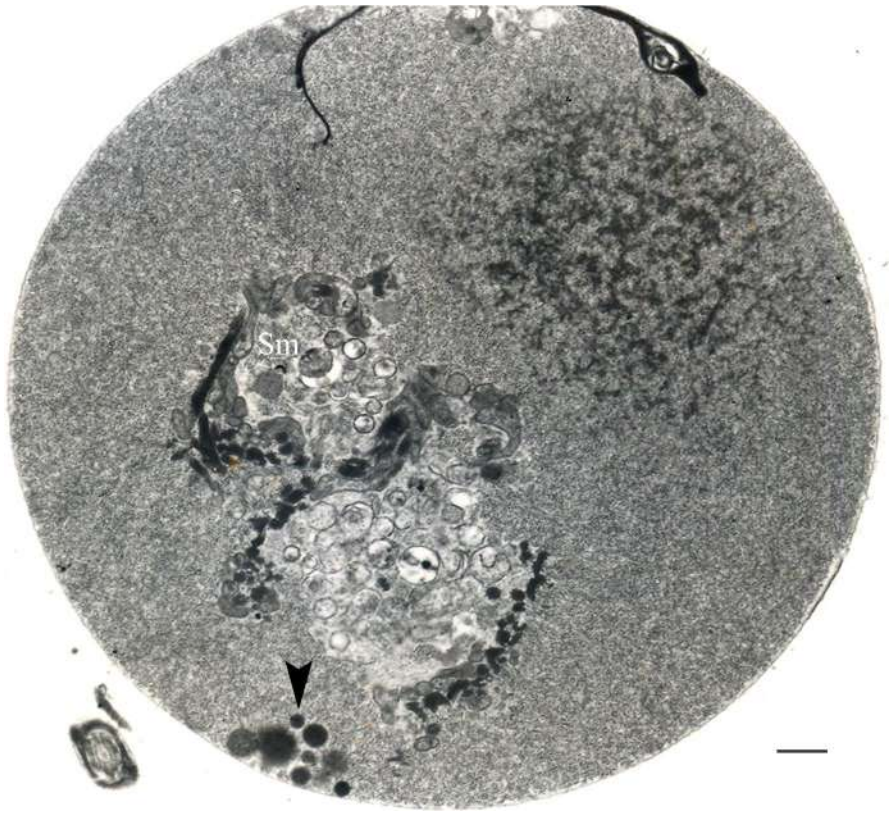


FIGURE 5 AFB1-treated rat epididymis. TEM of matrix-embedded spermatozoa, in the lumen, which have undergone almost complete disintegration and dissolution resulting in a fibrous/homogeneous dense material and a lipofuscin-like material (arrowhead). Epididymosomes are found in the dense matrix which entangles the disintegrating spermatozoa (Sm). Scale bar 80 nm (TEM: transmission electron micrograph)

and animals subjected to experiments. But this article having been published much before anything was known about aposomes and epididymosomes, there is no mention about the source of the dense material. We have bridged this gap by reporting the presence of varying numbers of abundant dense masses in the lumen, from caput epididymidis onwards. Spermatozoa were found entangled in the dense masses, and these spermatozoa were in different degrees of disintegration (Faisal & Akbarsha, 2008). Quite a few reports

support the role for a dense material in the disintegration of spermatozoa in epididymal milieu (Alexxander, 1972; Martan, 1969; Olson et al., 2004; Simeone & Young, 1931). Therefore, the dense material which in turn formed from the aposomes could be a device to mass-ubiquitinate the misshapen spermatozoa and to seclude the viable spermatozoa from the ubiquitination system, to facilitate the removal of misshapen spermatozoa.

Thus, this study links aposomes of epididymal epithelial principal cell origin to the dense matrix, which entangles the misshapen spermatozoa towards a sperm quality control mechanism, and suggests a newer pathological role for epididymosomes present in the aposomes, apart from their known physiological roles, to contribute to disintegration/dissolution of dead/defective spermatozoa. The precise biochemical and molecular mechanisms underlying disintegration/dissolution of misshapen spermatozoa by epididymosomes and how the viable spermatozoa get excluded from the dense matrix are worthy of further investigation.

ACKNOWLEDGEMENTS

The study was supported by the Department of Science and Technology (DST), Government of India, New Delhi (No. SR/SO/AS-59/2004).The instrumentation facility under the FIST scheme of DST (No.SR/FST/LSI-112/2002) and the Special Assistance Programme (SAP) of University Grants Commission (UGC), Government of India, New Delhi (No.F.3-5/2007 (SAP-II), to the Department of Animal Science, Bharathidasan University, are gratefully acknowledged. The ultracut and TEM facility of Wellcome Trust Research Centre, Christian Medical College and Hospital, Vellore, India, is heartily acknowledged.

CONFLICT OF INTEREST

The authors have no potential conflicts of interest to disclose.

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article.

ORCID

Mohammad Abdulkader Akbarsha https://orcid.org/0000-0002-4313-9606

REFERENCES

Agnes, V. F., & Akbarsha, M. A. (2001). Pale vacuolated epithelial cells in epididymis of aflatoxin-treated mice. *Reproduction*, *122*(4), 629–641. https://doi.org/10.1530/rep.0.1220629

Agnes, V. F., & Akbarsha, M. A. (2003). Spermatotoxic effect of aflatoxin B1 in the albino mouse. *Food and Chemical Toxicology*, *41*(1), 119–130. https://doi.org/10.1016/S0278-6915(02)00171-0

Akbarsha, M. A., Faisal, K., & Radha, A. (2015). The epididymis: Structure and function. In S. K. Singh (Ed.), *Mammalian endocrinology and male reproductive biology* (pp. 115–166). Boca Raton, FL: CRC Press.

Alexxander, N. J. (1972). Vasectomy: Long-term effects in the rhesus monkey. *Reproduction*, *31*(3), 399–406. https://doi.org/10.1530/jrf.0.0310399

Arienti, G., Carlini, E., De Cosmo, A. M., Di Profio, P., & Palmerini, C. A. (1998). Prostasome-like particles in stallion semen. *Biology of Reproduction*, *59*(2), 309–313.

Baskaran, S., Selvam, M. K. P., & Agarwal, A. (2020). Exosomes of male reproduction. In G. S. Makowski (Ed.), *Advances in clinical chemistry* (vol. *95*, pp. 149–163). Cambridge, MA: Elsevier.

Belleannée, C. (2015). Extracellular microRNAs from the epididymis as potential mediators of cell-to-cell communication. *Asian Journal of Andrology*, *17*(5), 730. https://doi.org/10.4103/1008-682X.155532

Caballero, J. N., Frenette, G., Belleannée, C., & Sullivan, R. (2013). CD9-positive microvesicles mediate the transfer of molecules to bovine spermatozoa during epididymal maturation. *PLoS One*, *8*(6), 1–12. https://doi.org/10.1371/journal.pone.0065364

Clermont, Y. (1962). Quantitative analysis of spermatogenesis of the rat: A revised model for the renewal of spermatogonia. *American Journal of Anatomy*, *111*(2), 111–129. https://doi.org/10.1002/aja.1001110202

Cooper, T., & Hamilton, D. (1977). Observations on destruction of spermatozoa in the cauda epididymidis and proximal vas deferens of non-seasonal male mammals. *American Journal of Anatomy*, *149*(1), 93–109. https://doi.org/10.1002/aja.1001490107

Cornwall, G. A. (2009). New insights into epididymal biology and function. *Human Reproduction Update*, *15*(2), 213–227. https://doi.org/10.1093/humupd/dmn055

Dacheux, J.-L., & Dacheux, F. (2002). Protein secretion in the epididymis. In B. Robaire & B. T. Hinton (Eds.), *The epididymis: From molecules to clinical practice* (pp. 151–168). New York, NY: Springer.

de Souza, A. P. B., Schorr-Lenz, Â. M., Lucca, F., & Bustamante-Filho, I. C. (2018). The epididymis and its role on sperm quality and male fertility. *Animal Reproduction*, *14*(Supplement 1), 1234–1244.

Faisal, K., & Akbarsha, M. A. (2008). A dense matrix of epididymal origin to process and remove defective spermatozoa: Observation in AFB1-treated rat. *Journal of Endocrinology and Reproduction*, *12*, 53–56.

Faisal, K., & Akbarsha, M. A. (2016). Spermatotoxic effect of methanol extract of *Quassia amara* L.: Impact on expression of specific genes concerned with ubiquitination-proteasome degradation pathway. *Journal of Endocrinology and Reproduction*, *20*, 123–134.

Faisal, K., Periasamy, V. S., Sahabudeen, S., Radha, A., Anandhi, R., & Akbarsha, M. A. (2008). Spermatotoxic effect of aflatoxin B1 in rat: Extrusion of outer dense fibres and associated axonemal microtubule doublets of sperm flagellum. *Reproduction*, *135*(3), 303–310. https://doi.org/10.1530/REP-07-0367

Frenette, G., Girouard, J., D'Amours, O., Allard, N., Tessier, L., & Sullivan, R. (2010). Characterization of two distinct populations of epididymosomes collected in the intraluminal compartment of the bovine cauda epididymis. *Biology of Reproduction*, *83*(3), 473–480.

Frenette, G., Légaré, C., Saez, F., & Sullivan, R. (2005). Macrophage migration inhibitory factor in the human epididymis and semen. *Molecular Human Reproduction*, *11*(8), 575–582. https://doi.org/10.1093/molehr/gah197

Frenette, G., Lessard, C., Madore, E., Fortier, M. A., & Sullivan, R. (2003). Aldose reductase and macrophage migration inhibitory factor are associated with epididymosomes and spermatozoa in the bovine epididymis. *Biology of Reproduction*, *69*(5), 1586–1592. https://doi.org/10.1095/biolreprod.103.019216

Frenette, G., Lessard, C., & Sullivan, R. (2002). Selected proteins of "prostasome-like particles" from epididymal cauda fluid are transferred to epididymal caput spermatozoa in bull. *Biology of Reproduction*, *67*(1), 308–313.

Frenette, G., & Sullivan, R. (2001). Prostasome-like particles are involved in the transfer of P25b from the bovine epididymal fluid to the sperm surface. *Molecular Reproduction and Development: Incorporating Gamete Research*, *59*(1), 115–121. https://doi.org/10.1002/mrd.1013

Frenette, G., Thabet, M., & Sullivan, R. (2006). Polyol pathway in human epididymis and semen. *Journal of Andrology*, *27*(2), 233–239. https://doi.org/10.2164/jandrol.05108

Frohlich, O., & Young, L. G. (1996). Molecular cloning and characterization of EPI-1, the major protein in chimpanzee (Pan troglodytes) cauda epididymal fluid. *Biology of Reproduction*, *54*(4), 857–864. https://doi.org/10.1095/biolreprod54.4.857

Gatti, J.-L., Métayer, S., Belghazi, M., Dacheux, F., & Dacheux, J.-L. (2005). Identification, proteomic profiling, and origin of ram epididymal fluid exosome-like vesicles. *Biology of Reproduction*, *72*(6), 1452–1465. https://doi.org/10.1095/biolreprod.104.036426

Gorczyca, W., Melamed, M. R., & Darzynkiewicz, Z. (1998). Analysis of apoptosis by flow cytometry. In T. S. Hawley & R. G. Hawley (Eds.), *Flow cytometry protocols* (pp. 217–238). New York, NY: Springer.

Grigor'eva, A. E., Dyrkheeva, N. S., Bryzgunova, O. E., Tamkovich, S. N., Chelobanov, B. P., & Ryabchikova, E. I. (2017). Contamination of exosome preparations, isolated from biological fluids. *Biomeditsinskaya Khimiya*, *63*(3), 91–96. https://doi.org/10.18097/PBMC20176301091

Harper, M. (1994). Gamete and zygote transport. *The Physiology of Reproduction*, *1*, 123–188.

Hermo, L., & Jacks, D. (2002). Nature's ingenuity: Bypassing the classical secretory route via apocrine secretion. *Molecular Reproduction and Development: Incorporating Gamete Research*, *63*(3), 394–410. https://doi.org/10.1002/mrd.90023

Hinton, B. T., & Palladino, M. A. (1995). Epididymal epithelium: Its contribution to the formation of a luminal fluid microenvironment. *Microscopy Research and Technique*, *30*(1), 67–81. https://doi.org/10.1002/jemt.1070300106

Hoogendijk, C. F., Kruger, T. F., Bouic, P. J., & Henkel, R. R. (2009). A novel approach for the selection of human sperm using annexin V-binding and flow cytometry. *Fertility and Sterility*, *91*(4), 1285–1292. https://doi.org/10.1016/j.fertnstert.2008.01.042

Jainudeen, M., & Hafez, B. (2000). Reproductive failure in males. In B. Hafez & E. S. E. Hafez (Eds.), *Reproduction in farm animals* (pp. 279–289). Baltimore, MA: Lippincott Williams & Wilkins.

Martan, J. (1969). Epididymal histochemistry and physiology. *Biology of Reproduction*, *1*(suppl_1), 134–154. https://doi.org/10.1095/biolreprod1.supplement_1.134

Menkveld, R. (2010). Clinical significance of the low normal sperm morphology value as proposed in the fifth edition of the WHO Laboratory Manual for the Examination and Processing of Human Semen. *Asian Journal of Andrology*, *12*(1), 47–58. https://doi.org/10.1038/aja.2009.14

Minelli, A., Moroni, M., Martinez, E., Mezzasoma, I., & Ronquist, G. (1998). Occurrence of prostasome-like membrane vesicles in equine seminal plasma. *Reproduction*, *114*(2), 237–243. https://doi.org/10.1530/jrf.0.1140237

Nixon, B., De Iuliis, G. N., Hart, H. M., Zhou, W., Mathe, A., Bernstein, I. R., Anderson, A. L., Stanger, S. J., Skerrett-Byrne, D. A., Jamaluddin, M. F. B., Almazi, J. G., Bromfield, E. G., Larsen, M. R., & Dun, M. D. (2019). Proteomic profiling of mouse epididymosomes reveals their contributions to post-testicular sperm maturation. *Molecular & Cellular Proteomics*, *18*(Supplement 1), S91–S108. https://doi.org/10.1074/mcp.RA118.000946

Olson, G. E., Winfrey, V. P., NagDas, S. K., & Melner, M. H. (2004). Region-specific expression and secretion of the fibrinogen-related protein, fgl2, by epithelial cells of the hamster epididymis and its role in disposal of defective spermatozoa. *Journal of Biological Chemistry*, *279*(49), 51266–51274. https://doi.org/10.1074/jbc.M410485200

Reilly, J. N., McLaughlin, E. A., Stanger, S. J., Anderson, A. L., Hutcheon, K., Church, K., Mihalas, B. P., Tyagi, S., Holt, J. E., Eamens, A. L., & Nixon, B. (2016). Characterisation of mouse epididymosomes reveals a complex profile of microRNAs and a potential mechanism for modification of the sperm epigenome. *Scientific Reports*, *6*(1), 1–15. https://doi.org/10.1038/srep31794

Rejraji, H., Sion, B., Prensier, G., Carreras, M., Motta, C., Frenoux, J.-M., & Drevet, J. R. (2006). Lipid remodeling of murine epididymosomes and spermatozoa during epididymal maturation. *Biology of Reproduction*, *74*(6), 1104–1113. https://doi.org/10.1095/biolreprod.105.049304

Rejraji, H., Vernet, P., & Drevet, J. R. (2002). GPX5 is present in the mouse caput and cauda epididymidis lumen at three different locations. *Molecular Reproduction and Development: Incorporating Gamete Research*, *63*(1), 96–103. https://doi.org/10.1002/mrd.10136

Robaire, B., & Hermo, L. (1988). Efferent ducts, epididymis, and vas deferens: Structure, functions, and their regulation. *The Physiology of Reproduction*, *1*, 999–1080.

Roussel, J., Stallcup, O., & Austin, C. (1967). Selective phagocytosis of spermatozoa in the epididymis of bulls, rabbits, and monkeys. *Fertility and Sterility*, *18*(4), 509–516. https://doi.org/10.1016/s0015-0282(16)36369-5

Rowlison, T., Ottinger, M. A., & Comizzoli, P. (2018). Key factors enhancing sperm fertilizing ability are transferred from the epididymis to the spermatozoa via epididymosomes in the domestic cat model. *Journal of Assisted Reproduction and Genetics*, *35*(2), 221–228. https://doi.org/10.1007/s10815-017-1083-3

Sharma, U., Conine, C. C., Shea, J. M., Boskovic, A., Derr, A. G., Bing, X. Y., Belleannee, C., Kucukural, A., Serra, R. W., Sun, F., Song, L., Carone, B. R., Ricci, E. P., Li, X. Z., Fauquier, L., Moore, M. J., Sullivan, R., Mello, C. C., Garber, M., & Rando, O. J. (2016). Biogenesis and function of tRNA fragments during sperm maturation and fertilization in mammals. *Science*, *351*(6271), 391–396. https://doi.org/10.1126/science.aad6780

Simeone, F. A., & Young, W. C. (1931). A study of the function of the epididymis: IV. The fate of non-ejaculated spermatozoa in the genital tract of the male guinea-pig. *Journal of Experimental Biology*, *8*(2), 163–175.

Sullivan, R. (2015). Epididymosomes: A heterogeneous population of microvesicles with multiple functions in sperm maturation and storage. *Asian Journal of Andrology*, *17*(5), 726. https://doi.org/10.4103/1008-682X.155255

Sullivan, R., & Mieusset, R. (2016). The human epididymis: Its function in sperm maturation. *Human Reproduction Update*, *22*(5), 574–587. https://doi.org/10.1093/humupd/dmw015

Sullivan, R., & Saez, F. (2013). Epididymosomes, prostasomes, and liposomes: Their roles in mammalian male reproductive physiology. *Reproduction*, *146*(1), R21–R35. https://doi.org/10.1530/REP-13-0058

Sullivan, R., Saez, F., Girouard, J., & Frenette, G. (2005). Role of exosomes in sperm maturation during the transit along the male reproductive tract. *Blood Cells, Molecules, and Diseases*, *35*(1), 1–10. https://doi.org/10.1016/j.bcmd.2005.03.005

Sutovsky, P., Moreno, R., Ramalho-Santos, J., Dominko, T., Thompson, W. E., & Schatten, G. (2001). A putative, ubiquitin-dependent mechanism for the recognition and elimination of defective spermatozoa in the mammalian epididymis. *Journal of Cell Science*, *114*(9), 1665–1675.

Thimon, V., Frenette, G., Saez, F., Thabet, M., & Sullivan, R. (2008). Protein composition of human epididymosomes collected during surgical vasectomy reversal: A proteomic and genomic approach. *Human Reproduction*, *23*(8), 1698–1707. https://doi.org/10.1093/humrep/den181

Vito, P., Lacaná, E., & D'Adamio, L. (1996). Interfering with apoptosis: Ca2+-binding protein ALG-2 and Alzheimer's disease gene ALG-3. *Science*, *271*(5248), 521–525.

Yanagimachi, R., Kamiguchi, Y., Mikamo, K., Suzuki, F., & Yanagimachi, H. (1985). Maturation of spermatozoa in the epididymis of the Chinese hamster. *American Journal of Anatomy*, *172*(4), 317–330. https://doi.org/10.1002/aja.1001720406

Zhou, W., Anderson, A. L., Turner, A. P., De Iuliis, G. N., McCluskey, A., McLaughlin, E. A., & Nixon, B. (2017). Characterization of a novel role for the dynamin mechanoenzymes in the regulation of human sperm acrosomal exocytosis. *MHR: Basic Science of Reproductive Medicine*, *23*(10), 657–673. https://doi.org/10.1093/molehr/gax044

Zhou, W., De Iuliis, G. N., Dun, M. D., & Nixon, B. (2018). Characteristics of the epididymal luminal environment responsible for sperm maturation and storage. *Frontiers in Endocrinology*, *9*, 59. https://doi.org/10.3389/fendo.2018.00059

How to cite this article: Faisal K, Akbarsha MA. Role of aposomes and epididymosomes in sperm quality control: A light and transmission electron microscopic study in an experimental rat model. *Andrologia*. 2021;53:e13862. https://doi.org/10.1111/and.13862

Hindawi
Journal of Toxicology
Volume 2021, Article ID 9578474, 12 pages
https://doi.org/10.1155/2021/9578474

Research Article

In Vivo and *In Vitro* Toxicity Profiles of Hexane Extract of *Alpinia malaccensis* Rhizome in Rat and Cell Line Models

T. Somarathna,[1,2] M. G. Thammitiyagodage,[2] K. K. D. S. Ranaweera,[3] G. A. S. Premakumara,[4] M. A. Akbarsha,[5,6] B. Kadalmani,[7,8] and N. S. Weerakkody[1]

[1]*Department of Agricultural & Plantation Engineering, Faculty of Engineering Technology, The Open University of Sri Lanka, Nawala, Sri Lanka*
[2]*Animal Centre, Medical Research Institute, No: 527, Dr Danister de Silva Mawatha, Borella, Sri Lanka*
[3]*Department of Food Science and Technology, Faculty of Applied Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka*
[4]*Deapartment of Basic Sciences & Social Science, Faculty of Nursing, University of Colombo, Colombo 3, Sri Lanka*
[5]*Mahatma Gandhi-Doerenkamp Center, Bharathidasan University, Tiruchirappalli, India*
[6]*Departmentof Biotechnology & Research Co-ordinator, National College (Autonomous), Tiruchirappalli 620001, India*
[7]*Department of Animal Science, School of Life Sciences, Bharathidasan University, Tiruchirappalli, India*
[8]*UGC National Centre for Alternatives to Animal Experiments, Bharathidasan University, Tiruchirappalli, India*

Correspondence should be addressed to N. S. Weerakkody; nweer@ou.ac.lk

Received 19 January 2020; Revised 30 July 2020; Accepted 1 January 2021; Published 18 January 2021

Academic Editor: Mahmoud Elsabahy

Copyright © 2021 T. Somarathna et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The objective of the study was to evaluate the potential toxicity of crude *n*-hexane extract of *Alpinia malaccensis* rhizome. The *in vivo* acute oral toxicity was evaluated by administering a single oral dose of the extract at 0, 300, or 2000 mg/kg body weight to female Wistar rats according to modified OECD Test Guideline 423. For the *in vitro* cytotoxicity study, A549, HepG2, 3T3, and COS-7 cell lines were exposed to different doses of *A. malaccensis* extract and cell viability was assessed adopting MTT assay followed by AO/EB staining, Hoechst staining, and comet assay with a view to compare the cellular and molecular mechanisms underlying the toxicity, if any. It was found that administration of 2000 mg/kg bw dose in *in vivo* oral acute toxicity study did not produce significant toxicity or mortality. No significant ($p < 0.05$) differences were observed for body weight and hematological and biochemical parameters compared to control after 14 days of treatment. No changes in behavior, body weight, hematological and biochemical parameters, and aspects of histopathology were observed when compared to the control. Thus, the possible oral lethal dose for *A. malaccensis* extract is above 2000 mg/kg body weight. The *in vitro* cytotoxicity analysis showed nontoxicity concentrations of the extract to be 2, 1.4, 30, and 1.4 μg/mL for A549, HepG2, 3T3, and COS-7 cells, respectively, where no apoptotic/necrotic cell death and DNA damage were observed. In conclusion, the extract of rhizome of *A. malaccensis* did not produce apparent cytotoxicity or acute oral toxicity, confirming the scope to use *A. malaccensis* as a safe food preservative and a natural therapeutic product after further subacute and chronic toxicity studies.

1. Introduction

Medicinal plants have been widely used in conventional or traditional medicinal practices as a reliable cure to diseases since prehistoric days. The World Health Organization also has recognized plants as a reliable source of therapeutics [1]. The therapeutic property of the medicinal plants lies in the bioactive compounds they contain. It is believed that the plant-derived compounds, when used as therapeutics, are less harmful compared to the synthetic drugs [2]. Thus, the medicinal plants offer great scopes to discover newer drugs. Nevertheless, surveys have indicated that many plants used in traditional medicines can potentially produce adverse effects [3, 4], and some of them have been shown to contain toxic compounds [5]. Therefore, not all medicinal plants are safe. Hence, there is pertinent need to assess the toxicity of medicinal plants to ensure safety.

Alpinia malaccensis, popularly known as Malacca ginger and "Rankihiriya," belongs to the family Zingiberaceae. It is a perennial South Asian medicinal plant cultivated widely in the tropical regions of Asia including Indo-China, Bangladesh, and Sri Lanka [6]. *A. malaccensis* has traditionally been used in the preparation of medicines to cure nausea, vomiting, and wounds and also as a seasoning ingredient in the processing of meat [7]. Somarathna et al. [8] evaluated the bioactive chemical constituents of the hexane crude extract of *A. malaccensis* rhizome and found 82.87% 1'-acetoxychavicol acetate (1'ACA) as the major active chemical compound. Crude *A. malaccensis* extract as well as the pure compound 1'ACA showed strong antibacterial and anti-biofilm activities against *Listeria monocytogenes* and *Staphylococcus aureus* [8, 9].

However, Primahana et al. [10] reported that transmethyl cinnamate found in *A. malaccensis* produces moderate toxicity to brine shrimp at 120.47 μg/mL LC_{50}. Further, Thu et al. [11] reported that fruit of *A. malaccensis* induces cytotoxicity in A549 and HepG2 cell lines at 4.8 and 5.5 μg/mL doses, respectively. It was suggested that the inconsistency of the chemical composition may be due to the differences in the plant parts used, age, variety, edaphic factors, and geographical regions where cultivated [12, 13]. Also, Chan et al. [14] reported that the rhizome accumulates more secondary chemicals than the other plant parts. Therefore, evaluation of toxicological aspects of *A. malaccensis* rhizome will be a rewarding area of research.

The present study was aimed at investigating the potential toxicity of *n*-hexane extract, which would contain the most nonpolar compounds, of rhizome of *A. malaccensis* by finding the acute *in vivo* toxicity using the rat model and *in vitro* cytotoxicity using cell lines.

2. Materials and Methods

2.1. Plant Collection and Authentication. A. malaccensis plants were collected from the medicinal garden of the Nature Secret (Pvt) Ltd, Millewa, Horana, Sri Lanka, and a voucher specimen (2012/APE/02)was deposited in the herbarium of Department of Agricultural Plantation Engineering, the Open University of Sri Lanka. The plant was identified using the key to species by Dassanayake and Forsberg [15].

2.2. Extraction of A. malaccensis. Fresh *A. malaccensis* rhizomes were washed in running water, and the outer skin was peeled off. The rhizome was sliced and oven-dried at 40°C for 24 h. The dried slices were pulverized using a grinder (National Super Blender, Taiwan, Model MX-TIIOPN) for 1 minute at 30 seconds interval in each cycle for five times. The powder was stored at −20°C until use. *n*-Hexane was used as the solvent for the extraction. The extract was prepared by adding 10 g of *A. malaccensis* powder to 100 mL of *n*-hexane and agitated (160 rpm) for 24 h at 30°C in a rotary shaker (Stuart® orbital shaker SSL1, UK). The mixture was centrifuged at 4500 ×g for 10 min (Centurion Scientific Ltd, UK), and the supernatant was filtered using #1 Whatman filter paper. The filtrate was evaporated under vacuum at 40°C using a rotary evaporator (KIA RV 5, Switzerland) and filter-sterilized through a 0.45 μm filter unit (Millex® HA, Germany). The filtrate was N_2-fluxed under heat (40°C) for 3 h until a hexane-free extract was obtained. Finally, the concentrated extract was stored at 4°C until use.

2.3. Cell Culture. Human lung carcinoma cell (A549), hepatocarcinoma cell (HepG2), normal mouse fibroblast cell (3T3), and monkey kidney tissue cell (COS-7) were obtained from the National Center for Cell Science (NCCS), Pune, India. The cells were maintained in DMEM medium supplemented with 10% fetal bovine serum (FBS, Invitrogen, USA) and with 20 mL each of penicillin and streptomycin as antibiotics, in a humidified atmosphere of 5% CO_2 and 95% air, in a CO_2 incubator (Thermo Scientific, USA) [16]. The cytotoxicity studies were conducted at Mahatma Gandhi-Doerenkamp Center for Alternatives at Bharathidasan University, Tiruchirappalli, India.

2.4. MTT Assay for Assessment of Cell Viability. Cell viability, which would reveal the cytotoxic property of the extract, was evaluated using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) colorimetric assay with slight modifications [17]. Cells were seeded in 96-well plates at 5×10^3 cells/well and incubated for 24 h at 37°C. The cells were treated with the extract at increasing concentrations within 0–1000 μg/mL for 24 h, at 37°C. The extract was quantitatively dissolved in minimum quantity of DMSO and diluted in the culture medium to prepare the stock solution, which was then made up in the culture medium so as to have the final extract at DMSO concentration of 0.1%. This concentration of DMSO is known not to affect the cell viability [18]. DMSO at this concentration was also used as the solvent control. Experiments with each extract concentration were conducted in triplicates on the same batch of cells. After 24 h incubation, 20 mL of MTT (Sigma-Aldrich, St. Louis, MO, USA) solution (5 mg/mL in PBS) was added to each well and incubated for 3 h at 37°C. The medium was then removed, and 100 mL of DMSO was added to each well to dissolve the purple formazan product. The absorbance was measured at 570 nm (measurement) and 630 nm (reference) using a 96-well plate reader (Bio-Rad, Hercules, CA, USA). The percentage inhibition was calculated from these data using the following formula, and IC_{50}, defined as concentration of the test substance at which cell viability is decreased to 50%, was calculated.

$$\text{Percentage of cell inhibition} = \frac{\text{mean OD (control)} - \text{mean OD (treatment)}}{\text{mean OD (control)}} x\ 100. \quad (1)$$

2.5. Morphological Assessment of Cell Death Using AO/EB Fluorescent Assay. The morphological characteristics of cells in respect of apoptosis and necrosis were assessed by AO/EB staining [19]. The A549, HepG2, 3T3, and COS-7 cells were cultured in 6 well plates and incubated for 24 h with IC_{50} and

no toxicity concentrations of the extract, as found in the MTT assay. The treated and untreated cells were centrifuged (3000 rpm for 4 min) and incubated with acridine orange (AO) and ethidium bromide (EB) solutions (1 part of 100 mg/mL each of AO and EB in PBS) and observed in a fluorescent microscope (Carl Zeiss, Jena, Germany) using a UV filter (450–490 nm). Three hundred cells per sample were counted, in duplicate, and scored as viable or dead, and if dead whether by apoptosis or necrosis as judged from nuclear morphology and cytoplasmic organization. Then, the percentages of apoptotic and necrotic cells were calculated. The morphological features of interest were photographed.

2.6. Assessment of Nuclear Morphological Features Using Hoechst 33528 Staining. The nuclear morphological features of the cells were assessed using Hoechst 33528 staining [20]. The cells were cultured in 6-well plates and treated with *A. malaccensis* at its IC_{50} concentration and no toxicity concentration, for 24 h. After incubation, the treated and control cells were harvested and stained with Hoechst 33258 (1 mg/mL in PBS) for 5 min at room temperature. A drop of cell suspension was placed on a glass slide and covered with a cover slip. Three hundred cells, each in triplicate, were observed at x400 in the fluorescent microscope fitted with a 377–355 nm filter. The percentage of cells reflecting pathological changes was calculated.

2.7. Genotoxicity Assessment Using Comet Assay. Genotoxicity assessment was performed using comet assay where single-cell gel electrophoresis was performed to quantify the DNA damage [21]. The cells were treated with the IC_{50} concentration and no toxicity concentration of the extract for 24 h. The harvested cells were suspended in low melting point agarose in PBS and pipetted out to microscope slides on precoated layer of normal melting point agarose. Another layer of medium melting agarose was laid on top of the low melting agarose. Slides were immersed in prechilled lysis buffer (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris, 0.2mMNaOH (pH 10), and Triton X-100) and incubated overnight at 4°C in order to lyse the cells and allow DNA unwinding. Then, the slides were exposed to alkaline buffer (300 mM NaOH, and 1 mM Na_2-EDTA (pH > 13)) for 20 min to allow DNA unwinding and then subjected to electrophoresis at 7 V. The slides were washed with neutralization buffer (0.4 M Tris, pH 7.5) for 2 min and observed in the fluorescent microscope. The triplicate data, each from one hundred and fifty cells, from each treatment group, were collected manually. The images were used to evaluate the degree of DNA damage representing the fraction of total DNA in the tail, according to Gayathri et al. [16].

2.8. Acute Oral Toxicity Study Using Wistar Rats

2.8.1. Animals. The acute oral toxicity test was carried out using female Wistar rats. The number of animals and sex used for each test were determined considering both Organization of Economic Cooperation and Development (OECD) guidelines and the opinion of the Ethics Review Committee of Medical Research Institute (2016/22), Sri Lanka, based on the 3R concept. All animals were maintained under standard laboratory conditions including 20°C to 24°C temperature, 50% to 70% relative humidity, and a light regimen of 12 h light and dark throughout the experimental period. Standard size, polycarbonate, and transparent cages were used for animal housing. Three rats from the same sex were kept in one cage. Sterilized wood shavings were used as the bedding material. All animals were fed with MRI rabbit and rat formula prepared according to WHO guideline given by Saboudry [22]. The formulations were prepared at the Medical Research Institute using locally available ingredients. All rats were acclimatized to laboratory conditions for a period of 7 days prior to dosing.

2.8.2. Acute Oral Toxicity Test. Acute oral toxicity test of the crude *n*-hexane extract of rhizome of *A. malaccensis* was carried out according to the method described in OECD Test Guideline 423. Three-month-old female Wistar rats, weighing 150–200 g, were randomly divided into three groups, control ($n = 3$) and two treatment groups ($n = 3$ each). The animals were maintained in an air-conditioned and light-controlled room with access to water and feed *ad libitum* throughout the experiment. Animals were kept fasting overnight with free access to water the day prior to starting the experiment. Animals were individually weighed. Rats in the control group were administered olive oil (1 mL). Doses of 300 and 2000 mg/kg BW of crude extract were used for the limit test. The administered volume was adjusted to 1 mL/kg BW per rat.

After administration of *A. malaccensis* extract, rats were observed for first 30 min, and first 24 h with special attention during first 4 h, and thereafter daily for 14 days. The rats were weighed and observed for signs of toxicity including mortality, changes in behavioral pattern (salivation, lethargy, and sleep), physical appearance, injury, pain, and signs of illness during the observation period. In addition, the body weight, feed intake, and water consumption were recorded throughout the experimental period. Animals were mildly sedated using gaseous anesthesia, and 1 mL of blood was collected by the lateral tail vein puncture technique for biochemical and hematological analysis. The serum creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and blood urea nitrogen (BUN) were measured with commercially available estimation kits (Pointe Scientific, Colombo) using a semiautomated biomedical analyzer (Stat Fax 3300, Ramsey, MN, USA). At the end of the experiment, the rats were humanely euthanized by overdosing gaseous anesthesia, and the liver, kidney, lung, heart, and spleen were collected. The relative organ weights of rats were recorded and also examined macroscopically. The organs were preserved in a fixation medium of 10% solution of formalin for histopathological study.

2.9. Histopathological Study. Portions of formalin-fixed liver, kidney, lung, heart, and spleen were dehydrated with alcohol, embedded in paraffin wax, cut into 4-5 μm thick sections, and stained with hematoxylin and eosin. Slides were examined in a light microscope at x40 magnification. The microscopic features of the organs of control and treated rats were compared. The study was carried out in the Veterinary Institute, Gannoruwa, Sri Lanka.

2.10. Statistical Analysis. All values are expressed as mean ± SEM. Comparisons between groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests using SPSS statistical software. A p value of <0.05 is considered significant.

3. Results

3.1. Cytotoxic Property of Crude n-Hexane Extract of A. malaccensis. The cytotoxic property of *A. malaccensis* *n*-hexane extract was analyzed against A549, HepG2, 3T3, and COS-7 cells at different concentrations to determine the respective IC_{50} by MTT assay. The IC_{50} values of different concentrations of *A. malaccensis* extract are graphically represented in Figure 1. The IC_{50} values in respect of A549, HepG2, 3T3, and COS-7 cells were 7.25, 22.5, 62.75, and 8.25 μg/mL, respectively. The extract showed 0% inhibition at concentrations 2, 1.4, 30, and 1.4 μg/mL, for A549, HepG2, 3T3, and COS-7 cells, respectively, and these concentrations are considered as nontoxic.

3.2. Microscopic Features of Apoptosis and/or Necrosis. Microscopic features of apoptosis were observed adopting AO/EB staining and are shown in Figure 2(a). In general, dead cells are permeable to EB and fluoresce red, whereas live cells are permeable to AO only and therefore fluoresce green. The viability and membrane integrity of the cells were determined based on the fluorescence pattern. The morphological changes observed in the treated cells were classified based on the fluorescence emission as follows: (i) viable cells having highly organized nuclei fluoresced green; (ii) early apoptotic cells which showed nuclear condensation fluoresced orange green; (iii) late apoptotic cells with the highly condensed chromatin or fragmented chromatin fluoresced orange to red; and (iv) necrotic cells fluoresced orange to red without chromatin fragmentation. Data on cells indicating apoptotic and necrotic morphologies, induced on treatment with the IC_{50} concentration and no toxicity concentrations of *A. malaccensis* for 24 h, and collected from manual counting are presented in Figure 2(b), which revealed that *A. malaccensis* at IC_{50} is efficient in bringing about early apoptosis (less than 50%), but little necrosis was produced. However, *A. malaccensis* at no toxicity concentration did not induce any remarkable apoptosis or necrosis (less than 10%) for the cell lines tested (Figure 2).

3.3. Microscopic Features of Nucleus. Hoechst 33528 staining was adopted to find the morphological changes in the nucleus as caused by treatment of IC_{50} and no toxicity concentrations of *A. malaccensis*, and the features are shown in Figure 3(a). Data collected from manual counting of cells with normal and abnormal nuclear features are shown in Figure 3(b). In the control cells, the nuclear chromatin was full while after treatment with the IC_{50} of the extract, changes such as chromatin marginalization, condensation, and fragmentation were noticed. These observations revealed that exposure of *A. malaccensis* to IC_{50} concentration alone led to chromatin fragmentation which is a characteristic feature of apoptosis. However, treatment with no toxicity concentration (IC_0) of the extract indicated no difference with the control.

3.4. Identification of DNA Damage as Revealed in Comet Assay. Comet assay was adopted to detect the cellular DNA lesions or genotoxicity. Comet assay is very sensitive of strand breaks in DNA. DNA damage was analyzed based on DNA tail size, shape, and migration pattern, and the data are shown in Figure 4(a). *A. malaccensis* at IC_{50} caused moderate damage to DNA. However, at the no toxicity concentration, there was only negligible damage to DNA. Since the tail length and density reflected the extent of strand breaks in DNA, the percentage of DNA in the tail provided a quantitative measure of DNA damage as shown in Figure 4(b).

3.5. Acute Oral Toxicity as Revealed in Animal Experiments. A single dose of 300 or 2000 mg/kg of *A. malaccensis* extract of the rhizome administered through oral route did not produce any mortality in rats during the 14-day observation period. Further, with regard to the behavioral patterns, rats treated 2000 mg of *A. malaccensis* extract were cleaning their face very frequently for the first 4 hours. However, after 4 h, the behavioral pattern was the same as in the control group.

3.6. Organ Weights of Tested Rats. Data on the organ weights between control and treated rats are shown in Table 1. The mean body weight of rats that received the two doses of *A. malaccensis* extract was not significantly different from the control group. The rats treated with the *A. malaccensis* extract at a dosage of 2000 mg/kg bw had liver weights significantly ($p < 0.05$) higher than control liver weight.

3.7. Biochemical Responses. Data on serum biochemical parameters of the rats are shown in Table 2. No significant difference ($p > 0.05$) in serum biochemical parameters was observed between rats treated *A. malaccensis* and control. Packed cell volume percentage (PCV%) was not significantly different between treated and control groups. Serum AST (u/L) and ALT (u/L) levels were not different between groups.

3.8. Hematological Responses. Data with regard to hematological parameters of rats are presented in Table 3. No significant difference ($p > 0.05$) was observed between treated and control groups.

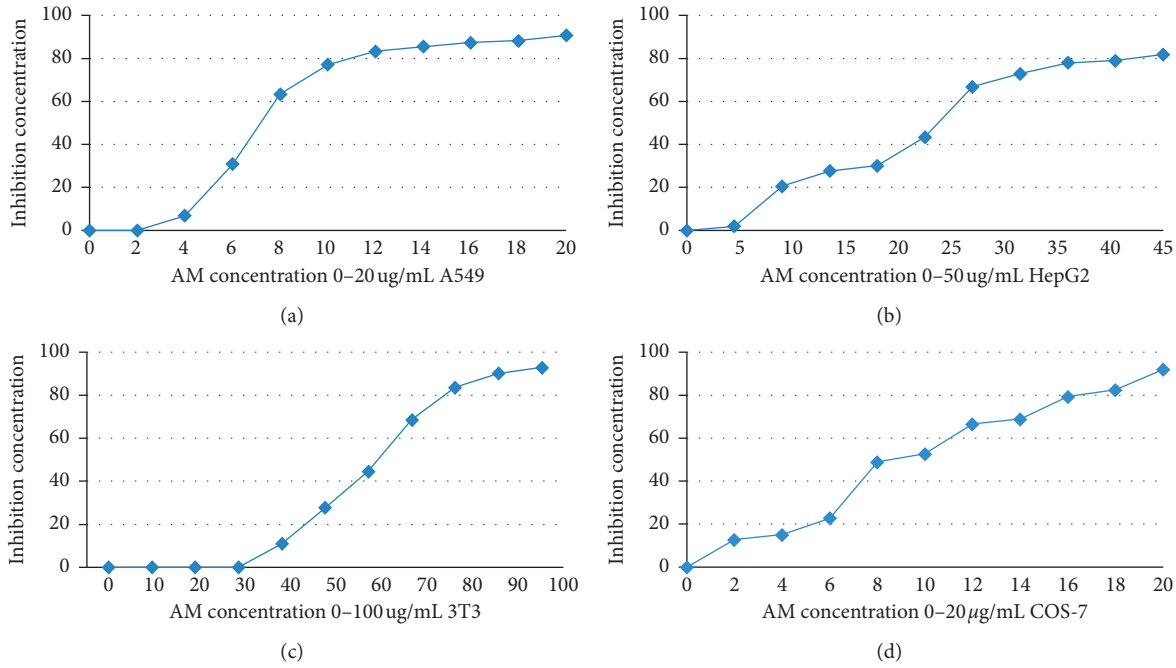


Figure 1: Dose response curve of *A. malaccensis* extract against different cell lines.

3.9. Histopathological Observations of Organs. The results of histopathological examination of liver sections of rats treated with olive oil (control) and different doses of the extract are shown in Figure 5. The liver tissue displayed normal hepatic cords and portal vein (Figure 5(a)) in the control groups. Liver tissue of rats administered the extract at 300 mg/kg did not show any alteration (Figure 5(b)), while the tissues of rats treated with 2000 mg/kg body weight showed mild congestion (Figure 5(c)).

The results of histopathological examination of kidney sections are shown in Figure 6. The kidney tissues presented intact tubules in control rats (Figure 6(a)). Rats administered the extract at 300 mg/kg body weight showed normal glomeruli and tubules (Figure 6(b)), while those in the 2000 mg/kg group showed glomeruli with mild lymphocytic infiltration (Figure 6(c)).

The histopathological examination of heart muscle sections of control rats revealed no pathological changes (Figure 7(a)). The rats in extract treatment at 300 mg/kg group also did not show any abnormality (Figure 7(b)), while those in the 2000 mg/kg group showed mild congestion (Figure 7(c)).

Lung sections of control rats are shown in Figure 8(a). The rats administered 300 and 2000 mg/kg extract did not show any perceivable microscopic changes (Figure 8(b)).

4. Discussion

Evaluation of toxicity, if any, of *A. malaccensis n*-hexane extract is the principal objective of this research since there is little scientific and clinical data on the effectiveness and the safety of *A malaccensis* rhizome extract. Single oral dose administration of 2000 mg/kg body weight of crude *A. malaccensis n*-hexane extract did not induce mortality or clinical signs of toxicity in rats throughout the observation period of 14 days. This indicated that the LD_{50} value of *A. malaccensis* extract is greater than 2000 mg/kg body weight. Although all treated rats showed normal behavior during 24 h, some animals showed sign of mild distress during the first 4 h and drank too much water during the first 3 h after oral administration of the extract. According to Teo et al. [23], changes in the body weight can be considered as markers of adverse effects upon oral administration of drugs and chemicals. More than 10% body weight loss from the initial body weight is considered as significant ($p > 0.05\%$) [24]. In our study, none of the animals showed such reduction in body weight, but their body weights increased during the observation period indicating that the plant extract did not produce any adverse effect on the body weight of rats in terms of acute oral toxicity. Similar observations were reported by Karunarathne et al. [13] when they administered 2000 mg/kg body weight of crude *Alpinia galanga n*-hexane extract on rats, which is a plant extract of the same genus having 1'ACA as the major chemical compound. Furthermore, no change of organs was observed in the treated rat with the exception being the liver of rats treated with 2000 mg/kg bw day of *A. malaccensis* crude extract. Assessment of liver and kidney function is a very vital index in evaluating the toxicity of the plant extracts. The serum urea, creatinine, and liver enzyme (ALT, AST, and ALP) concentrations of the blood were the indices used to evaluate the function of kidney [25]. The results in respect of BUN, creatinine, ALT, and AST after 14 days of oral administration were not significantly different from the control group and revealed that there was no abnormality of the kidney for all the treated rats. Hematopoietic system is one of the most susceptible targets of toxic compounds, especially the bone marrow where the production of red blood cell occurs [26]. Acute administration of the plant

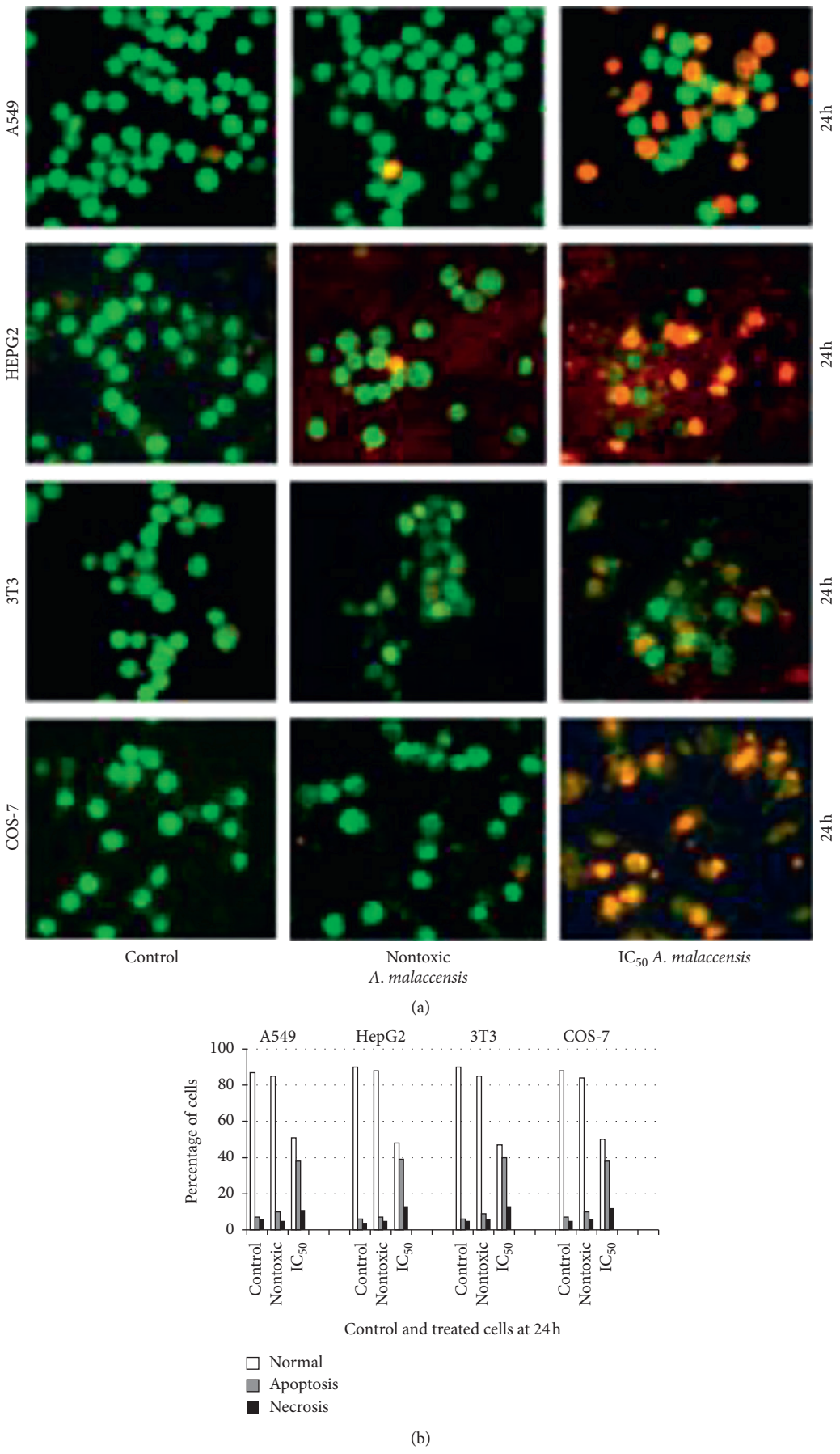
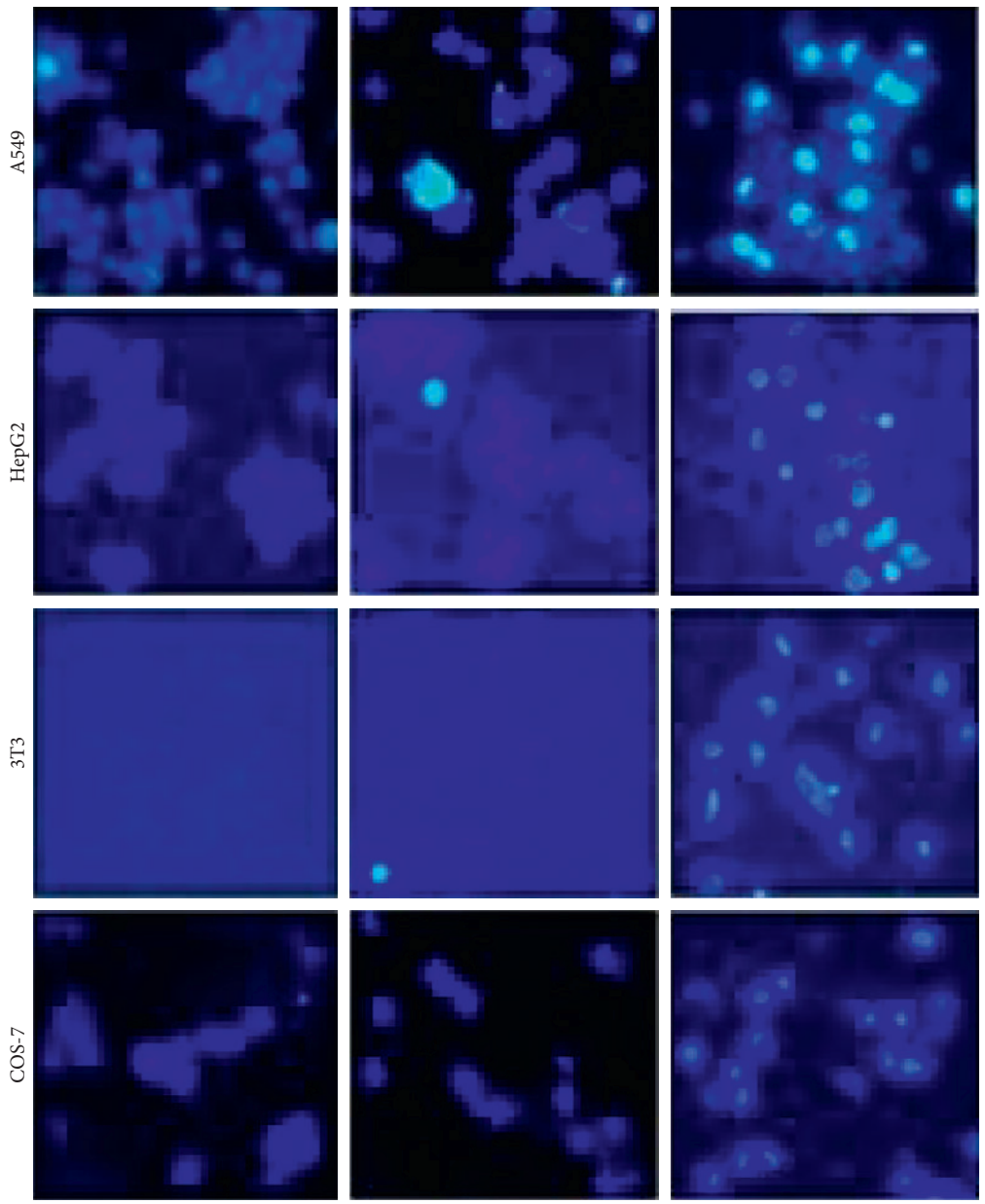
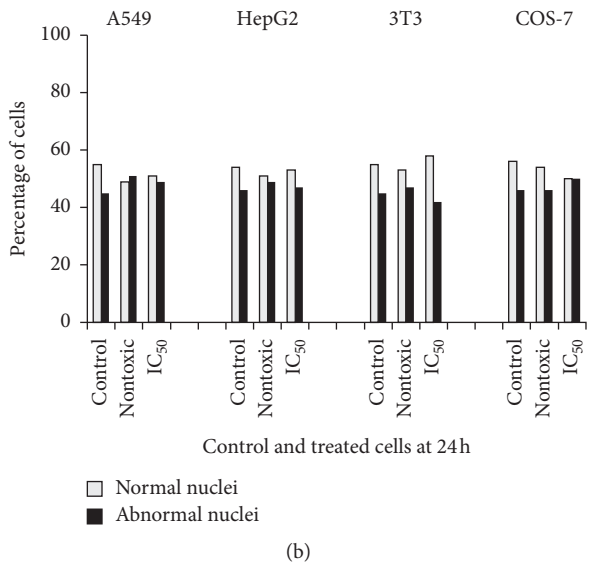


Figure 2: Assessment of apoptosis and necrosis. (a) Cells exposed to A. malaccensis extract and observed adopting AO/EB staining. Control cells are viable and fluoresce uniformly in green; cells in apoptosis fluoresce from yellow to orange; necrotic cells are swollen and fluoresce in bright red. (b) Percentage of normal, apoptotic, and necrotic cells. Data are expressed as the average of three independent observations.



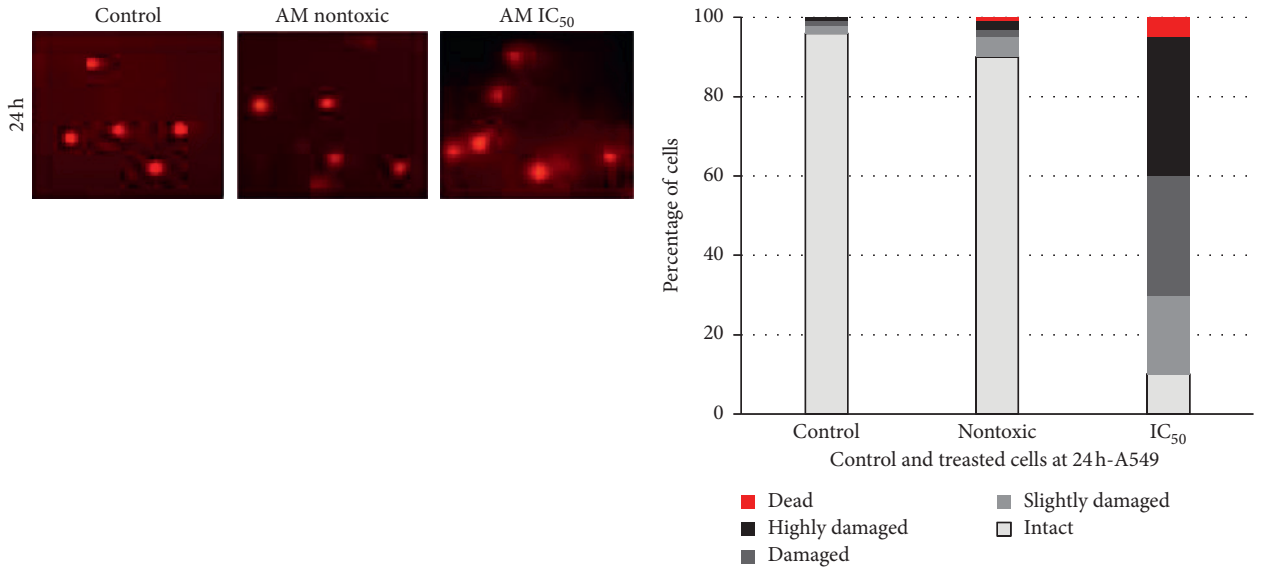
(a)

Figure 3: Continued.



(b)

Figure 3: (a) Nuclear features of cells as revealed in Hoechst staining. (b) Percentage of cells with normal and abnormal nuclei. Data are expressed as average of three independent observations.



(a) (b)

Figure 4: Assessment of DNA damage in A549 cells adopting comet assay. Data for the other cells are not shown since all showed similar trend. (a) DNA damage induced by *A. malaccensis* extract. Treated cells in which DNA has undergone strand breaks look like comets. (b) DNA damage as defined according to the DNA in the tail. The multiple parts of each column (from the bottom to the top): intact (0–20%), slightly damaged (20–40%), damaged (40–60%), highly damaged (60–80%), and dead (80–100%). Data are expressed as average of three independent experiments.

Table 1: Organ weights (g) of female rats.

Organ	Weight in grams	*A. malaccensis* extract (mg/kg)	
	Control	300	2000
Liver	6.05 ± 0.40^{b}	6.09 ± 0.42^{b}	7.12 ± 0.18^{a}
Left kidney	0.56 ± 0.06	0.53 ± 0.01	0.67 ± 0.06
Right kidney	0.57 ± 0.09	0.54 ± 0.38	0.66 ± 0.03
Lungs	0.98 ± 0.08	1.00 ± 0.05	1.16 ± 0.05
Heart	0.68 ± 0.04	0.68 ± 0.04	0.75 ± 0.04
Spleen	0.44 ± 0.04	0.42 ± 0.02	0.50 ± 0.02

Data with different lower case letters are significantly different ($p < 0.05$).

Table 2: Serum biochemical parameters of female rats.

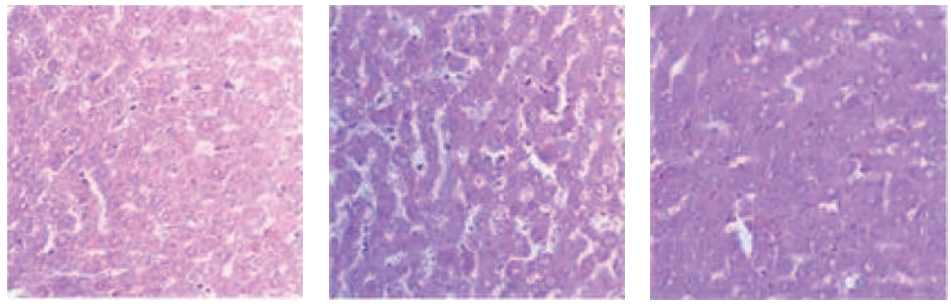
Biochemical parameter	*A. malaccensis* extract (mg/kg)		
	Control	300	2000
PCV (%)	43.00 ± 1.41*	43.66 ± 0.47	43.66 ± 0.47
ALT(u/L)	34.33 ± 2.50	31.06 ± 1.55	28.40 ± 5.39
AST (u/L)	117.00 ± 2.08	117.00 ± 0.00	119.65 ± 1.05
BUN (urea/mg)	15.53 ± 2.69	15.23 ± 2.20	16.03 ± 1.10
Creatinine (mg/dL)	0.63 ± 0.09	0.93 ± 0.09	0.87 ± 0.12

*Results are expressed as mean ± SEM of 3 samples.

Table 3: Serum hematological values of female rats.

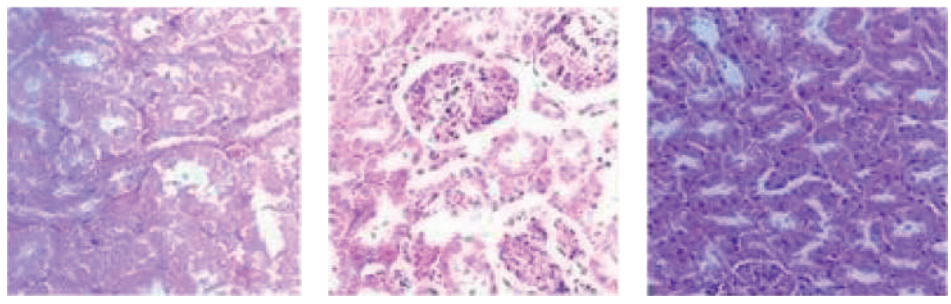
Hematological parameter	Control	*A. malaccensis* extract	
		300 mg/kg	2000 mg/kg
Hb (g/dL)	12.60 ± 0.14*	14.00 ± 0.84	13.13 ± 0.71
WBC (×103 mm)	9.00 ± 0.71	11.20 ± 1.57	8.66 ± 0.30
RBC (106/L)	6.78 ± 0.89	7.20 ± 0.21	6.53 ± 0.41
Neutrophils (109/L)	16.33 ± 0.76	15.66 ± 5.43	13.00 ± 0.81
Lymphocytes (109/L)	81.33 ± 8.99	82.66 ± 5.55	83.66 ± 0.41
Erythrocytes (109/L)	0.33 ± 0.47	0.33 ± 0.47	1.00 ± 0.81
Monocytes (109/L)	2.00 ± 0.82	1.33 ± 0.47	2.33 ± 0.47

*Results are expressed as mean ± SEM of 3 samples.



(a) (b) (c)

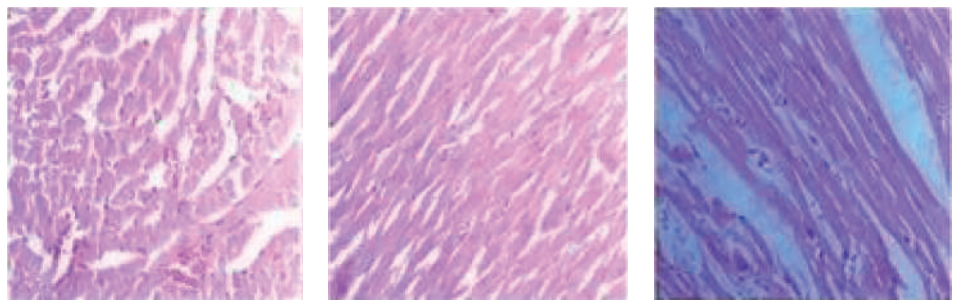
Figure 5: Photomicrographs of liver sections of rat. (a) Control. (b) *A. malaccensis* 300 mg/kg. (c) *A. malaccensis* 2000 mg/kg. Scale bar, 10 μm.



(a) (b) (c)

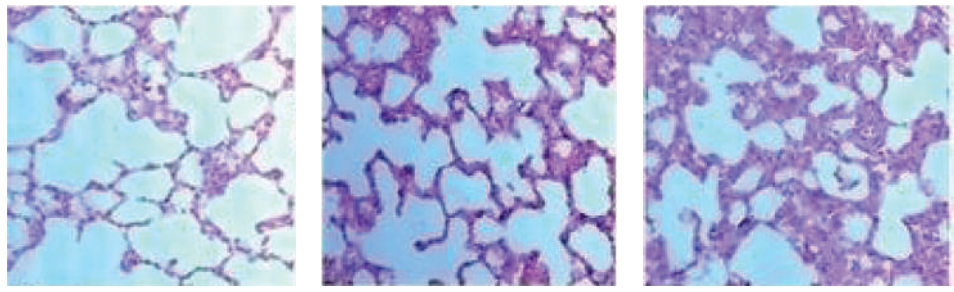
Figure 6: Photomicrographs of the kidney sections of rat. (a) Control. (b) *A. malaccensis* 300 mg/kg. (c) *A. malaccensis* 2000 mg/kg. Scale bar, 10 μm.

extract did not cause any significant changes in the hematological profile of rats which received the *A. malaccensis* extract at different doses, suggesting that the plant extract does not affect the hematopoietic system. The treatment did not bring about any serious histopathological change in the liver, kidney, heart, and spleen. Therefore, it is to be inferred that the plant extract, at least up to 2000 mg/kg, is not toxic in respect to acute oral administration. However, before a final decision, subacute and chronic toxicity testing of *A. malaccensis* will be highly relevant.



(a) (b) (c)

Figure 7: Photomicrographs of the heart sections of rat. (a) Control. (b) *A. malaccensis* 300 mg/kg. (c) *A. malaccensis* 2000 mg/kg. Scale bar, 10 μm.



(a) (b) (c)

Figure 8: Photomicrographs of the lung sections of rat. (a) Control. (b) *A. malaccensis* 300 mg/kg. (c) *A. malaccensis* 2000 mg/kg. Scale bar, 10 μm.

In vitro studies are equally important in deciphering if a material is toxic or not. Different cell lines might exhibit different sensitivities towards a compound if it is cytotoxic. Therefore, use of more than one cell line is considered necessary in the detection of cytotoxic effects [27]. So, we found the IC_{50} values of *A. malaccensis n*-hexane extract for A549, HepG2, 3T3, and COS-7 cell lines which were 7.25, 22.5, 62.75, and 8.25 μg/mL, respectively, and the zero-toxic concentration (IC_0) was 2, 1.4, 30, and 1.4 μg/mL, respectively. This is important since so far no toxicity data have been reported in literature for *A. malaccensis* [28].

Further, the mode of cell death, whether apoptosis or necrosis, induced by the plant extract was assessed. Double staining of AO/EB for nontoxic concentration of *A. malaccensis* showed nuclear and morphological changes of apoptosis only in less than 10% of the cells. However, more than 10% apoptosis was observed for cells treated the IC_{50} concentration of *A. malaccensis* extract. Similar observations were made for Hoechst staining. However, comet assay revealed that 7.25 μg/mL of *A. malaccensis* IC_{50} induced severe damage to DNA of A549 cell. In contrast, plant extracts at non-cytotoxic concentration(s) did not induce DNA damage in A549 cell. Thus, our results clearly demonstrated that *A. malaccensis* at nontoxic doses did not exert genotoxicity in A549 cells. In fact, the extract at these nontoxic concentrations could be used as preservative of food stored for human consumption without any adverse effect on human health. Thus, considering the nontoxic concentrations, acceptable daily intake (ADI) of the extract could be approximately calculated using NOAEC (no-observed adverse effect concentration) divided by uncertainty factor (usually10 to extrapolate from animal to human and 10 for interindividual differences in sensitivity). NOAEC is defined as the concentration at which there is no risk (nontoxic level) [29]. The approximate ADI value for *A. malaccensis n*-hexane extract is 55.41 mg/day (data not shown). However, bioavailability of the extract(s), animal data, or PBPK (physiologically based biokinetic model) is necessary to derive an appropriate ADI for humans. Therefore, we recommend further investigations on subchronic and chronic toxicity testing to find the reliability of the estimated ADI of the plant extract when used as food preservative and/or therapeutic. Put together, the most nonpolar (*n*-hexane) extract of *A. malaccensis* offers great potential as a food preservative as well as therapeutic which could be further established through clearly directed studies.

5. Conclusion

The oral toxicity studies in the rat model revealed that *A. malaccensis n*-hexane extract at a single dose of 2000 mg/kg body weight did not produce any serious adverse effect with respect to general behavior, body weight, feed intake, biochemical parameters, and organ histology. In addition, cytotoxicity analysis showed that nontoxic concentration of *A. malaccensis* (2, 1.4, 30, and 1.4 μg/mL, respectively) does not affect cell viability or the DNA. Therefore, these concentrations may be practiced for human consumption

without any adverse health effect. However, we recommend subchronic and chronic toxicity tests to further exonerate the adverse effects by repeated administration of *A. malaccensis*. Further, it is possible that higher doses of *A. malaccensis* extract would potentially damage DNA, and therefore, we recommend further research towards standardization of *A. malaccensis* as preservative of food meant for human consumption and/or as a therapeutic.

Data Availability

The data of organ weights, serum biochemical parameters, serum hematological values of female rats, *in vitro* inhibition concentration of *A. malaccensis* on different cell lines, morphological assessment on apoptosis and necrosis, nuclear features of cells, assessment of DNA damage, and photomicrographs of internal organ sections used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

The authors wish to thank Dr. Tilusha Manchanayaka from Veterinary Institute, Gannoruwa, Sri Lanka, for histopathological investigations, Mr. W. G. S. S. Kumara, Senior Medical Laboratory Technician, from the Animal Centre, Medical Research Institute, Sri Lanka, and Gowdhami Balakrishnan from Bharathidasan University, India, for the technical support. The authors also wish to thank the National Research Council of Sri Lanka for financial support with grant no. 12-054 and India Science and Research Fellowship (ISRF) Program of DST, India, which provided a travel grant to Thikshani Somarathna (TS) to carry out the cytotoxicity study in India.

References

[1] World Health Organization, *WHO Traditional Medicine Strategy 2002–2005*, World Health Organization (WHO), Geneva, Switzerland, 2002.

[2] A. Karimi, M. Majlesi, and M. Rafieian-Kopaei, "Herbal versus synthetic drugs; beliefs and facts," *Journal of Nephropharmacology*, vol. 4, no. 1, pp. 27–30, 2015.

[3] V. Ertekin, M. A. Selimoğlu, and S. Altinkaynak, "A combination of unusual presentations of *Datura stramonium* intoxication in a child: rhabdomyolysis and fulminant hepatitius," *The Journal of Emergency Medicine*, vol. 28, no. 2, pp. 227-228, 2005.

[4] S. Koduru, D. S. Grierson, and A. J. Afolayan, "Antimicrobial activity of *Solanum aculeastrum*," *Pharmaceutical Biology*, vol. 44, no. 4, pp. 283–286, 2006.

[5] S. Sirikantaramas, M. Yamazaki, and K. Saito, "Mechanisms of resistance to self-produced toxic secondary metabolites in plants," *Phytochemistry Reviews*, vol. 7, no. 3, pp. 467–477, 2008.

[6] G. Raj, D. P. Pradeep, C. Yusufali, M. Dan, and S. Baby, "Chemical profiles of volatiles in four Alpinia species from Kerala, South India," *Journal of Essential Oil Research*, vol. 25, no. 2, pp. 97–102, 2012.

[7] M. N. I. Bhuiyan, J. U. Chowdhury, J. Begum, and N. C. Nandi, "Essential oils analysis of the rhizomes of *Alpinia conchigera Griff.* and leaves of A*lpinia malaccensis* (Burm.f.) Roscoe from Bangladesh," *African Journal Plant Science*, vol. 4, no. 6, pp. 197–201, 2010.

[8] T. Somarathna, W. M. A. D. B. Fernando, K. K. D. S. Ranaweera, G. A. S. Premakumara, T. Abeysinghe, and N. S. Weerakkody, "Antimicrobial activity and phytochemical screening of *Alpinia malaccensis* (Ran-kiriya) against food-borne bacteria," *Journal of Applied Microbiology*, vol. 125, no. 5, pp. 1276–1285, 2018.

[9] T. Somarathna, G. A. S. Premakumara, M. D. Akbarsha, and K. Balamuthu, "In vitro antibacterial activity of selected underutilized plants and cytotoxic property of *Terminalia catappa*," *International Journal of Pharmacy and Pharmaceutical Sciences*, vol. 9, no. 12, pp. 218–225, 2017.

[10] G. Primahana, T. Ernawati, N. L. P. Dewi, I. D. Dwiyatmi, A. Darmawan, and M. Hanafi, "Synthesis of 2-allylphenyl cinnamate and brine shrimp lethality test activity evaluation," *Procedia Chemistry*, vol. 16, pp. 694–699, 2015.

[11] N. B. Thu, T. N. Trung, N. M Khoi et al., "Articles: screening of Vietnamese medicinal plants for cytotoxic activity," *Natural Product Sciences*, vol. 16, no. 1, pp. 43–49, 2010.

[12] A. Janssen, J. Scheffer, and A. Svendsen, "Antimicrobial activity of essential oils: a 1976–1986 literature review. Aspects of the test methods," *Planta Medica*, vol. 53, no. 5, pp. 395–398, 1987.

[13] P. U. H. S. Karunarathne, M. G. Thammitiyagodage, and N. S. Weerakkody, "Safety evaluation of galangal (*Alpinia galanga*) extract for therapeutic use as an antimicrobial agent," *International Journal of Pharmacutical Science. and Research*, vol. 9, no. 11, pp. 4582–4590, 2018.

[14] E. Chan, Y. Lim, and M. Omar, "Antioxidant and antibacterial activity of leaves of Etlingera species (Zingiberaceae) in Peninsular Malaysia," *Food Chemistry*, vol. 104, no. 4, pp. 1586–1593, 2007.

[15] M. Dassanayake and R. Forsberg, *A Revised Handbook to the Flora of Ceylon*, Amerindian Publishing, Washington, DC, USA, 1983.

[16] L. Gayathri, R. Dhivya, D. Dhanasekaran, V. S. Periasamy, A. A. Alshatwi, and M. A. Akbarsha, "Hepatotoxic effect of ochratoxin A and citrinin, alone and in combination, and protective effect of vitamin E: in vitro study in HepG2 cell," *Food and Chemical Toxicology*, vol. 83, no. 83, pp. 151–163, 2015.

[17] T. Mosmann, "Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays," *Journal of Immunological Methods*, vol. 65, no. 1-2, pp. 55–63, 1983.

[18] F. Nemati, A. Dehpouri, B. Eslami, V. Mahdavi, and S. Mirzanejad, "Cytotoxic properties of some medicinal plant extracts from mazandaran, Iran," *Iranian Red Crescent Medical Journal*, vol. 15, no. 11, 2013.

[19] D. L. Spector and R. D. Goldman, *Cell: A Laboratory Manual. Culture and Biochemical Analysis of Cells*, Cold Spring Harbor Laboratory Press, Long Island, NY, USA, 1998.

[20] S. A. Latt, G. Stetten, L. A. Juergens, H. F. Willard, and C. D. Scher, "Recent developments in the detection of deoxyribonucleic acid synthesis by 33258 Hoechst fluorescence," *Journal of Histochemistry & Cytochemistry*, vol. 23, no. 7, pp. 493–505, 1975.

[21] N. P. Singh, M. T. McCoy, R. R. Tice, and E. L. Schneider, "A simple technique for quantitation of low levels of DNA damage in individual cells," *Experimental Cell Research*, vol. 175, no. 1, pp. 184–191, 1988.

[22] M. Saboudry, *A Breeding and Care of laboratory Animals*, pp. 18-19, World Health Organization, Geneva, Switzerland, 1993.

[23] S. K. Teo, D. I. Stirling, S. D. Thomas, A. M. Hoberman, M. S. Christian, and V. D. Khetani, "The perinatal and postnatal toxicity of D-methylphenidate and D,L-methylphenidate in rats," *Reproductive Toxicology*, vol. 16, no. 4, pp. 353–366, 2002.

[24] D. Williamson, G. Bray, and D. Ryan, "Is 5% weight loss a satisfactory criterion to define clinically significant weight loss?" *Obesity (Silver Spring, Md.)*, vol. 23, no. 12, pp. 2319-2320, 2015.

[25] R. Chavda, K. R. Vadalia, and R. Gokani, "Hepatoprotective and antioxidant activity of root bark of *Calotropis procera* R.Br (asclepediaceae)," *International Journal of Pharmacology*, vol. 6, no. 6, pp. 937–943, 2010.

[26] M. Kifayatullah, M. S. Mustafa, P. Sengupta, M. M. R. Sarker, A. Das, and S. K. Das, "Evaluation of the acute and sub-acute toxicity of the ethanolic extract of *Pericampylus glaucus* (Lam.) Merr. in BALB/c mice," *Journal of Acute Disease*, vol. 4, no. 4, pp. 309–315, 2015.

[27] K. Khairunnisa and D. Karthik, "Evaluation of in-vitro apoptosis induction, cytotoxic activity of *Hymenodictyon excelsum* (roxb) wall in dalton's lymphoma ascites (DLA) and lung fibroblast-mouse l929 cell lines," *Journal of Applied Pharmaceutical Science*, vol. 4, no. 8, pp. 11–17, 2014.

[28] S. Sahoo, S. Singh, and S. Nayak, "Chemical composition, antioxidant and antimicrobial activity of essential oil and extract of *Alpinia malaccensis roscoe* (Zingiberaceae)," *International Journal of Pharmacy and Pharmaceutical Sciences*, vol. 6, no. 7, pp. 183–188, 2014.

[29] Food and Agriculture Organization (FAO) and World Health Organization (WHO), "Principles and Methods for the Risk Assessment of Chemicals in Food," *Chapter 7. Risk Characterization*, World Health Organization (WHO), Geneva, Switzerland, 2009.

Meeting Report

Setting the Trend for the Alternatives Movement in India: The Second National Conference of the Society for Alternatives to Animal Experiments-India

doi:10.14573/altex.2003183

Introduction

The Second National Conference of the Society for Alternatives to Animal Experiments (NCAAE-2019) was organized at National Center for Biopharmaceuticals (NFB), Guru Nanak Khalsa College, Mumbai, India, with Dr Pratiksha Palahe as the Organizing Secretary, on December 13-14, 2019. The theme *Trends in the Science of Alternatives* offered a platform for understanding the challenges in promoting alternatives to animal testing in India and other Asian countries. The conference with over 100 participants from all over India was successfully conducted with talks by invited speakers from India and abroad and by mid-career scientists. Posters were displayed and oral presentations were made by students. Speakers and delegates representing industry and regulators took part, so there was exchange of ideas between academics, animal protectionists, regulators and CROs. Pre- and post-conference workshops offered intensive hands-on training on different aspects of alternatives.

Inauguration

Dr Ganapati D. Yadav, Former Vice Chancellor, Institute for Chemical Technology, Mumbai, inaugurated the conference. While recalling the contribution of animal experiments to drug discovery and risk assessment, he emphasized the need to catch up with the trend in research methodologies, especially non-animal methods. "Scientists from biological sciences should work collaboratively, invoking artificial intelligence technology, and develop more alternatives for use in place of animal experiments," he said. Dr Kiran Mangaonkar, Principal, G.N. Khalsa College, welcomed the gathering. Dr Yogendra K. Gupta (President, SAAE-I) and Dr Mohammad A. Akbarsha (General Secretary, SAAE-I) briefly recalled the history of SAAE-I, its objectives and progress so far, and the objectives of the conference. Dr Pratiksha Palahe, Organizing Secretary, presented the program. A memorandum of understanding (MoU) was signed between Japanese Society for Alternatives to Animal Experiments (JSAAE) (represented by Dr Yasuyuki Sakai, President) and SAAE-I (represented by Dr Gupta). Dr Rubina Bose, Deputy Drug Controller General (West Zone, India) and Dr Smita Mahale, Director, National Institute of Research in Reproductive Health (under Indian Council for Medical Research), Mumbai, the guests of honor, offered felicitation. Ms. Shalmali Pendse, the Co-organizing Secretary, thanked all contributors.

Scientific Lectures

Dr **Thomas Hartung**, Director, Center for Alternatives to Animal Testing (CAAT) at Johns Hopkins University, Baltimore, MD, USA, delivered a virtual plenary lecture on "*Perspectives of non-animal models*". He stated that while the limitations of animal-based toxicology to predict human health issues are being increasingly recognized, shortcomings of traditional (human) cell culture are also evident. Issues around these include cell identity, differentiation, genetic stability and mycoplasma infection as well as non-homeostatic and non-physiological culture conditions. Large toxicological databases ("big data") and data-mining technologies ("artificial intelligence") now allow predictive computational approaches on a new scale, for example, using automated read-across (RASAR, i.e., read-across-based structure activity relationships). At the same time, the combination of cell culture with bioengineering has led to more organotypic cell culture technologies, such as 3D culture, human stem cell-derived systems, perfusion, co-cultures, combinations with scaffolds and sensors, etc. Increasingly, these approaches are combined to organ-on-chip or even multi-organ human-on-chip solutions. By recreating organ architecture and functionality and homeostasis of the cell environment, these models mirror the physiological situation more closely. The example of the human iPSC-derived mini-brain developed at CAAT illustrates this claim. Combined with an increased mechanistic base of reasoning (e.g., adverse outcome pathway (AOP) concepts, integrated testing strategies (ITS), and evidence-based methods of data evaluation and integration), these are revolutionary changes in how we can assess the biological effects of substances.

Dr **Christian Pellevoisin**, Scientific Director, EPISKIN Academy, Lyon, France, talked about "*Medical devices: ISO 10993 series moves to alternative methods*". He said that reconstructed human epidermis (RhE) allows validated full or partial replacement of different *in vivo* assays, is integrated in OECD Test Guidelines, and is used in screening and in support of weight of evidence approaches. Recently, RhE has been adapted to testing biocompatibility of medical devices, monitoring devices, and instruments used in diagnostics. The project conducted under Workgroup 8 (WG8) of the Technical Committee 194 (TC194) for biocompatibility of medical devices aims to replace the *in vivo* Draize test by an *in vitro* method using RhE models. The recent outcome of the round robin study with two RhE models in 17 lab-

oratories has led to a new standard (ISO 10993-23) that will be released in 2020. At the same time, several projects based on human reconstructed tissues for *in vitro* eye irritation, vaginal irritation or skin sensitization by medical devices are ongoing. The translation of these *in vitro* approaches to updated or new ISO 10993 standards will provide adapted non-animal approaches for risk assessment of medical devices.

Dr **Kwang-Mahn Kim**, Department of Dental Biomaterials & Bioengineering, Yonsei University College of Dentistry, Seoul, Republic of Korea, spoke on "*Alternative methods of biocompatibility test for medical devices used in dentistry*", in which he focused on biocompatibility testing for dental biomaterials. The dentin barrier test, murine local lymph node assay (LLNA), RhE test for skin irritation, and other biomimetic cytotoxicity tests are being assessed for biocompatibility testing. Dentin substitute materials, e.g., polyurethane discs, have been developed for the dentin barrier test and a test protocol for an *in vitro* oral mucosa irritation test using 3D culture of oral epithelial tissue has been defined. Additionally, the ISO TC 194/WG 8 round robin study for skin irritation test methods for medical devices will take testing of medical devices to the next level.

Dr **Albert P. Li**, President, CEO and Co-founder, *In Vitro* ADMET Laboratories LLC, Columbia, MD and Malden, MA, USA, addressed "In vitro *human experimental systems for the evaluation of xenobiotic properties: hepatocytes, enterocytes and IdMOC*". According to him, experimentation with laboratory animals does not always provide data that are translatable to humans because of species differences. This is particularly important in the evaluation of xenobiotic toxicity. The lecture addressed species differences in xenobiotic metabolism and human *in vitro* experimental systems that can replace *in vivo* animal experiments. He elaborated upon the applications of human hepatocytes, enterocytes, and the integrated multiple organ co-culture (IdMOC) system in the evaluation of xenobiotic metabolism and toxicity.

Dr **Jayasujatha Vethamanickam**, Safety Scientist, Safety and Environmental Assurance Centre, Unilever R&D, UK, in her talk on "*Non-animal approaches for cosmetic safety assessment: A case study*" stated that several countries, including India, have banned animal testing of cosmetics and their ingredients. The Bureau of Indian Standards (BIS) is currently updating the Methods of Tests for Safety Evaluation of Cosmetics to include more non-animal methods that have been accepted in other countries. As recently outlined by the International Cooperation on Cosmetics Regulation (ICCR), the key principle is that next generation risk assessments (NGRA) for cosmetic safety should be exposure-led and hypothesis-driven. A key metric in this case is the point-of-departure (PoD), the lowest concentration at which biological responses can be detected within a given set of non-animal assays. The ICCR principles were applied to a hypothetical safety assessment of 0.1% coumarin in face cream. Exposure calculations using specific consumer habits data were used to build a physiologically-based kinetic model and a battery of *in vitro* assays was used to determine PoDs for a variety of biological effects including genotoxicity, immunomodulatory effects and activity against a panel of biological targets. The PoDs from the *in vitro* assays were plotted against the calculated *in vivo* exposure to determine a margin of safety (MoS). From these results, it was concluded that coumarin is not genotoxic, does not bind to the 44 receptors tested or show any immunomodulatory effect. The case study demonstrated the importance of integrating exposure science with computational modeling and *in vitro* bioactivity data as the basis of non-animal safety assessments.

Dr **Eui-Bae Jeung**, Laboratory of Veterinary Biochemistry and Molecular Biology, College of Veterinary Medicine, Chungbuk National University, Republic of Korea, dwelt on "*Alternative developmental and neurological test based on stem cell*". The developmental toxicity test method (termed EBT) measuring the area of embryoid bodies (EBs) has undergone a validation study using a new predictive model. 61 chemicals were predicted with an average accuracy above 80%. The high-throughput developmental neurotoxicity test (DNT) method measuring cell viability and inhibition of neuronal differentiation based on GFP intensity using Sox1-GFP stem cells was optimized and challenged with developmental neurotoxicants. Together, the results suggest that EBT and DNT can be useful for evaluation of the toxicity of new chemicals or candidate drugs. A close collaboration between Korean and Indian research groups in the field of alternatives was proposed; the group has already signed a MoU for collaborative research efforts with the Department of Medical Elementology & Toxicology, Jamia Hamdard, New Delhi, India.

Dr **Dipti Kapoor**, representing People for the Ethical Treatment of Animals (PETA)-India, New Delhi, spoke on "*The need for non-animal recombinant antitoxins*". Most of the currently available antitoxins are harvested from the blood of immunized animals. This process can cause local and systemic adverse effects, including injection-site edema, thrombosis, phlebitis, abscesses, fistulas, and fibrosis. The resulting drugs derived from animal serum have well-documented safety issues including hypersensitivity reactions and serum sickness, and maintenance of reliable stockpiles for quick transport to patients in need of treatment is challenging. Several recombinant antibody-based therapeutics have been developed and approved for clinical use, and more are in the pipeline.

Dr **Brinda Poojary**, from the Research & Toxicology Department of Humane Society International, Hyderabad, India, spoke about "*Vaccine global regulatory harmonization*". Vaccines are among the greatest public health achievements. They have helped eradicate certain diseases and have increased life expectancy. The talk provided an overview of the role of animal models in vaccine production, the current scenario of working towards elimination of redundant tests, and the way forward towards alternative methods in the regulatory and industry sectors.

Dr **Abhay Deshpande**, Director, Global Innovation and Strategy, Jai Research Foundation, Vapi, Gujarat, India, delivered a lecture on "*Novel alternative methods: Global efforts bearing fruits*". He spoke on the different contributions of research groups, agencies and organizations, industry and regulators to the development and acceptance of new approach methods (NAM).

Dr **Barry Hardy**, President, Open Tox Association, and CEO at Edelweiss Connect Inc., GmbH, Basel, Switzerland, gave a virtual presentation on "*International collaborations advancing the development and applications of new alternative methods in predictive toxicology and risk assessment*". He introduced OpenRiskNet as an open e-infrastructure providing resources and ser-

vices to a variety of communities requiring risk assessment, including chemicals, cosmetic ingredients, therapeutic agents and nanomaterials. He also discussed the challenges of proposed activities to bring people and diverse resources to the same table to accelerate international collaboration that will support conversation and knowledge activities, leading to strong and rewarding outcomes.

Dr **Suresh Poosala**, Director, Reagene Bio-Sciences, Hyderabad, India, delivered a talk on "*3D bio-printing to promote drug discovery and replace animals in research*". The 3D bio-platform aims to de-risk clinical and product failures early on by improving the translatability of many assays as a cost-effective and highly predictive alternative to testing in animals. Functional units that reflect human pathophysiology are being generated. The translational assay platform may be custom-designed for specific needs to address targeted questions.

Dr **Sameer Jhadav**, Department of Chemical Engineering, Indian Institute of Technology, Mumbai, India, spoke on "*Bioassay for characterizing sperm motility*". Two bio-assays to measure sperm motion were introduced. The first involves a microfluidic device to measure the chemotactic response of sperm, the second characterizes the motion of sperm having immobilized heads.

Dr **Anne Gourmelon**, Principal Administrator – Test Guidelines Programme, Environment, Health and Safety Division, Environment Directorate, Organization for Economic Cooperation and Development (OECD), delivered a virtual talk on "*OECD works on transition to combinations and defined approaches for complex hazard endpoints*". The OECD develops test guidelines that enable industries that follow GLP principles to generate data that are accepted across member countries, including India, in the Mutual Acceptance of Data system. In the last decade, OECD has adopted numerous non-animal methods for simple toxicities. For more complex and systemic toxicities, a number of projects are now directed towards the combination of *in vitro* methods that represent key events in AOPs and *in silico* approaches.

Dr **Yasuyuki Sakai**, Department of Chemical System Engineering and Department of Bioengineering, Graduate School of Engineering, University of Tokyo, Japan, and President of Japanese Society for Alternatives to Animal Experiments (JSAAE), spoke on "*Latest activities and future directions of JSAAE for Asian cooperation toward 3Rs*". The JSAAE promotes 3Rs research in Japan through a wide variety of activities such as hosting of the 6th World Congress on Alternatives and Animal Use in Life Sciences in Tokyo (2007) and contributing to the development of guidelines in cooperation with Japanese Center for the Validation of Alternative Methods (JaCVAM). JSAAE now has MoU with counterpart societies in Korea, EU and USA and is planning to set up MoU with Chinese counterparts. To strengthen communication among Asian communities, the Society is planning to set up an Asian Consortium of Alternatives to Animal Experiments.

Dr **Hajime Kojima**, representing JaCVAM, National Institute of Health Sciences (NIHS), Tokyo, Japan, explained how JaCVAM has been instrumental in promoting the development and evaluation of alternatives to test methods in Japan. JaCVAM's mission is to promote the use of alternative methods to animal testing in regulatory studies while ensuring protection of the public by assessing safety of chemicals and other materials. JaCVAM plays an active role in the application and approval process for permission to manufacture and sell pharmaceuticals and other products as well as in revising standards for cosmetic products. JaCVAM participates in International Cooperation on Alternative Test Methods (ICATM) activities, under which it has organized a number of international validation studies that have led to the issuance of fourteen test guidelines and one guidance document by the OECD. Dr Kojima recommended the establishment of a center for validation in India.

Dr **Sheikh Raisuddin**, Department of Medical Elementology & Toxicology, Jamia Hamdard (Institute of Eminence & Deemed University), New Delhi, India, addressed "*School level curriculum intervention for awareness about animal welfare and alternatives*". Under the initiative of PETA, the National Council of Educational Research and Training (NCERT) and Central Board of Secondary Education (CBSE) have agreed to incorporate a humane-education program in school curricula. STRAW (Stray Relief and Animal Welfare) India contributes content relating to empathy for people, compassion for animals, and care for the environment to school textbooks and teachers' manuals. Animations and computer-aided dissection have taken firm root in the school education system in India. It also has been agreed by the school boards to phase out use of leather shoes as part of school uniform. In higher education, the University Grants Commission (UGC), Medical Council of India (MCI), Pharmacy Council of India (PCI), Veterinary Council of India (VCI), and National Council of Teacher Education (NCTE) also support animal welfare and alternatives in their curricula. In 2014, UGC issued directives to discontinue dissection and animal experimentation in laboratory courses at undergraduate and post-graduate levels. In his talk, Dr Raisuddin applauded the role of Bombay Society for the Prevention of Cruelty to Animals (BSPCA) including the Bai Sakarbai Dinshaw Petit Hospital for Animals.

Presentations by mid-career scientists

Dr **Shilpy Sharma**, from Department of Biotechnology, Savitribai Phule Pune University, Pune, talked on "*Alternatives to animal experiments for studying type 2 diabetes mellitus and associated metabolic disorders*". She reported a modality for rapid screening of compounds for protection of pancreatic β-cells against human islet amyloid polypeptide (hIAPP) using recombinant INS-1E cells and on the generation of functional human pancreatic islets using dental pulp-derived stem cells to study the insults caused by high glucose, lipid and hIAPP. Dr **Syed Ziaur Rahman**, Faculty In-Charge, Alternatives to Animal Experimentation Lab, Department of Pharmacology, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, India, shared his experience on "*Teaching and learning of experimental pharmacology by alternatives*". In his institute, animal experiments have been taught using computer-based simulators since 2006. Undergraduate students learn the basic concepts of pharmacology with the help of around 53 software-based pharmacology experiment teaching modules. Dr **Banappa S. Unger**, Pharmacology and Toxicology Division, National Institute for Traditional Medicine, under Indian Council for Medical Research (ICMR-NITM), Belagavi, Karnataka, India, shared his

team's success in using zebrafish as an alternative animal model to assess the side effects of atypical antipsychotic medication for psychiatric disorders, especially olanzapine. Dr **Lekha Divya**, Department of Zoology, Central University of Kerala, Kasaragod, Kerala, India, shared her experience with the red ant as an alternative model for toxicological evaluation of nanomaterials and nanowastes in non-target organisms.

Dr **Sreejith P. Panicker**, Department of Zoology, University of Kerala, Thiruvananthapuram, India, highlighted the conduct and outcome of the pre-conference workshops on alternative animal models organized under the aegis of SAAE-I (zebra fish and echinoderms to study tissue regeneration, conducted by Dr Mohammed Idris from Centre for Cellular and Molecular Biology (CCMB), Hyderabad, India, and his team, on November 25-29, 2019) and *in vitro* toxicity evaluation adopting integrated discrete multiple organ co-culture (IdMOC) conducted on December 9-11, 2019, by Dr Albert P. Li, *In Vitro* ADMET Laboratories Inc, Columbia, MD, USA). Others associated as speakers/trainers were Dr Sheikh Raisuddin, Department of Medical Elementology & Toxicology, Jamia Hamdard (Deemed University), New-Delhi (Topic: Potential of invertebrates as test organisms); Dr Mohammad A. Akbarsha, National College (Autonomous), Tiruchirappalli, India (Topics: i) Animal models of lesser sentience; and ii) Animal cell culture techniques and tools); Dr Prabha D. Nair, from Sree Chitra Tirunal Institute for Medical Sciences & Technology, Thiruvananthapuram, India (Topic: Science of tissue regeneration- the case of the cartilage); and Dr Anil Kumar, from Sree Chitra Tirunal Institute for Medical Sciences & Technology, Thiruvananthapuram, India (Topic: 3D Bioprinting using multiple cell types).

Preconference and Post-Conference Workshops

The pre-conference workshops conducted at Department of Zoology, University of Kerala, Thiruvananthapuram, India, have been covered *vide supra*. Another preconference workshop on *Adverse Outcome Pathways* (AOP), a joint venture of Centre for Predictive Human Model Systems and Edelweiss Connect, Switzerland, and Humane Society International, India, was hosted by the National Facility for Biopharmaceuticals, Mumbai, India, on December 12, 2019. Around 100 participants attended. Dr **Surat Parvatam**, Research Associate, Centre for Predictive Human Model Systems, AIC-CCMB, HIS-India, and Dr **Tatyana Doktrova**, Senior Toxicologist, Edelweiss Connect, Switzerland, were the speakers/trainers for the session. Dr **Brinda Poojary**, Campaign Manager, Research and Toxicology Department, HIS-India, spoke about the call for proposals under the theme of AOP. Dr **Pratiksha Palahe**, Head, R&D, National Facility for Biopharmaceuticals, Mumbai, was the Organizing Secretary.

Post-conference workshops "*Alternatives and skin irritation test according to OECD TG-439 using reconstructed human epidermis (RhE)*" were held on December 15-16, 2019 at National Facility for Biopharmaceuticals, Mumbai, India, and on December 18-19, 2019, at Department of Elementology & Toxicology, Jamia Hamdard, New Delhi. Each was a joint venture of the respective institute with EPISKIN Academy, Lyon, France, and SAAE-I. Dr Pratiksha Palahe and Dr Sheikh Raisuddin, respectively, extended the laboratory support, and Dr Christian Pellevoisin, Scientific Director, Episkin Academy, was the key resource person in both events.

Poster and oral presentation competition

A research poster presentation competition was held. Eight posters were selected for detailed oral presentation, of which three received oral presentation gold medals and three received poster presentation gold medals, and the remaining two were conferred consolation prizes. Travel cash awards were presented to Dr Shilpy Sharma and Dr Lekha Divya.

Conclusion

Overall, the second SAAE-India conference generated invigorated and enthusiastic response from stakeholders in India and abroad. It is hoped that the conferences and workshops in the future will be organized with more scope for presentations by all stakeholders, especially the young researchers.

Acknowledgements

The conference was supported financially by Science and Engineering Research Board (SERB), Department of Science and Technology, Government of India, New Delhi, Humane Society International, Doerenkamp-Zbinden Foundation, PETA-India, HiMedia Laboratories Pvt Ltd., Thermo Scientific, Jai Research Foundation, and Episkin Academy. The awards were sponsored by Episkin Academy- Loreal combine, France. Open Tox and Edelweiss Connect, Switzerland also collaborated in the endeavor. The National Centre for Alternatives to Animal Experiments, Bharathidasan University, Tiruchirappalli, India, was an academic partner. Additional academic support came from Dr Abhijit Majumder, Indian Institute of Technology, Mumbai, Dr Deepak Modi, National Institute for Research in Reproductive Health, Mumbai, and Dr Prajakta Dandekar, Institute for Chemical Technology, Mumbai. Great administrative and facility supports were availed from Sardar Gurinder Singh Bawa, Chairman, G.N. Khalsa College Management Committee, and Dr Kiran Mangaonkar, Principal, Guru Nanak Khalsa College. The technical support of Mr Dattaray Sawant and Mr. Tuskar Kamble, NFB, Mumbai, is gratefully acknowledged.

Pratiksha Palahe[1], Shalmali Pendse[1], Vinal Pardhi[1], Vikas Jha[1], Valencia D'souza[1], Sreejith P. Panicker[2], Sheikh Raisuddin[3,5] and Mohammad A. Akbarsha[4,5]

[1]National Facility for Biopharmaceuticals, G.N. Khalsa College, Matunga, Mumbai, India; [2]Department of Zoology, University of Kerala, Kariavattom, Thiruvananthapuram, India; [3]Department of Medical Elementology and Toxicology, Jamia Hamdard (Deemed University), New Delhi, India; [4]National College (Autonomous), Tiruchirappalli, India; [5]Society for Alternatives to Animal Experiments, Bharathidasan University, Tiruchirappalli, India

Journal of Ethnopharmacology 267 (2021) 113540

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

Upregulation of *Cyp19a1* and *PPAR-γ* in ovarian steroidogenic pathway by *Ficus religiosa*: A potential cure for polycystic ovary syndrome

Udhayaraj Suriyakalaa [a,b,c], Rajamanickam Ramachandran [a,b], Jaffar Ali Doulathunnisa [d], Smilin Bell Aseervatham [e], Devaraj Sankarganesh [b,f], Soundararajan Kamalakkannan [g], Balamuthu Kadalmani [h], Jayaraman Angayarkanni [b], Mohammad Abdulkader Akbarsha [i], Shanmugam Achiraman [a,*]

[a] Department of Environmental Biotechnology, Bharathidasan University, Tiruchirappalli, 620024, Tamilnadu, India
[b] Department of Microbial Biotechnology, Bharathiar University, Coimbatore, 641046, Tamilnadu, India
[c] PG and Research Department of Biotechnology, Hindusthan College of Arts and Science, Coimbatore, 641028, Tamilnadu, India
[d] State Key Laboratory of Bioelectronics, School of Biological Science and Medical Engineering, Southeast University, Nanjing, 210096, China
[e] PG and Research Department of Biotechnology and Bioinformatics, Holy Cross College (Autonomous), Tiruchirappalli, 620002, Tamilnadu, India
[f] Department of Biotechnology, School of Bio- and Chemical Engineering, Kalasalingam Academy of Research and Education, Krishnankoil, 626126, Tamilnadu, India
[g] Department of Zoology, Bishop Heber College, Tiruchirappalli, 620017, Tamilnadu, India
[h] Department of Animal Science, Bharathidasan University, Tiruchirappalli, 620024, Tamilnadu, India
[i] National College (Autonomous), Tiruchirappalli, 620001, Tamilnadu, India

ARTICLE INFO

Keywords:
Ficus religiosa
Infertility
PCOS
Estrous cycle
Propionic acid
Steroidogenesis

ABSTRACT

Ethnopharmacological relevance: Quite a few plants are in use to treat female infertility and associated problems. Availing the cues from traditional knowledge, phytochemical studies and ethnopharmacological evidences, the aphrodisiac plant *Ficus religiosa* (*F. religiosa*) is widely in use to cure infertility in women. For instance, the juice of leaf and aerial root of *F. religiosa* is reported to normalize the dysregulated menstrual cycle in women. Besides, it is believed that regular circumambulation of *F. religiosa* during the early hours of the morning helps women in alleviating infertility which could be attributed to the potential phytovolatiles released from *F. religiosa*. However, the evidences for therapeutic potential of *F. religiosa* in treating female infertility are arbitrary and mostly anecdotal.

Aim of the study: The present study was aimed at examining if extracts of fresh and/or dry leaf of *F. religiosa* would cure polycystic ovary syndrome (PCOS) in the rat model.

Methods: Rats were divided into seven groups; control (Group I), PCOS-induced (P.O, Letrozole −1 mg/kg BW for 21 days) and untreated (Group II), PCOS-induced and treated with the leaf extracts of *F. religiosa* (Groups III–VI), and, PCOS-induced and treated with pioglitazone (Group VII). The estrous intervals, body and organ weights (ovary and uterus), and serum hormones (testosterone, luteinizing hormone [LH], estrogen, and progesterone) were measured, and the expression of *Cyp19a1* (aromatase), and Peroxisome Proliferator-Activated Receptor-γ (*PPAR-γ*) were assessed in the experimental rats. The levels of 3β-hydroxysteroid dehydrogenase (3β-HSD), 17β-hydroxysteroid dehydrogenase (17β-HSD), and antioxidants (MDA, GSH, GPx, SOD, and CAT) were also quantified. Besides, the putative volatile compounds in the esterified leaf extracts were identified using Gas Chromatography-Mass Spectrometry (GC-MS).

Results: Letrozole treatment induced irregular estrous and altered weight of organs and hormonal milieu, which were reverted to normal in leaf extracts-treated PCOS-induced rats. Remarkably, fresh leaf treatment up-regulated *Cyp19a1*and *PPAR-γ* and increased the levels of 3β-HSD and 17β-HSD. We found 3-acetoxy-3-hydroxy-propionic acid in fresh and dry leaf extracts, which we attribute to efficacy of the extracts in alleviating PCOS.

Conclusion: Put together, our findings suggest the leaves of *F. religiosa* as potential in alleviating PCOS, mainly due to the presence of putative volatile molecules. Further screening of the leaves of *F. religiosa* is recommended to identify other key molecules and to develop a systematic therapeutic intervention for PCOS.

* Corresponding author.
E-mail addresses: achiramans@gmail.com, achiramans@bdu.ac.in (S. Achiraman).

https://doi.org/10.1016/j.jep.2020.113540
Received 24 October 2019; Received in revised form 3 August 2020; Accepted 29 October 2020
Available online 3 November 2020
0378-8741/© 2020 Elsevier B.V. All rights reserved.

1. Introduction

The development of ovum and its discharge from the follicle are impaired due to hormonal imbalance which in turn leads to menstrual irregularities, and eventually causes ovulation disorders including PCOS. These are among the most common endocrinopathies in women in the reproductive age. It is essentially caused due to hyperandrogenism and oligo-/an-ovulation which in turn would results in polycystic ovary disease (PCOD/PCOS), an established aspect of female infertility (Sankarganesh et al., 2019). The co-morbidities of PCOS include insulin resistance, obesity, type 2 diabetes, hypercholesterolemia, and oxidative stress (Elsheikh and Murphy, 2008). Chemotherapeutic intervention of PCOS, which is non-invasive and cost-affordable, invariably ends up with adverse side effects, whereas surgery is not only invasive and risky but also expensive (Althuis et al., 2005; Brinton et al., 2004; Calderon-Margalit et al., 2009; Mital et al., 2012; Terry et al., 2006). A handy approach to female infertility problems in general and PCOS in particular is herbal medicine among which *F. religiosa* is assuring.

F. religiosa (Order Rosales; Family Moraceae) is popularly known as sacred fig, bodhi tree, peepal tree, etc. In India, temples host this tree and people circumambulate the tree during the early hours of the day (Chandrakanth et al., 1990). It is an aphrodisiac plant and, hence, all the parts are in use in the treatment of various aspects of infertility, and thus it is believed to be medicinally important (Kirtikar and Basu, 1993; Panda, 2005; Prasad et al., 2006). Especially, the leaves reportedly regulate the irregular menstrual cycle and rectify delayed onset of puberty (Chopra, 1958; Ghimire and Bastakoti, 2009). In the Ayurvedic system of medicine, *F. religiosa* is used in formulations of Rasayanas, which contain antioxidants and rejuvenators, and prescribed for the treatment of vaginal and uterine disorders, and menstrual irregularities (Chopra, 1958). The above pharmacological applications of *F. religiosa* are based on anecdotal leads, but scientific evidence to support the aphrodisiac and fertility-enhancing properties of *F. religiosa* is still lacking. Therefore, it is appropriate to find scientific evidence for the potential of *F. religiosa* to alleviate female infertility using an established animal model (e.g., rat model of PCOS).

PCOS essentially concerns the ovary where impairment of follicular development and imbalance of steroid hormones are noticed (Sun et al., 2013). Clinical and biochemical hyperandrogenism and multiple follicular cysts in the ovary are the hallmarks of PCOS (Dunaif and Thomas, 2001). At the molecular level, up- or down-regulation of *Cyp19a1* and *PPAR-γ* is pivotal in the pathogenesis of PCOS (Gu and Baek, 2009; He et al., 2012; Hong and Qu, 2009; Qin and Rosenfield, 1998; Xita et al., 2009). Letrozole (a non-steroidal aromatase inhibitor)-induced PCOS model is appropriate to look at pathogenesis of PCOS and investigate therapeutic modalities. In its mechanism of action, letrozole inhibits/blocks the conversion of testosterone into estrogen resulting in excess androgen and induces all aspects of human PCOS (Kafali et al., 2004). The theca cells of the ovary express cytochrome P450c17, which is involved in the synthesis of androgens (dehydroepiandrosterone and androstenedione) from pregnenolone and progesterone. The androgens thus formed will be converted in the granulosa cells of ovary into estrogens by cytochrome P450 aromatase (*Cyp19a1*) (Mendelson and Kamat, 2007; Payne and Hales, 2004). Concomitantly, ovaries also secrete androgens directly into circulation. But, unlike in men, ovarian androgens in women do not engage in the negative feedback of LH. Therefore, ovarian androgen production is not controlled (Baptiste et al., 2010). Albeit several genes (*Cyp11α, Cyp17, Cyp19, LHR, FSHR, IRS, and PPAR-γ*) are involved in the manifestation of PCOS, *PPAR-γ* is one among the candidate genes (He et al., 2012). In this sense, thiazolidinediones (pioglitazone, rosiglitazone, troglitazone, etc.), a group of *PPAR-γ* agonists, are the first-line therapeutics for PCOS (Stout and Fugate, 2005). It has been proved that pioglitazone benefits the alleviation of PCOS by improving the antioxidant status, instead of modulating the metabolic reactions (Rezvanfar et al., 2012a).

Numerous studies have spotted *PPAR-γ* in the ovary, but information regarding its downstream activation of other genes/proteins is scanty (Banerjee and Komar, 2006). It was reported that *PPAR-γ* activation inhibits aromatase expression in cultured granulosa cells of rat (Lovekamp-Swan et al., 2003), and granulosa cells of humans (Mu et al., 2000). In contrast, positive influence of *PPAR-γ* over aromatase has not been reported in other studies (Banerjee and Komar, 2006; Komar et al., 2001; Komar and Curry Jr, 2003). Aromatase activity was unaffected by rosiglitazone while the expression and synthesis of testosterone were down-regulated (Chen et al., 2009; Rak-Mardyla and Karpeta, 2014). This concept being not adequately supported, it is pertinent to study the effect of *PPAR-γ* on aromatase and estrogen regulation in PCOS condition.

Hence, the present study aims at examining if *PPAR-γ* would modulate aromatase activity and estrogen level in PCOS condition, and to elucidate the pharmacological action of *F. religiosa* in the regulation of estrous cycle and hormonal imbalance using letrozole-induced PCOS rat model (hereinafter, PCOS rat).

2. Materials and methods

2.1. Collection of plant material and extraction

The leaves of *F. religiosa* were collected from Bharathidasan University campus, Tiruchirappalli, India, and authenticated by the Botanical Survey of India, Southern Regional Centre, Coimbatore, India. The reference specimen (No: BSI/SRC/5/23/2019/Tech/253) is preserved in the institution herbarium. The leaves were washed, shade-dried, pulverized as a coarse powder, using a mixer grinder, and sieved. Twenty grams of the dry leaf powder was extracted with 200 mL of distilled water (10% extract) over a period of 48 h on a mechanical shaker. Aqueous extraction of fresh leaf was performed according to Adeneye et al. (2008) with minor modification. In brief, freshly plucked leaves (20 g) were ground using a mixer grinder with 200 mL of distilled water, and considered as 10% extract. Both the extracts were separately filtered using a Buchner funnel and filter paper. The dry leaf extract was lyophilized in a freeze dryer (CHRIST ALPHA 2plus, Germany). The lyophilized dry leaf extracts were reconstituted in distilled water at two different concentrations for further use, wherein fresh leaf extract was prepared then and there. Both the fresh and dry leaf extracts prepared as mentioned above were lyophilized separately and used in trans-esterification process for volatile analysis.

2.2. Experimental animals and ethics statement

Ten-to-twelve weeks old female albino Wistar rats (*Rattus norvegicus*) weighing 150–180 g were procured from Kerala Veterinary and Animal Sciences University (KVASU), Kerala, India, and housed in polypropylene cages at 25 ± 2 °C with controlled humidity (45%–55%) and light-dark cycle (12:12 h). The animals had free access to feed (Amrut Feed, Bangalore, India) and water *ad libitum*. The procedure involving animals was approved by the Institutional Animal Ethics Committee, Bharathidasan University, Tiruchirappalli-24 (BDU/IAEC/47/2013/09.04.2013), and performed in adherence to the guidelines of the committee.

2.3. Determination of estrous cycle

Estrous stages were determined by microscopic analysis of vaginal smear using the relative proportion of nucleated epithelial cells, cornified cells, and leucocytes. It was performed each day morning between 7:00 and 9:00 am (Achiraman and Archunan, 2006). The length of the estrous cycle of rats is 4–6 days and consists of four stages, *viz.* pro-estrus, estrus, met-estrus, and di-estrus. A pro-estrus vaginal lavage was confirmed from predominantly nucleated epithelial cells; in estrus sample, a large number of cornified epithelial cells were found; in met-estrus, the proportion of nucleated epithelial cells, cornified cells

and leucocytes were found to be roughly equal; a di-estrus vaginal smear contained leucocytes at a large number. The rats with the concurrence of 4–5 days of the estrous cycle were included for the experiment.

2.4. Induction of PCOS

To induce PCOS, letrozole (Letroz®, Sun Pharmaceutical Industries *Ltd*, Mumbai) was dissolved in 0.5% carboxymethyl cellulose (CMC), and administered to the rats at 1 mg/kg BW concentration daily through oral route consecutively for 21 days. The control group received 0.5% of CMC as vehicle (Kafali et al., 2004).

2.5. Experimental design

The rats were divided into seven groups of six each. Group I was vehicle control for letrozole, and received CMC (oral, p.o.). The remaining six groups of rats were orally administered with 1 mg/kg BW of letrozole dissolved in 0.5% CMC at the volume of 2.0 mL/kg BW (which contain letrozole at above mentioned concentration) once daily for 21 days). Group II was PCOS-induced but untreated and served as PCOS control. Group III was administered 10% fresh leaf extract of *F. religiosa* (1.0 mL/kg BW, oral); Group IV was kept in an empty cage sprayed with the fresh leaf extract (10%), and subsequently transferred to another cage with husk, where cotton dipped in fresh leaf extract was placed in a closed Petri dish in expectation that the extract would release the active volatile compound(s) in a consistent manner, through the small holes on the top of the Petri dish; Group V was treated with standard PCOS drug (pioglitazone) (20 mg/kg BW p.o.). The remaining two groups were given 100 mg/kg BW (low dose), and 300 mg/kg BW (mid-dose) of aqueous dry leaf extract (p.o.), respectively. The given doses were determined based on the available literature and found to be non-toxic (Singh et al., 2011; Krishnaraju et al., 2006). All the treatments were continued for 30 days post-letrozole administration. At the end of the experiment (day 52), the rats were deprived of food overnight and sacrificed.

2.6. Determination of body weight and relative organ weight

The bodyweight of the animals was recorded regularly before the onset (day 0), post-induction of PCOS (day 22), and at the end of the treatment (day 51). Instantly after sacrifice, the weights of the organs of interest were recorded using an electronic balance (Denver, Germany). The relative organ weights were calculated using the following formula (Sharif et al., 2015):

Relative organ weight (%) = [Absolute organ weight/Body weight] × 100.

2.7. Blood collection and tissue processing

In preparation of 2.6 above, the rats were anesthetized with an overdose of ketamine (90–120 mg/kg BW), and blood was drawn directly from the heart using a syringe fitted with #22 needle. The blood was transferred to conical centrifuge tube, allowed to clot and centrifuged (3000 rpm for 15 min at 25 °C). The serum was separated and stored at −20 °C. The ovaries and uterine horns were excised, cleaned free of fat, and weighed. The ovaries, liver, and adipse tissues to be used in gene expression studies were further flash-frozen in liquid nitrogen and stored at −80 °C.

2.8. Hormone assays

The serum concentrations of testosterone (Product code-3725-300), LH (Product code-625-300), estrogen (Product code-4925-300), and progesterone (Product code-4825-300) were determined using enzyme-linked immunosorbent assay (ELISA) adopting the manufacturer's protocols (ELISA kits, Monobind Inc, USA).

Table 1
Details of the primer and the size of their amplification product are as follows:

Gene name	Sequence	Desired size
***PPAR γ* F**	5'- GCC AAG GCG AGG GCG ATC-3'	180 bp
***PPAR γ* R**	5'-CAG GGA TCG AAA CTG GCA CC-3'	
***Cyp19a1* F**	5'-CAG CAA GCG TGG GCT GC-3'	
***Cyp19a1* R**	5'-TCA CCA CGT CCA CGT AGC C-3'	215 bp

2.9. Steroidogenic enzyme assays

2.9.1. 3β-Hydroxysteroid dehydrogenase (3β-HSD)

The 3β-HSD activity was determined by the method of Bergmeyer (1974). In brief, the ovarian tissue was homogenized using ice-cold homogenization buffer (pH 7.2) and centrifuged at 12000 rpm for 30 min at 4 °C. The resultant supernatant was used to assay 3β-HSD. The reaction mixture contained 0.6 mL pyrophosphate buffer (100 mM), 0.2 mL NAD (0.5 mM), 2.0 mL distilled water, and 0.1 mL pregnenolone (0.1 mM). The supernatant was added to the reaction mixture, and the absorbance was measured every 20 s for 5 min, at 340 nm, in a spectrophotometer, against the blank.

2.9.2. 17β-Hydroxysteroid dehydrogenase (17β-HSD)

The 17β-HSD activity of the ovarian tissue extract was determined by the method of Bergmeyer (1974). The reaction mixture consisted of 0.6 mL pyrophosphate buffer (100 mM), 0.2 mL NADPH (0.5 mM), 2.0 mL distilled water, and 0.1 mL of 1,4-androstenedione-3,17-dione (0.8 mM). The absorbance was measured at 30 s intervals for over 5 min, at 340 nm, in a spectrophotometer, against the blank.

2.10. RNA isolation, reverse transcriptase polymerase chain reaction (RT-PCR) and quantitative PCR (qPCR)

Total ovarian RNA was extracted using TRIzol RNAase reagent kit (EZ one step RNA reagent, cat#BS410A, Biobasics, Canada) following the standard protocol prescribed by the manufacturer. The concentration and purity of RNA were assessed using a Biophotometer (Biophotometer plus, Eppendorf, Germany). Subsequently, 2 μg of total RNA was reverse transcribed into complementary DNA (cDNA) using a Verso cDNA synthesis kit (Cat. No #AB-1453/B, Thermo Scientific, Massachusetts, USA) in a Bio-Rad thermal cycler (Model: S1000TM, thermal cycler with dual 48/48 fast reaction module, Bio-Rad Laboratories, California, USA). Reverse transcription was achieved using the following temperature regimens: 72 °C for 5 min, 4 °C for 10 min, 42 °C for 60 min, 72 °C for 10 min, and 92 °C for 2 min. Real-time PCR (qPCR) was performed in triplicate using a Real-Time PCR system (LightCycler 96®, Roche, Basel, Switzerland). The total volume of the reaction mixture was 20 μL, to which 10 μL of 2 × SYBR Premix Ex Taq and Tli RNase H Plus (TaKaRa Bio Inc, Cat. #RR420A), 1.0 μL of each primer (forward and reverse- 3 pmol each), 2.0 μL of cDNA, and 6.0 μL of sterile water were added. The primers were designed using online software (Oligoanalyzer) within the coding domain sequence, and reverse complementarity and similarity searches were done in BLAST. The primers were purchased from Shrimpex Biotech Services Pvt. Ltd (Chennai, India). 18S rRNA and β-actin were used as the endogenous reference to normalize gene expression, and the $2^{-\Delta\Delta CT}$ method was used to determine the differences in messenger RNA (mRNA) content between samples (Table 1).

2.11. Lipid peroxidation and antioxidant assays

The level of lipid peroxidation and antioxidant status of the ovary were analyzed using the standard protocols as follows: Lipid peroxidation (MDA) (Ohkawa et al., 1979), reduced glutathione (GSH) (Moron et al., 1979), glutathione peroxidase (GPX) (Rotruck et al., 1973), superoxide dismutase (SOD) (Marklund and Marklund, 1974), and

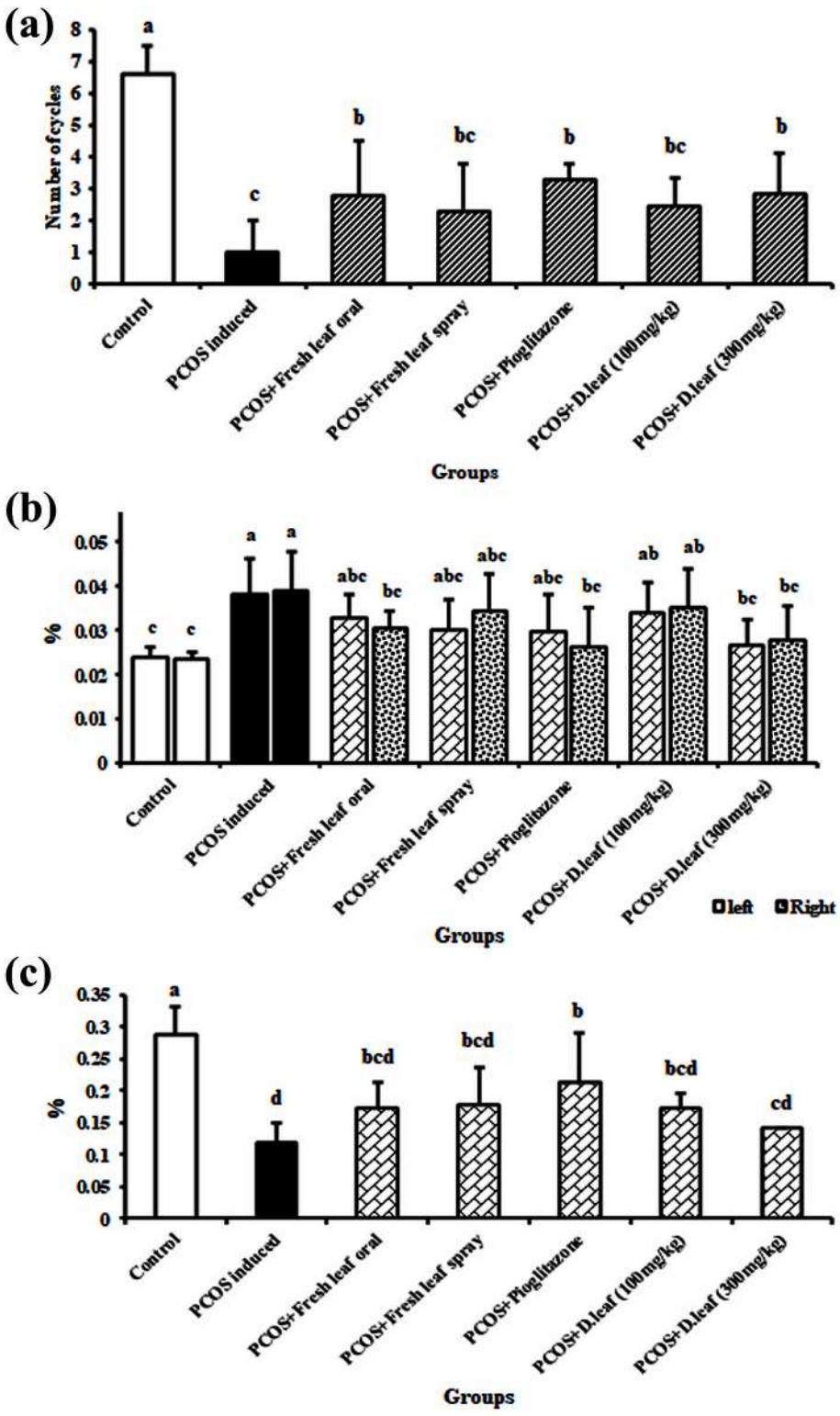


Fig. 1. Reproductive status and organ weights of the experimental rats. (a) Number of estrous cycles, (b) Ovarian weight, and (c) Uterus weight. Values are expressed as mean ± SD (N = 6). Bars with different alphabets are significantly different from each other, and those with same alphabets are not significantly different ($P < 0.05$).

catalase (CAT) (Sinha, 1972).

2.12. GC-MS)

2.12.1. Preparation of sample

The lyophilized aqueous extracts were subjected to a direct trans-esterification process before GC-MS analysis. In brief, 0.5 g of each leaf samples (dry and fresh) was mixed with 4.0 mL of 1.09 M methanolic HCl (0.738 mL of acetyl chloride made up to 10 mL using methanol) and 4.0 mL of methanol. The tubes were then placed in a water bath for 1 h at 80 °C. To this, 4.0 mL of distilled water and 4.0 mL of hexane were added, and vortexed for 15 s. Subsequently, the solutions

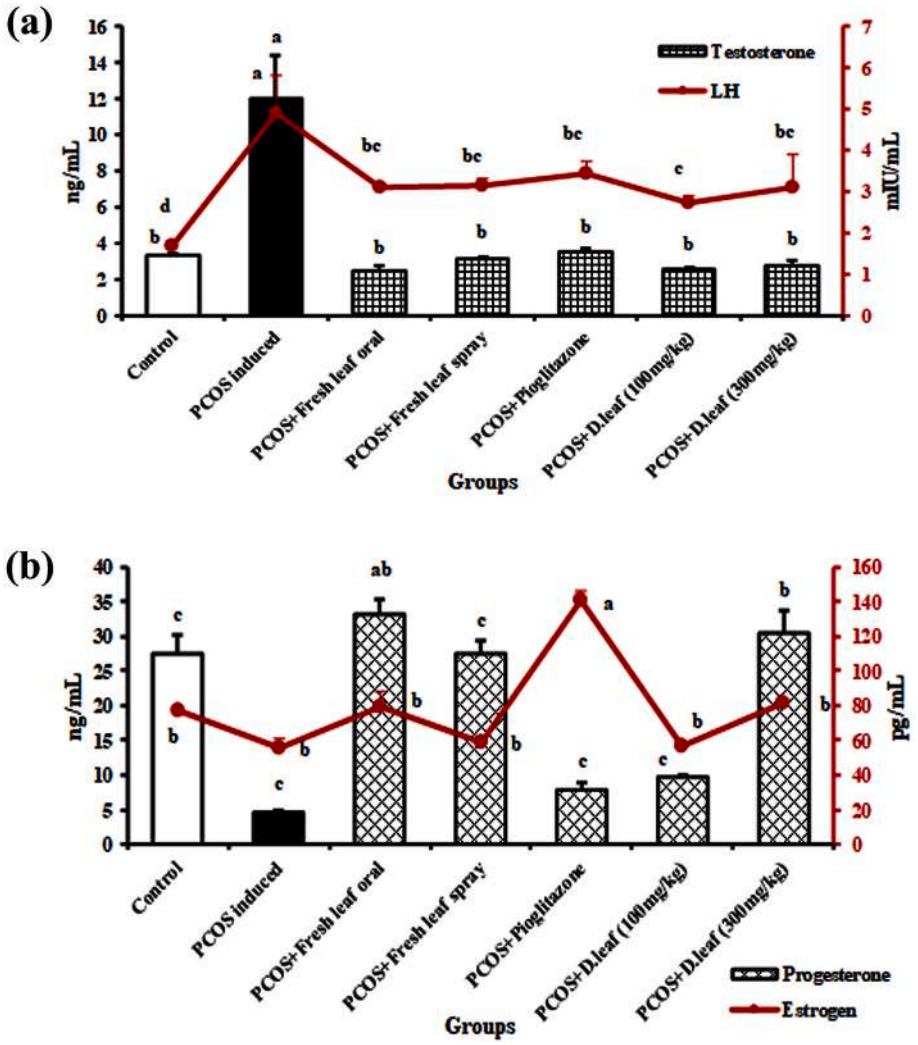


Fig. 2. Serum hormone levels in the experimental rats. (a) Testosterone and LH, (b) Progesterone and Estrogen. Values are expressed as mean ± SD (N = 6). Bars with different alphabets are significantly different from each other and those with same alphabets are not significantly different ($P < 0.05$).

were centrifuged at 1300×*g* for 3 min. The resultant upper phase was transferred to GC-MS vial and used in the analysis.

2.12.2. GC-MS condition

The extracts of fresh and dry leaves were analyzed using GC-MS (QP-2010, Shimadzu, Japan). Two microlitres of the extracts were injected individually into the injector port (280 °C) of the GC-MS (QP-2010, Shimadzu, Japan) in split mode (split ratio 1:30). GC consisted of a 30-m glass capillary column (RTx-5MS), which had a film thickness of 0.25 mm (0.2 mm i.d., coated with crossbond diphenyl dimethyl polysiloxane). The ion source and transfer line temperatures were 300 °C and 240 °C, respectively. The temperature programs used for fresh and dry leaf extracts were as follows: 40 °C for 5 min, increased at a rate of $10°C^{-1}$, and held at 240 °C for 5 min for fresh leaf extract; 40 °C for 5 min, increased at the rate of $15°C^{-1}$, and held at 205 °C for 4 min for dry leaf extract. The scan was delayed for a few min to avoid the solvent peak. Electron ionization was performed at 70 eV using helium as carrier gas (1 mL/min). The m/z was set between 30 and 600. The unknown compounds showing up in the chromatogram were identified based on probability matching using the library (NIST05).

2.13. Statistical analysis

The statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS version 17 (SPSS Inc., Chicago, IL, USA) and individual comparisons were obtained by Duncan's Multiple Range Test. The values were expressed as mean ± SD of six animals, where significance was declared at $P < 0.05$ (Zar, 1984).

3. Results

3.1. Effect of F. religiosa treatment in the regulation of estrous cycle

The duration of estrous cycle of control rats was 4–5 days and irregular in PCOS rats. The rats, after letrozole administration, exhibited either extended di-estrus or extended met-estrus, whereupon the total number of cycles over the duration of study decreased ($P < 0.05$). The estrous cycle of leaf extracts- and pioglitazone-treated rats was restored to regular (Fig. 1a). However, the difference between treatment groups was not significant.

3.2. Effect of F. religiosa treatment on ovarian and uterine weights of rats

PCOS rats showed a significant increase in ovarian weight compared

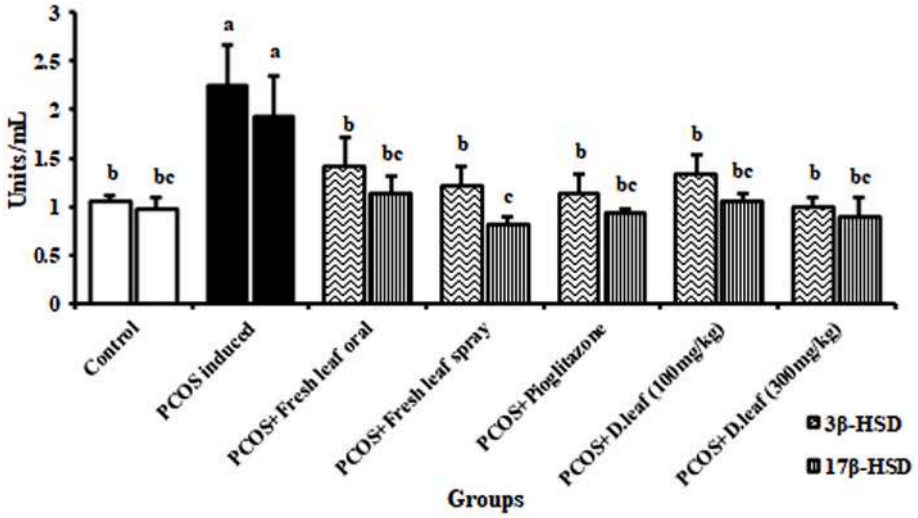


Fig. 3. Activity levels of steroidogenic enzymes (3β-HSD and 17β-HSD) in the ovary of the experimental rats. Values are expressed as mean ± SD (N = 6). Bars with different alphabets are significantly different from each other and those with same alphabets are not significantly different ($p < 0.05$).

to control. In contrast, treatment with leaf extracts or pioglitazone substantially revoked the weight towards normal. In particular, pioglitazone was more efficient in restoring the ovarian weight than extracts of dry and fresh leaves ($P < 0.05$) (Fig. 1b). The results in respect to uterus were opposite to the ovary. The weight of uterus remarkably decreased in PCOS rats and increased with leaf extract treatments (Fig. 1c).

3.3. Effect of F. religiosa treatment on serum hormonal profile of PCOS rats

The untreated PCOS rats showed higher LH and testosterone levels than control rats, which was decreased with leaf extracts and pioglitazone treatments ($P < 0.05$). Treatment of fresh leaf (oral as well as spray) was more efficient than pioglitazone (Fig. 2a). The level of progesterone decreased in PCOS rats compared to control ($P < 0.05$). However, treatment of fresh leaf (oral as well as spray), and dry leaf (300 mg/kg BW) extracts improved progesterone to significant level. In contrast, neither pioglitazone nor dry leaf extract (100 mg/kg BW) was able to improve progesterone level (Fig. 2b). Similar to progesterone, estrogen level was also decreased in PCOS rats, but insignificantly. However, pioglitazone treatment increased the estrogen level in PCOS rats ($P < 0.05$). As expected, an increase in estrogen was observed in PCOS rats treated with fresh leaf extract and dry leaf extract (300 mg/kg BW), but the change was insignificant (Fig. 2b). The decreased estrogen level in the PCOS group was in view of the metestrus state of the animal. Since in the control group, the samples were collected from animals in estrus stage there was an insignificant difference between the PCOS and control groups in terms of estrogen level.

3.4. Effect of F. religiosa treatment on ovarian steroidogenic enzymes of PCOS rats

The 3β-HSD activity was increased to significant level in PCOS rats, and decreased on treatment with fresh and dry leaf extracts, and pioglitazone ($P < 0.05$). The data from fresh leaf extract spray, pioglitazone, and dry leaf extract (300 mg/kg BW) treated rats were comparable with those of normal rats (Fig. 3). The 17β-HSD activity was also increased in PCOS rats, which was reduced subsequently on treatment of fresh leaf (oral and spray), dry leaf extracts, and pioglitazone (Fig. 3).

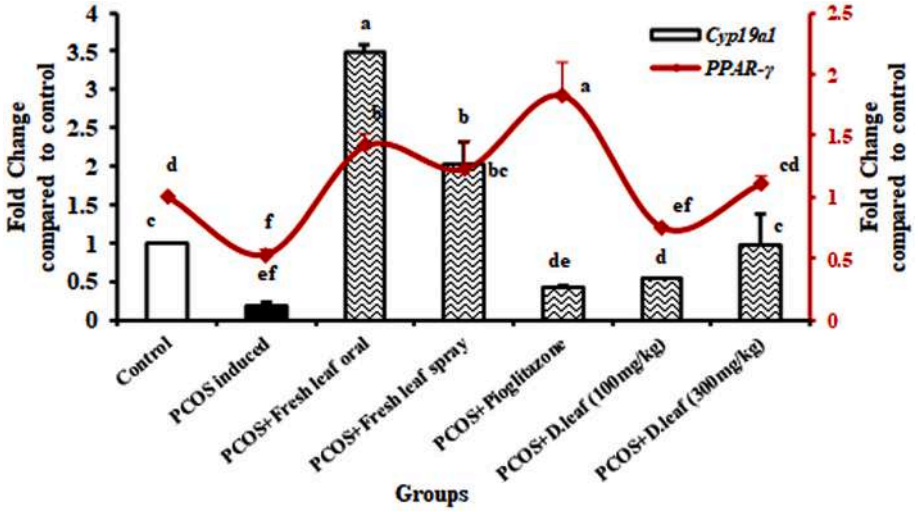


Fig. 4. qPCR data of expression of *Cyp19a1* (aromatase) and *PPAR-γ* in the ovary of the experimental rats. Results were compared with control, and β-actin was used as an internal standard. Each bar is mean ± SD of triplicate values. Groups with different alphabets are significantly different from each other, and those with same alphabets are not significantly different ($P < 0.05$).

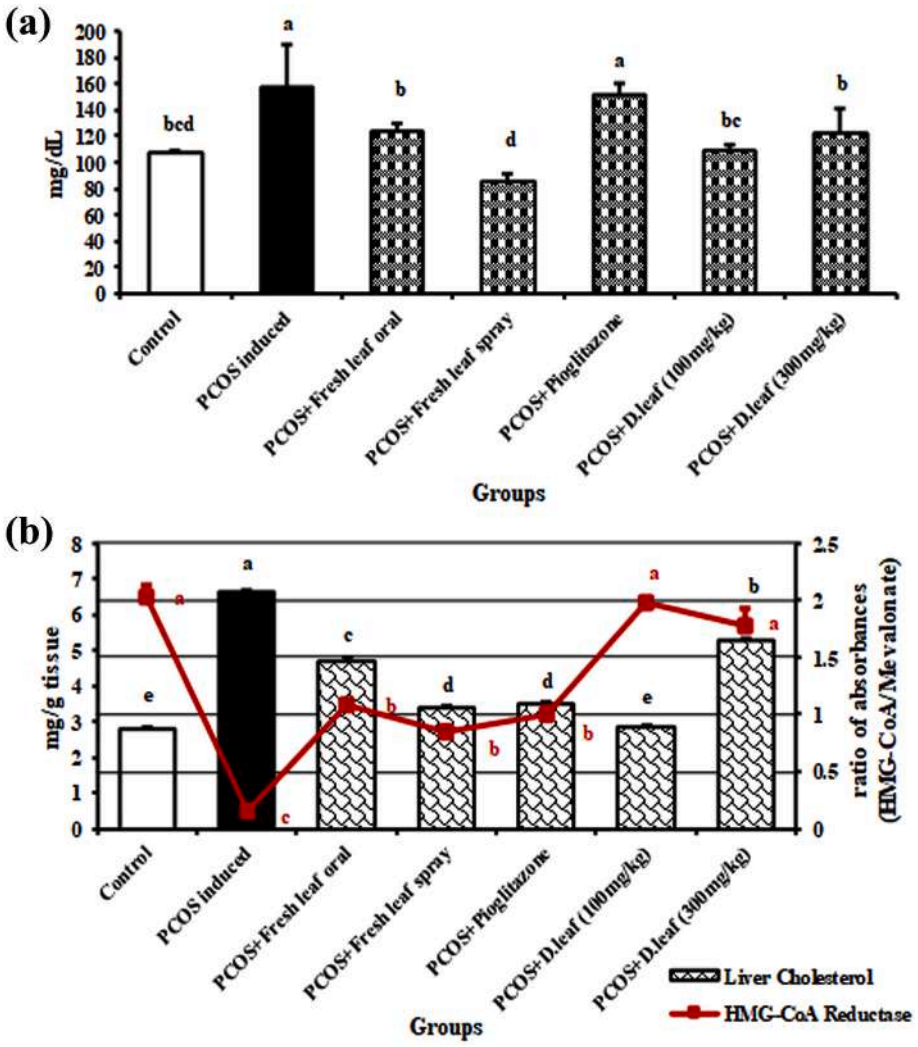


Fig. 5. The lipid profile analyzed in the experimental rats. (a) Serum cholesterol; (b) Liver cholesterol and HMG-CoA Reductase activity. Values are expressed as mean ± SD (N = 6). Bars with different alphabets are significantly different from each other, and those with same alphabets are not significantly different ($p < 0.05$).

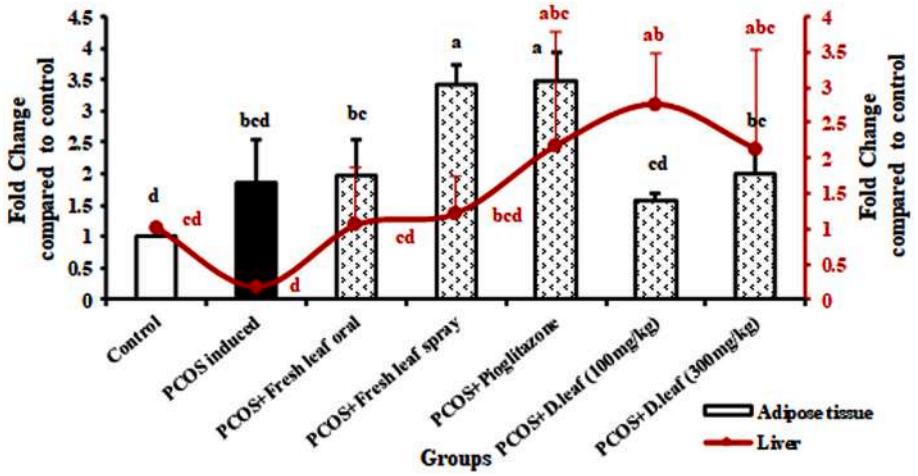


Fig. 6. *PPAR-γ* mRNA in the liver and adipose tissues of experimental rats. Results were compared with control, and β-actin was used as an internal standard. Each bar contains the triplicate values of mean ± SD. Bars with different alphabets are significantly different from each other, and bars with same alphabets do not differ ($p < 0.05$).

Table 2
Status of enzymatic (SOD, CAT, GPx) and non-enzymatic antioxidants (GSH & MDA) in the ovarian tissue of experimental rats.

GROUPS	MDA	GSH	SOD	CAT	GPx
Control	5.36 ± 0.93^{cd}	4.83 ± 0.90^{ab}	48.34 ± 2.94^{c}	29.38 ± 2.26^{bcd}	24.60 ± 4.45^{a}
PCOS induced	9.39 ± 0.32^{b}	2.46 ± 0.91^{c}	22.54 ± 2.76^{d}	10.47 ± 0.15^{e}	5.32 ± 1.05^{c}
PCOS + Fresh leaf (E); Oral	4.29 ± 0.94^{cd}	4.38 ± 1.80^{abc}	47.31 ± 5.11^{c}	33.59 ± 5.98^{b}	28.23 ± 2.75^{a}
PCOS + Fresh leaf (E); Spray	8.83 ± 2.28^{b}	4.22 ± 0.89^{abc}	65.37 ± 3.32^{a}	54.38 ± 2.74^{a}	24.02 ± 2.97^{a}
PCOS + Pioglitazone	14.17 ± 0.46^{a}	5.15 ± 1.07^{a}	47.29 ± 3.15^{c}	32.29 ± 2.56^{bc}	14.99 ± 2.77^{b}
PCOS + Dried leaf (E).(100 mg/kg BW)	5.68 ± 1.48^{c}	2.81 ± 0.30^{c}	55.17 ± 3.03^{b}	27.38 ± 3.10^{cd}	14.51 ± 0.52^{b}
PCOS + Dried leaf (E).(300 mg/kg BW)	5.96 ± 0.49^{c}	3.05 ± 0.16^{bc}	41.00 ± 2.00^{c}	28.09 ± 2.12^{bcd}	11.47 ± 1.28^{b}

Values are expressed as mean ± SD of 6 animals. E, Extract. Values with different superscript alphabets imply significant difference from each other and same superscript alphabets imply insignificant difference ($P < 0.05$).

3.5. Effect of F. religiosa treatment on ovarian Cyp19a1 (aromatase) and PPAR-γ

Cyp19a1 was changed several-fold in the experimental rats. In PCOS rats, *Cyp19a1* was down-regulated compared to control. Interestingly, it was increased manifold in fresh leaf extract treated rats (oral and spray). Also, the expression of *Cyp19a1* observed in dry leaf extract (300 mg/kg BW) treated rats was similar to control, followed by dry leaf extract (100 mg/kg BW) and pioglitazone treatment (Fig. 4).

The ovarian mRNA of *PPAR-γ* also revealed a considerable down-regulation in PCOS rats compared to other groups. Treatment with pioglitazone and fresh leaf (oral and spray) extracts caused significant upregulation of *PPAR-γ*. Even though the treatment of dry leaf extract (300 mg/kg BW) up-regulated the mRNA transcript of *PPAR-γ*, the expression level was near to control (Fig. 4).

3.6. Effect of F. religiosa treatment on serum and liver lipid profile

Serum total cholesterol was increased to significant level ($P < 0.05$) in PCOS rats and decreased on treatment with fresh leaf (spray), and dry leaf (100 mg/kg BW) extracts. Serum total cholesterol was decreased in rats treated with fresh (oral) and dry leaf (300 mg/kg BW) extracts. Among all the treatments, fresh leaf (spray and oral) extracts restored the cholesterol level close to normal (Fig. 5a). The significantly increased liver cholesterol level of PCOS rats was reduced in treatment with fresh leaf (spray), pioglitazone, and dry leaf extract (100 mg/kg BW) ($P < 0.05$) (Fig. 5b). The liver cholesterol level of the experimental groups also matched the results of HMG-CoA reductase activity, as illustrated in Fig. 5b. It further implies that the ratio of HMG-CoA to mevalonate was significantly ($P < 0.05$) lower in PCOS rats, which positively concurs with the increased activity of the enzyme, concomitantly with cholesterol content of the liver. However, treatment with fresh and dry leaf extracts caused decrease of activity of HMG-CoA comparable to PCOS rats.

3.7. Expression analysis of PPAR-γ in liver and adipose tissue of experimental rats through qPCR

PPAR-γ expression was down-regulated in the liver of PCOS-induced rats, which was up-regulated on pioglitazone treatment, next to leaf extracts treatment (Fig. 6). In contrast, parametrial adipose tissue of PCOS rats revealed an up-regulation of *PPAR-γ*. Fresh leaf extract and pioglitazone treatments further increased the expression of *PPAR-γ* compared to PCOS rats, and all other treatment groups. Besides, all other treatment groups showed an up-regulation in *PPAR-γ*, compared to control and PCOS rats (Fig. 6). It is prudent to consider the effectiveness of fresh leaf spray and pioglitazone treatments in expressing *PPAR-γ* in the adipose tissue.

3.8. Leaf extract of F. religiosa counteracts PCOS by influencing ovarian antioxidants

The overproduced free radicals resulting from letrozole administration to the rats spiked the lipid peroxidation, which was evidenced by increased MDA levels. The treatment with pioglitazone exacerbated this condition over letrozole, indicating its adverse side-effect. On the other hand, letrozole treatment decreased the level of non-enzymatic (GSH) and enzymatic antioxidants such as SOD, CAT, and GPx. Though the dry leaf extract and pioglitazone modulated both types of antioxidants (enzymatic and non-enzymatic) towards normal, wherein high efficiency was attributable only to fresh leaf extract (Table 2).

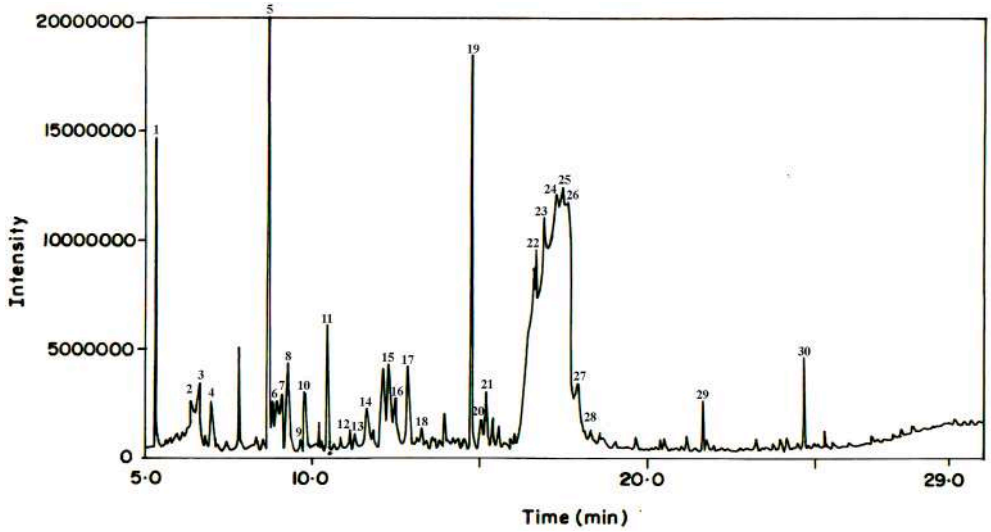


Fig. 7. GC-MS chromatogram of fresh leaf extract of *F. religiosa*.

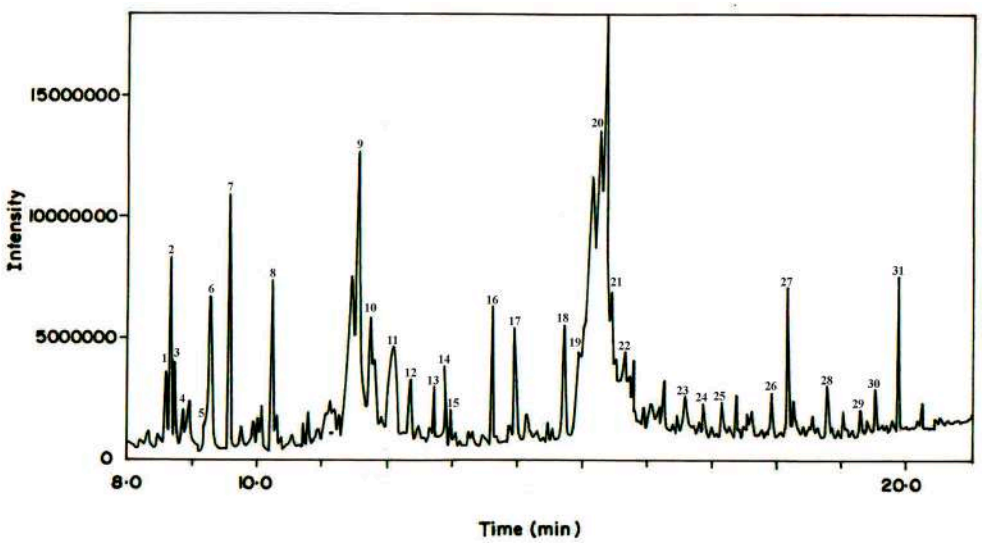


Fig. 8. GC-MS chromatogram of dry leaf extract of *F. religiosa*.

Table 3
The compounds identified in the esterified fresh leaf extract of *F. religiosa* and their properties.

Peak No.	m/z values	RT	% peak area	Compound name	Mol weight (Da)	Mol formula
1	113	5.306	3.15	Oxime methoxy phenyl	151	$C_8H_9NO_2$
2	94	6.375	1.09	Phenyl alpha-d-sedoheptuloside	286	$C_{13}H_{18}O_7$
3	47	6.498	1.49	2,2-Diethoxyethanol	134	$C_6H_{14}O_3$
4	28	6.993	1.02	2-Butenedioic acid (E)-dimethyl ester (Fumaric acid)	144	$C_6H_8O_4$
5	103	8.679	5.80	3-acetoxy 3-hydroxy propionic acid	162	$C_6H_{10}O_5$
6	84	8.764	0.59	Ethyl pipecolinate	157	$C_8H_{15}NO_2$
7	79	9.028	0.52	2,4-Heptadienoic acid, methyl ester	140	$C_8H_{12}O_2$
8	79	9.081	0.58	2,4-Heptadienoic acid, methyl ester	140	$C_8H_{12}O_2$
9	105	9.29	2.28	Benzoic acid	122	$C_7H_6O_2$
10	109	9.769	1.37	1,2-Benzenediol (Catechol)	110	$C_6H_6O_2$
11	43	10.42	1.64	2,6-Dimethyl-4H-pyran-4-one	124	$C_7H_8O_2$
12	95	11.225	0.24	4-(4-Methyl-[1,3,2]dioxaborinan-2-yloxy)	208	$C_{10}H_{13}BO_4$
13	60	11.586	1.39	Alpha-L-galactopyranoside	178	$C_7H_{14}O_5$
14	60	12.075	3.04	Methyl-beta-D-arabinopyranoside	164	$C_6H_{12}O_5$
15	100	12.261	2.34	5-Hydroxypipecolic acid	145	$C_6H_{11}NO_3$
16	60	12.454	1.38	Methyl pentopyranoside	164	$C_6H_{12}O_5$
17	112	12.818	2.36	2H-Pyran-3,4,5-triol, tetrahydro-2-methoxy-6-methyl	178	$C_7H_{14}O_5$
18	132	13.241	0.25	4H-pyran-4-one, 3-acetyl-2,6-dimethyl	168	$C_8H_8O_4$
19	143	14.753	5.11	Citric acid, trimethyl ester	234	$C_9H_{14}O_7$
20	113	15.06	0.81	D-Allose	180	$C_6H_{12}O_6$
21	143	15.375	0.26	1,2,3-Propanetricarboxylic acid, 1-hydroxy-trimethyl ester	234	$C_9H_{14}O_7$
22	87	16.583	10.83	Methyl (methyl 4-O-methyl-alpha-d-mannopyranoside)	236	$C_9H_{16}O_7$
23	111	16.929	15.79	3-thiophenecarboxylic acid	128	$C_5H_4O_2S$
24	103	17.083	5.79	Alpha-D-galactopyranosiduronic acid	222	$C_8H_{14}O_7$
25	69	17.225	21.10	Acrylsaeure, 3-(1-methoxycarbonylpropyl)-cyclopropyl-, methyl ester, trans	226	$C_{12}H_{18}O_4$
26	60	17.642	5.39	Methyl beta-D-glucopyranoside	194	$C_7H_{14}O_6$
27	97	17.775	1.28	Hexadecanedinitrile	248	$C_{16}H_{28}N_2$
28	60	17.927	1.57	alpha.-D-Galactopyranoside, methyl	194	$C_7H_{14}O_6$
29	159	21.629	0.61	l-(+)-Ascorbic acid 2,6-dihexadecanoate	652	$C_{38}H_{68}O_8$
30	213	24.662	0.90	2,4-pentadienoic acid, 5-(8-hydroxy-1,5-dimethyl-3-oxo-6-oxabicyclo [3.2.1]oct-8-yl)-3-methyl	294	$C_{16}H_{22}O_5$

RT-Retention time; **Mol Weight-**Molecular weight; **Mol formula-**Molecular formula.

3.9. Volatile compounds in fresh and dry leaves of F. religiosa as revealed by GC-MS analysis

The GC-MS chromatograms of esterified aqueous fresh and dry leaf extracts are depicted in Figs. 7 and 8, and the compounds identified are listed in Tables 3 and 4. GC-MS analysis of fresh leaves revealed as many as 30 volatile compounds (Table 3), in that the relative peak areas of a few compounds (3-acetoxy 3-hydroxy propionic acid, citric acid, methoxyphenyl oxime, pipecolinate, benzenediol, etc.) were increased. The dry leaf extract contained 31 volatile compounds, among which, propionic acid, citric acid, hexadecanoic acid (palmitic acid), and octadecanoic acid (stearic acid) were prominent. Also, a few other amino acids, such as phenylalanine and aspartic acid, were identified in the dry leaf extract (Table 4).

4. Discussion

Steroid hormones are essential to control sexual and reproductive endocrine functions and to regulate the hypothalamic-pituitary-ovarian axis, ovarian folliculogenesis, ovulation, and embryo implantation

Table 4
The compounds identified in the esterified dry leaf extract of *F. religiosa* and their properties.

Peak No.	m/z values	RT	% peak area	Compoud name	Mol weight (Da)	Mol formula
1	133	8.593	1.34	4-Ethylbenzoic acid, 2-butyl ester	206	$C_{13}H_{18}O_2$
2	103	8.661	3.68	3-acetoxy 3-hydroxy propionic acid	162	$C_6H_{10}O_5$
3	84	8.735	1.61	Ethyl pipecolinate	157	$C_8H_{15}NO_2$
4	108	8.842	1.15	2-(4-Methylglycoxymethylphenyl)propan-2-ol	224	$C_{13}H_{20}O_3$
5	102	9.192	0.91	L-Aspartic acid, dimethyl ester	161	$C_6H_{11}NO_4$
6	126	9.279	5.08	3-aminoformylbenzaldehyde	149	$C_8H_7NO_2$
7	133	9.575	2.02	4-Ethylbenzoic acid, 2-butyl ester	206	$C_{13}H_{18}O_2$
8	43	10.227	4.36	2,6-Dimethyl-4H-pyran-4-one	124	$C_7H_8O_2$
9	100	11.549	13.50	5-Hydroxypipecolic acid	145	$C_6H_{11}NO_3$
10	52	11.808	2.53	Methyl pentopyranoside	164	$C_6H_{12}O_5$
11	82	12.094	6.97	2H-Pyran-3,4,5-triol, tetrahydro-2-methoxy-6-methyl	178	$C_7H_{14}O_5$
12	84	12.358	1.52	N-(.gamma.-L-Glutamyl)phenylalanine	294	$C_{14}H_{18}N_2O_5$
13	87	12.717	0.86	Butanedioic acid, ethyl methyl ester	160	$C_7H_{12}O_4$
14	127	12.891	1.42	Trimethyl 1,2,3-propanetricarboxylate	218	$C_9H_{14}O_6$
15	67	12.968	0.66	4h-Pyran-4-one, 3-acetyl-2,6-dimethyl	166	$C_9H_{10}O_3$
16	143	13.619	2.65	Citric acid, trimethyl ester	234	$C_9H_{14}O_7$
17	228	13.96	1.74	2,4-pentadienoic acid, 5-(8-hydroxy-1,5-dimethyl-3-oxo-6-oxabicyclo [3.2.1]oct-8-yl)-3-methyl	294	$C_{16}H_{22}O_5$
18	43	14.718	3.77	9-oxa-bicyclo [3.3.1]nonane-1,4-diol	158	$C_8H_{14}O_3$
19	103	14.95	4.26	2,5-Bis(methoxycarbonylmethyl)tetrahydrofuran	216	$C_{10}H_{16}O_5$
20	56	15.269	12.53	Piperazine, 3,5-dimethyl-1-nitroso	143	$C_6H_{13}N_3O$
21	68	15.351	14.87	3-cis-Methoxy-5-trans-methyl-1R-cyclohexanol	144	$C_8H_{16}O_2$
22	111	15.44	1.72	3-thiophenecarboxylic acid	128	$C_5H_4O_2S$
23	95	16.576	1.07	2-Propenoic acid, 3-(4-hydroxyphenyl)-, methyl ester	178	$C_{10}H_{10}O_3$
24	111	16.867	0.59	2(4H)-benzofuranone, 5,6,7,7A-TE	196	$C_{11}H_{16}O_3$
25	119	17.145	0.27	P-menth-1-ene-3,3-D2	140	$C_{10}H_{16}D_2$
26	103.1	17.919	0.69	Hexadecanoic acid, methyl ester	270	$C_{17}H_{34}O_2$
27	237.3	18.164	3.20	n-Hexadecanoic acid (Palmitic acid)	256	$C_{16}H_{32}O_2$
28	162	18.772	0.93	L-Phenylalanine, N-acetyl-methyl ester	221	$C_{12}H_{15}NO_3$
29	74	19.275	0.77	Heptacosanoic acid, methyl ester	424	$C_{28}H_{56}O_2$
30	265.4	19.504	0.77	Octadecanoic acid (stearic acid)	284	$C_{18}H_{36}O_2$
31	152	19.867	2.55	3,4,4-Trimethyl-3-[(1z)-3-methyl-1,3-butadienyl]-2-oxocyclohexyl acetate	264	$C_{16}H_{24}O_3$

RT-Retention time; **Mol Weight-**Molecular weight; **Mol formula-**Molecular formula.

(Santos et al., 2013). Abnormal follicular maturation or acceleration of follicular atresia was reported to elevate intra-ovarian androgen levels. The excess intra-ovarian androgens may result from either increased circulating androgens or abnormal steroidogenesis, which may lead to follicular cysts and PCOS condition (Jadhav et al., 2013; Kafali et al., 2004). In the present study, letrozole-treated rats showed elevated testosterone levels and decreased estrogen and progesterone levels in serum, thereby confirming the induction of PCOS.

The key regulators of steroidogenesis (*Cyp17 and Cyp19*) are involved in the pathogenesis of PCOS. Therefore, most often, *Cyp19* (aromatase) is inhibited and is considered as one of the important intra-ovarian disturbances in PCOS. Aromatase is an important enzyme encoded by *Cyp19a1*. So, in the present study, aromatase was inhibited by letrozole to establish a PCOS model. The administration of letrozole caused hyperandrogenism. The quantitative expression study of *Cyp19a1* in ovarian tissues revealed mRNA down-regulation in PCOS rats compared to control, which was perhaps due to the inhibition of aromatase by letrozole. At this stage, the negative feedback of LH cannot occur, due either to the increased androgen or the low levels of estrogen. This will eventually lead to hyper-secretion of LH, which would further act on the LH receptors in the theca cells. Altogether, P450C17α, a key enzyme in steroid biosynthesis, is activated and exacerbates hyper-androgenism. Further, a decrease in the progesterone level was also reported in PCOS rats (Jadhav et al., 2013; Rezvanfar et al., 2012b). In line with these reports, decrease in progesterone was observed in the present study. We correlate the decrease of progesterone with decrease in uterine weight (may be due to deteriorated endometrium) in PCOS rats, as these parameters were found to be normal in control rats.

As expected, treatment of fresh leaf extracts to PCOS rats up-regulated the *Cyp19a1* in the ovary to significant levels. Similar results were obtained in pioglitazone-treated rats. *PPAR-γ* activators also influence the synthesis of steroid hormones in ovary (Huang et al., 2008), which signifies the role of *PPAR-γ* in steroidogenesis. It is further substantiated that treatment of fresh leaf extract and pioglitazone hampered the synthesis of androgen by reducing the levels of steroidogenic enzymes (3β-HSD and 17β-HSD). PCOS rats showed a significant increase in 3β-HSD and 17β-HSD, indicating higher level of androgen synthesis. It is understood that thiazolidinediones directly act on the steroidogenic enzymes in place of the nuclear receptor PPAR (Gasic et al., 2001; Mu et al., 2000). In particular, it has been shown that thiazolidinediones decrease the activity of 3β-HSD, which converts pregnenolone to progesterone (Gasic et al., 1998). The potential increase of serum progesterone resulted from leaf extract treatment has also increased the uterine weight, showing normal reproductive physiology. Management of the abnormal androgen will provide a tangible approach to treat PCOS. Treatment with *F. religiosa* leaf extracts and pioglitazone restored all these conditions to normal. In support to our findings, it has been shown that pioglitazone as a potent *PPAR-γ* agonist has the potential to cure all aspects of PCOS condition by balancing the steroid hormone levels, which in turn substantiates the association between *PPAR-γ* and steroidogenesis (Rezvanfar et al., 2012a). There are also reports that agonists of *PPAR-γ* (thiazolidinediones) increase the serum progesterone titre (Lebovic et al., 2004; Yazawa et al., 2010).

In PCOS women, treatment with synthetic *PPAR-γ* ligands alleviates both endocrine and metabolic disorders (Brannian et al., 2008; Iuorno and Nestler, 1999). In the present study, results of *Cyp19a1* (aromatase) expression were also directly correlated with expression of *PPAR-γ*. These results were substantiated by other studies, which reported a concerted decrease of the mRNA levels of aromatase and *PPAR-γ* during the hyperandrogenic condition (Faut et al., 2011; Velez et al., 2015). Similar to aromatase, *PPAR-γ* was also upregulated by the treatment of fresh leaf extract. This observation indicated the influence of *PPAR-γ* over aromatase. It has been reported that *PPAR-γ* can directly induce aromatase in the ovary; further, the level of *PPAR-γ* was down-regulated

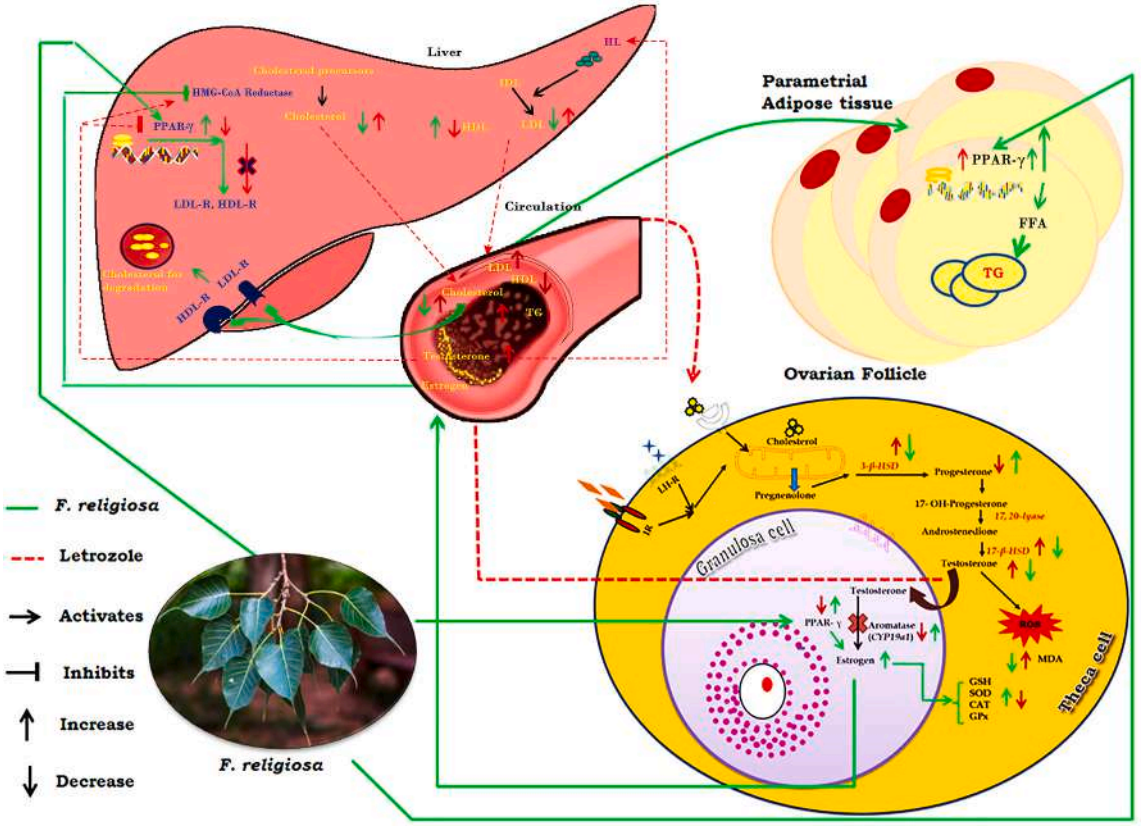


Fig. 9. The mechanism of PCOS induction by Letrozole, and the mechanism of action by the extracts of *Ficus religiosa* on the experimental rats in ameliorating PCOS. HL- Hepatic Lipase; IDL- Intermediate Density Lipoprotein; LDL- Low Density Lipoprotein; HDL- High Density Lipoprotein; LDL-R- LDL Receptor; HDL-R- HDL Receptor; TG- Triglycerides; FFA- Free Fatty Acids.
Red colour (upwards and downwards arrows/dotted lines/inhibitory lines) denotes the action of Letrozole.
Green colour (upwards and downwards arrows/Solid lines/inhibitory line) denotes the effect of *F. religiosa* in PCOS rats.
In parametrial adipose tissue, double arrows (↑ ↑) are used to emphasize the increased expression of PPAR-γ by *F. religiosa* compared to PCOS rats.

during infusion of high levels of LH and *vice versa* (Banerjee and Komar, 2006). These results are consistent with our findings in PCOS rats, in which hyper-secretion of LH and down-regulation of *PPAR-γ* was observed. Since pioglitazone is a *PPAR-γ* agonist, its treatment up-regulated *PPAR-γ* in the ovary of PCOS-induced rats. Further, it facilitates restoration of the steroidogenic status to normal. *PPAR-γ* may regulate the expression of genes required for follicular development, ovulation, oocyte maturation, and maintenance of corpus luteum (Froment et al., 2006). It also maintains energy balance in the ovary (Dupont et al., 2008). Several studies have expounded the relationship between PCOS and polymorphism of *PPAR-γ* encoding genes (He et al., 2012; San-Millan and Escobar-Morreale, 2010). Faut et al. (2011) attempted to identify the interference of hyperandrogenism on folliculogenesis, and reported impairment of *PPAR-γ* system in the follicles of PCOS rats. The *PPAR-γ* mRNA and protein levels in ovary of ovulation-stimulated rats was significantly decreased in an acute hyperandrogenic condition. As evidenced, modulation of *PPAR-γ* system would be the fundamental tool in treating PCOS (Faut et al., 2011).

Besides reproductive physiology, *PPAR-γ* also plays a role in lipid homeostasis. In this sense, the expression of *PPAR-γ* in adipose tissue and liver has been extensively studied using qPCR. The mRNA expression of *PPAR-γ* in adipose tissue got up-regulated in PCOS rats compared to control rats. These findings underscore that, unlike ovary and liver, letrozole up-regulates *PPAR-γ* in parametrial adipose tissue indicating the tissue-specific action of letrozole. In the present study, *PPAR-γ* was down-regulated in the liver of PCOS rats. To the best of our knowledge, this is the first study to examine the expression pattern of *PPAR-γ* in the liver of PCOS rats. Treatment with fresh leaves of *F. religiosa* (spray) and pioglitazone has significantly up-regulated the *PPAR-γ* expression in the liver and adipose tissue of PCOS rats. It has been shown that pioglitazone cures PCOS through directly improving the antioxidant status rather than modulating metabolic reactions (Rezvanfar et al., 2012b).

The production of ROS and antioxidants and steroidogenesis are interlinked. Many reports unravel the influence of estrogen over antioxidant status (Behl, 2002). For instance, when estrogen was supplemented to aging females, the increased level of lipid peroxidation was decreased significantly, indicating the positive influence of estrogen (Kumar et al., 2011). It has been reported that estrogen protects the kidney and heart of rodents from oxidative stress (Persky et al., 2000; Turgay et al., 2011). In the present study, an increase in MDA level in the ovary reveals an inverse correlation with the estrogen level. This indicates that the hyper-androgenic state is responsible for this condition through overproduction of ROS during testosterone synthesis. In our perspective, hyperandrogenism, coupled with apoptosis, in granulosa cells might be a culminating factor, which increases lipid peroxidation during PCOS condition. Treatment with *F. religiosa* leaf extract has significantly reduced the level of lipid peroxidation in PCOS rats, which also correlates with the improved estrogen level.

Interestingly, we have found that the very many compounds profiled through GC-MS analysis in all the extracts of *F. religiosa* have the potential to activate the central nervous system (CNS) and *PPAR-γ*. Specifically, 3-acetoxy 3-hydroxy propionic acid, a carboxylic acid, was relatively high in fresh leaf extract compared to dry leaf extract. In fact, propionic acid activates *PPAR-γ* and inhibits NF-κB (Al-Lahham et al., 2010). This action was similar to that of the binding of prostaglandins, a high affinity ligand of *PPAR-γ*. The detailed mechanisms of this binding

have been reviewed (Al-Lahham et al., 2010; Shiue et al., 2013). The upregulation of *PPAR-γ* in the present study might be due to the presence of the high amount of propionic acid in the fresh leaf of *F. religiosa*. Further, being a volatile compound, propionic acid (3-Acetoxy 3-Hydroxy Propionic acid) may also favor the efficient up-regulation of *PPAR-γ* in fresh leaf extract-treated rats (spray). It has been already reported that propionic acid activates *PPAR-γ*, similar to thiazolidinediones (Al-Lahham et al., 2010). Besides, substantial evidence was also provided by an Indian patent, which mentioned that derivatives of propionic acid could be used against PCOS (Lab, 2008). Thus, the leaf of *F. religiosa* has the potential to induce the expression of *PPAR-γ*, through which polycystic ovary syndrome and associated complications are ameliorated.

5. Conclusion

In the present study, we have demonstrated that the leaf extracts of *F. religiosa*, in particular the fresh leaf extract, stimulated the expression of *PPAR-γ* concomitantly with *Cyp19a1*, and directly alleviates the steroid imbalances with regular orchestrated estrous cycle in PCOS rats (Fig. 9). Irregular estrous cycle and multiple ovarian cysts are the primary hallmarks of PCOS, which were found in the present study, after 21 days of letrozole-administration. The presence of multiple ovarian cysts is evidenced by the histopathology (data not shown) and substantiated by the obvious increase and decrease in the weight of ovaries and uterus, respectively. At the outset, the alterations towards the betterment of all the above-mentioned preliminary data indicate the potential of leaf extract of *F. religiosa* to cure PCOS. However, confirmation of the potential candidate compounds in the fresh leaf of *F. religiosa* with parallel pharmacological studies would pave the way to develop *F. religiosa*-based therapeutics for PCOS without any adverse side effects.

Declaration of competing interest

The authors declare that they have no competing interests.

Acknowledgements

US thanks Science and Engineering Research Board (SERB), New Delhi for the National Post-Doctoral Fellowship (Ref No: PDF/2015/001041), and Council of Scientific and Industrial Research (No: 09/475 (0188)/2012-EMR-1), New Delhi, for the Senior Research Fellowship. SA acknowledges the Research Award of University Grants Commission (UGC), New Delhi, and the instrumentation facilities received through UGC-SAP, and DST-FIST. DS acknowledges UGC, New Delhi for Dr.D.S. Kothari Post-Doctoral Fellowship (Grant No. F.4-2/2006 (BSR)/BL/17-18/0444).

References

Achiraman, S., Archunan, G., 2006. 1-Iodo-2methylundecane, a putative estrus-specific urinary chemo-signal of female mouse (*Mus musculus*). Theriogenology 66 (8), 1913–1920. https://doi.org/10.1016/j.theriogenology.2006.05.010.

Adeneye, A.A., Adeleke, T.I., Adeneye, A.K., 2008. Hypoglycemic and hypolipidemic effects of the aqueous fresh leaves extract of *Clerodendrum capitatum* in Wistar rats. J. Ethnopharmacol. 116 (1), 7–10. https://doi.org/10.1016/j.jep.2007.10.029.

Al-Lahham, S.A.H., Peppelenbosch, M.P., Roelofsen, H., Vonk, R.J., Venema, K., 2010. Biological effects of propionic acid in humans; metabolism, potential applications and underlying mechanisms. Biochim. Biophys. Acta Mol. Cell Biol. Lipids 1801 (11), 1175–1183. https://doi.org/10.1016/j.bbalip.2010.07.007.

Althuis, M.D., Moghissi, K.S., Westhoff, C.L., Scoccia, B., Lamb, E.J., Lubin, J.H., Brinton, L.A., 2005. Uterine cancer after use of clomiphene citrate to induce ovulation. Am. J. Epidemiol. 161 (7), 607–615. https://doi.org/10.1093/aje/kwi084.

Banerjee, J., Komar, C.M., 2006. Effects of luteinizing hormone on peroxisome proliferator-activated receptor γ in the rat ovary before and after the gonadotropin surge. Reproduction 131 (1), 93–101. https://doi.org/10.1530/rep.1.00730.

Baptiste, C.G., Battista, M.C., Trottier, A., Baillargeon, J.P., 2010. Insulin and hyperandrogenism in women with polycystic ovary syndrome. J. Steroid Biochem. Mol. Biol. 122 (1–3), 42–52. https://doi.org/10.1016/j.jsbmb.2009.12.010.

Behl, C., 2002. Oestrogen as a neuroprotective hormone. Nat. Rev. Neurosci. 3 (6), 433–442. https://doi.org/10.1038/nrn84.

Bergmeyer, M., 1974. Steroid dehydrogenase. In: Methods of Enzymatic Analysis. Academic Press, New York, pp. 476–477.

Brannian, J.D., Eyster, K.M., Weber, M., Diggins, M., 2008. Pioglitazone administration alters ovarian gene expression in aging obese lethal yellow mice. Reprod. Biol. Endocrinol. 6, 10. https://doi.org/10.1186/1477-7827-6-10.

Brinton, L.A., Lamb, E.J., Moghissi, K.S., Scoccia, B., Althuis, M.D., Mabie, J.E., Westhoff, C.L., 2004. Ovarian cancer risk after the use of ovulation-stimulating drugs. Obstet. Gynecol. 103 (6), 1194–1203. https://doi.org/10.1097/01.AOG.0000128139.92313.74.

Calderon-Margalit, R., Friedlander, Y., Yanetz, R., Kleinhaus, K., Perrin, M.C., Manor, O., Harlap, S., Paltiel, O., 2009. Cancer risk after exposure to treatments for ovulation induction. Am. J. Epidemiol. 169 (3), 365–375. https://doi.org/10.1093/aje/kwn318.

Chandrakanth, M.G., Gilless, J.K., Gowramma, V., Nagaraja, M.G., 1990. Temple forests in India's forest development. Agrofor. Syst. 11, 199–211. https://doi.org/10.1007/BF00045899.

Chen, Q., Sun, X., Chen, J., Cheng, L., Wang, J., Wang, Y., Sun, Z., 2009. Direct rosiglitazone action on steroidogenesis and proinflammatory factor production in human granulosa-lutein cells. Reprod. Biol. Endocrinol. 7, 147. https://doi.org/10.1186/1477-7827-7-147.

Chopra, R., 1958. Chopra's Indigenous Drugs of India, 2ed. Dhur and Sons, Calcutta.

Dunaif, A., Thomas, A., 2001. Current concepts in the polycystic ovary syndrome. Annu. Rev. Med. 52, 401–419. https://doi.org/10.1146/annurev.med.52.1.401.

Dupont, J., Chabrolle, C., Rame, C., Tosca, L., Coyral-Castel, S., 2008. Role of the peroxisome proliferator-activated receptors, adenosine monophosphate-activated kinase, and adiponectin in the ovary. PPAR Res. 176275. https://doi.org/10.1155/2008/176275.

Elsheikh, M., Murphy, C., 2008. Polycystic Ovary Syndrome. Oxford University Press.

Faut, M., Elia, E.M., Parborell, F., Cugnata, N.M., Tesone, M., Motta, A.B., 2011. Peroxisome proliferator-activated receptor gamma and early folliculogenesis during an acute hyperandrogenism condition. Fertil. Steril. 95 (1), 333–337. https://doi.org/10.1016/j.fertnstert.2010.07.1083.

Froment, P., Gizard, F., Defever, D., Staels, B., Dupont, J., Monget, P., 2006. Peroxisome proliferator-activated receptors in reproductive tissues: from gametogenesis to parturition. J. Endocrinol. 189 (2), 199–209. https://doi.org/10.1677/joe.1.06667.

Gasic, S., Bodenburg, Y., Nagamani, M., Green, A., Urban, R.J., 1998. Troglitazone inhibits progesterone production in porcine granulosa cells. Endocrinology 139 (12), 4962–4966. https://doi.org/10.1210/endo.139.12.6385.

Gasic, S., Nagamani, M., Green, A., Urban, R.J., 2001. Troglitazone is a competitive inhibitor of 3β-hydroxysteroid dehydrogenase enzyme in the ovary. Am. J. Obstet. Gynecol. 184 (4), 575–579. https://doi.org/10.1067/mob.2001.111242.

Ghimire, K., Bastakoti, R.R., 2009. Ethnomedicinal knowledge and healthcare practices among the Tharus of Nawalparasi district in central Nepal. For. Ecol. Manag. 257, 2066–2072. https://doi.org/10.1016/j.foreco.2009.01.039.

Gu, B.H., Baek, K.H., 2009. Pro12Ala and His447 His polymorphisms of PPAR-γ are associated with polycystic ovary syndrome. Reprod. Biomed. Online 18 (5), 644–650. https://doi.org/10.1016/S1472-6483(10)60008-9.

He, J., Wang, L., Liu, J., Liu, F., Li, X., 2012. A meta-analysis on the association between PPAR-γ Pro12Ala polymorphism and polycystic ovary syndrome. J. Assist. Reprod. Genet. 29 (7), 669–677. https://doi.org/10.1007/s10815-012-9772-4.

Hong, Q., Qu, J.Y., 2009. Changes of aromatase P450 in polycystic svary syndrome. Journal of International Reproductive Health/Family Planning 28 (2), 121–123.

Huang, Z., Zhou, X., Nicholson, A., Gotto, A., Hajjar, D., Han, J., 2008. Activation of peroxisome proliferator-activated receptor-α in mice induces expression of the hepatic low-density lipoprotein receptor. Br. J. Pharmacol. 155 (4), 596–605. https://doi.org/10.1038/bjp.2008.331.

Iuorno, M.J., Nestler, J.E., 1999. The polycystic ovary syndrome: treatment with insulin sensitizing agents. Diabetes Obes. Metabol. 1 (3), 127–136. https://doi.org/10.1046/j.1463-1326.1999.00026.x.

Jadhav, M., Menon, S., Shailajan, S., 2013. Anti-androgenic effect of *Symplocos racemosa* Roxb. against letrozole induced polycystic ovary using rat model. Journal of Coastal Life Medicine 1 (4), 309–314. https://doi.org/10.12980/jclm.1.2013c79.

Kafali, H., Iriadam, M., Ozardal, I., Demir, N., 2004. Letrozole-induced polycystic ovaries in the rat: a new model for cystic ovarian disease. Arch. Med. Res. 35 (2), 103–108. https://doi.org/10.1016/j.arcmed.2003.10.005.

Kirtikar, K.R., Basu, B.D., 1993. Indian Medicinal Plants, 2ed. Periodical experts book agency, NewDelhi, India.

Komar, C.M., Braissant, O., Wahli, W., Curry Jr., T.E., 2001. Expression and localization of PPARs in the rat ovary during follicular development and the periovulatory period. Endocrinology 142 (11), 4831–4838. https://doi.org/10.1210/endo.142.11.8429.

Komar, C.M., Curry Jr., T.E., 2003. Inverse relationship between the expression of messenger ribonucleic acid for peroxisome proliferator-activated receptor γ and P450 side chain cleavage in the rat ovary. Biol. Reprod. 69 (2), 549–555. https://doi.org/10.1095/biolreprod.102.012831.

Krishnaraju, A.V., Rao, T.V.N., Sundararaju, D., Vanisree, M., Tsay, H., Subbaraju, G.V., 2006. Biological screening of medicinal plants collected from Eastern Ghats of India using Artemia salina (brine shrimp test). Int. J. Appl. Sci. Eng. 4 (2), 115–125.

Kumar, P., Kale, R., Baquer, N.Z., 2011. Estradiol modulates membrane-linked ATPases, antioxidant enzymes, membrane fluidity, lipid peroxidation, and lipofuscin in aged rat liver. J Aging Res 580245. https://doi.org/10.4061/2011/580245.

Lab, D.R., 2008. Novel Alkoxy Propionic Acid Compounds and Their Derivatives, Process for Their Preparation and Pharmaceutical Compositions. Patent No. IN218448.

Lebovic, D.I., Kir, M., Casey, C.L., 2004. Peroxisome proliferator-activated receptor-gamma induces regression of endometrial explants in a rat model of endometriosis. Fertil. Steril. 82 (3), 1008–1013. https://doi.org/10.1016/j.fertnstert.2004.02.148.

Lovekamp-Swan, T., Jetten, A.M., Davis, B.J., 2003. Dual activation of PPARα and PPARγ by mono-(2-ethylhexyl) phthalate in rat ovarian granulosa cells. Mol. Cell. Endocrinol. 201 (1–2), 133–141. https://doi.org/10.1016/S0303-7207(02)00423-9.

Marklund, S., Marklund, G., 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem. 47 (3), 469–474. https://doi.org/10.1111/j.1432-1033.1974.tb03714.x.

Mendelson, C.R., Kamat, A., 2007. Mechanisms in the regulation of aromatase in developing ovary and placenta. J. Steroid Biochem. Mol. Biol. 106 (1–5), 62–70. https://doi.org/10.1016/j.jsbmb.2007.05.001.

Mital, P., Shefal, J., Dinesh, J., Bhavesh, P., Nandini, P., Priti, V., Pragya, R., 2012. Prevalence of different factors responsible for infertility. Res. J. Recent Sci. 1, 207–211.

Moron, M.S., Depierre, J.W., Mannervik, B., 1979. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochim. Biophys. Acta 582 (1), 67–78. https://doi.org/10.1016/0304-4165(79)90289-7.

Mu, Y.M., Yanase, T., Nishi, Y., Waseda, N., Oda, T., Tanaka, A., Takayanagi, R., Nawata, H., 2000. Insulin sensitizer, troglitazone, directly inhibits aromatase activity in human ovarian granulosa cells. Biochem. Biophys. Res. Commun. 271 (3), 710–713. https://doi.org/10.1006/bbrc.2000.2701.

Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95 (2), 351–358. https://doi.org/10.1016/0003-2697(79)90738-3.

Panda, H., 2005. Herbs Cultivation and Medicinal Uses, 2ed. National institute of industrial research, New Delhi.

Payne, A.H., Hales, D.B., 2004. Overview of steroidogenic enzymes in the pathway from cholesterol to active steroid hormones. Endocr. Rev. 25 (6), 947–970. https://doi.org/10.1210/er.2003-0030.

Persky, A.M., Green, P.S., Stubley, L., Howell, C.O., Zaulyanov, L., Brazeau, G.A., Simpkins, J.W., 2000. Protective effect of estrogens against oxidative damage to heart and skeletal muscle in vivo and *in vitro*. Proc Soc Exp Biol Med 223 (1), 59–66. https://doi.org/10.1111/j.1525-1373.2000.22308.x.

Prasad, P.V., Subhaktha, P.K., Narayana, A., Rao, M.M., 2006. Medico-historical study of "asvattha" (sacred fig tree). Bull Indian Inst Hist Med Hyderabad 36 (1), 1–20.

Qin, K.N., Rosenfield, R.L., 1998. Role of cytochrome P450c17 in polycystic ovary syndrome. Mol. Cell. Endocrinol. 145 (1–2), 111–121. https://doi.org/10.1016/S0303-7207(98)00177-4.

Rak-Mardyla, A., Karpeta, A., 2014. Rosiglitazone stimulates peroxisome proliferator-activated receptor gamma expression and directly affects in vitro steroidogenesis in porcine ovarian follicles. Theriogenology 82 (1), 1–9. https://doi.org/10.1016/j.theriogenology.2014.02.016.

Rezvanfar, M.A., Ahmadi, A., Saadi, H.A.S., Baeeri, M., Abdollahi, M., 2012a. Mechanistic links between oxidative/nitrosative stress and tumor necrosis factor alpha in letrozole-induced murine polycystic ovary: biochemical and pathological evidences for beneficial effect of pioglitazone. Hum. Exp. Toxicol. 31 (9), 887–897. https://doi.org/10.1177/0960327111426589.

Rezvanfar, M.A., Ahmadi, A., Shojaei-Saadi, H.A., Baeeri, M., Abdollahi, M., 2012b. Molecular mechanisms of a novel selenium-based complementary medicine which confers protection against hyperandrogenism-induced polycystic ovary. Theriogenology 78 (3), 620–631. https://doi.org/10.1016/j.theriogenology.2012.03.008.

Rotruck, J.T., Pope, A.L., Ganther, H.E., Swanson, A.B., Hafeman, D.G., Hoekstra, W.G., 1973. Selenium: biochemical role as a component of glutathione peroxidase. Science 179 (4073), 588–590. https://doi.org/10.1126/science.179.4073.588.

Sankarganesh, D., Suriyakalaa, U., Ramachandran, R., Achiraman, S., Sankarganesh, A., Angayarkanni, J., 2019. Urinary volatile metabolomics as a viable alternative diagnostic tool for polycystic ovary syndrome: an exploratory hypothesis. Med. Hypotheses 124, 121–124. https://doi.org/10.1016/j.mehy.2019.02.009.

San-Millan, J.L., Escobar-Morreale, H.F., 2010. The role of genetic variation in peroxisome proliferator-activated receptors in the polycystic ovary syndrome (PCOS): an original case control study followed by systematic review and meta-analysis of existing evidence. Clin. Endocrinol. 72 (3), 383–392. https://doi.org/10.1111/j.1365-2265.2009.03679.x.

Santos, E.L.W., Dias, B.H.M., Andrade, A.C.R.d., Pascoal, A.M.H., Vasconcelos Filho, F.E.d., Medeiros, F.d.C., Guimaraes, S.B., 2013. Effects of acupuncture and electroacupuncture on estradiol-induced inflammation and oxidative stress in health rodents. Acta Cir. Bras. 28 (8), 582–588. https://doi.org/10.1590/s0102-86502013000800005.

Sharif, H.B., Mukhtar, M.D., Mustapha, Y Gabi Baba, Lawal, A.O., 2015. Acute and subchronic toxicity profile of *Euphorbia pulcherrima* methanol extract on Wistar albino rats. Advances in Pharmaceutics 539646, 9. https://doi.org/10.1155/2015/539646.

Shiue, Y.L., Chen, L.R., Tsai, C.J., Yeh, C.Y., Huang, C.T., 2013. Emerging roles of peroxisome proliferator-activated receptors in the pituitary gland in female reproduction. Biomarkers and Genomic Medicine 5, 1–11. https://doi.org/10.1016/j.gmbhs.2013.04.008.

Singh, D., Singh, B., Goel, R.K., 2011. Traditional uses, phytochemistry and pharmacology of *Ficus religiosa*: a review. J. Ethnopharmacol. 134 (3), 565–583. https://doi.org/10.1016/j.jep.2011.01.046.

Sinha, A.K., 1972. Colorimetric assay of catalase. Anal. Biochem. 47 (2), 389–394. https://doi.org/10.1016/0003-2697(72)90132-7.

Stout, D.L., Fugate, S.E., 2005. Thiazolidinediones for treatment of polycystic ovary syndrome. Pharmacotherapy 25 (2), 244–252. https://doi.org/10.1592/phco.25.2.244.56943.

Sun, J., Jin, C., Wu, H., Zhao, J., Cui, Y., Liu, H., Wu, L., Shi, Y., Zhu, B., 2013. Effects of electro-acupuncture on ovarian P450arom, P450c17α and mRNA expression induced by letrozole in PCOS rats. PLoS One 8 (11), e79382. https://doi.org/10.1371/journal.pone.0079382.

Terry, K.L., Willett, W.C., Rich-Edwards, J.W., Michels, K.B., 2006. A prospective study of infertility due to ovulatory disorders, ovulation induction, and incidence of breast cancer. Arch. Intern. Med. 166 (22), 2484–2489. https://doi.org/10.1001/archinte.166.22.2484.

Turgay, M., Turgay, F., Devrim, E., Kucuksahin, O., Caydere, M., Durak, I., 2011. The effects of dexamethasone on oxidant/antioxidant status in kidneys of rats administered mercuric chloride. Bratisl. Lek. Listy 113 (1), 10–13. https://doi.org/10.4149/bll_2012_002.

Velez, L.M., Heber, M.F., Ferreira, S.R., Abruzzese, G.A., Reynoso, R.B., Motta, A., 2015. Effect of hyperandrogenism on ovarian function. Reproduction 149 (6), 577–585. https://doi.org/10.1530/REP-15-0041.

Xita, N., Lazaros, L., Georgiou, I., Tsatsoulis, A., 2009. The Pro12Ala polymorphism of the PPAR-gamma gene is not associated with the polycystic ovary syndrome. Hormones (Basel) 8 (4), 267–272. https://doi.org/10.1007/BF03401274.

Yazawa, T., Inaoka, Y., Okada, R., Mizutani, T., Yamazaki, Y., Usami, Y., Kuribayashi, M., Orisaka, M., Umezawa, A., Miyamoto, K., 2010. PPAR-γ Coactivator-1α regulates progesterone production in ovarian granulosa cells with SF-1 and LRH-1. Mol. Endocrinol. 24 (3), 485–496. https://doi.org/10.1210/me.2009-0352.

Zar, J., 1984. Biostatistical Analysis, 2ed. Pentice-Hall, Englewood Cliffs, New Jersey, USA.

Inorganica Chimica Acta 510 (2020) 119783

Contents lists available at ScienceDirect

Inorganica Chimica Acta

journal homepage: www.elsevier.com/locate/ica

Research paper

Folate-engineered mesoporous silica-encapsulated copper (II) complex [Cu(L)(dppz)]$^{+}$: An active targeting cell-specific platform for breast cancer therapy

Thankaraj Salammal Sheena[a,1], Rajakumaran Dhivya[b,d,1], Venkatesan Rajiu[a], K. Jeganathan[a,*], Mallayan Palaniandavar[c], Ganesan Mathan[d,*], Mohammad Abdulkader Akbarsha[b,e,*]

[a] Centre for Nanoscience and Nanotechnology, Department of Physics, Bharathidasan University, Tiruchirappalli 620 024, India
[b] Mahatma Gandhi Doerenkamp Center, Bharathidasan University, Tiruchirappalli 620 024, India
[c] Department of Chemistry, Bharathidasan University, Tiruchirappalli 620 024, India
[d] Department of Biomedical Science, Bharathidasan University, Tiruchirappalli 620 024, India
[e] Research Coordinator, National College (Autonomous), Tiruchirappalli 620 001, India

ARTICLE INFO

Keywords:
Copper (II) complex
Mesoporous silica
Folate targeting
Breast cancer therapy

ABSTRACT

Aspects of inorganic medicinal chemistry aim at developing metal coordinated complexes that are efficient therapeutic agents for cancer. However, intrinsic toxicity of these complexes impedes their effective clinical use. In order to reduce the toxicity and improve the pharmacokinetics and biodistribution, we developed a nano-vector candidate and encapsulated in it a metal complex (mononuclear copper(II) complex [Cu(L)(dppz)]$^{+}$ (LH = 2-[(2-dimethylaminoethylimino)methyl]phenol, coordinated ligand, dppz = dipyrido[3,2-*a*:2′,3′-*c*] phenazine) which is an efficient targeted delivery platform based on folate-receptor. The *in-vitro* cytotoxicity of (FA@MSNPs-[Cu(L)(dppz)]$^{+}$) to the breast cancer cells MCF-7 and MDA-MB-231, and normal breast epithelial cell HBL-100 have been evaluated. The complex (FA@MSNPs-[Cu(L)(dppz)]$^{+}$), compared to free [Cu(L)(dppz)]$^{+}$, is highly cytotoxic to the breast cancer cells, in view of its efficient cellular uptake, and the delivery is mediated by FA receptor which produced only negligible cytotoxicity to the normal breast epithelial cell, HBL-100. Also, the mechanism of breast cancer cell death brought about by [Cu(L)(dppz)]$^{+}$ loaded mesoporous silica nanoparticles (MSNPs) is primarily apoptosis whereas they are not toxic to normal breast epithelial cells. Hence, folate-conjugated MSNPs encapsulated with [Cu(L)(dppz)]$^{+}$ has potential to be developed as a target-specific drug for breast cancer. This metal complex-loaded platform opens up newer opportunities to accommodate multiple metal-based drugs for cell-specific cancer therapy.

1. Introduction

Recent developments in material science hold promise to overcome one of the big challenges of modern medicine- delivery of drugs specifically to the defective cells. The key to this call is the development of nano-particulate drug carriers that are capable of reaching different tissues, crossing physiological barriers and enhancing cell specificity of the carrier [1–3]. Recent studies have shown that MSNPs are useful in drug delivery in view of their high flexibility, efficient release mechanisms, large drug load potential, large surface area, manageable surface functionality and suitability to deliver hydrophobic substances [4–8]. Further, the US-FDA has recently approved “C-dots” (Cornell dots), the silica-based nano-particles, for phase I clinical trial [9,10].

Development of specific ligands which selectively target cells and bind their surface offers great scope to enhance the cellular uptake so as to contribute to drug delivery systems and thus to enhance the therapeutic efficacy. Folic acid (FA) is a non-immunogenic receptor-specific ligand. It has earned reputation as a ligand for targeted anticancer drug delivery. This is because folate receptors are over-expressed on the surface of human cancer cells [11,12]. Their conjugation chemistry is so simple that they can be easily grafted by amide reactions onto mesoporous silica particles [13]. Folic acid-conjugated mesoporous silica has

* Corresponding authors at: Centre for Nanoscience and Nanotechnology, Department of Physics, Bharathidasan University, Tiruchirappalli 620 024, India (K. Jeganathan). Department of Biomedical Science, Bharathidasan University, Tiruchirappalli 620 024, India (G. Mathan). Mahatma Gandhi Doerenkamp Center, Bharathidasan University, Tiruchirappalli 620 024, India (M.A. Akbarsha).
E-mail addresses: kjeganathan@yahoo.com (K. Jeganathan), mathan@bdu.ac.in (G. Mathan), akbarbdu@gmail.com (M.A. Akbarsha).
[1] These two authors contributed equally.

https://doi.org/10.1016/j.ica.2020.119783
Received 17 March 2020; Received in revised form 20 May 2020; Accepted 20 May 2020
Available online 24 May 2020
0020-1693/ © 2020 Elsevier B.V. All rights reserved.

been reported to be a potential carrier for cisplatin [14]. This is supported by several recent reports which claim that FA-coupled MSNPs are capable of improving the efficiency of delivery and efficacy as anticancer drugs [15–17]. While these studies are promising, the use of folate-directed cancer treatment in human subjects still needs further development and testing [18–20].

We have been engaged in synthesis of metal complexes with special emphasis on copper complexes, and testing them for cytotoxic potential against cancer cells. In our earlier work, we synthesized and characterized the mononuclear copper (II) complex $[Cu(L)(dppz)]^+$ [LH = 2-[(2-dimethylaminoethylimino) methyl]phenol and dppz = dipyrido[3,2-*a*:2′,3′-*c*]phenazine. This complex was found to be cytotoxic to human breast cancer cell MCF-7 and cervical cancer cell ME-180 [21]. The mechanism underlying loss of viability of breast cancer cells (MCF-7 and MDA-MB-231) was later shown to be due to damage caused to DNA consequent upon the intercalation of the complex with the DNA. This would result in generation of excessive ROS, which in turn would lead to intrinsic apoptosis, with a critical role to mitochondria, with a small percentage of cells succumbing to necrosis. This was revealed in the morphological changes in the cells, DNA damage, change in mitochondrial transmembrane potential, expression of apoptosis-associated proteins and extent of ROS generation [21]. These observations suggested that our copper (II) complex is effective in damaging DNA of MCF-7 and MDA-MB-231 breast cancer cells. Therefore, this copper complex, the design, synthesis, characterization and cytotoxic property to cancer cells of which have been described elaborately in our earlier reports [21,22], proved to be worthy of further investigation. But a cause of concern has been the susceptibility, to a certain extent, of normal cells to this copper complex. Thus, we were guided to a mode of drug delivery that will target only the tumor cells and spare the normal cells. We have now successfully developed folate-conjugated MSNPs encapsulating the copper (II) complex (FA@MSNPs-$[Cu(L)(dppz)]^+$) for targeted drug delivery in the context of breast cancers, sparing the normal cells.

2. Materials and methods

Tetraethylorthosilicate (TEOS), cetyltrimethylammonium bromide (CTAB), sodium hydroxide (NaOH), 3-aminopropyltriethoxysilane (APTES), folic acid (98%), and *n*-hydroxysuccinimide (98%) were purchased from Sigma-Aldrich. 1-(3-dimethylaminopropyl)-3-ethylcarbodiamide hydrochloride (98%) was obtained from Alfa Aesar, UK. Ammonium nitrate was procured from Fisher Scientific, Mumbai, India. All other chemicals were of analytical grade and used as received without further purification.

2.1. Preparation of bare MSNPs

The $[Cu(L)(dppz)]^+$ complex was prepared according to Jaividya et al. [21] The schematic illustration of procedure for $[Cu(L)(dppz)]^+$ loaded folate-conjugated MSNPs is shown in Scheme 1. *Meso*-structure silica was prepared according to the procedure already reported in the literature [6,23]. To begin with, CTAB (0.5 g) was dissolved in 240 mL of deionized water at room temperature and constantly stirred to obtain a clear solution. 1.75 mL of (2 M) aqueous solution of sodium hydroxide (NaOH) was introduced to the surfactant solution and the temperature of the solution was raised to 80 °C, after which TEOS (2.5 mL) was added in drops. The resultant mixture was stirred for 2 h which produced a white precipitate. The solution was washed with ethanol and the MSNPs were dried at room temperature under vacuum. The excess CTAB surfactant was removed from the mesopores by ion exchange method by dispersing 100 mg MSNPs in an alcoholic solution of ammonium nitrate (250 mg ammonium nitrate in 50 mL of 95% ethanol) followed by heating the mixture at 80 °C and refluxed for 6 h. The solution was washed with ethanol and the MSNPs were dried at room temperature under vacuum.

2.2. Amine-functionalized MSNPs

MSNPs (300 mg) were suspended in 20 mL of toluene containing 300 μL of APTES and the suspension was refluxed with constant stirring at 50 °C for 8 h under N_2 atmosphere. The resultant sediment was centrifuged at 5000 rpm for 10 min, washed repeatedly with ethanol, and dried at 45 °C using a rotary evaporator.

2.3. Folic acid-conjugated MSNPs

In order to activate the carboxyl groups, present in the folic acid, 100 mg of folic acid was mixed in 25 mL of DMSO. Then, 38 mg of *n*-hydroxysuccinimide and 68 mg of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride were added to the mixture and stirred for 24 h. Subsequently, MSNPs-NH_2 spheres (50 mg) were added to 20 mL of toluene and the activated FA solution was allowed to react, under stirring, overnight at room temperature. After the reaction, the mixture was centrifuged and washed twice with toluene to obtain FA@NH_2-MSNPs spheres.

2.4. Drug loading

The conjugated mixture FA@NH_2-MSNPs (20 mg) was added with $[Cu(L)(dppz)]^+$ (2 mg) against DMSO solution for 24 h to load the drug molecules in the mesopores. The drug-loaded mesopores were separated out by repeated centrifugation, and the resultant pellet was isolated. The unloaded drugs remaining on surface of MSNPs was removed by repeated washing. In order to delineate the unloaded drug, the supernatant solution was analyzed by spectrophotometric (Shimadzu UV-1800 spectrophotometer) absorbance measurement at 382 nm with reference to a calibration curve of $[Cu(L)(dppz)]^+$ in DMSO. The encapsulation efficiency of drug was determined adopting the Eq. (1).

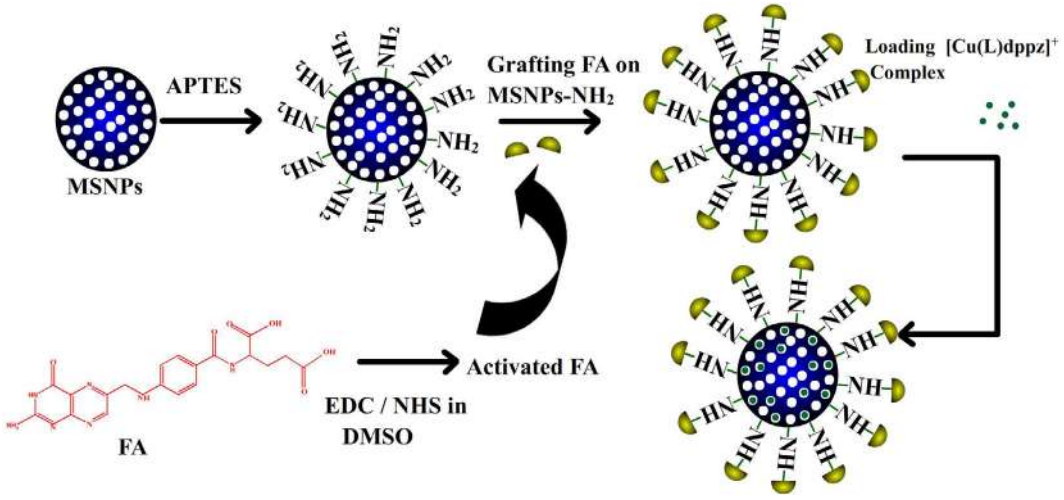
$$\textit{Encapsulation Encapsulation}\ (\%) = \frac{\textit{Amount of drug i n MSNPs}}{\textit{Initial Weight of}\ [Cu(L)(dppz)]^{+}\textit{Complex}} \times 100 \quad (1)$$

2.5. Characterization

Zeta (ξ) potential values were obtained using Malvern Zetasizer Nano ZS with a He–Ne laser beam. All measurements were made at a wavelength of 633.8 nm. The surface morphology of prepared MSNPs was examined by FESEM (FEI-Quanta FEG 200) and TEM (FEI Tecnai F20) at an acceleration voltage of 200 kV. The surface modification and the functional fingerprint present in the MSNPs, MSNPs-NH_2, and FA@NH_2-MSNPs were analyzed using FTIR (BRUKER IFS 66 V).

2.6. Cytotoxicity assay

Cell viability assay, adopting MTT tetrazolium salt colorimetry, as described by Mosmann et al., was performed to measure the cytotoxicity of the complexes [24]. The FA@MSNPs-$[Cu(L)(dppz)]^+$ was dissolved in 100 % dimethyl sulfoxide (DMSO) and diluted to 0.02 % DMSO. This, at 2.5 μg/mL concentration, was added to the wells 24 h after seeding 5000 cells (MCF-7/MDA-MB-231/HBL-100, all obtained from National Center for Cell Science, Pune, India) per well of 96-well plate. DMSO (0.02 %) was used as the solvent control. HBL-100 cell was treated with $[Cu(L)(dppz)]^+$ in the concentration range of 0–2 μM. In another experiment the cells were treated with FA@MSNPs at a concentration range of 0–100 μg/mL to find if FA@MSNPs itself would be cytotoxic. After 24 and 48 h incubation, 20 μL of MTT solution [5 mg/mL in phosphate-buffered saline (PBS)] was added to each well. The plates were wrapped with aluminum foil and incubated for 3 h at 37 °C. The resultant formazan crystal was dissolved in 100 μL of 100% DMSO. The absorbance was read at 570 nm (measurement) and 630 nm



Scheme 1. Methods for preparation of FA@MSNPs-$[Cu(L)(dppz)]^+$ NPs.

(reference) in a 96-well plate reader (Bio-Rad, Hercules, California, USA). Data were collected for three replicates, each in triplicate. The three average values were used to calculate the means and the standard deviations. The percentage inhibition was calculated from this data using the Eq. (2),

$$\text{percentage inhibition (\%)} = \frac{\text{Untreated cells (Control)} - \text{Treated } cells}{\text{Untreated cells (Control)}} \times 100 \quad (2)$$

From the values thus obtained, the IC_{50} for the respective durations of treatment, i.e., 24 and 48 h, for the three cell types were derived from the curves obtained by plotting percentage inhibition against concentration.

2.7. Acridine orange (AO) and ethidium bromide (EB) fluorescent assay for cell death

The procedure of Spector et al., was adopted to perform AO and EB staining [25]. MCF-7, MDA-MB-231 and HBL-100 cells were cultured separately in 6-well plates and treated with FA@MSNPs-$[Cu(L)(dppz)]^+$ at 2.5 μg/mL concentration, for 24 and 48 h. DMSO (0.02%) was used as solvent control. The treated and untreated cells (25 μL of suspension containing 5000 cells) were incubated with acridine orange and ethidium bromide solution (1 part of 100 μg/mL each of acridine orange and ethidium bromide in PBS) and examined in a fluorescent microscope (Carl Zeiss, Jena, Germany) using a UV filter (450–490 nm). Three hundred cells per sample were counted, in triplicate for each time point, and scored as viable or dead, and if dead whether by apoptosis or necrosis as judged based on morphology of nucleus and organization of cytoplasm. The percentages of cells in apoptosis and necrosis were calculated. Images of interest were photographed [26].

2.8. Hoechst staining

MCF-7, MDA-MB-231 and HBL-100 cells were cultured separately in 6-well plates and treated with 2.5 μg/mL of FA@MSNPs-$[Cu(L)(dppz)]^+$ for 24 and 48 h. The treated and control cells were stained with Hoechst 33,258 stain (1 mg/mL, aqueous) for 5 min at room temperature [27]. A drop of cell suspension was loaded on a glass slide, and a cover slip was laid over to reduce light diffraction. At random 300 cells, in triplicate to calculate the average, were observed in the fluorescent microscope fitted with a 377–355 nm filter. The percentage of cells that reflected pathological changes was calculated [28]. Data were collected for three replicates each and used to calculate the means and the standard deviations.

2.9. Western blot analysis

MCF-7, MDA-MB-231 and HBL-100 cells were treated for 24 h with FA@MSNPs-$[Cu(L)(dppz)]^+$ at 2.5 μg/mL concentration, when 0.02% DMSO was used as control. Cell lysates, each equivalent of 40 μg protein, were resolved over 10% polyacrylamide gel and then transferred onto the PVDF membrane. Blocking was done using 5% nonfat dry milk and probed with p53 primary monoclonal antibody in blocking buffer for overnight at 4 °C. The membrane was then challenged with the secondary antibody-alkaline phosphatase conjugate (Merck, India). Detection was done using NBIT/BCI kit (Merck, India). Anti-β-actin antibody (Santa Cruz, USA) was used to spot the β-actin loading control. Data were collected for three replicates each. The respective means and the standard deviations were calculated.

2.10. Statistics

Data are expressed as means ± standard deviations (SD). Paired sample *t*-test from GraphPad Prism-6.0 was conducted to find the significance of the differences between means. *P*- values less than 0.05 were considered significant.

3. Results and discussion

One of the biggest challenges of successful chemotherapy is to deliver drugs specifically to the defective cells. The advances in nanotechnology and targeted drug delivery are of special importance in cancer therapy. The current cancer treatments based on chemotherapy are typically associated with serious side-effects due to non-specific uptake of the drug by healthy cells. Furthermore, many cancer drug candidates are hydrophobic, which renders direct administration a challenge. These issues emphasize a clear need for the development of cancer cell-specific drug carrier systems capable of delivering poorly water-soluble drugs, which is the reason behind focusing nanomedicine research for cancer therapies [29]. Among the different types of particles being pushed forward in nanomedicine, MSNPs have been emphasized since they provide enormous relative surface area, tunable pore sizes and flexible surface functionalization, thus serving as an efficient drug carrier system [30].

In the present investigation, it was found that the growth inhibitory effect of free $[Cu(L)(dppz)]^+$ on normal breast epithelial cells is equally high as in cancer cells under the same treatment conditions necessitating targeted delivery of $[Cu(L)(dppz)]^+$to cancer cells sparing normal cells (Figs. S1 & S2). Thus, folic acid-coupled mesoporous silica nanoparticles encapsulated with copper (II) complex were generated.

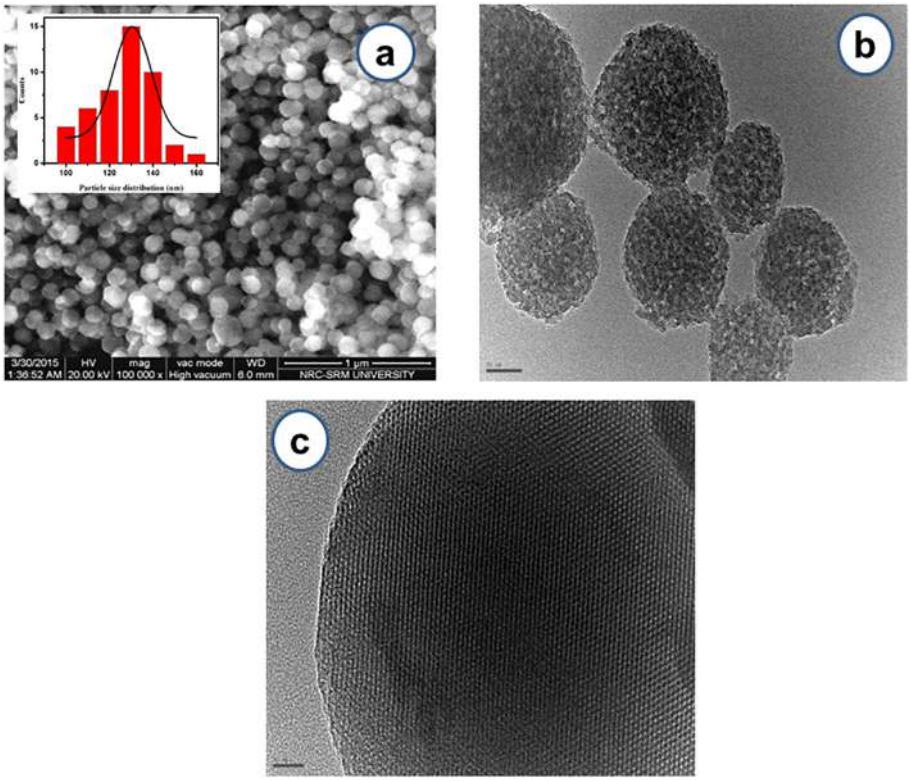


Fig. 1. (a) FESEM micrograph of MSNPs. The inset figure shows histogram profile of the corresponding FESEM image; (b) Transmission Electron Micrographs (TEM) of MSNPs; (c) The magnified image shows regular mesopores of MSNPs.

FA@MSNPs-$[Cu(L)(dppz)]^{+}$ were developed in order to deliver the copper (II) complex to cancer cells adopting folate receptor targeting. The particle size range of interest for targeted intracellular delivery applications in the case of MSNPs is about 50–200 nm, since larger particles cannot easily bypass physical membranes in the body, whereas smaller MSNPs particles are difficult to synthesize in view of their inherent mesoporosity [31]. Here we developed the homogenous MSNPs nanostructure with well-defined spherical morphology and validated from SEM and TEM measurements as shown in Fig. 1. The inset shows the analyzed histogram of the MSNPs which illustrates uniform size distribution with mean diameter around 130.66 nm.

The particle size obtained from TEM image is well corroborated with the SEM results also; the arrays of mesopores could be clearly seen in Fig. 1(c). In addition, surface modification of MSNPs was characterized by Zeta potential measurements. Before the CTAB removal MSNPs offer a positive surface potential. After the core removal, existence of silanol groups on MSNPs the surface charge tuned to be (−16.5 mV) which enabled the complete removal of CTAB as shown in Fig. 2. Later, due to the amine modification on the surface of the MSNPs, the ξ potential had become positive (Fig. 2). Furthermore, folate decorated on MSNPs-NH_2 through amidation reaction Scheme 1 exhibited positive surface potential that is expected to confer a long shelf life. The amine modification on the surface of the MSNPs modified the surface charge potential from negative to positive as revealed in zeta potential measurements. Also, FA@NH_2-MSNPs clearly indicated that the dispersion is highly stable and expected to have a long shelf life (Fig. 2).

Furthermore, FTIR spectroscopy was used to ascertain the formation of the FA@NH_2-MSNPs. The intense absorption bands from 3428 to 3446 cm^{-1} show the stretching vibration of –OH groups in the adsorbed water. Two peaks at 2924 and 2854 cm^{-1} are associated with the presence of CTAB in the MSNPs channels. After the core removal the peaks disappeared, which shows the successive removal of CTAB from the mesopores. The strong absorption peaks observed in 1080 cm^{-1},

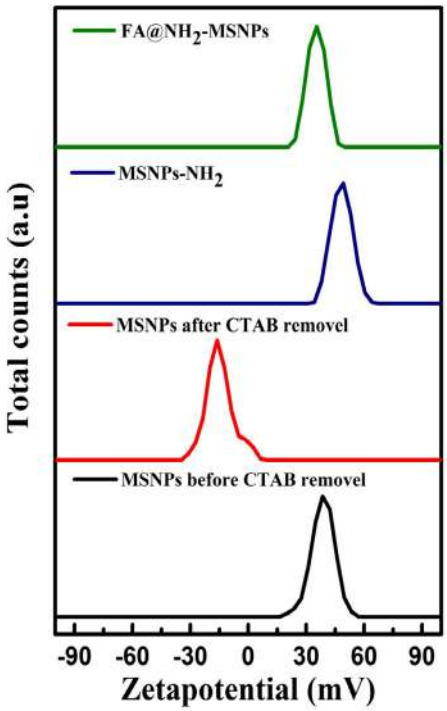


Fig. 2. Zeta potentials of MSNPs, NH_2-MSNPs and FA@NH_2-MSNPs in deionized water.

1082 cm^{-1}, 1084 and 1090 cm^{-1} owe to the asymmetric and the stretching vibration of Si-O-Si. The bands appearing at 1633 and 1641 cm^{-1} are attributed to the OH bending vibration. The band from 950 to 964 cm^{-1} visualizes the stretching vibration of the surface Si-O-groups. The peaks from 457 to 465 cm^{-1} evidence the tetrahedral Si-O

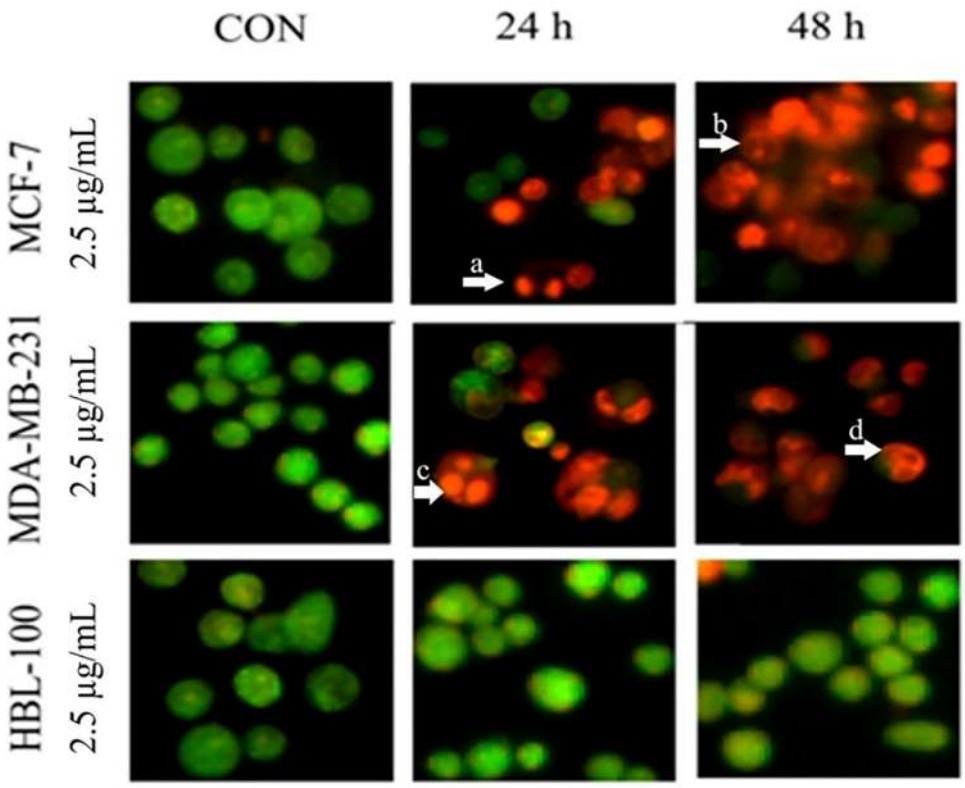


Fig. 3. Control and FA@MSNPs-[Cu(L)(dppz)]$^{+}$ treated MCF-7, MDA-MB-231 and HBL-100 cells stained with acridine orange and ethidium bromide (AO/EB). Note indication of morphological changes relating to apoptosis in MCF-7 and MDA-MB-231 cells and not in HBL-100 normal breast epithelial cells. (Arrow marks: a – apoptotic bodies, b – cytoplasmic blebbing, c - nuclear fragmentation and d- early apoptosis).

bending modes. After the amine functionalization NH_2 vibration band at 1633 cm^{-1} overlapped with OH vibration. The well-resolved vibrational peak at 1512 cm^{-1} is assigned to the N—H stretching vibrations of an aminopropyl group which also witnesses the amine functionalization on the pore surface [32]. The peak appearing in the region around 1410 cm^{-1} shows the characteristic symmetric stretching vibration of carboxylate COO^{-} of FA molecule [33]. The intensive functional group at 1640 cm^{-1} indicates amide stretching vibration which reflects on folic acid grafted on the surface of aminated MSNPs (Fig. S3).

Finally, the DNA damaging agent [Cu(L)(dppz)]$^{+}$ was loaded into the FA@NH_2-MSNPs with loading efficiency of ~89%. Chemotherapeutic drugs are well-known for their adverse side effects such as fatigue, nausea, and hair loss, which result from the way they work to kill fast dividing cells in the body rather than cancerous cells specifically [35]. Thus, cancer targeting is a highly desirable aspect in cancer therapy in order to reduce the negative effects to healthy cells. In the present study, it is shown that multifunctional folic acid-conjugated MSNPs loaded with [Cu(L)(dppz)]$^{+}$ can be efficiently trapped by breast cancer cells facilitated by folate receptor targeting with a potency of 10-fold increased uptake when compared to the normal breast epithelial cells, whereas free [Cu(L)(dppz)]$^{+}$ has been shown to be more or less equally cytotoxic to both breast cancer and normal breast epithelial cells. Furthermore, FA@MSNPs-[Cu(L)(dppz)]$^{+}$ has been demonstrated to be efficacious by its ability to bring about apoptotic morphological changes and desired genetic level changes only in the breast cancer cells sparing the normal cells at the same treatment conditions. Thus, we have demonstrated that the multifunctional MSNPs enhance the anticancer efficacy of [Cu(L)(dppz)]$^{+}$ through folate-mediated targeting to breast cancer cells, thus rendering [Cu(L)(dppz)]$^{+}$ to be an efficient anticancer agent.

3.1. Effect of FA@MSNPs-[Cu(L)(dppz)]$^{+}$ on breast cancer and normal breast epithelial cells

Efficient delivery of [Cu(L)(dppz)]$^{+}$ through nanocarriers into cancer cells can increase the specificity of inhibiting growth of the tumor cells sparing the healthy cells. Thus, to examine the cytotoxic effect of MSNPs used as nanocarriers in this study, cytotoxicity of FA@NH_2-MSNPs to MCF-7, MDA-MB-231 and HBL-100 cells before drug loading was analyzed adopting MTT assay. The results revealed that FA@NH_2-MSNPs in DMSO is not cytotoxic to the cells under the measured concentration range i.e., up to 100 µg mL^{-1} (Fig. S4). Thereafter, the efficacy of FA@NH_2-MSNPs in targeting the cancer cells after drug loading was analyzed. Unlike FA@NH_2-MSNPs, significant inhibition of cell growth was observed when the cells were treated with [Cu(L)(dppz)]$^{+}$ loaded nanoparticles.

We incubated FA@MSNPs-[Cu(L)(dppz)]$^{+}$ at different concentrations with MCF-7 and MDA-MB-231 cells, for 24 and 48 h. The IC_{50} value of FA@MSNPs-[Cu(L)(dppz)]$^{+}$ for MCF-7 was 1.1 ± 0.1 µg mL^{-1} for 24 h and 0.98 ± 0.2 µg mL^{-1} for 48 h and for MDA-MB-231 it was 1.7 ± 0.1 µg mL^{-1} and 1.2 ± 0.1 µg mL^{-1} for 24 h and 48 h, respectively (Table S1). It has been already shown in our earlier study [22] that the free [Cu(L)(dppz)]$^{+}$ is cytotoxic to the breast cancer cells, MCF-7 and MDA-MB-231, at respective very low concentrations (IC_{50} = 0.25 ± 0.02 µg mL^{-1}, and 0.64 ± 0.1 µg mL^{-1}, respectively, at 48 h). The free [Cu(L)(dppz)]$^{+}$, when tested on normal breast epithelial cell, HBL-100, showed equally high cytotoxicity with IC_{50} value of 0.76 ± 0.1 µgmL^{-1} under the same treatment conditions as for breast cancer cells (Fig. S1). On the other hand, FA@MSNPs-[Cu(L)(dppz)]$^{+}$ is cytotoxic to MCF-7 and MDA-MB-231 cells (IC_{50} = 0.98 ± 0.1 and 1.2 ± 0.1 µgmL^{-1}, respectively) after incubation for 48 h, to significant levels, and less cytotoxic to HBL-100 cells (IC_{50} = 13.7 ± 0.1 µgmL^{-1}). The calculated IC_{50} values for the inhibition of cell growth by FA@MSNPs-[Cu(L)(dppz)]$^{+}$ and free [Cu(L)(dppz)]$^{+}$ reveal that cancer cells are 10-fold more sensitive to FA@MSNPs-[Cu(L)(dppz)]$^{+}$ compared to normal cells, while both types of

cells are almost equally sensitive to free $[Cu(L)(dppz)]^+$ (Table S2). It has also been reported that the multifunctional MSNPs promote the anticancer efficacy of clinically used drug doxorubicin through folate-mediated targeting to cancer cells and subsequent stimuli-triggered drug release [34]. All these results reveal that the enhanced drug delivery efficacy of multifunctional nanoparticles combined with the co-operative effects of folate-mediated targeting holds promise for the cure of folate receptor-positive tumors.

3.2. Efficacy of FA@MSNPs-$[Cu(L)(dppz)]^+$ to target cancer cells compared to normal cells under the same treatment conditions

Acridine orange and ethidium bromide (AO & EB) and Hoechst staining were adopted to infer how the cancer and normal cells respond to the FA@MSNPs-$[Cu(L)(dppz)]^+$ under the same treatment conditions, with apoptosis or necrosis. Typical apoptotic morphologies such as condensed nuclei and formation of apoptotic bodies in treated cancer cells whereas no such changes were noticed in untreated control and normal cells (Fig. 3).

Hoechst 33258 staining revealed that in the untreated control cells and normal cells, the nuclei are stained in less intensity, and the color is homogenous compared to treated cancer cells in which the blue emission is highly intense. Condensed chromatin could be visualized in many treated cancer cells and some of the cells formed apoptotic bodies (Fig. 4). Data in respect of cells indicating necrotic and apoptotic morphologies, induced on treatment with the FA@MSNPs-$[Cu(L)(dppz)]^+$ for 24 and 48 h followed by staining with AO & EB and Hoechst 33258, collected from manual counting of cells, are presented in Figure S5 & S6.

Western blotting revealed that there is significant up-regulation of p53 tumor suppressor protein in the FA@MSNPs-$[Cu(L)(dppz)]^+$ treated cancer cells whereas only negligible change is found in the untreated and normal cells (Fig. 5). Taken together, we have shown the

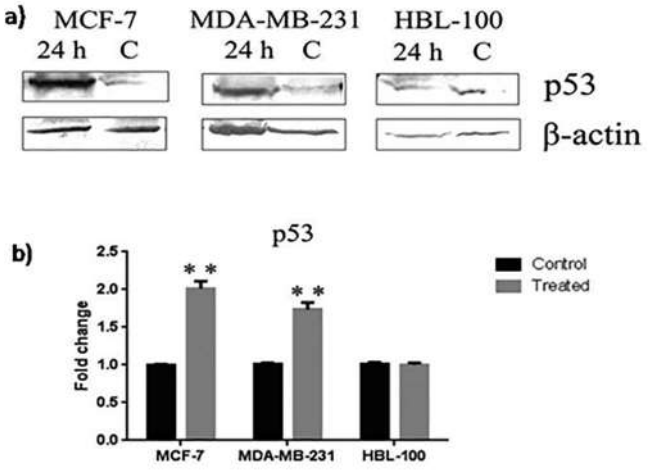


Fig. 5. a) Western blot analysis showing the expression of p53 in MCF-7, MDA-MB-231 and HBL-100 cells on treatment with FA@MSNPs-$[Cu(L)(dppz)]^+$ at 2.5 μg/mL. b) Western blot analysis data showing up-regulation of p53 tumor suppressor protein in the FA@MSNPs-$[Cu(L)(dppz)]^+$ treated cancer cells.

efficiency of $[Cu(L)(dppz)]^+$ loaded nanoparticles to effectively bring about primarily apoptotic cell death in breast cancer cells without inflicting any morphological, molecular or protein level changes in respect of induction of apoptosis in the normal breast epithelial cells under the same treatment conditions.

4. Conclusion

The growth inhibitory effect of free $[Cu(L)(dppz)]^+$ on normal

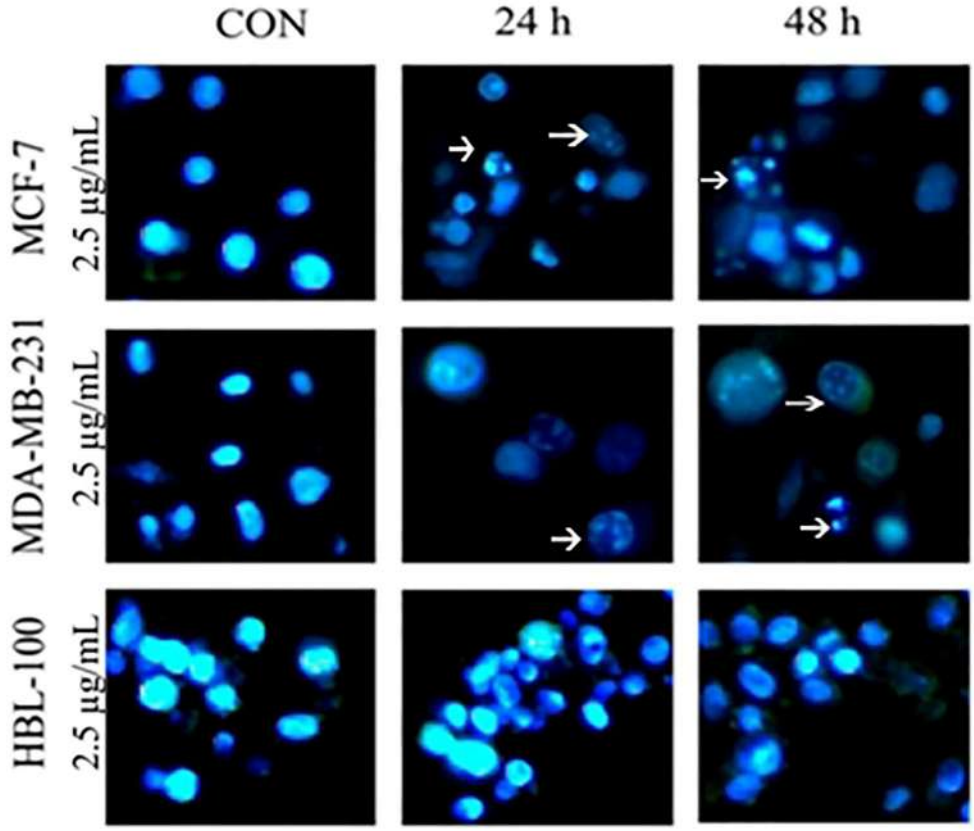


Fig. 4. Control and FA@MSNPs-$[Cu(L)(dppz)]^+$ treated MCF-7, MDA-MB-231 and HBL-100 cells stained with Hoechst 33258. Note indication of nuclear changes relating to apoptosis in MCF-7 and MDA-MB-231 cells and not in HBL-100 normal breast epithelial cells. Arrow marks indicate fragmentation of nucleus in MCF-7 and MDA-MB-231 cells treated with FA@MSNPs-$[Cu(L)(dppz)]^+$.

breast epithelial cells is equally high as in cancer cells under the same treatment conditions, necessitating targeted delivery of $[Cu(L)(dppz)]^+$ to cancer cells, sparing normal cells. Thus, folic acid-conjugated MSNPs loaded with copper (II) complex (FA@MSNPs-$[Cu(L)(dppz)]^+$) has been developed in order to deliver it to cancer cells in a targeted manner. MTT assay revealed that the multifunctional folic acid-conjugated MSNPs loaded (89%) with the DNA damaging agent $[Cu(L)(dppz)]^+$ can be efficiently trapped by breast cancer cells facilitated by folate receptor targeting with a potency of 10-fold increased uptake when compared to the normal breast epithelial cells. To conclude, the targeted delivery system delivers the DNA damaging agent $[Cu(L)(dppz)]^+$ preferentially to the cancer cells, overcoming the major limitation of the complex that both cancer cells and normal cells are affected, thereby providing a platform for efficacious cancer therapy. Thus, the system FA@MSNPs-$[Cu(L)(dppz)]^+$ is worthy of further preclinical and clinical investigation and can be developed for therapeutic application in breast cancer, and probably other cancers also.

CReadiT authorship contribution statement

Thankaraj Salammal Sheena: Conceptualization, Writing - original draft. **Rajakumaran Dhivya:** Conceptualization, Writing - original draft. **Venkatesan Rajiu:** Conceptualization. **Kulandaivel Jeganathan:** Supervision. **Mallayan Palaniandavar:** Methodology. **Ganesan Mathan:** Supervision. **Mohammad Abdulkader Akbarsha:** Investigation, Writing - review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The financial assistance from the Doerenkamp-Zbinden Foundation, Switzerland, is heartily acknowledged. K.J. acknowledges the financial support from Department of Science and Technology (DST) under the scheme of FIST and Nanomission (Contract No.SR/NM/NS-1502/2014). T.S.S acknowledges the meritorious fellowship from the University Grants Commission (UGC), Govt. of India. We thank Dr. Annapoorni Rangarajan, Professor of Molecular Reproduction, Development & Genetics, Indian Institute of Science, Bangalore, for technical support. Professor M. Palaniandavar acknowledges Indian National Science Academy (INSA), New Delhi for the INSA Senior Scientist award.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ica.2020.119783.

References

[1] S. Vandghanooni, J. Barar, M. Eskandani, Y. Omidi, TrAC Trends in Analytical Chemistry, vol. 123, 2020, pp. 115759.
[2] S. Rajkumar, M. Prabaharan, Colloids and Surfaces B: Biointerfaces, vol. 186, 2020, pp. 110701.
[3] M. Varache, I. Bezverkhyy, G. Weber, L. Saviot, R.m. Chassagnon, F. Baras, F. Bouyer, Langmuir, vol. 35, 2019, pp. 8984-8995.
[4] D.P. Ferris, Y.-L. Zhao, N.M. Khashab, H.A. Khatib, J.F. Stoddart, J.I. Zink, J. Am. Chem. Soc. 131 (2009) 1686–1688.
[5] S. Angelos, N.M. Khashab, Y.-W. Yang, A. Trabolsi, H.A. Khatib, J.F. Stoddart, J.I. Zink, J. Am. Chem. Soc. 131 (2009) 12912–12914.
[6] J. Lu, M. Liong, J.I. Zink, F. Tamanoi, Small 3 (2007) 1341–1346.
[7] Y. Cheng, Y. Zhang, W. Deng, J. Hu, Colloids and Surfaces B: Biointerfaces, vol. 186, 2020, pp. 110744.
[8] C. Giménez, C. de la Torre, M. Gorbe, E. Aznar, F.l. Sancenón, J.R. Murguía, R. Martínez-Máñez, M.D. Marcos, P. Amorós, Langmuir, vol. 31, 2015, pp. 3753-3762.
[9] M. Benezra, O. Penate-Medina, P.B. Zanzonico, D. Schaer, H. Ow, A. Burns, E. DeStanchina, V. Longo, E. Herz, S. Iyer, J. Clin. Investig. 121 (2011) 2768–2780.
[10] J.M. Rosenholm, J. Zhang, M. Linden, C. Sahlgren, Nanomedicine 11 (2016) 391–402.
[11] Y. Wu, P. Ge, W. Xu, M. Li, Q. Kang, X. Zhang, J. Xie, Materials Science and Engineering: C, vol. 108, 2020, pp. 110386.
[12] M. Wang, J. Long, S. Zhang, F. Liu, X. Zhang, X. Zhang, L. Sun, L. Ma, C. Yu, H. Wei, ACS Biomater. Sci. Eng. 6 (2020) 1565–1572.
[13] J. Gu, W. Fan, A. Shimojima, T. Okubo, Small 3 (2007) 1740–1744.
[14] L. Pasqua, F. Testa, R. Aiello, S. Cundari, J.B. Nagy, Microporous Mesoporous Mater. 103 (2007) 166–173.
[15] Y. Li, S. Wang, F.X. Song, L. Zhang, W. Yang, H.X. Wang, Q.L. Chen, Colloids and Surfaces A: Physicochemical and Engineering Aspects, vol. 590, 2020, pp. 124470.
[16] D. Dong, W. Gao, Y. Liu, X.-R. Qi, Cancer Lett. 359 (2015) 178–186.
[17] S. Zhao, S. Sun, K. Jiang, Y. Wang, Y. Liu, S. Wu, Z. Li, Q. Shu, H. Lin, Nano-Micro Letters, vol. 11, 2019, pp. 32.
[18] S. Franzen, Expert Opinion on Drug Delivery 8 (2011) 281–298.
[19] G.L. Zwicke, G. Ali Mansoori, C.J. Jeffery, Nano Reviews, vol. 3, 2012, pp. 18496.
[20] B. Farran, R.C. Montenegro, P. Kasa, E. Pavitra, Y.S. Huh, Y.-K. Han, M.A. Kamal, G. P. Nagaraju, G.S.R. Raju, Materials Science and Engineering: C, vol. 107, 2020, pp. 110341.
[21] P. Jaividhya, R. Dhivya, M.A. Akbarsha, M. Palaniandavar, J. Inorg. Biochem. 114 (2012) 94–105.
[22] R. Dhivya, P. Jaividhya, A. Riyasdeen, M. Palaniandavar, G. Mathan, M.A. Akbarsha, Biometals 28 (2015) 929–943.
[23] F. Muhammad, M. Guo, W. Qi, F. Sun, A. Wang, Y. Guo, G. Zhu, J. Am. Chem. Soc. 133 (2011) 8778–8781.
[24] T. Mosmann, J. Immunol. Methods 65 (1983) 55–63.
[25] M. Spector, R.D. Goldman, L.A. Leinwand, A Laboratory Manual vol, (1998) 1.
[26] R.S. Kumar, S. Arunachalam, V. Periasamy, C. Preethy, A. Riyasdeen, M. Akbarsha, Polyhedron 27 (2008) 1111–1120.
[27] S.A. Latt, G. Stetten, L.A. Juergens, H.F. Willard, C.D. Scher, J. Histochem. Cytochem. 23 (1975) 493–505.
[28] V. Rajendiran, R. Karthik, M. Palaniandavar, H. Stoeckli-Evans, V.S. Periasamy, M.A. Akbarsha, B.S. Srinag, H. Krishnamurthy, Inorg. Chem. 46 (2007) 8208–8221.
[29] P. Pant, C. Gupta, S. Kumar, A. Grewal, S. Garg, A. Rai, Journal of Materials NanoScience 7 (2020) 1–18.
[30] M. Manzano, M. Vallet-Regí, Advanced Functional Materials, vol. 30, 2020, pp. 1902634.
[31] H. Meng, M. Liong, T. Xia, Z. Li, Z. Ji, J.I. Zink, A.E. Nel, ACS Nano 4 (2010) 4539–4550.
[32] S.R. Gayam, P. Venkatesan, Y.-M. Sung, S.-Y. Sung, S.-H. Hu, H.-Y. Hsu, S.-P. Wu, Nanoscale 8 (2016) 12307–12317.
[33] X. Liu, F. Fu, K. Xu, R. Zou, J. Yang, Q. Wang, Q. Liu, Z. Xiao, J. Hu, J. Mater. Chem. B 2 (2014) 5358–5367.
[34] K. Nurgali, R.T. Jagoe, R. Abalo, Frontiers in Pharmacology, vol. 9, 2018, pp. 245.
[35] Q.-w. Zhang, L. Liu, C.-y. Gong, H.-s. Shi, Y.-h. Zeng, X.-z. Wang, Y.-w. Zhao, Y.-q. Wei, PloS one, vol. 7, 2012, pp. e50946.

Toxicology in Vitro 75 (2021) 105201

Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

Potential application of two cobalt (III) Schiff base complexes in cancer chemotherapy: Leads from a study using breast and lung cancer cells

Balakrishnan Gowdhami, M.Sc,M.Phil [a,c], Subramanian Ambika, M.Sc,Ph.D [b], Balakrishnan Karthiyayini, M.Tech [c,d], Venkatesan Ramya, M.Sc [a], Balamuthu Kadalmani, M.Sc, Ph.D [a,*], R.T.V. Vimala, M.Sc,Ph.D [e], Mohammad A. Akbarsha, M.Sc,Ph.D [f,g,**]

[a] Department of Animal Science, Bharathidasan University, Tiruchirappalli, Tamilnadu, India
[b] Department of Chemistry, Bishop Heber College, Tiruchirappalli, Tamilnadu, India
[c] National Centre for Alternatives to Animal Experiments, Bharathidasan University, Tiruchirappalli, Tamilnadu, India
[d] Department of Microbiology, Bharathidasan University, Tiruchirappalli, Tamilnadu, India
[e] Department of Biotechnology, Bharathidasan University, Tiruchirappalli, Tamilnadu, India
[f] Mahatma Gandhi-Dorenkamp Centre, Bharathidasan University, Tiruchirappalli, Tamilnadu, India
[g] Department of Biotechnology & Research Coordinator, National College (Autonomous), Tiruchirappalli, Tamilnadu, India

ARTICLE INFO

Editor: Dr P Jennings

Keywords:
Cobalt (III) Schiff base complexes
Apoptosis
DNA damage
ROS
Immunofluorescence
RT-PCR

ABSTRACT

Cobalt (III) Schiff base complexes are of attraction in the context of their potential application in cancer therapy. The aim of this study has been to find the mechanism of action of cobalt (III) Schiff base complexes **1** and **2,** the synthesis and characterization of which have already been reported, in inhibiting growth of human breast cancer cell MCF-7 and lung cancer cell A549. The already proclaimed anti-proliferative effect of the cobalt complexes was ascertained using MTT cytotoxicity assay. More assays such as Acridine orange & Ethidium bromide staining, AnnexinV-Cy3 staining, Hoechst staining, comet assay, and Reactive Oxygen Species (ROS) assay- all supported the cytotoxic property of the complexes. Moreover, the expression levels of mRNA of pro- and anti-apoptotic genes also supported the effectiveness of cobalt complexes by modifying the ratio of Bax: Bcl-2. In addition, the cobalt complexes induced apoptosis in MCF- 7 and A549 cells through modulation of pro-apoptotic, anti-apoptotic, and ROS modulatory gene expressions. The present study validates the scientific evidence for antiproliferative efficacy of cobalt complexes against MCF-7 and A549 cells. Thus, this study takes cobalt complexes **1** and **2** to a step higher towards their use as anticancer agents.

1. Introduction

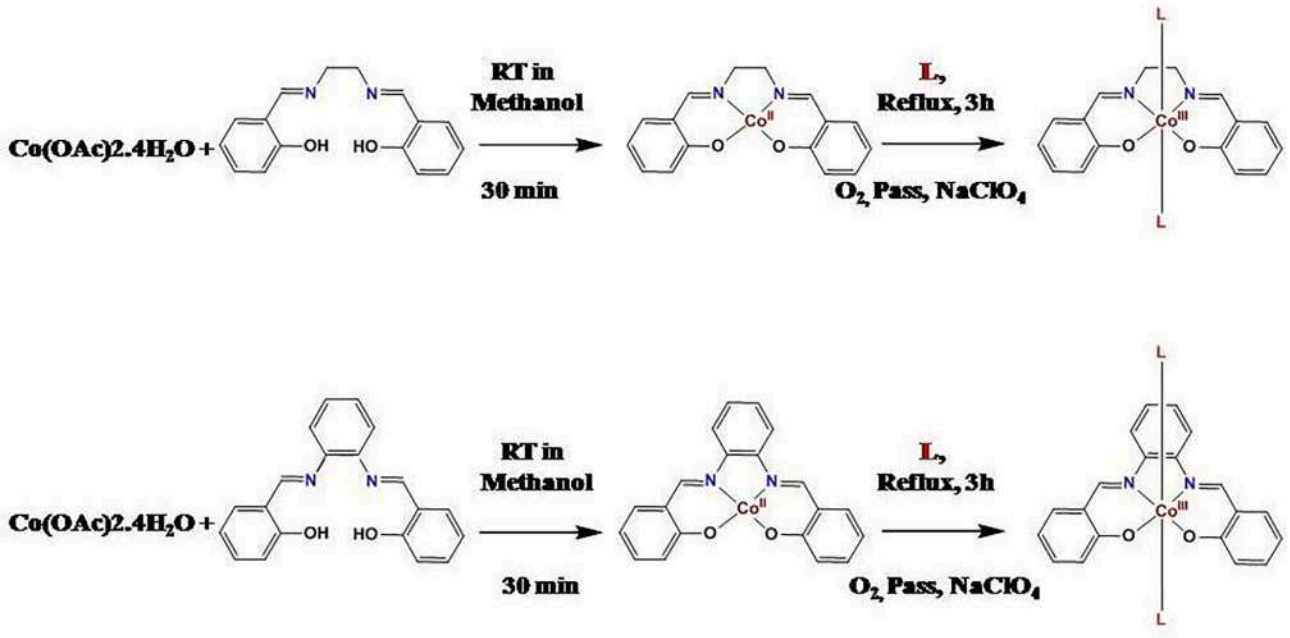
Breast and lung cancers are serious threat to millions of human lives. There is no ensured therapy for breast and lung cancers (Raine et al., 2010). Every other chemotherapeutic has been shown to produce adverse side effects in normal tissues and physiology (Schirrmacher, 2019). For example platinum complexes have been in use in the treatment and management of several cancer types including breast and lung cancer over decades (Huang et al., 2017). Despite the proven therapeutic efficacy of platinum complexes, limited aqueous solubility, side effects such as oto- and nephrotoxicity, myelosuppression, and poor selectivity towards healthy cells remain major issues (Laurell and Pierre, 2015). For this reason, during the recent year, there is extensive search for novel treatment regimes using non-platinum complexes such as Fe, Pd, Ru, Cu, Bi, Zn, and Co.

Cobalt is an essential element involved in a number of physiological processes inside the cell (Munteanu and Suntharalingam, 2015). Therefore, we presumed cobalt and cobalt-based complexes to have lesser side effects than the conventional platinum-based complexes. The coordination chemistry of cobalt are already established, especially cobalt complexes containing Schiff base ligands are known to exhibit better cytotoxic activity against cancer cells such as MCF-7 and A549 than cisplatin (King et al., 2017; Munteanu and Suntharalingam, 2015). Cobalt.

(III) Schiff base complexes act as strong chelating agents that facilitate the coordinated metal complexes in different oxidation states. Metal

* Correspondence to: B. Kadalmani, Department of Animal Science, Bharathidasan University, Tiruchirappalli 620024, India.
** Correspondence to: M. A. Akbarsha, National College, Tiruchirappalli 620001, India.
E-mail addresses: kadalmani@bdu.ac.in (B. Kadalmani), akbarbdu@gmail.com (M.A. Akbarsha).

https://doi.org/10.1016/j.tiv.2021.105201
Received 8 February 2021; Received in revised form 21 May 2021; Accepted 17 June 2021
Available online 19 June 2021
0887-2333/© 2021 Published by Elsevier Ltd.



Scheme 1. Synthesis of Cobalt (III) Schiff Base complexes **1** and **2** (Ambika et al., 2019).

Table 1
IC50 values (μM) of cobalt complexes**1** and **2** and cisplatin for MCF-7, A549 and 3 T3 cells incubated for 24, 48 and 72 h. Each data represents mean and standard deviation of triplicate data.

Cell line	Compound	24 h	48 h	72 h
MCF-7 (μM)	Complex **1**	35.26 ± 0.27	19.18 ± 0.45	08.56 ± 0.07
	Complex **2**	15.28 ± 0.29	8.46 ± 0.05	03.55 ± 0.08
	Cisplatin	38.10 ± 0.40	24.82 ± 0.37	12.47 ± 0.04
A549 (μM)	Complex **1**	79.40 ± 0.56	46.41 ± 0.54	19.53 ± 0.06
	Complex **2**	65.37 ± 0.30	31.32 ± 0.17	10.78 ± 0.18
	Cisplatin	60.15 ± 0.37	40.34 ± 0.21	22.38 ± 0.21
3 T3 (μM)	Complex **1**	144.87 ± 0.15	94.38 ± 0.16	52.16 ± 0.06
	Complex **2**	155.14 ± 0.27	105.34 ± 0.09	85.15 ± 0.09
	Cisplatin	165.04 ± 0.34	134.83 ± 0.47	115.12 ± 0.09

complexes are assumed to pass through the cell membrane and induce single−/double-standard DNA breaks through hydrolytic cleavage, thereby enhancing the cytotoxic property (Veeralakshmi et al., 2015). Of note, DNA-damaging agents have a long history of use in cancer chemotherapy (Dhivya et al., 2015). The DNA break induced by other Schiff base complexes such as Copper(II) complexes with vanillin Schiff base derivative (Lu et al., 2017), CdCl2(C14H21N3O2) complex (Hajrezaie et al., 2015), and lanthanide complexes (Andiappan et al., 2018) are known to induce accumulation of reactive oxygen species (ROS) inside the cell, leading to induction of apoptosis to result in cell death. Therefore, Cobalt (III) Schiff base complexes are also expected to accumulate ROS inside the cell and induce apoptosis-mediated cell death. Apoptosis and cell cycle inhibition are important events for tumor suppression. This could be achieved in number of ways including ROS generation, activation of p53 gene expression pathway, and/or DNA intercalation that would lead to cell membrane damage. Previously, we had reported the synthesis of two novel Cobalt (III) Schiff base complexes *viz* (trans-[Co(salen)(DA)2](ClO4) (complex **1**) and trans-[Co (salophen)(DA)2](ClO4) (complex **2**), where the salen and salopen were N,N′- bis(salicylidene)ethylenediamine, and *N*,*N*′-bis(salicylidene)-1,2-phenylenediamine, DA: dodecylamine) respectively (Ambika et al., 2019) Furthermore, in our previous report we had observed the DNA intercalation and groove binding ability of complex **1** and **2**. Both the cobalt.

(III) Schiff base complexes exhibited *in vitro* cytotoxic property against human small cell lung carcinoma cells (A549) with IC50 80 μM and 65 μM for complexes **1** and **2,** respectively. Furthermore, we had suggested based on AO/EB staining the mode of cell death in A549 lung cancer cells as apoptosis, and arrest of cell cycle (greatly in G2-M phase) by flow cytometry (Ambika et al., 2019).

In the present research we have expanded the cytoxicity effect to breast cancer cells breast cancer cells (MCF-7), alongside confirming the previously reported lung cancer cells (A549). In addition we had investigated the cytotoxic effect of these novel Cobalt (III) Schiff base complexes in normal mouse fibroblast (3 T3) cell. In addition, we had studied the signaling mechanisms involved and profiled the expression of key genes that are responsible for the cytotoxicity and apoptosis. Furthermore, we have attempted to validate the mode of cell death in MCF-7, A549 and 3 T3 cells as apoptosis, by morphologically examining the micronuclei induction, cellular ROS generation, chromosome abnormalities, DNA damage and by studying the molecular expression levels of proapoptotic genes such as BID, BAX, Cas-3, Cas-8 and the anti-apoptotic genes such as Bcl-2 and GRP-78 in MCF-7 breast cancer and A549 small cell lung cancer cells treated with the cobalt (III) Schiff base complexes. Overall, in the present research, we had expanded the scope of cytotoxicity of the novel Cobalt (III) Schiff base complexes to breast and lung cancers and validated apoptosis as the mode of induction of cell death as revealed in the gene expression profiles at molecular level.

2. Materials and methods synthesis of cobalt (III) complex

Two cobalt (III) Schiff base complexes, trans-[Co(salen)(DA)2](ClO4) **(1)** and trans- [Co(salophen)(DA)2] (ClO4) **(2)** (where salen: *N*,*N*′-bis(salicylidene)ethylenediamine, salopen: *N*,*N*′-bis(salicylidene)-1,2phenylenediamine, DA: dodecylamine) were prepared as previously reported (Ambika et al., 2019) and the schematic of the synthesis is presented in Scheme 1.

2.1. Cell culture

Human breast cancer cell MCF-7, human lung cancer cell A549, and mouse embryonic fibroblast cell 3 T3 were obtained from National Centre for Cell Science, Pune, India. The cells were cultured in DMEM

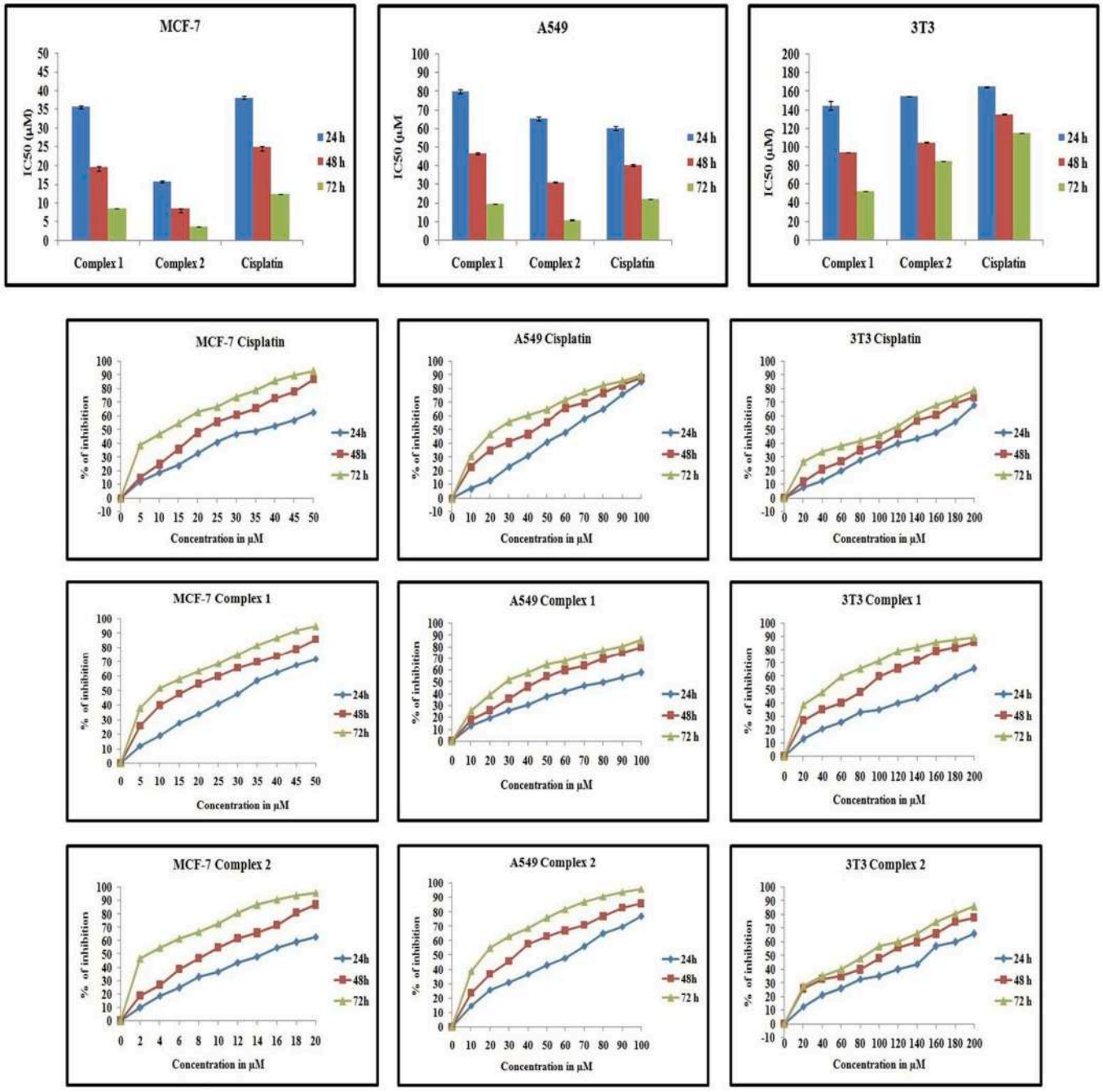


Fig. 1. **(A)** Graph showing IC50 values (μM) of cobalt complexes **1** and **2** and cisplatin for MCF-7, A549 and 3 T3 cells incubated for 24, 48 and 72 h. (Data represent means and standard deviations of three replicates each). **(B)** Cytotoxic effect of cobalt complexes **1** and **2** on MCF-7, A549 and 3 T3 cells incubated for 24, 48 and 72 h (Data represent means and standard deviations of three replicates each).

medium to which 10% FBS was added. The antibiotics streptomycin and penicillin (Sigma Chemical Co., St. Louis, Mo, USA) (each 100 U/mL) were mixed with the medium. The cells were maintained in a humidified atmosphere with 5% of CO2 at 37 °C in a Thermo Scientific (USA) incubator.

2.2. MTT assay

The Inhibitory concentration (IC50) of the cobalt (III) Schiff base complexes were determined by MTT [3-(4, 5-dimethylthiazol2-yl)-2, 5-diphenyltetrazolium bromide]. Briefly, the synthesized complexes (**1** & **2**) were dissolved in 0.02% DMSO (Sigma Chemical Co., St. Louis, Mo, USA). MCF-7, A549 and 3 T3 cells were treated with the complexes (dose range: 0–50 and 0–20 μM for complexes 1 and 2, respectively) for 24, 48, and 72 h. DMSO (0.02%) and cisplatin (dose range: 0–50, 0–100, 0–200 μM for MCF-7, A549 and 3 T3 cells, respectively) served as negative and positive controls, respectively. Post-treatment (24, 48 and 72 h), 20 μL of MTT solution [5 mg/mL MTT dissolved in phosphate-buffered saline (PBS)] was added and incubated in a 96 well plate (seeded with 5000 cells per well) for 4 h at 37 °C. By the end of 4 h incubation, and the purple color formazan crystals were dissolved in 100 μL of 100% DMSO . The absorbance was recorded at 570 nm (sample) and 630 nm (reference). The experiments were repeated three times and the results were presented as means ± SD. The percentage inhibition of the cells (MCF-7, A549 and 3 T3) was calculated using the following formula:

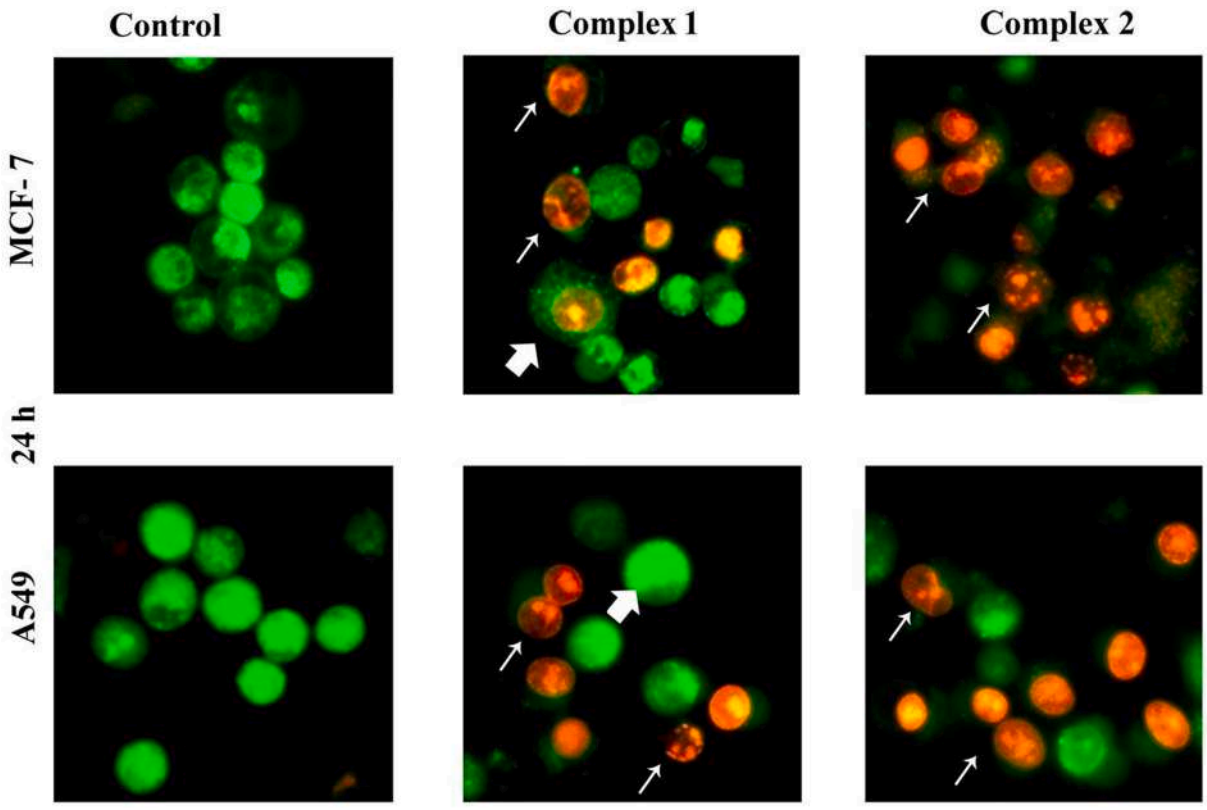


Fig. 2. Effect of control and cobalt complexes **1** and **2** treated MCF-7 and A549 cells stained with AO/EtBr. Small arrows point to cells in apoptosis and big arrows point to necrotic cells.

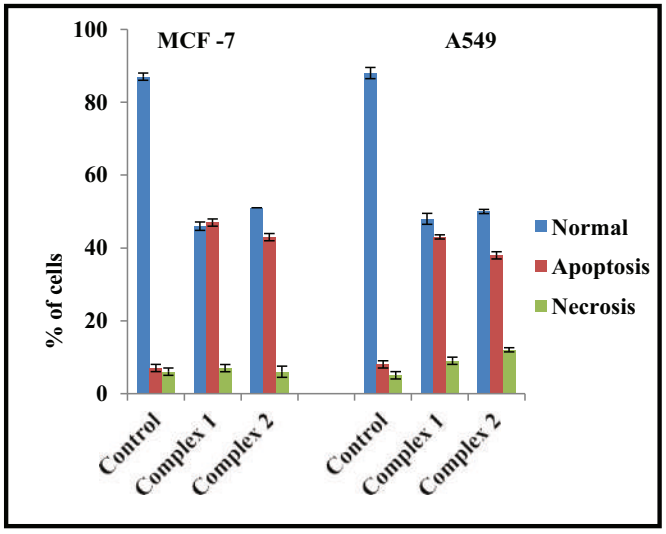


Fig. 3. Percentage of normal, apoptotic and necrotic cells at 24 h treatment with cobalt complexes **1** and **2** (Data represent means and standard deviations of three replicates each).

2.3. Acridine orange (AO) and Ethidium bromide (EB) fluorescent staining assay

Acridine orange (AO) and Ethidium bromide (EB) fluorescent staining assay was performed using the protocol described earlier (Spector and Goldman, 2006). Briefly, MCF-7 and A549 cells treated with the cobalt (III) Schiff's base complexes at the 24 h IC50 concentration were cultured in a sterile 6-well tissue culture plate (Tarson, India). By the end of 24 h, one part each of 100 µg/mL AO and EB in PBS were used to stain the control and treated cells (density of $5*10^4$ cells/well). Based on the cytoplasmic organization and nuclear morphology, apoptosis or necrosis was deciphered. DMSO at 0.02% was used as negative control. The viable and dead cells were sorted using microscope (Axio Observer 7, Carl Zeiss, Germany).

2.4. Hoechst 33528 staining

The staining was performed based on the procedure of Latt and Wohlleb (1975). MCF-7 and A549 cells were treated with the cobalt (III) Schiff's base complexes for 24 h. The control and treated cells were stained using Hoechst 33258 (1 mg/mL) and kept for incubation 5 min at room temperature (Rajendiran et al., 2007). The cells were suspended onto a glass slide with a cover slip over laid. Approximately, 100 cells were evaluated in a fluorescent microscope (Axio Observer 7, Carl Zeiss, Germany) at 400× magnification. The morphological changes were observed and the results were confirmed in triplicate experiments.

$$\text{Percentage inhibition} = \frac{[\text{Mean OD of untreated cells(control)-Mean OD of treated cells}]}{\text{Mean OD of untreated cells(control)}}$$

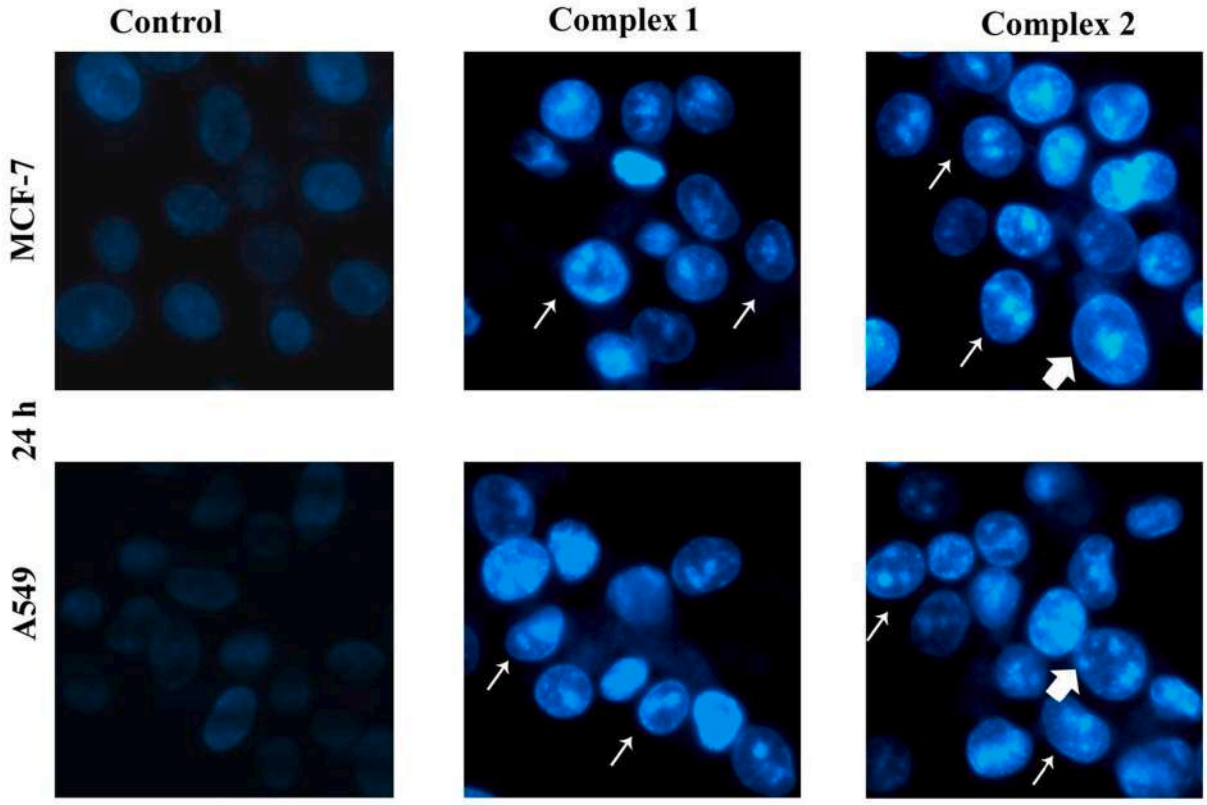


Fig. 4. Control and cobalt complexes **1** and **2** treated MCF-7 and A549 cells stained with Hoechst 33258. Small arrows point to cells in apoptosis and big arrows point to necrotic cells.

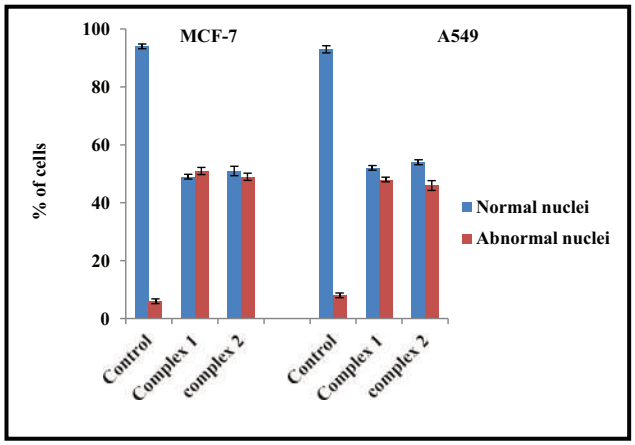


Fig. 5. Percentages of cells with normal and abnormal nuclei at 24 h treatment of cobalt complexes **1** and **2** (Data represent means and standard deviations of three replicates each).

2.5. Assessment of mitochondrial membrane potential (JC1 staining)

JC-1 dye was used to assess the mitochondrial membrane potential ($\Delta\Psi$ m) (Reers et al., 1991). Seeded MCF-7 and A549 cells (density of $5*10^4$ cells/ well) were incubated with the complexes at the IC50 concentration for 24 h and 48 h. Then, the cells were kept for incubation with the dye for 20 min. The fluorescence pattern was evaluated in the fluorescent microscope. The experiments were repeated thrice to obtain data for calculation of means and the standard deviations.

2.6. AnnexinV-Cy3 staining

Phosphatidyl serine translocation from inner to outer leaflet of plasma membrane is one of the earliest changes during apoptosis (Spector and Goldman, 2006). The presence of phosphatidyl serine has been identified by AnnexinV-Cy3 staining. The cultured MCF-7 and A549 cells were treated with 24 h IC50 concentrations of the complexes for 24 h. The cells were washed with PBS and 1× binding buffer (0.1 M HEPES/NaOH, 1.4 M NaCl, 25 mM CaCl, pH 7.4). Afterwards, 50 μL each of of Ann-Cy3 and 6-CFDA were used to stain the cells and incubated for 10 min in the dark. Excess stains were removed by washing. The cells were then observed randomly in the fluorescence microscope. This assay was used to distinguish live cells (green), necrotic cells (red), and apoptotic cells (red nuclei with green cytoplasm). Finally, the percentage of apoptotic and necrotic cells were calculated. The experiments were repeated thrice to calculate the means and the standard deviations.

2.7. Comet assay

Comet assay was performed based on the protocol described by Singh et al. (1988). MCF-7 and A549 cells were treated with the complexes at the respective IC50 concentrations for 24 h, wherein DMSO at 0.02% concentration was used as the negative control. Microscope slides were layered with normal melting point agarose. The seeded cells were embedded in low melting point agarose as a second layer and cooled for 10 mins. The slides were submerged overnight in lysis buffer (100 mM Na2EDTA, Triton X-100, 0.2 mM NaOH [pH 10.01], 2.5 M NaCl, and 10 mM Tris) to lyse the cells and allow DNA unfolding. Then, the slides were incubated in alkaline buffer (1 mM Na2-EDTA, 300 mM NaOH, [pH = 13]) for 20 min. After the incubation, the slides were washed and stained using EtBr. Finally, 100 cells were observed in the fluorescent

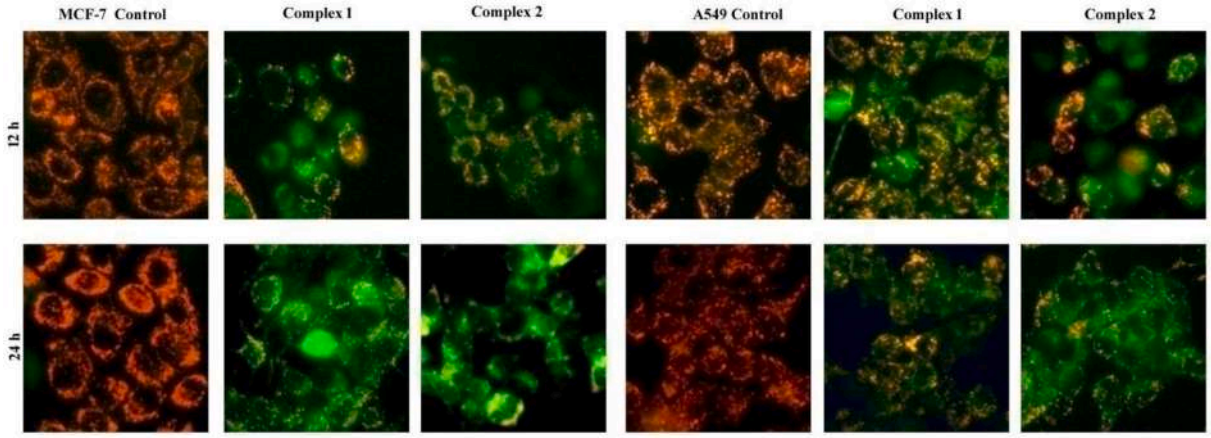


Fig. 6. JC-1 staining of control and cobalt complexes **1** and **2** treated MCF-7 and A549 cells incubated for 12 and 24 h to reflect on mitochondrial transmembrane potential.

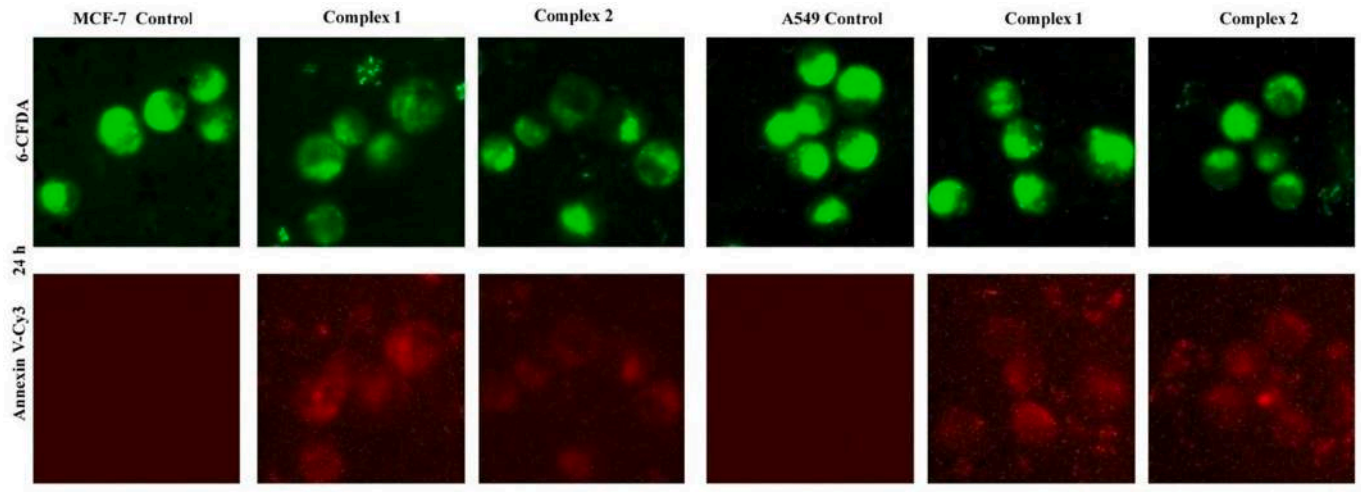


Fig. 7. Annexin V-Cy3–6-CFDA staining of cobalt complexes **1** and **2** treated MCF-7 and A549 cells.

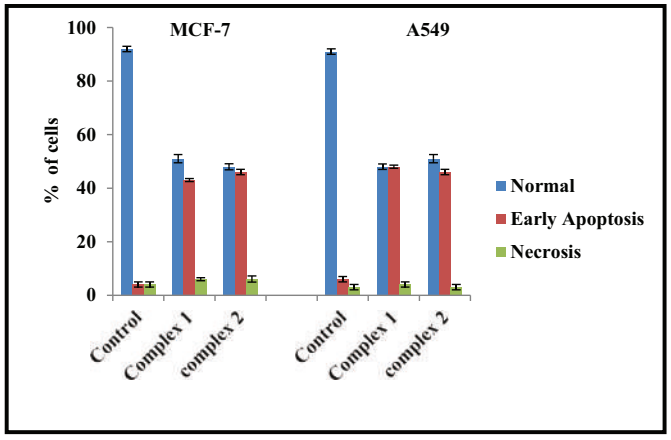


Fig. 8. Percentages of MCF-7 and A549 cells which have succumbed to death induced by cobalt complexes **1** and **2** (Data represent means and standard deviations of three replicates each).

microscope. CASP software was used to calculate the extent and percentage of DNA damage. The experiments were repeated thrice to calculate the means and the standard deviations.

2.8. ROS assay by DCFH-DA staining

ROS is generated due to the oxidative stress by oxidizing 2′, 7′-dichlorofluorescin diacetate (DCFH-DA) into 2′, 7′-dichlorofluorescin (Wang and Joseph, 1999). MCF-7 and A549 cells were treated with the complexes at the 24 h IC50 for 12 h. H2O2 (0.02%) was used as positive control. Finally, DCFH-DA (5 μM) was aliquoted into the wells and incubated for 30 min at 37 °C. The samples were then subjected to measurement of ROS generation based on excitation (485 nm) and emission (520 nm) wavelengths. The experiments were repeated thrice and the data were used to calculate the means and the standard deviations.

2.9. Immunofluorescence assay

MCF-7 and A549 cells were treated and incubated with the complex (at IC50 concentration) for 24 h. After the treatment, cells were fixed in 4% paraformaldehyde for 15′ at room temperature. The cells were washed twice and treated with the blocking buffer (5% BSA in PBS + 0.1% Triton X-100) for 1 h. After washing with the blocking buffer, cells were treated with the primary antibody (Bcl-2 and Bax) at 4 °C and incubated overnight. After washing, cells were incubated with the secondary antibody (Bcl-2 and Bax conjugated with FITC) at room temperature for 30 min in the dark. The cells were then stained with 4, 6-

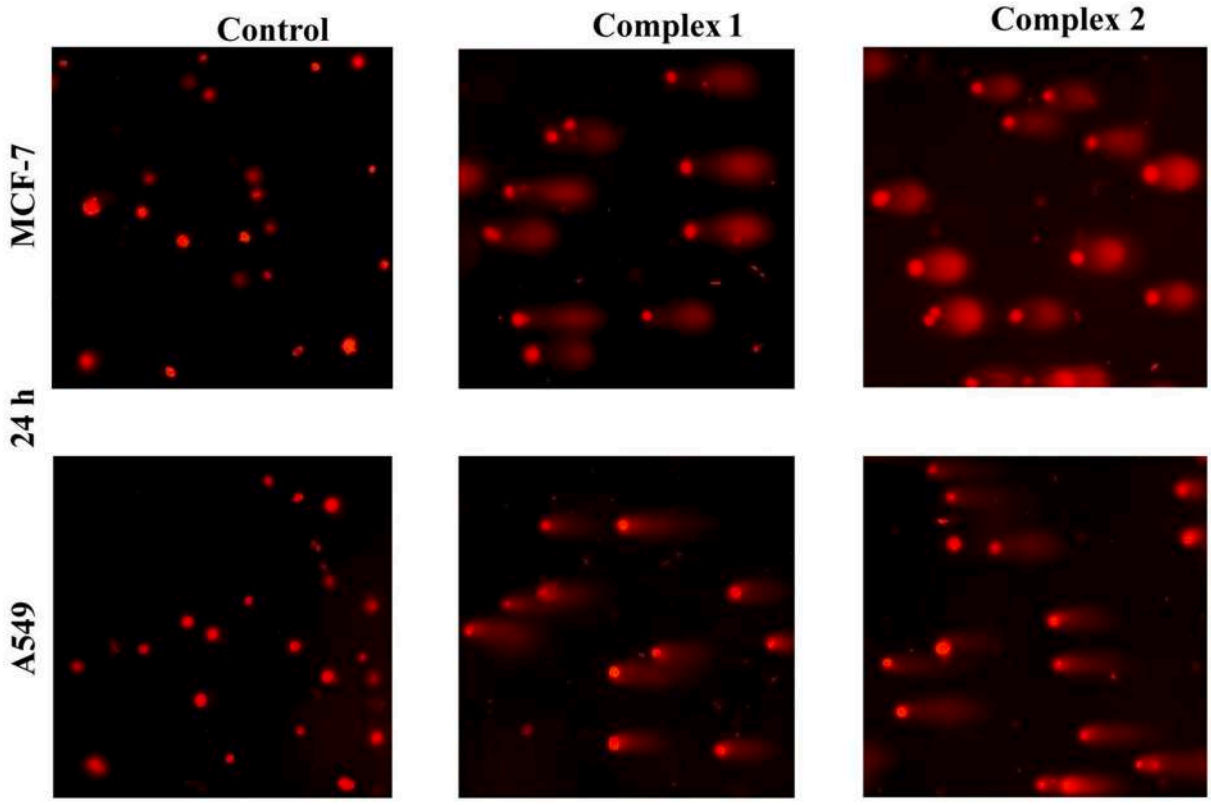


Fig. 9. Comet analysis of control and cobalt complexes **1** and **2** treated MCF-7 and A549 cells.

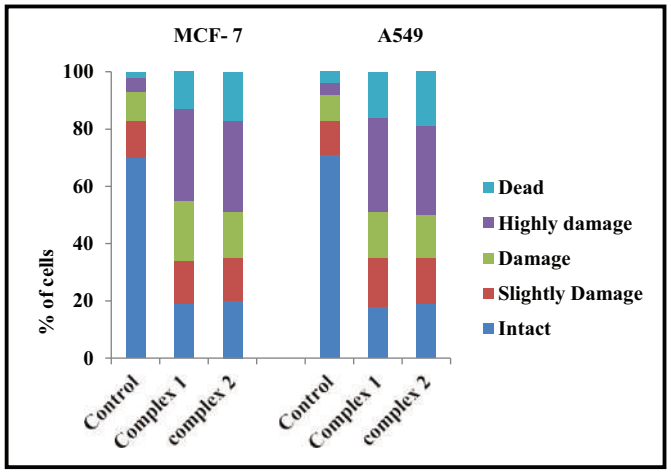


Fig. 10. Effect of treatment of cobalt complexes **1** and **2** on induction of DNA damage in MCF-7 and A549 cells. Classification: intact (0–20%), slightly damaged (20–40%), damaged (40–60%), highly damaged (60–80%), and dead (80–100%) (Each data point represents mean and standard deviation of three triplicates).

diamidino-2- phenylindole (DAPI; Sigma, USA) and observed in the fluorescent microscope.

2.10. Gene expression analysis by RT-PCR

RNA was isolated by Trizol method from control and cobalt (III) Schiff's base complexes treated MCF-7 and A549 cells at the 24 h IC50 for 12 h. RNase-free DNase I (2 units) was incubated with the isolated RNAs at 37 °C for 30 min and then centrifuged. The RNA pellets were collected and mixed with distilled water. Multiscribe reverse transcriptase, random primers, dNTP and 10× RT buffer were added with the isolated RNA to synthesize cDNA and incubated at 25 °C for 10 min and then at 37 °C for 120 min. SYBR Green Master Mix (iScript cDNA Kit) and 7900 RT-PCR detection system (Roche, Light cycler 96, Germany) were used. RT-PCR was performed under the following conditions: first step, 95 °C for 15 min; ensuing 40 cycles, 94 °C for 0.5–1 min, 50–58 °C for 0.5–1 min, and 72 °C for 1 min. GAPDH was used as control.

2.11. Statistics

Data are presented as means ± standard deviations. Statistical analyses were performed and graphs were prepared using Microsoft Excel™ (Microsoft Corporation, USA) running on Windows™ 10 platform.

3. Results

3.1. Cytotoxic effect of the cobalt (III) Schiff base complexes

The *in vitro* cytotoxic efficacy of the two cobalt (III) Schiff base complexes on MCF-7, A549 and 3 T3 cells was investigated by MTT assay. The cells were exposed to the complexes **1** and **2** to determine the IC50 for 24, 48 and 72 h. Cisplatin was used as the positive control. The IC50 of the complexes **1** and **2** for 24,48 and 72 h confirmed that the complexes inflict cytotoxic effect on MCF-7 cells at the concentrations as low as 08.56 ± 0.07 and 03.55 ± 0.88 μM for complexes **1** and **2**, respectively, over 72 h treatment. From the cytotoxicity studies using MTT assay, the complex **2** revealed to be more efficient in inflicting the antiproliferative activity than complex **1** in MCF-7 cells (Table 1). Similarly, the complex **2** was found to be more efficient than complex **1** with respect to A549 cells as well. However, both the complexes inflicted cytotoxicity in normal 3 T3 cells but at very high concentrations, 2 to 4 times that for MCF-7 and A549 (Table 1; Fig. 1A and B). Importantly, the complexes killed the cells at concentrations closer to or

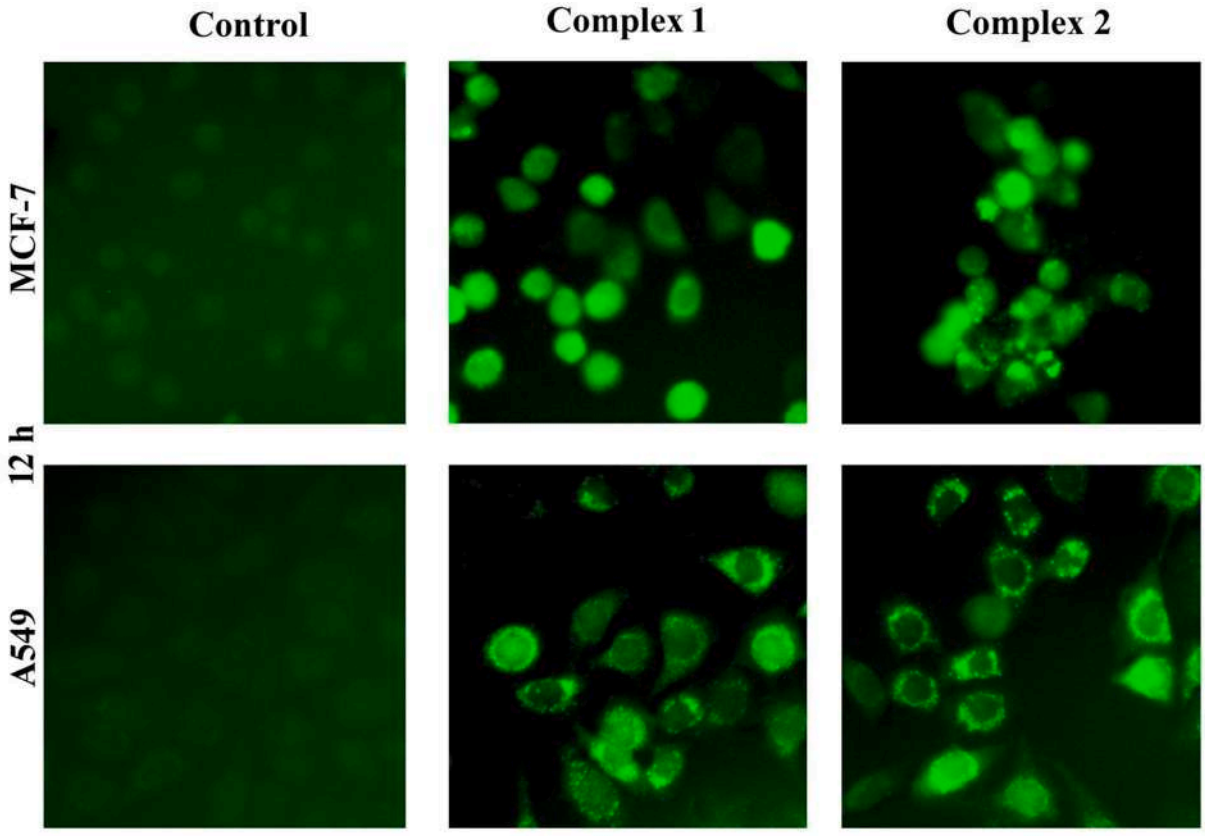


Fig. 11. DCFH-DA fluorescence staining representing the extent of ROS generation in MCF-7 and A549 cells exposed to cobalt complexes **1** and **2** incubated for 12 h.

lesser than cisplatin, the positive control. To sum up, the results, until now, show that the two cobalt (III) Schiff base complexes induce cell death in breast and lung cancer cells at concentrations much lower than for the normal fibroblast cells.

3.2. The cobalt (III) Schiff base complexes induce apoptosis in cancer cells, produced due to DNA damage and ROS generation, as revealed in different assays

In the AO/EtBr staining of cobalt (III) Schiff base complexes treated MCF-7 and A549 cells, bright green fluorescence indicated live cells, reddish-orange fluorescence indicated cells in apoptosis and swollen cells with red fluorescing nuclei revealed necrosis. The data indicating apoptotic and necrotic changes of MCF-7 and A549 cells after the treatment with the complexes **1** and **2** are illustrated in the Figs. 2 and 3. The percentage of normal, apoptotic and necrotic cells were manually counted and the data are presented as mean ± SD.

Hoechst 33528 staining was performed to demonstrate the onset of apoptosis in cobalt.

(III) Schiff base 1 & 2 treated MCF-7 and A549 cells. The abnormal cells appeared with condensed chromatin, binucleation and membrane blebbing towards apoptotic body formation (Fig. 4). The data showed that on treatment cells with normal and abnormal nuclei were close to 50% each and the response of MCF-7 cells showed to be slightly higher than A549 (Fig. 5).

Changes in the oxidation–reduction reaction disrupt the mitochondrial membrane potential (MMP) which is one of the earliest indicators of apoptosis. To authenticate apoptotic cell death, alteration in MMP of MCF-7 and A549 cells exposed to cobalt (III) Schiff base complexes **1** and **2** was validated using JC-1 dye. Treated and control cells were visualized in the fluorescent microscope to detect the disruption of MMP. Metabolically active mitochondria of normal MCF-7 and A549 cells fluoresced red while mitochondria of treated cells induced into apoptosis fluoresced green (Fig. 6).

An early event in apoptotic cell death, relocation of phosphatidylserine from the inner to the outer leaflet of the plasma membrane, was revealed in MCF-7 and A549 cells treated with the two cobalt complexes when subjected to Annexin V-Cy3 staining. In this fluorescent staining technique annexin V negative, 6-CFDA positive viable cells decreased in abundance; AnnexinV positive, 6-CFDA positive early apoptotic cells increased considerably; annexin V positive, 6- CFDA negative necrotic cells also increased to a certain extent. And these responses were of higher incidence in MCF-7 cells than A549 cells, and more with complex **1** than complex **2**, though the differences in response between cell types and complex treatment are not statistically significant (Fig. 7). Thus, cobalt complexes **1** and **2** induce the apoptotic pathway of cell death. MCF-7 cell lines were found to have higher apoptotic percentage than A549 cells as mentioned in the AO/EtBr and Hoechst staining assays (Fig. 8).

3.3. DNA damage induced by the complexes

Comet assay helps detect single- and double-strand breaks as an aspect of genotoxicity. In this study, effect of the two cobalt complexes on DNA of MCF-7 and A549 cells has been revealed in the comet assay in which DNA damage induced at the IC50 is specified by the appearance of the DNA tails or comets (Fig. 9) where densitometric parameters were analyzed by the CASP software (length and area of DNA tail). From the data, extensive DNA damage was revealed (as observed from the length of comets using CASP software) and the comets were longer in treated MCF-7 and A549 cells priming them to succumb to apoptosis (Fig. 10).

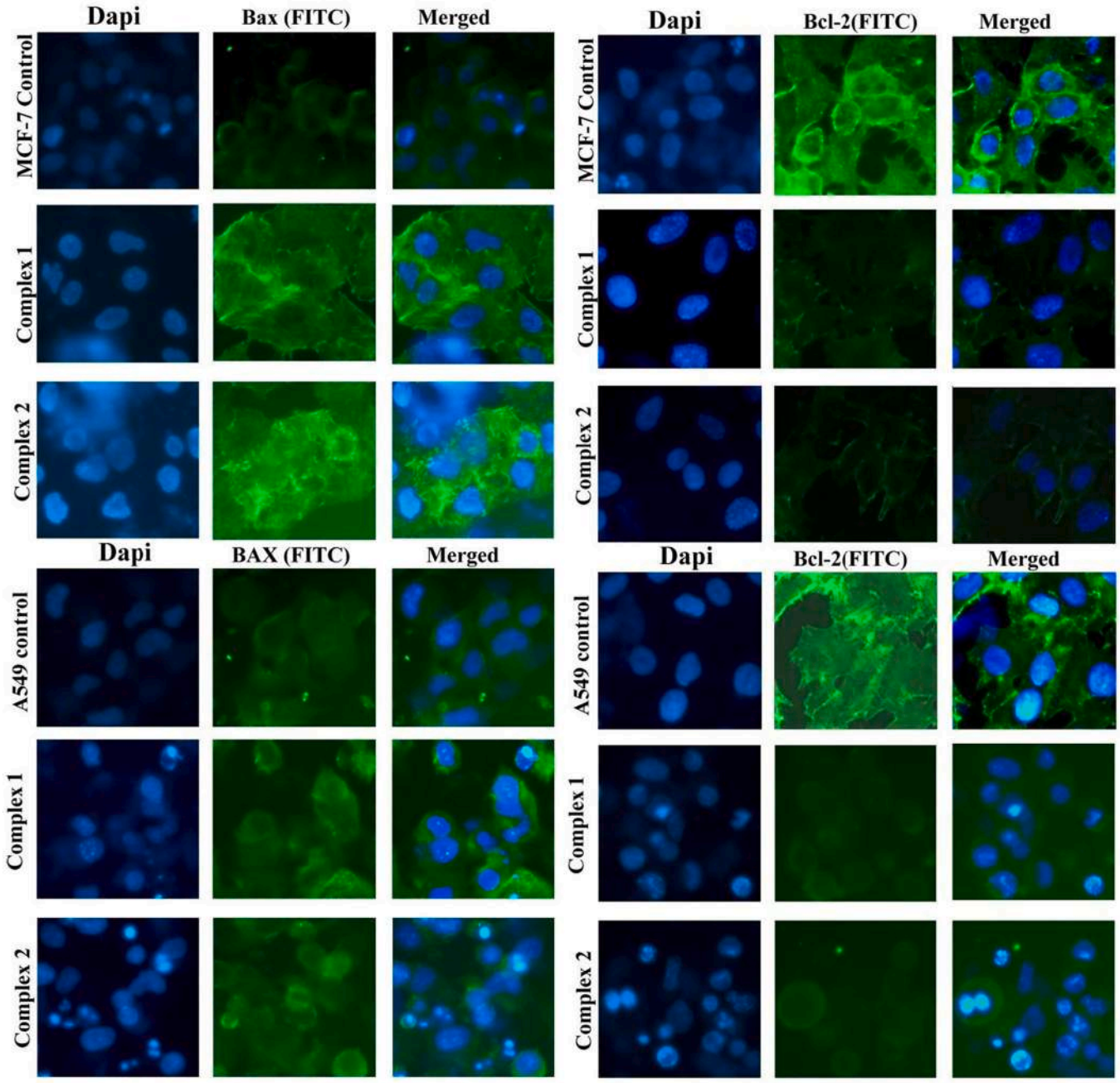


Fig. 12. Up-regulation of Bax expression (left panel) and down-regulation of Bcl-2 expression (right panel) in MCF-7 and A549 cells treated with cobalt complexes **1** and **2** where the cells were stained with Dapi as nuclear stain and FITC [fluorochrome conjugated secondary antibody] for protein expression.

3.4. Oxidative stress mediated by the complexes

Generally, anti-proliferative effect of chemotherapeutics increases the level of ROS and disrupts the homeostasis of cancer cells. Increased ROS is considered as a mediator of apoptosis (Ahamed et al., 2016). Therefore, our study tested the effect of the cobalt complexes **1** and **2** at the 24 h IC50 concentration to which the cells were exposed for 12 h and evaluated using the fluorescent DCFH-DA. After treating the complexes with MCF-7 and A549 cells, elevated amounts of intracellular ROS in terms of fluorescence intensity were observed in complexes **1** and **2** treated cells .The amounts of intracellular ROS generated by the cobalt complexes **1** and **2** were higher in the MCF-7 than A549 cells (Fig. 11).

3.5. Molecular expression analysis of Bax and Bcl2 by immunofluorescence assay

Bcl-2 and Bax are the large family of proteins that maintain the cellular homeostasis by functioning as anti-apoptotic (Bcl-2) and pro-apoptotic (Bax or Bak) molecules to induce apoptosis. Therefore, our study examined the expression of Bax and Bcl-2 proteins by treating the two cobalt complexes on MCF-7 and A549 cells. The result revealed that Bcl-*2* protein expression in treated MCF-7 and A549 cells decreased whereas Bax protein expression increased (Fig. 12). Based on the immunofluroscence data, it could be observed that Bax expression was up-regulated, and Bcl-2 expression was down-regulated in treated MCF-7 and A549 cells wherein the change was more intense in complex **2**-treated than complex **1**-treated cells.

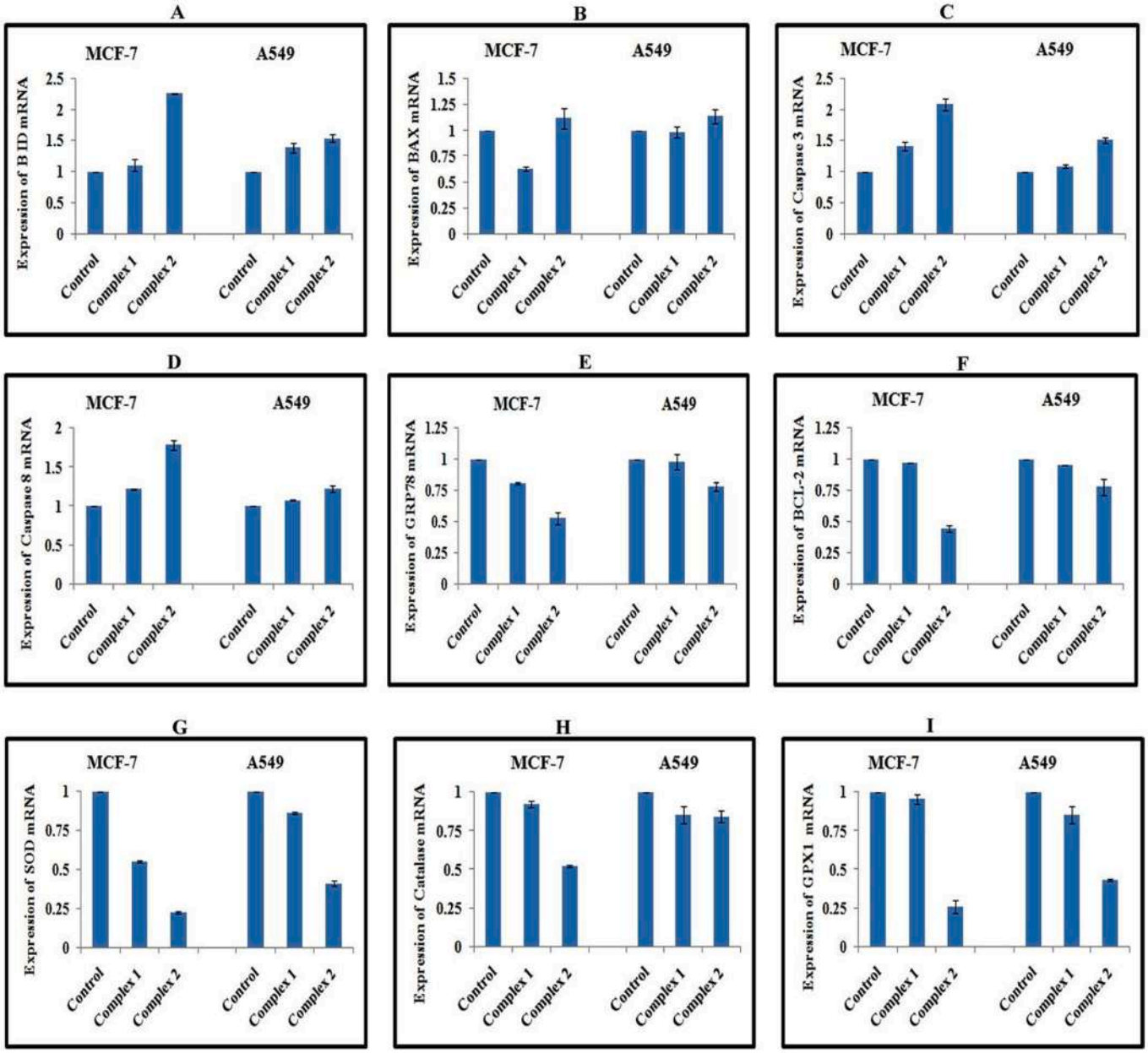


Fig. 13. A. Pro- and anti-apoptotic gene expression profiles of MCF-7 and A549 cells exposed to cobalt complexes **1** and **2**. A) BID; B) Bax; C) Caspase-3; D) Caspase-8; E) GRP78; F) BCl2. GAPDH was used as control. Each data point represents mean and standard deviation of three replicates. B. Antioxidant gene expression profiles of MCF-7 and A549 cells exposed to cobalt complexes **1** and **2**. G) SOD; H) Catalase; I) GPX1. GAPDH was used as control. Each data point represents mean and standard deviation of three replicates.

3.6. Gene expression analysis by RT-PCR

Essential knowledge about the activation pathway of apoptosis is required for designing the chemotherapeutic strategies. Sensitivity of cancer cells in terms of apoptosis to some chemotherapeutic agents is associated with proapoptotic and antiapoptotic protein ratio (For example: Bcl-2 to BAX ratio). Therefore, our study focused on proapoptotic (BID, BAX, Cas-3, and Cas-8) and anti-apoptotic (Bcl-2 and GRP-78) gene expressions in terms of fold change by treating of MCF-7 and A-549 cells with the two cobalt complexes for 24 h at the IC50. The result in respect of complex **1** showed that BID, BAX, caspase-3 and caspase– 8 expressions were increased by 1.10, 0.63, 1.41- and 1.22 times while Bcl-2 and GRP-78 expressions were decreased to 0.97 - and 0.81%. The result in respect of complex **2** showed that BID, BAX, caspase- 3 and caspase- 8 expressions were remarkably up-regulated 2.27, 1.12, 2.10- and 1.79- folds while Bcl-2 and GRP-78 expressions were notably down-regulated to 0.44- and 0.53% in MCF-7 cells. Similarly, the result in respect of complex **1** showed that BID, BAX, caspase-3 and caspase-8 expressions were markedly up-regulated by 1.38, 0.98, 1.09- and 1.07-folds while Bcl- 2 and GRP-78 expressions were down-regulated to 0.95 and 0.98%. The result in respect of complex **2** showed that BID, BAX, caspase- 3 and caspase- 8 expressions were markedly up- regulated 1.53, 1.14, 1.50- and 1.23-folds while Bcl-2 and GRP-78 expressions were down- regulated to 0.78 and 0.78% in A549 cells (Fig. 13A).

Antioxidant family of enzymes, which includes SOD, CAT, and GPX1, plays a critical role in the protective mechanism against oxidative stress. Therefore, our study tested antioxidant gene (*CAT, GPX1*, and *SOD*) expressions of MCF-7 and A549 cells by treating with the two cobalt complexes for 24 h at the respective IC50. The expression levels of CAT, GPX1, and SOD in MCF-7 and A549 cells were decreased compared to

control (GAPDH), wherein complex **2** produced higher effect than complex **1**, and the response of MCF-7 was higher than A549 (Fig. 13B).

4. Discussion

A cancer chemotherapeutic drug should possess antiproliferative effect to inhibit the growth of the cancer cell and execute specific mode (s) of cell death. For the past few decades, cobalt complexes have been examined as cytotoxic agents to take over the commercially available platinum-based drugs which produce adverse side effects in non-target tissues (Ambika et al., 2019). Previously, we had reported the synthesis and cytotoxic effect of cobalt (III) Schiff base complexes (complex **1** and **2**) and their anti-cancer and anti-angiogenic properties in A549 (human small cell lung carcinoma) cell. We found the intrinsic DNA binding constants (K*b*) of complexes 1 and 2 by plotting of [DNA]/ (εa-εf) *versus* [DNA]. The K*b* values (1.31×10^4 and 3.15×10^4 M^{-1} for complex **1** and complex **2** respectively) (Ambika et al., 2019) were lower than classical intercalator Ethidium Bromide (EB) (Kb = 4.94×10^5 M^{-1}) (Bloomfield et al., 1974). Both the complexes intercalated with DNA. Based on the observation, we assumed that the salicylaldehyde aromatic moiety is involved in the intercalation. This suggested that cellular DNA might be a target for the complexes **1** and **2**. Since targeting cellular DNA is likely to be significant for the cytotoxic and antitumor properties of the complexes **1** and **2,** it became necessary to evaluate the cytotoxic effect in various cancer cells to generalize the effect to more cell types. In this background, the present paper reports our findings on evaluation of the cobalt (III) Schiff base complexes for their *in vitro* growth inhibition and cell death induction properties in MCF-7 breast cancer cells along with confirmation of our previous report in A549 cells, adopting a series of experiments.

Initially, MTT assay was conducted to confirm the antiproliferative effect of the cobalt complex against MCF-7 and A549 cells. It differentiates the viable and dead cells and facilitates determine the IC50 concentrations. The result suggested that coordination compounds of cobalt might be playing role in the antiproliferative effect due to the hydrophobic forces in DNA interaction (Vignesh et al., 2014).

Further, testing cytological changes using various staining methods (AO & EtBr and Hoechst 33258) indicated that the complexes induce apoptosis as revealed in the morphological changes such as chromatin fragmentation, cytoplasmic condensation, membrane blebbing, and formation of apoptotic bodies. The relatively small percentage of cells undergoing necrotic death showed features such as cell swelling, chromatolysis and cellular lysis. Therefore, the different morphological features would reveal the different modes of cell death *viz.*, apoptosis, and necrosis. Especially, Hoechst 33258 staining revealed abnormal nuclei that are shrunk or crumpled, accompanied by nuclear condensation. The complexes understandably interact with AT rich regions which are selective for DNA interaction resulting in the compact chromatin of apoptotic nuclei (Kissinger et al., 1987).

The data from annexin V-Cy3 assay further substantiated the triggering of apoptotic death. The fluorescence pattern revealed the membrane phospholipid phosphatidylserine translocation from inner phase of the plasma membrane the outer surface, which is an early event in apoptosis. Annexin V has the highest binding affinity with phosphatidylserine and functions as a probe for caspase-dependent pathway. To substantiate this understanding of triggering of apoptosis, comet assay was performed to analyze DNA damage as a result of interaction and/or binding with DNA and proteins (Suzuki and Matsubara, 2011). Further, DNA damage would be caused due to cellular abnormal conditions, such as oncogene expression and hypoxia, thereby provoking p53 tumor suppressor protein. Activation of p53 enhances p21 expression which in turn brings up release of apoptogenic factors that would prime cell death (Cory and Adams, 2002). Our result revealed high incidence of DNA damage due to the effect of the cobalt (III) complexes in MCF-7 and A549 cells which would in turn turn on events in apoptosis.

In addition to the perceived direct effect of the treatment, it is known that DNA could be damaged owing to the production and elevated level of ROS. Here mitochondria are the main centre that balance the redox equilibrium thereby maintain viability of the cells but at the same time involve in the production of ROS (Kowaltowski et al., 2009; Teodoro et al., 2011). Towards validation of this, our study has confirmed increased level of ROS in metal complex-treated cells. It is known that increased level of ROS would lead to alteration of MMP, disruption of cell cycle progression and activation of the signaling pathways including the NF- kB (Morgan and Liu, 2011), NRF2, Wnt (de Sá Junior et al., 2017), and EGFR. Therefore, it is suggested that in the present context of cobalt (III) Schiff base complexes there is an association between ROS generation, DNA damage, and mitochondrial pathway-dependent cell death (Hussain et al., 2005) in MCF-7 and A549 cells.

ROS-mediated cell death is governed by two factors concerned with maintenance of homeostatic conditions: (i) ROS generation in mitochondria; and (ii) inhibition of antioxidant system. We found both these changes to happen. ROS type, time, location, and concentration reflect the cellular responses induced by the chemotherapeutics (Li et al., 2011). It is an established fact that increase of ROS levels would force the cancer cells, which are inherently defective, to succumb to cell death (Kong et al., 2000; Pelicano et al., 2004). Therefore, the complexes used in this study have stimulated cell death at different targets through similar mechanisms (Lowe et al., 1993). Thus, the direct effect of binding of the complexes with DNA and crucial proteins, damaging the molecules, and indirect effect of increase of ROS and decrease of antioxidant mechanisms would set into motion the modulation of pro-apoptotic (Cas-3, Cas-8, BID, and BAX), and anti-apoptotic (Bcl-2 and GRP-78) molecules.

Generally, ROS are generated due to stimulation of internal and external factors. In the present study it has been observed that the elevated cellular ROS and down-regulated expression of antioxidant genes (SOD, CAT, and GPX1) induced by the cobalt (III) complexes. The principal basis of ROS production might be inhibition of antioxidant system caused by ROS interacting enzymes [hydrogen peroxide (H2O2), superoxide radical (O^{2-}), singlet oxygen ($^1O2$), and hydroxyl radica1 (-OH)] which are all cytotoxic (Waris and Ahsan, 2006). Therefore, ROS accumulation would alter various signaling pathways using different transcription factors (Prasad et al., 2017). Cumulative oxidative stress leads to deleterious effects that will affect macromolecules (Ziech et al., 2010) resulting in the production of secondary reactive intermediates thereby causing irreversible DNA damage (Marnett, 2000), inflammation, and apoptosis (Thamilarasan et al., 2016).

Further, in this study we found that expression levels of pro-apoptotic genes (BAX, Cas-3, Cas- 8, and BID) were increased, whereas expression levels of anti-apoptotic genes (Bcl-2 and GRP- 78) were decreased in MCF-7 and A549 cells treated with the cobalt (III) Schiff base complexes. Increase in the expression of bax disturbs the mitochondrial membrane which leads to p53- mediated apoptosis (Becker et al., 2008). We further observed that caspase-3 and caspase-8 activities were higher in treated MCF-7 and A549 cells. It was reported that caspases play an important role in initiation as well as execution of extrinsic pathway of apoptosis (Nair et al., 2014).

On other hand, the antiapoptotic role of Bcl-2 is associated with chemoresistance mechanism that allows the cancer cells to survive. Bcl-2 can also link with the membrane protein of endoplasmic reticulum involved in intracellular Ca^{2+} regulation, which has effect on cell proliferation (Bagur and Hajnóczky, 2017). Additionally, expression level of antiapoptotic and proapoptotic Bcl-2 family members need to be balanced in order to ascertain the homeostatic conditions (Cory and Adams, 2002). In our study, the result shows that expression of pro-apoptotic gene bax was increased, while the expression of anti-apoptotic gene bcl-2 was decreased in MCF-7 and A549 cells exposed to the cobalt (III) complexes. Previous studies have found that vulnerability of cells to apoptotic death could be caused due to the increased level of Bax and decreased level of Bcl-2 (Lee et al., 2012; Zhu et al., 2013). In support of this, the immunofluorescence assay demonstrated

decrease of BCl2 fluorescence and increase of Bax fluorescence in the cobalt (III) complexes-treated MCF-7 and A549 cells. It is also suggested that induction of mitochondrial apoptosis pathway by the cobalt (III) complexes might be controlled through the BCl2 and Bax proteins since a high ratio of Bax to BCl2 can lead to the changes in MMP consequent upon release of cytochrome *c* which brings up cellular apoptosis (Teijido and Dejean, 2010).

Apart from this, one of the Bcl-2 family member is Bid, an abundant pro-apoptotic protein, which is involved in death receptor-mediated apoptosis. It relocates its truncated form, tBid, to release cytochrome *c*, the apoptosis-associated protein. Bid interacts with membrane lipids that could be important for mitochondrial targeting (Grinberg et al., 2002; Lutter et al., 2000; Zha et al., 2000). Additionally, GRP78, a prosurvival factor, interacts with intermediates of apoptotic pathway that limits activation of caspases leading to apoptosis inhibition and facilitation of cell survival (Reuter et al., 2008). The function of GRP78 is linked to maintenance of ER integrity and stress-induced autophagy, required for cancer cell survival (Wang et al., 2009). In support of this, our study reports down-regulation of GRP78 expression. In contrast, the cobalt (III) complexes **1** and **2** produced the best result indicating extensive death of both MCF-7 and A549 cells, the incidence of which is higher in the former. There is always a strong connection between DNA binding affinity of a compound/complex and its anti-cancer activity, and this binding affinity is one of the predominant contributory factors for the Co(III) Sciff base complexes 1 and 2 in the present context to be promising anticancer candidates.

5. Conclusions

The present study reiterates the antiproliferative property of the two cobalt (III) Schiff base complexes synthesized using a combinatorial approach. Cobalt (III) complexes inhibited breast (MCF-7) and lung (A549) cancer cells with little, if any, deleterious effect in normal cells (3 T3 in the present context). Fluorescent staining indicated that the cobalt (III) Schiff base complexes triggered apoptosis in the breast and lung cancer cells through DNA binding and damage, alteration of mitochondrial membrane potential, and oxidative stress. Overall, the results lead to the conclusion that in as much as both complex 1 and 2 are efficacious, the complex **2** is more effective than complex **1** due to its higher binding affinity with the DNA. Therefore, it is suggested that cobalt (III) Schiff base complexes can be further investigated as alternative cytotoxic / anti-cancer chemotherapeutic agent in the place of cisplatin.

Declaration of Competing Interest

The authors declare no conflicts of interest.

Acknowledgments

We would like to show our sincere gratitude to the Dr. R. Thirumurugan, Co-ordinator of National Centre for Alternatives to Animal Experiments (NCAAE) for his continuous support, encouragement, and guidance during the course of this research. This work was financially supported by National Centre for Alternatives to Animal Experiments (NCAAE) under UGC- CPEPA scheme, Government of India (F.No.2-1/2013 (NS/PE)). The research facility provided by Mahatma Gandhi-Doerenkamp Centre established by Doerenkamp-Zbinden Foundation and Department of Science and Technology, Government of India, under DST-Promotion of University Research and Scientific Excellence (PURSE) scheme-Phase II, Rashtriya Uchchatar Shiksha Abhiyan (RUSA)-2.O by Department of Animal Science is heartily acknowledged.

References

Ahamed, M., Akhtar, M.J., Alhadlaq, H.A., Alshamsan, A., 2016. Copper ferrite nanoparticle-induced cytotoxicity and oxidative stress in human breast cancer MCF-7 cells. Colloids Surf. B: Biointerfaces 142, 46–54.
Ambika, S., Manojkumar, Y., Arunachalam, S., Gowdhami, B., Meenakshi Sundaram, K. K., Solomon, R.V., Venuvanalingam, P., Akbarsha, M.A., Sundararaman, M., 2019. Biomolecular interaction, anti-Cancer and anti-Angiogenic properties of cobalt(III) Schiff Base complexes. Sci. Rep. 9, 2721.
Andiappan, K., Sanmugam, A., Deivanayagam, E., Karuppasamy, K., Kim, H.-S., Vikraman, D., 2018. In vitro cytotoxicity activity of novel Schiff base ligand–lanthanide complexes. Sci. Rep. 8, 3054.
Bagur, R., Hajnóczky, G., 2017. Intracellular Ca2+ sensing: its role in calcium homeostasis and signaling. Mol. Cell 66, 780–788.
Becker, K., Marchenko, N., Palacios, G., Moll, U.M., 2008. A role of HAUSP in tumor suppression in a human colon carcinoma xenograft model. Cell Cycle 7, 1205–1213.
Bloomfield, V.A., Victor, B., Crothers, D.M., Crothers, D.M., Tinoco Jr., I., 1974. Physical Chemistry of Nucleic Acids. HarperCollins Publishers.
Cory, S., Adams, J.M., 2002. The Bcl2 family: regulators of the cellular life-or-death switch. Nat. Rev. Cancer 2, 647–656.
de Sá Junior, P.L., Câmara, D.A.D., Porcacchia, A.S., Fonseca, P.M.M., Jorge, S.D., Araldi, R.P., Ferreira, A.K., 2017. The roles of ROS in cancer heterogeneity and therapy. Oxidative Med. Cell. Longev. 2017.
Dhivya, R., Jaividhya, P., Riyasdeen, A., Palaniandavar, M., Mathan, G., Akbarsha, M.A., 2015. In vitro antiproliferative and apoptosis-inducing properties of a mononuclear copper (II) complex with dppz ligand, in two genotypically different breast cancer cell lines. Biometals 28, 929–943.
Grinberg, M., Sarig, R., Zaltsman, Y., Frumkin, D., Grammatikakis, N., Reuveny, E., Gross, A., 2002. tBID Homooligomerizes in the mitochondrial membrane to induce apoptosis. J. Biol. Chem. 277, 12237–12245.
Hajrezaie, M., Paydar, M., Looi, C.Y., Moghadamtousi, S.Z., Hassandarvish, P., Salga, M. S., Karimian, H., Shams, K., Zahedifard, M., Majid, N.A., Ali, H.M., Abdulla, M.A., 2015. Apoptotic effect of novel Schiff based CdCl2(C14H21N3O2) complex is mediated via activation of the mitochondrial pathway in colon cancer cells. Sci. Rep. 5, 9097.
Huang, C.-Y., Ju, D.-T., Chang, C.-F., Reddy, P.M., Velmurugan, B.K., 2017. A review on the effects of current chemotherapy drugs and natural agents in treating non–small cell lung cancer. Biomedicine 7.
Hussain, S., Hess, K., Gearhart, J., Geiss, K., Schlager, J., 2005. In vitro toxicity of nanoparticles in BRL 3A rat liver cells. Toxicol. in Vitro 19, 975–983.
King, A.P., Gellineau, H.A., Ahn, J.-E., MacMillan, S.N., Wilson, J.J., 2017. Bis (thiosemicarbazone) complexes of cobalt (III). Synthesis, characterization, and anticancer potential. Inorg. Chem. 56, 6609–6623.
Kissinger, K., Krowicki, K., Dabrowiak, J.C., Lown, J.W., 1987. Molecular recognition between oligopeptides and nucleic acids. Monocationic imidazole lexitropsins that display enhanced GC sequence dependent DNA binding. Biochemistry 26, 5590–5595.
Kong, Q., Beel, J., Lillehei, K., 2000. A threshold concept for cancer therapy. Med. Hypotheses 55, 29–35.
Kowaltowski, A.J., de Souza-Pinto, N.C., Castilho, R.F., Vercesi, A.E., 2009. Mitochondria and reactive oxygen species. Free Radic. Biol. Med. 47, 333–343.
Latt, S.A., Wohlleb, J.C., 1975. Optical studies of the interaction of 33258 Hoechst with DNA, chromatin, and metaphase chromosomes. Chromosoma 52 (4), 297–316.
Laurell, G., Pierre, P.V., 2015. Hearing loss after cisplatin: oxidative stress pathways and potential for protection. In: Free Radicals in ENT Pathology. Springer, pp. 217–241.
Lee, J.S., Jung, W.-K., Jeong, M.H., Yoon, T.R., Kim, H.K., 2012. Sanguinarine induces apoptosis of HT-29 human colon cancer cells via the regulation of Bax/Bcl-2 ratio and caspase-9-dependent pathway. Int. J. Toxicol. 31, 70–77.
Li, Z.-Y., Yang, Y., Ming, M., Liu, B., 2011. Mitochondrial ROS generation for regulation of autophagic pathways in cancer. Biochem. Biophys. Res. Commun. 414, 5–8.
Lowe, S.W., Schmitt, E.M., Smith, S.W., Osborne, B.A., Jacks, T., 1993. p53 is required for radiation- induced apoptosis in mouse thymocytes. Nature 362, 847–849.
Lu, C., Eskandari, A., Cressey, P.B., Suntharalingam, K., 2017. Cancer stem cell and bulk Cancer cell active copper(II) complexes with vanillin Schiff Base derivatives and naproxen. Chemistry 23, 11366–11374.
Lutter, M., Fang, M., Luo, X., Nishijima, M., Xie, X.-S., Wang, X., 2000. Cardiolipin provides specificity for targeting of tBid to mitochondria. Nat. Cell Biol. 2, 754–756.
Marnett, L.J., 2000. Oxyradicals and DNA damage. Carcinogenesis 21, 361–370.
Morgan, M.J., Liu, Z.-G., 2011. Crosstalk of reactive oxygen species and NF-κB signaling. Cell Res. 21, 103–115.
Munteanu, C.R., Suntharalingam, K., 2015. Advances in cobalt complexes as anticancer agents. Dalton Trans. 44, 13796–13808.
Nair, P., Lu, M., Petersen, S., Ashkenazi, A., 2014. Apoptosis initiation through the cell-extrinsic pathway. In: Methods in Enzymology. Elsevier, pp. 99–128.
Pelicano, H., Carney, D., Huang, P., 2004. ROS stress in cancer cells and therapeutic implications. Drug Resist. Updat. 7, 97–110.
Prasad, S., Gupta, S.C., Tyagi, A.K., 2017. Reactive oxygen species (ROS) and cancer: role of antioxidative nutraceuticals. Cancer Lett. 387, 95–105.
Raine, R., Wong, W., Scholes, S., Ashton, C., Obichere, A., Ambler, G., 2010. Social variations in access to hospital care for patients with colorectal, breast, and lung cancer between 1999 and 2006: retrospective analysis of hospital episode statistics. Bmj 340.
Rajendiran, V., Karthik, R., Palaniandavar, M., Stoeckli-Evans, H., Periasamy, V.S., Akbarsha, M.A., Srinag, B.S., Krishnamurthy, H., 2007. Mixed-ligand copper(II)-phenolate complexes: effect of coligand on enhanced DNA and protein binding, DNA cleavage, and anticancer activity. Inorg. Chem. 46, 8208–8221.

Reers, M., Smith, T.W., Chen, L.B., 1991. J-aggregate formation of a carbocyanine as a quantitative fluorescent indicator of membrane potential. Biochemistry 30, 4480–4486.
Reuter, S., Eifes, S., Dicato, M., Aggarwal, B.B., Diederich, M., 2008. Modulation of anti-apoptotic and survival pathways by curcumin as a strategy to induce apoptosis in cancer cells. Biochem. Pharmacol. 76, 1340–1351.
Schirrmacher, V., 2019. From chemotherapy to biological therapy: a review of novel concepts to reduce the side effects of systemic cancer treatment. Int. J. Oncol. 54, 407–419.
Singh, N.P., McCoy, M.T., Tice, R.R., Schneider, E.L., 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. Experimental Cell Research 175, 184–191.
Spector, D.L., Goldman, R.D., 2006. Basic Methods in Microscopy: Protocols and Concepts from Cells: A Laboratory Manual.
Suzuki, K., Matsubara, H., 2011. Recent advances in p53 research and cancer treatment. J. Biomed. Biotechnol. 2011.
Teijido, O., Dejean, L., 2010. Upregulation of Bcl2 inhibits apoptosis-driven BAX insertion but favors BAX relocalization in mitochondria. FEBS Lett. 584, 3305–3310.
Teodoro, J.S., Simões, A.M., Duarte, F.V., Rolo, A.P., Murdoch, R.C., Hussain, S.M., Palmeira, C.M., 2011. Assessment of the toxicity of silver nanoparticles in vitro: a mitochondrial perspective. Toxicol. in Vitro 25, 664–670.
Thamilarasan, V., Sengottuvelan, N., Sudha, A., Srinivasan, P., Chakkaravarthi, G., 2016. Cobalt (III) complexes as potential anticancer agents: physicochemical, structural, cytotoxic activity and DNA/protein interactions. J. Photochem. Photobiol. B Biol. 162, 558–569.
Veeralakshmi, S., Nehru, S., Sabapathi, G., Arunachalam, S., Venuvanalingam, P., Kumar, P., Anusha, C., Ravikumar, V., 2015. Single and double chain surfactant–cobalt (III) complexes: the impact of hydrophobicity on the interaction with calf thymus DNA, and their biological activities. RSC Adv. 5, 31746–31758.
Vignesh, G., Senthilkumar, R., Paul, P., Periasamy, V.S., Akbarsha, M.A., Arunachalam, S., 2014. Protein binding and biological evaluation of a polymer-anchored cobalt (III) complex containing a 2, 2′-bipyridine ligand. RSC Advances 4 (101), 57483–57492.
Wang, H., Joseph, J.A., 1999. Quantifying cellular oxidative stress by dichlorofluorescein assay using microplate reader. Free Radic. Biol. Med. 27, 612–616.
Wang, M., Wey, S., Zhang, Y., Ye, R., Lee, A.S., 2009. Role of the unfolded protein response regulator GRP78/BiP in development, cancer, and neurological disorders. Antioxid. Redox Signal. 11, 2307–2316.
Waris, G., Ahsan, H., 2006. Reactive oxygen species: role in the development of cancer and various chronic conditions. J. Carcinogene. 5, 14.
Zha, J., Weiler, S., Oh, K.J., Wei, M.C., Korsmeyer, S.J., 2000. Posttranslational N-myristoylation of BID as a molecular switch for targeting mitochondria and apoptosis. Science 290, 1761–1765.
Zhu, A., Zhou, H., Xia, J., Jin, H., Wang, K., Yan, J., Zuo, J., Zhu, X., Shan, T., 2013. Ziyuglycoside II- induced apoptosis in human gastric carcinoma BGC-823 cells by regulating Bax/Bcl-2 expression and activating caspase-3 pathway. Braz. J. Med. Biol. Res. 46, 670–675.
Ziech, D., Franco, R., Georgakilas, A.G., Georgakila, S., Malamou-Mitsi, V., Schoneveld, O., Pappa, A., Panayiotidis, M.I., 2010. The role of reactive oxygen species and oxidative stress in environmental carcinogenesis and biomarker development. Chem. Biol. Interact. 188, 334–339.

This is an open access article published under an ACS AuthorChoice License, which permits copying and redistribution of the article or any adaptations for non-commercial purposes.

ACS OMEGA

http://pubs.acs.org/journal/acsodf

Article

Characterization of Hen's Egg White To Use It as a Novel Platform To Culture Three-Dimensional Multicellular Tumor Spheroids

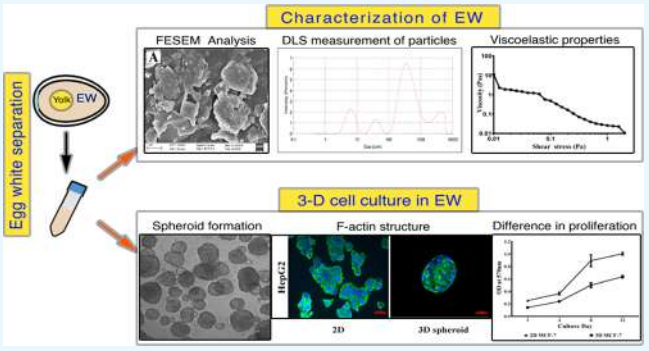
Perumalsamy Balaji, Anbazhagan Murugadas, Arunachalam Ramkumar, Ramasamy Thirumurugan, Sellathamby Shanmugaapriya, and Mohammad Abdulkader Akbarsha*

Cite This: https://dx.doi.org/10.1021/acsomega.0c02508

Read Online

ACCESS | Metrics & More | Article Recommendations

ABSTRACT: We are standardizing protocols to develop egg white (EW) as a cost-effective platform for culture of three-dimensional (3-D) multicellular tumor spheroids for application in understanding tumor microenvironments and drug screening. In this article, we describe several physical and physiological characteristics of EW to use it as 3-D cell culture platform. Field emission scanning electron microscopy revealed the presence of different microstructures. Hydrodynamic size distribution data indicated nano- and micron-sized particles. Rheological measurements revealed the viscosity and viscoelastic behavior appropriate for maintaining cell viability and supporting 3-D cell growth under high-sheer conditions. It was found that thereis no autofluorescence, a requirement for imparting transparency and for microscopic observations of the spheroids. The EW facilitated the development of 3-D tumor spheroids, with an emphasis of difference in cell proliferation and intercellular cytoskeletal organization between two-dimensional and 3-D spheroid cultures. Put together, EW proves to be a cost-affordable and simple platform for 3-D culture of tumor spheroids.



1. INTRODUCTION

Two-dimensional (2-D) cell culture is an important technique practiced in cancer biology research to understand the molecular mechanisms of tumor development and invasion and to find appropriate drug targets. Even though it has been in extensive use during the past several decades, certain limitations have affected its use as an effective investigative tool in cancer biology. Importantly, 2-D culture does not always ensure productivity, whereupon cost and failure rates increase.[1] Going a little deep into the issue, cells cultured in the 2-D format on a rigid substrate are deficient in cell−cell and cell−extracellular matrix (ECM) communications causing stretched cytoskeletal elements. This in turn affects the cellular polarity and leads to aberrant gene and protein expressions.[1−4] To overcome these limitations, models resulting from three-dimensional (3-D) cultures have come up. In 3-D culture systems, cells are cultured by embedding in a complex 3-D native ECM-like substrate providing cell−cell as well as cell−ECM communications, which help in maintaining the intercellular junctional complexes and cell polarity. This facilitates the cells to simulate the functional and physiological characteristics of in vivo tumors.[5,6] Especially, the 3-D multicellular tumor spheroid (MTS) models facilitate a better understanding of tumor growth, invasiveness, tumor cell architecture, cell-signaling mechanisms, tumor cell dormancy, angiogenesis, and so forth than other 3-D models.[7,8] Importantly, properties of solid tumors, such as spatial organization of cells, expression of genes, different zones of proliferation, and involvement of local microenvironments with the disease, can be recaptured by culturing cells in 3-D MTS.[9−11]

Use of a hydrogel/scaffold is an attractive approach to culture cells in the 3-D MTS format as it provides mechanical as well as structural supports to the cells in a way similar to the native tissue. The microstructures and interconnected pores present in hydrogels/scaffolds enable retention of water and efficient transport of nutrients and wastes.[12] Our study aims at using whole egg white (EW) from hen (*Gallus gallus domesticus*; White Leghorn breed) as an easy-to-prepare and cost-affordable platform as an alternative to hydrogels/scaffolds to produce 3-D MTS. The composition of EW has already been adequately characterized. It is composed of 9.7−10.6% protein by weight, which contributes to the develop-

Received: May 28, 2020
Accepted: July 23, 2020

Downloaded via 157.50.85.210 on August 3, 2020 at 17:10:12 (UTC).
See https://pubs.acs.org/sharingguidelines for options on how to legitimately share published articles.

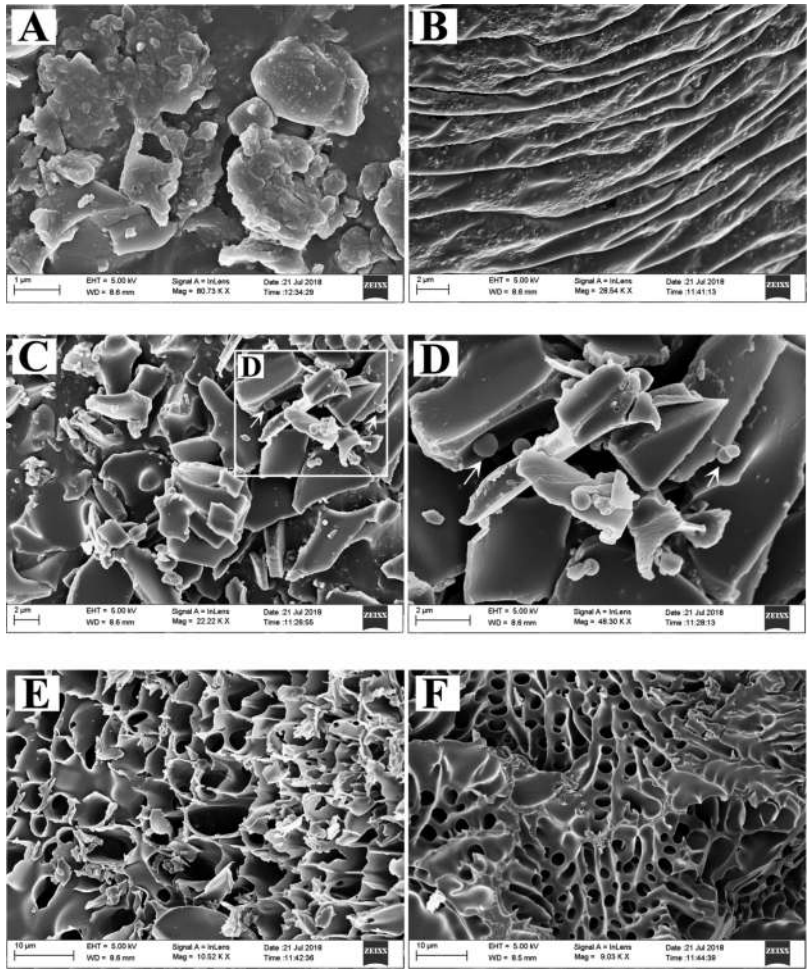


Figure 1. Microstructure of the EW analyzed by SEM. Different morphological features were observed. (A,B) Aggregated and self-assembled morphologies, respectively. Flakes and sphere structures are presented in (C). (D) Inset in (C). (E,F) Two different porous structures observed in EW.

ment of embryo.[13,14] More importantly, extracts and proteins from EW support cell growth and proliferation.[15,16] Therefore, EW can serve as a suitable material to culture cells in the 3-D format. Furthermore, the nontoxic property, biocompatibility, and biodegradability of EW are added advantages to use it as a 3-D cell culture platform.[17−19] We conducted a preliminary study of an EW cryogel bioscaffold for use in 3-D MTS and found advantages such as extensive porosity, homogeneous pore size, and amide linkages during cross-linking, in addition to cost-affordability.[20]

The present study, developing further on our earlier findings, aimed at characterizing the morphological features, particle size distribution, rheological properties, and optical properties of EW toward its application as a convenient platform to culture cells in the 3-D MTS format. The effect of shear stress while handling EW was addressed. The different cell types cultured as 3-D MTS in EW were analyzed adopting light microscopy. The proliferation efficiencies between the 2-D culture and 3-D MTS were compared over the period of incubation. The difference in the structure of the cytoskeleton between the 2-D and 3-D MTS models was analyzed.

2. RESULTS AND DISCUSSION

2.1. Microstructural Characteristics of EW. The surface morphological features of the EW were analyzed using field emission scanning electron microscopy (FESEM) measurements. The results showed that the EW contains different morphologies with different ranges of sizes, nano- to micrometers, among which interconnected aggregated structures were predominant (Figure 1A). Furthermore, self-assembled morphologies (Figure 1B), flakes (Figure 1C), sphere-like structures (Figure 1D), and porous structures of different sizes (Figure 1E,F) were also observed. The spheres measured a diameter between 0.22 and 1.5 μm and the average sphere size was 0.65 μm (Figure 1C). The dimensions of flakes ($L \times W$) were in the range of 1.68 × 1.02 μm to 6.81 × 5.48 μm (Figure 1C,D). Also, the diameters of two different porous structures, shown in Figure 1E,F, were between 2.28 μm and 7.47 μm with an average pore size of 4.5 μm and 0.78 μm × 3.25 μm with an average pore size of 2.33 μm, respectively.

It is well known that hen's EW, a thick fluid, contains 11% proteins. Ovalbumin, conalbumin, ovomucoid, ovotransferrin, and lysozyme are the major proteins. The other constituents are fat 0.2%, ash 0.8%, and water 88%. The different morphologies observed in scanning electron microscopy (SEM) analysis are reflections of the different compositions.[13] Ovalbumin constitutes 54% of total dry mass. EW albumins undergo aggregation at this concentration. Also, it is known that EW albumins can undergo aggregation in the pH range 4−9 at room temperature.[21−23] Moreover, the polar and

https://dx.doi.org/10.1021/acsomega.0c02508
ACS Omega XXXX, XXX, XXX−XXX

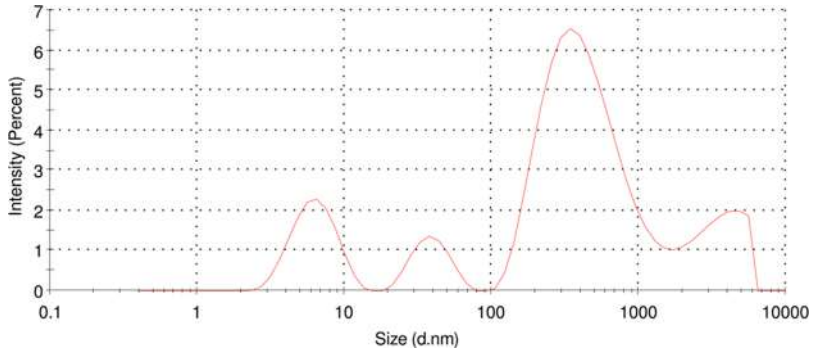


Figure 2. Hydrodynamic size distribution of EW analyzed by DLS measurement.

nonpolar proteins present in EW may be responsible for the different structures formed in EW. Especially, the polar nature of the structural protein ovomucin is thought be the reason for aggregation of EW to filamentous and fibroid structures. To the best of our knowledge, this is the first report of elucidation of the different morphologies present in the raw EW of hen where previous findings reveal only the structure of proteins in their purest form. Furthermore, the hydrodynamic size distribution of the EW was assessed adopting dynamic light scattering (DLS). The average size was found to be 485.7, 3568, and 6.469 nm (Figure 2). The results obtained from the particle size measurement confirm the presence of nano- to micron-sized particles in the EW and substantiate the data obtained from FESEM analysis (Figure 1).

2.2. Rheological Characteristics. ECM is a macromolecular structure that provides mechanical and structural supports to cells in tissues and maintains tissue polarity via the cell−ECM interaction. More precisely, the viscoelasticity of the ECM determines the fate of tissue homeostasis. Therefore, it is necessary to provide in vivo relevant physiological force to the cells in order to maintain their original tissue homeostasis under laboratory conditions. In our study, the measurement of viscosity against the shear stress shows gel-like elastic behavior at the low stress level. At high shear stress, the overall viscosity of EW decreased to near 0.1 Pa s, and a fluid-like behavior was observed (Figure 3).

The study of viscoelasticity in oscillation frequency sweep mode confirms the transition from a gel-like behavior into a fluid-like under the constant strain. The result shows frequency-dependent storage (G') and loss (G'') modules. The storage module (G'), responsible for elastic characteristics, was initially higher than the loss module (G'') at low frequencies ($G' > G''$). It was decreased significantly below the loss module (G'') in response to increased frequencies and, as a result, the viscous behavior prevails over the elastic nature of EW (Figure 4). The result justifies that the elastic behavior

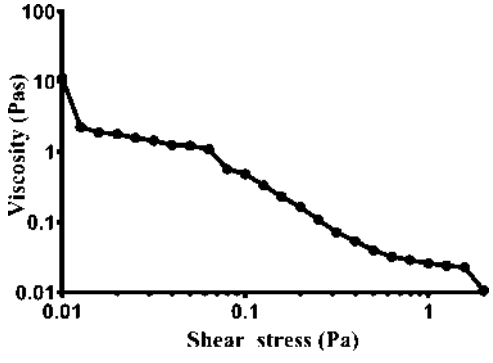


Figure 3. Apparent viscosity measurement in relation to shear stress shows high viscosity at low shear stress, which decreased with increased shear stress.

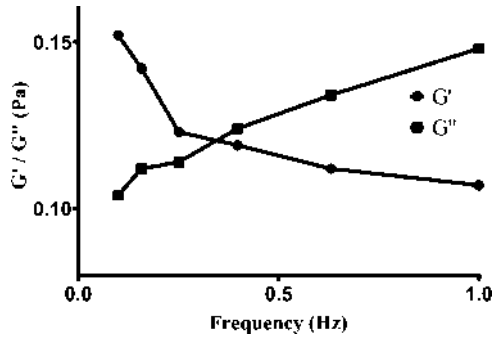


Figure 4. Frequency-dependent storage (G') and loss modules (G'') exhibit a viscous behavior with increased frequencies prevailed over the elastic behavior observed at the rest.

maintained at rest will keep the cells in the viscous state, which is advantageous to culture the cells as spheroids. As seen in SEM analysis, most of the particles in EW that were revealed as macromolecules were aggregates that help to maintain EW in a highly viscous form. Under the deformation or shearing, these macromolecules may disintegrate into smaller particles. At this stage, the material shows shear-thinning flow behavior, which may be useful to transfer the EW using syringes. Because the EW has this property, the cells can get mixed homogeneously with the EW, whereupon syringes and microtips can be used to handle the cell−EW suspension. The viscosity of EW, as found in the experiment, was 11.2 Pa G', which is advantageous for mammalian cell culture applications. Collagen matrices affording $G' \approx 4-60$ Pa are appropriate to culture 3D soft tissue.[24]

Importantly, the resurgence of viscosity after stress was measured by shearing the material at 500 s^{-1} for 30 s. It was observed that the viscosity of EW was established immediately after shearing stopped (Figure 5). However, measurement of dynamic complex viscosity, η^*, versus angular frequency showed a decrease in the structural strength of the EW material after shearing. In this experiment, constant strain was applied to decrease the structural strength of the EW material and to study the equilibrium between internal structural breakdown and rebuilding. The result shows a decrease in η^* under the effect of increasing frequency (before shearing), and

https://dx.doi.org/10.1021/acsomega.0c02508
ACS Omega XXXX, XXX, XXX−XXX

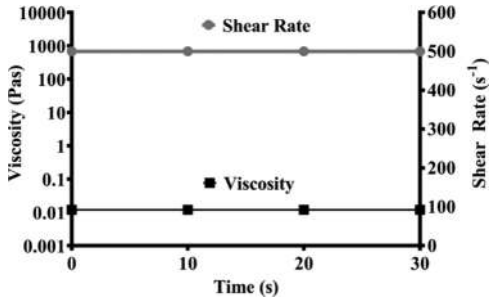


Figure 5. Evaluating the EW viscosity after shearing the gel at 500 s^{-1} for 30 s demonstrates instant recovery of viscosity after shearing stopped.

thereafter, start of structural build up upon deformation is slowed (after shearing) (Figure 6). However, complex viscosity

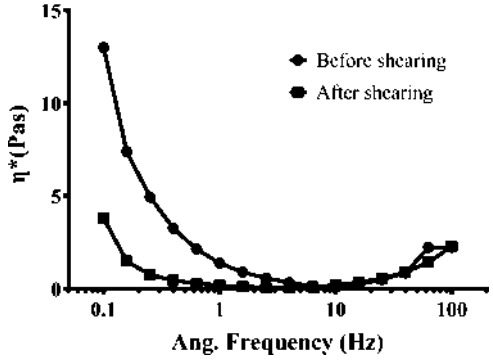


Figure 6. Dynamic complex viscosity, η^*, of EW measured before and after shearing. The results represent the lack of complete recovery of η^*, describing the structural deformation after shearing.

was not fully recovered and the reduction may possibly be due to disruption of the internal network structure present in the EW. This reduction in complex viscosity after shearing may affect the strength of the material while dealing with the EW during experiment and it might affect homogeneous spheroid formation. Overall, the results from the rheological experiments facilitate the conclusion that the EW possesses viscoelastic properties favorable to culture the cells. Especially, transition from an elastic state to a fluid-like state and shear stress viscosity that is 11.2 Pa G' observed in EW are suitable to mimic physiological ECM for culture of soft tissues.[24] On the other hand, the disruption in the internal structure of EW observed under shearing conditions may affect its function as a cell culture platform.

2.3. Injectability of EW for Cell Culture. The effect of sheer stress on viability of cells while handling cell−EW suspension was measured using syringes of different volumes and needles of different gauge sizes. The results from the MTT viability assay indicated that viability of cells was not affected even in the case of smallest needle used (#26) (Figure 7). The fluid-like behavior of EW as inferred from the rheological observation may keep the cells viable even after high shear. It is believed that EW may reduce the volume of suspension under high shear stress, and the high composition of macromolecules present in EW may contribute to this phenomenon. The 1−300 μL pipette tip, routinely used in laboratory experiments, is equivalent to a #20 needle.[25]

2.4. Optical Properties. Visualization of 3-D cultured cells, without disturbing the culture conditions, is an important

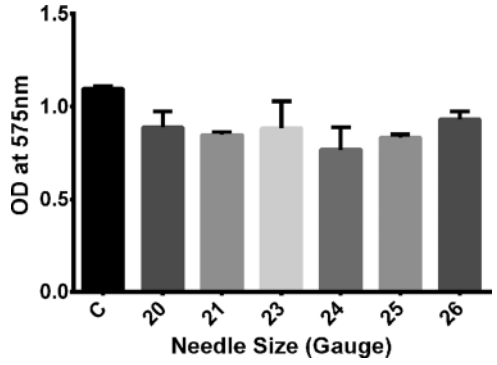


Figure 7. Injectability of EW for cell culture. The results after the MTT assay show that the viability was not affected by injection with syringes with needles of different gauge sizes. Error bars represent mean ± SD (n = 6).

aspect in developing a transparent cell culture medium. It depends greatly on optical properties of the material used in the 3-D culture. In our study, the optical property of EW was analyzed by measuring the absorbance and fluorescence spectra. The results from the UV−visible spectral analysis show that the peak observed at 300 nm corresponds to the high absorbance light (Figure 8A). Therefore, it is concluded that the optical detection near 300 nm may be limited in the EW. However, it is noted that the wavelength of visible light used in most of the optical microscopes ranges from 400 to 700 nm. Thus, the absorbance at 300 nm may not hinder

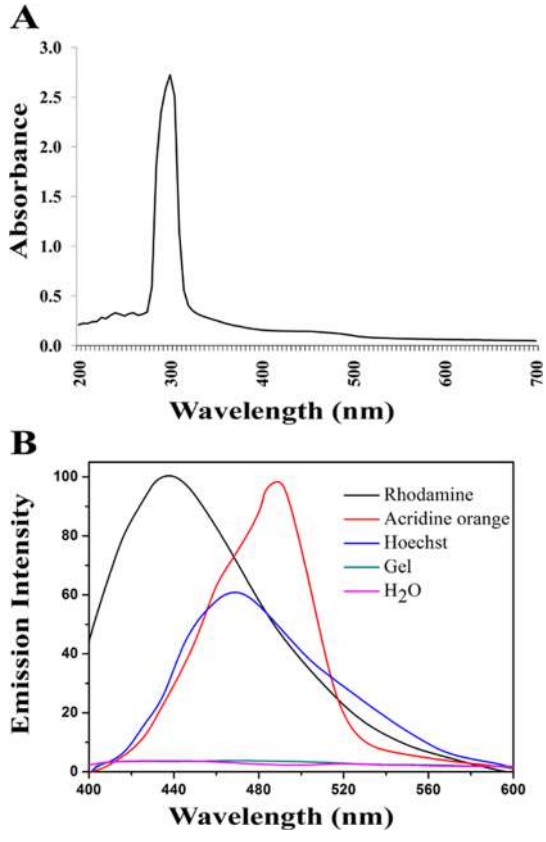


Figure 8. Optical properties of EW. (A) Absorbance spectrum of EW. The peak was recorded at 300 nm, corresponding to the high absorbance at 300 nm. (B) No autofluorescence was recorded in the fluorescence spectral analysis of EW compared to pure water.

https://dx.doi.org/10.1021/acsomega.0c02508
ACS Omega XXXX, XXX, XXX−XXX

visualization in the optical microscope and be helpful in experimenting with live cell imaging. In addition, the property of autofluorescence of EW was measured by scanning the fluorescence spectra after excitation at 350, 480, and 511 nm wherein hoechst 33258, acridine orange, and rhodamine 123 were used as the respective reference dyes. The excitation wavelength chosen in our study is based on a wide range of fluorescent dyes used in biological imaging under this wavelength range. No fluorescent emission was observed on excitation at different wavelengths (Figure 8B). Thus, it is evident that the EW does not fluoresce and, so, suitable for most of the fluorescence-based microscopic observations and fluorescence-related quantitative methods.

2.5. Biocompatibility. The biocompatibility of EW was tested using A549, MCF7, HepG2, and HEK293 cells. During the experiments, different proportions of EW and growth medium were mixed and added to wells of a 96-well culture plate. The total volume was set as 200 μL and EW/culture medium concentrations were maintained at five proportions: 0:200, 50:150, 100:100, 150:50, and 200:0 for groups 1, 2, 3, 4, and 5, respectively. The results from the MTT assay after 48 h of incubation with EW showed that the percentages of viability of all four cell lines were significantly increased at 50 μL and 100 μL EW concentrations compared to the untreated or 0 μL and 200 μL. The rate of viability was observed to be higher at 100 μL EW than the other concentrations (Figure 9). The

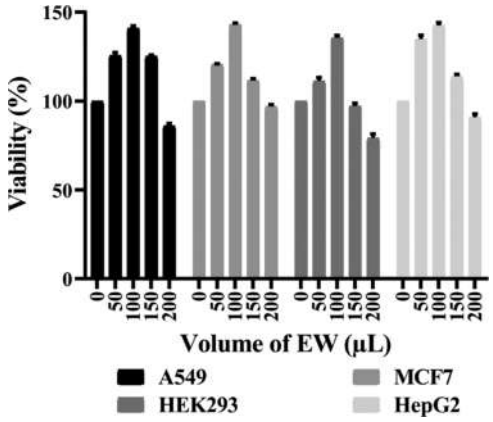


Figure 9. Biocompatibility of EW with A549, MCF7, HepG2, and HEK293 cells adopting the MTT assay. The results show difference in percentage of viability against different volumes of EW and higher viability at 100 μL EW compared to other concentrations. Error bars represent mean ± SD (n = 3).

viability for all four cells was decreased at a volume of 200 μL of EW. The alkaline pH of EW is also a factor that would affect cell viability. The alkaline pH of isolated EW (pH 9−10) was reduced to 7−8 by adding the growth medium when beginning the cell culture. It is believed that while using a low volume of EW, the alkalinity of EW may get neutralized by adding culture media as in groups 2 and 3. However in groups 4 and 5, less or no medium was added, which means less chance of neutralizing the pH. The results reveal that increase of the EW concentration affects the cell viability, and the alkaline pH must be neutralized with growth media for cell culture experiments. Most importantly, the EW contains numerous proteins that promote cell proliferation when the pH gets neutralized.

2.6. Light Microscopic Morphological Features of Spheroids Grown in EW. The cells generally appear polygonal or cuboidal when cultured in a monolayer. However, when cultured as a spheroid, their morphology is cell-line specific.[10,26,27] Four morphological forms have been reported: grape-bunch-like, round, mass-like, and stellate.[28] In our study, different cell types of human and mouse origins such as MCF7, MDA-MB-231, 3T3, A549, HEK293, A431, HepG2, and SiHa were cultured as 3-D spheroids using EW. The MCF7, 3T3, A431, and HepG2 spheroids appeared as highly organized masses, whereas MDA-MB-231, A549, HEK293, and SiHa spheroids appeared grape-bunch-like (Figures 10 and 11). The mass-like spheroids generally have cells exhibiting high cell−cell adhesion and disorganized nuclei filling the core. The grape bunch-like spheroids generally exhibit poor cell−cell adhesion and disorganized nuclei.[28,29] Despite the fact that spheroid formation is cell line specific, the original characteristics of cells such as proliferation, migration, and invasion are not affected by the spheroid morphology.[30,31] Importantly, the material and the physiomechanical properties of the culture system may exert a profound effect on spheroid morphology.[31] Evidently, the property of different internal morphologies with multiple scale structures, that is, nano to macro, and the apparent viscosity related to the ECM of soft tissues observed in EW may contribute to the control of spheroid organization by facilitating cell−cell and cell−ECM interactions. In addition, the stimuli received from cell−cell and cell−ECM interactions determine the polarity of cells in spheroids.[32−34] Measurement of the diameter of spheroids on days 3, 6, and 10 revealed that the spheroid size increased over the culture period. It was noted that the size of MCF7, MDA-MB-231, 3T3, and HEK293 spheroids reached almost 100 μm on day 10, whereas A549 and SiHa spheroids were very small on this day (Table 1).

2.7. Cell Proliferation. The results from the MTT assay indicate that EW facilitates proliferation of A549, MCF7, and HepG2 cells (Figure 12). In 3-D culture, the cell proliferation increased gradually even if the cells were cultured continuously for 10 days. The results show that cell growth in 3-D culture was slower than that in its monolayer counterpart. The results obtained from the quantification of genomic DNA are in good agreement with the MTT assay results (Figure 13). This is consistent with previous reports.[35,36] The results indicate that in 2-D culture all cells seeded on the plastic surface contact the medium, so they can proliferate and produce daughter cells till nutrients provided for and space allowed. The dead cells formed due to the crowding and the necrotic cells can be easily removed by changing the medium, and new daughter cells can replace that particular area, whereas in 3-D culture, the EW provides a larger area for cell proliferation all around, resulting in more counts of cells by improving and maintaining the proliferation rate. Also, the cells in the spheroids may pile up as layers one over the other to define an external proliferating zone and internal quiescent zone containing all states of cells such as viable, dead, and necrotic. Therefore, there is ability to maintain cell proliferation, but it is lesser with the 3-D format than 2-D. The mechanical stimuli of EW and the choice of cell lines can also affect the proliferation rate.[27,37−39] Also, the slow cell proliferation in 3-D culture is related to the differences in the gene expression pattern of growth promoting and restricting genes.[30,40,41]

2.8. Cytoskeletal Organization. The development of in vitro structural configuration resembling the native tissue

https://dx.doi.org/10.1021/acsomega.0c02508
ACS Omega XXXX, XXX, XXX−XXX

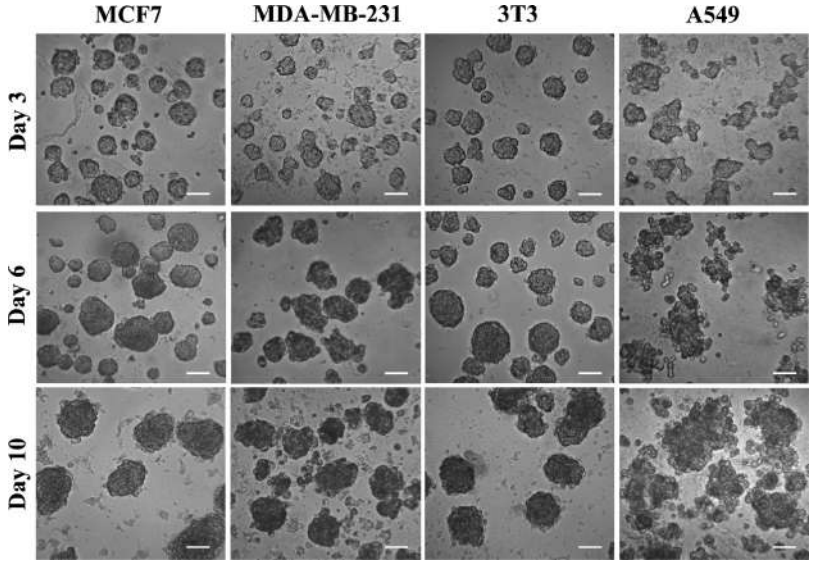


Figure 10. Light microscopic observation of spheroids of MCF7, MDA-MB-231, 3T3, and A549 cells on culture days 3, 6, and 10. Scale bar represents 50 μm.

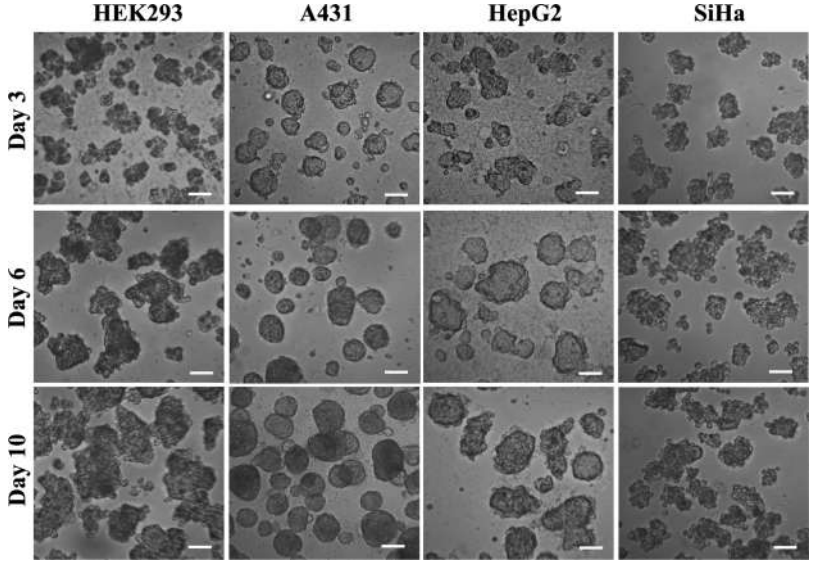


Figure 11. Light microscopic observation of spheroids of HEK293, A431, HepG2, and SiHa cells on culture days 3, 6, and 10. Scale bar represents 50 μm.

Table 1. Spheroid Diameter Measured from Microscopic Observations[a]

cell line	day 3	day 6	day 10
MCF7	51.99 ± 8.81	69.15 ± 7.64	97.47 ± 9.89
MDA-MB-231	52.10 ± 8.19	71.54 ± 5.99	92.11 ± 6.20
3T3	68.33 ± 1846	92.75 ± 19.38	103.54 ± 6.21
A549	59.04 ± 17.15	65.06 ± 5.67	69.40 ± 10.44
HEK293	42.18 ± 9.34	62.30 ± 10.21	94.94 ± 13.11
A431	67.00 ± 8.847	76.40 ± 6.74	80.43 ± 5.87
HepG2	54.22 ± 13.93	83.76 ± 5.64	85.26 ± 11.44
SiHa	57.93 ± 7.52	60.84 ± 7.02	73.35 ± 6.31

[a] μm, mean ± SE of 10 measurements each.

involves a series of cellular events that are closely related to dynamic cytoskeletal organization. Actin, the major component of the intercellular junctional cytoskeleton, contributes to cell–cell contact, cell maturity, tissue structure, and functionality.[42] In the present study the junctional filamentous actin (F-actin) filaments were intensely aggregated in the cortex and at the intercellular junctions throughout A549, MCF7, and HepG2 spheroids (Figure 14). The formation of junctional F-actin has been associated with the enhanced cellular adhesion and signal transduction indicating the preservation of the in vivo-like cytoarchitecture in the spheroids.[43] In contrast, the monolayer culture of all three cells shows stress fibers of F-actin in the cytoplasm at the middle section of monolayers. The results show prominent F-actin formation localized at the cell base in monolayer cells where cells attach to the surface of the tissue culture plates, instead of the cell–cell contact points. This subsequently decreases cell–cell adhesion and cell signaling, which consequently affects tissue-specific functions.[44]

2.9. Merits of EW over Hydrogels/Scaffolds for Culture of 3-D MTS. The EW as a medium to culture 3-D MTS has advantages as well as limitations. Unlike polysaccharides (e.g., alginate and chitosan), cellulose (e.g.,

https://dx.doi.org/10.1021/acsomega.0c02508
ACS Omega XXXX, XXX, XXX−XXX

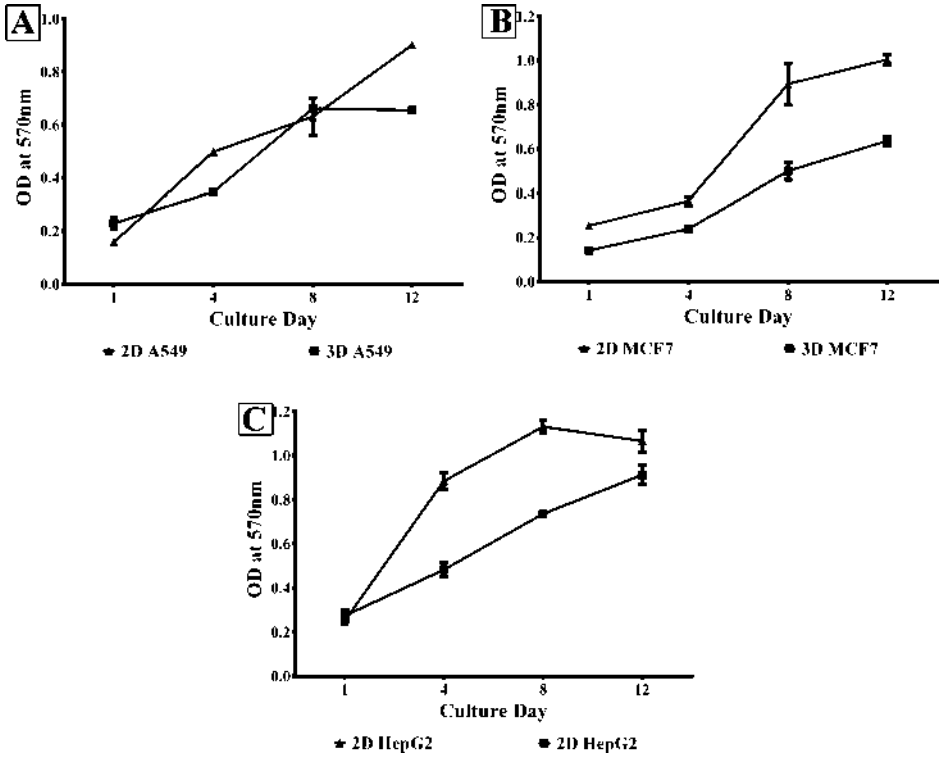


Figure 12. Comparison of proliferation efficiency between 2D culture and 3D spheroids of A549 (A) and MCF7 (B) and HepG2 (C) cells on days 1, 4, 8, and 12 adopting the MTT assay. Error bars represent mean ± SD (n = 3).

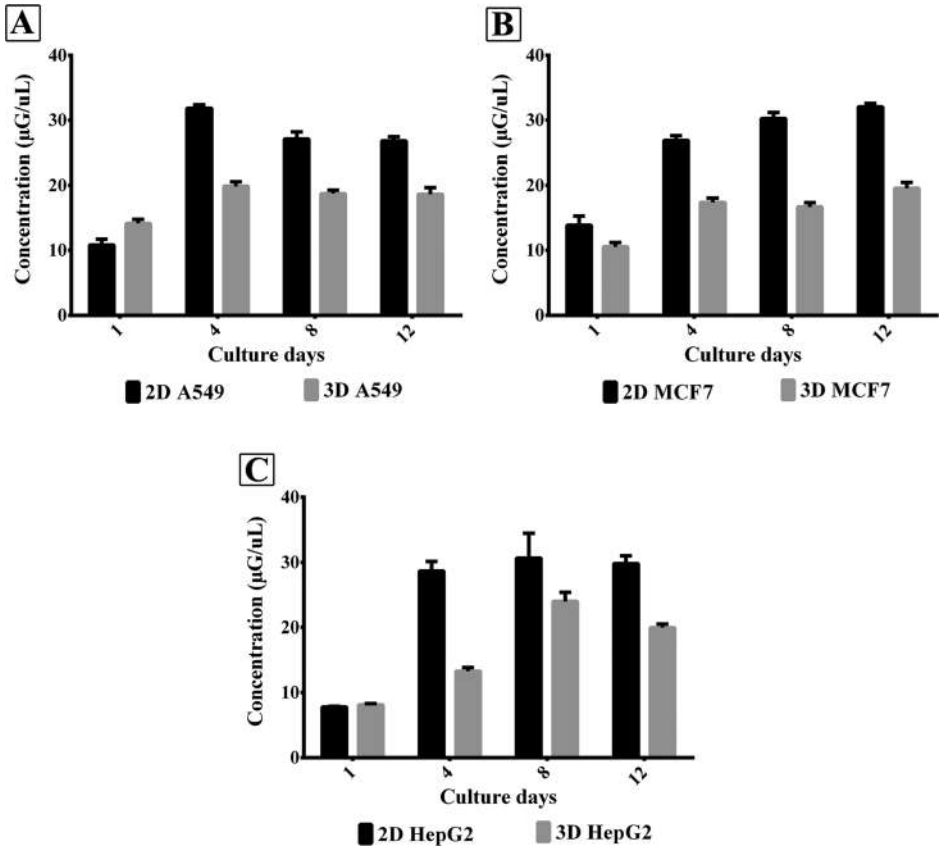


Figure 13. Difference in the rate of proliferation between 2D culture and 3D spheroids of A549 (A) and MCF7 (B) and HepG2 (C) cells on days 1, 4, 8, and 12 assessed by measuring the gDNA. Error bars represent mean ± SD (n = 3).

https://dx.doi.org/10.1021/acsomega.0c02508
ACS Omega XXXX, XXX, XXX−XXX

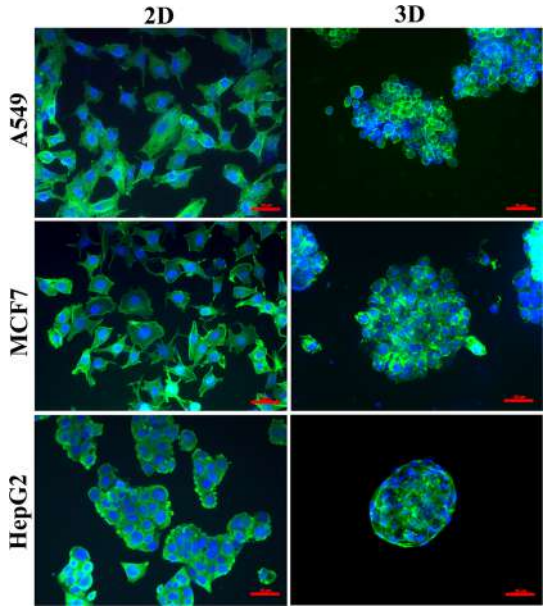


Figure 14. Immunostaining and comparison of junction marker F-actin between spheroid and monolayer cultures. Images from fluorescent staining show significant junctional F-actin (green color) at the points of cell−cell contact throughout 3-D cultured spheroids, whereas in cells cultured in monolayers, a large amount of F-actin cytoskeleton is distributed in the cell base area contacting the dish surface. Green and blue colors represent staining of F-actin and nuclear counterstain, respectively. Scale bar is 50 μm.

nanofibrillar cellulose), and synthetic materials (e.g., polyethylene glycol), EW is formed of proteins and so provides space, nutrients, and support to the cells in culture. Furthermore, the nano- to micron-level structures observed in EW may provide more binding moieties to the cells, which can potentially regulate cell functions. Also, the physiological properties of EW such as viscoelasticity can be modified simply by means of heat and cross-linking chemistry in such a way that EW can be used as a cost-effective bioink.[17,19,20] Nevertheless, as of now, it is difficult to find the specific endogenous factor(s) from among the mixture of EW proteins, which promote cell growth. Likewise, the impact of batch-to-batch variability on the reproducibility of the outcome requires further analysis.

3. CONCLUSIONS

This study deciphers hen's EW as a platform appropriate to grow 3-D MTSs. Investigation of the properties of microstructure, rheology, optical measurements, and biocompatibility are the important aspects to be concerned with in the context of a novel material for cell culture applications. The SEM analysis and particle size distribution measurement of EW showed different microstructures and confirm the nano- to micron-level particle size distribution. The observed rheological characteristics of EW provide information on viscoelasticity, and the results indicate that viscosity that exists in the EW is suitable to culture soft tissues. The reversible gelation property offers less resistance to the flow helping to reduce the shear stress. This fluid-like behavior was found to be suitable to handle the cells embedded in EW using microtips without affecting the viability of cells. As EW does not produce any absorbance peak between 400 and 700 nm, the observation of cells through a light microscope was not hindered. Also, the lack of autofluorescence in EW was of advantage to use it with a wide variety of fluorescent reagents. The protein-rich EW showed biocompatibility with the cells, and the specific volume of the EW practiced provides nutrient to the cells and promotes cell proliferation. 3-D MTS was successfully developed using different cell lines. The morphology, proliferation, cytoskeleton molecules such as F-actin involved in the important function of cell−cell, and cell−ECM interactions were compared between 3-D and 2-D cell cultures and the pre-eminence of the spheroid model over flat culture was evaluated in detail. Overall, this study indicates that the microstructures, advantageous rheological properties, and other important characteristics of the EW facilitate the formation of spheroids of different cell types. More importantly, this cost-affordable and easy-to-isolate EW, having bioactive substances that act as the in vivo-like ECM, is of advantage for the growth of cells as 3-D MTS without any additional ECM components.

4. MATERIALS

4.1. Cell Lines and Maintenance. Human breast cancer cells MCF7 and MDA-MB-231, human lung cancer cell A549, human embryonic kidney cell HEK293, human epidermoid carcinoma cell A431, human liver carcinoma cell HepG2, human cervical cancer cell SiHa, and mouse fibroblast cell 3T3 were obtained from National Center for Cell Science (NCCS), Pune, India. The cells were cultured in high-glucose DMEM and RPMI 1640 media (Sigma-Aldrich, USA), supplemented with 10% fetal bovine serum, and 2% penicillin, and streptomycin as antibiotics (Gibco, Thermo Scientific, USA). Cells were maintained in the exponential phase at 37 °C in a humidified atmosphere of 5% CO_2 in a CO_2 incubator (Thermo Scientific, USA).

4.2. Reagents and Plasticwares. Trypsin EDTA (0.25%) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were supplied by HiMedia, India. The dyes Alexa Fluor 532 phalloidin, hoechst-33258, acridine orange, and rhodamine 123 were purchased from Invitrogen, Thermo Fisher, USA. The GenElute genomic DNA prep kit was supplied by Sigma-Aldrich, USA. All other chemicals and reagents used were of analytical grade, obtained from EMPLURA, Sigma-Aldrich, USA and HiMedia, India. Tissue culture plasticware products were obtained from HiMedia, India.

4.3. Egg White. Chicken (*Gallus gallus domesticus*; White Leghorn breed) eggs were purchased from a local vendor. The egg shell was punctured using an egg hole puncher and EW was collected in a sterile beaker. The EW was gently stirred and refrigerated at 8 °C.

5. METHODS

5.1. SEM Analysis. The morphological features of the EW were analyzed using a field emission scanning electron microscope. The lyophilized samples were sputter-coated with gold (Emitech, SC7620) for FESEM micrograph measurements (Carl Zeiss, Germany). Particle size, pore size, and their size distribution profiles were determined from at least 50 measurements on SEM images using image analysis software (ImageJ 1.50i).

5.2. DLS and Zeta Potential Measurements. The hydrodynamic size distribution in EW was analyzed in a Malvern zeta sizerNano ZS90 apparatus at 25 °C, illuminating

https://dx.doi.org/10.1021/acsomega.0c02508
ACS Omega XXXX, XXX, XXX−XXX

the sample by 633 nm radiation from a solid state He−Ne laser, and the scattered light was collected at an angle of 90°. All measurements were made in triplicate.

5.3. Rheological Measurements. The rheological properties of EW were measured at room temperature with a strain-controlled rheometer (Physica MCR 301, Anton Paar, Austria) equipped with a parallel-plate geometry. The diameter of the measuring plate was 12.5 mm. The steady-state viscosity of EW was measured against the shear stress range of 0.01−100 Pa. The viscoelastic properties of the EW were characterized using frequency sweep measurements from 0.1 to 100 rad/s at a constant strain of 30%.

5.4. Injectability of EW for Cell Culture. The injectability of EW to dispense the cells was measured according to Bhattacharya et al.[25] Cells were initially cultured routinely in T25/T75 flasks for 48 h prior to the injection. Cell culture in EW was initiated by mixing A549 cells with the EW at a density of 2.5×10^3 cells per 200 μL in a 96-well plate using a 1 mL syringe and needles of different gauge sizes (#20, 21, 23, 24, 25, and 26). After transfer, the cells were cultured for another 24 h, and thereafter, the viability was checked using the MTT assay (elaborate protocol is provided in Section 5.6).[45] The mean OD values from six replicate experiments were used to plot the graph.

5.5. Optical Properties. The optical property of EW was characterized to determine the transparency of EW in terms of observing cellular behavior in microscopic detection. The absorbance and fluorescence properties of EW were recorded according to Bhattacharya et al.[25] with a slight modification. UV−vis absorbance of EW was recorded in the range of 200−700 nm using a UV−vis spectrometer (Multiskan GO, Thermo Scientific, USA). The fluorescence spectra of EW after excitation at 350, 480, and 511 nm were measured using a spectrofluorimeter (Jasco, USA). The excitation wavelengths were most commonly used in biological imaging.[46,47] Hoechst 33258 (10 μg/mL), acridine orange (6 μg/mL), and rhodamine 123 (10 mM) were used as the reference for each excitation wavelength. Purified water was used as the negative control.

5.6. Biocompatibility Characterization. For the analysis of biocompatibility, EW was infused with different cell types such as A549, MCF7, HepG2, and HEK293. Different proportions of EW and growth media were mixed and added to wells of the 96 culture plate. The total volume in each well was set to be 200 μL and EW/culture media were prepared in five proportions, 0:200, 50:150, 100:100, 150:50, and 200:0 for groups 1, 2, 3, 4, and 5, respectively. After 48 h incubation at 37 °C with 5% CO_2, 20 μL of the MTT working solution (5 mg/mL in PBS) was transferred to wells of the 96 culture plate. After incubation for 4 h at 37 °C, the formazan crystals were dissolved in 100 μL of dimethyl sulfoxide (DMSO). The absorbance of the purple color was determined at 570 nm with a reference wavelength of 630 nm (iMark, Bio-Rad, USA). Data were collected for three replicates each and used to calculate the respective means. The average OD570 of untreated cells was set as 100%. Then, the percentage of viability in EW-exposed samples was measured by comparison of the average OD_{570} values of treated cells with the OD_{570} value of the untreated cells. The results of biocompatibility analysis are presented as means ± SD of three experimental replicates.

5.7. 3-D and 2-D Cultures. The EW was used to support the culture of different cell types in the 3-D spheroid culture format. The cells MCF7, MDA-MB-231, 3T3, A549, HEK293, A431, HepG2, and SiHa were cultured in 2-D and 3-D formats. In order to conduct 2-D cell culture, the cells maintained in the culture flasks were detached using trypsin. 5×10^4 cells were seeded in the wells of standard 24-well cell culture plates, which were already fed with the culture medium, and incubated at 37 °C and 5% CO_2. The medium was replaced every 2 days. The number of cells seeded in 2-D was maintained the same throughout the study.

The cells for 3-D culture were prepared as for standard monolayer cell culture. After trypsinization, 1×10^5 cells were mixed with EW to achieve typically 1000 cells/μL. The cell−EW mixture was added onto 24-well ultralow attachment plates. An equal volume of growth medium was added on top of the cell−EW mixture and cultured at 37 °C and 5% CO_2. The medium was replaced every 2 days without disturbing the cells.

5.8. Morphological Analysis of Spheroids. Formation of spheroids of MCF7, MDA-MB-231, 3T3, A549, HEK293, A431, HepG2, and SiHa cells was monitored using an inverted light microscope (Axiovert 40 CFL, Carl Zeiss, Germany) and images were obtained on days 3, 6, and 10. The profile shape of the spheroids was classified into four categories—round, mass, grape, and stellate according to Edmondson *et al.*[48] The diameters of spheroids from each well were measured using Carl Zeiss Zen 2012 software during the light microscopic observation. Diameters of more than 10 spheroids were used to calculate the average spheroid size in each group.

5.9. Analysis of Cell Proliferation. The proliferation efficiency of cells in spheroids and 2-D format was quantified using the MTT assay and determination of genomic DNA. At specific intervals (days 1, 4, 8, and 12), A549, MCF7, and HepG2 cells were cultured as spheroids as well as in the monolayer format, analyzed, and compared.

5.9.1. MTT Assay. The MTT assay was used to measure the proliferation efficiency of cells according to Mosmann.[45] The spheroids and 2-D cultures were subjected to a standard protocol reported by Balaji *et al.*[20] with slight modifications. With respect to spheroid culture, after the specific intervals (days 1, 4, 8, and 12), 50 μL of the working MTT solution from the stock (5 mg/mL) was added to the particular wells of a 24-well plate. After 3 h incubation in a 5% CO_2 incubator (95% humidity, 37 °C), the whole content, including the spheroids, was centrifuged at 1000 rpm (3−4 min). The formazan crystals in the cells were dissolved in 1 mL of DMSO; 200 μL of the solution was transferred to the 96-well plate to measure the absorbance.

Similarly, the cells in 2D culture were treated with 50 μL of the working MTT solution. After 3 h incubation, the formazan crystals were dissolved by adding 1 mL of DMSO; 200 μL of the solution was transferred to the 96-well plate to measure the absorbance at 570 nm with 630 nm as reference in a plate reader (iMark, Bio-Rad, USA).

5.9.2. DNA Quantification Assay. Proliferation efficiency of the cells cultured in 2-D and 3-D formats was furthermore analyzed by measuring the gDNA content using the GenElute mammalian genomic DNA prep kit. After the specific incubation period (1, 4, 8, and 12 days), the cells were collected, lysed, pooled, and subjected to processing as prescribed by the manufacturer. The concentration of the extracted gDNA was measured using a UV−vis spectrometer (Multiskan GO, Thermo Scientific, USA).

https://dx.doi.org/10.1021/acsomega.0c02508
ACS Omega XXXX, XXX, XXX−XXX

5.10. F-Actin Staining. The morphology and cytoskeletal organization of the spheroids and the cells cultured as 2-D were analyzed by capturing the F-actin orientation by phalloidin staining. Briefly, the cultured spheroids or cells were fixed in 4% formalin for 15 min at 4 °C. Following washing with PBS, the cells were permeabilized with 0.1% Triton X-100 and incubated overnight with Alexa Fluor 488-labeled phalloidin to stain the filamentous actin. The spheroids were then counterstained with Hoechst 33258 and visualized using a fluorescent microscope (Axio Observer 7, Carl Zeiss, Germany). The fluorescent emission was captured at 525 and 461 nm for phalloidin and hoechst, respectively.

5.11. Statistical Analysis. The data were subjected to statistical analysis using PRISM version 6.0 software (GraphPad Software, La Jolla, CA, USA). Data obtained from the injectability analysis were subjected to one-way ANOVA, and the results are presented as means ± SD of six experimental replicates. Data obtained from the MTT assay and biocompatibility characterization were subjected to two-way ANOVA, and the results are presented as means ± SD of three experimental replicates. The data from SEM analysis and diameter measurement of spheroids were analyzed using Microsoft Office Excel 2007, where $p < 0.05$ was considered statistically significant.

AUTHOR INFORMATION

Corresponding Author

Mohammad Abdulkader Akbarsha − *National College (Autonomous), Tiruchirappalli 620001, India; Mahatma Gandhi-Doerenkamp Centre for Alternatives, Bharathidasan University, Tiruchirappalli 620 024, India;* orcid.org/0000-0002-4313-9606; Phone: +919790995854; Email: akbarbdu@gmail.com

Authors

Perumalsamy Balaji − *Department of Biomedical Science and National Centre for Alternatives to Animal Experiments, Bharathidasan University, Tiruchirapalli 620024, India*

Anbazhagan Murugadas − *Department of Pediatrics, School of Medicine, Emory University, Atlanta, Georgia 30322, United States; National Centre for Alternatives to Animal Experiments, Bharathidasan University, Tiruchirappalli 620 024, India*

Arunachalam Ramkumar − *Department of Environmental Biotechnology and National Centre for Alternatives to Animal Experiments, Bharathidasan University, Tiruchirapalli 620024, India*

Ramasamy Thirumurugan − *Department of Animal Science and National Centre for Alternatives to Animal Experiments, Bharathidasan University, Tiruchirapalli 620024, India*

Sellathamby Shanmugaapriya − *Department of Biomedical Science, Bharathidasan University, Tiruchirapalli 620024, India*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.0c02508

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was carried out at Mahatma Gandhi-Doerenkamp Center for Alternatives to Use of Animals in Life Science Education, Bharathidasan University, Tiruchirappalli—620024, established by Doerenkamp-Zbinden Foundation, Switzerland. This work was partially supported by National Center for Alternatives to Animal Experiments (NCAAE), funded by UGC, Government of India [F.no. 2-1/2013(NS/PE)].

ABBREVIATIONS

DMSO, dimethyl sulfoxide; MTT, 3-4,5-dimethylthiazole-2-yl,2,5-diphenyl tetrazoliumbromide; G', storage module; G'', loss module; F-actin, filamentous actin

REFERENCES

(1) Lv, D.; Hu, Z.; Lu, L.; Lu, H.; Xu, X. Three-dimensional cell culture: A powerful tool in tumor research and drug discovery. *Oncol. Lett.* **2017**, *14*, 6999−7010.

(2) Kelm, J. M.; Timmins, N. E.; Brown, C. J.; Fussenegger, M.; Nielsen, L. K. Method for generation of homogeneous multicellular tumor spheroids applicable to a wide variety of cell types. *Biotechnol. Bioeng.* **2003**, *83*, 173−180.

(3) Bazzoun, D.; Lelièvre, S.; Talhouk, R. Polarity proteins as regulators of cell junction complexes: implications for breast cancer. *Pharmacol. Ther.* **2013**, *138*, 418−427.

(4) Delarue, M.; Montel, F.; Vignjevic, D.; Prost, J.; Joanny, J.-F.; Cappello, G. Compressive stress inhibits proliferation in tumor spheroids through a volume limitation. *Biophys. J.* **2014**, *107*, 1821−1828.

(5) Pampaloni, F.; Reynaud, E. G.; Stelzer, E. H. K. The third dimension bridges the gap between cell culture and live tissue. *Nat. Rev. Mol. Cell Biol.* **2007**, *8*, 839−845.

(6) Ma, H.-l.; Jiang, Q.; Han, S.; Wu, Y.; Tomshine, J. C.; Wang, D.; Gan, Y.; Zou, G.; Liang, X.-J. Multicellular tumor spheroids as an in vivo−like tumor model for three-dimensional imaging of chemotherapeutic and nano material cellular penetration. *Mol. Imag.* **2012**, *11*, 487−498.

(7) Vidi, P.-A.; Bissell, M. J.; Lelièvre, S. A. Three-dimensional culture of human breast epithelial cells: the how and the why. In *Epithelial Cell Culture Protocols*; Randell, S., Fulcher, M., Eds.; Humana Press: Totowa, NJ, 2012; pp 193−219.

(8) Infanger, D. W.; Cho, Y.; Lopez, B. S.; Mohanan, S.; Liu, S. C.; Gursel, D.; Boockvar, J. A.; Fischbach, C. Glioblastoma stem cells are regulated by interleukin-8 signaling in a tumoral perivascular niche. *Cancer Res.* **2013**, *73*, 7079−7089.

(9) Durand, R. E.; Raleigh, J. A. Identification of nonproliferating but viable hypoxic tumor cells in vivo. *Cancer Res.* **1998**, *58*, 3547−3550.

(10) Lin, R.-Z.; Chang, H.-Y. Recent advances in three-dimensional multicellular spheroid culture for biomedical research. *Biotechnol. J.* **2008**, *3*, 1172−1184.

(11) Baker, B. M.; Chen, C. S. Deconstructing the third dimension: how 3D culture microenvironments alter cellular cues. *J. Cell Sci.* **2012**, *125*, 3015−3024.

(12) Drury, J. L.; Mooney, D. J. Hydrogels for tissue engineering: scaffold design variables and applications. *Biomaterials* **2003**, *24*, 4337−4351.

(13) Stevens, L. Egg white proteins. *Comp. Biochem. Physiol., Part B: Biochem. Mol. Biol.* **1991**, *100*, 1−9.

(14) Mine, Y. Recent advances in the understanding of egg white protein functionality. *Trends Food Sci. Technol.* **1995**, *6*, 225−232.

(15) Ruan, G.-P.; Wang, J.-X.; Pang, R.-Q.; Yao, X.; Cai, X.-M.; Wang, Q.; Ma, L.-H.; Zhu, X.-Q.; Pan, X.-H. Treatment with chicken-egg-white or whole-egg extracts maintains and enhances the survival and differentiation of spleen cells. *Cytotechnology* **2012**, *64*, 541−551.

https://dx.doi.org/10.1021/acsomega.0c02508
ACS Omega XXXX, XXX, XXX−XXX

(16) Mizunoya, W.; Tashima, A.; Sato, Y.; Tatsumi, R.; Ikeuchi, Y. The growth-promoting activity of egg white proteins in the C2C12 myoblast cell line. *Anim. Sci. J.* **2015**, *86*, 194−199.
(17) Kaipparettu, B. A.; Kuiatse, I.; Tak-Yee Chan, B.; Benny Kaipparettu, M.; Lee, A. V.; Oesterreich, S. Novel egg white-based 3-D cell culture system. *Biotechniques* **2008**, *45*, 165−171.
(18) Mohan, N.; Nair, P. D.; Tabata, Y. A 3D biodegradable protein based matrix for cartilage tissue engineering and stem cell differentiation to cartilage. *J. Mater. Sci.: Mater. Med.* **2009**, *20*, 49−60.
(19) Jalili-Firoozinezhad, S.; Rajabi-Zeleti, S.; Mohammadi, P.; Gaudiello, E.; Bonakdar, S.; Solati-Hashjin, M.; Marsano, A.; Aghdami, N.; Scherberich, A.; Baharvand, H.; Martin, I. Facile fabrication of egg white macroporous sponges for tissue regeneration. *Adv. Healthcare Mater.* **2015**, *4*, 2281−2290.
(20) Balaji, P.; Murugadas, A.; Shanmugaapriya, S.; Abdulkader Akbarsha, M. Fabrication and characterization of egg white cryogel scaffold for three-dimensional (3D) cell culture. *Biocatal. Agric. Biotechnol.* **2019**, *17*, 441−446.
(21) Putzeys, P.; Brosteaux, J. The scattering of light in protein solutions. *Trans. Faraday Soc.* **1935**, *31*, 1314−1325.
(22) Heller, W.; Klevens, H. Molecular weights of proteins as determined by light scattering. *Physical Review*; American Physical Society, One Physics Ellipse: College Park, Maryland USA, 1945; p 61.
(23) Abdou, A.; Kim, M.; Sato, K. Functional proteins and peptides of hen's egg origin. *Bioactive Food Peptides in Health and Disease*; IntechOpen: London, U.K., 2013; pp 115−116.
(24) Miron-Mendoza, M.; Seemann, J.; Grinnell, F. The differential regulation of cell motile activity through matrix stiffness and porosity in three dimensional collagen matrices. *Biomaterials* **2010**, *31*, 6425−6435.
(25) Bhattacharya, M.; Malinen, M. M.; Lauren, P.; Lou, Y.-R.; Kuisma, S. W.; Kanninen, L.; Lille, M.; Corlu, A.; GuGuen-Guillouzo, C.; Ikkala, O.; Laukkanen, A.; Urtti, A.; Yliperttula, M. Nanofibrillar cellulose hydrogel promotes three-dimensional liver cell culture. *J. Controlled Release* **2012**, *164*, 291−298.
(26) Friedrich, J.; Eder, W.; Castaneda, J.; Doss, M.; Huber, E.; Ebner, R.; Kunz-Schughart, L. A. A reliable tool to determine cell viability in complex 3-d culture: the acid phosphatase assay. *J. Biomol. Screening* **2007**, *12*, 925−937.
(27) Weiss, M. S.; Bernabé, B. P.; Shikanov, A.; Bluver, D. A.; Mui, M. D.; Shin, S.; Broadbelt, L. J.; Shea, L. D. The impact of adhesion peptides within hydrogels on the phenotype and signaling of normal and cancerous mammary epithelial cells. *Biomaterials* **2012**, *33*, 3548−3559.
(28) Kenny, P. A.; Lee, G. Y.; Myers, C. A.; Neve, R. M.; Semeiks, J. R.; Spellman, P. T.; Lorenz, K.; Lee, E. H.; Barcellos-Hoff, M. H.; Petersen, O. W.; Gray, J. W.; Bissell, M. J. The morphologies of breast cancer cell lines in three-dimensional assays correlate with their profiles of gene expression. *Mol. Oncol.* **2007**, *1*, 84−96.
(29) Härmä, V.; Virtanen, J.; Mäkelä, R.; Happonen, A.; Mpindi, J.-P.; Knuuttila, M.; Kohonen, P.; Lötjönen, J.; Kallioniemi, O.; Nees, M. A comprehensive panel of three-dimensional models for studies of prostate cancer growth, invasion and drug responses. *PLoS One* **2010**, *5*, No. e10431.
(30) Luca, A. C.; Mersch, S.; Deenen, R.; Schmidt, S.; Messner, I.; Schäfer, K.-L.; Baldus, S. E.; Huckenbeck, W.; Piekorz, R. P.; Knoefel, W. T.; Krieg, A.; Stoecklein, N. H. Impact of the 3D microenvironment on phenotype, gene expression, and EGFR inhibition of colorectal cancer cell lines. *PLoS One* **2013**, *8*, No. e59689.
(31) Hongisto, V.; Jernström, S.; Fey, V.; Mpindi, J.-P.; Kleivi Sahlberg, K.; Kallioniemi, O.; Perälä, M. High-throughput 3D screening reveals differences in drug sensitivities between culture models of JIMT1 breast cancer cells. *PLoS One* **2013**, *8*, No. e77232.
(32) Petersen, O. W.; Ronnov-Jessen, L.; Howlett, A. R.; Bissell, M. J. Interaction with basement membrane serves to rapidly distinguish growth and differentiation pattern of normal and malignant human breast epithelial cells. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 9064−9068.
(33) Griffith, L. G.; Swartz, M. A. Capturing complex 3D tissue physiology in vitro. *Nat. Rev. Mol. Cell Biol.* **2006**, *7*, 211−224.
(34) Cawkill, D.; Eaglestone, S. S. Evolution of cell-based reagent provision. *Drug Discovery Today* **2007**, *12*, 820−825.
(35) Khaitan, D.; Chandna, S.; Arya, M.; Dwarakanath, B. Establishment and characterization of multicellular spheroids from a human glioma cell line; Implications for tumor therapy. *J. Transl. Med.* **2006**, *4*, 12.
(36) Gurski, L. A.; Jha, A. K.; Zhang, C.; Jia, X.; Farach-Carson, M. C. Hyaluronic acid-based hydrogels as 3D matrices for in vitro evaluation of chemotherapeutic drugs using poorly adherent prostate cancer cells. *Biomaterials* **2009**, *30*, 6076−6085.
(37) Semino, C. E.; Merok, J. R.; Crane, G. G.; Panagiotakos, G.; Zhang, S. Functional differentiation of hepatocyte-like spheroid structures from putative liver progenitor cells in three-dimensional peptide scaffolds. *Differentiation* **2003**, *71*, 262−270.
(38) Park, J. S.; Woo, D. G.; Sun, B. K.; Chung, H.-M.; Im, S. J.; Choi, Y. M.; Park, K.; Huh, K. M.; Park, K.-H. In vitro and in vivo test of PEG/PCL-based hydrogel scaffold for cell delivery application. *J. Controlled Release* **2007**, *124*, 51−59.
(39) Wang, X.; Sun, L.; Maffini, M. V.; Soto, A.; Sonnenschein, C.; Kaplan, D. L. A complex 3D human tissue culture system based on mammary stromal cells and silk scaffolds for modeling breast morphogenesis and function. *Biomaterials* **2010**, *31*, 3920−3929.
(40) Birgersdotter, A.; Sandberg, R.; Ernberg, I. Gene expression perturbation in vitro–a growing case for three-dimensional (3D) culture systems. *Semin. Cancer Biol.* **2005**, *15*, 405−412.
(41) Gurski, L. A.; Petrelli, N. J.; Jia, X.; Farach-Carson, M. C. 3D Matrices for Anti-Cancer Drug Testing and Development. *Oncol. Issues* **2017**, *25*, 20−25.
(42) Chu, Y.-S.; Thomas, W. A.; Eder, O.; Pincet, F.; Perez, E.; Thiery, J. P.; Dufour, S. Force measurements in E-cadherin-mediated cell doublets reveal rapid adhesion strengthened by actin cytoskeleton remodeling through Rac and Cdc42. *J. Cell Biol.* **2004**, *167*, 1183−1194.
(43) Zhang, J.; Betson, M.; Erasmus, J.; Zeikos, K.; Bailly, M.; Cramer, L. P.; Braga, V. M. Actin at cell-cell junctions is composed of two dynamic and functional populations. *J. Cell Sci.* **2005**, *118*, 5549−5562.
(44) Abu-Absi, S. F.; Friend, J. R.; Hansen, L. K.; Hu, W.-S. Structural polarity and functional bile canaliculi in rat hepatocyte spheroids. *Exp. Cell Res.* **2002**, *274*, 56−67.
(45) Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **1983**, *65*, 55−63.
(46) Tsien, R. Y.; Waggoner, A. Fluorophores for confocal microscopy. In *Handbook of biological confocal microscopy*; Pawley, J., Ed.; Springer: Boston, MA, 1995; pp 338−352.
(47) Liu, J.; Liu, C.; He, W. Fluorophores and their applications as molecular probes in living cells. *Curr. Org. Chem.* **2013**, *17*, 564−579.
(48) Edmondson, R.; Broglie, J. J.; Adcock, A. F.; Yang, L. Three-dimensional cell culture systems and their applications in drug discovery and cell-based biosensors. *Assay Drug Dev. Technol.* **2014**, *12*, 207−218.

https://dx.doi.org/10.1021/acsomega.0c02508
ACS Omega XXXX, XXX, XXX−XXX

SCIENTIFIC REPORTS
nature research

Check for updates

OPEN

In vitro study on aspects of molecular mechanisms underlying invasive aspergillosis caused by gliotoxin and fumagillin, alone and in combination

Loganathan Gayathri[1,2,3], Mohammad A. Akbarsha[4,5] & Kandasamy Ruckmani[1,2]✉

Gliotoxin (GT) and fumagillin (FUM) are mycotoxins most abundantly produced by *Aspergillus fumigatus* during the early stages of infection to cause invasive aspergillosis (IA). Therefore, we hypothesized that GT and FUM could be the possible source of virulence factors, which we put to test adopting in vitro monoculture and the novel integrated multiple organ co-culture (IdMOC) of A549 and L132 cell. We found that (i) GT is more cytotoxic to lung epithelial cells than FUM, and (ii) GT and FUM act synergistically to inflict pathology to the lung epithelial cell. Reactive oxygen species (ROS) is the master regulator of the cytotoxicity of GT, FUM and GT + FUM. ROS may be produced as a sequel to mitochondrial damage and, thus, mitochondria are both the source of ROS and the target to ROS. GT-, FUM- and GT + FUM-induced DNA damage is mediated either by ROS-dependent mechanism or directly by the fungal toxins. In addition, GT, FUM and GT + FUM may induce protein accumulation. Further, it is speculated that GT and FUM inflict epithelial damage by neutrophil-mediated inflammation. With respect to multiple organ cytotoxicity, GT was found to be cytotoxic at IC_{50} concentration in the following order: renal epithelial cells < type II epithelial cells < hepatocytes < normal lung epithelial cells. Taken together, GT and FUM alone and in combination contribute to exacerbate the damage of lung epithelial cells and, thus, are involved in the progression of IA.

Abbreviations

DMSO	Dimethyl sulfoxide
FUM	Fumagillin
GT	Gliotoxin
IC_{50}	Concentration at which 50% of cells are dead
IdMOC	Integrated discrete multiple organ co-culture
IA	Invasive aspergillosis
MTT	3-4, 5-Dimethylthiazole-2-yl, 2,5-diphenyl tetrazolium bromide
ROS	Reactive oxygen species

Aspergillus fumigatus, the abundantly distributed saprophytic fungus, is a weak pathogen. Generally, *A. fumigatus* releases air-borne, dormant, buoyant microscopic conidiospores in copious amounts, so that a person would

[1]Department of Pharmaceutical Technology, University College of Engineering, Anna University-BIT Campus, Tiruchchirappalli, Tamil Nadu 620024, India. [2]Centre for Excellence in Nanobio Translational Research (Autonomous), University College of Engineering, Anna University-BIT Campus, Tiruchchirappalli, Tamil Nadu 620024, India. [3]Department of Biotechnology and Bioinformatics, Holy Cross College (Autonomous), Tiruchchirappalli, Tamil Nadu 620002, India. [4]National College (Autonomous), Tiruchchirappalli, Tamil Nadu 620001, India. [5]Mahatma Gandhi-Doerenkamp Centre for Alternatives, Bharathidasan University, Tiruchchirappalli, Tamil Nadu 620 024, India. ✉email: ruckmani@aubit.edu.in

Mycotoxin	Level of GT in lungs	Level of GT in serum	Experimental model	Reference
GT	3,976 ± 1,662 ng/g	36.5 ± 30.28 ng/ml	Mice	[2]
GT	–	166 to 785 ng/ml	Patients with cancer	[2]
GT	40–63 µg/kg (sputum)	33–47 µg/kg	Patients with pulmonary aspergillosis	[11]
Bis(methylthio)gliotoxin	0.19–13.68 mg/L (bronchoalveolar lavage or sputum)	–	Patients with IA	[12]
GT	–	0.19–0.25 µg/ml	Patients with high risk for IA	[13]
Bis(methylthio)gliotoxin	–	0.46–49.84 µg/ml	Patients with high risk for IA	[11]

Table 1. Summary of Serum levels of GT in mice and human.

potentially inhale hundreds of spores every day[1]. After successful permeation of airway mucosa, the spores encounter the airway epithelial cells where the first line immune response of the host is likely to begin[1,2]. After vigorous hyphal growth for 24 h, the fungus may produce some secondary metabolites that breach the epithelial/endothelial barrier. Once the epithelial layer of the alveoli is damaged, the fungus enters the endothelium of blood vessels to become an angiotrophic fungus whereupon the disease becomes invasive and spreads to other organs[2,3]. However, immunocompetent healthy individuals are able to deal with the fungal conidia by the several immune mechanisms that prevent the germination and growth of hyphae whereas in immunocompromised persons conidial germination and mycelial development in the lung epithelial cells can cause severe/fatal disease called Invasive Aspergillosis (IA)[1,3].

Reports have shown that IA is now a major cause of death at leukemia treatment, bone marrow transplantation and solid-organ transplantation centers and it is responsible for 30% of fungal infections in patients dying of cancer wherein the mortality rate of leukemia patients is 80 to 90%, even when given antifungal therapy[2,3]. Thus, anti-*A. fumigatus* therapy remains challenging as a consequence of high mortality from IA[4]. One of the major reasons for this failure could be poor understanding of the colonization, pathobiology and virulence factors of *A. fumigatus* because every pathogen is capable of developing strategies to disseminate hyphal growth and evade host immune surveillance during infection[3,4]. In order to fight against the first-line host innate immune response, *A. fumigatus* adopts a strategy that involves discharge of mycotoxins to damage the epithelial/endothelial barriers of the respiratory tract. For instance, it has been reported that *A. fumigatus* relies on secreted proteases, lipases, and/or toxins for their survival benefits but none of these have been explored for pathogenesis of IA except mycotoxins[5].

Mycotoxins are secondary metabolites of fungi which are not vital for their lifecycle but offer competitive survival advantage over the host immunity. *A. fumigatus* produces a variety of mycotoxins/secondary metabolites such as gliotoxin, fumagillin, fumitremorgin, verruculogen, restrictocin, helvolic acid, etc. Although the pathogenicity of IA is multifactorial, gliotoxin has been proved to be a virulent factor of *A. fumigatus* but the specific roles of the other mycotoxins are not well defined[1,3,4]. Indeed, the combined action of two or more of these mycotoxins may produce synergistic effects against the host's defense mechanisms. In the light of the fact that two or more mycotoxins would interact synergistically or additively and produce more serious adverse effects than single compounds[6,7], it is pertinent that this issue in respect of highly virulent mycotoxins produced by A. *fumigatus* is worthy of being addressed.

Gliotoxin (GT), a hydrophobic metabolite, belongs to the class of epipolythiodioxopiperazine compounds characterized by a quinoid moiety and disulfide bridge across the piperazine ring which is essential for their toxicity[8]. GT is a well-studied immune-suppressive mycotoxin that is produced against the first line immune response of epithelial barrier of the host. GT is known to induce apoptosis in leukocytes, and inhibit phagocytosis, respiratory burst, and T-cell and B-cell responses stimulated by the host. Also, GT has received considerable attention as a pathogenic and putative virulence factor as revealed in the following observations: (i) GT was detected in the lung and serum of cancer patients suffering from IA as well as in mice with experimentally induced IA (Table 1); (ii) up to 93% of *A. fumigatus* strains isolated from cancer patients suffering from IA produce GT[9]; and (iii) GT was found to be produced much faster at 37 °C under high levels of oxygen which is close to that of the environment of host's lung[10].

Fumagillin (FUM) is a cyclohexane derivative, and identified as a potent angiogenesis inhibitor that reduces the proliferation of endothelial cells for blood vessel formation to avoid infiltration of host's immune cells to the site of infection[14]. Further, FUM is also involved in IA during hyphal invasion and thereby damage the epithelial layer, inhibit neutrophils and slow down beating of cilia of ciliated epithelial cells[14–17]. In fact, *A. fumigatus* produces both GT and FUM during the early stages of IA, within 30 to 72 h[14,15]. This may facilitate the fungus escape the immune surveillance and propagate the disease so as to become invasive. Despite the fact that the fungal virulence/pathogenicity is mediated by mycotoxins, the relatively unexplored frontier of the lung epithelial interface of either of the mycotoxins alone and/or the two in combination is an untouched medically important area of research that may open up avenues to find new therapies for IA[18].

Therefore, in this study we aimed at finding the molecular mechanism by which GT and FUM alone, as well as in combination, affect the epithelial cell barrier of the lung, adopting in vitro approach. Human origin A549 (lung carcinoma) and L132 (normal) lung epithelial cells that are the most established models to study IA were chosen to explore cytotoxicity, genotoxicity, cell cycle arrest, mechanism of cell death and pro-inflammatory responses against GT and FUM individually as well as in combination. As the disease progresses to invasiveness, we further

analyzed organ-specific toxicity of GT in A549 (lung), L132 (lung), HepG2 (liver) and HEK2913 (kidney) epithelial cells by adopting a novel co-culture technique, integrated discrete multiple organ co-culture (IdMOC).

Results

Cytotoxic effect of GT and FUM alone and in combination on lung epithelial cell. Exposure to GT caused dose-dependent increase in cytotoxicity at the concentration range 0–10 μM and the measured IC_{50} was 2.7 and 4.25 μM for A549 and L132, respectively (Fig. 1a). As shown in Fig. 1b, FUM at the concentration range 0–60 μM resulted in significant increase of cytotoxicity in dose-dependent manner, and the IC_{50} was 40 μM and 50 μM for A549 and L132, respectively. Based on these individual IC_{50} values cells, GT and FUM were combined at 10%, 20%, 30%, 40% and 50% of the respective IC_{50} concentrations as shown in Fig. 1c. The combination of GT and FUM at 20% of the respective IC_{50} (GT 0.54 μM + FUM 8 μM) against A549 cells and 40% of the respective IC_{50} (GT 1.7 μM + FUM 20 μM) against L132 cells significantly increased cytotoxicity to 50%. Figure 1d–g show that individual doses of GT and FUM alone, at the precise concentrations used in combinatorial experiment could not significantly increase 50% cytotoxicity against A549 and L132 cells. This is evidence to the effect that the combinatorial effect is only because of the combination of GT + FUM and not due to individual doses in the combination of GT and FUM against A549 and L132 cells. Also, according to Table 2, the combinations of GT + FUM used in the study showed synergistic interaction at the particular ratio. Therefore, we have used the following dose for treatment purpose throughout the study: IC_{50} concentration of GT (2.7 μM), FUM (40 μM) and GT + FUM (0.54 μM + 8 μM) against A549 and IC_{50} concentration of GT (4.25 μM), FUM (50 μM) and GT + FUM (1.7 μM + 20 μM) were used against L132 cells.

Effect of GT and FUM alone and in combination on induction of reactive oxygen species (ROS) and oxidative stress gene expression in lung epithelial cells. The ability of GT and FUM, alone and in combination, to induce oxidative stress was evaluated. Accumulation of ROS resulted in oxidation of H_2-DCF to become highly fluorescent DCF, which was observed as green fluorescence in both A549 and L132 cells (Fig. 2a). The results in Fig. 2b and c demonstrate time-dependent increase of ROS in both A549 and L132 cells. H_2O_2 was used as the positive control for ROS, and the significant increase of ROS was compared with data of test mycotoxins.

Further, we analyzed the changes in transcript levels of antioxidant/stress-responsive genes such as Forkhead Box O1 (FOXO1), Catalase, Superoxide dismutase 1 (SOD1), Glutathione S-Transferase P1 (GSTP1), Glutathione reductase (GR), Glutathione peroxidase 1 (GPx1) and Glutathione peroxidase 2 (GPx2). As shown in Fig. 2d and e, expression of stress-responsive transcription factor FOXO was elevated to significant level and, consequently, the expression of anti-oxidant genes that are related to FOXO transcription are also significant in A549 and L132 cells exposed to GT, FUM and GT + FUM for 24 h.

Effect of GT and FUM alone and in combination, on mitochondrial trans-membrane potential (ΔΨm). JC-1 is a probe which indicates loss of ΔΨm by shift in fluorescence emission from red to green. As shown in Fig. 3, control (untreated) cells fluoresced red and cells treated with GT, FUM and GT + FUM fluoresced only green indicating that ΔΨm was lost completely in both A549 and L132 cells within 24 h.

Evidence for accumulation of unfolded protein on treatment of GT, FUM and in combination in lung epithelial cells. Induction of GRP78 and HSP70 transcript levels was evaluated to confirm the accumulation of unfolded proteins in the lumen of the endoplasmic reticulum (ER). Figure 4a and b revealed significant increase in expression of GRP78 and HSP70 chaperons in A549 and L132 cells exposed to the respective concentrations of GT, and FUM each alone and in combination for 24 h. This confirms that GT and FUM each alone and in combination may increase the level of unfolded protein inside the cells and thus pose a chance to induce ER stress.

Genotoxicity of GT and FUM alone and in combination revealed in comet assay. As shown in Fig. 5a, both GT and FUM caused significant damage to DNA wherein the impact was more with GT than FUM and, in addition, the combination of GT + FUM also caused significant damage to DNA of A549 and L132 cells. Thus, the genotoxic potential of GT, FUM and GT + FUM against lung epithelial cells was confirmed. Since the tail length and density reflected the extent of strand breaks in DNA, the percentage of DNA in the tail provided a quantitative measure of significant DNA damage as shown in Fig. 5b and c.

Induction of cell cycle arrest in lung epithelial cell by GT and FUM alone and in combination. The results showed that exposure of A549 and L132 cells to GT for 24 h resulted in significant increase in the proportion of cells in S-phase (30–39%) with concomitant decrease in G2/M-phase. Exposure to FUM caused significant increase of cells in G1-phase (55–63%) with concomitant decrease of cells in S-phase compared to control group. As a combination, GT + FUM produced significant decrease of cells in G1, S, G2/M phases and increase in the sub-G1 phase in both the cell lines (Fig. 6a–d).

Mode of cell death in lung epithelial cell induced by GT and FUM alone and in combination. Annexin V FITC-A *vs* Propidium Iodide-A plots of A549 (Fig. 7a) and L132 (Fig. 7b) cells show population of cells corresponding to viable and non-apoptotic (Annexin V^- PI^-), early (Annexin V^+ PI^-), and late (Annexin V^+ PI^+) apoptotic cells. In the control (untreated) A549 (Fig. 7c) and L132 (Fig. 7d) samples, majority of cells (98%) were viable and non-apoptotic (Annexin V^- PI^-). In contrast, when A549 (Fig. 7c) and L132

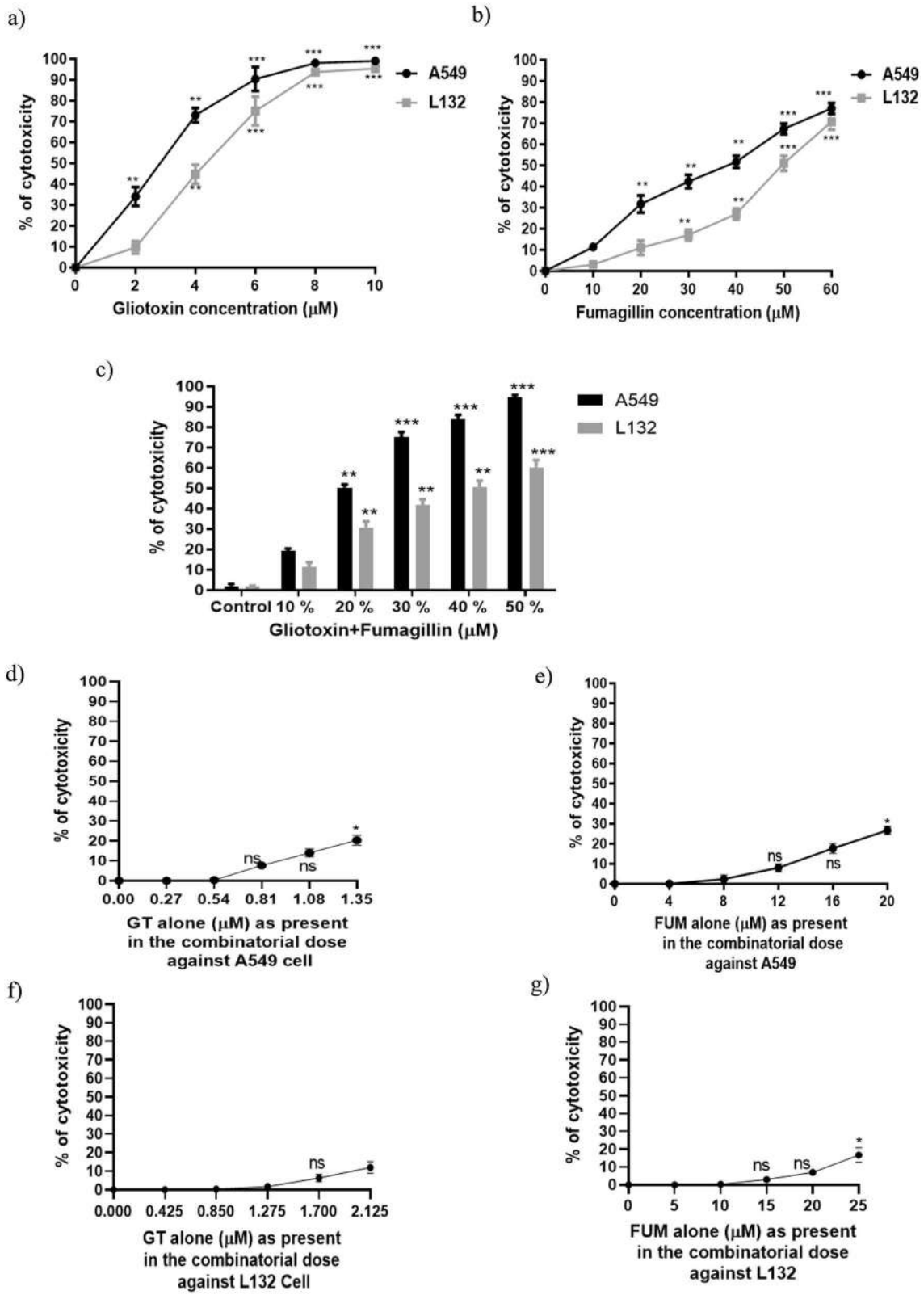


Figure 1. Cytotoxic effect of GT and FUM alone and in combination against A549 and L132 cells following 24 h of exposure. (**a**) Dose–response curve of GT at different concentrations. (**b**) Dose–response curve of FUM at different concentrations. (**c**) Combined cytotoxicity of GT + FUM. (**d**) Dose–response curve of GT alone as present in the combinatorial dose for A549 cell. (**e**) Dose–response curve of FUM alone as present in the combinatorial dose for A549 cell. (**f**) Dose–response curve of GT alone as present in the combinatorial dose for L132 cell, (**g**) Dose–response curve of FUM alone as present in the combinatorial dose for- L132 cell. Data are expressed as mean ± SD of three independent experiments for each dose point (n = 18 for panels (a, d, e, f, g), n = 21 for panel b, n = 36 for panel c). Significance between control and treatment groups was tested by adopting two way ANOVA (ns = not significant, *p < 0.5, **p < 0.01 and ***p < 0.001).

Mycotoxin	Cell line	Dose effect parameter			CI values of combinatorial dose				
		D_m (μM)	m	r	10%	20%	30%	40%	50%
GT	A549	2.51	3.18	0.98					
FUM	A549	35.56	1.54	0.99					
GT	L132	3.86	3.26	0.99					
FUM	L132	49.88	2.75	0.99					
GT + FUM	A549	7.96	2.63	0.97	0.44	0.44	0.41	0.40	0.269
GT + FUM	L132	20.83	1.56	0.99	0.43	0.55	0.72	0.84	0.91

Table 2. Synergistic interaction of GT and FUM in A549 and L132 cells. Dm and m values are used for calculating the CI value, i.e., CI < 1, CI = 1 and CI > 1 represent synergism, additivity and antagonism, respectively. Here, Dm, m and r stand for median-effect dose, kinetic order and regression coefficient of the fitting function. The ratios of GT + FUM is 1:14.8 and 1:11.76 against A549 and L132, respectively. CompuSyn software was used for calculation and simulation.

(Fig. 7d) cells treated with GT, FUM and GT + FUM in combination for 24 h, the percentage of Annexin V^- PI^- cells decreased and early apoptotic cell population (Annexin V^+ PI^-) increased significantly (Fig. 7c,d). Also, increase in Annexin V^+ PI^+ population was observed which indicates late apoptotic cells. In addition, GT, FUM and GT + FUM-mediated induction of apoptotic cell death was confirmed by microscopic analysis of Annexin V FITC/PI staining assay in A549 (Fig. 8a) and L132 (Fig. 8b) cells. In conclusion, GT, FUM and GT + FUM induced apoptotic mode of cell death in lung epithelial cells.

Evidence for induction of intrinsic-pathway of apoptosis by GT and FUM alone and in combination at molecular level. In order to understand the pathway of apoptosis, transcript levels of BAK, BAX, BID, Bcl-2, caspase-8 and caspase-3 were determined. As shown in Fig. 9a and b expression levels of anti-apoptotic Bcl-2 and the initiator caspase (caspase-8) of extrinsic pathway were decreased but expression levels of BAK, BAX, BID and caspase-3 were significantly increased in both A549 and L132 cells. Results of caspase 3 and 8 enzyme activity assays, shown in Fig. 9c and d, confirm that GT, FUM and GT + FUM induced the activity of caspase 3 but not caspase 8 in A549 and L132 cells. Thus, caspase activity analysis supports caspase 3 and 8 RNA expression analysis.

Secretion of pro-inflammatory cytokines by A549 and L132 epithelial cells against GT, FUM and GT + FUM. As shown in Fig. 10a, expression levels of IL4, IL-8 and IL-10 were significantly increased against GT whereas IL-8 alone was increased against FUM in A549 cell. In the case of L132 cell, IL-6 and IL-8 cytokine expression levels were significantly increased against GT whereas IL-8 alone was induced by FUM (Fig. 10b). As a combination GT + FUM did not induce expression of any cytokine within 24 h. Apart from up-regulation of a few cytokines as above, GT, FUM alone as well as in combination down-regulated the expressions of a few cytokines such as IL-1β, IL-17, IFN-γ, TNF-α and GM-CSF (Fig. 10a,b).

Organ-specific cytotoxicity of GT as evidenced by IdMOC. IdMOC is a novel cell culture system that can be utilized for the evaluation of toxicity, especially for studying the comparative cytotoxicity of an agent towards multiple cell types. Therefore, in one set, A549, HepG2, and HEK293 cells in duplicates and in the other set, L132, HepG2 and HEK293 cells in duplicates were co-cultured and exposed to GT under identical conditions in the concentration range 0–7 μM for 24 h (Fig. 10c,d). The IC_{50} values for HepG2 (3 μM) and HEK293 (2.1 μM) were higher than for A549 (2.5 μM); and IC_{50} values of L132 (4.2 μM) and HepG2 (3 μM) were higher than for HEK293 (2.2 μM). The rank order of the multiple cell types towards GT cytotoxicity, from the most sensitive to the most resistant, based on the different IC_{50} values are as follows: IC_{50}: HEK293 > A549 > HepG2 > L132.

Discussion

One of the open questions in the context of IA is, how the secreted fungal mycotoxins tend to become virulent factors of *A. fumigatus* such as to damage lung epithelial cells[19]. This issue has not yet been explored with clarity, and a clear understanding can enable us to harness the defensive and offensive capabilities of *A. fumigatus* during IA. In this paper, the molecular mechanism of GT-, FUM- and GT + FUM-induced lung epithelial injury, including immune response, has been studied. Upon germination, the fungal hyphae should breach the lung alveolar surface that consists of type I and type II epithelial cells in which type I cells cover 95% of the surface and engage in gas exchange whereas type II cells cover only 5% of the surface but engage crucially in the secretion of surfactant proteins and innate immune response[2,19]. Therefore, it is important to select such cell types to study the molecular mechanism of mycotoxins to relate with IA adopting in vitro techniques. A549 is a lung carcinoma epithelial cell that represents standard model of type II cells which is capable of revealing the interaction between the pathogenic factors and the host whereas L132 is an embryonic lung epithelial cell used extensively for pulmonary toxicity studies that also manifests the phenotypes of both type I and type II alveolar cells[20,21]. Therefore, the cytotoxic potentials of GT, FUM and GT + FUM were studied in A549 and L132 cells. Results from cytotoxicity studies revealed that GT more seriously attacks epithelial cells than FUM. Previous in vitro cytotoxicity assays against A549 have also shown that GT is more cytotoxic than FUM (Table 3). Comparatively,

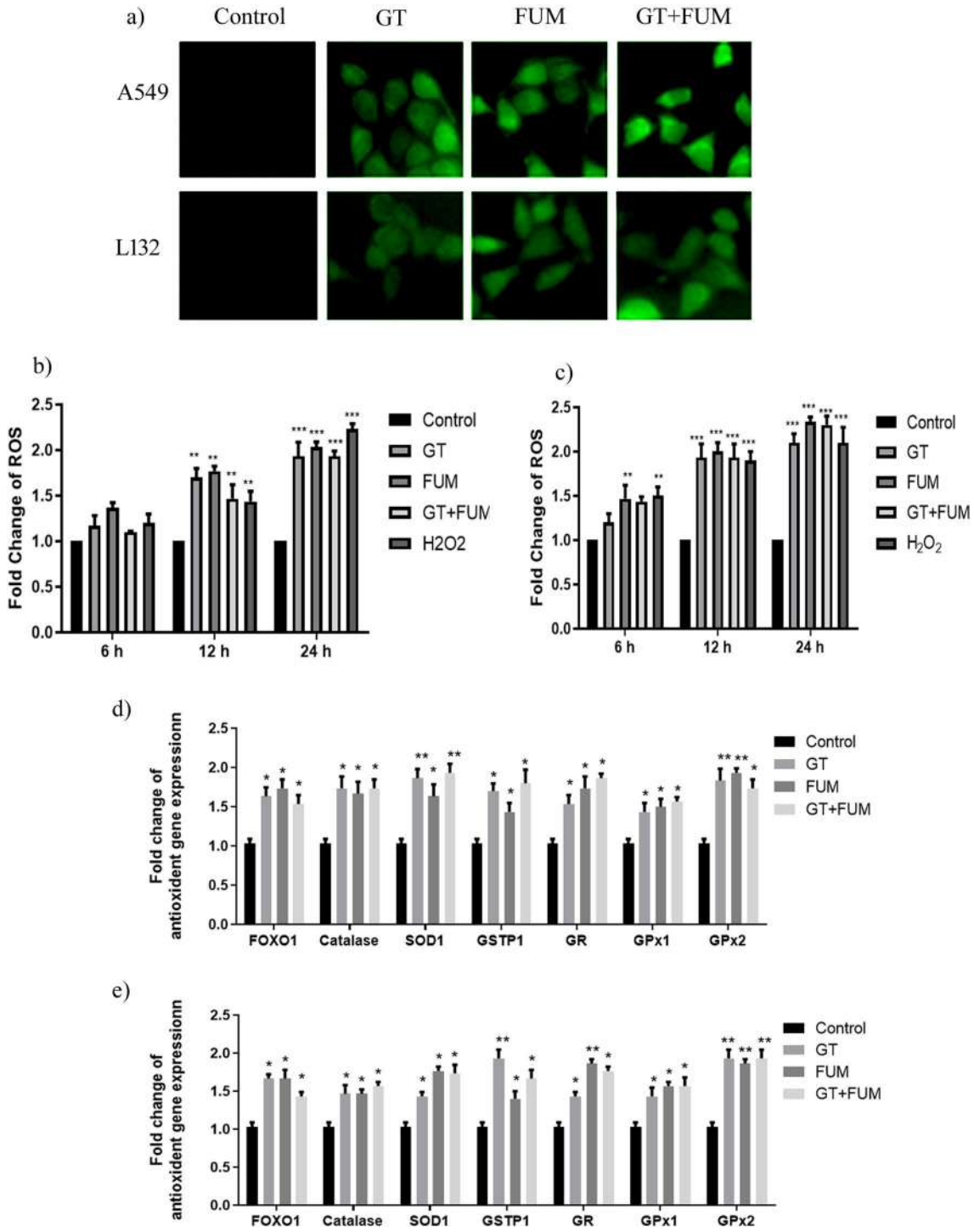


Figure 2. Effect of GT& FUM alone and in combination on induction of reactive oxygen species (ROS) and oxidative stress gene expressions. (**a**) Representative images of ROS generation after 24 h of exposure to GT, FUM and GT + FUM in A549 and L132 cells. (**b**, **c**) Quantification of intracellular ROS generation after 6 h, 12 h and 24 h of exposure to GT, FUM and GT + FUM in A549 and L132 cells, respectively. ROS level is expressed as fold change in the fluorescence in comparison with control (untreated). (**d**, **e**) Expression levels of oxidative stress related genes in A549 and L132 cells, respectively. Data are expressed as mean ± SD of three independent experiments for each dose point (n = 45 for panels b & c, n = 84 for panels **d** and **e**). Significance between control and treatment groups was tested by adopting two way ANOVA (*p < 0.5 **p < 0.01 and ***p < 0.001).

between cell lines, lung epithelial carcinoma cell (A549) is damaged faster at lower concentrations of GT, FUM and GT + FUM than normal lung epithelial cell L132. We speculate that the cancerous nature of A549 is responsible for the change in the sensitivity towards GT and FUM. On the other hand, combination of GT and FUM at

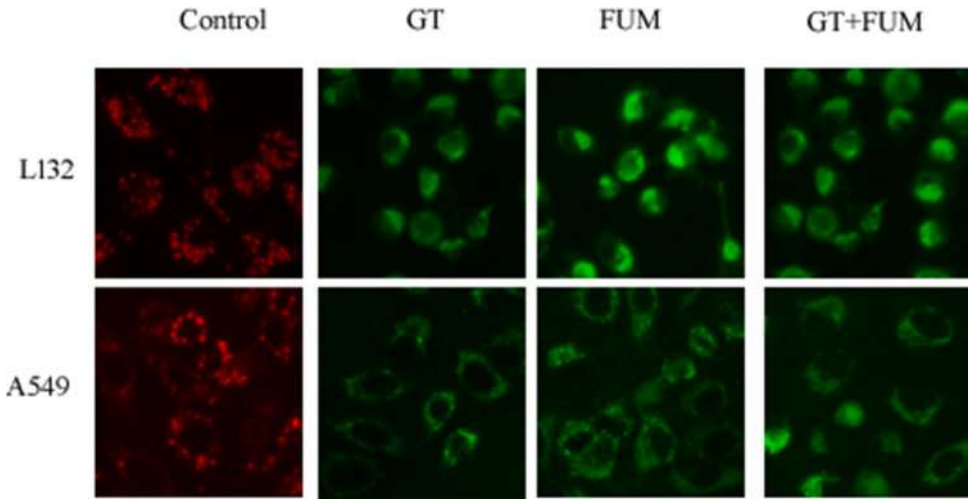


Figure 3. Photomicrographs of L132 and A549 cells showing the effect of GT, FUM alone and in combination on the change in mitochondrial trans-membrane potential at 24 h. Red color indicates high mitochondrial membrane polarization in control (untreated); Green color indicates depolarization of mitochondria in mycotoxin treated cells. Results are representative of three independent experiments.

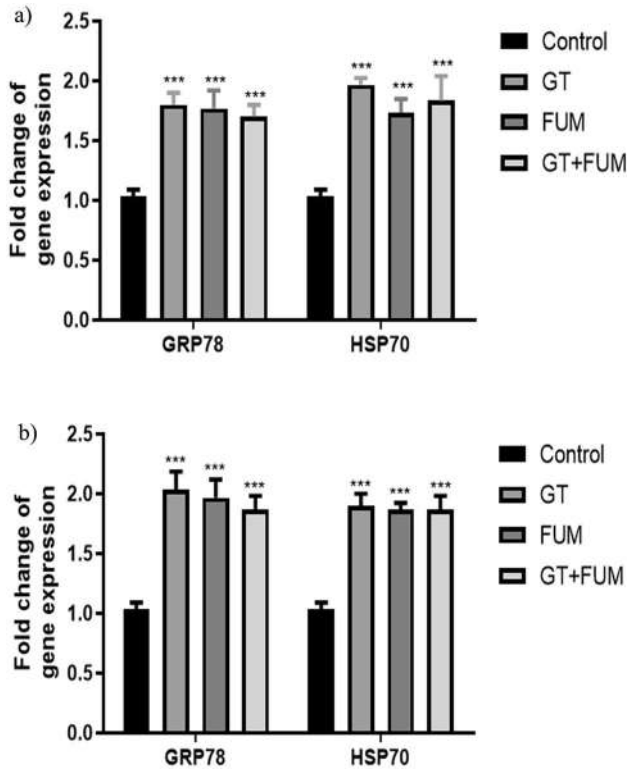


Figure 4. Effect of GT & FUM alone and in combination on gene expression of GRP78 and HSP70 chaperons. (**a**, **b**) Expression levels of GRP78 and HSP70 in A549 and L132 cells, respectively, after 24 h. Data are expressed as mean ± SD of three independent experiments for each dose point (n = 24 for panels a & b). Significance between control and treatment groups was tested by adopting two way ANOVA (***$p < 0.001$).

20% and 40% of the respective IC_{50} concentrations acting on A549 and L132 cells led to increase in cytotoxicity compared to individual toxins at their respective IC_{50} concentrations.

Though the clinical presentation of IA is relatively rapid, ranging from days to a few weeks for neutropenic host, non-neutropenic patients such as solid organ transplant recipients (particularly lung and heart–lung transplant recipients), patients with AIDS, COPD, and the critically ill intensive care unit (ICU) patients are relatively indolent and the condition progresses over weeks rather than over days (up to 12 weeks)[27–29]. Therefore, total net

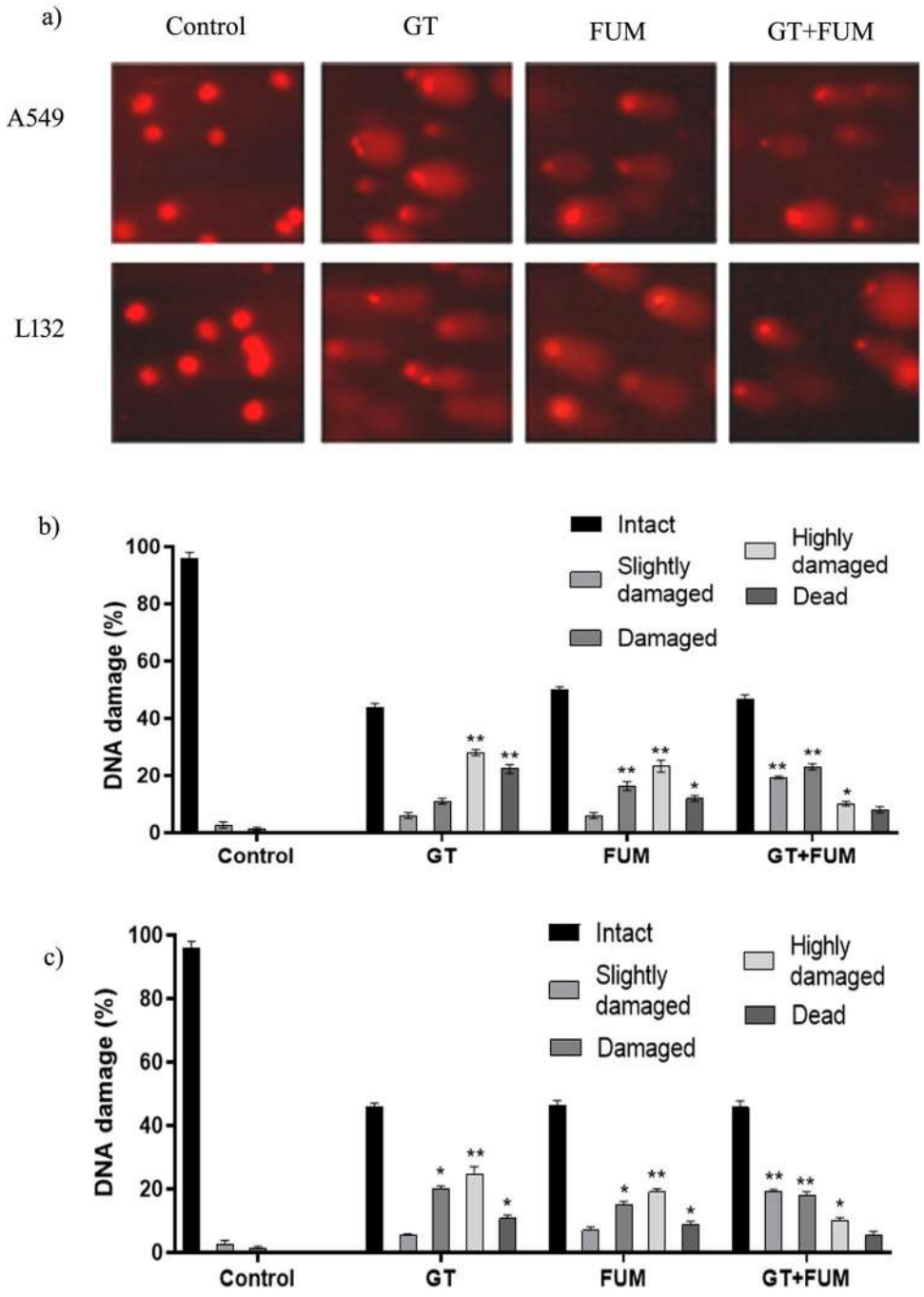


Figure 5. Assessment of DNA damage induced by GT & FUM alone and in combination after 24 h treatment in A549 and L132 cells. (**a**) Representative images of A549 and L132 cells showing DNA damage. (**b**, **c**) DNA damage (intact—0 to 20%, slightly damaged—20 to 40%, damaged—40 to 60%, highly damaged—60 to 80%, and dead—80 to 100%) as defined according to the DNA in the tail of A549 and L132 cells, respectively. Data are expressed as mean ± SD of three independent experiments for each dose point (n = 60 for panels **b** and **c**). Significance between control and treatment groups was tested by adopting two way ANOVA ((*$p < 0.5$, **$p < 0.01$).

immunosuppression of the host usually reflects the progression of fungal spread in lungs during the course of IA[27–29]. Thus, the concentration of mycotoxins produced by *A. fumigatus* may also vary between host's immune surveillances. In addition, production of mycotoxins may vary between species and even within a given species[30]. Hence, it is nearly impossible to speculate conclusively about fungi producing a toxin at a particular concentration under certain conditions. At least any such statement must remain subject to severe uncertainties[30].

However, there are a few in vitro reports to endorse the concentration of GT and FUM produced by *A. fumigatus* in which, clinical strains of *A. fumigatus* at 1×10^5 cfu can produce GT at the maximum of 21.35 µg/

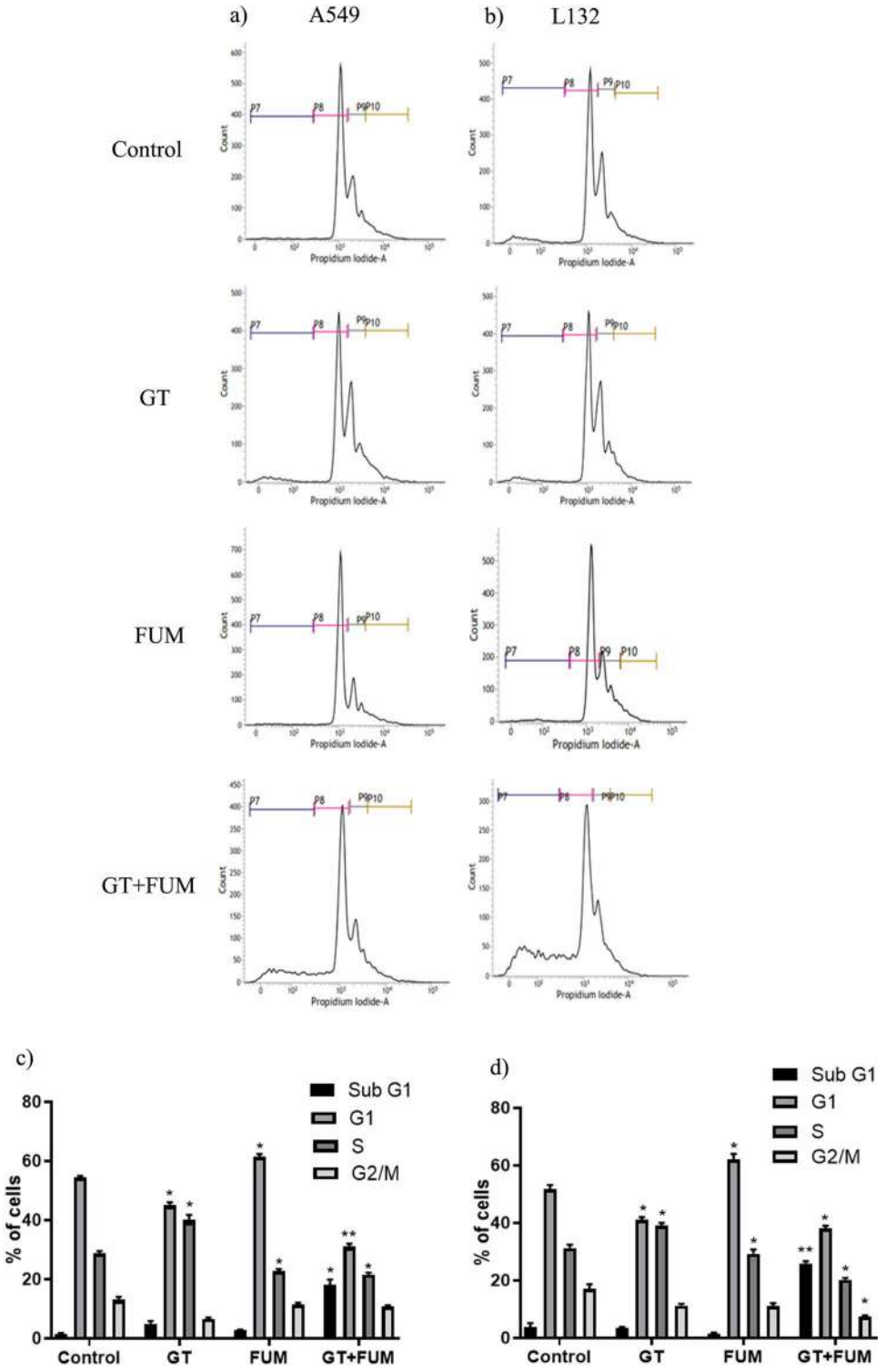


Figure 6. Assessment of cell cycle arrest induced by GT & FUM alone and in combination after 24 h treatment in A549 and L132 cells. (**a**, **b**) Representative contour plots of propidum iodide *vs* cell count for cell cycle distribution of A549 and L132 cells, respectively. Representation of P7, P8, P9 and P10 on the plot indicates Sub-G1, G1, S and G2/M respectively. (**c**, **d**) Bar diagram represents, percentage of cells in Sub-G, G1, S and G2/M phase of A549 and L132 cells, respectively. Data are expressed as mean ± SD of three independent experiments for each dose point (n = 48 for panels **c** and **d**). Significance between control and treatment groups was tested by adopting two way ANOVA (*$p < 0.5$, **$p < 0.01$).

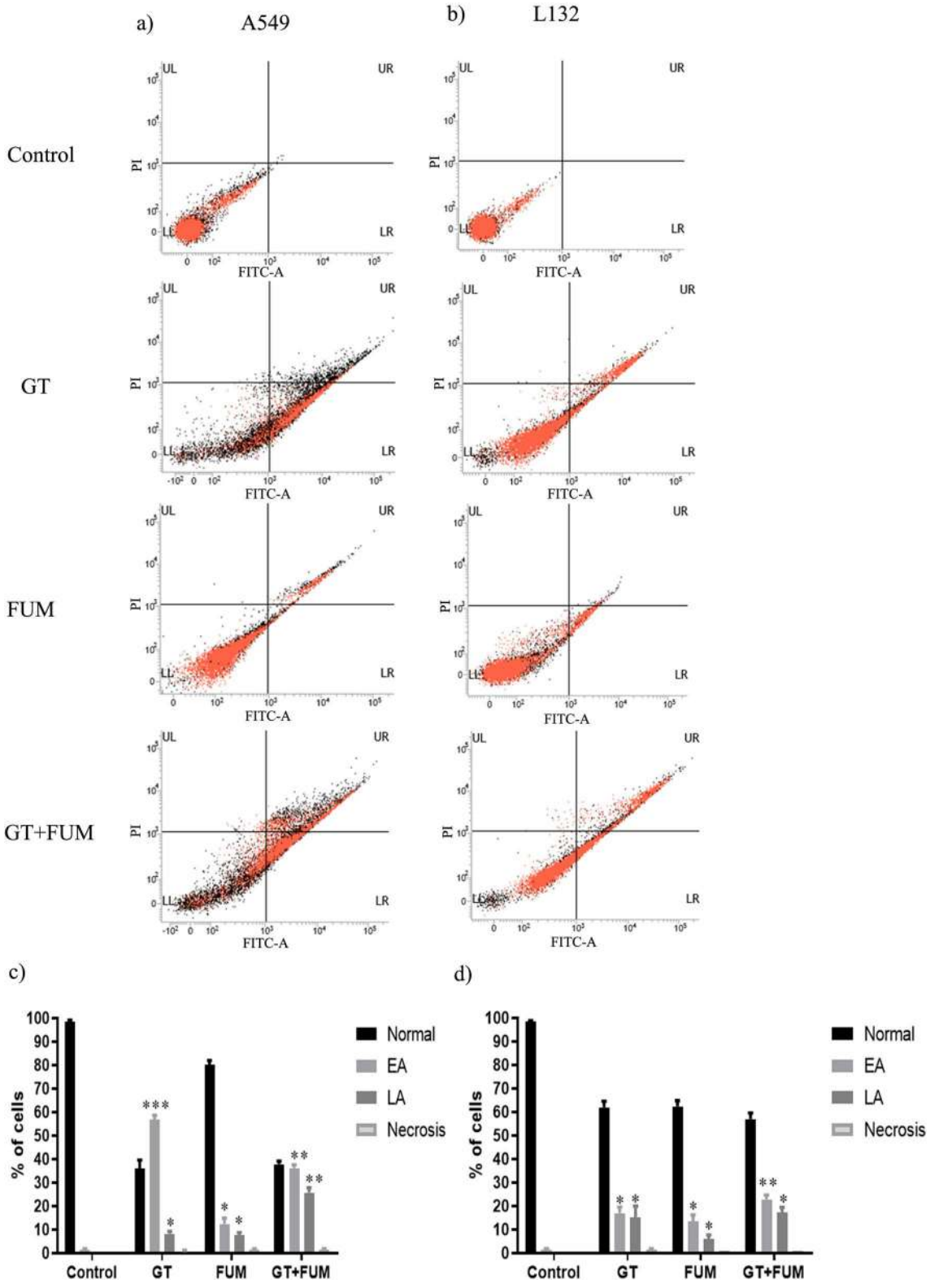


Figure 7. Assessment of apoptosis induced by GT & FUM alone and in combination after 24 h treatment in A549 and L132 cells. (**a**, **b**) Representative contour plots of Annexin V-FITC *vs* propidium iodide of A549 and L132 cells, respectively. Representation of LL, LR, UR and UL in the plot indicates normal, early apoptosis (EA), late apoptosis (LA) and necrosis cell populations respectively. (**c**, **d**) Bar diagram represents, percentage of normal, EA, LA and necrosis cells of A549 and L132 cells, respectively. Data are expressed as mean ± SD of three independent experiments for each dose point (n = 48 for panels **c** and **d**). Significance between control and treatment groups was tested by adopting two way ANOVA (*$p < 0.5$, **$p < 0.01$).

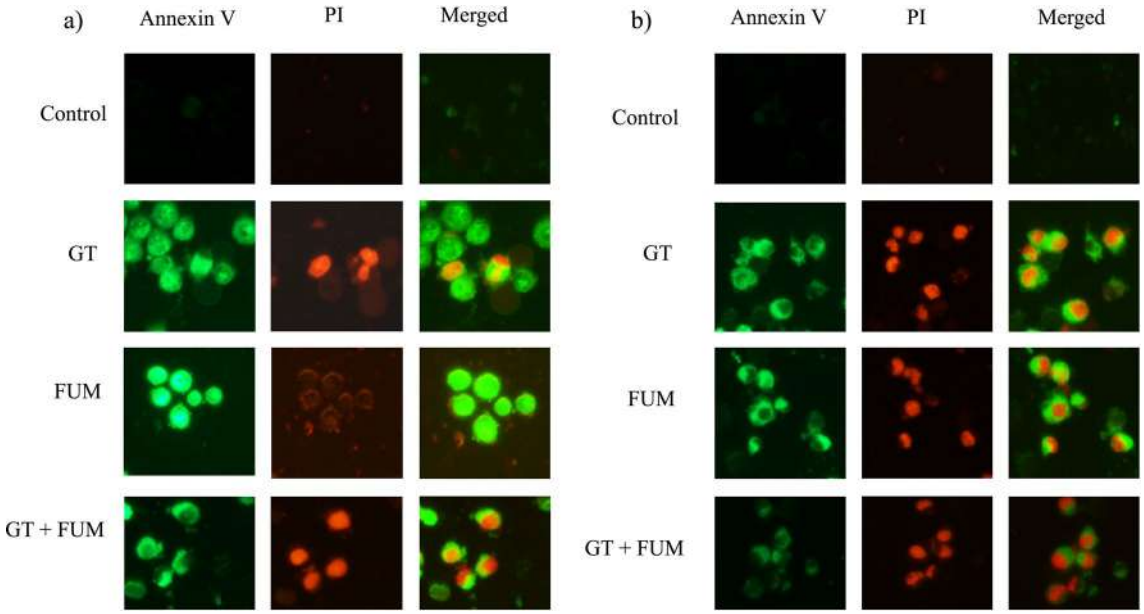


Figure 8. Photomicrographs showing the effect of GT & FUM alone and in combination on the mode of cell death as revealed by Annexin V/PI staining after 24 h. (**a**) Representative images of A549 cells. (**b**) Representative images of L132 cells. Normal cell are Annexin V^- PI^- (no fluorescence emission observed under FITC filter and TRITC filter), EA cells are Annexin V^+ PI^- (green fluorescence observed under FITC filter and no fluorescence under TRITC filter), LA are Annexin V^+ PI^+ (green fluorescence observed under FITC filter and Red fluorescence under TRITC filter), Necrosis (Annexin V^- PI^+ (no fluorescence under FITC filter and red fluorescence observed under TRITC filter).

mL and mean of 5.75 µg/mL in RPMI 1,640 medium at 37°C[9] and environmental strains of *A. fumigatus* at 9.3×10^6 cfu can produce 365 µg/mL of FUM in YES medium at 25 °C[22]. These concentrations reveal toxin producing capacity of *A. fumigatus* under favorable conditions but this may or may not be realistic in true in vivo pathophysiological condition. In addition, we can at least understand the diagnostic concentrations of GT from the Table 1 but, for FUM, no such measure is available so far to directly compare the in vivo concentrations with the studied concentrations. Therefore, our study was intended to find the half maximal cytotoxic concentrations (IC_{50}) of GT, FUM and GT + FUM instead of solely relying on particular concentrations at in vivo conditions (Table 1). Therefore, our study shows that at a defined concentration GT and FUM alone and in combination can act synergistically to induce epithelial damage to lungs.

In general, mycotoxins use ROS as a weapon to alter the cellular redox signaling to initiate destructive signals against cell endurance[6]. In order to counteract the imbalance in redox state, the host's body would kick off stress-related or antioxidant gene expressions to maintain cellular homeostasis[31]. As hypothesized, we found GT, FUM and GT + FUM to significantly induce cellular level of ROS in lung epithelial cells within 24 h and also concurrent increase in the gene expression of FOXO1, which is an important transcription factor that regulates cellular stress response and promotes cellular antioxidant defense systems such as catalase, SOD1, GSTP1, GR, GPx1 and GPx2 within 24 h in both A549 and L132 cells. Although antioxidant enzyme expressions are increased, adequate changes to reduce the level of generated ROS are not evidenced in Fig. 2b and c. To conclude further about the action of GT, FUM and GT + FUM on cellular antioxidant system, protein level enzyme activities of antioxidants are required to be studied.

Further, the elevated level of ROS guided us to find the healthy state of mitochondria that play a vital role in the sudden burst of ROS upon treatment of mycotoxins. We found an increase in mitochondrial membrane depolarization in both A549 and L132 cells by GT, FUM and GT + FUM. This shows the possibility that collapse of mitochondrial membrane is mediated by ROS or the production of ROS is followed by mitochondrial membrane depolarization. This comprehension is supported by the concept "Reactive Oxygen Species (ROS)-induced ROS-release" (RIRR) in which the excessive ROS can collapse mitochondrial membrane that would lead to temporary increase in ROS generation by the electron transfer chain[32]. Later, ROS is released to cytoplasm to inflict damage to the neighboring mitochondria. This mitochondrion-to-mitochondrion ROS-signaling signifies positive signals for better ROS production which would lead to momentous mitochondrial and cellular injuries[32].

Further, the singlet oxygen of ROS can readily react with proteins, DNA and lipid molecules of the cell. Thus, oxidative stress/pathological conditions of the cell can build unfolded proteins to accumulate in the lumen of the ER, which we studied indirectly by means of GRP78 and HSP70 expression analyses. Both GRP78 and Hsp70 are chaperons that are up-regulated inside the cells to increase the folding capacity of unfolded proteins and to assist a wide range of folding processes, including refolding of mis-folded and aggregated proteins in the ER, respectively[33,34]. The increase in GRP78 and Hsp70 that we found confirms that the ROS that is generated reacts with protein molecules and also alters the post-translational process that occurs inside the lumen of ER after treating the cells with GT, FUM and GT + FUM. Previous in vivo and in vitro studies have demonstrated an

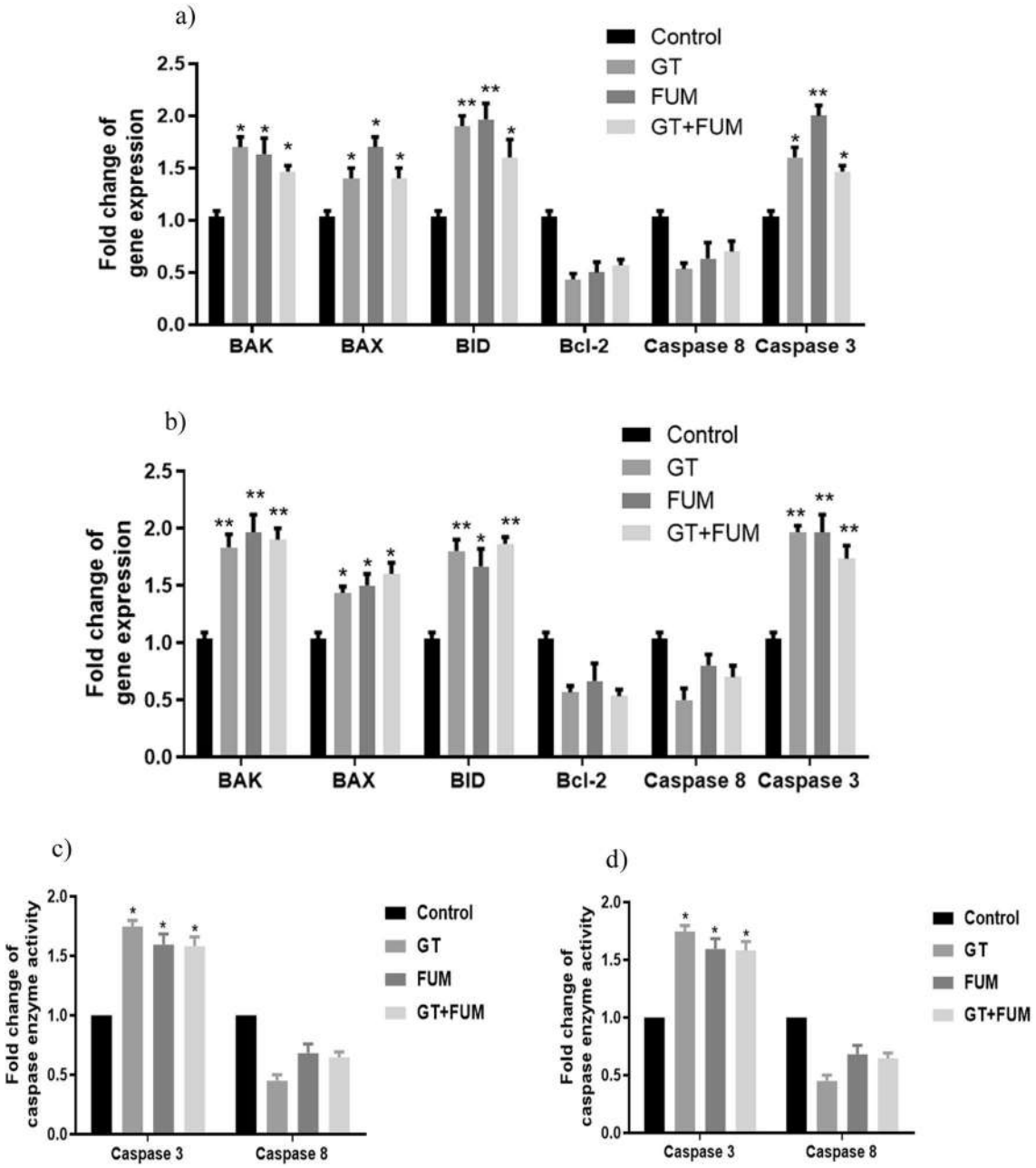


Figure 9. Molecular level induction of apoptosis-related genes by GT, FUM alone and in combination after 24 h. (**a**, **b**) Gene expression profile in A549 and L132 cells respectively. (**c**, **d**) Detection of caspase-3 and -8 enzyme activities in A549 and L132 cells respectively. Data are expressed as mean ± SD of three independent experiments for each dose point (n = 72 for panels **a** and **b**; n = 24 for panels **c** and **d**). Significance between control and treatment groups was tested by adopting two way ANOVA (*$p < 0.5$, **$p < 0.01$).

increase in ER stress markers in murine lungs and tracheal epithelial cells challenged with *A. fumigatus* antigens[35]. We found genotoxic potential of GT and FUM against the epithelial cell lines A549 and L132, evidencing single strand DNA breaks. These results are consistent with the earlier studies on genotoxicity of GT and FUM[36,37]. In addition, the combination of GT + FUM at low concentrations showed DNA-damaging potential which is threatening compared to individual mycotoxins at corresponding concentrations. Though the protagonist of the entire event caused by the test mycotoxins is putatively identified as ROS, direct DNA damage is also likely to happen. Further investigations using strong antioxidants are needed to find how precisely GT, FUM and GT + FUM-treatments cause DNA damage. Further, causation of S and G1 phase arrest by these cytolethal mycotoxins, GT, FUM and GT + FUM, during cell cycle is evidenced. Looking into the intricate mechanism of action, the data indicate that both GT and FUM enter the cell cytoplasm to bring about cell cycle arrest. Thereupon, GT induces cell cycle arrest by interfering with the DNA doubling S-phase through imminent DNA damage. This is followed by induction of cell cycle arrest by FUM at G1 phase, indicating that it interferes with

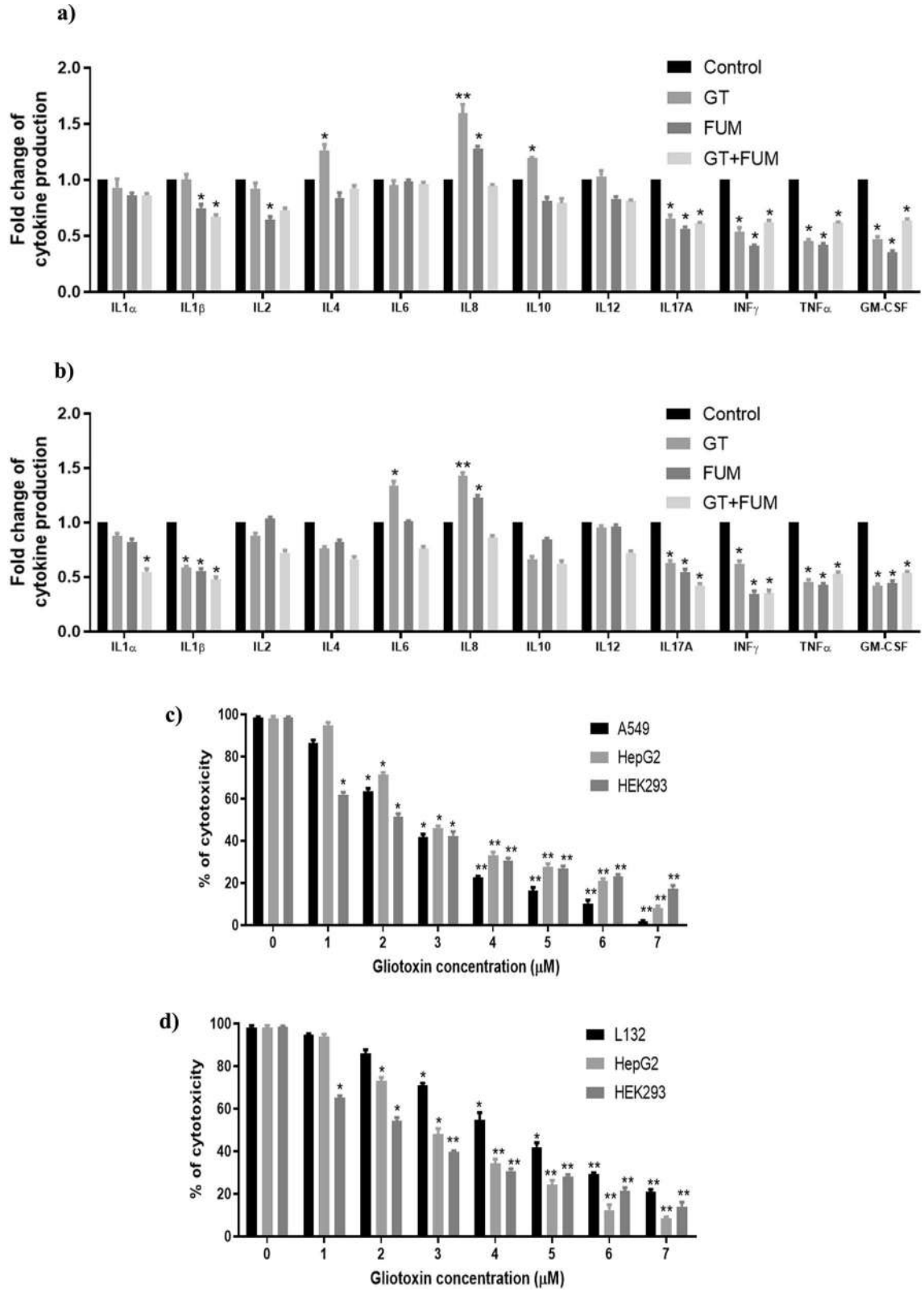


Figure 10. Secretion of inflammatory cytokines by epithelial cells following exposure of GT & FUM alone and in combination for 24 h and organ-specific cytotoxicity of GT adopting IdMOC technique. (**a**, **b**) Inflammatory cytokines secreted by A549 and L132 cells, respectively. (**c**) Organ-specific cytotoxicity of GT in A549 cell co-cultured with HepG2 and HEK293 cells after 24 h; (**d**) Organ-specific cytotoxicity of GT in L132 cell co-cultured with HepG2 and HEK293 cells after 24 h. Data are expressed as mean ± SD of three independent experiments for each dose point (n = 144 for panels **a** and **b**; n = 72 for panels **c** and **d**). Significance between the control and the treatment groups was tested by adopting two way ANOVA (*$p < 0.5$, **$p < 0.01$).

Cell type	IC_{50} value/time/assay	Toxic effect	Reference
GT			
A549	0.1 µg/ml (0.3 µM) /24 h/neutral red assay	Cytotoxicity	[22]
A549	0.16 µg/ml (0.4 µM)/48 h/MTT assay	Cytotoxicity	[23]
A549	0.16 µg/ml (0.0005 mM)/24 h/MTT assay	Cytotoxicity	[24]
A549	0.04 µg/ml (0.12 µM)/not mentioned/MTT	Cytotoxicity	[25]
A549	0.097 µg/ml (0.3 µM)/24 h/MTT assay	Cytotoxicity	[26]
FUM			
A549	16.5 µg/ml (35.9 µM)/24 h/neutral red assay	Cytotoxicity	[22]
A549	3.2 µg/ml (6.97 µM)/not mentioned/MTT assay	Cytotoxicity	[25]

Table 3. Summary of cytotoxicity studies on GT and FUM against A549 cells.

the transcriptional and post-transcriptional events that are crucial for the G1 to S-phase transition. The difference in the outcomes of previous studies on GT-induced effect on cell cycle analysis and our study may be due the difference in concentrations[23,38] whereas the outcomes of cell cycle analysis in respect of FUM are similar[39]. The GT + FUM combination produced significant decrease of A549 and L132 cells in S, G2, and M phases and increase of cells in sub-G1 phase, which point to inhibition of mitosis and induction of apoptosis. This indicates that GT + FUM induce DNA damage but the cells which are not able to repair the damage succumb to apoptosis. These results support our data on genotoxic potential of GT, FUM and GT + FUM against A549 and L132 cells.

Apparently, intracellular cues such as increased cytotoxicity, ROS generation, stress signaling pathways, mitochondrial membrane damage and cell cycle arrest can lead to cell death. The results of Annexin V/PI flow cytometry confirmed that both GT and FUM alone as well as in combination induced apoptosis rather than necrosis in both A549 and L132 epithelial cells. Our results are consistent with previous reports in respect of GT and FUM[23,24,40]. Apoptosis can be initiated by one of two pathways, caspase-8-associated death receptor pathway, i.e. extrinsic pathway, and mitochondria-mediated intrinsic pathway. Binding of ligands with death receptors will activate a series of downstream factors, including caspase-8 which is a critical mediator of the extrinsic pathway. Intrinsic pathway of apoptosis is initiated via mitochondrial membrane pore formation where the stress signals cause the binding of cytoplasmic proteins BAX and BID with mitochondrial membrane protein BAK. At the culmination of both of these apoptotic pathways, initiation of executioner caspase 3-dependent proteolytic cascade is considered a key factor of apoptotic cell death[6]. Our expression analysis revealed decrease of anti-apoptotic Bcl-2 protein and increase of pro-apoptotic BAX, BID and BAK, validating the participation of mitochondria in the cell death. Decrease in the RNA expression/activity of caspase 8 and increase in the expression/activity of caspase 3 authenticate that caspase-dependent mitochondria-mediated intrinsic pathway of apoptosis is induced by GT, FUM and GT + FUM in lung epithelial cells. Our results with regard to apoptosis are consistent with previous findings in respect of GT and FUM[41,42].

Though we found generation of ROS to be a crucial event in the molecular mechanism of cell death induced by GT, FUM and GT + FUM, we still have question on the production of intracellular ROS in response to GT and FUM. Previous studies let us believe that the structure of GT can exist in either disulfide or dithiol forms, depending on the reducing or oxidizing environment[43,44]. Further, GT exists naturally in an oxidized disulphide form but upon cellular uptake GT is chemically reduced to the dithiol form by intracellular glutathione (GSH). Thus, GT is increasingly accumulated in a cell in a glutathione-dependent manner[43,44]. However, induction of ROS followed by apoptosis is observed in many cell lines because reduced-GT can be oxidized to GT, thus, releasing ROS, and it can also form mixed disulfides by thiol groups with proteins or antioxidants such as glutathione[45,46]. Then, oxidation of the intracellular toxin results in rapid efflux from the cell which also occurs when glutathione levels decrease following induction of apoptotic cell death by the toxin[43,44]. Further, reduced dithiol form of GT has the ability to chelate Zn^{2+} ions from intracellular metalloenzymes and target thioredoxin redox system[47,48]. Also, GT is involved in activation of phospholipase D which catalyzes hydrolysis to produce the signal molecule phosphatidic acid, soluble choline and actin cytoskeleton rearrangement[49,50]. On the other hand in case of FUM, total glutathione content is significantly higher in cell lines insensitive (human umbilical vein endothelial cells) to FUM than in sensitive cell lines (human colon cancer cell line)[51]. Also, FUM has been demonstrated to affect not only methionine aminopeptidases proteins (family of intracellular proteolytic enzymes) but also all the subsequent activities necessary for the effective functioning of proteins related to cell viability and growth[41]. These studies clearly show that glutathione, the hall mark of cellular redox homeostasis, is affected by both GT and FUM, and the structural advantages of theses mycotoxins could be the reason for the generation of intracellular ROS and thus affect several intracellular proteins and induce apoptosis. Thus, GT and FUM in combination can act synergistically against cellular glutathione redox system.

Further, lung epithelial cells can initiate an inflammatory response against mycotoxins to disrupt fungal growth. Previous studies on immunological responses either for *A. fumigatus* or culture filtrate of *A. fumigatus* showed increase of cytokines IL-4, IL-10, IL-6, IL-8, IFN-g and TNF-α[1,52]. Although, it is important to find stimulatory effect of GT, FUM and GT + FUM involved in the inflammation of epithelial cells which act as a first-line defense signals that release pro-inflammatory cytokines to attract neutrophils, macrophages and dendritic cells at the site of infection. In this way we project the mycotoxins to act as virulence factors without any fungal antigen moiety to provoke innate immune response of host epithelial cells rather as a physical barrier

of lung. As revealed in the results, IL-8 is the common pro-inflammatory signal produced by both A549 and L132 cells against GT and FUM. IL-8 is a chemokine that contributes to the effective recruitment and activation of neutrophils at the site of infection. This clearly shows that both type 1 and type 2 epithelial cells enable neutrophil-mediated-inflammatory responses against GT and FUM. These results are consistent with previous studies showing production of IL-8 upon conidial germination and hyphal growth of *A. fumigatus* inside epithelial cells. Apart from IL-8, GT induced IL-4 and IL-10 in A549 cell. T helper 1 (Th1) anti-inflammatory cytokines IL-4 and IL-10 are involved in the maintenance of homeostasis and, in particular, in controlling pro- and anti-inflammatory cytokines involved in infectious, allergic and autoimmune diseases. In addition, these anti-inflammatory cytokines limit the immune response to self- and non-T-self antigens, protecting the host from excessive Th1 pathology and preventing tissue damage[1]. Further, previous reports have shown that immune response initiated by IL-4 and IL-10 activates T helper 2 (Th2) cell differentiation and inhibits Th1 and Th17 response. IL-4 and IL-10 deficient mice show lower *A. fumigatus* burden and increased survival rates compared to wild type mouse in invasive pulmonary aspergillosis[1]. Thus, we speculate that exposure of GT would even enhance neutrophil recruitment at the site of infection to cause neutrophil-mediated inflammatory tissue damage and, thus, IL-4 and IL-10 cytokines are increased by epithelial cells to prevent the excessive inflammatory response of Th1 to avoid self-tissue damage. On the other hand, in L132 cell, production of IL-6 cytokine was also significant apart from IL-8. The IL-6 is an important proinflammatory cytokine involved in regulation of Th17 response during *A. fumigatus* infection. Further, Th17cells activate neutrophil migration towards the infected area and thereby increase inflammation. Over all, the results clearly show that recruitment of neutrophil to the site of infection could be the innate immune response of epithelial cells against the fungal mycotoxins GT and FUM. Moreover, these toxins have been proved for their ability to inhibit the function of neutrophils. This could be one of the reasons for neutropenia patients to manifest frequent IA infection due to fewer neutrophils to fight the pathogen[27–29]. An earlier study has shown similar results in LPS induced A549 cells against GT[46]. In addition, the combination of GT + FUM at a lower concentration could not induce any cytokine expression within 24 h which is perhaps a good strategy to bring about epithelial cell death without inflammatory response or may take more than 24 h to alter the immunological response. This brings up the dangerous effect of GT + FUM as a combination to damage epithelial cells invasively, without any resistance from the host immune system. However, the molecular mechanisms by which these toxins induce cytokine expression remains to be studied. Further, in vivo investigation about immune regulation mechanism might reveal some potential targets for diagnosis and treatment of pulmonary fungal diseases.

Once the epithelial layer of alveoli is damaged, *A. fumigatus* arrives at the endothelium of the blood vessel to become an angiotrophic fungus and, thus, the disease becomes invasive and spreads to other organs. Disseminated aspergillosis is defined as the involvement of at least two non-contiguous organ sites. *Aspergillus* may disseminate from lungs to invariably every organ[28]. Disseminated disease has been described in 9–36% of kidney recipients, 15–20% of lung recipients, 20–35% of heart recipients and 50–60% of liver recipients with IA[28]. Therefore, we followed up with study of multiple organ toxicity of GT, adopting IdMOC, a novel cell culture system that is appropriate in the context of evaluation of comparative cytotoxicity of an agent towards multiple cell types. IA is the most severe form of disease that occurs when the infection spreads rapidly from lungs to liver, heart, kidneys or skin. So far, GT is the only mycotoxin that has been identified in the sera of IA patients, and considered as a virulent factor of *A. fumigatus*. Thus, the presence of GT in the sera of IA patients justifies evaluation of multiple organ toxicity or organ-specific toxicity. As shown in the results, A549 (representing lung epithelial cell) and HEK (representing renal epithelial cell) are likely to be more sensitive and liable for GT-induced cytotoxicity than HepG2 (representing liver epithelial cells) and L132 (representing both types 1 and II lung epithelial cells) which are fairly sensitive. Studies on potential toxicokinetic variations between cell models can explain the sensitivity issues. Also, future studies that associate neuronal cells and skin cells are required to relate the clinical symptoms of brain and skin damage in IA patients[28].

In conclusion, our study shows that at a specific concentration GT and FUM interact synergistically to induce cytotoxicity in lung epithelial cells. The molecular mechanism of toxicity of GT, FUM and GT + FUM to the lung epithelial cells greatly depends on ROS-mediated, ER-stress-induced, mitochondria-mediated, caspase-dependent intrinsic pathway of apoptosis. ROS are produced after mitochondrial damage and, thus, mitochondria are both the source of ROS and target of ROS. GT-, FUM- and GT + FUM-induced DNA damage is mediated either by an ROS-dependent course or by a direct attack. In addition, GT, FUM and GT + FUM may induce unfolded protein accumulation. Although there are signs of indication that GT and FUM can induce neutrophil recruitment at the site of infection as revealed in the expression of cytokines in epithelial cells (Fig. 11) and GT would damage epithelial cells by neutrophil-mediated inflammatory response, further studies are needed to confirm the hypothesis made out in the study. GT is identified to be highly cytotoxic to renal epithelial cells at low concentration and type II epithelial cells at high concentration. It is already known in an in vivo model that GT is a virulence factor and FUM is a likely factor in cellular damage during infection of the host by *A. fumigatus*[14,53]. This is further substantiated by the down-regulation of cytokines that are generally involved in the regulation of immune system as well as in the control of the fungal pathogenesis. Taken together, these findings indicate that GT and FUM alone and in combination are capable of damaging lung epithelial cells and, thus, these mycotoxins could be an attractive target for drug development for IA.

Materials and methods

Materials. GT (CAS Number 67-99-2; ≥ 98% purity) and FUM (CAS Number 23110-15-8; ≥ 90% purity) were obtained from Sigma Aldrich Chemical Company (St. Louis, MO, USA), DMEM, Phosphate-Buffered Saline (PBS), trypsin–EDTA, penicillin and streptomycin were obtained from Hyclone laboratories (South Logan, Utah). F etal bovine serum (FBS) was obtained from Gibco (UK). 3-(4,5-dimethylthiazol-2-yl)-2,5-di-

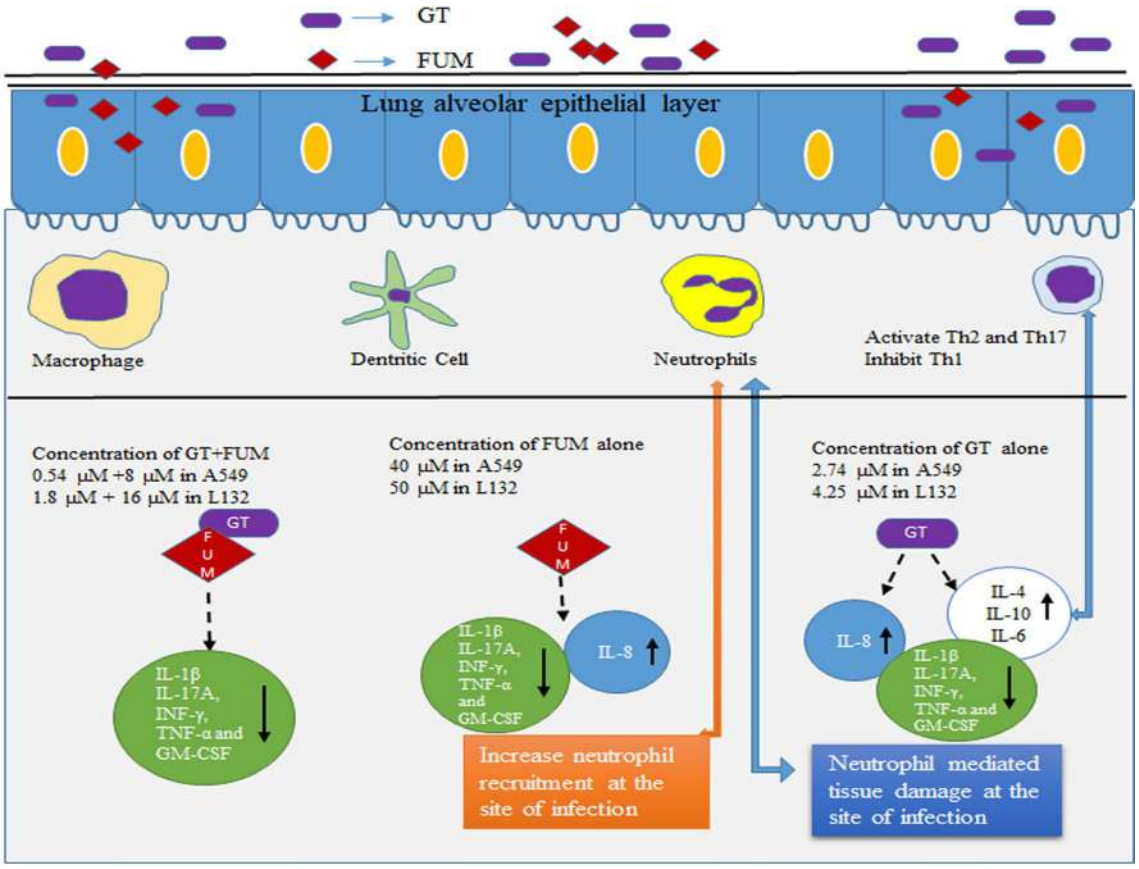


Figure 11. Represents how GT, FUM and GT + FUM act as virulent factors to provoke/evade immune surveillance of host without any fungal antigen.

phenyltetrazolium bromide (MTT), AnnexinV, Propidium Iodide (PI) and RNase I were obtained from Sigma Aldrich Chemical Company (St. Louis, MO, USA). All other chemicals and reagents were of analytical grade.

Methods

Cell culture. Human lung epithelial carcinoma cell A549, normal lung epithelial cell L132, human hepatocarcinoma cell HepG2 and human embryonic kidney cell HEK293 were obtained from National Centre for Cell Science (NCCS), Pune, India. The cells were maintained in DMEM medium supplemented with 10% FBS, and with 100 U/mL each of penicillin and streptomycin as antibiotics in a humidified atmosphere of 5% CO_2 and 95% air, in a CO_2 incubator (Eppendorf, India).

Integrated discrete multiple organ co-culture (IdMOC). The IdMOC 96 well plates were gift from Dr. Albert P. Li, President and CEO of In Vitro ADMET Laboratories LLC, Columbia, USA. The concepts and applications of IdMOC have been reviewed[54].

Cytotoxicity assay. Colorimetric cytotoxicity assay was performed according to Mosmann et al.[55] A549 and L132 cells were seeded in 96 well plate at 5×10^3 cells/well and treated with different concentrations of GT, FUM and combination of the two, dissolved in dimethyl sulfoxide (DMSO) for 24 h, at 37 °C. DMSO was used as the solvent control. After 24 h incubation, 20 µL of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] solution (5 mg/mL in PBS) was added to each well, and incubated for 3 h at 37 °C. The medium was then removed and the purple formazan product was dissolved in 100 µL of DMSO. The absorbance was measured at 570 nm (measurement) and 630 nm (reference) using a 96-well plate EnSpire multimode reader (Perkin Elmer, Waltham, MA, USA). Data were collected for triplicates and used to calculate the respective means and the standard deviations. The percentage viability (*visa vi* cytotoxicity) was calculated from this data using the following formula:

$$Percentage\ \ viability\ (visa\ vi,\ \ cytotoxicity) = Mean\ \ OD\ \ of\ \ untreated\ \ \ cells\ (control) - Mean\ \ OD\ \ of\ \ treated\ \ cells / Mean\ \ OD\ \ of\ \ untreated\ \ cells\ (control) \times 100.$$

From the values thus obtained, the IC_{50} was calculated. IC_{50} is defined as concentration of the test substance at which cell viability is decreased to 50%, meaning 50% cells are dead.

Isobologram method of toxicity assessment of binary combination of mycotoxins. The types of interaction of GT and FUM mixtures were determined by isobologram analysis using CompuSyn software (version 1.0.1 [2004]; CompuSyn, Inc.) established by Chou and Talalay[56]. In this analysis, combination index (CI) is a quantitative parameter used to evaluate the types of interaction and the corresponding influential level among binary or more compounds. CI < 1 indicates synergism, CI = 1 indicates additive effect and > 1 indicates antagonism of the compounds (in the present case, mycotoxin combinations).

Reactive oxygen species (ROS) assay for the assessment of oxidative stress. The generation of intracellular ROS was measured using the fluorescent probe 2′,7′-dichlorofluorescein diacetate (DCFH-DA) according to Wang and Joseph[57]. To evaluate mycotoxins-induced DCF oxidation, 1×10^6 counts of A549 and L132 cells, as the case may be, were seeded in each of the 6 wells of six well plates and treated with the positive control (H_2O_2) and IC_{50} concentration of GT and FUM alone and the two in combination at 20% and 40% of the respective IC_{50} values, for 6 h, 12 h and 24 h against A549 and L132 cells, respectively. After that, the wells were loaded with 5 µM of DCFH-DA and incubated for 30 min at 37 °C. ROS generation was measured in EnSpire multimode reader (Perkin Elmer, Waltham, MA, USA) at an excitation wavelength of 485 nm and emission wavelength of 520 nm. Results are expressed as the ratio mycotoxin-induced DCF fluorescence/control fluorescence.

Single-cell gel electrophoresis (comet assay) for genotoxicty assessment. DNA damage was detected adopting the comet assay[58]. The technique combines DNA gel electrophoresis with florescent microscopy to visualize migration of DNA from individual agarose-embedded cells. The cells were treated with the IC_{50} concentration of GT and FUM, alone and in combination at 20% and 40% of the respective IC_{50} values, for 24 h against A549 and L132 cells, respectively. The harvested cells were suspended in low melting point agarose in PBS and pipetted out to microscope slides pre-coated with a layer of normal melting point agarose. The slides were immersed in pre-chilled lysis buffer (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris, 0.2 mM NaOH [pH 10], and Triton X-100) and incubated overnight at 4 °C in order to lyse the cells and to permit DNA unwinding. Thereafter, the slides were exposed to alkaline buffer (300 mM NaOH, 1 mM Na_2-EDTA, [pH > 13]) for 20 min at 20 V to allow DNA unwinding. The slides were washed with buffer (0.4 M Tris, pH 7.5) to neutralize excess alkali before staining with EB. The EB-stained slides were observed in a fluorescent microscope (Nikon, Japan). One hundred and fifty cells, in triplicate, from each treatment group were digitized and analyzed using CASP software. The extent of DNA damage was assessed from the shape and area of the comet tail. The images were used to determine the DNA content of individual nuclei and to evaluate the degree of DNA damage representing the fraction of total DNA in the tail.

JC1 staining for assessment of mitochondrial trans-membrane potential. Mitochondrial trans-membrane potential ($\Delta\Psi m$) was measured using the fluorescent probe JC-1 (5,5′,6,6′-Tetrachloro-1,1′,3,3′-tetraethyl-imidacarbocyanine iodide) by adopting the method of Reers et al.[59]. The cells were grown in glass cover slips placed in the wells of 6-well plates and treated with IC_{50} concentrations of GT and FUM alone and the two in combination at 20% and 40% of the respective IC_{50} values, for 24 h, against A549 and L132 cells. The cells were stained with JC-1 dye after 24 h exposure. The mitochondrial depolarization patterns of the cells were observed in the fluorescent microscope (Nikon, Japan) and the fluorescence pattern of cells (red, normal; green, mitochondrial membrane depolarized) were observed and recorded.

Flow cytometry analysis for cell cycle arrest. Cell cycle arrest was detected using flow cytometry[60]. The cells were treated with the IC_{50} concentrations of GT and FUM alone and in combination at 20% and 40% of the respective IC_{50} concentrations, for 24 h, against A549 and L132 cells. The cells were harvested, washed with PBS, centrifuged at 1,000 rpm for 5 min, and fixed with 1 mL 70% ice cold ethanol overnight at 4 °C. Later, the ethanol was removed and 10 µL of RNase A (10 mg/mL) was added and incubated for 30 min. Again, cells were washed with PBS and re-suspended in 1 mL PBS with 50 µL Propidium Iodide (1 mg/mL) for 30 min in dark. The cells were analyzed using the BD FACSverse flow cytometer (Becton–Dickinson), and a peak fluorescence gate was used to discriminate the aggregates. A total of 20,000 events were acquired and three replicates were used per experimental condition. The percentage of cells in each phase was determined in reference to untreated cells.

Flow cytometry analysis of apoptosis. Detection of apoptosis by flow cytometry was conducted using the protocol given in manufacturer's instruction (BD Biosciences, cat no: 556547). The cells were treated with the IC_{50} concentration of GT and FUM alone and in combination at 20% and 40% of the respective IC_{50} values, for 24 h against A549 and L132 cells. The cells were harvested, washed with PBS, centrifuged at 1,000 rpm for 5 min and re-suspended in100 µL binding buffer. Five microliters of Annexin-V and Propidium Iodide were added and incubated for 15 min at room temperature in dark. The stained cells were measured at 515 nm fluorescence emission using FACSverse flow cytometer (BD) and the data were processed by FACSverse software.

Annexin V-FITC/PI binding assay for the assessment of cell death. Annexin V-FITC/PI staining was performed to assess the morphological characteristics of cell death by apoptosis or necrosis[61]. The A549 and L132 cells were cultured in 6-well plates and treated with IC_{50} concentrations of GT and FUM alone and in combination at 20% and 40% of the respective IC_{50} values, for 24 h, respectively. The treated and untreated cells were centrifuged at 3,000 rpm for 5 min, incubated with Annexin V-FITC/PI and examined under FITC filter

for green emission of Annexin V-FITC and TRITC filter for red emission of PI using an inverted fluorescent microscope (Nikon, Japan).

Real time PCR for gene expression analysis. Total RNA were isolated from untreated and treated A549 and L132 cells (2×10^6 in T_{25} culture flask) using the TRIzol Reagent (Invitrogen; USA) and quantified using a NanoDrop spectrophotometer (Bio Drop Duo, UK). First strand cDNA was synthesized with 1 µg of RNA and oligo(dTs) to a final volume of 20 µL according to the manufacturer's instruction (Thermo Scientific, USA). qPCR was performed as 10 µL reaction mix with a final concentration of 1×SYBR Green (Roche), 0.5 µL of each primer, and the cDNA that was synthesized in the earlier step. To detect non-specific amplification, negative controls (no template control and minus RT control (only with RNA) were included in the assays. The amplicons were sequenced to confirm their identity**.** The reactions were carried out using a single step real time PCR machine (Roche, USA) under the following conditions: initial denaturation at 95 °C for 5 min, followed by 30 cycles of 3 step amplification at 95 °C for 10 s, 50/60 °C for 30 s and 72 °C for 10 s.

Primers for the apoptosis and stress-related genes were selected from the earlier reports[62–66]. GAPDH served as the internal control to normalize the RNA level. Three technical repeats and experimental replicates were performed for each gene.

Caspase-3 & -8 activity assay for apoptotic cell death. Caspase-3 and -8 activities were determined using assay kit, according to the manufacturer's instructions (Cat. No. K106-100 & K113-25 Biovision, Inc., Milpitas, CA, USA). A549 and L132 cells were cultured in 6-well plates and treated with IC_{50} concentrations of GT and FUM alone and in combination at 20% and 40% of the respective IC_{50} values, for 24 h. The control and treated cells were lysed in sample lysis buffer (Biovision, Inc.). The homogenates were then centrifuged at 10,000×*g* and 4 °C for 10 min and the supernatant was collected for protein determination. The cell lysates were then exposed to DEVD-pNA and IETD-pNA substrate conjugate provided in the kit for caspase-3 and -8, respectively, for 1 h at 37 °C. The sample was measured at 400–405 nm in multimode plate reader (Model: Enspire, Perkin Elmer, CA, USA).

Multi-analyte ELISA array for detection of inflammatory cytokines. The Human Inflammatory Cytokines Multi-Analyte ELISArray kit (Cat. No. MEH-004A; Qiagen, Valencia, CA, USA) was used to detect a panel of 12 cytokines according to the manufacturer's protocol. The ELISA panel included: Interleukin (IL)-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-17A, interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α) and granulocyte–macrophage colony-stimulating factor (GM-CSF)[67].

Integrated discrete multiple organ toxicity assay using IdMOC technology for organ-specific toxicity. IdMOC 96 well plates with 16 containing wells, each with 6 inner wells, were used to discretely co-culture A549, HepG2, and HEK293 cells (Plate 1) and L132, HepG2, and HEK293 (Plate 2). In each inner well 10,000 cells were plated in 80 µL of the medium specific for each cell type. The cells were cultured in an incubator kept at 37 °C, and a highly humidified atmosphere of 95% air and 5% carbon dioxide for 24 h before treatment. On the day of treatment, the medium was removed from all inner wells of the IdMOC plate and replaced with 2.5 mL DMEM containing various concentrations of GT. Treatment was performed in triplicate for an incubation period of 24 h. After 24 h, 50 µL of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] solution (5 mg/mL in PBS) was added to each well, and incubated for 3 h at 37 °C. The medium was removed and the purple formazan product was dissolved in 50 µL of DMSO. The absorbance was measured at 570 nm (measurement) and 630 nm (reference) using a 96-well plate EnSpire multimode reader (Perkin Elmer, Waltham, MA, USA)[7]. Data were collected for triplicates and used to calculate the respective means and the standard deviations. The percentage viability (*visa vi* cytotoxicity) was calculated from this data using the following formula:

$$\begin{aligned} &\textit{Percentage of viability}\,(\textit{visa vi, cytotoxicity}) = \textit{Mean OD of untreated cells}\,(\textit{control}) \\ &- \textit{Mean OD of treated cells}/\textit{Mean OD of untreated cells}\,(\textit{control}) \times 100. \end{aligned}$$

Statistical analysis. Results were expressed as mean ± SD of three independent experiments. Difference between groups was analyzed by Two way ANOVA from GraphPad Prism-6.0; ns = not significant; $^{*}p<0.5$, $^{**}p<0.01$ and $^{***}p<0.001$ were considered statistically significant at the respective levels.

Received: 5 April 2020; Accepted: 6 August 2020
Published online: 02 September 2020

References

1. Dagenais, T. R. & Keller, N. P. Pathogenesis of *Aspergillus fumigatus* in invasive aspergillosis. *Clin. Microbiol. Rev.* **22**, 447–465 (2009).
2. McCormick, A., Loeffler, J. & Ebel, F. *Aspergillus fumigatus*: contours of an opportunistic human pathogen. *Cell. Microbiol.* **12**, 1535–1543 (2010).
3. Lewis, R. E. *et al.* Detection of gliotoxin in experimental and human aspergillosis. *Infect. Immun.* **73**, 635–637 (2005).
4. Latgé, J. P. Aspergillus fumigatus and aspergillosis. *Clin. Microbiol. Rev.* **12**, 310–350 (1999).
5. Hof, H. & Kupfahl, C. Gliotoxin in *Aspergillus fumigatus*: an example that mycotoxins are potential virulence factors. *Mycotoxin Res.* **25**, 123–131 (2009).
6. Gayathri, L. *et al.* Hepatotoxic effect of ochratoxin A and citrinin, alone and in combination, and protective effect of vitamin E: in vitro study in HepG2 cell. *Food. Chem. Toxicol.* **83**, 151–163 (2015).

7. Gayathri, L. *et al.* Metabolism-dependent cytotoxicity of citrinin and ochratoxin A alone and in combination as assessed adopting integrated discrete multiple organ co-culture (IdMOC). *Toxicol. In Vitro.* **46**, 166–177 (2018).
8. Gardiner, D. M., Waring, P. & Howlett, B. J. The epipolythiodioxopiperazine (ETP) class of fungal toxins: distribution, mode of action, functions and biosynthesis. *Microbiology* **151**, 1021–1032 (2005).
9. Kupfahl, C. *et al.* Gliotoxin production by clinical and environmental *Aspergillusfumigatus* strains. *Int. J. Med. Microbiol.* **298**, 319–327 (2008).
10. Kosalec, I., Pepeljnjak, S. & Jandrlic, M. Influence of media and temperature on gliotoxin production in *Aspergillus fumigatus* strains. *Arh. Hig. Rada. Toksikol.* **56**, 269–273 (2005).
11. Hmood, B. A. Detection of gliotoxin in patients with pulmonary aspergillosis. *JUB* **25**, 639–650 (2017).
12. Vidal-García, M. *et al.* Production of the invasive aspergillosis biomarker bis (methylthio) gliotoxin within the genus *Aspergillus*: in vitro and in vivo metabolite quantification and genomic analysis. *Front. Microbiol.* **9**, 1246 (2018).
13. Domingo, M. P. *et al.* Bis (methyl) gliotoxin proves to be a more stable and reliable marker for invasive aspergillosis than gliotoxin and suitable for use in diagnosis. *Microbiol. Infect. Dis.* **73**, 57–64 (2012).
14. Guruceaga, X. *et al.* A possible role for fumagillin in cellular damage during host infection by *Aspergillus fumigatus*. *Virulence* **9**, 1548–1561 (2018).
15. Amitani, R. *et al.* Purification and characterization of factors produced by *Aspergillus fumigatus* which affect human ciliated respiratory epithelium. *Infect. Immun.* **63**, 3266–3271 (1995).
16. Fallon, J. P., Reeves, E. P. & Kavanagh, K. Inhibition of neutrophil function following exposure to the *Aspergillus fumigatus* toxin fumagillin. *J. Med. Microbiol.* **59**, 625–633 (2010).
17. Kamei, K. & Watanabe, A. Aspergillus mycotoxins and their effect on the host. *Med. Mycol.* **143**, 95–99 (2005).
18. Wiesner, D. L. & Klein, B. S. Lung epithelium: barrier immunity to inhaled fungi and driver of fungal-associated allergic asthma. *Curr. Opin. Microbiol.* **40**, 8–13 (2017).
19. Osherov, N. Interaction of the pathogenic mold *aspergillus fumigatus* with lung epithelial cells. *Front. Microbiol.* **3**, 346 (2012).
20. Roehlecke, C. *et al.* Resistance of L132 lung cell clusters to glyoxal-induced apoptosis. *Histochem. Cell. Biol.* **114**, 283–292 (2000).
21. Panngom, K. *et al.* Preferential killing of human lung cancer cell lines with mitochondrial dysfunction by nonthermal dielectric barrier discharge plasma. *Cell. Death. Dis.* **4**, e642 (2013).
22. Bünger, J. *et al.* Cytotoxicity of occupationally and environmentally relevant mycotoxins. *Toxicology* **202**, 199–211 (2004).
23. Hung, M., Van, L., Song, Y. W. & Cho, S. K. Effects of the combination of gliotoxin and adriamycin on the adriamycin-resistant non-small-cell lung cancer A549 cell line. *Mar. Drugs* **16**, 105 (2018).
24. Peterson, D. E., Collier, J. M., Katterman, M. E., Turner, R. A. & Riley, M. R. Cytotoxicity of bacterial-derived toxins to immortal lung epithelial and macrophage cells. *Appl. Biochem. Biotechnol.* **160**, 751–763 (2010).
25. Schulz, T., Senkpiel, K. & Ohgke, H. Comparison of the toxicity of reference mycotoxins and spore extracts of common indoor moulds. *Int. J. Hyg. Environ. Health.* **207**, 267–277 (2004).
26. Kreja, L. & Seidel, H. J. On the cytotoxicity of some microbial volatile organic compounds as studied in the human lung cell line A549. *Chemosphere* **49**, 105–110 (2002).
27. Kosmidis, C. & Denning, D. W. The clinical spectrum of pulmonary aspergillosis. *Thorax* **70**, 270–277 (2015).
28. Munoz, P., Guinea, J. & Bouza, E. Update on invasive aspergillosis: clinical and diagnostic aspects. *Clin. Microbiol. Infect.* **12**, 24–39 (2006).
29. Husain, S. & Camargo, J. F. Invasive Aspergillosis in solid-organ transplant recipients: guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. *Clin. Transplant.* **33**, e13544 (2019).
30. Mayer, S., Engelhart, S., Kolk, A. & Blome, H. The significance of mycotoxins in the framework of assessing workplace related risks. *Mycotoxin Res.* **24**, 151–164 (2008).
31. Klotz, L. O. *et al.* Redox regulation of FoxO transcription factors. *Redox Biol.* **6**, 51–72 (2015).
32. Zorov, D. B., Juhaszova, M. & Sollott, S. J. Mitochondrial ROS-induced ROS release: an update and review. *Biochim. Biophys. Acta Bioenerg.* **1757**, 509–517 (2000).
33. Pfaffenbach, K. T. & Lee, A. S. The critical role of GRP78 in physiologic and pathologic stress. *Curr. Opin. Cell Biol.* **23**, 150–156 (2011).
34. Radons, J. The human HSP70 family of chaperones: where do we stand?. *Cell Stress Chaperones* **21**, 379–404 (2016).
35. Margalit, A., Kavanagh, K. & Carolan, J. C. Characterization of the proteomic response of A549 cells following sequential exposure to *Aspergillus fumigatus* and *Pseudomonas aeruginosa*. *J. Proteome Res.* **19**, 279 (2020).
36. Stanimirovic, Z., Stevanovic, J., Bajic, V. & Radovic, I. Evaluation of genotoxic effects of fumagillin by cytogenetic tests in vivo. *Mutat. Res.* **628**, 1–10 (2007).
37. Nouri, M. A., Al-Halbosiy, M. M., Dheeb, B. I. & Hashim, A. J. Cytotoxicity and genotoxicity of gliotoxin on human lymphocytes in vitro. *JKSUS* **27**, 193–197 (2015).
38. Baust, H. *et al.* Evidence for radiosensitizing by gliotoxin in HL-60 cells: implications for a role of NF-κ B independent mechanisms. *Oncogene* **22**, 8786–8796 (2003).
39. Sin, N. *et al.* The anti-angiogenic agent fumagillin covalently binds and inhibits the methionine aminopeptidase, MetAP-2. *Proc. Natl. Acad. Sci* **94**, 6099–6103 (1997).
40. Kidoikhammouan, S. *et al.* Blocking of methionine aminopeptidase-2 by TNP-470 induces apoptosis and increases chemosensitivity of cholangiocarcinoma. *J. Cancer Res Ther.* **15**, 148 (2019).
41. Guruceaga, X. *et al.* Fumagillin, a mycotoxin of Aspergillus fumigatus: biosynthesis, biological activities, detection, and applications. *Toxins* **12**, 7 (2020).
42. Zheng, J. *et al.* Gliotoxin produced by Aspergillus fumigatus induces apoptosis of human bronchial epithelial cells via the Bcl-2 pathway. *Int. J. Clin. Exp. Med.* **10**, 8854–8865 (2017).
43. Guilford, F. T. & Hope, J. Deficient glutathione in the pathophysiology of mycotoxin-related illness. *Toxins* **6**, 608–623 (2014).
44. Bernardo, P. H., Brasch, N., Chai, C. L. & Waring, P. A novel redox mechanism for the glutathione-dependent reversible uptake of a fungal toxin in cells. *J. Biol Chem.* **278**, 46549–46555 (2003).
45. Chen, J. *et al.* Reduced-gliotoxin induces ROS-mediated anoikis in human colorectal cancer cells. *Int. J. Oncol.* **52**, 1023–1032 (2018).
46. Johannessen, L. N., Nilsen, A. M. & Løvik, M. Mycotoxin-induced depletion of intracellular glutathione and altered cytokine production in the human alveolar epithelial cell line A549. *Toxicol. Lett.* **168**, 103–112 (2007).
47. Saleh, A. A. *et al.* Systems impact of zinc chelation by the epipolythiodioxopiperazinedithiolgliotoxin in *Aspergillus fumigatus*: a new direction in natural product functionality. *Metallomics* **10**, 854–866 (2018).
48. Choi, H. S., Shim, J. S., Kim, J. A., Kang, S. W. & Kwon, H. J. Discovery of gliotoxin as a new small molecule targeting thioredoxin redox system. *Biochem. Biophys. Res. Commun.* **359**, 523–528 (2007).
49. Jia, X. *et al.* Gliotoxin promotes *Aspergillus fumigatus* internalization into type II human pneumocyte A549 cells by inducing host phospholipase D activation. *Microbes Infect.* **16**, 491–501 (2014).
50. Zhang, C. *et al.* Gliotoxin induces cofilin phosphorylation to promote actin cytoskeleton dynamics and internalization of *Aspergillus fumigatus* into type II human pneumocyte cells. *Front. Microbiol.* **10**, 1345 (2019).
51. Frottin, F. *et al.* MetAP1 and MetAP2 drive cell selectivity for a potent anti-cancer agent in synergy, by controlling glutathione redox state. *Oncotarget* **7**, 63306 (2016).

52. Chen, F. *et al.* Transcriptome profiles of human lung epithelial cells A549 interacting with *Aspergillus fumigatus* by RNA-Seq. *PLoS ONE* **10**, e0135720 (2015).
53. Sugui, J. A. *et al.* Gliotoxin is a virulence factor of *Aspergillus fumigatus*: gliP deletion attenuates virulence in mice immunosuppressed with hydrocortisone. *Eukaryot. Cell* **6**, 1562–1569 (2007).
54. Gayathri, L., Dhanasekaran, D. & Akbarsha, M. A Scientific concepts and applications of integrated discrete multiple organ co-culture technology. *J. Pharmacol. Pharmacother.* **6**, 63 (2015).
55. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **65**, 55–63 (1983).
56. Chou, T. C. Theoretical basis, experimental design, and computerized simulation of synergism and antagonism in drug combination studies. *Pharmacol. Rev.* **58**, 621–681 (2006).
57. Wang, H. & Joseph, J. A. Quantifying cellular oxidative stress by dichlorofluorescein assay using microplate reader. *Free Radic. Biol. Med.* **27**, 612–616 (1999).
58. Singh, N. P. A., McCoy, M. T., Tice, R. R. & Schneider, E. L. Simple technique for quantitation of low levels of DNA damage in individual cells. *Exp. Cell Res.* **175**, 184–191 (1988).
59. Reers, M., Smith, T. W. & Chen, L. B. J-aggregate formation of a carbocyanine as a quantitative fluorescent indicator of membrane potential. *Biochemistry* **30**, 4480–4486 (1991).
60. Darzynkiewicz, Z. & Juan, G. DNA content measurement for DNA ploidy and cell cycle analysis. *Curr. Protoc. Cytom.* **1**, 5–7 (1997).
61. Rieger, A. M., Nelson, K. L., Konowalchuk, J. D. & Barreda, D. R. Modified annexin V/propidium iodide apoptosis assay for accurate assessment of cell death. *JoVE* **50**, e2597 (2011).
62. Dubrovskaya, V. A. & Wetterhahn, K. E. Effects of Cr(VI) on the expression of the oxidative stress genes in human lung cells. *Carcinogenesis* **19**, 1401–1407 (1998).
63. Speckmann, B. *et al.* Proinflammatory cytokines down-regulate intestinal selenoprotein P biosynthesis via NOS2 induction. *Free Radic. Biol. Med.* **49**, 777–785 (2010).
64. Ahamed, M. *et al.* Oxidative stress mediated apoptosis induced by nickel ferrite nanoparticles in cultured A549 cells. *Toxicology* **283**, 101–108 (2011).
65. Aueviriyavit, S., Phummiratch, D., Kulthong, K. & Maniratanachote, R. Titanium dioxide nanoparticles-mediated in vitro cytotoxicity does not induce Hsp70 and Grp78 expression in human bronchial epithelial A549 cells. *Biol. Trace Elem. Res.* **149**, 123–132 (2012).
66. Silva, J. D. *et al.* Influence of the *Paracoccidioides brasiliensis14*-3-3 and gp43 proteins on the induction of apoptosis in A549 epithelial cells. *Mem. Inst. Oswaldo Cruz* **110**, 476–484 (2015).
67. Galoian, K. *et al.* Effect of cytostatic proline rich polypeptide-1 on tumor suppressors of inflammation pathway signaling in chondrosarcoma. *Mol. Clin. Oncol.* **5**, 618–624 (2016).

Acknowledgements

We heartily acknowledge the gift of IdMOC 96 well plates from Dr. Albert P. Li, CEO, In Vitro ADMET Laboratories, USA. We thank, DST supported National Facility for Drug Development for academia, pharmaceutical and allied industries, Anna University Tiruchirappalli, and Doerenkamp-Zbinden Foundation, Switzerland, for the instrumentation support.

Author contributions

L.G. contributed to the idea, design and implementation of the research, analysis of the results and writing of the manuscript. M.A.A. contributed to the final version of the manuscript. K.R. supervised the whole project. All authors have read and approved the manuscript.

Funding

Financial assistance from the Department of Science and Technology- Science and Engineering Research Board (DST-SERB), Government of India (Project No. PDF/2016/002111), is heartily acknowledged.

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to K.R.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2020

SCIENTIFIC REPORTS
nature research

Check for updates

OPEN

In vitro study on aspects of molecular mechanisms underlying invasive aspergillosis caused by gliotoxin and fumagillin, alone and in combination

Loganathan Gayathri[1,2,3], Mohammad A. Akbarsha[4,5] & Kandasamy Ruckmani[1,2]✉

Gliotoxin (GT) and fumagillin (FUM) are mycotoxins most abundantly produced by *Aspergillus fumigatus* during the early stages of infection to cause invasive aspergillosis (IA). Therefore, we hypothesized that GT and FUM could be the possible source of virulence factors, which we put to test adopting in vitro monoculture and the novel integrated multiple organ co-culture (IdMOC) of A549 and L132 cell. We found that (i) GT is more cytotoxic to lung epithelial cells than FUM, and (ii) GT and FUM act synergistically to inflict pathology to the lung epithelial cell. Reactive oxygen species (ROS) is the master regulator of the cytotoxicity of GT, FUM and GT + FUM. ROS may be produced as a sequel to mitochondrial damage and, thus, mitochondria are both the source of ROS and the target to ROS. GT-, FUM- and GT + FUM-induced DNA damage is mediated either by ROS-dependent mechanism or directly by the fungal toxins. In addition, GT, FUM and GT + FUM may induce protein accumulation. Further, it is speculated that GT and FUM inflict epithelial damage by neutrophil-mediated inflammation. With respect to multiple organ cytotoxicity, GT was found to be cytotoxic at IC_{50} concentration in the following order: renal epithelial cells < type II epithelial cells < hepatocytes < normal lung epithelial cells. Taken together, GT and FUM alone and in combination contribute to exacerbate the damage of lung epithelial cells and, thus, are involved in the progression of IA.

Abbreviations

DMSO	Dimethyl sulfoxide
FUM	Fumagillin
GT	Gliotoxin
IC_{50}	Concentration at which 50% of cells are dead
IdMOC	Integrated discrete multiple organ co-culture
IA	Invasive aspergillosis
MTT	3-4, 5-Dimethylthiazole-2-yl, 2,5-diphenyl tetrazolium bromide
ROS	Reactive oxygen species

Aspergillus fumigatus, the abundantly distributed saprophytic fungus, is a weak pathogen. Generally, *A. fumigatus* releases air-borne, dormant, buoyant microscopic conidiospores in copious amounts, so that a person would

[1]Department of Pharmaceutical Technology, University College of Engineering, Anna University-BIT Campus, Tiruchchirappalli, Tamil Nadu 620024, India. [2]Centre for Excellence in Nanobio Translational Research (Autonomous), University College of Engineering, Anna University-BIT Campus, Tiruchchirappalli, Tamil Nadu 620024, India. [3]Department of Biotechnology and Bioinformatics, Holy Cross College (Autonomous), Tiruchchirappalli, Tamil Nadu 620002, India. [4]National College (Autonomous), Tiruchchirappalli, Tamil Nadu 620001, India. [5]Mahatma Gandhi-Doerenkamp Centre for Alternatives, Bharathidasan University, Tiruchchirappalli, Tamil Nadu 620 024, India. ✉email: ruckmani@aubit.edu.in

Mycotoxin	Level of GT in lungs	Level of GT in serum	Experimental model	Reference
GT	3,976 ± 1,662 ng/g	36.5 ± 30.28 ng/ml	Mice	[2]
GT	–	166 to 785 ng/ml	Patients with cancer	[2]
GT	40–63 µg/kg (sputum)	33–47 µg/kg	Patients with pulmonary aspergillosis	[11]
Bis(methylthio)gliotoxin	0.19–13.68 mg/L (bronchoalveolar lavage or sputum)	–	Patients with IA	[12]
GT	–	0.19–0.25 µg/ml	Patients with high risk for IA	[13]
Bis(methylthio)gliotoxin	–	0.46–49.84 µg/ml	Patients with high risk for IA	[11]

Table 1. Summary of Serum levels of GT in mice and human.

potentially inhale hundreds of spores every day[1]. After successful permeation of airway mucosa, the spores encounter the airway epithelial cells where the first line immune response of the host is likely to begin[1,2]. After vigorous hyphal growth for 24 h, the fungus may produce some secondary metabolites that breach the epithelial/endothelial barrier. Once the epithelial layer of the alveoli is damaged, the fungus enters the endothelium of blood vessels to become an angiotrophic fungus whereupon the disease becomes invasive and spreads to other organs[2,3]. However, immunocompetent healthy individuals are able to deal with the fungal conidia by the several immune mechanisms that prevent the germination and growth of hyphae whereas in immunocompromised persons conidial germination and mycelial development in the lung epithelial cells can cause severe/fatal disease called Invasive Aspergillosis (IA)[1,3].

Reports have shown that IA is now a major cause of death at leukemia treatment, bone marrow transplantation and solid-organ transplantation centers and it is responsible for 30% of fungal infections in patients dying of cancer wherein the mortality rate of leukemia patients is 80 to 90%, even when given antifungal therapy[2,3]. Thus, anti-*A. fumigatus* therapy remains challenging as a consequence of high mortality from IA[4]. One of the major reasons for this failure could be poor understanding of the colonization, pathobiology and virulence factors of *A. fumigatus* because every pathogen is capable of developing strategies to disseminate hyphal growth and evade host immune surveillance during infection[3,4]. In order to fight against the first-line host innate immune response, *A. fumigatus* adopts a strategy that involves discharge of mycotoxins to damage the epithelial/endothelial barriers of the respiratory tract. For instance, it has been reported that *A. fumigatus* relies on secreted proteases, lipases, and/or toxins for their survival benefits but none of these have been explored for pathogenesis of IA except mycotoxins[5].

Mycotoxins are secondary metabolites of fungi which are not vital for their lifecycle but offer competitive survival advantage over the host immunity. *A. fumigatus* produces a variety of mycotoxins/secondary metabolites such as gliotoxin, fumagillin, fumitremorgin, verruculogen, restrictocin, helvolic acid, etc. Although the pathogenicity of IA is multifactorial, gliotoxin has been proved to be a virulent factor of *A. fumigatus* but the specific roles of the other mycotoxins are not well defined[1,3,4]. Indeed, the combined action of two or more of these mycotoxins may produce synergistic effects against the host's defense mechanisms. In the light of the fact that two or more mycotoxins would interact synergistically or additively and produce more serious adverse effects than single compounds[6,7], it is pertinent that this issue in respect of highly virulent mycotoxins produced by A. *fumigatus* is worthy of being addressed.

Gliotoxin (GT), a hydrophobic metabolite, belongs to the class of epipolythiodioxopiperazine compounds characterized by a quinoid moiety and disulfide bridge across the piperazine ring which is essential for their toxicity[8]. GT is a well-studied immune-suppressive mycotoxin that is produced against the first line immune response of epithelial barrier of the host. GT is known to induce apoptosis in leukocytes, and inhibit phagocytosis, respiratory burst, and T-cell and B-cell responses stimulated by the host. Also, GT has received considerable attention as a pathogenic and putative virulence factor as revealed in the following observations: (i) GT was detected in the lung and serum of cancer patients suffering from IA as well as in mice with experimentally induced IA (Table 1); (ii) up to 93% of *A. fumigatus* strains isolated from cancer patients suffering from IA produce GT[9]; and (iii) GT was found to be produced much faster at 37 °C under high levels of oxygen which is close to that of the environment of host's lung[10].

Fumagillin (FUM) is a cyclohexane derivative, and identified as a potent angiogenesis inhibitor that reduces the proliferation of endothelial cells for blood vessel formation to avoid infiltration of host's immune cells to the site of infection[14]. Further, FUM is also involved in IA during hyphal invasion and thereby damage the epithelial layer, inhibit neutrophils and slow down beating of cilia of ciliated epithelial cells[14–17]. In fact, *A. fumigatus* produces both GT and FUM during the early stages of IA, within 30 to 72 h[14,15]. This may facilitate the fungus escape the immune surveillance and propagate the disease so as to become invasive. Despite the fact that the fungal virulence/pathogenicity is mediated by mycotoxins, the relatively unexplored frontier of the lung epithelial interface of either of the mycotoxins alone and/or the two in combination is an untouched medically important area of research that may open up avenues to find new therapies for IA[18].

Therefore, in this study we aimed at finding the molecular mechanism by which GT and FUM alone, as well as in combination, affect the epithelial cell barrier of the lung, adopting in vitro approach. Human origin A549 (lung carcinoma) and L132 (normal) lung epithelial cells that are the most established models to study IA were chosen to explore cytotoxicity, genotoxicity, cell cycle arrest, mechanism of cell death and pro-inflammatory responses against GT and FUM individually as well as in combination. As the disease progresses to invasiveness, we further

analyzed organ-specific toxicity of GT in A549 (lung), L132 (lung), HepG2 (liver) and HEK2913 (kidney) epithelial cells by adopting a novel co-culture technique, integrated discrete multiple organ co-culture (IdMOC).

Results

Cytotoxic effect of GT and FUM alone and in combination on lung epithelial cell. Exposure to GT caused dose-dependent increase in cytotoxicity at the concentration range 0–10 μM and the measured IC_{50} was 2.7 and 4.25 μM for A549 and L132, respectively (Fig. 1a). As shown in Fig. 1b, FUM at the concentration range 0–60 μM resulted in significant increase of cytotoxicity in dose-dependent manner, and the IC_{50} was 40 μM and 50 μM for A549 and L132, respectively. Based on these individual IC_{50} values cells, GT and FUM were combined at 10%, 20%, 30%, 40% and 50% of the respective IC_{50} concentrations as shown in Fig. 1c. The combination of GT and FUM at 20% of the respective IC_{50} (GT 0.54 μM + FUM 8 μM) against A549 cells and 40% of the respective IC_{50} (GT 1.7 μM + FUM 20 μM) against L132 cells significantly increased cytotoxicity to 50%. Figure 1d–g show that individual doses of GT and FUM alone, at the precise concentrations used in combinatorial experiment could not significantly increase 50% cytotoxicity against A549 and L132 cells. This is evidence to the effect that the combinatorial effect is only because of the combination of GT + FUM and not due to individual doses in the combination of GT and FUM against A549 and L132 cells. Also, according to Table 2, the combinations of GT + FUM used in the study showed synergistic interaction at the particular ratio. Therefore, we have used the following dose for treatment purpose throughout the study: IC_{50} concentration of GT (2.7 μM), FUM (40 μM) and GT + FUM (0.54 μM + 8 μM) against A549 and IC_{50} concentration of GT (4.25 μM), FUM (50 μM) and GT + FUM (1.7 μM + 20 μM) were used against L132 cells.

Effect of GT and FUM alone and in combination on induction of reactive oxygen species (ROS) and oxidative stress gene expression in lung epithelial cells. The ability of GT and FUM, alone and in combination, to induce oxidative stress was evaluated. Accumulation of ROS resulted in oxidation of H_2-DCF to become highly fluorescent DCF, which was observed as green fluorescence in both A549 and L132 cells (Fig. 2a). The results in Fig. 2b and c demonstrate time-dependent increase of ROS in both A549 and L132 cells. H_2O_2 was used as the positive control for ROS, and the significant increase of ROS was compared with data of test mycotoxins.

Further, we analyzed the changes in transcript levels of antioxidant/stress-responsive genes such as Forkhead Box O1 (FOXO1), Catalase, Superoxide dismutase 1 (SOD1), Glutathione S-Transferase P1 (GSTP1), Glutathione reductase (GR), Glutathione peroxidase 1 (GPx1) and Glutathione peroxidase 2 (GPx2). As shown in Fig. 2d and e, expression of stress-responsive transcription factor FOXO was elevated to significant level and, consequently, the expression of anti-oxidant genes that are related to FOXO transcription are also significant in A549 and L132 cells exposed to GT, FUM and GT + FUM for 24 h.

Effect of GT and FUM alone and in combination, on mitochondrial trans-membrane potential (ΔΨm). JC-1 is a probe which indicates loss of ΔΨm by shift in fluorescence emission from red to green. As shown in Fig. 3, control (untreated) cells fluoresced red and cells treated with GT, FUM and GT + FUM fluoresced only green indicating that ΔΨm was lost completely in both A549 and L132 cells within 24 h.

Evidence for accumulation of unfolded protein on treatment of GT, FUM and in combination in lung epithelial cells. Induction of GRP78 and HSP70 transcript levels was evaluated to confirm the accumulation of unfolded proteins in the lumen of the endoplasmic reticulum (ER). Figure 4a and b revealed significant increase in expression of GRP78 and HSP70 chaperons in A549 and L132 cells exposed to the respective concentrations of GT, and FUM each alone and in combination for 24 h. This confirms that GT and FUM each alone and in combination may increase the level of unfolded protein inside the cells and thus pose a chance to induce ER stress.

Genotoxicity of GT and FUM alone and in combination revealed in comet assay. As shown in Fig. 5a, both GT and FUM caused significant damage to DNA wherein the impact was more with GT than FUM and, in addition, the combination of GT + FUM also caused significant damage to DNA of A549 and L132 cells. Thus, the genotoxic potential of GT, FUM and GT + FUM against lung epithelial cells was confirmed. Since the tail length and density reflected the extent of strand breaks in DNA, the percentage of DNA in the tail provided a quantitative measure of significant DNA damage as shown in Fig. 5b and c.

Induction of cell cycle arrest in lung epithelial cell by GT and FUM alone and in combination. The results showed that exposure of A549 and L132 cells to GT for 24 h resulted in significant increase in the proportion of cells in S-phase (30–39%) with concomitant decrease in G2/M-phase. Exposure to FUM caused significant increase of cells in G1-phase (55–63%) with concomitant decrease of cells in S-phase compared to control group. As a combination, GT + FUM produced significant decrease of cells in G1, S, G2/M phases and increase in the sub-G1 phase in both the cell lines (Fig. 6a–d).

Mode of cell death in lung epithelial cell induced by GT and FUM alone and in combination. Annexin V FITC-A *vs* Propidium Iodide-A plots of A549 (Fig. 7a) and L132 (Fig. 7b) cells show population of cells corresponding to viable and non-apoptotic (Annexin V^- PI^-), early (Annexin V^+ PI^-), and late (Annexin V^+ PI^+) apoptotic cells. In the control (untreated) A549 (Fig. 7c) and L132 (Fig. 7d) samples, majority of cells (98%) were viable and non-apoptotic (Annexin V^- PI^-). In contrast, when A549 (Fig. 7c) and L132

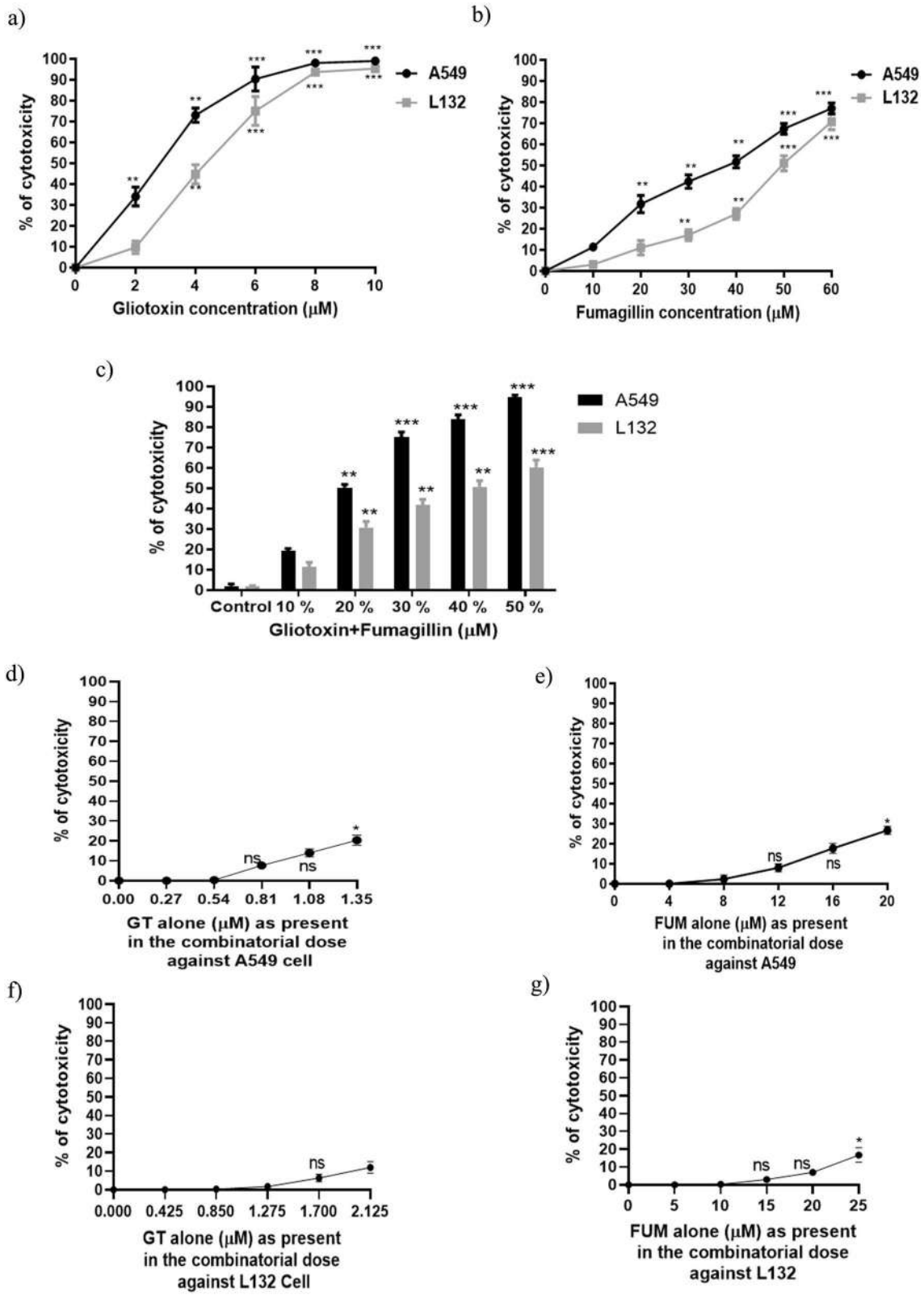


Figure 1. Cytotoxic effect of GT and FUM alone and in combination against A549 and L132 cells following 24 h of exposure. (**a**) Dose–response curve of GT at different concentrations. (**b**) Dose–response curve of FUM at different concentrations. (**c**) Combined cytotoxicity of GT + FUM. (**d**) Dose–response curve of GT alone as present in the combinatorial dose for A549 cell. (**e**) Dose–response curve of FUM alone as present in the combinatorial dose for A549 cell. (**f**) Dose–response curve of GT alone as present in the combinatorial dose for L132 cell, (**g**) Dose–response curve of FUM alone as present in the combinatorial dose for- L132 cell. Data are expressed as mean ± SD of three independent experiments for each dose point (n = 18 for panels (a, d, e, f, g), n = 21 for panel b, n = 36 for panel c). Significance between control and treatment groups was tested by adopting two way ANOVA (ns = not significant, *p < 0.5, **p < 0.01 and ***p < 0.001).

Mycotoxin	Cell line	Dose effect parameter			CI values of combinatorial dose				
		D_m (μM)	m	r	10%	20%	30%	40%	50%
GT	A549	2.51	3.18	0.98					
FUM	A549	35.56	1.54	0.99					
GT	L132	3.86	3.26	0.99					
FUM	L132	49.88	2.75	0.99					
GT + FUM	A549	7.96	2.63	0.97	0.44	0.44	0.41	0.40	0.269
GT + FUM	L132	20.83	1.56	0.99	0.43	0.55	0.72	0.84	0.91

Table 2. Synergistic interaction of GT and FUM in A549 and L132 cells. Dm and m values are used for calculating the CI value, i.e., CI < 1, CI = 1 and CI > 1 represent synergism, additivity and antagonism, respectively. Here, Dm, m and r stand for median-effect dose, kinetic order and regression coefficient of the fitting function. The ratios of GT + FUM is 1:14.8 and 1:11.76 against A549 and L132, respectively. CompuSyn software was used for calculation and simulation.

(Fig. 7d) cells treated with GT, FUM and GT + FUM in combination for 24 h, the percentage of Annexin V^- PI^- cells decreased and early apoptotic cell population (Annexin V^+ PI^-) increased significantly (Fig. 7c,d). Also, increase in Annexin V^+ PI^+ population was observed which indicates late apoptotic cells. In addition, GT, FUM and GT + FUM-mediated induction of apoptotic cell death was confirmed by microscopic analysis of Annexin V FITC/PI staining assay in A549 (Fig. 8a) and L132 (Fig. 8b) cells. In conclusion, GT, FUM and GT + FUM induced apoptotic mode of cell death in lung epithelial cells.

Evidence for induction of intrinsic-pathway of apoptosis by GT and FUM alone and in combination at molecular level. In order to understand the pathway of apoptosis, transcript levels of BAK, BAX, BID, Bcl-2, caspase-8 and caspase-3 were determined. As shown in Fig. 9a and b expression levels of anti-apoptotic Bcl-2 and the initiator caspase (caspase-8) of extrinsic pathway were decreased but expression levels of BAK, BAX, BID and caspase-3 were significantly increased in both A549 and L132 cells. Results of caspase 3 and 8 enzyme activity assays, shown in Fig. 9c and d, confirm that GT, FUM and GT + FUM induced the activity of caspase 3 but not caspase 8 in A549 and L132 cells. Thus, caspase activity analysis supports caspase 3 and 8 RNA expression analysis.

Secretion of pro-inflammatory cytokines by A549 and L132 epithelial cells against GT, FUM and GT + FUM. As shown in Fig. 10a, expression levels of IL4, IL-8 and IL-10 were significantly increased against GT whereas IL-8 alone was increased against FUM in A549 cell. In the case of L132 cell, IL-6 and IL-8 cytokine expression levels were significantly increased against GT whereas IL-8 alone was induced by FUM (Fig. 10b). As a combination GT + FUM did not induce expression of any cytokine within 24 h. Apart from up-regulation of a few cytokines as above, GT, FUM alone as well as in combination down-regulated the expressions of a few cytokines such as IL-1β, IL-17, IFN-γ, TNF-α and GM-CSF (Fig. 10a,b).

Organ-specific cytotoxicity of GT as evidenced by IdMOC. IdMOC is a novel cell culture system that can be utilized for the evaluation of toxicity, especially for studying the comparative cytotoxicity of an agent towards multiple cell types. Therefore, in one set, A549, HepG2, and HEK293 cells in duplicates and in the other set, L132, HepG2 and HEK293 cells in duplicates were co-cultured and exposed to GT under identical conditions in the concentration range 0–7 μM for 24 h (Fig. 10c,d). The IC_{50} values for HepG2 (3 μM) and HEK293 (2.1 μM) were higher than for A549 (2.5 μM); and IC_{50} values of L132 (4.2 μM) and HepG2 (3 μM) were higher than for HEK293 (2.2 μM). The rank order of the multiple cell types towards GT cytotoxicity, from the most sensitive to the most resistant, based on the different IC_{50} values are as follows: IC_{50}: HEK293 > A549 > HepG2 > L132.

Discussion

One of the open questions in the context of IA is, how the secreted fungal mycotoxins tend to become virulent factors of *A. fumigatus* such as to damage lung epithelial cells[19]. This issue has not yet been explored with clarity, and a clear understanding can enable us to harness the defensive and offensive capabilities of *A. fumigatus* during IA. In this paper, the molecular mechanism of GT-, FUM- and GT + FUM-induced lung epithelial injury, including immune response, has been studied. Upon germination, the fungal hyphae should breach the lung alveolar surface that consists of type I and type II epithelial cells in which type I cells cover 95% of the surface and engage in gas exchange whereas type II cells cover only 5% of the surface but engage crucially in the secretion of surfactant proteins and innate immune response[2,19]. Therefore, it is important to select such cell types to study the molecular mechanism of mycotoxins to relate with IA adopting in vitro techniques. A549 is a lung carcinoma epithelial cell that represents standard model of type II cells which is capable of revealing the interaction between the pathogenic factors and the host whereas L132 is an embryonic lung epithelial cell used extensively for pulmonary toxicity studies that also manifests the phenotypes of both type I and type II alveolar cells[20,21]. Therefore, the cytotoxic potentials of GT, FUM and GT + FUM were studied in A549 and L132 cells. Results from cytotoxicity studies revealed that GT more seriously attacks epithelial cells than FUM. Previous in vitro cytotoxicity assays against A549 have also shown that GT is more cytotoxic than FUM (Table 3). Comparatively,

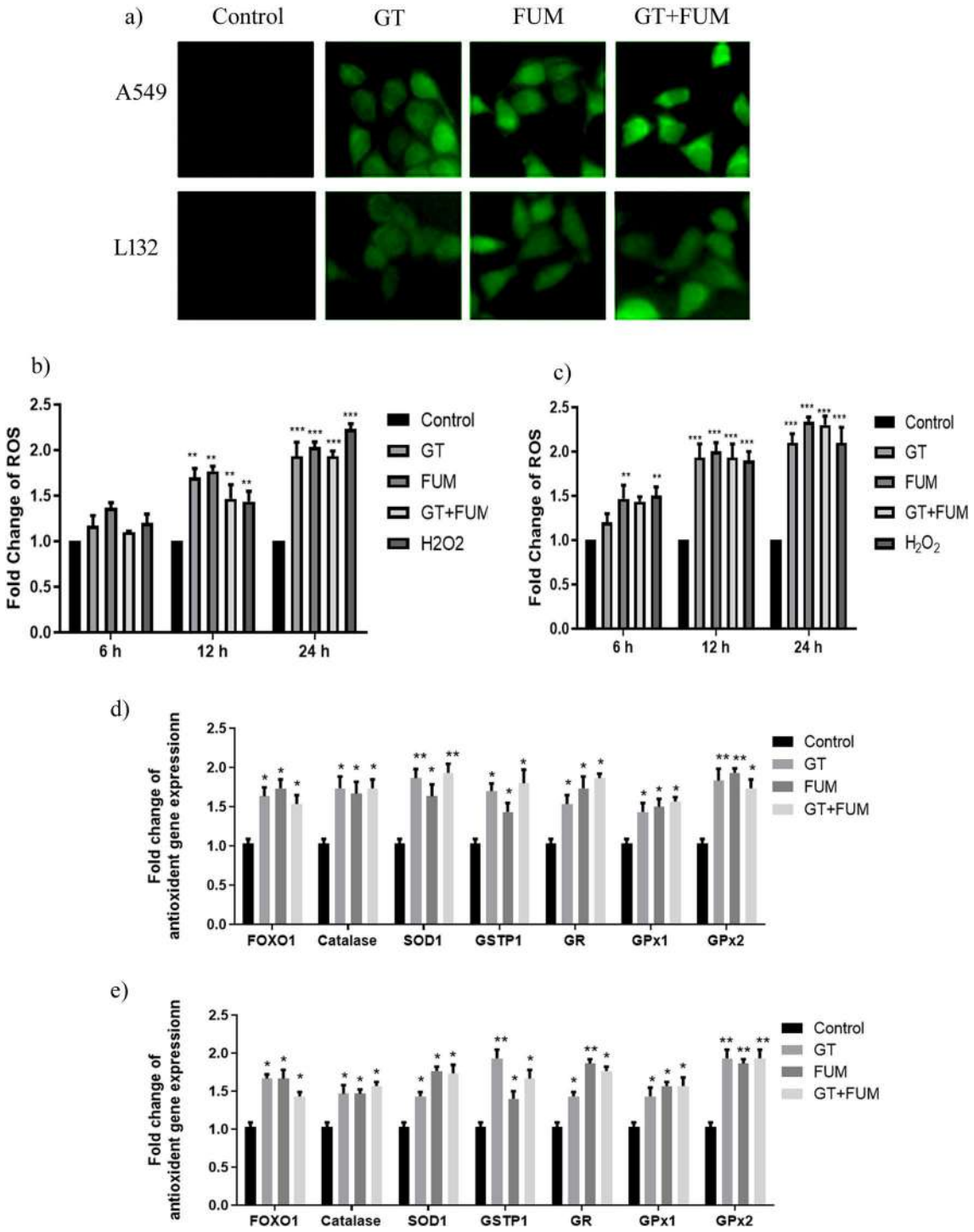


Figure 2. Effect of GT& FUM alone and in combination on induction of reactive oxygen species (ROS) and oxidative stress gene expressions. (**a**) Representative images of ROS generation after 24 h of exposure to GT, FUM and GT + FUM in A549 and L132 cells. (**b**, **c**) Quantification of intracellular ROS generation after 6 h, 12 h and 24 h of exposure to GT, FUM and GT + FUM in A549 and L132 cells, respectively. ROS level is expressed as fold change in the fluorescence in comparison with control (untreated). (**d**, **e**) Expression levels of oxidative stress related genes in A549 and L132 cells, respectively. Data are expressed as mean ± SD of three independent experiments for each dose point (n = 45 for panels b & c, n = 84 for panels **d** and **e**). Significance between control and treatment groups was tested by adopting two way ANOVA (*p < 0.5 **p < 0.01 and ***p < 0.001).

between cell lines, lung epithelial carcinoma cell (A549) is damaged faster at lower concentrations of GT, FUM and GT + FUM than normal lung epithelial cell L132. We speculate that the cancerous nature of A549 is responsible for the change in the sensitivity towards GT and FUM. On the other hand, combination of GT and FUM at

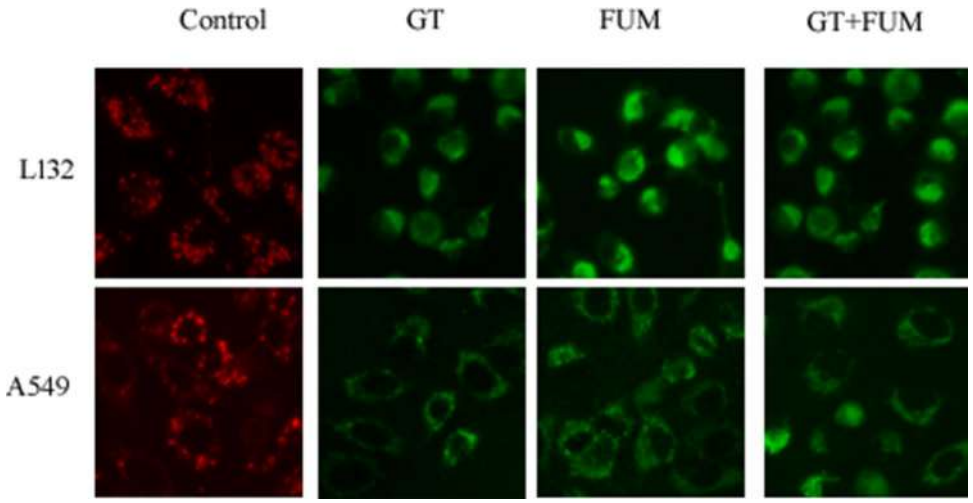


Figure 3. Photomicrographs of L132 and A549 cells showing the effect of GT, FUM alone and in combination on the change in mitochondrial trans-membrane potential at 24 h. Red color indicates high mitochondrial membrane polarization in control (untreated); Green color indicates depolarization of mitochondria in mycotoxin treated cells. Results are representative of three independent experiments.

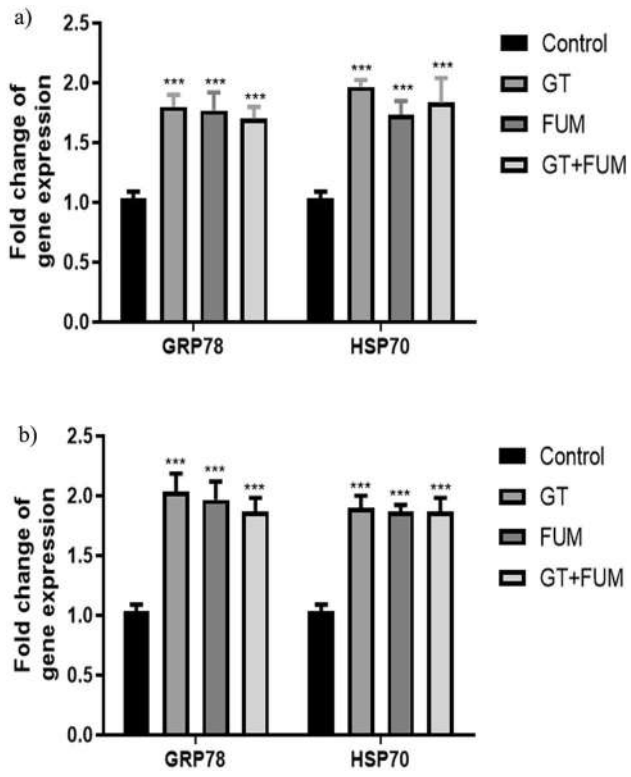


Figure 4. Effect of GT & FUM alone and in combination on gene expression of GRP78 and HSP70 chaperons. (**a**, **b**) Expression levels of GRP78 and HSP70 in A549 and L132 cells, respectively, after 24 h. Data are expressed as mean ± SD of three independent experiments for each dose point (n = 24 for panels a & b). Significance between control and treatment groups was tested by adopting two way ANOVA (***$p < 0.001$).

20% and 40% of the respective IC_{50} concentrations acting on A549 and L132 cells led to increase in cytotoxicity compared to individual toxins at their respective IC_{50} concentrations.

Though the clinical presentation of IA is relatively rapid, ranging from days to a few weeks for neutropenic host, non-neutropenic patients such as solid organ transplant recipients (particularly lung and heart–lung transplant recipients), patients with AIDS, COPD, and the critically ill intensive care unit (ICU) patients are relatively indolent and the condition progresses over weeks rather than over days (up to 12 weeks)[27–29]. Therefore, total net

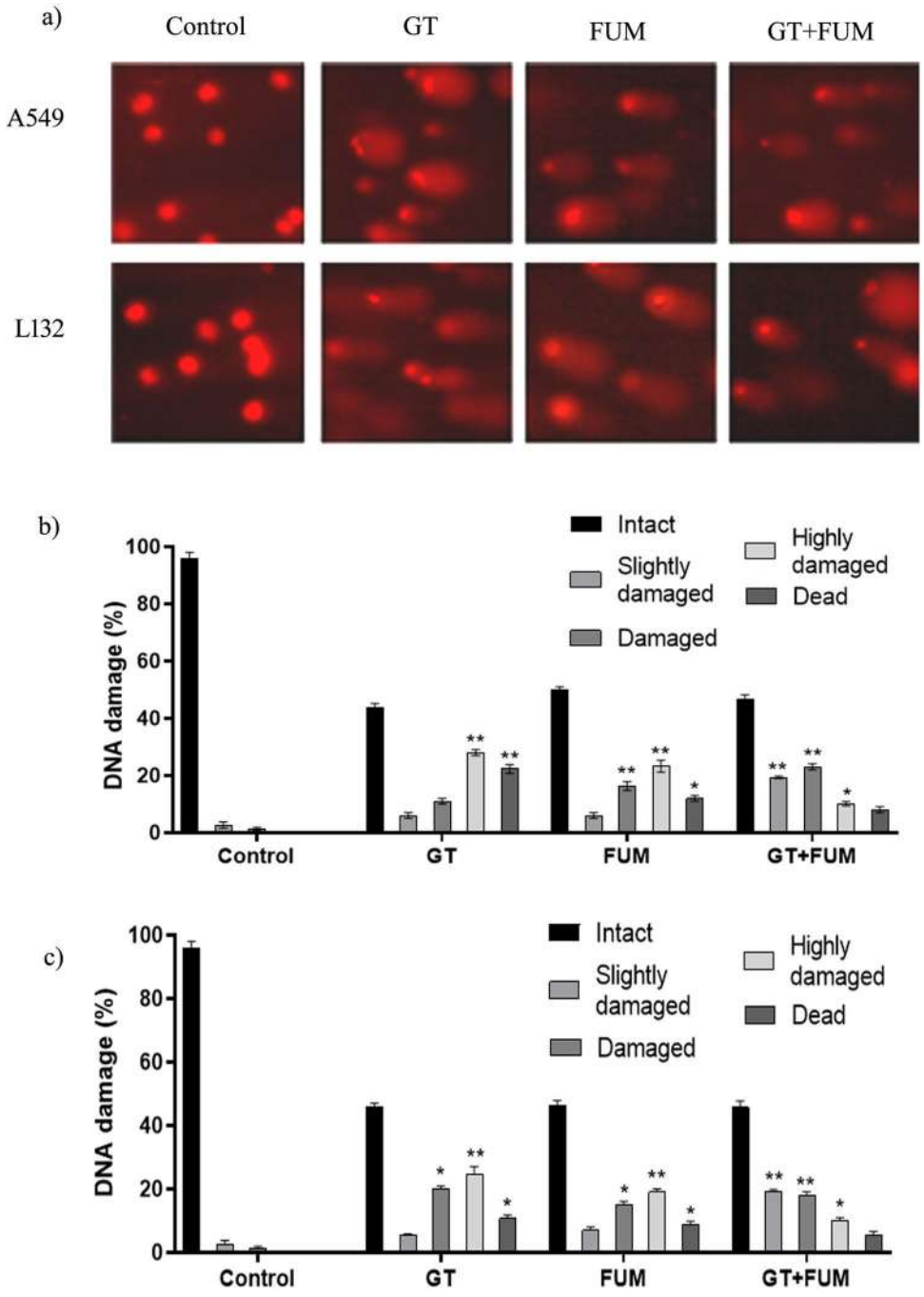


Figure 5. Assessment of DNA damage induced by GT & FUM alone and in combination after 24 h treatment in A549 and L132 cells. (**a**) Representative images of A549 and L132 cells showing DNA damage. (**b**, **c**) DNA damage (intact—0 to 20%, slightly damaged—20 to 40%, damaged—40 to 60%, highly damaged—60 to 80%, and dead—80 to 100%) as defined according to the DNA in the tail of A549 and L132 cells, respectively. Data are expressed as mean ± SD of three independent experiments for each dose point (n = 60 for panels **b** and **c**). Significance between control and treatment groups was tested by adopting two way ANOVA ((*$p < 0.5$, **$p < 0.01$).

immunosuppression of the host usually reflects the progression of fungal spread in lungs during the course of IA[27–29]. Thus, the concentration of mycotoxins produced by *A. fumigatus* may also vary between host's immune surveillances. In addition, production of mycotoxins may vary between species and even within a given species[30]. Hence, it is nearly impossible to speculate conclusively about fungi producing a toxin at a particular concentration under certain conditions. At least any such statement must remain subject to severe uncertainties[30].

However, there are a few in vitro reports to endorse the concentration of GT and FUM produced by *A. fumigatus* in which, clinical strains of *A. fumigatus* at 1×10^5 cfu can produce GT at the maximum of 21.35 µg/

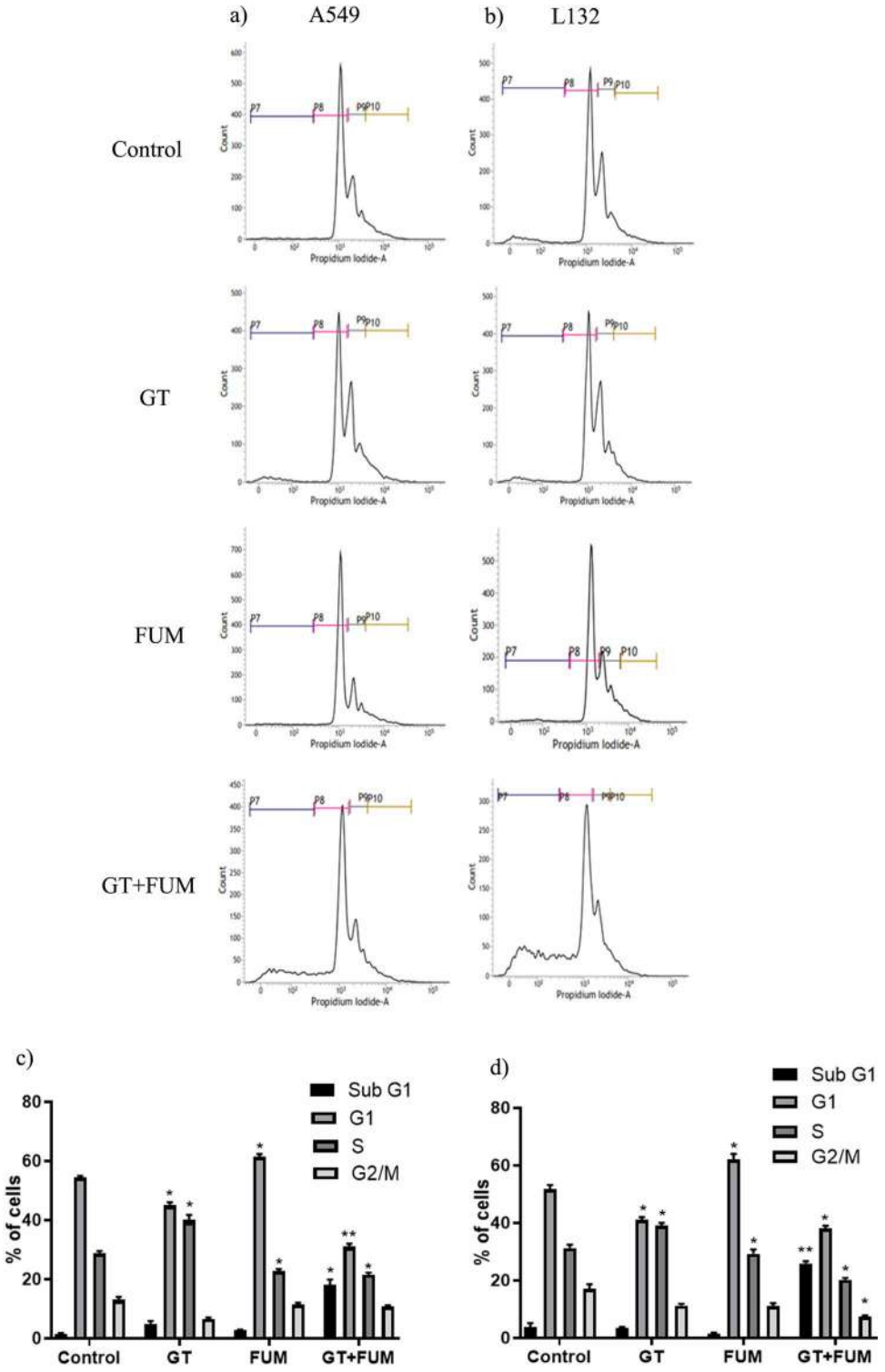


Figure 6. Assessment of cell cycle arrest induced by GT & FUM alone and in combination after 24 h treatment in A549 and L132 cells. (**a**, **b**) Representative contour plots of propidum iodide *vs* cell count for cell cycle distribution of A549 and L132 cells, respectively. Representation of P7, P8, P9 and P10 on the plot indicates Sub-G1, G1, S and G2/M respectively. (**c**, **d**) Bar diagram represents, percentage of cells in Sub-G, G1, S and G2/M phase of A549 and L132 cells, respectively. Data are expressed as mean ± SD of three independent experiments for each dose point (n = 48 for panels **c** and **d**). Significance between control and treatment groups was tested by adopting two way ANOVA (*$p < 0.5$, **$p < 0.01$).

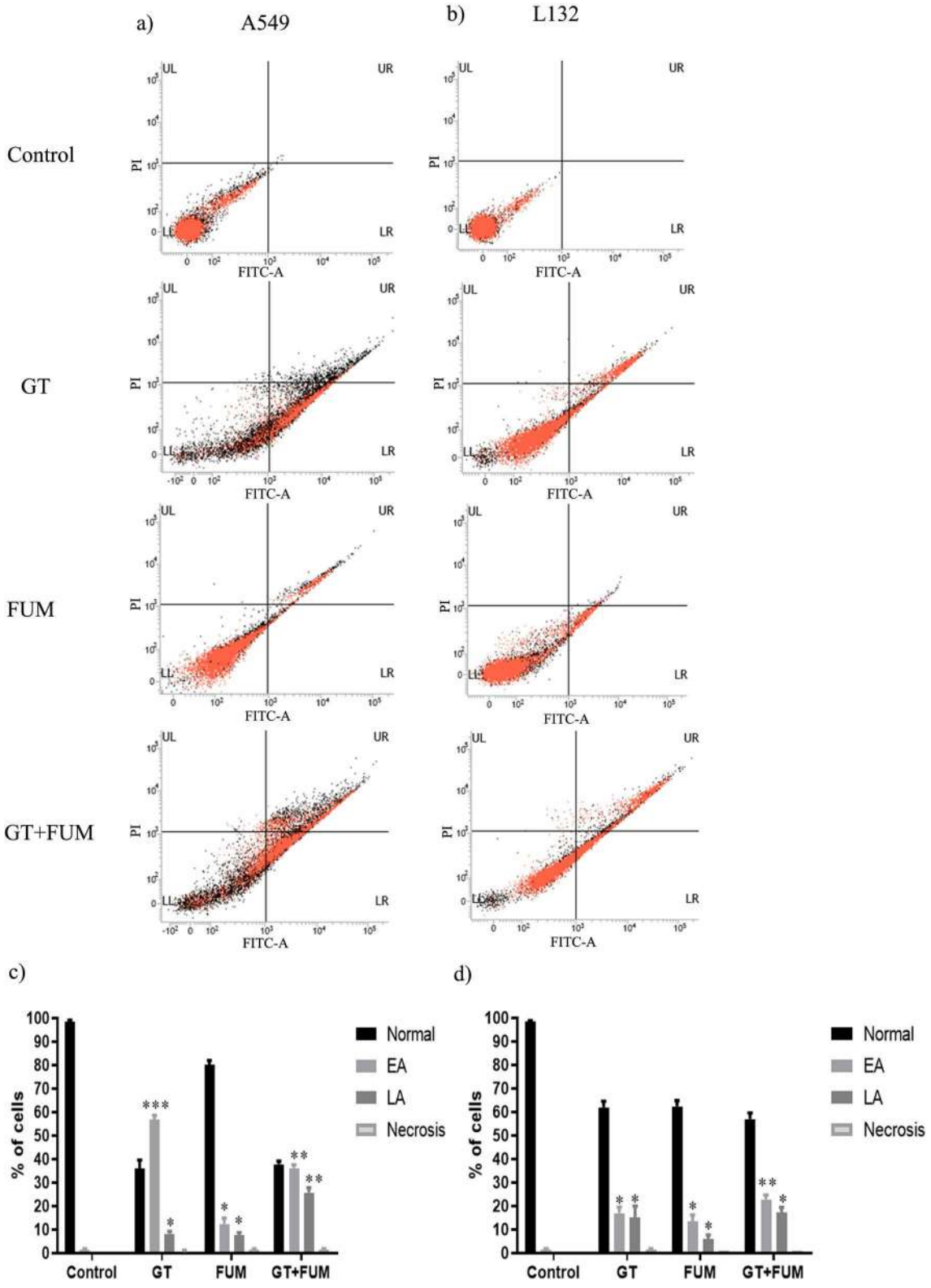


Figure 7. Assessment of apoptosis induced by GT & FUM alone and in combination after 24 h treatment in A549 and L132 cells. (**a**, **b**) Representative contour plots of Annexin V-FITC *vs* propidium iodide of A549 and L132 cells, respectively. Representation of LL, LR, UR and UL in the plot indicates normal, early apoptosis (EA), late apoptosis (LA) and necrosis cell populations respectively. (**c**, **d**) Bar diagram represents, percentage of normal, EA, LA and necrosis cells of A549 and L132 cells, respectively. Data are expressed as mean ± SD of three independent experiments for each dose point (n = 48 for panels **c** and **d**). Significance between control and treatment groups was tested by adopting two way ANOVA (*$p < 0.5$, **$p < 0.01$).

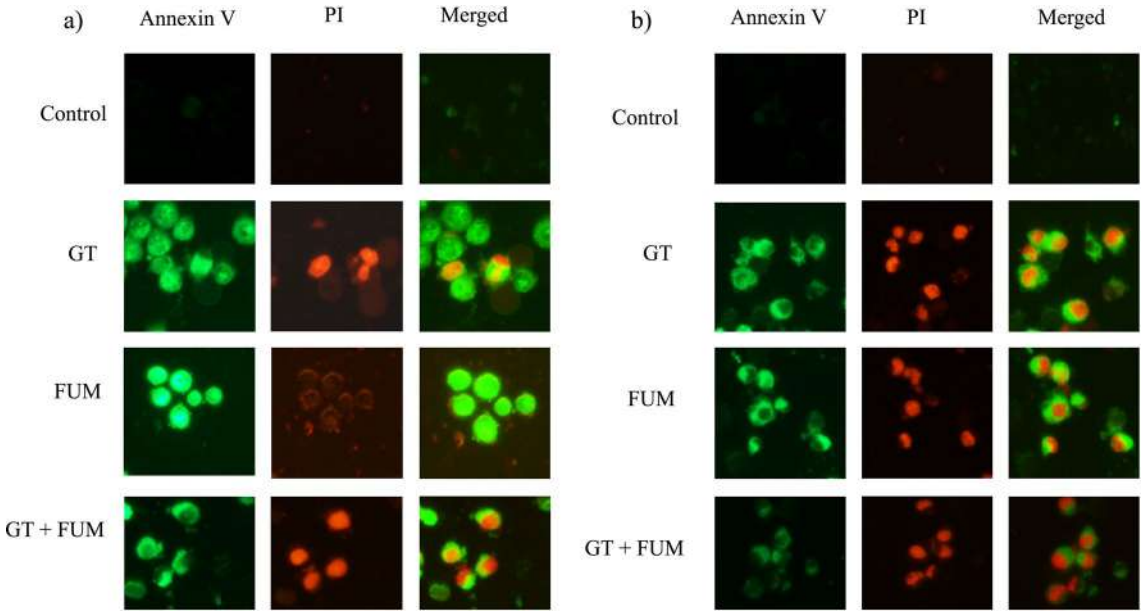


Figure 8. Photomicrographs showing the effect of GT & FUM alone and in combination on the mode of cell death as revealed by Annexin V/PI staining after 24 h. (**a**) Representative images of A549 cells. (**b**) Representative images of L132 cells. Normal cell are Annexin V^- PI^- (no fluorescence emission observed under FITC filter and TRITC filter), EA cells are Annexin V^+ PI^- (green fluorescence observed under FITC filter and no fluorescence under TRITC filter), LA are Annexin V^+ PI^+ (green fluorescence observed under FITC filter and Red fluorescence under TRITC filter), Necrosis (Annexin V^- PI^+ (no fluorescence under FITC filter and red fluorescence observed under TRITC filter).

mL and mean of 5.75 µg/mL in RPMI 1,640 medium at 37°C[9] and environmental strains of *A. fumigatus* at 9.3×10^6 cfu can produce 365 µg/mL of FUM in YES medium at 25 °C[22]. These concentrations reveal toxin producing capacity of *A. fumigatus* under favorable conditions but this may or may not be realistic in true in vivo pathophysiological condition. In addition, we can at least understand the diagnostic concentrations of GT from the Table 1 but, for FUM, no such measure is available so far to directly compare the in vivo concentrations with the studied concentrations. Therefore, our study was intended to find the half maximal cytotoxic concentrations (IC_{50}) of GT, FUM and GT + FUM instead of solely relying on particular concentrations at in vivo conditions (Table 1). Therefore, our study shows that at a defined concentration GT and FUM alone and in combination can act synergistically to induce epithelial damage to lungs.

In general, mycotoxins use ROS as a weapon to alter the cellular redox signaling to initiate destructive signals against cell endurance[6]. In order to counteract the imbalance in redox state, the host's body would kick off stress-related or antioxidant gene expressions to maintain cellular homeostasis[31]. As hypothesized, we found GT, FUM and GT + FUM to significantly induce cellular level of ROS in lung epithelial cells within 24 h and also concurrent increase in the gene expression of FOXO1, which is an important transcription factor that regulates cellular stress response and promotes cellular antioxidant defense systems such as catalase, SOD1, GSTP1, GR, GPx1 and GPx2 within 24 h in both A549 and L132 cells. Although antioxidant enzyme expressions are increased, adequate changes to reduce the level of generated ROS are not evidenced in Fig. 2b and c. To conclude further about the action of GT, FUM and GT + FUM on cellular antioxidant system, protein level enzyme activities of antioxidants are required to be studied.

Further, the elevated level of ROS guided us to find the healthy state of mitochondria that play a vital role in the sudden burst of ROS upon treatment of mycotoxins. We found an increase in mitochondrial membrane depolarization in both A549 and L132 cells by GT, FUM and GT + FUM. This shows the possibility that collapse of mitochondrial membrane is mediated by ROS or the production of ROS is followed by mitochondrial membrane depolarization. This comprehension is supported by the concept "Reactive Oxygen Species (ROS)-induced ROS-release" (RIRR) in which the excessive ROS can collapse mitochondrial membrane that would lead to temporary increase in ROS generation by the electron transfer chain[32]. Later, ROS is released to cytoplasm to inflict damage to the neighboring mitochondria. This mitochondrion-to-mitochondrion ROS-signaling signifies positive signals for better ROS production which would lead to momentous mitochondrial and cellular injuries[32].

Further, the singlet oxygen of ROS can readily react with proteins, DNA and lipid molecules of the cell. Thus, oxidative stress/pathological conditions of the cell can build unfolded proteins to accumulate in the lumen of the ER, which we studied indirectly by means of GRP78 and HSP70 expression analyses. Both GRP78 and Hsp70 are chaperons that are up-regulated inside the cells to increase the folding capacity of unfolded proteins and to assist a wide range of folding processes, including refolding of mis-folded and aggregated proteins in the ER, respectively[33,34]. The increase in GRP78 and Hsp70 that we found confirms that the ROS that is generated reacts with protein molecules and also alters the post-translational process that occurs inside the lumen of ER after treating the cells with GT, FUM and GT + FUM. Previous in vivo and in vitro studies have demonstrated an

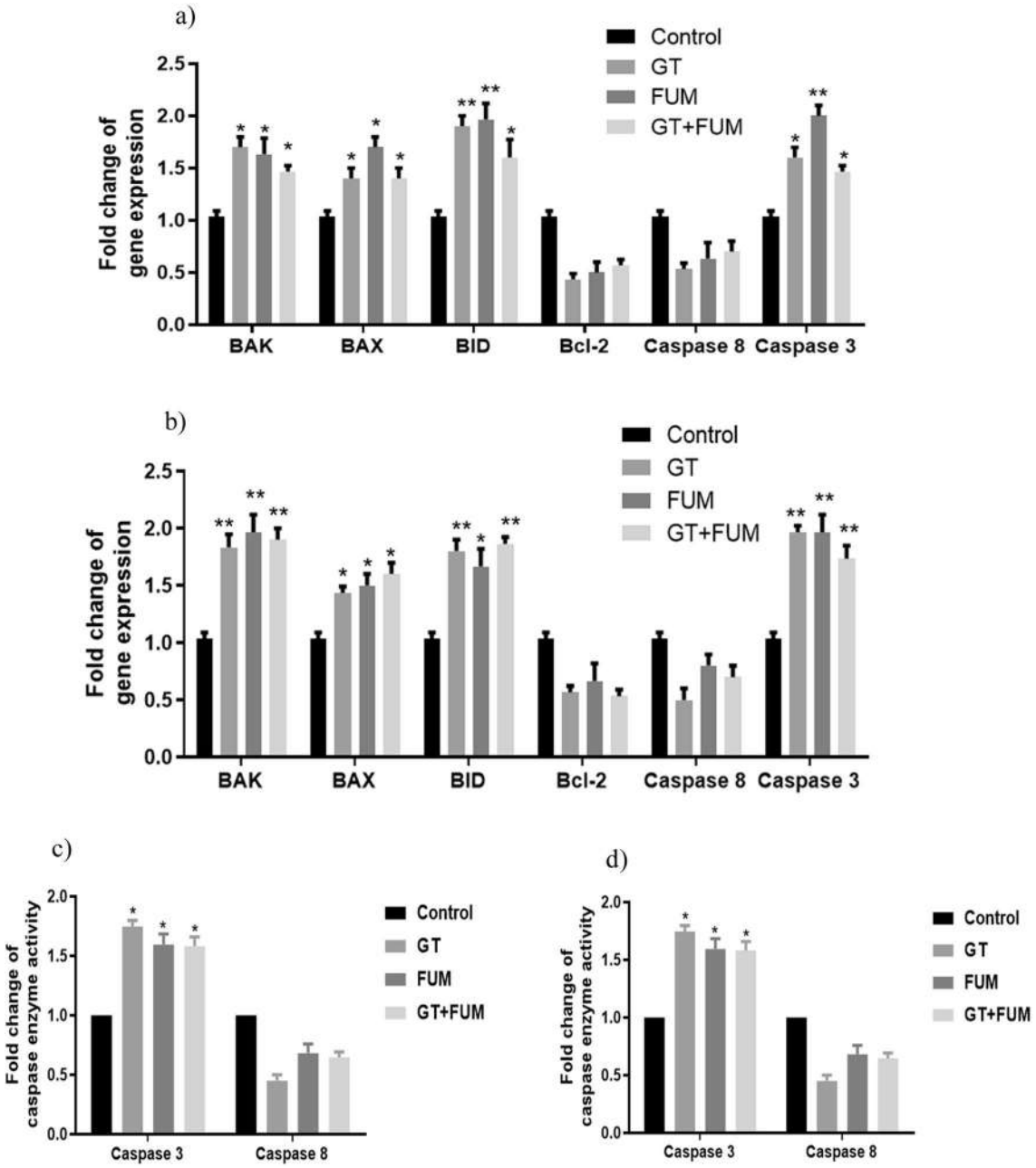


Figure 9. Molecular level induction of apoptosis-related genes by GT, FUM alone and in combination after 24 h. (**a**, **b**) Gene expression profile in A549 and L132 cells respectively. (**c**, **d**) Detection of caspase-3 and -8 enzyme activities in A549 and L132 cells respectively. Data are expressed as mean ± SD of three independent experiments for each dose point (n = 72 for panels **a** and **b**; n = 24 for panels **c** and **d**). Significance between control and treatment groups was tested by adopting two way ANOVA (*$p < 0.5$, **$p < 0.01$).

increase in ER stress markers in murine lungs and tracheal epithelial cells challenged with *A. fumigatus* antigens[35]. We found genotoxic potential of GT and FUM against the epithelial cell lines A549 and L132, evidencing single strand DNA breaks. These results are consistent with the earlier studies on genotoxicity of GT and FUM[36,37]. In addition, the combination of GT + FUM at low concentrations showed DNA-damaging potential which is threatening compared to individual mycotoxins at corresponding concentrations. Though the protagonist of the entire event caused by the test mycotoxins is putatively identified as ROS, direct DNA damage is also likely to happen. Further investigations using strong antioxidants are needed to find how precisely GT, FUM and GT + FUM-treatments cause DNA damage. Further, causation of S and G1 phase arrest by these cytolethal mycotoxins, GT, FUM and GT + FUM, during cell cycle is evidenced. Looking into the intricate mechanism of action, the data indicate that both GT and FUM enter the cell cytoplasm to bring about cell cycle arrest. Thereupon, GT induces cell cycle arrest by interfering with the DNA doubling S-phase through imminent DNA damage. This is followed by induction of cell cycle arrest by FUM at G1 phase, indicating that it interferes with

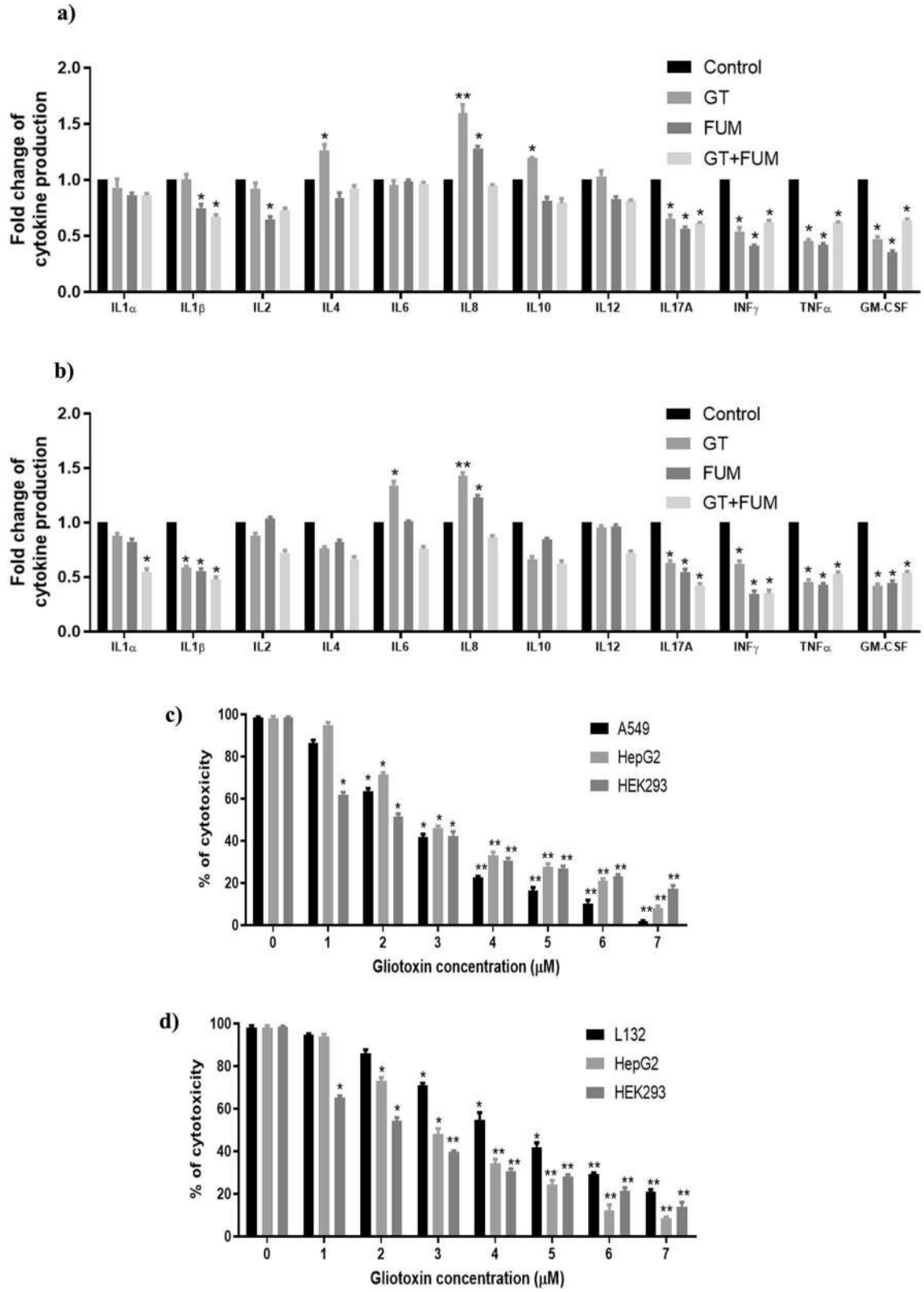


Figure 10. Secretion of inflammatory cytokines by epithelial cells following exposure of GT & FUM alone and in combination for 24 h and organ-specific cytotoxicity of GT adopting IdMOC technique. (**a**, **b**) Inflammatory cytokines secreted by A549 and L132 cells, respectively. (**c**) Organ-specific cytotoxicity of GT in A549 cell co-cultured with HepG2 and HEK293 cells after 24 h; (**d**) Organ-specific cytotoxicity of GT in L132 cell co-cultured with HepG2 and HEK293 cells after 24 h. Data are expressed as mean ± SD of three independent experiments for each dose point (n = 144 for panels **a** and **b**; n = 72 for panels **c** and **d**). Significance between the control and the treatment groups was tested by adopting two way ANOVA (*$p < 0.5$, **$p < 0.01$).

Cell type	IC_{50} value/time/assay	Toxic effect	Reference
GT			
A549	0.1 µg/ml (0.3 µM) /24 h/neutral red assay	Cytotoxicity	[22]
A549	0.16 µg/ml (0.4 µM)/48 h/MTT assay	Cytotoxicity	[23]
A549	0.16 µg/ml (0.0005 mM)/24 h/MTT assay	Cytotoxicity	[24]
A549	0.04 µg/ml (0.12 µM)/not mentioned/MTT	Cytotoxicity	[25]
A549	0.097 µg/ml (0.3 µM)/24 h/MTT assay	Cytotoxicity	[26]
FUM			
A549	16.5 µg/ml (35.9 µM)/24 h/neutral red assay	Cytotoxicity	[22]
A549	3.2 µg/ml (6.97 µM)/not mentioned/MTT assay	Cytotoxicity	[25]

Table 3. Summary of cytotoxicity studies on GT and FUM against A549 cells.

the transcriptional and post-transcriptional events that are crucial for the G1 to S-phase transition. The difference in the outcomes of previous studies on GT-induced effect on cell cycle analysis and our study may be due the difference in concentrations[23,38] whereas the outcomes of cell cycle analysis in respect of FUM are similar[39]. The GT + FUM combination produced significant decrease of A549 and L132 cells in S, G2, and M phases and increase of cells in sub-G1 phase, which point to inhibition of mitosis and induction of apoptosis. This indicates that GT + FUM induce DNA damage but the cells which are not able to repair the damage succumb to apoptosis. These results support our data on genotoxic potential of GT, FUM and GT + FUM against A549 and L132 cells.

Apparently, intracellular cues such as increased cytotoxicity, ROS generation, stress signaling pathways, mitochondrial membrane damage and cell cycle arrest can lead to cell death. The results of Annexin V/PI flow cytometry confirmed that both GT and FUM alone as well as in combination induced apoptosis rather than necrosis in both A549 and L132 epithelial cells. Our results are consistent with previous reports in respect of GT and FUM[23,24,40]. Apoptosis can be initiated by one of two pathways, caspase-8-associated death receptor pathway, i.e. extrinsic pathway, and mitochondria-mediated intrinsic pathway. Binding of ligands with death receptors will activate a series of downstream factors, including caspase-8 which is a critical mediator of the extrinsic pathway. Intrinsic pathway of apoptosis is initiated via mitochondrial membrane pore formation where the stress signals cause the binding of cytoplasmic proteins BAX and BID with mitochondrial membrane protein BAK. At the culmination of both of these apoptotic pathways, initiation of executioner caspase 3-dependent proteolytic cascade is considered a key factor of apoptotic cell death[6]. Our expression analysis revealed decrease of anti-apoptotic Bcl-2 protein and increase of pro-apoptotic BAX, BID and BAK, validating the participation of mitochondria in the cell death. Decrease in the RNA expression/activity of caspase 8 and increase in the expression/activity of caspase 3 authenticate that caspase-dependent mitochondria-mediated intrinsic pathway of apoptosis is induced by GT, FUM and GT + FUM in lung epithelial cells. Our results with regard to apoptosis are consistent with previous findings in respect of GT and FUM[41,42].

Though we found generation of ROS to be a crucial event in the molecular mechanism of cell death induced by GT, FUM and GT + FUM, we still have question on the production of intracellular ROS in response to GT and FUM. Previous studies let us believe that the structure of GT can exist in either disulfide or dithiol forms, depending on the reducing or oxidizing environment[43,44]. Further, GT exists naturally in an oxidized disulphide form but upon cellular uptake GT is chemically reduced to the dithiol form by intracellular glutathione (GSH). Thus, GT is increasingly accumulated in a cell in a glutathione-dependent manner[43,44]. However, induction of ROS followed by apoptosis is observed in many cell lines because reduced-GT can be oxidized to GT, thus, releasing ROS, and it can also form mixed disulfides by thiol groups with proteins or antioxidants such as glutathione[45,46]. Then, oxidation of the intracellular toxin results in rapid efflux from the cell which also occurs when glutathione levels decrease following induction of apoptotic cell death by the toxin[43,44]. Further, reduced dithiol form of GT has the ability to chelate Zn^{2+} ions from intracellular metalloenzymes and target thioredoxin redox system[47,48]. Also, GT is involved in activation of phospholipase D which catalyzes hydrolysis to produce the signal molecule phosphatidic acid, soluble choline and actin cytoskeleton rearrangement[49,50]. On the other hand in case of FUM, total glutathione content is significantly higher in cell lines insensitive (human umbilical vein endothelial cells) to FUM than in sensitive cell lines (human colon cancer cell line)[51]. Also, FUM has been demonstrated to affect not only methionine aminopeptidases proteins (family of intracellular proteolytic enzymes) but also all the subsequent activities necessary for the effective functioning of proteins related to cell viability and growth[41]. These studies clearly show that glutathione, the hall mark of cellular redox homeostasis, is affected by both GT and FUM, and the structural advantages of theses mycotoxins could be the reason for the generation of intracellular ROS and thus affect several intracellular proteins and induce apoptosis. Thus, GT and FUM in combination can act synergistically against cellular glutathione redox system.

Further, lung epithelial cells can initiate an inflammatory response against mycotoxins to disrupt fungal growth. Previous studies on immunological responses either for *A. fumigatus* or culture filtrate of *A. fumigatus* showed increase of cytokines IL-4, IL-10, IL-6, IL-8, IFN-g and TNF-α[1,52]. Although, it is important to find stimulatory effect of GT, FUM and GT + FUM involved in the inflammation of epithelial cells which act as a first-line defense signals that release pro-inflammatory cytokines to attract neutrophils, macrophages and dendritic cells at the site of infection. In this way we project the mycotoxins to act as virulence factors without any fungal antigen moiety to provoke innate immune response of host epithelial cells rather as a physical barrier

of lung. As revealed in the results, IL-8 is the common pro-inflammatory signal produced by both A549 and L132 cells against GT and FUM. IL-8 is a chemokine that contributes to the effective recruitment and activation of neutrophils at the site of infection. This clearly shows that both type 1 and type 2 epithelial cells enable neutrophil-mediated-inflammatory responses against GT and FUM. These results are consistent with previous studies showing production of IL-8 upon conidial germination and hyphal growth of *A. fumigatus* inside epithelial cells. Apart from IL-8, GT induced IL-4 and IL-10 in A549 cell. T helper 1 (Th1) anti-inflammatory cytokines IL-4 and IL-10 are involved in the maintenance of homeostasis and, in particular, in controlling pro- and anti-inflammatory cytokines involved in infectious, allergic and autoimmune diseases. In addition, these anti-inflammatory cytokines limit the immune response to self- and non-T-self antigens, protecting the host from excessive Th1 pathology and preventing tissue damage[1]. Further, previous reports have shown that immune response initiated by IL-4 and IL-10 activates T helper 2 (Th2) cell differentiation and inhibits Th1 and Th17 response. IL-4 and IL-10 deficient mice show lower *A. fumigatus* burden and increased survival rates compared to wild type mouse in invasive pulmonary aspergillosis[1]. Thus, we speculate that exposure of GT would even enhance neutrophil recruitment at the site of infection to cause neutrophil-mediated inflammatory tissue damage and, thus, IL-4 and IL-10 cytokines are increased by epithelial cells to prevent the excessive inflammatory response of Th1 to avoid self-tissue damage. On the other hand, in L132 cell, production of IL-6 cytokine was also significant apart from IL-8. The IL-6 is an important proinflammatory cytokine involved in regulation of Th17 response during *A. fumigatus* infection. Further, Th17cells activate neutrophil migration towards the infected area and thereby increase inflammation. Over all, the results clearly show that recruitment of neutrophil to the site of infection could be the innate immune response of epithelial cells against the fungal mycotoxins GT and FUM. Moreover, these toxins have been proved for their ability to inhibit the function of neutrophils. This could be one of the reasons for neutropenia patients to manifest frequent IA infection due to fewer neutrophils to fight the pathogen[27–29]. An earlier study has shown similar results in LPS induced A549 cells against GT[46]. In addition, the combination of GT + FUM at a lower concentration could not induce any cytokine expression within 24 h which is perhaps a good strategy to bring about epithelial cell death without inflammatory response or may take more than 24 h to alter the immunological response. This brings up the dangerous effect of GT + FUM as a combination to damage epithelial cells invasively, without any resistance from the host immune system. However, the molecular mechanisms by which these toxins induce cytokine expression remains to be studied. Further, in vivo investigation about immune regulation mechanism might reveal some potential targets for diagnosis and treatment of pulmonary fungal diseases.

Once the epithelial layer of alveoli is damaged, *A. fumigatus* arrives at the endothelium of the blood vessel to become an angiotrophic fungus and, thus, the disease becomes invasive and spreads to other organs. Disseminated aspergillosis is defined as the involvement of at least two non-contiguous organ sites. *Aspergillus* may disseminate from lungs to invariably every organ[28]. Disseminated disease has been described in 9–36% of kidney recipients, 15–20% of lung recipients, 20–35% of heart recipients and 50–60% of liver recipients with IA[28]. Therefore, we followed up with study of multiple organ toxicity of GT, adopting IdMOC, a novel cell culture system that is appropriate in the context of evaluation of comparative cytotoxicity of an agent towards multiple cell types. IA is the most severe form of disease that occurs when the infection spreads rapidly from lungs to liver, heart, kidneys or skin. So far, GT is the only mycotoxin that has been identified in the sera of IA patients, and considered as a virulent factor of *A. fumigatus*. Thus, the presence of GT in the sera of IA patients justifies evaluation of multiple organ toxicity or organ-specific toxicity. As shown in the results, A549 (representing lung epithelial cell) and HEK (representing renal epithelial cell) are likely to be more sensitive and liable for GT-induced cytotoxicity than HepG2 (representing liver epithelial cells) and L132 (representing both types 1 and II lung epithelial cells) which are fairly sensitive. Studies on potential toxicokinetic variations between cell models can explain the sensitivity issues. Also, future studies that associate neuronal cells and skin cells are required to relate the clinical symptoms of brain and skin damage in IA patients[28].

In conclusion, our study shows that at a specific concentration GT and FUM interact synergistically to induce cytotoxicity in lung epithelial cells. The molecular mechanism of toxicity of GT, FUM and GT + FUM to the lung epithelial cells greatly depends on ROS-mediated, ER-stress-induced, mitochondria-mediated, caspase-dependent intrinsic pathway of apoptosis. ROS are produced after mitochondrial damage and, thus, mitochondria are both the source of ROS and target of ROS. GT-, FUM- and GT + FUM-induced DNA damage is mediated either by an ROS-dependent course or by a direct attack. In addition, GT, FUM and GT + FUM may induce unfolded protein accumulation. Although there are signs of indication that GT and FUM can induce neutrophil recruitment at the site of infection as revealed in the expression of cytokines in epithelial cells (Fig. 11) and GT would damage epithelial cells by neutrophil-mediated inflammatory response, further studies are needed to confirm the hypothesis made out in the study. GT is identified to be highly cytotoxic to renal epithelial cells at low concentration and type II epithelial cells at high concentration. It is already known in an in vivo model that GT is a virulence factor and FUM is a likely factor in cellular damage during infection of the host by *A. fumigatus*[14,53]. This is further substantiated by the down-regulation of cytokines that are generally involved in the regulation of immune system as well as in the control of the fungal pathogenesis. Taken together, these findings indicate that GT and FUM alone and in combination are capable of damaging lung epithelial cells and, thus, these mycotoxins could be an attractive target for drug development for IA.

Materials and methods

Materials. GT (CAS Number 67-99-2; ≥ 98% purity) and FUM (CAS Number 23110-15-8; ≥ 90% purity) were obtained from Sigma Aldrich Chemical Company (St. Louis, MO, USA), DMEM, Phosphate-Buffered Saline (PBS), trypsin–EDTA, penicillin and streptomycin were obtained from Hyclone laboratories (South Logan, Utah). F etal bovine serum (FBS) was obtained from Gibco (UK). 3-(4,5-dimethylthiazol-2-yl)-2,5-di-

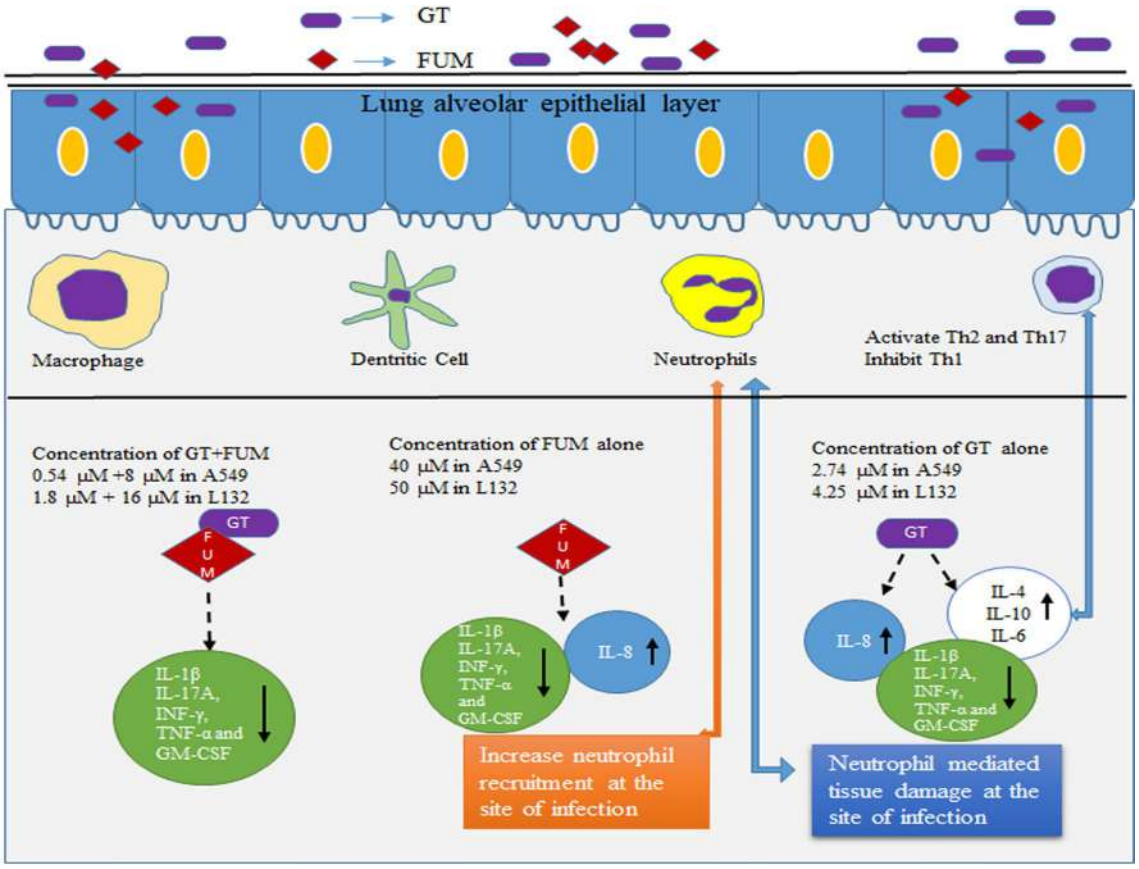


Figure 11. Represents how GT, FUM and GT + FUM act as virulent factors to provoke/evade immune surveillance of host without any fungal antigen.

phenyltetrazolium bromide (MTT), AnnexinV, Propidium Iodide (PI) and RNase I were obtained from Sigma Aldrich Chemical Company (St. Louis, MO, USA). All other chemicals and reagents were of analytical grade.

Methods

Cell culture. Human lung epithelial carcinoma cell A549, normal lung epithelial cell L132, human hepatocarcinoma cell HepG2 and human embryonic kidney cell HEK293 were obtained from National Centre for Cell Science (NCCS), Pune, India. The cells were maintained in DMEM medium supplemented with 10% FBS, and with 100 U/mL each of penicillin and streptomycin as antibiotics in a humidified atmosphere of 5% CO_2 and 95% air, in a CO_2 incubator (Eppendorf, India).

Integrated discrete multiple organ co-culture (IdMOC). The IdMOC 96 well plates were gift from Dr. Albert P. Li, President and CEO of In Vitro ADMET Laboratories LLC, Columbia, USA. The concepts and applications of IdMOC have been reviewed[54].

Cytotoxicity assay. Colorimetric cytotoxicity assay was performed according to Mosmann et al.[55] A549 and L132 cells were seeded in 96 well plate at 5×10^3 cells/well and treated with different concentrations of GT, FUM and combination of the two, dissolved in dimethyl sulfoxide (DMSO) for 24 h, at 37 °C. DMSO was used as the solvent control. After 24 h incubation, 20 µL of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] solution (5 mg/mL in PBS) was added to each well, and incubated for 3 h at 37 °C. The medium was then removed and the purple formazan product was dissolved in 100 µL of DMSO. The absorbance was measured at 570 nm (measurement) and 630 nm (reference) using a 96-well plate EnSpire multimode reader (Perkin Elmer, Waltham, MA, USA). Data were collected for triplicates and used to calculate the respective means and the standard deviations. The percentage viability (*visa vi* cytotoxicity) was calculated from this data using the following formula:

$$Percentage\ \ viability\ (visa\ vi,\ \ cytotoxicity) = Mean\ \ OD\ \ of\ \ untreated\ \ \ cells\ (control) - Mean\ \ OD\ \ of\ \ treated\ \ cells / Mean\ \ OD\ \ of\ \ untreated\ \ cells\ (control) \times 100.$$

From the values thus obtained, the IC_{50} was calculated. IC_{50} is defined as concentration of the test substance at which cell viability is decreased to 50%, meaning 50% cells are dead.

Isobologram method of toxicity assessment of binary combination of mycotoxins. The types of interaction of GT and FUM mixtures were determined by isobologram analysis using CompuSyn software (version 1.0.1 [2004]; CompuSyn, Inc.) established by Chou and Talalay[56]. In this analysis, combination index (CI) is a quantitative parameter used to evaluate the types of interaction and the corresponding influential level among binary or more compounds. CI < 1 indicates synergism, CI = 1 indicates additive effect and > 1 indicates antagonism of the compounds (in the present case, mycotoxin combinations).

Reactive oxygen species (ROS) assay for the assessment of oxidative stress. The generation of intracellular ROS was measured using the fluorescent probe 2′,7′-dichlorofluorescein diacetate (DCFH-DA) according to Wang and Joseph[57]. To evaluate mycotoxins-induced DCF oxidation, 1×10^6 counts of A549 and L132 cells, as the case may be, were seeded in each of the 6 wells of six well plates and treated with the positive control (H_2O_2) and IC_{50} concentration of GT and FUM alone and the two in combination at 20% and 40% of the respective IC_{50} values, for 6 h, 12 h and 24 h against A549 and L132 cells, respectively. After that, the wells were loaded with 5 µM of DCFH-DA and incubated for 30 min at 37 °C. ROS generation was measured in EnSpire multimode reader (Perkin Elmer, Waltham, MA, USA) at an excitation wavelength of 485 nm and emission wavelength of 520 nm. Results are expressed as the ratio mycotoxin-induced DCF fluorescence/control fluorescence.

Single-cell gel electrophoresis (comet assay) for genotoxicty assessment. DNA damage was detected adopting the comet assay[58]. The technique combines DNA gel electrophoresis with florescent microscopy to visualize migration of DNA from individual agarose-embedded cells. The cells were treated with the IC_{50} concentration of GT and FUM, alone and in combination at 20% and 40% of the respective IC_{50} values, for 24 h against A549 and L132 cells, respectively. The harvested cells were suspended in low melting point agarose in PBS and pipetted out to microscope slides pre-coated with a layer of normal melting point agarose. The slides were immersed in pre-chilled lysis buffer (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris, 0.2 mM NaOH [pH 10], and Triton X-100) and incubated overnight at 4 °C in order to lyse the cells and to permit DNA unwinding. Thereafter, the slides were exposed to alkaline buffer (300 mM NaOH, 1 mM Na_2-EDTA, [pH > 13]) for 20 min at 20 V to allow DNA unwinding. The slides were washed with buffer (0.4 M Tris, pH 7.5) to neutralize excess alkali before staining with EB. The EB-stained slides were observed in a fluorescent microscope (Nikon, Japan). One hundred and fifty cells, in triplicate, from each treatment group were digitized and analyzed using CASP software. The extent of DNA damage was assessed from the shape and area of the comet tail. The images were used to determine the DNA content of individual nuclei and to evaluate the degree of DNA damage representing the fraction of total DNA in the tail.

JC1 staining for assessment of mitochondrial trans-membrane potential. Mitochondrial trans-membrane potential ($\Delta\Psi m$) was measured using the fluorescent probe JC-1 (5,5′,6,6′-Tetrachloro-1,1′,3,3′-tetraethyl-imidacarbocyanine iodide) by adopting the method of Reers et al.[59]. The cells were grown in glass cover slips placed in the wells of 6-well plates and treated with IC_{50} concentrations of GT and FUM alone and the two in combination at 20% and 40% of the respective IC_{50} values, for 24 h, against A549 and L132 cells. The cells were stained with JC-1 dye after 24 h exposure. The mitochondrial depolarization patterns of the cells were observed in the fluorescent microscope (Nikon, Japan) and the fluorescence pattern of cells (red, normal; green, mitochondrial membrane depolarized) were observed and recorded.

Flow cytometry analysis for cell cycle arrest. Cell cycle arrest was detected using flow cytometry[60]. The cells were treated with the IC_{50} concentrations of GT and FUM alone and in combination at 20% and 40% of the respective IC_{50} concentrations, for 24 h, against A549 and L132 cells. The cells were harvested, washed with PBS, centrifuged at 1,000 rpm for 5 min, and fixed with 1 mL 70% ice cold ethanol overnight at 4 °C. Later, the ethanol was removed and 10 µL of RNase A (10 mg/mL) was added and incubated for 30 min. Again, cells were washed with PBS and re-suspended in 1 mL PBS with 50 µL Propidium Iodide (1 mg/mL) for 30 min in dark. The cells were analyzed using the BD FACSverse flow cytometer (Becton–Dickinson), and a peak fluorescence gate was used to discriminate the aggregates. A total of 20,000 events were acquired and three replicates were used per experimental condition. The percentage of cells in each phase was determined in reference to untreated cells.

Flow cytometry analysis of apoptosis. Detection of apoptosis by flow cytometry was conducted using the protocol given in manufacturer's instruction (BD Biosciences, cat no: 556547). The cells were treated with the IC_{50} concentration of GT and FUM alone and in combination at 20% and 40% of the respective IC_{50} values, for 24 h against A549 and L132 cells. The cells were harvested, washed with PBS, centrifuged at 1,000 rpm for 5 min and re-suspended in100 µL binding buffer. Five microliters of Annexin-V and Propidium Iodide were added and incubated for 15 min at room temperature in dark. The stained cells were measured at 515 nm fluorescence emission using FACSverse flow cytometer (BD) and the data were processed by FACSverse software.

Annexin V-FITC/PI binding assay for the assessment of cell death. Annexin V-FITC/PI staining was performed to assess the morphological characteristics of cell death by apoptosis or necrosis[61]. The A549 and L132 cells were cultured in 6-well plates and treated with IC_{50} concentrations of GT and FUM alone and in combination at 20% and 40% of the respective IC_{50} values, for 24 h, respectively. The treated and untreated cells were centrifuged at 3,000 rpm for 5 min, incubated with Annexin V-FITC/PI and examined under FITC filter

for green emission of Annexin V-FITC and TRITC filter for red emission of PI using an inverted fluorescent microscope (Nikon, Japan).

Real time PCR for gene expression analysis. Total RNA were isolated from untreated and treated A549 and L132 cells (2×10^6 in T_{25} culture flask) using the TRIzol Reagent (Invitrogen; USA) and quantified using a NanoDrop spectrophotometer (Bio Drop Duo, UK). First strand cDNA was synthesized with 1 µg of RNA and oligo(dTs) to a final volume of 20 µL according to the manufacturer's instruction (Thermo Scientific, USA). qPCR was performed as 10 µL reaction mix with a final concentration of 1×SYBR Green (Roche), 0.5 µL of each primer, and the cDNA that was synthesized in the earlier step. To detect non-specific amplification, negative controls (no template control and minus RT control (only with RNA) were included in the assays. The amplicons were sequenced to confirm their identity**.** The reactions were carried out using a single step real time PCR machine (Roche, USA) under the following conditions: initial denaturation at 95 °C for 5 min, followed by 30 cycles of 3 step amplification at 95 °C for 10 s, 50/60 °C for 30 s and 72 °C for 10 s.

Primers for the apoptosis and stress-related genes were selected from the earlier reports[62–66]. GAPDH served as the internal control to normalize the RNA level. Three technical repeats and experimental replicates were performed for each gene.

Caspase-3 & -8 activity assay for apoptotic cell death. Caspase-3 and -8 activities were determined using assay kit, according to the manufacturer's instructions (Cat. No. K106-100 & K113-25 Biovision, Inc., Milpitas, CA, USA). A549 and L132 cells were cultured in 6-well plates and treated with IC_{50} concentrations of GT and FUM alone and in combination at 20% and 40% of the respective IC_{50} values, for 24 h. The control and treated cells were lysed in sample lysis buffer (Biovision, Inc.). The homogenates were then centrifuged at 10,000×*g* and 4 °C for 10 min and the supernatant was collected for protein determination. The cell lysates were then exposed to DEVD-pNA and IETD-pNA substrate conjugate provided in the kit for caspase-3 and -8, respectively, for 1 h at 37 °C. The sample was measured at 400–405 nm in multimode plate reader (Model: Enspire, Perkin Elmer, CA, USA).

Multi-analyte ELISA array for detection of inflammatory cytokines. The Human Inflammatory Cytokines Multi-Analyte ELISArray kit (Cat. No. MEH-004A; Qiagen, Valencia, CA, USA) was used to detect a panel of 12 cytokines according to the manufacturer's protocol. The ELISA panel included: Interleukin (IL)-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-17A, interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α) and granulocyte–macrophage colony-stimulating factor (GM-CSF)[67].

Integrated discrete multiple organ toxicity assay using IdMOC technology for organ-specific toxicity. IdMOC 96 well plates with 16 containing wells, each with 6 inner wells, were used to discretely co-culture A549, HepG2, and HEK293 cells (Plate 1) and L132, HepG2, and HEK293 (Plate 2). In each inner well 10,000 cells were plated in 80 µL of the medium specific for each cell type. The cells were cultured in an incubator kept at 37 °C, and a highly humidified atmosphere of 95% air and 5% carbon dioxide for 24 h before treatment. On the day of treatment, the medium was removed from all inner wells of the IdMOC plate and replaced with 2.5 mL DMEM containing various concentrations of GT. Treatment was performed in triplicate for an incubation period of 24 h. After 24 h, 50 µL of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] solution (5 mg/mL in PBS) was added to each well, and incubated for 3 h at 37 °C. The medium was removed and the purple formazan product was dissolved in 50 µL of DMSO. The absorbance was measured at 570 nm (measurement) and 630 nm (reference) using a 96-well plate EnSpire multimode reader (Perkin Elmer, Waltham, MA, USA)[7]. Data were collected for triplicates and used to calculate the respective means and the standard deviations. The percentage viability (*visa vi* cytotoxicity) was calculated from this data using the following formula:

$$\begin{aligned} &Percentage\ of\ viability\ (visa\ vi,\ cytotoxicity) = Mean\ OD\ of\ untreated\ cells\ (control) \\ &- Mean\ OD\ of\ treated\ cells/Mean\ OD\ of\ untreated\ cells\ (control) \times 100. \end{aligned}$$

Statistical analysis. Results were expressed as mean ± SD of three independent experiments. Difference between groups was analyzed by Two way ANOVA from GraphPad Prism-6.0; ns = not significant; $^{*}p<0.5$, $^{**}p<0.01$ and $^{***}p<0.001$ were considered statistically significant at the respective levels.

Received: 5 April 2020; Accepted: 6 August 2020
Published online: 02 September 2020

References

1. Dagenais, T. R. & Keller, N. P. Pathogenesis of *Aspergillus fumigatus* in invasive aspergillosis. *Clin. Microbiol. Rev.* **22**, 447–465 (2009).
2. McCormick, A., Loeffler, J. & Ebel, F. *Aspergillus fumigatus*: contours of an opportunistic human pathogen. *Cell. Microbiol.* **12**, 1535–1543 (2010).
3. Lewis, R. E. *et al.* Detection of gliotoxin in experimental and human aspergillosis. *Infect. Immun.* **73**, 635–637 (2005).
4. Latgé, J. P. Aspergillus fumigatus and aspergillosis. *Clin. Microbiol. Rev.* **12**, 310–350 (1999).
5. Hof, H. & Kupfahl, C. Gliotoxin in *Aspergillus fumigatus*: an example that mycotoxins are potential virulence factors. *Mycotoxin Res.* **25**, 123–131 (2009).
6. Gayathri, L. *et al.* Hepatotoxic effect of ochratoxin A and citrinin, alone and in combination, and protective effect of vitamin E: in vitro study in HepG2 cell. *Food. Chem. Toxicol.* **83**, 151–163 (2015).

7. Gayathri, L. *et al.* Metabolism-dependent cytotoxicity of citrinin and ochratoxin A alone and in combination as assessed adopting integrated discrete multiple organ co-culture (IdMOC). *Toxicol. In Vitro.* **46**, 166–177 (2018).
8. Gardiner, D. M., Waring, P. & Howlett, B. J. The epipolythiodioxopiperazine (ETP) class of fungal toxins: distribution, mode of action, functions and biosynthesis. *Microbiology* **151**, 1021–1032 (2005).
9. Kupfahl, C. *et al.* Gliotoxin production by clinical and environmental *Aspergillusfumigatus* strains. *Int. J. Med. Microbiol.* **298**, 319–327 (2008).
10. Kosalec, I., Pepeljnjak, S. & Jandrlic, M. Influence of media and temperature on gliotoxin production in *Aspergillus fumigatus* strains. *Arh. Hig. Rada. Toksikol.* **56**, 269–273 (2005).
11. Hmood, B. A. Detection of gliotoxin in patients with pulmonary aspergillosis. *JUB* **25**, 639–650 (2017).
12. Vidal-García, M. *et al.* Production of the invasive aspergillosis biomarker bis (methylthio) gliotoxin within the genus *Aspergillus*: in vitro and in vivo metabolite quantification and genomic analysis. *Front. Microbiol.* **9**, 1246 (2018).
13. Domingo, M. P. *et al.* Bis (methyl) gliotoxin proves to be a more stable and reliable marker for invasive aspergillosis than gliotoxin and suitable for use in diagnosis. *Microbiol. Infect. Dis.* **73**, 57–64 (2012).
14. Guruceaga, X. *et al.* A possible role for fumagillin in cellular damage during host infection by *Aspergillus fumigatus*. *Virulence* **9**, 1548–1561 (2018).
15. Amitani, R. *et al.* Purification and characterization of factors produced by *Aspergillus fumigatus* which affect human ciliated respiratory epithelium. *Infect. Immun.* **63**, 3266–3271 (1995).
16. Fallon, J. P., Reeves, E. P. & Kavanagh, K. Inhibition of neutrophil function following exposure to the *Aspergillus fumigatus* toxin fumagillin. *J. Med. Microbiol.* **59**, 625–633 (2010).
17. Kamei, K. & Watanabe, A. Aspergillus mycotoxins and their effect on the host. *Med. Mycol.* **143**, 95–99 (2005).
18. Wiesner, D. L. & Klein, B. S. Lung epithelium: barrier immunity to inhaled fungi and driver of fungal-associated allergic asthma. *Curr. Opin. Microbiol.* **40**, 8–13 (2017).
19. Osherov, N. Interaction of the pathogenic mold *aspergillus fumigatus* with lung epithelial cells. *Front. Microbiol.* **3**, 346 (2012).
20. Roehlecke, C. *et al.* Resistance of L132 lung cell clusters to glyoxal-induced apoptosis. *Histochem. Cell. Biol.* **114**, 283–292 (2000).
21. Panngom, K. *et al.* Preferential killing of human lung cancer cell lines with mitochondrial dysfunction by nonthermal dielectric barrier discharge plasma. *Cell. Death. Dis.* **4**, e642 (2013).
22. Bünger, J. *et al.* Cytotoxicity of occupationally and environmentally relevant mycotoxins. *Toxicology* **202**, 199–211 (2004).
23. Hung, M., Van, L., Song, Y. W. & Cho, S. K. Effects of the combination of gliotoxin and adriamycin on the adriamycin-resistant non-small-cell lung cancer A549 cell line. *Mar. Drugs* **16**, 105 (2018).
24. Peterson, D. E., Collier, J. M., Katterman, M. E., Turner, R. A. & Riley, M. R. Cytotoxicity of bacterial-derived toxins to immortal lung epithelial and macrophage cells. *Appl. Biochem. Biotechnol.* **160**, 751–763 (2010).
25. Schulz, T., Senkpiel, K. & Ohgke, H. Comparison of the toxicity of reference mycotoxins and spore extracts of common indoor moulds. *Int. J. Hyg. Environ. Health.* **207**, 267–277 (2004).
26. Kreja, L. & Seidel, H. J. On the cytotoxicity of some microbial volatile organic compounds as studied in the human lung cell line A549. *Chemosphere* **49**, 105–110 (2002).
27. Kosmidis, C. & Denning, D. W. The clinical spectrum of pulmonary aspergillosis. *Thorax* **70**, 270–277 (2015).
28. Munoz, P., Guinea, J. & Bouza, E. Update on invasive aspergillosis: clinical and diagnostic aspects. *Clin. Microbiol. Infect.* **12**, 24–39 (2006).
29. Husain, S. & Camargo, J. F. Invasive Aspergillosis in solid-organ transplant recipients: guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. *Clin. Transplant.* **33**, e13544 (2019).
30. Mayer, S., Engelhart, S., Kolk, A. & Blome, H. The significance of mycotoxins in the framework of assessing workplace related risks. *Mycotoxin Res.* **24**, 151–164 (2008).
31. Klotz, L. O. *et al.* Redox regulation of FoxO transcription factors. *Redox Biol.* **6**, 51–72 (2015).
32. Zorov, D. B., Juhaszova, M. & Sollott, S. J. Mitochondrial ROS-induced ROS release: an update and review. *Biochim. Biophys. Acta Bioenerg.* **1757**, 509–517 (2000).
33. Pfaffenbach, K. T. & Lee, A. S. The critical role of GRP78 in physiologic and pathologic stress. *Curr. Opin. Cell Biol.* **23**, 150–156 (2011).
34. Radons, J. The human HSP70 family of chaperones: where do we stand?. *Cell Stress Chaperones* **21**, 379–404 (2016).
35. Margalit, A., Kavanagh, K. & Carolan, J. C. Characterization of the proteomic response of A549 cells following sequential exposure to *Aspergillus fumigatus* and *Pseudomonas aeruginosa*. *J. Proteome Res.* **19**, 279 (2020).
36. Stanimirovic, Z., Stevanovic, J., Bajic, V. & Radovic, I. Evaluation of genotoxic effects of fumagillin by cytogenetic tests in vivo. *Mutat. Res.* **628**, 1–10 (2007).
37. Nouri, M. A., Al-Halbosiy, M. M., Dheeb, B. I. & Hashim, A. J. Cytotoxicity and genotoxicity of gliotoxin on human lymphocytes in vitro. *JKSUS* **27**, 193–197 (2015).
38. Baust, H. *et al.* Evidence for radiosensitizing by gliotoxin in HL-60 cells: implications for a role of NF-κ B independent mechanisms. *Oncogene* **22**, 8786–8796 (2003).
39. Sin, N. *et al.* The anti-angiogenic agent fumagillin covalently binds and inhibits the methionine aminopeptidase, MetAP-2. *Proc. Natl. Acad. Sci* **94**, 6099–6103 (1997).
40. Kidoikhammouan, S. *et al.* Blocking of methionine aminopeptidase-2 by TNP-470 induces apoptosis and increases chemosensitivity of cholangiocarcinoma. *J. Cancer Res Ther.* **15**, 148 (2019).
41. Guruceaga, X. *et al.* Fumagillin, a mycotoxin of Aspergillus fumigatus: biosynthesis, biological activities, detection, and applications. *Toxins* **12**, 7 (2020).
42. Zheng, J. *et al.* Gliotoxin produced by Aspergillus fumigatus induces apoptosis of human bronchial epithelial cells via the Bcl-2 pathway. *Int. J. Clin. Exp. Med.* **10**, 8854–8865 (2017).
43. Guilford, F. T. & Hope, J. Deficient glutathione in the pathophysiology of mycotoxin-related illness. *Toxins* **6**, 608–623 (2014).
44. Bernardo, P. H., Brasch, N., Chai, C. L. & Waring, P. A novel redox mechanism for the glutathione-dependent reversible uptake of a fungal toxin in cells. *J. Biol Chem.* **278**, 46549–46555 (2003).
45. Chen, J. *et al.* Reduced-gliotoxin induces ROS-mediated anoikis in human colorectal cancer cells. *Int. J. Oncol.* **52**, 1023–1032 (2018).
46. Johannessen, L. N., Nilsen, A. M. & Løvik, M. Mycotoxin-induced depletion of intracellular glutathione and altered cytokine production in the human alveolar epithelial cell line A549. *Toxicol. Lett.* **168**, 103–112 (2007).
47. Saleh, A. A. *et al.* Systems impact of zinc chelation by the epipolythiodioxopiperazinedithiolgliotoxin in *Aspergillus fumigatus*: a new direction in natural product functionality. *Metallomics* **10**, 854–866 (2018).
48. Choi, H. S., Shim, J. S., Kim, J. A., Kang, S. W. & Kwon, H. J. Discovery of gliotoxin as a new small molecule targeting thioredoxin redox system. *Biochem. Biophys. Res. Commun.* **359**, 523–528 (2007).
49. Jia, X. *et al.* Gliotoxin promotes *Aspergillus fumigatus* internalization into type II human pneumocyte A549 cells by inducing host phospholipase D activation. *Microbes Infect.* **16**, 491–501 (2014).
50. Zhang, C. *et al.* Gliotoxin induces cofilin phosphorylation to promote actin cytoskeleton dynamics and internalization of *Aspergillus fumigatus* into type II human pneumocyte cells. *Front. Microbiol.* **10**, 1345 (2019).
51. Frottin, F. *et al.* MetAP1 and MetAP2 drive cell selectivity for a potent anti-cancer agent in synergy, by controlling glutathione redox state. *Oncotarget* **7**, 63306 (2016).

52. Chen, F. *et al.* Transcriptome profiles of human lung epithelial cells A549 interacting with *Aspergillus fumigatus* by RNA-Seq. *PLoS ONE* **10**, e0135720 (2015).
53. Sugui, J. A. *et al.* Gliotoxin is a virulence factor of *Aspergillus fumigatus*: gliP deletion attenuates virulence in mice immunosuppressed with hydrocortisone. *Eukaryot. Cell* **6**, 1562–1569 (2007).
54. Gayathri, L., Dhanasekaran, D. & Akbarsha, M. A Scientific concepts and applications of integrated discrete multiple organ co-culture technology. *J. Pharmacol. Pharmacother.* **6**, 63 (2015).
55. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **65**, 55–63 (1983).
56. Chou, T. C. Theoretical basis, experimental design, and computerized simulation of synergism and antagonism in drug combination studies. *Pharmacol. Rev.* **58**, 621–681 (2006).
57. Wang, H. & Joseph, J. A. Quantifying cellular oxidative stress by dichlorofluorescein assay using microplate reader. *Free Radic. Biol. Med.* **27**, 612–616 (1999).
58. Singh, N. P. A., McCoy, M. T., Tice, R. R. & Schneider, E. L. Simple technique for quantitation of low levels of DNA damage in individual cells. *Exp. Cell Res.* **175**, 184–191 (1988).
59. Reers, M., Smith, T. W. & Chen, L. B. J-aggregate formation of a carbocyanine as a quantitative fluorescent indicator of membrane potential. *Biochemistry* **30**, 4480–4486 (1991).
60. Darzynkiewicz, Z. & Juan, G. DNA content measurement for DNA ploidy and cell cycle analysis. *Curr. Protoc. Cytom.* **1**, 5–7 (1997).
61. Rieger, A. M., Nelson, K. L., Konowalchuk, J. D. & Barreda, D. R. Modified annexin V/propidium iodide apoptosis assay for accurate assessment of cell death. *JoVE* **50**, e2597 (2011).
62. Dubrovskaya, V. A. & Wetterhahn, K. E. Effects of Cr(VI) on the expression of the oxidative stress genes in human lung cells. *Carcinogenesis* **19**, 1401–1407 (1998).
63. Speckmann, B. *et al.* Proinflammatory cytokines down-regulate intestinal selenoprotein P biosynthesis via NOS2 induction. *Free Radic. Biol. Med.* **49**, 777–785 (2010).
64. Ahamed, M. *et al.* Oxidative stress mediated apoptosis induced by nickel ferrite nanoparticles in cultured A549 cells. *Toxicology* **283**, 101–108 (2011).
65. Aueviriyavit, S., Phummiratch, D., Kulthong, K. & Maniratanachote, R. Titanium dioxide nanoparticles-mediated in vitro cytotoxicity does not induce Hsp70 and Grp78 expression in human bronchial epithelial A549 cells. *Biol. Trace Elem. Res.* **149**, 123–132 (2012).
66. Silva, J. D. *et al.* Influence of the *Paracoccidioides brasiliensis14*-3-3 and gp43 proteins on the induction of apoptosis in A549 epithelial cells. *Mem. Inst. Oswaldo Cruz* **110**, 476–484 (2015).
67. Galoian, K. *et al.* Effect of cytostatic proline rich polypeptide-1 on tumor suppressors of inflammation pathway signaling in chondrosarcoma. *Mol. Clin. Oncol.* **5**, 618–624 (2016).

Acknowledgements

We heartily acknowledge the gift of IdMOC 96 well plates from Dr. Albert P. Li, CEO, In Vitro ADMET Laboratories, USA. We thank, DST supported National Facility for Drug Development for academia, pharmaceutical and allied industries, Anna University Tiruchirappalli, and Doerenkamp-Zbinden Foundation, Switzerland, for the instrumentation support.

Author contributions

L.G. contributed to the idea, design and implementation of the research, analysis of the results and writing of the manuscript. M.A.A. contributed to the final version of the manuscript. K.R. supervised the whole project. All authors have read and approved the manuscript.

Funding

Financial assistance from the Department of Science and Technology- Science and Engineering Research Board (DST-SERB), Government of India (Project No. PDF/2016/002111), is heartily acknowledged.

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to K.R.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2020

molecules

MDPI

Article

Induction of Redox-Mediated Cell Death in ER-Positive and ER-Negative Breast Cancer Cells by a Copper(II)-Phenolate Complex: An In Vitro and In Silico Study

Vaiyapuri Subbarayan Periasamy [1,2], Anvarbatcha Riyasdeen [1], Venugopal Rajendiran [3], Mallayan Palaniandavar [4], Hanumanthappa Krishnamurthy [5], Ali Abdullah Alshatwi [2] and Mohammad Abdulkader Akbarsha [6,7,*]

1 Department of Animal Science, Bharathidasan University, Tiruchirappalli 620024, India; vsperrys@gmail.com (V.S.P.); anfariyas@gmail.com (A.R.)
2 Department of Food and Nutrition, College of Food and Agricultural Sciences, King Saud University, Riyadh 11451, P.O. Box 2460, Saudi Arabia; alshatwi@ksu.edu.sa
3 Department of Chemistry, School of Basic and Applied Sciences, Central University of Tamil Nadu, Thiruvarur 610005, India; rajendiran@cutn.ac.in
4 Department of Chemistry, Bharathidasan University, Tiruchirappalli 620024, India; palaniandavarm@gmail.com
5 National Centre for Biological Sciences, Tata Institute for Fundamental Research, Bangalore 560065, India; krishna@ncbs.res.in
6 Mahatma Gandhi-Doerenkamp Center (MGDC) for Alternatives to Use of Animals in Life Science Education, Bharathidasan University, Tiruchirappalli 620024, India
7 Research Co-ordinator, National College (Autonomous), Tiruchirappalli, 620001, India
* Correspondence: akbarbdu@gmail.com; Tel.: +91-979-0995-854; Fax: +91-431-2481-997

Academic Editors: Diego Muñoz-Torrero and Beatriz De Pascual-Teresa
Received: 26 August 2020; Accepted: 29 September 2020; Published: 1 October 2020

Abstract: This research was aimed at finding the cytotoxic potential of the mixed ligand copper(II) complex [Cu(tdp)(phen)](ClO_4)—where H(tdp) is the tetradentate ligand 2-[(2-(2-hydroxyethylamino) -ethylimino)methyl]phenol, and phen is 1,10-phenanthroline—to two genotypically different breast cancer cells, MCF-7 ($p53^+$ and ER^+) and MDA-MB-231 ($p53^-$ and ER^-). The complex has been already shown to be cytotoxic to ME180 cervical carcinoma cells. The special focus in this study was the induction of cell death by apoptosis and necrosis, and its link with ROS. The treatment brought about nuclear fragmentation, phosphatidylserine externalization, disruption of mitochondrial trans-membrane potential, DNA damage, cell cycle arrest at sub-G1 phase, and increase of ROS generation, followed by apoptotic death of cells during early hours and a late onset of necrosis in the cells surviving the apoptosis. The efficacy of the complex against genotypically different breast cancer cells is attributed to a strong association through p53-mitochondrial redox—cell cycle junction. The ADMET properties and docking of the complex at the active site of Top1 are desirable attributes of a lead molecule for development into a therapeutic. Thus, it is shown that the copper(II)–phenolate complex$[Cu(tdp)(phen)]^+$ offers potential to be developed into a therapeutic for breast cancers in general and ER-negative ones in particular.

Keywords: anticancer agents; apoptosis; breast cancer; cell cycle arrest; copper(II)-phenolate complex; necrosis; ROS

1. Introduction

Therapeutic potential of metal-based complexes has been of great interest in the drug discovery scenario due to their unique electronic, thermodynamic and kinetic properties such as redox activity, metal–ligand exchange interactions, high electron affinity, and 3D geometry, which favor targeting specific biomolecules [1]. These unique pharmacodynamic characters of metal-based complexes have become an attractive therapeutic concept for the past few decades towards development of novel targeted drug molecules [2]. Currently, platinum-based analogues are among the most practiced main-line metal-based drugs for treatment of a variety of cancers including breast cancer [3,4]. However, these drugs are not very selective to cancers since normal cells are also affected, which leads to substantial dose-limiting acute and chronic toxicities [5,6]. It is generally believed that non-platinum drugs have the potential to overcome this shortcoming [7,8].

Complexes of metals such as platinum, gold, palladium, ruthenium, rhodium, copper, and lanthanum, with aromatic N-containing ligands (pyridine, pyrazinamide, nicotinamide, imidazole, nicotinic acid, 1,10-phenanthroline, and their derivatives), have been shown, using in vitro and in vivo models, to offer promise as anticancer, antibacterial, antiviral, and antifungal agents [9–19]. Among these non-Pt compounds, copper complexes are particularly attractive for designing lead anticancer agents, owing to unique pharmacodynamic properties, enhanced bioavailability, and less toxicity [20–22]. Copper is an essential, endogenous metal and is found in the many proteins and metalloenzymes that are involved in enhancement of bioavailability and driving cellular redox reactions that target specific pathways without producing side effects [23,24].

Aiming at these desired pharmacodynamic profiles, diimines, tetradentate ligands and substituted derivatives as ligands are currently attractive in the discovery of metal-based drugs for different cancers [25,26]. These coordinated or transition metal complexes have been found to be potent as cell cycle inhibitors [27–29], DNA topoisomerase inhibitors [30,31], pro- and anti-apoptotic protein modulators (p53, Bax and Bcl-2) [32–34], etc. Particularly, these metal complexes bring about cancer cell death through inhibition of DNA synthesis [14,26,28,35], alteration of mitochondrial membrane potential and/or suppression of inhibitors of apoptosis [26,36,37]. In addition, diimine ligands of transition metals such as copper are advantageously tuned by increased transport of the metal complexes into the cells [38,39].

In this study, we used copper(II) coordinated by 1,10-phenanthroline (phen) chelator, which can exhibit effective and targeted activity due to the ability of the diimine ligand to engage in DNA recognition. Unique 3D geometry and electronic and other molecular mechanics of this specially designed complex can selectively regulate the membrane permeability, bind with DNA and induce cell death (i.e., apoptosis) [9]. Also, the typical structure of diimines provides for the ability of the complex to participate as a DNA intercalator and/or nucleo-cytoplasmic disruptor through apoptotic mechanism [28,29].

The currently available main line cancer chemotherapeutics are endowed with potential to induce apoptosis in various cancers. Programmed cell death (including apoptosis), as a distinct series of cellular pathways, offers unique targets for chemotherapeutic intervention [40–44]. The direct molecular link between cancer pathogenesis and dysregulation of programmed cell death (PCD) can be used as a therapeutic target by triggering PCD in cancers with drugs such as copper complexes. Especially, cell death mechanisms such as apoptosis, autophagy and necro-apoptosis are different kinds of programmed cell death (PCD) and brilliant cell physiological mechanisms by which cells with irreparably damaged DNA are removed without any inflammatory response [40,43].

Most of the studies emphasize that copper phenolate complexes can act as programmed cell death (apoptosis) modulators via triggering different signal transduction pathways (such as p53 and/or caspase-dependent apoptosis), which provide various hypothetical opportunities to control and/or select pathways in order to find the mode of action [34,37,45,46].

Especially, estrogen receptor (ER) status is one of the principal foci for treatment of breast cancer. The presence or absence of ER in the breast cancer cell is a valuable prognostic factor that

provides predictive value to the potential benefits of target-based therapies [47]. Approximately 70% of metastatic breast cancers are ER-positive, and the patients tend to have a greater chance of effective tumor response and longer survival than patients with ER-negative cancers [48–50].

Some time back, our group synthesized a number of copper(II)–phenolate complexes and evaluated the in vitro anti-proliferative activity with respect to the cervical carcinoma cell ME180 [51]. Since this cell contains HPV18 DNA [24], we picked up interest in finding the efficacy of these copper(II)–phenolate complexes in cancer cells that are not transformed by viral DNA and, hence, chose to conduct the study in breast cancer cells that are genotypically different among themselves.

In the present study, we elucidated the cell death-inducing property of one of the copper(II) –phenolate complexes, $[Cu(tdp)(phen)]^+$, in MCF-7 (ER-positive and p53-positive) and MDA-MB-231 (ER-negative and p53-negative) cells, adopting different methods with special reference to screening of apoptosis. This copper complex brought about extensive changes in both the cell types, indicating apoptosis during the early hours of treatment and necrosis during the late hours, with the molecular mechanisms of action apparently selective for each of the two cell types. In silico prediction of ADMET and molecular docking indicate desirable features.

2. Materials and Methods

2.1. Copper Complexes

The H(tdp) and $[Cu(tdp)(phen)]ClO_4 \cdot CH_3OH$ were synthesized and characterized according to the procedures and methods described in our previous paper [51]. For comprehending the structure–activity relationship, the crystal structure of the complex is shown in Figure 1.

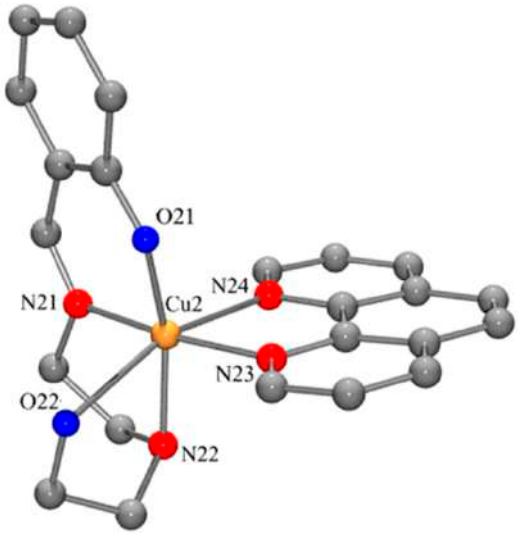


Figure 1. Ball-and-stick representation of the crystal structure of $[Cu(tdp)(phen)](ClO_4) \cdot CH_3OH$ in which atoms are shown as spheres of arbitrary diameter. Hydrogen atoms are omitted for purpose of clarity [51].

2.2. Cell Culture

Breast cancer cells MCF-7 (ER-positive and p53-positive) and MDA-MB-231 (ER-negative and p53-negative) were obtained from National Centre for Cell Science (NCCS), Pune, India. The cells were cultured in DMEM (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (Sigma-Aldrich) and 100 U/mL penicillin and 100 µg/mL streptomycin as antibiotics (Himedia, Mumbai, India), in T25 or T75 flasks and 6, 12, 24, or 96-well cell culture plates (TPP, Trasadingen, Schaffhausen, Switzerland), depending on the context, at 37 °C, in a humidified atmosphere of 5% CO_2

in a CO_2 incubator (Heraeus, Hanau, Germany). All experiments were conducted using cells from passage 15 or less.

2.3. Cytotoxicity Assay

MTT assay was performed as described in the previous study [52]. The copper complex was prepared as stock solutions, to suit the universal 96-well plate map, at different concentrations in nM or μM, dissolved in 100% DMSO (Sigma-Aldrich, St. Louis, MO, USA) or dH_2O. Working solutions were prepared in the culture medium, at a final DMSO concentration of 0.02%, where DMSO was used as the solvent, and added to the wells 24 h after seeding of 5 to 7×10^4 cells per well in 200 μL of fresh culture medium. DMSO (0.02%) was used as the solvent control. Light microscopic cytological changes were monitored and photographed following exposure to different concentrations of the complex for 24 and 48 h using an inverted microscope (Carl Zeiss, Jena, Germany). After the respective periods of treatment, 20 μL of MTT solution [5 mg/mL in phosphate-buffered saline (PBS)] was added to each well, and the plates were wrapped with aluminum foil and incubated for overnight at 37 °C. The purple formazan crystal that formed was dissolved in 100 μL of 100% DMSO. The absorbance of the color was monitored at 570 nm (measurement) and 630 nm (reference) using a 96-well microplate reader (Bio-Rad, Hercules, CA, USA). Data were collected for four replicates each and used to calculate the median effect dose, i.e., IC_{50} (Dm value), sigmoidity of the dose-effect curve (m value) and linear correlation coefficient of the median-effect plot (r value), using Calcusyn software (Biosoft, Cambridge, UK).

2.4. AO & EB Fluorescent Probe for Assessment of Cell Death

Acridine orange (AO) and ethidium bromide (EB) dual staining was performed as described by Spector et al. [53]. The cells treated with the complex at the respective IC_{50} concentrations for 24 and 48 h were incubated with AO and EB solution (1 part each of 100 μg/mL AO and EB in PBS) and mixed gently, and examined in a fluorescent microscope (Carl Zeiss, Jena, Germany) using a UV filter (450–490 nm). Three hundred cells per sample were counted in duplicate for each dose point. Cells were scored as viable, apoptotic or necrotic, as judged from nuclear morphology and membrane integrity, and the respective percentages of apoptotic and necrotic cells were then calculated. The cells of interest were photographed.

2.5. Annexin V-Cy3 Apoptosis Assay

Phosphatidylserine translocation from inner to outer leaflet of the plasma membrane is one of the early features of apoptosis. Cell surface phosphatidylserine was detected by phosphatidylserine-binding protein annexin V conjugated with Cy3 using the commercially available annexin V-Cy3 apoptosis detection kit (APOAC, Sigma-Aldrich, St. Louis, MO, USA). Cells treated with 24 h IC_{50} of the complex for 6, 12 and 24 h were washed with cold phosphate-buffered saline and incubated with 50 μL of the double label-staining solution (containing 1 μg/mL annexin-Cy3 and 100 μM 6-carboxyfluorescein diacetate) for 10 min at room temperature in the dark. The cells were then washed with 1× binding buffer, and 300 cells at random were observed in the fluorescent microscope. The combination of 6-carboxyfluorescein diacetate (6-CFDA) with annexin V conjugated with Cy3 facilitated detection of live cells (green), necrotic cells (red), and apoptotic cells (red nuclei and green cytoplasm). The percentage of cells reflecting cell death (apoptotic and necrotic, separately) was calculated. Data were collected from two individual experiments, each in duplicate, and used to calculate the respective means and the standard deviations.

2.6. Measurement of ROS

Intracellular reactive oxygen species (ROS) was determined by using the fluorescent probe 2′,7′-dichlorofluorescein diacetate (DCFH-DA) [54]. DCFH-DA readily diffuses through the cell membrane and is enzymatically hydrolyzed by intracellular esterases to form non-fluorescent 2′,7′-dichlorofluorescein (DCFH), which is then rapidly oxidized to form highly fluorescent 2′,7′-dichlorofluorescin (DCF) in the presence of ROS. The DCF fluorescence intensity is indicative of

the amount of ROS formed intracellularly. Here, after treating the cells with IC_{50} of the complex for 6, 12 and 24 h, the medium was poured out, the cells were washed with PBS and then incubated with 10 μM DCFH-DA in the loading medium. After DCFH-DA was removed, the cells were washed with PBS and lysed by sonication for 1 min at room temperature. Immediately, the fluorescence intensity (relative fluorescence units) was measured at 485 nm excitation and 530 nm emission in a Jasco (Easton, MD, USA) F 6500 spectrofluorometer.

2.7. Assay of Mitochondrial Trans-Membrane Potential (JC-1 Staining)

Mitochondrial trans-membrane potential was measured using the fluorescent probe JC-1, which produces green fluorescence in the cytoplasm and red to orange fluorescence when concentrated in mitochondria that are metabolically active and so maintain a negative internal potential [53]. JC-1 staining was performed according to the manufacturers' protocol (Sigma, St. Luis, MI, USA). Cells treated for 6 and 12 h with 24 h IC_{50} of the complex in 12-well plates were incubated for 30 min with JC-1 (2 μg/mL) prepared in the culture medium. The adherent cell layer was then washed with PBS and examined in the fluorescent microscope using a UV filter (450–490 nM). Three hundred cells per sample were counted, in duplicate, for each dose- and time point. Orange-red fluorescence of cells indicated intact mitochondrial membrane whereas green fluorescence indicated loss of mitochondrial membrane integrity. These specific fluorescent patterns were indicative of live and dead cells, respectively (if dead, apoptotic or necrotic).

2.8. Alkaline Single-Cell Gel Electrophoresis (Comet) Assay

DNA damage was quantified adopting comet assay [55]. Cells treated with the complex, at the 24 h IC_{50} concentration, for 12 and 24 h, were trypsinized and brought to suspension. Two hundred micro-liters of 1% normal agarose in PBS at 65 °C was dropped gently on to a fully frosted microslide, covered immediately with a cover slip and placed over frozen ice pack for about 5 min. The cover slip was removed after the gel had set. The cell suspension was mixed with 1% low melting agarose at 37 °C in 1:3 (*v/v*) ratio. One-hundred micro-liters of this mixture was applied quickly on top of the gel, coated over the micro-slide and allowed to set as before. A third coating of 100 μL 1% low melting agarose was laid on the gel containing the cell suspension and allowed to set. The slides were prepared in tetraplicate for each cell fraction. After solidification of agarose, the cover slips were removed and the slides were immersed in ice-cold lysis solution (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris, 0.1% Triton X-100; pH adjusted to 10 using NaOH pellets) and placed in a refrigerator at 4 °C for 16 h. The slides, after being removed from the lysis solution, were placed horizontally in an electrophoresis tank. The reservoirs were filled with electrophoresis buffer (300 mM NaOH, 1 mM Na_2EDTA, pH > 13) until the slides were just immersed in it. The slides were allowed to stand in the buffer for about 20 min (to allow DNA unwinding) after which electrophoresis was carried out at 0.8 v/cm for 15 min. After electrophoresis, the slides were removed, washed thrice in neutralization buffer (0.4 M Tris, pH 7.5) and gently dabbed to dry. The nuclear DNA was stained with 20 μL EB (50 μg/mL). Photographs were obtained in the fluorescent microscope (450–490 nm). One-hundred-and-fifty cells from each treatment were digitalized and analyzed using CASP comet assay software. The images were used to determine the DNA content of individual nuclei and evaluate the degree of DNA damage representing the fraction of total DNA in the tail. Cells were assigned to five classes, intact (0–20%), slightly damaged (20–40%), damaged (40–60%), highly damaged (60–80%), and dead (80–100%).

2.9. Cell Cycle Analysis

Flow cytometer-based DNA content analysis is an important tool to detect cell death/cell cycle arrest. Cell cycle analysis was performed as per an established protocol [56]. The cells were seeded in T_{25} flasks at a density of 1×10^6 cells/flask. After 24 h, the complex, at the respective IC_{50} concentrations, was added to the flasks and incubated for 24 and 48 h. The cells were trypsinized, harvested, fixed in 80% cold ethanol, and stored for 24 hr at 4 to 8 °C. The cells were rehydrated with PBS and centrifuged

at 4000 rpm for 5 min. Further, the cell pellets were resuspended in propidium iodide (50 µg/mL). Cell cycle distribution was analyzed using FACScan (Becton-Dickinson, Sanjose, CA, USA) with 15 mw, 488 nm argon ion laser. Propidium iodide signals were collected using 585/42 band pass filter. The data were analyzed using Cell Quest software.

2.10. Western Blot Analysis

Total proteins from untreated and treated cells were extracted, quantified and subjected to electrophoresis, followed by Western blot analysis [57,58] using antibodies of specific apoptotic markers *viz.*, p53, Bcl-2 and Bax, with β-actin as the loading control (Sigma-Aldrich, St. Louis, MO, USA). In T_{75} flasks, 5×10^6 cells were seeded and treated with the complex at the 24 h IC_{50} concentration for 6, 12, and 24 h. The cells were washed in cold PBS and lysed in RIPA buffer with 1% Triton X-100, 50 mM Tris, pH 7.6, and 150 mM NaCl containing 2 mM phenylmethylsulfonyl fluoride. Total protein of the lysate was quantified adopting Bradford assay [57]. Subsequently, the proteins were separated adopting sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electroblotted to a nitro-cellulose membrane. The loading and transfer of equal amounts of protein were confirmed by staining the membrane with PonceauS. The membranes were blocked for 3 h using 5% non-fat dry milk powder in Tris-buffered saline (TBS: 10 mM Tris-HCl pH 7.4, 100 mM NaCl) and then immunoblotted for 3 h with the primary antibody at appropriate dilutions. The membranes were washed with TBS/0.05% Tween-20 and incubated with secondary antibodies conjugated with peroxidase-conjugate (Bangalore Genei, Bangalore, India) and/or alkaline phosphatase for 1 h. After extensive washing, the reaction product was developed using diaminobenzidine (DAB) substrate. The Western blot image was digitized, normalized, and subjected to image analysis using ImageJ (NIH, Bethesda, MD, USA) software (Open Access Public Domain).

2.11. ADMET and Molecular Docking Studies

Different ADMET and molecular properties such as physicochemical parameters, pharmacokinetics, lipophilicity, water solubility, carcinogenicity, mutagenicity, and relevant descriptors of the copper complex were predicted using Discovery Studio 3.5. For the molecular docking study, 2D structure of the copper complex was drawn using ChemDraw tools. Further ligand preparation, including 3D structure conversion and minimization, was carried out as per the Discovery Studio v3.5 protocol. The 3D structures of topoisomerase I (Top1) with DNA complex (PDB id: 1SC7) were retrieved from the RCSB Protein Data Bank. Target protein preparation and minimization were carried out as described in the Discovery Studio protocol. The optimal target position and different interactive bonds of the copper complex with Top1 were determined using the Molegro Virtual Docker software (trial version). Further, bonding interactions between the copper complex and Top1 were visualized by Discovery Studio Visualizer.

2.12. Statistical Analysis

The results were analyzed for IC_{50} values, sigmoidity, correlation and mean ± standard deviation using Calcusyn software and Microsoft Excel 2000 software. For analysis of paired samples, Student's *t*-test was conducted, and p value <0.05 was considered statistically significant. The ROS data were subjected to Mann Whitney non-parametric test at 90% confidence limit using Graphpad Prism software.

3. Results

3.1. Cytotoxic Potential of the Complex as Revealed in MTT Assay

The cytotoxic potential of the complex on human breast cancer cell lines was determined as the dose value of exposure of the complex required to reduce survival of the cells to 50% (IC_{50}). The copper complex produced time- and concentration-dependent cytotoxic effects in both breast cancer cells. The IC_{50} values, sigmoidity and correlation are presented in Table 1. The IC_{50} value for the complex was low for both the cell lines at 48 h treatment compared to 24 h treatment. Further, at 48 h time point it was relatively low for

MDA-MB-231 cells (1.0 ± 0.9 μM), compared to MCF-7 cells (1.2 ± 0.8 μM), though the difference was not statistically significant.

Table 1. In vitro MTT cytotoxicity assay for the complex against human breast carcinoma cell lines MCF-7 and MDA-MB-231 [IC_{50} values (Dm), sigmoiditiy (m) and correlation (r)].

Cell Lines	24 h			48 h		
	IC_{50} (μM)	m	r	IC_{50} (μM)	m	r
MCF-7	1.6 ± 0.8	2.9	0.8	1.2 ± 0.8	2.8	0.8
MDA-MB-231	1.9 ± 1.2	3.1	0.9	1.0 ± 0.9	2.2	0.9

3.2. Indications of Apoptosis and Necrosis as Revealed in AO-EB Staining

After the cells were exposed to the respective IC_{50} concentrations of the complex for 24 and 48 h, and stained with AO & EB, the cells were assigned to four patterns according to the fluorescence emission and nature of chromatin condensation in the nuclei (Figure 2): (i) viable (uniformly green-fluorescing nuclei with a highly organized structure), (ii) early apoptotic (still had intact membranes but DNA fragmentation was initiated and had green-fluorescing nuclei, and peri-nuclear chromatin condensation was visible as bright green patches or fragments), (iii) late apoptotic (with bright orange fluorescing nuclei with condensed or fragmented chromatin), and (iv) necrotic cells (uniformly bright red-fluorescing nuclei with no indication of chromatin fragmentation but the cells were swollen to a large size).

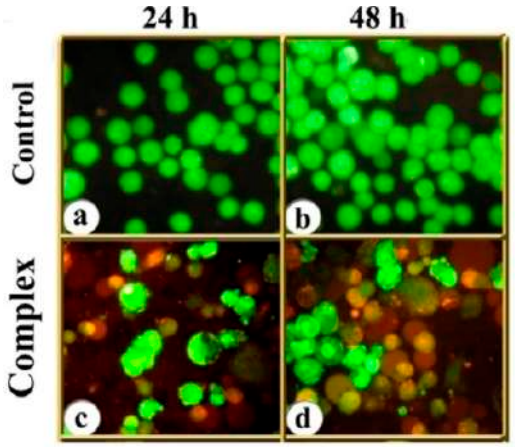


Figure 2. MCF-7 cells treated with the complex for 24 and 48 h and AO & EB stained. (**a**,**b**) control cells (viable; uniform green-fluorescence); (**c**,**d**) cells treated with the complex; pre-apoptotic (yellow-fluorescence) and apoptotic (bright orange fluorescence) cells. Necrotic cells are swollen and fluoresce bright-red (400×).

Over all, the results indicated that treatment with the copper complex induced both MCF-7 and MDA-MB-231 cells to take to death through apoptosis as well as necrosis (Figure 3), and the response was dependent on the cell type, concentration of the complex, and duration of the treatment.

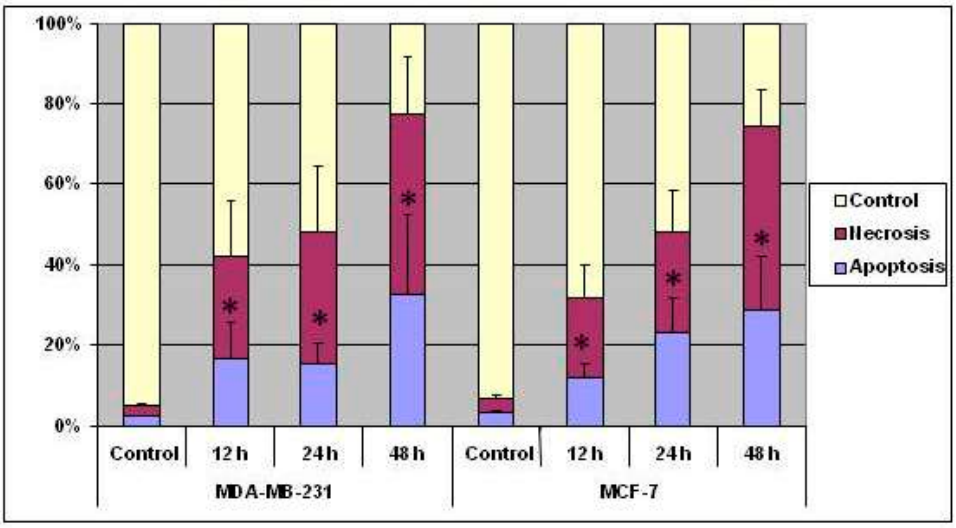


Figure 3. Data show the response of MCF-7 and MDA-MB-231 cells, in terms of apoptosis (AO & EB staining), to treatment with the copper complex. The percentages of cells in apoptosis and necrosis are indicated by the histograms. The data shown are the means from triplicates. Vertical bars represent standard error of the mean. *p*-values obtained between control and complex-treated cells using *t*-test where '*' indicates ($p \leq 0.05$)—significant.

3.3. Indications of Early Apoptotic Changes as Revealed in Annexin V-Cy3 and 6-CFDA Staining

The cells treated with the complex were subjected to dual staining with annexin V-Cy3 and 6-CFDA to obtain evidence for early apoptotic changes. Annexin V binds to phosphatidylserine moieties that become exposed on the outer surface of the cell membrane during apoptosis, whereas 6-CFDA staining serves as a marker for viable cells. This combination helps to differentiate early apoptotic cells (annexin V-positive, 6-CFDA-positive), necrotic cells (annexin V-positive, 6-CFDA-negative), and viable cells (annexin V-negative, 6-CFDA-positive). Both MCF-7 and MDA-MB-231 cells treated with the complex exhibited significant incidence of apoptosis (Figures 4 and 5).

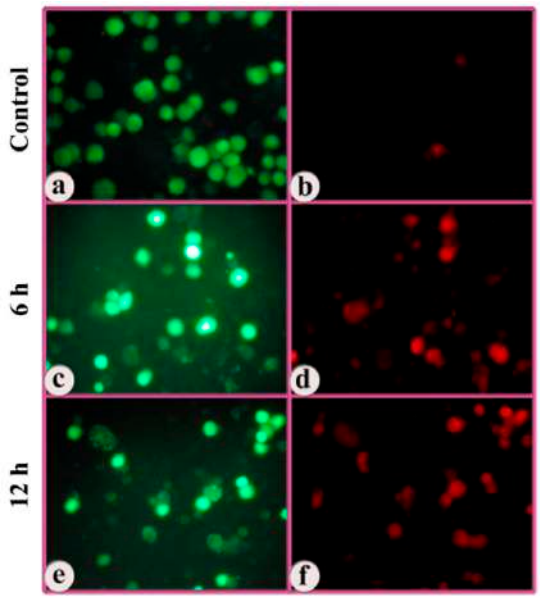


Figure 4. Fluorescent photomicrographs showing annexin V-Cy3 apoptosis assay on MCF-7 breast cancer cells treated with the complex. (**a**,**b**) control (untreated); (**c**,**d**) treated with the complex for 6 h; (**e**,**f**) treated with the complex for 12 h.

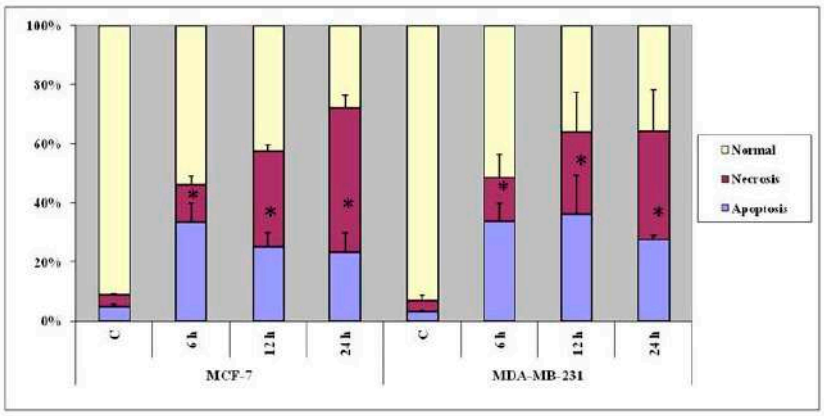


Figure 5. Data show the response of MCF-7 and MDA-MB-231 cancer cells, in terms of apoptosis (annexin V-Cy3 staining), to treatment with the complex. The percentages of cells in apoptosis and necrosis are indicated by the histograms. The data shown are means from triplicates. Vertical bars represent standard error of the mean. *p*-values obtained between control and treated cells with the complex using *t*-test where '*' indicates ($p \leq 0.05$)—significant.

Here again, it appeared that the mode of cell death was dependent on incubation time from two perspectives, (i) with an increase in incubation time, more cells died; and (ii) with lesser incubation time, the incidence of apoptosis was more than necrosis, and with longer incubation time, more cells took to necrosis than apoptosis. Within this generalization, the incidence of apoptosis was more in $p53^{-}$ & ER^{-} MDA-MB-231 cells than $p53^{+}$ & ER^{+} MCF-7 cells, but this difference was not statistically significant.

3.4. Changes in ROS Level

MCF-7 and MDA-MB-231 cells treated with the complex at 24 h IC_{50} concentration for 6, 12 and 24 h were subjected to analysis of cellular ROS levels. The treatment induced both the cell types to generate high amounts of ROS, in a manner duration-dependent with regard to MCF-7 cells. However, data were tested at 90% confidence level. For MCF-7 cell significance was revealed only for the 24 h time point, whereas for MDA-MB-231, data is significant at all three time points (Figure 6).

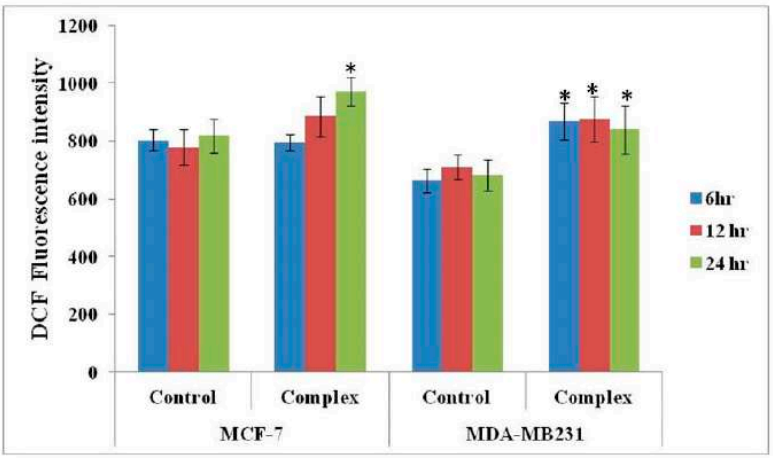


Figure 6. Cellular ROS levels in MCF-7 and MDA-MB-231 cancer cells treated with the complex at 24 h IC_{50} for 6, 12 and 24 h. *p*-values obtained between control and treated cells with the complex using Mann Whitney non-parametric test where '*' indicates ($p \leq 0.05$)—significant.

3.5. Change in the Mitochondrial Membrane Potential as Revealed in JC-1 Staining

The fluorescent cationic dye JC-1 was used to detect the change in mitochondrial trans-membrane potential, which is an early event in the induction of apoptosis. In healthy, non-apoptotic cells the dye

accumulates and aggregates within the mitochondria, resulting in bright orange to red fluorescence. In apoptotic cells, due to collapse of mitochondrial trans-membrane potential, JC-1 remains outside the mitochondria, in the cytosol, in the green-fluorescent monomeric form. The results revealed that there was dramatic change in mitochondrial trans-membrane potential in both MCF-7 and MDA-MB-231 cells on treatment with the copper complex, as revealed in the intense green fluorescence of the cytosol in a duration-dependent manner, indicating the onset of an early event in the apoptosis cascade (Figure 7).

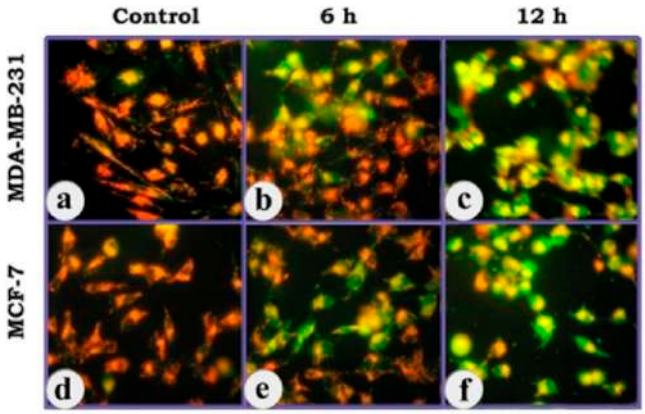


Figure 7. Photomicrographs of breast cancer cells (MCF-7) showing the mitochondrial trans-membrane potential as revealed in JC-1 staining. (**a**,**d**) control; JC-1 dye accumulation in the mitochondria of healthy cells as aggregates (red- to orange-fluorescence); (**b**,**e**) cells treated with the complex for 6 h; (**c**,**f**) cells treated with the complex for 12 h. (400×). Due to collapse of mitochondrial transmembrane potential, the JC-1 dye remains in the cytoplasm in the monomeric form and emits green fluorescence (6 & 12 h).

3.6. DNA Damage as Revealed in Comet Assay

The DNA content of individual nuclei and the degree of DNA damage representing the fraction of total DNA in the tail were determined adopting comet assay, and the cells were assigned to five classes, referred to as *vide supra.* A significant percentage of cells showed increased length and density of comet tails in a time-dependent manner as compared to untreated cells (Figure 8).

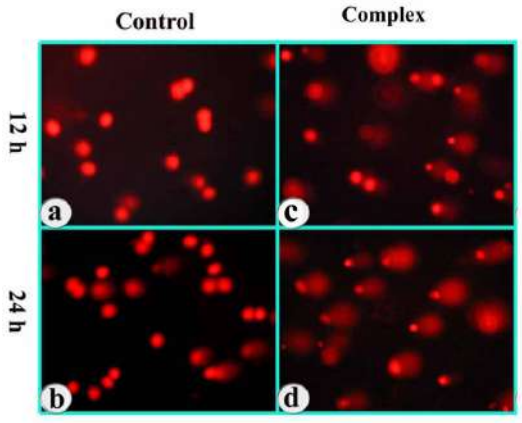


Figure 8. Images of DNA double-strand breaks (comets) at 12 and 24 h treatment of MCF-7 cells with the complex. Left panel (**a**,**b**)—control; right panel (**c**,**d**)—treated. The duration-dependence of the DNA damage is revealed.

As seen in Figure 9, a fairly high percentage of cells treated with the complex got assigned to 'damaged', 'highly damaged' and 'dead' categories at 12 and 24 h treatment, the response increasing in

a duration-dependent manner. Here, the 'dead' and 'highly damaged' cell populations were more among ER-negative MDA-MB-231 cells than ER-positive MCF-7 cells.

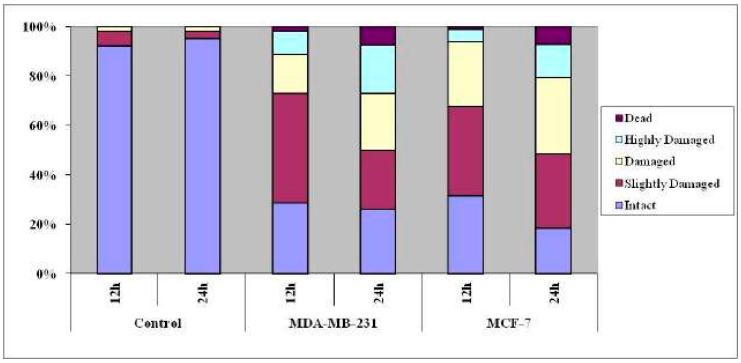


Figure 9. Results of comet assay in MCF-7 and MDA-MB-231 cancer cells treated with the complex for 12 and 24 h. The 100% stacked column compares the percentage of comet DNA.

3.7. Change in Cell Cycle Progression as Revealed in Flow Cytometric Analysis

Since the cells treated with the complex indicated a fairly good response in terms of apoptosis, the complex was analyzed for impact on the cell cycle progression, adopting flow cytometry. The DNA of 10,000 cells of each sample was evaluated, and the results revealed duration-dependent distribution of both the breast cancer cells belonging to the treatment groups different from the respective controls (Figure 10). As seen in Table 2, there was significant increase of cells in the S- and G_2/M phases when treated with the complex, showing that there was significant arrest of cell division at these respective phases. Importantly, this response was more dramatic in MDA-MB-231 cells than MCF-7 cells.

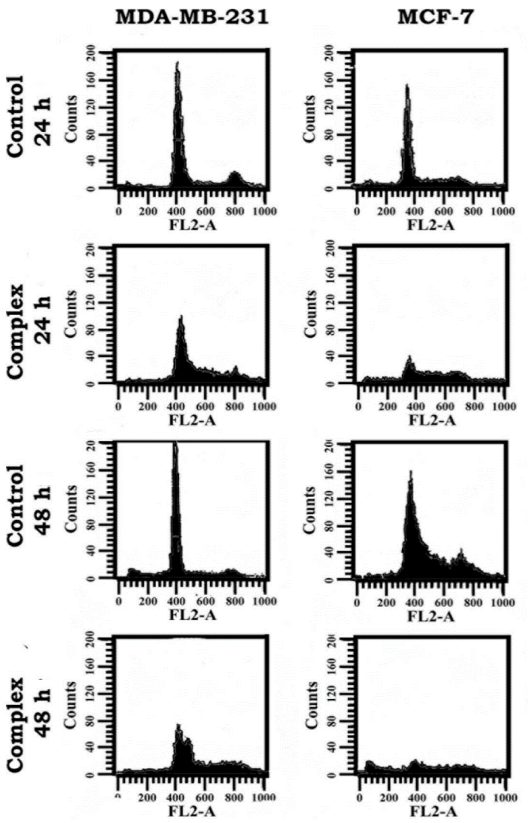


Figure 10. Histograms derived from flow cytometry data showing the effect of the complex on cell cycle distribution of breast cancer cells (MDA-MB-231 & MCF-7) for 24 and 48 h treatment.

Table 2. Table showing DNA content of cells (MCF-7 and MDA-MB-231) distributed among different phases (sub-G0, G0+G1, S, G2+M) of cell cycle as revealed in FACS analysis. The data shown are means ± SD from three independent replicates.

Complexes	Duration	Sub-G0	SD	G0+G1	SD	S	SD	G2+M	SD
MDA-MB-231									
Control	24 h	5.05	±0.6	80.77	±0.29	4.47	±0	7.46	±0.3
[Cu(tdp)phen]+		1.82	±0.2	59.84	±0.46	19.4	±0.1	14.9	±0.2
Control	48 h	5.38	±0.7	83.88	±1.09	3.31	±0.2	5.96	±0.4
[Cu(tdp)phen]+		3.69	±0.3	53.59	±1.02	17.1	±0.2	20.9	±0.4
MCF-7									
Control	24 h	2.74	±0.3	77.39	±0.72	6.74	±0.4	9.89	±0.5
[Cu(tdp)phen]+		5.35	±1.8	53.28	±8.88	16.4	±2.5	17.4	±3.7
Control	48 h	2.41	±0.4	54.84	±3.4	22.2	±1.8	16.8	±1.8
[Cu(tdp)phen]+		25.72	±4.5	21.63	±0.31	15.8	±1	28.3	±2.7

3.8. Change in Molecular Indicators of Apoptosis

The expression of p53 (tumor suppressor protein) and two Bcl-2 family members, Bcl-2 (anti-apoptotic) and Bax (pro-apoptotic), was evaluated adopting Western blot technique (Figure 11). Using p53 wild-type MCF-7 and p53-mutant MDA-MB-231 cells, a strong correlation was found between p53 status and the response of the cells to the complex in terms of apoptosis. In the p53-positive MCF-7 cells, the treatment produced a slight increase in the p53 protein expression at 6 and 12 h time points, which harmonized with Bax up-regulation and Bcl-2 down-regulation. In the p53-mutant MDA-MB-231 cells, p53 protein expression was increased at all three time points with a corresponding increase of Bax protein, but Bcl-2 protein expression also remained fairly high.

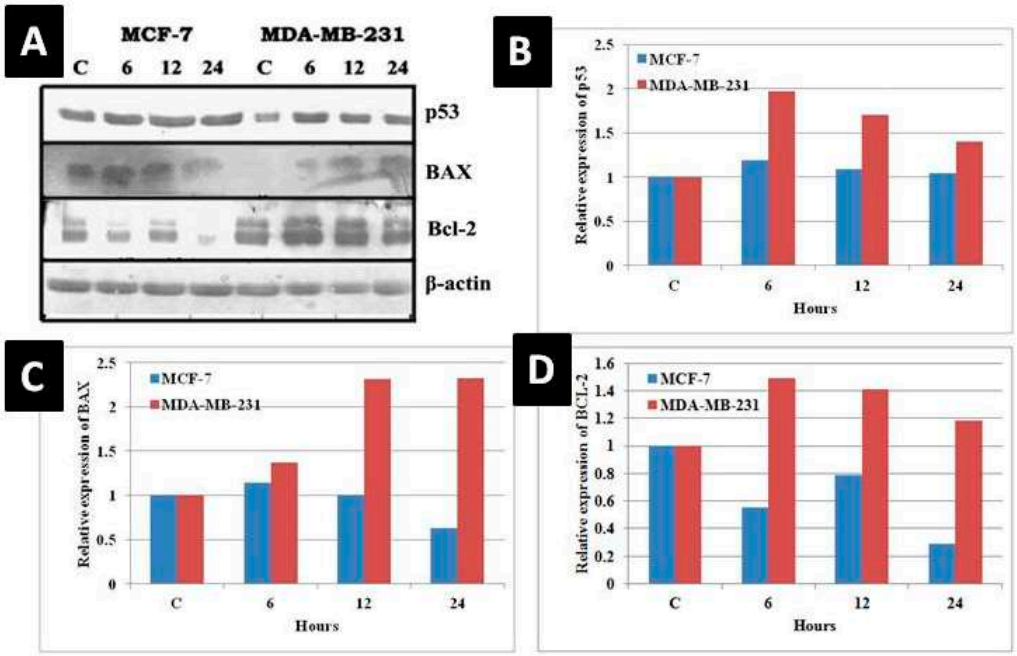


Figure 11. Western blot analysis of modulation of pro- and anti-apoptotic proteins due to treatment of MCF-7 and MDA-MB-231 cells with the complex. (**A**) The blot was probed with antibodies for p53, Bcl-2, and Bax, where β-actin was the loading control. (**B**–**D**) The densitometry graphs represent the relative expression of p53, BAX and Bcl-2 proteins, respectively.

3.9. ADMET and Molecular Docking Studies

In silico-based molecular and ADMET properties such as distribution parameters (AlogP98); solubility; absorption level; absorption parameter (PSA-2D); plasma protein binding (PPB); blood brain barrier (BBB); cytochrome P450 2D6 (CYP2D6); hepatotoxicity; Ames_Prediction; Ames_Score; TOPKAT FDA Carcinogenicity, Female Rat; and TOPKAT FDA Carcinogenicity, Male Rat, compared and interpreted based on the references, were predicted for our copper complex using Drug Discovery Studio 3.5 (Table 3). The values of molecular properties, such as AlogP and solubility, can help us evaluate and predict the hydrophilicity or lipophilicity of a compound. The predicted partition coefficient AlogP98 (3.588) and aqueous solubility (score = −7.391) of our copper complex assign to the hydrophobic scale, which can be favorable for membrane permeability. The predicted parameter scores of absorption level (scale = 0) and PSA-2D (31.909) indicate good absorption in the human intestine. The plasma protein binding (PPB) (score = 2.89411) suggests that our copper complex likely binds to carrier proteins in the blood. The predicted BBB value (score = 0.45) is a clear indication of penetrability through the blood brain barrier. The prediction of the complex based on Ames (v3.1) score (−2.33954), hepatotoxicity (−2.52683), and CYP2D6 enzyme inhibition score (−2.30385) shows it to be in the non-toxic range. TOPKAT carcinogenicity in female rat (v3.1) and TOPKAT carcinogenicity in male rat model (v3.1) predictions indicate the complex to be non-carcinogenic. Overall, these pharmacokinetic descriptors give an acceptable range defined for a lead compound providing for further drug development.

Table 3. In silico ADMET properties of the copper complex deciphered adopting Discovery Studio prediction software.

Descriptors	Score
ADMET AlogP98 (partition coefficient)	3.588
ADMET Aqueous Solubility	−7.391
ADMET Absorption Level	0
ADMET PSA2D—(Fast polar surface area)	31.909
ADMET EXT PPB	2.89411
ADMET BBB	0.45
ADMET EXT CYP2D6	−2.30385
ADMET EXT Hepatotoxicity	−2.52683
TOPKAT Ames mutagenicity	Non-Mutagen
TOPKAT Ames mutagenicity score	−2.33954
TOPKAT Carcinogenicity in female rat	Non-Carcinogen
TOPKAT Carcinogenicity in male rat	Non-Carcinogen

The molecular docking of copper complex into active site of Top1 enzyme was performed using Discovery Studio 3.5 and Molegro Virtual Docker software (trial version). The final higher docking score was selected in terms of the ligand–protein–DNA complex with the lowest binding energy (−7.8 Kcal/mol). Bond interactions and distance (Å) of the copper complex with amino acid residues in the active site of Top1 enzyme are presented in Figure 12. It was found that Pi-sigma and Pi–Pi T-shaped interaction between the copper complex and the selective active site of Top1 enzyme occurs at a specific site on Leu A:716 and ALA A:175, respectively. Another important non-covalent bond, i.e., Pi–anion interaction with DNA molecule, was found between tdp and phen of the complex with DG C:12 site of DNA. Overall, the docking results indicate that this critical conformational pose of the ligand–protein–DNA complex might play a role in enzyme inhibition and DNA intercalation.

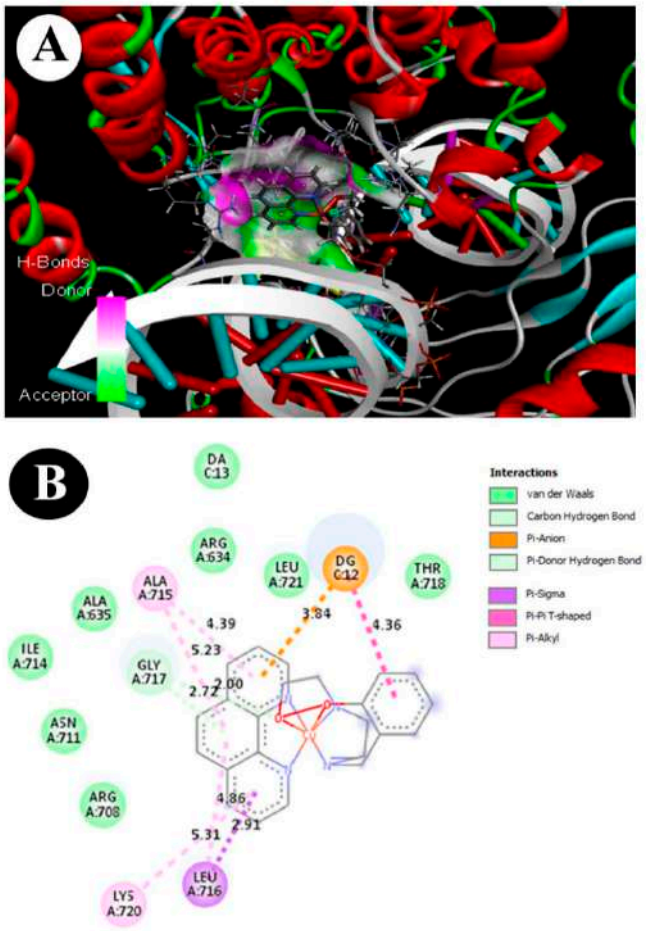


Figure 12. Molecular docking analysis of the copper complex with Top1 in DNA. (**A**) 3D view of copper complex with Top1 in DNA. (**B**) 2D view of copper complex interaction with Top1 in DNA.

4. Discussion

In general, tumors develop due to deregulation of cell proliferation, which is invariably associated with the inability of cells to undergo PCD [59,60]. Several cytotoxic drugs mediate cell death by modulating key elements of the apoptosis cascade and/or the cellular stress response [61]. The primary objective of the present study has been to find if the copper phenolate complex $[Cu(tdp)(phen)]^+$ would be cytotoxic to breast cancer cells of very different origins and which manifest in different forms, the p53-positive & ER-positive MCF-7 and p53-negative & ER-negative MDA-MB-231.

It is an attempt to find if this complex would kill either or both the breast cancer cells, and in the latter case, if the underlying mechanisms would be different between the two since two crucial genes, *p53* and *ER* are involved. The 24 and 48 h IC_{50} values reveal a concentration- and time-dependent cytotoxic effect of the complex. Also, the results suggest that both MDA-MB-231 (p53-negative & ER-negative) and MCF-7 (p53-positive & ER-positive) breast cancer cells are sensitive to the complex by mechanisms selective to each. It is likely that the coordinated structural feature of this complex determines this selectivity. The coordinated phen contributes to partial DNA interaction to manifest the cytotoxic property [26,62–64], and the Schiff base ligand would encourage intercalation of phen in the minor groove by engaging in hydrogen-bonding interactions between coordinated -NH- and -OH with the functional groups positioned on the edge of DNA bases [45,65,66].

In our study, the nuclear morphological and cell surface externalization staining results clearly revealed the relationship between the cytotoxic property of a substance with the early- and late events in apoptosis. Annexin V is a marker for early events in apoptosis, so our copper complex induced the early events of apoptosis cascade, where phosphatidylserine is translocated from the inner to

outer leaflet of the plasma membrane. On the other hand, changes such as chromatin fragmentation, shrinkage of cells, and formation of apoptotic bodies as revealed in AO-EB staining are a clear indication of late apoptotic events. Thus, a major percentage of the affected cells manifested cytological changes consistent with apoptosis inclusive of both early and late events. This is indicated in the phen present in the complex, which is an effective inducer of apoptosis [26,34–36,45].

The shift from preponderance of apoptosis to necrosis, as found in this study, is dependent on the concentration of the complex as well as incubation time; higher doses and longer incubation times are required to switch from apoptosis to necrosis. Importantly, necrosis is as effective a role-player in cancer therapy as apoptosis [67]. The shift from apoptosis to necrosis by several copper complexes is due to an interlinked phenomenon of oxidative stress and disruption of redox homeostasis [45].

Reactive oxygen species, including superoxide anion radical, hydrogen peroxide and hydroxyl radical, are produced within cells under normal aerobic growth conditions, but their production is increased under the influence of external stresses. Several recent reports suggest a role for ROS in drug-induced apoptosis through engagement of downstream proteins involved in the execution of apoptosis [25,68]. Modulation of oxidative stress is an important strategy in anticancer drug development. Intracellular ROS is considered a key mediator of apoptosis and, therefore, production of ROS at toxic levels or at a threshold may provide opportunity for exploitation to kill cancer cells [69]. In this study, the pattern of intracellular ROS level was altered on treatment, differentially between MCF-7 and MDA-MB-231 cells, whereupon apoptosis was induced in both cases. Thus, in the copper(II) phenolate complex-induced cell death, in this study, an imminent role for ROS is indicated. In most cell types, mitochondria produce substantial quantities of ROS when the cell is subjected to stress [70,71].

Concomitant with alteration in ROS levels, there was loss of mitochondrial membrane potential. The ROS production is further derived from the already reported plasmid DNA cleavage effect by $[Cu(tdp)(phen)]^+$ in the presence of reducing agent ascorbic acid and also without an added reductant. In fact, the complex $[Cu(tdp)(phen)]^+$ exhibited conversion of the supercoiled (SC) form of plasmid DNA to nicked circular (NC) and linear circular (LC) forms through the generation of freely diffusible hydroxyl radical, a reactive oxygen species (ROS), directly responsible for initiation of the cleavage reaction and thus cleaved plasmid DNA in the absence of a reducing agent [51]. Thus, the copper complex brings about evidence to the effect that free oxy-radical species can play an important role in directing the course of cell death. Therefore, it is suggested that the copper complex modulates the intra-cellular redox system and, thereby, provides for accumulation of ROS differentially between MCF-7 and MDA-MB-231 cells, oxidative DNA damage, and cell death.

The activation of endogenous endonucleases, with subsequent cleavage of DNA into oligonucleosomal fragments, is a hallmark of apoptosis [72]. Copper complexes, which are highly oxidized, have been reported to induce DNA strand breaks and base damage in various cancer cells [37,45,51,64,70]. The results of the comet assay in this study indicate DNA damage, thus supporting the inference arrived at from morphological analyses, loss of mitochondrial membrane potential and alteration of intracellular ROS levels, and a majority of affected cells succumbed to death by apoptosis [73].

The results in terms of copper complex-induced nuclear, cell surface and mitochondrial changes are in good correlation with the incidence of apoptosis as revealed in the increase of sub-G1 population. Chemical agents that damage DNA are known to act via modulation of redox system, to produce growth arrest and induce apoptotic cell death [28,29,34,35,51,65,70]. Cell cycle progression is regulated in an orderly manner from G1 to M, through S and G2, by phase-specific regulation of the levels of cyclins, cyclin-dependent kinases (CDKs) and tubulin, and also their inhibitors, which have strong association with apoptosis [74,75]. The data emanating from analysis of cell cycle in this study reveal that there was perturbation in the percentage of cells distributed among the various phases of cell cycle, which is driven by the nature of the complex, the cell type and the duration-dependence.

The different patterns of cell death in this regard in the two different breast cancer cells strongly suggest that the mechanism of action of the complex may be genotype-selective (i.e., p53-positive/negative and ER-positive/negative). Numerous investigations have correlated oxidative stress with different p53-directed

cell fates, such as cell cycle arrest, DNA repair and apoptosis [76]. For example, generation of excess ROS in mitochondria, resulting from treatment with chemotherapeutic agents, leads to apoptosis [77,78]. For p53 to effectively regulate the basal levels of ROS, a redox-active regulation loop should exist, which would serve as a channel for cross talk between the basal levels of cellular ROS and p53 and to keep the fluctuations of ROS and p53 within physiological ranges [40,41,77,78].

It is an established fact that p53 is an important regulator of transcription, and more than 300 genes are regulated by this transcription factor. The p53-mediated apoptosis, in response to DNA damage, is predominantly attributable both to transcriptional activation of genes that encode pro-apoptotic proteins (such as Bax) and transcriptional repression of genes that encode anti-apoptotic proteins (such as Bcl-2) [79,80]. The results of this study indicate both ER-positive and ER negative breast cancer cells as susceptible to p53-associated response of our copper complex modulation, fairly different between ER-positive MCF-7 cell and ER-negative MDA-MB-231 cell. This is evidenced by reasonably different shifts in p53 expression and Bax/Bcl-2 protein ratios in the two breast cancer cells. When correlated with the all other parameters (cytotoxicity, morphological changes, annexin V-positivity, DNA damage, change in mitochondrial trans-membrane potential and S-phase & G2/M arrest), both types of cells are sensitive to the complex but via molecular mechanisms essentially cell type-specific. Moreover, estrogen receptor-dependent modulation of gene expression is a potential target for chemotherapeutic agents. Estrogen receptor is directly associated with the control of cell proliferation and cell death induction [81]. However, the ER-p53 sensitivity assessment needs more in-depth gene expression profile analyses that will help us draw a clear conclusion about the mode of action of the copper complex.

More than one pathway, with a redox system as the mediator in both cases, is apparently associated with the course towards apoptosis. Especially, p53 has an integral role in maintaining a mitochondria-mediated redox mechanism as well as DNA repair mechanism. In this context, topoisomerase I is an upstream target to p53 and has direct association with mitochondria-mediated apoptosis and cell cycle inhibition. Several studies have reported that human topoisomerase I (Top1—PDP: 1SC7) is one of the important molecular targets for a number of anticancer drugs. For example, analogues of camptothecins, indolocarbazoles, and indenoisoquinolines inhibit and/or intercalate with Top1-DNA complex [82–84]. In our molecular docking studies, a strong binding affinity of copper complex towards Top1 enzyme-DNA complex (−8.5 kcal/mol) was found. Particularly, Pi–sigma and Pi–Pi T-shaped interactions between copper complex and Top1 enzyme occur on Leu A:716 and ALA A:175, respectively. Moreover, Pi–anion interaction with DNA (DG C:12 region) where the residues i.e., Leu A:716, ALA A:175 and DG C:12, might have a key role in the enzyme inhibition and DNA intercalation (Figure 8). High affinity and strong specificity of the copper complex with Top1–DNA complex might play an important role in the inhibition of cancer cell activities such as replication, and alteration in transcription, redox mechanism and DNA repair mechanism with association to the cell death mechanism. Thus, the mechanism of cell death as induced by the copper complex appears to be essentially p53-mediated with at least a redox system in common. From these perspectives, it will be pertinent to work further on the mechanisms of cell death induced by the copper complex adopting ER- and/or p53-based comparative molecular studies. Investigation along this direction is in progress.

Thus, this study shows that a mixed ligand copper(II) complex, $[Cu(tdp)(phen)]^{+}$) with a diimine co-ligand (phen), is capable of dealing with two genotypically different breast cancer cells in different manners, and is rather selective, culminating in apoptosis and necrosis. This is interesting since ER-negative breast cancer cells have so far found very poor prognostic strategies, and the present finding can go a long way in this direction, provided the complex is subjected to further testing at the molecular level. It is concluded that the copper complex offers potential to be developed as a target-specific anticancer agent for treatment of breast cancers in general and ER-negative ones in particular.

Author Contributions: V.R. performed the chemistry portion of the research. M.P. supervised the chemistry research and corrected the chemistry part of the manuscript. V.S.P. and A.R. carried out the biological research and analyzed the data. H.K. conducted the cell cycle analysis and interpreted the data. A.A.A. and M.A.A. participated in the design of the experiments, supervision and writing of the biology part of the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by SERB, New Delhi, as a Start-Up Research Grant (Young Scientists)—Chemical Sciences (SB/FT/CS-187/2011) to V.R. The APC was met from Researchers Supporting Project No RSP-2020/178, King Saud University, Riyadh, Saudi Arabia, by A.A.A.

Acknowledgments: M.P. acknowledges the Senior Scientist Award from Indian National Science Academy (INSA), New Delhi. M.A.A. acknowledges in vitro research facility at Mahatma Gandhi-Doerenkamp Center which was established with fund from Doerenkamp-Zbinden Foundation, Switzerland. The flow cytometry facility of NCBS, Bangalore, is also acknowledged.

Conflicts of Interest: The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

References

1. Ndagi, U.; Mhlongo, N.; Soliman, M.E. Metal complexes in cancer therapy—An update from drug design perspective. *Drug Des. Devel. Ther.* **2017**, *11*, 599–616. [CrossRef] [PubMed]
2. Frezza, M.; Hindo, S.; Chen, D.; Davenport, A.; Schmitt, S.; Tomco, D.; Dou, Q.P. Novel metals and metal complexes as platforms for cancer therapy. *Curr. Pharm. Des.* **2010**, *16*, 1813–1825. [CrossRef] [PubMed]
3. Kelland, L. The resurgence of platinum-based cancer chemotherapy. *Nat. Rev. Cancer* **2007**, *7*, 573–584. [CrossRef] [PubMed]
4. Dilruba, S.; Kalayda, G.V. Platinum-based drugs: Past, present and future. *Cancer Chemother. Pharmacol.* **2016**, *77*, 1103–1124. [CrossRef] [PubMed]
5. Oun, R.; Moussa, Y.E.; Wheate, N.J. The side effects of platinum-based chemotherapy drugs: A review for chemists. *Dalton Trans.* **2018**, *47*, 6645–6653. [CrossRef] [PubMed]
6. Manohar, S.; Leung, N. Cisplatin nephrotoxicity: A review of the literature. *J. Nephrol.* **2018**, *31*, 15–25. [CrossRef]
7. Nardon, C.; Boscutti, G.; Fregona, D. Beyond platinums: Gold complexes as anticancer agents. *Anticancer Res.* **2014**, *34*, 487–492.
8. Ott, I.; Gust, R. Non platinum metal complexes as anti-cancer drugs. *Arch. Pharm.* **2007**, *340*, 117–126. [CrossRef]
9. Zhao, G.; Lin, H. Metal complexes with aromatic N-containing ligands as potential agents in cancer treatment. *AntiCancer Agents Med. Chem.* **2005**, *5*, 137–147. [CrossRef]
10. Kuduk-Jaworska, J.; Puszko, A.; Kubiak, M.; Pelczynska, M. Synthesis, structural, physico-chemical and biological properties of new palladium(II) complexes with 2,6-dimethyl-4-nitropyridine. *J. Inorg. Biochem.* **2004**, *98*, 1447–1456. [CrossRef]
11. Hu, J.; Liao, C.; Mao, R.; Zhang, J.; Zhao, J.; Gu, Z. DNA interactions and in vitro anticancer evaluations of pyridine-benzimidazole-based Cu complexes. *Med. Chem. Comm.* **2018**, *9*, 337–343. [CrossRef] [PubMed]
12. Chen, Z.F.; Orvig, C.; Liang, H. Multi-target metal-based anticancer agents. *Curr. Top. Med. Chem.* **2017**, *17*, 3131–3145. [CrossRef] [PubMed]
13. Nobili, S.; Mini, E.; Landini, I.; Gabbiani, C.; Casini, A.; Messori, L. Gold compounds as anticancer agents: Chemistry, cellular pharmacology, and preclinical studies. *Med. Res. Rev.* **2010**, *30*, 550–580. [CrossRef] [PubMed]
14. Prosser, K.E.; Chang, S.W.; Saraci, F.; Le, P.H.; Walsby, C.J. Anticancer copper pyridine benzimidazole complexes: ROS generation, biomolecule interactions, and cytotoxicity. *J. Inorg. Biochem.* **2017**, *167*, 89–99. [CrossRef] [PubMed]
15. Tang, B.; Wan, D.; Lai, S.H.; Yang, H.H.; Zhang, C.; Wang, X.Z.; Zeng, C.C.; Liu, Y.J. Design, synthesis and evaluation of anticancer activity of ruthenium (II) polypyridyl complexes. *J. Inorg. Biochem.* **2017**, *173*, 93–104. [CrossRef] [PubMed]
16. Zheng, K.; Wu, Q.; Wang, C.; Tan, W.; Mei, W. Ruthenium(II) complexes as potential apoptosis inducers in chemotherapy. *AntiCancer Agents Med. Chem.* **2017**, *17*, 29–39.

17. Li, W.; Jiang, G.B.; Yao, J.H.; Wang, X.Z.; Wang, J.; Han, B.J.; Xie, Y.Y.; Lin, G.J.; Huang, H.L.; Liu, Y.J. Ruthenium(II) complexes: DNA-binding, cytotoxicity, apoptosis, cellular localization, cell cycle arrest, reactive oxygen species, mitochondrial membrane potential and western blot analysis. *J. Photochem. Photobiol. B* **2014**, *140*, 94–104. [CrossRef]
18. Xu, L.; Zhong, N.J.; Xie, Y.Y.; Huang, H.L.; Jiang, G.B.; Liu, Y.J. Synthesis, characterization, in vitro cytotoxicity, and apoptosis-inducing properties of ruthenium(II) complexes. *PLoS ONE* **2014**, *9*, e96082. [CrossRef]
19. Misirlic-Dencic, S.; Poljarevic, J.; Isakovic, A.M.; Sabo, T.; Markovic, I.; Trajkovic, V. Current development of metal complexes with diamine ligands as potential anticancer agents. *Curr. Med. Chem.* **2020**, *27*, 380–410. [CrossRef]
20. Santini, C.; Pellei, M.; Gandin, V.; Porchia, M.; Tisato, F.; Marzano, C. Advances in copper complexes as anticancer agents. *Chem. Rev.* **2014**, *114*, 815–862. [CrossRef]
21. Shobha Devi, C.; Thulasiram, B.; Aerva, R.R.; Nagababu, P. Recent advances in copper intercalators as anticancer agents. *J. Fluoresc.* **2018**, *28*, 1195–1205. [CrossRef] [PubMed]
22. Portugal, J.; Barceló, F. Noncovalent binding to DNA: Still a target in developing anticancer agents. *Curr. Med. Chem.* **2016**, *23*, 4108–4134. [CrossRef] [PubMed]
23. Festa, R.A.; Thiele, D.J. Copper: An essential metal in biology. *Curr. Biol.* **2011**, *21*, R877–R883. [CrossRef]
24. Tapiero, H.; Townsend, D.M.; Tew, K.D. Trace elements in human physiology and pathology. Copper. *Biomed. Pharmacother.* **2003**, *57*, 386–398. [CrossRef]
25. Sharma, M.; Ganeshpandian, M.; Majumder, M.; Tamilarasan, A.; Mukhopadhyay, R.; Islam, N.S.; Palaniandavar, M. Octahedral copper(II)-diimine complexes of triethylenetetramine: Effect of stereochemical fluxionality and ligand hydrophobicity on Cu(II)/Cu(I) redox, DNA binding and cleavage, cytotoxicity and apoptosis-inducing ability. *Dalton Trans.* **2020**, *49*, 8282–8297. [CrossRef] [PubMed]
26. Ramakrishnan, S.; Shakthipriya, D.; Suresh, E.; Periasamy, V.S.; Akbarsha, M.A.; Palaniandavar, M. Ternary dinuclear copper(II) complexes of a hydroxybenzamide ligand with diimine coligands: The 5,6-dmp ligand enhances DNA binding and cleavage and induces apoptosis. *Inorg. Chem.* **2011**, *50*, 6458–6471. [CrossRef]
27. Maciel, L.L.F.; de Freitas, W.R.; Bull, E.S.; Fernandes, C.; Horn, A.; de Aquino Almeida, J.C.; Kanashiro, M.M. In vitro and in vivo anti-proliferative activity and ultrastructure investigations of a copper(II) complex toward human lung cancer cell NCI-H460. *J. Inorg. Biochem.* **2020**, *210*, 111166. [CrossRef]
28. Parsekar, S.U.; Singh, M.; Mishra, D.P.; Antharjanam, P.K.S.; Koley, A.P.; Kumar, M. Efficient hydrolytic cleavage of DNA and antiproliferative effect on human cancer cells by two dinuclear Cu(II) complexes containing a carbohydrazone ligand and 1,10-phenanthroline as a coligand. *J. Biol. Inorg. Chem.* **2019**, *24*, 343–363. [CrossRef]
29. Polloni, L.; Seni Silva, A.C.; Teixeira, S.C.; Azevedo, F.V.P.V.; Zóia, M.A.P.; da Silva, M.S.; Lima, P.M.A.P.; Correia, L.I.V.; do Couto Almeida, J.; da Silva, C.V.; et al. Action of copper(II) complex with β-diketone and 1,10-phenanthroline (CBP-01) on sarcoma cells and biological effects under cell death. *Biomed. Pharmacother.* **2019**, *112*, 108586. [CrossRef]
30. Haleel, A.; Mahendiran, D.; Veena, V.; Sakthivel, N.; Rahiman, A.K. Antioxidant, DNA interaction, VEGFR2 kinase, topoisomerase I and in vitro cytotoxic activities of heteroleptic copper(II) complexes of tetrazolo[1,5-a]pyrimidines and diimines. *Mat. Sci. Eng. C. Mater.* **2016**, *68*, 366–382. [CrossRef]
31. Ng, C.H.; Chan, C.W.; Lai, J.W.; Ooi, I.H.; Chong, K.V.; Maah, M.J.; Seng, H.L. Enantiomeric pair of copper(II) polypyridyl-alanine complexes: Effect of chirality on their interaction with biomolecules. *J. Inorg. Biochem.* **2016**, *160*, 1–11. [CrossRef] [PubMed]
32. Ma, T.; Xu, J.; Wang, Y.; Yu, H.; Yang, Y.; Liu, Y.; Ding, W.; Zhu, W.; Chen, R.; Ge, Z.; et al. Ternary copper(II) complexes with amino acid chains and heterocyclic bases: DNA binding, cytotoxic and cell apoptosis induction properties. *J. Inorg. Biochem.* **2015**, *144*, 38–46. [CrossRef] [PubMed]
33. Zhu, T.; Chen, R.; Yu, H.; Feng, Y.; Chen, J.; Lu, Q.; Xie, J.; Ding, W.; Ma, T. Antitumor effect of a copper (II) complex of a coumarin derivative and phenanthroline on lung adenocarcinoma cells and the mechanism of action. *Mol. Med. Rep.* **2014**, *10*, 2477–2482. [CrossRef]
34. Wu, J.; Chen, W.; Yin, Y.; Zheng, Z.; Zou, G. Probing the cell death signaling pathway of HepG2 cell line induced by copper-1,10-phenanthroline complex. *Biometals* **2014**, *27*, 445–458. [CrossRef] [PubMed]
35. Zhou, H.; Zheng, C.; Zou, G.; Tao, D.; Gong, J. G(1)-phase specific apoptosis in liver carcinoma cell line induced by copper-1,10-phenanthroline. *Int. J. Biochem. Cell. Biol.* **2002**, *34*, 678–684. [CrossRef]

36. Shi, X.; Fang, H.; Guo, Y.; Yuan, H.; Guo, Z.; Wang, X. Anticancer copper complex with nucleus, mitochondrion and cyclooxygenase-2 as multiple targets. *J. Inorg. Biochem.* **2019**, *190*, 38–44. [CrossRef]
37. Slator, C.; Barron, N.; Howe, O.; Kellett, A. [Cu(o-phthalate)(phenanthroline)] exhibits unique superoxide-mediated NCI-60 chemotherapeutic action through genomic DNA damage and mitochondrial dysfunction. *ACS Chem. Biol.* **2016**, *11*, 159–171. [CrossRef]
38. Gaál, A.; Orgován, G.; Mihucz, V.G.; Pape, I.; Ingerle, D.; Streli, C.; Szoboszlai, N. Metal transport capabilities of anticancer copper chelators. *J. Trace Elem. Med. Biol.* **2018**, *47*, 79–88. [CrossRef]
39. Mukherjee, N.; Podder, S.; Banerjee, S.; Majumdar, S.; Nandi, D.; Chakravarty, A.R. Targeted photocytotoxicity by copper(II) complexes having vitamin B6 and photoactive acridine moieties. *Eur. J. Med. Chem.* **2016**, *122*, 497–509. [CrossRef]
40. Baig, S.; Seevasant, I.; Mohamad, J.; Mukheem, A.; Huri, H.Z.; Kamarul, T. Potential of apoptotic pathway-targeted cancer therapeutic research: Where do we stand? *Cell Death Discov.* **2016**, *7*, e2058. [CrossRef]
41. Fulda, S. Targeting apoptosis signaling pathways for anticancer therapy. *Front. Oncol.* **2011**, *1*, 23. [CrossRef] [PubMed]
42. Jendrossek, V. The intrinsic apoptosis pathways as a target in anticancer therapy. *Curr. Pharm. Biotechnol.* **2012**, *13*, 1426–1438. [CrossRef]
43. Pfeffer, C.M.; Singh, A.T.K. Apoptosis: A target for anticancer therapy. *Int. J. Mol. Sci.* **2018**, *19*, 448. [CrossRef] [PubMed]
44. Yang, Y.; Karakhanova, S.; Hartwig, W.; D'Haese, J.G.; Philippov, P.P.; Werner, J.; Bazhin, A.V. Mitochondria and mitochondrial ROS in cancer: Novel targets for anticancer therapy. *J. Cell. Physiol.* **2016**, *231*, 2570–2581. [CrossRef] [PubMed]
45. Rochford, G.; Molphy, Z.; Kavanagh, K.; McCann, M.; Devereux, M.; Kellett, A.; Howe, O. Cu(II) phenanthroline-phenazine complexes dysregulate mitochondrial function and stimulate apoptosis. *Metallomics* **2020**, *12*, 65–78. [CrossRef] [PubMed]
46. Masuri, S.; Cadoni, E.; Cabiddu, M.G.; Isaia, F.; Demuru, M.G.; Moráň, L.; Buček, D.; Vaňhara, P.; Havel, J.; Pivetta, T. The first copper(II) complex with 1,10-phenanthroline and salubrinal with interesting biochemical properties. *Metallomics* **2020**, *12*, 891–901. [CrossRef]
47. Parton, M.; Krajewski, S.; Smith, I.; Krajewska, M.; Archer, C.; Naito, M.; Ahern, R.; Reed, J.; Dowsett, M. Coordinate expression of apoptosis-associated proteins in human breast cancer before and during chemotherapy. *Clin. Cancer Res.* **2002**, *8*, 2100–2108.
48. Michel, L.L.; von Au, A.; Mavratzas, A.; Smetanay, K.; Schütz, F.; Schneeweiss, A. Immune checkpoint blockade in patients with triple-negative breast cancer. *Target. Oncol.* **2020**, *15*, 415–428. [CrossRef]
49. Fedele, P.; Sanna, V.; Fancellu, A.; Cinieri, S. A clinical evaluation of treatments that target cell cycle machinery in breast cancer. *Expert Opin. Pharmacother.* **2019**, *20*, 2305–2315. [CrossRef]
50. Waks, A.G.; Winer, E.P. Breast cancer treatment: A review. *J. Am. Med. Assoc.* **2019**, *321*, 288–300. [CrossRef]
51. Rajendiran, V.; Karthik, R.; Palaniandavar, M.; Stoeckli-Evans, H.; Periasamy, V.S.; Akbarsha, M.A.; Srinag, B.S.; Krishnamurthy, H. Mixed-ligand copper(II)-phenolate complexes: Effect of coligand on enhanced DNA and protein binding, DNA cleavage, and anticancer activity. *Inorg. Chem.* **2007**, *46*, 8208–8221. [CrossRef] [PubMed]
52. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **1983**, *65*, 55–63. [CrossRef]
53. Spector, D.L.; Goldman, R.D.; Leinwand, L.A. *Culture and Biochemical Analysis of Cells. Cell: A Laboratory Manual*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, USA, 1998.
54. Wang, H.; Joseph, J.A. Quantifying cellular oxidative stress by dichlorofluorescein assay using microplate reader. *Free Radic. Biol. Med.* **1999**, *27*, 612–616. [CrossRef]
55. Gopalakrishna, P.; Khar, A. Comet assay to measure DNA damage in apoptotic cells. *J. Proteom.* **1995**, *30*, 69–73. [CrossRef]
56. Riccardi, C.; Nicoletti, I. Analysis of apoptosis by propidium iodide staining and flow cytometry. *Nat. Protoc.* **2006**, *1*, 1458–1461. [CrossRef]
57. Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254. [CrossRef]

58. Towbin, H.; Staehelin, T.; Gordon, J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proc. Natl. Acad. Sci. USA* **1979**, *76*, 4350–4354. [CrossRef]
59. Ouyang, L.; Shi, Z.; Zhao, S.; Wang, F.T.; Zhou, T.T.; Liu, B.; Bao, J.K. Programmed cell death pathways in cancer: A review of apoptosis, autophagy and programmed necrosis. *Cell Proliferat.* **2012**, *45*, 487–498. [CrossRef]
60. Elmore, S. Apoptosis: A review of programmed cell death. *Toxicol. Pathol.* **2007**, *35*, 495–516. [CrossRef]
61. Lowe, S.W.; Ruley, H.E.; Jacks, T.; Housman, D.E. p53-dependent apoptosis modulates the cytotoxicity of anticancer agents. *Cell* **1993**, *74*, 957–967. [CrossRef]
62. Kyzioł, A.; Cierniak, A.; Gubernator, J.; Markowski, A.; Jeżowska-Bojczuk, M.; Komarnicka, U.K. Copper(I) complexes with phosphine derived from sparfloxacin. Part III: Multifaceted cell death and preliminary study of liposomal formulation of selected copper(I) complexes. *Dalton Trans.* **2018**, *47*, 1981–1992. [CrossRef] [PubMed]
63. Loganathan, R.; Ganeshpandian, M.; Bhuvanesh, N.S.P.; Palaniandavar, M.; Muruganantham, A.; Ghosh, S.K.; Riyasdeen, A.; Akbarsha, M.A. DNA and protein binding, double-strand DNA cleavage and cytotoxicity of mixed ligand copper(II) complexes of the antibacterial drug nalidixic acid. *J. Inorg. Biochem.* **2017**, *174*, 1–13. [CrossRef] [PubMed]
64. Ramakrishnan, S.; Rajendiran, V.; Palaniandavar, M.; Periasamy, V.S.; Srinag, B.S.; Krishnamurthy, H.; Akbarsha, M.A. Induction of cell death by ternary copper(II) complexes of L-tyrosine and diimines: Role of coligands on DNA binding and cleavage and anticancer activity. *Inorg. Chem.* **2009**, *48*, 1309–1322. [CrossRef] [PubMed]
65. Gandin, V.; Porchia, M.; Tisato, F.; Zanella, A.; Severin, E.; Dolmella, A.; Marzano, C. Novel mixed-ligand copper(I) complexes: Role of diimine ligands on cytotoxicity and genotoxicity. *J. Med. Chem.* **2013**, *56*, 7416–7430. [CrossRef]
66. Molphy, Z.; Slator, C.; Chatgilialoglu, C.; Kellett, A. DNA oxidation profiles of copper phenanthrene chemical nucleases. *Front. Chem.* **2015**, *3*, 28. [CrossRef]
67. Wong, D.Y.Q.; Lim, J.H.; Ang, W.H. Induction of targeted necrosis with HER2-targeted platinum(IV) anticancer prodrugs. *Chem. Sci.* **2015**, *6*, 3051–3056. [CrossRef]
68. Gençkal, H.M.; Erkisa, M.; Alper, P.; Sahin, S.; Ulukaya, E.; Ari, F. Mixed ligand complexes of Co(II), Ni(II) and Cu(II) with quercetin and diimine ligands: Synthesis, characterization, anti-cancer and anti-oxidant activity. *J. Biol. Inorg. Chem.* **2020**, *25*, 161–177. [CrossRef]
69. Gorrini, C.; Harris, I.S.; Mak, T.W. Modulation of oxidative stress as an anticancer strategy. *Nat. Rev. Drug Discov.* **2013**, *12*, 931–947. [CrossRef]
70. Ng, C.H.; Kong, S.M.; Tiong, Y.L.; Maah, M.J.; Sukram, N.; Ahmad, M.; Khoo, A.S. Selective anticancer copper(II)-mixed ligand complexes: Targeting of ROS and proteasomes. *Metallomics* **2014**, *6*, 892–906. [CrossRef]
71. Guo, W.J.; Ye, S.S.; Cao, N.; Huang, J.; Gao, J.; Chen, Q.Y. ROS-mediated autophagy was involved in cancer cell death induced by novel copper(II) complex. *Exp. Toxicol. Pathol.* **2010**, *62*, 577–582. [CrossRef]
72. Saraste, A.; Pulkki, K. Morphologic and biochemical hallmarks of apoptosis. *Cardiovasc. Res.* **2000**, *45*, 528–537. [CrossRef]
73. Grasso, D.; Zampieri, L.X.; Capelôa, T.; Van de Velde, J.A.; Sonveaux, P. Mitochondria in cancer. *Cell Stress* **2020**, *4*, 114–146. [CrossRef] [PubMed]
74. Heijink, A.M.; Krajewska, M.; van Vugt, M.A. The DNA damage response during mitosis. *Mutat. Res.* **2013**, *750*, 45–55. [CrossRef] [PubMed]
75. Pietenpol, J.A.; Stewart, Z.A. Cell cycle checkpoint signaling: Cell cycle arrest versus apoptosis. *Toxicology* **2002**, *181–182*, 475–481. [CrossRef]
76. Wang, Z.; Sun, Y. Targeting p53 for novel anticancer therapy. *Transl. Oncol.* **2010**, *3*, 1–12. [CrossRef]
77. Dalla Via, L.; García-Argáez, A.N.; Martínez-Vázquez, M.; Grancara, S.; Martinis, P.; Toninello, A. Mitochondrial permeability transition as target of anticancer drugs. *Curr. Pharm. Des.* **2014**, *20*, 223–244.
78. Morisaki, T.; Katano, M. Mitochondria-targeting therapeutic strategies for overcoming chemoresistance and progression of cancer. *Curr. Med. Chem.* **2003**, *10*, 2517–2521. [CrossRef]
79. Merino, D.; Lok, S.W.; Visvader, J.E.; Lindeman, G.J. Targeting BCL-2 to enhance vulnerability to therapy in estrogen receptor-positive breast cancer. *Oncogene* **2016**, *35*, 1877–1887. [CrossRef]

80. Cory, S.; Adams, J.M. Killing cancer cells by flipping the Bcl-2/Bax switch. *Cancer Cell* **2005**, *8*, 5–6. [CrossRef]
81. Bailey, S.T.; Shin, H.; Westerling, T.; Liu, X.S.; Brown, M. Estrogen receptor prevents p53-dependent apoptosis in breast cancer. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 18060–18065. [CrossRef]
82. Tomicic, M.T.; Kaina, B. Topoisomerase degradation, DSB repair, p53 and IAPs in cancer cell resistance to camptothecin-like topoisomerase I inhibitors. *Biochim. Biophys. Acta Rev. Cancer* **2013**, *1835*, 11–27. [CrossRef] [PubMed]
83. Beretta, G.L.; Perego, P.; Zunino, F. Targeting topoisomerase I: Molecular mechanisms and cellular determinants of response to topoisomerase I inhibitors. *Expert. Opin. Ther. Targets* **2008**, *12*, 1243–1256. [CrossRef] [PubMed]
84. Sordet, O.; Khan, Q.A.; Kohn, K.W.; Pommier, Y. Apoptosis induced by topoisomerase inhibitors. *Anticancer Agents Med. Chem.* **2003**, *3*, 271–290. [CrossRef] [PubMed]

Sample Availability: Not available.

© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).

Biometals
https://doi.org/10.1007/s10534-021-00351-8

Cytotoxic cobalt (III) Schiff base complexes: in vitro anti-proliferative, oxidative stress and gene expression studies in human breast and lung cancer cells

Balakrishnan Gowdhami · Yesaiyan Manojkumar · R. T. V. Vimala · Venkatesan Ramya · Balakrishnan Karthiyayini · Balamuthu Kadalmani · Mohammad Abdulkader Akbarsha

Received: 22 April 2021 / Accepted: 12 November 2021
© The Author(s), under exclusive licence to Springer Nature B.V. 2021

Abstract Increasing cancer drug chemo-resistance, especially in the treatment of breast and lung cancers, alarms the immediate need of newer and effective anticancer drugs. Until now, chemotherapeutics based on metal complexes are considered the most effective treatment modality. In the present study, we have evaluated the cytotoxic effect of two cobalt (III) Schiff base complexes based on the leads from complex combinatorial chemistry. Cobalt (III) Schiff base complexes (**Complex 3** = $Co(Ph\text{-}acacen)(HA)_2](-ClO_4)$ and **Complex 4** = $[Co(Ph\text{-}acacen)(DA)_2](-ClO_4)]$ (Ph-acacen, 1-phenylbutane-1,3-dione; DA, dodecyl amine; HA, heptylamine) were evaluated against human breast cancer cell MCF-7 and lung cancer cell A549 using MTT cell viability assay, cellular morphological changes studied by Acridine Orange and Ethidium Bromide (AO/EB), Dual fluorescent staining, Hoechst staining 33248, Comet assay, Annexin V-Cy3 and 6 CFDA assay, JC-1 staining, Reactive oxygen species (ROS) assay, Immunofluorescence assay, and Real-time reverse transcription-polymerase chain reaction (RT-qPCR). Treatment of cobalt (III) Schiff base complexes (**Complex 3 & 4**) affected the viability of the cancer cells. The cell death induced by the complexes was predominantly apoptosis, but necrosis also occurred to a certain extent. Complex **4** produced better cytotoxic effect than complex **3**, and MCF-7 cell was more responsive than A549. In that order, the complexes were more selective to cancer cell than normal cell, and more effective in overall performance than the

B. Gowdhami · V. Ramya · B. Kadalmani (✉)
Department of Animal Science, Bharathidasan University, Tiruchirappalli, Tamilnadu 620024, India
e-mail: kadalmani@bdu.ac.in

B. Gowdhami · B. Karthiyayini
National Centre for Alternatives to Animal Experiments, Bharathidasan University, Tiruchirappalli, Tamilnadu 620024, India

Y. Manojkumar
Department of Chemistry, Bishop Heber College, Tiruchirappalli, Tamilnadu, India

R. T. V. Vimala
Department of Biotechnology, Bharathidasan University, Tiruchirappalli, Tamilnadu 620024, India

B. Karthiyayini
Department of Microbiology, Bharathidasan University, Tiruchirappalli, Tamilnadu 620024, India

M. A. Akbarsha
Mahatma Gandhi-Doerenkamp Centre for Alternatives, Bharathidasan University, Tiruchirappalli, Tamilnadu 620024, India

M. A. Akbarsha (✉)
Department of Biotechnology & Research Coordinator, National College (Autonomous), Tiruchirappalli, Tamilnadu 620001, India
e-mail: akbarbdu@gmail.com

Published online: 22 December 2021

standard drug cisplatin. Therefore, we conclude that cobalt (III) Schiff base complexes, especially complex **4**, have the potential to be developed as effective drugs for treatment of cancers in general, and breast and lung cancers in particular.

Keywords Apoptosis · DNA damage · Reactive oxygen species · Immunofluorescence · RT-PCR

Introduction

Breast and lung cancers represent the leading causes of cancer mortality (Sung et al. 2021). Breast cancer is a heterogeneous disease that causes health problem to millions of women (Palanivel et al. 2020). Similarly, lung cancer is prevalent in people aged over 50 with smoking history (Jemal et al. 2004). At present surgery, chemotherapy and radiation are the main line therapies in practice for breast and lung cancers but only early stage cancers respond to these treatments, whereas metastatic cancers are still an insurmountable challenge. Further, the undesirable side effects and induction of resistance to the current chemotherapeutics such as paclitaxel, etoposide, and cisplatin pose problems in treating these diseases (Cornelio et al. 2009). Despite several anticancer drugs, the overall survival rate of lung and breast cancer patients remains poor, and there is a need for an effective cancer drug, which is an active line of investigation globally.

Synthetic drugs based on metal complex as the core are known to have good therapeutic efficacy as anticancer drugs (Garbutcheon-Singh et al. 2011). Therefore, towards development of newer anticancer drugs there is great focus on complexes with versatile functional groups and moieties. The selection of metal ions is based on several rationales among which development of cobalt-based complexes is of great interest since they are hydrophilic, soluble, bioavailable and of negligible in vivo toxicity (Riyasdeen, et al. 2012; Nagaraj and Arunachalam 2014). Further, cobalt complexes have been reported to induce the activities of antioxidant (SOD, CAT, GPx1) and apoptotic (caspases, endonuclease) enzymes (Aggett and Fairweather-Tait 1998; Tapiero et al. 2003). In addition, mixed-ligand cobalt (III) complexes have the ability to intercalate with and/or cleave DNA, scavenge free radicals and redox-regulate mitochondrial function (Turnlund et al. 1989; Marzano et al. 2009) and, thus, can be potential anticancer agents (Jagadeesan et al. 2013). Through these mechanisms the complexes affect the viability of cancer cells via protein inhibition, ROS generation, and DNA distortion. The mixed-ligand cobalt (III) complexes are excellently cytotoxic, and possess antitumor property (Zangrando et al. 2015; Liu et al. 2016; Park et al. 2016; Wang et al. 2017). Also, cobalt complexes can be synthesized by eco-friendly methods. Therefore, the advantages of cobalt complexes can be exploited in several medical applications including delivery of drugs. In this background, Manojkumar et al. (2019) brought up a few newer mixed cobalt (III) Schiff base complexes, characterized them fairly adequately, and conducted preliminary cytotoxicity studies using Acridine Orange(AO) and Ethidium Bromide (EB) fluorescent assay, Hoechst 33248 staining, Annexin V-Cy3 and 6-CFDA assay, and cell cycle analysis in A549 lung cancer cell (non-small cell lung carcinoma) with VERO cell (monkey kidney cell, normal) as normal control. Among the several complexes, *trans*-[Co (Ph-acacen) (HA) 2] (ClO4) [**3**] and *trans*-[Co (Ph-acacen) (DA) 2] (ClO4) [**4**] affected the viability of A549 cells selectively, and spared the VERO cells, pointing to the potential selective antitumor property of these complexes. Taking cues from this research we have expanded the scope in the present study to investigate further the anti-proliferative effect and the mode(s) of cell death induced by these complexes (complex **3**&**4**) in the human lung cancer cell (A549) and breast cancer cell (MCF-7) wherein 3T3 mouse embryonic fibroblast was used as the normal cell for finding selectivity, if any. In addition, the molecular mechanisms underlying the potential anticancer property of these complexes against human lung and breast cancer cells were studied by profiling the expression pattern of key apoptotic and antioxidant enzymes, using RT-qPCR.

In the present work, the analysis of the cytotoxic and antiproliferative properties of two different cobalt (III) Schiff base complexes in MCF-7 and A549 cells, was carried out. According to the results of their cytotoxicity and antiproliferative evaluation, in combination with fluorescence microscopy-based cellular antioxidant analysis and qPCR gene expression studies, newer light on their cytotoxic mechanism at the molecular level is shed. Thus, the cobalt (III)

complexes are capable of inducing apoptotic death in MCF-7 and A549 cells through intrinsic and extrinsic pathways of apoptosis leading to loss of cell membrane integrity, DNA damage, alteration in mitochondrial membrane potential, induction of oxidative stress, and modulation of gene expression that are involved in cellular stress response.

Materials and methods

Chemicals and reagents

MTT [3-(4, 5-dimethylthiazol2-yl)-2, 5-diphenyltetrazolium bromide], Streptomycin, Penicillin, Dimethyl sulfoxide (DMSO), Cisplatin, Acridine Orange (AO), and Ethidium Bromide (EB), Hoechst 33258, AnnexinV-Cy3, 6-Carboxyfluorescein diacetate (6-CFDA) and 4, 6-diamidino-2-phenylindole (DAPI) were procured from Sigma-Aldrich, St. Louis, MO, U.S.A. Chemicals used in buffers and other reagents were procured from Merck, India. Bcl-2 and Bax antibodies conjugated with FITC were obtained from Thermo Fisher Scientific, USA.

Synthesis of cobalt (III) complex

The two cobalt (III) Schiff base complexes, *trans*-[Co(Ph-acacen)$(HA)_2$](ClO_4) Complex [**3**] and *trans*-[Co(Ph-acacen)$(DA)_2$](ClO_4) Complex [**4**] (where acacen: *N*,*N*′-bis(acetyl acetone) ethylenediamine and Ph-acacen: *N*,*N*′-bis(1-phenylbutane-1,3-dione)ethylene diamine, HA: heptylamine and DA:dodecylamine), were prepared as described earlier (Manojkumar et al. 2019).

Cell culture

Human breast cancer cell MCF-7, human lung cancer cell A549 and mouse embryonic fibroblast cell 3T3 were obtained from National Center for Cell Science (NCCS), Pune, India. DMEM medium added with 10% FBS was used to culture the cells. Streptomycin and Penicillin (Sigma, St. Louis, USA), at 100 U/mL each, were used as antibiotics added to the medium. The culture was incubated in a humidified atmosphere of 5% carbon dioxide at 37 °C in a CO_2 incubator (Thermo Scientific, USA).

MTT assay

The MTT [3-(4, 5-dimethylthiazol2-yl)-2, 5-diphenyltetrazolium bromide] assay was used to determine the IC_{50} concentration of the complexes. Cobalt(III) Schiff base complexes **3** and **4** were dissolved in 0.1% DMSO (Sigma, St. Louis, USA) prepared in PBS, to make the stock solution and diluted in the culture medium to make the final DMSO concentration to be 0.01% (Manojkumar et al. 2019). The experiments were conducted for the different cell lines in separate 96 well culture plates. Five thousand cells were seeded in each well and exposed to the test solutions (complex **3**, 0–20 µM for MCF-7 and A549 cells; complex **4**, 0–10 µM for MCF-7, 0–20 µM for A549; and both complexes 0–200 µM for 3T3 cells) for 24, 48, and 72 h where 0.01% DMSO was used as the solvent control. Cisplatin was prepared first in saline (150 mM), according to Hall et al. (2014), and diluted in 0.01% DMSO to a final concentration of 4 mM, to match with the preparation of the complexes and used as the positive control (Dose range: 0–50, 0–100, 0–200 µM for MCF-7, A549 and 3T3 cells, respectively). Saline (150 mM) mixed with 0.01% DMSO was used as the negative control. After the treatment, 20 µL of MTT solution [5 mg/mL in phosphate buffered saline (PBS)] was aliquoted to each well and then the plates were kept for incubation for 4 h at 37 °C by covering it with aluminium foil. The purple-colored formazan product was dissolved in 100 µL DMSO. The absorbance was measured at 570 nm (sample) and 630 nm (reference) using a plate reader (Bio-Rad, Hercules, California, USA). Three replicates each were performed and the respective means and the standard deviations were calculated. The percentage inhibition was calculated using the formula,

$$\text{Percentage inhibition} = \frac{[\text{Mean OD of untreated cells (control)} - \text{Mean OD of treated cells}]}{\text{Mean OD of untreated cells (control)}} \times 100$$

The percentage inhibition was plotted against the concentration, and the IC_{50} for 24, 48 and 72 h were determined. In all experiments hereafter the IC_{50} was used.

Acridine Orange (AO) and Ethidium Bromide (EB) fluorescent assay for cell death

The staining was conducted according to the protocol of Spector and Goldman (1998). Treatment was performed with separately cultured (5×10^5 cells/well) in sterile 6 well plates (Tarson, India). MCF-7 and A549 cells were exposed to the 24 h IC_{50} of the complexes **3** and **4** where DMSO (0.01%) was used as control. Further, control and treated cells were stained with AO and EB solution (1 part of 100 mg/mL each of AO and EB in PBS) and observed in a fluorescent microscope (Carl Zeiss, Jena, Germany) fitted with UV filter (450–490 nm). Three hundred cells per sample, each in triplicate, were counted as viable or dead, and if dead whether by apoptosis or necrosis as judged from the profile of the cytoplasm and nuclear morphology (Kumar et al. 2008).

Hoechst 33528 staining

The method described by Latt and Wohlleb (1975) was followed. MCF-7 and A549 cells were treated with the 24 h IC_{50} of the complexes **3** and **4** for 24 h. After incubation, Hoechst 33258 (1 mg/mL) staining solution was added and incubated for 5 min at room temperature. The cell suspension was placed on a glass slide and a cover slip was laid over it. Randomly, 300 cells were visualized in a fluorescent microscope and the control *vs*. treated cells were counted (Rajendiran et al. 2007). The experiments were performed in triplicate and the data were used to find the means and the standard deviations.

AnnexinV-Cy3 and 6-CFDA staining

Phosphatidylserine translocation from inner to outer leaflet of the plasma membrane is one of the early features of apoptosis. AnnexinV-Cy3 staining was used to find the presence of phosphatidylserine at the cell surface (Spector and Goldman 1998). 6-CFDA was used to differentiate apoptotic cells from viable cells. MCF-7 and A549 cells, cultured in six well plates (5×10^5 cells/well), were incubated with the 24 h IC_{50} of the cobalt complexes **3** and **4** for 24 h. PBS and 1 × binding buffer (0.1 M Hepes (pH 7.4), 1.4 M NaCl, 25 mM $CaCl_2$) were used to wash the cell pellets and the washed pellets were suspended in 50 µL of Annexin V-Cy3 and 6-CFDA. Then, the plates were incubated for 10 min in the dark. Afterwards, the excess label was removed by washing the cells with binding buffer. A fluorescent microscope was used to observe Annexin V-Cy3 (red) and 6-CFDA (green) labeled cells. This assay allowed one to differentiate live cells (green), and early apoptotic cells (red and green). Three hundred cells from each well were enumerated at random, and the percentage of cells in live and early apoptotic cells was statistically analyzed. The experiments were performed in triplicates and the data were used to find the means and the standard deviations.

JC-1 staining

The fluorescent probe JC-1 was used for assessing mitochondrial membrane potential ($\Delta\Psi$ m). The dye, released from mitochondrial red JC-1, aggregates at the cytosol due to loss of $\Delta\Psi$, and emits green fluorescence (Reers, et al. 1991). MCF-7 and A549 cells cultured on cover slips were placed in micro titer plate and incubated with the cobalt complexes **3** and **4** at the respective 24 h IC_{50} for 12 and 24 h. Finally, the cells were incubated with the dye for 20 min. The fluorescence pattern (red, intact; green, drug-impacted) was qualitatively evaluated.

Comet assay

The assay was conducted as per the protocol of Singh et al. (1988). MCF-7 and A549 cells were treated with the respective 24 h IC_{50} of the cobalt complexes **3** and **4** for 24 h. After incubation, the cultured cells were embedded in low melting point agarose coated on microscope slides pre-coated with a layer of normal melting point agarose. The slides were cooled for 10 min and then submerged in lysis solution (100 mm Na_2EDTA, 2.5 m NaCl, 0.2 mm NaOH [pH 10], Triton X-100, and 10 mm Tris) and incubated overnight for lysing the cell and to allow DNA unfolding. Then, alkaline buffer (1 mm Na_2-EDTA, 300 mm NaOH [pH 13]) was applied on the slides for 20 min to allow DNA unwinding followed by 15 min of electrophoresis at 0.8 v/cm. Finally, the slides were washed before staining with Ethidium Bromide

(EB). One hundred cells were photographed in a fluorescent microscope and the comet length was measured according to Kumaravel et al. (2009). Finally, DNA damage was calculated using CASP software. The experiments were performed in triplicate and the data were used to find the means and the standard deviations.

Measurement of ROS by DCFH-DA staining

Oxidative stressors induce generation of ROS that can oxidize 2′, 7′-dichlorofluorescin diacetate (DCFHDA) to 2′, 7′-dichlorofluorescin (Wang and Joseph 1999). The treatment was performed with separately cultured MCF-7 and A549 cells treated with 24 h IC_{50} of cobalt complexes **3** and **4** for 12 h where H_2O_2 (0.02%) was used as the positive control. Then, DCFH-DA (5 µM) was added to the well and, finally, incubation was performed for 30 min at 37 °C. Based on the excitation (485 nm) and emission (520 nm) wavelengths, as measured in a spectrofluorometer [Jasco F 6000 (Easton, MD, USA)], the extent of ROS generation was determined. The experiment was performed in triplicate and then the means and the standard deviations were calculated.

Immunofluorescence assay for Bcl_2 and Bax proteins

MCF-7 and A549 cells were treated with 24 h IC_{50} of the cobalt complexes **3** and **4**, for 24 h, and fixed in 4% paraformaldehyde, prepared in 1xPBS, for 15 min at room temperature, washed twice in 1xPBS and further incubated with 0.1% Triton X-100 prepared in PBS and blocking buffer, 5% non-fat dry milk powder in Tris-buffered saline, for 1 h. After pipetting out the blocking buffer, cells were incubated overnight with the primary antibodies (anti-Bcl-2 and anti-Bax, respectively; Bangalore Genei, Bangalore, India; dilution: 10 µg/mL) at 4°C. After washing, the cells were incubated with the secondary antibodies anti-Bcl-2 (mouse monoclonal antibodies against Bcl-2; 10 µg/mL) and anti-Bax (rabbit polyclonal antibody against Bax; Bangalore Genei) conjugated with FITC at room temperature for 30 min in the dark. Finally, the cells were stained for 1–5 min using 4, 6-diamidino-2-phenylindole (DAPI; stock 20 mg/mL in water; Sigma, USA) and observed in a fluorescent microscope.

Expression analysis of apoptosis-related genes (RT-PCR)

Trizol method was used to isolate RNA from control and treated MCF-7 and A549 cells at the 24 h IC_{50} concentration for 24 h. To remove genomic DNAs, RNase-free DNase I (2 units) was added with the isolated RNA at 37 °C for 30 min. Then, the RNA (10 pg) was mixed with water. To synthesize cDNA, RNA was added with multiscribe reverse transcriptase, random primers (Table 1) (Bax, caspase 3, caspase 8, BID, Bcl-2, GRP78, SOD, Catalase, GPX) dNTP, and 10 × RT buffer at 25 °C for 10 min followed by 37 °C for 120 min. RT-PCR was performed using a SYBR Green Master Mix (iScript cDNA Kit) and 7900 RT-PCR detection system (Roche, Light cycler 96, Germany). RT-PCR was conducted at the following conditions: first step, 95 °C for 15 min; ensuing 40 cycles, 94 °C for 0.5–1 min, 50–58 °C for 0.5–1 min and 72 °C for 1 min. GAPDH was used as control (Murugadas et al. 2016).

Statistics

The data were subjected to statistical analysis using PRISM version 6.0 software (Graph-

Pad Software, La Jolla, CA, USA). Data obtained from the inject ability analysis were subjected to two-way ANOVA, and the results are presented as means ± SD; ns = not significant; $*p < 0.5$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$ were considered statistically significant.

Results

Antiproliferative activity of cobalt (III) complexes 3 and 4 as assessed by MTT assay

The cobalt complexes **3** and **4** were synthesized according to the method described by us earlier (Manojkumar et al. 2019). Figure 1 shows the proposed structure of the complexes 3 and 4.

The percentage inhibition of cobalt complexes **3**, **4** and cisplatin (positive control) at different time intervals (24, 48 and 72 h) and varying concentrations on MCF-7, A549 and 3T3 cells was determined by MTT assay. The cytotoxic activity was determined based on the exposure value of the complex required to

Table 1 RT-PCR primer sequences

Gene	Forward primer	Reverse primer
BID	5′- GGACACTGTGAACCAGGA-3′	5′- GGAAGCCAAACACCAGTA -3′
BAX	5′- CTACAGGGTTTCATCCAG -3′	5′- CCAGTTCATCTCCAATTCG -3′
Caspase 3	5′-CGTGTGGAGTATTTGGATG-3′	5′-GTACAGTCAGAGCCAACCTC-3′
Caspase 8	5′- CTGGGAAGGATCGACGATTA -3′	5′- CATGTCCTGCATTTTGATGG -3′
GRP 78	5′-AGGATGTGGGCACGGTGG-3′	5′-CATTCCAAGTGCGTCCGATGA-3′
BCL-2	5′- GTGGATGACTGAGTACCT -3′	5′- CCAGGAGAAATCAAACAGAG -3′
SOD	5′- TGGTCCTGTGAAGCTGATTG -3′	5′- TTGTCAGCTCCTGCAGTCAC -3′
Catalase	5′- TACTCAGAGGCACCGTCTTG -3′	5′- CTCCTCATCTCGGTGCATAA -3′
GPX	5′- AATGTGGCGTCACTCTGAGG -3′	5′- CAATTCTCCTGATGGCCAAA -3′
GAPDH	5′- GAGAAACCTGCCAAGTATG -3′	5′- GGAGTTGCTGTTGAAGTC -3′

reduce the survival rate of the cell to 50% (IC_{50}). The observed IC_{50} values of complexes **3** and **4** for MCF-7, A549 and 3T3 cells are presented in the Table 2.

The results indicate that both complexes **3** and **4** are efficient in dealing with both MCF-7 and A549 cells, but the complex **4** is more efficient than complex **3** [Table 2; Fig. 2A and B]. Interestingly, the normal 3T3 cells, when subjected to treatment with complexes **3** and **4**, showed highly appreciable survival rate when compared to cancer cells. The Selectivity Index (SI), calculated as IC_{50} of pure compound in a normal cell line / IC_{50} of the same pure compound in cancer cell line (Badisa et al. 2009), was the highest for MCF-7 cell, followed by A549 cell. Putting together, the results suggest that the cobalt (III) Schiff base complexes **3** and **4** induce cell death at doses lesser than the positive control drug cisplatin.

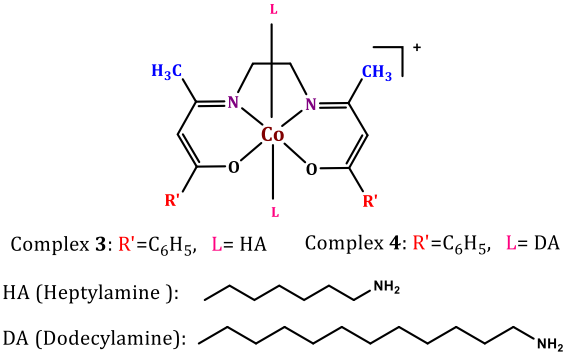


Fig.1 Structure of the complexes **3** and **4** (Adopted from Manojkumar et al. 2019)

Apoptosis-associated changes as revealed in AO/EB and Hoechst 33528 staining

The cell viability and apoptosis-associated changes in the cell membrane were studied by AO/EB staining method using fluorescent microscopy. Highly structured green fluorescing nuclei indicated viable and healthy cells. The affected cells manifested different grades of changes, early stage of apoptosis as indicated by green fluorescing nuclei with perinuclear chromatin condensation, late stage of apoptosis as indicated by slight orange fluorescing nuclei with highly condensed chromatin, and necrosis indicated by swollen cells with dark orange bulged sized fluorescing nuclei. Results of both qualitative (Fig. 3) and quantitative (Fig. 4) analysis using AO/EB indicate that the mode of cell death induced by complexes **3** and **4** (against the blank at 24 h IC_{50}) in MCF-7 and A549 cells was essentially apoptosis, although we could observe some marginal necrosis too. The changes in nuclear morphology of the MCF-7 and A549 cells treated with complexes **3** and **4** (at 24 h IC_{50}) were studied using Hoechst 33258.

Quantitative analysis showed that in the control group of MCF-7 cells, 91.5 ± 0.71% cells had normal nuclei, and 8.5 ± 0.29% cells had abnormal nuclei, whereas cobalt complexes **3** and **4** treated cells showed normal nuclei as follows:(**3**) = 48.35 ± 0.29%;(**4**) = 49.3 ± 1.24% (Fig. 5). Similarly, 92.65 ± 0.52% cells in control group of A549 cells had normal nuclei and 7.35 ± 0.48% cells had abnormal nuclei, whereas cobalt complexes **3** and **4** treated cells showed normal nuclei as follows: (**3**) = 51.25 ± 0.14%; (**4**) = 49.56 ± 2.35%. The

Table 2 IC_{50} values (μM) of cobalt complexes **3**, **4** and cisplatin for MCF-7, A549 and 3T3 cells incubated for 24, 48 and 72 h

Cell line	Compound	24 h (μM)	48 h (μM)	72 h (μM)
MCF-7	Complex **3**	09.6 ± 0.1 (14.4)*	04.4 ± 0.1 (21.7)	02.9 ± 0.2 (20.8)
	Complex **4**	05.4 ± 0.2 (27.3)	03.6 ± 0.2 (29.27)	01.6 ± 0.1 (50.1)
	Cisplatin	36.8 ± 0.3	18.7 ± 0.2	11.6 ± 0.1
A549	Complex **3**	17.9 ± 0.2 (7.7)	10.4 ± 0.1 (9.2)	09.6 ± 0.1 (6.3)
	Complex **4**	15.7 ± 0.1 (9.4)	10.1 ± 0.1 (10.4)	09.1 ± 0.1 (8.8)
	Cisplatin	61.2 ± 0.1	31.5 ± 0.1	14.4 ± 0.1
3T3	Complex **3**	138.4 ± 0.1	95.3 ± 0.1	60.3 ± 0.2
	Complex **4**	147.5 ± 0.1	105.4 ± 0.1	80.2 ± 0.1
	Cisplatin	160.4 ± 0.3	125.7 ± 0.1	115.1 ± 0.1

Each data represents mean and standard deviation of three replicates.

*Data in parenthesis, the Selectivity Index (SI = IC_{50} of pure compound in a normal cell line/ IC_{50} of the same pure compound in cancer cell line)

results indicate normal nuclear morphology in control cells, whereas close to 50% of the treated cells had abnormal nuclei such as chromatin condensation/ shrinkage, nuclear fragmentation into dot-like bodies, apoptotic body formation, etc. (Fig. 6). These observations substantiate the occurrence of cell death by apoptosis.

Change in mitochondrial trans-membrane potential (MMP)

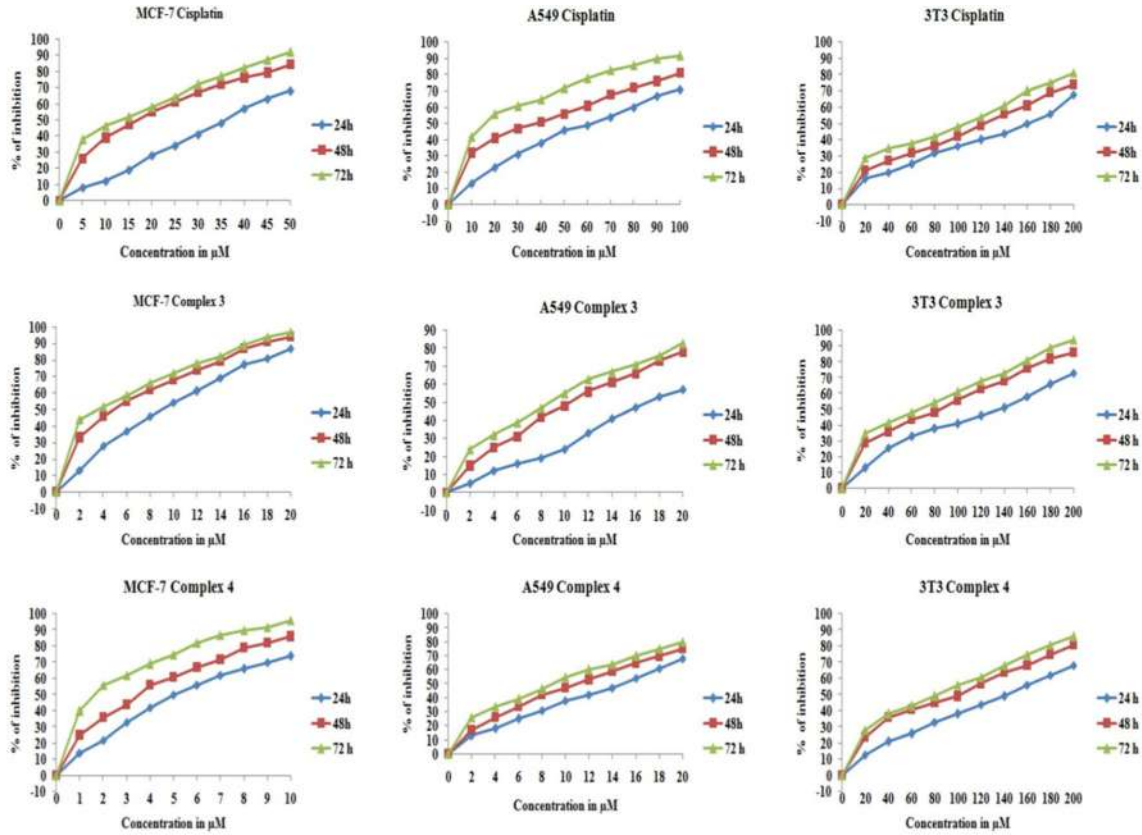
Changes in MMP ($\Delta\Psi m$) alter the electrochemical gradient which is an initial stage of apoptosis. Changes in MMP in MCF-7 and A549 cells after treatment with complexes **3** and **4** were assessed by JC 1 dye. In our study, red fluorescing cells were abundant among control cells, indicating that there was little, if any, alteration in MMP in both MCF-7 and A549 cells. Cells treated with cobalt complexes fluoresced green (loss of red fluorescence) and thus represented change in MMP, indicating a nearly step in apoptosis. Within this generalization, complex **4** produced more remarkable effect than complex **3** in both MCF-7 and A549 cells (Fig. 7).

DNA damage induced by the treatment, as revealed in comet assay

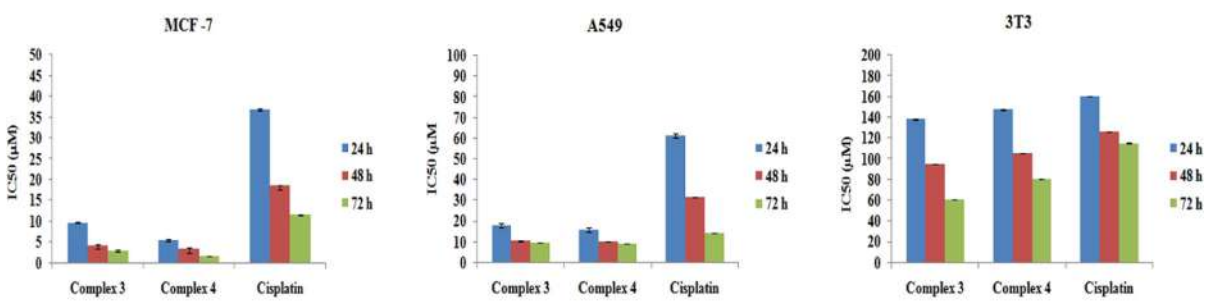
Comet assay revealed the extent of DNA damage induced in MCF-7 and A549 cells by cobalt complexes **3** and **4**. There were rarely a few comets in the control cells. The control group showed 98.3 ± 0.07% head and 1.7 ± 0.03% tail DNA for MCF-7 cells, and 97.4 ± 0.15% head and 2.6 ± 0.85% tail DNA for A549 cells. The incidence of comets was enormous in cobalt complexes **3** and **4** treated MCF-7 as well as A549 cells (Fig. 8). CASP software was used to analyze the geometric and densitometric parameters so as to estimate the amount of DNA in the head (intact DNA) and the tail (DNA with strand breaks) regions (Fig. 9). Additionally, the longer tails observed in treated MCF-7 and A549 cells reflected on induction of apoptosis by treatment of the cobalt complexes.

Apoptosis *vs* necrosis, as revealed in Annexin V-Cy3-CFDA staining

With the initiation of molecular events of apoptosis, phosphatidylserine is translocated from inner to outer leaflet of plasma membrane. When treated with the cobalt complexes **3** and **4**, incidence of early changes of apoptotic cell death was notably increased which was detected by using annexin V (red fluorescence) and 6-CFDA (green fluorescence). The result suggested that the complex **3** and **4** preponderantly activate apoptosis rather than necrosis (Fig. 10). The images show abundant early apoptotic cells (Annexin V-Cy3 positive, 6-CFDA positive), and a fairly good number of viable cells (Annexin V-Cy3 negative, 6-CFDA positive) (Fig. 11). Cobalt complex **4** produced higher incidence of apoptosis than complex **3** in both MCF-7 and A549 cells.



(a) Dose-dependent cytotoxic effect of cisplatin and cobalt complexes **3** and **4** on MCF-7 (50, 20, 10µM), A549 (100, 20, 20 µM) and 3T3 (200 µM) cells incubated for 24, 48 and 72 h (Each data represents mean and standard deviation of three replicates).



(b) Graph showing IC_{50} values (µM) of cobalt complexes **3**, **4** and cisplatin for MCF-7, A549 and 3T3 cells incubated for 24, 48 and 72 h (Each data represents mean and standard deviation of three replicates).

Fig. 2 (**a**) Dose-dependent cytotoxic effect of cisplatin and cobalt complexes **3** and **4** on MCF-7 (50, 20, 10 µM), A549 (100, 20, 20 µM) and 3T3 (200 µM) cells incubated for 24, 48 and 72 h (Each data represents mean and standard deviation of three replicates). (**b**) Graph showing IC_{50} values (µM) of cobalt complexes **3**, **4** and cisplatin for MCF-7, A549 and 3T3 cells incubated for 24, 48 and 72 h (Each data represents mean and standard deviation of three replicates)

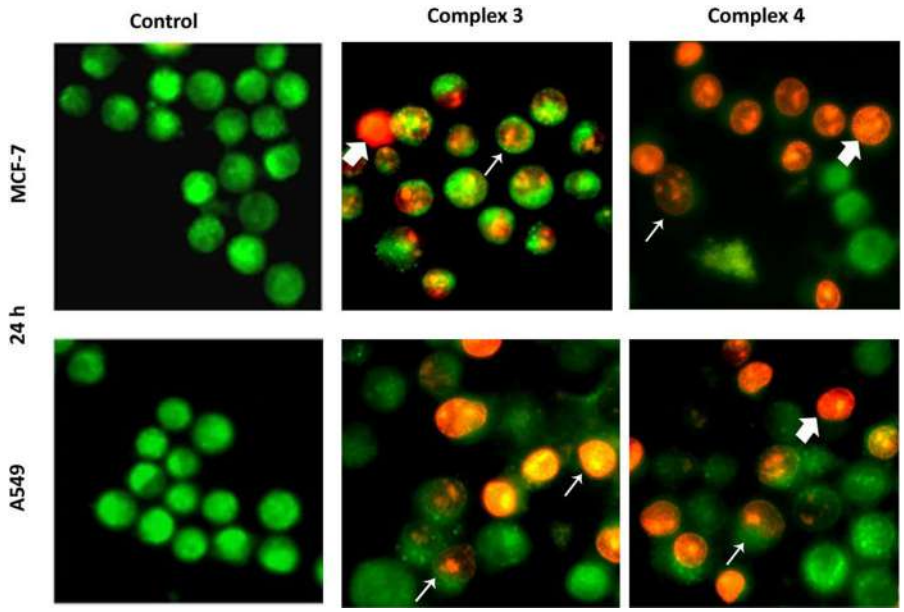


Fig. 3 Effect of control and cobalt complexes **3** and **4** on MCF-7 (Complex **3**: 9.6 and complex **4**: 5.4 μM) and A549 (Complex **3**: 17.9 and complex **4**: 15.7 μM) cells stained with AO/EB. Thin arrows point to cells in apoptosis and thick arrows point to necrotic cells

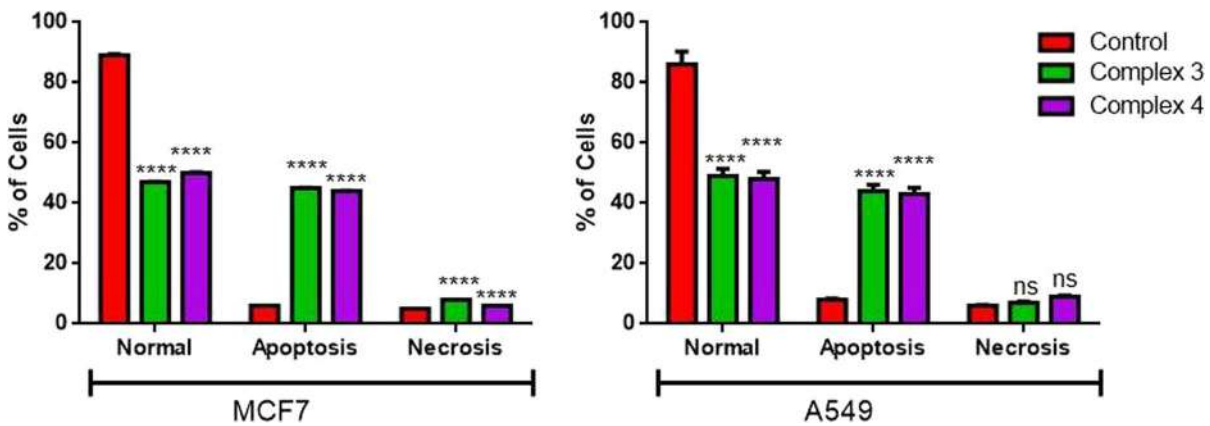


Fig. 4 Percentage of normal, apoptotic and necrotic cells at 24 h treatment with cobalt complexes **3** and **4** (Data represent means and standard deviations of three replicates each). Significance between control and treated was tested by adopting two way ANOVA (*ns* not significant, ****$p < 0.0001$)

Generation of ROS on treatment with cobalt (III) complexes

Mitochondria are the important sub-cellular targets of toxicity. A close relationship between oxidative stress and mitochondrial function has been observed. Hence, the IC_{50} of cobalt complexes **3** and **4**, exposed for 12 h, induced ROS generation in both MCF-7 and A549 cells (Fig. 12). Hence, we determined ROS generation using cobalt complexes **3** and **4** against MCF-7 and A549 cells at the respective IC_{50} for 12 h. ROS generation was detected using the fluorescent dye DCFH-DA and observed in the fluorescent microscope (Fig. 13).

Effect of treatment of the complexes on pro-apoptotic and anti-apoptotic protein expressions

Effect of the cobalt (III) complexes **3** and **4** on expression levels of Bcl-2 (anti-apoptotic protein) and

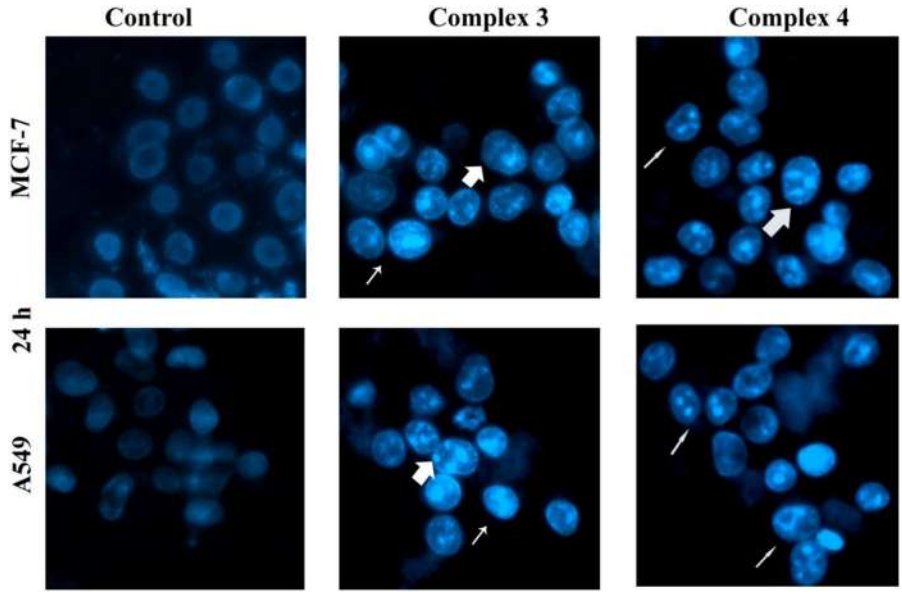


Fig.5 Effect of control and cobalt complexes **3** and **4** treated MCF-7 (Complex **3**: 9.6 and complex **4**: 5.4 μM) and A549 (Complex **3**: 17.9 and complex **4**: 15.7 μM) cells; stained with Hoechst 33258. Thin arrows point to cells in apoptosis and thick arrows point to necrotic cells

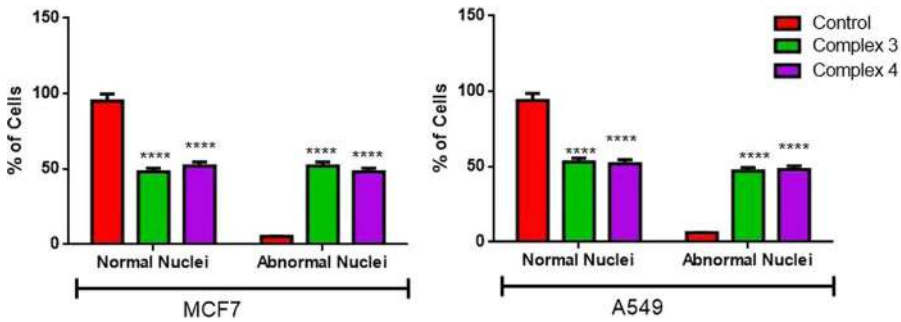


Fig.6 Bar diagram showing the percentage of cells with normal and abnormal nuclei at 24 h treatment of cobalt complexes **3** and **4** (Data represent means and standard deviations of three replicates each). Significance between control and treated was tested adopting two way ANOVA (****$p < 0.0001$)

Bax (pro-apoptotic protein) in MCF-7 and A549 cells was investigated. The fluorescence intensity of Bax was increased (Fig. 14), but that of Bcl-2 was reduced. Thus, expression of Bax was upregulated and that of Bcl_2 was down-regulated. Complex **4** produced more prominent result than complex **3** in both cell lines. Statistical treatment of the data confirmed that up- and down-regulation of Bax and Bcl-2 expressions, respectively, were significant ($p < 0.05$). Finally, the highest levels of Bax and Bcl-2 expressions were found in MCF-7 cells compared to A549 cells.

Effect of treatment of the complexes on expression of apoptosis-associated genes

We investigated the effect of cobalt (III) complexes on the expression of pro-apoptotic (BID, BAX, Cas-3, and Cas-8), anti-apoptotic (Bcl-2 and GRP-78) and antioxidant (CAT, GPX1, and SOD) genes in terms of fold change by treating MCF-7 and A549 cells with complexes **3** and **4** for 24 h at the respective IC_{50}. The respective gene expressions were normalized to the control group GAPDH. The expression of pro-

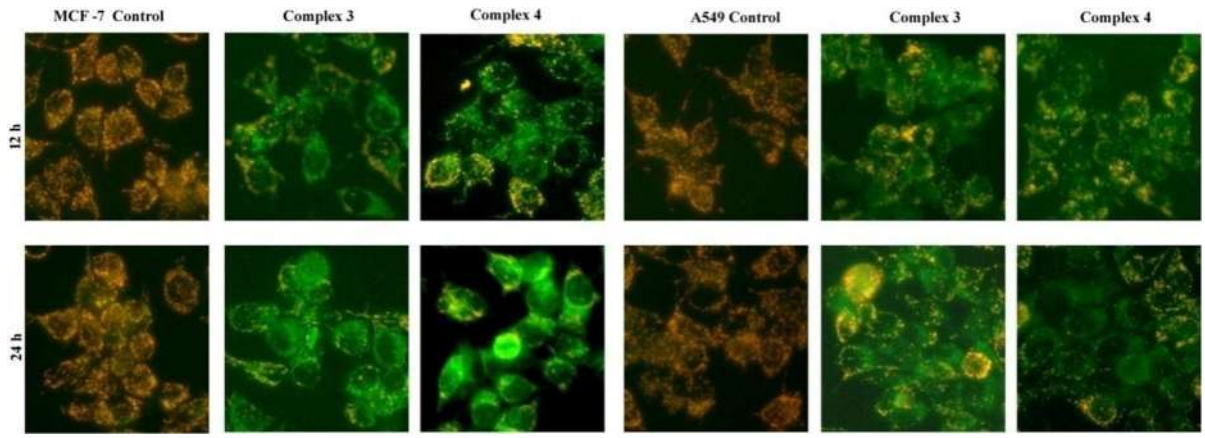


Fig.7 JC1 staining of control and cobalt complexes **3** and **4** treated MCF-7 (Complex **3**: 9.6 and complex **4**: 5.4 μM) and A549 (Complex **3**: 17.9 and complex **4**: 15.7 μM) cells incubated for 12 and 24 h to reflect on mitochondrial trans-membrane potential

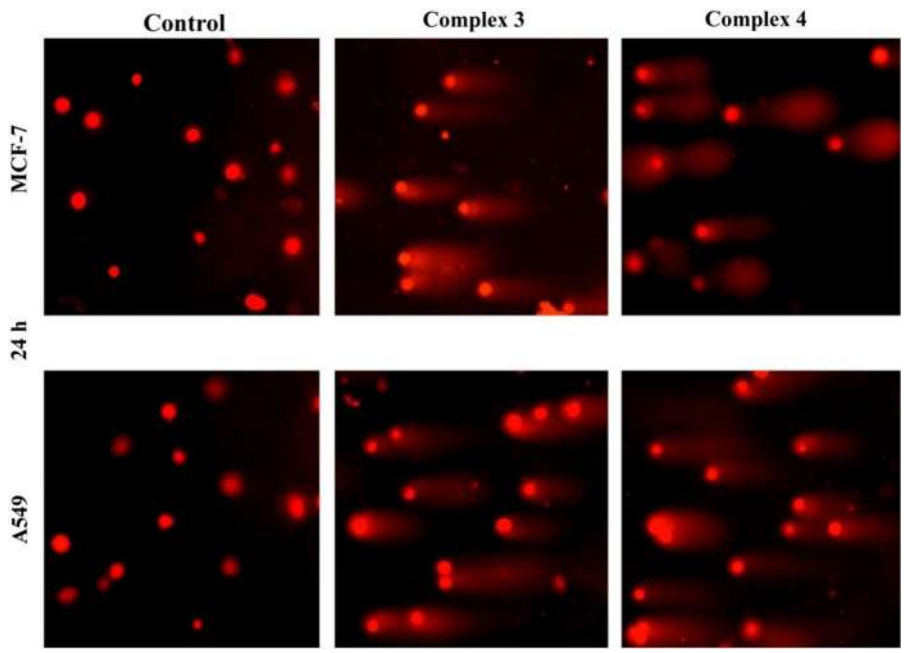


Fig.8 Comet analysis of control and cobalt complexes **3** and **4** treated MCF-7 (Complex **3**: 9.6 and complex **4**: 5.4 μM) and A549 (Complex **3**: 17.9 and complex **4**: 15.7 μM) cells

apoptotic genes was upregulated (fold change for MCF-7, Complex 3: BID (1.24), Bax (1.12), caspase-3 (1.60), and caspase- 8 (1.18) and complex 4: BID (2.33), Bax (1.92), caspase-3 (2.16), and caspase-8 (1.90) and A549, Complex 3: BID (1.10), Bax (1.098), caspase-3 (1.41), and caspase- 8 (1.083) and complex 4: BID (2.27), Bax (1.14), caspase-3 (2.10), and caspase–8 (1.56)) compared to the control (normalized as 1 for all groups) in treated MCF-7 and A549 cells. Compared to control, anti-apoptotic genes (fold change for MCF-7, complex 3: Bcl-2 (0.90) and GRP-78 (0.76), complex 4: Bcl-2 (0.65) and GRP-78 (0.60) and for A549, Complex 3: Bcl-2 (0.97) and GRP-78 (0.98), complex 4: Bcl-2 (0.78) and GRP-78 (0.81)) (Fig. 15).

The antioxidant genes on the other hand [(fold change for MCF-7, Complex 3: SOD (0.57), CAT (0.85), and GPX1 (0.94) and complex 4: SOD (0.22),

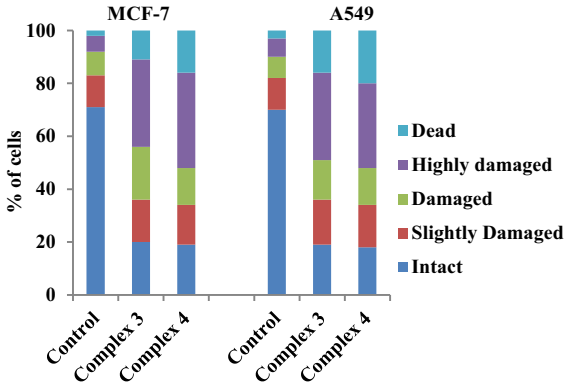


Fig. 9 Effect of treatment of cobalt complexes **3** and **4** on inducing DNA damage in MCF-7 and A549 cells. Classification: intact (0–20%), slightly damaged (20–40%), damaged (40–60%), highly damaged (60–80%), and dead (80–100%) (Each data point represents mean and standard deviation of three replicates)

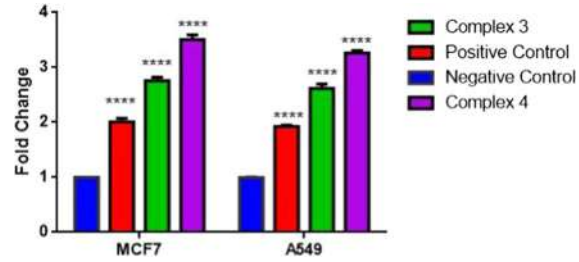


Fig.12 ROS generation in MCF-7 and A549 cells exposed to cobalt complexes **3** and **4** for 12 h as revealed by ROS assay (Data represents mean and standard deviation of three triplicates). Significance between control and treated was tested by adopting two way ANOVA (****$p < 0.0001$)

CAT (0.34), and GPX1 (0.43), and A549 Complex 3: SOD (0.55), CAT (0.35), and GPX1 (0.85) and complex 4: SOD (0.33), CAT (0.66), and GPX1

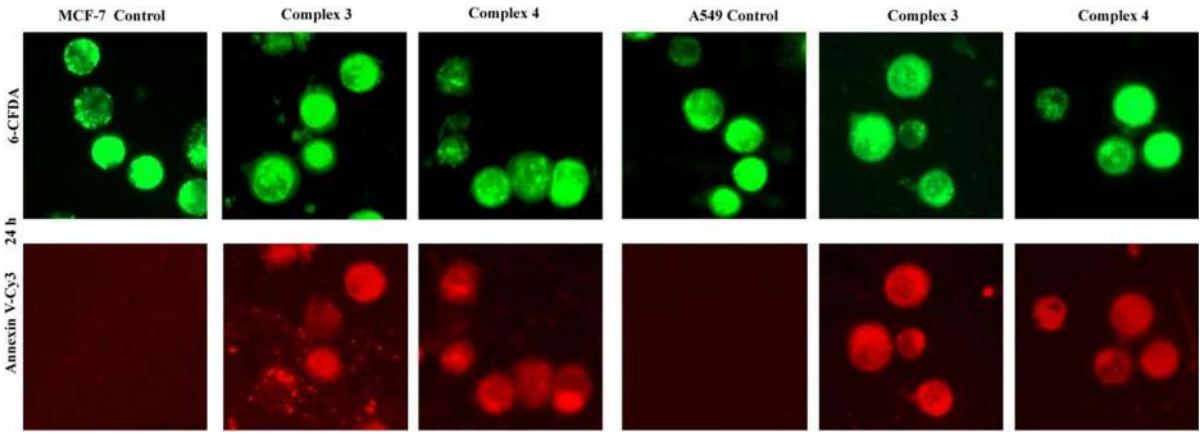


Fig.10 Annexin V-Cy3 -6-CFDA staining of cobalt complexes **3** and **4** treated MCF-7 (Complex **3**: 9.6 and complex **4**: 5.4 μM) and A549 (Complex **3**: 17.9 and complex **4**: 15.7 μM) cells

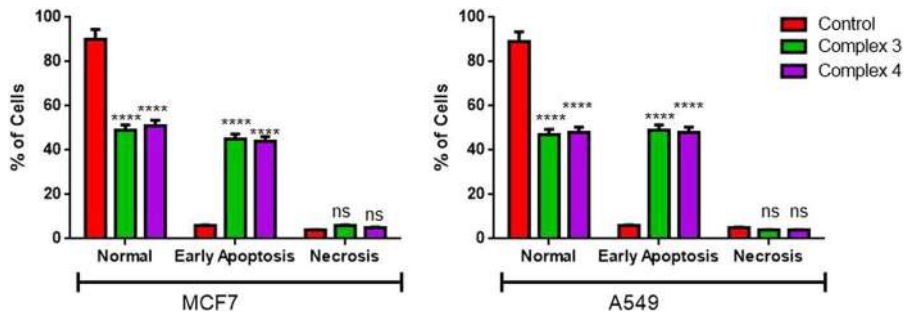


Fig.11 Bar diagrams showing the percentage of MCF-7 and A549 cells which have succumbed to cell death induced by cobalt complexes **3** and **4** (Each data point represents mean and standard deviation of three replicates). Significance between control and treated was tested by adopting two way ANOVA (*ns* not significant, ****$p < 0.0001$)

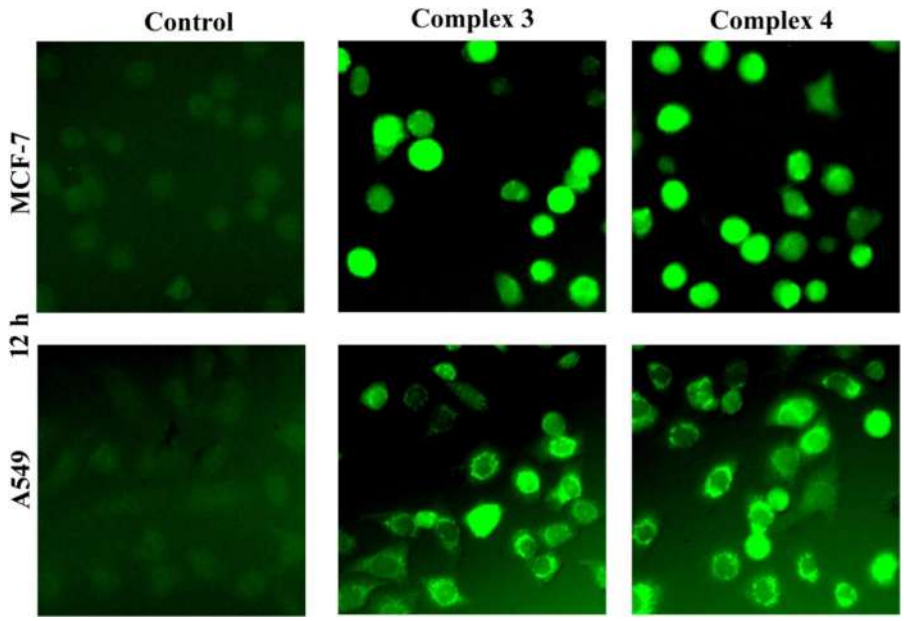


Fig.13 DCFH-DA fluorescence staining representing the level of ROS generation in MCF-7 (Complex **3**: 9.6 and complex **4**: 5.4 μM) and A549 (Complex **3**: 17.9 and complex **4**: 15.7 μM) cells exposed to cobalt complexes **3** and **4** for 12 h

(0.54)] were down-regulated (Fig. 16). The results also indicate that between complexes 3 and 4, the 4 is more effective in modulating the antioxidant genes and inducing apoptosis at a lower dose when compared to 3.

Discussion

Discovery of a promising lead drug molecule could be possible by bringing about structural modification of an existing compound that will enhance the functional capability of the compound (Venkatesan et al. 2019). Earlier, we had reported the synthesis and characterization of novel cobalt (III) Schiff base complexes **1**–**4** of which **3** and **4** proved to be more efficacious than **1** and **2**. These two complexes had been characterized adopting various techniques such as UV–Visible spectroscopy, infra-red (IR) spectroscopy, ESI–MS, and NMR spectroscopy (Manojkumar et al. 2019). In the absorption spectral study therein, we observed the binding ability of the complexes to calf thymus DNA through intercalation that resulted in hypochromism and red/blue shift of the bands due to stacking of the planar aromatic chromophore between the base pairs of DNA. Both the complexes showed a high energy band at wavelength 230–380 nm due to intra-ligand π–π* type transitions. Further, we observed lower DNA binding constant (Kb) values for complex **3** (Kb = 1.08×10^3 M^{-1}) and **4** (Kb = 4.50×10^3 $M^{-}1$) when compared to Ethidium Bromide (EB) (Kb = $4.94 \times 10^5 M^{-1}$), a classical intercalator, due to the presence of π–π*stacking of the Schiff base ligand phenyl ring with the calf thymus DNA base pairs. Furthermore, the conjugation of the long aliphatic chains in complex **3** (heptylamine) and **4** (dodecylamine) enhanced the hydrophobic interactions with calf thymus DNA. Thus, alteration in the structure from earlier version of the complexes was achieved by associating appropriately identified ligands heptylamine and dodecylamine, respectively, with cobalt (III) complexes **3** and **4**. This unique structural modification was anticipated to induce apoptotic death of cancer cells, and we envisioned to refine the properties such as increasing solubility, improving ability to be absorbed, and reducing toxicity and other associated benefits (Manojkumar, et al. 2019). However, the molecular mechanisms underlying induction of apoptosis in cancer cells by the complexes were unclear.

In the earlier study (Manojkumar et al. 2019), the cytotxic potential of complexes **3** and **4** was tested against A549, with VERO as normal control, and the assays included MTT, AO & EB, Hoechst 33248,

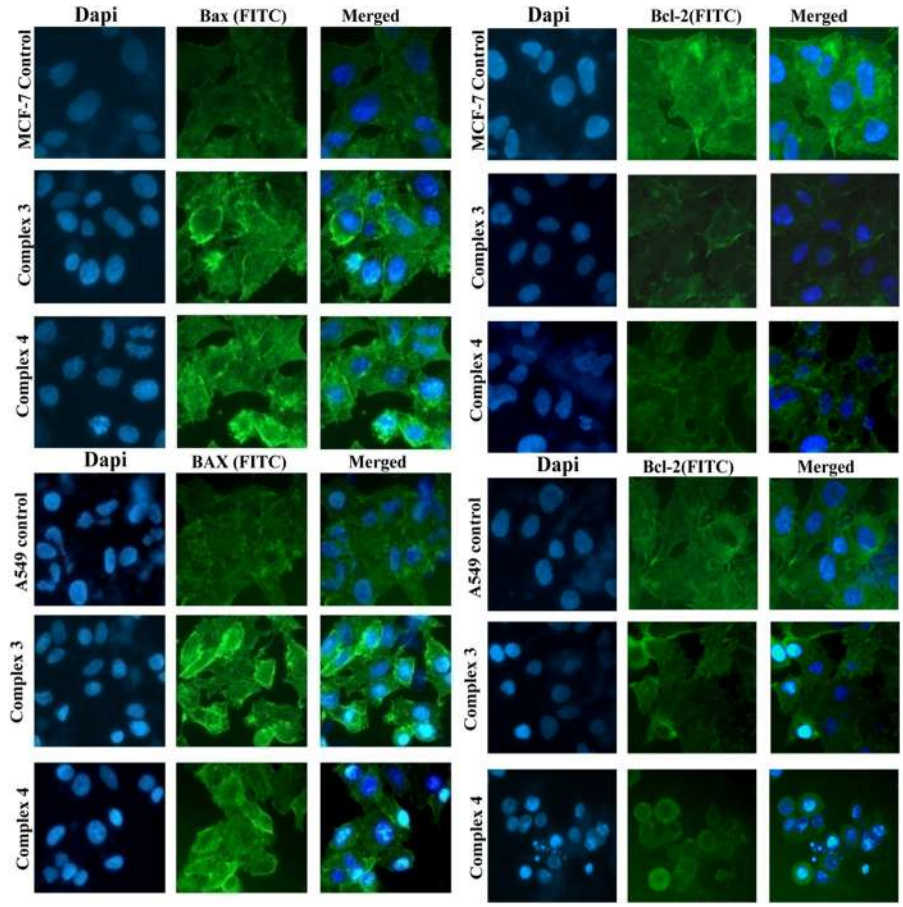


Fig.14 Up-regulation of Bax expression (Left panel) and down-regulation of Bcl-2 expression (Right panel) in MCF-7 (Complex **3**: 9.6 and complex **4**: 5.4 μM) and A549 (Complex **3**: 17.9 and complex **4**: 15.7 μM) cells treated with cobalt complexes **3** and **4** where the cells were stained with DAPI as nuclear stain and FITC [fluorochrome-conjugated secondary antibody] for protein expression over 24 h

Annexin V-Cy3 and 6-CFDA, and cell cycle analysis. In the present study, we have expanded the investigation to study the anticancer potential of the cobalt (III) complexes against MCF-7 and A549 cells and compared them to normal 3T3 cells where in our emphasis has been ROS, DNA damage and molecular mechanisms of apoptosis. Though 3T3 is mouse fibroblastic cell line, it has been in use as the normal control in the context of drug testing against cancer cell lines (Alabsi et al. 2012; Jastrzebska et al. 2013; Armania et al. 2013; Begum et al. 2013). Herein, experiments were designed to study the morphological and biochemical changes in MCF-7 and A549 cells in order to validate the cytotoxicity of the cobalt (III) complexes. Complex **3** and **4** treatment (at IC_{50} concentrations) reduced the survival percentage of MCF-7 and A549 cells. ROS-mediated pathway leading to mitochondrial cell death was observed at different time intervals. We have established that the predominant mode of cell death is apoptosis. Although necrosis was also observed the percentage of cells that underwent necrosis was negligible. It is well known that DNA damage is one of the principal causes of apoptosis. Moreover, cells treated with complexes **3** and **4** showed elevated productions of ROS, wherein possibly complexes **3** and **4** might be chelating the DNA through the various forces such as Vander Waal interactions or hydrophobic effects. Moreover, cells treated with complexes 3 and 4 showed elevated production of ROS, wherein possibly these complexes interact with DNA and other bio-macromolecule in the cell (Mahmoudi et al. 2009). This interaction is

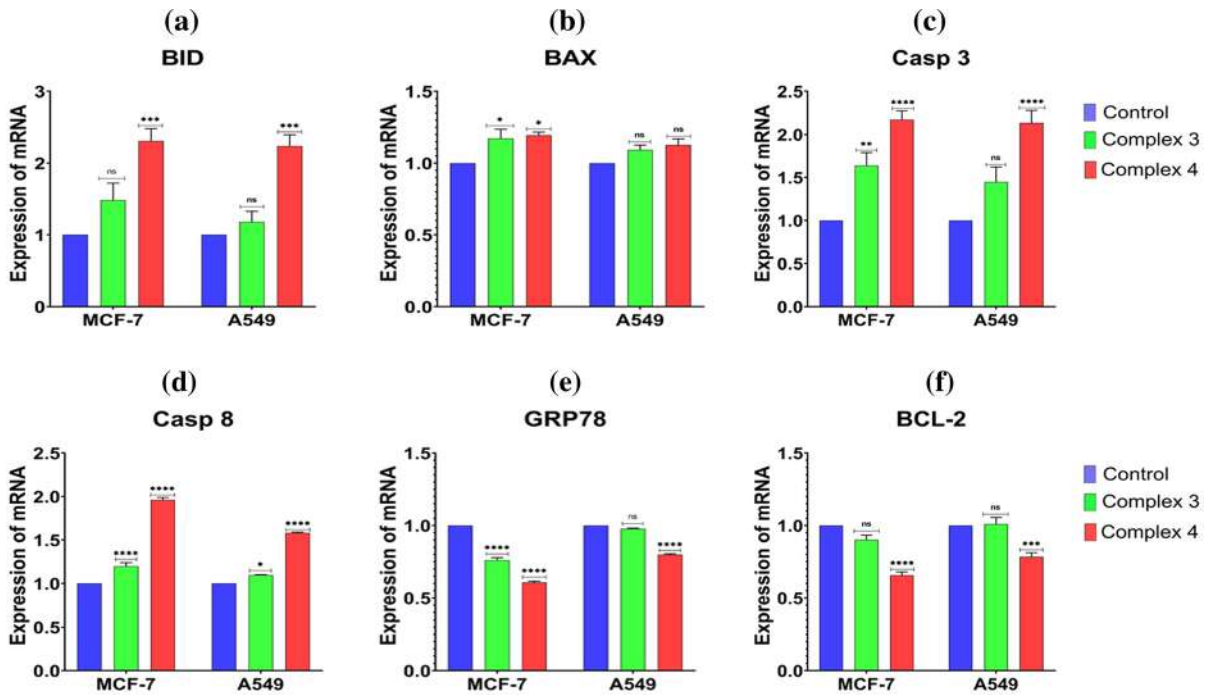


Fig.15 Pro- and anti-apoptotic gene expression profiles in MCF-7 (Complex **3**: 9.6 and complex **4**: 5.4 μM) and A549 (Complex **3**: 17.9 and complex **4**: 15.7 μM) cells exposed to cobalt complexes **3** and **4**. **A** BID; **B** Bax; **C** Caspase 3; **D** Caspase 8; **E** GRP78; **F** BCl_2. GAPDH was used as control. Each data point represents mean and standard deviation of three replicates. Significance between control and treated was tested by adopting two way ANOVA (*ns* not significant,*p < 0.5, **p < 0.01, ***p < 0.001, ****p < 0.0001)

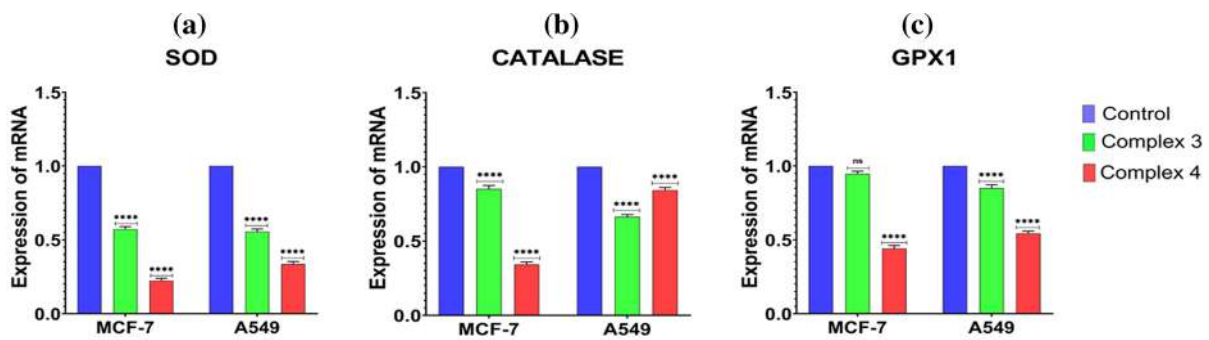


Fig.16 Antioxidant gene expression profiles in MCF-7 (Complex **3**: 9.6 and complex **4**: 5.4 μM) and A549 (Complex **3**:17.9 and complex **4**:15.7 μM) cells exposed to cobalt complexes **3** and **4**. **A** SOD; **B** CATALASE; **C** GPX1. GAPDH was used as control. Each data point represents mean and standard deviation of three replicates. Significance between control and treated was tested by adopting two way ANOVA (*ns* not significant, ****p < 0.0001)

responsible for production of excessive ROS thereby triggering the cancer cells to undergo apoptosis via ROS-mediated pathway by activating caspase gene expression and Bcl2/BAX modulation.

The chromatin fragmentation, cytoplasmic vacuolation, membrane blebbing, multi-nucleation and apoptotic body formation observed in MCF-7 and A549 cells upon treatment of complexes **3** and **4** in our study strongly suggest apoptosis as the principal mechanism of cell death (Ambika et al. 2019). Cobalt (III) complexes differ among themselves in respect of cytotoxic activity which could be due to the different

DNA binding interactions and anti-proliferative activities.

Comet assay is one of the simple and reliable techniques for making assessment of DNA damage (Riyasdeen et al. 2012). The result showed that the percent of tail DNA was high when MCF-7 and A549 cells were treated with the cobalt (III) complexes **3** and **4**. DNA damage is one of the principal causes/courses of apoptosis. Since the complexes induce production of ROS, they might be chelating the DNA through the various forces such as Vander Waal interactions, hydrophobic effects, etc., which reflect that the cells succumb to apoptotic death (Kumar and Arunachalam 2008). Further, cellular stress creates abnormal conditions such as hypoxia, and oncogene expression which would activate p53 tumor suppressor protein. The p53 (apoptotic protein) activation significantly increases p21 expression, subsequently releasing apoptogenic factors leading to cell death (Adams and Cory 2007).

One of the earliest events in apoptosis is the translocation of phosphatidylserine from inner to outer leaflet of the plasma membrane that can be further substantiated by AnnexinV-Cy3 staining (Mariño and Kroemer 2013). Over all, the result confirmed that the cobalt (III) complexes induce apoptotic cell death. In addition, apoptosis is correlated with the mitochondria's involvement in various apoptotic pathways. Thus, the study emphasizes that the cobalt (III) complexes exert anticancer activity thorough mitochondria. It involves in MMP changes resulting the mitochondrial swelling and permeabilization of the outer membrane (Kitsis and Molkentin 2010; Kumbar et al. 2020).

From another perspective, loss of MMP representing apoptosis might have a close relationship with high levels of ROS. Therefore, in this study, we examined the production of ROS in the cells treated with the cobalt (III) complexes adopting DCFH-DA. Since the ROS induce oxidative damage to biomolecules and invoke defense mechanism, which is considered as a major factor for cytotoxicity of the complexes. It can also disturb the cellular components such as DNA, proteins, lipids, etc., that ultimately leads to cell death (Pujalté et al. 2015; Sabella et al. 2014).

In many malignancies, ROS production has been shown to cause ER stress-mediated apoptosis. Hypoxia also causes endoplasmic reticulum (ER) stress, which can lead to cell death if the ER is irreversibly damaged or to cell survival if the stress is modest (Akman et al. 2021; Saleem et al. 2020; Lin et al. 2018, Iurlaro and Pinedo 2016).

ROS production and neutralizing antioxidants have to be balanced in order to maintain the homeostatic conditions of the living system (Murugadas, et al. 2016). Hence, our study emphasized the expression level of pro-apoptotic (BID, Bax, Cas-3, and Cas-8), anti-apoptotic (Bcl-2 and GRP-78) and antioxidant genes (CAT, GPX1, and SOD) induced by the complexes. Generally, CAT, GPX1, and SOD play roles in neutralizing the excess ROS. The enzymes produced by the expression of antioxidant genes that convert superoxide (SOD) into hydrogen peroxide which will be further catalyzed by CAT and GPX into water (Weydert and Cullen 2010). In our study, expression of antioxidant genes such as CAT, GPX1, and SOD was found to be down-regulated when treated with 24 h IC_{50} of the complexes whereas apoptotic gene expression was found to be up-regulated. Therefore, reduced expression of antioxidant genes as observed is not adequate to detoxify the ROS (Mwaanga et al. 2014). Henceforth, these findings reveal that the cobalt (III) complexes not only stimulate oxidative stress but also possess capability to limit antioxidant defense system. At the same time, antiapoptotic and proapoptotic gene expressions have to be balanced in order to maintain the homeostatic conditions. Thus, we examined Bcl-2 family members, including Bcl-2, GRP78 (antiapoptotic genes), Bax and BID (proapoptotic genes) to control the ability of apoptosis. Bax and BID gene expressions were found to be up-regulated and Bcl-2 gene and GRP78 expressions were found to be down-regulated. The exertion of these effects on the expression of Bax, in turn, stimulates cytochrome c release from mitochondria into the cytosol. This would trigger the caspase cascade through release of apoptogenic factors (Jänicke et al. 1998; Gowdhami et al. 2021). In the same way, death receptor-mediated apoptosis is initiated which would result in translocation of the *t*-form of Bid (*t*-Bid) to assist the release of apoptogenic proteins like cytochrome c (Youle and Strasser 2008). Bcl-2 and GRP78 play irreversible roles to inhibit apoptotic pathway intermediates and prevent caspase activation and subsequently inhibit apoptosis (Wang et al. 2014; Pyrko et al. 2007).

Thus, the research suggests that our cobalt (III) complexes induce Bax and BID gene expression and

reduce Bcl-2 and GRP78 expression thereby maintaining the biological system (Adams and Cory 2007; Sánchez-Pérez et al. 2010). These results authenticate the occurrence of apoptosis in MCF-7 and A549 cells following the exposure of cobalt (III) complexes **3** and **4** along the multiple apoptotic pathways.

Conclusion

Based on the observations herein, it can be concluded that our cobalt (III) complexes are capable of inducing apoptotic death in MCF-7 and A549 cells lead to loss of cell membrane integrity, DNA damage, alteration in mitochondrial membrane potential, translocation of phosphatidylserine, induction of oxidative stress, and modulation of gene expressions in apoptotic pathways. Moreover, it would be necessary to explore the various apoptotic mechanisms by ensuring the expression of antioxidant, proapoptotic, and antiapoptotic genes. The complex **4** is more potent than complex **3** due to its strong chelating activity with DNA. Furthermore, this complex is more effective with breast cancer cell than lung cancer cell. Overall, the present investigation shows that the cobalt (III) Schiff base complexes investigated herein can be effective and promising chemotherapeutic drugs for cancer, with greater emphasis on complex **4**.

Acknowledgements We express our sincere gratitude to the Dr. R. Thirumurugan, Co-ordinator of National Centre for Alternatives to Animal Experiments (NCAAE), for the continuous support, encouragement, and guidance during the course of this research. This work was financially supported by National Centre for Alternatives to Animal Experiments (NCAAE) under UGC-CPEPA scheme, Government of India (F.No.2-1/2013 (NS/PE)). The research facility provided by Mahatma Gandhi-Doerenkamp Centre, established by Doerenkamp-Zbinden Foundation, and the Department of Science and Technology, Government of India, under DST-Promotion of University Research and Scientific Excellence (PURSE) scheme-Phase II, Rashtriya Uchchatar Shiksha Abhiyan (RUSA)-2.O by the Department of Animal Science is heartily acknowledged. The authors thank the Management of Bishop Heber College (Autonomous), Tiruchirappalli-620 017, Tamil Nadu, India, for the support (F.No: MRP/1014/2020 dated : 23.12.2020) and facilities provided through Material Chemistry Lab, PG and Research Department of Chemistry and DST-FIST Instrumentation Centre (HAIF) at Bishop Heber College.

Funding University Grants Commission, F.No.2-1/2013 (NS/PE), Balakrishnan Gowdhami, National Centre for Alternatives to Animal Experiments (NCAAE) under UGC-CPEPA scheme, Balakrishnan Gowdhami, Department of Science and Technology, Ministry of Science and Technology, Mahatma Gandhi-Doerenkamp Centre, Mohammad Abdulkader Akbarsha,University Research, Balamuthu Kadalmani, Scientific Excellence (PURSE) scheme-Phase II, Balamuthu Kadalmani, Rashtriya Uchchatar Shiksha Abhiyan (RUSA)-2.O, Balamuthu Kadalmani, Doerenkamp-Zbinden Foundation, Mahatma Gandhi-Doerenkamp Centre, Mohammad Abdulkader Akbarsha.s

Declarations

Conflict of interest The authors declare no conflicts of interest.

References

Adams JM, Cory S (2007) The Bcl-2 apoptotic switch in cancer development and therapy. Oncogene 26:1324–1337

Aggett PJ, Fairweather-Tait S (1998) Adaptation to high and low copper intakes: its relevance to estimated safe and adequate daily dietary intakes. Am J Clin Nutr 67:1061S-1063S

Akman M, Belisario DC, Salaroglio IC, Kopecka J, Donadelli M, Enrico De Smaele ED, Riganti C (2021) Hypoxia, endoplasmic reticulum stress and chemoresistance: dangerous liaisons. J Exp Clin Cancer Res 40:28

Alabsi AM, Ali R, Ali AM, Al-Dubai SAR, Harun H, Abu Kasim NH, Alsalahi A (2012) Apoptosis induction, cell cycle arrest and in vitro anticancer activity of gonothalamin in a cancer cell lines. Asian Pac J Cancer Prev 13:5131–5136

Ambika S, Manojkumar Y, Arunachalam S, Gowdhami B, Sundaram KKM, Solomon RV, Venuvanalingam P, Akbarsha MA, Sundararaman M (2019) Biomolecular interaction, anti-cancer and anti-angiogenic properties of cobalt (III) Schiff base complexes. Sci Rep 9:2721

Armania N, Yazan LS, Ismail IS, Foo JB, Tor YS, Ishak N, Ismail N, Ismail M (2013) Dillenia suffruticosa extract inhibits proliferation of human breast cancer cell lines (MCF-7 and MDA-MB-231) via induction of G2/M arrest and apoptosis. Molecules 18:13320–13339

Badisa RB, Darling-Reed SF, Joseph P, Cooperwood JS, Latinwo LM, Goodman CB (2009) Selective cytotoxic activities of two novel synthetic drugs on human breast carcinoma MCF-7 cells. Anticancer Res 29:2993–2996

Begum S, Syed SA, Siddiqui BS, Sattar SA, Choudhary MI (2013) Carandinol: first isohopane triterpene from the leaves of *Carissa carandas* L. and its cytotoxicity against cancer cell lines. Phytochem Lett 6:91–95

Cornelio DB, Roesler R, Schwartsmann G (2009) Emerging therapeutic agents for cervical cancer. Recent Pat Anti-Cancer Drug Discov 4:196–206

Garbutcheon-Singh KB, Grant MP, Harper BW, Krause-Heuer AM, Manohar M, Orkey N, Aldrich-Wright JR (2011) Transition metal based anticancer drugs. Curr Top Med Chem 11:521–542

Gowdhami B, Ambika S, Karthiyayini B, Ramya V, Kadalmani B, Vimala RTV, Akbarsha MA (2021) Potential application of two cobalt (III) Schiff base complexes in cancer chemotherapy: leads from a study using breast and lung cancer cells. Toxicol Vitro 75:105201

Hall MD, Telma KA, Chang K-E, Lee TD, Madigan JP, Lloyd JR, Goldlust IS, Hoeschele JD, Gottesman MM (2014) Say no to DMSO: dimethylsulfoxide inactivates cisplatin, carboplatin, and other platinum complexes. Can Res 7:3913–3922

Iurlaro R, Pinedo CM (2016) Cell death induced by endoplasmic reticulum stress. FEBS J 283:2640–2652

Jagadeesan J, Langeswaran K, Gowthamkumar S, Balasubramanian MP (2013) Diosgenin exhibits beneficial efficiency on human mammary carcinoma cell line MCF-7 and against N-nitroso-N-methylurea (NMU) induced experimental mammary carcinoma. Biomed Prev Nutr 3:381–388

Jänicke RU, Sprengart ML, Wati MR, Porter AG (1998) Caspase-3 is required for DNA fragmentation and morphological changes associated with apoptosis. J Biol Chem 273:9357–9360

Jastrzebska E, Flis S, Rakowska A, Chudy M, Jastrzebski Z, Dybko A, Brzozka Z (2013) A microfluidic system to study the cytotoxic effect of drugs: the combined effect of celecoxib and 5-fluorouracil on normal and cancer cells. Microchim Acta 180:895–901

Jemal A, Tiwari RC, Murray T, Ghafoor A, Samuels A, Ward E, Feuer EJ, Thun MJ (2004) Cancer statistics. 2004. CA Cancer J Clin 54:8–29

Kitsis RN, Molkentin JD (2010) Apoptotic cell death "Nixed" by an ER–mitochondrial necrotic pathway. Proc Natl Acad Sci 107:9031–9032

Kumar RS, Arunachalam S, Periasamy V, Preethy C, Riyasdeen A, Akbarsha MA (2008) Synthesis, DNA binding and antitumor activities of some novel polymer–cobalt (III) complexes containing 1, 10-phenanthroline ligand. Polyhedron 27:1111–1120

Kumar RS, Arunachalam S (2008) Synthesis, micellar properties, DNA binding and antimicrobial studies of some surfactant–cobalt (III) complexes. Biophys Chem 136:136–144

Kumaravel TS, Vilhar B, Faux SP, Jha AN (2009) Comet Assay measurements: a perspective. Cell Biol Toxicol 25:53–64

Kumbar M, Patil SA, Toragalmath SS, Kinnal SM, Shettar A, Hosakeri JH (2020) Anticancer activity studies of novel metal complexes of ligands derived from polycyclic aromatic compound via greener route. J Organomet Chem 914:121219

Latt SA, Wohlleb JC (1975) Optical studies of the interaction of 33258 Hoechst with DNA, chromatin, and metaphase chromosomes. Chromosoma 52:297–316

Lin CL, Lee CH, Chen CM, Cheng CW, Chen PN, Ying TH, Hsieh YH (2018) Protodioscin induces apoptosis through ROS-mediated endoplasmic reticulum stress via the JNK/p38 activation pathways in human cervical cancer cells. Cell Physiol Biochem 46:322–334

Liu Y-H, Li A, Shao J, Xie C-Z, Song X-Q, Bao W-G, Xu J-Y (2016) Four Cu (II) complexes based on antitumor chelators: synthesis, structure, DNA binding/damage, HSA interaction and enhanced cytotoxicity. Dalton Trans 45:8036–8049

Mahmoudi M, Simchi A, Milani A, Stroeve P (2009) Cell toxicity of superparamagnetic iron oxide nanoparticles. J Colloid Interface Sci 336:510–518

Manojkumar Y, Ambika S, Arulkumar R, Gowdhami B, Balaji P, Vignesh G, Arunachalam S, Venuvanalingam P, Thirumurugan R, Akbarsha MA (2019) Synthesis, DNA and BSA binding, in vitro anti-proliferative and in vivo anti-angiogenic properties of some cobalt (III) Schiff base complexes. New J Chem 43:11391–11407

Mariño G, Kroemer G (2013) Mechanisms of apoptotic phosphatidylserine exposure. Cell Res 23:1247–1248

Marzano C, Pellei M, Tisato F, Santini C (2009) Copper complexes as anticancer agents. Anti-Cancer Agents Med 9:185–211

Murugadas A, Zeeshan M, Thamaraiselvi K, Ghaskadbi S, Akbarsha MA (2016) Hydra as a model organism to decipher the toxic effects of copper oxide nanorod: Ecotoxicogenomics approach. Sci Rep 6:1–14

Mwaanga P, Carraway ER, van den Hurk P (2014) The induction of biochemical changes in Daphnia magna by CuO and ZnO nanoparticles. Aquat Toxicol 150:201–209

Nagaraj K, Arunachalam S (2014) Synthesis, CMC determination, nucleic acid binding and cytotoxicity of a surfactant–cobalt (III) complex: effect of ionic liquid additive. New J Chem 38:366–375

Palanivel S, Murugesan A, Yli-Harja O, Kandhavelu M (2020) Anticancer activity of THMPP: downregulation of PI3K/S6K1 in breast cancer cell line. Saudi Pharm J 28(4):495–503

Park KC, Fouani L, Jansson PJ, Wooi D, Sahni S, Lane DJ, Palanimuthu D, Lok HC, Kovačević Z, Huang ML (2016) Copper and conquer: copper complexes of di-2-pyridylketone thiosemicarbazones as novel anti-cancer therapeutics. Metallomics 8:874–886

Pujalté I, Passagne I, Daculsi R, de Portal C, Ohayon-Courtès C, L'Azou B (2015) Cytotoxic effects and cellular oxidative mechanisms of metallic nanoparticles on renal tubular cells: impact of particle solubility. Toxicol Res 4:409–422

Pyrko P, Schönthal AH, Hofman FM, Chen TC, Lee AS (2007) The unfolded protein response regulator GRP78/BiP as a novel target for increasing chemosensitivity in malignant gliomas. Can Res 67:9809–9816

Rajendiran V, Karthik R, Palaniandavar M, Stoeckli-Evans H, Periasamy VS, Akbarsha MA, Srinag BS, Krishnamurthy H (2007) Mixed-ligand copper (II)-phenolate complexes: effect of coligand on enhanced DNA and protein binding, DNA cleavage, and anticancer activity. Inorg Chem 46:8208–8221

Reers M, Smith TW, Chen LB (1991) J-aggregate formation of a carbocyanine as a quantitative fluorescent indicator of membrane potential. Biochemistry 30:4480–4486

Riyasdeen A, Periasamy VS, Paul P, Alshatwi AA, Akbarsha MA (2012) Chloroform extract of Rasagenthi Mezhugu, a Siddha formulation, as an evidence-based complementary and alternative medicine for HPV-positive Cervical Cancers. Evidence-Based Complement Alternative Med 136527:1–10

Sabella S, Carney RP, Brunetti V, Malvindi MA, Al-Juffali N, Vecchio G, Janes SM, Bakr OM, Cingolani R, Stellacci F

(2014) A general mechanism for intracellular toxicity of metal-containing nanoparticles. Nanoscale 6:7052–7061

Saleem MZ, Nisar MA, Alshwmi M, Din SRU, Gamallat Y, Khan M, Ma T (2020) Brevilin a inhibits STAT3 signaling and induces ROS-dependent apoptosis, mitochondrial stress and endoplasmic reticulum stress in MCF-7 breast cancer cells. OncoTargets Therapy. 13:435–450

Sánchez-Pérez Y, Morales-Bárcenas R, García-Cuellar CM, López-Marure R, Calderon-Oliver M, Pedraza-Chaverri J, Chirino YI (2010) The α-mangostin prevention on cis-platin-induced apoptotic death in LLC-PK1 cells is associated to an inhibition of ROS production and p53 induction. Chem Biol Interact 188:144–150

Singh NP, McCoy MT, Tice RR, Schneider EL (1988) A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res 175:184–191

Spector DL, Goldman RD, Leinwand LA (1998) Cell: a laboratory manual. Culture and Biochemical Analysis of Cells. CSHL Press, New York

Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F (2021) Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Cancer J Clin 71(3):209–249. https://doi.org/10.3322/caac.21660

Tapiero H, Dá T, Tew K (2003) Trace elements in human physiology and pathology. Copper Biomed Pharmacother 57:386–398

Turnlund J, Keyes WR, Anderson HL, Acord LL (1989) Copper absorption and retention in young men at three levels of dietary copper by use of the stable isotope 65Cu. Am J Clin Nutr 49:870–878

Venkatesan R, Karuppiah PS, Arumugam G, Balamuthu K (2019) β-Asarone exhibits antifungal activity by inhibiting ergosterol biosynthesis in Aspergillus niger ATCC 16888. Proc Nat Acad Sci India Sect B 89:173–184

Wang H, Joseph JA (1999) Quantifying cellular oxidative stress by dichlorofluorescein assay using microplate reader. Free Radical Biol Med 27:612–616

Wang X, Chen M, Zhou J, Zhang X (2014) HSP27, 70 and 90, anti-apoptotic proteins, in clinical cancer therapy. Int J Oncol 45:18–30

Wang Y-T, Fang Y, Zhao M, Li M-X, Ji Y-M, Han Q-X (2017) Cu (II), Ga (III) and In (III) complexes of 2-acetylpyridine N (4)-phenylthiosemicarbazone: synthesis, spectral characterization and biological activities. Med Chem Commun 8:2125–2132

Weydert CJ, Cullen JJ (2010) Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cells and tissue. Nat Protoc 5:51–66

Youle RJ, Strasser A (2008) The BCL-2 protein family: opposing activities that mediate cell death. Nat Rev Mol Cell Biol 9:47–59

Zangrando E, Islam M, Islam A-AM, Sheikh MC, Tarafder MTH, Miyatake R, Zahan R, Hossain MA, (2015) Synthesis, characterization and bio-activity of nickel (II) and copper (II) complexes of a bidentate NS Schiff base of S-benzyl dithiocarbazate. Inorg Chim Acta 427:278–284

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Journal of Chemical Ecology
https://doi.org/10.1007/s10886-021-01311-9

Staphylococcus pasteuri (BCVME2) Resident in Buffalo Cervical Vaginal Mucus: A Potential Source of Estrus-Specific Sex Pheromone(s)

Mahalingam Srinivasan[1] · Rengasamy Lakshminarayanan Rengarajan[1] · Dharmadurai Dhanasekaran[2] · Mohammad Abdulkader Akbarsha[3] · Govindaraju Archunan[1,4]

Received: 28 July 2021 / Revised: 28 July 2021 / Accepted: 24 August 2021
© The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2021

Abstract
Mammals have microbes resident in their reproductive tract, some of which can be pathogenic while others may play a role in protecting the tract from infection. Volatile compounds play a role as sex pheromones that attract males for coitus during female estrus or heat. It is likely that these compounds themselves are secondary metabolites of bacterial flora resident in the vagina. In order to substantiate this hypothesis, bacteria were isolated from cervico-vaginal mucus (CVM) of buffalo during various phases of the estrous cycle and identified, using morphological, biochemical and molecular characteristics, as *Bacillus* during preestrus and diestrus, and as *Staphylococcus* during all three phases of the estrous cycle. Populations of *Staphylococcus* differed between different phases of the estrous cycle, the predominant forms being *S. warneri* (BCVMPE1_1) during preestrus, *S. pastueri* (BCVME2) during estrus and *S. epidermis* (BCVMDE3) during diestrus. Mice were used as chemosensors to differentiate the estrus-specific *S. pasteuri* (BCVME2) from the others. Chemical analysis showed that *S. pasteuri* (BCVME2) produced acetic, propanoic, isobutyric, butyric, isovaleric and valeric acids. In addition, it was shown that *S. pasteuri* (BCVME2) volatiles influenced the sexual behaviors, flehmen and mounting, of the bull. Thus, *S. pasteuri* (BCVME2) is a potential source of vaginal pheromone(s) during estrus in buffalo.

Keywords Bacteria · Estrous cycle · Vaginal mucus · HS-GC–MS · Volatile compounds

Introduction

The cervico-vaginal mucus (CVM) microbial community is associated with reproductive health and fertility in mammals. For example, cervical secretions are known to play crucial roles in the reproductive success of cows (Adnane et al. 2018). CVM of a healthy cow contains aerobic as well as anaerobic microbiota, especially those belonging to the Enterobacteriaceae family, as well as *Staphylococcus* sp., *Streptococcus* sp., *Enterococcus* sp. and *Lactobacillus* sp. (Otero et al. 1999; Wang et al. 2013). CVM microbiota are influenced by hormonal, immunological and nutritional factors, and also antibiotics and hormones that are used as drugs for treatment of the host (Otero et al. 2006). The dynamics of microbiota in the uterus and vagina have been well studied in cows under various pathophysiological conditions (Ault et al. 2019; Bicalho et al. 2017a, b, c; Galvao et al. 2019). In buffalo, the association of CVM microbiota with hematological parameters, such as counts of white blood cells, lymphocytes, monocytes, granulocytes, etc., during the different phases of the estrous cycle have been reported (Joshi et al. 2020). The role of CVM bacteria in reproduction and pheromone release in bovines has been reviewed (Srinivasan et al. 2021). However, elaborate investigation on the contribution of estrus-specific bacteria to buffalo pheromone(s) has not yet been investigated.

Research has revealed that a culture-independent method using next-generation sequencing (NGS) is appropriate for describing the diversity of the CVM microbiome with particular reference to the buffalo estrous cycle (Srinivasan

✉ Govindaraju Archunan
garchu56@yahoo.co.in

[1] Department of Animal Science, Bharathidasan University, Tiruchirappalli-620024, Tamil Nadu, India

[2] Department of Microbiology, Bharathidasan University, Tiruchirappalli-620024, Tamil Nadu, India

[3] Research Co-ordinator & Department of Biotechnology, National College (Autonomous), Tiruchirappalli-620001, Tamil Nadu, India

[4] Dean-Research, Marudupandiyar College, Thanjavur 613403, Tamil Nadu, India

Published online: 20 September 2021

et al. 2019). Proteobacteria, Actinobacteria, Firmicutes, Bacteroidetes and Tenericutes are the most common CVM of buffalo. Among these five families, Firmicutes is highly abundant during estrus compared to the other phases (Srinivasan et al. 2019). The volatile chemicals of CVM can vary among phases of the estrous cycle, and it is well known that bulls are able to differentiate estrus and non-estrus cows based on pheromone(s) present in the vaginal secretion (Karthikeyan and Archunan 2013; Rajanarayanan and Archunan 2004; Sankar and Archunan 2004).

Male dogs and mice differentiate estrus and non-estrus cows by sniffing the odor of vaginal fluid, urine, saliva, feces and milk of the latter (Fischer-Tenhagen et al. 2013; Rameshkumar et al. 2008; Sankar and Archunan 2005). Bovine bulls exhibit flehmen and mounting behaviors after perceiving specific volatiles from females during estrus (Karthikeyan et al. 2013; Rajanarayanan and Archunan 2004; Sankar and Archunan 2004). Flehmen is the most distinct and important aspect of premating behavior, and has been used to identify and quantify pheromone production in buffaloes and cows (Karthikeyan et al. 2013; Rajanarayanan and Archunan 2004; Sankar and Archunan 2004).

Acetic acid and propanoic acid have been identified in cow vaginal mucus (Sankar and Archunan 2011) and oleic acid in buffalo vaginal secretion (Karthikeyan and Archunan 2013) during estrus; all are thought to act as female sex pheromones. Interestingly, acetic acid has been found in buffalo urine, with a substantial increase in concentration during estrus, relative to other phases of the estrous cycle (Muniasamy 2016). The bacterial populations present in CVM are thought to affect sexual attraction of ewes, while fatty acids produced by microbes may act as pheromones as well as estrus indicators in buffaloes (Ungerfeld and Silva 2005).

Although buffalo CVM has been shown to produce estrus-specific volatiles that affect reproductive behavior of male buffalo during mating, the source of buffalo CVM pheromone compounds is not well established. Further, evidence for production by female buffalo of volatiles that elicit male attraction is poor. The present study was, therefore, intended to: i) identify the bacterial community in buffalo CVM, through a culture-based method, during different phases of the estrous cycle, (ii) screen estrus-specific bacteria using mice as a model sensor, iii) find if bacterial secretions contained volatile compounds, and iv) evaluate bull buffalo behavior in response to estrus-specific bacterial compounds of CVM, for support that bacterial volatiles are pheromones that elicit specific premating behaviors in males.

Materials and Methods

Animal and Sample Collection

Twelve healthy female buffaloes were used and their phases of the estrous cycle monitored as per the protocol of Rajanarayanan and Archunan (2004). CVM collection was carried out based on the procedure and protocol of Laguardia-Nascimento et al. (2015).

Isolation and Characterization of Bacteria

For isolation of bacteria, the CVM of all 12 buffaloes, at each of the three phases of the estrous cycle, were collected; i.e., 36 CVM samples, 12 for each phase. For further analysis, the CVM of specific phases of four buffaloes were pooled, and 0.5 ml homogenized in 4.5 ml of sterile distilled water. An aliquot of 0.1 ml of homogenate was serially diluted and plated in Nutrient-MacConkey-De Man, Rogosa and Sharpe- and Mannitol salt-agar by a standard plate count method (Cassoli et al. 2016). Plates were incubated at 37 °C for 24–48 h. Following incubation, bacterial colonies were counted and expressed in log values [Log CFU/ml, i.e. Log10 (CFU / (dilution factor x aliquot)]. Morphologically distinct colonies were picked and further processed. Primary characterization was based on colony morphology, including color, size and shape. Isolates were subjected to Gram staining and biochemical tests for catalase, oxidase, sugar fermentation and hemolysis.

Extraction of Genomic DNA and PCR Amplification of 16S rRNA Gene

Genomic DNA of bacterial isolates was extracted based on the method of Vingataramin and Frost (2015). The quality of gDNA was evaluated on 1.0% agarose gel. A fragment of the 16S rRNA gene was amplified by PCR with the primers 27F AGAGTTTGATCMTGGCTCAG and 1492R CGGTTACCT TGTTACGACTT. PCR reactions were executed in a volume of 30 µl containing 15 µl 2 × PCR premix, 2 µl template DNA, 0.5 µl of each primer and 12 µl sterile distilled water. Cycling parameters used were as follows: initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s and extension at 72 °C for 90 s, and a final elongation at 72 °C for 7 min (Coombs and Franco, 2003).

Sequencing and Phylogenetic Analysis of 16S rRNA Gene

Eurofins Genomics India Pvt. Ltd (Bangalore, India) conducted the 16S rRNA gene sequencing. Amplicons were

purified using a pre-sequencing kit and sequenced on ABI 3730xl automated sequencer (Perkin Elmer Applied Biosystems, Foster City, CA, USA) and ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA polymerase used as per protocols recommended by the manufacturer.

The dataset of 16S rRNA gene sequences was prepared for a BLAST search. All individual representative sequences were performed at NCBI (Altschul et al. 1990). Sequences were obtained from BLAST searches based on E-value, in which identity scores differed depending on size of the gene family. Nucleotide compositional parameters were predicted from BioEdit 7.0.4 (Hall 2011). Besides BLAST results, 16S rRNA gene sequences of the most closely related taxa were retrieved from the GenBank database and aligned using the ClustalW tool implemented in MEGA X (Kumar et al. 2018). MEGA X was used to reconstruct the phylogenetic tree adopting a neighbor-joining method using bootstrap values based on 1000 replications (Kim et al. 2019). Gene sequences of 16S rRNA were deposited in the NCBI database using BankIt (http://www.ncbi.nlm.nih.gov).

Isolation of Volatiles from Bacterial Culture

Bacterial colonies representing CVM of the three phases of the estrous cycle were inoculated in 250 ml of LB (Luria Bertani) broth and incubated in a rotary shaker at 150 rpm for 48 h. The culture was centrifuged at 5000 × g for 10 min (Tredwell et al. 2011). The supernatant was subjected to further study.

Discrimination of Bacterial Volatiles Using Mice

Bacterial odor was screened using mice as an odor detector (Sankar and Archunan 2005). Twenty four male mice (12 weeks old) were used for odor preference in a Y-maze apparatus made of tin sheet and glass plate (Sankar and Archunan 2005). The study was conducted according to a standard protocol (Achiraman and Archunan, 2006). Bacterial supernatants (100 µl) were presented in 2 ml vials with minute pores. Mice were introduced into the central chamber at a common place. The number of visits over 15 min and the duration of each visit were recorded. For each sample, three mice (replicates) were used as test animals. Use of mice in this experiment was approved by the Institutional Ethics Committee (IAEC) of Bharathidasan University (**BDU/IAEC/2017/NE/13Dt.21.03.2017).** Finally, potential odor-producing bacterial isolates were selected and subjected to analysis by headspace gas chromatography-mass spectrometry (HS-GC/MS).

Identification of Bacterial Volatiles by HS-GC/MS

The supernatant (1 ml) collected from the *S. pasteuri* culture was subjected to HS-GC/MS analysis using an Agilent mass spectrometer (Agilent Technologies, DE, USA) coupled to a GC system (5977 MSD, 7679A Headspace Sampler). The GC was equipped with a DB-VRX column (20 m × 180 µm × 1 µm). Helium was the carrier gas at a flow of 1 ml.min^{-1}. The column oven was programmed as follows: initial temperature 40 °C for 3 min, then to 150 °C at 7 °C.min^{-1}, held for one minute, then to 240 °C at 60 °C. min^{-1}. Samples (1 µl) were injected in split mode (30 s) with a split ratio of 10:1. Compounds were ionized at 70 eV in positive ion mode. Peak identification used mass spectral interpretation and comparisons with the National Institute of Standard and Technology (NIST) library (Boots et al. 2014).

Behavioral Observations of Bulls to CVM Bacterial Volatiles

Non-estrus female buffaloes (n = 6) and bulls (n = 6) were selected for behavioral studies. The supernatant of bacterial cultures of estrus CVM, non-estrus CVM and LB broth were tested. Each sample (5 ml) was individually soaked onto cotton and rubbed on the vulvar region of non-estrus buffalo. Bulls were allowed to sniff test animals, and their behavior observed for 30 min (Karthikeyan and Archunan 2013). Flehmen and mounting behaviors exhibited by the bull in response to the test samples were recorded.

Statistical Analysis

Analysis of variance (ANOVA) was carried out on behavioral data using the SPSS (V.2.3) package. Images were plotted in GraphPad Prism (V.8.0.2).

Results

Isolation of Bacteria from Buffalo CVM

Bacteria isolated from CVM during preestrus, estrus and diestrus were present as 38 CFU/ml, 51 CFU/ml and 36 CFU/ml, respectively, as determined by adopting the serial dilution method. Thus, cumulatively, as many as 125 bacterial colonies were observed covering all three phases. Among them, six isolates were chosen, based on morphological characters such as smoothness and color (white or creamy yellow) for higher level analyses. Biochemical characterization of estrus bacteria indicated that they were Gram positive, non-hemolytic, and fermented glucose, fructose, maltose, and sucrose with acid (Table S1).

Molecular characterization of bacterial isolates showed that expression of the *Staphylococcus* community differed between the three phases as follows: *Staphylococcus warneri* (BCVMPE1_1) during preestrus, *S. pasteuri* (BCVME2) during estrus and *S. epidermidis* (BCVMDE3) during diestrus. With regard to bacilli resident in CVM, *B. subtilis* (BCVMPE1) and *B. toyonensis* (BCVMPE2) were limited to preestrus, whereas *B. proteolyticus* (BCVMDE5) showed up only during diestrus. Sequences have been deposited at NCBI under accession numbers: MT789231.1 (BCVMPE1_1), MT705012.1 (BCVMPE2), MT598012.1 (BCVMPE1), MT598013.1 (BCVME2), MT598014.1 (BCVMDE3), and MT705014.1 (BCVMDE5).

Construction of the Phylogenetic Tree

Pairwise 16S rRNA gene sequence similarities were determined using BLASTn analysis. We assigned a species based on > 98% from the query coverage. Variations in nucleotide composition were identified for CVM bacterial isolates (in percentage as well as numbers). The G + C base pair was high, above 52% (Table S2). NCBI-BLAST identified isolates having > 98% similar sequences at inter- and intra-specific levels. Results showed that the tree could be grouped into two major clusters. The first consisted of various species of *Staphylococcus*, while the second consisted of *Bacillus* species (Fig. 1A). Molecular taxonomic characterization

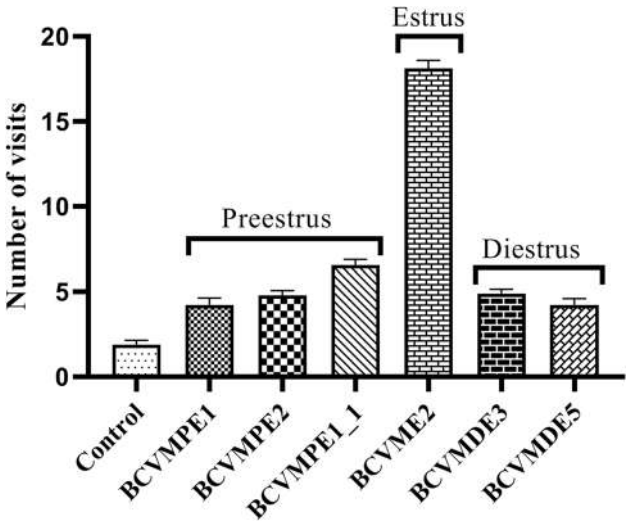


Fig. 2 Ability of mice to differentiate buffalo cervical vaginal mucus (CVM) bacteria by sniffing bacterial volatile samples. Bars show the number of visits by mice towards bacterial volatile(s) from buffalo CVM. Data are Mean ± SEM

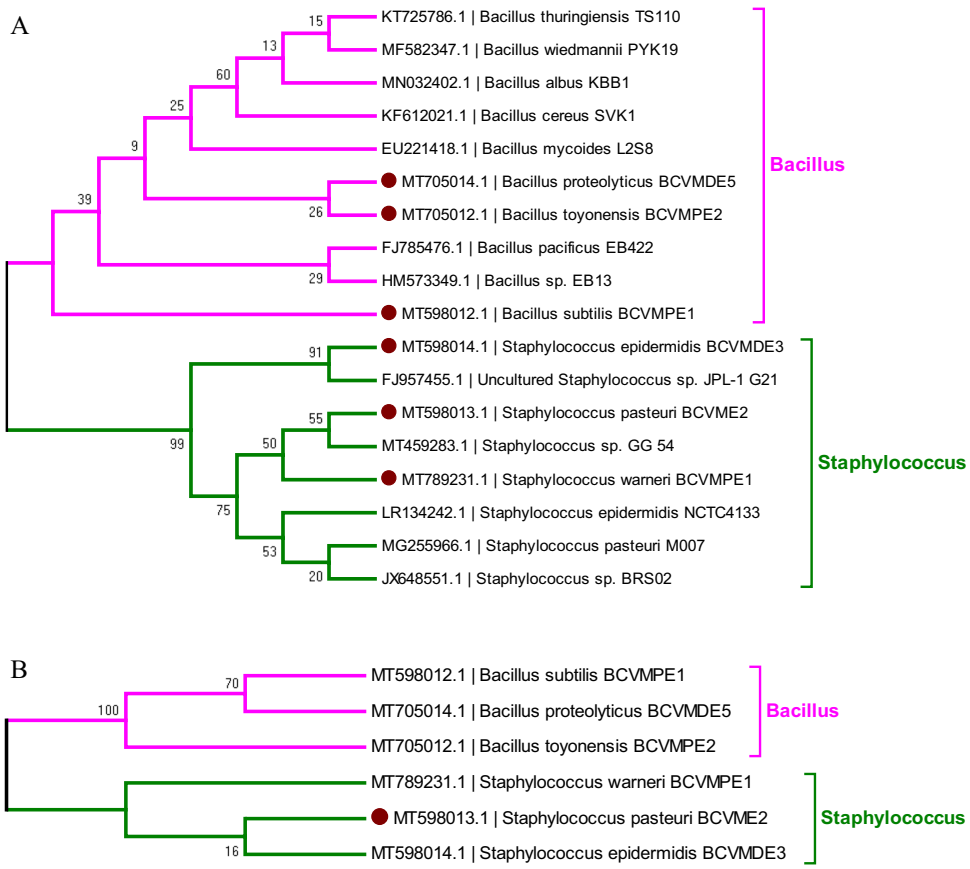


Fig. 1 Phylogenetic tree. A) Inter- and intra-specific evolutionary relationships of strains isolated from buffalo cervical vaginal mucus (CVM). B) Highlights of the specific expression of bacterial isolates in CVM during the estrus phase

showed differential expression in respect to the three phases of estrous cycle (Fig. 1B).

Discrimination of Bacterial Volatiles from CVM Using Mouse Behavioral Assay

There were differences among bacterial volatiles across different phases of the estrous cycle. Mice were attracted more to samples from estrus-specific bacteria than to non-estrus bacteria (Fig. 2). Mice visited and spent more time at estrus-specific bacterial volatiles than to non-estrus bacterial volatiles ($p < 0.001$). Estrus *S. pasteuri* (BCVME2) was the most attractive to mice.

Identification of volatiles produced by bacteria as revealed in HS-GC/MS

HS-GC/MS analysis (Fig. 3) of cell-free supernatant of estrus-specific bacteria isolated from CVM revealed six compounds, acetic acid, propanoic acid, isobutyric acid, butyric acid, isovaleric acid and valeric acid, were produced by *S. pasteuri* (Table 1). Acetic acid was the most abundant, followed by isovaleric acid, valeric acid, isobutyric acid, butyric acid, and propanoic acid.

Bull Behavior Conditioned by *S. pasteuri* Volatiles

Bulls were most attracted to intact estrus CVM followed by supernatant of *S. pasteuri,* LB broth, and non-estrus CVM. The frequency of flehmen responses exhibited by bulls was highest to the intact estrus CVM (5.83 ± 0.72) and *S. pasteuri* volatiles (4.66 ± 0.49). By contrast, bulls showed lesser

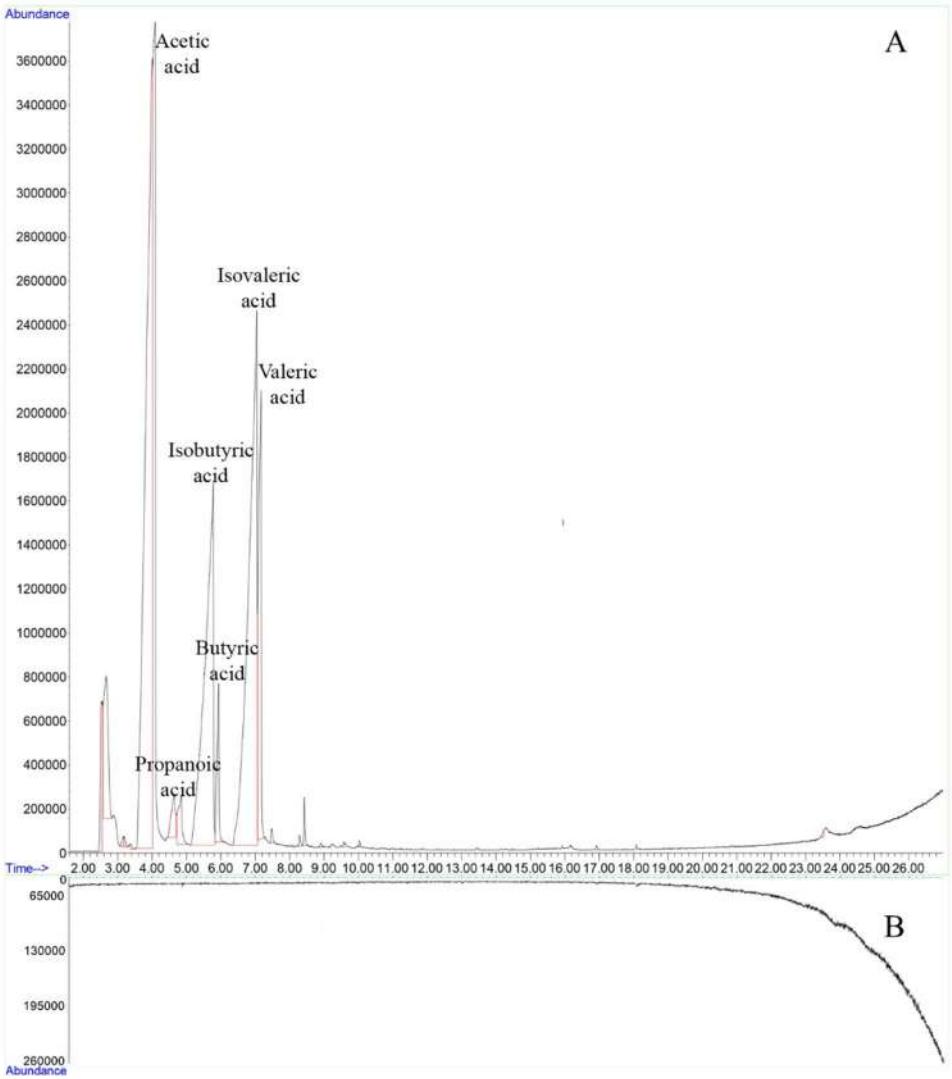
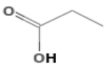
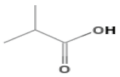
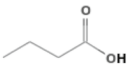
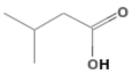
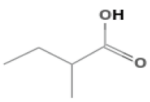


Fig. 3 Mass chromatogram of bacterial volatiles. A) S*taphylococcus pasteuri;* B) LB broth

Table 1 Volatile compounds of *Staphylococcus pasteuri* (BCVME2) isolated from buffalo cervical vaginal mucus.

Volatile	Mol weight	Structure
Acetic acid	60.05	
Propanoic acid	74.07	
Isobutyric acid	88.10	
Butyric acid	88.10	
Isovaleric acid	102.13	
Valeric acid	102.13	

attraction to non-estrus CVM (0.67 ± 0.42), and no response to LB broth. Likewise, mounting behavior was high toward intact estrus CVM (4.83 ± 0.79) and *S. pasteuri* volatiles (4.5 ± 0.67). However, bulls showed less response to non-estrus CVM (0.5 ± 0.50) and no response to LB broth. Flehmen and mounting behaviors observed during exposure to estrus CVM samples and *S. pasteuri* secretory substances were similar (Fig. 4).

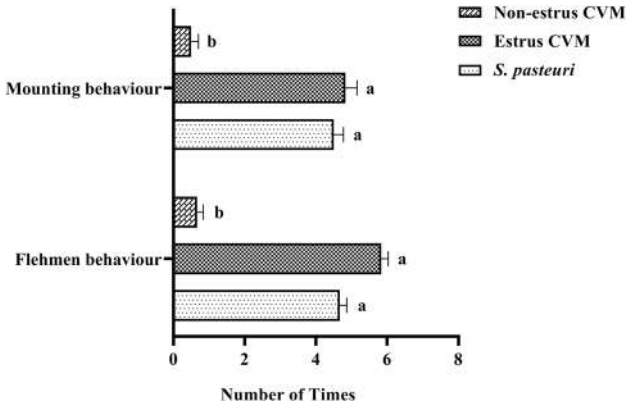


Fig. 4 Behavioral assay. Bull buffalo behavior to non-estrus females treated with volatile extracts from non-estrus and estrus buffalo cervical vaginal mucus (CVM) and S*taphylococcus pasteuri*. Values are Means ± SEM; n = 6. Bars with same letter indicate no difference

Discussion

Our previous study showed differences in the CVM bacterial community with respect to various phases of the estrous cycle (Srinivasan et al. 2019), with the Firmicutes phylum most abundant during the estrus phase. It is likely that expression of certain specific bacterial genes is higher during estrus than during other phases. The present investigation revealed expression of specific gene(s) in *S. pasteuri* and that CVM bacterial strains are influenced differently during estrus in buffalo. It has also been reported that the vaginal bacterial population is affected by cyclical variation during the estrous cycle (Otero et al. 1999, 2000).

In the present study, *S. pasteuri* were observed in buffalo CVM during estrus. Similar studies have been reported in various animals. Association of vaginal *Simonsiella* spp to the estrus phase has been documented in lions and leopards (Callealta et al. 2018). It is noteworthy that *Streptococcus* and *Staphylococcus* are the most dominant bacteria in healthy cows during the reproductive cycle (Amin et al. 1996). *Staphylococcus* (70%) and *Escherichia* (15%) are observed most frequently during natural and induced estrus of ewes (Orihuela et al. 2019), and all isolates showed high sensitivity to ciprofloxacin antimicrobials that evaluated vaginitis regulation (Mohammed et al. 2017). The present finding supports the previous report of the presence of *Staphylococcus pasteuri*, which belongs to phylum Firmicutes, and is abundant during estrus (Srinivasan et al. 2019).

In addition, bacteria such as *Bacillus* spp., *Staphylococcus* spp., and *Streptococcus* spp., have been identified in the vagina of ewes (Manes et al. 2010) as well as cows (Otero et al. 1999, 2000; Zambrano-Nava et al. 2011). These reports match with the present finding of bacterial community identified in the CVM of buffalo. On the other hand, the major bacterial genera in the vagina of Gyr and Nellore breeds of cows include *Aeribacillus*, *Bacillus*, *Clostridium*, *Bacteroides* and *Ruminococcus* (Giannattasio-Ferraz et al. 2019). The present study also depicted the presence of *Bacillus* spp. in buffalo exclusively during the preestrus and diestrus phases. Notably, *Lactobacilli* were of very low abundance in bovine vaginal microbiota, confirmed by culture-dependent

and culture-independent methods (Otero et al. 2000; Quereda et al. 2020; Srinivasan et al. 2019). The present study revealed the absence of *Lactobacillus* sp., in buffalo CVM. Overall, the results from our study are consistent with previous reports.

Mice were used to screen volatile samples. Among seven samples tested using mice, *S. pasteuri* volatiles were more attractive than the other cultures. These results are consistent with an earlier report that mice can discriminate between estrus and non-estrus samples (Rameshkumar et al. 2008; Sankar and Archunan 2005). A recent study provides evidence that mice are highly sensitive to volatiles produced by microbes (Peixoto et al. 2018).

GC/MS analysis indicated acetic acid, propanoic acid, isobutyric acid, butyric acid, isovaleric acid and valeric acid were produced by *S. pasteuri*. It is known that short-chain fatty acids, such as acetic, propanoic and butyric, are relatively abundant in colonic lumen due to bacterial fermentation, which regulates entero-pathogenic colonization of host immunomodulation in humans (Smith et al. 2013; Wrigley 2004). A recent study showed that acetic, valeric, caproic and myristoleic acids are in relatively high levels in milk of estrus cows (Zebari et al. 2019). Acetic and propanoic acids have been identified in feces of cow, and varied in relation to time of ovulation (Mozūraitis et al. 2017).

Since estrus-specific *S. pasteuri* produces volatiles, we focused on testing the role of these substances in bull behavior, in order to support its estrus-specificity and use as a reliable estrus indicator. Interestingly, bulls exhibited the reproductive behaviors of flehmen and mounting in response to *S. pasteuri* secretory substances. The present study also corroborated previous findings that an estrus-specific pheromone in feces has an effect on flehmen and mounting behaviors in buffalo (Karthikeyan et al. 2013). It has been reported that bulls exhibit these reproductive behaviors in response to the presence of a mixture of volatiles in the CVM of estrus cows (Karthikeyan and Archunan 2013; Rajanarayanan and Archunan 2004; Sankar and Archunan 2004). In cows, acetic acid, propanoic acid, and 1-iodoundane have been reported to be vaginal pheromones (Sankar and Archunan 2004), while oleic acid is thought to be an estrus indicator, which may stimulate flehmen and mounting behavior in bulls (Karthikeyan and Archunan 2013). The present findings suggest that volatile compounds of bacterial origin may act as attractants for bulls and induce the olfactory system to elicit flehmen and mounting behaviors to maintain health and management during reproduction. Since *S. pasteuri* secretes acetic, propanoic, isobutyric, butyric, isovaleric, and valeric acids, these compounds may be the major bacterial volatiles that induce a buffalo bull's sexual behavior. For instance, acetic acid, 2-butanone and oleic acid improve Zebu bull sexual behavior and total sperm production (Mondal et al., 2019). Our study reports the presence, in the buffalo vaginal fluid, of volatile compounds secreted by CVM bacteria that facilitate the expression of male reproductive behaviors. This suggests that these volatiles are putative pheromone compounds produced during estrus in buffalo.

Further studies to find the mechanisms associated with the specific effects of *S. pasteuri* volatiles are in progress. Characterization of the protein profile of *S. pasteuri* from buffalo CVM during estrus, to understand host-bacterial interactions and their role in chemical communication could help reproduction management in buffaloes.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10886-021-01311-9.

Acknowledgements GA acknowledges the award of BSR Faculty Fellowship by the UGC, New Delhi, [F. No: 18-1/2011 (BSR) dt: 04.01.2017]. The laboratory facility made available by DST-FIST II & DST-PURSE schemes of Government of India is also gratefully acknowledged.

Authors' contributions MS: Conceptualization, investigation, data interpretation, writing the original manuscript, review & editing; RLR: Data interpretation; DD: Contributed to conceptualization, and also isolation of bacteria and culture methods; MAA: Data analysis, review & editing; GA: Conceptualization, supervision, review & editing.

Funding Not applicable.

Availability of data and materials Not applicable.

Code availability Not applicable.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication All authors have consented for the article communication and publication.

Conflicts of interest/Competing interests The authors declare no conflicts of interest.

References

Achiraman S, Archunan G (2006) 1-Iodo-2methylundecane, a putative estrus-specific urinary chemo-signal of female mouse (Mus musculus). Theriogenology 66:1913–1920. https://doi.org/10.1016/j.theriogenology.2006.05.010

Adnane M, Meade KG, O'Farrelly C (2018) Cervico-vaginal mucus (CVM) - an accessible source of immunologically informative biomolecules. Vet Res Commun 42:255–263. https://doi.org/10.1007/s11259-018-9734-0

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Amin J, Zaria L, Malgwi R (1996) Vaginal aerobic bacterial flora of apparently healthy cattle in various stages of the reproductive cycle in the Sahel region of Nigeria Bull. Anim Heal Prod Africa 44:15–18

Ault TB et al (2019) Uterine and vaginal bacterial community diversity prior to artificial insemination between pregnant and nonpregnant postpartum cows. J Anim Sci 97:4298–4304. https://doi.org/10.1093/jas/skz210

Bicalho MLS, Lima S, Higgins CH, Machado VS, Lima FS, Bicalho RC (2017a) Genetic and functional analysis of the bovine uterine microbiota. Part II: Purulent vaginal discharge versus healthy cows. J Dairy Sci 100:3863–3874. https://doi.org/10.3168/jds.2016-12061

Bicalho MLS, Machado VS, Higgins CH, Lima FS, Bicalho RC (2017b) Genetic and functional analysis of the bovine uterine microbiota. Part I: Metritis versus healthy cows. J Dairy Sci 100:3850–3862. https://doi.org/10.3168/jds.2016-12058

Bicalho MLS, Santin T, Rodrigues MX, Marques CE, Lima SF, Bicalho RC (2017c) Dynamics of the microbiota found in the vaginas of dairy cows during the transition period: Associations with uterine diseases and reproductive outcome. J Dairy Sci 100:3043–3058. https://doi.org/10.3168/jds.2016-11623

Boots AW et al (2014) Identification of microorganisms based on headspace analysis of volatile organic compounds by gas chromatography-mass spectrometry. J Breath Res 8:027106. https://doi.org/10.1088/1752-7155/8/2/027106

Callealta I, Ganswindt A, Goncalves S, Mathew A, Lueders I (2018) Detection of Simonsiella spp. in the Vagina of Lions and Leopard in Oestrus. Reprod Domest Anim 53:1605–1608. https://doi.org/10.1111/rda.13298

Cassoli LD, Lima WJ, Esguerra JC, Da Silva J, Machado PF, Mourao GB (2016) Do different standard plate counting (IDF/ISSO or AOAC) methods interfere in the conversion of individual bacteria counts to colony forming units in raw milk? J Appl Microbiol 121:1052–1058. https://doi.org/10.1111/jam.13227

Coombs JT, Franco CM (2003) Isolation and identification of actinobacteria from surface-sterilized wheat roots. Appl Environ Microbiol 69:5603–5608. https://doi.org/10.1128/AEM.69.9.5603-5608.2003

Fischer-Tenhagen C, Tenhagen BA, Heuwieser W (2013) Ability of dogs to detect cows in estrus from sniffing saliva samples. J Dairy Sci 96:1081–1084. https://doi.org/10.3168/jds.2012-5683

Galvao KN, Bicalho RC, Jeon SJ (2019) Symposium review: The uterine microbiome associated with the development of uterine disease in dairy cows. J Dairy Sci 102:11786–11797. https://doi.org/10.3168/jds.2019-17106

Giannattasio-Ferraz S et al (2019) A common vaginal microbiota composition among breeds of Bos taurus indicus (Gyr and Nellore). Braz J Microbiol 50:1115–1124. https://doi.org/10.1007/s42770-019-00120-3

Hall T (2011) BioEdit: an important software for molecular biology. GERF Bull Biosci 2:60–61

Joshi CG, Bagatharia SB, Joshi M, Patil DB, Suthar VS, Mahesh P (2020) Vaginal microbiota during estrous cycle and its plausible association with certain hematological parameters in bubalus bubalis. Indian J Vet Sci Biotech 15:54–58. https://doi.org/10.21887/ijvsbt.15.4.11

Karthikeyan K, Archunan G (2013) Gas chromatographic mass spectrometric analysis of estrus specific volatile compounds in buffalo vaginal mucus after initial sexual foreplay. J Buffalo Sci 2:1–7. https://doi.org/10.6000/1927-520x.2013.02.01.1

Karthikeyan K, Muniasamy S, SankarGanesh D, Achiraman S, Ramesh Saravanakumar V, Archunan G (2013) Faecal chemical cues in water buffalo that facilitate estrus detection. Anim Reprod Sci 138:163–167. https://doi.org/10.1016/j.anireprosci.2013.02.017

Kim J, Disanto F, Kopelman NM, Rosenberg NA (2019) Mathematical and simulation-based analysis of the behavior of admixed taxa in the neighbor-joining algorithm. Bull Math Biol 81:452–493. https://doi.org/10.1007/s11538-018-0444-0

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35:1547–1549. https://doi.org/10.1093/molbev/msy096

Laguardia-Nascimento M et al (2015) Vaginal microbiome characterization of nellore cattle using metagenomic analysis. PLoS ONE 10:e0143294. https://doi.org/10.1371/journal.pone.0143294

Manes J, Fiorentino MA, Kaiser G, Hozbor F, Alberio R, Sanchez E, Paolicchi F (2010) Changes in the aerobic vaginal flora after treatment with different intravaginal devices in ewes. Small Rumin Res 94:201–204. https://doi.org/10.1016/j.smallrumres.2010.07.021

Mohammed KM, Nabih AM, Darwish GM (2017) Efficacy of antimicrobial agents on vaginal microorganisms and reproductive performance of synchronized estrus ewes. Asian Pacific J Reprod 6:121–127. https://doi.org/10.12980/apjr.6.20170305

Mondal S, Bhakat M, Singh A, Mohanty TK, Abdullah M, Yadav SK, Kumar R (2019) Effect of cattle-specific estrus molecules on libido and semen production of zebu bulls under tropical climate. Trop Anim Health Prod 51:1823–1827. https://doi.org/10.1007/s11250-019-01880-8

Mozūraitis R, Kutra J, Borg-Karlson A-K, Būda VJJods, (2017) Dynamics of putative sex pheromone components during heat periods in estrus-induced cows. J Dairy Sci 100:7686–7695. https://doi.org/10.3168/jds.2016-12376

Muniasamy S (2016) Detection of estrus from urinary chemo-signals in murrah buffalo (Bubalus bubalis). Bharathidasan University

Orihuela A, Fierros-Garcia A, Hallal-Calleros C, Robles-Castro SR, Ungerfeld R (2019) Vaginal biota number is smaller in tailed than docked hair ewes (Ovis aries), but is not affected by copulation. Trop Anim Health Prod 51:993–995. https://doi.org/10.1007/s11250-018-1753-x

Otero C, de Ruiz CS, Ibanez R, Wilde OR, Holgado AAPD, Nader-Macias ME (1999) Lactobacilli and enterococci isolated from the bovine vagina during the estrous cycle. Anaerobe 5:305–307. https://doi.org/10.1006/anae.1999.0245

Otero C, Saavedra L, Silva de Ruiz C, Wilde O, Holgado AR, Nader-Macias ME (2000) Vaginal bacterial microflora modifications during the growth of healthy cows. Lett Appl Microbiol 31:251–254. https://doi.org/10.1046/j.1365-2672.2000.00809.x

Otero MC, Morelli L, Nader-Macias ME (2006) Probiotic properties of vaginal lactic acid bacteria to prevent metritis in cattle. Lett Appl Microbiol 43:91–97. https://doi.org/10.1111/j.1472-765X.2006.01914.x

Peixoto L, Salazar LTH, Laska M (2018) Olfactory sensitivity for mold-associated odorants in CD-1 mice and spider monkeys. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 204:821–833. https://doi.org/10.1007/s00359-018-1285-x

Quereda JJ et al (2020) Vaginal microbiota changes during estrous cycle in dairy heifers. Front Vet Sci 7.https://doi.org/10.3389/fvets.2020.00371

Rajanarayanan S, Archunan G (2004) Occurrence of flehmen in male buffaloes (Bubalus bubalis) with special reference to estrus. Theriogenology 61:861–866. https://doi.org/10.1016/j.theriogenology.2003.07.004

Rameshkumar K, Achiraman S, Karthikeyan K, Archunan G (2008) Ability of mice to detect estrous odor in bovine urine: Roles of hormones and behavior in odor discrimination. Zool Sci 25:349–354. https://doi.org/10.2108/zsj.25.349

Sankar R, Archunan G (2004) Flehmen response in bull: role of vaginal mucus and other body fluids of bovine with special reference

to estrus. Behav Processes 67:81–86. https://doi.org/10.1016/j.beproc.2004.02.007

Sankar R, Archunan G (2005) Discrimination of bovine estrus-related odors by mice. J Ethol 23:147–151. https://doi.org/10.1007/s10164-004-0140-4

Sankar R, Archunan G (2011) Gas chromatographic/mass spectrometric analysis of volatile metabolites in bovine vaginal fluid and assessment of their bioactivity. Int J Anal Chem 2011:256106. https://doi.org/10.1155/2011/256106

Smith PM et al (2013) The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. Science 341:569–573. https://doi.org/10.1126/science.1241165

Srinivasan M, Dhanasekaran D, Archunan G (2019) Vaginal microbiome analysis of buffalo (Bubalus bubalis) during estrous cycle using high-throughput amplicon sequence of 16S rRNA gene. Symbiosis 78:97–106. https://doi.org/10.1007/s13199-018-00595-y

Srinivasan M, Adnane M, Archunan G (2021) Significance of cervicovaginal microbes in bovine reproduction and pheromone production – A hypothetical review. Res Vet Sci 135:66–71. https://doi.org/10.1016/j.rvsc.2021.01.003

Tredwell GD, Edwards-Jones B, Leak DJ, Bundy JG (2011) The development of metabolomic sampling procedures for Pichia pastoris, and baseline metabolome data. PLoS ONE 6:e16286. https://doi.org/10.1371/journal.pone.0016286

Ungerfeld R, Silva L (2005) The presence of normal vaginal flora is necessary for normal sexual attractiveness of estrous ewes. Appl Anim Behav Sci 93:245–250. https://doi.org/10.1016/j.applanim.2004.11.014

Vingataramin L, Frost EH (2015) A single protocol for extraction of gDNA from bacteria and yeast. Biotechniques 58:120–125. https://doi.org/10.2144/000114263

Wang Y, Ametaj BN, Ambrose DJ, Ganzle MG (2013) Characterisation of the bacterial microbiota of the vagina of dairy cows and isolation of pediocin-producing Pediococcus acidilactici. BMC Microbiol 13:19. https://doi.org/10.1186/1471-2180-13-19

Wrigley DM (2004) Inhibition of Clostridium perfringens sporulation by Bacteroides fragilis and short-chain fatty acids. Anaerobe 10:295–300. https://doi.org/10.1016/j.anaerobe.2004.05.006

Zambrano-Nava S, Boscan-Ocando J, Nava J (2011) Normal bacterial flora from vaginas of Criollo Limonero cows. Trop Anim Health Prod 43:291–294. https://doi.org/10.1007/s11250-010-9701-4

Zebari HM, Rutter SM, Bleach ECL (2019) Fatty acid profile of milk for determining reproductive status in lactating Holstein Friesian cows. Anim Reprod Sci 202:26–34. https://doi.org/10.1016/j.anireprosci.2019.01.004

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Bulletin of Pure and Applied Sciences
Vol.40A (Zoology), No.1,
January-June 2020: P.116-126

Print version ISSN 0970 0765
Online version ISSN 2320 3188
DOI 10.5958/2320-3188.2021.00015.2

Content Available online at: https://www.bpasjournals.com/zoology

Original Research Article

Antioxidant and Antihyperglycemic Potential of *Luffa acutangula* Fruit Extract in Streptozotocin-induced Diabetic Rats

[1]Rajarajan Thatchinamoorthi*, [2]Kumar Ganesan, [3]Moses Rajasekara Pandian

Author's Affiliation:
[1]Post Graduate and Research Department of Biotechnology & Microbiology, National College (Autonomous), Tiruchirappalli, Tamilnadu620001, India
[2] School of Chinese Medicine, LKS Faculty of Medicine, The University of Hong Kong, Sassoon Road, Hong Kong, China.
[3]Department of Zoology, Arignar Anna Government Arts College, Namakkal. Tamilnadu637001, India.

***Corresponding author:**
Dr. Rajarajan Thatchinamoorthi,
Assistant Professor, PG Research Department of Biotechnology &Microbiology, National College (Autonomous), Tiruchirappalli, Tamilnadu620 001, India.

E-mail: biorajarajan@gmail.com

Article Info:
Received on 11.01.2021
Accepted on 29.04.2021
Published on 15.06.2021

ABSTRACT:
Our earlier study demonstrates that the treatment of acrude extract of *Luffaacutangula* (L.) Roxb. (Family: Cucurbitaceae), a folkloric hypoglycaemic plant widely used in the southern part of India which reduces glucose level in blood under high glucose load, and in STZ-provoked diabetes in rats. This study was carried out to establish the antioxidant effects of crude extracts of *L. acutangula* (50 and 100 mg/kg) in STZ-provoked diabetic animals. Regular treatment of the fruit extracts of *L. acutangula* (50 and 100 mg/kg) for 28 days ensued noteworthy decrease in the levels of plasma TBARS, hydroperoxide, and ceruloplasmin and a substantial increase in plasma GSH, vitamins C and α-tocopherol.Based on the findings, *L. acutangula*is potential antihyperglycemic agents and restored all the antioxidant factors close to normal range. Thus, our outcomes open new prospects for advanced studies, including, a clinically based study to appraise the antidiabetic potential of the herbs.

Keywords: *Luffaacutangula,* Streptozotocin, Antioxidant Enzymes, Antidiabetic Effect.

INTRODUCTION

Diabetes is a chronic metabolic syndrome, described by a complex and heterogeneous etiology, with greater risk factors at the levels of social, behavioral, environmental, and genetic predisposition (Ganesan Xu, 2019; Thatchinamoorthi et al., 2021). It is linked to serious impacts, which severely impair peoples' quality of life and is attributed to several life-threatening problems. However, the early diagnosis and initiation of the treatment may avert or impede the onset of long-term impediments.

How to cite this article: Thatchinamoorthi R, Ganesan K, Pandian MR (2021). Antioxidant and Antihyperglycemic Potential of *Luffa acutangula*Fruit Extract in Streptozotocin-induced Diabetic Rats. *Bulletin of Pure and Applied Sciences-Zoology,* 40A(1), 116-126.

It is projected to be an extremely widespread non-communicable disease by 2025 (Ganesan et al., 2018a).In the Indian traditional medicine, many herbs are employed as antidiabetics. In practice, it is well established that the traditional system of medicine is an alternative strategy to contemporary medicine (Jayachandran et al., 2018). According to the World Health Organization, the practice of herbal medicine is required to be helpful, particularly in nations where the modern therapy of diabetes is not satisfactory (Zhang et al., 2018, 2020).

Nowadays several studies have been attention on oxidative stress, which triggers various diseases including, diabetes mellitus from the inequality between free radical generation and systems of radical hunting components (Ganesan et al., 2020a; Kumar et al., 2006; Kumar et al., 2009). In diabetes, glucose toxicity, auto-oxidation, and protein glycationmay produce enormous amounts of free radicals, resulting in lipid peroxidation (Ganesan et al., 2018b; Ganesan et al., 2017; Ganesan and Xu, 2017a).

Recent investigations have demonstrated the increased lipid peroxidation in various diabetic animal models (Ganesan et al., 2013; Ganesan et al., 2020b; Sharmila Banu et al., 2007), impaired the metabolism of glutathione (Sukalingam et al., 2018), and reduced ascorbic acid level (Ganesan et al., 2006; Ganesan et al., 2007d). The quantities of lipid peroxidation in the cell are generally defense by several cellular functions viz., enzymatic, and non-enzymatic scavenging mechanisms (Ganesan and Xu, 2017c, d). The effectiveness of these defense mechanisms is distorted during diabetes and other disease conditions(Ganesan and Xu, 2017b, e)indicating that inadequate free radical scavenging may perform important functions resulting in various tissue damages (Ganesan et al., 2008b; Ganesan et al., 2007c).Type 2 diabetes is nowadays assertive with a high rate of 3-4 new incidents every 10 seconds and is being diagnosed at a younger age of the adolescents (Ganesan et al., 2007e). The intake of exogenous insulin is greatly recognized as the safest choice of prescription for therapy in a diabetic individual. However, the linked mission and their effect particularly on insulin resistance people in DM are still inadequate.

Luffa acutangula (L.) Roxb. (family: Cucurbitaceae) has been extensively used by various populations in India, South Asia and various parts of the world for therapy of several ailments, including, diabetes, bacterial and fungal infections, dysentery, headache, renal stone, leprosy, and ringworm infection. Various experimental studies have also shown that the crude extracts of *L. acutangula* exhibited potent antioxidant (Sulaiman et al., 2013), hepato protective (Jadhav et al., 2010; Ulaganathan et al., 2010), gastro protective (Pimple et al., 2012), cardio and nephro protective (Jadhav et al., 2013), antihyperglycemic and antihyperlipidemic (Sharmin et al., 2012), antidiabetic (Raj et al., 2012) anticancer (Shendge and Belemkar, 2018; Vanajothi et al., 2012), analgesic (Quanico et al., 2008), anti-inflammatory (Gill et al., 2011), antibacterial (Nallappan, 2016), anthelmintic (Rahman, 2014), antimicrobial (Hunashal and D., 2012), and CNS depressant (Misar et al., 2004).

Phytochemical analyses have also been screened and detected around 50 compounds, including alkaloids, flavonoids, triterpene, anthraquinones, proteins, saponins, volatile compounds, and other active phytoconstituents (Jun-kai, 2002; Nagarajaiah and Prakash, 2015; Shendge and Belemkar, 2018). Our preliminary study outcome was greatly promising as it showed the glucose levels in blood, which was significantly reduced after treatment of crude fruit extracts of *L. acutangula*in normal, and high glucose load situations and STZ-provoked diabetes in rats. To our understanding, inadequate studies have been undertaken to emphasize on the antioxidant potential of *L. acutangula* fruit extract and thus this study describes the antidiabetic effect of *Luffaacutangula* and examines the impact of the test agent on blood antioxidants.

MATERIALS AND METHODS

Plant materials

The fruit of *L.acutangula* was obtained from the neighboring market in Namakkal, Tamilnadu, India, and authenticated by a taxonomist, and the Voucher Herbarium specimens were kept in the Rapinat Herbarium, Tiruchirappalli, Tamilnadu for future references. The dried raw fruits of *L.acutangula* (1Kg) were skinned, cleaned, cut into tiny pieces, and stirred in warring mixer using distilled water (2 liters). The extraction of the fruits was undertaken at a room temperature (26 ± 1^{0}C) with continual stirring during the night. The obtained homogenate was percolated using cheesecloth and spined at 4000 rpm for 15 min. The obtained supernatant was the tested drug(yield 21% w/w) was stored at 4^{o}C until use.

Animals

The investigation was undertaken on both sexes of Wistar albino rats (180-220g). The rats were kept in the Animal unit, Muthayammal College of Arts and Science, Namakkal. Animals were initially adapted for a week in lab environments and supplied a standard chow diet with water ad libitum. The animal studies were undertaken according to the animal guidance of the NIH (Council, 2011). The animals were distributed into 5 groups containing 6 animals each. Group I received as vehicle alone (gum acacia (2%), control. Group II served streptozotocin (STZ). Group III and IV served the crude extracts of fruit (50mg, 100mg/kg b.w, p.o) mixed in a vehicle and administered a single dose of STZ. Group V received glibenclamide (0.6mg/kg/ b.w, p.o) and administered a single dose of STZ. Initial food, water intake and body weight were measured. After end of the treatment, blood was drawn at 30m, 60m, and 90m time interval for the quantification of glucose in the plasma, and the testing was continued to examine the effect of fruit extract on antioxidant status in the plasma of STZ induced diabetic rats.

Induction of diabetes

The rats were established diabetes by the intraperitoneal administration of a STZ (60mg/kg, which was kept in citrate buffer, 0.1M, pH 4.5). After Forty-eight hours, blood was drawn, and levels of plasma glucose were examined to validate the development of diabetes. The animals with glucose levels at above 260mg/dl were used for the experiments.

Biochemical analysis

After 28 days of fruit extract treatment, the animals were sacrificed, and blood was drawn into heparinized tubes. Then, the plasma was obtained, followed by the determination of biochemical parameters: plasma glucose by glucose Oxidase method (Trinder, 1969),OGTT (du Vigneaud and Karr, 1925), plasma TBARS (Niehaus and Samuelsson, 1968), hydroperoxide (Jiang et al., 1992), ceruloplasmin (Ravin, 1961), reduced glutathione (GSH)(Beutler et al., 1963), α-tocopherol (vitamin E)(Baker et al., 1951) and ascorbic acid (vitamin C)(Roe and Kuether, 1943). The percentage of glycemic changes was determined by using the formula-

$$\text{Percentage of glycemic changes} = \frac{Gx - Go}{Go}$$

Where

G_o - initial glycemic values;

G_x- Glycemic values at x time interval (hours).

Statistical analysis

The values were shown as Mean ± SD. The data were analyzed using one way-ANOVA and group means were compared with Duncan's multiple comparison test (DMRT) with 95% confidence. For the statistical analysis, SPSS software package was used.

RESULTS

The effect of fruit extracts of *L.acutangula* (50, 100 mg/kg) in STZ diabetic rats is shown in Table 1. The plasma glucose levels in STZ induced diabetic rats was 240–260 mg/dl during fasting conditions. The initial significant decrease of blood sugar was noticed 2h after treatment of *L.acutangula* fruit extract. Similarly, a decrease in body weight was detected in STZ diabetic rats, but when the animals were administered orally with *L.acutangula* fruit extract (50,100 mg/ kg), the decrease in body weight was significantly improved(Table 2).

Table 1: Effect of fruit extracts of *L. acutangula* on blood glucose in normal and experimental groups

Groups	Treatment (g/kg b.w.)	Blood glucose (mg/dl)			
		Fasting	1h	2h	3h
I	Control	64.2 ± 5.27	66.1 ± 3.9^{a} (2.96)	65.3 ± 2.4^{a} (1.71)	64.3 ± 5.2^{a} (0.16)
II	Diabetic control	253.5 ± 9.2^{a}	249.5± 5.8^{bc} (–1.58)	246.8 ± 9.9^{b} (–2.64)	244.2 ± 6.8^{b} (–3.67)
III	Diabetic + *L. acutangula* 50	247.1 ± 8.3^{a}	242.6 ± 6.4^{b} (–1.82)	231.5 ± 9.5^{c} (–6.31)	221.5 ± 8.3^{c} (–10.36)
IV	Diabetic + *L. acutangula* 100	250.3 ± 8.8^{a}	247.9 ± 8.9^{bc} (–0.96)	224.6 ± 11.2^{cd} (–10.27)	213.8 ± 7.6^{d} (–14.58)
V	Diabetic + glibenclamide 0.6	252.4 ± 9.2^{a}	245.8 ± 9.4^{c} (–2.61)	219.6 ± 10.3^{d} (–12.96)	209.4 ± 8.4^{d} (–17.03)

Values are expressed as Means ± SD. Values in parenthesis show the percentof glycemic alteration. Values not sharing a common superscript vary significantly at $p<0.05$, Duncan's Multiple Range Test (DMRT)

Table 2: Effect of fruit extracts of *L. acutangula* on body weight changes, food, and water intake in experimental groups.

Groups	Treatment (g/kg b.w.)	Bodyweight		Bodyweight changes (g)	Food intake (g/week)	Water intake (L/week)
		Initial	Final			
I	Control	182.5 ± 5.5	221.5 ± 5.6^{a}	+ 39.0 ± 5.8	85.5 ± 5.34^{a}	4.89 ± 0.67^{a}
II	Diabetic control	185.8 ± 5.7	170.8 ± 6.8^{b}	-15.0 ± 4.8	70.4 ± 4.34^{b}	7.57 ± 0.96^{b}
III	Diabetic + *L. acutangula* 50	180.8 ± 4.8	183.3 ± 4.6^{c}	+2.5 ± 3.4	78.7 ± 4.89^{ab}	6.54 ± 0.37^{bc}
IV	Diabetic + *L. acutangula* 100	188.2 ± 3.9	185.3 ± 5.6^{c}	+2.9 ± 2.6	86.4 ± 5.34^{a}	6.51 ± 0.98^{bc}
V	Diabetic + glibenclamide 0.6	181.7 ± 3.8	187.5 ± 6.2^{c}	+5.8 ± 4.1	87.5 ± 4.58^{a}	5.94 ± 0.67^{c}

Values are expressed as Means ± SD. The values not sharing a common superscript vary significantly at $p<0.05$, Duncan's Multiple Range Test (DMRT)

Table 3confirms that changes in the levels of plasma glucose, thiobarbituric acid reactive substances, ceruloplasmin, and hydroperoxide in normal and experimental animals. All these parameters were significantly increased in experimental diabetic groups when compared to control groups. Treatment of *L.acutangula* fruit extracts (50,100 mg/ kg), or glibenclamide (standard drug, 0.6 mg/kg) induced a significant decrease in the content of plasma ceruloplasmin and hydroperoxide when compared to STZ diabetic animal groups. Table 4indicates the levels of the plasma GSH, vitamin E and vitamin C, which were considerably decreased in STZ induced diabetic rats when compared to normal animal groups, Administration of *L.acutangula* fruit extracts (50,100 mg/ kg, p.o.), or glibenclamide (0.6 mg/kg, p.o.) drastically elevated the levels of GSH, vitamin E and vitamin Cwhen compared to STZ induced diabetic animals.

Journal of Stress Physiology & Biochemistry, Vol. 18, No. 3, 2022, pp. 84-92 ISSN 1997-0838
Original Text Copyright © 2022 by Nagalakshmi and Anand

ORIGINAL ARTICLE

A Comparative Assessment on Physicochemical Properties of *Senna auriculata* Leaves and Flowers of the Natural and Polluted Sources

R. Nagalakshmi* and S. P. Anand

[1] *PG and Research Department of Botany, National College, Affiliated to Bharathidasan University, Tiruchirappalli-620001, Tamil Nadu, India*

*E-Mail: ***nagalakshmi.93botany@gmail.com***

Received March 16, 2022

Background: The standardization of herbal medicines is an essential step in evaluating the quality of medicines based on drug concentration, physical and chemical standards. Due to the fact that the plant of *Senna auriculata* L. has a great medicinal potential with information about the compounds present in the various parts of the plant, it was considered important to study the preliminary physicochemical properties.

Results: Samples A & B were collected from natural habitats and Samples C & D were collected from polluted areas. Physicochemical properties of powdered plant material of *S. auriculata* include extractive value, ash value, loss on drying, and fluorescence behaviour were determined as per standard methods. The water-soluble extractive value in the leaves and flowers of samples A and B was greater than samples C and D. In terms of alcohol soluble extractive value, both samples A and B had a high percentage yield in their leaves and flowers, whereas samples C and D had a somewhat lower percentage yield. The leaves and flowers of samples A and B had lower moisture content percentages, whereas samples C and D had somewhat higher moisture content percentages.

Conclusions: The results of the physicochemical analysis suggest that samples A and B were far preferable to samples C and D in terms of quality. According to the findings of this study, collecting medicinal plants from their natural habitat is preferred since contaminated environments might impact the physicochemical properties of the plant material.

Key words: Senna auriculata, Physicochemical properties, Fluorescence behaviour, Natural sources, Polluted sources

Traditional medicine has been used for thousands of years with great contribution to human health by those skilled in the art, mainly as primary health care for a group of people and it has also retained its worldwide recognition (WHO, 2002). The traditional medical systems in India consist mainly of Ayurveda, Sidda and Unani. The systems are heavily dependent on medicinal plants. Due to its many biomedical uses, such as antibacterial, antifungal, cardioprotective, hepatoprotective, antidiabetic (Sujatha *et al*., 2018; Nagalingam *et al*., 2019; Pavunraj *et al*., 2019). The beneficial effects of medicinal plants and their maximum use can be achieved by other aspects, such as botany, ethnobotany, pharmacognosy, pharmacology, phytochemistry, biochemistry, biodiversity etc. Herbal drugs have been used around the world since the dawn of mankind and are of tremendous importance in international trade. The clinical, pharmaceutical and economic value of herbal medicines continues to grow, although it varies widely from country to country. From the traditional scientific study of remedies, many natural products have reached us and most of them are obtained from plants. Even if some of these crude pharmaceuticals have been gathered in less quantities for local use by local people and healers, several other raw medicines are harvested in larger numbers and marketed as raw materials for several herbal industries (Uniyal *et al*., 2006).

The basis of herbal products, which may contain powdered plant substances or extracts, remedies, and plant fatty oils. Extraction, fractionation, purification, concentration, and other physical or biological processes produce them. These are prepared by physical or biological processes. Standardization of herbal medicines is an important stage in determining the quality of medicines based on drug concentration, physical and chemical specifications. Each drug has its unique physicochemical characteristics that, like fingerprints, assist in understanding the drug and recognizing it from other drugs in the same category. With the commercialization of herbal medicine, it is vital to ensure the safety, uniqueness, and efficacy of medicinal plants and herbal products. *S. auriculata* bark, flowers, leaves, roots, and unripe fruit combinations are used to make "Avarai Panchaga Choornam," which is used to treat blood glucose levels, urinary infections, conjunctivitis, and ophthalmia (Deshpande *et al*., 2013; Khader *et al*., 2017). Another beverage preparation known as "kalpa herbal tea" has *S. auriculata* (dried flowers) as its main component and is often used by those suffering from diabetes, urinary tract illnesses, and constipation (Thabrew *et al*., 2004). Many herbal products are made from leaves and flowers of *S. auriculata,* such as senna tablets, pickle, tea, idly powder, tonic, avaram poo sooranam, soup powder, diabetic food supplement. Due to the fact that the plant of *Senna auriculata* (L.) Roxb. has a great medicinal potential with information about the compounds present in the various parts of the plant, it was considered important to study the physicochemical properties of the plant.

MATERIAL AND METHODS

Plant material

In the present investigation we have selected one of the important medicinal plants, *Senna auriculata* (L.) Roxb. Tanner's cassia is a common name for such an evergreen plant. In the Ayurvedic and Siddha systems of medicine, the flower, buds, leaves, stem, root, and unripe fruit are recommended for treatment. It is commonly used to treat rheumatoid arthritis, conjunctivitis, and diabetes. The taxonomic classification is given in Table 1.

Collection and identification

S. auriculata leaves and flowers were taken from natural and contaminated sources in June 2021. For natural sources, two samples (A and B) were collected from unpolluted regions of site 1 (Pachaimalai hills) and site 2 (Kolli hills). Two samples (Samples C and D) were collected from the roadside in industrial regions. Site 3 is located on the Avur road, while Site 4 is located on the Samayapuram kariyamanickam road. The Botanical Survey of India (BSI), Southern Regional Centre, Coimbatore, validated the authenticity of the plant specimen (BSI/SRC/5/23/2021/Tech-166).

Physicochemical analysis

Physicochemical evaluation will help assess the

quality of the plant powder. Physicochemical properties of powdered plant material of *S. auriculata* include extractive value, (alcohol soluble extractive value, water-soluble extractive value), ash value (total ash value, water- soluble ash, acid insoluble ash), loss of drying, Fluorescence behavior were determined as per standard methods (WHO, 2000; Kokate *et al*., 2002; Indian Pharmacopoeia, 2010).

Determination extractive value

The extractive value is the number of active elements extracted from plant material using solvents. It is generally calculated as the alcohol-soluble extractive value and the water-soluble extractive value.

Alcohol soluble extract value

Five grams of plant powder was macerated with 100 mL of alcohol in a conical flask. The flask was shaken repeatedly for the first 6 hours before being left to stand for 18 hours. The contents were quickly filtered through a dry filter paper. A 25ml filtrate sample was transferred to a tarred flat-bottomed dish and dried in 105^0 C water bath for 6 hours. The dried extract was cooled in desiccators for 30 minutes, weighed immediately and the value was used to calculate the extractive value and expressed in percentage.

Water-soluble extract value

Five grams of air-dried material were macerated in 100 ml of water for 24 hours in a reflux conical flask, shaken repeatedly for 6 hours, and allowed to stand for 18 hours. After rapidly filtering the solution, 25 ml of the filtrate was transferred to a tarred flat bottom dish and dried over a water bath. The extracts were subsequently dried in a hot air oven at 105 °C, and the residue was quantified. The percentage of extractive values for different solvents was calculated using the formula given below:

Extractive value (% w/w) = [(weight of residue ×100) / (25×weight of sample)] × 100

Determination ash value

Incineration of crude drugs results in the loss of organic material in the form of carbon dioxide and the formation of ash containing inorganic material such as carbonates, phosphates, sodium, potassium, calcium, and magnesium silicates. The ash value is a significant criterion for determining inorganic components and assessing the quality and purity of the crude drug in powdered form.

Total ash

The total ash is the total amount of material that remains after burning. This includes both physiological ash (obtained from the plant tissues) and non-physiological ash (ashes are the remains of foreign objects attached to the surface of plants). Four grams of air-dried powdered plant material were precisely weighed and placed in a silicon crucible that had previously been burned and tarred. The material was spread equally and burnt, gradually increasing the temperature (>450 C) until it became white, confirming the lack of carbon. The substance was then weighed after cooling in a desiccator. The percentage of total ash concerning the air-dried powdered drugs was calculated using the expression given below:

Total ash (% w/w) = (weight of ash/weight of sample) × 100

Acid-insoluble ash

The acid-insoluble ash measures the amount of silica, especially sand and siliceous earth. In a crucible containing the total ash obtained by the above method, 25ml of 2N dilute HCl was added, then was covered with a watch-glass and boiled gently for 5 minutes. Rinse the watch glass with 5 ml of hot water and then pour this liquid into the crucible. The residue was collected on an ash-less filter paper, washed with hot water until the filtrate was neutral. Dry on a hot plate and burn under constant weight. Allow the residue to cool in a suitable desiccator for 30 minutes and then weigh. The percentage of acid-insoluble ash obtained in this way was calculated as follows:

Acid insoluble ash (% w/w) = (Weight of acid-insoluble ash/ Weight of sample) × 100

Water-soluble ash

Water-soluble ash is the difference between total ash and residue after treatment with water. The water-soluble ash is used to estimate the number of inorganic elements. The total ash (the ash obtained by the above method) was boiled with 25ml of water for 5 minutes. The insoluble matter was collected in a Gooch crucible

and washed with hot water and heated at a low temperature not exceeding 450 ˚C for 15 min. Let the residue cool in a suitable desiccator for 30 minutes and then weigh.The weight of insoluble matter is subtracted from the weight of the ash taken for analysis. The difference in weight is the weight of the water-soluble matter in the ash. Use the following expression to calculate the percentage of water-soluble ash:

Water-soluble ash (% w/w) = (Weight of water-soluble ash/ Weight of sample) × 100

Determination loss on drying

For the determination of loss on drying, one gram of dried powdered leaf was accurately weighed in a tarred Petri dish. The powder was distributed as evenly as practicable, by gentle sidewise shaking. The dish was dried in an oven at 100 – 105 °C for 1 hour. It was cooled by desiccators and again weighed. The loss on drying was calculated regarding the amount of the dried powder taken.

Loss on drying (%w/w) =

$$\frac{\text{Difference between weight before and after drying}}{\text{Weight of the sample before drying}} \times 100$$

Fluorescence analysis

The fluorescence analysis of plant powder may serve to evaluate the purity when the plant powders were treated with various acids and alkalis like HCl, H_2SO_4, HNO_3, HCl in methanol, and NaOH. The mixture was mixed well, allowed to stand for a few minutes, and filtered. The filtrate was observed under daylight as well as in UV light radiation at 365 nm and the colour exhibited was noted.

RESULTS AND DISCUSSION

The therapeutic efficacy of a medicinal plant is directly related to its quality. Adulterants in raw plant drugs and powdered drugs must be recognised as well. For this reason, several physiochemical properties can be used to standardise and maintain the 'quality assurance' of plant drugs. The following physicochemical evaluations were carried out quantitatively according to the procedures given in materials and methods: extractive value, ash value, loss on drying, and fluorescence behaviour.

The extractive values are useful for estimating the chemical ingredients present in a crude drug and also for determining which constituents are soluble in a specific solvent. Extraction of any crude with a specific solvent yields a solution containing various phytoconstituents. The presence or absence of a certain component has a significant impact on the therapeutic value of the plant extract. The composition of these phytoconstituents in the specific solvent can provide preliminary information on the quality of a certain medicine sample (Tatiya *et al*., 2012). The water soluble extract values are critical in the evaluation of crude drugs. It can be used to denote poor quality, adulteration with any undesired material, or improper processing of the crude drug during the drying, storage etc. Table 2 reveals that the water soluble extractive value in the leaves and flowers of samples A and B was greater in percentage yield, but samples C and D had a slightly lower percentage yield. In terms of alcohol soluble extractive value, both samples A and B had a high percentage yield in their leaves and flowers, whereas samples C and D had a somewhat lower percentage yield. These findings highlighted that a slight decrease in percentage yield was mostly related to a lack of adequate nutrient supply. When the extractive values were compared, the alcohol soluble extractive value was greater than the water soluble extractive value. The presence of polar components such as sugar, acids, gum, and some inorganic compounds is indicated by the water-soluble extractive value. The presence of polar elements such as phenols, alkaloids, resins, steroids, glycosides, and flavonoids is indicated by the alcohol soluble extractive value (Jain *et al*., 2011; Kripa *et al*., 2016). The overall data demonstrated that alcohol is an option for extraction to produce the best results.

Ash values are significant quantitative standards for assessing the quality, identification, and purity of crude drugs, particularly in powdered form (Patnia *et al*., 2012; Swamy and Mulla, 2010). The residue that remains after burning of plant material is known as ash value, and it simply indicates inorganic salts that are naturally found in crude medicine, adhering to it, or purposely added to it as a form of adulteration. The ash value of a crude drug is used to determine its quality and purity. It shows the presence of contaminants such as carbonate,

oxalate, and silicate. Three methods were used to calculate the ash value: total ash, acid-insoluble ash, and water-soluble ash. The total ash is the amount of ash that remains after ignition. This includes both 'physiological ash,' which is formed from plant tissue, and 'non-physiological ash,' which is the residue of extraneous matter adhering to the plant (Purohit *et al*., 2005). Acid insoluble ash is mostly composed of silica and indicates contamination by earthy material (Chanda, 2014). When acid insoluble ash is taken, a portion of the ash content is acid insoluble and so may be physiologically important as salt in the body. It also suggested that the plant was highly digestible when consumed (Ibrahim *et al*., 2010). Water soluble ash is the portion of total ash that is water soluble. The amount of inorganic component contained in drugs is estimated using water soluble ash. The ash values of *S. auriculata* leaves and flowers are presented in Table 3. These observations revealed the presence of silica, calcium oxalate, carbonates, silicates, and phosphates of sodium, calcium, potassium, and magnesium in *S. auriculata* ash.

A typical method for determining the moisture content of a powdered material is the loss on drying test. This might be triggered by water and any other volatile matter that can be driven out under certain conditions. The results of physicochemical investigation revealed that the percentage of moisture in natural and polluted samples varied. The leaves and flowers of samples A and B had lower moisture content percentages, whereas samples C and D had somewhat higher moisture content percentages (Table 4). This finding implies that the proportion of moisture in plants in contaminated environments is higher. The dry weight percent was seen to rise with an increase in the rate of pollution under greater stress environments. The amount of water or moisture contained in plant material determines the rate of deterioration. If the water content is high, fungal and bacterial growth can easily deteriorate the plant (Pandey and Tripathi 2014). Excess moisture can cause enzymatic activity to break down essential ingredients, which can increase the growth of yeast and fungi during storage (African Pharmacopoeia, 1986). The less in moisture level of drugs, the less probable bacterial, fungal, or yeast growth would arise while storage (Purwantiningsih *et al*., 2011)

Fluorescence analysis is another critical pharmacognostic parameter. The fluorescence analysis of leaf powder of *S. auriculata* under day light as well as UV light are shown in Tables 5 & 6. The fluorescence behaviour of flower powder of *S. auriculata* from natural & polluted sources is noted in Tables 7 & 8. In daylight, several components are fluorescence in the visible range. Fluorescence is produced by ultraviolet light in many natural compounds that do not fluoresce conspicuously in daylight. If the chemical is not luminous on its own, it may usually be converted into fluorescent derivatives or metabolic byproducts by utilizing various reagents. As a result, crude pharmaceuticals are frequently assessed qualitatively in this manner, and it is an important criterion for crude drug pharmacognostic evaluation (Khandelwal, 2008; Zhao *et al*., 2011).

Table 1: Taxonomical classification

Kingdom:	Plantae
Division:	Magnoliophyta
Class:	Magnoliopsida
Sub Class:	Rosidae
Order:	Fabales
Family:	Fabaceae
Sub Family:	Caesalpiniaceae
Genus:	*Senna*
Species:	*S. auriculata*

Table 2 Extractive values of *Senna auriculata* leaves and flowers

Samples	Extractive value (%w/w)			
	Leaves		Flowers	
	Alcohol soluble	Water-soluble	Alcohol soluble	Water-soluble
A	18.6	12.5	16.4	13.1
B	13.2	10.7	11.2	10.4
C	07.5	04.9	13.6	08.5
D	10.4	07.3	10.7	05.1

A & B - Natural sources; C & D - Polluted sources. # Values are mean of three determinations

Table 3 Ash values of *Senna auriculata* leaves and flowers

Samples	Ash value (%w/w)					
	Leaves			Flowers		
	Total ash	Acid-insoluble	Water-soluble	Total ash	Acid-insoluble	Water-soluble
A	09.7	1.5	2.5	10.8	2.4	2.7
B	11.3	2.4	1.7	10.7	2.7	2.6
C	12.2	4.3	4.9	11.2	4.2	5.0
D	12.6	4.7	4.3	12.4	5.0	4.9

A & B - Natural sources; C & D - Polluted sources. # Values are mean of three determinations.

Table 4 L.O.D value of *Senna auriculata* leaves and flowers

Samples	L.O.D value (%w/w)	
	Leaves	Flowers
A	10.3	7.9
B	10.7	8.6
C	16.0	12.1
D	11.2	12.7

Note: - A & B - Natural sources; C & D - Polluted sources. # Values are mean of three determinations.

Table 5 Fluorescence behaviour of *Senna auriculata* leaves from natural sources

Treatment	Observation under	
	Day light	UV light
Sample A		
Powder as such	Green	Green
Powder+1N HCl	Brownish green	Black
Powder+H_2SO_4	Dark green	Dark brown
Powder+HNO_3	Brownish green	Dark green
Powder+1N NaOH in methanol	Green	Green
Sample B		
Powder as such	Greyish green	Green
Powder+1N HCl	Brown	Black
Powder+H_2SO_4	Dark green	Dark brown
Powder+HNO_3	Dark green	Dark brown
Powder+1N NaOH in methanol	Green	Green

Table 6 Fluorescence behaviour of *Senna auriculata* leaves from polluted sources

Treatment	Observation under	
	Day light	UV light
Sample C		
Powder as such	Greyish green	Brown
Powder+1N HCl	Brown	Black green
Powder+H_2SO_4	Dark green	Brown
Powder+HNO_3	Yellow green	Greyish green
Powder+1N NaOH in methanol	Light Brown	Black green
Sample D		
Powder as such	Brown	Brown
Powder+1N HCl	Black	Dark green
Powder+H_2SO_4	Brown	Dark brown
Powder+HNO_3	Yellow green	Black green
Powder+1N NaOH in methanol	Greyish green	Green

Table 7 Fluorescence behaviour of *Senna auriculata* flowers from natural sources

Treatment	Observation under	
	Day light	UV light
Sample A		
Powder as such	Yellowish green	Yellowish green
Powder+1N HCl	Light orange	Brown
Powder+H_2SO_4	Yellowish orange	Dark brown
Powder+HNO_3	Orange	Blackish orange
Powder+1N NaOH in methanol	Yellow	Yellow
Sample B		
Powder as such	Yellowish orange	Yellowish orange
Powder+1N HCl	Brown	Dark brown
Powder+H_2SO_4	Light orange	Black
Powder+HNO_3	Orange	Brown
Powder+1N NaOH in methanol	Yellow	Yellow

Table 8 Fluorescence behaviour of *Senna auriculata* flowers from polluted sources

Treatment	Observation under	
	Day light	UV light
Sample C		
Powder as such	Yellowish brown	Brown
Powder+1N HCl	Light orange	Blackish orange
Powder+H_2SO_4	Yellowish orange	Brown
Powder+HNO_3	Orange	Black
Powder+1N NaOH in methanol	Yellow	Dark brown
Sample D		
Powder as such	Yellowish orange	Brown
Powder+1N HCl	Orange	Dark brown
Powder+H_2SO_4	Yellowish orange	Blackish orange
Powder+HNO_3	Orange	Black
Powder+1N NaOH in methanol	Brown	Dark brown

CONCLUSION

All the above data suggests that samples A and B were far preferable than samples C and D in terms of quality. This is due to the fact that samples A and B were collected from relatively pollution-free natural habitats, whereas samples C and D were collected from polluted environments. According to the findings of this study, collecting medicinal plants from their natural habitat is preferred since contaminated environments

might impact the physicochemical properties of the plant material.

CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

REFERENCES

African Pharmacopoeia. (1986) General Methods of Analysis Pharmacopoeia, 11(1st Ed.). pp 121-208.

Chanda S. (2014) Importance of pharmacognostic study of medicinal plants: an overview. *J. pharmacogn. phytochem.* 2(5): 69-73.

Deshpande S., Kewatkar S., and Paithankar V. (2013) Antimicrobial activity of saponins rich fraction of *Cassia auriculata* Linn against various microbial strains. *Int. Curr. Pharm. J.* 2(4): 85–87.

Ibrahim J., Ajaegbu V.C., and Egharevba H.O. (2010) Pharmacognostic and Phytochemical Analysis of *Commelina benghalensis* L. *Ethnobot. leafl*. 14(5): 610–615.

Indian Pharmacopoeia. (2010) Government of India Ministry of Health & Family Welfare, Ghaziabad, Vol. III.

Jain S., Sharma C., Khatri P., Jain A., and Vaidya A. (2011) Pharmacognostic and phyto chemical investigations of the leaves of *Zizyphus xylopyrus* (Retz) willd. *Int. J. Pharm. Pharm. Sci.* 3(2): 122-5.

Khader S.Z.A, Ahmed S.S.Z., Balasubramanian S.K., Arunachalam T.K., Kannappan G., Mahboob M.R., Ponnusamy P., and Ramesh K. (2017) Modulatory effect of dianthrone rich alcoholic flower extract of *Cassia auriculata* L. on experimental diabetes. *Integr. Med. Res.* 6 (2): 131-140.

Khandelwal K. (2008) Practical pharmacognosy. Pragati Books Pvt. Ltd, Nagpur.

Kokate C.K., Purohit A.P., and Gokhale S.B. (2002) Text Book of Pharmacognosy, 18th Ed. Pune: Nirali Prakashan.

Kripa K.G., Sangeetha R., and Chamundeeswari D. (2016) Pharmacognostical and physicochemical evaluation of the plant *Leucas aspera*. *Asian J. Pharm. Clin. Res.* 9(2): 263-8.

Nagalingam M., Vikramathithan M., Dhanesh G.A., and Rajeshkumar S. (2019) Evaluation of herbal and chemical-based mouthwash against oral pathogens. *Drug Invent. Today*. 11(1): 147-151.

Pandey A., and Tripathi A. (2014) Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. *J. pharmacogn. phytochem.* 2(5): 115-119.

Patnia S., Saha A.N., Meena H., Pandey H.K., and Manchanda A. (2012) Physicochemical, phytochemical and elemental analysis of stem bark and roots of *Berberis asiatica*. *Adv. App. Sci. Res.* 3(6): 3624-3628.

Pavunraj M., Rajeshkumar S., Bhuvana L., and Babujanarthanam R. (2019) Preparation of *Cassia auriculata* plant extracts using different solvents and its antibacterial and antifungal activity against clinical pathogens. *Drug Invent. Today*. 11(1): 142-146.

Purohit A.P., Kokate C.K., and Gokhale S.B. (2005) Pharmacognosy 13th Edition, Nirali prakashan India. pp 256-259.

Purwantiningsih, Purwantin I., and Djoko S. (2011) Identification of standard parameters of kepel leaves [*Stelechocarpus Burahol* (Bl.) Hook. F. and Th.] And the extract as raw material for antihyperuricemic medicaments. *Asian J. Pharm. Clin. Res.* 4(1): 149-53.

Sujatha J., Asokan S., and Rajeshkumar S. (2018) Phytochemical analysis and antioxidant activity of chloroform extract of *Cassis alata*. *Res J Pharm Technol.* 11(2): 439-444.

Swamy P., and Mulla S.K. (2010) Preliminary pharmacognostical and phytochemical evaluation of *Portulaca quadrifida* Linn". *Int. J. Pharmtech Res.* 2(3): 1699-1702.

Tatiya A., Surana S., Bhavsar S., Patil D., and Patil Y. (2012) Pharmacognostic and preliminary phytochemical investigation of *Eulophia herbacea* Lindl. Tubers (Orchidaceae). *Asian Pac. J. Trop. Dis.* 2(Suppl 1): S50 –S55

Thabrew M.I., Munasinghe T.M.J., Senarath S., and Yapa R.M.S.C. (2004) Effects of *Cassia auriculata*

and *Cardiospermum halicacabum* teas on the steady state blood levels of theophylline in rats. *Drug Metabol Drug Interact*. 20(4): 263-272.

Uniyal S.K., Singh K.N., Jamwal P., and Lal B. (2006) Traditional use of medicinal plants among the tribal communities of Chhota Bhangal, Western Himalaya. *J. Ethnobiol. Ethnomedicine.* 2(1): 1–8.

WHO, (2000) World Health Organization: General guidelines for methodologies on research and evaluation of traditional medicine, Geneva.

WHO, (2002) World Health Organization: Traditional Medicine Strategy Report.

Zhao Z., Liang Z., and Guo P. (2011) Macroscopic identification of Chinese medicinal materials: traditional experiences and modern understanding. *J. Ethnopharmacol.* 134(3): 556-564.

ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/358283782

A COMPARATIVE STUDY ON HEAVY METAL ANALYSIS OF SENNA AURICULATA (L.) ROXB. OBTAINED FROM NATURAL AND POLLUTED SOURCES BY ATOMIC ABSORPTION SPECTROSCOPY

Chapter · February 2022

CITATIONS
0

READS
276

2 authors:

Anand S. P
National College, Tiruchirappalli
50 PUBLICATIONS **320** CITATIONS
SEE PROFILE

Nagalakshmi Ramamoorthy
National College, Tiruchirappalli
6 PUBLICATIONS **0** CITATIONS
SEE PROFILE

Some of the authors of this publication are also working on these related projects:

A COMPARATIVE STUDY ON HEAVY METAL ANALYSIS OF SENNA AURICULATA (L.) ROXB. OBTAINED FROM NATURAL AND POLLUTED SOURCES BY ATOMIC ABSORPTION SPECTROSCOPY View project

Pharmacological screening of Medicinal plants View project

All content following this page was uploaded by Nagalakshmi Ramamoorthy on 03 February 2022.

The user has requested enhancement of the downloaded file.

ISBN: 978-93-91768-50-8

Advances in Plant Science

Volume II

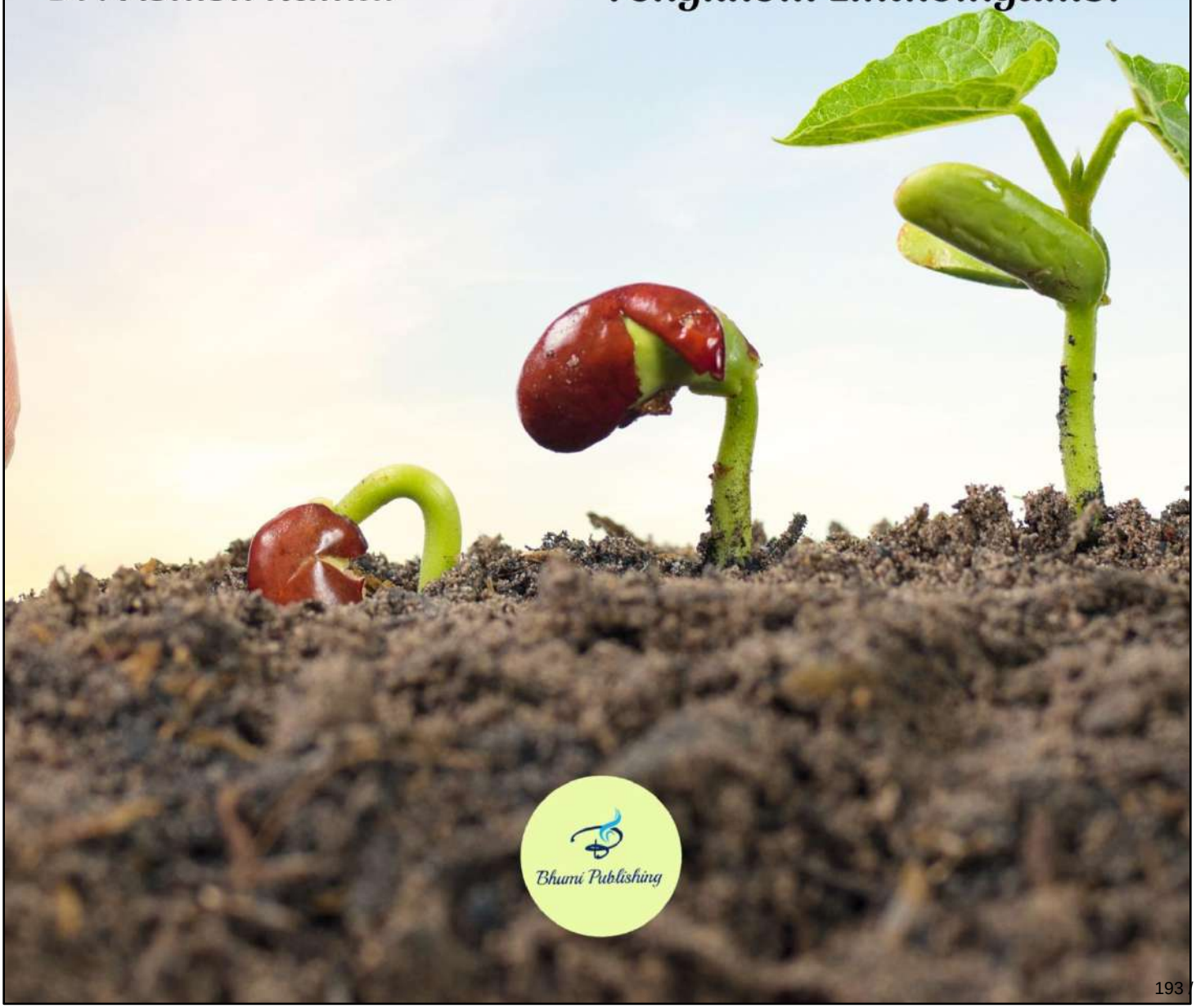
Editors

Dr. Laxmikant N. Borkar

Dr. Jeetendra Sainkhediya

Dr. Ashish Kumar

Yengkhom Linthoingambi



Bhumi Publishing

Advances in Plant Science

Volume II

(ISBN: 978-93-91768-50-8)

Editors

Dr. Laxmikant N. Borkar
Department of Botany,
S. S. Jaiswal College,
Arjuni Morgaon, Dist- Gondia, M.S.

Dr. Jeetendra Sainkhediya
Department of Botany,
Govt. P. G. College,
Sendhwa, Dist. Barwani, M.P.

Dr. Ashish Kumar
Department of Biotechnology,
Sant Gahira Guru Vishwavidyalaya,
Sarguja Ambikapur, Chhattisgarh

Yengkhom Linthoingambi Devi
Department of Genetics and Plant
Breeding, College of Agriculture,
IGKV, Raipur, Chhattisgarh

Bhumi Publishing

ISBN: **978-93-91768-50-8**

© Copyright reserved by the publishers

Publication, Distribution and Promotion Rights reserved by Bhumi Publishing, Nigave Khalasa, Kolhapur

Despite every effort, there may still be chances for some errors and omissions to have crept in inadvertently.

No part of this publication may be reproduced in any form or by any means, electronically, mechanically, by photocopying, recording or otherwise, without the prior permission of the publishers.

The views and results expressed in various articles are those of the authors and not of editors or publisher of the book.

Published by:

Bhumi Publishing,

Nigave Khalasa, Kolhapur 416207, Maharashtra, India

Website: www.bhumipublishing.com

E-mail: bhumipublishing@gmail.com

Book Available online at:

https://www.bhumipublishing.com/books/

PREFACE

We are delighted to publish our book entitled ***"Advances in Plant Science Volume II"****. This book is the compilation of esteemed articles of acknowledged experts in the fields of plant science providing a sufficient depth of the subject to satisfy the need of a level which will be comprehensive and interesting. It is an assemblage of variety of information about advances and developments in plant science. With its application oriented and interdisciplinary approach, we hope that the students, teachers, researchers, scientists and policy makers will find this book much more useful.*

The articles in the book have been contributed by eminent scientists, academicians. Our special thanks and appreciation goes to experts and research workers whose contributions have enriched this book. We thank our publisher Bhumi Publishing, India for compilation of such nice data in the form of this book.

Finally, we will always remain a debtor to all our well-wishers for their blessings, without which this book would not have come into existence.

- Editors

Advances in Plant Science Volume II

CONTENTS

Sr. No.	Book Chapter and Author(s)	Page no.
1.	**A COMPARATIVE STUDY ON HEAVY METAL ANALYSIS OF *SENNA AURICULATA* (L.) ROXB. OBTAINED FROM NATURAL AND POLLUTED SOURCES BY ATOMIC ABSORPTION SPECTROSCOPY** Ramamoorthy Nagalakshmi and Subramaniam Parvathi Anand	1 – 12
2.	**TREE CROWN EXTRACTION FROM AERIAL IMAGERY USING DEEP LEARNING** Mamita Kalita and Avinash Chouhan	13 – 21
3.	***COELASTRACEAE* AND *SCENEDESMACEAE* FROM BANGANGA WATER RESERVOIR** Prakash B. Jadhavar	22 – 26
4.	**PHYTOCHEMICAL AND ANTIMICROBIAL ANALYSIS OF *TECOMA STANS* FROM PARBHANI DISTRICT** Arshia Parveen	27 – 33
5.	**ECONOMIC IMPORTANCE OF PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR)** Kahekashan Tanveer Mirza	34 – 38
6.	**A COMPREHENSIVE OVERVIEW ON DIETARY SUPPLEMENTS** Swarnima Pandey and Sushant Kumar	39 – 51
7.	**SEED SURFACE CHARACTERISTICS AND PRELIMINARY PHYTOCHEMICAL ANALYSIS OF SOME LAMIACEAE (LABIATAE) SEEDS** P. P. Ulhe	52 – 59

8.	**BENEFITS AND PHARMACOLOGICAL PROPERTIES OF CUMIN- AN UPDATED REVIEW** Bhagyashali J. Pawar and Anita S. Wagh	60 – 67
9.	**ANTIBACTERIAL ACTIVITY OF BIOPESTICIDES AGAINST SEED BORNE FUNGI OF JOWAR** L. R. Rathod and S. L. Gaikwad	68 – 74
10.	**PHYTOCHEMISTRY, ETHNOMEDICINAL USE AND PHARMACOLOGICAL PROFILE OF *ADHATODA VASICA* NEES - AN ETHNOMEDICINAL PLANT** Shalini Lal, Nuzhat Afroz and Binit Baraik	75 – 90
11.	**PLANT PATHOLOGY: AN OVERVIEW** R. R. Tembhurne	91 – 96
12.	**CHROMATOGRAPHY: A POPULAR TECHNIQUE USED FOR STUDYING CHEMICAL ANALYSIS IN A MIXTURE** Ramesh Singh	97 – 102

A COMPARATIVE STUDY ON HEAVY METAL ANALYSIS OF *SENNA AURICULATA* (L.) ROXB. OBTAINED FROM NATURAL AND POLLUTED SOURCES BY ATOMIC ABSORPTION SPECTROSCOPY

Ramamoorthy Nagalakshmi* and Subramaniam Parvathi Anand

PG and Research, Department of Botany,

National College, Affiliated to Bharathidasan University,

Tiruchirappalli-620021, Tamil Nadu, India

*Corresponding author E-mail: nagalakshmi.93botany@gmail.com

Abstract:

Heavy metals are the most harmful contaminants because of their persistence and proclivity to accumulate in biota. The objective of this study was to compare the heavy metal concentrations of *Senna auriculata* (L.) Roxb. with soil samples collected from natural and polluted sources. Heavy metals such asPb, Cr, Cd, Zn, Cu, and Fe were determined by using atomic absorption spectroscopy. The results revealed that the majority of the heavy metal levels of the analyzed soil samples were below the limit values. However, The Cd level exceeded the standard in polluted areas such as site-3 soil sample (9.65mg/kg) and site-4 soil sample (6.74 mg/kg). The Zn level exceeded the permissible limit in the site-3 soil sample (115.84 mg/kg). Analytical results of *S. Auriculata* show that samples A and B (natural sources) had heavy metal contents below the permissible level except for cadmium. Cadmium is not detected in sample B. while the samples from polluted sources had heavy metal contents higher than the permissible level except cadmium and copper. The levels of lead, and zinc in sample C, as well as iron and chromium in sample D, of both leaves and floral extract, exceeded the standard limit.These results showed that the majority of heavy metals have been accumulated above the standard levels in the plant parts of *S.auriculata* collected from the polluted sources when compared to the natural sources. This could be the result of Heavy metal pollution from industrial sources navigating their way from soil to plants. These exceeded levels of these heavy metals become toxic to humans on consumption.Therefore, to utilize and gain the values of the pharmacological effects of this experimental plant, the source obtained from the unpolluted natural environment is preferable.

Keywords: Heavy metals, natural source, permissible limit, polluted source, *Senna auriculata*

Introduction:

Environmental pollution is a significant ecological issue since it directly or indirectly impacts people. The main causes of environmental pollution are urbanization, industrialization, and the growth of contemporary technologies. Many organic and inorganic substances that are discharged into the environment can be dangerous. When a chemical exceeds its tolerated limit, it is classified as contamination. Heavy metals are the most harmful contaminants because of their persistence and proclivity to accumulate in biota. The topic of heavy metal pollution in the soil is gaining attention in most major cities throughout the world. As a result, the environment may experience geo accumulation, bioaccumulation, and bio-magnification. Rapid industrialization and disorderly urbanization, together with the ongoing use of enormous quantities of fertilizers and pesticides, resulting in the accumulation of toxic compounds in soil, water, and air (Kumar *et al*., 2015, Rodriguesa *et al.,* 2017). Exhaust emissions and fossil fuel combustion have also been recognized as the primary sources of metallic pollution, particularly in roadside soil. Arsenic, cadmium, lead, and mercury is among the most dangerous elements in the environment, according to the Environmental Protection Agency (EPA) by Goyer (2004). Among different heavy metals lead, cadmium, copper, zinc, nickel, iron, and chromium are the major metallic pollutants of the roadside soil (Silva *et al*., 2005). Even in low concentrations, lead, and cadmium is harmful. Therefore, Lead, Cadmium, Chromium, Zinc, Iron, and Copper are focused in this study.

Heavy metals can be absorbed by plants from the soil. Plant consumption by humans and animals can be ascribed to heavy metal uptake by plant roots from polluted soil as well as direct deposition of pollutants from the atmosphere on plant surfaces (Zhuang *et al*., 2009). The interaction of soil-plant roots is crucial in regulating the transit of heavy metals from the soil to plant components (Sharma *et al*., 2009). Heavy metals' toxicity to humans is determined by their dosage, rate of emission, and duration of exposure. In low amounts, copper and zinc are vital trace metals for living organisms; yet, in excessive concentrations, they can cause toxicity. Heavy metal excesses in plants and animals can have a wide range of acute and chronic consequences on a wide range of organisms in various habitats.With the commercialization of herbal medicine, ensuring the safety, uniqueness, and efficacy of medicinal plants and herbal products has become critical. The safety of a medicine is defined by its pharmacological-toxicological profile and the adverse effects induced by contaminants in bulk and dosage forms, therefore toxic metal content should always be kept to a minimum. The detection of highly hazardous heavy metals in plant extracts is critical for food safety and quality management. The reason for this is, of course, that pharmaceuticals grown in the wild are more difficult to control

for all potential sources of pollution. As expected, the possibility of the deposition of toxic pollutants on plants cannot be disregarded. Little is known about the harmful metal status of these herbal medicinal plants produced in contaminated areas. The danger is that using herbs cultivated in polluted places could expose humans and animals to hazardous contaminants. The detection of heavy metals in plants is critical. Since the absorption of harmful trace metals by humans, this, even in moderate doses over time, can cause organ failure and prolonged arrhythmia. Several attempts have been made to estimate heavy metals and harmful trace elements in plants and medicinal formulations (Kishan *et al.*, 2014) but none have been made in *Senna auriculata* (L.) Roxb. *Senna auriculata* leaves and flowers are used to make a variety of herbal products such as senna tablets, pickles, tea, idly powder, tonic, avaram poo soornam, soup powder, diabetic food supplement, etc. Therefore, it was imperative to analyze the heavy metals (Pb, Cd, Cr, Zn, Fe, and Cu) in *Senna auriculata* leaves and flowersobtained from natural and polluted sources.

Materials and Methods:

Study species

In the present study, we chose one of the most important medicinal plants *Senna auriculata* (L.) Roxb. is a member of the Fabaceae family. It is an evergreen shrub commonly known as Tanner's cassia. The flower, buds, leaves, stem, root, and unripe fruit are used for treatments in the Ayurvedic and Siddha systems of medicine. It is frequently used in the treatment of rheumatoid arthritis, conjunctivitis, and diabetes.

***Senna auriculata* (L.) Roxb.**

Study sites

Soil and plant samples were collected from both natural and polluted sources for this investigation. Two samples (samples A and B) were taken from unpolluted areas of site 1 (Pachaimalai hills) and site 2 (Kolli hills) for natural sources.Two samples (Sample C and D) were obtained from the roadside in industrial areas of the Tiruchirappalli district. Site 3 is on the avur road, and site 4 is on the samayapuramkariyamanickam road. Many industries are located within 500 meters of this region.

Collection and identification

Senna auriculata leaves and flowers, as well as soil samples, were collected from the four study locations in June 2021. The plant species were identified and authenticated by the Botanical Survey of India, Southern Regional Centre,and Coimbatore (BSI/SRC/5/23/2021/Tech-166).

Heavy metal analysis

Apparatus

Heavy metal concentrations in powdered samples were evaluated by Atomic Absorption Spectroscopy (THERMO SCIENTIFIC- ice 3000) at the National College Instrumentation Facility (NCIF), National College, Trichy.

Working Principle and Instrumentation of AAS

Atomic-absorption spectroscopy measures the absorption of ground-state atoms in a gaseous state. Atoms absorb ultraviolet or visible light and progress to higher levels of electronic energy. The absorbance of the analyte determines its concentration. After calibrating the equipment with a known concentration of a standard substance, the concentration is usually measured using the working curve. An AAS is made up of the following functional components: the light source: A 'hollow cathode lamp,' which has a tungsten anode and a barrel-shaped hollow cathode constructed of the component to be resolved, is the most frequent source of light. These are placed in a glass tube that is filled with an inert gas (neon or argon). An atomizer, which can be a flame or an electrothermal or cold vapor/hydride producing framework, is used to atomize the sample, and a monochromator is used to choose the specific wavelength of light (spectral line) that is absorbed by the sample. The use of a specific light allows for the assurance of the selected element in the presence of others. The monochromator transmits the light onto a detector, which is typically a photomultiplier tube. This calculates the amount of light absorbed and a recorder to record and produce an electrical sign according to the light intensity.

Chemicals

All chemicals and reagents used in this study were of analytical grades. Nitric acid (HNO_3), Hydrochloric acid (HCl), Perchloric acid ($HClO_4$).Stock standard solutions for each metal lead (Pb), cadmium (Cd), zinc (Zn), iron (Fe), and copper (Cu) with a concentration of 1000 ppm. The standard solutions for all the heavy metals under study were prepared in deionized water.

Preparation of soil samples

Each soil sample was homogenized and air-dried in an oven at 105°C. The sieved material was then placed in a 300ml beaker and heated at 230°C with 15ml of nitric acid (HNO_3, 69%) and 25ml of perchloric acid ($HClO_4$,58%). The digested solution was filtered using Whatman No. 42 filter paper after being changed to ash, and the volume was increased to 50ml in a volumetric flask. An atomic absorption spectrophotometer is used to determine the metal concentration (Mehmet 2008).

Preparation of plant samples

The plant samples were air-dried at room temperature for 5-7 days before being ground into a fine powder. Uddin *et al*. (2016) stated that the most efficient digesting procedure for plant material was the wet digestion method. A total of 2.0 g of each sample was weighed and placed in a conical flask. The conical flask was then filled with a 9 ml combination of nitric-hydrochloric acids HNO_3 (65%) andHCl (37%) in a ratio of 1:3. The mixture was then gently heated over a water bath at 95 °C for 4–5 hours, or until the sample was completely dissolved. The digest was allowed to cool at room temperature before being filtered through a Whatman No. 42 filter paper and diluted with deionized water to a final volume of 50 ml. The experiment was done 3 times with each sample being aspirated twice.

Analytical procedure

Heavy metals such as lead, chromium, cadmium, zinc, copper, and iron were evaluated quantitatively on digested samples using atomic absorption spectroscopy (THERMO SCIENTIFIC- ice 3000). Table 1 lists the instrumental conditions used during heavy metal analysis. All measurements on samples and standard solutions are done in triplicate. The mean and standard deviation of the heavy metal values from three replicates were calculated using Microsoft Excel.

Table 1: Instrumental condition of AAS for heavy metal analysis

AAS Parameter	Elements					
	Pb	**Cr**	**Cd**	**Zn**	**Cu**	**Fe**
Wavelength (nm)	283.3	357.9	228.8	213.9	324.8	248.3
Slit width (nm)	0.7	0.7	0.7	0.7	0.7	0.2
Current (A)	10	12	3.5	5.0	15.0	30
Flame	AA	AA	AA	AA	AA	AA

AA - Air-acetylene

Results and Discussion:

In the present study Heavy metalslike Pb, Cr, Cd, Zn, Cu, and Fe were determined in the plant parts (leaves and flowers) of *Senna auriculata* and the soil samples from natural and polluted sources. The contents of these elements in the samples as a means oftriplicate.

Heavy metal contents in the soil samples

The mean concentrations of heavy metals in the soil samples that were collected from 4 study sites are presented in Table 2. The results revealed that the majority of the heavy metal levels of the analyzed soil samples were below the limit values given in Table 4. However, the Cd and Zn levels of some soil samples were above the standard levels. The Cd level exceeded the standard in polluted areas such as site-3 soil sample (9.65mg/kg) and site-4 soil sample (6.74 mg/kg). The Zn level exceeded the permissible limit in the site-3 soil sample (115.84 mg/kg).

Table 2: Heavy Metal concentrations in soil samples from natural and polluted sources

Elements	Natural sources		Polluted sources	
	Site - 1	Site - 2	Site - 3	Site- 4
Pb	3.09±0.28	0.07±0.21	27.44±0.02	23.95±0.30
Cr	0.97±0.02	2.73±0.04	15.82±0.07	27.69±0.15
Cd	0.28±0.37	0.04±0.02	9.65±3.21	6.74±0.02
Zn	10.6±0.01	9.02±2.37	115.84±0.25	89.35±0.29
Cu	8.02±0.62	9.76±0.01	25.89±0.03	13.32±0.03
Fe	10.23±0.03	17.8±0.21	54.03±0.74	98.46±0.42

Concentrations (Mean ± SD) (mg/kg),SD – Standard deviation; ND – not detected.

Site 1- Pachaimalai hills,Site 2 – Kollihills, Site 3 - Avur road,

Site 4 - Samayapuramkariyamanickam road.

The high Cd and Zn value of the samples from the polluted sources are attributable to the paint, chemicals, and electrical industries and some cement factories within 300m distance from the sample collection site. The dumping of industrial wastes or the deposition of pollutants into the atmosphere raises the total concentration of Cd in soils (Weggler et al., 2004). In developed countries, there is concern about anthropogenic Cd accumulations in the environment, and it is categorized as a potentially hazardous element in terms of soil biological activity, plant metabolism, and human and ecosystem health (Kabata-Pendias 2001).Zinc occurs naturally in soil (about 70mg kg1 in crustal rocks), however, Zn concentrations are increasing unnaturally as a result of anthropogenic inputs. Most of the Zn is added during industrial processes such as mining, coal and waste incineration, and steel production. Heavy metal pollution from industrial sources navigates its way to plants via soil and water.

Heavy metal contents in *Senna auriculata*

The mean concentrations of metals in leaves and flowers of*Senna auriculata*have been presented in Table 3.

Table 3: Heavy metal concentrations in *S. auriculata* from natural and polluted sources

Elements	Plant parts	Natural sources		Polluted sources	
		Sample - A	Sample - B	Sample - C	Sample - D
Pb	Leaves	1.86±0.03	0.01±0.01	13.46±0.02	7.43±0.26
	Flowers	1.02±0.01	ND	12.78±0.03	7.38±0.37
Cr	Leaves	0.63±0.01	1.07±0.03	1.89±0.01	3.84±0.01
	Flowers	0.42±0.01	0.91±0.26	1.65±0.04	3.06±0.02
Cd	Leaves	0.05±0.03	ND	0.32±0.01	0.21±0.02
	Flowers	0.03±0.01	ND	0.27±0.03	0.19±0.02
Zn	Leaves	2.63±0.02	1.56±0.01	59.05±0.12	39.24±0.01
	Flowers	0.87±0.02	1.38±0.04	56.12±0.65	38.96±0.03
Cu	Leaves	1.98±0.01	3.48±0.02	9.52±0.03	6.02±0.01
	Flowers	1.46±0.02	3.35±0.01	9.46 ±0.02	5.73±0.01
Fe	Leaves	4.87±0.27	5.48±0.03	11.92±0.01	19.28±0.22
	Flowers	3.84±0.43	5.07±0.02	10.72±0.01	18.02±0.24

All data are the mean ± SD of three replicates (mg/kg).

SD – Standard deviation; ND – not detected.

Table 4: Permissible limits for heavy metals

Sources	Elements(mg/kg)					
	Pb	**Cr**	**Cd**	**Zn**	**Cu**	**Fe**
Soil	85	100	0.8	100	36	150
Plants	10	2	0.3	50	10	15

According to WHO, (1996; 2005; 2007)

Analytical results show that samples A and B (natural sources) had heavy metal contents below the permissible level except for cadmium. Cadmium is not detected in sample B. while the samples from polluted sources had heavy metal contents higher than the permissible level except cadmium and copper. The levels of lead, and zinc in sample C, as well as iron and chromium in sample D, of both leaves and floral extract, exceeded the standard limit (Fig. 1). This could be due to the presence of paint, battery, cement, electrical, chemical, and steel manufacturing companies on site3 and site 4.Similar results were reported by Hussain and Khan (2010) in *Taraxacum officinale*, Ghani (2010) in *Zea mays*, andJuan *et al.* (2020) in *Jatropha curcas L.*

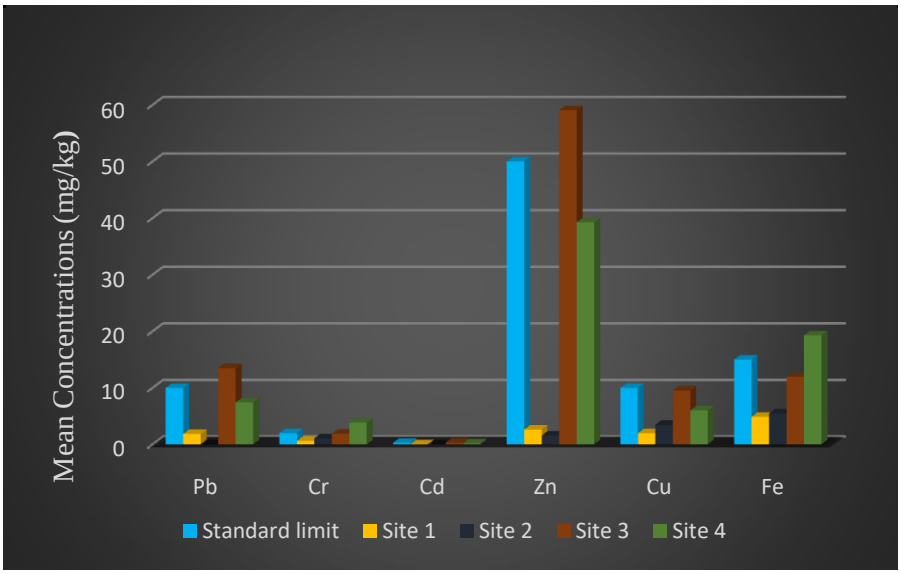


Figure 1: Heavy metal contents in *Senna auriculata* from natural and polluted sources

Lead (Pb) is a non-essential toxic heavy metal that is widespread and causes a variety of negative effects in living organisms. Although it is a natural element, anthropogenic activities such as mining, fossil fuel burning, and manufacturing, paints, batteries contribute due to its higher concentration in the soils (Tchounwou *et al*., 2012). Pb is carcinogenic, harms the respiratory and digestive systems, and inhibits the immune system. This metal is especially dangerous to children since it harms their intelligence and nervous systems (Borges *et al*., 2003).

Due to immunomodulatory, oxidative, and inflammatory pathways, Pb exposure can cause urinary tract and cardiovascular illnesses. Zinc functions as a cofactor in several enzymes involved in macronutrient metabolism and cell reproduction. Blast furnace slag and sewage, mining wastes, soil coal and fly ash, and the usage of commercial products such as zinc-containing fertilizers, fungicides, and wood preservatives, Galvanizing, plating iron and steel, luminous paints, batteries, deodorants, flame retardants, and other zinc industrial effluents are the most prominent sources of anthropogenic zinc in the soil. Excessive intake can result in arteriosclerosis, stomach cramps, pancreatic damage, and caustic effects on the skin, liver failure, renal failure, and anemia (Duruibe *et al*., 2007). Chromium is essential for metabolic operations in the body, however, the hexavalent form is toxic to humans (Khurshid and Iqbal, 1984). Because of its anti-corrosive properties, it is widely utilized in industries such as metal surface plating, leather tanning, glassware cleaning, textile production, and so on. Acute chromium poisoning results in nausea, vomiting, acute renal failure, irritation, contact dermatitis, eczema, allergies, contact dermatitis, eczema, and reproductive damage, among other things.

Iron (Fe) is a vital element. It is thought to exist in soils mostly as oxides and hydroxides as minute particles or in association with the surfaces of other minerals.Iron is a necessary component of haemoglobin, the red pigment in our blood that transports oxygen throughout our bodies.It can cause conjunctivitis, choroiditis, and retinitis. Chronic inhalation of high amounts of iron oxide fumes orspecks of dust can cause siderosis, a type of pneumoconiosis. Excessive iron oxide inhalation can raise the risk of lung cancer. The iron and steel industry, sewage, and iron mine dust, iron sulphate fertilizer, and herbicide are all anthropogenic sources of iron. The accumulation of heavy metals in medicinal plants has been reported to be based on various factors such as climatic factors, plant species, and soil pollution, apart from other environmental factors (Sovljanski *et al*., 1989). Heavy metal deposition in edible and medicinal plants required careful examination to prevent higher heavy metal concentrations from reaching the customer (Steenkamp *et al.,* 2000). The morphology of medicinal plants does not ensure their safety from contamination, especially when plants are harvested from contaminated environments (Olowoyo *et al*., 2012). According to Chaiyarat *et al*. (2011) selective site selection, combined with appropriate soil management, can assist to reduce heavy metal accumulation by medicinal plants.

Conclusion:

These results showed that the majority of heavy metals have been accumulated above the standard levels in the leaves and flowers of *Senna auriculata* collected from the polluted sources

when compared to the natural sources. This could be the result of Heavy metal pollution from industrial sources navigating their way from soil to plants. These exceeded levels of these heavy metals become toxic to humans on consumption. Based on this finding, it is suggested that medicinal plants used for human consumption or to manufacture herbal products and standardized extracts should be collected from an unpolluted natural environment is preferable.

References:

Borges, D.L.G., Veiga, M.A.M.S., Frescura, V.L.A, Welz, B. and Curtis, A.J. (2003): Cloud-point extraction for the determination of Cd, Pb and Pd in blood by electrothermal atomic absorption spectrometry, using Ir or Ru as permanent modifiers. Journal of Analytical Atomic Spectrometry, 18(5): 501-507.

Chaiyarat, R., Suebsima, R., Putwattana, N., Kruatrachue, M. and Pokethitiyook, P. (2011): Effects of soil amendments on growth and metal uptake by *Ocimumgratissimum* growth in Cd/Zn-contaminated soil. Water, Air, and Soil Pollution, 214(1): 383–392.

Duruibe, J.O., Ogwuegbu, M.O.C. and Egwurugwu J.N. (2007): Heavy metal pollution and human biotoxic effects. International Journal of Physical Sciences, 2(5): 112-118.

Ghani A. (2010): Toxic Effects of Heavy Metals on Plant Growth and Metal Accumulation in Maize (*Zea mays* L.): Iranian Journal of Toxicology, 3(3): 325-334.

Goyer, R. (2004): Issue paper on the human health effects of metals. Washington, DC: US Environmental Protection Agency.

Hussain, I. and Khan, L. (2010): Comparative Study on Heavy Metal Contents in *Taraxacum Officinale.* International Journal of Pharmacognosy and Phytochemical Research, 2(1): 15-18.

Juan, F.G.M., González Caro, M.D.C., López Barrera, M.D.C., Miguel, T.G., Douglas, B. and Paloma, Á.M. (2020): Metal Accumulation by *Jatrophacurcas* L. Adult Plants Grown on Heavy Metal-Contaminated Soil. Plants,9(4): 418.

Kabata-Pendias, A. and Mukherjee, A.B. (2007): Trace Elements in Soils and Plants, Springer: Berlin, Heidelberg, Germany. 57-65.

Khurshid, J.S. and Iqbal H.Q. (1984): The role of inorganic elements in the human body. Nucleus, 21(4): 3-23.

Kishan, P.S., Bhattacharya, S. and Sharma, P. (2014): American Eurasian assessment of heavy metal contents of some Indian medicinal plants. Journal of Agricultural and Environmental Sciences,14(10): 1125–1129.

Kumar, N., Kumar, S., Bauddh, K., Dwivedi, N., Shukla, P., Singh, D.P. and Barman S.C. (2015): Toxicity assessment and accumulation of metals in radish irrigated with battery manufacturing industry effluent. International Journal of Vegetable Science, 21(4): 373-375.

Mehmet, Y.N. (2008): Determination of lead, cadmium and copper in roadside soil and plants in Elazig, Turkey. Environ Monit Assess,136(1-3): 401– 410.

Olowoyo, J.O., Okedeyi, O.O., Mkolo, N.M., Lion, G.N. and Mdakane, S.T.R. (2012): Uptake and translocation of heavy metals by medicinal plants growing around a waste dump site in Pretoria, South Africa. South African Journal of Botany, 78: 116–121.

Rodriguesa, A.A.Z., De Queiroz, M.E.L.R., Oliveira, A.F., Heleno, A.A.F.F., Zambolim, L., Freitasa, J.F. and Morais, E.H.C. (2017): Pesticide residue removal in classic domestic processing of tomato and its effects on product quality. Journal of Environmental Science and Health, 52(12): 850-857.

Sharma, R.K., Agrawal, M. and Marshall, F.M. (2009): Heavy metals in vegetables collected from production and market sites of a tropical urban area of India. Food and Chemical Toxicology,47(3): 583–591.

Silva, A.L.O., Barrocas, P.R.G., Jacob, S.C. and Moreira, J.C. (2005): Dietary intake and health effects of selected toxic elements. Brazilian Journal of Plant Physiology, 17(1): 79-93.

Sovljanski, R., Obradovie, Kisgeci, S., Lazie, S. and Macko, V. (1989): The heavy metal contents and quality of hops canoes treated by pesticides during the vegetation. ActaHorticulturae, 249: 81-88.

Steenkamp, V., Von arb, M. and Stewart, M.J. (2000): Metal concentrations inplants and urine from patients treated with traditional remedies. Forensic Science International, 114(2): 89–95.

Tchounwou, P.B., Yedjou, C.G., Patlolla, A.K. and Sutton, D.J. (2012): Heavy Metal Toxicity and the Environment. In: Luch A. (eds): Molecular,Clinical and Environmental Toxicology. ExperientiaSupplementum 101: 133-164.

Uddin, A.B.M.H., Khalid, R.S., Mohamed, A., Abdualrahman, Abdualkader, M., Kasmuri, A. and Abbas S.A. (2016): Comparative study of three digestion methods for elemental analysis in traditional medicine products using atomic absorption spectrometry. Journal of Analytical Science and Technology,7(1): 1-7.

Weggler, K., McLaughlin, M.J. and Graham, R.D. (2004): Effect of chloride in soil solution on the plant availability of bio solid-borne cadmium. Journal of Environmental Quality, 33(2): 496-504.

WHO, (1996): Permissible limits of heavy metals in soil and plants. Genava, Switzerland.

WHO, (2005): Quality Control Methods for Medicinal Plant Materials. Geneva, Switzerland.

WHO, (2007): Guidelines for assessing quality of herbal medicines with reference to contaminants and residues. Geneva, Switzerland.

Zhuang, P., Zou, B., Li, N.Y. and Li, Z.A. (2009): Heavy metal contamination in soils and food crops around Dabaoshan mine in Guangdong, China: implication for human health. Environmental Geochemistry and Health, 31(6): 707–715.

TREE CROWN EXTRACTION FROM AERIAL IMAGERY USING DEEP LEARNING

Mamita Kalita*[1] and Avinash Chouhan[2]

[1]Department of Botany, Gauhati University, Guwahati, Assam, India

[2]North Eastern Space Applications Centre, NESAC, Umiam, Ri-Bhoi, Meghalaya, India

*Corresponding author E-mail: mamita.bot@gmail.com

Abstract:

This study presents an application of deep learning in very high-resolution, remotely sensed data. The study highlights pixel-wise segmentation using deep learning to extract tree crown features from UAV imagery. UAV imagery object detection or segmentation is always challenging because of very different object views, i.e., top view, lack of available data, and minimal object size. We choose this task to demonstrate automatic tree crown extraction in very high resolution to bypass manual digitization tasks requiring a massive workforce and time and are primarily unsuitable for high-resolution imagery such as UAV. The canopy mapping will give us the idea of vegetation coverage over a space. However, also it can help us derive the biomass of an area with further insight into its biodiversity. The vast size of imagery in terms of dimensions and size also creates a problem for segmentation tasks. In this study, we discuss all the issues and challenges that the aerial imagery segmentation task has and highlight possible solutions and approaches. The objective of our study is to accurately extract canopy cover and analyze its efficiency, which is not possible by other classification or segmentation methods. Due to UAV's high temporal and spatial resolution, forest health study and canopy closure monitoring can be done.

Keywords: Deep Learning, Segmentation, Aerial images, UAV, Remote Sensing.

Introduction:

UAV remote sensing and deep learning

Aerial photography and its use is not a new science. UAV-RS surveys help us overcome traditional fieldwork methods, which are time consuming, tedious, exhaustive, and impractical for carrying out small periodic surveys. It has been used for decades; however, the advancement made in its use and practical applications in almost all possible fields has been recent. These

lightweight UAVs can be directly controlled from the ground and fly below the cloud cover. UAVs are also known as remotely piloted vehicles (RPV), Remote controlled (RC) helicopters, remotely operated aircraft (ROA), unmanned vehicle systems (UVS), which are designed to operate without humans on board. UAV was started as a goal in military surveillance of hostile areas. UAV can be used for precision farming in agriculture to get accurate damages or problems in the field. The UAV flight is widely recognized nowadays due to its ability to characterize landscape at any elevation and slope.

Further, a low weight UAV gives us an alternative platform through which remote sensing technology can be applied effectively over an area of a tiny scale. UAV provides us the platform to study the structural properties of land use features. It also helps us know the changes in an area over a short period, and the study can be done effectively within a brief period. UAVs can be of two types fixed wing and rotary wings. The following study has been done using a rotary type of UAV. It has wide applications in the field of forestry as well. The payload capacity of the UAV is 1.5-2 kg, and various sensors like multispectral, thermal, and hyper spectral, optical, or LIDAR can be used in flight as per user requirements. UAV's can be used for both manuals as well as autonomous flights. UAV remote sensing (UAV-RS) is widely used for large scale monitoring, vegetation structure, to urban modeling.

Deep Learning also has enormous potential in various applications in remotely sensed data for classification, segmentation, and object detection. Early methods relied on Object based Image Analysis (OBIA). However, we have focused on segmentation using deep learning to generate tree crowns from high resolution aerial images. Deep learning has been used in object based tree crown delineation from high resolution imagery (Whiteside et al., 2011). Also, an index based approach for vegetation extraction from satellite images can be made (Hebbar et al., 2018). We have explored various deep architecture for this task and found that deep Learning based architecture is most suitable for semantic segmentation on VHR aerial data. This is motivated to use the advantage that deep learning has over OBIA for segmentation tasks on aerial data. Texture algorithms have been applied to extract tree crown (Gomes and Maillard, 2015), watershed segmentation, marked point process, and template matching. Generated map is precious as this gives the capability to generate a very high resolution map without manual digitization. Support vector machine and principal component analysis for individual tree crown extraction from hyper spectral imagery (La *et al.*, 2015). It is very significant for remote sensing as these tasks require massive person hour efforts.

Currently, most case studies focus on CCTV data or moving vehicles with standard RGB and RGB-D cameras. The study through UAV helps us to overcome the labor cost. Also, it can

be flown with a minimum interval of time or as per user requirements. Point cloud based segmentation (Disney and Calders, 2018), an object based image analysis tool (Sara *et al.,* 2012) for canopy extraction. A deep convolution neural network for image net classification can be done (Krizhevsky *et al.,* 2012). From a computation perspective, our inference should be efficient and feasible regarding time and memory requirements for training and testing. The ability to train end to end using stochastic gradient descent is also beneficial, more manageable, and repeatable. Long et al. has extended this work to semantic segmentation using a convolution neural network. Further, encoder-decoder based architecture for medical image segmentation has been proposed (Ronneberger *et al.,* 2015).

Materials and Methods:

Study area

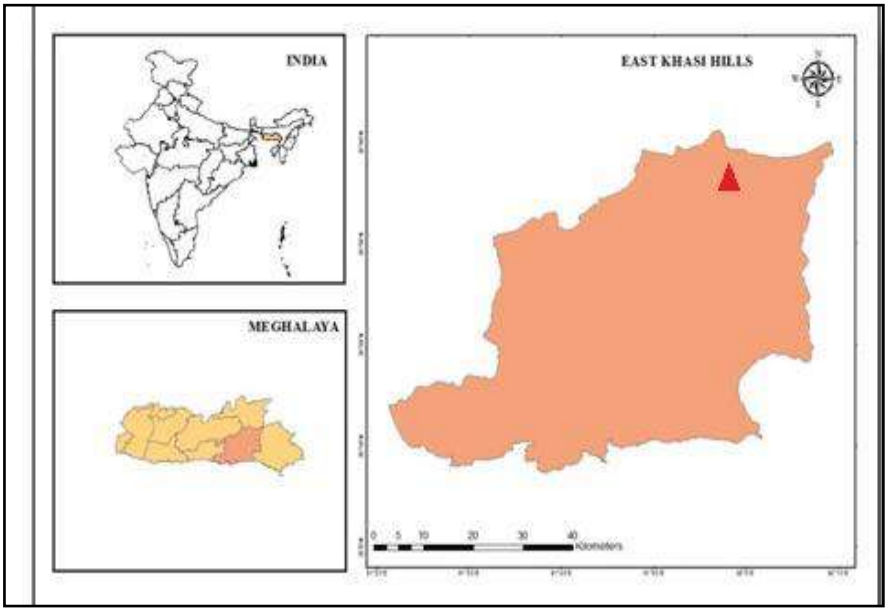


Figure 1: Map showing location of the study area

The study has been done in an area located in East Khasi Hills of Meghalaya (Fig.1). The state is characterized by a tropical climate and can provide us with our desired features of canopy cover. The state's geographical area is around 22,429 sq. km, and the total forest cover is 8510 sq. km. Meghalaya (the abode of clouds) is situated between Bangladesh in the south and the Brahmaputra valley in the north, a North-Eastern region of India. The state includes Khasi, Garo, Jaintia hills, Assam ranges at its border and lies between the latitudes of 25°02' and 26°07' N and longitudes 89°49'and 92°50'E. The state has Tropical wet evergreen, Tropical Semi-evergreen, Tropical moist deciduous, Subtropical broadleaved hill, and Subtropical pine forests.

Pines, Bamboo, Sal, are pretty rampant in this region. The soil type is sandy loamy, reddish-brown in color, and the soil pH ranges from 4.6 to 5.8. The maximum temperature is 25°C during summers while 16°C in winters. The minimum temperature of Meghalaya is 15°C during summers, while the same in winters is 4°C. The annual rainfall in the state varies from 4000mm to 11,436mm. Mawsynram and Cherrapunji, located in the southern part, are the world's rainiest spots. The study area covers varied land use features. The area has sparse forest cover with agricultural and human settlements. The region's climate was characterized by average annual precipitation of 2000 mm.

UAV data aquisition

A hex copter UAV has been used with a resolution of 5 cm. The targeted areas were captured in multiple UAV flights of 40 minutes each. The UAV flight was done in areas having spatial variability so that the results could be validated much more accurately. The UAV flight requires an area of interest (AOI) with required ground sample distance (GSD), and the platform for control stations is provided in the ground with the help of a remote controller showing real-time data such as position waypoints, altitude, speed, and distances. DJI Matrice 600 hex copter UAV is chosen for the study having flying endurance of 50-60 minutes with a range of 5-10 km. The UAV flight was done at an altitude of 100 m, covering a range of 5-6 km. A low weight UAV of 5 kg is used in the following study with a multispectral sensor named Parrot sequoia. A multispectral sensor with four 1.2 panchromatic bands such as Green (550 nm), Red (660nm), Red edge (735nm), and NIR (790nm) is used. Absolute GPS coordinates were utilized in this study to obtain mosaic. A tiff file has been utilized to process Arcgis and further, Pix 4Dsoftware was used to transform the multispectral images into ortho mosaic data and 3D map. However, in our study, we have directly used the RGB images obtained from UAV. More than 200 images have been used resulting from UAV flight. These RGB images form the basis of our study in canopy extraction.

Data pre-processing and dataset preparation

In the UAV survey, we have captured very high resolution (4000×3000 pixels) images of various locations. Each survey results in 100-150 such images. These images are used to generate orthomosaic of the areas as there is no public dataset available for drone imagery with ground truth suitable for our task, so we have generated our dataset. Our training dataset consists of 800 images and manually annotated ground truth. We divided them into two sets: The training and validation sets. The training set consists of 500 images, and the validation set consists of 300

images. We intentionally choose dissimilar validation set images. These results in more generalized learning of trained models and avoid overfitting. As processing on that significant scale is complicated, we divided every image into patches of 512×512 pixels. We trained our model with these patches. For the testing dataset, we selected our study area orthomosaic image other than the training dataset and divided it into patches of 512×512 pixels. In this way, we got 200 images in our test dataset.

We trained our model without using any pertained weight. Our earlier approaches to use transfer learning with Resnet [10] pre-trained weight failed as it always results in non-convergence of learning. So we initialize our network weight randomly. We train our network for 500 epochs. We trained our network on a 6 GB Nvidia graphics card. In training to avoid our model to overfit, we use a drop out of 20% in each layer. We have used data augmentation like rotation and brightness change in the original image to generalize learning.

Model Architecture

We have used CNN architecture influenced by the Encoder-Decoder network. Each encoder has a corresponding decoder in this network and is followed by the final pixel-wise classification layer. Our network uses a VGG style encoder-decoder (Simonyan at al., 2014) where upsampling in the decoder network has been done using transposed convolutions. In addition, it employs additive skip connections from the encoder to the decoder.

In the encoder path, we perform the down sampling of the image using the max pooling operation. Decreasing the size of the image is required as we have a limited capacity of GPU resources. This operation also creates multi-scale features of the image.

There is a hierarchy of decoders for each encoder in a decoder network. Decoder used transposed convolution for upsampling the image, followed by a series of convolution operations and non-linearity. There are several interpolation methods for upsampling, e.g., nearest neighbor or bilinear. However, we have used transposed convolution to have learnable parameters instead of predefined parameters. For training, we have used manually generated ground truth on aerial images.

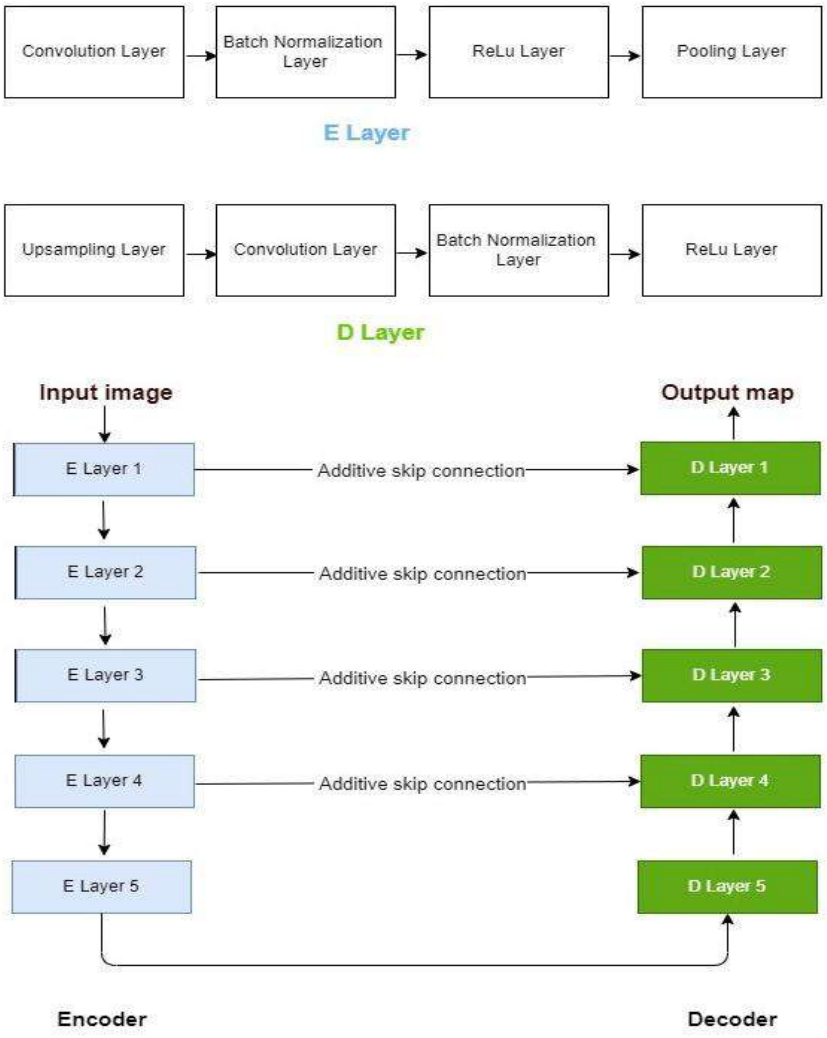


Figure 2: A figure showing network architecture

Results and Discussion:

We have used two matrices-Accuracy and Intersection over union (IOU) to easure the performance of our model.

The matrices calculated are as such:

$$\text{Accuracy} = \frac{(\text{TP} + \text{TN})}{(\text{TP} + \text{TN} + \text{FP} + \text{FN})}$$

TP, TN, FP, and FN represent several true positives, true negatives, false positives, and false negatives

$$IOU = \frac{\text{Area of overlap}}{\text{Area of union}}$$

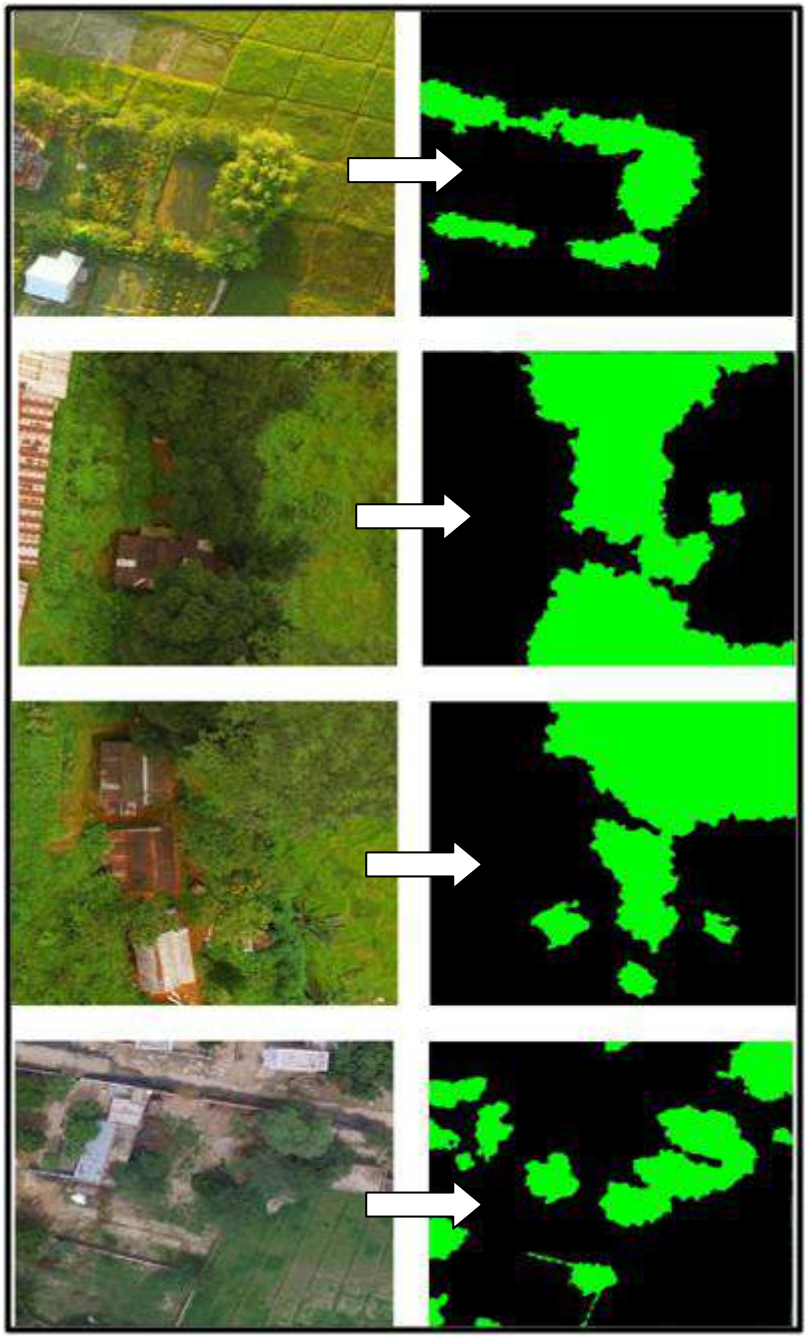


Figure 3: Input RGB (left) and generated output (right) showing canopy extraction

Our model has achieved 86.02 % accuracy and 77.3% IOU on training datasets. This study shows how efficiently green cover can be discriminated against and tree canopy cover extracted. The algorithm used in the study enables us to do large-scale forest canopy mapping and to use it in forest monitoring applications further. Various studies have been done regarding the use of UAVs in various fields. The use of unmanned aerial vehicles (UAV) has been rapidly increasing reliability, flexibility, low-cost maintenance, and accessible flight handling. The data

generated from UAVs help study various features due to their high-resolution data. Also, the time taken to generate the data is significantly less and user friendly. UAV's have applications ranging from aerial imaging to research and development. It covers almost all fields ranging from surveying and mapping to precision agriculture. The main drawback of satellite imagery is that it lacks coarse ground resolution. Nevertheless, UAV imagery allows us to overcome the drawback of resolution and provides an excellent resolution for ground study.

Conclusion:

The objective to extract canopy cover of a varied range and differentiate it effectively through deep learning algorithms has been achieved. UAV survey in a region like northeast India having difficult terrain accessibilities is greatly improved by UAV-RS. Detailed canopy structure analysis can lead us to know the structural dynamics of forest cover and help us evaluate forest loss. Canopy cover gives us estimates of stock in an area. Further tree density can also be determined and its changes due to various natural and anthropogenic events. Deep learning has been used in one way or another in various fields of object identification. However, it has been limited in use in the field of forestry.

Further, only a few data have been documented on tree crown extraction using deep learning. The periodic acquisitions of UAV imagery are used to estimate the forest's biophysical properties. Remote sensing has high potential in forestry, and it has been widely used over the last few decades. The study can further be enhanced by including the NIR band for model training.

References:

BL B., S. N., and R. Hebbar, (2018): Tree Crown Detection and Extraction from High Resolution Satellite Images in an Urban Area, International Conference on Design Innovations for 3Cs Compute Communicate Control (ICDI3C): Bangalore, 8-11, 10.1109/ICDI3C.2018.00011.

Disney M, Calders K, (2018): Extracting individual trees from lidar point clouds using trees, 10.1111/2041-210X.13121.

He K., X. Zhang, S. Ren, and J. Sun, (2016): Deep Residual Learning for Image Recognition, IEEE Conference on Computer Vision and Pattern Recognition (CVPR): Las Vegas, NV, 2016, 770-778, 10.1109/CVPR.2016.90.

Krizhevsky, Alex and Sutskever, Ilya and Hinton, Geoffrey, (2012): ImageNet Classification with Deep Convolutional Neural Networks, Neural Information Processing Systems, 25. 10.1145/3065386.

La, HP, Eo, Y.D., Chang, A. (2015): Extraction of individual tree crown using the hyperspectral image and LiDAR data, KSCE J Civ Eng 19, 1078–1087.

Long, Jonathan and Shelhamer, Evan and Darrell, Trevor, (2015): Fully convolutional networks for semantic segmentation, 3431-3440. 10.1109/CVPR.2015.7298965.

Marilia Ferreira Gomes, Philippe Maillard, (2015): Detection of Tree Crowns in Very High Spatial Resolution Images, 10.5772/62122.

Ronneberger, Olaf and Fischer, Philipp and Brox, Thomas, (2015): U-Net: Convolutional Networks for Biomedical Image Segmentation, LNCS. 9351. 234-241. 10.1007/978-3-319-24574-4_28.

Sara A. Goeking, Greg C. Liknes, Erik Lindblom, John Chase, Dennis M.Jacobs, and Robert Benton, (2012): A Gis-Based Tool For Estimating Tree Canopy Cover On Fixed-Radius Plots Using High-Resolution Aerial Imagery, Forest Inventory and Analysis (FIA): symposium, Baltimore, U.S. Department of Agriculture, Forest Service, Northern Research Station, 237-241.

Simonyan, Karen, and Andrew Zisserman, (2014): Very deep convolutional networks for large-scale mage recognition. arXiv preprint arXiv:1409.1556.

Whiteside, Tim and Boggs, Guy and Maier, Stefan, (2011): Extraction of Tree Crowns from High-Resolution Imagery over Eucalypt Dominant Tropical Savannas, Photogrammetric Engineering and Remote Sensing. 77. 813-824. 10.14358/PERS.77.8.813.

COELASTRACEAE AND *SCENEDESMACEAE* FROM BANGANGA WATER RESERVOIR

Prakash B. Jadhavar

Department of Botany,

Smt. S.K. Gandhi Arts, Amolak Science and

P. H. Gandhi Commerce College,

Kada, Tq. Ashti, Dist. Beed 414 202

Corresponding author E-mail: p.bjadhavar@gmail.com

Abstract:

Osmanabad district is part of the Godavari River Basin in Maharashtra, whereas Manjra, Sina, Terna, Bori, Benitura and Banganga are the main rivers which are flowing through the district. The author has earlier described the algal taxa belonging to Chlorophyceae, and cyanophyaceae from the Banganga project of Osmanabad district in Maharashtra. Present paper deals with Coelastraceae and Scenedesmaceaeasthe addition of taxa from the study area. During the present study, the algal members of Chlorophyaceae were noted from the study area and presented in the present communication.

Keywords: Chlorophyaceae, Algae, Flora, Banganga.

Introduction:

The Banganga Project is built up on Banganga River near Songiri and Bhongiri village of Bhoomtehsil in Osmanabad district. It is mainly used for irrigation and drinking water purpose. The study of the algal flora of the water reservoir was carried out to explore the diversity of algae of the water reservoir. The algal structure ranges from unicellular mucilaginous colonies to multicellular compact forms which show considerable diversity in form and adaptation to their distinctive environment (Krishnamurthy, 2000). During the present study, the Coelastraceae and Scenedesmaceae algal taxa were observed.

Materials and Methods:

The random sampling technique has been used for the collection of algal samples at the Banganga Project. Collections were made for 2 consecutive years (2016-2018), from October to

January. The algal samples were preserved in 4% formalin. Identification of taxa was carried out by using Prescott (1951), Pal *et al.* (1962), Philipose (1967), Patel and Satyanarayan (1976), Patel and Satyanarayan (1976), Prasad and Misra (1992), Hajarika and Datta (1994), Krishnamurthy (2000), Hosmani (2010) and other relevant literature.

Results and Discussion:

During the study, 10 taxa of Coelastraceae and Scenedesmaceae were observed which are described as under.

Family: Coelastraceae

Genus: *Coelastrum* Naegeli

The colonyis usually a hollow sphere, rarely polygonal to pyramidal, and of 4 to 32 or more cells. Cells spherical, ovoid or pyramidal are closely adjoined and compressed or interconnected by narrow processes to form small or large- intercellular spaces. A cell wall is composed of an inner cellulose layer and an outer pectic layer which is often locally thickened to form polar outgrowths or lateral processes that connect the cells. Chloroplasts arecup-shaped to diffuse and with a pyrenoid.

1) *Coelastrum cambricum* Archer var. ***intermedium*** (Bohlin) G.S.West

Prasad and Misra, 1992, p 30, pl 4, f 5

Colonies spherical, consisting of 32 cells; cells spherical in middle and sub- spherical at periphery with slightly thick, blunt and rounded projections, intercellular spaces more or less triangular; chloroplast parietal with one pyrenoid. . Colony 62.5 µ in diameter, cells 15 µ.

2) *C. microporum* Naegeli

Philipose, 1967, p 228, f 135 (a)

Colonies more or less spherical and of 8-16 cells with small intercellular spaces. Cells soherical to ovoid, enclosed by delicate gelatinous sheaths and interconnected by almost imperceptible gelatinous processes. Cells with sheath 6.25 µ in diameter. Colonies 35 µ in diameter.

3) *C. proboscideum* Bohlin.

Prasad and Misra, 1992, p 31, pl 4, f 7

Colonies pyramidal or cubical consisting of 16 cells; cells truncatel conical, enclosed by a gelatinous sheath and joined along lower lateral walls, cell wall slightly thickened at poles; Chloroplast single, parietal with one pyrenoid. Colony 25 µ in diameter, cells 10 µ.

4) *C. scabrum* Reinsch

Philipose, 1967, p 231, f 140 (a)

Colony 16 celled, less spherical. Cells angular globose with three or morewart-like truncate processes from the outer surface. Cells 10 µ in diameter, colony 27.5 µ.

Family: Scenedesmaceae

Genus: *Crucigenia* Morren

Colony free-floating, consisting of a plate of 4 to 16 trapezoid or rhomboid cells lying in one plane about a small or large central space; chloroplast a parietal plate with a one pyrenoid in each cell; colony enclosed by a thin, inconspicuous gelatinous envelope which often causes the families to adhereto one another and to form colonial complexes.

5) *Crucigenia tetrapedia* (Kirchner) W.et G.S.West

Philipose, 1967, p 240, f 151 (a)

Colony 4 celled, quadrate with a minute rectangular space at centre. Cells flattened and triangular with rounded ends. Outer side of cells always concave. Cells 10 µ.

Genus: *Scenedesmus* Meyen

Colony flat (rarely curved) plate of usually 2 to 8 (rarely 16 to 32) cells which are always in multiples of two. Cells acicular, ellipsoid, ovoid or cyllendrical, arranged in one or two rows and in lateral contact. Cell wall smooth or granulate, with or without lateral ridges, lateral teeth or spines. Chloroplast single and parietal and often filling the cell, and with a single pyrenoid.

6) *Scenedesmus acutiformis* Schroeder

Philipose, 1967, p 260, f 169 (a)

Colony 4 celled. Cells cylindrical fusiform and arranged in a single linear series. Cell wall smooth. Median cells with a lateral longitudinal ridge extending from pole to pole on each

side. Terminal cells with two or four ridges. Poles of cells acute and without teeth or spines, but sometimes with a minute papilla. Cells 7.5 µ broad, 15 µ long.

7) *S. arcuatus* (Lammermann) Lammermann

Philipose, 1967, p 256, f 166 (d)

Colonies 8 celled, curved and with small intercellular spaces.Cells in eight celled colonies in two series, ablong avoid, sometimes slightly angular at the base due to mutual pressure. Cell wall smooth, without teeth or spines. Cells 7.5 µ broad, 17.5 µ long.

8) *S. armatus* (Chodat) G.M.Smith var. ***major*** G.M.Smith

Philipose, 1967, p 266, f 171 (k)

Colonies Four celled. Cells oblong, ellipsoid and arranged in linear series. Terminal cells with a single long spine from each pole. All cells with a median lateral longitudinal rib which is sometimes indistinct. Four celled colony 10 µ broad, 20 µ long. Cells 7.5 µ broad, 25 µ long.

9) *S. armatus* (Chodat) G.M.Smith var. ***bicaudatus*** (Guglielmetti) Chodat

Philipose, 1967, p 262, f 171 (e)

Colony 2 celled. Differs from the type in having a long spine from one of the poles of the terminal cell only, the spines of the two terminal cells alternating with each other. Cells 5 µ broad, 12.5 µ long, and spine 7.5 µ long.

10) *S. bijuga* (Turp.) Lagerheim

Prescott, 1951, p 276, pl 63, f 2

Colony composed of 4 cells in a single flat series; cells ovate or oblong, without teeth or spine, cells 5 µ in diameter, 12.5 µ long.

References:

Hajarika, A.K. and A.C. Datta, (1994): Limnological studies of two fresh water ponds of Guwahati. Assam. Env. Eco.12 (1): 26-29

Hosmani P. Shankar (2010): Phytoplankton divesity in lakes of Mysore District Karnataka State, India. The Ecoscan 4 (1): 53-57.

Krishnamurthy, V. (2000): Algae of India neighboring Countries I, Chlorophycota- Oxford and IBH Publishing Co. Pvt. Ltd.

Pal, B.P., Kundu B.C., Sundaralingam V.S. and Venkataraman, G.S. (1962): Charyophyta. I.C.A.R., New Delhi.

Patel, R.J. and K. Satyanarayan (1976): Desmids of Pachmarhi (M.P.):, India. Vidya.J. Guj. Univ. 19: 55-63.

Patel, R.J. and K. Satyanarayan (1976): Desmids of Pachmarhi (M.P.):, India. Vidya.J. Guj. Univ. 19: 55-63.

Philipose, M.T. (1967): Chlorococcales, Indian Council of Agricultural Research, New Delhi.

Prasad, B.N. and Misra, P.K. (1992): Fresh water algal flora of Andman and Nicobar Islands, Vol. II, Bishen Singh Mahendra Pal Singh, Dehra Dun.

Prescott G.W. (1951): Algae of the Western Great Lakes Area, Cranbrook Institute of Science, Michigan.

PHYTOCHEMICAL AND ANTIMICROBIAL ANALYSIS OF *TECOMA STANS* FROM PARBHANI DISTRICT

Arshia Parveen
Department of Chemistry
B. Raghunath ACS College, Parbhani, M. S. India
Corresponding author E-mail: arshiairfanmalik@gmail.com

Abstract:

Tecoma Stan is a well-known medicinal plant. The Plant metabolites are the promising choice over modern synthetic drugs for its minimum side effects and remedy for several diseases. The medicinal values of the phytochemicals have remarkable biological activities such as antimicrobial, antioxidant, anti-inflammatory, antidiabetic and wound healing etc. *Tecoma stans* is a flowering perennial shrub belonging to the family Bignoniaceae was subjected to phytochemical screening.The present investigation revealed that the methanolic and ethanolic extract has maximum amount of phytochemical and proved to be highly antimicrobial therapeutic activity.

Keywords: *Tecom stans*, phytochemical screening, therapeutic activity, biological activities

Introduction:

Natural phytochemicals from plant sources either as pure compound or as standardized extracts provide unlimited availability of new drug leading to challenges in the medical field [1]. According to the World Health Organization (WHO) nearly 20,000 medicinal plants exist in 91 countries [2], antifertility [3], preparation of dyes [4] etc. *Woodfordia fruticosa* is known for its medicinal value and used extensively in Atavas and Arishtas for self-generation of alcohol [5] and they are commonly used for treatment of rheumatism, leucorrhea, liver disorder, asthma and inflammatory action [6]. Traditionally, the crude extracts of various parts of the plant have taken a momentum since 1990s [7], food and feed [8] and curative agents [9, 10]. *Tecoma stans* or yellow bells from family Bignoneacea. It is a semi evergreen ornamental tropical shrub which is used traditionally for reducing blood glucose. Almost all the parts of *Tecoma stans* are of medicinal importance and used traditionally for the treatment of various ailments. The plants leave, bark and root have been used as a muscle relaxant mild cardio tonic and of chloretic

activities. As pharmacological uses *Tecoma stans* have been used as herbal medicine treatment for diabetes [11], Digestive problems, controls of yeast infection as powerful diuretic, vermifuge and tonic [12]. Flower and leaves have some medicinal value for the treatment of various cancer [13]. Its leaves are used traditionally in Mexico to control Diabetes [14]. Hence an attempt is made in the present study to analyze the functional group of phytoactive compounds present in the flower extract in ethanol of *Tecoma stans.*

Pharmacological activities:

Antioxidant activity:

Springob *et al.* reported Antioxidant activity and both phenolic compound and flavonoid total content were determined for callus tissue of *T. stans* cultured in either a set photoperiod or in darkness [15].

Anti-Inflammatory activity:

Sawapna Chaudhary *et al* evaluated the anti-inflammatory activity of chloroform root extract of *Tecoma stans*. Chloroform extract was analyzed for anti-inflammatory activity against carrageenan-induced paw edema method in Wistar albino rats [16].

Antidiabetic Activity:

Tecoma stans aqueous extract (TAE) of leaves is widely used as a traditional antidiabetic remedy in Mexico. Tecomine was shown to be one of the compounds responsible for the hypoglycemic action. Aguilar-Santamaria et al evaluated in vivo and in vitro intestinal α-glycosidase inhibition as the possible mode of action of TAE on type 2 diabetes mellitus (DM2) animal models [17].

Wound healing activity:

Das *et al.* evaluated the methanolic bark extract of Tecoma stan linn for wound healing activity in albino rats.Administration of methanol extract of the bark has shown more significant wound healing activity in excision and incision wound models and support the popular use of plant to open wound in folk medicine [18].

Materials and Methods:

Tecoma stans is a herbal medicine used for treatment of diabetes, digestive problems, control of yeast infections, as powerful diuretic, vermifuge and tonic. Preliminary phytochemical screening of this plant revealed the presence of tannins, flavonoids, alkaloids, quinones and traces of saponins and amino acids.

Collection of plant material:

The *Tecoma stans* plant was collected in the month of November 2021 from B. Raghunath College Surrounding area, Dist- Parbhani. Authentication of the plant material was made by Dr. Sunil Modak, Professor in Dept. of Botany, B.Raghunath College, Dist- Parbhani. The botanical classification is

Kingdom: Plantae

Unranked: Angiosperms

Unranked: Eudicots

Unranked: Asterids

Order: Lamiales

Family: Biognoniaceae

Genus: *Tecoma*

Species *stanus*

Figure 1: Sample of *Tecoma stans*

Preparation of extract:

The leaf and stem where detached and dried in shade. About 100 grams of dried leaf and flowers were grinded sieved to powder. A known quantity of powdered leaf and stem were subjected to extraction with two different solvents methanol and ethanol. Later the extracts were filtered with Whatsmann filter paper No. 1 and subjected to various phytochemical analyses [19, 20] as per standard.

Figure 2: Preparation of extract *Tecoma Stan* stem and leaves

Results and Discussion:

The result obtained in the phytochemical screening of the *Tecoma stans* plant (Table 1 and2) alcoholic extracts of the leaf and stem showed the presence of most of the secondary metabolites studied flavonoids, tannins, saponins, phenolic compounds, alkaloids, terpenoids, steroids and glycosides which would be the active principle of the plant. Plant phenolic compounds flavonoids, alkaloids, tannins, saponinsfrom plant extract and plant-based steroids have been found to possess potent antioxidantand antimicrobialand antidiabetics' properties.

Table 1: Phytochemical analysis of the ethanolic extract of *Tecoma stans*

Sr. No.	Phytochemicals	ethanolic extract of *Tecoma stans* leaf	ethanolic extract of *Tecoma stans* stem
1	**Flavonoids**	+	+
2	**Tannins**	-	-
3	**Saponins**	+	+
4	**Phenolic gr.**	-	+
5	**Alkaloids**	+	+
6	**Terpenoids**	-	+
7	**Steroids**	+	+
8	**Glycosides**	+	+

Table 2: Phytochemical analysis of the methanolic extract of *Tecoma stans*

Sr. No.	Phytochemicals	Methanolic extract of leaf	Methanolic extract of stem
1	**Flavonoids**	+	+
2	**Taninins**	+	+
3	**Saponins**	+	+
4	**Phenolic gr.**	+	+
5	**Alkaloids**	+	+
6	**Terpenoids**	+	+
7	**Steroids**	+	+
8	**Glycosides**	+	+

Antimicrobial activity:

The methanol extracts of the leaves and stem bark of Bignoniaceae Linn *Tecoma stans* was studied for their antimicrobial activity using a wide range of Gram-positive and Gram-negative bacteria. It was observed that the extracts of stem bark generally showedbetter antimicrobial activity than those of the leaves and some organisms were selectively more sensitive to the extracts than others [21].The antimicrobial efficacy of was determined by agar disc diffusion method [22, 23]. To check the susceptibility pattern of against selected bacteria nutrient agar medium was inoculated with specific organisms.The different dilutions of the extracts are made and by disc method followed from standard procedure we measured the antimicrobial study of the *Tecoma Stans.* The extract of plant is found to be antimicrobial active and it is measure against *Bacillus Subtalis*, *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa* by zone of clearance in mm. It is observe that the extract shows good antimicrobial activity.

Table 3: Antimicrobial study

Sr. No.	Micrrganism	Leaf Extract	Stem Extract
1.	***Escherichia coli***	2 mm	2 mm
2.	***Staphylococcus aureus***	1 mm	1.5 mm
3.	***Bacillus subtilis***	3 mm	3.5 mm
4.	***Pseudomonas aeruginosa***	2 mm	2.5 mm

Figure 3: Zone of Clearance for measurement of Antimicrobial activity

Conclusion:

The present study was carried the phytoconstituents followed by the antimicrobial activity of extract of *Tecoma stans*. The study leads to a broader perspective for industries in usage of a new class of preservative agent for good pharmacological and therapeutic agents.

Further studies are crucial towards isolation, identification, purification, characterization and biological activity of specific bioactive compounds of this plant for industrial drug formulation, identification, activity of specific bioactivecompounds of this plant for industrial drug formulation.

References:

1. Saidharan S, Chen Y, Yoga and Latha L (2014): Extraction, isolation and characterization of bioactive compounds from plant extracts. African Journal of Traditional Complementary and Alternative Medicines. 2014: 1 – 16.
2. Lewis WH and Elvin Lewes MPF. (1997): Medicinal Botany, Plants affecting Mans Health. John Wiley and Sans. Newyork. 515 – 517.
3. Jigna Paretch and Sumitra Chanda (2007): Brazailian Journal of Microbiology. 38: 204 207.
4. Ram P, Rastogi and Mehrotta BN. (1970): A compendium of Indian medicinal plants. Publication and information Directorate (CSIR), New Delhi. 2: 710 – 712.
5. Finose A and Devaki K, (2011): Phytochemical and Chromatographic studies in the flowers of Woodfordia fructicosa (L) kurz. Asian Journal of Plant Science and Research. 1(3): 81 – 85.
6. Heeshma Kushalani, Pratima Tattee and Kamalinder Singh (2006): Indian Journal of Pharmaceutical Science. 528 – 529.
7. Cowam MM. (1999): Plant products as antimicrobial agents. Clinical Microbial Rev. 12: 564 – 582.
8. Moerman DE. (1996): An analysis of the food plants and Drug plants of native North America. Journal of Ethnopharmacology. 52: 1 – 22.
9. Anand M, Basavaraju R. (2021): A review on phytochemistry and pharmacological uses of Tecoma stans (L.) Juss. ex Kunth. J Ethnopharmacol. 30;265:113270
10. Archana Singh, Nagori B.P., Kumkum Mathur (2013): Tecoma stan: An Important Medicinal Plant. Int. J. Pharma. Erud. 3(2), 13-21
11. Angel Josabad Alonso-Castro , Rocio Zapata-Bustos, José Romo-Yañez, Paul Camarillo-Ledesma, Maricela Gómez-Sánchez, Luis A Salazar-Olivo (2010): The antidiabetic plants Tecoma stans (L.) Juss. ex Kunth (Bignoniaceae) and Teucrium cubense Jacq (Lamiaceae) induce the incorporation of glucose in insulin-sensitive and insulin-resistant murine and human adipocytes. J Ethnopharmacol., 2010 Jan 8;127(1):1-6

12. Gupta, V., Umesh Dhaked , Ratohor, D.S., Chaudhary, A., (2010): J. Natural Consciental, 1: pp.112-116.
13. Kirtikar, K.R. , Basu B.D. (1980): Indian Medicinal Plants: Dehradun (Bishen Singh , Mahendra Pal Singh) 2nd edn. pp. 1366-1368
14. Costantino, L., Raimondi, L. , Parisino , R. Bruniti , T., Pessotto, P.; (2003): I. Pharmaco. 58: pp. 781-785
15. Kunth J. E. (2009): Journal of ethnopharmacology. 124: 284-8.
16. Chaudhary S. Cellular and molecular immunology and immunotherapy. 2009: 37-9.
17. Aguilar Santamaria L., Ramirez G., icasio P., Alegria-Reyes C.,HerreraArellano A. Journal of Ethanopharmacology. 2009; 24:284-288.10
18. Das C., Dash S., Sahoo D. C., Mohanty A. (2010): An evaluation of methanolic bark extract of Tecoma stan linn, for wound healing in albino rats. International Journal of Pharmacy and Technology. Volume II.735-42.
19. Ram P, Rastogi and Mehrotta BN. (1970): A compendium of Indian medicinal plants. Publication and information Directorate (CSIR), New Delhi. 2: 710 – 712.
20. Devika R and Justin Kovilpillai. (2012): Phytochemical screening of studies of bioactive compound of Tagetes erecta. International Journal of Pharma and Biosciences 3(4): 596.
21. Ghaddam M., Bahadoram S. Daru. (2007): 15 (3): 123-8.
22. Bauer, AW., Kirby, WMW., Sheries, JC. (1966): Antibiotic susceptibility testing by a standardized single disc method. Am J Clin Pathol. 45: 493-6.
23. Sowjanya, P., Srinivasa, B., and Sri Devi, B. (2015): Preliminary phytochemical and antibacterial investigations of the methanol extract of Alternanthera philoxeroides (Mart.) Griseb. European journal of pharmaceutical and medical research. 2(4): 347-356.

ECONOMIC IMPORTANCE OF PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR)

Kahekashan Tanveer Mirza

Department of Botany,

Yeshwant Mahavidyalaya, Nanded 431 602

Corresponding author E-mail: kahekashan10@gmail.com

Plant growth promoting rhizobacteria (PGPR) are a collection of bacteria that strongly colonize plant roots and enhance plant growth and yield (Wu *et al*., 2005). The mechanisms by which PGPR promote plant growth are not completely understood, but are considered to consist of the capacity to produce phytohormones against phytopathogenic microorganisms through the production of siderophores (Sharoona *et al*., 2006; Egambardiyeva, 2007) and the synthesis of antibiotics, enzymes and/ or fungicidal compounds (Ahmad *et al*., 2006). Considerable increase in growth and yield of agronomical important crops in response to inoculation with PGPR has been reported (Biswas *et al*., 2000; Asghar *et al*., 2002). *Pseudomonas* strains could affect seed germination and seedling growth (Shaukat *et al*., 2006). Strains of *Pseudomonas putida* and *Pseudomonas fluorescens* were reported to increase root and shoot elongation in canola (Glick *et al*., 1997). Therefore, it has been shown that *Pseudomonas* has the potential for agriculture utilization and could be used as biofertilizers (Cakmakc *et al*., 2006). Fluorescent *Pseudomonas*, capable of multiple mechanisms for biocontrol of phytopathogens and plant growth promotion, are being used widely (Pierson and Weller, 1994; Banasco *et al*., 1998; Dileep *et al*., 1998) as they produce a wide variety of antibiotics, chitinolytic enzymes, growth promoting hormones, siderophores, HCN and catalase. The use of PGPR has become a common employ in many regions of the world. Larger use of PGPR is possible in agriculture for biocontrol of plant pathogens and biofertilization (Siddiqui, 2006; Lugtenberg and Kamilova, 2009; Das *et al*., 2010; Saharan and Nehra, 2011). The bacterial strains isolated from *Lolium perenne* rhizosphere are capable of acting as plant growth promoting bacteria and show various plant growth promoting activities (Shoebitz *et al*., 2007)

Pseudomonads are Gram-negative bacteria comprising chemoheterotrophs predominantly inhabiting the soil and having versatile functions. They are potential colonizers of rhizosphere of many crops like cereals, pulses, oilseeds and vegetables (Johri *et al.,* 1997). Both fluorescent and

non-fluorescent species constitute the genus *Pseudomonas.* The fluorescent species produce diffusible yellow-green pigment and fluoresce under low-wavelength UV radiation.

The Marathwada region is well known for its hot summer and general dryness throughout the year except during the monsoon season. The Marathwada region comprises eight districts i.e. Aurangabad, Beed, Hingoli, Jalna, Latur, Nanded, Osmanabad and Parbhani. It is situated between 17.5° to 20.5° latitude and 75.5° to 78.5° longitude and at an average height of about 300 m above mean sea level (MSL). It is one of the four divisions of Maharashtra State. The main river Godavari of the region flows from west to east collect waters from several large and small tributaries. In spite of these water resources, the region suffers from heavy drainage due to west-east slope ultimately affecting the agriculture to a considerable extent. Some of the important cereal crops of the region are Bajra - pearl-millet (*Pennisetum typhoides* Burm.), Jowar (*Sorghum vulgare* pers.), Maize - corn (*Zea mays* L.,) and Wheat (*Triticum aestivum* L.)

Wheat is widespread and significant staple food crops for the people of the India it grown on about 26 million hectors which is about 20 per cent area under cereal sand the annual production is about 69 million tonnes of grain (Rangaswami and Mahadevan, 1999).

Safflower is one of the oilseeds of India, china, Egypt and other Far Eastern countries. In India it is grown in Maharashtra, Madhya Pradesh, Andhra Pradesh, Karnataka, Tamil nadu and other states and total area under this crop is about 2.3 lakh hectors (Rangaswami and Mahadevan, 1999).

In the biogeochemical cycles of both inorganic and organic nutrients in the soil and in the preservation of soil health and quality, soil micro-organisms are very important (Jeffries *et al.,* 2003). The use of some PGPR as growth promoter and natural bio-control has been developed and commercialized (Dey *et al.,* 2004; Herman *et al.,* 2008; Minorsky, 2008). Biological control of plant diseases is gaining attention due to increased pollution concerns because of pesticides use for crop protection and the consequent development of pathogen resistance to such chemicals.

Soil borne pathogens have disturbing effect on plant health and yield. For successful disease Management it is important to found the most helpful and economical ways to protect the plant from various pests or diseases (Zahir *et al* 2004). In recent years, the use of PGPR as an inducer of systemic resistance in crop plant against different pathogen has been verified under field condition (Wei *et al.*, 1991 and 1996; Vidhayasekharan and Muthamilan, 1999; Viswanathan and Samiyappan, 1999).

Genetic analysis has shown a large diversity of soil bacteria, while the individuals have the low culturability. Bacterial species identification is traditionally based on functions rather than genetics, and thus agricultural and natural soil bacterial communities are to a large extent composed of unknown species. These are a reservoir of undiscovered genetics resources for mankind and merits protection in them. Molecular and biochemical techniques of estimating abundance and number of each species are mean of approaching an understanding of the community composition and diversity of soil bacterial communities at the genetic level.

The indirect effect of PGPR on plant growth is exerted by preventing harmful effects of plant pathogens by the production of secondary metabolites including HCN (Owen and Zlor, 2001), ammonia, antibiotics, and volatile metabolites. Plant growth promoting rhizobacteria actively colonize in plants rhizospHere and avoid the deleterious effects of phytopathogens (Rangajaran *et al*., 2003; Saikia *et al*., 2005). Biological control of a large array of pHytopathogens by the induction of systemic resistance has received much importance in last few decades (Jetiyanon *et al*., 2003). The ability of the antagonistic rhizobacteria is highly influenced by their morphological characteristics to inhibit the pathogens. High morphological activities results in production of more

Secondary metabolites to suppress the pathogens. The diversity of rhizobacteria in the suppressive soils is high as compared to conductive soil. In this respect the suppressive soil provide more chances to screen the antagonistic microbe that can be a potent biocontrol agent (Garbeva *et al*., 2004) Fluorescent *Pseudomonads* have emerged as most potential group implicated for promoting plant growth and biological control of plants diseases. *Pseudomonas fluorescens* are reported antagonist to various plant pathogens and also recommended for seed dressing.

References:

Ahmad, F., Ahmad, I., Khan, M.S. (2006). Screening of free-living rhizosheric bacteria for their multiple plant growth promoting activities. Microbiology Research, 36, 1-9.

Asghar, H. N., Zahir, Z. A., Arshad, M. and Khaliq, A. (2002). Relationship between in vitro production of auxins by rhizobacteria and their growth promoting activities in Brassica juncea. L. Biology and Fertility of Soils, 35, 231–237.

Banasco, P., Fuente, L., De, La., Gaultieri, G., Noya, F., Arias, A. (1998). *Fluorescent Pseudomonas* sp. as biocontrol agents against forage legume root pathogenic fungi. Soil Biology and Biochemistry, 10(10-11), 1317-1323.

Biswas, J. C., Ladha, J.K. and Dazzo, F.B. (2000). Rhizobia inoculation improves nutrient uptake and growth of lowland rice. Soil Science Society of America Journal, 64: 1644–1650.

Cakmakc, R. I., Aydin, D. F., Sahin, A. F. (2006). Growth promotion of plants by plant growth promoting rhizobacteria under green house and two different field soil conditions. Soil Biology and Biochemistry, 38, 1482-1487.

Das, N., Schneider, D., Chen, and Smith N. A. (2010). SEMAFOR 1.0: A probabilistic frame-semantic parser. Technical Report CMU-LTI-10-001, Carnegie Mellon University.

Dey, R., Pal K. K., Bhatt D. M., Chauhan S. M. (2004). Growth promotion and yield enhancement of peanut (*Arachis hypogaea* L.) by application of plant growth-promoting rhizobacteria. Microbiol. Res., 159: 371-394.

Dileep, C., Kumar, B., Dileep S., Dube H. C. (1998). Promotion of plant growth and yield by two rhizoplane fluorescent *Pseudomonads.* Indian. J Exp Biol., 36:399-402.

Egambardiyeva, D. (2007). The effect of plant growth promoting bacteria on growth and nutrient uptake of maize in two different soils. *Applied Soil Ecology* 36, 184-189.

Herman, M. A. B., Nault, B. A., Smart, C. D. (2008). Effects of plant growthpromoting rhizobacteria on bell pepper production and green peach apHid infestations in New York. Crop Protect. 27: 996-1002.

Garbeva, P., Veen J. A. V., Elsas J. D.V. (2004). Microbial diversity in soil: selection of microbial populations by plant and soil type and implications for disease suppressiveness. Annu. Rev. Phytopathol., 42: 243–270.

Glick BR, Liu C, Ghosh S, Dumbroff .EB, (1997) Soil Bio. Biochem, , 29.Characterization of plant growth-promoting traits of bacteria isolated fromthe rhizosphere of grapevine grown in alkaline and acidic soils.

Jeffries, P., Gianinazzi, S., Perotto, S., Turanu, K. and Barea, M. (2003). The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. Biology and Fertility of Soils, 37: 1-16.

Jetiyanon, K., Fowler, W. D., Kloepper, J. W. (2003). Broad-spectrum protection against several pathogens by PGPR mixtures under field conditions in Thailand. Plant Dis., 87:1390-1394.

Johri, B. N., Rao, C. V. S. and Goel, R. (1997), Fluorescent pseudomonads in plant disease management. In : Biotechnological Approaches in Soil Microorganisms for Sustainable Crop Production, Ed. Dadarwal, K. R., Scientific Publishers, Jodhpur, India, pp. 193-221.

Minorsky P.V. (2008). On the inside. Plant Physiol., 146: 323-324.

Owen, A., and Zlor, R. (2001). Effect of cyanogenic rhizobacteria on the growth of velvet leaf (*Abutilon theopHrasti*) and Corn (*Zea mays*) in autoclaved soil and the influence of supplemented glycine. Soil Biochem., 33: 801-809.

Rangaswami. , G. Mahadevan A. (1998) Diseases of Crop Plants in India. Edition, 4. Publisher, Prentice Hall India,

Rangajaran S., Saleena L. M., Vasudevan P., Nair S. (2003). Biological suppression of rice diseases by *Pseudomonas* spp. under saline soil conditions. Plant Soil, 251:73–82.

Saharan, B. S., Nehra, V. (2011). Plant growth promoting rhizobacteria: a critical review. *Life Sci Med Res* 2011: LSMR-21.

Saikia R., Singh, B. P., Kumar R., Arora, D. K. (2005). Detection of pathogenesis related Proteins-chitinase and b-1, 3-glucanase in induced chickpea. Curr. Sci., 89:659–663

Shaharoona, B., Arshad, M., Zahir, Z.A., Khalid, A. (2006). Performance of *Pseudomonas* sp. containing ACC-deaminase-1 for improving growth and yield of maize (*Zea mays* L.) in the presence of nitrogenous fertilizer. Soil Biology and Biochemistry, 38, 2971-2975.

Shaukat, S. S. and Siddiqui, I. A.,(2003), The influence of mineral and carbon sources on biological control of charcoal rot fungus, *Macrophomina phaseolina* by fluorescent pseudomonads in tomato. Letters in Appl. Microbiol., 36 (6): 392.

Shoebitz, M., Ribaudo, C.M., Pardo, M.A., Cantore, M.L., Ciampi, L., Cura, J. A. (2007). Plant growth promoting properties of a strain of *Enterobacter ludwigii* isolated from *Lolium perenne* rhizosphere. Biochemistry Journal, 41(9), 1768-1774.

Siddiqui, Z., 2006. PGPR: Prospective Biocontrol Agents of Plant Pathogens. PGPR: Biocontrol and Biofertilization 111,142.

Pierson, E. A., Weller, D. M. (1994). Use of mixture of *fluorescent Pseudomonas* to suppress take-all and improve growth of wheat. Journal of Phytopathology, 84(9): 940-947.

Vidhayasekaran, P., and Muthamilan M. (1999). Evaluation of power formulation of *Pseudomonas flourescens* pf1 for controlof rice sheath blight. Biocontrol sci. technol., 9: 67-74.

Viswanathan, R., Samiyappan, R. (1999). Red rot disease in sugarcane: a major constraint for the Indian sugar Industry. Sugar Cane, 5, 9–15.

Wei, G., Kloepper, J. W., Tuzun, S. (1991). Induction of systemic resistance of cucumber to *Colletotrichum orbiculare* by select strains of plant growth-promoting rhizobacteria. Phytopathology, 81:1508-1512.

A COMPREHENSIVE OVERVIEW ON DIETARY SUPPLEMENTS

Swarnima Pandey*[1] and Sushant Kumar[1]

[1]Faculty of Pharmacy, Hygia college of Pharmacy,

Affiliated to Dr. APJ-AKTU, Lucknow, UP, India

[2]Faculty of Pharmacy, Uttar Pradesh University of Medical Sciences,

Saifai, Etawah, U.P., India 206 130

*Corresponding author E-mail: yesgoldi@gmail.com

Introduction:

The diet is believed to be much richer than before. However, the abundance of the food industry and the ignorance of the basic principles of nutrition have led to a large population leading to an unbalanced diet that is high in calories, high in fat and low in protein, vitamins and minerals. This long-term situation leads to the development of various degenerative diseases. This is where dietary supplements come in to solve the problem. Dietary supplements are Termed as those products which contain one or more concentrated nutrients. Its purpose is to supplement an individual's daily diet when the individual's diet is unbalanced and does not belong to the general food category rather than medicine. A dietary supplement is a medicinal product or specialty food product and is not intended for a specific group of people. Dietary supplements provide the body with the ingredients it needs to keep it in good physical and mental condition. In this way, the human body is not exhausted and at the same time avoids injury and fatigue.Dietary supplements have grown in number over the last few decadeswhich are formulated as tablets or powders. Side effects of dietary supplements are listed which increases with their consumption or overdose.

People who systematically consume dietary supplements may be consuming more vitamins and other nutrients than the organism can tolerate. You need to know that there is. This poses a health risk from overdose of dietary supplements. The problem is exacerbated as people can get these supplements themselves without a prescription or medical supervision. The purpose of this summary report is to show the relationship between dietary supplements and the human body. It helps protect and promote health. Medical and nursing journals and books were searched through the electronic databases MEDLINE, IATROTEK, and their respective libraries.

Keywords: Nutrition, dietary supplements, and health.

Review literature:

Maughan *et al.* revealed disclosed supplements enable more consistent and focused training by promoting recovery between training sessions, reducing training interruptions due to illness or injury, and improving competitive performance often regarded as facilitating coordination. Studies have shown that the use of supplements is widespread among athletes, but the use of some of these products is backed by solid research, and some are even harmful to athletes. There is a possibility. Certain sports foods, such as energy bars and sports drinks, play a real role, and some protein supplements and meal replacements may also help. Lin *et al.* revealed the use of herbs and dietary supplements (HDS) alone or with medications may increase the risk of side effects for the patient. This review aims to assess documented HDS drug interactions and contraindications contraindications.

Sobal *et al.* revealed about Vitamin/mineral supplements are often used by athletes as ergogenic aids to improve performance. This paper reviews studies of the prevalence, patterns, and explanations for vitamin/mineral supplement use among athletes. Fifty-one studies provided quantitative prevalence data on 10,274 male and female athletes at several levels of athletic participation in over 15 sports. The overall mean prevalence of athletes' supplement use was 46%. Most studies reported that over half of the athletes used supplements (range 6% to 100%), and the larger investigations found lower prevalence levels. Elite athletes used supplements more than college or high school athletes.

Blendon et al. revealed about about vitamin / mineral supplements is often used by athletes as a performance-enhancing aid to improve performance. This article reviews prevalence studies, patterns, and explanations for the use of vitamin / mineral supplements in athletes. Fifty-one studies provided quantitative prevalence data from 10,274 male and female athletes at various levels of participation in more than 15 sports. The average overall prevalence of dietary supplement use was 46%. Most studies reported that more than half of athletes consumed supplements (range, 6% to 100%), and larger studies found lower prevalence values.

Louise *et al.* revealed about Dietary supplements disclosed for health purposes. Based on the results of 4,444 different national polls, including the opinions of dietary supplement users and non-users, a significant proportion of Americans surveyed regularly take dietary supplements as part of their daily health care. Dwyer *et al.* told about many of the scientific and regulatory challenges that exist in dietary supplement safety, quality, and efficacy research are the same in all countries as the market becomes more and more global.

Emily *et al.* revealed these studies have not previously summarized the number of studies on the prevalence of dietary supplement (DS) use among military personnel. This article provides a systematic literature review on this subject. Berman *et al.* revealed *Citrus aurantium*

extract is an herbal weight loss product sold as a safe alternative to Ephedra, but *C. aurantium* can also have a negative effect on health. *C. aurantium* contains synephrine (oxedrin), which is structurally similar to adrenaline. Although there are no adverse events due to the use of *C. Synephrine*, an aurantlum product, can increase blood pressure in humans and other animal species and exacerbate cardiovascular events.

Scope of dietary supplement:

Dietary Supplements: The FDA regulates both finished dietary supplements and nutritional ingredients. The FDA regulates dietary supplements differently than "traditional" foods and medicines. According to the Food Supplement Health Education Act (DSHEA) of 1994:

- Food supplements and food supplement manufacturers and distributors are prohibited from selling inferior or mislabeled products. This means that these companies are responsible for assessing product safety and labeling prior to marketing to ensure that
- meets all the requirements of DSHEA and FDA regulations. ..
- The FDA is responsible for taking action against post-launch mixed or mislabeled dietary supplements.
- Example:
- Yogurt probiotics for intestinal health.
- Foods / cereals / snacks fortified with water-soluble fiber, vitamins and minerals.
- Omega 3 Milk to Prevent Heart Disease
- Canola Oil with Low Triglycerides to Lower Cholesterol Levels
- Autowheat, Bran, Obaco, Lignin for Heart Disease and Colon Cancer • Pre Controls Testicle Flora Biotics Oligofluctose
- Reducing Stanol (Benecol) Cholesterol

Classification of dietary supplement:

In accordance with the law, labeling of these substances must include:

• Declaration of a dietary supplement after the product name

• Recommended dose of product for daily consumption

• A statement that dietary supplements are not a substitute for a balanced and diverse diet. • Declaration on how to keep products out of the reach of children

According to the National Institute of Health Sciences, dietary supplements fall into two categories according to their intended use.

1. Dietary supplements as food. They complement a normal diet. 2.2. Food for special nutritional purposes as a drink. Due to its special composition, it is aimed at the special nutrition of a particular population group. For example, it targets healthy babies, children aged 2 to 5 years, and

people in a special category with disabilities. For groups of people who are in a metabolic or specific physiological state. Supplements can also be distinguished according to their origin: supplements of natural or synthetic origin. They are categorized as follows and are comparable in the nature or form they are available to.

• Whether vitamin and mineral supplements are combined in the form of multivitamins or multiminerals. • Liquid or tablet protein supplements with or without carbohydrates, fats, vitamins and minerals. • Amino acids of all shapes and compositions. • Dietary supplements that help you gain weight

• Meal exchange in the form of powder, waffles, or biscuits. • Carbohydrate supplements with or without electrolytes and vitamins. • Natural anabolic dietary supplements that are not "prohibited substances" • "Activator" supplements for growth hormone and other hormones • Supplements for basic fatty acids. • Foods or food ingredients such as yeast, garlic, seaweed and royal jelly. • Herbs Protein Food Supplements

Protein and Amino Acid Supplements are widely marketed to athletes and habitually active consumers as muscle growth and performance-enhancing products, and high-protein, low-carbohydrate diets are traditionally used for weight loss has been done. However, knowledge of nutritional importance and the effects of dietary supplements and sports supplements vary significantly between athletes and lifestyle users, especially with respect to individual levels of physical activity and overall nutritional and metabolic status. Because proteins help ensure growth in infancy, support muscle and bone metabolism, ensure normal nervous system maintenance and development, and maintain muscle mass and physical fitness in old age, humans It is a lifelong essential nutritional ingredient in the diet. Athletes have proper protein synthesis and energy production, as well as proper immune function and good bowel integrity under multi-stress conditions of targeted, more frequent, more intense, and / or longer training routines. Physiological protein requirements may increase to maintain. Protein requirements increase with increasing intensity and duration of athletic performance. Therefore, protein should be consumed with meals before and after actual performance and regularly during the day to ensure an efficient supply of essential or essential amino acids. For example, branched-chain amino acid (BCAA; valine, leucine, isoleucine) supplements are widely used by athletes and have been suggested to reduce muscle soreness and improve exercise performance after strenuous exercise. BCAA supplementation can play a role in regulating the production of neurotransmitters in the brain and causing fatigue during exercise. Due to its rapid digestion and absorption, whey protein sup.

Formulation available in market of protein dietary supplements and their composition (ingredients):

Protein content per 100g of product was 80-90g in 11.7% composition:

Sr. No.	Ingredients	Concentration
1	Protein Concentrate	58.33%
2	Protein Isolate	50%
3	Milk Protein Concentrate	23.33%
4	Milk Protein Isolate	21.66%
5	Micellar Casein	20%

The purpose of this study was to investigate the composition of protein supplements in terms of protein content, source, and cost. All dietary supplement stores in Vadodara have been identified and all protein supplements from their website are listed. Details of these products were collected from each brand's official website. Products that did not provide nutrition labeling were excluded. 59 products from 15 brands were surveyed in the form of powders (83.05%), bars (13.56%) and beverages (3.39%). For powders that needed to be reconstituted by adding to the beverage in addition to milk or water, the serving size varied from 24g to 72g. The potion size of the bar is 5080g and the drink size is 414429ml.

Future aspects:

The popularity and little unregulated sales of herbal preparations in the United States over the past few decades have raised concerns about their potential health consequences. Scientific research on the physiological and pathological effects of herbs is relatively sparse. The recent

Ephedra sinica ban (l) by the Food and Drug Administration, long after its clinical association with stroke, heart attack, hypertension, and psychological problems, indicates the need for a reassessment of the regulatory environment for Chinese herbs increase. There is also a need for more basic and clinical research in this area. *C. aurantium* (also known as Seville orange or sour orange) is a small citrus tree about 5 meters high with fragrant white flowers. *C. aurantium* is too acidic to eat, but ripe fruits can be eaten in Iran, and in Mexico fresh fruits can be eaten with salt and pepper paste. Unripe fruits are sometimes pickled and used as spices. *C. aurantium* rind is often used for jams, and dried pods are used to flavor Belgian beer called Bouquet Garni and Orange Muscat. Essential oils from the dry skin of immature fruit flavors Curacao, Cointreau and Triple Sec. Flowers are used in tea, and the essential oil of flowers called neroli is used in the water of orange blossoms, which is used to flavor perfumes, liqueurs and sweets. The most common use of *C. aurantium* is medicinal rather than cooking. Dried immature fruits are mainly used in Asian herbal medicine to treat digestive problems. It is called Zhishi in Chinese, Kijitsu in Japanese, and Chisil in Korean. Dried skins of immature or ripe fruits are also used in Western herbal medicines to stimulate appetite and gastric secretions (as opposed to recent sales of Daidai-containing products as a weight loss aid). It is a common ingredient in Swedish bitters and other gastrointestinal remedies. Flowers are sometimes used as a mild sedative in folk remedies. Therefore, there is a history of benign human consumption

Although it is a fruit of *C. aurantium*, the culinary or medicinal use of herbs is restricted and daily intake will not increase significantly.

Active Ingredients and Pharmacology:

The most effective active ingredients in the fruits of Daidai are synephrine (also known as synephrine or oxedrin) and octopamine. *C. Daidai* also contains flavonoids such as limonene, hesperidin, neohesperidin, naringin and tangaletin. Furanocoumarin is also available. Structurally, C. The active ingredient of aurantium is closely associated with the endogenous neurotransmitter and ephedrine (Figure 1). Synephrine is structurally similar to epinephrine, and octopamine is similar to noradrenaline (only the number of hydroxyl groups in the aromatic ring is different; see 6). Ilsynephrine (Phenylephrine, Neosynephrine) is closely related to synephrine. Phenylephrine is an alpha adrenergic receptor agonist used in conventional medicine as a decongestant nasal spray and mydriatic. It doesn't exist in C, so I won't explain it here. Daidai Synephrine and octopamine are both endogenous trace bioamines and are widely distributed in vertebrates, including plants, bacteria, invertebrates, and humans. Octopamine is found in the sympathetic nerves in the same area as norepinephrine, but synephrine and m-synephrine are found only in the adrenal glands. A recent study of healthy men and women found that plasma in all 16 subjects contained octopamine, 15 subjects contained synephrine, and 6 subjects contained octopamine and the precursor of synephrine, tyramine understand. Octopamine and synephrine

were detected in the platelets of most subjects, but tyramine was not. In rats, poctopamine was found in the adrenal glands, heart, spleen, vas deferens, brain, liver, kidneys, colon, bladder and lungs. Dopamine beta-hydroxylase converts tyramine to octopamine. This biosynthesis is enhanced by inhibition of monoamine oxidase. Phenylethanolamine methyltransferase breaks down octopamine into synephrines. Synephrine has an alpha-adrenergic effect and activates about 3 (although) ~ 1 or ~ 2) Adrenaline receptor. Octopamine appears to be a selective ~ 3-adrenergic receptor agonist (10). Both synephrine and octopamine appear to inhibit cAMP production. The function of endogenous synephrines and octopamines is not well explained. Synephrine, octopamine, and tyramine, formerly known as "fake neurotransmitters," can actually be true neurotransmitters. These amines affect platelet-mediated signaling events and may contribute to the pathophysiology of migraine and other types of headache.

Compound d, Imoctopamine, exhibits an antifat-producing effect on angiotensin II-induced fluid intake in rats. This effect is clearly mediated by et2adrenoreceptors because it is blocked by yohimbine. Synephrine had antidepressant effects in a mouse model using immobility trials, but the effects were not dose-dependent. No effect was seen at the lowest (0.3 mg / kg) or highest dose (30 mg / kg), and the effects at 3 mg / kg and 10 mg / kg were similar. The effect of synephrine was reversed by administration of the ol antagonist prazosin, so it seems that alpha adrenergic receptors are involved. In a later study by the same group, S (+) p-synephrine was more effective than R () pseudosynephrine in reducing immobility in the tail hang test. Effects on Weight Loss and Lipolysis There is little evidence to support the use of

C. Daidai is included in commercial weight loss products, but is used for weight loss. The combination product was tested for weight loss in the only clinical trial using Daidai. A double-blind, randomized, placebo-controlled, three-group study of 23 subjects with a body weight index of> 25 kg / m2 was conducted under the guidance of an exercise physiologist and a 1800 kcal American Heart Association Step I diet. Strength circuit training program 3 days a week. The combination tested contained 975 mg of *C. Daidai* extract (6% synephrine alkaloid), 528 mg caffeine, 900 mg St. John's wort (3% Hypericum erectum), taken daily for 6 weeks. Measurements of the results included weight, fat loss, and mood. Twenty subjects completed the study. This study reports that treated subjects lost significant body weight (1.4 kg) compared to the placebo group (lost 0.9 kg) and the control group (lost 0.04 kg). However, the publication table seems to suggest that the differences are important only when compared to the baseline, and not when compared.

Effects on weight loss and lipolysis:

Little proof helps the usage of *C. aurantium* for weight reduction, in spite of its inclusion in over-the- counter weight-loss products. The best scientific trial of *C. aurantium* for weight

reduction examined a aggregate product. A double-blind, randomized, placebo-controlled, three-armed take a look at of 23 topics with frame mass index >25 kg/m2 as compared remedy, placebo, and no remedy as an accessory to a 1800 kcal American Heart Association Step I food regimen and a weight circuit schooling exercising application three days every week beneathneath the course of an exercising physiologist . The examined aggregate contained 975 mg *C. aurantium* extract (6% synephrine alkaloids), 528 mg caffeine, and 900 mg St. John`s wort (three% hypericum [sic]), taken each day for six weeks. Outcome measures covered weight, fats loss, and mood. Twenty topics finished the take a look at. The take a look at reviews that dealt with topics 'misplaced a extensive quantity of weight (1.four kg) as compared with the placebo organization (which misplaced 0.nine kg) and the manipulate organization (which misplaced 0.04 kg). However, the desk withinside the guide seems to signify that the variations are extensive best as compared with baseline however now no longer in contrast with the opposite corporations on the quit of the trial.

The remedy institution misplaced 2.9% of fat, while there has been no full-size alternate within side the placebo or manipulates groups. No full-size modifications have been visible in any institution in a Profile of Mood States Questionnaire, blood lipids, blood pressure, coronary heart rate, electrocardiogram, serum chemistries, or urinalyses. The handled institution skilled a full-size growth in basal metabolic rate, while the placebo institution skilled a full-size lower in basal metabolic rate. There became no alternate within side the untreated manipulate institution. No facet outcomes have been reported.

Assuming that the 3% hypericum clearly approach 0.3% hypericin, the dose of St. John`s wort (Hypericum perforatum) on this specific product might be a healing antidepressant dose. There isn't anyt any scientific proof that St. John's wort facilitates weight loss, however despair can simply predispose to overeating.

This product carries a beneficiant quantity of caffeine, the equal of approximately four cups of espresso or 10 cups of tea. Caffeine has a thermogenic impact, and this impact is synergistic with different sympathomimetic agents. Even one hundred mg caffeine has a thermogenic impact lasting 1-2 hrs, and dosages >six hundred mg/day boom 24-hr electricity expenditure below respiration chamber conditions.

Beta-three adrenoreceptor agonists do have lipolytic consequences withinside the fats cells of rats, hamsters, and dogs, however they're a great deal much less energetic in human fats cells. Octopamine became stronger than synephrine (however a ways much less mighty than norepinephrine) for exciting lipolysis in adipocytes from rats, hamsters, or dogs; however, the impact became now no longer sizable in fats cells from guinea pigs or humans (10). Octopamine became completely lipolytic in adipocytes from the lawn dormouse and Siberian hamster.

In summary, the most effective posted trial of a C. aurantiumcontaining weight loss product discovered that the product became now no longer advanced to placebo for weight loss. There isn't anyt any proof that synephrine and octopamine in degrees that could be discovered in weight loss merchandise might have any lipolytic impact on human adipocytes.

Adverse Effects and Drug Interactions

C. aurantium might be predicted to have sympathomimetic consequences, however *C. aurantium* extracts have now no longer been related to unfavourable consequences to date. A thinly defined case document related a big myocardial infarction in a 28-yearold male to the abuse of synephrine tablets .

C. aurantium, grapefruit (*C. paradisi*), and pomelo (*C. maximi*) comprise numerous flavonoids that have an effect on drug metab- olism, together with 6',7'-dihydroxybergamottin, that's used to selectively block intestinal cytochrome P450 isoenzyme CYP3A4 in bioavailability research. C. aurantium, however now no longer grapefruit, additionally carries a furocoumarin, bergapten, that still inhibits CYP3A4 in cultured intestinal epithelial cells, however the impact is weaker than that of 6',7'- dihydroxybergamottin .

CYP3A4 metabolizes extra than 1 / 4 of pharmaceuticals, and grapefruit juice will increase blood degrees of many drugs. C. aurantium, predictably, additionally will increase drug degrees, and as it carries bergapten in addition to 6',7'-dihydroxybergamottin, might also additionally have a fair more potent impact than grapefruit juice.

A current scientific pharmacokinetics have a look at discovered that *C. aurantium* juice, however now no longer grapefruit juice, considerably elevated plasma degrees of simultaneously administered indinavir. Another scientific have a look at discovered that *C. aurantium* juice affected felodipine pharmacokinetics in addition to grapefruit juice, growing most awareness and AUC (place below the awareness-time curve) with out affecting' terminal removal half-lifestyles . And a 3rd pharmacokinetics have a look at discovered that each *C. aurantium* juice and grapefruit juice elevated the bioavailability of dextromethorphan

Background: Multiple sclerosis (MS) constitutes a persistent modern demyelinating sickness which negatively influences the significant frightened system. MS signs and symptoms detrimentally have an effect on the nice of lifestyles, in addition to the lifestyles expectancy of MS sufferers. In this aspect, the existing have a look at objectives to seriously summarize and examine the presently to be had scientific research focusing at the capability useful consequences of nutritional dietary supplements on controlling MS symptomatology and relapse.

Methods: PubMed database became comprehensively searched, the usage of relative key phrases to pick out scientific trials that investigated the useful consequences of nutritional

supplementation towards MS symptomatology and progression. forty scientific trials have been discovered, which have been divided into categories.

Results: Nutritional fame of MS sufferers, in addition to supplementation were recommended as capability elements affecting progression. Several giant research have documented a systematically excessive occurrence of diet A, B12 and D3 deficiency among MS sufferers. At present, scientific facts have recommended that maximum of the nutritional dietary supplements below have a look at might also additionally exert antioxidant and anti inflammatory properties, enhancing despair symptomatology and nice of lifestyles overall. However, malnutrition chance in MS sufferers has now no longer been safely explored so as for extra unique conclusions to be drawn. The dietary supplements which can have a nice impact on MS are vitamins, fatty acids, antioxidants.

Adverse effects and drug interactions:

C. aurantium would be expected to have sympathomimetic effects, but *C. aurantium* extracts have not been associated with adverse effects to date. A thinly described case report linked alarge myocardial infarction in a 28-yearold male to the abuse of synephrine tablets.

C. aurantium, grapefruit *(C. paradisi),* and pomelo *(C. maximi*) contain several flavonoids that affect drug metab- olism, including 6',7'-dihydroxybergamottin, which is used to selectively block intestinal cytochrome P450 isoenzyme CYP3A4 in bioavailability studies. C. aurantium,but not grapefruit, also contains a furocoumarin, bergapten, that also inhibits CYP3A4 in cultured intestinal epithelial cells, but the effect is weaker than that of 6',7'- dihydroxybergamottin .

CYP3A4 metabolizes more than a quarter of pharmaceuticals, and grapefruit juice increases blood levels of many drugs. C. aurantium, predictably, also increases drug levels, and because it contains bergapten as well as 6' ,7'-dihydroxybergamottin, may have an even stronger effect thangrapefruit juice.

A recent clinical pharmacokinetics study found that *C. aurantium* juice, but not grapefruit juice, significantly increased plasma levels of concurrently administered indinavir . Another clinical study found that *C. aurantium* juice affected felodipine pharmacokinetics similarly to grapefruitjuice, increasing maximum concentration and AUC (area under the concentration-time curve) without affecting' terminal elimination half-life . And a third pharmacokinetics study found that both *C. aurantium* juice and grapefruit juice increased the bioavailability of dextromethorphan

Background: Multiple sclerosis (MS) constitutes a chronic progressive demyelinating disease which negatively affects the central nervous system. MS symptoms detrimentally affect the quality of life, as well as the life expectancy of MS patients. In this aspect, the present study aims to critically summarize and evaluate the currently available clinical studies focusing on the

potential beneficial effects of dietary supplements on controlling MS symptomatology and relapse.

Methods: PubMed database was comprehensively searched, using relative keywords to identify clinical trials that investigated the beneficial effects of dietary supplementation against MS symptomatology and progression. 40 clinical trials were found, which were divided into categories.

Results: Nutritional status of MS patients, as well as supplementation has been suggested as potential factors affecting progression. Several substantial studies have documented a systematically high prevalence of vitamin A, B12 and D3 deficiency amongst MS patients. At present, clinical data have suggested that most of the dietary supplements under study may exert antioxidant and anti-inflammatory properties, improving depression symptomatology and quality of life overall. However, malnutrition risk in MS patients has not been adequately explored in order for more precise conclusions to be drawn. The supplements that may have a positive effect on MS are vitamins, fatty acids, antioxidants, phytochemicals and melatonin.

Conclusion:

Individuals who systematically take nutritional dietary supplements have to be knowledgeable that they could take extra vitamins than their organism is capable of tolerate. Upper tolerable tiers are the ones, which if the attention of the substance is exceeded, then, aspect consequences and toxicity begin to seem to the human organism. People might not be conscious that there are dangers which can rise up from the ones formulations. So, they regularly devour immoderate amounts. The hassle turns into extra critical for, they are able to acquire those materials with the aid of using themselves, without a prescription and scientific supervision. Therefore, customers of dietary dietary supplements ought to examine cautiously the labels on dietary supplements and enriched meals and keep away from taking more than one doses that exceed the Recommended Dietary benefits (RDAs). In instances of doubt, human beings have to are looking for the recommendation of a of a consultant earlier than they pick out a nutritional supplement

Several nutritional dietary supplements can also additionally lower infection and fatigue, additionally growing additionally autoimmunity tolerance in MS patients, and as a consequence enhancing excellent of existence and existence expectancy. Currently, there's no powerful medical indication for making use of nutritional supplementation as complementary remedy in opposition.

References:

Antonio, J. and Stout, J.R. (2001). Sports Supplements. Philadelphia, PA: Lippincott Williams andWilkins.

Ballantyne, C.S., Phillips, S.M., MacDonald, J.R., Tarnopolsky, M.A. and MacDougall, J.D. (2000) The acute effects of androstenedione supplementation in healthy young males. Canadian Journal of Applied Physiology, 25, 68–78.

Barrett, B. (2003). Medicinal properties of Echinacea: a critical review. Phytomedicine 10, 66–86.

Barnett, C., Costill, D.L., Vukovich, M.D., Cole, K.J., Goodpaster, B.H., Trappe, S.W. and Fink, W.J. (1994) Effect of L-carnitine supplementation on muscle and blood carnitine content and lactate accumulation during highintensity sprint cycling. International Journal of Sport Nutrition, 4, 280–288.

Brown, G.A., Vukovich, M.D., Sharp, R.L., Reifenrath, T.A., Parsons, K.A. and King, D.S. (1999). Effect of oral dehydroepiandsterone on serum testosterone and adaptations to resistance training. Journal of Applied Physiology, 87, 2274–2283.

Brown, G.A, Vukovich, M.D., Martini, E.R., Kohut, M.L., Franke, W.D., Jackson, D.A. and King, D.S. (2000a). Endocrine responses to chronic androstenedione intake in 30–56 year old men. Journalof Clinical Endocrinology and Metabolism, 85, 4074–4080.

Brown, G.A., Vukovich, M.D., Reifenrath, T.A., Uhl, N.L., Parsons, K.A., Sharp, R.L. and King, D.S. (2000b). Effects of anabolic precursors on serum testosterone concentrations and adaptationsto resistance training in young men. International Journal of Sport Nutrition and Exercise Metabolism, 10, 340–359.

Brown, G.A., Vukovich, M.D., Martini, E.R., Kohut, M.L., Franke, W.D., Jackson, D.A. and King,

D.S. (2001a). Endocrine and lipid responses to chronic androstenediolherbal supplementation in 30–58 year old men. Journal of the American College of Nutrition, 20, 520–528.

Brown, G.A., Vukovich, M.D., Martini, E.R., Kohut, M.L., Franke, W.D., Jackson, D.A. and King,

D.S. (2001b). Effects of androstenedione-herbal supplementation on serum sex hormone concentrations in 30–59 year old men. International Journal of Vitamin and Nutrition Research,71, 27–31.

Brown, G.A., Martini, E.R., Roberts, B.S., Vukovich, M.D. and King, D.S. (2002). Acute hormonal responses to sublingual androstenediol intake in young men. Journal of Applied Physiology, 92,142–146.

Brown, G.A., Dewey, J.C., Brunkhorst, J.A., Vukovich, M.D. and King, D.S. (in press). Changes in serum testosterone and estradiol concentrations following acute androstenedione

ingestion inyoung women. Hormone and Metabolic Research.

Burke, L.M. and Inge, K. (1994). Protein requirements for training and 'bulking up'. In Clinical Sports Nutrition (edited by L.M. Burke and V. Deakin), pp. 124–150. Sydney, NSW: McGraw-Hill.

Castell, L.M. and Newsholme, E.A. (1997). The effects of oral glutamine supplementation on athletes after prolonged exhaustive exercise. Nutrition, 13, 738–742.

Castell, L.M., Poortmans, J.R. and Newsholme, E.A. (1996). Does glutamine have a role in reducing infections in athletes? European Journal of Applied Physiology, 73, 488– 490.

Haller CA, Benowitz NL. Adverse cardiovascular and central nervous system events associated with dietary supplements containing ephedra alkaloids. N Engl J Med 343:1833-1838, 2000.

Hosseinimehr SJ, Tavakoli H, Pourheidari G, Sobhani A, Shafiee A. Radioprotective effects of citrus extract against gamma-irradiation in mouse bone marrow cells. J Radiat Res (Tokyo) 44:237-241, 2003.

Facciola S. Cornucopia II: A source book of edible plants. Vista, CA: Kampong Publications, 1998.

Kiple KF, Ornelas KC, Eds. The Cambridge World History of Food. Cambridge, UK: Cambridge University Press, Vol. 2:pp1822, 1826, 2000.

Bisset NG, Wichtl M. Herbal Drugs and Phytopharmaceuticals. Stuttgart, Germany: Medpharm GmbH Scientific Publishers, and Boca Raton, FL: CRC Press, pp91-95, 1994.

Airriess CN. Selective inhibition of adenylyl cyclase by octopamine via a human cloned ot2A-adrenoceptor. Br J Pharmacol 122:191-198, 1997.

SEED SURFACE CHARACTERISTICS AND PRELIMINARY PHYTOCHEMICAL ANALYSIS OF SOME LAMIACEAE (LABIATAE) SEEDS

P. P. Ulhe

Department of Botany,

Narayanrao Kale Smruti Model College,

(Art, Commerce and Science) Karanja (gh.) Dist - Wardha

Coresponding author E-mail: pallavi_ulhe27@rediffmail.com

Abstract:

The family Lamiaceae also known as Labiatae. It is also known as mint family. This family is a flowering plant family. For the study of seed morphology and preliminary phytochemical observations about 04 species were studied of Lamiaceae. In Lamiaceae, *Ocimum sanctum*, *Ocimum americanum*, *Ocimum basilicum* and *Salvia officinalis* were well studied. In the morphological observations seed surface irregular with grooves and furrows, some fibrous, cellular deposition on the surface. In the preliminary phytochemical observations with aqueous extract of seed powder treated with various chemical for detection of alkaloids, carbohydrates, reducing sugars, steroids, glycosides, flavonoids, terpenoides, saponins protein, tannins, amino acids, volatile oil or essential oil etc. Biuret and Xanthoproteic test were also done for detection of protein. Phytochemical analysis were done for correct identity and evaluation. This study is also useful to isolate the pharmacologically active principles present in the drug. Scanning electron microscopy (SEM) study is useful for detection of variation on seed surface. The micromorphological observation helps to identify the seed also. The family shows fragrance also.

Keywords: Phytochemical, preliminary screening, seed morphology, SEM, Lamiaceae (Labiatae)

Introduction:

Seed coat morphology is an important study for seed identification because seed identification is important in farming. Seed is a mature ovule. Outer coat of seed is known as seed coat, a small embryonic plant enclosed in a covering and usually with some stored food. For study of seed coat characters all the parameters are very important like seed size, shape, colour, hilum shape, hilum size, surface features, seed weight etc. The scanning electron microscopy is a modern technique for surface identification. Seeds have also the medicinal value. Lamiaceae is

one of the most important angiospermic family and having an aromatic smell. About 16 members of this family were reported from vidharbha region (MS) India (Dhore and Joshi, 1988). Seeds of this family are small, minute. Phytochemical analysis of some members of Lamiaceae family shows various chemical constituents which is present in stem, leaves and seeds also. The Lamiaceae seed having various medicinal values which is used on wound, bronchitis, liver disease, gastric disorders etc.

Figure 1: *Ocimum sanctum* Linn.

Figure 2: *Ocimum basilicum* Linn.

Figure 3: *Ocimum americanum* Linn.

Figure 4: *Salvia officinalis* Linn.

Figure 1 to 4: Habit of plant *Ocimum sanctum* Linn., *Ocimum basilicum* Linn., *Ocimum americanum* Linn., *Salvia officinalis* Linn. respectively

Materials and methods:

Sample collection:

Some seeds of Lamiaceae family like *Ocimum sanctum* Linn., *Ocimum americanum* Linn., *Ocimum basilicum* Linn., and *Salvia officinalis* Linn. were collected from various local regions in Amravati district and BSIP institute of Lucknow.

For seed coat study all the seeds parameters were studied using dissecting and binocular microscope. Digital weighing balance was used for weighing the seeds in mg. The

morphological observations of seeds were done followed by their photography, using 1 cm. scale.

Seed coat morphology (SEM):

To study the seed coat morphology scanning electron microscopy is most important. For this purpose, the individual seeds were dipped in alcohol for 5-10 min., to remove the dust from them. The seed mounted on pin type stubs using double sided adhesive tape or conductive silver paint to prevent charging of the surface during scanning and then coated with a very thin layer of gold in a polaron sputter coating unit. For spermoderm study of seed photomicrograph were taken in the scanning electron microscope (SEM) (LEO 430) at Birbal Sahani Institute of paleobotany, Lucknow.

Preliminary phytochemical tests:

The preliminary phytochemical analysis is most important for detection of various chemical constituents. Trease and Evans (1989) test were done. Qualitative phytochemical analysis of the crude powder of the 04 plants for the identification of phytochemicals like alkaloids, carbohydrates, reducing sugars, steroids, glycosides, flavonoides, terpenoides, saponine, protein, tannins, amino acids, volatile oil or essential oil. Preliminary photochemical test were done using aqueous extract.

Qualitative test:

Test for protein: About 04 seed samples were used for detection of protein .For this process Biuret and Xanthoproteic test were done.

In Biuret test: 1ml seed pulp/powder in a test tube treated with 2 ml solution of 20% NaOH and a drop of 1% $CuSO_4$.Then the test tube was shaken for few minutes. The pulp turns violet which indicates the presence of protein.

In Xanthoproteic test: 1 ml seed pulp in a test tube treated with 2 to 3ml of Conc. nitric acid. The pulp turns yellow colouration which clearly indicated the presence of protein. The concentration of protein in seeds was given in symbols (+,++,+++) are as follows.

Observations:

Scanning Electron Microscopy (SEM) study:

In Scanning electron microscopy (SEM) investigation shows that in *Ocimum sanctum* Linn. seed oblong shape with small, granular bodies deposited all over the surface. These granulated deposition present all over the surface. Hilar region slightly tapered.

Ocimum americanum seed shows irregular surface. Seed surface contain cellular thread like fibrous network all over the surface. Surface irregular, granulated. Hilar region circular, granulated. *Ocimum basilicum* seed shows irregular, cellular network, granulated deposition.

Salvia officinalis seeds also shows deep, irregular, thick cellular network. Surface shows no. of thick ridges and deep furrows are present. Some granulated small bodies present on the surface. Surface more irregular than other *Ocimum* species.

Figure 5: *Ocimum sanctum* Linn.

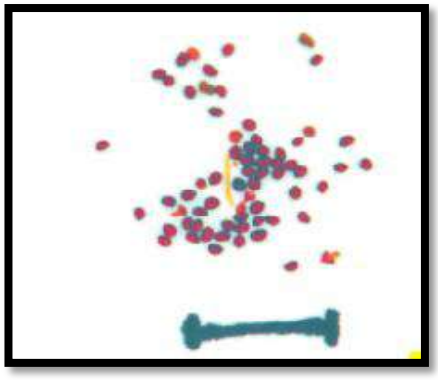


Figure 6: *Ocimum americanum* Linn.

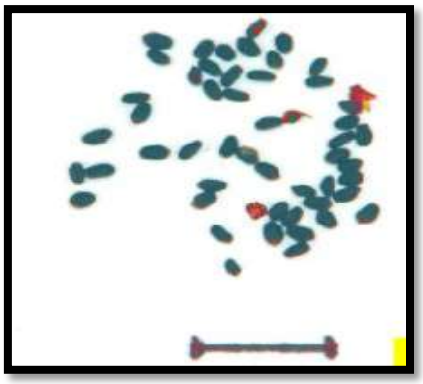


Figure 7: *Ocimum basilicum* Linn.

Figure 8: *Salvia officinalis* Linn.

Figure 5 to 8: *Ocimum sanctum* Linn., minute seeds, *Ocimum americanum* Linn .minute, oblong seeds *Ocimum basilicum* Linn .seeds oblong ovate .*Salvia officinalis* Linn .obovoid, brown seeds respectively

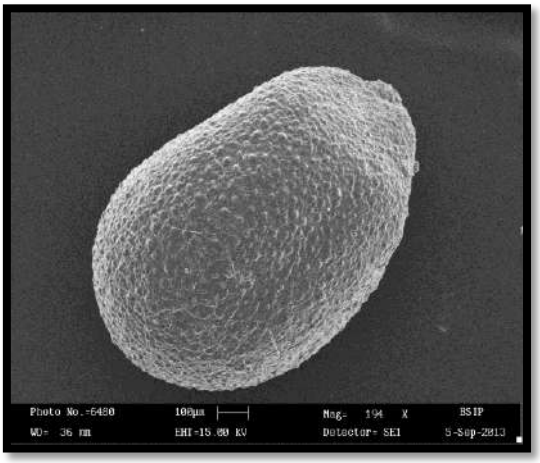


Figure 9: *Ocimum sanctum* Linn. 194X

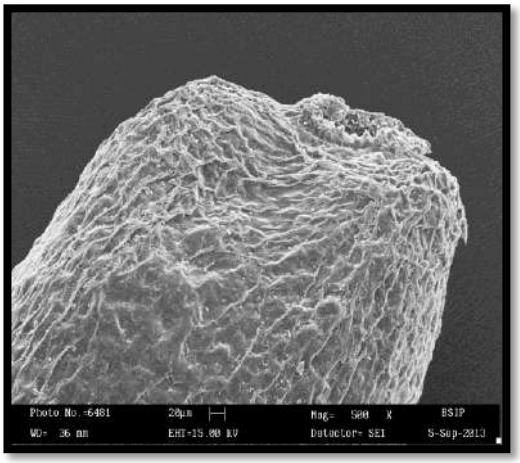


Figure 10: *Ocimum americanum* Linn. 500X

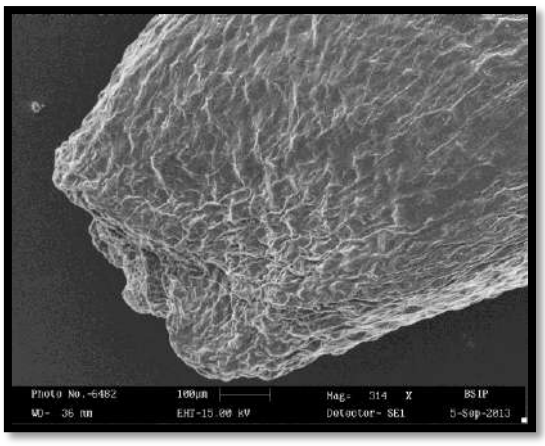


Figure 11: ***Ocimum basilicum*** **Linn.314X**

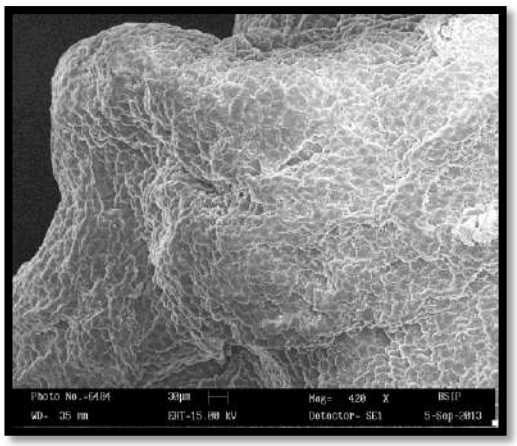


Figure 12: ***Salvia officinalis*** **Linn.420X**

Figure 9 – 12: ***Ocimum sanctum*** **Linn. 194X, oblong seeds with granular deposition,** ***Ocimum americanum*** **Linn. 500X. irregular rough surface,** ***Ocimum basilicum*** **Linn314X. irregular deep ridges and furrows** ***Salvia officinalis*** **Linn.420X .irregular cellular ridges and furrows respectively through scanning electron microscope study**

Table 1: Protein test

Sr.No.	Botanical name	Family	Biuret test	Xanthoproteic test
1	*Ocimum sanctum* Linn.	Lamiaceae (Labiatae)	+	+
2	*Ocimum americanum* Linn.	Lamiaceae (Labiatae)	+	+++
3	*Ocimum basilicum* Linn.	Lamiaceae (Labiatae)	+++	++
4	*Salvia officinalis* Linn.	Lamiaceae (Labiatae)	++	++

Present **(+)**, Absent **(-)**

From the above observation we detected protein concentration in seed samples of *Ocimum sanctum* Linn., *Ocimum americanum* Linn., *Ocimum basilicum* Linn. and *Salvia officinalis* Linn.etc. The Biuret test and Xanthoproteic test shows *Ocimum basilicum* Linn. have more quantity as compare to others *Ocimum sanctum* Linn. have less quantity.

In phytochemical observation the qualitative chemical tests revealed the presence of sugars,steroids,glycosoides,flavonoides,terpenoides,saponine,protein,tannin,amino acids, volatile oil or essential oil using the seed extract treated with various chemicals for detection of phytoconstituents. Using the preliminary phytochemical analysis shows presence or absence of various chemical constituents in them.

Table 2: Phytochemical study of following plant (seed) extracts

Sr No.	Phytochemicals	*Ocimum sanctum*	*Ocimum americanum*	*Ocimum basilicum*	*Salvia officinalis*
01	Alkaloids	+	+	+	+
02	Carbohydrates	+	+	+	+
03	Reducing sugars	-	-	-	+
04	Steroids	+	+	+	+
05	Glysoides	+	+	+	+
06	Flavonoides	+	+	+	+
07	Terpenoids	+	+	+	+
08	Saponine	+	+	+	+
09	Protein	+	+	+	+
10	Tannins	+	+	+	+
11	Amino acids	+	+	+	+
12	Volatile oil or essential oil	_	_	+	+

Present **(+)**, Absent **(-)**

Table 3: Medicinal uses of seeds

Sr. No.	Name of seed sample	Local name	Medicinal uses
1	*Ocimum sanctum* Linn.	Krishna tulas	Seeds yield an essential oil containing eugenal, carvacrol, methyl eugenal and caryophyllene. Antibacterial and insecticidal properties. Seeds are mucilaginous, demulcent, useful in complaint of the urinary systems.(Sharma 2003)
2	*Ocimum basillicum* Linn.	Sabja	Seeds mucilaginous , Dimulcent, aphrodiasiac and diuretic properties. Useful in catarrh, chronic diarrhoea, dysentery, gonorrhea, naphritis, cystitis and internal piles (NIIR Board of consultant and engineers)
3	*Salvia officinalis* Linn.	Kamar kas	Anthilmintic, diuretic
4	*Ocimum americanum* Linn.	Tulsi	Aromatic ,antibacterial

In *Ocimum sanctum*, *Ocimum americanum* and *Ocimum basilicum* shows presence of alkaloids, carbohydrates, steroids, glycosides, flavonoides, terpenoids, saponine, protein, tannins, amino acids etc. In *Salvia officinalis* shows presence of carbohydrates, reducing sugars, steroids, glycosides, flavonoides, terpenoides, tannins, volatile or essential oil. Reducing sugars absent in *Ocimum sanctum, Ocimum americanum, Ocimum basilicum* etc . Volatile oil or essential oil absent in *Ocimum sanctum, Ocimum americanum* etc. Above study help to determine various chemical constituents present in it.

Discussion:

From the above observations we studied the seed morphological and preliminary phytochemical analysis of some seeds of Lamiaceae. In SEM observations of *Ocimum sanctum* shows more granulated deposition than *Ocimum americanum* and *Ocimum basilicum*. *Ocimum americanum* shows more fibrous network. *Ocimum basilicum* shows compact cellular network with fibrous deposition on the surface. *Salvia officinalis* shows presence of deep ridges and furrows on the surface, more irregular than other species. On the above observation the important micromorphological characters were well studied. According to Chaung and Heckard (1972) the seed coat pattern is diversified among species and furnishes an important feature for classification.

The preliminary phytochemical studies of 4 seed samples were studied. The qualitative analysis of seed samples were used for detection of alkaloids, carbohydrates, reducing sugars, steroids, glycosides, flavonoides, terpenoids, saponine, protein, tannins, aminoacids, volatile oil or essential oil etc.

In Biuret and Xanthoproteic test in which *Ocimum americanum* and *Ocimum basilicum* shows more protein content than others. So seed identification is an important feature for detection of micromorphological characters and detection of various chemical constituents. Seed identification both externally and internally provide various information and reliable criteria about unknown seeds. Seed shows high medicinal value, used in preparation of various drugs. Various chemical compositions present inside the seed are effective for drug preparations. All the above study helps for seed identification, drugs preparation on various types of diseases.

So identification, detection and analysis are most important for basic research for various purposes and economic use also.

References:

Board of consultants and engineers (2005).National institute of industrial research. Hand book on Unani medicines with formulae, processes, uses and analysis Asia pacific business press Inc. Delhi India.1-678.

Chaung T.I. Heckard L.R. *(*1972).Seed coat morphology in *Cordylanthus* sp. (Scrophulariaceae) and its taxonomic significance. Amer.J.Bot.258-265.

Choudhary G.B., Behra M., Jena P.K., Tripathy S.K. *(*2011). Pharmacogonistical and phytochemical investigation of various tulsi plants available in south eastern odisha. International journal of research in pharmaceutical and biomedical sciences vol. *(*2) 605-610.

Dhore M.M. Joshi P.A. *(*1988). Flora of Melghat Tiger Reserve, directorate, project tiger, Melghat, Dist- Amravati, Maharashtra.

Harborne J.B.. *(*1994). Phytochemical methods: A guide to modern techniques of plant analysis, Chapmann and Hall pub. New York.

Ramu.G, Krishna Mohan G. Jayaveera K.N. Dhanapal S.P. Santhikumar G. *(*2012) Priliminary phytochemical and antioxidant study of hydro alcoholic extracts from selected genera of Indian Lamiaceae. Asian pacific journal of tropical biomedicine. 685-688.

Sharma, R. (2003). Introduction. Medicinal plants of India. An encyclopaedia Daya publishing house XXX.

Shirsat R. Suradkar S. Koche D. *(*2014). Priliminary phytochemistry and antimicrobial activity of *Salvia plebian* R.Br. and *Colebrookea oppositifolia* smith. International journal of pure and applied sciences and technology 20(1), 21-24.

Thakor A.B. (2009).A study of seed morphology of Malvaceae plants in Gujrat state, India. International journal of plant sciences Vol.4. 2:626-629

Treses G.E. Evans W.C. (1989). Pharmacogonosy 11th edition. Brailliar Tridel and Macmillan publishers, London.

BENEFITS AND PHARMACOLOGICAL PROPERTIES OF CUMIN - AN UPDATED REVIEW

Bhagyashali J. Pawar*[1] and Anita S. Wagh[2]

[1]Shreeyash Institute of Pharmacy,

Satara Parisar, Aurangabad (M. S.)

[2]Srinath College of Pharmacy, Aurangabad (M. S.)

*Corresponding author E-mail: pawarbhagyashali50@gmail.com

Abstract:

Cumin is an ancient spice which has been used in Indian cooking as far back as 5000 years ago, use in Egypt, Greeks and Europe. Cumin is the best source of natural iron and commonly known as Jeera. It is usually used both as whole seed or ground in any curry dish which makes it one of the most important of all Indian spices and as flavouring agent. Despite of its flavouring property, cumin has been found to possess various pharmacological activities such as antiseptic, astringent, diuretics, stimulants, anticancer, antimicrobial, antidiabetic, antifertility, antileptics, antioxidants and Immunomodulatory also helps to improve amnesia and mental function. Its various pharmacological activities may be due to the presence of different chemical constituents such as volatile oil, fixed oil, proteins, amino acids, a variety of flavonoid glycosides including derivatives of apigenin and luteolin. Hence in this review we are highlightening the important Pharmacological and medicinal properties of cumin seeds.

Keywords: Cumin, Pharmacology, Phytochemicals, Volatile oil

Introduction:

Cumin (*Cuminum cyminum*) is a small annual herbaceous plant native to the Mediterranean region, where it is cultivated extensively. Cumin is a member of the aromatic plant family (Umbelliferae). The seeds of the plant are used in cooking to add flavour to spicy dishes. The dried seeds resemble those of caraway, but are straighter in form and have a coarser taste and odour [1]. Major cumin seed producers include Egypt, Iran, India, and Morocco [2].The unites States is one of the largest producers of cumin oil. This spice should not be confused with sweet cumin, which is a common name for anise (*Pimpinella anisum)* [3]. Black cumin *(Bunium persicum*) has smaller and sweeter seeds than C. cyminum, but is not commercially important. They are also used as an appetite stimulant and to ease several stomach disorders [4]. Its

micronutrients include about 1.4 mg iron, 38 mg potassium and 8 mg magnesium. Magnesium serves a host of functions, including promoting heart health, controlling blood pressure and aiding the absorption of calcium [5]. A cumin seed has been found to possess essential oils such as cuminaldehyde (4-isopropylbenzaldehyde), pyrazines, 2-methoxy-3-sec-butylpyrazine, 2 ethoxy-3-isopropylpyrazine, and 2-methoxy-3-methylpyrazine [6]. Moreover, Cumin seeds contain possess numerous phyto-chemicals that are known to have antioxidant, carminative and anti-flatulent properties. The active principles in the cumin may increase the motility of the gastro-intestinal tract as well as increase the digestion power by increasing gastro-intestinal enzyme secretions. This spice is an excellent source of minerals like Iron, calcium, copper, potassium, manganese, selenium, zinc and magnesium. It also contains very good amounts of B-complex vitamins such as thiamin, vitamin B-6, niacin, riboflavin, and other vital anti-oxidant vitamins like vitamin E, vitamin A and vitamin C.

Geographical distribution:

World scenario:

Cuminum cyminum is a yearly plant. The estimated world production is around 300,000 tons. Today cumin production is mainly concentrated in Central and South Asia. Nowadays, India is the largest producer (70% of world production), exporter and consumer of cumin seed in the world. India scenario: India holds a major position in the world production of Cumin. Rajasthan produces 56% while as production from Gujarat accounts for 44% [7].

Scientific classification, local and vernacular name:

Botanically cumin is *Cuminum cyminum* and a member of Apiaceae family (Parsley family)

Scientific classification:

Kingdom: Plantae

Subkingdom: Viridiplantae

Division: Tracheophyta

Sub division: Spermatophytina

Class: Magnoliopsida

Order: Apiales

Family: Apiaceae (Umbeliferar)

Genus: Cuminum – cumin

Species: *cuminum cymimum*-cumin

Vernacular names:

English: cumin, white cumin; Persian: Zirah-sabz or cravich; Spanish: Comino; French: cumin; Italian: cumino; German: Kreuzkummel; Romische kummel

Local names:

Hindi: Jeera, Jira, SafedJeera; Marathi: Pandharejire; Sanskrit: Ajaji, jiraka, Ajajika; Gujarati: Jirautmi, Jirn, Jiraugi, Jeeru; Malayalam: Jeerakam; Oriya: Dalajira, jira

Morphology of *Cuminum cyminum*:

Cumin is the dried seed of the herb *Cuminum cyminum,* a member of the Parseley family. The cumin plant grows to 30-50cm tall andis harvested by hand. It is an annual herbaceous plant with a slender, glabrous, branched stem that is 20-30 cm tall and has a diameter of 3-5cm. Each branch has 2 to 3 sub-branches. All the branches attain the same height so the plant has a uniform canopy [8].

Figure 1: Image of Cumin Plant and Seed

The stem is colored grey or dark green. The leaves are 5-10 cm long, pinnate or bipinnate with thread like leaflets. The flowers are small, white or pink and borne in umbels, each umbel has 5 to 7 umbellts [8]. The fruit is a lateral fusiform or ovoid achene 4-5 mm long containing two mericarps with a single seed. Cumin seeds have 8 ridges with oil canals.

Traditional uses:

Cumin traditionally used to reduce inflammation, decrease urination, prevent gas and suppress muscle spasms. It has also used as an aid for Indigestion, Jaundice, diarrhea and flatulence. Cumin powder has been used as a poultice and suppository and has been smoked in a pipe and taken orally [9-14]. Inaddition, cumin has been used historically for the treatment of toothaches and Epilepsy in Iran [15]. Cumin is a major component of curry and chilli powders and has been used to flavor a variety of food products [16]. The oil which is derived by steam distillation [17]. It is used to flavor a variety of food products like alcoholic beverages, desserts and condiments. It is also used as afragrant component of creams, lotions and perfumes [16].

Phytochemical:

Cumin seeds contain up to 5% of a volatile oil composed primarily of aldehydes (up to 60%). In addition, the seeds yield about 22% fats, numerous free amino acids, and a variety of flavonoid glycosides, including derivatives of apigenin and luteolin [10-18]. The cuminaldehyde content varies considerably, depending on the source of the oil (fresh vs ground seeds). Fine grinding of the seed can result in the loss of up to 50% of the volatile oil with the greatest loss occurring within 1 hour of milling [16]. Monoterpene hydrocarbons are another major component of the oil, sesquiterpenes are minor constituents [11, 17].

The chief components of the characteristics aroma of untreated whole seeds are 3p-menthen-7al and cuminaldehyde in combination with other related aldehydes. Cumin also contains safrole, a mutagen, which is degraded by cooking [19].Cumin seeds contain2 to4% essential volatile oils, also called cumin oil.

The main phytochemical present in the cumin oil are as follows;
Cuminaldehyde, Cinnamaldehyde, 8-Cineole,Cuminalcohol,Limonene,Linalool,Perillaldehyde, Terpinen-4-ol, Terpenoid aldehydes – Cuminic aldehyde and somericmenthadiencarboxaldehydes..Monoterpenes –Beta-pinene, p-cymene and Gamma-Terpinene.

Pharmacological properties:

Antioxidant effects:

Cumin oils as well as their aqueous and solvent derived extracts have significant antioxidant activity in several test methods. These effects are documented as their ability to prominently quench hydroxyl radicals, 1.1-diphenyl-2-picrylhydrazyl (DPPH) radicals and liquid peroxides. The other assays employed were ferric thiocyanate method in linoleic acid system, Fe2+ ascorbate-induced rat liver microsomal liquid peroxidation (LPO), soybean lipoxygenase dependent lipid peroxidation and ferric reducing ability [20]. The cumin oil exhibited high antioxidant activity which has been attributed largely to thresence of monoterpene alcohols, flavonoids and other polyphenolic compounds [21].

Antidiabetic effects:

The antidiabetic effects of cumin products are amply documented [1]. In a glucose tolerance test conducted in rabbits, cumin significantly increased the area under the glucose tolerance curve and hyperglycemic peak [2]. A methanolic extract of cumin seeds reduced the bloodglucose and inhibited glycosylated hemoglobin, creatinine, blood urea nitrogen and improved serum insulin and glycogen (liver and skeletal muscle) content in alloxan and

streptozotocin (STZ) diabetic rats [3, 4]. The collateral benefits included decreased creatinine, urea nitrogen and improved insulin and glycogen in tissue and skeletal muscles, accompanied by a reduction in rat tail tendon collagen-linked fluorescence and pepsin digestion which are implicated in the pathogenesis of diabetic microvascular complications [4]. In another study, an aqueous extract of cumin prevented *in vitro* glycation of total soluble protein, α-crystallin, and delayed the progression and maturation of STZ-induced cataract in rats. Cumin prevented loss of chaperone activity in diabetic rats and also attenuated the structural changes of α-crystallin in lens, which is a long-lived protein and is susceptible to several post-translational modifications in certain diabetic conditions [5]. Eight-week sub-acute administration of cumin to STZ-diabetic rats reduced hyperglycemia and glucosuria accompanied by an improvement in body weight, blood urea and reduced excretion of urea and creatinine [6]. Oral administration of cumin also showed hypoglycemic effect in normal rabbit, resulting in significant decrease in the area under the glucose tolerance curve [2] .The biologically active constituent of cumin seed oil was characterized as cuminaldehyde which inhibited aldose reductase and alpha-glucosidase isolated from rat [7]. In hyperglycemia associated with diabetes, the use of aldose reductase inhibitors has shown efficacy in attenuating diabetic complications.

Hyperlipidemia is an associated complication of diabetes mellitus. Oral administration of cumin to alloxan diabetic rats reduced body weight, plasma and tissue cholesterol, phospholipids, free fatty acids and triglycerides. Histological observations demonstrated significant decrease in fatty changes and inflammatory cell infiltrates in diabetic rat pancreas [3]. Cumin suppressed alcohol and thermally oxidized oil induced hyperlipidemia. It decreased aspartate transaminase (AST), alkaline phosphatase (ALP) and γ-glutamyltransferase (GGT) activities and decreased the tissue (liver and kidney) levels of cholesterol, triglycerides and phospholipids and prevented the changes in the composition of fatty acids in the plasma of rats administered with alcohol and/or thermally oxidized oil. The activity of phospholipase A and C decreased significantly [9, 10]. Hypocholesterolemic effect of methanolic extract of cumin is also documented in ovariectomized rat in relation to its anti-osteoporotic effect [11]. Cumin added to hypercholesterolemic diet decreased serum and liver cholesterol in rats [12].

Antimicrobial effects:

The antimicrobial action of cumin both oil and aqueous has assessed against a wide range of valuable and pathogenic gram-positive and gram-negative microbial strains. Cumin seed oil and alcoholic extract inhibited the growth of *Klebsiellapneumoniae* and its clinical isolates and caused improvement in cell morphology, capsule expression and decreased urease activity. Cumin has also found the biofilm-formation preventive properties against Streptococcus mutans

and *Streptococcus pyogenes* [13, 14]. Cumin has shown the anti-fungal activity against food, soil, animal and human pathogens, yeasts, aflatoxins and mycotoxin producers [15-17].

Anticarcinogenic/antimutagenic:

The dietary supplements of cumin have prevented the occurrence of rat colon cancer induced by a colon-specific carcinogen and also decrease the activity of β-glucuronidase and mucinase enzymes. In cumin-colon treated rats, the levels of cholesterol, cholesterol/phospholipids ratio and 3-methylglutaryl COA reductase activity were reduced [18, 19]. The other inhibition activities of dietary cumin in mice are benzopyrene-induced for stomach tumorigenesis, 3-methylcholanthrene induced uterine cervix tumorigenesis, and 3-methyl-4-dimethyaminoazobenzene induced hepatomas.

Immunomodulatory:

In a recent study, oral treatment with cumin showed immunomodulatory properties in normal and immune-suppressed animals via modulation of T lymphocytes' expression in a dose-dependent manner. It stimulated the T cells' (CD4 and CD8) and Th1 cytokines' expression in normal and cyclosporine-A induced immune-suppressed mice. In restraint stress-induced immune-suppressed animals, the active compound of cumin countered the depleted T lymphocytes, decreased the elevated corticosterone levels and size of adrenal glands and increased the weight of thymus and spleen [19].

Gastrointestinal disorders:

Cumin is extremely good for digestion and related problems. The very smell (aroma) of it, which comes from an aromatic organic compound called Cuminaldehyde, the main component of its essential oil, activates our salivary glands in our mouth (the mouth watering flavor), facilitating the primary digestion of the food [20]. Next is Thymol, a compound present in cumin, which does same to the glands which secrete acids, bile and enzymes responsible for complete digestion of the food in the stomach and the intestines, due to its Stimulating properties. Cumin is also Carminative i.e. relieves from you from gastroubles and thereby improves digestion and appetite. Due to its essential oils, magnesium and sodiumcontent, it promotes digestion and also gives relief in stomach-ache when taken with hot water (like aqua ptycotis and mint) [21]. Perfusion of an aqueous extract of cumin via the stomach of pentobarbitone-anesthetized rats under the aspirin-induced gastric mucosal injury showed an increased acid secretion by a cholinergic mechanism [22]. Aqueous and solvent derived extracts of cumin increased amylase, protease, lipase and phytase activities [23].

Anti-osteoporotic:

Cumin seeds are reported to be estrogenic. The presence of phytoestrogens in cumin has been shown and also related to its anti-osteoporotic effects. In the animals receiving a methanolic extract of cumin, a significant reduction in urinary calcium excretion and augmentation of calcium content and mechanical strength of bones was found. Animals showed greater bone and ash densities and improved microarchitecture, with no adverse effects like body weight gain and weight of atrophic uterus [24].

References:

1. Srinivasan K. Plant foods in the management of diabetes mellitus: Spices as beneficial antidiabetic food adjuncts. Int J Food SciNutr 2005;56:399-414.
2. Roman-Ramos R, Flores-Saenz JL, Alarcon-Aguilar FJ. Anti-hyperglycemic effect of some edible plants. J Ethnopharmacol 1995;48:25-32.
3. Dhandapani S, Subramanian VR, Rajagopal S, Namasivayam N. Hypolipidemic effect of *Cuminum cyminum* L. on alloxan-induced diabetic rats. Pharmacol Res 2002;46:251-5.
4. Jagtap AG, Patil PB. Antihyperglycemic activity and inhibition of advanced glycation end product formation by *Cuminum cyminum* in streptozotocin induced diabetic rats. Food ChemToxicol 2010;48:2030-6
5. Kumar PA, Reddy PY, Srinivas PN, Reddy GB. Delay of diabetic cataract in rats by the antiglycating potential of cumin through modulation of á-crystallin chaperone activity. J NutrBiochem 2009;20:553-62.
6. Willatgamuva SA, Platel K, Sarawathi G, Srinivasan K. Antidiabetic influence of dietary cumin seeds (*Cuminum cyminum*) in streptozotocin induced diabetic rats. Nutr Res 1998;18:131-42
7. Lee HS. Cuminaldehyde: Aldose Reductase and alpha-glucosidase inhibitor derived from *Cuminum cyminum* L. seeds. J Agric Food Chem 2005;53: 2446-53
8. Aruna K, Rukkumani R, Varma PS, Menon VP. Therapeutic role of *Cuminum cyminum* on ethanol and thermally oxidized sunflower oil induced toxicity. Phytother Res 2005;19:416-21
9. Kode A, Rajagopalan R, Penumathsa SV, Menon VP. Effect of ethanol and thermally oxidized sunflower oil ingestion on phospholipid fatty acid composition of rat liver: Protective role of *Cuminum cyminum* L. Ann NutrMetab 2005;49:300-3.
10. Shirke SS, Jagtap AG. Effects of methanolic extract of *Cuminum cyminum* on total serum cholesterol in ovariectomized rats. Indian J Pharmacol 2009;41:92-3.

11. Sambaiah K, Srinivasan K. Effect of cumin, cinnamon, ginger, mustard and tamarind in induced hypercholesterolemic rats. Nahrung 1991;35:47-51
12. Derakhshan S, Sattari M, Bigdeli M. Effect of cumin (*Cuminum cyminum* L.) seed essential oil on biofilm formation and plasmid integrity by Klebsiellapneumoniae. Pharmacog Mag 2010; 6: 57-61. 69.
13. Shayegh S, Rasooli I, Taghizadeh M, Astaneh SD. Phytotherapeutic inhibition of supragingival dental plaque. Nat Prod Res 2008; 22:428- 39.
14. De ML, De FV, Fratianni F, Nazzaro F. Chemistry, antioxidant, antibacterial and antifungal activities of volatile oils and their components. Nat Prod Commu 2009; 4: 1741-50
15. Hajlaoui H, Mighri H, Noumi E, Snoussi M, Trabelsi N, Ksouri R. Chemical composition and biological activities of Tunisian *Cuminum cyminum* L. essential oil: A high effectiveness against Vibrio spp. strains. Food ChemToxicol 2010; 48: 2186-92
16. Skrinjar MM, Mandi AI, Misan AC, Sakac MB, Saric LC, Zec MM. Effect of mint (Mentha piperita L.) and Caraway (Carumcarvi L.) on the growth of some toxicogenic Aspergillus species and aflatoxin B1 production. Proc Nat SciMaticaSrpska Novi Sad 2009; 116:131-9.
17. Nalini N, Sabitha K, Vishwanathan P, Menon VP. Influence of spices on the bacterial (enzyme) activity in experimental colon cancer. J Ethnopharmacol 1998; 62: 15-24.
18. Nalini N, Manju V, Menon VP. Effect of spices on lipid metabolism in 1, 2-dimethylhydrazine-induced rat colon carcinogenesis. J Med Food 2006; 9: 237-45.
19. Chauhan PS, Satti NK, Suri KA, Amina M, Bani S. Stimulatory effects of *Cuminum cyminum* and flavonoid glycoside on cyclosporine-A and restraint stress induced immune-suppression in swiss albino mice. ChemBiolInterac 2010;185:66-72
20.http://articles.timesofindia.indiatimes.com/2012-08-03/diet/31210170_1_cumin-health-benefits-lentil preparations.
21. Dhandapani S, Subramaninan VR, Rajagopal S, Namasivayam N. Pharmacol Res 2002; 46: 251–255.
22. Vasudevan K, Vembar S, Veeraraghavan K, Haranath PS. Influence of intragastric perfusion of aqueous spice extracts on acid secretion in anesthetized albino rats. Indian J Gastroenterol. 2000;19:53–6.
23. Milan KSM, Dholakia H, Tiku PK, Vishveshwaraiah P. Enhancement of digestive enzymatic activity by cumin (Cuminumcymonum L.) and role of spent cumin as a bionutrient. Food Chem.2008;110:678–83.
24. Shirke SS, Jadhav SR, Jagtap AG. Methanolic extract of *Cuminum cyminum* inhibits ovariectomy-induced bone loss in rats. ExpBiol Med. 2008;233:1403–10.

ANTIBACTERIAL ACTIVITY OF BIOPESTICIDES AGAINST SEED BORNE FUNGI OF JOWAR

L. R. Rathod*[1] and S. L. Gaikwad[2]

[1]Department of Botany,

[2]Department of Physics,

Mahatma Phule Arts, Science, Commerce College, Panvel

*Corresponding author E-mail: lrathod78@yahoo.com

Abstract:

The effect of biocontrol agents, antagonists *Pseudomonas fluorescence* and *Bacillus subtilis* and effect of were observed on dominant fungi like *Aspergillus flavus* (Link), *Fusarium moniliforme* (J. Schled.), and *Alternaria Alternata* (Fr. Keissl). The present investigation and seeds can be stored for a longer time and disease can be stored for a longer time and disease can be controlled. Hence this investigation will be useful in increasing the productivity of Jowar seeds.

Keywords: Antagonists, *Pseudomonas fluorescence* and *Bacillus subtilis* and Seed borne Pathogens.

Introduction:

Biocontrol offers a solution to many of the persistent problems in agriculture including problems of health hazards, environmental pollution, residual effects, overreliance on pesticides/fungicides, resource limitations, etc. Biocontrol mechanism to suppress fungal pathogens by *Trichoderma* spp., *Pseudomonas* and *Bacillus spp*. generally involves the production of antibiotics, siderophores, volatile compounds, hydrocyanic acid (HCN), enzymes phytohormones and subtilin, phosphatidylethanolamine, and lysylphosphatidylglycerol. The effect of biocontrol agents i.e., bacterial species including *Pseudomonas fluorescens* and *Bacillus subtilis* were tested by dual culture technique. The Petri plates were incubated with pathogenic fungi and biocontrol agents. The zone of inhibition was measured concerning its growth and results were recorded. Biological control of seed-borne pathogens continues to inspire research and development in many fields. However, the interrelationships of many environmental variables can result in multiple interactions among organisms and their environment, several of which might contribute to effective biological control as a biological control agent, *P. fluorescens* has been shown to have a beneficial effect on plant growth and health (Anuratha and Gnanamanickam, 1990; Shanmugam *et al.*, 2002; Chin-A-Woeng *et al.*, 2003). Furthermore,

natural products and chemical compounds were discovered as a result of basic research into the molecular mechanisms of pathogenesis and we use the term biological control in the broader sense as we describe the current status of research, commercial development, and application of biocontrol strategies targeted at plant pathogens (De Boer, 1999; Reddy *et al.*, 2007, 2009). Cook and Baker (1983), Georgakopoulos *et al.* (2002), Crowley (2006), Girija and Manojkumar (2005) and Roy Manidipa1 *et al.* (2013) studied the biological control of Plant Pathogens. The mechanisms of biological control of plant pathogens by antagonistic bacteria and fungi have been the subjects of many studies in the past two decades (Janisiewicz *et al.*, 2000; Fravel, 2005; Megha *et al.*, 2007). Mechanisms of biocontrol of root and soil-borne pathogens are as a result of the direct action of antagonists on plant pathogens, through antibiosis, predation or parasitism, induced resistance of the host plant, and direct competition for space and limited resources (Janisiewicz *et al.*, 2000; Gupta *et al.*, 2001; Nagarajkumar *et al.*, 2004; Romero, 2004; Lwin and Ranamukhaarachchi, 2006).

Materials and Methods:

Dual culture technique:

Antagonistic properties of bacterial strains (*Pseudomonas fluorescens* and *Bacillus subtilis*) were tested against *Aspergillus flavus* (Link), *Fusarium moniliforme* (J. Schled.), and *Alternaria alternata* (Fr. Keissl) on PDA plates using a dual culture technique (Skidmore and Dickinsom, 1976). Agar blocks (5 days old, 5mm diameter) containing 5 days old mycelium were placed in the center of the PDA plate. A loop full 24 hours old culture of bacterial strain was inoculated at 2cm juxtaposed to the pathogen on each plate. The fungal pathogen was inoculated centrally on the PDA plate but uninoculated by bacteria strain served as control. The plates were incubated at 28± 10C for 5 days and colony growth inhibition (%) was calculated. Observe the development of the inhibition zone. The diameter of the mycelial growth was measured when controlled plates showed the maximum growth, after 8 days of incubation. The percentage inhibition of mycelial growth was calculated as per the formula given by Vincent (1947).

Experimental Results:

Table 1 shows that the growth of *Aspergillus flavus* was 4.0 cm in presence of *Pseudomonas fluorescens*, while 4.5 cm in the presence of *Bacillus subtilis.* The percent growth of inhibition of *Pseudomonas fluorescens* and *Bacillus subtilis* were 46.66% and 40.00%

respectively. The growth of *Aspergillus flavus* on the control plate was 7.5 cm. *Pseudomonas fluorescens* inhibit the maximum growth of *Aspergillus flavus* as compared to *Bacillus subtilis.*

The growth of *Fusarium Fusarium moniliforme* (J. Schled) in presence of *Pseudomonas fluorescens* was 2.5 cm and the percentage growth of inhibition was 58.33. In presence of *Bacillus subtilis* growth of the fungus was 3.5 cm and the percentage growth of inhibition was 41.66. The growth of *Fusarium moniliforme* on the control plate was 6.0 cm from Table 2 and Fig 1. So, it is clear that *Pseudomonas fluorescens* inhibits the maximum growth of *Fusarium moniliforme* as compared to *Bacillus subtilis.*

Table 3 reveal that the growth of *Alternaria Alternata* in presence of *Pseudomonas fluorescens* was 2.0 cm and the percentage growth inhibition was 50.00 while in the presence of *Bacillus subtilis* growth was 3.0 cm and the percentage of growth inhibition was 25.00. The growth of *Alternaria Alternata* on the control plate was 4.0 cm. The percentage of growth inhibition was maximum by *Pseudomonas fluorescens.*

Table 1: Effect of Bacterial species on the growth of *Aspergillus flavus* Link. in Dual culture

Sr. No.	Bacterial antagonists	Growth of *Aspergillus flavus* against bacterial species in cm	% of growth inhibition
1	***Pseudomonas fluorescens***	4.0	46.66
2	***Bacillus subtilis***	4.5	40.00
3	Control	7.5	-
	S.E. ±	0.89	2.35
	C.D. at p=0.01	8.83	149.60
	C.D. at p=0.05	3.85	29.86

Table 2: Effect of Bacterial species on the growth of *Alternaria Alternata* (Fr. Keissl) in Dual culture

Sr. No.	Bacterial antagonists	Growth of *Alternaria alternata* against bacterial species in cm	% of growth inhibition
1	***Pseudomonas fluorescens***	2.0	50.00
2	***Bacillus subtilis***	3.0	25.00
3	Control	4.0	-
	S.E. ±	0.46	8.86
	C.D. at p=0.01	4.56	564.02
	C.D. at p=0.05	19.17	112.61

Discussion:

Vincent (1947) studied the Distortion of fungal hyphae in the presence of certain inhibitors. Agrawal (1976) observed a Technique for the detection of seed-borne fungi. Skidmore and Dickinsom (1976) observed colony interaction of hypal interference between *Septoria nodorium* and Phylloplane fungi. Loper (1988): studied the Role of fluorescent siderophore production in biological control of *Pythium ultimum* by a *Pseudomonas fluorescens* strain. Anuratha and Gnanamanickam (1990) studied the biological control of bacterial wilt caused by Pseudomonas solanacearum. Smith Handelsman and Goodman (1997) studied Modeling dose-response relationships in biological control: partitioning host responses to the pathogen and biocontrol agent. Vidhyasekaran and Muthamilan (1999) evaluated a powder formulation of *Pseudomonas fluorescens*. Janisiewicz *et al.* (2000) studied the mechanism of biological control of postharvest diseases on fruits Whipps (2001) studied Microbial interactions and biocontrol in the rhizosphere. Gupta *et al.* (2001) observed Antibiosis mediated necrotrophic effect of *Pseudomonas* against two fungal pathogens. Georgakopoulos *et al.* (2002) evaluated the Biological control of cucumber and sugar beet damping-off caused by Pythium ultimun with bacterial and fungal antagonists. Shanmugam *et al.* (2002) observed the Interaction of *Pseudomonas fluorescens* with Rhizobium for their effect on the management of peanut root rot. Chin-A-Woeng *et al.* (2003) studied Phenazines and their role in biocontrol by Pseudomonas bacteria. Suryakala *et al.* (2004) studied Chemical characterization and in vitro antibiosis of siderophores of rhizosphere fluorescent pseudomonads. Wandersman and Delepelaire (2004) observed Bacterial iron sources: From siderophores to hemophores. Nagarajkumar *et al.* (2004) studied the involvement of secondary metabolites and extracellular lytic enzymes produced by *Pseudomonas fluorescens* in inhibition of *Rhizoctonia solani*, the rice sheath blight pathogen. Romero *et al.* (2004). Isolated and evaluation of antagonistic bacteria towards the cucurbit powdery mildew fungus *Podosphaera fusca*. Manwar *et al.* (2004). Observed Siderophore production by a marine *Pseudomonas aeruginosa* and its antagonistic action against phytopathogenic fungi. Fravel (2005) studied the Commercialization and implementation of biocontrol. Girija Ganeshan and Manojkumar (2005) screened *Pseudomonas fluorescens*, a potential bacterial antagonist to control plant diseases. Crowley (2006) studied Microbial siderophores in the plant rhizospheric. Lwin and Ranamukhaarachchi (2006) studied the Development of biological control of *Ralstonia solanacearum* through antagonistic microbial populations. de Bruijn *et al.* (2007) Genome-based discovery, structure prediction, and functional analysis of cyclic lipopeptide antibiotics in *Pseudomonas* species. Megha, *et al.*

(2007) observed Multiple Beneficial Functions of Fluorescent Pseudomonads of Western Ghats of Uttar Kannada Reddy *et al.* (2007) screened Antifungal metabolites of *Pseudomonas fluorescens* isolated from the rhizosphere of rice crop. Reddy and Rao (2009) observed Biochemical and PCR-RAPD characterization of *Pseudomonas fluorescens* produced antifungal compounds inhibit the rice fungal pathogens in vitro. Roy Manidipal *et al.* (2013) studied Pseudomonads: Potential Biocontrol agents of Rice Diseases.

Conclusion:

Seed act as a carrier for the transport of seed-borne pathogens harmful to the plants. The abundance of seed viability of seed-borne fungi is responsible for loss in seed viability and poor growth of the crop, which brings about varied pathogenic effects on plants. Biological control is an ecology-conscious, cost-effective, and sustainable alternative method in disease management. As reported by many researchers that *Pseudomonas* and *Bacillus* produce different antibiotics which suppress the disease-causing pathogens. The ability of bacterial siderophores and antibiotics to suppress phytopathogens and seed-borne pathogens could be of significant agronomic importance. We conclude by describing prospects for using biological control to limit the damage of seed-borne pathogens in both conventional and organic agriculture.

References:

Agrawal, V. K. (1976). Technique for the detection of seed-borne fungi. Seed Res. 4: 24-31.

Anuratha, C. S. Gnanamanickam, S. S. (1990). Biological control of bacterial wilt caused by *Pseudomonas solanacearum* in India with antagonistic bacteria. *Plant Soil*. 124:109-116.

Chin-A-Woeng T.F., Bloemberg, G. V. and Lugtenberg, B. J. (2003). Phenazines and their role in biocontrol by *Pseudomonas* bacteria. New Phytol 157:503-523.

Crowley, D. E. (2006). Microbial siderophores in the plant rhizospheric. In: Barton LL and Abadía J (eds) Iron Nutrition in Plants and Rhizospheric Microorganisms. Springer, Dordrecht, pp 169-198.

de Bruijn I, de Kock MJD, Yang, M. de Waard, P. van Beek, T. A. and Raaijmakers, J. M. (2007). Genome-based discovery, structure prediction, and functional analysis of cyclic lipopeptide antibiotics in *Pseudomonas* species. Mol Microbiol 63:417-428.

Fravel, D. R. (2005). Commercialization and implementation of biocontrol. *Annual Review of Phytopathology*. 43:337-59.

Georgakopoulos, D. G., Fiddaman, P., Leifert, C. and Malathrakis, N. E. (2002). Biological control of cucumber and sugar beet damping-off caused by *Pythium ultimun* with bacterial and fungal antagonists. *Journal of Applied Microbiology.* 2002; 92:1078-86.

Girija Ganeshan and A. Manojkumar (2005). *Pseudomonas fluorescens*, a potential bacterial antagonist to control plant diseases. *Journal of Plant Interactions.* (3): 123-134.

Gupta, C. P., Dubey, R. C., Kang, S. C., and Maheshwari, D. K. (2001). Antibiosis mediated the necrotrophic effect of *Pseudomonas* GRC2 against two fungal pathogens, *Current Science,* 81, 91-94.

Janisiewicz, W. J., Tworkoski, T. J. and Sharer, C. (2000): Characterizing the mechanism of biological control of postharvest diseases on fruits with a simple method to study competition for nutrients. *Phytopathology.* 90(11):1196-2000.

Loper, J. E. (1988): Role of fluorescent siderophore production in biological control of *Pythium ultimum* by a *Pseudomonas fluorescens* strain. Phytopathology 78:166-172.

Lwin, M., and Ranamukhaarachchi, S. L. (2006). Development of biological control of *Ralstonia solanacearum* through antagonistic microbial populations. *International Journal of Agriculture and Biology.* 8: 657-660.

Manwar, A. V., Khandelwal, S. R., Chaudhuri, B. L., Meyer, J. M. and Chincholkar, S. B. (2004). Siderophore production by a marine *Pseudomonas aeruginosa* and its antagonistic action against phytopathogenic fungi, *Applied Biochem. Biotechnol.*, 118, 243-252.

Megha, Y. J., Alagawadi, A. R. and Krishnaraj, P. U. (2007). Multiple Beneficial Functions of Fluorescent Pseudomonads of Western Ghats of Uttar Kannada District, *Karnataka J.Agric.Sci.*, 20(2), 305-309.

Nagarajkumar, M., Bhaskaran, R. and Velazhahan, R. (2004). Involvement of secondary metabolites and extracellular lytic enzymes produced by *Pseudomonas fluorescens* in inhibition of *Rhizoctonia solani*, the rice sheath blight pathogen, *Microbiology Research*, 159(1), 73-81.

Reddy, K. R. N., Anita, C.D. and Reddy, M.S. (2007). Antifungal metabolites of *Pseudomonas fluorescens* isolated from the rhizosphere of rice crop, *J.Mycol.Pl.Pathol.*, 37(2), 280-284.

Reddy, P. B., and Rao, K. S. (2009). Biochemical and PCR-RAPD characterization of *Pseudomonas fluorescens* produced antifungal compounds inhibit the rice fungal pathogens in vitro, *Electronic J. of Envl., Agrl. and food Chem.*, 8(10), 1062-1067.

Romero, D., Pérez-García, A., Rivera, M. E., Cazorla, F. M. and de Vicente, A. (2004). Isolation and evaluation of antagonistic bacteria towards the cucurbit powdery mildew fungus *Podosphaera fusca* . *Appl. Microbiol. Biotechnol*. 64: 263–269.

Roy Manidipa1, Sharma Gauri Dutta and Ramana Ch.Venkata (2013). Pseudomonads: Potential Biocontrol agents of Rice Diseases. *Research Journal of Agriculture and Forestry Sciences.* Vol. 1(9), 19-25.

Shanmugam, V., Senthil, N., Raguchander, T. Ramanathan, A. Samiyappan, R. (2002). Interaction of *Pseudomonas fluorescens* with *Rhizobium* for their effect on the management of peanut root rot. Phytoparasitica 30:169-176.

Skidmore, A. M., and Dickinsom, C. H. (1976). Colony interaction of hypal interference between *Septoria nodorium* and Phylloplane fungi. *Trans. Brit. Mycol. Sco.* 66:57-64.

Smith, K. P., Handelsman, J. Goodman, R. M. (1997). Modeling dose-response relationships in biological control: partitioning host responses to the pathogen and biocontrol agent. Phytopathology 87, 720–729.

Suryakala, D., Maheshwari Devi, P.V. and Lakshmi, K. V. (2004). Chemical characterization and in vitro antibiosis of siderophores of rhizosphere fluorescent pseudomonads, *Indian Journal of Microbiology*, 44(2), 105-108.

Vidhyasekaran, P. Muthamilan, M. (1999). Evaluation of a powder formulation of *Pseudomonas fluorescens* Pf1 for control of rice sheath blight. Biocontrol Science and Technology 9, 67–74.

Vincent, J.M. (1947). Distortion of fungal hyphae in the presence of certain inhibitors. *Nature.* 159: 850.

Wandersman, C. and Delepelaire, P. (2004). Bacterial iron sources: From siderophores to hemophores. *Annu Rev. Microbiol*. 58:611-647.

Whipps, J. M. (2001). Microbial interactions and biocontrol in the rhizosphere. *J. Exp. Bot.* 52:487-511.

PHYTOCHEMISTRY, ETHNOMEDICINAL USE AND PHARMACOLOGICAL PROFILE OF *ADHATODA VASICA* NEES - AN ETHNOMEDICINAL PLANT

Shalini Lal*[1], Nuzhat Afroz[2] and Binit Baraik[3]

[1]Department of Botany, Dr. Shyama Prasad Mukherjee University,
Ranchi, Jharkhand, India, 834008

[2]Department of Botany, Ranchi University, Ranchi, Jharkhand, India, 834008

[3]Department of Botany, J. N. College, Ranchi University, Ranchi, Jharkhand, India, 834002

*Corresponding author E-mail: shalini.kumari@gmail.com

Abstract:

Medicinal plants are important for pharmacological research and drug development. It provides raw materials for the herbal medicine trade and the pharmaceutical industry. Herbal medicines are derived from the leaves, roots or other parts of the medicinal plants. A large number of modern pharmaceutical drugs are derived from plant secondary metabolites. *Adhatoda vasica* Nees, is a well-known medicinal plant belonging to family Acanthaceae, used for the treatment of various diseases, primarily for the respiratory tract ailments. A significant amount of research has been carried out to explore the chemistry and pharmacological effect of *Adhatoda vasica*. The plant is known to contain pyrroloquinazoline alkaloids and its derivatives, such as vasicine and vasicinone. It shows various pharmacological activities like antimicrobial, antiulsar, anti inflammatory, anti cancer, anti antitussive, andanti-oxidant activities etc. This review describes the phytochemistry, ethnomedicinal uses, and pharmacological activities of *Adhatoda vasica*. Shortly it also describes the genetic diversity studies, biotechnological applications such as micropropation and elicitation studies for the propagation and enhanced metabolites production of this plant.

Keyword: *Adhatoda vasica*, Phytochemistry, pharmacological activity, tissue culture, molecular studies.

Introduction:

Medicinal plants have been used as a source of drugs by mankind for several thousand years to cure different illnesses. India has a rich heritage of traditional medicine and health care system. The Indian system of medicine heavily relies on medicinal plants and Ayurveda have

their roots in folk medicine (Ravishankar and Shukla, 2007). Among the variety of modern medicines too, many of them are produced directly or indirectly from medicinal plants. Studying medicinal plants helps to understand plant diversity, phytochemistry and their pharmacological properties. The medicinal effects of plants depend on the presence of secondary metabolites that are affected by environmental stress.

***Adhatoda vasica* Nees**

Adhatoda vasica Nees belongs to the family Acanthaceae, is a small evergreen shrub with many long opposite branches having large lance-shaped, opposite and exstipulate leaves (Figure 1 and 2). The leaves, flowers, fruits and roots are used as medicinal parts .The leaves of *Adhatoda vasica* contains phytochemicals such as alkaloids, tannins, saponins, phenolics and flavonoids etc. The leaves carry an unpleasant smell and have a bitter taste. The flowers are spikes or panicles, small irregular zygomorphic, bisexual, hypogynous and either white or purple in colour. It has capsular four seeded fruits. The plant is used for the treatment of various diseases and disorders, primarily for the respiratory tract ailments.

Figure 1: *Adhatoda vasica* plant

Figure 2: *Adhatoda vasica* leaf and inflorescence

Origin and distribution

Adhatoda vasica Nees is native to India. It's distributed throughout India up to an altitude of 1,300 m and mainly found in lower Himalayas. It is also found in few parts of Sri-Lanka, Bhutan, Pakistan, Afghanistan, and is additionally introduced to other countries like China, Hong kong, Taiwan, Ethiopia etc. This plant is also found in tropical regions of Southeast Asia and a couple of part in Germany and Sweden**.**

Phytochemical analysis

The phytochemical analysis of ethanolic leaf extract of *Adhatoda vasica* revealed the presence of alkaloids, flavonoids, terpenoids, saponins, phenols and steroids (Bajpai *et al.*, 2015, Sudevan *et al.,* 2019). Six different quinazoline alkaloids (vasicoline, vasicolinone, vasicinone,

vasicine, adhatodine and anisotine) have been detected in the leaf of *Adhatoda vasica* (Jha *et al.,* 2012). Quantification of Vasicine and Vasicine acetate by HPLC-DAD analysis showed their contents to be 0.2293% and 0.0156%, respectively, on dry weight basis of the leaves (Duraipandiyan *et al.,* 2015). Eleven compounds such as, 1,2,3, trimethyl benzene (1.51 %), borneol (58.60%), ethanonaphthalene (2.82%), 1,1,4a trimethyl-5,6-dimethylenedecahydro naphthalene (5.28 %), 2,tert-butyl-1,4- dimethoxy benzene (6.50%), bicyclo[jundec-4-ene,4,11-trimethyl-8-methylene (14.56%), hexa- methyl dewar benzene (0.87%), alphacaryophyllene (1.95%), cycloproplejazulene (1.48 %), caryophyllene oxide (2.35%) and 2-naphthalenemethanol (1.46%) has also been identified from the essential oil obtained by hydrodistillation of *Adhatoda vasica* leaves (Sarkera *et al.,* 2011). A rapid, efficient, and sensitive method has been developed for the estimation of vasicine (Madhukar *et al.,* 2014). A wide range of fatty acids and the heterocyclic compound has also been identified from leaves, shoot and flowers of *Adhatoda vasica* (Khan and Bhadauria, 2017, Jayapriya and Shoba, 2015)**.**

Ethnomedicinal use of *Adhatoda vasica*

Plants have been used since ancient times for the treatment of various diseases. Traditionally, *A. vasica* is used for the treatment of various ailments, which is described in table 1.

Pharmacological activity

During the last 2-3 decade, several scientific studies on the phytochemistry, pharmacological activities and molecular biology of *Adhatoda vasica* have been carried out. A wide range of phytochemical constituents have been isolated from this plant which show pharmacological activities like antimicrobial, antiulcer, anti inflammatory, anti cancer, anti antitussive**,** anti-oxidant activities etc.

Anti-ulcer activity

The anti-ulcer activity of *Adhatoda vasica* leaves using two ulcer models: Ethanol-induced and Pylorus ligation plus aspirin-induced models have been studied. *Adhatoda vasica* leaf powder showed a considerable degree of anti-ulcer activity in experimental rats when compared with a control. The highest degree of activity (80%) was observed in the ethanol-induced ulceration model. Results of the study suggest that in addition to its classically established pharmacological activities, the plant also has immense potential as an anti-ulcer agent of great therapeutic relevance (Shrivastava *et al.,* 2006).

Table 1: Ethnomedicinal use of *Adhatoda vasica*

Ethnomedicinal use	Parts used	Mode of administration
Bronchitis, cough and cold	Leaf	5–6 leaves mixed with ginger is boiled half to its original amount and taken with honey for curing cough (Raj *et al.,* 2018). Leaf juice taken for several days as expectorant to treat chronic bronchitis, cough and cold (Dutta *et al.*, 2014). Flower powder used for cough and cold (Singh *et al.,* 2017).
Diabetes	Leaf Root	Juice of leaves is used with an empty stomach. A decoction of roots (~ 50 g) with cow milk (125 mL) is taken daily in the morning (Kumar *et al.,* 2019).
Stomachache and fever.	Leaf	Leaf buds (5–10) decoction 100 ml is taken thrice a day for treatment of stomachache and fever (Singh *et al.,* 2017). Decoction prepared from leaves of *Adhatoda vasica* is used orally for treatment of fever (Venkatachalapathi *et al.,* 2018).
Tuberculosis.	Leaf	Juice obtained from crushed leaves is orally taken (Kabir *et al.,* 2014).
Nose bleeding, dysentery and blood vomiting	Flower Leaf	Fresh flowers and leaves are boiled in water and decoction is prepared which is consumed once in a day (Sajem and Gosai, 2006)
Itching and Skin infections.	Leaf	Leaves are boiled and the solution is used for taking bath for curing itching (Raj *et al.,* 2018). Young leaves are made into a paste and applied over infected area in skin (Kabir *et al.*, 2014)**.**
Cut and wound	Leaf	Leaf extract or juice applied for treatment of cut and wounds (Singh *et al.,* 2017).
Snakebite	Leaf	Paste is applied externally (Sulochana *et al.,* 2015)**.**
Teeth cleaning	Stem	Stem used for cleaning teeth (Singh *et al.,* 2017).

Antibacterial Activity

Vasicine acetate obtained by acetylation of Vasicine isolated from ethanolic extract of the leaves of *A. vasica* showed antibacterial activity against *M. Luteus*, *E. Aerogenes*, *S. epidermidis*, and *P. aeruginosa*. Vasicine acetate exhibited good zone of inhibition against bacteria: 10 mm

against *E. aerogenes*, 10 mm against *S. epidermidis*, and 10 mm against *P. aeruginosa*. Vasicine acetate showed minimum inhibitory concentration values against bacteria: *M. luteus* (125 μg/mL), *E. aerogenes* (125 μg/mL), *S. epidermidis* (125 μg/mL), and *P. aeruginosa* (125 μg/mL) (Duraipandiyan *et al.,* 2015). Sudevan *et al.,* also reported antibacterial activity against *Salmonella* and *Escherichia coli* (Sudevan *et al.,* 2019). Vasicine showed moderate activity against *M. luteus*, and *S. Typhimurium* (Shahwar *et al.,* 2012). Antimicrobial activity of isolated compounds was assessed by using the micro dilution method. The strong antibacterial activity was exhibited by vasicine at 20 µg/ml dose against *E. coli* (Singh and Sharma 2013). Phytol, a diterpene molecule, isolated and characterized from *Adhatoda vasica* showed antibacterial activity against *Bacillus licheniformis*, a pathogenic bacteria which cause high mortalities and economic losses among the ornamental fish farms of India (Saha and Bandyopadhyay 2020). Copper Oxide/Carbon nanocomposites developed using the leaf extract of *Adhatoda vasica* showed significant antibacterial activity against the pathogenic bacterial strains *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Staphylococcus aureus (*Bhavyasree and Xavier 2020). Nithya and Sundrarajan, prepared noble metal such as Ag and Au doped CeO_2 nanoparticles by ionic liquid assisted hydrothermal method in the presence of *Adhatoda vasica* leaves extract and used as an antibacterial and anticancer agent. The AgAu loaded CeO_2 nanoparticles exhibited the highest zone of inhibition against *E. coli* and *S. aureus* strains when compared with pristine CeO_2, Ag loaded CeO_2, and Au loaded CeO_2 nanoparticles (Nithya and Sundrarajan 2020).

Antifungal activity

The nanocomposites prepared by mixing the copper sulphate penta hydrate solution with the plant extractposses antifungal activity against the fungi *Aspergillus niger* and *Candida albicans (*Bhavyasree and Xavier 2020). In another experiment, vasicine showed antifungal activity against *C. albicans* (Singh and Sharma 2013).

Antioxidant activity

Adhatoda vasica is known to posses significant antioxidant and free radical scavenging activities In an experiment ethanolic leaf extract of *Adhatoda vasica* showed antioxidant capacity, with inhibition of the 1,1-diphenyl2-picrylhydrazyl (DPPH) radical, scavenging nitric oxide, superoxide and hydroxyl radicals. Moreover, it also displayed a concentration-dependent reducing power activity and potent inhibitory effects on ferric ion-induced lipid peroxidation in bovine brain extract (Bajpai *et al.,* 2015). Vasicine isolated from the ethanolic extract exhibited significant antioxidant activity. In an experiments vasicine showed significant antioxidant

activity as infered by the concentration dependent increase of the reduction of Fe^{3+} in FRAP assay and significant scavenging of free radicals in DPPH assay (Shahwar *et al.*, 2012). The radical scavenging activity of Vasicine acetate obtained by acetylation of Vasicine isolated from ethanolic extract of the leaves of *A. vasica* was the maximum at 1000 μg/mL (66.15%) (Duraipandiyan *et al.*, 2015). Vasicinone, a quinazoline alkaloid from *Adhatoda vasica* treatment also lowered the ROS levels in A549 cells and have potential free radical scavenging (DPPH, Hydroxyl) activity and ferric reducing power in cell free systems (Dey *et al.*, 2018). These findings suggest the biological efficacy of *A. vasica* as a potential source of natural antioxidants.

Anti-inflammatory activity

The anti-inflammatory activity was tested by using carrageenan and CFA-model induced paw oedema in rats. The observed results revealed that vasicine showed most potent anti-inflammatory effects (59.51%) at the dose of 20.0 mg/kg at 6 h after carrageenan injection. Maximum inhibition rate was observed of vasicinone (63.94%) at the dose of 10.0 mg/kg at 4 days after CFA injection. All the five alkaloids viz., vasicine, vasicinone, vasicine acetate, 2-acetyl benzyl amine, vasicinolone present in the chloroform fraction demonstrated significant anti-inflammatory activities (Singh and Sharma 2013)**.** Sudevan *et al.,* also reported significant anti-inflammatory activity of the active components present in *Adhatoda* plant extract (Sudevan *et al.,* 2019).

Anticancer/Cytotoxic Activity

One of the important aims of drug discovery for cancer is to find therapeutic agents from natural products that are effective and safe for cancer treatment. Vasicine acetate obtained by acetylation of Vasicine isolated from ethanolic extract of the leaves of *A. vasica* showed prominent cytotoxic activity against A549 lung adenocarcinoma cancer cell line (Duraipandiyan *et al.*, 2015). An alkaloid, 2-acetyl-benzylamine *Adhatoda vasica*, posses significant cytotoxic properties against leukemia cells MOLM-14 and NB-4. It induced cell cycle arrest at G2/M phase in MOLM-14 cells and G0/G1 phase in NB-4 cells (Balachandran *et al.,* 2017). The anticancer activity of the *Adhatoda vasica* extract is found to be effective in human cervical cancer cell line (HeLa) has also been reported in in-vitro analysis (Sudevan *et al.,* 2019). Recently it was observed that the CeO_2, Ag/CeO_2, Au/CeO_2 and $Ag\text{-}Au/CeO_2$ nanoparticles prepared by ionic liquid assisted hydrothermal method in the presence of *Adhatoda vasica* leaves extract exhibited anticancer activity against the HeLa cells (Nithya and Sundrarajan 2020). The findings proved that these compounds could be developed as a potential therapeutic agent against cancer. Dey *et al.,* investigated the anti-proliferative effect of vasicinone and its underlying mechanism against A549 lung carcinoma cells. The A549 cells upon treatment with various

doses of vasicinone showed significant decrease in cell viability. Vasicinone treatment caused DNA fragmentation, LDH leakage, and disruption of mitochondrial potential, and lower wound healing ability in A549 cells. Moreover it was also observed that the vasicinone treatment lead to down regulation of Bcl-2, Fas death receptor and up regulation of PARP, BAD and cytochrome c, which suggest the anti-proliferative nature of vasicinone which mediated apoptosis through both Fas death receptors as well as Bcl-2 regulated signalling (Dey *et al.*, 2018).

Antimutagenic activity

Environmental and occupational exposure with cadmium affects the renal system adversely. In a study, the antioxidant and anticlastogenic efficacy of *A. vasica* against cadmium chloride (CdCl2)-induced renal oxidative stress and genotoxicity in Swiss albino mice has been evaluated. A single intra-peritoneal dose of CdCl2 resulted in significant increase in chromosomal aberration and micronuclei formation. Cadmium intoxication altered the antioxidant levels and enhanced malondialdehyde **(**MDA) formation significantly. Oral administration of *A. vasica* at two doses (50 and 100 mg/kg BW) for seven consecutive days showed significant suppression of mutagenic effects of $CdCl_2$ in plant-pretreated groups. The antimutagenic efficacy of *A. vasica* can be attributed to its restoring effects on antioxidant status and suppression of MDA level formation. Prophylactic pretreatment of *A. vasica* extract in cadmium-intoxicated mice showed marked inhibition of lipid peroxidation (LPO) and xanthine oxidase (XO) activity (Jahangir *et al.*, 2006)**.**

Antitussive activity

Antitussive drugs are amongst the most widely used medications worldwide. In traditional system of medicine *A. vasica* has been used for its antitussive activity. Leaf extract used to treat cold, cough, whooping cough, as an expectorant and to treat chronic bronchitis. It was reported that the *Adhatoda vasica* leaves extract posses good antitussive activity. The antitussive activity of *Adhatoda vasica* extract has been evaluated in anaesthetized guinea pigs and rabbits and in anaesthetized guinea pigs. Intravenously, it was as active as codeine on mechanically and electrically induced coughing in rabbits and guinea-pigs. After oral administration to the guinea-pig the antitussive activity was similar to codeine against coughing induced by irritant aerosols (Dhuley 1999). A pectic arabinogalactan has also been isolated from *Adhatoda vasica* by aqueous extraction and precipitation with ethanol. Oral administration of arabinogalactan inhibited the number of coughs induced by citric acid in guinea pigs and slightly decreased the values of specific airway resistance (Chattopadhyay *et al.*, 2011). Similarly, ethyl acetate and methanolic extract of leaves of *Adhatoda vasica* also posses

antitussive activity in Ammonium hydroxide and Sulphur dioxide induced cough models in mice. It was found that both extracts of *Adhatoda vasica* showed anti-tussive activity and obtained percentage inhibition of cough reflex is approximately comparable as standard drug Codiene phosphate (Srivastava and Choudhary 2016).

Immunomodulatory

Vinothapooshan and Sundar reported the immunomodulatory properties of different extract of leaves of *Adhatoda vasica* in experimental animals. Oral administration of extracts in adult male Wister rats significantly increased the percentage neutrophil adhesion to nylon fibers. The observed results at different doses were significant when compared to control groups (Vinothapooshan and Sundar, 2011). This indicated that the extract could be useful as chemotherapy for neutrophils. In another study, effect of *Adathoda vasica* extract in antibody production, no. of antibody producing plasma cell, release of anti-inflammatory cytokines in serum on mice was studied. The leaf extract had long term effect on humoral immune system and inhibitory potential on inflammatory response (Adhikary *et al.*, 2014).

Radio modulatory effect

Modulatory effect of ethanolic extract of *A. vasica* against radiation-induced changes in terms of histological alterations in testis, reduced glutathione (GSH), lipid peroxidation (LPO), acid and alkaline phosphatases levels, and chromosomal alterations in Swiss albino mice has been studied. *A. vasica* extract pretreated irradiated animals exhibited a significant increase in GSH content and decrease in LPO level. Similarly, a significant increase in the serum alkaline phosphatase activity and decrease in acid phosphatase activity was observed in *A. vasica* extract pretreated irradiated animals. *Adhatoda* pretreatment also significantly prevented radiation-induced chromosomal damage in bone marrow cells. Finding from these studies suggests that *Adhatoda* plant extract has significant radioprotective effects (Kumar *et al.*, 2005, Kumar *et al.*, 2007).

Anti Alzheimer

Alzheimer's disease (AD) is a neurodegenerative disease affecting older adults and is characterized by a progressive decline of memory and cognition. Various studies have been carried out to find therapeutic approaches for Alzheimer's disease. However, the proper treatment option is still not available. The herbal therapy is now anticipated to control AD progression and help to relieve the symptoms. Medicinal plants used in different systems of medicine particularly exhibit their powerful role in the management and cure of memory disorders (Akram and Nawaz 2017). *Adhatoda vasica* showed inhibitory effect on acetylcholinesterase (AChE). Additionally, itsstrong and reversible interaction against AChE, make them effective, new and promising

agents for treatment of AD in the future (Ali *et al.,* 2013, Ali *et al.,* 2016). Based on these studies researcher suggested that *Adhatoda vasica* can be used directly or indirectly for the development of efficient drug for the treatment of Alzheimer disease.

Anti-tuberculosis activity

Aqueous extract of leaves of *A. vasica*, was tested *in vitro* for its activity against two multi-drug resistant (MDR) isolates (DKU-156 and JAL-1236) and susceptible strain *M. tuberculosis* H37Rv using Lowenstein Jensen (L-J) medium and colorimetric BacT/ ALERT 3D system. Extract exhibited anti-tuberculosis activity against MDR isolate DKU-156, JAL-1236 and sensitive *M. tuberculosis* H37Rv in L-J medium. In BacT/ALERT also, extract of this plant showed significant inhibition against *M. Tuberculosis* (Gupta *et al.,* 2010). Vasicine acetate and 2-acetyl benzylamine isolated from hexane extract of *A. vasica* leaves significantly inhibited pathogenic bacteria *M. Tuberculosis* (Ignacimuthu *et al.,* 2010).

Hepatoprotective activity

Adhatoda vasica leaf extract showed significant hepatoprotective effect at doses of 50-100 mg/kg, on liver damage induced by D-galactosamine in rats (Bhattacharyya *et al.,* 2005). Hepatoprotective activity of vasicinone isolated from leaves of *Adhatoda vasica* has been reported in Carbon Tetrachloride (CCl_4)-induced acute hepatotoxicity model in mice. CCl_4 treatments lead to significant increase in serum glutamic oxaloacetate transaminase (SGOT), serum glutamic pyruvates transaminase (SGPT), alkaline phospatase (ALP) levels. Pre-treatment with vasicinone significantly decreased these enzyme levels. Furthermore, these biochemical observations were also confirmed by histopathological examinations of liver sections and were comparable with the standard hepatoprotective drug Silymarin which served as a positive control (Sarkar *et al.,* 2014). Similarly, hepatoprotective activity of aqueous extract of *Adhatoda vasica* on total protein, bilirubin, aspartate aminotransferase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) in CCl_4 intoxicated rats has also been studied. Administration of CCl_4 showed significantly increase liver marker enzymes in serum and significantly decreased total protein. *A. vasica* at 250 mg/kg and 500 mg/kg body weight showed significant increase in total protein when compared to CCl_4 treated rats. Leaf extract lowered enzymes levels, which is a designation of hepatoprotective action of the extract (Kumar *et al.,* 2015).

Antidiabetic

In an experiment it was observed that the methanolic extract from the leaves of *Adhatoda vasica* showed sucrase inhibitory activity. Compounds vasicine and vasicinol showed high sucrase inhibitory activity, and the IC50 values were 125μM and 250μM, respectively (Gao *et al.,* 2008). The effect of ethanolic extracts of leaves and roots was also studied in alloxan

induced diabetic animals. Oral administration of leaves extract to normal and experimental diabetic rats produced a significant reduction in blood glucose levels as compared to the root extract (Gulfraz *et al.,* 2011). These finding suggest use of the *A. vasica* extract as an antidiabetic agent and show a possibility that compounds present in this plant could be a useful treatment for metabolic disorders.

Antiviral activity

The preliminary report on antiviral activity of *Adhatoda vasica*, against Herpes Simplex Viruses shows its antiviral activity. The Herpes viruses (HSV) are important pathogens that can cause mild to severe viral infections in human (Koelle and Corey 2008). Methanolic and ethanoloic extract from the leaves of *Adhatoda vasica* significantly inhibits plaque formation in HSV infected tissue culture, which suggest that this herbal extract has potent anti-viral agents against herpes simplex viruses that can be exploited for development of an alternative remedy for HSV infections (Chavan *et al.,* 2013).

Genetic diversity in *Adhatoda vasica*

Systematic analysis of genetic diversity and relationship among cultivars is essential for development of appropriate conservation and breeding program. DNA-based molecular marker techniques, such as random amplification of polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) has been used to unravel the genetic variability and relationships across thirty-two wild accessions of *Adhatoda vasica*. Amplification of genomic DNA using 38 primers (18 RAPD and 20 ISSR) yielded 434 products, of which 404 products were polymorphic revealing 93.11 % polymorphism (Kumar *et al.,* 2014). Garg *et al.,* also reported genetic variation amongst the accessions of *Adhatoda vasica* collected from different sub-climatic zones of India by RAPD (Garg *et al.,* 2015). Genetic diversity study in India using Amplified Fragment Length Polymorphism (AFLP) fingerprinting also suggested a very high genetic variation in *Adhatoda vasica* (Varma and Shrivastava 2018).

In vitro* regeneration and production of secondary metabolites in *Adhatoda vasica

Conventionally, *A. vasica* is propagated through seed germination and stem cuttings. But poor seed germination and season dependent propagation via stem cuttings limit the frequency of propagation. Tissue culture technique provides a high efficiency regeneration system for successful regeneration of adventitious shoots for ex situ preservation as well as genetic improvement studies for pharmaceutical uses and future research investigations in medicinal plants. A novel protocol for indirect shoot organogenesis of *Adhatoda vasica* was also developed using petiole and leaves explants. Media with concentrations of cytokinins in combination with auxins were used to induce callus formation. Although both petiole and leaf segment explants

produced callus, higher potential for organogenic differentiation from callus derived from petiole explants was observed on MS medium supplemented with 0.25 mg l^{-1} NAA and 0.25 mg l^{-1} TDZ (Mandal and Laxminarayana 2014). Prajila and Indulekha, induced callus from axillary bud explants of *Adhatoda* and the callus showed shoot organogenesis in MS+2,4-D 0.1 mg/l and BAP 4.0 mg/l and root was induced in MS+ 2,4-D 0.1mg/l (Prajila and Indulekha 2016). Plant cell cultures, shoot cultures, root cultures and transgenic roots obtained through biotechnological means are promising potential alternative sources for the production of high-value secondary metabolites of industrial importance (Rao and Ravishankar 2002). It was observed that the *in vitro* cultured samples produced relatively higher vasicinone than that of the *in vivo* counterparts. In vivo leaves and stems produced comparatively lower quantities of vasicinone (2.41% and 1.93% of dry weight, respectively) as compared to leaves, stems and calli (6.4%, 2% and 5.22 % of dry weight, respectively) (Panigrahi *et al.*, 2017).

Madhukar *et al.*, optimized tissue culture protocol for quinazoline alkaloids that can be used at industrial level using bioreactors for large scale production and constant supply of vasicine. The addition of extra 28 mM KNO_3 and 100 mM NaCl in MS medium supplemented with 2,4-dichlorophenoxyacetic acid, benzyladenine and indole acetic acid (1ppm each) produces faster biomass and higher amount of quinazoline alkaloids (Madhukar *et al.*, 2014).

Enhanced secondary metabolites production in *Adhatoda vasica* by Elicitation

Plants and/or plant cells *in vitro*, show physiological and morphological responses to microbial, physical or chemical factors which are known as ‘elicitors’. Elicitation is a process of inducing or enhancing synthesis of secondary metabolites by the plants to ensure their survival, persistence and competitiveness (Namdeo 2007). Effect of elicitors to improve the productivity of useful metabolite in *Adhatoda vasica* cell culture has also been studied by some author. Increased vasicine production in cell suspension culture of *A. vasica* supplemented with α-naphthyl acetic acid and Mannitol at concentration as 3mg 100ml-1 and 0.32M 100ml-1 respectively has been reported (Pa and Mathew 2012). Pyrroloquinazoline alkaloids are medicinally important compounds, determined by HPLC from cell cultures of *Adhatoda vasica*. Anthranilate synthase functions as rate-limiting factor for the biosynthesis of pyrroloquinazoline alkaloids. Tryptophan and sorbitol enhanced the production of vasicinone, vasicine, 2-acetyl benzyl amine and other pyrroloquinazoline alkaloids by stimulating the anthranilate synthase activity in cell cultures of *A. vasica* (Singh *et al.*, 2017).

Conclusion:

For the last few years, there has been an increasing trend and awareness in medicinal plants research. *Adhatoda vasica* Nees, belonging to family Acanthaceae, is a well-known medicinal plant used for the treatment of various diseases and disorders, primarily for the respiratory tract ailments.It contains pyrroloquinazoline alkaloids and its derivatives, such as vasicine and vasicinone, which is responsible for its pharmacological activities. A significant amount of research has already been carried out during the past few decades in exploring the chemistry and pharmacological effect of *Adhatoda vasica*. But further extensive research and development work still needed for their better therapeutic utilization.

Acknowledgement:

The authors are thankful to the Head, PG Department of Botany, Dr. Shyama Prasad Mukherjee University, Ranchi, Jharkhand for their valuable support and providing necessary facilities for writing the review article.

References:

Adhikary R, Majhia A, Mahantia S, Bishayi B. (2014): Immunomodulatory and anti-oxidant properties of methanolic extract of *Adhatoda vasica* Nees leaf after particulate antigen stimulation in mice, *Journal of Pharmacy Research*,8(10):1520-1537.

Akram M, Nawaz A. (2017): Effects of medicinal plants on Alzheimer's disease and memory deficits. *Neural Regen Res*, 12(4):660-670.

Ali SK, Hamed AR, Soltan MM, El-Halawany AM, Hegazy UM, Hussein AA. (2016): Kinetics and molecular docking of vasicine from *Adhatoda vasica*: An acetylcholinesterase inhibitor for Alzheimer's disease, *South African Journal of Botany*, 104:118–124.

Ali SK, Hamed AR, Soltan MM, Hegazy UM, Elgorashi EE, El-Garf IA, Hussein AA. (2013): In-vitro evaluation of selected Egyptian traditional herbal medicines for treatment of Alzheimer disease. *BMC Complement Altern Med*, 13:121.

Bajpai VK, Agrawal P, Bang BH, Park YH. (2015): Phytochemical analysis, antioxidant and antilipid peroxidation effects of a medicinal plant, *Adhatoda vasica*. *Frontiers in Life Science*, 8(3):305-312.

Balachandran C, Arun Y, Sangeetha B, Duraipandiyan V, Awale S, Emi N, Ignacimuthu S, Perumal PT. (2017): *In vitro* and *in vivo* anticancer activity of 2-acetyl-benzylamine isolated from *Adhatoda vasica* L. leaves, *Biomed Pharmacother*, 93:796-806.

Bhattacharyya D, Pandit S, Jana U, Sen S, Sur TK. (2005): Hepatoprotective activity of *Adhatoda vasica* aqueous leaf extract on D-galactosamine-induced liver damage in rats, *Fitoterapia*, 76(2):223-5.

Bhavyasree PG, Xavier TS. (2020): Green synthesis of Copper Oxide/Carbon nanocomposites using the leaf extract of *Adhatoda vasica* Nees, their characterization and antimicrobial activity, *Heliyon*, 6(2): e03323.

Chattopadhyay N, Nosalova G, Saha S, Bandyopadhyay SS, Fleskova D, Ray B. (2011): Structural features and antitussive activity of water extracted polysaccharide from *Adhatoda vasica*., *Carbohydr Polym*, 83 (4): 1970–1974.

Chavan R, Gohil D, Shah V, Kothari S, Chowdhary A. (2013): Anti-viral activity of indian medicinal plant *Justicia adhatoda* against herpes simplex virus: An *In-Vitro* study, *International Journal of Pharma and Bio Sciences*, 4(4):769 – 778.

Datta T, Patra AK, Dastidar SG. (2014): Medicinal plants used by tribal population of Coochbehar district, West Bengal, India-an ethnobotanical survey, *Asian Pac J Trop Biomed*, 4(1): S478-82.

Dey T, Dutta P, Manna P, Kalita J, Boruah HPD, Buragohain AK, Unni B. (2018): Anti-Proliferative Activities of Vasicinone on Lung Carcinoma Cells Mediated via Activation of Both Mitochondria-Dependent and Independent Pathways, *Biomol Ther (Seoul)*, 26(4):409-416.

Dhuley JN. (1999): Antitussive effect of *Adhatoda vasica* extract on mechanical or chemical stimulation-induced coughing in animals, *J Ethnopharmacol*, 67(3):361-365.

Duraipandiyan V, Al-Dhabi NA, Balachandran C, Ignacimuthu S, Sankar C, Balakrishna K. (2015): Antimicrobial, antioxidant, and cytotoxic properties of vasicine acetate synthesized from vasicine isolated from *Adhatoda vasica* L., *Biomed Res Int*, 2015:727304.

Gao H, Huang YN, Gao B, Li P, Inagaki C, Kawabata J. (2008): Inhibitory effect on α-glucosidase by *Adhatoda vasica* Nees., *Food Chem*, 108(3):965-72.

Garg M, Tamboli ET , Singh M, Chester K, Abdin MZ, Chandna R, Ahmad S. (2015): RAPD Based assessment of genetic diversity of *Adhatoda vasica* leaves from different Sub Continents of India, *Indian Journal of Pharmaceutical Education and Research*, 49(3):231-239.

Gulfraz M, Ahmad A, Asad MJ, sadiq A, Afzal U, Imran M, Anwar P, Zeenat A, Abbasi KS, Maqsood S, Qureshi RU. (2011): Antidiabetic activities of leaves and root extracts of *Justicia adhatoda* Linn against alloxan induced diabetes in rats, *African Journal of Biotechnology*, 10(32):6101-6106.

Gupta R, Thakur B, Singh P, Singh HB, Sharma VD, Katoch VM, Chauhan SV. (2010): Anti-tuberculosis activity of selected medicinal plants against multi-drug resistant *Mycobacterium tuberculosis* isolates, *Indian J Med Res*, 131:809-13.

Ignacimuthu S, Shanmugam N. (2010): Antimycobacterial activity of two natural alkaloids, vasicine acetate and 2-acetyl benzylamine, isolated from Indian shrub *Adhatoda vasica* Ness. Leaves,*J Biosci*, 35(4):565-70.

Jahangir T, Khan TH, Prasad L, Sultana S. (2006): Reversal of cadmium chloride-induced oxidative stress and genotoxicity by *Adhatoda vasica* extract in Swiss albino mice, *Biol Trace Elem Res*, 111(1-3):217-28.

Jayapriya G, Shoba FG. (2015): GC-MS analysis of bio-active compounds in methanolic leaf extracts of *Justicia adhatoda* (Linn.), *Journal of Pharmacognosy and Phytochemistry*, 4(1): 113-117.

Jha DK, Panda L, Lavanya P, Ramaiah S, Anbarasu A. (2012): Detection and confirmation of alkaloids in leaves of *Justicia adhatoda* and bioinformatics approach to elicit its anti-tuberculosis activity, *Appl Biochem Biotechnol*, 168(5):980-90.

Kabir MH, Hasan N, Rahman MM, Rahman MA, Khan JA, Hoque NT, Bhuiyan MR, Mou SM, Jahan R, Rahmatullah M. (2014): A survey of medicinal plants used by the Deb barma clan of the Tripura tribe of Moulvibazar district, Bangladesh, *J Ethnobiol Ethnomed*,10:19.

Khan AM, Bhadauria S. (2017): Isoloation of some potential phytocompounds from *Adhatoda vasica* through Gas Chromatography-Mass Spectroscopy Analysis, *Asian J Pharm Clin Res*, 10(12): 328-332.

Koelle DM, Corey L. (2008): Herpes Simplex: Insights on Pathogenesis and Possible Vaccines, *Annu Rev Med*, 59:381–95.

Kumar A, Aswal S, Chauhan A, Semwal RB, Kumar A, Semwal DK. (2019): Ethnomedicinal Investigation of Medicinal Plants of Chakrata Region (Uttarakhand) Used in the Traditional Medicine for Diabetes by Jaunsari Tribe, *Nat Prod Bioprospect*, 9(3):175-200.

Kumar A, Mishra P, Singh SC, Sundaresan V. (2014): Efficiency of ISSR and RAPD markers in genetic divergence analysis and conservation management of *Justicia adhatoda* L., a medicinal plant, *Plant Systematics and Evolution*, 300:1409–1420.

Kumar A, Ram J, Samarth RM, Kumar M. (2005): Modulatory influence of *Adhatoda vasica* Nees leaf extract against gamma irradiation in Swiss albino mice, *Phytomedicine*, 12(4):285-93.

Kumar M, Dandapat S, Sinha MP. (2015): Hepatoprotective Activity of *Adhatoda vasica* and *Vitex negundo* Leaf Extracts Against Carbon Tetrachloride Induced Hepatotoxicity in Rats, *Advances in Biological Research*, 9 (4): 242-246.

Kumar M, Samarth R, Kumar M, Selvan SR, Saharan B, Kumar A. (2007): Protective effect of *Adhatoda vasica* Nees against radiation-induced damage at cellular, biochemical and chromosomal levels in Swiss albino mice, *Evidence-based Complementary and Alternative Medicine*, 4(3):343-350.

Madhukar G, Tamboli ET, Rabea P, Ansari SH, Abdin MZ, Sayeed A. (2014): Rapid, sensitive, and validated UPLC/Q-TOF-MS method for quantitative determination of vasicine in *Adhatoda vasica* and its *in vitro* culture, *Pharmacogn Mag*, 10(1): S198-205.

Mandal J, Laxminarayana U. (2014): Indirect shoot organogenesis from leaf explants of *Adhatoda vasica* Nees., *Springerplus*, 3:648.

Namdeo AG. (2007): Plant cell elicitation for production of secondary metabolites: A review, *Pharmacognosy Rev*, 1: 69-79.

Nithya P, Sundrarajan M. (2020): Ionic liquid functionalized biogenic synthesis of AgAu bimetal doped CeO_2 nanoparticles from *Justicia adhatoda* for pharmaceutical applications: Antibacterial and anti-cancer activities, *J Photochem Photobiol B*, 202:111706.

Pa R, Mathew L. (2012): The Effect of elicitors on the production of Vasicine from *Justicia adhatoda* L. cell suspension cultures, *Int J Pharm Sci Res*, 3(10); 3923-3926.

Panigrahi J, Gantait S, Patel IC. (2017): Concurrent production and relative quantification of vasicinone from *in vivo* and *in vitro* plant parts of Malabar nut (*Adhatoda vasica* Nees), *3 Biotech*, 7(5):280.

Prajila P, Indulekha P. (2016): Shoot organogenesis in *Justicia adhatoda* (L). Nees. *International Journal of Botany Studies*, 1(7): 21-22.

Raj AJ, Biswakarma S, Pala NA, Shukla G, Vineeta, Kumar M, Chakravarty S, Bussmann RW. (2018): Indigenous uses of ethnomedicinal plants among forest-dependent communities of Northern Bengal, India, *J Ethnobiol Ethnomed*, 14(1):8.

Rao SR, Ravishankar GA. (2002): Plant cell cultures: chemical factories of secondary metabolites. *Biotechnol Adv*, 20:101–153.

Ravishankar B, Shukla VJ. (2007): Indian Systems of Medicine: A Brief Profile, *Afr J Trad CAM* , 4(3): 319 – 337.

Saha M, Bandyopadhyay PK. (2020): *In vivo* and *in vitro* antimicrobial activity of phytol, a diterpene molecule, isolated and characterized from *Adhatoda vasica* Nees. (Acanthaceae), to control severe bacterial disease of ornamental fish, *Carassius auratus*, caused by *Bacillus licheniformis* $PKBMS_{16}$, *Microb Pathog*, 141:103977.

Sajem AL, Gosai K.(2006): Traditional use of medicinal plants by the Jaintia tribes in North Cachar Hills district of Assam, northeast India, *J Ethnobiol Ethnomed*, 2:33.

Sarkar C, Bose S, Banerjee S. (2014): Evaluation of hepatoprotective activity of vasicinone in mice, *Indian J Exp Biol*, 52(7):705-11.

Sarkera AK, Chowdhury JU, Bhuiyan HR. (2011): Chemical Composition and Antimicrobial Activity of Essential Oil Collected from *Adhatoda vasica* Leaves, *Bangladesh J Sci Ind Res*, 46(2), 191-194.

Shahwar D, Raza MA, Tariq S, Riasat M, Ajaib M.(2012): Enzyme inhibition, antioxidant and antibacterial potential of vasicine isolated from *Adhatoda vasica* Nees., *Pak J Pharm Sci* , 25(3):651-6.

Shrivastava N, Srivastava A, Banerjee A, Nivsarkar M.(2006): Anti-ulcer activity of *Adhatoda vasica* Nees*., J Herb Pharmacother*, 6(2):43-9.

Singh A, Nautiyal MC, Kunwar RM, Bussmann RW. (2017): Ethnomedicinal plants used by local inhabitants of Jakholi block, Rudraprayag district, western Himalaya, India, *J Ethnobiol Ethnomed*, 13(1):49.

Singh B, Sharma RA. (2013): Anti-inflammatory and antimicrobial properties of pyrroloquinazoline alkaloids from *Adhatoda vasica* Nees., *Phytomedicine*, 20(5):441-5.

Singh B, Sahu PM, Sharma RA. (2017): Effect of elicitors on the production of pyrroloquinazoline alkaloids by stimulating anthranilate synthase activity in *Adhatoda vasica* Nees cell cultures, *Planta*, 246(6):1125-1137.

Srivastava S, Choudhary GP. (2016): Anti-Tussive Activity of Ethyl Acetate and Methanol Extracts of *Adhatoda vasica* Nees., *Int J Pharm Sci Res*, 7(10): 4180-83.

Sudevan S, Parasivam R, Sundar S, Velauthan H, Ramasamy V. (2019): Investigation of anti-inflammatory and anti-cancer activity of *Justicia adathoda* metabolites, *Pak J Pharm Sci*, 32(4):1555-1561.

Sulochana AK, Raveendran D, Krishnamma AP, Oommen OV. (2015) Ethnomedicinal plants used for snake envenomation by folk traditional practitioners from Kallar forest region of South Western Ghats, Kerala, India, *J Intercult Ethnopharmacol*, 4(1):47-51.

Varma A, Shrivastava N. (2018): Genetic structuring in wild populations of two important medicinal plant species as inferred from AFLP markers, *Plant Biosystem*, 152(5): 1088-1100.

Venkatachalapathi A, Sangeeth T, Ali MA, Tamilselvi SS, Paulsamy S, Al-Hemaidc FMA. (2018): Ethnomedicinal assessment of Irula tribes of Walayar valley of Southern Western Ghats, India, *Saudi J Biol Sci*, 25(4):760-775.

Vinothapooshan G, Sundar K. (2011): Immunomodulatory activity of various extracts of Adhatoda vasica Linn. in experimental rats, *African Journal of Pharmacy and Pharmacology*, 5(3):306-310.

PLANT PATHOLOGY: AN OVERVIEW

R. R. Tembhurne

Department of Botany,

Sangola College, Sangola, Dist.-Solapur

Corresponding author E-mail: ramesh_tembhurne@rediffmail.com

Introduction:

A plant is in health reflects all its organs or parts performing their functions in natural and regular manner. It is also true that when any of the organ or parts not doing their proper work, say growth, reproduction in natural and regular manner, then plant is said to be diseased, irrespective of cause or causes of its abnormal condition. Therefore disease involves malfunctioning or malforming of various plants processes either in growing or in dormant stages. Hence the term disease is applied to any deviation from natural growth and to produce permanent visible changes on economic value. The word pathology has been derived from greek words Pathos means suffering and logos means discourse or study. Thus plant pathology is the study of the plant diseased. Also plant pathology is a discourse or study about suffering plant. Plant pathology is area of botanical science which deals with the diseases or troubles of the plant thus the field of plant pathology comparises both the art of treating the sick plant and science of understanding the nature of diseased plant. Phytopathology refers to the study of plant disease and covers scientific activity concerned with plant diseases. Therefore disease is defined by various workers in different ways. According to modern conception, disease is an iteraction among host, parasite, and environment. A dictionary meaning is any departure from health, properly marked symptoms, malady, illness, disorders and acoording to Stakman and Harrar (1957) plant disease is a physiological disorder or structural abonormality that is harmful to the plant or to any of its parts or products that reduces the economic value.

Nature and concept of plant diseases:

It is imperative to defined and discus the meanings of certain terms commonly used in phytopathology before we discus the basic principles and concepts of the plant pathology. It is essential to include these definitions and terms in an introductory chapter, since an understanding of these terms is required before the reader can be made aware of the details of the processes involved in plant disease. Phytopathology (phyton-plant, pathos-suffering, logos-knowledge or study) is the study of the diseases of plants and covers the entire field of biological and scientific activity concerned with the understanding of this complex phenomenon. Phytopathology is thus the study of the nature, development and control of plant disease.

Disease is a complex phenomenon is difficult to define in a few words. We have to keep in mind what Locke said in Human Understanding, through definitions will serve to explain the names of substances as they stand for our ideas, yet they leave them not without great imperfection as they stand for things. According to the modern conception, disease is an interaction among the host parasite and environment. A simple dictionary meaning of disease is any departure from health, presenting marked symptoms, malady, illness, disorder. Diseased plants are distinguished by changes in their morphological structure or physiological processes which are brought about by unfavorable environment or by parasitic agencies. Several definitions of diseases in plants have been proposed. Some examples are a series of harmful physiological processes caused by continuous irritation of the plant by a primary agent a harmful deviation from the normal functioning of physiological processes a continuous impairment of metabolism. Stakman and Harrar in 1975 defined plant disease as a physiological disorder or structural abnormality that is harmful to the plant or to any of its part or products that reduces the economic value.

Simple interest disease:

This denotes a disease the increase of which is mathematically analogous to simple interest in money. There is only one generation of disease in the course of one epidemic. A simple interest disease develops from a common source of inoculum, which is the capital is constant and often there is one generation of infection in a season. In the increase of simple interest diseases, the log [1/(1-x)] is potted against time (where x = the proportion of diseased tissue), thus allowing for the fact that with increasing infection, the number of plants susceptible to infection decreases (Fig. 1.1). Soil brone diseases such as vascular wilts and soil borne smuts which infect seedlings and subsequently sporulate in the inflorescences of the mature plants provided that the pathogen does not spread from the smutted heads are examples of simple interest diseases.

Compound interest diseases:

This denotes a disease the increase of which is mathematically analogous to compound interest money. There are several or many generations of the pathogen in the life of the crop, that is the capital is increased by the amount of interest. In plotting the amount of disease against time, the transformation log (x/1-x) is used where x represents the proportion of infected susceptible tissue or of infected plants if the pathogen develops in a systematic manner (Fig. 1.2). Late blight of potato, stem rust of wheat or powdery mildew of wheat are examples of compound interest diseases.

If we know that a particular disease is of the simple interest type, suitable control measures can be applied. For example sanitation affects the simple interest disease more than the compound interest type. If the disease is of the former type, sanitation, including the eradication

of the inoculum of the pathogen form planting material and sites should be insisted upon. If it is of the compound interest type, and the epidemic spreads rapidly and lasts long the infection rate should be reduced by a timely application of fungicides. The stress should shift from sanitation to reducing the infection rate in proportion to the increase in the rate of spread and duration of the epidemic.

Disease can be identified by the visual signs shown by the diseased plants called symptoms, by identifying the causal organism associatd with the host plants. The perticular organism which causes disease is called as pathogen. The ability of the pathogen to cause disease is known as pathogenecity. The chain of events inducing disease is called as pathogenesis. When the causal organism invades it penetrates the host cell. The initial invasion or entry of pathogen into host is known as penetration. When the pathogen reaches the host tissue and established further. The establishment of disease in the host plant is known as infection. The period which require infection and appearance of disease known as incubation period. Resistant is the extent to which the plant is able to prevent the entry or the growth of the pathogen whereas succeptibility is the extent to which the plant is damaged by the spathogen. The effects or changes which are produced externally or internally in the host are known as symptoms.

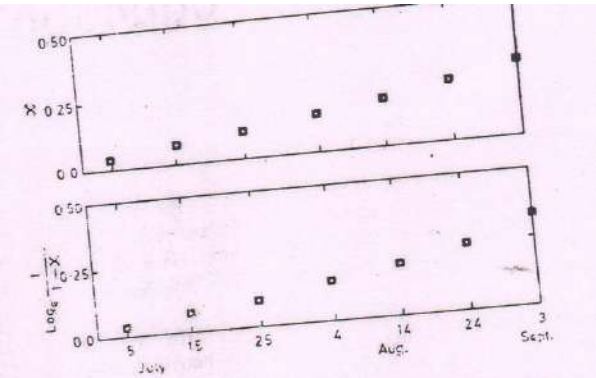


Fig. 1.1 The progress of an epidemic of wilt cotton caused by *Fusarium oxysporum* f. *vasinfectum*. In the upper graph, x is plotted against time; the lower part plots $\text{Log}_e [1/(1-x)]$ against time. (Redrawn from J.E. Van der Plank. *Plant Diseases—Epidemics and Control*, Academic Press, New York and London, 1963, p. 34.)

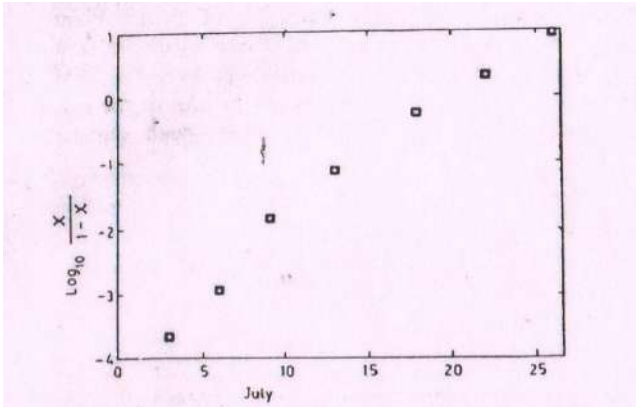


Fig. 1.2 Progress of stem rust of wheat caused by *Puccinia graminis tritici*. (Redrawn from J.E. Van der Plank. *Plant Diseases—Epidemics and Control*. Academic Press. New York and London, 1963, p. 32.)

Causes of disease:

When a parasitic micro-organism enters into the causal complex of a disease, it is commonly considered to be the cause of the disease. However we know that sometimes a variation in the environment also makes a plant liable to infection. The micro-organism is thus not the sole causal factor. After a micro-organism has successfully infected a susceptible plant, the subsequent interaction between the plant and micro-organism (which results in a disease) is also subject to environmental influences. Thus, strictly speaking it is not correct to refer to a

micro-organism as the cause of a given disease since this implies that it is the sole cause. The term causal organism is proper since it implies that the organism is a part of the causal complex. Most of the causal organism is parasites. A parasite is not synonymous with the pathogen. In some cases, organisms are parasitic without becoming causal factors in the disease. Many parasitic establishments do not become pathogenic if the parasitic compensates for the damage done done by its presence. The mycorrhizal fungus is certainly parasitic on the roots of trees but it is not pathogenic. The root nodule bacterium (*Rhyzobium leguminosarum*) is another example. In other cases the by-products of a strict saprophyte are an important part of the causal complex. Leaf tip unjury to oats may be due to the absorption of griseofulvin from soil Brian et. al. in 1951. Milo root rot is caused by a powerful toxin produced by the fungus (*Periconia circinata*) growing in the root zone Oswald in 1955, Leukel in 1948.

Thrower in 1966 modified a definition offered by the British Mycological Society in 1950 and defined a parasite as an organism or virus existing in an intimate association with another living organism from which it derives an essential part of the materials for its existence. In a literal sense a pathogen is any agent which causes damage. According to A guide to the use of terms in plant pathology prepared by the Federation of British Plant Pathologists in 1973, a pathogen is an organism or virus capable of causing disease in a particular host or range of hosts. Most but not all pathogens are also parasites in that they derive the materials they need for existence from a living plant (the host or the suscept) as distinct from saprophytes which derive these materials from dead organic matter.

Importance of plant disease:

The late blight of potato, a disease caused by the fungus, *Phytopthora infestans* is a famous example of what a plant disease can do to change the course of history. In 1845 this disease destroyed the potato crop of Ireland where potato constituted the staple diet of the majority in rural areas. The disease had started in Ireland, England and parts of the continental Europe as early as 1830 and was causing some damage every year, resulting in food shortage. When the late blight of epidemic destroyed the potato crop in 1845 there was famine in Ireland. The demographic data are highly variable (cf. Hampson in 1992) but it was reported that in 1840 the population of Ireland was 8 million which was reduced to 4 million after the famine. Hundreds of thousands perished from hunger and disease. There was large scale migration of the population to other countries including the North American continent where 6 million are reported to have migrated between 1847 and 1854. There are opinions contradicting the belief that the entire catastrophe in Ireland was due to potato famine. Socio-political conditions including the failure of the government to manage the situation were also equally, if not more responsible. But the fact remains that this single disease forced man to realize the importance of plant disease. As a result scientific investigations were taken up the cause of disease was

identified concept of fungus as cause of a disease was finally established and extensive use of chemicals for plant disease control came into existence. The late blight epidemic not only brought the science of plant pathology to limelight it caused many social and political changes in the affected countries. Free trade in England was permitted and import of food grains and other foodstuff was allowed. In order to protect shipping the country had to strengthen its navy which became world's strongest navy.

Wheat rust has been another disease that has appeared in epidemic form from time to time in many countries. This disease has forced the farmers in many parts of the world to change their cropping pattern. Wheat has been replaced by corn (maize) or rye because it was regularly destroyed by rust. This caused change in the food habit of the population in the affected areas. In the last years of the Second World War IN 1943 Bengal had to face a serious famine. One of the reasons to which this famine has been attributed was the loss in yield of the rice crop (major diet of the population) due to attack of Helminthosporium leaf spot which had been affecting the crop for the last several years. Situation was similar to the Irish potato famine but not so catastrophic. In this case also many reasons other than loss of rice crop are listed.

In the middle of the nineteenth century coffee and tea were equally consumed in England because these were available in plenty from such occupied countries as India, Srilanka and Malaysia. Sri Lanka (Ceylon) used to produce the maximum coffee in the world. In 1867 coffee rust attacked the plantastions in Sri Lanka and by 1893 the export of coffee from Sri Lanka had declined by 93 % . The economic crisis forced the planters to cut down coffee plants and take to tea planting. Export of the tea revived the economy to some extent and at the same time consumption of tea increased in England. When coffee rust was spreading in Sri Lanka the science of plant pathology was just developing and control measures for the disease were non known. Tea was also attacked by a blight but by that time chemical control measures were known and the situation did not deteriorate. The system of monoculture in coffee plantation of Sri Lanka was considered as contributory factor in devastations caused by the coffee rust which was not prevalent in coffee growing countries of South America. Coffee rust was first seen in the Western Hemisphere in 1979 in Brazil where it is now spreading. In Brazil coffee trees are often surrounded by others kinds of trees. The decline of coffee cultivation in Sri Lanka gave a boost to coffee industry in Brazil which became a major coffee exporting country in the world. Nearness of this country to USA could be one reason for popularity of this beverages in USA. These instances of plant disease epedemics are worth mentioning because they left their effect not only in the country concerned but also in other countries.

In India wheat rusts had been considered to cause a loss of over Rs. 40 million annually. In the years of epidemics there have been losses amounting to Rs. 500 million or more. Although

introduction of dwarf high yielding varieties has reduced the losses to great extent even now the farmers lose 8-10 % of the expected yield due to rusts. The loose smut of wheat is estimated to cause an average loss of 3 % (about Rs. 50 million) every year. The Molya disease caused by a nematode (cereal cyst nematode) is another example. This disease of wheat and barley prevalent in most parts of Rajasthan, causes a loss of Rs. 30 million in barley and Rs. 40 Million in wheat every year. Different smuts of sorghum are responsible for an annual loss of Rs. 100 million. Five to 75 % loss in chickpea due to Ascochyta blight was reported form Rajasthan during 1982. Wilt of pigeonpea causes 5-10 % loss every year in U. P. and Bihar. At a time when there is shortage of pulses in the countru control of only wilt and sterility mosaic of pigeonpea could increase the production by 15-16 %. Of about 10 % crop losses in the country due to nematodes, there is a loss of about Rs. 20 million every year in coffee alone due to the attack of *Partylenchus coffeae*. Other plant diseases such as red rot and wilt of sugarcane, potato viruses, rice blast and blight, Karnal bunt of wheat, root knot of tomato, eppplant and cucurbits, apple scab, mango malformation, bunchy top of banana, and sandal spike are responsible for huge losses.

In addition to direct loss in yield and monetary returns to the farmers, the plant diseases affect the society in many other ways. When foodgrains are attacked by fungi they may contain toxin (such as aflatoxins) which cause insanity, paralysis, stomach disorders and liver cancer. The money spent on management of plant diseases is also a loss because in absence of diseases this money could be saved. The expenditure on raising the crop before it is attacked by the pathogens is also a waste. When there is less production transport industry may suffer due to lack of goods for transport. Industries that consume raw agricultural materials (cotton, jute, oilseeds, vegetables and fruits for processing) face difficulty in utilizing their installed capacity when there is less production due to plant diseases. In order to make up for the loss of foodgrains and other agricultural products such oilseeds the Governments have to import these commodities which means loss of foreign exchange at the disposal of the Government. Plant disease management requires use of toxic chemicals. Excessive use of such chemicals may lead to environmental pollution affecting human health.

References:

Alexopoulos C. J. Mims C.W. and Blackwel. M. (1996). Introductory Mycology John Wiley and Sons Inc.

Clifton.A. (1958). Introduction to Bacteria McGraw Hill book Co. New York

Kumar H. D.(1988).Introductory Phycology Affiliated East – West Press Ltd. New Delhi

Mandahar C. L.(1978). Introduction to Plant Viruses Chand and Co. Ltd. Delhi

Rangaswamy G. and Mahadevan A.Diseases of crop plants in India Prentice Hall of India Pvt. Ltd. New Delhi

CHROMATOGRAPHY: A POPULAR TECHNIQUE USED FOR STUDYING CHEMICAL ANALYSIS IN A MIXTURE

Ramesh Singh

Department of Botany

Dr. B. R. Ambedkar, Government Girls Postgraduate College, Fatehpur (U.P)

Corresponding author E-mail: rameshgdcbotany1980@gmail.com

Chromatography is one of the popular laboratory technique used in chemical analysis by separating the components of a given mixture as a result of their differential distribution between stationary and mobile phases. The term" chromatography" have been derived from the Greek word Chroma meaning color and graphein meaning to write. The mixture is dissolved in a fluid solvent (Gas or Liquid) called the mobile phase, which carries it through a system (A column, a capillary tube and a plate or sheet) on which a material called the stationary phase is fixed. Chromatography was first devised by a famous Russian Botanist Mikhail Tswett (1872-1919) in the year 1901 during his research on plant pigments. He developed the technique and coined the term Chromatography, primarily for the separation of Plant pigments such as Chlorophylls, carotenes and xanthophylls. Since these components separate in bands of different colors (green, yellow orange etc.) they directly inspired the name of the technique .Later on Chromatography technique developed substantially as a result of the work of Archer John Porter Martin and Richard Laurence Millington Synge during the 1940s and 1950s for which they won the Nobel prize in the field of chemistry in1952. They established the principles and basic techniques of partition chromatography and their work encouraged the rapid development of several chromatographic methods: Paper chromatography, gas chromatography and what become known as high performance liquid chromatography. The interaction between the mobile phase and the stationary phase results in the separation of various compounds from the mixture

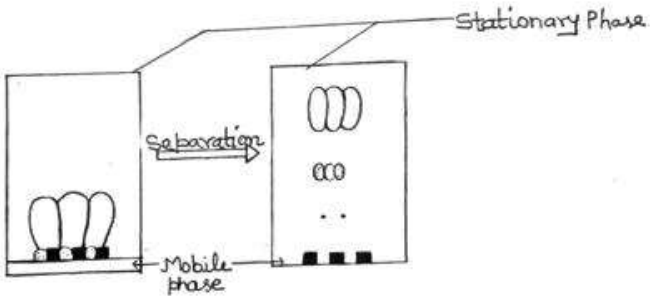


Figure 1: Diagram Shows basic principle of Chromatography

Types of Chromatography- On the basis of functioning /working principles chromatography is of following types-

1. Adsorption chromatography- In this type of chromatography seperation is based on adsorption of chemicals to the surface of a support.

2. Partition Chromatography- Here separation of components from a mixture is based on the partitioning of chemicals into a layer of the stationary phase.

3. Ion- Exchange chromatography- Here separation of ions is based on their binding to fixed charges on a support. It is used for the separation of proteins, RNA and DNA.

4. Gel exclusion chromatography- Here separation of chemicals based on their size and ability to enter a porous support .It is used for the separation of proteins, nucleic acids, polysaccharides and lipids.

5. Affinity chromatography- Here separation of chemicals is based on their interactions with a biologically related binding agent. It is used for the separation of immunoglobulins, cellular enzymes and mRNA

Modes of carrying out chromatography- There are basically three modes that's are generally used /carrying out the process of chromatography. These carrying modes are called as chromatograms. On the basis of these different types of modes that are used in separation, chromatography is divided into following categories.

1. Paper (Called as Paper chromatography)
2. Thin layer (Called as Thin layer chromatography)
3. Column (Called as Column Chromatography)
4. Liqulid (Liquid chromatography/H.P.L.C)

1. Paper Chromatography:

It is a simple method for the separation of smaller molecules from one another. the mixture of the molecules to be separated are applied to the sheets of suitable paper, which are subsequently placed in a vessel which contains a suitable solvent .A distinction is made between ascending and descending paper chromatography according to weather the solvent migrates on the paper from below to above. This technique of chromatography is used for the separation of amino acids, nucleotides and other lower molecular weight metabolic products.

2. Adsorption chromatography:

In this chromatography the solute molecules binds directly to the stationary phase. The components which have more affinity towards mobile phase elutes out first and the components which have more affinity for the stationary phase elutes later no any two components have same

affinity towards mobile phase and stationary phase. Some Solid substances which are known as adsorbents have power to hold molecule at their surface.

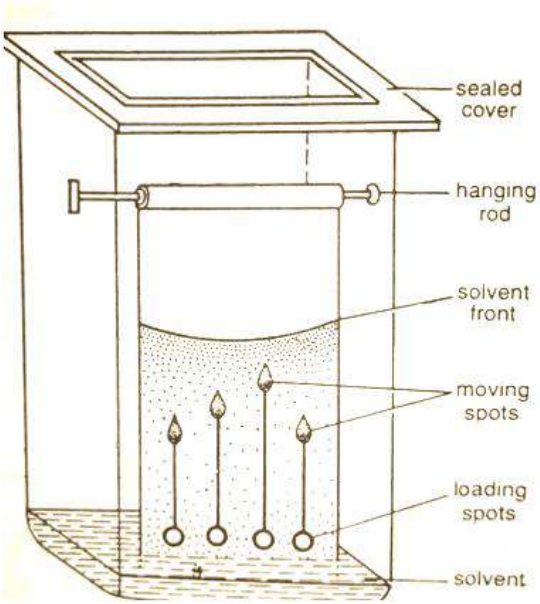


Figure 2: Paper chromatography

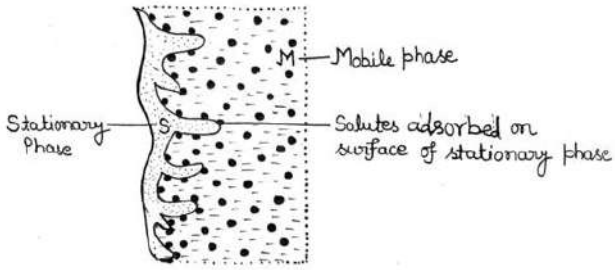


Figure 3: Adsorption chromatography

This holding force is due to weak non ionic attractive forces of the Vander wall and hydrogen bonding which occur only at specific adsorption beds.

Advantages of adsorption chromatography:

This technique retains and separates some compounds that cannot be separated by other methods, like separation of geometrical isomers.

Disadvantages of adsorption chromatography:

- Very strong retention of some solutes.
- It may cause catalytic changes in solutes.
- Solid support may have range of chemical and physical environment that cause nonsymmetrical peaks and variable retention times.

Applications of adsorption chromatography:

- To separate unsaturated hydrocarbons from the mixture.
- To separate and isolate lipids, steroids and fats.
- Use in identification of carbohydrates.
- Use to separate stereo-isomers.

Table 1: Some adsorbents and their possible application

Name of adsorbent	Name of components to be used in separation from mixture
Silicic acid	Steroides, aminoacids and Lipids
Charcol	Peptide sand carbohydrates
Aluminium oxide	Steroides, ester, alkaloids
Magnesium carbonate	Porphyrines
Calcium Phosphate	Proteins, Poly nucleotides
Cellulose	Proteins

3. Column Chromatography:

In column chromatography an insoluble medium is packed into a glass tube. The length and width of this tube affects the separation of molecules .the molecules to be separated is applied to the top of the column and their migration is started by adding a solvent. The characteristic separation which results depends on the choice of solvent and carrier material.

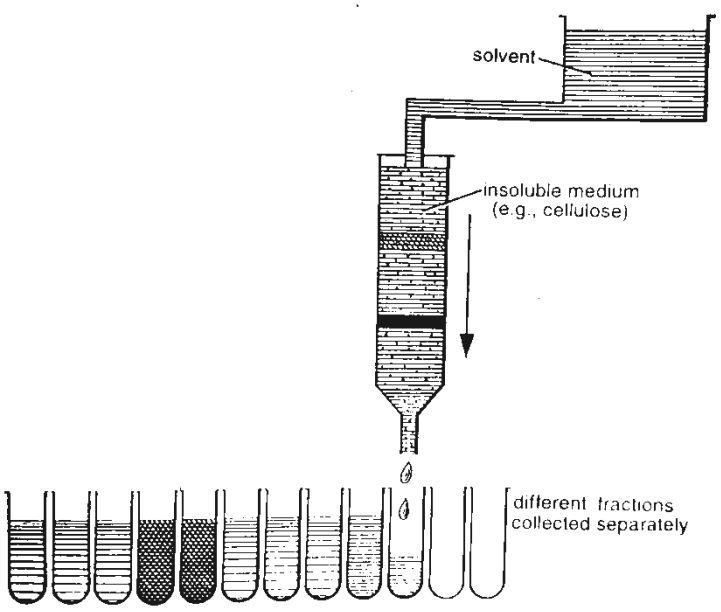


Figure 4: Demonstration of Column Chromatography

A positively charged carrier binds to the negatively charged molecules. Other carrier contains pores which are penetrated by the smaller molecules which are therefore slowed down the solution which flows from the column is collected in small fractions, which contain the separated classes of molecules Column chromatography is important for the separation of mixtures of proteins, that is for the isolation of enzymes such as cytochrome C or RNA Polymerase. Good Column is characterized a maximum good peak and less width the peak. The

efficiency of a column and its subsequent ability to separate or resolve solute zones can be determined in two ways like resolution index and theoretical plates

4. High performance / pressure liquid chromatography (HPLC):

It is the chromatography that results in high resolution (Sharp Peaks) with rapid separation of components of mixture like in a few minutes and needs very small sample material like 0.01-0.1ml. The basic components of HPLC are as pump, injecton, column, dectector, recorder or data system like computer etc. It is used for all types of chromatography like size exclusion, ion exchange and affinity chromatography. Solvents to be used in HPLC should be-

- Filtered through micro filters to remove impurities.
- Should be de gassed to avoid presence of air bubbles.
- HPLC grade refers to the quality of the mobile phase ingredients and solution not the sample. They should be pure.
- Excellent UV-Vis Transparency.
- The solvents commonly used for HPLC are often flammable and moderately toxic. Consequently most of the solvents should be stored in a secure metal cabinet.

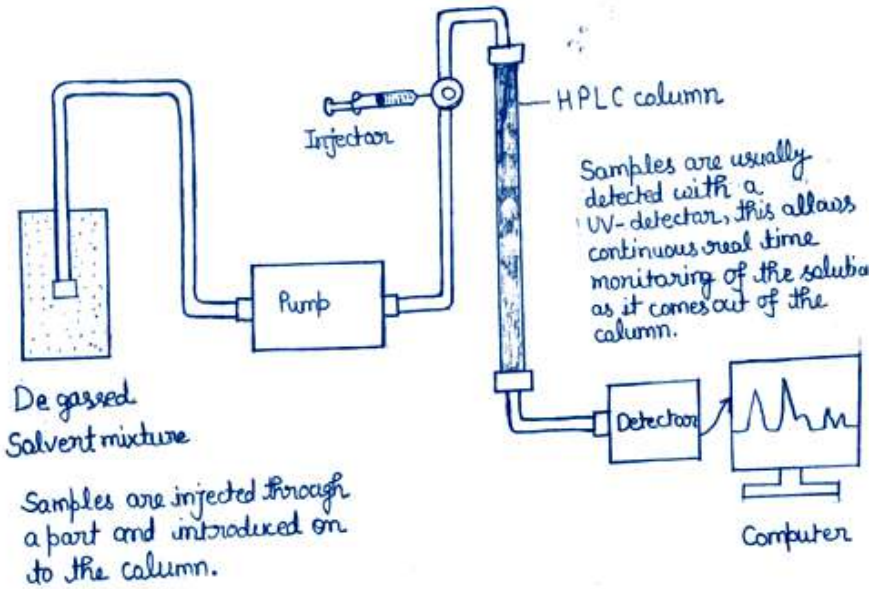


Figure 5: Various components of H.P.L.C chromatography

HPLC columns are normally made of stainless steel and are 50-300mm long with an internal diameter of 2-5mmFor selection of right column knowledge of sample (Number of compounds present, sample matrix, concentration range, solubility etc.) and goal for the separation (weather want to have maximum resolution of all compounds, partial resolution, fast

analysis of a single compound, sensitivity etc) should be well known. The stationary phase packed in the column should be small particle (approx, 3-10 micron) and large surface area.

Applications of HPLC Chromatography:

- Chemistry and Biochemistry research analyzing complex mixtures.
- Used in purifying chemical compounds.
- Quality control to ensure the purity of raw materials.
- Analyzing air and water pollutants.
- Monitoring materials that may jeoparadise occupational safety or health.
- Monitoring pesticides levels in the environment.
- To survey food and drug products.
- To determine the amount of such chemical compounds found in new drugs in pharmaceuticals.

Applications of Chromatography:

Chromatography is such a vital technique ,that is used in various fields like Pharmaceutical industry ,Chemical industry, Food industry ,Forensic science as well as various molecular studies for human welfare .This is also used for analysis of presence of pollutants in environment as well as other such purposes. So it is very useful technique for human kind by various ways.

References:

1. Batra. Virendra (2019). Plant Cell Biology Oxford Book Company, Jaipur Rajsthan.
2. Ettre LS, Zlatkis A. (2011). 75 Years of Chromatography: A Historical Dialogue. Elsevier.
3. Harper, Douglas. "Chromatography". Online Etymology Dictionary.
4. Hostettmann K, Marston A, Hostettmann M (1998). Preparative Chromatography Techniques Applications in Natural Product Isolation (Second ed.). Berlin, Heidelberg:
5. McMurry J (2011). Organic chemistry: with biological applications (2nd ed.). Belmont, CA: Brooks/Cole. pp. 395.
6. Verma, P.S and Agarwal V.K. (1998). Cell Biology, Genetics,Molecular Biology,Evolution and Ecology. S.Chand and company LTD, New Delhi. 14th Ed.
7. http://frndzzz.com/Advantages-and-Disadvantages-of-Paper-Chromatography
8. https://www.slideshare.net/shaisejacob/paper-chromatography-ptnew?next_slideshow=1
9. https://www.slideshare.net/shaisejacob/paper-chromatography-ppt-new
10. https://www.biochemden.com/paper-chromatography/

Advances in Plant Science Volume II

ISBN: 978-93-91768-50-8

About Editors

Dr. Laxmikant N. Borkar, (M.Sc., M.Phil., Ph.D.) is working as an Assistant Professor and Head in Department of Botany, S. S. Jaiswal College Arjuni Morgaon Dist- Gondia, Maharashtra, India. He has 13 years of teaching experience. He has published 28 research papers in Reputed National and International Journals. He has attended more than 30 National and International conference / Workshops /seminar/ symposia and presented Paper. His area of interest is Cyto-genetics, mycology and Melittopalynology. He is a life member of the Indian Science congress Association, Indian association for Angiosperm Taxonomy. Dr. Borkar has published some book chapters in various research and syllabus books. He workes as an Editor for the "Agriculture Science: research and Review" pubished by Bhmi Publishign, India in 2021."

Dr. Jeetendra Sainkhediya has completed M.Sc. (Botany), in 2009 with specialization in Taxonomy from Holkar Science College, Indore DAVV M.P. and M. Phil. (Botany) from Vikram University, Ujjain (M.P.) in the year 2010. He has been awarded her Ph.D. degree from Botany in "Flora of Harda district of Madhya Pradesh, India" in DAVV, Indore, M.P. He has successfully completed her Minor Research Project of three years during Ph.D. research work funded by M.P. State Biodiversity, Bhopal. He has her credit of more than 50 research/review papers in various national and international Journal of repute, and presented more than 15 research papers in various Seminar & Conferences, and written articles in various Magazine related to environment and serving as Editor of IRWJMSR (International Research World Journal of Multidisciplinary Scientific Research) and Sustainability, Reviewer of many Journals and Editorial Board Member of several International Journal. As a resource person he has delivered lectures on related to Ecology, ethno botany, Herbal medicine and Plant taxonomy. He has been awarded youth teacher award 2019 and Best research paper presentation award 2019 and Best researcher award 2021and many others. He is writing many books e.g. "SENDHWA KILE KA ETIHAS" in 2018 and "Some medicinal plants of Barwani district of Madhya Pradesh, India utilization and conservation" and" Some Plants used by Bhils in Western Madhya Pradesh specially diarrhea and dysentery" in 2022 etc.

Dr. Ashish Kumar is currently working as an Assistant Professor & Head in the Department of Biotechnology, Sant Gahira Guru University Sarguja, Ambikapur Chhattisgarh. He has received his Ph.D. degree in Biotechnology with an emphasis on Plant Molecular Genetics from Pt. Ravishankar Shukla University, Raipur, Chhattisgarh, India. He has completed his post-doctorate from the Institute of Plant Sciences, the Volcani Center, Ministry of Agriculture and Rural Development Govt. of Israel. He is awarded a doctoral and post-doctoral fellowship from University Grants Commission, New Delhi. He has received accolades for excellent professional achievements such as the Vice Chancellor's Best Employee Award and the Senior Scientist Award by the Microbiologist Society of India among many others. Dr. Ashish has worked as Senior Research Fellow at Indian Institute of Vegetable Research (IIVR), Varanasi, and as Research Associate at Central Research Institute for Dryland Agriculture (CRIDA), Hyderabad. Dr. Ashish has published several book chapters, reviews, and research papers in reputed national and international journals. He has completed one research project funded by Chhattisgarh Council of Science & Technology, Raipur, India. He has reviewed research proposals for several funding agencies such as C-COST, DBT, and DST, New Delhi India. Dr. Ashish has more than eight years of teaching and fifteen years of research experience in various reputed national and international research institutes. His field of research interests includes Plant Molecular Biology and Biotechnology.

Yengkhom Linthoingambi Devi is currently working as Research Scholar in the Department of Genetics and Plant Breeding, College of Agriculture, IGKV, Raipur, Chhattisgarh. She did her M.Sc. from CPGAS, CAU, Barapani, Meghalaya. She had published 3 research papers, 8 popular articles, 5 book chapters with few others in review and had attended a number of conferences, seminars, trainings, webinars etc. She had cleared ICAR-NET, CSIR NET, DBT-NET and UGC-NET in different subjects. She is a recipient of gold medal and several awards like best Young Breeder Award, Best Oral and Poster Presentation and others. Her fields of research interests are genetic aspects and breeding work regarding qualitative and quantitative improvement of plants. She is a recipient of National Fellowship for Schedule Caste students and also a member of Indian Society of Genetics and Plant Breeding and Lifetime Member of ATDS Society.

Bhumi Publishing

ISBN 978-93-91768-50-8
9 789391 768508

View publication stats

OPEN ACCESS

Haya: The Saudi Journal of Life Sciences
Abbreviated Key Title: Haya Saudi J Life Sci
ISSN 2415-623X (Print) |ISSN 2415-6221 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article

Free Radical Scavenging and Antioxidant Activity of *Capparis Zeylanica* Linn

S.Sivagamasundari[1*], Ananad S.P[1], Doss A[2]

[1]Department of Botany, National College (Autonmous), Trichy
[2]PG & Research Department of Botany, V.O.Chidambaram College, Thoothukudi Tamilnadu, India

DOI: 10.36348/sjls.2021.v06i09.002 | **Received:** 04.08.2021 | **Accepted:** 09.09.2021 | **Published:** 30.09.2021

***Corresponding author:** S.Sivagamasundari

Abstract

The aim of the present research was to evaluate the antioxidant potential of ethanol and aqueous extracts of *Capparis zeylania*. The free radical scavenging activity was found to be high in ethanol extract for DPPH, hydroxyl, superoxide, ABTS and reducing power assays in a concentration dependent manner followed by aqueous extract. The present results supported that the leaf extracts (ethanol and aqueous) of *C.zeylanica* could serve as potential alternative source antioxidants and can be explored as a good therapeutic agent in the free radical induced diseases.

Key words: Ascorbic acid, Trolox, Free radicals, ROS, Antioxidant.

Copyright © 2021 The Author(s): This is an open-access article distributed under the terms of the Creative Commons Attribution **4.0 International License (CC BY-NC 4.0)** which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited.

INTRODUCTION

Medicinal herbs are an indispensable part of the traditional medicine practiced all over the world due to low costs, easy access and ancestral experience [1]. Now-a-days, vast numbers of people in developing and developed countries depend on plant based healthcare, skin therapy, commercial economic uses, and cultural development. The World Health Organization (WHO) reported that 80% of the people rely on traditional medicine, which is clearly elucidated by the 19.4 billion USD global revenue for herbal remedies in 2010 [2]. Therefore, the medicinal plants used in traditional medical treatments are significant in both developing and industrialized countries.

Capparis zeylanica Linn. (Caparadaceae) is commonly known as Indian caper, is a climbing shrub found throughout India and has been used as a 'Rasayana' drug in the traditional Ayurvedic system of medicine. It is a many branched thorny, sub-scandent climbing shrub. Plants are 2-3 m in height, armed with 3-6 mm long, leaves are elliptic or broadly lanceolate, base rounded, apex mucronate, flower profuse, pinkish white, later turning pink, berries are globular or elliposide, 3-4 cm in diameter, and seeds are globase, embedded in white pulp. The plant distributed throughout the major parts of India, Bangladesh and some parts of Pakistan. Leaves are used as folk medicine and as ingredient in various Ayurvedic preparations. Traditionally it is use as Antidote to snake bite, to cure swelling of testicle, small pox, boils, cholera, colic, hemiplagia, neuralgia, sores, pneumonic and pleurisy [3]. In North India, the leaves are widely used as counter- irritant, febrifuge and as a cataplasm in swellings and piles [4]. *Capparis* species has been reported to anthelmintic, antimicrobial activity [5] and anti-inflammatory activity [6]. In the present study, the crude extracts (ethanol and aqueous) of *C.zeylanica* were investigated for their antioxidant properties of DPPH, Hydroxyl, Superoxide, ABTS and reducing power assays.

MATERIALS AND METHODS

Plant collection

The fresh plant parts were collected from Kolli hills, Namakkal District, Tamil Nadu, and India. The collected plant was authenticated by Dr. S.P.Anand, Assistant Professor of Botany, PG & Research Department of Botany, National College, Tiruchirappalli, Tamilnadu, India and the voucher specimens were deposited at the herbarium of Department of Botany, National College (Autonomous), Tiruchirappalli-1.

Preparation of extracts

Plant material

Fresh and health leaves were collected from Kolli hills, Tamilnadu, India. The leaves were washed thoroughly in distilled water and the surface water was removed by air drying under shade. The leaves were powdered with the help of mechanical blender and used for extraction.

Preparation of extracts

Fifty gram of the coarse powder of *C.zeylanica* leaf was extracted successively with 100 ml of ethanol and aqueous by cold maceration methods. All the extracts were filtered through Whatman No. 41 filter paper separately and all the extracts were concentrated in a rotary evaporator. All the concentrated extracts were subjected for in vitro antioxidant activity.

Chemicals

Ethanol, DPPH, Ascorbic acid and DMSO were of AR grade from Himedia (Mumbai, India), TCA, Ferric chlorite, Gallic acid, Quecetin, Potassium ferric cyanide, Sodium nitroprusside, Sulfanilamide, Naphthylethylenediamine dihydrochloride, Sodium carbonate, Hydrogen peroxide, Folin phenol reagent, Aluminum chloride and Potassium acetate were from SD Fine Chemicals (Mumbai, India).

DPPH assay

DPPH scavenging activity was carried out by the method of Blois [7]. Different concentrations of crude extracts were dissolved in DMSO (dimethyl sulfoxide) and taken in test tubes in triplicates. Then 5 ml of 0.1mM ethanol solution of DPPH (1, 1, Diphenyl-2-Picrylhydrazyl) was added to each of the test tubes and were shaken vigorously. They were then allowed to stand at 37^{o}C for 20 minutes. The control was prepared without any extracts. Methanol was used for base line corrections in absorbance (OD) of sample measured at 517nm. A radical scavenging activity was expressed as 1% scavenging activity and was calculated by the formula:

Percentage of radical scavenging activity = Control OD – Sample OD/ Control OD

Hydroxyl radical scavenging activity

As per the modified method of Halliwell *et al.* [8] the scavenging ability for hydroxyl radical was estimated. Using dilute deionized water, Stock solutions of EDTA (1 mM), $FeCl_3$ (10 mM), H_2O_2 (10 mM), Ascorbic Acid (1 mM), and Deoxyribose (10 mM) were made. The assay was performed by adding 0.1 ml EDTA, 0.36 ml of deoxyribose 0.01 ml of $FeCl_3$, 0.1 ml H_2O_2, 1.0 ml of the extract of unlike concentration (50, 100, 200, 400 & 800 µg/ml) dissolved in distilled water, 0.33 ml of phosphate buffer (50 mM, pH 7.9), 0.1 ml of ascorbic acid in sequence. The mixture was then incubated at 37^{o}C for 1 hour. 1.0 ml of the incubated mixture was mixed with 1.0 ml of 10% TCA and 1.0 ml of 0.5% TBA (in 0.025 M NaOH containing 0.025% BHA) to develop the pink chromogen measured at 532 nm. The hydroxyl radical scavenging action of the extract is reported as % inhibition of deoxyribose. The degradation is determined by using the subsequent equation.

Hydroxyl radical scavenging activity = (A0 - A1) / A0 X 100

Where, A0 is the absorbance of the control and A1 is the absorbance of the test samples and reference. All the tests were performed in triplicates and the results were averaged.

Superoxide anion radical scavenging

The superoxide anion scavenging activity was computed as depicted by Srinivasan *et al.* [9]. The superoxide anion radicals were generated in 3.0 ml of Tris - HCl buffer (16 mM, pH 8.0) containing 0.5 ml of NBT (0.3 mM), 0.5 ml NADH (0.936 mM) solution, 1.0 ml extract of different concentrations (50, 100, 200, 400 & 800 µg/ml) and 0.5 ml Tris - HCl buffer (16 mM, pH 8.0). The reaction was started by adding 0.5 ml PMS solution (0.12 mM) to the mixture, incubated at 25^{o}C for 5 min and the absorbance was measured at 560nm against a blank sample, ascorbic acid. The percentage inhibition was calculated by using the following equation.

Superoxide radical scavenging activity = (A0 - A1) / A0 X 100

Where, A0 is the absorbance of the control and A1 is the absorbance of the test samples and reference. All the tests were performed in triplicates and the results were averaged.

ABTS Radical Cation

ABTS assay was based on the slightly modified method of Huang *et al.* [10]. ABTS radical cation ($ABTS^{+}$) was made by reacting 7 mM ABTS solution with 2.45 mM potassium persulphate. This mixture is allowed to stand in the dark at room temperature for 12-16 hrs before use. The $ABTS^{+}$ Solution were concentrated with ethanol to an absorbance of 0.70 + 0.02 at 734 nm. Subsequent to addition of 100 µL of sample or trolox standard to 3.9 ml of diluted ABTS+ solution, absorbance was worked out. This was done at 734 nm by Genesys 10S UV-VIS (Thermo scientific) precisely following 6 minutes. Results were expressed as trolox equivalent antioxidant capacity (TEAC).

ABTS radical cation activity = (A0 - A1) / A0 X 100

Where, A0 is the absorbance of the control and A1 is the absorbance of the test samples and reference. All the tests were executed in triplicates and the end results were averaged.

Reducing power assay

Reducing activity was carried out by using the method of Oyaizu [11]. Different concentrations of crude extracts were prepared with DMSO and taken in test tube as triplicates. To test tubes 2.5 ml of sodium phosphate buffer and 2.5 ml of 1% Potassium ferric cyanide solution was added. These contents were mixed well and were incubated at 50^{o}C for 20 minutes. After incubation 2.5ml of 10% Trichloroacetic acid (TCA)

was added and were kept for centrifugation at 3000rpm for 10 minutes. After centrifugation 5ml of supernatant were taken and to this 5ml of distilled water was added. To this about 1ml of 1% ferric chlorite was added and was incubated at 35°C for 20 minutes. The OD (absorbance) was taken at 700nm and the blank was prepared by adding every other solution but without extract and ferric chloride (0.1%) and the control was prepared by adding every other solution but without extract. The reducing power of the extract is linearly proportional to the concentration of the sample.

RESULTS AND DISCUSSION

Reactive oxygen species (ROS) including superoxide radicals, hydroxyl radicals, singlet oxygen and hydrogen peroxide are often generated as byproducts of biological reaction or from exogenous factor. Plants are rich in antioxidants; so much attention has been directed towards the development of ethnomedicines as they contain phenols, flavonoids, alkaloids, tannins, vitamins, terpenoids, and many more phytochemicals responsible for different pharmacological activities. The antioxidant activity of *C.zeylanica* have been evaluated by measuring their DPPH, Hydroxyl, Superoxide, ABTS, reducing power ability and ascorbic acid/Trolox content using crude extracts of leaves. The 1,1-diphenyl-2-picryl hydroxyl (DPPH) radical was repeatedly used as the model system to evaluate the ability of antioxidant properties of several natural compounds such as extracts of plants in a relatively short time. The method is based on the reduction of methanol DPPH solution in the presence of a hydrogen donating antioxidant due to formation of the non-radical form DPPH –H by the reaction. The extract was able to reduce the stable radical DPPH to the yellow colored diphenyl picrylhydrazine. It has been found that cysteine, glutathione, ascorbic acid, tocopherol, poly-hydroxy aromatic compounds reduce and decolorize DPPH by their hydrogen donating ability [7]. DPPH free radical scavenging properties of ethanol and aqueous extracts of leaf of *C.zeylanica* are shown in table 1. The scavenging effect of standard (ascorbic acid), and crude extracts exhibited increases with the increase in the concentration. Among the two extracts studied, ethanol extract of *C.zeylanica* reveled the highest DPPH radical scavenging activity.

Table-1: Effect of Ethanol and Aqueous extracts of *C.zeylanica* on DPPH assay

Concentration (µg/ml)	Antioxidant activity (%)		
	Ethanol	Aqueous	Ascorbic acid
50	32.33 ± 0.57	24.03 ± 0.25	25.81 ± 0.10
100	41.0 ± 1.0	31.23 ± 0.49	35.08 ± 0.46
200	60.46 ± 0.80	45.73 ± 0.30	58.51 ± 0.13
400	70.16 ± 0.28	59.33 ± 0.25	66.43 ± 0.24
800	81.9 ± 0.85	75.71 ± 0.41	79.23 ± 0.43
IC_{50}	38.01 ± 0.18	40.2 ± 0.24	36.7 ± 0.28

Each value is expressed as percentage of activity mean ± standard deviation (n=3)

The hydroxyl radical scavenging activity of the ethanol and aqueous extracts was investigated (Table 2). The crude extracts exhibited strong concentration-dependent scavenging abilities for the hydroxyl radical. Ethanol extract was found to be the most powerful scavenger of the hydroxyl radical, with an inhibition of up to 80.7 % at a concentration of 800µg/ml. The moderate scavenger was found to be aqueous, the inhibition of which, however, reached 63.8 % at 800µg/ml. The results showed that the extracts obtained by cold-extraction had excellent scavenging activities for the hydroxyl radical. Hydroxyl radicals are highly strong reactive oxygen species, and there is no specific enzyme to defense against them in human body [12]. Therefore, it is important to discover some chemicals with good scavenging capacity on these reactive oxygen species. In this study, the hydroxyl radical scavenging ability of ethanolic and aqueous extracts were compared with ascorbic acid showed more pronounced hydroxyl radical scavenging activity in a dose dependent manner.

Table-2: Effect of Ethanol and Aqueous extracts of *C.zeylanica* on Hydroxyl assay

Concentration (µg/ml)	Ethanol	Aqueous	Ascorbic acid
50	25.83 ±0.37	19.16 ± 0.30	21.43 ± 0.14
100	45.4 ± 0.45	27.56 ± 0.30	38.54 ± 0.23
200	68.96 ± 0.57	38.2 ± 0.26	45.09 ± 0.76
400	75.43 ± 0.37	55.66 ± 0.35	73.70 ± 0.29
800	80.7 ± 0.36	63.8 ± 0.26	79.61 ± 0.48
IC_{50}	32.8 ± 0.45	39.1 ± 0.54	29.6 ± 0.21

Each value is expressed as percentage of activity mean ± standard deviation (n=3)

Superoxide anions convert to oxygen and hydrogen peroxide by superoxide dismutase, or they react with nitric oxide to form peroxynitrite. Hydrogen peroxide can be converted into water and oxygen by catalase. Therefore, superoxide scavenging capacity in the human body is very important as the first line of

protection against oxidative stress [13]. The superoxide anion radical scavenging activities (%) of crude extracts (ethanol and aqueous) *C.zeylanica* were significantly equal to standard. Superoxide radical scavenging capacities of the crude extracts tested varied from 18.6 to 74.3 % which represents a variation of standard ascorbic acid. Ethanol extract showed the highest antioxidant capacity (74.3%) followed by aqueous (65.53%) (Table 3).

Table-3: Effect of Ethanol and Aqueous extracts of *C.zeylanica* on Superoxide anion radical scavenging assay

Concentration (µg/ml)	Ethanol	Aqueous	Ascorbic acid
50	27.06 ± 0.40	18. 46 ± 0.25	27.43 ± 0.34
100	44.6 ± 0.49	25.56 ± 0.15	43.41 ± 0.28
200	56.4 ± 0.00	46.7 ± 1.91	56.13 ± 0.21
400	64.53 ± 0.25	56.6 ± 0.26	66.54 ± 0.29
800	74.3 ± 0.3	65.53 ± 0.15	79.39 ± 1.02
IC_{50}	36.5 ± 0.27	38.6 ± 0.23	31.8 ± 0.65

Each value is expressed as percentage of activity mean ± standard deviation (n=3)

The scavenging potential of the crude extracts (ethanol and aqueous) for the ABTS radical were analyzed and compared (Table 4). As can be seen, the scavenging effect of the crude extracts increased with increasing concentration. As in the case of DPPH radical scavenging, ethanol exhibited the highest ABTS antiradical properties, followed by aqueous with an inhibition of 68.0 % for the ABTS radical at 800µg/ml. The order of ABTS radical scavenging activity of all extracts was similar to that observed for DPPH. The differences in the ABTS scavenging activities exhibited by the various extracts indicated that the extracting solvent and extraction method influenced the antioxidant ability of the extracts. The ABTS radical action is reactive towards most antioxidants including phenolics, thiols and Vitamin C. During this reaction, the blue ABTS radical cation is converted back to its colorless neutral form. The reaction may be monitored spectrophotometrically. The scavenging activity of ABTS+ radical by the plant extract was found to be appreciable; this implies that the plant extract may be useful for treating radical related pathological damage especially at higher concentration [14].

Table-4: Effect of Ethanol and Aqueous extracts of *C.zeylanica* on ABTS assay

Concentration (µg/ml)	Ethanol	Aqueous	Trolox
50	29.83 ± 0.25	18.43 ± 0.32	28.42 ± 0.27
100	41.63 ± 0.32	31.36 ± 0.25	42.54 ± 0.11
200	56.56 ± 0.35	50.33 ± 0.23	59.51 ± 0.27
400	65.5 ± 0.4	61.33 ± 0.577	66.32 ± 0.93
800	71.8 ± 0.3	68.0 ± 0.7	70.45 ± 0.21
IC_{50}	34.78 ± 0.45	35.8 ± 0.04	32.7 ± 0.71

Each value is expressed as percentage of activity mean ± standard deviation (n=3)

Reducing power assay is often used to evaluate the ability of natural antioxidants to donate electron or hydrogen [15]. Samples with high reducing power were reported to have a better ability to donate electrons. It has been widely accepted that the higher level of absorbance at 700 nm indicates greater reducing power of the test sample [16]. In this assay, samples were assayed in redox-linked reaction, whereby the antioxidants present in the samples act as the reductants, while the reagent containing excess of ferric ions act as oxidants. Reduction of the ferric chloride into ferrous complex can be measured at 700 nm. The intensity of the colour is related to the amount of antioxidant reductants present in the sample. Many research efforts have been revealed that there was a direct correlation between antioxidant activity and reducing power [16]. Table 5 indicates the reductive capabilities of the ethanol and aqueous extracts of *C.zeylanica*. In the concentration range investigated, the crude extracts showed reducing power that increased linearly with concentration. At 50, 100, 200, 400 & 800 mg/ml, reducing power of ethanol extract of *C.zeylanica* were found to be 0.183, 0.290, 0.418, 0.594 & 0.721. Among the two extracts tested, the ethanol extract exhibited significant reducing capacities.

Table-5: Effect of Ethanol and Aqueous extracts of *C.zeylanica* on reducing power

Concentration (µg/ml)	Ethanol	Aqueous	Ascorbic acid
50	0.183 ± 0.002	0.098 ± 0.003	0.245 ± 0.02
100	0.290 ± 0.004	0.159 ± 0.001	0.324 ± 0.31
200	0.418 ± 0.03	0.269 ± 0.002	0.487 ± 0.87
400	0.594 ± 0.02	0.412 ± 0.002	0.671 ± 0.03
800	0.721 ± 0.03	0.543 ± 0.003	0.765 ± 0.05

Each value is expressed as percentage of activity mean ± standard deviation (n=3)

CONCLUSION

In the present study, the ethanol and aqueous extracts of *C.zeylanica* exhibited significant antioxidant activity. Among the two extracts used in the antioxidant activity, ethanol extracts of *C.zeylanica* showed maximum and good activity. *C.zeylanica* is thought to be used as additives for food products and pharmaceutical industries with appropriate antioxidant properties and an antioxidant in future studies of experimental animal models, against free radicals generated in response to oxidative stress; also, it is thought that these data will be reference for future studies.

REFERENCES

1. Machado, T.B., Pinto, A.V., Pinto, M.C., Leal, I.C., Silva, M.G., Amaral, A.C., Kuster, R.M., & Santos, K.R. (2003). In vitro activity of Brazilian Medicinal Plants, naturally occurring naphthoquinones and their analogues, against methicillin-resistant *Staphylococcus aureus*. *Int J Antimicrob Ag, 21*; 284-289.
2. Ujowundu, C.O., Okafor O.E., Agha N.C., Nwaogu L.A., Igwe, K.O., & Igwe C.U. (2010). Phytochemical and chemical composition of *Combretum zenkeri* leaves. *J Med Plants Res*, 4; 965–968.
3. Chopra, R.N., Nayer, S.L., & Chopra, I.C. (1992). Glossary of Indian medicinal plants, *CSIR Publication, New Delhi*, 50-52.
4. Kirtikar, K.R., & Basu B.D. (1987). Indian Medical Plants. International Book Publication & *Distrubution, Dehradun, 1*; 195-201.
5. Mali, R.G., Hundiwale, J.C., Sonawane, R.S., Patil R.N., & Hatapakki, B.C. (2004). Evaluation of *Capparis deciduas* for Anthelmintic and Anti-microbial activities. *Ind J Nat Prod*, 20;10- 12.
6. Chaudhary, S.R., Chavan, M.J., & Gaud, R.S. (2004). Anti-inflammatory and analgesic activity of *Capparis zeylanica* root extracts. *Ind J Nat Prod*, 20; 36-39.
7. Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, *181*(4617), 1199-1200.
8. Halliwell, B., Gutteridge, J. M., & Aruoma, O. I. (1987). The deoxyribose method: a simple "test-tube" assay for determination of rate constants for reactions of hydroxyl radicals. *Analytical biochemistry*, *165*(1), 215-219.
9. Srinivasan, R. M. J. N., Chandrasekar, M. J. N., Nanjan, M. J., & Suresh, B. (2007). Antioxidant activity of Caesalpinia digyna root. *Journal of Ethnopharmacology*, *113*(2), 284-291.
10. Huang, M. H., Huang, S. S., Wang, B. S., Wu, C. H., Sheu, M. J., Hou, W. C., ... & Huang, G. J. (2011). Antioxidant and anti-inflammatory properties of Cardiospermum halicacabum and its reference compounds ex vivo and in vivo. *Journal of ethnopharmacology*, *133*(2), 743-750.
11. Oyaizu, M. Studies on products of browning reaction. Antioxidant activities of products of browning reaction prepared from glucoamine. *Jap. J. Nutri*, *986*(44), 307-315.
12. Liu C.Z., Yu J.C., Zhang, X.Z., Wang, T., & Han J.X. (2005). On changes of activity of antioxidases in hippocampus of rats with multi-infarct dementia and the intervention effects of acupuncture China. *J Trad Chi Med Pharm*, 20;724–726.
13. Kim I.S., Yang M., Lee O.H. & Kang S.N. (2011). The antioxidant activity and the bioactive compound content of *Stevia rebaudiana* water extracts. LWT-Food Sci Technol. 44(5):1328–1332.
14. Wang, M., Li, J., Rangarajan, M., Shao, Y., La, Voie, E.J., Huang, T., & Ho, C. (1998). Antioxidative phenolic compounds from sage (*Salvia officinalis*). *J Agric Food Chem*, 46; 4869-4873.
15. Dorman, H. J. D., Peltoketo, A., Hiltunen, R., & Tikkanen, M. J. (2003). Characterisation of the antioxidant properties of de-odourised aqueous extracts from selected Lamiaceae herbs. *Food chemistry*, *83*(2), 255-262.
16. Duh, P. D. (1998). Antioxidant activity of burdock (Arctium lappa Linné): Its scavenging effect on free-radical and active oxygen. *Journal of the American Oil Chemists' Society*, *75*(4), 455-461.

International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 17-08-2021, Accepted: 02-09-2021, Published: 18-09-2021
Volume 6, Issue 5, 2021, Page No. 651-656

Physico-chemical, phytochemical and FT-IR analysis of ethanolic leaves extract of *Cardiospermum halicacabum* L.

Nagalakshmi Ramamoorthy*, Anand Subramaniam Parvathi, Justin Raj Arockiam
PG and Research, Department of Botany, National College, Affiliated to Bharathidasan University, Tiruchirappalli, Tamil Nadu, India

Abstract
In the present study was carried out to investigate the Physico chemical, phytochemical and FTIR analysis of *Cardiospermum halicacabum* L. leaves. Physico chemical parameters such as Extractive value, Ash value, Loss on drying and fluorescence analysis was determined quantitatively. It will be helpful in assessing the quality of the plant powder. Preliminary phytochemical screening of ethanolic leaves extract of *C. halicacabum* showed the presence of alkaloids, carbohydrates, glycosides, flavonoids, phenol, proteins, amino acids, saponins and tannins. The quantitative analysis of phytochemicals such as alkaloids, flavonoids, phenols, proteins and tannins were also estimated from ethanol extract by various methods. The alkaloid content was found to be 3.23mg/g and the protein content was found to be 2.82mg/g. Total flavonoid content 11.08 mg/g, total phenolic content 14.07mg/g and the tannin content was found to be 1.73 mg/g. The results showed that Ethanolic extract of *C. halicacabum* leaves contain more phenolic compounds which can be beneficial in the therapeutic approach. The FTIR analysis results showed that the ethanol leaf extract of *C. halicacabum* having the presence of Hydroxyl group, Alcohols, Amides, Alkanes Aldehydes, Alkenes, Aromatic compounds, Nitro compounds, Carboxylic acids, Alkyl and Aryl halides, which shows major peaks at 3888.85, 3784.86, 3709.89, 3450.j76, 1036.61, 821.27, 772.24, 658.02 and 560.83 respectively. The results of the FTIR analysis are significant with the preliminary qualitative analysis.

Keywords: *Cardiospermum halicacabum*, ethanolic extract, FT-IR analysis, functional groups, phytochemical screening

Introduction
Herbal medicine is based on traditional medicine and exists all over the world. Each of these traditional medicines has its origin and a private basic philosophy (Vogel, 2003) [43]. India is one of the countries with the greatest medical and cultural diversity in the world (Archna Sharma *et al.*, 2013) [5]. Here Ayurveda, Siddha and Unani are among the most important traditional medical systems. The systems are heavily dependent on medicinal plants. India has a rich source of medicinal plants, around 8000, distributed in 16 agro-climatic zones (Rajalaxmi Nayak *et al.*, 2015) [33]. Herbal medicines make up the majority of complementary medicines. Since the cost of most Western medicines is beyond human affordability, millions of the world's population in developing countries depend entirely on herbal medicines. Herbal medicines are on the advance in developing countries, and herbal medicines are now firmly established in medical care (Mukherjee *et al.*, 2003) [26]. Phytochemicals are naturally found in medicinal plants in all the parts namely, leaves, stems, flowers, fruits, seeds, bark, and roots which play an important role in curing diseases. The phytochemical components are extremely important to facilitate the search for the particular effectiveness of the plant in medicine. Bioactive compounds normally accumulate as secondary metabolites (flavonoids, alkaloids, terpenoids, steroids, saponins, etc.) are present in all plant cells, but their concentration varies depending on the parts of the plant, the season, the climate, and the phase of growth (Raza *et al.*, 2013) [34]. Many of these were the basis for the development of new lead chemicals for pharmaceuticals. *Cardiospermum halicacabum* L. is one of the most important medicinal plants of the Sapindaceae family. The common names for this plant are Balloon vine, Puff-ball, Heartseed Vine, Heart pea, Love in a puff (Krishna Murti *et al.*, 2010) [20]. "Mudakattan" is a green backyard healer traditionally used for various diseases such as rheumatism, lumbago, cough, hyperthermia, nervous disease, limb stiffness, and snakebite, etc. (Jayabalan., 2006, Jeyadevi *et al.*, 2013, Patil *et al.*, 2011, Ponmari *et al.*, 2011, Raza *et al.*, 2013) [16, 17, 30, 31, 34]. *Cardiospermum halicacabum* L. contains certain phytochemicals that support its role in the medicinal field. It has anti-inflammatory (Sheeba & Asha, 2009, Selloum *et al.*, 2003) [38, 37], antidiarrheal (Kurian., 1995, Venkat Rao *et al.*, 2006) [21, 41], antiparasitic, antipyretic (Asha & Ushpangadan, 1999) [6], antifungal (Khunkitt *et al.*, 2000, Veeramani *et al.*, 2015) [19, 40], anxiolytic activity (Raza *et al.*, 2013) [34], adulticidal activities, urinary tract infections suppression and antihyperglycemic properties (Krishna Murti *et al.*, 2010, Patil *et al.*, 2010) [20, 29]. While there is traditional and experimental evidence to support various claims and benefits of these plants, proper evaluation and use are still required. A full investigation is inevitable, for this reason that we study the leaves of this plant using strict scientific protocols in order to capitalize on the enormous economic potential of this culture, which can be determined by its consumption. The employment opportunity for a farm labourer is sufficient.

Materials and Methods

Collection and Processing of Plant Material

Cardiospermum halicacabum L. was collected in February 2021 in Rettaimalai, Tiruchirappalli District**,** Tamil Nadu, and South India. The collected plants were washed with water to remove soil and dust. The plant material was dried for four to five days in the shade and cut into small pieces**.** Then it was pulverized into a coarse powder. The powder was used to extract the active principles.

Preparation of the Plant Extract

The coarse powder was placed in the Soxhlet apparatus and extracted successively with ethanol solvent. The extract was then filtered and evaporated on a rotary evaporator. The dried extracts were stored in the refrigerator for further experiments.

Physicochemical Properties

Physicochemical properties such as the percentage of extraction values, total ash, acid-insoluble ash, water-soluble ash; values were calculated according to the guidelines of the World Health Organization (WHO, 1998). In fluorescence analysis the plant powders have been treated with various acids and alkalis such as HCl, H_2SO_4, HNO_3, HCl in methanol, and NaOH, observed in both daylight and ultraviolet light to evaluate the purity of the plant powder.

Qualitative Phytochemical Screening

The plant extract was qualitatively determined for the presence of alkaloids, carbohydrates, glycosides, proteins and free amino acids, fixed oils, phenolic compounds, tannins, flavonoids, saponins, and sterols according to the standard method provided by Harborne, (1984) [13].

Quantitative phytochemical studies

Assessment of the Alkaloids

The alkaloid content of raw drugs was estimated by the Indian Pharmacopoeia, (1996) [15]. Five grams of the extract was weighed and transferred to a 250 ml beaker. An aliquot of 8 ml of 20% Acetic acid and 32 ml of ethanol was placed in the same beaker, covered, and left to stand for 4 hours. After 4 h, the extract was filtered and it was concentrated to a quarter of the original volume using a water bath. A few ml of concentrated ammonium hydroxide was added to the concentrate until the precipitation was complete. The entire solution was able to settle. The precipitate was collected by filtration and weighed.

Assessment of the Total Flavonoids

The total flavonoid content of leaf extract was estimated using the aluminum chloride method described by (Hossain & Nagooru, 2011) [14]. 1 ml of extract was mixed with 4 ml of deionized water and 0.3 ml of 10% sodium nitrate. The solution was allowed to stand for 5 min. 0.3 ml of 10% strength aluminum chloride solution was added to this solution. The solution is left to stand for one minute. A 2 ml aliquot of 1M sodium hydroxide solution was added to this. The test tube was shaken well to mix the reagents and allowed to stand for a while. The OD was measured at 510 nm. Rutin as the standard for creating the standard curve. Using the standard curve, the total flavonoid content of the extract was obtained.

Assessment of the Phenols

The total polyphenols present in the extracts were estimated using the Folin's ciocalteau method (Bray & Thorpe, 1954) [8]. One ml of extract was placed in a test tube, into which 1 ml of Folin phenol reagent and 2 ml of 20% sodium carbonate solution were added. 1 ml of 80% methanol was used as a blank and all of the above reagents were added. One ml of Catechol solution was used as the standard for creating the standard curve. The test tubes were shaken well and heated in a boiling water bath for 1 minute. After cooling, the solution turns blue and dilutes to 25 ml with distilled water. The absorbance of the solution was read at 650 nm against the 80% methanol-solvent blank value.

Assessment of the Tannins

Tannins were determined using the Folin-Ciocalteu method (Govindappa *et al.,* 2011) [11]. Approximately 0.1 ml of the sample extract was placed in a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent, 1 ml of 35% sodium carbonate solution, and dilutes to 10 ml with distilled water. The mixture was stirred well and kept at room temperature for 30 minutes. A range of tannin reference standard solutions (20, 40, 60, 80, 100 µg/ ml) was prepared in a manner equivalent to that described above. The absorbance for the test and standard solutions was measured against the blank value at 700 nm with a UV/ visible spectrophotometer.

Assessment of the Proteins

The protein content of the extract was determined using the method of Lowry *et al.,* (1951) [22]. 5.5 ml of alkaline copper tartrate reagent was added to each test tube and allowed to stand for 15min. Add a 0.5 ml aliquot of Folin phenol was mixed thoroughly and allowed to stand at room temperature for 30 minutes. The absorbance at 650 nm was measured. The value from the standard graph was used to calculate the protein concentration in the plant material.

FTIR Analysis

The Fourier Transform Infrared (FTIR) spectrophotometer is possibly the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds. The wavelength of the absorbed light is characteristic of the attraction, as will be seen within the annotated spectrum. The chemical bonds in a molecule can be determined from the infrared absorption spectrum. Dry powder made from ethanolic plant material extracts was used for the FTIR analysis. 10 mg of the dry extract powder was encapsulated in 100 mg of KBr pellet, to prepare translucent sample disks. The powder sample was placed in an FTIR spectroscope (PERKIN ELMER, IR) with a scanning range of 500 to 4000 cm^{-1} with a resolution of 4 cm^{-1}. The probable functional groups have been identified based on the maximum values of the IR radiation range (Ashokkumar & Ramaswamy 2014; Mohrig *et al.,* 2006; Nandiyanto *et al.,* 2019) [7, 25, 27].

Results

The Physicochemical parameters such as extractive value, ash value, loss on drying, and fluorescence analysis were quantified and the results were tabulated in Table 1. Fluorescence analysis of plant powders treated with various acids and alkalis such as HCl, H_2SO_4, HNO_3, HCl in methanol and NaOH, observed the powders in daylight as

well as in ultraviolet light presented in Table 2. The phytochemical analysis of the *Cardiospermum halicacabum* leaf extract was analyzed for compounds such as carbohydrates, glycosides, alkaloids, phytosterols, fixed oils, saponins, proteins, and amino acids, phenolic compounds, flavonoids, and tannins. A preliminary phytochemical examination of the ethanol leaf extract revealed the presence of eight compounds i.e. Carbohydrates, glycosides, alkaloids, saponins, phenolic compounds, proteins, flavonoids, and tannins, as well as the absence of phytosterols, and fixed oils which are listed in Table 3. The quantitative phytochemical analysis of the ethanolic leaf extract of *C. halicacabum* L. is represented in Table 4. The results showed that the phenol content was more in ethanolic extract of C. halicacabum L. leaf and was followed by flavonoids, alkaloids, proteins, and tannins, which are shown in Fig. 1. The FTIR spectrum of the extract of *C. halicacabum* leaves is shown in Fig. 2. The results of the FTIR analysis showed that the *C. halicacabum* ethanol leaf extract contains hydroxyl group, alcohols, amides, alkanes aldehydes, alkenes, aromatic compounds, nitro compounds, carboxylic acids, alkyl, and aryl halides.Table 5 shows the major peaks at 3888.85, 3784.86, 3709.89, 3450.76, 1036.61, 821.27, 772.24, 658.02 and 560.83 respectively.

Discussion

The results of the analysis of physicochemical parameters showed that the water-soluble extractive value was higher than the alcohol-soluble extractive value. This result agrees with the result of (Patil *et al.,* 2010) [29]. Apart from total ash, water-soluble ash value was higher in plant powder, which was followed by acid-insoluble ash value. The ash or residue produced by an organic chemical compound. A ruler is used to measure the amount of inorganic matter that is present as an impurity. The water-soluble ash is used to determine the presence of anhydrous material. Many flavonoids showed characteristic colors under ultraviolet light. In the case of non-fluorescent compounds, the fluorescence can be imparted by impurities present in the sample. Therefore, some raw materials are often qualitatively assessed in this way and it is an important parameter of pharmacognostic evaluation (Ansari, 2006, Gupta *et al.,* 2006) [4, 12]. Phytochemical screening of the plant was reported as a positive test for the presence of various phyto-components such as alkaloids, flavonoids, and tannins (Annadurai *et al.,* 2013, Mohaddesi *et al.,* 2016, Sagadevan *et al.,* 2013) [2, 23, 36]. The whole plant extracts (i.e. petroleum ether, alcohol, and water) from *C. halicacabum* (Linn) contain tannins, flavonoids, saponins, sterols, and triterpenes (Kurian., 1995, Venkat Rao *et al.,* 2006) [21, 41]. The alcoholic extract of the root of this plant contains fixed oils, fats, proteins, flavonoids, phenols, tannins, carbohydrates, saponins, phytosterols, and triterpenoids (Dhayabaran *et al.,* 2012) [10]. Chloroform extract from this plant has reported the presence of carbohydrates, steroids, terpenoids, glycosides, alkaloids, phenols, and tannins. The ethyl acetate extract from this plant contains carbohydrates, steroids, terpenoids, phenols, tannins, proteins, amino acids, glycosides, flavonoids, and alkaloids. The methanolic extract of this plant contains carbohydrates, amino acids, phenols, tannins, alkaloids, glycosides and the aqueous extract of this plant contains carbohydrates, flavonoids, amino acids, proteins, steroids, terpenoids, phenols, tannins, glycosides, alkaloids (Aishwarya *et al.,* 2014, Deepan *et al.,* 2012, Parameshappa *et al.,* 2012) [1, 9, 28]. Vadivazhagi, (2020) [39] examined the extract of the total phytochemicals from the *C. halicacabum* leaf using Petroleum ether and qualitatively identified secondary metabolites. The extracts had a high phenol content and showed strong radical scavenging activity and adequate iron reduction (Annamalai *et al.,* 2011) [3]. A similar result was found in Jeyadevi *et al.,* (2013) [18], the ethanolic extract from the leaf of *C. halicacabum* L. contains more phenol with 17.94 mg/g and 14.97 mg/g of flavonoid. Phytochemical screening of the plant was reported as a positive test for the presence of various phyto-components such as alkaloids, flavonoids, and tannins. The amount of total phenol in various extracts was found to be between 29.697 ± 0.232 to 187.372 ± 0.615 mg pyrogallol equivalent/g (Mohaddesi *et al.,* 2016) [23]. Previous research shows the results from FTIR analysis of *C. halicacabum* leaves confirmed the presence of amines, lipids, amino acids, proteins, halogen derivatives, lactams, sulfur compounds, alkanes, and iodine compounds. (Prabakaran *et al.,* 2014, Viji *et al.,* 2010) [32, 42]. FT-IR analysis of the methanolic extract of *C. halicacabum* leaves shows the presence of alcohol, isocyanides, alkyl Compound, alkane, primary alcohol, chloric components (Mohammed Junaid *et al.,* 2020, Sabeerali *et al.,* 2018) [24, 35]. The results of the FTIR analysis are significant with the preliminary qualitative analysis**.**

Conclusion

Extractions of any crude drug with a certain solvent result in a solution that contains various phyto-components. The composition of these phyto-components in the respective solvent provides initial information about the quality of a drug sample. The presence or absence of a certain component plays an important role in the decision about the medicinal properties of the plant. The ethanolic extract from the leaves of *C. halicacabum* L. contains phyto-compounds such as phenols, flavonoids, tannins, saponins, etc. It contains more phenolic compounds than other compounds, which can be beneficial in a therapeutic approach.

Table 1: Physicochemical parameters of *C. halicacabum* L. Leaves

Parameters	Observation
Extractive value	
a. Water Soluble (%)	16.03
b. Alcohol Soluble (%)	13.5
Ash value	
a. Total Ash (%)	9.8
b. Acid Insoluble Ash (%)	1.7
c. Water Soluble Ash (%)	4.10
Loss on drying (%)	12.04

Table 2: Fluorescence analysis of *C. halicacabum* L. leaves

Treatment	Observation under	
	Daylight	UV light
Powder as such	Green	Green
Powder+1N HCl	Brownish green	Black
Powder+H_2SO_4	Dark green	Dark brown
Powder+HNO_3	Brownish green	Brown
Powder+1N NaOH in	Methanol Green	Green

Table 3: Preliminary phytochemical analysis of an ethanolic extract of *C. halicacabum* L. leaves

Phytoconstituents	Ethanol extract
Carbohydrates	+
Glycosides	+
Alkaloids	+
Phytosterol	-
Fixed oils	-
Saponins	+
Proteins and amino acids	+
Phenolic compounds	+
Flavonoids	+
Tannins	+

Note: (+) = Present and (-) = Absent

Table 4: Quantitative phytochemical analysis of an ethanolic extract of *C. halicacabum* L. leaves

Phytoconstituents	Quantity (mg/g) dry. wt.
Alkaloids	3.23
Proteins	2.82
Flavonoids	11.08
Phenols	14.07
Tannins	1.73

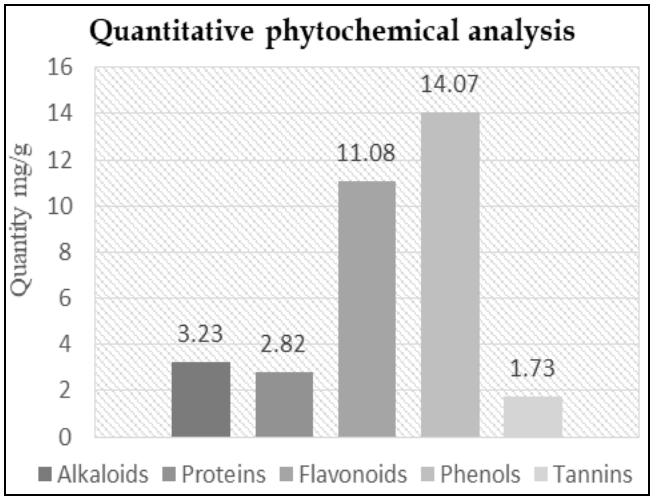


Fig 1: Quantitative phytochemical analysis of an ethanolic extract of *C. halicacabum* L. leaves

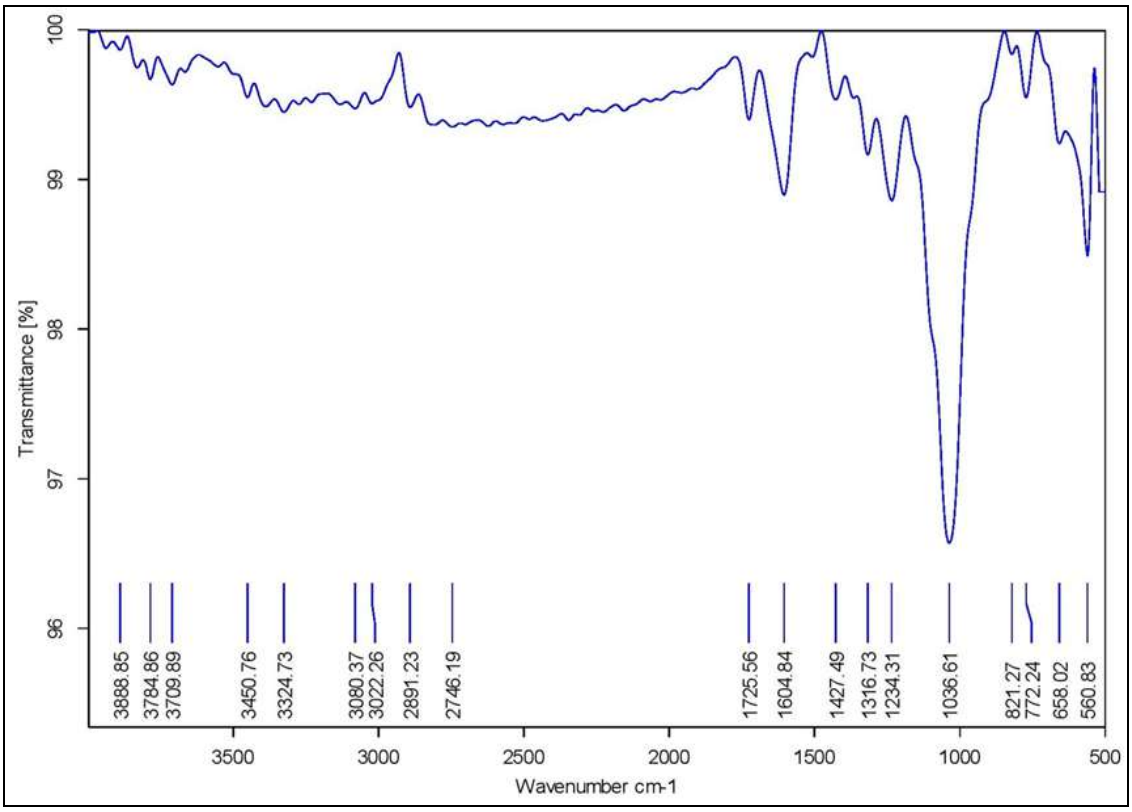


Fig 2: FTIR spectrum of ethanol extract of *C. halicacabum* L leaves.

Table 5: FTIR Interpretation of compounds of ethanol extract of *C. halicacabum* L.

Frequency range	Molecular motion	Functional group
3888.85 - 3709.89	O-H (Non-bonded)	hydroxyl group
3450.76	O-H stretch	Alcohol
3324.73	N-H stretch	Amides
3080.37 - 3022.26	C-H stretch	Aromatic compounds
2891.23	C-H stretch	Aldehydes, Alkanes
2746.19 - 1725.56	O-H stretch, C=O stretch	Carboxylic acids
1604.84	C=C stretch	Aromatic compounds, Alkenes
1427.49	C-H stretch	Alkenes
1316.73	NO_2 stretch	Nitro Compounds
1234.31 - 1036.61	C-F stretch	Alkyl & Aryl Halides
821.27 - 772.24	=C-H bend	Alkenes
658.02 - 560.83	C-Br stretch, C-Cl stretch	Alkyl & Aryl Halides

References

1. Aishwarya V, Sheik Abdulla S, Dheeba B, Renuka R. *In vitro* antioxidant and anticancer activity of *cardiospermum halicacabum* l. against each cell line. International Journal of Pharmacy and Pharmaceutical Sciences,2014:6(8):263-268.
2. Annadurai A, Elangovan V, Velmurugan S, Ravi Kumar R. Preliminary phytochemical screening and antibacterial of *Cardiospermum halicacabum* L. Pelagia Research Library,2013:4(5):302-308.
3. Annamalai A, Ponmari G, Sathishkumar R, Lakshmi PTV. Effect of drying treatment on the contents of antioxidants in *Cardiospermum halicacabum* Linn. International Journal of Pharma and Bio Sciences,2011:2(1):304-313.
4. Ansari SH. Essentials of Pharmacognosy. 1st Ed. Birla Publications Pvt. Ltd. New Delhi, 2006, 48-51.
5. Archna Sharma RA, Sharma, Hemlata S. Phytochemical and Pharmacological Profile of *Abutilon Indicum* L. Sweet: A Review. International Journal of Pharmaceutical sciences Review and Research,2013:20(1):120-127.
6. Asha VV, Ushpangadan P. Antipyretic activity of *Cardiospermum halicacabum.* Indian Journal of Experimental Biology,1999:37(4):411-414.
7. Ashokkumar R, Ramswamy M. Phytochemical screening by FTIR spectroscopic analysis of leaf extracts of selected Indian Medicinal plants. International Journal of Current Microbiology and Applied Sciences,2014:3(1):395-406.
8. Bray HG, Thorpe WV. Analysis of phenolic compounds of interest in metabolism. Methods in Biochemistry Analysis,1954:1:27-52.
9. Deepan T, Alekhya V, Saravanakumar P, Dhanaraju MD. Phytochemical and Anti-Microbial Studies on the Leaves Extracts of *Cardiospermum halicacabum* Linn. Advances in Biological Research,2012:6(1):14-18.
10. Dhayabaran D, Florance J, Krishnadas N, Indumathi M. Anticonvulsant activity of alcoholic root extract of C. *halicacabum*. Revista Brasileira de Farmacognosia,2012:22(3):623-629.
11. Govindappa M, Naga Sravya S, Poojashri MN, Sadananda TS, Chandrappa CP. Antimicrobial, Antioxidant and *in virto* Anti-inflammatory activity of Ethanol extract and Active Phytochemical screening of *Wedelia trilobata* (L.) Hitchc. Journal of Pharmacognosy and Phytochemistry,2011:3(3):43-51.
12. Gupta AK, Misra N. Hepatoprotective activity of aqueous ethanolic extract of *Chamomile capitula* in paracetamol intoxicated albino rats. American Journal of Pharmacology and Toxicology,2006:1(1):17-20.
13. Harborne JB. Phytochemical Methods- A guide to modern techniques of plant analysis. (2nd Ed.). Chapman and Hall, London, New York, 1984, 4-120.
14. Hossain MA, Nagooru MR. Biochemical profiling and total flavonoids contents of leaves crude extract of endemic medicinal plant *Corydylineterminalis* L. Kunth. Pharmacognosy Journal,2011:3(24):25-30.
15. Indian pharmacopoeia. Ministry of Health and family welfare, Government of India, New Delhi, 1996, A48-A54.
16. Jayabalan N. Plant Biotechnology, APH Publishing Corporation, India, 2006, 214.
17. Jeyadevi R, Sivasudha T, Rameshkumar A, James Harnly M, Long Ze L. Phenolic profiling by UPLC–MS/MS and hepato protective activity of *Cardiospermum halicacabum* against CCl_4 induced liver injury in Wistar rats. Journal of Functional Foods,2013:5(1):289-298.
18. Jeyadevi R, Sivasudha T, Ramesh kumar A, Dinesh kumar L. Anti-arthritic activity of Indian leafy vegetable *Cardiospermum halicacabum* in wistar rat and UPLC-QTOF-MS/MS identification of the putative active phenolic components. Inflammation research, 2013:62(1):115-116.
19. Khunkitti W, Fujimaki Y, Aoki Y. *In vitro* antifilarial activity of extracts of the medicinal plant *Cardiospermum halicacabum* against Brugiapahangi. Journal of Helminthology,2000:74(3):241-246.
20. Krishna Murti, Mayank A, Panchal, Vijay L, Gajera V. Pharmacological properties of *C.halicacabum* - A review. Pharmacologyonline,2010:2:1005.
21. Kurian JC. Plants that heal. Pune: Oriental Watchman Publishing House, 1995, 190.
22. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. Journal of Biological Chemistry,1951:193(1):265-275.
23. Mohaddesi B, Dudhrejiya A, Chauhan M. Pharmacognostical and physico-chemical evaluations of *Cardiospermum halicacabum* L. seeds. Research Journal of Pharmacognosy,2016:3(1):35-41.
24. Mohammed Junaid Hussain D, Sathish Kumar K, Darul Raiyaan GI, Mohamed Khalith SB, Sundarapandian S, Kantha Deivi A. Effect of Solvents on Phytochemical Composition and Antioxidant Activity of *Cardiospermum halicacabum* (L.) Extracts Pharmacognosy Journal,2020:12(6):1241-1251.
25. Mohrig JR, Hammond CN, Schatz PF. "Infrared Spectroscopy" in Techniques in Organic Chemistry. Freeman: New York, 2006.
26. Mukerjee PK, Verpoorte R. In GMP for Botanicals; Regulatory and Quality issues on phytomedicines, 2003, 99-112.
27. Nandiyanto BD, Oktiani R, Ragadhita R. How to Read and Interpret FTIR Spectroscope of Organic Material. Indonesian Journal of Science & Technology, 2019:4(1):97-118.
28. Parameshappa B, Sultan AB, Sen S, Chakraborty R, Vinod KG, Vidya SG *et al*. Acetaminophen-induced nephrotoxicity in rats: Protective role of *Cardiospermum halicacabum*. Pharmaceutical Biology, 2012:50(2):247-253.
29. Patil AG, Joshi Patil KA, Phatak DA, Naresh Chandra AK. Pharmacognostical and physicochemical studies on the leaves *of C. halicacabum* L. Pharmacognosy, 2010:2(5):44-49.
30. Patil AG, Joshi KA, Patil DA, Chandra N. Pharmacological standardization and HPTLC fingerprint of *Cardiospermum halicacabum* L. Stem. Research Journal of Pharmaceutical, Biological and Chemical Sciences,2011:2(2):343-52.
31. Ponmari G, Sathish kumar R, Lakshmi PTV. Effect of drying treatment on the contents of antioxidant in *C. halicacabum* L. International Journal of Pharmacy and Biological Sciences,2011:2(1):304-314.
32. Prabakaran S, Pugazhendy K, Revathi A. Phytochemical investigation and functional group

screening of *Cardiospermum halicacabum* and *Pisonia alba* by FT-IR spectroscopic analysis. International journal of modern research and reviews,2014:2(9):282-286.
33. Rajalaxmi N, Ashok P, Samanta L, Santilata S. Phytochemical Analysis of Antioxidants from *Abutilon indicum L*. and *Paederia foetida L*. Supported by TLC, FTIR and NMR Studies. International Journal of Science and Research,2015:4(5):1312-1318.
34. Raza SA, Hussain H, Riaz, Mahmood S. Review of beneficial and remedial aspects of *Cardiospermum halicacabum* L. African Journal of Pharmacy and Pharmacology,2013:7(48):3026-3033.
35. Sabeerali A, Selvarasuvasuki M, Ganapathy Murugan AL. Identification of biological components from potential bone healer medicinal plants. Journal of Drug Delivery & Therapeutics,2018:8(3):32-41.
36. Sagadevan P, Suresh SN, Rathish kumar S, Gayathri S, Vithya E. Anti-cancer activity of methanolic leaf extracts of *Andrographis paniculata* (Nees) and *Cardiospermum halicacabum* L Against human breast cancer cell line. International Journal of Pharmaceutical and Life Sciences,2013:4(9):2983-2986.
37. Selloum L, Bouriche H, Tigrine C, Boudoukha C. Anti-inflammatory effect of rutin on rat paw oedema, and on neutrophils chemotaxis and degranulation. Experimental and Toxicologic Pathology, 2003:54(4):313-318.
38. Sheeba MS, Asha VV. *Cardiospermum halicacabum* ethanol extract inhibits LPS induced COX-2, TNF and iNOS expression, which is mediated by NF-_B regulation, in RAW264.7 cells. Journal of Ethnopharmacology,2009:124(1):39-44.
39. Vadivazhagi MK. Anti-oxidation and Anti-microbial efficiency of *Cardiospermum halicacabum* leaf. A Journal of Composition Theory,2020:13(3):834-847.
40. Veeramani C, Numaira S, Chandramohana G, Alsaifa A, Pugalendi KV. Influence of *Cardiospermum halicacabum* leaf extract on membrane- bound ATPases in streptozotocin-induced diabetic rat. African Journal of Traditional, Complementary, and Alternative Medicines,2015:12(3):68-74.
41. Venkat Rao N, Chandra Prakash K, Shanta Kumar SM. Pharmacological investigation of *Cardiospermum halicacabum* (Linn) in different animal models of diarrhea. Research paper,2006:38(5):346-349.
42. Viji M, Murugesan S. Phytochemical Analysis and Antibacterial Activity of Medicinal Plant *Cardiospermum halicacabum* Linn. Journal of Phytology,2010:2(1):68-77.
43. Vogel GH. Drug discovery and evaluation. Pharmacological assays, 2003, 202.
44. WHO. World Health Organization: Quality control methods for herbal materials. Geneva, 1998, 24.

Journal of Stress Physiology & Biochemistry, Vol. 17, No. 4, 2021, pp. 85-92 ISSN 1997-0838
Original Text Copyright © 2021 by Nagalakshmi, Anand, Raj and Bavya

ORIGINAL ARTICLE

Phytochemical Screening and Functional Groups Identification of *Cardiospermum halicacabum* L. Leaves

Ramamoorthy Nagalakshmi[1]* Subramaniam Parvathi Anand[1],

Arockiam Justin Raj[1], Subramanian Bavya[2]

[1] *PG and Research Department of Botany, National College, Affiliated to Bharathidasan University, Tiruchirappalli-620001, Tamil Nadu, India.*

[2] *Department of Botany, St. Joseph's College, Affiliated to Bharathidasan University, Tiruchirappalli-620002, Tamil Nadu, India.*

*E-Mail: ***nagalakshmi.93botany@gmail.com***

Received July 12, 2021

Background: The phytochemical components are extremely important to facilitate the search for the particular effectiveness of the plant in medicine. Identification of the chemical nature of phytochemical compounds present in medicinal plants will provide information about the various functional groups responsible for its medicinal properties. Thus, the need to investigate the phytochemicals from various plants has been exponentially increasing. *Cardiospermum halicacabum* L. is one of the most important medicinal plants belonging to the Sapindaceae family. This investigation was administered to study phytochemical and FT-IR analysis of a various solvent extracts of *Cardiospermum halicacabum* L. leaves.

Results: The preliminary phytochemical analysis of *Cardiospermum halicacabum* L. leaves extract revealed the presence of carbohydrates, glycosides, alkaloids, phytosterol, fixed oils, saponins, proteins, and amino acids, phenolic compounds, flavonoids, and tannins. As a result, the phytochemical analysis of aqueous extracts revealed the presence of more phyto components followed by petroleum ether, and chloroform extracts. FT-IR spectroscopy confirmed the existence of various chemical components such as alcohol, alkanes, aromatic compound, carboxylic acid, aldehydes, alkenes, amides, nitro compounds and alkyl halides in the leaves extract of *C. halicacabum* L. The functional groups observed in the various extracts probably indicate the presence of carbohydrates, glycosides, alkaloids, amino acids, phenols, flavonoids, tannins, and saponins. The results obtained from FTIR analysis are significant with the preliminary qualitative analysis.

Conclusions: Extractions of any crude drug with a certain solvent result in a solution that contains various phyto-components. The composition of these phyto-components in the respective solvent provides initial information about the quality of a drug sample. The presence or absence of a certain component plays an important role in the decision about the medicinal properties of the plant. As a result, the phytochemical analysis of aqueous extracts revealed the presence of more phyto-components followed by petroleum ether, and chloroform extracts which can be beneficial in a therapeutic approach.

Key words: Cardiospermum halicacabum, Various solvents, FT-IR analysis, Functional groups, Phytochemical screening.

Today, in this modern world highly effective synthetic drugs are readily available but have a long list of possible bad side effects. Because of this, many people rely on herbal medicines which can provide new inputs into the drug development strategy. Herbal medicine is based on traditional medicine and exists all over the world. Each of these traditional medicines has its origin and a private basic philosophy (Vogel, 2003). India is one of the countries with the greatest medical and cultural diversity in the world (Archna Sharma *et al*., 2013). Herbal medicines are on the advance in developing countries, and herbal medicines are now firmly established in medical care (Mukerjee and Verpoorte, 2003).

The medicinal properties of any plant are due to the presence of a variety of secondary metabolites in them. Phytochemicals are naturally found in medicinal plants in all the parts namely, leaves, stems, and roots which play an important role in curing diseases. The phytochemical components are extremely important to facilitate the search for the particular effectiveness of the plant in medicine. Thus, the need to investigate the phytochemicals from various plants has been exponentially increasing.

Fourier transform infrared spectroscopy is perhaps the most generally utilized technique for classifying chemical components and has been used in several countries as a necessary method for identifying drugs for the pharmacopeia (Subashini *et al*., 2015). FTIR spectroscopy is considered to be a reliable and sensitive method for detecting functional groups in plant extracts, and it has been determined in the IR range of 400-4000 cm^{-1} (Florence and Jeeva, 2015). This may be the most important and authoritative method to determine the types of chemical groups (functional groups) present in a compound. The wavelength of the fascinated light is a characteristic of the chemical bond which might be seen in the annotated spectrum (Sahayaraja *et al*., 2015). Identification of the chemical nature of phytochemicals contained in medicinal plants provides information about the various functional groups responsible for their medicinal properties.

Cardiospermum halicacabum L. is one of the most important medicinal plants of the Sapindaceae family. The local names for this plant are Balloon vine, Puff-ball, Heartseed Vine, Heart pea, Love in a puff (Krishna Murti *et al*., 2010). *C. halicacabum* contains certain phytochemicals that support its role in the medicinal field. It has anti-inflammatory, antidiarrheal, antiparasitic, antipyretic, antifungal, anxiolytic activity, adulticidal activities, urinary tract infections suppression, and antihyperglycemic properties (Asha and Ushpangadan, 1999; Khunkitti *et al*., 2000; Selloum *et al*., 2003; Sheeba and Asha, 2009; Veeramani et al., 2015). Its chemical constituents are well documented (Harborne, 1998; Deepan *et al*., 2012; Sagadevan *et al*., 2013; Annadurai *et al*., 2013; Mohaddesi *et al*., 2016; Vadivazhagi, 2020). The purpose of this study is to use FT-IR analysis to determine the functional groups of various *Cardiospermum halicacabum* leaf extracts.

MATERIALS AND METHODS

Collection and processing of plant material

Cardiospermum halicacabum L. was collected in February 2021 in Rettaimalai, Tiruchirappalli District, Tamil Nadu, and South India. The collected plants were washed with water to remove soil and dust. The plant material was dried for four to five days in the shade and cut into small pieces, then grind into a coarse powder. The powder was used to extract the active ingredients.

Extraction of the plant material

The coarse powder was placed in the Soxhlet apparatus and extracted successively with various solvents such as aqueous, petroleum ether, and chloroform. The extract was then filtered and evaporated on a rotary evaporator. The dried extracts were stored in the refrigerator for further experiments.

Preliminary phytochemical studies

The leaf extract of various solvents was qualitatively determined for the presence of alkaloids, carbohydrates, glycosides, proteins and free amino acids, fixed oils, phenolic compounds, tannins, flavonoids, saponins, and sterols according to the standard method provided by Harborne, (1998).

FTIR analysis

The Fourier Transform Infrared Spectrophotometer (FTIR) is perhaps the most powerful tool for identifying the different types of chemical bonds (functional groups) present in compounds. The wavelength of the absorbed light is characteristic of the attraction, which can be seen across the entire spectrum. Chemical bonds in a molecule can be determined based on its IR absorption spectrum. Dry powder from various solvent extracts of plant material was utilized for the FTIR examination. 10 mg of the dry extract powder was packed in 100 mg of KBr pellet, to prepare translucent sample disks. The powder sample was placed in a Fourier transform IR spectroscope (PERKIN ELMER, IR) with a scanning range from 400 to 4000 cm $^{-1}$ with a resolution of 4 cm $^{-1}$.

RESULTS

Preliminary phytochemical studies

Preliminary phytochemical screening of the *C. halicacabum* leaf extract of various solvents (aqueous, petroleum ether, and chloroform) presented in Table 1. The preliminary phytochemical analysis of aqueous leaf extract revealed the presence of seven compounds i.e. carbohydrates, glycosides, alkaloids, fixed oils, saponins, phenolic compounds, tannins, and absence of phytosterols, proteins, and flavonoids. Petroleum ether leaf extract revealed the presence of 6 compounds i.e. carbohydrates, glycosides, phytosterol, saponins, proteins, and flavonoids. Preliminary phytochemical analysis of Chloroform and extract revealed the presence of 3 compounds such as alkaloids, fixed oils, and saponins.

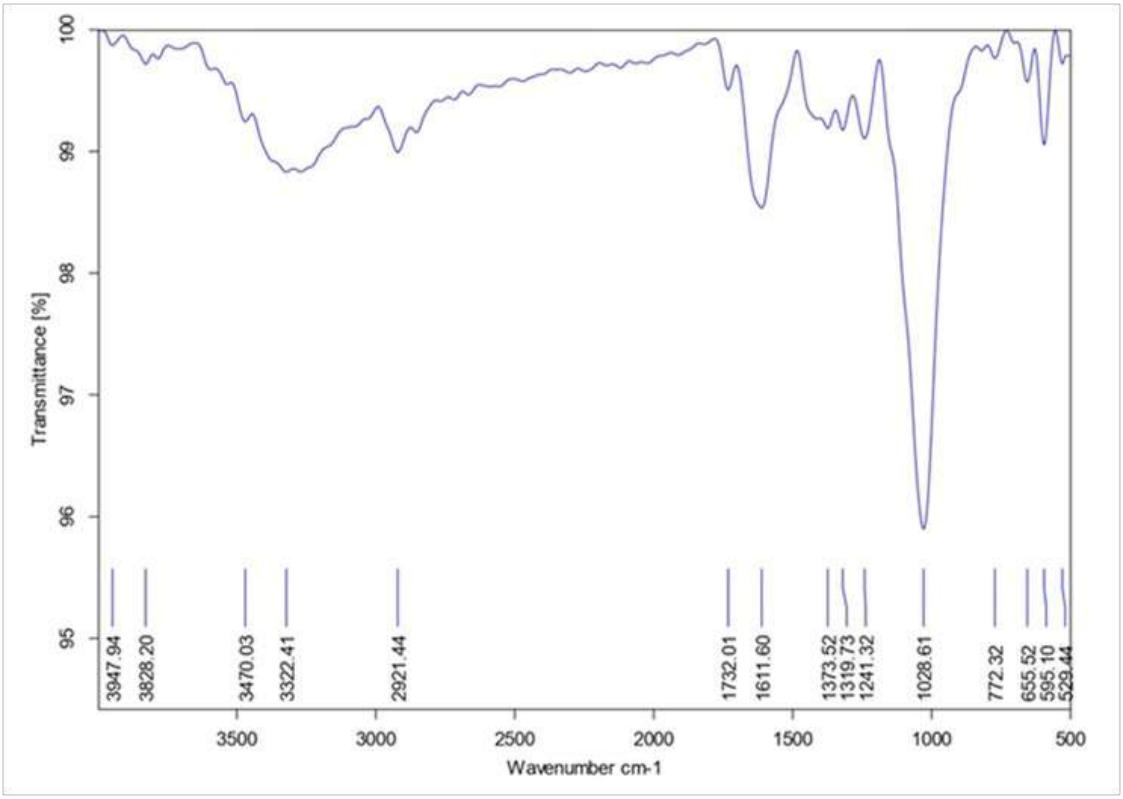


Figure 1. FT-IR spectrum of aqueous extract of *C. halicacabum* L.

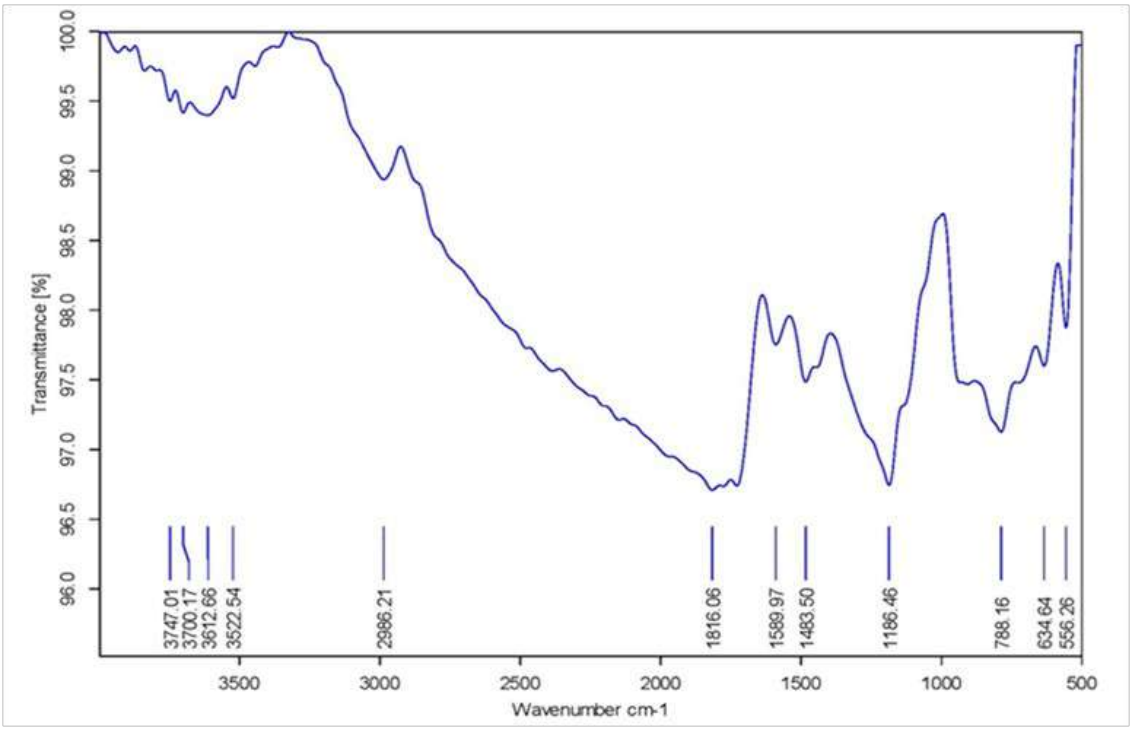


Figure 2. FT-IR spectrum of petroleum ether extract of *C. halicacabum* L.

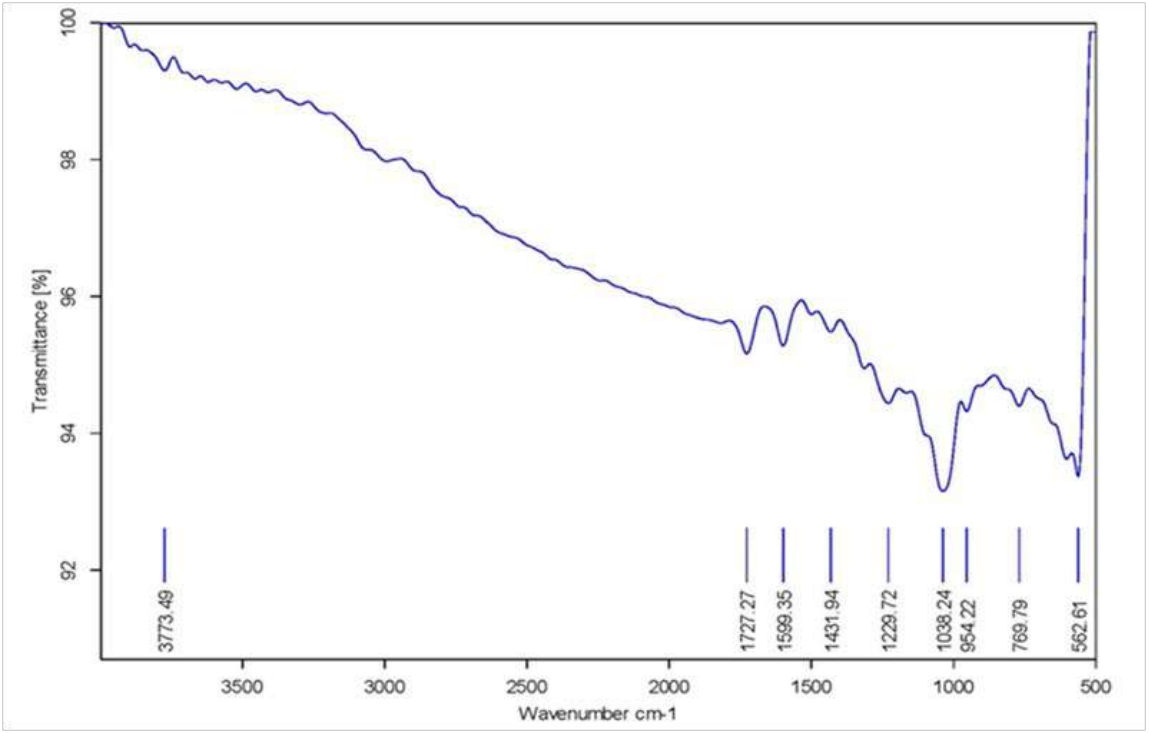


Figure 3. FT-IR spectrum of chloroform extract of *C. halicacabum* L.

Table 1 Preliminary phytochemical screening of *C. halicacabum* leaf extract

S.No	Components	Aqueous	Petroleum ether	Chloroform
1	Carbohydrates	+	+	-
2	Glycosides	+	+	-
3	Alkaloids	+	-	+
4	Phytosterol	-	+	-
5	Fixed oils	+	-	+
6	Saponins	+	+	+
7	Proteins and amino acids	-	+	-
8	Phenolic compounds	+	-	-
9	Flavonoids	-	+	-
10	Tannins	+	-	-

(+) = Present and (-) = Absent

Table 2 FT-IR frequency range and functional groups of aqueous extract of *C. halicacabum*

Frequency range	Molecular motion	Functional group
3947.94 - 3828.20	O-H Non-bonded	Hydroxyl group
3470.03	O-H stretch	Alcohols
3322.41	N-H stretch	Amines
2921.44	C-H stretch	Alkanes
1732.01	C-H stretch	Aldehydes
1611.60	C=C stretch	Aromatic compounds
1373.52 - 1319.73	NO2 stretch	Nitro compounds
1241.32 - 1028.61	C-F stretch	Alkyl & Aryl halides
772.32	C-H-Plane bend	Alkenes & Aromatics
655.52	C-Br stretch	Alkyl & Aryl halides
595.10 - 529.44	C-CI stretch	Alkyl & Aryl halides

Table 3 FT-IR frequency range and functional groups of petroleum ether extract of *C. halicacabum*

Frequency range	Molecular motion	Functional group
3747.01 - 3612.66	O-H Non-bonded	Hydroxyl group
3522.54	O-H stretch	Alcohols
2986.21	C-H stretch	Alkanes
1816.06	C-H stretch	Aldehydes
1589.97 -1483.50	C=C stretch	Aromatic compounds
1186.46	C-F stretch	Alkyl & Aryl halides
788.16	C-H-Plane bend	Alkenes & Aromatics
634.64	C-Br stretch	Alkyl & Aryl halides
556.26	C-I stretch	Alkyl & Aryl halides

Table 4 FT-IR frequency range and functional groups of chloroform extract of *C. halicacabum*

Frequency range	Molecular motion	Functional group
3773.49	O-H Non-bonded	Hydroxyl group
1727.27	C=O stretch	Carboxylic acids
1599.35	C=C stretch	Aromatic compounds
1431.94	C-H stretch	Alkenes
1229.72 - 1038.24	C-F stretch	Alkyl & Aryl halides
954.22	=C-H bend	Alkenes
769.79	C-H-Plane bend	Alkenes & Aromatics
562.61	C-Cl stretch	Alkyl & Aryl halides

FTIR spectral data interpretation

The FTIR spectrum is used to determine the functional groups of the active components present in the extract based on the spectrum values in the region of IR radiation (Mohrig *et al*., 2006; Ashokkumar and Ramaswamy, 2014; Nandiyanto *et al*., 2019). The FTIR spectrum of plant extracts (prepared in various solvents) of *C. halicacabum* was given in Fig 1 – 3. Data on the peak value and potential of functional groups obtained by Fourier-infrared spectroscopy-analysis of the presence in plant extracts (prepared in aqueous, petroleum ether, and chloroform) were presented in Tables 2 to 4. The FTIR spectrum results showed that the aqueous leaf extract having the presence of hydroxyl group, alcohols, amines, alkanes aldehydes, alkenes, aromatic compounds, nitro compounds, alkyl, and aryl halides which shows major peaks from 529.44 - 3947.94 respectively. Chloroform extract having the presence of hydroxyl group, carboxylic acids, alkenes, aromatic compounds, alkyl, and aryl halides which shows major peaks from 562.61- 3773.49 respectively. The spectrum of petroleum ether extract indicated the presence of hydroxyl group, alcohols, aldehydes, alkanes, alkenes, aromatic compounds, alkyl, and aryl halides which shows major peaks at 556.26 - 3747.01 respectively.

DISCUSSION

The extractions of any crude with a specific solvent create a solution that contains various phytocomponents. The composition of these phytocomponents in the respective solvent can be a means of providing preliminary information about the quality of a particular drug sample. The presence or absence of a particular component plays an important role in deciding the medicinal property of the plant extract. As a result, phytochemical analysis of the aqueous extracts showed the presence of more phytocomponents, followed by petroleum ether, and chloroform extracts. Alcoholic and aqueous extracts of *C. halicacabum* L. contain tannins, flavonoids, saponins, sterols, and triterpenes (Venkat Rao *et al*., 2006; Kurian, 1995). Parameshappa *et al*., (2012) reported that petroleum ether extract of the plant contains sterols, carbohydrates, flavonoids, and triterpenes; Chloroform extract contains alkaloids, coumarin, flavones, quinones, saponins, steroids, and tannins (Aishwarya *et al*., 2014).

The results of FT-IR spectroscopy confirm the presence of various chemical components such as alcohol, alkanes, aromatic compound, carboxylic acid, aldehydes, alkenes, amides, nitro compounds, and alkyl halides in the extract of *C. halicacabum* L. leaves (Viji *et al*., 2010). This result agrees with the result of Prabakaran *et al*., (2014). FT-IR analysis of the extract from methanolic leaves of *C. halicacabum* shows the presence of alcohol, isocyanides, alkyl compounds, alkanes, and components containing chlorine (Sabeerali *et al.*, 2018; Mohammed Junaid *et al.*, 2020). The functional groups observed in the various extracts likely indicate the presence of carbohydrates, glycosides, alkaloids, amino acids, phenols, flavonoids, tannins, and saponins. The consequences of the FTIR evaluation are considerable for the preliminary qualitative investigation.

ACKNOWLEDGEMENT

The authors gratefully acknowledge PG and Research Department of Botany, National College, providing the infrastructure and other facilities. All the authors wish to acknowledge the National College Instrumental Facility (NCIF), National College (Autonomous), Tiruchirappalli for their support and guidance during the FT-IR analysis.

CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

REFERENCES

Aishwarya V., Sheik abdulla S., Dheeba B., Renuka R. (2014) In vitro antioxidant and anticancer activity of *Cardiospermum halicacabum* L. Against eac cell line. *Int. J. Pharm. Sci.,* 6(8), 263-268.

Annadurai A., Elangovan V., Velmurugan S., Ravi Kumar R. (2013) Preliminary phytochemical screening and antibacterial of *Cardiospermum halicacabum* L. *Pelagia Research Library.,* 4(5), 302-308.

Archna Sharma R.A., Sharma, Hemlata Singh. (2013) Phytochemical and Pharmacological Profile of

Abutilon indicum L. Sweet: A Review. *Int. J. Pharma. Sci. Rev and Res.,* 20, 20-127.

Asha V.V. and Ushpangadan P. (1999) Antipyretic activity of *Cardiospermum halicacabum*. *Indian J. Exp. Biol.*, 37(4), 411-414.

Ashokkumar R.M. and Ramswamy (2014) Phytochemical screening by FTIR spectroscopic analysis of leaf extracts of selected Indian Medicinal plants. *Int. J. Curr. Microbiol. App. Sci.,* 3(1), 395-406.

Deepan T., Alekhya V., Saravanakumar P., Dhanaraju M.D. (2012) Phytochemical and Anti-Microbial Studies on the Leaves Extracts of *Cardiospermum halicacabum* Linn. *Adv. Biol. Res.,* 6(1), 14-18.

Florence A. R. and Jeeva S. (2015) FTIR and GC-MS spectral analysis of *Gmelina asiatica* L. Leaves. *Science Research Reporter*. 5(2), 125-136.

Harborne J. B. (1998) Phytochemical methods. In A guide to modern techniques of plant analysis 3 rd ed. London, Chapman and Hall, pp. 40-137.

Khunkitti W., Fujimaki Y. and Aoki Y. (2000) In vitro antifilarial activity of extracts of the medicinal plant *Cardiospermum halicacabum* against Brugiapahangi. *J. Helminthol.,* 74(3), 241-246.

Krishna Murti Mayank A., Panchal, Vijay L., Gajera V. (2010) Pharmacological properties of *C.halicacabum* - A review. *Pharmacology*. 2, 1005.

Kurian J.C. (1995) Plants that heal. Pune: Oriental Watchman Publishing House.

Mohaddesi B., Dudhrejiya A. and Chauhan M. (2016) Pharmacognostical and physicochemical evaluations of *Cardiospermum halicacabum* L. seeds. *Res. J. of Pharmacog*., 3(1), 35-41.

Mohammed Junaid H. D., Sathish Kumar K., Darul Raiyaan G.I., Mohamed Khalith S.B., Sundarapandian S., Kantha Deivi A. (2020) Effect of Solvents on Phytochemical Composition and Antioxidant Activity of *Cardiospermum halicacabum* (L.) Extracts. *Pharmacogn J.* 12(6), 1241-1251.

Mohrig J.R., Hammond C.N. and Schatz P.F. (2006) “Infrared Spectroscopy” in Techniques in Organic Chemistry. Freeman: New York,

Mukerjee P.K. and Verpoorte R. (2003) ‘In GMP for Botanicals; Regulatory and Quality issues on phytomedicines’ 1.

Nandiyanto B.D., Oktiani R. and Ragadhita R. (2019) How to Read and Interpret FTIR Spectroscopy of Organic Material. *Indo. J. of Sci & Technol.,* 4(1), 97-118.

Parameshappa B., Sultan A.B., Sen S., Chakraborty R., Vinod K.G., Vidya S.G., Sowmya L., Raju K.K., Sesh K.P.K.R., Lakshmi A.V.S.M. (2012) Acetaminophen-induced nephrotoxicity in rats: Protective role of *Cardiospermum halicacabum*. 50(2), 247-253.

Prabakaran S., Pugazhendy K. and Revathi A. (2014) Phytochemical investigation and functional group screening of *Cardiospermum halicacabum* and *Pisonia alba* by FT-IR spectroscopic analysis. Int. J. mod. res. rev., 2(9), 282-286.

Sabeerali A., Selvarasuvasuki M. and Ganapathy Murugan A.L. (2018) Identification of biological components from potential bone healer medicinal plants. *J. drug deliv. Ther*., 8(3), 32-41.

Sagadevan P., Suresh S.N., Rathish Kumar S., Gayathri S., Vithya E. (2013) Anti-cancer activity of methanolic leaf extracts of *Andrographis paniculata* (Nees) and *Cardiospermum halicacabum* L against human breast cancer cell line. *Int. J. of Pharm & Life sci.,* 4(9), 2983-2986.

Sahayaraja P.A., Gowri J., Dharmalingama V., Shobanaa R., Angelin Premab A.A. (2015) Phytochemical screening by FTIR spectroscopic analysis of leaf and stem extracts of *Wedelia biflora*. *Int. j. nano corros. sci. eng.,* 2(5), 322-334.

Selloum L., Bouriche H., Tigrine C., Boudoukha C. (2003) Anti-inflammatory effect of rutin on rat paw edema, and on neutrophils chemotaxis and degranulation. *Exp. Toxicol Pathol* 54(4), 313-318.

Sheeba M.S. and Asha V.V. (2009) *Cardiospermum halicacabum* ethanol extract inhibits LPS induced COX-2, TNF, and iNOS expression, which is mediated by NF-B regulation, in RAW264.7 cells. *J. Ethnopharmacol*., 124(1), 39-44.

Subashini M.S., Rajendran P., Ashok G., Kanthesh B.M. (2015) TLC, FTIR and GCMS analysis of leaves of *Gymnema sylvestre* R.Br from Kolli Hills, Tamil Nadu, India. *Int. J. Curr. Microbiol. App. Sci.,* 4(7), 757-764.

Vadivazhagi M.K. (2020) Anti-oxidation and Anti-microbial efficiency of *Cardiospermum halicacabum* leaf. *A Journal of Composition Theory.* 834-847.

Veeramani C., Al-Numaira S., Chandramohana G., Alsaifa A., Pugalendi K.V. (2015) Influence of *Cardiospermum halicacabum* leaf extract on membrane-bound ATPases in streptozotocin-induced diabetic rat. *Afr. J. Tradit. Complement. Altern. Med.,* 12(3), 68-74.

Venkat Rao N., Chandra Prakash K. and Shanta Kumar S.M. (2006) Pharmacological investigation of *Cardiospermum halicacabum* (Linn) in different animal models of diarrhea. *Research paper*. 38(5), 346-349.

Viji M. and Murugesan S. (2010) Phytochemical Analysis and Antibacterial Activity of Medicinal Plant *Cardiospermum halicacabum* Linn. Journal of Phytology.

Vogel G.H. (2003) Drug discovery and evaluation. Pharmacological assays 202.

SAARC J. Agric., 20(1): 121-130 (2022) *DOI: https://doi.org/10.3329/sja.v20i1.60556*

THE RELATIONSHIP BETWEEN HEAVY METALS CONCENTRATIONS IN SOIL AND PLANT (*Senna auriculata* (L.) Roxb.) OF THE HILLS AND ROADSIDES IN TIRUCHIRAPPALLI, INDIA

S.P. Anand and R. Nagalakshmi*

PG and Research Department of Botany, National College, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India

ABSTRACT

The relationship between heavy metal concentrations of soil and plant (*Senna auriculata*) in the hills and roadsides was studied. Atomic absorption spectroscopy was used for determination of metals such as lead (Pb), chromium (Cr), cadmium (Cd), zinc (Zn), copper (Cu), and iron (Fe) in the soil and plant (leaves and flowers) samples. The results showed that heavy metal concentrations in roadside soil and plant samples were much greater than that in hills. It is evident that concentration of a heavy metal in soil samples are considerably higher ($p<0.05$) than that in plant samples. Pearson correlation analysis shows that Pb in leaves and flowers correlated well with that in soil, $r>0.93$. Strong positive relationship exists between soil and plant for Cr content, ($r\geq0.97$). Cadmium and Zn contents in leaves and flowers correlated significantly with Cd, and Zn in soil, all $r>0.97$. The Cu concentration of leaves and flowers shows a positive correlation with Cu content in soil, all $r\geq0.93$. The Fe concentration of both plant leaves and flowers is found significantly and positively correlated with soil Fe, $r=0.99$. Overall results reveal that all the heavy metals are significantly and positively associated with soil and plant, implying that increasing concentration of heavy metals in soil is likely to increase their concentrations in *S. auriculata* plant.

Keywords: Heavy metals, Hills, Roadsides, *Senna auriculata*, Soil

INTRODUCTION

Heavy metals are the elements that have metallic characteristics and comparatively high density. Heavy metals in soils or plants cannot be destroyed or degraded. Further, with the growth of industrialization, heavy metals' presence has rapidly increased. Heavy metals are of two types – the one group consists of micronutrients

* Corresponding author: nagalakshmi.93botany@gmail.com

Received: 16.02.2022 *Accepted: 20.04.2022*

that plants essentially take a small amount for normal growth of plants, the elements are iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), molybdenum (Mo), and nickel (Ni), while the other metals such as cadmium (Cd), chromium (Cr), lead (Pb), arsenic (As) and cobalt (Co) are toxic heavy metals (Emamverdian et al., 2015). Plants absorb heavy metals that are present in soil solution. Because of the variability in soil qualities and plant growth characteristics, there is frequently no direct connection between total metal concentrations in soils and plants (Obrador et al., 2007). One of the reasons for the lack of a good correlation between metal concentrations in soils and plants is that it might, be influenced by soil contamination sources.

Heavy metal phytoavailability in soil may be measured by establishing a relationship between metal concentration in soil and accumulation in plant tissues (Chen et al., 2014). Heavy metal concentrations in various ecosystems are currently increasing in comparison to natural background levels due to rapid growth in industrial, agricultural, and urban activities. Roadside soils, plants, and organisms are absorbing significant quantities of toxic metals, primarily from automobile emissions and toxic substances transported. The principal metallic contaminants of roadside soil include lead, cadmium, copper, zinc, nickel, iron, and chromium, among other heavy metals (Silva et al., 2005). Lead and cadmium are toxic even in low quantities. As a result, the focus of this research is on Pb, Cd, Cr, Zn, Fe and Cu. Heavy-metal (e.g. Pb) emissions from automobiles are caused by fuel combustion, lubricating oil consumption, tyre wear, brake wear, road abrasion, and other factors (Weckwerth, 2001). The Cd emissions are mostly caused by lubricant oil use as well as tyre wear. Zinc emission arises due to tyre wear and galvanized components such as gasoline tanks. The most significant source of Cu emission is brake wear. Heavy metals can be transferred from roadside soils to plant uptake by atmospheric deposit or vehicle runoff (Viard et al., 2004; Nabulo et al., 2006).

Senna auriculata (L.) Roxb., a member of the Fabaceae family and often known as aavaram, was chosen as an important medicinal plant for this research. The leaves of these plants are therapeutic; they are eaten, boiled individually or with tea, or used in wound healing. *Senna auriculata* is an excellent choice for roadside landscaping. As a result, monitoring metal concentrations in these plant leaves are important, especially in industrially polluted and road side areas. Metal mobility between soil and plants is a critical topic in determining the environmental impact of metals (Anoliefo et al., 2006). Therefore, in the current study, a correlation analysis was performed to investigate the relationship of the concentrations of six heavy metals, namely Pb, Cr, Cd, Zn, Cu, and Fe in the leaves and flowers of *S. auriculata* with their concentrations in soils in the hills and roadsides.

MATERIALS AND METHODS

Collection and identification

Soil and plant samples were collected from hills (sites 1 and 2) and roadside near industrial regions (sites 3 and 4) in July 2021. Site 1 is Pachaimalai Hills, Site 2 is Kolli Hills, Site 3 is Avur Road, and Site 4 is Samayapuram Road. Botanical Survey of India (BSI/SRC/5/23/2021/Tech-166), Southern Regional Centre, Coimbatore recognized and authenticated the collected plant species.

Analytical procedure

Each soil sample was homogenized and oven-dried at 105°C. After sieving, the material was poured into a 300 ml beaker and heated at 230°C with15ml of nitric acid (HNO_3, 69%) and 25 ml of perchloric acid ($HClO_4$, 58%). After being turned to ash, the digested solution was filtered using Whatman No. 42 filter paper, and the volume was increased to 50ml in a volumetric flask. The metal concentration is determined using an atomic absorption spectrophotometer (Mehmet, 2008). The plant samples were air-dried at room temperature for 5-7 days before being ground into a fine powder. According to Uddin et al. (2016), the wet digestion process was the most efficient for plant materials. A sample weighing 2 g was taken into a conical flask. After that, a 9 ml mixture of nitric-hydrochloric acids HNO_3 (65%) and HCl (37%), in a 1:3 ratio, was added to the conical flask. The mixture was then gently heated at 95°C water bath for 4–5 hours, or for a period until the sample was dissolved. The digest was allowed to cool before being filtered through a Whatman No. 42 filter paper and diluted with deionized water to a final volume of 50 ml. The Stock 1000 ppm standard solutions for all of the heavy metals under test were made in deionized water (Urmila, 2016). Atomic Absorption Spectroscopy (THERMO SCIENTIFIC-ice 3000) was used to detect the heavy metal concentrations in soil and plant samples at National College Instrumentation Facility, National College Tiruchirappalli. All samples and standard solutions were measured in triplicate.

Calculation

The metal concentration which we get from AAS is in mg/L of the solution. This can be converted into mg per kg of the dry weight of soil and plant by the following formula:

$$\text{Concentration of the metal in sample} = \frac{\text{Measured concentration in AAS (mg)} \times \text{Volume (L)}}{\text{Weight of the sample (kg)}}$$

Statistical analysis

The mean and standard deviation of the heavy metal values from three replicates were calculated using Microsoft Excel. ANOVA was used to determine whether there was a significant difference between the mean concentrations of heavy metals in plant and soil samples collected from hills and roadsides. To assess the magnitude of the relationship between heavy metal concentrations in soil and plant samples, Pearson's correlation test was performed. The significance level was fixed at $p<0.05$.

RESULTS AND DISCUSSION

Heavy metal concentrations in soil samples

Table 1 shows the mean concentrations of heavy metals in soil samples of four study sites. The findings demonstrate that heavy metal concentrations in roadside samples are greater than in hill sources. The majority of the heavy metal levels in the soil samples studied were below the WHO limit values (2007). However, several soil samples had Cd and Zn levels that were higher than the recommended limits of 0.8 mg/kg and 100 mg/kg, respectively. Cd levels above the standard in roadside soil samples such as site-3 (9.65 mg/kg) and site-4 (6.74 mg/kg). The Zn content in the site-3 soil sample (115.84 mg/kg) exceeded the standard limits. The elevated Cd and Zn values of the roadside samples are due to fuel combustion from automobiles plus discharge of the paint, chemical, and electronic industries, as well as certain cement companies located within 300 m of the sample collection site. The disposal of industrial wastes or the release of pollutants into the atmosphere enhances the overall content of Cd in soils (Weggler et al., 2004). Anthropogenic Cd accumulations in the environment are a source of concern in industrialized nations, and it is classified as a potentially dangerous element in terms of soil biological activity, plant metabolism, and human and ecosystem health (Kabata-Pendias and Pendias, 2001). Zinc exists naturally in soil (approximately 70 mg kg^{-1} in crustal rocks), but Zn concentrations are rising unnaturally due to anthropogenic influences. The majority of Zn is released to the environment via industrial operations such as mining, coal and waste incineration, and steel manufacture. Large quantities of heavy metals are "absorbed" by roadside soils from several sources, including automobile emissions, coal-burning waste, and other activities (Jose et al., 2009, Saeedi et al., 2009). Heavy metals are found in fuels, fuel tank walls, engines and other vehicle components, catalytic converters, tyres, and brake pads, and road surface materials (Zehetner et al., 2009).

Table 1. Mean concentration of heavy metals in soil samples

Metals	Concentrations (Mean ± SD) (mg/kg)			
	Site - 1	Site - 2	Site - 3	Site- 4
Pb	3.09±0.28	0.07±0.21	27.44±0.02	23.95±0.30
Cr	0.97±0.02	2.73±0.04	15.82±0.07	27.69±0.15
Cd	0.28±0.37	0.04±0.02	9.65±3.21	6.74±0.02
Zn	10.6±0.01	9.02±2.37	115.84±0.25	89.35±0.29
Cu	8.02±0.62	9.76±0.01	25.89±0.03	13.32±0.03
Fe	10.23±0.03	17.8±0.21	54.03±0.74	98.46±0.42

Site 1: Pachaimalai hill, Site 2: Kolli hill, Site 3: Avur road, Site 4: Samayapuram road.

Heavy metal concentrations of *Senna auriculata* plants

The mean concentrations of heavy metals in leaves and flowers of *Senna auriculata* are presented in Table 2. Lead values in leaf and flower samples from the study sites ranged from 0.01 to 13.46 mg/kg and 1.02 to 12.78 mg/kg, respectively, compared to the WHO-recommended permissible limit of 10 mg/kg for plants. This criterion was satisfied by the determined The Pb levels in plant samples from hills are within the limit. However, the Pb concentration in the sample of site-3 (roadsides) was much greater than the permissible limits. The Cr concentration of leaves ranged from 0.63 to 3.84 mg/kg and in flowers the concentration varied from 0.42 to 3.06 mg/kg. The maximum Cr concentration of leaves and flowers (3.84 mg/kg and 3.06 mg/kg) was detected in site 4 sample (3.84 mg/kg and 3.06 mg/kg), which was much above the permissible limit (2 mg/kg). The Cd level in leaves was 0.05 to 0.32 mg/kg. The Cr values in flowers varied from 0.03 to 0.27 mg/kg. According to the WHO, the maximum permissible concentration of Cd in plants is 0.3 mg/kg. Thus, all the leaf and flower samples from hills and roadsides were found to have Cd concentrations below the acceptable limit, except for the flower sample from a roadside (site 3), which had a Cd content slightly higher than the permissible limit of 0.32 mg/kg.

Table 2. Mean concentration of heavy metals in *Senna auriculata*

Metals	Plant parts	Concentrations (Mean ± SD) (mg/kg)			
		Site 1	Site 2	Site 3	Site 4
Pb	Leaves	1.86±0.03	0.01±0.01	13.46±0.02	7.43±0.26
	Flowers	1.02±0.01	ND	12.78±0.03	7.38±0.37
Cr	Leaves	0.63±0.01	1.07±0.03	1.89±0.01	3.84±0.01
	Flowers	0.42±0.01	0.91±0.26	1.65±0.04	3.06±0.02
Cd	Leaves	0.05±0.03	ND	0.32±0.01	0.21±0.02
	Flowers	0.03±0.01	ND	0.27±0.03	0.19±0.02
Zn	Leaves	2.63±0.02	1.56±0.01	59.05±0.12	39.24±0.01
	Flowers	0.87±0.02	1.38±0.04	56.12±0.65	38.96±0.03
Cu	Leaves	1.98±0.01	3.48±0.02	9.52±0.03	6.02±0.01
	Flowers	1.46±0.02	3.35±0.01	9.46 ±0.02	5.73±0.01
Fe	Leaves	4.87±0.27	5.48±0.03	11.92±0.01	19.28±0.22
	Flowers	3.84±0.43	5.07±0.02	10.72±0.01	18.02±0.24

ND – not detected, *P<0.05

The Zn concentrations in leaves and flowers ranged from 1.56 to 59.05 mg/kg and 0.87 to 56.12 mg/kg, respectively. The greatest Zn level of leaves and flowers was observed in the site 3 sample, which was above the permissible limit (50 mg/kg). The Cu concentrations in *S. auriculata* leaves and flowers ranged from 1.98 to 9.52

mg/kg and 1.46 to 9.46 mg/kg, respectively, both of which were lower than the WHO permissible limit of 10 mg/kg. The Fe values in leaves varied from 4.87 to 19.28 mg/kg and that in flowers was from 3.84 to 18.02 mg/kg. According to the WHO, the maximum allowable level of Fe in plants is 15 mg/kg. The sample from site 4 contains higher concentrations of Fe than the other sites.

Except for cadmium, analytical results demonstrate that samples from site 1and2 (hills) showed heavy metal concentrations below the permissible limits. Cadmium was not detectable in the site 2 sample. Except for Cd and Cu, the heavy metal concentration of the roadside samples was greater than the acceptable limit. The Pb and Zn levels in the site 3 sample as well as Fe and Cr levels in the site 4 sample, of both leaves and flower extract are above the permissible limit. Therefore, the results indicate that the plant samples from the roadside had higher heavy metal contents than the ones from the hills. This is also true for heavy metal concentrations in soil samples from sites 3 and 4 (both roadsides) than in the hills sources (Table 2).

Table 3. Pearson correlation coefficient(r) between heavy metal concentrations in soil and *S. auriculata* leaves

	Pb soil	Cr soil	Cd soil	Zn soil	Cu soil	Fe soil	Pb leaves	Cr leaves	Cd leaves	Zn leaves	Cu leaves	Fe leaves
Pb soil	1											
Cr soil	0.818	1										
Cd soil	0.983	0.700	1									
Zn soil	0.992	0.741	0.998	1								
Cu soil	0.753	0.237	0.859	0.828	1							
Fe soil	0.765	0.996	0.635	0.680	0.152	1						
Pb leaves	0.940*	0.574	0.986*	0.975*	0.931*	0.500	1					
Cr leaves	0.643	0.966*	0.493	0.544	-0.019	0.985*	0.344	1				
Cd leaves	0.974*	0.667	0.998*	0.994*	0.882	0.599	0.992	0.453	1			
Zn leaves	0.973*	0.666	0.998*	0.994*	0.882	0.599	0.993	0.452	0.981	1		
Cu leaves	0.878	0.444	0.950*	0.930*	0.975*	0.364	0.988	0.199	0.963	0.964	1	
Fe leaves	0.777	0.997*	0.651	0.694	0.172	0.999*	0.518	0.981	0.615	0.615	0.383	1

* P<0.05

Table 4. Pearson correlation coefficient(r) between heavy metal concentrations in soil and *S. auriculata* flowers

	Pb soil	Cr soil	Cd soil	Zn soil	Cu soil	Fe soil	Pb flower	Cr flower	Cd flower	Zn flower	Cu flower	Fe flower
Pb soil	1											
Cr soil	0.818	1										
Cd soil	0.983	0.700	1									
Zn soil	0.992	0.741	0.998	1								
Cu soil	0.753	0.237	0.859	0.828	1							
Fe soil	0.765	0.996	0.635	0.680	0.152	1						
Pb flower	0.950*	0.598	0.991*	0.981*	0.920*	0.526	1					
Cr flower	0.682	0.978*	0.539	0.588	0.033	0.992*	0.421	1				
Cd flower	0.984*	0.705	0.999*	0.998*	0.856	0.641	0.989	0.545	1			
Zn flower	0.981*	0.691	0.999*	0.997*	0.865	0.626	0.992	0.529	0.999	1		
Cu flower	0.861	0.412	0.939*	0.917*	0.982*	0.331	0.976	0.216	0.936	0.943	1	
Fe flower	0.755	0.994*	0.624	0.669	0.137	0.999*	0.513	0.994	0.629	0.614	0.317	1

* $P<0.05$

Correlation between soil and *S. auriculata* for heavy metal concentrations

Correlation analysis was done to see the relationship of the concentrations of Pb, Cr, Cd, Zn, Cu and Fe in the leaves and flowers of *S. auriculata* with their concentrations in soils which shows a significant positive correlation (Tables 3 and 4).

The heavy metal levels in plant leaves correlated significantly ($p<0.05$) with its level in soils showing $r = 0.94$ for Pb, $r = 0.99$ for Cd, $r = 0.97$ for Cr, $r = 0.97$ for Zn, $r = 0.93$ for Cu and $r = 0.98$ for Fe, and in flowers the 'r' values for Pb, Cr, Cd, Zn, Cu and Fe are 0.95, 0.98, 0.99, 0.98, 0.92 and 0.99, respectively.

The correlation analysis reveals that increasing concentrations of heavy metals in the soil causes their increasing concentrations in *S. auriculata* (Fig. 1). Essien and Douglass (2012) found that Pb, Zn, Cu, Ni, Mn, and V in soils all had a significant influence on increasing selected metals (Pb, Zn, Cd) in plants at various coefficients. According to Gregor (2004), metal uptake by both roots and leaves increases as the available metal concentration in the external medium increases. Thus, in the present study, the plants grown in the roadside had a greater concentration of metals than the plants grown in the hills. This is consistent with the findings of Velikovic et al. (2016), who found that the concentration of heavy metals in the soil influences the concentration of heavy metals in vegetable crops produced on contaminated soil. Onder et al. (2007) also reported significant positive relationship between soil and plant heavy metal content. Furthermore, the presence of a positive interaction in the majority of the correlations implies that the absorption of one heavy metal in a plant

impacts the co-absorption of other heavy metals in the same plant which shows a synergistic relationship among them.

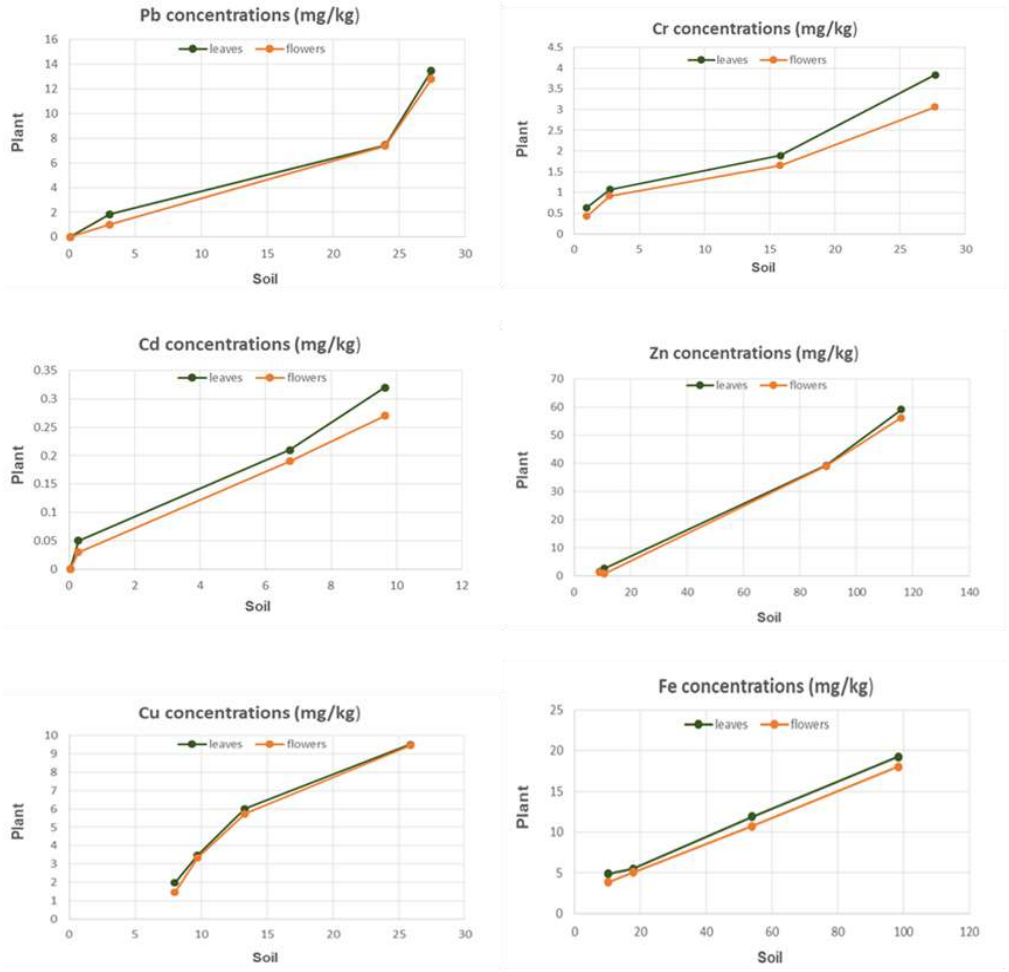


Figure 1. The relationship between soil and plant (*S. auriculata*) for heavy metal concentration

CONCLUSION

Heavy metals (Pb, Cr, Cd, Zn, Cu and Fe) are significantly and positively correlated with soil and plants implying those rising concentrations of heavy metals in soil increased concentrations of *S. auriculata*. Heavy metal concentrations in roadside soils and plants are relatively higher than that in hills.

REFERENCES

Anoliefo, G.O., Ikhajiagbe, B., Okonofhua, B.O. and Diafe, F.V. (2006). Eco-Taxonomic distribution of Plant species around motor mechanic workshops in Asaba and Benin City Nigeria: Identification of oil tolerant plant species. *African Journal of Biotechnology*, 4(19): 1757-1762.

Chen, Z., Ai, Y., Fang, C., Wang, K., Li, W., Liu, S., Li, C., Xiao, J. and Huang, Z. (2014). Distribution and phytoavailability of heavy metal chemical fractions in artifcial soil on rock cut slopes alongside railways. *Journal of Hazardous Materials*, 273: 165-173.

Emamverdian, A. Ding, Y., Mokhberdoran, F. and Xie, Y. (2015). Heavy metal stress and some mechanisms of plant defense response. *Scientific World Journal*, 2015: 1-8.

Essien, O.E. and Douglass E.E. (2012). Heavy metal transfer to vegetables from contaminated farmland adjoining sub urban animal park/market, Uyo. *African Journal of Agricultural Research*. 7(8): 1268-1275.

Gregor, M. (2004). Metal availability, uptake, transport and accumulation in plants. p. 1-27. In Prasad, M.N.V. (ed.), *Heavy metal stress in plants-from biomolecules to ecosystems*. Spinger-verlag, Berlin. Germany.

Jose Acosta, A., Faz, A. and Martinez-Martinez, S. (2009). Identification of heavy metals sources by multivariable analysis in a typical Mediterranean city (SE Spain). *Environmental Monitoring and Assessment*, 169(1-4): 519-530.

Kabata-Pendias, A. and Pendias, H. (2001). Trace Elements in Soils and Plants, 3rd Edition, CRC Press, Boca Raton, Pp. 403

Mehmet, Y.N. (2008). Determination of lead, cadmium and copper in roadside soil and plants in Elazig, Turkey. *Environmental Monitoring and Assessment*, 136(1-3): 401-410.

Nabulo, G., Oryem-Origa, H. and Diamond, M. (2006). Assessment of lead, cadmium, and zinc contamination of roadside soils, surface films, and vegetables in Kampala City, Uganda. *Environmental Research*, 101: 42-52.

Obrador, A., Alvarez, J.M., Lopez-Valdivia L.M., Gonzalez D., Novillo J. and Rico M.I. (2007). Relationships of soil properties with Mn and Zn distribution in acidic soils and their uptake by a barley crop. *Geoderma*, 137(3-4): 432-443.

Onder, S., Dursun, S., Gezgin, S. and Demirbas, A. (2007). Determination of heavy metal pollution in grass and soil of City Centre Green areas (Konya, Turkey). *Polish Journal of Environmental Studies*, 16(1): 145-154.

Saeedi, M., Hossein Zadeh, M., Jamshidi, A. and Pajooheshfar, S.P. (2009). Assessment of heavy metals contamination and leaching characteristics in highway side soils. Iran. *Environmental Monitoring and Assessment*, 151(1-4): 231-41.

Silva, A.L.O., Barrocas, P.R.G., Jacob, S.C. and Moreira, J.C. (2005). Dietary intake and health effects of selected toxic elements. *Brazilian Journal of Plant Physiology*, 17(1): 79-93.

Uddin, A.B.M.H., Khalid, R.S., Mohamed, A., Abdualrahman, Abdualkader, M., Kasmuri, A. and Abbas S.A. (2016). Comparative study of three digestion methods for elemental analysis in traditional medicine products using atomic absorption spectrometry. *Journal of Analytical Science and Technology*, 7(1): 1-7.

Urmila. (2016). Study of heavy metals contamination of soil and its effects on plants. Ph.D. Thesis, Maharshi Dayanand University.

Velickovic, Z., Ivankovic, N., Strikovic, V., Karkalic, R., Jovanovic, D., Bajic, Z. and Bogdanov, J. (2016). Investigation of soil properties influence on the heavy metals sorption by plants and possibilities for prediction of their bioaccumulation by response surface methodology. *Journal of the Serbian Chemical Society*, 81(8): 947-958.

Viard, B., Pihan, F., Promeyrat, S. and Pihan, J.C. (2004). Integrated assessment of heavy metal (Pb, Zn, Cd) highway pollution: Bioaccumulation in soil, Graminaceae and land snails. *Chemosphere*, 55: 1349-1359.

Weckwerth, G. (2001). Verification of traffic emitted aerosol components in the ambient air of Cologne (Germany). *Atmospheric Environment*, 35: 5525-5536.

Weggler, K., McLaughlin, M.J. and Graham, R.D. (2004). Effect of chloride in soil solution on the plant availability of bio solid-borne cadmium. *Journal of Environmental Quality*, 33(2): 496-504.

WHO. (2007). Guidelines for assessing quality of herbal medicines with reference to contaminants and residues. Geneva, Switzerland. Pp. 24-28.

Zehetner, F., Rosenfflner, U., Mentler, A. and Gerzabek, M.H. (2009). Distribution of road salt residues, heavy metals and polycyclic aromatic hydrocarbons across a highway-forest interface. *Water, Air, and Soil Pollution*, 198(1-4):125-132.

ORIGINAL RESEARCH PAPER
Volume - 10 | Issue - 03 | March - 2021 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

PRELIMINARY PHYTOCHEMICAL SCREENING AND *IN VITRO* ANTIMICROBIAL ACTIVITY AGAINST CLINICAL PATHOGEN OF MEDICINALLY IMPORTANT ORCHID *CALANTHE MASUCA* (D.DON) LINDL

Pharmacology

Dr. V. Nandagopalan Associate professor, PG & Research Department of Botany, National College, Tiruchirappalli-1, Tamil Nadu, India.

D. Kavitha* Research scholar, PG & Research Department of Botany, National College, Tiruchirappalli-1, Tamil Nadu, India.*Corresponding Author

ABSTRACT

Calanthe masuca (D.Done) Lindl is a potential medicinal plant of terrestrial *orchids*. Tribal peoples were used the plants for therapeutic purpose. The present study was to evaluate the phytochemical analysis and Antimicrobial Activity of *Calanthe masuca* in leaf extracts. Preliminary phytochemical analysis revealed that the presence of bioactive substance in leaf extract was found that Alkaloids, Flavanoids, Phenol, Tannins, Saponins, Steroids, Terpenoids, Carboxcylic acid, Glycoside and Mucilage. Antimicrobial activity by Agar well diffusion method again six pathogenic microbes such as *Staphylococcus aureus, Bacillus subtilis, E.coli* and *Pseudomonas aeruginosa* in bacteria, *Aspergillus niger* and *Candida albicans* in fungi by using different solvent such as Aqueous, Methanol and Petroleum ether. It was observered that Methanol extract showed highest activity against the pathogen such as *S.aureus* (13.5 mm), *B.subtilis* (12.4 mm), *E.coli* (12.5 mm) and *P.aeruginosa* (13.0 mm) and then followed by the Aqueous extract and low activity showed in petroleum ether extract. The methanol extract showed significant Antifungal activity against *Aspergillus niger* (14.0mm) and *Candida albicans* (13.0 mm) with higher concentration and Petroleum ether extract showed lower activity in fungi. The capacity of the crude concentrates of *Calanthe masuca* to suppress the development of microorganism which means that it has a wide range of antimicrobial potential utilized for various cures.

KEYWORDS

Calanthe masuca, Phytochemical, Methanol extract, Antimicrobial activity.

INTRODUCTION

Orchids are one of the biggest and most assorted gatherings of angiosperms comprising of almost 25,000 – 30,000 species having a place with 750 – 800 genera. They are found in practically all locales around the globe aside from Antarctica, yet their most prominent variety happens in tropical and sub-tropical districts. Orchids are reprobate gathering of blooming plants found in nature and are dispersed in all pieces of the world. The shape, size and shade of orchid blossoms display extraordinary scope of variety. They are significant therapeutically and furthermore fill in as environmental pointers. Orchids are developed for fancy reason, utilized as home grown prescriptions, food and has social incentive by various clans around the world[1]. Orchids are the blossoms of generally captivating and flawless of all qualities blossoms and shows a wide scope of variety in structure, size, shading and surface that make them remarkable among the Ornamental. They are for the most part developed for wonderful blossoms and are broadly known for their financial significance and extremely less for their restorative use[2]. In Phytochemical evaluation plant referred as secondary metabolites derived from Leaf, Stalk, Root, Pseudobulb and Seed. They are consists of Proteins, Carbohydrates, Alkaloids, Sterol, Mucilage, Flavanoids, Terpinoids, Glycosides, Saponins and Tannins. Phytochemical bioactive compound possess antioxidant, antitumor, anti-cancer, anti-inflammatory, antiatheroscleric, anticarcinogenic, antiphrastic, antibacterial, and antiviral activities[3]. Phytochemical screening and antioxidant interest of various components.

In bioactive compounds in plants are produced as secondary metabolites such as alkaloids, tri-terpenoids, flavonoids, stilbenoids, steroids and phenolic compounds, which may be stage specific or organ specific or tissue specific properties. Many orchid plants are traditionally used in herbal medicine as a remedy for microbial infections and other ailments. However, the potential of most of the orchid species for therapeutic use is yet to be scientifically explored[4,5]. Plant materials remain a significant asset to battle genuine infections on the planet. The most significant of these bioactive constituents of plants are Alkaloids, Tannins, Flavonoids and Phenolic mixes[6]. A huge number of *orchids* have been exactly utilized for treatment of various illnesses as diuretic, antirheumatic, mitigating, against cancer-causing, hypoglycaemic, antimicrobial, anticonvulsive, unwinding, neuro-defensive, and antivirus specialists. Plant concentrate of *O. latifolia* indicated action against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa. Candida* is additionally a causative specialist for a scope of mucosal contaminations, for example, oral thrush and vaginitis[7,8].

In the present study *Calanthe masuca* (D.Don) Lindl was taken and identified. The leaf part of the plant was taken in different solvent for extraction and subjected to Preliminary phytochemical compound and Antimicrobial activity.

METHODS AND MATERALS

Collection of the Plant

The fresh plant materials were collected from Kolli Hills in Solakadu area in Namakkal District of Tamil Nadu, India. The plant materials were washed thoroughly washed in two to three times with running water and one time sterile with distilled water. The leaf part only cut in to small pieces were shade dried and coarsely powdered separately and stored in well closed bottle for further analysis on the laboratory.

Plant Authentication

The collected plant specimen was authenticated by Botanical Survey of India, Coimbatore, Tamil Nadu, India. The Voucher number is (BSI/SRC/5/23/2019Tech/252). The Voucher specimen submitted on the Department of Botany, National College (Autonomous), Tiruchirappalli – 620 001, Tamil Nadu, India.

Preparation of plant extracts

The leaf of the plant was washed thoroughly 2-3 time with running water and once sterile with distilled water. Then the plant parts were shade two month dried and coarsely powdered separately and stored in air tight glass bottles for further analysis in laboratory. 50g of are dried powder of leaf sample was extracted with 100 ml of solvents such as Aqueous, Methanol and Petroleum ether with gentle stirring for 72h. In sample of leaf was collect in dark for 72h with intermittent shaking. After incubation the solution was filtered No.1 Whatmann filter paper and the filtrate was collected (crude extracts). It was then transferred to glass vials and kept at 4°C before use them.

Phytochemical screening

Phytochemical examination were accomplished for all the extracts as in line with the usual method followed by[9,10,11].

Qualitative Phytochemical Analysis

1. Test for Alkaloids- Mayer's test: To some ml of plant pattern extract, two drops of Mayer's reagent are brought alongside sides of the test tube. Appearance of white creamy precipitate to suggests the presence of alkaloids.

2. Test for Flavanoids- Lead acetate: To 0.5ml extract, 4 ml of 1% ammonia become brought and to this 1 ml of conc.H_2So_4 turned into brought. The formation of yellow shade suggests the presence of flavonoids.

3. Test for phenol- Ferric Chloride test: To 50 mg of extract, five ml of distilled water turned into brought and a few drops of 5% ferric

chloride answer had been brought. The formation of darkish green color suggests the presence of phenol.

4. Test for Tannins: Few drops of one% lead acetate were delivered to 5 ml of plant extract. Formation of yellow precipitate suggests the presence of tannins.

5. Test for Saponins- Foam test: To 0.5 mg of leaf extract was diluted with 20 ml distilled water and shaken properly in a graduated cylinder for15 min. The formation of foam to a period of 1cm indicated the presence of saponins and steroids.

6. Test for Steroids: To 1ml of extract dissolved in 1ml of acetic anhydride, 1ml of chloroform and 1ml of Conc. HCl separately. Formation of green coloration suggests the presence of steroids.

7. Test for Terpenoids - Salkowski test: To five ml of the extract changed into blended with 2 ml of chloroform and focused sulpheuric acid to form a layer. A reddish brown colour in the interface confirmed the presence of terpenoids.

8. Test for Glycosides- Bom- Trageru's check: To 1 ml of the extract, 1 ml of alpha napthol changed into introduced to which chloroform become delivered alongside the perimeters and it became searched for the improvement of colour and the end result become recorded. Development of violet colour suggests the presence of glycosides.

9. Carboxylic acid: To 1 ml plant extract turned into brought 2 ml of sodium bicarbonate answer. Colour adjustments arise shows the presence of carboxylic acid.

10. Test for Mucilages: A) Alcohol 95% test B) Extract turned into dissolved in distilled water and filtered. The filtrate was dealt with few mL of absolute alcohol with consistent stirring. A function white or cloudy precipitate shows the presence of gums and mucilages.

Microorganism Test

The test organism used included *Staphylococcus aureus* MTCC-902 (*S.aureus*), *Bacillus subtilis* MTCC-441*(B.subtilis), Escherichia.coli* MTCC443 *(E.coli), Pseudomonas aeruginosa*- MTCC-424 *(P.aeruginose), Candida albicans- isolated (C.albicans) and Aspergillus niger- isolated (A.niger).* In this microorganism were purchased from institute Microbial technology Chandihar, India.

Antibacterial Assay

The Antibacterial activity was determined by the Agar well diffusion method using Nutrient Agar Medium (Hi-media) for 24hrs old culture of microorganism maintained in Nutrient Agar Broth (Hi-media) of the organism is used[4]. Wells are cut and different concentration (500µl/ml, 250µl/ml, 100µl/ml, 50µl/ml) of various solvent extract such as Water, Methanol and Petroleum ether leaf extract of plant were taken[12]. Gentamicine antibiotic used as positive control. After inoculation, plates were incubated at 37°C for 24 hrs. Zone of inhibition formed were measured and calculated the mean diameter recorded[13].

Antifungal Activity

The Antifungal activity was determined by the Agar well diffusion method using Potato Dextrose Agar Medium 24hrs old fungal culture of microorganism of maintained in Potato Dextrose Agar medium (Hi-media) of the organism are used. Wells are cut and different concentration (500µl/ml, 250µl/ml, 100µl/ml, 50µl/ml) of various solvent extract such as Water, Methanol and Petroleum ether leaf extract of plant were taken[12,13]. Gentamicine antibiotic used as positive control. After inoculation, plates were incubated at 37°C for 24 hrs. Zone of inhibition formed were measured and calculated the mean diameter recorded[12].

RESULTS AND DISCUSSION

Phytochemical screening analysis

In the present study, qualitative Phytochemical screening of Aqueous, Methanol and Petroleum Ether leaf extracts of *Calanthe masuca*. The methanolic leaf extract showed strong presence of secondary metabolites such as Alkaloids, Flavonoids, Phenol, Tannins, Saponins, Steroids, Terpenoids and Carboxcylic acid (Table:1). The Aqueous leaf extract of showed Flavonoids, Phenol, Saponin and Carboxcylic acid[14]. In Petroleum Ether leaf extract showed the presence of Steroid. Whereas Glycoside and Mucilage both are absent in three solvent. So the leaf parts of *Calanthe masuca* are responsible for medicinal values[15]. The plant extracts revealed the presence of Alkaloid, Flavanoid, Tannin, Saponin and Terpenoid. The presence of Alkaloid, Tannin and Saponin enhanced the antimicrobial activity against the pathogenic microorganisms[17]. The secondary metabolites of various chemical compound present in the plant species are known to possess antimicrobial activities. Flavonoids are discovered to be powerful antimicrobial substances towards a huge variety of microorganisms, in all likelihood due to their potential to complex with extracellular and soluble proteins and to complex with bacterial mobile wall; more lipophilic flavonoids might also disrupt microbial membrane. Phenolics and polyphenols present in the plants are known to be toxic to microorganisms[18,15,16]. After, the presence of antimicrobial activity in higher plants is well established as they provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. The important medicinal plant of source of the development of potential new chemotherapeutic drugs and the *in vitro* antibacterial test form the basis.

Antimicrobial Screening

The antibacterial activity of different solvent of plant extracts (Water, Methanol and Petroleum ether) of *Calanthe masuca* were observed on gram positive and gram negative bacteria[7,18]. In the result of antibacterial measurement of various solvent extracts were maximum (13.5mm) with concentration of 500 µg/ml methanol extract of leaf plants of *Calanthe masuca* and lowest (3.5 mm) with concentration of 250 µg/ml petroleum ether extract. Where the zone of inhibition for each of the sample were compared with standard Antibiotic (Fig: 1). The methanol extract of *Calanthe masuca* shows the highest inhibition against all the pathogenic bacteria **(***S.aureus* 13.5 mm), (*B.subtilis* 12.4 mm), *(E. coli* 12.5 mm) *and* (*P.aeruginosa* 13.0 mm) (Fig-1 and table-2). In Aqueous extract and petroleum ether shows the minimal zone of inhibition in leaf extract.

Anti-fungal activities of the leaf extract (Aqueous, methanol and petroleum ether) showed activity against the tested fungal isolates (*Aspergillus niger* and *Candida albicans*) [19,20]. The susceptibility of these fungi using *Calanthe masuca* leaf extract showed significant results for most of these fungi have recently have been implicated in case of immune-compromised patients who frequently develop opportunistic infection. The methanol extract have highest activity against *Aspergillus niger* with inhibition of zone (14.0 mm, 11.0 mm, 4.4 mm and 3.3 mm) respectively. They are followed by water extract and least was observed petroleum ether extract (Fig; 2) (Table-3). In this distinction is attributed to the solubility of the active component in different solvents. On the other hand, methanol extract of *Calanthe masuca* leaves exhibited higher degree of inhibition against *Staphylococcus aureus* (13.5 mm) and *P.aeruginosa* (13.0 mm) than *Vanda tessellata* leaves extract (9.2 mm and 8.0 mm). In *Eria pseudoclavicaulis* leaf extract (*P.aeruginosa* 11.0 mm), *Bacillus subtilis* (9.0 mm), *E.coli* (s)[4,19,21]. It is an indication of the broad spectrum antimicrobial potential of *Calanthe masuca* which make the plant a candidate for bio prospecting for antibiotic and antifungal drugs.

The present study showed that leaf plant extracts of *Calanthe masuca* in methanol and water provided a highest zone of inhibition when compared to petroleum ether extracts were found to be less active against the tested organisms. The potential of compounds against the standard strains may be explored in order to develop therapeutics for microorganisms. The phytochemical analysis and Antimicrobial activities of *Calanthe masuca* of leaf extract reported for the first time and also terrestrial orchids shows high pharmacological activity such as Antibacterial and Antifungal properties. Whereas further more work are necessary to isolate the bioactive compounds from the plant to carry on pharmaceutical potential use.

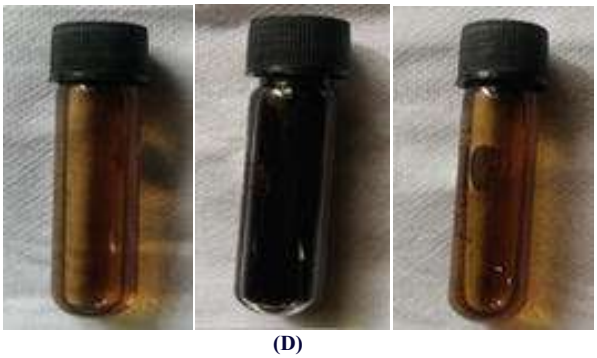
Table 1: Phytochemical constituents present in different solvent leaf extracts of Calanthe masuca

S.NO	Phytoconstituent	Aqueous extract	Methanol extract	Petroleum Ether extract
1.	Alkaloids	-	+	-
2.	Flavonoids	+	+	-
3.	Phenol	+	+	-
4.	Tannins	-	+	-
5.	Saponin	+	+	-
6.	Steroid	-	+	+
7.	Terpenoid	-	+	-
8.	Glycoside	-	-	-
9.	Carboxcylic acid	+	+	-
10.	Mucilage	-	-	-

PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr

(+=Present, - =Absent)

(A) (B) (C)



(D)

Fig1 : Procedure of extract preparation A), fresh leaf plant of *Calanthe masuca*, B) tried plant, C) coarse powdered by pulverizor, D) stock solution of extracts.

Table- 2: Inhibition zone of three different solvent extracts of *Calanthe masuca* against bacteria

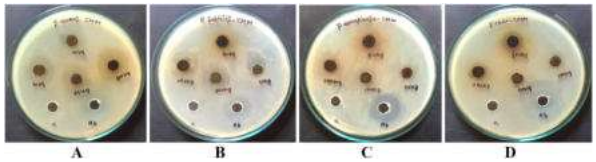
Name of the solvent	Conc. (mg l- 1)	Inhibition zone diameter (mm ± S.E)			
		Bacteria			
		Gram positive		Gram negative	
		S.aureus	*B.subtilis*	*E. coli*	*P.aeruginosa*
Water	500µg/ml	12.5±1.0	11.5±1.0	11.5±1.0	12.5±1.0
	250 µg/ml	7.5±0.5	10.5±0.5	9.5±0.5	10.0±1.0
	100 µg/ml	4.4±0.4	7.3±0.3	5.4±0.4	6.5±0.5
	50 µg/ml	3.3±0.3	3.2±0.2	4.3±0.3	5.4±0.4
	AB	9.0±1.0	9.0±1.0	12.0±1.0	8.0±1.0
Methaol	500µg/ml	13.5±1.5	12.4±1.0	12.5±1.0	13.0±1.0
	250 µg/ml	11±1.0	10.5±0.5	9.5±0.5	11.5±0.5
	100 µg/ml	9.5±0.5	9.4±0.4	5.3±0.3	7.4±0.4
	50 µg/ml	7.4±0.4	8.3±0.3	4.3±0.3	5.3±0.3
	AB	12.0±1.0	10.5±1.0	12.5±1.0	10.5±0.5
Petroleum Ether	500µg/ml	5.4±1.0	7.5±1.0	6.5±1.5	3.5±0.5
	250 µg/ml	3.5±0.5	6.5±0.5	4.5±0.5	0
	100 µg/ml	0	0	0	0
	50 µg/m	0	0	0	0
	AB	9.5±0.5	8.5±1.0	8.0±1.0	9.5±1.0

AB- Passtive control- Gentamicin, SE- Standard error, *S.aureus- Staphylococcus aureus, B.subtilis- Bacillus subtilis, E. coli- Escherichia coli, P.aeruginosa- Pseudomonas aeruginosa.*

Table-3: Inhibition zone of three different solvent extracts of *Calanthe masuca* against fungal

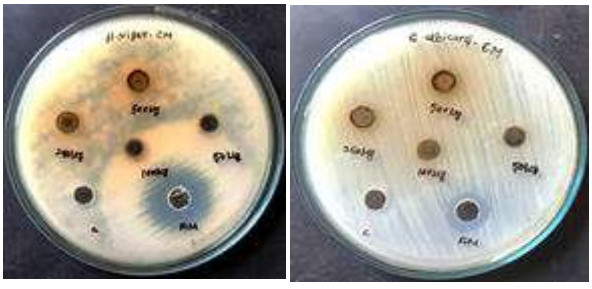
S. NO	Fungal organism	Zone of inhibition diameter in mm			
		Different solvent extract			
		Conc. (mgl- 1)	Water	Methanol	Petroleum ether
1.	*A. niger*	500µg/ml	13.0±1.0	14.0±2.0	10.5±1.0
		250 µg/ml	4.5±0.5	11.0±1.0	4.5±0.5
		100 µg/ml	3.4±0.4	4.4±0.4	0
		50 µg/ml	0	3.3±0.3	0
		AB	11.0±1.0	14.0±1.0	11.5±1.0
2.	*C.albicans*	500µg/ml	3.5±0.5	13.0±1.0	4.5±0.5
		250 µg/ml	0	9.5±0.5	0
		100 µg/ml	0	4.4±0.4	0
		50 µg/ml	0	2.1±0.1	0
		AB	5.0±1.0	5.0±1.0	6.0±1.0

AB- Positive control- Amphotericin, *A. niger- Aspergillus niger, C.albicans- Candida albicans*



A B C D

Fig:1- Antibacterial activity using plant extract of *Calanthe masuca* (A)*S.aureus*, (B) *B.subtilis*, (C)*E. coli*, (D)*P.aeruginosa*



E F

Fig: 2 Antifungal activity using plant extract of *Calanthe masuca* (E)*A. niger*, (F) *C.albicans.*

CONCLUSION

Calanthe masuca leaf is a terrestrial orchid has a therapeutic medicinal value and pharmaceutical potential use in tribal resident study area. The plant leaf part extract is collected for the research work. *Calanthe masuca* leaf extract were better effect against phytochemical screening and Antimicrobial activity. Three different solvent is used to evaluate. In phytochemical screening reveled that presence of secondary metabolites of the Alkaloids, Flavanoid, Phenol, Tannins, Saponin, Steroids and Terpenoid. Zone of inhibition is higher in concentration methanol leaf extract shows *Staphylococcus aureus, Bacillus subtilis, E.coli* and *Pseudomonas aeruginosa* in bacteria*, Aspergillus niger* and *Candida albicans* in fungi. Thus the indication of the broad spectrum antimicrobial potential of *Calanthe masuca* plant extracts the bioprospecting for antibiotic and antifungal drugs. The study showed the potential use of the leaf plant extract for developing new antimicrobial drug against pathogenic microorganism and antibiotic compound serve as novel drug for diseases cause pathogen

REFERENCES

1. Shanmugavalli N., Umashankar V and Raheem. Antimicrobial activity of *Vanilla planifolia*. Indian Journal of Science and Technolgy. 2009. 2(3): 73-40.
2. Katta J., Rampilla V and Khasim S.M. A Study on Phytochemical and Anticancer activities of Epiphytic *Orchid Aerids odorata* Lour. European Journal of Medicinal plants. 2019. 28(3): 1-21.
3. Arora M., Kaur G., Kahlon P.S., Mahayan A and Sembi J.K. Pharmacognostic Evaluation and Antimicrobial Activity of Endangered Ethno medicinal plant *Crepidium acuminatum* (D.Don) Szlach. 2017. 9(6): 56-63.
4. Sahaya Shibu B., Moin S., Servin Wesley P and Chitra Devi B. Preliminary Phytochemical Screening, Antibacterial and Antioxidant activity of *Eria pseudoclavicaulis* Blatt. An endemic orchid of Western Ghats. American Journal of Pharmtech Research. 2012. 2(6): 1-8.
5. Paul P., Chowdhury A., Deepanath and Bhattacharjee M K. Antimicrobial efficacy of *orchid* extracts as potential inhibitors of Antibitic resistant strains of *Escharichia coli*. Asian Journal of Pharmacutical clinical Research. 2013. 6(3): 108-111.
6. Radhika B., Murthy.J.V.V.S.N and Nirmala Grace D. Preliminary Phytochemical analysis and Antimicrobial activity against clinical pathogen of medicinally important Orchid *Cymbidium aloifolium* (L.) SW. 2013. 4(10): 3925-3931.
7. Avasthi AS., Ghosal S and Purkayastha S. Study of Antimicrobial Activity of *Orchids Latifolia*. 2013. 4(4): 638-646.
8. Marjoka A., Alam O and Huda M K. Phytochemical screening of three medicinal important epiphytic *orchids* of Bangladesh. Jahangimagar University. Biological Science. 2016. 5(1): 95-99.
9. Harborne J B. Phytochemical methods: A guide to modern techniques of plant analysis, 3rd edition. Chapman Hall Co., New York, 1998. 1-302.
10. Sofowra A. Medicinal Plants and Traditional Medicine in Africa. Ibadan, Nigeria: Spectrum Books Ltd.; 1993. P: 191-289.
11. Trease GE, Evans WC. Pharmacognosy. XIVth. ed. London: Mac Millian Publishing Ltd.; 1997. p. 568-78.
12. Bakul Bhattacharjee and Shahinul Islam SM. Assessment of Antibacterial and Antifungal activity of the extracts of *Rhynchostylis retusa Blume* A Medicinal Orchid. World Journal of Pharmacy and Phamaceutical Science. 2015. 4(2): 74-87.
13. Soumiya G and William C. Phytochemical and Antimicrobial properties of *Cymbidium aloifolium* (L.)SW.LEAF. Indo-Asian Journal of Multidisciplinary Research. 2017. 3(2): 1103-1109.
14. Ashok kumar *et al*. Preliminary Phytochemical Analysis of leaf and bark (mixture) extract of *Ficus infectoria* plant. The Pharma Innovation. 2011. (1): 5.
15. Sohag Sl., Hoque MM and Huda MK. Phytochemical screening and antioxidant of rare medicinal orchids *Luisia zeylanica* Lindl. Journal of Pharmacognosy and Phytochemistry. 2017. 6(4): 688-692.s
16. Mahendra A., Madhuri D and Theng A. Phytochemical Screening, Quantitative analysis

of Primary and Secondary metabolites of *Acacia arabica* bark. International Journal of Current Pharmaceutical Research. 2018. 10(2): 35-37.
17. Radhika B and Murthy JVVSN. Preliminary Phytochemical Analysis & In Vitro Bioactivity against Clinical Pathogen of medicinal important *orchid* of *Rhynchostylis retusa Blume*. American Journal of Pharmtech Research. 2013. 3(4): 1-11.
18. Shubhu J and Chowdappa S. Phytochemical analysis and Antibacterial activity of *Cymbidium aloifolium* L a medicinal *orchid* from Western Gharts of Karnataka, India. International Journal of Advance Scientific Research and Publication. 2016. 1-6.
19. Bhattacharjee B., Islam T., Rahman Z and Shahinul Islam SM. (2014). Antimicrobial activity and Phytochemical Screening of whole plant extracts of *Vanda Tessellata* (ROXB.) HOOK.EX.G.DON. World Journal of Pharmacy and Pharmaceutical Science. 4(1): 72-83.
20. Okhale SE., Ugbabe GE., Bamidele O., Ajoku GA and Egharevba HO. Phytochemical and antimicrobial studies on extractives of *Calyptrochilum emargintum* (SW) Schltr (*Orchidaceae*) growing in Nigeria. Journal of Medicinal Plants Research. 2014. 8(4): 223-228.
21. Ranjitha MC., Akarsh S., Prashith Kekuda TR., Darshini SM and Vidya P. Antibacterial activity of some plants of Karnataka, India. Journal of Pharmacognosy and Phytochemistry. 2016. 5(4): 95-99.

ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/360599905

International Journal of Botany Studies www.botanyjournals.com Phytochemical investigation antioxidant activities of Vanda spathulata (L.) spreng. and identification of bioactive c…

Article · May 2022

CITATIONS
0

READS
29

2 authors, including:

Jeline Rani
Bishop Heber College
4 PUBLICATIONS **0** CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:

Mosquito repellent preparation from animal waste View project

All content following this page was uploaded by Jeline Rani on 14 May 2022.

The user has requested enhancement of the downloaded file.

International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 03-11-2021, Accepted: 19-11-2021, Published: 04-12-2021
Volume 6, Issue 6, 2021, Page No. 728-740

Phytochemical investigation antioxidant activities of *Vanda spathulata* (L.) spreng. and identification of bioactive compounds using GC-MS

Jeline Rani J[1], Nandagopalan V[2], Azhagiyamanavalan Lakshmi Prabha[3]
[1] Assistant Professor, Department of Botany, Bishop Heber College (Autonomous), Trichy, Tamil Nadu, India
[2] Associate Professor, Department of Botany, National College (Autonomous), Trichy, Tamil Nadu, India
[3] Associate Professor, Department of Botany, School of Life Science, Bharathidasan University, Trichy, Tamil Nadu, India

Abstract
Vanda spathulata is known to be collected from the wild and used in horticulture and Ayurvedic medicine. *V. spathulata* are thought to have healing properties in treating asthma, depression and some memory enhancing antioxidant and anticancer property. To date, reports on phytochemical analyses were most available on endangered and medicinal orchids. The present study was conducted to determine the total phenolics, total flavonoids, and antioxidant activity of five different extracts prepared from leaves, stem and roots. Among the three plant parts Methanolic Leaf extract contained the highest total phenolics content with a value of 1.52 and total phenols 0.32 mg g-1 which is proved because of bioactive compounds identified by GC-MS. The compound from methanolic extract of leaf has 58 compounds are *5 Hydroxy methyl furfural* n-Hexadecanoic acid 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl 3'5' Dimethoxyacetophenone Dichloroacetic acid, tridec-2-ynyl ester Hexadecanoic acid, 2-hydroxy-1-(hydroxy methyl) ethyl ester, 12-Octadecadienoic acid (Z,Z) which has the antioxidant property, root methanolic extract the compound l-(+)-Ascorbic acid 2,6-dihexadecanoate used for synthesis of cancer compound. The aqueous extract of leaf, root and stem has 15 compounds were identified. There are four unknown which is not yet identified the compounds are aqueous root 1,1-Dibromo-2-Hexylcyclopropanev. 3-Cyclopentylpropionic acid from Stem aqueous extract, 3-chloroprop-2-enyl ester, 4-Bromo-4-Methyl-5-Oxo-Tetrahydro-Furan-2-Carboxylic Acid. Z,Z-3,13-Octadecedien-1-ol in aqueous leaf. A wide range of fatty acids, heterocyclic compound which are having anti-fungal, anti-inflammatory, antibiotic activity, skin conditioning property were identified so that it can be recommended as a plant of phytopharmaceutical importance.

Keywords: phytochemical analysis, GC-MS analysis, *V. spathulata*, antioxidant activity

Introduction

Orchidaceae is the largest, including most multifariousness family of flowering plants, consisting of more than 25,000 species belonging to 700 - 800 genera (Begum, 2000) [4]. It included both terrestrial and epiphytic orchids. Vanda, genus of about 50 species of colourful orchids (family Orchidaceae) distributed from East Asia to Australia. Many attractive hybrids have been developed by crossing species within the genus and also by crossing *Vanda* species with those of other orchid genera. Most species are epiphytic and have long sturdy stems that bear closely spaced straplike leaves. Some species have cylindrical leaves in a form known as terete. *Vanda* flowers usually are flat and have a short spur on the lip; the flowers are long-lasting and often fragrant. The plants grow well in warm temperatures with high humidity and require a well-draining potting medium. Due to their ornamental and medicinal importance they demand a very high price in the international market. In India, orchids from 10% of the world orchid flora with Himalayas as their natural home and the largest and commercially important flowering plants that over 22,500 species with 779 genera are distributed throughout the world. There are 1331 species belonging to 186 genera widely distributed throughout the country.

Different kinds of orchids are indigenous to Bangladesh; those are *Rhyncostylis* sp., *Pierardi* sp., *Arides* sp., *Dendrobium* sp., *Cymbidium* sp., *Arnada* sp., *Arathera* sp., *Bokthara* sp., *Eridis* sp., *Miltonia* sp., *Hoya* sp., *Vanda* sp. These orchids are found naturally growing or anchoring on the mango tree, wood apple tree, tamarind tree, rain tree, sissoo etc. (Kabir, 2012) [20]. In the world, the genus Dendrobium having more than 1100 species. They are widely distributed and cultivated in the world ranging from southern Asia to New Guinea and Australia (Puchooa, 2004) [4]. It is the most popular orchids all over the world, also in Bangladesh. Different characteristics of Dendrobium such as rapid growth, easiness of plantlet regeneration, beauty of the flower, and year round production in control flowering and long lasting of the flower stalk are very advantageous of this genus (Talukder *et al.*, 2003) [46].

Vanda spathulata are known for their golden yellow flowers. They have a wide endemic distribution in southern India and Sri Lanka, in dry to intermediate zones, with its distribution ranging from partially arid habitat at sea level to dry deciduous forests and scrub jungle at high altitudes over 1,000 meters. *V. spathulata* flower between September and January.*Vanda spathulata* (Spoon-Leaf Vanda) is an orchid found growing on trees in South India. It prefers to grow on small trees and bushes in open sun. Leaves occur in 2-ranks, up to 6 x 1.5 cm, oblong or spoon-shaped, 2-lobed at tip, leathery, base sheathing, stalkless. Flowers are borne in leaf opposed racemes with 6-8 flowers. Flowers are yellow, 4 cm across. Sepals are 2.5 cm, obovate, narrowed to the base.

Petals are more or less similar to the sepals. Lip is 3-lobed, spur up to 0.5 cm. Column is up to 1 cm. Spoon-Leaf Vanda is found in South India and Sri Lanka. In India It is found in Karnataka, Kerala and Tamil Nadu. Flowering: September-January. It is also used in folk and Ayurvedic medicine, dried flowers are powdered and the juice is used for a variety of illnesses such as asthma and depression. It is thought it may have some memory enhancing properties and its antioxidant activity has been investigated.

The major threats to *V. spathulata* are severe habitat destruction and over collection. It has caused significant population declines in some areas and even completes loss so that it is now restricted to small pockets. Specifically in Sri Lanka, the threatened status is largely due to increasing habitat loss as urbanization and development projects such as roads and irrigation in the drier zones *V. spathulata* grows in. Antioxidants play an important role in health care to prevent and scavenge free radicals; alleviate chronic diseases and degenerative ailments such as cancer, autoimmune disorders, hypertension, atherosclerosis; and delay the ageing process (Indrianingsih *et al* 2015). Knowledge of the phytochemical present and their localisation with appropriate reagents within a plant cell may help to explain or to predict a variety of herbal preparations. The goal in surveying plants for biologically active compounds should be to isolate the constituents responsible for a particular activity. Since the metabolomics of plants is rapidly developing, it will be a key technology in the system biology approach in studies of the interaction of the plant and its environment and also for studies of the activity of medicinal plants. Therefore, the aim of this study is to characterize the volatile compounds derived from the leaves, stem and root of *V. spathulata.*

Materials and Methods

Collection of material

The plant material was collected from the fully matured *V. spathulata* from Eastern Ghats of Kolli Hills in Namakkal District of Tamil Nadu, India during the period of January 2018 and authenticated in BSI, Coimbatore. The plant voucher number: BSI/SRC/5/23/2018/Tech./2180

Processing of plant material

The collected plant material of leaves, root and stem were washed with tap water followed by distilled water. Shade dried plant material were powdered (100-500µM) with mechanical blender. Five different solvents were taken for the analysis. The following solvents like Petroleum ether, Chloroform, Ethyl Acetate, Methanol and Aqueous. About 50g of plant powder was taken for the extraction using a soxhlet apparatus with 300ml of solvents. The process was run for 48 hrs after which the sample was concentrated using rotatory evaporator to powder form. The dried extracts were kept in a sterile bottle.

Preliminary and Quantitative phytochemical investigation

The Secondary metabolites such as alkaloids, Anthraquinones, triterpenes, saponins, phenols, and Flavonoids were screened according to the methods described by Harbone (1973). Determination of total phenolic extract (TPC), Total flavonoids content (TFC), saponins and alkaloids were estimated following the methods of Adedapo *et al.,* (2009) [2], Chang *et al*., (2002), Obadoni and Ochuko (2001) [33] and Okwu and Josiah (2006) respectively.

Antioxidant activity

DPPH radical scavenging activity

The antioxidant activity of the extract was evaluated by measuring the ability of the extracts to scavenge the free radical DPPH (Ollanketo *et al.,* 2002) [35]. For the purpose of comparing the oxidant activity in various extracts, the concentration of sample producing a 50% reduction of the radical absorbance (IC_{50}) was used as an index. To find this value the extract was diluted in series with ethanol and 2 mL of each added extract was added to 2 mL of 110 µM DPPH solutions. The solution was mixed using a vortex and the mixture was then incubated for 20 h in darkness at room temperature, after which the absorbance was measured at the wavelength of 517 nm using ethanol as a reference. The IC_{50} values were then found from a plot of percent inhibition (PI) versus the concentration. The values of PI were calculated using the following equation: PI (%) = *(At/*Ar) x 100, where At and Ar are absorbance of the test sample and the DPPH reference respectively.

Determination of inhibition of lipid peroxidation

Reaction mixture (0.5 mL) containing plant extract (0.1 mL, 25% w/v) in Tris-HCI buffer (40mM, pH 7.0), KCI (30 mM), ferrous ion (0.16 mM), and ascorbic acid (0.06 mM) were incubated for 1h at 37°C in the presence (2.5 to 25 µg mL^{-1}) and absence of the fruit extracts. The lipid peroxide formed was measured by TSARS formation (Ohkawa *et al.,* 1979) [34]. Incubation mixtures (0.4mL) were treated with sodium dodecyl sulfate (SDS; 8.1%, 0.2 mL), thiobarbituric acid (TBA; 0.8%, 1.5 mL), and acetic acid (20%, 1.5 mL, pH 3.5). The total volume was then made up to 4 mL with distilled water and kept in a water bath at 100 °C for 1h. After cooling, 1 mL of distilled water and 5 mL of a mixture of n-BuOH and pyridine (5:1) were added and vortexed. After centrifugation, the absorbance of the organic layer was measured at 532 nm. The PI of lipid peroxidation was determined by comparing the results of the test compound with those of the control not treated with the extract.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and test compound for hydroxyl radicals generated from the Fe^{3+}/ascorbate/EDTA/H_2O_2 system. The hydroxyl radical attacks deoxyribose, which results in thiobarbituric acid reacting substance (TBARS) formation (Elizabeth and Rao, 1990). The reaction mixture contained 2.8 mM deoxyribose, 0.1 mM $FeCl_3$ 0.1 mM EDTA, 1 mM H_2O_2, 0.1 mM ascorbic acid, 20 mM KH_2PO_4-KOH buffer (pH 7.4), and various concentrations of the extract (2.5 to 25 mg mL^{-1}) in a final volume of 1 mL. The reaction mixture was incubated for 1 h at 37 °C. Deoxyribose degradation was measured as TBARS and PI was calculated (Ohkawa *et al.,* 1979) [34].

Superoxide radical scavenging

The SOD activity was measured by the reduction of NBT according to a method reported by Sreejayan and Rao (1996). The non-enzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS/NADH) system generates superoxide radicals, which reduce nitro blue tetrazolium (NBT) to purple formazan. The 1 mL reaction

mixture contained phosphate buffer (20 mM, pH 7.4), NADH (73 µM*)*, NBT (50 µM*)*, PMS (15 µM) and various concentrations (2.5 to 25 µg mL^{-1}) of sample solution. After incubation for 5 min at ambient temperature, the absorbance at 562 nm was measured against an appropriate blank to determine the quantity of formazan generated.

Preparation of extracts

The collected *V. spathulata* leaves, nodal region and aerial roots were washed with distilled water carefully to remove the traces of impurities. All the above said plant parts were shade dried and pulverized separately by using mechanical grinder. The grinded powder of each were then subjected to successive extraction with organic solvents such as, Petroleum ether, Chloroform, Ethyl Acetate, Methanol and water by using Soxhlet apparatus. The extracts were then collected and distilled off on a hot plate and the last trace of the solvents was removed in vacuo and stored at 40° C. The residue of the Ethyl Acetate, Methanol and Aqueous extracts were performed using a gas-chromatograph-mass spectrograph (GC-MS).

Gas chromatography mass spectrometry analysis

GC-MS analysis was performed with GC Clarus 500 Perkin Elmer equipment. Compounds were separated on Elite-1capillary column (100% Dimethyl polysiloxane). Oven temperature was programmed as follows: isothermal temperature at 100° C for 1min, then increased to 22 0°C at the rate of 50° /min held for 9 min. Ionization of the sample components was performed in the EI mode (70eV). The carrier gas was helium (1ml/min) and the sample injected was 2µl. The detector was Mass detector turbo mass gold-Perk in Elmer. The total running time for GC was 28 min and software used was Turbo mass 5.2. The individual constituents were identified by comparing their mass spectra with the spectra of known compounds stored in the spectral data base, NIST and WILEY attached to the GC-MS instrument and reported.

Results and Discussion

Qualitative estimations of their bioactive constituents of *V. spathulata* leaves, root and stem extracts were summarized in the table-1. Qualitative estimation detected and confirmed the presence or absence of phytochemicals. The extracts showed the presence of flavonoids, steroids, phenol, saponins, alkaloids, tannin, Catechin and anthraquinone. In methanol extract of leaves had substantial amount of flavonoids, phenolic, and, saponin compounds 1.52, 0.32, 1.10 mg g-1, as well as steroids 0.52 were observed (Table 2). Similarly, the petroleum ether, chloroform, Ethyl acetate of leaf, stem and root, catechins and tannin were also detected respectively. Quantitative estimation (table-1a) revealed that the amount of secondary metabolites varied mg g^{-1} between the solvent system and that may be due to solubility and polarity of the solvents used for extraction and substances used for the plant extracts. Higher amount of TFC (18.8%), TPC (33.3%), saponins (5.8 %) and alkaloids (30.2 %) were registered by the methanolic and aqueous extract of all three parts (fig:1). Triterpenoids have been reported as having anti-hypertensive, hypocholesterolemic, hepatoprotective, and antihistamine effects, along with antitumor and antiangiogenic activity (Cor *et al*., 2018) [10]. The anticarcinogenic and antimutagenic potentials of tannins may be related to their antioxidative property, which is important in protecting against cellular oxidative damage, including lipid peroxidation. The generation of superoxide radicals was reported to be inhibited by tannins and related compounds (Chung *et al*., 1998) [8]. Phenols are one of the main natural compounds that already have been reported as compounds in orchids, in different tissues such as leaves, roots or stems (Minh *et al*., 2017) [28].

The DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to decolourise in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for visible deep purple colour. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolourised which can be quantitatively measured from the changes in absorbance. The scavenging activity more than 50% was observed with all the tested crude extracts at varied test substance's concentrations, The DPPH scavenging activity of the aqueous and methanolic extracts of leaf Vanda depicted in figure-2 respectively. It was observed that leaf followed by stem and roots methanolic extract having higher scavenging activity that of aqueous leaf extract (table 3). It was found to be that at the concentration of lC_{50} 6.52 µg mL^{-1} the methanolic leaf extract possesses the highly significantly (P>0.001; table3) DPPH scavenging activity leaf 8.42 µg mL^{-1} (84%) and stem IC_{50} 11.38 µg mL^{-1} (72.3%). However, root methanolic extracts registered lesser scavenging activity IC_{50} 16.57 µg mL^{-1}, but its exhibited DPPH activity between 25.3 to 57.0%.

The effect of antioxidants on DPPH by the leaf extract was due to their hydrogen donating ability and the leaf extract could contain greater amounts of phenolic compounds which lead to more potent radical scavenging effect than other parts tested. The extracts were effective in scavenging free radicals in a dose dependent manner. Among orchids, *Leucas zeylanica* exhibited stronger scavenging efficacy while *Prosopis pallida* displayed low scavenging potential as indicated by IC_{50} values. The lower IC_{50} value indicate better scavenging activity. At 100µg/ml concentration, a scavenging activity of 50% and higher was observed in case of extract of *L. zeylanica, D. nutantiflorum* and *C. breviscapa*. Reference antioxidant i.e., ascorbic acid scavenged DPPH radicals more efficiently than extracts. Though the scavenging efficacies of extracts were lesser than that of ascorbic acid, it is evident that the extracts showed hydrogen donating ability and therefore the extracts could serve as free radical scavengers, acting possibly as primary antioxidants (Chung *et al*., 2006) [9]. Orchids are shown to scavenge DPPH free radicals. Extracts from *Dendrobium aqueum* (Mukherjee *et al*., 2012) [29], *Coelogyne nervosa* (Shibu *et al.,* 2013) [42] and *Pholidota pallida* (Nagananda *et al*., 2014) [30] have shown to scavenge DPPH free radicals.

The ability of the different extracts of *V. spathulata* to inhibit lipid peroxidation was determined according to the method as described by Liu *et al.* 2000 [24]. Aqueous and methanolic extracts of leaf, stem and root showed highly significant inhibition (table-3) of the lipid peroxidation generated by Fe^{2+}/ascorbate in TBARS. The percentage of lipid peroxidation activity of different concentrations of the plant extracts is summarised. Superior free radical scavenging activity was observed in methanolic extracts of root (87.3%) at 25 µg mL^{-1} followed by stem (73%) at 10 µg mL^{-1}. There was a concentration dependent lipid

peroxidation scavenging activity. An assessment of the oxidative stress biomarkers [2-thiobarbituric acid reactive substances (TBARS), carbonyl derivatives content of protein oxidative modification, total antioxidant capacity in the plasma and equine erythrocytes after treatment with *Dendrobium parishii* Rchb. flower extract was done (Buyun *et al.*, 2019) [5]. Lipid peroxidation can generate a high amount of ROS, which, in turn, can be involved in cardiovascular diseases, diabetes mellitus, carcinogenesis, etc. (Ngoc *et al*.,2012) [32]. Therefore, the use of plants with high phenolic content and anti-lipid peroxidation activity might be beneficial for the prevention of many disorders.

Degradation of deoxyribose by hydroxyl radicals generated by Fe^{3+}/ascorbate/ EDTA / H_2O_2 system was found to be initiated by the extracts of leaf, stem and root (table-4). Both aqueous and Methanolic extracts recorded highly significant. Methanolic leaf extract (92.0%) registered higher hydroxyl radical scavenging activity when compared to stem (86.0%), root (77.0%), however, the highest hydroxyl scavenging activity with the lowest IC_{50} value 2.83 µg mL^{-1} was found in root extract. The results indicates that, the leaf methanolic extracts are good sources of electron donors, which may accelerate the degradation of deoxyribose by hydroxyl radicals generated by Fe^{3+}/ascorbate/ EDTA/H_2O_2 system, thus the highest hydroxyl scavenging activity. Moreover, orchids are well known for having bioactivities and potential sources of antioxidants (Stajner *et al*., 2009) [45]. A concentration dependent inhibition of superoxide radicals generated by riboflavin photoreduction method was noticed (table- 5). Leaf methanolic extract (97%) extract having higher superoxide scavenging activity. The IC_{50} value of the leaf extract was 11.99 µg ml^{-1} stem IC_{50} 0.96 µg ml^{-1}. Plant extracts and essential oils have been used for many thousands of years, in pharmaceuticals, alternative medicine and natural therapies and food preservation. The phenolic compounds present in the extract may contribute directly to antioxidative action (Duh *et al* 1999) [11]. These results indicate that polyphenols present in extract could be responsible for the beneficial effects. For the measurement of reductive ability, we investigated the Fe^{3+} to Fe^{2+} transformation in the presence of methanolic extract. The reducing power of the methanolic extract of *Holarrhena antidysenterica* increases with the increased concentration of the methanolic extract. Compelling evidences indicate that methanolic extract of *H. antidysenterica* stem bark contain high concentration of phenolic content. Previously reported literature indicated the significance of phenolic compounds in preventing the ROS effect [Veliogu *et al* 1998; Wang *et al.*, 1999 [49]; Gil *et al* 2000 [15]; Roginsky and Lissi 2000]

UV-FTIR analysis

Characterization of secondary metabolite fingerprint by chromatography and spectroscopy provide valuable information about qualitative and quantitative formulation of plant species and their pattern of recognition by chemometry. Spectroscopic (UV-VIS, FTIR) methods together or separate can be used in this sense as well as conventional methods (Aysal *et al*., 2007 and Ibrahim *et al*., 2008) [3, 19]. Fourier Transform Infrared Spectroscopy (FT-IR) is a high-resolution analytical technique to identify the chemical constituents and elucidate the structural compounds (Hashimoto and Kameoka 2008 and Hussain *et al*., 2007) [17, 18]. FT-IR offers a rapid and non- destructive investigation to fingerprint plant extracts or powders. In addition, UV-VIS and FT-IR spectroscopy is proved to be a reliable and sensitive method for detection of biomolecular composition (Komal Kumar and Devi Prasad 2011) [22]. Therefore, in the present study UV.VIS and FT-IR techniques are employed to evaluate the UV visible and IR finger print in methanolic and aqueous solvent extract of *Vanda spathulata*. Methanolic leaf extract showed the peak value ranges from 203.25, 206.95, 219.30, 221.75, 271.20, 340.55 and 586.35 nm with absorbance 0.6731, 0.2767, 0.1676, 0.1630, 0.0790, 0.0569 and 0.0083 (Table 6, 7). Aqueous extract showed the peak value ranges from 202.00, 205.75, 214.35, 219.30, 221.80, 266.20 and 587.60 nm with absorbance 0.1728, 0.0151, 0.0153, 0.0262, 0.224, 0.0080 and 0.0078 respectively (fig 3,4).

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of Infrared radiation. The results of FTIR analysis of leaf extracts of various solvents confirmed the presence of various functional groups. The FTIR analysis of Methanolic leaf extract revealed the presence of Alkyl halide, Ether, Aromatic, Alkyne, Acid, Amide and Alcohol which showed major peaks at 657.15, 1016.29, 1030.15, 1040.70, 1054.03, 1112.14, 1412.57, 2120.40, 2525.21, 2841.15, 2865.79, 2947.76, 2967.26, 3403.47 and 3696.45 respectively. The results of FTIR analysis of aqueous leaf extract revealed the presence of Alkyl halide, (Table 7, 8). Alkene and Amine which showed major peaks at 620.68, 1032.70, 1054.95, 1108.69, 1198.03, 1270.81, 1384.59, 1632.92 and 3444.41 respectively (fig 5, 6).

The FTIR analysis revealed the presence of alkaloids due to N-H stretching, polyphenols and flavonoids due to O-H stretching, terpenes due to C-H group (Maobe and Nyarango 2013) [26]. The functional groups present in test plant are aldehydes, alkenes, amines, amides, alcohols, phenols, aromatics, carboxylic acids and anhydride, esters and lactones, ethers, quinines and organic halogen compounds. All these compounds belong to secondary plant metabolites as per researcher explanations (Skoog *et al*., 2007; Paulraj *et al*., 2011) [44, 36]. These were confirmed by FT-IR spectrophotometer study that predicted the presence of the groups: O-H, N-H, C-H, C-Cl, C=C, nitrates and silicates stretching. The presence of characteristic functional groups of carboxylic acids, anhydrides, alcohols, phenols, amines, amides, esters, ethers, sulphur derivatives, glycosides, nitrates, nitriles, isonitriles, organic halogens and carbohydrate could be responsible for the various medicinal properties of *Micrococca mercurialis* (Maobe and Nyarango 2013) [26].

Gas chromatography

GC-MS analysis was carried out in crude extracts of the Vanda *spathulata* leaves, using the solvents aqueous and methanol extract. The result showing the GC-MS profile of the compounds identified as eighty-three compounds which is proved that the therapeutic property of plant table more than given in the table with their molecular structure also given. The chromatograms of respective compounds were compared with the mass spectra of the NIST library and their therapeutic action and interaction were discussed hereunder respectively. The peaks in the chromatogram were integrated and were compared with the database of spectrum of known components stored in the GC-MS

library. These active principles with their retention time (RT), molecular formula, Molecular weight (MW) and relative percentage (peak area %) are presented in detailed tabulations given in Table 10. Phytochemical analysis by GCMS analysis of the leaf, stem and root for both aqueous and methanolic extract revealed the presence phytochemical compounds. The highest peak value ranges from leaf methanolic extract 14.52% 5-hydroxy methyl furfural, 7.96% n-Hexadecanoic acid, 7.88%-4 H-Pyran-4-one, 2, 3-dihydro-3,5-dihydroxy-6-methyl respectively. Stem extract 6.96% salicin, 3.27% which is used for synthesis of anticancer compounds, methanolic root extract has 13.16% 4-((1E)-3-Hydroxy-1-prophenyl)-2-methoxyphenol etc.

Some of the constituents revealed by GC–MS are biologically active compounds. Nonadecene leads to antimicrobial properties. Tetracosane showed cytotoxic activity against cancer cell lines (Uddin *et al.,* 2012) [47]. Tetradecanol possesses antibacterial, antidiabetic, and antitumor activities (Xu *et al.,* 2013) [50]. Tetradecanoic acid and hexadecanoic acid have antioxidant and antimicrobial activities (Abubakar, *et al.,* 2016) [1]. Many phenol derivatives present in the extracts were also proven to have antioxidant and anticancer activities (Lam *et al*., 2015) [23]. 5-hydroxymethylfurfural has a role as a plant metabolite and a bacterial metabolite. An organic compound known as 5-hydroxymethylfurfural (HMF) is formed from reducing sugars in honey and various processed foods and Major metabolite excreted in urine, good wine storage time-temperature marker, treatment of Sickle cell disease. Anti-oxidative, anti-allergic, anti-inflammatory, anti-hypo toxic, anti-sickling and anti-hyper uricemic effects (shapla *et al.,* 2018) [41].

HMF is a six-carbon heterocyclic organic compound containing both aldehyde and alcohol (hydroxymethyl) functional groups. The ring of the structure is centred on furan moieties, whereas the two functional groups, i.e., formyl and hydroxy-methyl groups, are linked at the second and fifth positions, respectively. HMF is a solid, yellow substance that has a low melting point but is highly soluble in water (Roychoudhury *et al.,* 1976) [40]. Octadecadienoic acid is the compound which is used for the treatment or prevention of cardiac arrhythmias. Anti-arrhythmias drugs may affect the polarisation-repolarisation phases of the action potential, its excitability or refractoriness, or impulse conduction or membrane responsiveness within cardiac fibres. Tetradecane has the *in vitro* activity against *Plasmodium falciparum*, *Trypanasoma brucei* (Howarth, 2003).

Conclusion

Aqueous and methanolic extracts of Vanda plant parts *viz*., leaf, stem, root and were assessed for their free radical scavenging activities against DPPH radical, inhibition of lipid peroxidation, hydroxyl radical scavenging activity, and superoxide radical. Orchids are known to contain a host of phytochemicals, including alkaloids, triterpenoids and phenolic compounds (Gutierrez 2010) [16]. Phytochemical analysis of *Vanda tessellata* revealed several phytocompounds such as flavonoids, tannins, saponins, terpenoids, steroids and alkaloids (Ghani *et al.,* 1994 and Sirisha *et al*., 2013) [14, 43].

Antioxidant activity of the extracts was tested at a concentration ranging between 2.5 and 25 µg mL^{-1} against free radicals. IC_{50} value represents the concentration of test extract where the inhibition of test activity reached 50% and mean performed on these antioxidant results were compared with the activity between the different plant parts. The overall results showed that, leaf extract registered superior antioxidant activity level followed by leaf>stem>root methanolic extract superior over the aqueous extract in scavenging free radicals.

One of the key factors influencing the extraction efficiency of bioactive compounds from plant extracts is an extraction solvent. Besides, the concentration of the crude drug, temperature, plant parts used for the extraction of secondary metabolites and rate of diffusion are the other factors that influence the efficacy of the extract. Orchids are known to contain a host of phytochemicals, including alkaloids, triterpenoids and phenolic compounds. Gas chromatography-mass spectrometry (GC-MS), analyses were done to find out the active principles in root Ghana *et al.,* 1994 and Chowdry *et al.,* 2014.

Gas chromatography-mass spectrometry (GC-MS), analyses were done to find out the active principles in root extracts of *Vanda tessellata* in two different solvents. GC-MS analyses revealed a battery of different compounds, many of which are with multi-therapeutic properties was coincide with (*Saxena et al.,* 2013 and Thanh Van Ngo *et al.,* 2017) [25]. Leaf and stem extracts of *V. spathulata* in two different solvents. GC-MS analyses revealed a battery of different compounds, many of which are with multi-therapeutic properties. Both methanolic and aqueous extract a total of 83 analytes are revealed. The data indicate that the methanol extracts performed better and are a more promising therapeutic agent. The extracts can be used in various pharmaceutical applications. Further this plant extract can be used further studies like anticancer activity.

Table 1: Preliminary phytochemical analysis of *Vanda spathulata extracts*

Phytochemicaltests	Petroleum ether			Chloroform			Ethyl acetate			Methanol			Aqueous		
	L	R	S	L	R	S	L	R	S	L	R	S	L	R	S
Steroids	+	+	+	+	+	+	+	-	+	++	+	+	+	+	+
Triterpenoids	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Reducing Sugar	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sugar	++	++	++	++	++	+	++	-	++	+	+	+	+	-	-
Alkaloids	-	-	+	-	-	+	-	-	-	+++	++	++	++	++	++
Phenols	-	-	-	-	-	-	-	-	-	+++	+++	++	++	++	++
Catechins	+	+	-	+	+	+	-	-	+	+	+	+	+	+	+
Flavonoids	-	-	-	-	-	-	-	-	-	+++	++	++	++	++	++
Saponins	+	+	+	+	+	+	-	-	+	++	+	+	++	+	+
Tannins	-	-	+	-	-	++	++	++	+++	-	-	-	-	-	-
Anthraquinones	-	-	-	-	-	+	+	-	-	++	+	+	+	+	+
Aminoacids	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

L=Leaves, R=Root, S-Stem; (+++) Intense, (++) Medium, (+)Less, (-)Absent

Table 2: Quantitative estimation of phytochemicals in *V. spathulata* leaf Methanol and aqueous extracts

S. no	Quantitative estimation of leaf extract *V. spathulata*		
	Secondary metabolites	**Methanol**	**Aqueous**
1	Total flavonoids	1.52±0.19	0.28±0.18
2	Total phenols	0.32±0.09	0.24±0.05
3	Saponins	1.10±0.17	1.04±0.19
4	Alkaloids	0.21±0.01	0.16±0.07
5	steroids	0.52±0.01	0.46±0.01
6	tannins	0.38±0.01	0.31±0.01
7	Catechins	0.28±0.01	0.25±0.01

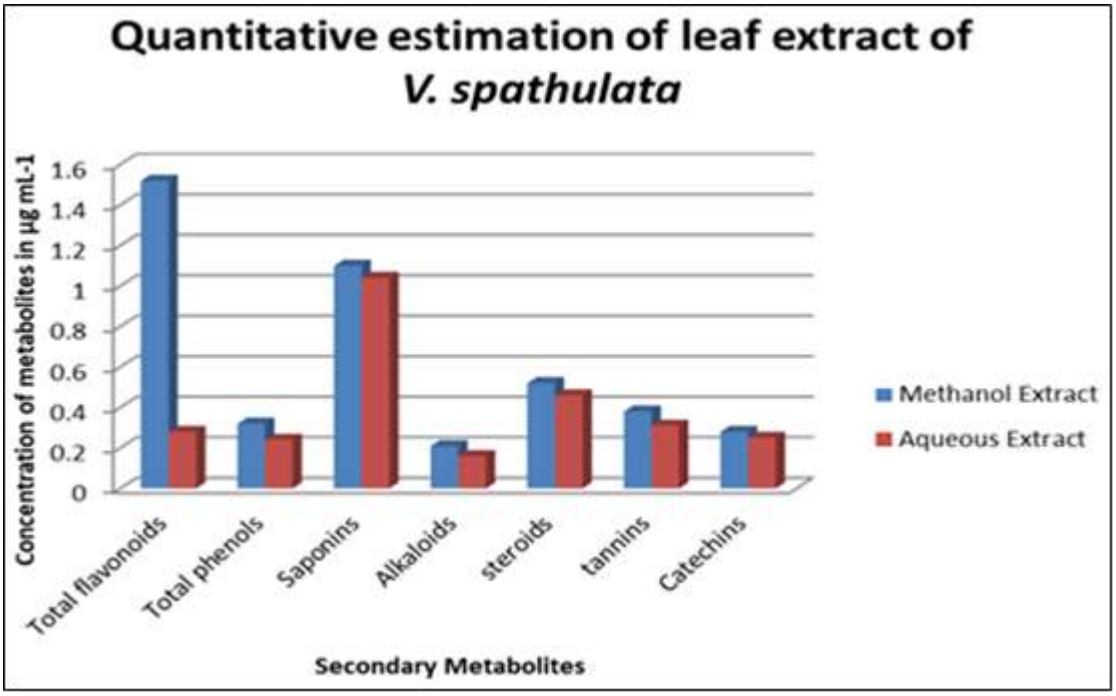


Fig 1: Quantitative estimation of phytochemicals in *V. spathulata* leaf Methanol and aqueous extracts

Table 3: DPPH radical scavenging activity of *V. spathulata* extracts.

Extract conc. µg mL^{-1}	DPPH Radical Scavenging activity*					
	Aqueous Extract			Methanol Extract		
	R	**S**	**L**	**R**	**S**	**L**
2.5	8.0±1.0	12.3±0.6	23.3±0.3	25.33±0.3	18.00±0.6	22.33±0.3
5.0	13.7±0.7	20.3±0.9	43.0±1.2	30.33±0.3	23.67±0.7	53.00±0.6
7.5	37.3±0.7	23.7±0.3	54.7±0.6	33.67±0.7	47.33±0.7	64.67±0.3
10.0	45.7±1.2	31.3±0.7	71.7±0.7	41.33±0.9	55.67±0.9	71.67±0.9
12.5	54.7±0.3	41.0±0.3	77.3±1.0	51.00±1.0	64.67±0.3	87.33±0.7
25.0	62.3±1.0	47.0±1.2	81.0±0.3	57.00±1.2	72.33±0.9	91.00±0.6
IC_{50}	14.51	22.08	7.94	16.57	11.38	6.52
R^2	0.87	0.92	0.82	0.93	0.87	0.82

L: Leaf; S: stem; R: Root extracts. *Data are expressed as mean percentage ± S.E. and IC_{50} (µg mL^{-1}).

Table 4: Lipid peroxidation activity of *V. spathulata* extracts.

Extract conc. µg mL^{-1}	Lipid peroxidation*					
	Aqueous Extract			Methanol Extract		
	R	**S**	**L**	**R**	**S**	**L**
2.5	18.3±0.9	23.3±0.7	26.0±0.9	19.00±0.7	17.00±0.6	16.00±0.6
5.0	24.0±0.7	33.7±0.9	34.3±1.0	23.00±0.6	19.33±0.7	44.33±0.7
7.5	38.7±1.2	40.7±1.0	50.0±1.0	33.33±0.7	23.33±0.9	53.00±0.6
10.0	43.7±1.0	44.0±0.9	58.0±0.3	43.67±0.9	41.00±0.6	68.00±0.6
12.5	58.7±0.7	54.0±1.2	66.7±1.2	50.67±0.3	47.67±0.7	72.67±0.9
25.0	63.7±0.3	59.0±0.3	77.3±0.7	54.00±0.3	53.67±0.7	87.33±0.3
IC_{50}	13.89	14.56	9.65	16.49	17.76	8.50
R^2	0.89	0.90	0.91	0.87	0.89	0.88

L: Leaf; S: stem; R: Root extracts.
*Data are expressed as mean percentage ± S.E. and IC_{50} (µg mL^{-1}).

Table 5: Superoxide dismutase activity of *V. spathulata* extracts.

Extract conc. µg mL^{-1}	SOD activity*					
	Aqueous Extract			Methanol Extract		
	R	S	L	R	S	L
2.5	5.7±0.6	6.7±1.2	11.7±1.2	5.67±0.3	9.67±0.7	11.67±0.7
5.0	8.7±1.0	11.3±0.6	19.0±0.3	8.67±0.3	18.33±0.3	19.00±0.6
7.5	11.3±0.3	14.7±1.2	24.7±1.0	11.33±0.9	24.67±0.3	34.67±0.3
10.0	24.3±1.0	25.7±0.3	30.3±0.7	24.33±0.7	35.67±0.3	40.33±0.6
12.5	39.3±0.7	41.7±0.7	52.7±0.3	39.33±0.7	51.67±0.9	62.67±0.3
25.0	51.7±0.3	59.3±0.3	84.7±0.6	61.67±0.3	69.33±0.7	94.67±0.3
IC_{50}	21.14	19.29	14.13	19.26	15.63	11.99
R^2	0.94	0.97	0.98	0.97	0.96	0.98

L: Leaf; S stem; R: *Data are expressed as mean percentage ± S.E. of inhibition and IC_{50} (µg mL^{-1}).

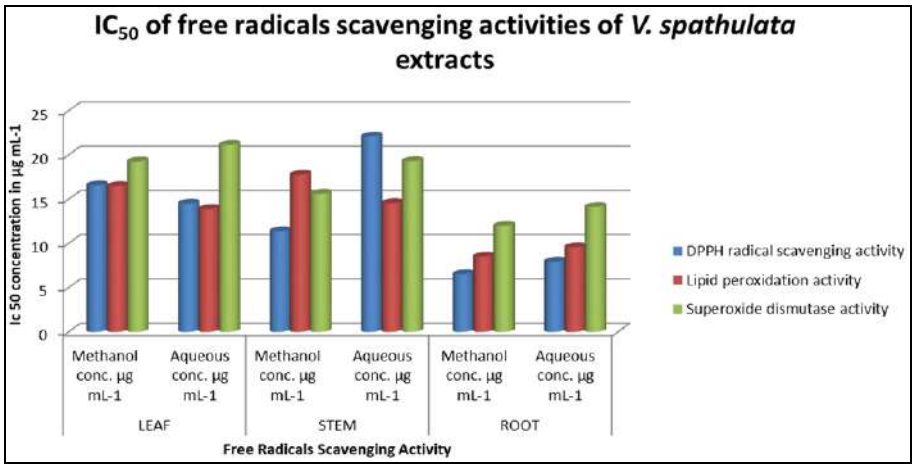


Fig 2: IC_{50} of free radicals scavenging activities of *V. spathulata* extracts.

Table 6: IC_{50} of free radicals scavenging activities of *V. spathulata* extracts.

Free radicals scavenging activities	LEAF		STEM		ROOT	
	Methanol conc. µg mL^{-1}	Aqueous conc. µg mL^{-1}	Methanol conc. µg mL^{-1}	Aqueous conc. µg mL^{-1}	Methanol conc. µg mL^{-1}	Aqueous conc. µg mL^{-1}
DPPH radical scavenging activity	16.57	14.51	11.38	22.08	6.52	7.94
Lipid peroxidation activity	16.49	13.89	17.76	14.56	8.50	9.56
Superoxide dismutase activity	19.26	21.14	15.63	19.29	11.99	14.13

Table 7: UV-VIS peak values of Methanolic leaf extract of *Vanda spathulate*

Sl. No.	Peak Value	Absorbance
1	203.25	0.6731
2	206.95	0.2767
3	219.30	0.1676
4	221.75	0.1630
5	271.20	0.0790
6	340.55	0.0569
7	586.35	0.0083

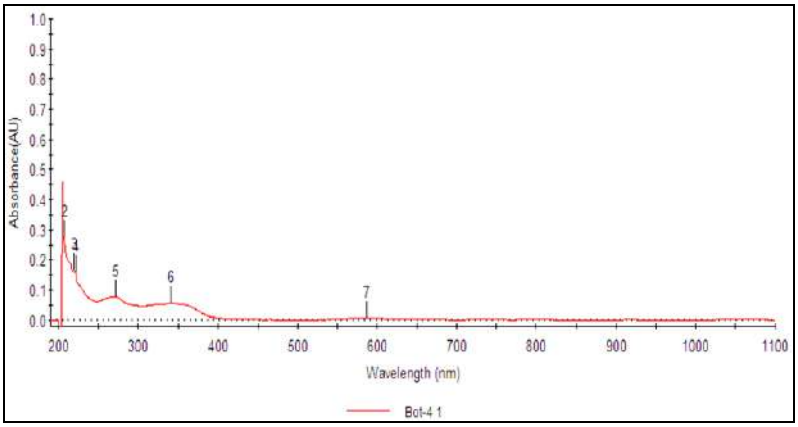


Fig 3: UV-VIS spectral analysis of Methanolic leaf extract of *Vanda spathulata*

Table 8: UV-VIS peak values of Aqueous leaf extract of *Vanda spathulate*

Sl. No.	Peak value	Absorbance
1	202.00	0.1728
2	205.75	0.0151
3	214.35	0.0153
4	219.30	0.0262
5	221.80	0.0224
6	266.20	0.0080
7	587.60	0.0078

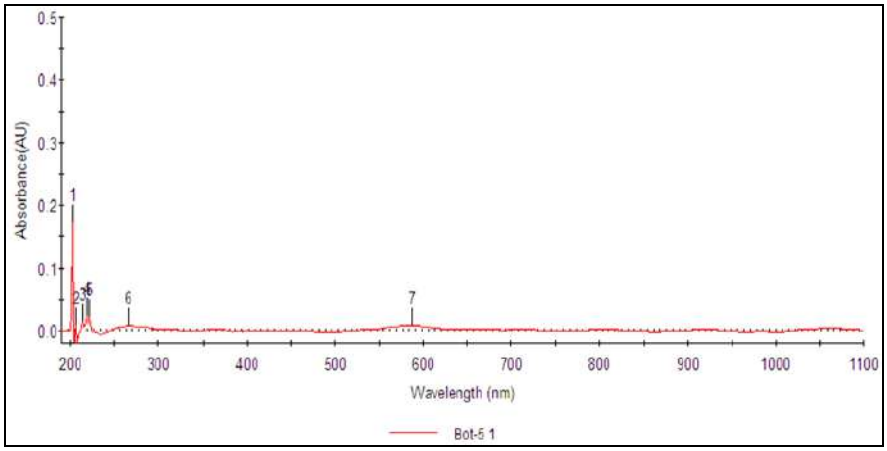


Fig 4: UV-VIS spectral analysis of Aqueous leaf extract of *Vanda spathulata*

Table 9: FTIR spectral analysis of Methanolic leaf extract of *Vanda spathulate*

Extract	Peak Value	Functional group	Functional group name
Methanol	657.15	C-Cl	Alkyl Halide
	1016.29	C-F	Alkyl Halide
	1030.15	C-O	Ether
	1040.70	C-O	Ether
	1054.03	C-O	Ether
	1112.14	C-O	Ether
	1412.57	C=C	Aromatic
	1453.34	C=C	Aromatic
	2120.40	-CΞC-	Alkyne
	2525.21	O-H	Acid
	2841.15	O-H	Acid
	2865.79	O-H	Acid
	2947.76	O-H	Acid
	2967.26	O-H	Acid
	3403.47	N-H	Amide
	3696.45	O-H	Alcohol

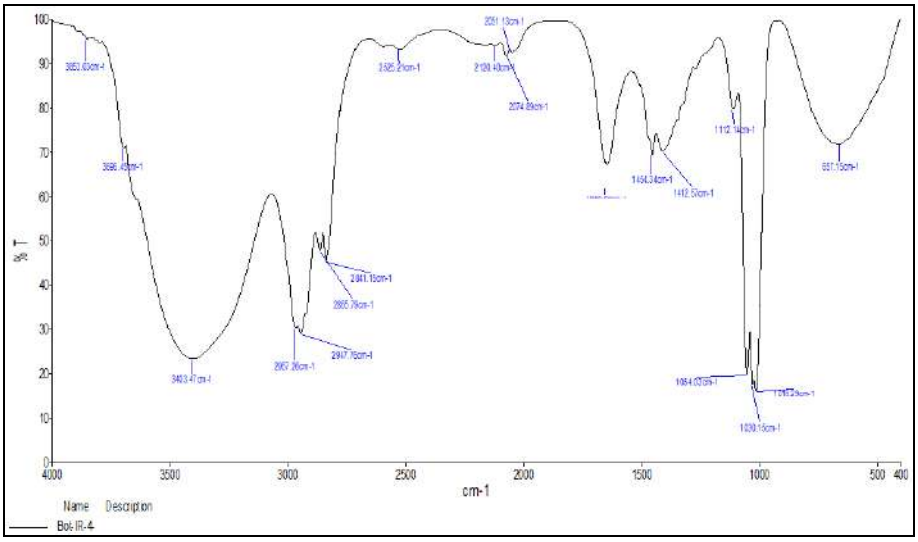


Fig 5: FTIR spectral analysis of Methanolic leaf extract of *Vanda spathulata*

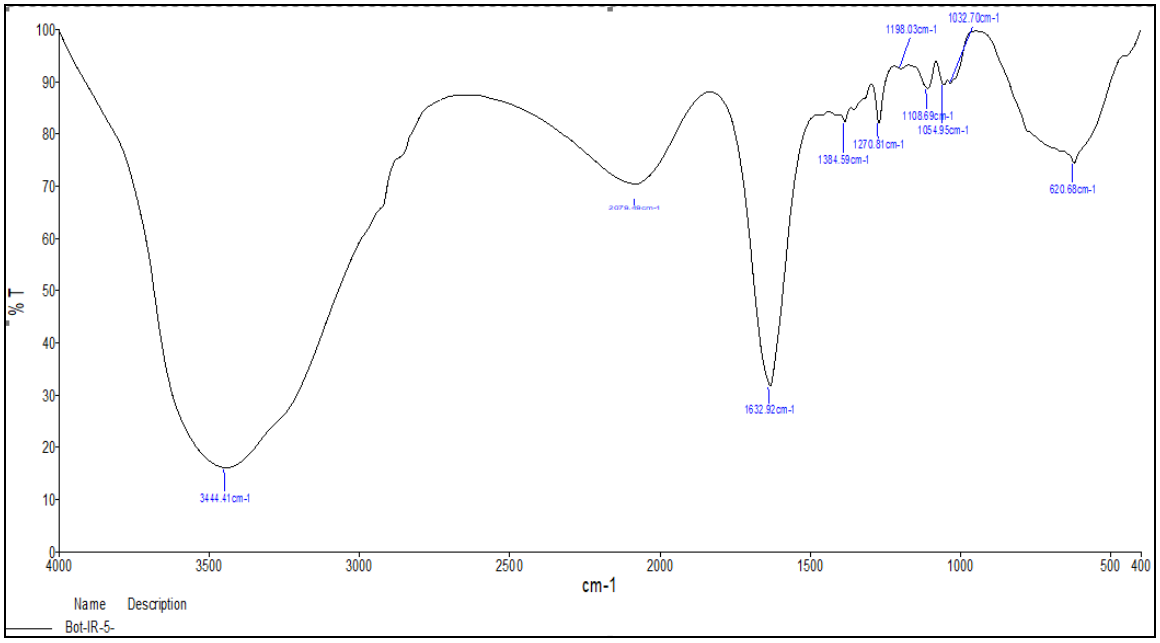


Fig 6: FTIR spectral analysis of Aqueous leaf extract of *Vanda spathulata*

Table 10: FTIR spectral analysis of Aqueous leaf extract of *Vanda spathulate*

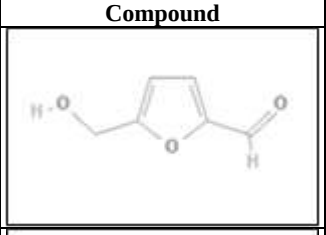
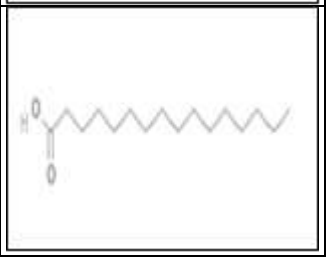
Extract	Peak Value	Functional group	Functional group name
Aqueous	620.68	C-Cl	Alkyl Halide
	1032.70	C-F	Alkyl Halide
	1054.95	C-F	Alkyl Halide
	1108.69	C-F	Alkyl Halide
	1198.03	C-F	Alkyl Halide
	1270.81	C-F	Alkyl Halide
	1384.59	C-F	Alkyl Halide
	1632.92	C=C	Alkene
	3444.41	N-H	Amine

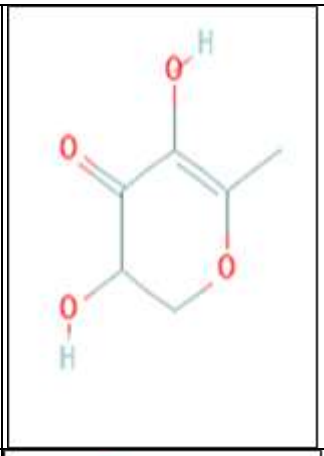
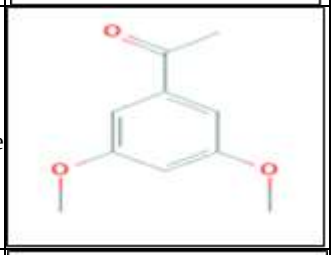
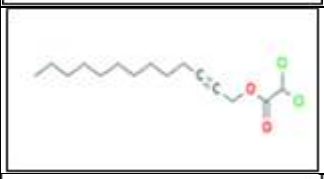
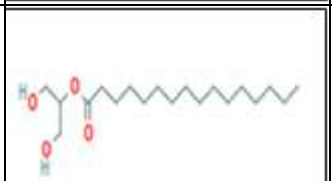
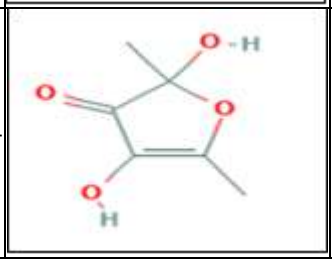
Table 11: GCMS Profiling of Methanolic leaf extract of *Vanda spathulata*

Sl. no.	Rt. Time	Name of the Compound	Mol. Wt	Molecular Formula	Peak Area %
1.	4.080	2-Furanmethanol	98	C5H6O2	1.47
2.	5.320	2-Cyclopenten-1-One, 2-Hydroxy	98	C5H6O2	0.71
3.	6.420	2,4-Dihydroxy-2,5-Dimethyl-3(2H)-Furan-3-One	144	C6H8O4	2.49
4.	8.810	2,4(1h,3h)-Pyrimidinedione, 5-Methyl-	126	C5H6N2O2	5.95
5.	8.810	Thymine	126	C5H6N2O2	5.95
6.	9.970	1,3-Dioxane-5-D, 2-(1,1-Dimethylethyl)-4-Methyl-, [2s(2.Alpha.,4.Alpha.,5.Alpha.)]	159	C9H17DO2	0.82
7.	10.240	4H-Pyran-4-One, 2,3-Dihydro-3,5-Dihydroxy-6-Methyl	144	C6H8O4	7.88
8.	11.535	Undecane, 4,7-Dimethyl- 4,7-Dimethylundecane $$ 4,7-Dimethylundecane 4,7-Dimethylundecane	184	C13H28	0.63
9.	12.060	:Benzofuran, 2,3-Dihydro- 2,3-Dihydrobenzofuran 2,3-Dihydro-1-Benzofuran 1-Benzofuran 2,3-Dih	120	C8H8O	1.09
10.	12.240	5-Hydroxymethylfurfural	126	C6H6O3	14.52
11.	12.490	(E)-Hex-3-Enyl (E)-2-Methylbut-2-Enoate	182	C11H18O2	1.09
12.	12.645	Butanedioic Acid, Hydroxy-, Dimethyl Ester	162	C6H10O5	1.34
13.	13.470	Vitispirane	192	C13H20O	0.65
14.	14.125	2-Methoxy-4-Vinylphenol, 2-Methoxy-4-Vinylphenol (Vinylguaiacol), 4-Ethenyl-2-Methoxyphenol- 4-H	150	C9H10O2	1.29
15.	14.930	Phenol, 2,6-Dimethoxy	154	C8H10O3	0.68
16.	15.735	5-Oxoproline, Dl-Pyroglutaminsaeure	129	C5H7NO3	0.85
17.	15.905	1-Tetradecanol	214	C14H30O	1.05
18.	16.080	Decane,	142	C10H22	4.07
19.	16.080	Tetradecane, Isotetradecane N-Tetr	198	C14H30	4.07
20.	19.410	3',5'-Dimethoxyacetophenone	180	C10H12O3	7.59
21.	19.800	Naphtho[1,2-C]Furan-3-Ol, 1,3,3a,4,6,7,8,9,9a,9b-Decahydro-6,6,9a-Trimethyl-, (3.Alpha.,3a.Alpha.,9a.Beta.,9b.Alph	236	C15H24O2	0.32
22.	19.800	Benzene, (1-Ethyloctadecyl)- (1-Ethyloctadecyl)Benzene (1-Ethyloctadecyl)Benzene (1-Ethyloct	358	C26H46	0.32
23.	19.935	1,2-Benzenedicarboxylic Acid, Diethyl Ester Phthalic Acid -Benzenedicarboxylic Acid	222	C12H14O4	0.64

		Diethyl Ester			
24.	20.045	Trichloroacetic Acid, Undecyl Ester	316	C13H23Cl3O2	1.53
25.	20.195	Decane, 2,3,7-Trimethyl- 2,3,7-Trimethyldecane 2,3,7-Trimethyldecane	184	C13H28	1.25
26.	20.700	Megastigmatrienone	190	C13H18O	0.7
27.	21.105	3,4-Altrosan	162	C6H10O5	0.85
28.	21.555	Octanamide, N-Hydroxy	159	C8H17NO2	0.32
29.	22.005	4-Methyl-2-Pentyl Methylphosphonofluoridate	182	C7H16FO2P	0.87
30.	22.060	(E)-2,6-Dimethoxy-4-(Prop-1-En-1-Yl)Phenol	194	C11H14O3	0.42
31.	22.135	3-Hydroxy-2-Methyl-5-(Prop-1-En-2-Yl) Cyclohexanone	168	C10H16O2	0.42
32.	22.825	4-((1E)-3-Hydroxy-1-Propenyl)-2-Methoxyphenol	180	C10H12O3	1.05
33.	22.890	1,2-Ethanediamine, N,N-Dimethyl-N'-2-Pyridinyl-N'-(2-Thienylmethyl)- 1,2-Ethanediamine, N,N-Dimethyl-N'	261	C14H19N3S	0.44
34.	23.185	:2,3,4,5-Tetrahydroxypentanal Tetrahydropyran-2,3,4,5-Tetraol Pentose (+)-Xylose (D)-Xylose	150	C5H10O5	0.88
35.	23.390	1-Nonene, 4,6,8-Trimethyl	168	C12H24	0.31
36.	23.770	1-Nonadecene	266	C19H38	2.15
37.	23.900	Nonadecane,	268	C19H40	1.34
38.	24.200	Cyclohexanecarboxylic Acid, 4-Pentyl-, 4-Cyanophenyl Ester, Trans	299	C19H25NO2	0.68
39.	24.860	9-Aza-1-Methylbicyclo[3.3.1]Nonan-3-One	153	C9H15NO	0.6
40.	25.150	Carbonic Acid, Nonyl Prop-1-En-2-Yl Ester	228	C13H24O3	0.72
41.	25.815	(1-Pentylhexyl)Cyclohexane, Cyclohexane, (1-Pentylhexyl)	238	C17H34	0.37
42.	26.015	Hexadecanoic Acid, Methyl Ester- Methyl Hexadecanoate - Palmitic Acid Methyl Ester	270	C17H34O2	0.81
43.	26.610	N-Hexadecanoic Acid	256	C16H32O2	7.96
44.	27.105	Trans-Sinapyl Alcohol	210	C11H14O4	0.92
45.	27.150	1-Nonadecene	266	C19H38	2.5
46.	27.255	Spiro[Cyclopentane-1,2'(1'h)-Quinoxaline], 3'-(4-Morpholinyl)-6',8'-Dinitro-3',4'-Dihydro-2'-(Morpholin-4-Yl)	361	C16H19N5O5	0.71
47.	28.575	Sinapic Acid Methyl Ester	238	C12H14O5	0.39
48.	28.785	Cyclooctanone, 2-(4-Hydroxy-2-Butenyl)-2-Nitro-, (E)-2-(4-Hydroxy-2-Butenyl)-2-Nitrocycloctanone	241	C12H19NO4	0.3
49.	29.280	9,12-Octadecadienoic Acid (Z,Z)	280	C18H32O2	2.37
50.	29.375	Dichloroacetic Acid, Tridec-2-Ynyl Ester	306	C15H24Cl2O2	4.29
51.	29.470	(1S,2R,4R,7R)-4-Isopropyl-7-Methyl-3,8-Dioxatricyclo[5.1.0.02,4]Octane	168	C10H16O2	0.61
52.	29.800	2-Octyn-1-Ol	126	C8H14O	0.72
53.	30.230	(Trans)-2-Nonadecene	266	C19H38	0.77
54.	34.595	Hexadecanoic Acid, 2-Hydroxy-1-(Hydroxymethyl)Ethyl Ester	330	C19H38O4	3.68
55.	34.850	1,2-Benzenedicarboxylic Acid 1,2-Benzenedicarboxylic Acid, Bis(2-Ethylhexyl) Ester 1, 2-Benzenedica	390	C24H38O4	1.11
56.	37.205	Octadecanoic Acid, 2,3-Dihydroxypropyl Ester	358	C21H42O4	0.48
57.	39.605	3-Ethyl-8-Methyl-2-Oxatetracyclo[4.4.0.0(1,4).0(6,8)]Decane	178	C12H18O	0.78

Table 12: GCMS Profiling of Methanolic leaf extract of *Vanda spathulate*

Sl. No.	Peak Area	Name of the Compound	Molecular Structure of the Compound	Biological Activities
1.	***14.52***	***5 Hydroxy methyl furfural***		Food additives -flavoring agent, Anti-oxidative, anti-allergic, anti-inflammatory, anti-hypotoxic, anti-sickling and anti-hyper uricemic effects (shapla *et al*., 2018)
2.	7.96	n-Hexadecanoic acid		Inhibitor of Phospholipase A2, Anti-inflammatory agent, treatment of Rheumatism (Aparna, 2012), DNA topoisomerase-Inhibitory activity, Cytotoxicity against human colorectal carcinoma cells (HCT-116) (Ravi and Kannabiran, 2017), Anti-microbial activity, Anti-inflammatory, Anti-oxidant, Hypocholesterolemic, Nematicide, Pesticide, Anti-androgenic flavor, Hemolytic, 5-alpha reductase inhibitor, potent mosquito larvicide (Liu *et al*., 2009, Abubakar & Majinda, 2016)

3.	7.88	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl		Anti-microbial compound (Udaweediye *et al*., 2016), Reactive oxygen-scavenging activity, Anti alpha-glucosidase activity in patients with diabetes, Anti mutagenic activity against arylamine, Inhibit colon cancer cell growth through the inhibition of NFkB (Ban *et al*., 2007). Anti-oxidant (Cechovska *et al*., 2011), Anti-fungal activity against wood-degrading fungi (Teoh *et al*., 2011), Radical scavenger activity, Antiproliferation & pro-apoptotic effects, Affects sympathetic nerve activity (Beppu *et al*., 2012). Strong anti-oxidant (Yu *et al*., 2013), Anti-oxidant capacity, Metal Chelation Activity, Anti-proliferation, Pro-apoptotic activity, Affect sympathetic nerve activity & metabolism of rats (Zhou *et al*., 2014). Antifungal activity (Teoh and Don, 2014). Increase the level of endogenous anti-oxidant enzymes in male wistar rats (Olaniyan *et al*., 2018).
4.	7.59	3'5' Dimethoxyacetophenone		Inhibitory activity toward Acetyl Cholineesterase (AChE) and Butyrylcholinesterase (BuChE) (Shen *et al*., 2013). Anti-oxidant (Alghamdi *et al*., 2018).
5.	5.95	Thymine		Anti-oxidant capacity (Subki *et al*), Essential metabolic Cofactor (Raschke *et al*., 2007), Anti-tumor agent, Inhibition of Cell proliferation on A431 vulvar epidermal carcinoma cell line (Bouazza *et al*., 2008)
6.	4.29	Dichloroacetic acid, tridec-2-ynyl ester		Used for Cosmetic treatments, topical medication for the chemoblation of warts, Salts of DCA inhibits the enzyme puruvate dehydrogenase kinase, activity against Brain Cancer calls (Roy *et al*., 2019)
7.	4.07	Tetradecane		*In vitro* activity against Plasmodium falciparum, Trypanasoma brucei (Howarth, 2003)
8.	3.68	Hexadecanoic acid, 2-hydroxy-1-(hydroxy methyl) ethyl ester		Hemolytic, Pesticide, flavour, Anti-oxidant (Tyagi and Agarwal 2017) Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Anti androgenic, Flavor, Hemolytic, 5-Alpha reductase Inhibitor (Lalitha *et al*., 2015), Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Antiandrogenic flavor, Hemolytic, Alphareductase inhibitor (Chidambarampillai & Mohan, 2013)
9.	2.5	1-Nonadecene		Antifungal (Kumari *et al*., 2019), Antifungal, Antibacterial and Anti-viral properties (Udaweediye *et al*., 2016), Antibacterial activity (Gherraf *et al*., 2013)
10.	2.49	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one		Flavouring agent (Schwab 2013, Chukwu *et al*., 2017, Hauck, 2003), Caramel like smell and flavour (Blank, 1996)

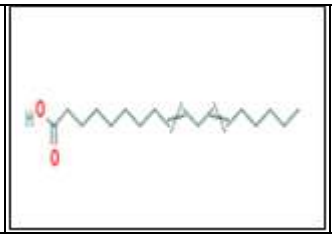
11.	2.37	9,12-Octadecadienoic acid (Z,Z)		Anti-inflammatory, Hypocholesterolemic anti-arthritic (Jones 2002, Petricevich *et al*., 2017, Singh and Preethi, 2019, Starlin *et al*., 2019), Cancer preventive, Hepato protective, Nematicide, Insectifuge, Antihistmainic, Antieczemic, antiacne, 5-Alpha reductase inhibitor, Antiandrogenic, Antiarthritic, Anticoronary, Insectifuge (Sudha *et al*., 2013, Parthipan *et al*., 2016)

Table 13: GCMS Profiling of Aqueous Leaf extract of *Vanda spathulate*

Sl. No.	Peak area %	Name of the compound
1.	35.73	1,3,5-Tris(cyclohexyl)hex-1-ene
2.	24.72	Z,Z-3,13-Octadecedien-1-ol
3.	18.17	OCTANOIC ACID, 4-CHLORO-, CHLOROMETHYL ESTER
4.	14.32	4-tert-Butylcyclohexyl ethylphosphonofluoridate
5.	7.06	1,1'-BICYCLOHEXYL, 4-ETHOXY-4'-PROPYL-

References

1. Abubakar MN, Majinda RT. GC-MS Analysis and Preliminary Antimicrobial Activity of *Albizia adianthifolia* (Schumach) and *Pterocarpus angolensis* (DC). Medicines,2016:3:3.
2. Adedapo AA, Jimoh FO, Koduru S, Masika PJ, Afolayan AJ. Assessment of the medicinal potentials of the methanol extracts of the leaves and stems of *Buddleja saligna*. BMC CAM,2009:9:21.
3. Aysal P, Ambrus AD, Lehotay SJ, Cannavan A. Validation of an efficient method for the determination of pesticide residues in fruits and vegetables using ethyl acetate for extraction. J. Environ. Sci. Heal,2007:42:481-90.
4. Begum F. Training Courses on Orchid Production in Bangladesh organized by Hortex, 2000.
5. Buyun L, Tkachenko H, Kurhaluk N, Gyrenko O, Kovalska L, Osadowski Z. Assessment of oxidative stress biomarkers in the equine blood after *in vitro* incubation with leaf extract obtained from Dendrobium parishii Rchb.F. Agrobiodiversity for Improving Nutrition, Health, and Life Quality,2019:3:416-427.
6. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J. Food. Drug. Anal,2013:10:178-182.
7. Chowdhury MA, Rahman MM, Chowdhury MR, Uddin MJ, Sayeed MA, Hossain MA. Antinociceptive and cytotoxic activities of an epiphytic medicinal orchid: *Vanda tessellata* Roxb. BMC Complementary and Alternative Medicine,2014:14:464.
8. Chung KT, Wong TY, Wei CI, Huang YW, Lin Y. Tannins and human health: a review. Crit. Rev. Food Sci. Nutr,1998:38(6):421-464.
9. Chung Y, Chien C, Teng K, Chou S. Antioxidative and mutagenic properties of Zanthoxylum ailanthoides Sieb and zucc. Food Chemistry,2006:97:418-25.
10. Cor D, Knez Ž, Knez Hrnčič M. Antitumour, Antimicrobial, Antioxidant and Antiacetylcholinesterase Effect of Ganoderma Lucidum Terpenoids and Polysaccharides: A Review. Molecules,2018:23(3):649.
11. Duh PD, Tu YY, Yen GC. Antioxidant activity of aqueous extract of harnjyur (Chyrsanthemum morifolium Ramat). Lebensmittel-Wissenschaft and Technologie,1999:32:269-277.
12. Elizabeth S, Rao MNA. Oxygen radical scavenging activity of curcumin. Int. J. Pharm,1990:58:237-240.
13. Ghani A, Rastogi Ram, Mehrota BN. Compendium of Indian Medicinal plants. CDRI Lucknow and National Institute of science and communication,1990-1994:5:757.
14. Ghani A, Rastogi Ram, Mehrota BN. Compendium of Indian Medicinal plants, CDRI Lucknow and National Institute of science and communication,1990-1994:5:757.
15. Gil MI, Tomas-Barberan FA, Hess PB *et al*. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. Journal of Agricultural and Food Chemistry,2000:48:4581-4589.
16. Gutierrez RMP. Orchids: A review of uses in traditional medicine, its phytochemistry and pharmacology. J Med Plants Res,2010:4(8):592-638.
17. Hashimoto A, Kameoka T. Applications of infrared spectroscopy to biochemical, food, and agricultural processes. Applied Spectroscopy Reviews,2008:43:416-51
18. Hussain K, Ismail Z, Sadikun A, Ibrahim P. Evaluation of Metabolic Changes in Fruit of Piper armentosum in various Seasons by Metabolomics using Fourier Transform Infrared (FTIR) Spectroscopy. Int. J. Pharm. Clinical Res,2007:1(2):68-71.
19. Ibrahim M, Hameed AJ, Jalbout A. Molecular Spectroscopic Study of River Nile Sediment in the Greater Cairo Region. Applied Spectroscopy,2008:62(3):306-11.
20. Kabir MI, Mortuza MG, Islam MO. Morphological features growth and development of Dendrobium sp. orchid as influenced by nutrient spray. Journal of Environmental Science and Natural Resources,2012:5(1):309-18.
21. Kim HK, Choi YH, Erkelens C, LeFeber AWM, Verpoorte R. Metabolic fingerprinting of *Ephedra* species using ^{1}H NMR spectroscopy and principal component analysis. Chem. Pharm. Bull,2005:53:105-109.
22. Komal Kumar J, Devi Prasad AG. Identification and comparison of biomolecules in medicinal plants of Tephrosia tinctoria and Atylosia albicans by using FTIR. Romanian J Biophys,2011:21(1):63-71.
23. Lam Y, Ng TB, Yao RM, Shi J, Xu K, Sze SCW, Zhang KY. Evaluation of chemical constituents and

important mechanism of pharmacological biology in Dendrobium plants. Evid.-Based Complement. Altern. Med, 2015, 1-25.
24. Liu F. Antioxidative and free radical scavenging activities of selected medicinal herbs. Life Sci,2000:66:725-35. doi: 10.1016/S0024-3205(99)00643-8.
25. Mamta Saxena, Jyoti Saxena, Rajeev Nema, Dharmendra Singh, Abhishek Gupta. Phytochemistry of Medicinal Plants. Journal of Pharmacognosy and Phytochemistry,2013:1(6):168-182.
26. Maobe MAG, Nyarango RM. Fourier Transformer InfraRed Spectrophotometer Analysis of Warburgia ugandensis Medicinal herb used for the Treatment of Diabetes, Malaria and Pneumonia in Kisii Region, Southwest Kenya. Global J Pharmacology,2013:7(1):61-68.
27. Mei WY, Dong ZY, Zhang ZJ, Lin XY, Shen Y, Zhou D *et al*. Extensive *denovo* variation in rice induced by introgression from wild rice (*Zizania latifolia*). Genetics,2005:170:1945-1956.
28. Minh TN, Tuyen PT, Khang DT, Quan NV, PTT, Ha NT *et al*. Potential use of plant waste from the moth orchid (Phalaenopsis sogo yukidian V3) as an antioxidant source. Foods,2017:6(10):85.
29. Mukherjee S, Phatak D, Parikh J, Jagtap S, Shaikh S, Tupe R. Antiglycation and antioxidant activity of a rare medicinal orchid Dendrobium aqueum Lindl. Medicinal Chemistry and Drug Discovery,2012:2(2):46-54.
30. Nagananda GS, Patil A, Kambli JV, Rajath S. Phytochemical Evaluation and *in vitro* Free Radical Scavenging Activity of Cold and Hot Successive Pseudobulb Extracts of Medicinally Important Orchid Pholidota pallida Lindl. Advances in Bioresearch,2014:5(1):100-105.
31. Ngo TV, Scarlett CJ, Bowyer MC, Ngo PD, Vuong QV. Impact of different extraction solvents on bioactive compounds and antioxidant capacity from the root of Salacia chinensis L. Journal of Food Quality, 2017, 1-8.
32. Ngoc TM, Khoi NM, Nhiem NX *et al*. Xanthine oxidase inhibitory activity of constituents of Cinnamomum cassia twigs. Bioorganic and Medicinal Chemistry Letters,2012:22(14):4625-4628.
33. Obadoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. Glob. J. Pure Appl. Sci,2001:86:2003-2008.
34. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochem,1979:95(2):351-358.
35. Ollanketo M, Peltoketo A, Hartonen K, Hiltunen R, Riekkola ML. Extraction of sage by pressurized hot and conventional methods: antioxidant activity of the extracts. Euro. Food Res. Tech,2002:215:158-163.
36. Paulraj K, Irudayaraj V, Johnson M, Patric D. Phytochemical and anti-bacterial activity of epidermal glands extract of Christella parasitica (L.) H. Lev. Asian Pac. J. Trop Biomed,2011:1:8-11.
37. Prescott LM, Harley PJ, Klein AD. Microbiology. 7th ed. McGraw Hill Publisher, Singapore, 2008, 94-122.
38. Puchooa D. Comparison of different culture media for the *in vitro* culture of *Dendrobium* (Orchidaceae). Int. J. Agril. Biol,2004:6(5):884-888.
39. Roginsky V, Lissi EA. Review of methods to determine chain-breaking antioxidant activity in food. Food Chemistry,2005:92:235-254.
40. Roychoudhury R, Jay E, Wu R. Terminal labeling and addition of homopolymer tracts to duplex DNA fragments by terminal deoxynudeotidyl transferase. Nucleic Acids Res,1976:3:101-116.
41. Shapla UM, Solayman M, Alam N, Khalil MI, Gan SH. 5-Hydroxymethylfurfural (HMF) levels in honey and other food products: Effects on bees and human health. Chemistry Central Journal,2018:12(1):35-41. doi: 10.1186/s13065-018-0408-3.
42. Shibu SB, Devi CB, Moin S, Wesley SP. Evaluation of bioactive potential of Coelogyne nervosa A.Rich. - An endemic medicinal orchid of Western Ghats, India. Asian Journal of Pharmaceutical and Clinical Research,2013:6(S1):114-118.
43. Sirisha JV, Sailakshmi K, Vijayal K. Human red blood cell (HRBC) membrane stabilizing activity of leaves of pet-ether extract of *Vanda tessellate* Roxb. International Ayurvedic Medicinal Journal,2013:1:5-8.
44. Skoog A, Holler EJ, Crouch SR. Principles of instrumental analysis. 6 Edition, 2007, 1039.
45. Stajner D, Popovic BM, Canadanovic-Brunet J, Goran A. Exploring Equisetum arvense L. Equisetum ramosissimum L. and Equisetum telmateia L. as source of natural antioxidants. Phyther Res,2009:23:546-550.
46. Talukder SK, Nasiruddin KM, Yesmin S, Hassan L, Begum R. Shootproliferation of *Dendrobium* orchid with BAP and NAA. Journal of Biological Science,2003:3(11):1058-1062.
47. Uddin SJ, Grice D, Tiralongo E. Evaluation of cytotoxic activity of patriscabratine, tetracosane and various flavonoids isolated from the Bangladeshi medicinal plant *Acrostichum aureum*. Pharm. Biol,2012:50:1276-1280.
48. Velioglu VS, Mazza G, Gao L *et al*. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. Journal of Agricultural and Food Chemistry,1998:46:4113-4117.
49. Wang M, Shao Y, Li J *et al*. Antioxidative phenolic glycosides from sage (Salvia offi cinalis). Journal of Natural Products,1999:62:454-456.
50. Xu J, Han QB, Li SL, Chen XJ, Wang XN, Zhao ZZ *et al*. Chemistry, bioactivity and quality control of Dendrobium, a commonly used tonic herb in traditional Chinese medicine. Phytochem. Rev,2013:12:341-367.

View publication stats

International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 14-08-2021, Accepted: 29-08-2021, Published: 14-09-2021
Volume 6, Issue 5, 2021, Page No. 497-503

Hormonal influence of *Mentha arvensis* green silver nanoparticle's on mung bean

A Vasuki, V N andagopalan, A Lakshmi Prabha*
[1] Research Scholar, Department of Botany, Bharathidasan University, Trichy, Tamil Nadu, India
[2] Associate Professor, Department of Botany, National College, Trichy, Tamil Nadu, India
[3] Professor and Head of the Department of Botany, Bharathidasan University, Trichy, Tamil Nadu, India

Abstract
This paper evaluate *Mentha arvensis* silver Nanoparticles treat as a nutrient hormone for green gram seeds. This green synthesis silver nanoparticle enhance the growth of green gram seeds germination, root and stem length. Treated seeds were give a best result while compare to control seeds. 1.0ul to 10 ul used concentration, in this concentration 8 ul concentration of AgNps presenting the highest value (Growth and length) compare up to10ul AgNps and control. XRD value shows the peak presenting in 38 min graph, in 8 ul concentration high peak present in XRD at 38 minutes. So *Mentha arvensis* nanoparticles presence in the root of treated green gram seeds confirmed by XRD.

Keywords: mentha arvensis, AgNps, XRD

Introduction

Basic studies on ethylene regulation opened for applied research in the field of micropropagation somatic embryogenesis and secondary metabolites production. Nanotechnology opens a wide scope of novel application in the field of Biotechnology and agricultural, because AgNps have a unique physiochemical properties. Plant tissue culture is an important for conservation, mass propagation, genetic manipulation, bioactive compounds production and plant improvement. There for the present chapter highlights the significant role of AgNo3 and AgNp in plant growth and development.

Nanoparticle in various aspects through the integration of nanotechnology into plant tissue culture and highlights of using Nps as promoter for the production of secondary metabolites. Wide range investigation into the large scale production of the compounds attained from Np-treated plant culture.

Mentha arvensis

Mentha arvensis species of flowering plant in Lamiaceae family. Its common names include Field Mint, Wild Mint or Corn Mint, it is better known by its scientific name Mentha arvensis. They are also found in the eastern regions of Himalaya, various regions of North America and Eastern Siberia. These plants grow well at riverbanks, edges of marshes and moist prairies. It is quite easy to grow these plants from seeds. These seeds can be sown during fall; however, one can also sow them in spring after refrigerating them for 2 to 3 months.

Fig 1

Scientific Classification
Kingdom: Plantae
Clade: angiosperms
Clade: Eudicots
Clade: Asterids
Order: Lamiales
Family: Lamiaceae
Genus: Mentha
Species: M. arvensis
Binomial name: *Mentha arvensis*

Aim

1. Nanoparticle producing from Mentha arvensis leaves
2. Using AgNps as a growth hormone for green gram in tissue culture
3. AgNps Confirmation in green gram root by using XRD

Materials and Method

Synthesis of silver nanoparticles

Chemical used

The preparation of 1 mM aqueous extract of silver nanoparticle. 2 ml of Methanolic plant extract and 9 ml of 1mM Silver nitate extract for the synthesis of silver nanoparticle, in 15 tubes and one tube was maintained for control without adding silver nitrate solution. It was incubated for 24 hrs at light condition. The formation of synthesized silver nanoparticles from methanol plant extract was used for centrifuge. Collected the pellet for further study and discard the supernatant. The pellet was dried to evaporate the solvent and kept in micro oven. Finally collected the pellet as a semisolid in nature. The silver nanoparticles was confirmed by color changes and it is characterized by UV Visible Spectrophotometer, FTIR for chemical analysis and SEM

Micropropagation

Pure green gram seeds were collected and it was sterilized with running tap water for 5 min. After that the seeds were undergoes a surface sterilization to free from contamination. Bavastin and mercuric chloride, 70% ethanol to remove the microbes and dust particles. The Preparation of 10 mg green synthesized silver nanoparticle (AgNps) diluted with 25ml of distilled water for root induction. After surface sterilized distilled water green gram seeds were dried in sterilized filter paper.

The preparation of MS medium for 150 ml from stock solution showed. After that the medium was 5ml in each tube distributed for 16 test tubes and kept in autoclaving process for sterilization under 121°C for 45 mins. The various concentration of 1µl,2 µl,3 µl,4 µl,5 µl,6 µl,7 µl, 8µl, 9µl and 10 µl silver nanoparticles solution was added on Basal MS medium surface, one MS medium Basal tube maintained as a control without adding silver nitrate solution. Dried seeds were inoculated in MS medium containing each tube. The test tubes were maintained at room temperature 21°C-23°C for 3-10 days.

Result

Synthesized of Silver nanoparticle

The mixture of silver nanoparticle solution and *Mentha arvensis* extract was dark green before the reaction occurs. After that added a 1mM of silver nitrate solution into the extract. The reaction of mixture became pale yellow to dark brown within 24 h when treated with the leaf extract of *Mentha arvensis* at room temperature. The formation of dark brown color shows a AgNps extract

Fig 2

Uv-vis spectroscopy

The size of the synthesized silver nanoparticles was identified by using the spectral analysis. Shown the stable level of absorbance peak at 330 nm in the sample

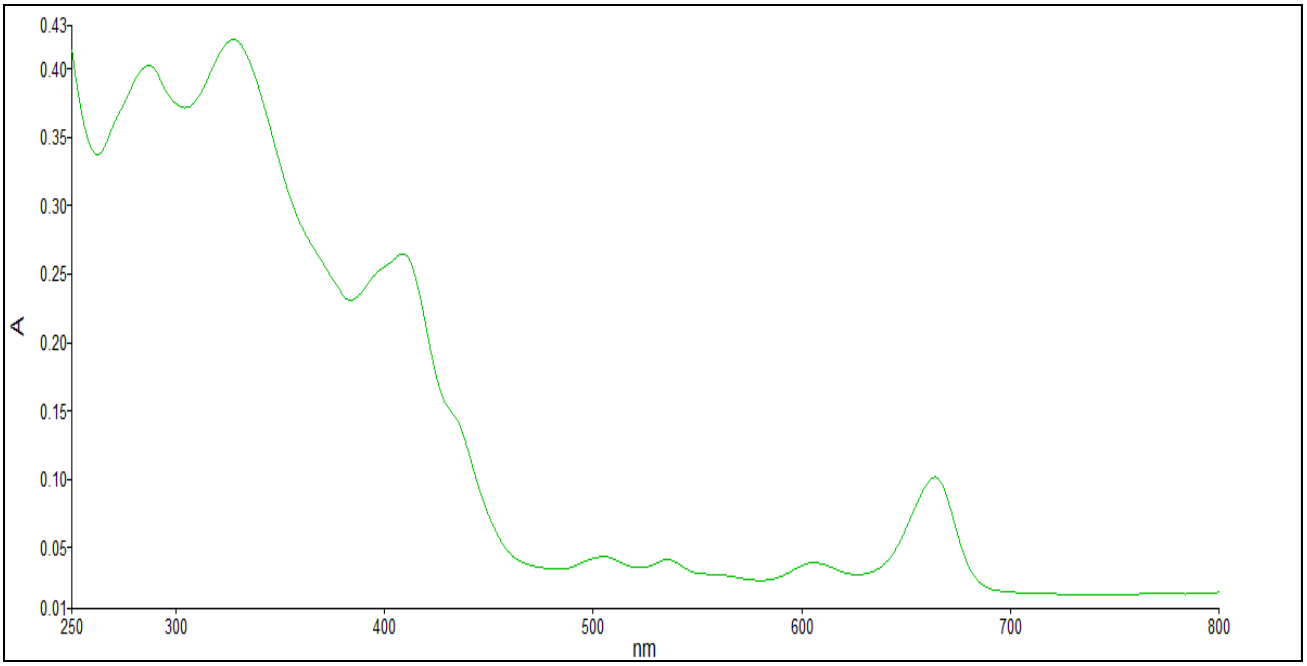


Fig 3: UV Visible Spectral analysis of Synthesized AgNPs from Mentha arvensis extract.

Ftir

Fourier transform infrared spectroscopy analysis showed an absorption peaks of reduced silver at 1699 cm−1.The stretching vibration of C=C obtained at 1621cm−1 and the single absorbance peak located at 1159 cm−1 is assigned to C-O Polyols,

while 3377 and 1735 cm−1 corresponds to N-H and C=O stretching vibration shows in peak.

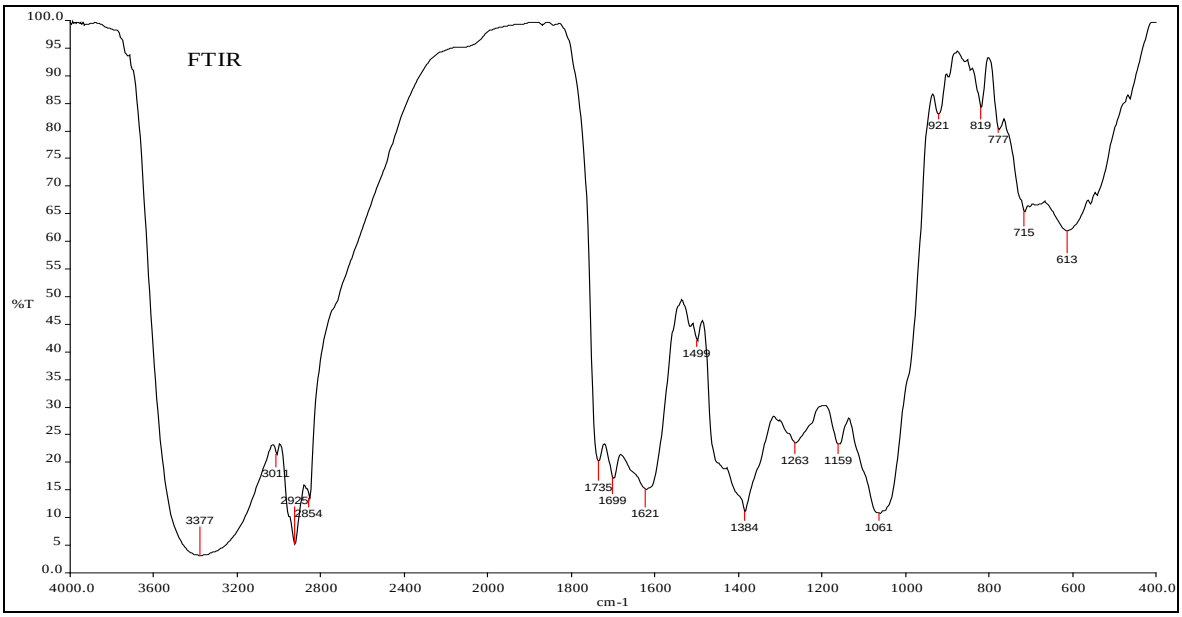


Fig 4: FTIR analyses for AgNo3 synthesis of *Mentha arvensis* extract.

SEM

SEM analysis represents the better particle size in 1000nm. In 1µm shown at 30000 x magnifications, 3µm shown at 15000 x magnifications. The crystal like structure of silver nanoparticle are formed in SEM analysis shows.

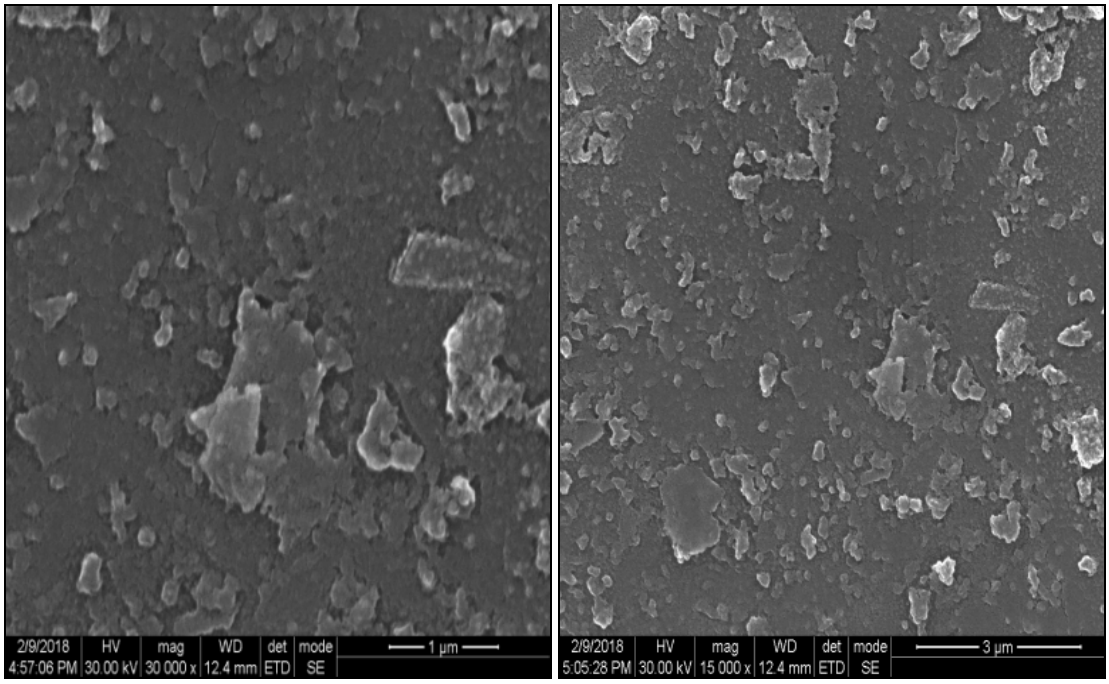


Fig 5: The SEM Micrograph of synthesized silver nanoparticles from *Mentha arvensis* extract (A)1µm of 30000x magnification (c) 3 µm of 15000x

Hormonal study in plant tissue culture

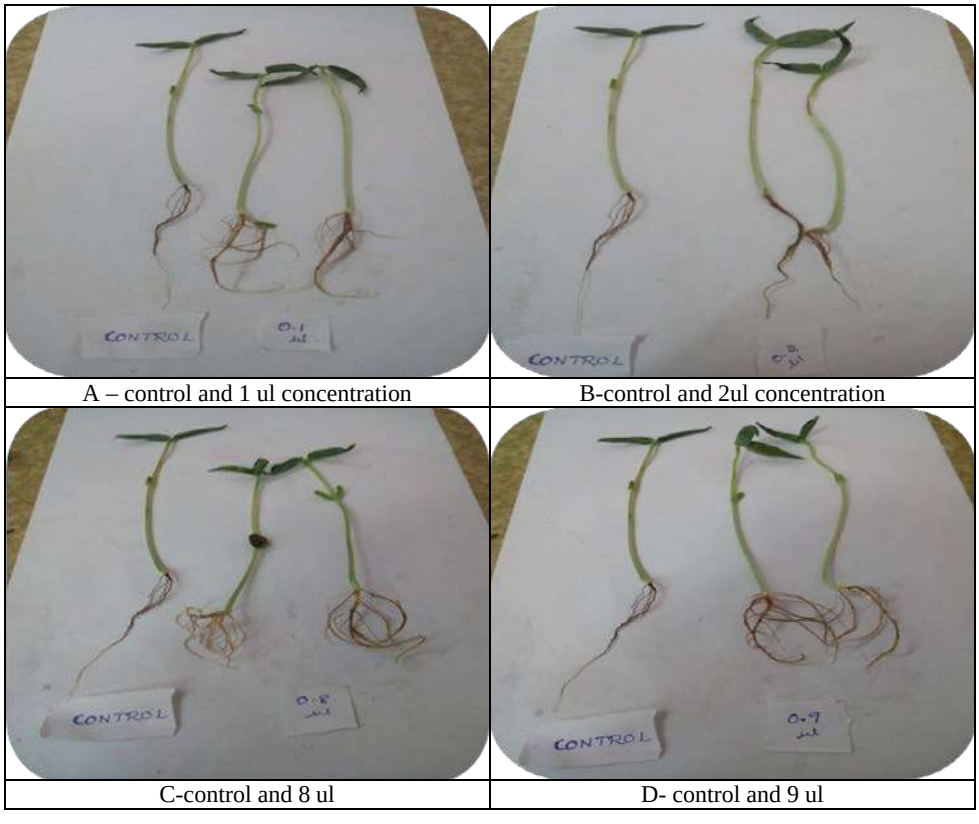
The growth of root forming structures is good in various concentration levels shown, It shows positive results in the concentration level of 1µl 2µl, 8µl and 9µl. In 8 µl shows a better result while comparing to the other level of concentration. To address this issue through Nano biotechnology, we ingeniously build a concept of silver nanoparticles (AgNPs) as a "Nano bullets" can act for a dual mode like root enhancer and pathogen destroyer on the target site. As the result, the action duality of hormone stabilized AgNPs was manifested to three-fold enhanced root growth compared than controls and it increased the root forming capabilities against root growth inhibiting phytopathogens.

The Measurement of whole plant and its part of leaves, roots and stem.

Table 1: The growth of seeds germination at various concentration level after 10 days.

S.No	Conc (ul)	Plant Height Value (cm)	Root Height Value (cm)	No of Roots(cm)	Leaves (Nos)	Leaves Height (cm)	Stem Width	Height Of Stem (cm)	Seed Germination (nos)
1	Control	18.2	7.5	7	2	2.5	0.1	11.2	1
2	1	17	7	10	2	2.4	0.2	10	2
3	2	17.2	7	6	2	2.7	0.3	10.5	2
4	3	14	7	5	2	1.7	0.1	7.5	2
5	4	17.5	6.4	8	2	2.4	0.2	10	2
6	5	17.4	6	8	2	2.5	0.2	10	2
7	6	15.5	5.5	8	2	2.4	0.2	10	2
8	7	14.5	7	6	2	2.2	0.1	17.9	1
9	8	16.5	6	10	2	2.2	0.1	10	2
10	9	16.8	4.5	7	2	2.3	0.1	10.9	2
11	10	17.1	6.5	6	2	2	0.1	11.2	1

Fig 6: Control to 10 ul concentration growth of green gram for 10 days



A – control and 1 ul concentration | B-control and 2ul concentration

C-control and 8 ul | D- control and 9 ul

Fig 7

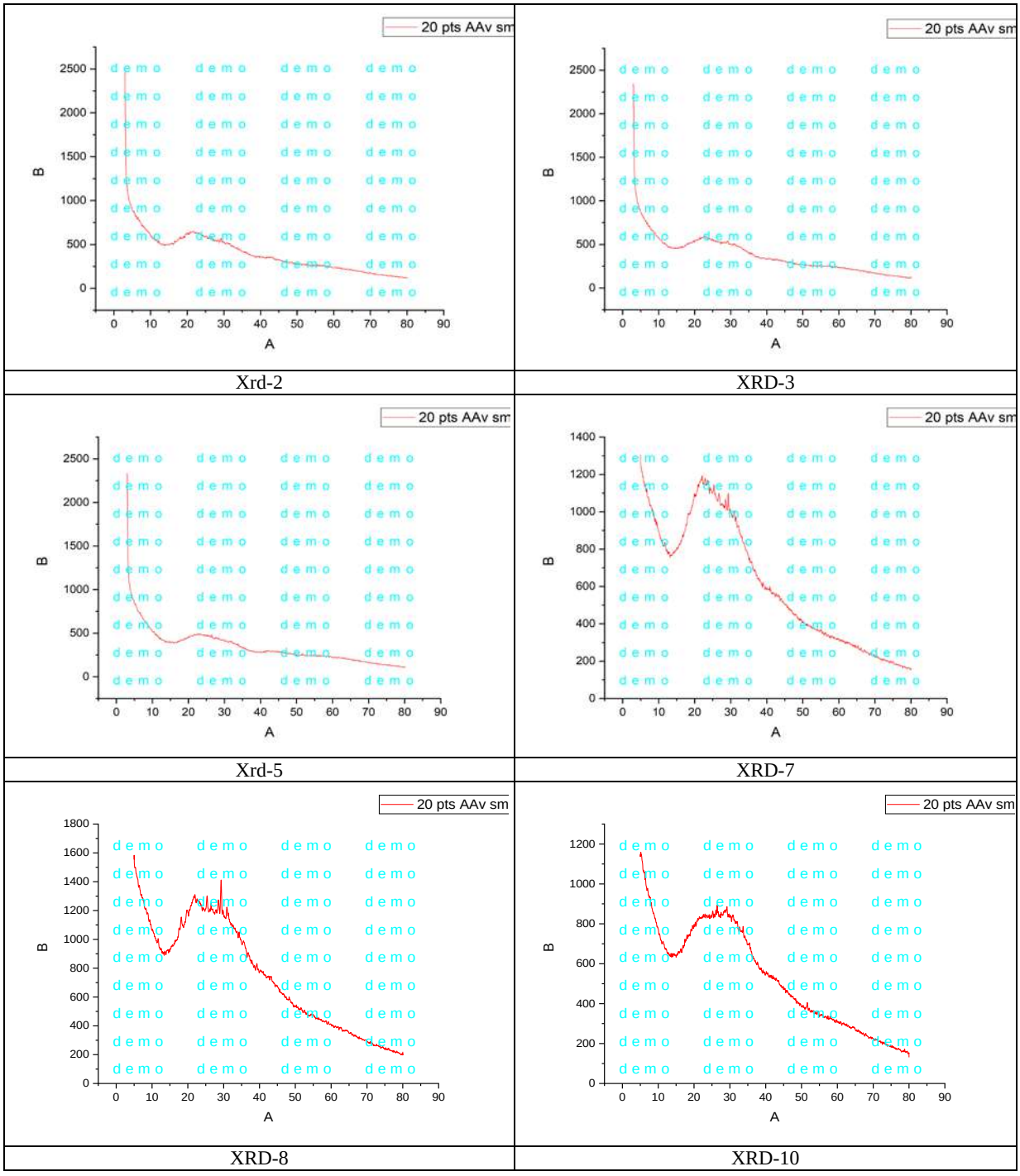


Fig 8: XRD- Results for Root

Table 2: SPSS value for 10 concertation.

Concertations	Plant height	Root height	Number of root	Number of Leaves	Leaves height	Stem width	Height of Stem	Seed Germination
0.0	.888862^{i}	.65574^{h}	1.00664^{a}	.35119^{f}	.65574^{e}	.00577^{a}	.55076^{i}	.11269^{d}
1.0	.28868^{d}	.20817^{d}	.11547^{c}	.11547^{b}	.45826^{d}	.01155^{f}	.11547^{a}	.57735^{j}
2.0	.60828^{h}	.26458^{e}	.17321^{e}	.15275^{c}	.20000^{b}	.02309^{c}	.37859^{g}	.15275^{e}
3.0	.17321^{c}	.30551^{f}	.30551^{i}	.20817^{e}	.22913^{c}	.00577^{a}	.30551^{f}	.57735^{j}
4.0	.28868^{d}	.11547^{a}	.15275^{d}	.20817^{e}	.45826^{d}	.01528^{g}	.15275^{b}	.10017^{c}
5.0	.32146^{g}	.26458^{e}	.25166^{g}	.15275^{c}	.10000^{a}	.00577^{a}	.57735^{j}	.2977^{i}
6.0	.27285^{e}	.11547^{a}	.20817^{f}	.11547^{b}	.10000^{a}	.02367^{d}	.23094^{e}	.05774^{b}
7.0	.17321^{c}	.15275^{c}	.25166^{g}	.17321^{d}	.10000^{a}	.00000	.20817^{d}	.01155^{a}
8.0	.11547^{b}	.41633^{g}	.26458^{h}	.05774^{a}	.10000^{a}	.01000^{b}	.15275^{c}	.20817^{f}
9.0	1.0969^{a}	.20817^{d}	.20817^{f}	.05774^{a}	.10000^{a}	.04853^{e}	.41633^{h}	.23094^{g}
10.0	.30551^{f}	.13229^{b}	.05774^{b}	.11547^{b}	.10000^{a}	.04583^{e}	.20817^{d}	.26458^{h}

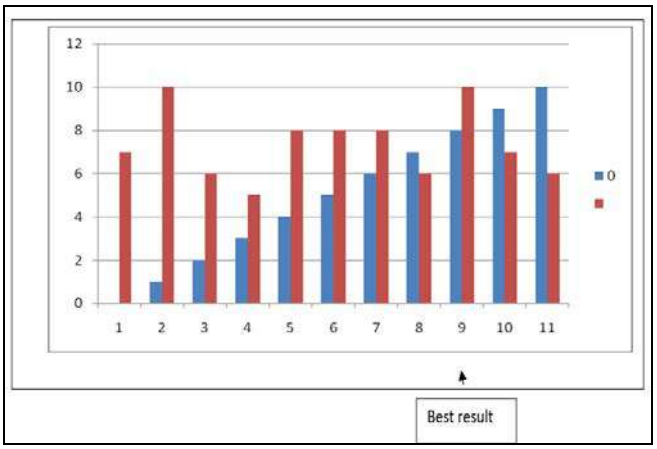


Fig 9: Graph for Root height concentration

0-12 height in cm, 1-11- Agnps concentration I n (ul) Graph for Root height (1 -is control, 2- 1ul, 3- 2ul,4- 3 ul,5- 4ul,6-5ul,7-6ul, 8-7ul, 9 is-8ul, 10 -9 ul, 11-10 ul)

Discussion

In this study shows a presence of secondary metabolites in the crude extract of methanolic from *Mentha arvensis.* Synthesis of silver ions in aqueous solution of silver complex during the reaction with plant extract have been confirmed by color changes. Silver nanoparticle confirmation by UV, SEM, FTIR, XRD.

In hormonal study of plant tissue culture, the consideration of synthesized silver nanoparticle as a hormone and it is to be administrated for root induction in MS medium. The synthesized AgNPs of plant extract can act as a hormone and possess a better root formation in green gram seeds. It is tested in various types of concentrations. The lower concentration of hormone gave a bulge root formation shown, while comparing to the higher concentration level 8 ul of Agnps showing the best result in XRD high peak. So here we considered the lower concentration level of AgNps hormonal analysis is well. Finally it is act as plant growth hormones. XRD value shows the peak presenting in 38 min graph. In XRD 38 minutes presenting peak giving the best result for AgNps. So this *Mentha arvensis* nanoparticles presence in the root of treated green gram seeds confirmed by XRD.

References

1. Afruja Sultana. Cytotoxic and anti-oxidant activity of petroleum ether fraction of Mentha arvensis leaf extract, 2016, 1-52.
2. Ankita Sharma, Sapan Patel. Preliminary phytochemical screening and quantitative analysis of secondary metabolites of *Mentha arvensis* and *Azadirachta indica*,2018:3:114-118.
3. Akbar Dara M, Mubashir H Masoodia, Adil F Walia, Mudasir A Mira, Nida S Shapoob. Antioxidant potential of methanol Root extract of *Mentha arvensis* L. from Kashmir Region,2014:4(03):050-057.
4. Debleena Roy. Phytochemical analysis of field grown and tissue cultured Mentha arvensis L. plants with special reference to antioxidative potentials,2017:4:7.
5. Deepak Dwivedi, Gaurav Khandelwal, Rakesh Kumar Patidar, Vinod Singh. Antimicrobial Activity of *Mentha Arvensis* Against Clinical Isolates Of Human Cariogenic Pathogens- An *In-Vitro* Study, 2012:3(5):1355-1360.
6. Durai Mahendran, Natesan Geetha, Perumal Venkatachalam. Role of Silver nitrate and Silver Nanoparticles on Tissue culture medium and enhanced the plant growth and Development. *In vitro* plant breeding towards Novel Agronomic Traits, 2019, 59-74.
7. Eidla Mikaelle Maciel do Nascimento, Fabíola Fernandes Galvão Rodrigues, Adriana Rolim Campos, José Galberto Martins da Costa. Phytochemical Prospection, Toxicity and Antimicrobial Activity of *Mentha arvensis* (Labiatae) from Northeast of Brazil, 2009.
8. Farnaz Malik, Shahzad Hussain, Alia Sadiq, Ghazala Parveen, Amina Wajid, Shazia Shafat *et al*. Phytochemical analysis, anti-allergic and anti-inflammatory activity of *Mentha arvensis* in animals,2009:6(9):613-619.
9. Fatih Mehmet Kilinc, Nesrin Bozhan, Israt Jahan, Ahmet onay. Plant Tissue culture Goes Nano: Nanoparticles- Mediated Secondary Metabolite Production. II. International Green Biotechnology Congress, 2019.
10. Gupta Sandeep, Ahirwar Dheeraj, Jhade Deenanath, Sharma Neeraj Kumar, Ahirwar Bharti. Pharmacognostic Standardization, Physico and Phytochemical Evaluation of Aerial Parts of *Mentha arvensis* Linn, 2010.
11. Johnson M, Wesely E, Kavitha M, Uma V. Antibacterial activity of leaves and inter-nodal callus extracts of *Mentha arvensis* L, 2011, 196-200.
12. Kamala Priya MR, Priya R Iyer. Anticancer studies of the synthesized gold nanoparticles against MCF 7 breast cancer cell lines.
13. Kumar Chandan, Subash Vishwakarma, Caroline Jeba R. Sheikh Khushbu. Anticancer Activity of *Mentha Arvensis*, 2014.
14. Ling Zhang, Shu-gen Xu, Wei Liang, Jun Mei, Yue-ying Di, Hui-hua Lan *et al*,2015:14(11):2099-2106.
15. Nidhi Sharma, Jacob D. assessment of reversible contraceptive efficacy of methanol extract of Mentha arvensis L.leaves in male albino mice,2002:80:9-13.
16. Nidhi Sharma, Jacob D. Antifertility investigation and toxicological screening of the petroleum ether extract of the leaves of Mentha arvensis L. in male albino mice,2001:75:5-12.
17. Nripendra Nath Biswas, Mohammed Khadem Ali. Antioxidant, antimicrobial, cytotoxic and analgesic activities of ethanolic extract of *Mentha arvensis* L, 2014, 4(10).
18. Prajna Paramita Banerjee, Arindam Bandyopadhyay, Singapura Nagesh Harsha, Rudragoud S Policegoudra, Shelley Bhattacharya, Niranjan Karak *et al*. *Mentha arvensis* (Linn.)-mediated green silver Nanoparticles trigger caspase 9-dependent cell Death in MCF7 and MDA-MB-231 cells, 2017.
19. Ramesh L Londonkar, Pramod V Poddar. Studies on activity of various extracts of Mentha arvensis Linn against drug induced gastric ulcer in mammals,2009:1:82-88.
20. Punuri Jayasekhar Babu, Pragya Sharma, Bibhuti Bhusan Borthakur, Ratul Kumar Das, Pradip Nahar,

Utpal Bora. a Synthesis of Gold Nanoparticles Using Mentha arvensis Leaf Extract, 2015.
21. Raja Muthuramalingam Thangavelu, Dharanivasan Gunasekaran, Michael Immanuel Jesse, Mohammed Riyaz SU, Deepan Sundarajan, Kathiravan Krishnan. Nano biotechnological approach using plant rooting hormones synthesized silver nanoparticle as a "Nano bullets" for the dynamic applications in horticulture -An i*n vitro* and *ex vitro* study, 2016.
22. Senthamarai Manogaran, Navaneetha Krishnan, Vinitha Sree Senthilkumar, Sowmiya Manivannan, Vijayalekshmi Veerababhu, Kilavan Packiam Kannan. Green synthesis of silver nanoparticles and their antimicrobial property of endophytic fungi isolated from *Mentha arvensis* L. and *Psidium guajava* L, 2015.
23. Siva Kumar T, Rathimeena T, Thangapandian V, Shankar T. Silver Nanoparticles Synthesis of *Mentha arvensis* Extracts and Evaluation of Antioxidant Properties,2015:1(2):22-28.
24. Singh Amarjeet, Sharma Pankaj, Lamba HS, Lamba Avneet Kaur. Study of anticataract activity of *Sesbania grandiflora Linn & Mentha arvensis Linn*,2016:1(2):22-29.
25. Srinivas pidugu, Arun T. Antibacterial activity and phytochemical screening of *Mentha arvensis* Linn.against proteus mirabilis from urinary tract infected patients,2012:4:1735-1744.
26. Vineet Soni, Anju Sharma, Pyare Lal Swarnkar. Clonal propagation and evaluation of peroxidase activity during *in vitro* rhizogenesis in Mentha arvensis L, 2016, 53-60.

International Research Journal of Plant Science (ISSN: 2141-5447)
Vol. 12(5) pp. 01-5, June, 2021
Available online @ https://www.interesjournals.org/plant-science.html
DOI: http:/dx.doi.org/10.14303/irjps.2021.27
Copyright ©2021 International Research Journals

Research Article

Evaluation of phytochemical compound in leaf extract of calanthe masuca (d.don) lindl., using UV-VIS, FTIR and GCMS analysis - An orchidaceae member

Kavitha D*[1], Nandagopalan V[2]

[1,2]PG & Research Department of Botany, National College, Tamil Nadu, India.

Correspondence email: shalini.sdofficial@gmail.com

Abstract

The present investigation was focused on the UV-VIS spectrum, FT-IR and GC-MS Analysis of *Calanthe masuca* (D.Don) Lindl. The organic solvent methanol was taken for extraction. The leaf part of *Calanthe masuca* which belongs to the Orchidaceae family. The UV-VIS profile showed different peaks ranging from 220-970 nm with different absorption respectively. The FTIR spectrum confirmed the presence of compound such as alcohol, alkane, carboxylic acid, aldehyde, allene, cyclic alkene, alky halide, aromatic compound, isothiocyanate and alkenes in leaf methanol extract. The results of the GC-MS analysis provide for leaf extract which have dissolved to shows different peaks. The major peak of Leaf extract are 3-Tetradecanynoic acid, 9,12- Octadecadienoic acid (Z,Z)-, n-Hexadecanoic acid, L-proline, 1-acetyl, Neophytodiene. Therefore this plant had potential bioactive compounds.

Keywords: Bioactive compound, *Calanthe masuca*, UV-VIS, FTIR and GC- MS analysis.

Introduction

Orchids are generally to development of the flowering plant ant it belongs to the angiosperm species and are widely known for their economic importance medicinal therapeutic use. The first documentary evidence to summit the Chenese and to medicinal uses of orchids (Paudel et al., 2018). The earliest reported that use of the medicinal orchids at 28th century B.C. When Shen-nung described as the *Bletilla striata* and *Dendrobium* species of *Materia Medica*. The literal meaning of the term orchid (*órkhis*), in Greek words is testicles and it was Theophrastus who first coined the term as the anatomy of plant resembles testicles. Orchids have been subjected for phytochemical and pharmacological studies (Thomas et al., 2013).

The India is the richest habitats of orchids and was comprise of about 2500 species in 167 genera (Haridas et al., 2016). The orchid's species certain constituent such as alkaloids, flavonoids etc suggest medicinal properties **(**Jyoti et al., 2018). In India, some orchid's plants like *Eulophia campestris, Orchis latifolia, and Vanda roxburgii* were drawn the attention of scientific community and because of the medicinal properties. Some orchids have deal with the phytochemistry and medicinal uses of orchids. Biological activity naturally present in this plants. They act as natural system of plants aroma colour and flavor (Dong Chen et al., 2015).

The important role in cure treating human disease such as cancer, coronary heart diseases, diabetes and infectious diseases. Therapeutic phytochemical compounds and various secondary metabolites such as flavonoids, carotenoids, alkaloids, anthocyanidins, phenolics, tannins, carboxylic acids, terpenoids, amino acids and inorganic acids has a different bio-potential of medicinal plants. The identification of bioactive compound from the medicinal plants using various instrumentation techniques such as UV-VIS, FTIR and GC-MS analysis (Theng and Korpenwar, 2015; Antony Sandosh et al., 2103; Phukan et al., 2017).

The plant *Calanthe masuca (D.Don) Lindl* is belongs into family Orchidaceae. The genus *Calanthe* was described in 1821 by Robert Brown in Botanist's Repository. *Calanthe masuca* is an endangered terrestrial orchid. There is no report on compound isolation in the *Calanthe masuca* leaf extract. The present studies into identify the bioactive compound by the Spectral analysis such as UV-VIS, FTIR and GCMS analysis. This study was carried out in order to secure some standards for standardization of these crude drugs.

Materails and Methods

Plant material collection & authentication

The plant leaf materials of *Calanthe masuca* were collected from the Kolli Hills of Namakkal District, Tamil Nadu, and

India. The leaf parts of the plant were washed thoroughly 2-3 times with running water and once sterile with distilled water. The fresh plant material were shade into two month dried and coarsely powdered separately and stored in air tight glass bottles for further analysis in laboratory. The plant was authenticated at Botanical survey of India (BIS), Southern circle, Coimbatore, India. The Voucher number is BSI/SRC/5/23/2019 Tech/252. The Herbarium was stored in the National College, Tiruchirappalli.

Plant sample extraction

100g of dried leaf powder was soxhlet extracted with Methanol solvents for about 12-24h the crude extract of methanol solvent were evaporated by the vacuum rotary evaporator pressure reduced. The sample extract were collected in air tight container and refrigerator at 4°C until further analysis.

UV-VIS

The plant extract were centrifuged into 3000 rpm for 10 min and after filtered that through the Whatmann No.1 filter paper. In this sample were diluted into 1:10 with the same solvent. The plant extract have been scanned for the wave length ranging at 200nm to 1100 nm using Perkin Elmer spectrophotometer and the characteristic peaks were detected. The peak values at the UV-VIS were recorded (Theng & Korpenwar, 2015).

FTIR analysis

The FTIR analyses have been performed using Perkin Elmer spectrophotometer system. This was used to deck the characteristic peaks in ranging from 400- 4000 cm^{-1} and their functional groups. The peak values of the FTIR were recorded and were as each and every analysis was repeated twice for the spectrum confirmation (Jain et al., 2016).

GC-MS analysis

The analysis at unidentified constituents of GC-MS plays major role in plant origin. The leaf methanol extract containing different compounds of Calanthe masuca was subjected for (GC-MS) analysis. Instruments and chromatographic circumstance GC-MS examination was carried out on GC clarus 500 Perkin Elmer system containing a Aoc-2oi auto analyst and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument retaining the subsequent condition; column Elite-1 attached silica capillary column 30x0.25mmxIDx1µm of capillary column, composed of (100% dimethyl poly siloxane), the operational electron impact mode at 70 eV; and the helium (99.99%) have been as transporter gas at a persistent flow of 1 ml/minute and an injection capacity of 0.5 EI was employed (split ratio of 10:1) inject or temperature 250°C; ion –source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/ minutes, to 200°C, minutes, then 5°C/ minutes to 280°C/ min, finish with a 9 minutes isothermal at 280°C. Mass spectra have been occupied at 70 eV; and the scan intermission of 0.5 seconds and the fragments from 45 to 450 Da. To evaluate constituent is identified in the mass detector. The spectrum of the recognized constituents stored in NIST library and concludes the name and molecular weight (Sathish et al., 2012; Antony Sandosh et al., 2103).

RESULT AND DISCUSSION

UV-VIS analysis

The UV-VIS analysis preformed for identification of phytocontituents present in methanol leaf extract of Calanthe masuca. The UV- visible spectra have been performed to identify the compounds containing σ-bonds, π-bonds and lone pair of electrons, chromophores and aromatic rings. The qualitative UV-VIS profile of methanol leaf extract of Calanthe masuca was taken at the wavelength of 200 nm to 1100 nm due to the sharpness of the peaks and proper baseline. The methanol leaf extract were showed the peaks at (Table 1) 220.55, 364.10, 397.55, 531.70 and 664.50 nm with the absorption 4.000, 1.0848, 1.3319, 0.1693 and 0.2762 nm respectively (Figure 1) (Theng & Korpenwar, 2015; Jain et al., 2016;

Sathish et al., 2012; Elufioye & Mada, 2018).

FTIR analysis

The FTIR spectrums have been used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The results of FTIR peak values and functional groups were represented in (Table 2 and Figure 2). The FTIR spectrum confirmed the presence of alcohols, alkane, carboxylic acid, aldehyde, allene, cyclic alkene, aliphatic ether, amine, flurocompound, alkyl halide and isothiocyanate in leaf methanol extracts. Hence, the methanol extracts subjected to FTIR analysis is used for the identification of chemical constituents' presents in *Calanthe masuca* is proved to be a reliable and sanative method for detection of biomolecular composition (Raphael Marandi et al., 2018; John Peter Paul and Amster Regain Lawrence, 2017; Perraudin et al., 2006).

Table 1: UV-VIS Peak values of methanol leaf extract of *Calanthe masuca.*

S.NO	Wavelength (nm)	Absorption Peak(O.D)
1	220.55	4.0000
2	364.10	1.0848
3	397.55	1.3319
4	531.70	0.1693
5	664.50	0.2762

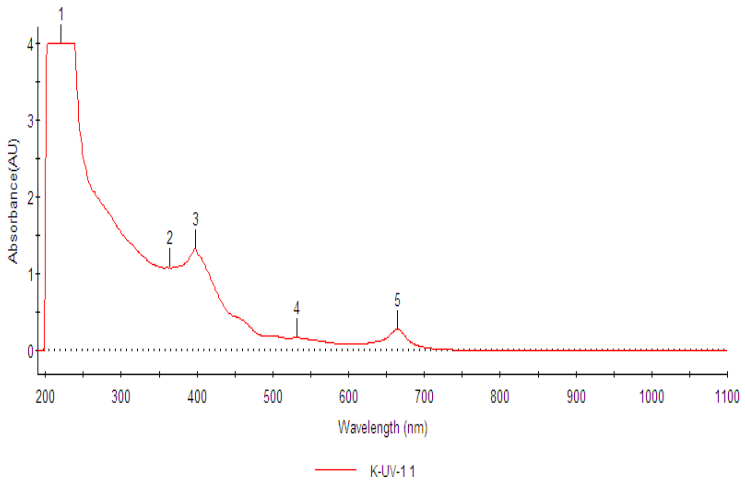


Figure 1. UV-VIS spectra of methanol leaf extract of *Calanthe masuca*.

Table 2: FTIR Peak values functional groups of methanol leaf extract of *Calanthe masuca.*

S.NO	Peak Value	Functional group	Functional group Name	Vibrations
1	656.33	C-Br	Alkyl Halide	Stretching
2	1018.94	C-F	Flurocompound	Stretching
3	1030.62	C-N	amine	Bending
4	1049.62	C-O	esters	Stretching
5	1112.96	C-O	Aliphatic ether	Stretching
6	1413.32	O-H	Carboxylic acid	Stretching
7	1452.98	C-H	Alkane	Bending
8	1646.13	C=C	Cyclic alkene	Stretching
9	2050.20	C=C=C	allene	Stretching
10	2523.60	C-H	aldehyde	Stretching
11	2837.87	O-H	Carboxylic acids	Stretching
12	2864.13	O-H	Carboxylic acids	Stretching
13	2947.58	C-H	Alkane	Stretching
14	3401.49	O-H	alcohol	Stretching

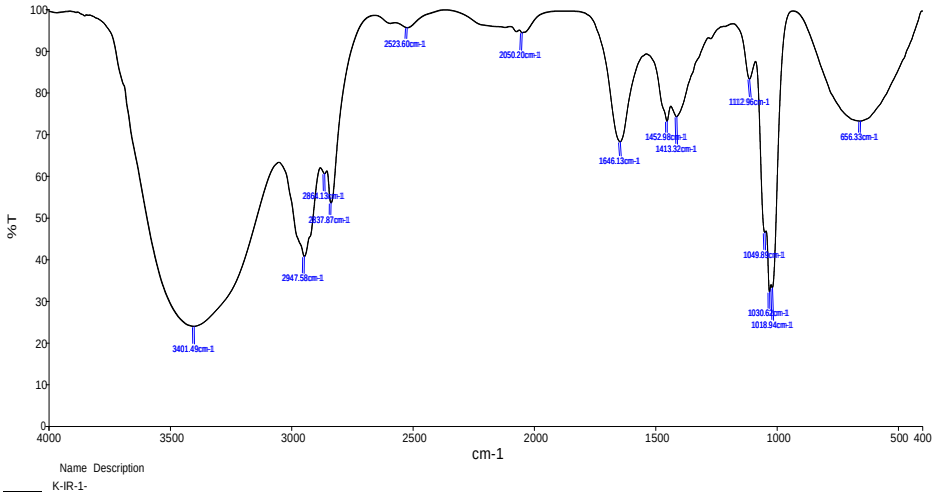


Figure 2. FTIR spectra of methanol leaf extract of *Calanthe masuca.*

GC-MS analysis

The chemical constituents identified by the GC-MS analysis of the methanol leaf extract of *Calanthe masuca* was enumerated along with their molecular formula, retention time and peak area (Table 3 and Figure 3) (Perraudin et al., 2006; Haridas et al., 2016). The GC-MS analysis of *Calanthe masuca* revealed the presence of methanol leaf extract 20 compounds identified in the methanol extract (Perraudin et al., 2006). The chromatogram of methanol plant leaf extract shows 5 prominent peaks as L-Proline, 1-Acety- ($C_7H_{11}NO_3$)

Table 3: Phytochemical Compounds identified in the methanol leaf extract of *Calanthe masuca* by GC-MS Analysis.

S.NO	R/ T	Peak Area %	Name of the compound	Molecular Formula	Molecular Weight
1	9.415	1.14	1-pyrazolecarboxamide	$C_4H_5N_3O$	111
2	12.038	8.91	L-Proline, 1-Acetyl	$C_7H_{11}NO_3$	157
3	19.042	0.63	Ethanone, 1-(3,4-dimethoxyphenyl)-	$C_{10}H_{12}O_3$	180
4	24.405	0.14	Cyclopentane,1,1,3,3-tetramethyl-	C_9H_{18}	126
5	24.515	6.04	Neophytadiene	$C_{20}H_{38}$	278
6	24.621	0.67	2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	$C_{20}H_{40}$	280
7	24.937	1.32	3,7,11,15-Tetramethyl-2-hexadecen-1-	$C_{20}H_{40}O$	296
8	25.250	1.71	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296
9	26.007	0.61	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270
10	26.666	15.85	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256
11	28.222	0.33	Hexadecanoic acid	$C_{16}H_{32}O_2$	294
12	28.680	1.86	8,11-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	256
13	28.780	1.06	3,6-octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294
14	28.954	0.34	Dodecnal	$C_{12}H_{24}O$	184
15	29.192	1.94	2-hexadecan-1-ol,3,7,11,15-tetramethyl-,[R-R*,R*-(E)]]-	$C_{20}H_{40}O$	296
16	29.369	24.92	9,12-Octadecadienoic acid, methyl ester	$C_{18}H_{32}O_2$	280
17	29.455	26.16	3-Tetradecanynoic acid	$C_{14}H_{24}O_2$	224
18	29.780	3.35	Octadecanoic acid	$C_{18}H_{36}O_2$	284
19	33.827	0.42	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	$C_{12}H_{23}O_2$	213
20	38.301	2.61	Squalene	$C_{30}H_{50}$	410

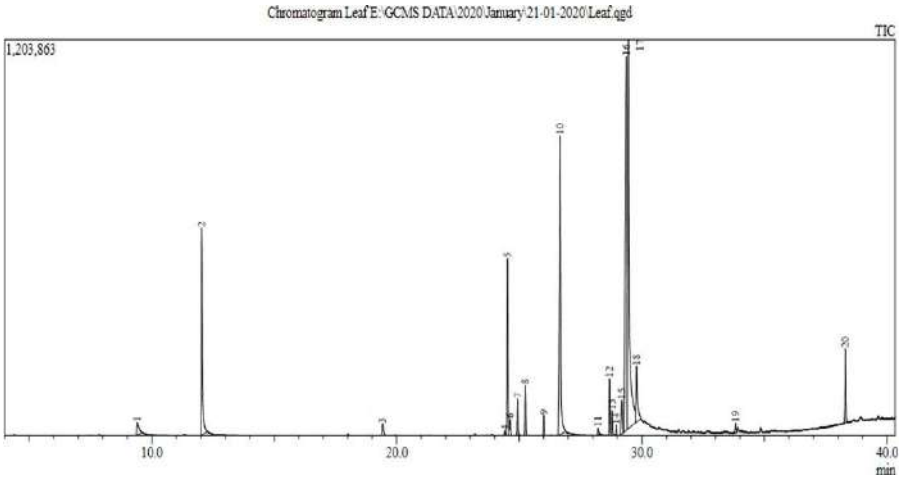


Figure 3. GC-MS Chromatogram of methanol leaf extract of *Calanthe masuca.*

with retention time of 12.038 and peak area of (8.91%), Neophytadiene ($C_{20}H_{38}$) with retention time of 24.515 and peak area of (6.04 %), n-Hexadecanoic acid ($C_{16}H_{32}O_2$) with retention time of 26.666 and peak area of (15.85%), 9,12-Octadecadienoic acid, methyl ester ($C_{18}H_{32}O_2$) with retention time of 29.369 and peak area of (24.92%) and 3-Tetradecanynoic acid ($C_{14}H_{24}O_2$) with retention time of 29.455 and peak area of (26.16) (Thomas et al., 2013; Katta et al., 2020).

It contains five major peaks along with many small peaks indicating presence of major compounds (Table 3). Structural assignment of GC retention data of compounds have been based on spectral matching with NIST library (National Institute of Standards and Technology). The small peaks have been attributed to the compounds present in small quantities as well as disintegrated major compounds. The peaks related to low retention times are mainly low polar plant compounds (Keerthiga and Anand, 2015; Swathi Krishna et al., 2017; Mistra et al., 2018). The GCMS analysis of the methanol leaf extract resulted many compounds which have diverse use in anti-diabetic, antibacterial, antifungal, antioxidant, anti-inflammatory Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Anliandrogenic, Flavor, Hemolytic, 5 Alpha reductase inhibitor, anti-tumor and anticancer properties have been identified. In addition to these, the plant is extensively used as a fracture medicine by tribal people of the area.

Conclusion

This investigation has given preliminary information to determine the chemical composition of *Calanthe masuca*

using spectral of UV-VIS, FTIR and GC-MS techniques. UV spectrophotometric analysis is a simple, rapid and accurate method for the determination of bioactive compound present in crude drug powder of medicinal plants. The FTIR spectral analysis has been shows the presence of characteristic functional groups in methanol leaf extracts of *Calanthe masuca*. In the present study 20 bioactive compounds have been identified from methanol leaf extract of *Calanthe masuca* by Gas Chromatogram-Mass spectrometry (GC-MS) analysis. Whereas *Calanthe masuca* plant extract contains various bioactive compounds 3-Tetradecanynoic acid, 9,12-Octadecadienoic acid (Z,Z)-, n-Hexadecanoic acid, L-proline, 1-acetyl, Neophytodiene and it has identify pharmaceutical importance. The presence of these bioactive compounds in *Calanthe masuca* plant cure specific diseases such as anticarcinogenic, antidiabetic, antimicrobial, antioxidant and anti-inflammatory properties.

References

Antony Sandosh T, Paul John Pater M & Yesu Raj J(2013). Phytochemical Analysis of *Stylosanthes fruticose* using UV-VIS, FTIR and GC-MS. Res J Chem Sci. 3(4): 14-23.

Dong Chen N, Fu Chen N, Li J, Yun Cao C, Mel Wang J, & Ping Huang H(2015). Similarity Evaluation of Different Origins And Species Of *Dendrobium* By GC-MS And FTIR Analysis Of Polysaccarides. Intern J Analytical Chem. Pp. 1-8.

Elufioye TO, & Mada OO(2018). GC-MS, FTIR, UV Analysis andin vitro Antioxidant Activity of a Nigeria Poly Herbal Mixture: Pax Herbal Bitters. Free Radicals and Antioxidants. 8(2): 74-81.

Haridas R, Manorama S, & Thomas B(2016). Phytochemical characterization of potential bioactive compounds from medicinal Orchid: *Malaxis rheedei* sw. Intern J Pharmaco Screening Methods. 6(2): 53-56.

Jain PK, Soni A, Jain P, & Bhawsar J(2016). Phytochemical analysis of *Mentha spicata* plant extract using UV-VIS, FTIR and GC-MS technique. Journal of chemical and Pharmaceutical Research. 8(2): 1-6.

John Peter Paul J, & Amster Regain Lawrence R(2017). Phytochemical analysis of *Sargassum linearifolium* (Turner) C.Ag. (Brown seaweed) using UV-VIS, FTIR and HPLC. Intern J Herbal Medicine. 5(6): 14-17.

Jyoti DA, Mohammad A, Manoj K, & Raiesh K(2018). FTIR analysis for screening variation in antimicrobial activity of fresh and dried leaf extract of *Rhynchostylis retusa*: A threatened orchid species of Assam, North east India. International research Journal of Pharmacy. 4(7): 187-189.

Kalaiarasan A, & Ahmed John S(2011). GC-MS Analysis of *Bulbophyllum* Kaitense Rechib. Pseudobulbs Estern Ghats of India. Intern J Chem and App. 3(3): 215-220.

Katta J, Rampilla V, & Mohamad KS(2020). Evaluation of Phytochemical and Pharmacological aspects of epiphytic orchid *Luisia zeylanica* Lindl. Intern J Pharmaceu Sci and Res. 11(3): 1333-1349.

Keerthiga, & Anand SP(2015). Bioactive Compound Evaluation of ethanol extract from *Geodorum densiflorum* (Lam.) Schltr.By GC-MS Analysis. International Journal of Pharmacological Research. 5(6): 139-144.

Mistra D, Rout SK, & Priyadarshani Kar A(2018). Phytochemical Screening and GC-MS Analysis of Methanol Extract of the leaves of *Nerium oleander* Linn. Acta Scientific Pharmaceutical Science. 2: 11-14.

Paudel MR, Chand MB, Pant B, & Pant B(2018). Antioxidant and cytotoxic activities of *Dendrobium moniliform* extracts and the detection of related compounds by GC-MS. BMC Complementary and Alternative Medicine. 18; 134: 1-9.

Perraudin F, Popovici J, & Bertrand C(2006). Analysis of headspace-solid micro extracts from flower of *Maxillaria tenuifolia* Lindl. By GC-MS. Electronic Journal of Natural Substances. 1: 1-5.

Phukan H, Bora CR, & Mitra PK(2017). Phytochemical Screening and GC-MS Analysis of Methanolic leaf extract of an Endemic plant *Kayea assamica*. Journal of Pharmacy and Biological Science. 12(5): 7-16.

Raphael Marandi R, John Britto S, & Prabhat Soreng K(2018). FT-IR, HPLC, GC-MS and wis of *Peucedanum dhana* buch.-Ham.Ex CB Clarke (Bhojraj): A rare and endangered medicinal plant of Chotanagpur, Jharkhand. International Journal of Research in Pharmacy and Pharmaceutical Sciences. 3(1): 119-126.

Sathish S, Janakiraman N, & Johnson M(2012). Phytochemical Analysis of *Vitex altissima* L. using UV-VIS, FTIR and GC-MS. International Journal of Pharmaceutical Science and Drug Research. 4(1): 56-62.

Swathi Krishna C, Chandra R, & Khaleel KM(2017). Phytochemical Screening and GC-MS Studies of *Syzgium dhaneshiana* leaf and bark extracts. International Journal of Pharmacuetical Science and Research., 8(5): 2277-2281.

Theng KB, & Korpenwar A(2015). Phytochemical analysis of ethanol extract of *Amipelosisus latifolia* (Roxb) Planch tuberous root using UV-VIS, FTIR AND GC-MS. International Journal of Pharmaceutical Science and Research. 6(9): 3936-3942.

Thomas E, Aneesh TP, Thomas DG, & Anandan(2013). GC-MS Analysis of Phytochemical compounds present in the rhizomes of *Nervilia aragoana* gaud. Asian J Pharma and clinic Res. 6(3): 68-74.

International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X; Impact Factor: RJIF 5.12
Received: 04-04-2021, Accepted: 02-05-2021, Published: 19-05-2021
Volume 6, Issue 3, 2021, Page No. 192-195

Evaluate the antibacterial activity by synthesis of silver nanoparticles from pogostemon *Benghalensis* (Burm. F.) Kuntze aerial part

T Manikandan[1], V Nandhagopalan[2], A Lakshmi Prabha[3*]

[1] Research Scholar, Department of Botany, Bharathidasan University, Tiruchirapalli, Tamil Nadu, India

[2] Associate Professor, Department of Botany, National College, Tiruchirapalli, Tamil Nadu, India

[3] Professor and Head, Department of Botany, Bharathidasan University, Tiruchirapalli, Tamil Nadu, India

Abstract

In recent years green synthesis of silver Nanoparticles has been increased for diverse application. In this study we proposed lamiaceae family plant. Pogostemon benghalensis for synthesis of silver nanoparticles and their efficacy in antibacterial activity against five humen pathogens. The direct method was chosen for silver nanoparticles synthesis using water extract of Pogostemon *Benghalensis*. Green synthesized silver nanoparticles were characterized by UV-Vis spectroscopy, FTIR, SEM, EDS. The UV-Vis spectro depicts surface Plasmon resonance peak present at 450nm. The three dimensional structure of silver nanoparticles was seen through SEM analysis. The observed FT-IR peaks showed functions groups and the stretch of bonds that responds to Agnps synthesis. The synthesized nanoparticles showed remarkable antibacterial activity against three gram positive bacteria strains Staphylococcus aureus, Enterococcus faecalis, Enterococcus phyogenes and two gram negative bacterial Strains Salmonella typhi, Enterobacter aerogenes. The highest antimicrobial activity against Enterococcus pyogenes (16mm) and the lowest antibacterial against Enterococcus faecalis (7mm). The present suggest that Agnps can be used for potential of antibiotic drugs.

Keywords: pogostemon *Benghalensis*, AgNPs, FTIR, SEM

Introduction

Nanobiotechnology is an emerging field of contemporary modern research that involves the manipulation of materials at the atomic or molecular level for their diverse properties [1–3] Nanomaterials find their applications in several fields of biomedical sciences, such as gene delivery, biosensing, image analysis, and drug delivery. Nanostructures, such as silver, gold, CuO, and Zn Opossess the the rapeutical potential and hence, used for various biomedical applications including as antimicrobial agents [4–10]. Therefore, studies in the field of nanotechnology and material science are rapidly growing to develop and screen wide ranging materials by using a number of methods [11, 12]. Alternatively, biogenic synthesis
of AgNPs that uses plants and microbes has gained wide acceptance because these approaches are simple, rapid, efficient, and cost-effective. [13, 14, 15]. Phyto fabrication of AgNPs depends on the available functional groups in plants or plant extracts that might act as reducing and capping agent [16] *Pogostemon cablin* Benth. (Patchouli) is a commercial. Medicinal plant belongs to the family Lamiaceae. Its raw materials are consumed in the pharmaceutical, fragrance, cosmetic, flavor and perfumery industries [17]. The multidisciplinary field of nanotechnology mainly concern to synthesis and design nano materials and device with in the range of 1-100nm. But the range of size of nanoparticles in some literature grater than 100nm [18]. A large of traditional medicinal plants used in the treatment of cut and injuries, blood pressure, skin diseases, dysentery, blood deficiency, jaundice, urinary trouble, cough, asthma, influenza, malaria etc [19]. These plants are as source of bio-reductant and stabilizers and reported to contain alkaloids, glycosides, tannins, saponnins and aromatic compounds [20, 21]. The most common major materials used for manufacturing nano -products at present is silver, then followed by carbon, Titanium, silicon, Zinc and Gold, Silver nano particles are used as anti-bacterial agents, as catalytic and also as optical sensor. By using plant extract methods, a lot of groups are involved to synthesis silver nanoparticles from plants like Aloe *vera* [22], To synthesis silver nano particles by plant extract biosynthesis method from leaves of *Pogostemon benghalensis. (Family-Lamiaceae, Assamese vernacular name-shukloti)* and study their characterization, this traditional medicinal plant is selected for the applications used in cut and injuries by local peoples. Mainly leaves of this plants use by local women's after delivering a child. It is a shrub with a height of 4 to 5ft, leaves are elliptic. The whole plants use as medicinal purpose. Leaves, Young shoots and juice of leaves are used to cure stomach problems, increase digestive power, stop bleeding and mainly for menstrual disorders.

Materials and Methods

Silver Nanoparticle Synthesis

Materials and Methods

Plant material and preparation of Extracts

Aerial parts of *Pogostemon benghalensis* were dried for 25 days under the shadow at room temperature and ground into fine powder. 5g of powder was used to make aqueous extract using 100ml of distilled water. It was gently heated and filtered through a filter paper

Synthesis of Silver Nanoparticles

1mM aquesous solution of silver nitrate ($AgNO_3$) was prepared and used for the synthesis of silver nanoparticles. 1000 ml of leaf extract was taken in a separate conical flask

and 100µl of 1mM $AgNO_3$ was added than heated until the colour changed from golden to brown.

Characterization of Silernanoparticles by UV- Visible spectroscopy analysis

Bioreduction of aqueous Ag^+ ions can easily be followed by UV - Vis spectrophotometer and one of the most important features in optical absorbance spectra of metal nanoparticles is surface Plasmon band, which is due to collective electron oscillation around the surface mode of the excitation of their surface Plasmon response (SPR), when dissolved in water. The colour of the solution changes from colour less to brownish yellow with in 5 minutes at microwave and than to dark brown.

Characterization of Silver nanoparticles by Fourier Transform Tnfrared Spectroscopy (FTIR)

Silver nanoparticles synthesized after 5 hour of reaction of 1Mm $AgNO_3$ solution with *p.benghalensis* leaves extract were centrifuged at 10,000 rpm for 45 mins at room temperature after which the pellet was re dispersed in sterile distilled water was repeated three times to ensure better separation of free entities from the nanoparticles. The purified pellet was then tried and subjected to Fourier transform infrared (FTIR, IR affinity-1, Shimadzu corporation, Tokyo, japan) spectroscopy measurent using the potassium bromide (KBr) pellet technique in diffuse reflection mode at a resolution of $4cm^{-1}$ The nanoparticle powder was mixed with KBr and exposed to an infrared source of 400 cm^{-2} to 4000 cm^{-2}

Scanning Electron Microscope (SEM with EDAX analysis

In Scanning Electron Microscope analysis, the energy of electron beam current was continuously adjusted from 1 pA to 1µA to suit the type of examination in progress. SEM analysis was performed using Zeiss – SEM machine. Thin film of the samble was prepared on a corbon coated copper grid by just dropping very small amount of the sample on the grid. The film of the SEM grid was allowed to dry by putting it under a mercury lamp for 5 min. Tha each sample was analysed by the SEM. The presence of silver was confirmed by EDAX

Antibacterial activity of *Pogostemon benghalensis* leaf aqueous extract from AgNPs synthesis

The antibacterial assays were done by disc diffusion method.

The antibacterial assays of *Pogostemon benghalensis* leaf extract fraction and silver nanoparticles synthesized from aerial part extracts were performed. Fresh overnight culture of inoculums 100µl of each bacterium were spread on to Nutrient agar plates. Sterile discs of 5mm diameter contain 25µl, 50µl, 75µl and 100µl sample (10 mg/1ml conc) and AgNPs sample (1mg/1ml conc.) with for discs were plate and five antibiotic discs were placed in separate plate for positive control. The inoculated plates were incubated at 37^0c for 24 h. The antibacterial activity was evaluated by measuring the diameter of the inhibition zone of the test microorganism

Result

Silver nanoparticles (AgNPs) were synthesiszed using *Pogostemon benghalensis* plant extract. The AgNPs showed Yellowish-brown color in aqueous solution due to excitation of Surface Plasmon vibrations. Reduction of silver ions to solver nanoparticles could be followed by color change (Fig.1). AgNPs were investigated to evaluate their Antibacterials activity against Gram Positive bacteria (Staphylococcus *aureus*, Enterococcus *faecalis*, Enterococcus *pyogenes*), Gram Negative bacteria (Salmonella *typhi*, *Enterobacter aerogenes*) using disc diffusion method (Fig2). The Highest zone of inhibition was observed in Enterococcus *pyogenes* (16mm) and Lowest zone inhibition was observed in Enterococcus *faecalis* (7mm). All these results indicates that the AgNPs can be used as control the bacterial growth. So *Pogostemon benghalensis* AgNPs used as therapeutic purpose.

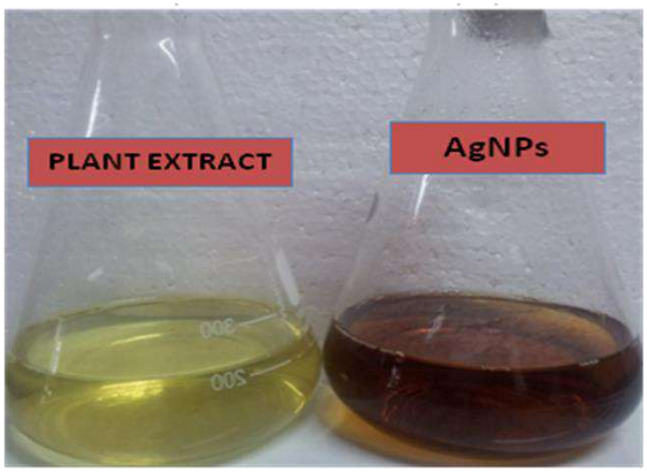
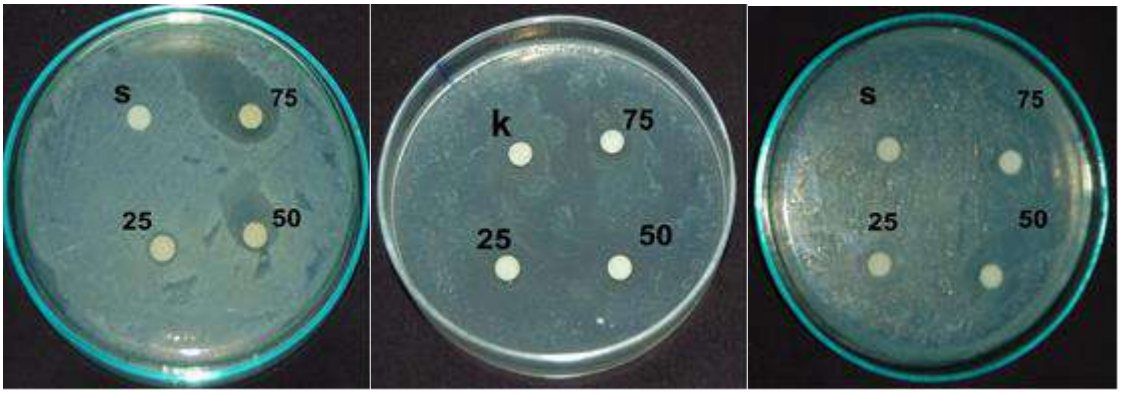
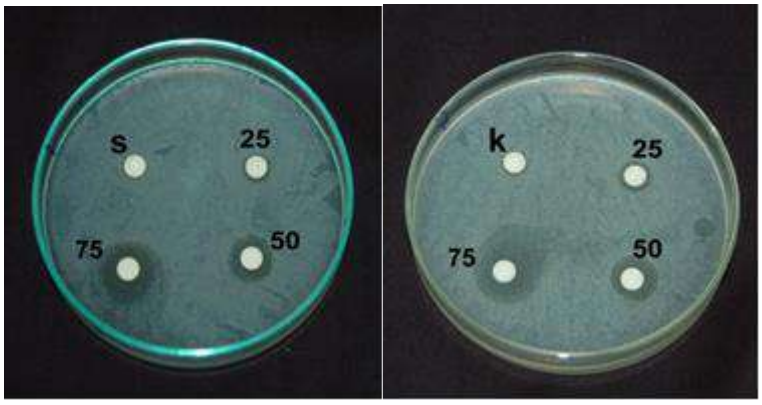


Fig 1: Reeduction of Silver Nanoparticles indicating by colour change



Staphylococcus aureus Salmonella typhi Enterococcus faecalis



Enterococcus pyogenes Enterobactor aerogenes

Fig 2: Antibacterial activity of AgNPs synthesis from *pogostemon benghalensis* Aerials part aqueous extract

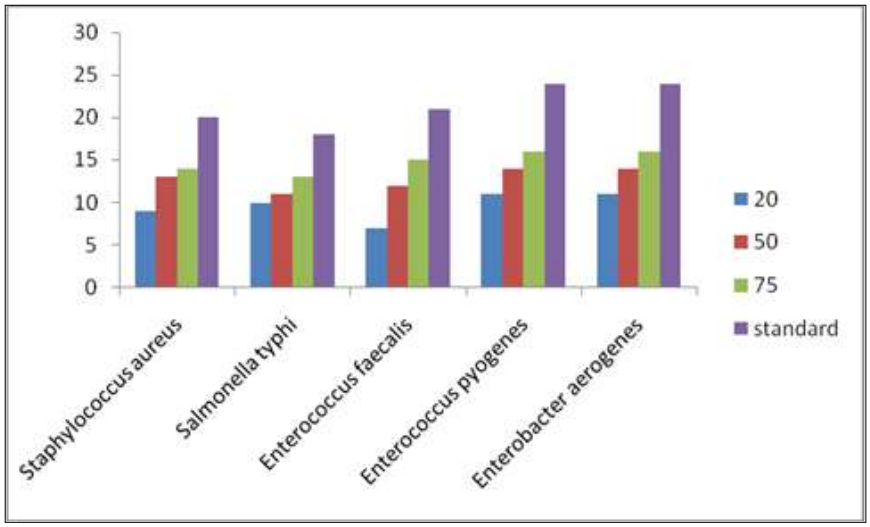


Fig 3

Table 1: Antibacterial activity of AgNPs synthesis from *pogostemon benghalensis* Aerials part aqueous extract

Bacteria name	Zone of inhibition (mm)			Antibiotic disc
	25µl	50µl	75µl	Standard disc
Staphylococcus aureus	9 mm	13 mm	14 mm	20
Salmonella typhi	10 mm	11 mm	13 mm	18
Enterococcus faecalis	7 mm	12 mm	15 mm	21
Enterococcus pyogenes	11 mm	14 mm	16 mm	24
Enterobacter aerogenes	8 mm	10 mm	13 mm	23

Conclusion

In conclusion, the present study reveals that the AgNps inhibits the growth of all the tested pathogenic gram-positive and gram-negative bacteria, which indicates that the Ag+ with plant extract may contain a broad range of antibacterial activity. The AgNps can be used as biocompatible, effective therapeutic drug against bacterials.

Acknowledgment

The authors thank the University research fellowship for support and TM thank Bharathisadan University for providing fellowship

Conflict of interests

The authors have nothing to declare

Reference

1. Saxena A, Tripathi RM, Zafar F, Singh P. *Mat.* Lett,2012:67:91.
2. Akhtar MS, Panwar J, Yun YS. *ACS Sust. Chem. Eng,2013:*1:591.
3. Rudramurthy GR, Swamy MK, Sinniah UR, Ghasemzadeh, *Molecules, 2016:*21:836.
4. She B, Wan X, Tang J, Deng Y, Zhou X, C Xiao. Sci. Adv. Mater,2016*:*8:1074.
5. Tao G, Wang Y, Liu L, Chang H, Zhao P, He H, *Sci. Adv. Mater,2016:*8:1547 (2016).
6. He H, Caic R, Wang Y, Tao G, Guo P, Zuo H, *et al. Int. J. Biol. Macromol,2017:*104:457.
7. Singh S, Biswas K, Chowdhury S, Mohanta YK, J. Bandyopadhyay, *Mater. Focus,2017:*6:297.
8. Al-Ajmi MF, Hussain A, Alsharaeh E, Ahmed F, Amir S, Anwar MS, *et al. Sci. Adv. Mater,2018:*10:349.
9. Mohanta YK, Biswas K, Bandyopadhyay J, Tamang A, De D, Mohanta D, Panda SK, *et al. Mater. Focus,2018:*7:94 (2018).
10. Yang M, Wang Y, Cai R, Tao G, Chang H, Ding C, *Sci. Adv. Mater,2018:*10:761.
11. Vahabi K. Mansoori GA, Karimi S, *Insciences J., 2011:*1:65.
12. Akhtar MS, Ahmad U. *Rev. Adv. Sci. Eng,2014:*3:197.
13. Jain N, Bhargava A, Majumdar S, Tarafdar JC, Panwar J, *Nanoscale,2011:*3:635.

14. Swamy MK, Sudipta KM, Jayanta K, Balasubramanya S Nanosci, 2015a, 5:73.
15. Swamy MK, Akhtar MS, Mohanty SK, Sinniah UR. *Spectrochim*. Acta Part A,2015b:151:939.
16. Chiguvare H, Oyedeji OO, Matewu R, Aremu O, Oyemitan IA, Oyedeji O, *et al*. Molecules,2014*:*21:774.
17. Swamy MK, Sinniah UR. Molecules,2015:20:8521.
18. Bian F, Zhang X, Wang Z, Wu Q, Hu H, Xu j,*Chin.Phys.Lett*,2008:25(12):4463-4465
19. Sikmon JE, Morales MR, Phippe WB, Vieira RF, Janick J. (Ed.). Z. H.). ASHS press, Alexandria, VA, 1990, 495.
20. Mallikarjuna, Narasimha G, Dillip GR, Praveen B, Shreedhar B, Sree Lakshmi C *et al*. Digest Journal of Nanomaterials and Biostructures,2011:6(1):181-186.
21. Mano Priya K, Karunai Salvia B, John Paul J, Digest Journal of Nanomaterials and Biostructures, 2011:6(2):869-877.
22. Handra S. P., Chaudhary M., Pasricha R., Ahmad A., Sastry M., Biotechnol. Prog, 2006:22:577.

Jundishapur Journal of Microbiology
Published online 2022 April

Research Article
Vol. 15, No.1 (2022)

In Vitro Anticancer Activity of Methanolic Leaf Extract of *Vanda Spathulata* (L.) Spreng - A Medicinally Important Orchid

***Jeline Rani J[1], Nandagopalan V[2]**

[1]Assistant Professor, Department of Botany, Bishop Heber College (Autonomous), Trichy – 17
[2]Associate Professor, Department of Botany, National College (Autonomous), Trichy – 1
*Corresponding author

Abstract

Phytochemicals found in plant extracts have previously been shown to have anticancer and chemo preventive properties. Although *Vanda spathulata* is a rich source of phytocompounds, nothing is known about its anticancer properties. The goal of this research was to see whether *V. spathulata* leaf extracts had anticancer properties by looking at how they affected MCF-7 cell growth and apoptosis. (Human Breast Adenocarcinoma). Leaves were extracted using the solvent Methanol. MTT was used to investigate anticancer activity (3- (4,5-dimethylthiazol-2yl) -5- (3-carboxymethoxyphenyl) -2- (4-sulfophenyl) -2H-tetrazolium) assay on breast cancer cells MCF-7. Five strengths of methanolic leaf extract (12.5, 25, 50, 100, and 200 g/mL) were tested for their cytotoxicity. When methanolic leaf extract concentrations were increased, cell viability decreased significantly, and cytotoxicity increased, as assessed by MTT. In MCF 7, the IC50 values of methanolic leaf extract of *V. spathulata* extract were 113.07g/mL.

Keywords: Anticancer activity, Phytochemicals, plant extract, Cytotoxicity, MTT assay

Received 2022 February 2; **Revised** 2022 March 20; **Accepted** 2022 April 24

1. Introduction

For the most part, the global burden of cancer continues to grow as a consequence of the ageing and growing of the global population, as well as an increase in cancer-causing behavior, particularly smoking, in economically developing nations. Despite all of the sophisticated pharmacological and surgical therapies available, body part malignant neoplastic illness remains the third most often diagnosed cancer globally, with a prevalence of 1.4 million (9.7%), followed by lung carcinoma (13.0%) and breast carcinoma (11.9%). (WHO 2013, GLOBOCAN 2015). Effective, if expensive, medicines are available for a few malignancies, while none are available for others. As a result, we must dive further into this subject to find safer and more practical treatment modalities that not only restrict the spread of body part malignant neoplastic illness but also ensure patient well-being by having the fewest side effects in comparison to today's chemotherapeutical regimens. Plants are a valuable source of natural products that include phytochemicals, also known as secondary metabolites, which are used in a variety of pharmacological and therapeutic applications. A large number of metabolites are used to treat a range of ailments, including cancer and other cellular abnormalities (Scheck et al., 2006, Ramasamy et al., 2013). We wish to focus on various plants and their phytochemical elements in light of recent breakthroughs in the area of phytomedicine and its beneficial immunomodulatory and anticancer effects. Plants are employed because they are cost-effective, readily accessible, and have minimal toxicities (Patel et al., 2010, Shukla et al., 2015). Scientists must now concentrate only on the scientific confirmation of plant species used in folk

medicine, as shown by ethnobotanical surveys. Identification of bioactive substances and their medicinal benefits are subsequent tasks after promising plant species have been identified. (Greenwell & Rahman, 2015).

Vanda spathulata (L.) Spreng an exquisite and plant life orchidaceous plant ordinarily referred to as as Svarna-pushpa bandaa or bandaa was taken for this study. Each flower and leaves methanolic extract were reported to possess anti-depressant and Anti-Oxidant activity (Khan et al., 2018). Dried and fine-grained flowers of *V. spathulata* given for consumption and want to treat asthma attack, maniac troubles, Depression, Neotropic as a pacifier and liver tonic. Juice of the plant is employed to treat the gall, abate hysteria and improve immediate memory learning and show the protecting impact in Neurotoxicity (Dasari *et al*., 2013, De and Medhi, 2015). The purpose of this research was to use the MTT test technique to investigate the anticancer activity of plant extract. There is no earlier report on the study using *Vanda spathulata* (L.) Spreng till date.

2. MATERIALS AND METHODS

2.1 Collection of the Plant Specimen

One of the Kolli Hills in the Namakkal District of Tamil Nadu, India, was where *Vanda spathulata* was found and gathered Ziplock polythene bags were used to gather and maintain plant materials, which were then positioned in Ice packs. Plant material was washed multiple times with refined water to eliminate sand and other flotsam and jetsam from the lab. Using a sterile cutting edge, the leaves were chopped into little pieces and then dried in the shade. After two weeks of complete drying, leaves were coarsely powdered by using blender and the content was stored in clean closed bottles for further analysis.

2.2 Authentication of the Plant Specimen

Plant Survey of India (BSI), Coimbatore, Tamil Nadu, India, perceived and approved the acquired plant example. BSI/SRC/5/23/2018/Tech./2180 is the voucher number. The voucher specimen was sent to the Department of Botany at Tiruchirappalli National College (Autonomous), Tamil Nadu, India.

2.3 Processing of plant material

The plant leaves were gathered and rinsed with tap water and then distilled water. With a mechanical blender, shade dried plant material was pulverised (100-500µM) Methanol was the solvent taken for the analysis. About 50g of leaf powder was taken for the extraction using a soxhlet apparatus with 300ml of solvent. The process was run for 48 hrs after which the sample was concentrated using rotatory evaporator to powder form. The dried extracts were kept in a sterile bottle.

2.4 *In vitro* cytotoxic effect determination by MTT assay

National Center for Cell Sciences (NCCS) cells used to be stored in DMEM (Dulbecco's Modified Eagle Medium) at Pune, India (Sigma Aldrich, USA). sodium bicarbonate and l-glutamine in MEM with 10% FBS, as well as anti-toxin arrangements containing Penicillin (100U/ml), Streptomycin (100µg/ml) and Amphotericin B (2.5µg/ml) were utilized to develop the cell line in a 25 cm2 tissue culture flask (Merck, Germany). A humidified CO2 incubator with a 5% concentration of CO2 was used to incubate refined cell lines at 37°C. (NBS Eppendorf, Germany). An inverted phase contrast microscope was used to see the cells up close, and then the MTT test was used to measure the health of the cells.

2.4.1 In a 96-well plate, cells are seeded.

Trypsinized cells from a two-day-old blended monolayer were suspended in 10% development medium. Hatching took done at 37 degrees Celsius in a humidified CO2 hatchery with a 100µl cell suspension. (5x103 cells/well).

Compound stock preparation

A cyclomixer was used to separate 1mg of methanolic leaf separate in 1mL of DMEM. The model approach was separated through a 0.22 µm Millipore needle channel to guarantee sterility.

2.4.2 Evaluation of Cytotoxicity

5% DMEM was sequentially weakened multiple times by two-overlap weakening (200µg, 100µg, 50 µg, 25µg, and 12.5µg in 500µl of 5% DMEM) and every grouping of 100 l was applied in three-fold to the comparing wells in a humidified 5 percent CO2 hatchery and brooded at 37oC for three days. The results were then analyzed. There were also untreated cells available for comparison.

2.4.3 Direct microscopic observation cytotoxicity assay

Rearranged stage contrast tissue culture magnifying instrument (Olympus CKX41 with Optika Pro5 CCD camera) was utilized to see the entire plate following 24 hours of treatment, catching minuscule pictures. There were cytotoxicity signals assuming there were noticeable changes in cell shape including adjusting or contracting of cells, granulation, and vacuolization in the cytoplasm.

2.4.4 MTT Method for Cytotoxicity Assay

15 mg MTT (Sigma, M-5655) was separated in 3 ml PBS and a short time later disinfected by filtering through the undissolved material. Plates were incubated for four hours at 37oC in a humidified, 5% CO2 incubation facility with 30l of reconstituted MTT plan in each test and control well. It was necessary to destroy the supernatant before dissolving the formazan precious stones in 100µl of MTT Solubilization Solution (DMSO, Sigma Aldrich USA) and stirring the solution well. A microplate per user was utilized to quantify the absorbance at 540 nm.

% Cytotoxicity using the following formulas:

% Cytotoxicity = 100 - [Abs (sample) /Abs (control)] x100.

% Cell Viability = [Abs (sample) /Abs (control)] x100.

2.5 Statistical analysis

Three distinct experiments each included three sets of tests. When utilizing MS-Windows based graph pad Instat (version 3), we were able to visually estimate the quantity of extract required to suppress cancer development by 50%, or the IC50. Mean± standard deviation was used to illustrate the findings.

3 Results and Discussion

Cancer is an abnormal development of tissue in which cells divide uncontrollably, but in a self-directed manner, driving in an expansion in the quantity of cells that separation. Anticancer treatment is becoming more popular. The usage of cultured tissues and cells has expanded as a result of in vitro cytotoxicity testing technologies that limit the use of laboratory animals (Kanchana and Balakrishna, 2011). The development and identification of novel anticancer lead compounds with minimal immune system side effects has been a key focus of immunotherapy research. Despite several attempts, multidrug resistance remains a serious problem in cancer chemotherapy, which has lately been the topic of much research (Xu et al., 2009). Many scientists focused their efforts on natural substances found in plants, marine creatures, and microbes in order to achieve this goal. The

cytotoxicity of a variety of therapeutically relevant plant extracts was examined. In vitro cytotoxicity of medicinal plant extracts was shown to be variable (Rajesh et al., 2019). Despite the fact that there are numerous different sorts, chest illness is the most broadly perceived harmful development among women all over the planet. This is a kind of breast cell that rapidly multiplies and rapidly increases in size. Some 1% to 5% of breast cancer cases have started in other breast tissues in the past 30 years, with 50% to 75% of cases starting in the breast's lymph nodes and 10% to 15% starting in the lymph nodes (Dillon et. al., 2010). Breast cancer fatality rates have decreased in recent years as a result of better detection and treatment. (http://www.imaginis.com/general-information-on-breast-cancer/what-is-breast-cancer-2). The National Cancer Institute has recognised the most regularly used herbs as having cancer-preventive effects (Jaikumar and Jasmine, 2016). As a result, the current research intends to analyze the cytotoxicity of methanolic leaf extract using a simple and inexpensive approach, as well as to assess the cytotoxic impact of the MCF-7 breast cancer cell line. Because it preserves many desirable features of mammary epithelial cells, the Michigan Cancer Foundation-7 (MCF-7) cell line is important for in vitro bosom malignant growth research, such as the capacity to process estrogen through estrogen receptors (Son *et al*., 2003).

The cytotoxicity of methanolic leaf extract of *V. spathulata* against breast cancer and normal cells is summarized in Table 1. The plant extract was shown to have potential antitumor action against cell lines. The cytotoxicity of the plant extract was tested using human breast adenocarcinoma (MCF-7) cell lines at five different doses (12.5, 25, 50, 100 and 200 µg/mL). The MTT test showed that expanding the grouping of the plant separate diminished cell suitability and expanded cytotoxicity. In MCF 7, the IC50 values of plant extract were reported to be 113.07µg/mL. The research discovered that when concentrations rise, cell growth inhibition (cytotoxicity) and cell viability decrease. The findings of this research correspond with those of Mona Orangi *et al*., (2016), showed that the methanolic fractions of *Scrophularia anthracis* cause MCF-7 cells to undergo apoptosis at increasing concentrations, indicating that these fractions might be utilized to create anticancer compounds.

Table.1: Effect of varying concentrations of plant extract on cytotoxicity of MCF-7 (Breast cancer) cell lines as determined by MTT assay.

Activity	**MCF 7 (Breast cancer cell line)**					
	Cell control	**12.5 (µg/mL)**	**25 (µg/mL)**	**50 (µg/mL)**	**100 (µg/mL)**	**200 (µg/mL)**
Viability (%)	100	90.80	81.17	70.49	51.13	20.62
Cytotoxicity (%)	0	9.20	18.13	29.51	48.87	79.38
LC_{50} Value (µg/mL)	113.07					

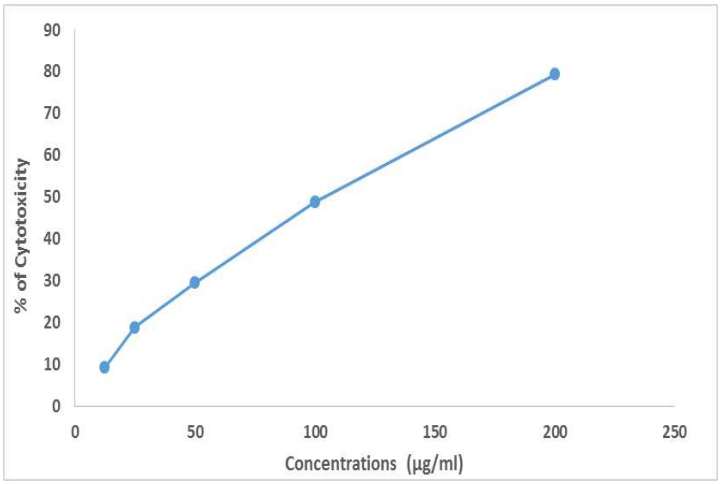
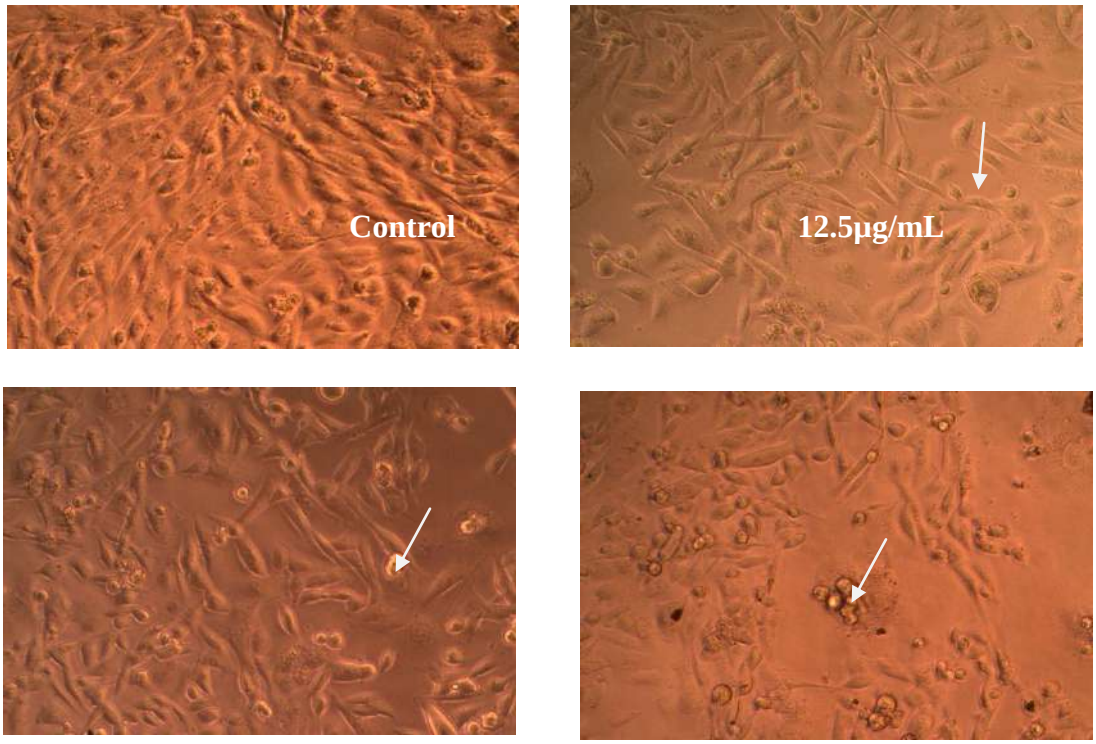


Figure.1: Effect of varying concentrations of plant extract on cytotoxicity of MCF-7 (Breast cancer) cell lines as determined by MTT assay.

3.1 Examining the morphology

Figure 1 shows how *Vanda spathulata* methanolic leaf removal affects the morphology of MCF-7 cells following treatment with different concentrations. When the concentration of plant extract (upto200µg/mL) was raised, the morphological parameters of the tested cell lines altered substantially, and this change was proportional to the applied concentration. They started to shrink and lose their capacity to stick to the culture plate's surface as a result. Furthermore, in compared to the control morphology, cells seemed rounded and totally floated at the maximum applied concentration. Inducing cell toxicity and apoptosis (morphology change) in MCF-7 cells has anticancer action.



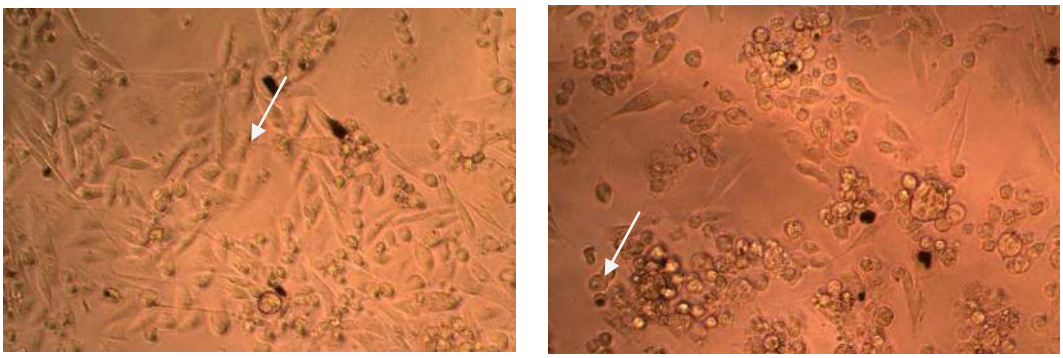


Figure. 2: Effect of varying concentrations of plant extract on cytotoxicity of MCF-7 (Breast cancer) cell line as determined by MTT assay (Arrows indicate representative apoptotic cells).

Cell lines treated with varying concentrations of methanolic leaf concentrate were compared to untreated cells over the course of a 24-hour period. When contrasted with untreated malignant growth cells, the state of plant separates treated disease cells changed extensively. The methanolic leaf extricate treated cells had lost their film honesty, despite the fact that it was as yet flawless at lower focuses. At more prominent dosages, in any case, the leaf extricates treated cells uncovered a critical contrast from the benchmark group. Nuclear chromatin condensation and aggregation, as well as apoptosis, started to occur when cells shrank and lost their capacity to adhere to the culturing plate. These morphological alterations in cell lines seen in the study were consistent with earlier research suggesting that this kind of change in cells might be a potential cause of cell death (Nida Nayyar Farshori *et al*., 2013). In contrast to the control morphology, which was proportionate to the applied concentration, the examined cell lines' morphological features were significantly altered, and they were now completely afloat.

Conclusion

Cell culture is a crucial method and one of the most important tools for studying the structure and function of in vivo cells. The in vitro cell-based test offers mechanistic information for a variety of cancer research studies, mostly in medication development. In this work, In order to test the leaf extract's anticancer properties, researchers used the MCF-7 cancer cell line. The MTT (3- 4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) experiment was performed to investigate the methanolic leaf extract's in vitro anticancer activities. MCF-7 cells were cytotoxic to the methanolic leaf extract of *V. spathulata*, with an IC_{50} value of 113.07µg/mL. Finally, the methanolic leaf extract of *V. spathulata* has anticancer action in MCF-7 cells via producing cell toxicity and apoptosis (morphology change).

Author contributions

All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Conflicts of Interest

The authors declared no potential conflicts of interest to the research, authorship, and/or publication of this article

References

1. De, L. C., and R. P. Medhi. (2015) Orchid–a diversified component of farming systems for profitability and livelihood security of small and marginal farmers. *J Glob Biosci* 4(2):1393-1406.
2. Dillon, D. A., Guidi, A. J., & Schnitt, S. J. (2010) chapter 28: Pathology of Invasive Breast Cancer, in Harris JR, Lippman ME, Morrow M, Osborne CK. Diseases of the Breast, 4th edition, *LippincottWilliams & Wilkins*.
3. GLOBOCAN (2015) - Estimated Cancer Incidence, Mortality and Prevalence About Colorectal Carcinoma, Worldwide in 2015. Available from: http://www.globocan.iarc.fr/pages/fact_sheets_cancer.aspx?cancer=colorectal. [Last accessed on 2019 Jun 19].
4. Greenwell, M., & Rahman, P. K. S. M. (2015). Medicinal plants: their use in anticancer treatment. *International journal of pharmaceutical sciences and research*, *6*(10), 4103.
5. Jaikumar, B., & Jasmine, R. P. G. (2016). A Review on a few medicinal plants possessing anticancer activity against human breast cancer. *International Journal of Pharm Tech Research*, *9*, 3, 333-365.
6. Kanchana, A., & Balakrishna, M, (2011). Anti-cancer effect of saponins isolated from *Solanum trilobatum* leaf extract and induction of apoptosis in human larynx cancer cell lines. *International journal of pharmacy and pharmaceutical sciences, 3,* 356-364.
7. Khan, H., Belwal, T., Tariq, M., Atanasov, A. G., & Devkota, H. P. (2019). Genus Vanda: A review on traditional uses, bioactive chemical constituents and pharmacological activities. *Journal of ethnopharmacology*, 229, 46-53.
8. Mona Orangi, Ardalan Pasdaran, Dariush Shanehbandi, Tohid Kazemi, Bahman Yousefi, Behnaz-Alsadat Hosseini, and Behzad Baradaran. (2016) Cytotoxic and Apoptotic Activities of Methanolic Subfractions of Scrophularia oxysepala against Human Breast Cancer Cell Line. Evidence-Based Complementary and Alternative Medicine, 1-10.
9. Nida Nayyar Farshori, Ebtesam Saad Al-Sheddi, Mai Mohammad Al-Oqail, Javed Musarrat, Abdulaziz Ali Al-Khedhairy, Maqsood Ahmed Siddiqui. (2013) Anticancer Activity of Petroselinum sativum Seed Extracts on MCF-7 Human Breast Cancer Cells. Asian Pacific Journal of Cancer Prevention. 14; 5719- 5723.
10. Patel B, Das S, Prakash R, Yasir M (2010) Natural bioactive compound with anticancer potential. *Int J Adv Pharma Sci* 1:32–41.
11. Rajaneekar Dasari, D. Sathyavati , Sampath Kumar Belide, P.Jayachandra Reddy, K.Abbulu (2013) Evaluation of antioxidant activity of two important memory enhancing medicinal plants *Celtis timorensis* and *Vanda spathulata. Asian Journal of Pharmaceutical and Clinical Research* 6(2):153-155.
12. Rajesh A, Doss A, Tresina PS and Mohan VR (2019) In-vitro anticancer activity of few plant extracts against MCF-7, MDA-MB468 and MDA-MB231 cell lines. The Pharma Innovation Journal; 8(3): 38-41
13. Ramasamy TS, Yu JS, Selden C, Hodgso H, Cui W (2013) Application of three-dimensional culture conditions to human embryonic stem cell-derived definitive endoderm cells enhances hepatocyte differentiation and functionality. *Tissue Eng Part A* 19:360–7.

14. Scheck AC, Perry K, Hank NC, Clark WD. (2006) Anticancer activity of extracts derived from the mature roots of Scutellaria baicalensis on human malignant brain tumor cells. *BMC Complement Altern Med* 6:27.
15. Shukla S, Braz MA. (2015) Anticancer potential of medicinal plants and their phytochemicals: a review. *J Bot* 38:199, http://dx.doi.org/10.1007/s40415-015-0135-0.
16. Son,Y.O., Kimb, J., Lima, J. C., Chunga,Y., Chungb, G. H., & Lee, J. C. (2003). Ripe fruits of *Solanum nigrum* L. inhibits cell growth and induces apoptosis in MCF-7 cells. *Food and Chemical Toxicology, 41*, 1421–1428.
17. WHO; IARC (2012) (International Agency for Research on Cancer); Latest World Cancer Statistics, Global Cancer Burden Rises to 14.1 Million New Cases in 2012, 12 th December 2013 Press Release No. 233. Available from: http://www.iarc.fr/en/media-centre/pr/2013/pdfs/pr223_E.pdf. [Last accessed on 2015 Jun 17] Back to cited text no. 1
18. Xu, H., Yao, L., Sun, H., & Wu, Y. (2009). Chemical composition and antitumor activity of different polysaccharides from the roots of *Actinidia eriantha*. *Carbohydrate polymers, 78*, 316-322.

Taylor & Francis
Taylor & Francis Group

Materials Technology
Advanced Performance Materials

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ymte20

One step preparation of green reduced copper oxide nanorods using *Citrus sinensis* L. peel extracts and evaluation of their photocatalytic degradation of Rose Bengal dye and antibacterial activity

Krishnan S, Ayyadurai V, Ramar K, Saranya A, Maheshwaran P & Murugesan S

To cite this article: Krishnan S, Ayyadurai V, Ramar K, Saranya A, Maheshwaran P & Murugesan S (2021): One step preparation of green reduced copper oxide nanorods using *Citrus sinensis* L. peel extracts and evaluation of their photocatalytic degradation of Rose Bengal dye and antibacterial activity, Materials Technology, DOI: 10.1080/10667857.2021.1929720

To link to this article: https://doi.org/10.1080/10667857.2021.1929720

Published online: 25 May 2021.

Submit your article to this journal

Article views: 26

View related articles

View Crossmark data

Full Terms & Conditions of access and use can be found at
https://www.tandfonline.com/action/journalInformation?journalCode=ymte20

MATERIALS TECHNOLOGY
https://doi.org/10.1080/10667857.2021.1929720

Taylor & Francis
Taylor & Francis Group

Check for updates

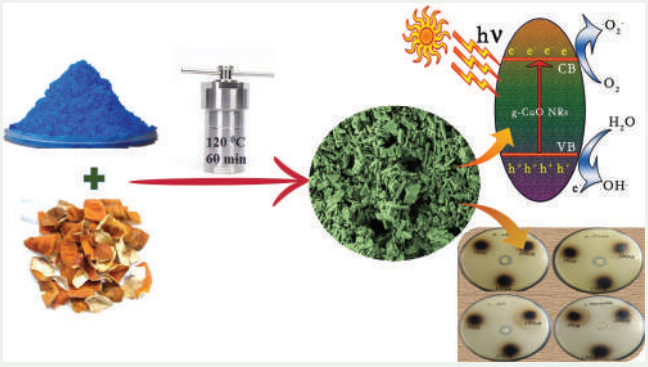
One step preparation of green reduced copper oxide nanorods using *Citrus sinensis* L. peel extracts and evaluation of their photocatalytic degradation of Rose Bengal dye and antibacterial activity

Krishnan S[a], Ayyadurai V[b], Ramar K[c], Saranya A[d], Maheshwaran P[d] and Murugesan S[a]

[a]Phytochemistry Laboratory, Department of Botany, Periyar University, Salem, India; [b]PG and Research Department of Botany, National College (Autonomous), Tiruchirappalli, India; [c]PG and Research of Department of Chemistry, Jamal Mohamed College (Autonomous), Tiruchirappalli, India; [d]Department of Chemistry, PGP College of Arts and Science, Namakkal, India

ABSTRACT

Modern development in nanoscience and nanotechnology has contributed to the varied uses of metal and metal oxide nanoparticles in numerous arena of sciences, research organisations and industries. Among all metal oxides, copper oxide nanoparticles (CuO NPs) has reached more responsiveness as a result of its unique properties and applications. Herein, we report a one-step hydrothermal preparation of a photocatalytically stable copper oxide nanorods (g-CuO NRs) in the presence of *Citrus sinensis L*. peel extracts. The as prepared g-CuO NRs was used for photocatalytic activity in degradation of Rose Bengal (RB) under visible light irradiation. Upon 30 min of visible light irradiation, the g-CuO NRs photocatalyst exhibits an excellent photocatalytic activity (94.5%) for degradation RB. In addition, the g-CuO NRs were further tested for their antibacterial activity against gram-negative *Escherichia coli, Klebsiella pneumoniae* and gram-positive *Staphylococcus aureus, Streptococcus pneumoniae* bacteria and the test results showed improved antibacterial activity with the increase concentration of g-CuO NRs. The outcome of this work highlights the g-CuO NRs to be an interesting material for treatment of organic dyes and biomedical applications.



ARTICLE HISTORY
Received 9 February 2021
Accepted 8 May 2021

KEYWORDS
Green synthesis; g-CuO nanorods; photocatalyst; antibacterial activity

Introduction

The foremost important problem intimidating the existing world is contamination in various ecosystems [1,2]. Owing to the development of textile industries and dye houses, huge amounts of waste discharges are mixed with aquatics. Textile discharges containing a variety of synthetic organic contaminate dyes, posing serious health risks to humans and other living things [3,4]. The dye-polluted wastewater is very hazardous, causing genetically irreversible illnesses such as breast and colon cancer, heart disease, and haemophilia, etc [5–7]. There are many approaches have been applied to address the problem of wastewater contamination [8]. Among others, photocatalysis is an advanced method for dye degradation due to its simple experimental setup, low cost and eco-friendly nature [9].

Currently, semiconducting properties of the materials have appealed growing consideration for their extensive ecological, attractive photocatalytic, photoelectrochemical, optical and chemical properties such as low toxicity and chemical stability [10–14]. It has been a reliable aim chased by chemists to convert chemicals powerfully with solar energy. Also the biological nanoparticles are prepared with the assistance of medicinal plants as reducing agents and is an attractive investigation field by preparing various types of

CONTACT Murugesan S drsmbtjas@periyaruniversity.ac.in Phytochemistry Laboratory, Department of Botany, Periyar University, Salem, Tamil Nadu, India

© 2021 Informa UK Limited, trading as Taylor & Francis Group

metal/metal oxide nanoparticles like, calcium, copper, gold, iron, silver and zinc [15–20]. Among them, CuO NPs have received great attentions in many applications due to its optoelectronic, catalytic, sensing, and superconducting properties [21]. Aside the previously mentioned usage, CuO NPs showed exclusive anticancer, antimicrobial and antioxidant efficacy which renders them a promising tool for biomedical applications. These properties have initiated exhaustive research into photocatalysts and antibacterial for their possible applications in the fields of environmental remediation [22–25].

Different methods available to prepare CuO NPs, namely, sol–gel technique, sonochemical, alkoxide-based route, electrochemical methods, precipitation-pyrolysis, microwave irradiations, solid-state reaction method, thermal decomposition of precursor, etc. However, these existing techniques sometime contain harmful aspects such as contamination with impurities, use of strong acid or strong alkali solutions, or vacuum environments. Therefore, an environmentally benign fabrication process is desired to obtain high-quality products via a low-cost method. Hydrothermal synthesis of the green reduced CuO NPs as a new green technology method has the potential to solve these problems. The synthesis of green assisted copper oxide (CuO) nanomaterial using plants source are eco-friendly, cost-effective and more stable. However, the research going on with this technique is very seldom. The reason green assisted plant extracts work so well in the preparation of nanomaterial is because they act as reducing agents as well as capping agents. Different plant/parts of plant have been exploited for the fabrication of copper oxide NPs are shown in Table 1.

In recent years, many researches have shown promising results on the activity of nanoparticles as a potent antimicrobial substance that can act either individually or combined with other materials [32,33]. Recently, the work on Ag_3PO_4/TiO_2 heterostructure exhibited high antibacterial activity towards *E.coli* bacterial strain [34]. Likewise, incorporation of nanocrystalline titania as an integral part of silicone network structure was applied for reduction in *S. aureus* biofilm formation and the mechanism of inhibition of bacterial colonisation is envisaged to involve disruption and puncturing of the bacterial membrane [35]. The antibacterial activities of AgNPs anchored to the thiol-group of functionalised polymer were reported and the results demonstrate the nanohybrid structure play a synergistic role against *E.coli* strain [36]. Similarly, the solar disinfection by photocatalysis to destroy bacteria by W, Nd and Zn doped titania materials were studied. The antimicrobial characteristic of the free radicals was explored to investigate the inhibition of the growth rate of E. coli bacteria the results showed that the hydroxide-free radicals have high toxicity for microorganism [37]. The anti-microbial nanoparticles have been reported for titania-coated nickel ferrite, neodymium-doped titania coated-nickel ferrite and neodymium-doped titania coated-nickel ferrite composite nanoparticles. The doped composite materials acts better activity against the E. coli pathogen in comparison with undoped TiO_2 nanoparticles [38–40].

In this work, we report a green route for preparation of g-CuO nanorods photocatalyst using *Citrus sinensis L.* peel extract for the efficient degradation of Rose Bengal (RB) dye under visible light irradiation. Four bacterial strains including two gram-negative *E. coli, K. pneumoniae* and two gram-positive *S. aureus, S. pneumoniae* microorganisms bacteria were used to investigate the in vitro antibacterial property of g-CuO nanorods. To the best of our knowledge, there is no report of *Citrus sinensis L.* peel extract mediated green synthesis of g-CuO nanorods and application in photocatalytic and antibacterial activity to date.

Experimental procedures

Materials and reagents

All reagents were analytical grade purchased from Sigma-Aldrich and used without further purification. All solutions were prepared with 18.2 MΩ cm deionised Milli-Q water.

Preparation of plant extract

Fresh peel of *Citrus sinensis L.* were collected from local fruit juice shop near Periyar University campus, Salem District, Tamilnadu, India. The peel was properly cleaned with tap water followed by deionised water to avoid dust and other contaminated particles and kept in an oven at a temperature of 60°C for 10 days and then the dried peel were powdered well using a mixer grinder and the powder was stored in dark at ambient temperature. To prepare the aqueous extract, around 30 g of the dried peel powder were taken in a 250 ml of beaker having 150 mL of double distilled water and refluxed at 70°C for 30 min under magnetic stirring. The mixture was then centrifuged (Sigma 2–16PK centrifuge) for 10 min at 7000 rpm and then filtered using Whatman No.1 filter paper. The clear solutions of plant extracts thus obtained were stored in a refrigerator until further use.

Preparation of g-CuO nanorods

The CuO NRs reduced and stabilised with the extract of *Citrus sinensis L.* peel were synthesised following a methodology previously described [41,42]. 1 M of copper nitrate ($Cu(NO_3)_2.3H_2O$) were dissolved in 20 ml of deionised water. Afterwards, 30 ml of *Citrus*

Table 1. Summary of plant-mediated CuO nanoparticles.

S. No.	Plant name	Family	Part used	Phytochemical constituents	Size (nm)	Ref.
1	Punica granatum	Lythraceae	Peel extract	Alcohol, phenols, amide, and othe molecules containing carbonyl group	40	[26]
2	Tribulus terrestris	Zygophyllaceae	Fruit extract	Alkaloids, flavonoids, tannins, ascorbic acid and phenolic compounds	5–22	[27]
3	Cordia sebestena	Boraginaceae	Flower extract	Phenol, flavonoid, tannin, phytosterol, coumarin, fumaric acid, a-tocopherol	20–35	[28]
4	Hibiscus rosa sinensis	Malvaceae	Flower extract	Tannins and phenolic proteins, triterpenoids, 2, 3-hexanediol, nHexadecanoic acid, 1,2-Benzenedicarboxylic acid and squalene.	12	[29]
5	Eucalyptus globulus	Myrtaceae	Leaf extract	Phenols, terpenoids, flavonoids and tannins	5–24	[30]
6	Pterolobium hexapetalum	Fabaceae	Leaf extract	Phenols, flavonoid, terpenoids, tannins, alkaloids, carbohydrates, and glycosides	10–50	[31]

sinensis L. peel extract were added to the solution for the reduction of copper ions. The pH value 7.0 adjusted for the mixture by the addition of NaOH (1 N) solution. Then, binary mixture was heated at 120°C in a sealed flask for 1 h in the hotplate for 60 min and kept at room temperature. The reaction showed colour change from green to dark brown solution. The prepared CuO NRs were separated after centrifugation at 5000 rpm for 1 h and the product was re-dispersed by ultrasonication for 15 min and then dried in an oven for 3 h. Finally, they were calcinated at 300°C for 5 h and the final product was stored at room temperature for further characterisation and applications studies.

Characterisation techniques

The crystal phase of the as-prepared CuO NRs photocatalyst was carried out using X-ray diffraction (RIGAKU Powder X-Ray Diffractometer) and the data were collected in the 2θ range of 10 to 80°. Scanning electron microscope (SEM, JEOL, JSM-5600) and a transmission electron microscope (TEM, FEI, Tecnai G20) were performed in order to investigate the size, size distribution and morphology of the synthesised nanoparticles. The EDX spectroscopy was conducted using Oxford 6587, Oxford Instruments. FTIR measurements were carried out, in a range of 4000 to 450 cm^{-1} by Frontier FTIR, PerkinElmer, USA. UV-Vis Diffuse reflectance spectroscopy (UV-Vis DRS) was recorded in the range 200–800 nm with a Perkin Elmer Lambda 25 spectrometer. The solid-state photoluminescence measurements were carried out using a spectrofluorimeter (Varian, Cary Eclipse Fluorescence Spectrophotometer).

Photocatalytic degradation of methylene blue (MB) dye

The photocatalytic activity of the synthesised green assisted g-CuO NRs catalyst was evaluated through photodegradation of Rose Bengal (RB) dye solution under solar light illumination. In order to assess the degradation process, 30 mg of the catalyst was dispersed in 50 mL of RB solution under ultrasonication for 1 min. Before illumination, the suspension mixture was gently agitated for 30 min in dark in order to achieve adsorption-desorption equilibrium of the dye with the catalyst. Afterwards, the resulting solution was kept under direct sunlight. At given intervals, 3 mL of the suspension was withdrawn and centrifuged to remove the dispersed catalyst powder (Note that the change in dye concentrations was used to determine the adsorption level of dye in dark.). The concentration of the clean transparent solution was determined by using UV-Vis spectrophotometer (Shimadzu, UV-2450). The concentration of remaining dye after photocatalytic experiment was determined by measuring the absorbance of dye. The photocatalytic efficiency rate was calculated using the relation degradation (%) = $[(C_t\text{-}C_0)/C_0]$ x 100. Where C_t and C_0 are the concentrations of RB dye after time 't' and at the start, respectively.

Antibacterial activity

The antibacterial activities of g-CuO NRs were assessed by agar well diffusion method [9,43]. Four different human pathogenic bacterial strains such as gram positive *Staphylococcus aureus, Streptococcus pneumoniae* and gram negative *Escherichia coli, Klebsiella pneumonia* bacteria strain were used as the test microorganisms. The tested bacterial species were obtained from microbial type culture collection centre, Chandigarh, India. A set of three concentrations (50, 100 and 150 μg ml^{-1} in sterile distilled water) of the prepared nanorods were added to the, respectively, labelled wells. The zone of inhibition (ZOI) around the well were measured after 24 h.

Results and discussion

XRD analysis

The crystal structure of the g-CuO NRs was determined by X-ray diffraction (XRD). As evident from Figure 1, all diffraction peaks were intense which indicates the synthesised g-CuO NRs was highly crystalline. The typical diffraction peaks were well matched with the standard data of CuO (JCPDS Card No. 10–0399). All diffraction peaks were assigned to monoclinic phase. It is notable that different diffraction peaks corresponding to the planes (120), (220), (040), (230), (201), (240), (150), (041), (250) and (200), respectively. The presence of carbon is due to the acids, alcohols, proteins and many other phytochemicals that already exists in the plant extracts.

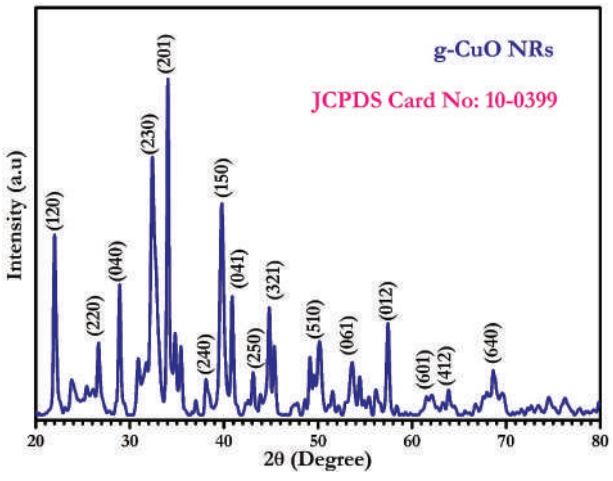


Figure 1. XRD patterns of green synthesised g-CuO NRs.

Furthermore, the crystallite size of g-CuO NRs is determined using Debye–Scherrer's formula [44]. The lattice parameters obtained for synthesised g-CuO NPs were a = 9.502, b = 11.97, and c = 3.240. The calculated crystallite size of g-CuO NRs was been found to be 34 nm.

Morphological studies

The morphology, structure, and size of the g-CuO NRs were investigated using FE-SEM and HR-TEM. As shown in Figure 2(a-c), as-obtained g-CuO has a rod-shape and irregular structures with particle size range from 60 to 80 nm. Also, observed, the images indicated that the NPs were both agglomerated and monodispersed with almost spherical morphologies. Such variation in particle shape and size distribution is associated with the chemical composition of the *Citrus sinensis L*. peel extract. Furthermore, elemental distributions of the green assisted nanorods was analysed by EDX spectroscopy. From the EDX spectrum (Figure 2 (d)), the elemental compositions were recorded, which they are confirming the presence of Cu, O and C elements in the g-CuO NRs. No other signals were observed, indicating the good purity of g-CuO NRs. The TEM images of the g-CuO NRs at different magnifications are shown in Figure 3. TEM micrographs (Figure 3(a-e)) reveals that the g-CuO are rod-like structure with average size of 65 nm. Figure 3(f) shows the representative selected area electron (SAED) pattern for g-CuO NRs achieved with the peel extract. SAED patterns show various diffraction rings of monoclinic g-CuO. The ring pattern shows that these g-CuO NRs are randomly oriented, and the strong intensities reveal the high crystallinity of the sample. All of the obtained rings in SAED pattern may be attributed to the diffraction peaks of monoclinic structure of g-CuO NRs indicating that the obtained nanorods are in the pure phase of CuO, supporting with the XRD results.

Fourier transform infrared (FTIR) analysis

FTIR spectroscopy was performed to identify the presence of functional groups on the surface of g-CuO NRs. The FTIR spectrum (Figure 4) showed main characteristic bands at 3401, 2971, 1608, 1400, 1272, 1056, 568 and 499 cm^{-1} respectively. The FTIR spectrum of plant extract assisted g-CuO NRs shows that the broad absorption band at 3401 cm^{-1} attributes to the hydroxyl (OH) functional group in alcohols and phenolic compounds. These bands are attributed to the protein residue present on the surface of NPs after synthesis process. In the present work, we assumed that the proteins present in the leaf extract was responsible for the reduction acetate group of the metal salt to NPs and acted as stabilising and capping agents for g-CuO NPs The small band at approximately 2971 cm^{-1} is due to O = C = O stretching vibration. Band at 1608 cm^{-1} can be assigned to aromatic bending of alkene group (C = C). The absorption peak around at 1272 cm^{-1} may be correspond to –OH bending vibrations of Cu–OH. The main characteristic band of g-CuO at 1400 and 1056 cm^{-1} are assigned to C – O stretching vibrations. The peaks at 568 and 499 cm^{-1} are indicated that the formation of characteristic stretching vibrations in monoclinic Cu-O.

Optical spectroscopy

In order to determine the light absorption properties and bandgap energy of the prepared g-CuO NRs, UV-Visible diffuse reflectance spectroscopy (UV-Visible DRS) was performed in the range of 200 to 800 nm as showed in Figure 5(a). The presence of molecules such as proteins in the leaf extract was responsible for the reduction of Cu metal salt to form g-CuO NPs. The UV–Vis spectrum of the g-CuO NRs dispersed in water exhibiting the maximum absorption peaks at about 380 nm. In the spectrum, the peak at 380 nm are due to surface plasmon absorption of metal oxide. The surface plasmon absorption in the metal oxide nanoparticles is due to the collective oscillation of the free conduction band electrons which is excited by the incident electromagnetic radiation. This type of resonance is seen when the wavelength of the incident light far exceeds the particle diameter. Surface plasmon absorption band with a maximum at 380 nm indicates the formation of g-CuO NRs.

Energy band gap is usually attained from optical edge of absorption spectrum, which is nothing but the minimum energy necessary to excite an electron from the maximum occupied molecular orbital to the minimum unoccupied molecular orbital. From the absorption spectrum the band gap energy was found using Tauc's plot formula [45], $\alpha h\nu = A(h\nu\text{-}Eg)^n$. Where h, ν, α and C are Plank's constant, photon frequency, absorption coefficient and a constant, respectively. By extrapolating the linear region of $(\alpha h\nu)^2$ versus the photon energy axis the optical band gaps can be estimated. Figure 5(b) shows expected band gap energy and it was found to be 1.87 eV. This banned band arrangement induced fresh energy level in the band gap of g-CuO NRs.

Figure 5(c) shows the PL excitation and emission spectrum of g-CuO NRs. The PL property is a potent instrument to examine the optical properties of the metal oxides semiconductor, particularly the PL emission properties, owing to most of applications of optoelectronic devices. The PL property was influenced by the formation, particle size and morphology of the g-CuO NRs. Generally, the reported emission spectrum of g-CuO NRs consists of many bands in the range 200–700 nm. The spectrum showed UV as well as visible emissions, exhibit UV emission peak at 300 nm and visible emission peak in the violet region

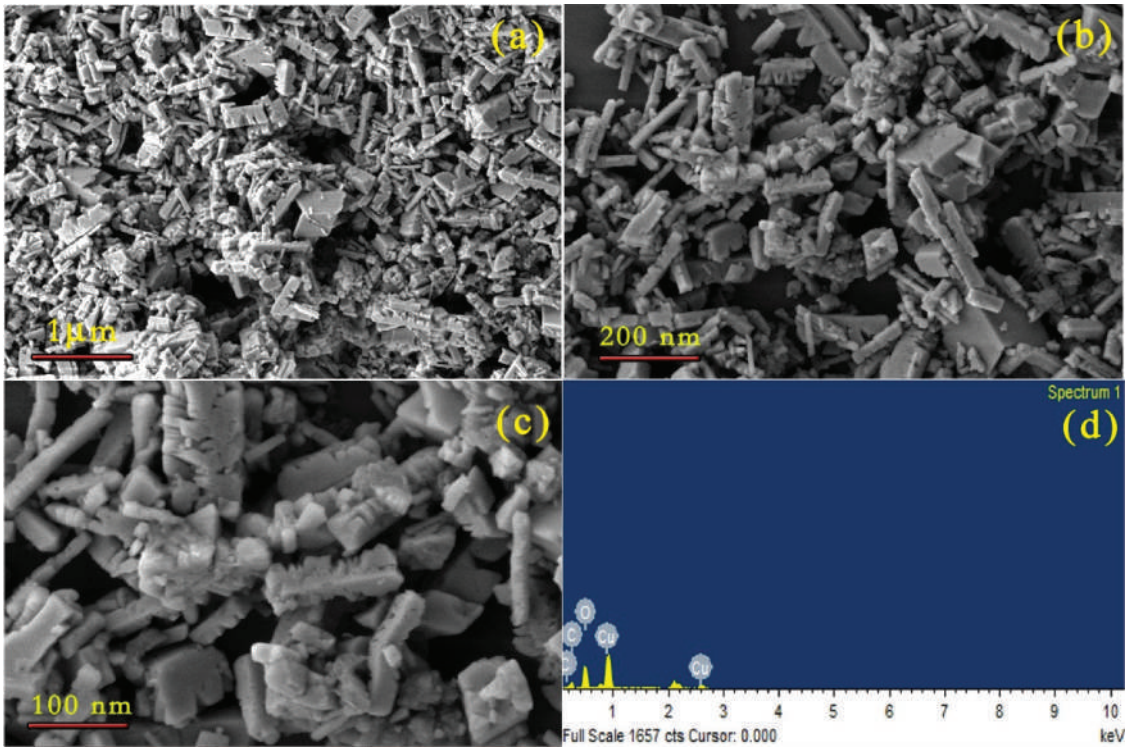


Figure 2. (a-c) FE-SEM images and (d) EDS spectrum of g-CuO NRs.

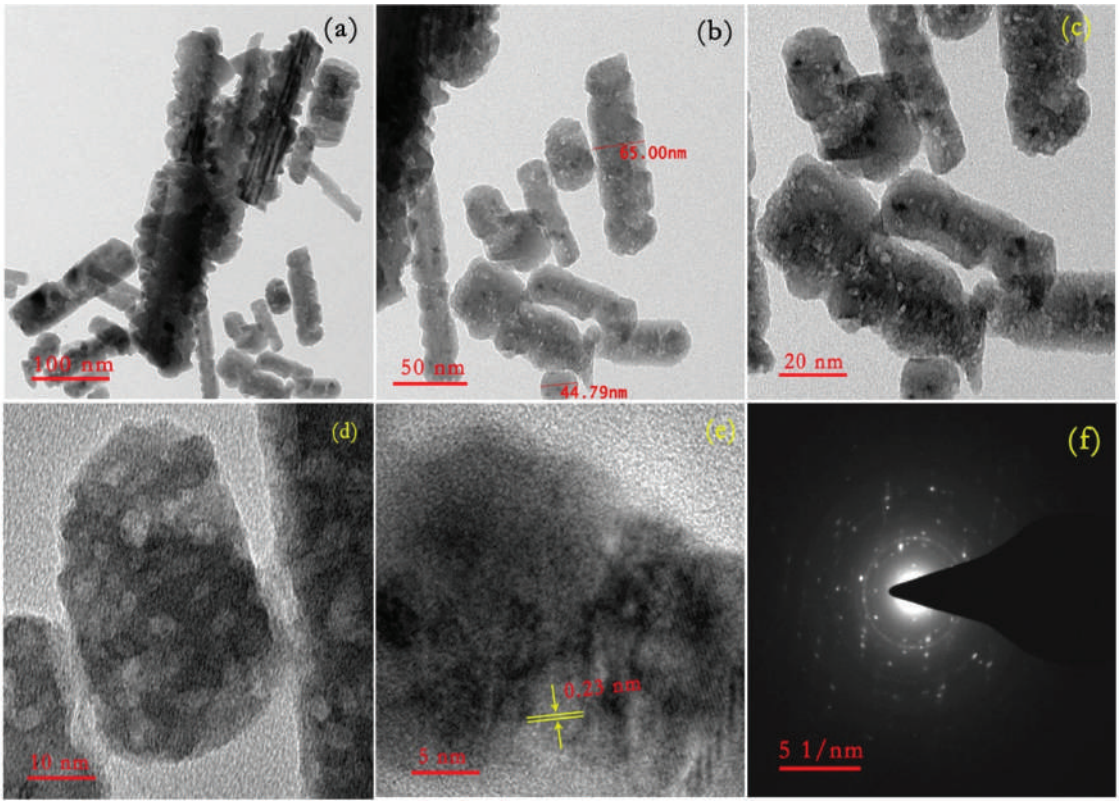


Figure 3. (a-e) HR-TEM images and (f) SAED pattern of g-CuO NRs.

(400 nm), respectively. The origin of UV emission in g-CuO NRs is owing to the recombination of electron–hole pair in free-excitons [46]. The photoluminescence blue bands at 413 nm were investigated by transition vacancy of oxygen and interstitial oxygen. This photoluminescence spectrum is useful for understanding the electron transition energies within g-CuO NRs through determining the emission peaks and using them to estimate the corresponding electronic energy levels.

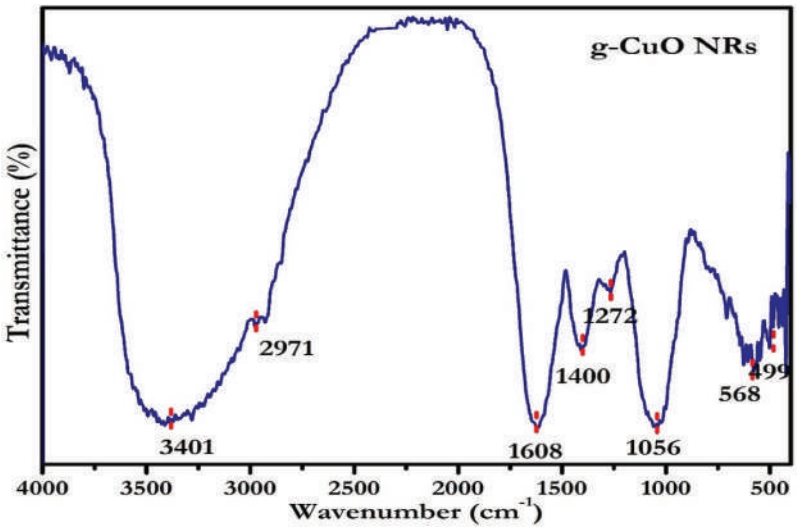


Figure 4. Fourier transform infrared spectroscopy (FTIR) spectrum of g-CuO NRs.

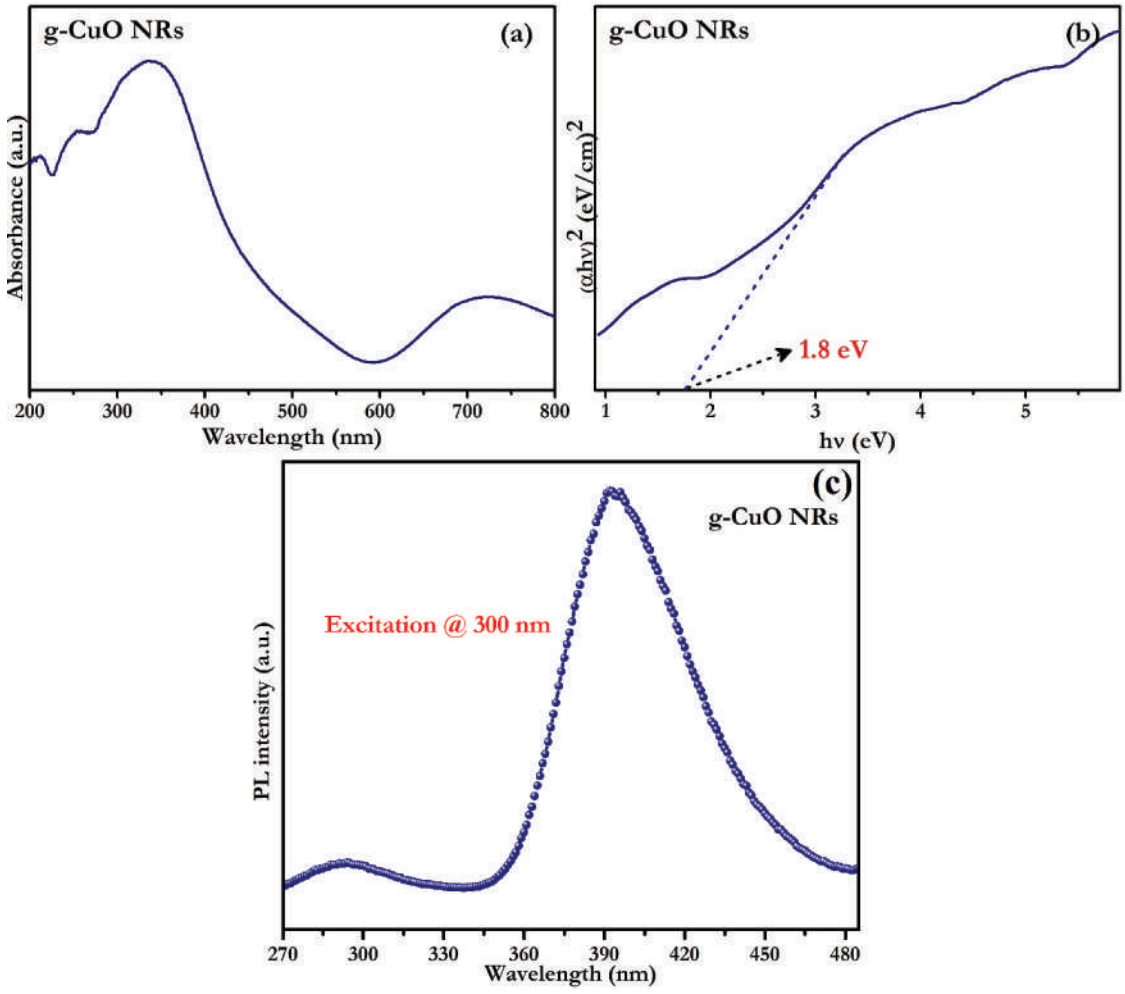


Figure 5. (a) UV–vis diffuse reflectance spectrum, (b) band gaps and (c) Photoluminescence Spectrum of g-CuO NRs.

Photocatalytic studies

To highlight the potential catalytic efficiency of green-assisted g-CuO NRs towards environmental remediation and elude the wastewater contaminant, photocatalytic tests of Rose Bengal (RB) aqueous solution was investigated under sunlight irradiation (Figure 6(a-c)). Under optimal research circumstances, normally 50 mL sample of wastewater containing 0.1 g of g-CuO nanophotocatalyst and 10 ppm of RB was placed into the beaker. Prior to illumination, the suspensions were

magnetically stirred in the darkness for half an hour to reach the adsorption/desorption equilibrium. After sunlight irradiation for interim times, the suspension was tested and the degradation process of RB was examined through a UV-Vis spectroscopy. The control experiment displays that the absorbance of RB is extremely slow without photocatalyst (Figure 6(a)). The synthesised g-CuO NRs from pytoreduction exhibited photocatalytic activity against RB dye and this dye degradation demonstrated that the nanorods of bio-synthesised g-CuO could efficiently degrade 94.5% within 30 min under sunlight irradiation (Figure 6(b)) indicating that the RB is stable enough to be the probe for the investigation of the photocatalytic properties of the as-prepared photocatalysts. Usually, photocatalytic degradation processes are carried out only in wastewater because the radicals can only react with the RB dye dissociated. Therefore, the degradation of dye is likely related to their dissociation degree of RB dye in wastewater.

Furthermore, the kinetics of dye degradation was estimated and the results are shown in Figure 6(d). The kinetics models of the pseudo-first-order model were tested to determine the kinetics rate in the degradation process of RB dye onto the g-CuO NRs and are commonly expressed as the flowing equation, $\ln(C_t/C_0) = kt$. Where k is the photodegradation rate constant (min^{-1}) and C_0 and C_t are the concentrations (mg/L) of dye after self-photolysis and at different irradiation times, respectively. The results showed linear curve fit between $\ln(C_t/C_0)$ and reaction time t of different dyes follows a pseudo-first-order kinetics behaviour [47,48]. The correlation coefficients (R^2) were higher than 0.9576, which indicates that the photodegradation of dyes fits well with the kinetic model. The rate constants of without and g-CuO NRs are 0.0004 and 0.3236 min^{-1}, respectively.

A recycling test was conducted for RB degradation in order to check the stability of g-CuO NRs for practical application. In this experiment, the removed photocatalyst after photodegradation process was washed with water and acetone for five times followed by drying at 100°C and then used for the further cycle. The results of recycling experiments are presented in Figure 7. The as-prepared g-CuO photocatalyst showed relatively higher photocatalytic activity in photodegradation of RB after four repeated cycles of reactions. That is to say, the photocatalytic activity changes little from the first time to the fourth time. The slight decrease of photocatalytic activity after four cycles was probably due to a small loss of g-CuO NRs from the samples during the photodegradation and recycling process.

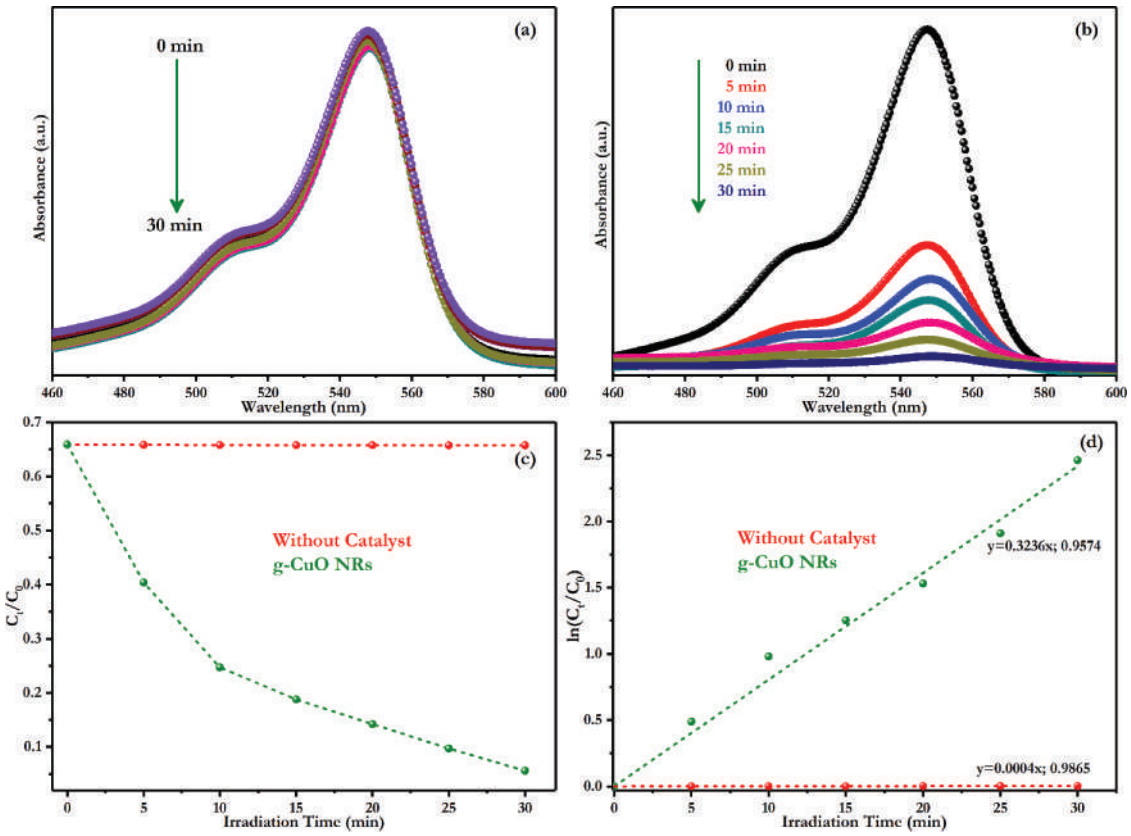


Figure 6. UV-vis spectra of photodegradation of RB dye using (a) Blank and (b) g-CuO NRs at different sunlight exposure time, (c) Photodegradation efficiency curves of RB degradation using without and with g-CuO NRs, (d) Plots of $\ln(C_t/C_0)$ versus irradiation time for the photodegradation processes of RB using without and g-CuO NRs.

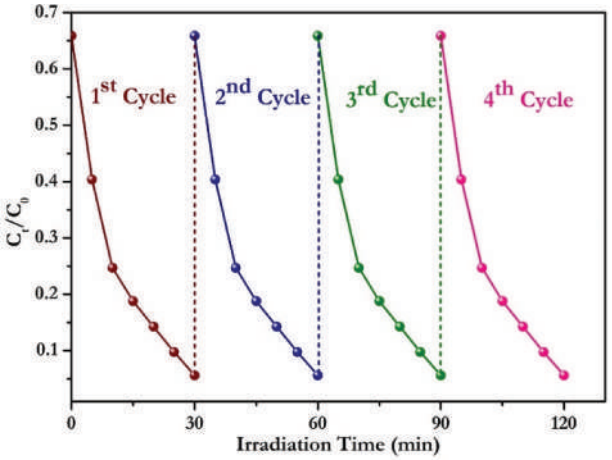


Figure 7. Recycled photocatalytic activity of g-CuO NRs for degradation of RB dye under sunlight irradiation.

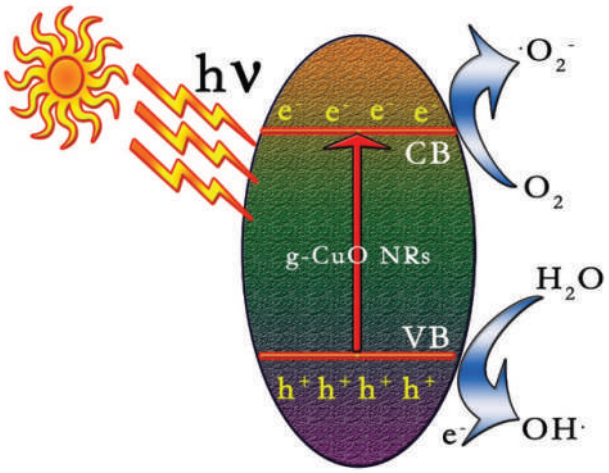


Figure 8. Possible mechanism for photocatalytic degradation of RB dye using g-CuO NRs.

In general, photocatalytic activity is a quite difficult behaviour that contains numerous steps: (i) generation of the electron–hole pairs (related to photon absorption), (ii) separation and diffusion of the charge carriers to the active surface sites, (iii) reaction of holes with defects or with the RB on the surface-solution interphase, and (iv) electron scavenging. Hence, the perceived different quantum efficiencies could be owing to the dissimilar periods of the electron–hole pairs generated by the photons interrupting on the g-CuO catalysts. Based on g-CuO NRs results, a probable reaction mechanism of g-CuO NRs function in the photocatalytic elimination of RB is depicted in Figure 8. Under sunlight irradiation, electrons transfer from valence bond (VB) to the conduction band (CB) in the illuminated g-CuO semiconductor is led to electron-hole pair formation which in turn generates superoxide radical anion (O_2^-) and hydroxyl radicals ($^{\bullet}OH$) in the presence of H_2O and O_2 molecules [49]. The relevant reaction formulas are shown as follows:

$$g - CuO + hv \rightarrow h^+ + e^-$$

$$h^+ + OH^- or H_2O \rightarrow OH$$

$$e^- + O_2 \rightarrow O_2^-$$

$$OH^-/O_2^- + RBdyes \rightarrow DegradedProducts$$

Antibacterial activity

The antibacterial activities of g-CuO NRs was studied with two pathogens of gram-negative *E.coli, K. pneumoniae* and two gram-positive *S. aureus, S. pneumoniae* bacteria. Figure 9 shows the zone of growth inhibition on bacteria due to the effect of g-CuO NRs for three different concentrations and the diameters of inhibition zone (in mm) are tabulated in Table 2. Increase in the size of zone of inhibition was noticed for all bacteria tested with increasing concentrations of g-CuO NRs indicating that the g-CuO NRs is a potent antimicrobial agent carrying more capability to kill the tested organisms. The maximum growth inhibition zones for *S. aureus* and *S. pneumoniae* are 10.30 and 9.24 mm at the concentration of 150 μg ml^{-1}, whereas inhibition zones for gram-negative *E. coli* and *K. pneumoniae* are 9.22 and 10.36 mm, respectively. It can be observed that, the g-CuO NRs exhibited better antimicrobial activity for *S. aureus and K. pneumoniae* pathogens as compared to that of *S. pneumoniae* and *E. coli bacterial pathogens* and it can be concluded that these nanorods are more active against both gram-positive and gram-negative bacterial strains. The effective antibacterial activity for g-CuO NRs was due to the adherence of nanosize of g-CuO on the bacteria membrane, degrades lipopolysaccharide molecules, causes large increases in membrane permeability, and then enters inside the bacterial cell, resulting in DNA damage and leads to bacterial cell death.

Conclusion

The g-CuO nanorods were prepared in a facile manner and characterised using various techniques and the results confirmed that the formation of highly pure and well-dispersed g-CuO NRs with average particle size of 60–80 nm. The as-obtained nanorods exhibited better photocatalytic activity in degradation of RB under sunlight irradiation. Moreover, it was demonstrated that the g-CuO NRs was stable enough to be recycled several times. Furthermore, g-CuO NRs were used to study the antibacterial activity for gram-positive and gram-negative bacteria that have shown better results at three different concentrations. Therefore, the green prepared g-CuO nanorods may find

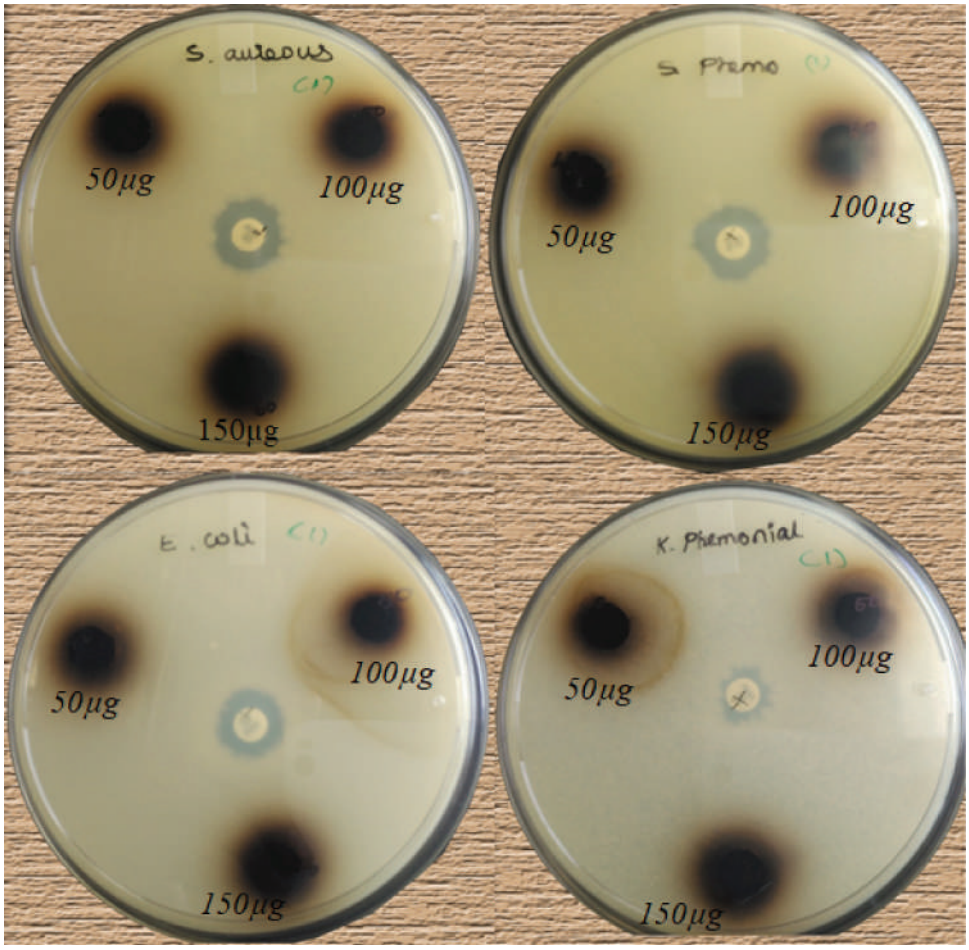


Figure 9. Growth inhibition of gram-positive (*S. aureus* and *S. pneumoniae*) and gram-negative (*E. coli* and *K. pneumoniae*) bacteria strains caused by g-CuO NRs.

Table 2. Diameters of the inhibition zone of as-prepared g-CuO NRs.

	Zone of inhibition (in mm) g-CuO NRs (Average of three measurements).		
	Concentration of the g-CuO NRs		
Bacterial strains	50(µg ml^{-1})	100(µg ml^{-1})	150(µg ml^{-1})
S. aureus	7.21 ± 0.19	8.45 ± 0.24	10.30 ± 0.14
S. pnemoniae	6.36 ± 0.43	7.50 ± 0.52	9.24 ± 0.51
E. coli	7.45 ± 0.75	7.11 ± 0.42	9.22 ± 0.26
K. pnemoniae	8.81 ± 0.11	9.81 ± 0.13	10.36 ± 0.53

promising applications in wastewater treatment and various biomedical applications.

Disclosure of potential conflicts of interest

No potential conflict of interest was reported by the author(s).

References

[1] Wächter P. The ecological rift – capitalism's war on the earth. Env Polit. 2012;21:533–535.
[2] Layzer JA. Natural experiments: ecosystem-based management and the environment. Choice Rev. 2009;46:46–3798–46–3798. Online.
[3] Chaukura N, Gwenzi W, Tavengwa N, et al. Biosorbents for the removal of synthetic organics and emerging pollutants: opportunities and challenges for developing countries. Environ Dev. 2016;19:84–89.
[4] Khandare RV, Govindwar SP. Phytoremediation of textile dyes and effluents: current scenario and future prospects. Biotechnol Adv. 2015;33:1697–1714.
[5] Jabeen N, Jabeen A. To identify hazards and risks (Health and safety) in the textile dyeing industry. Asian J Home Sci. 2017;12:182–187.
[6] Bizani E, Fytianos K, Poulios I, et al. Photocatalytic decolorization and degradation of dye solutions and wastewaters in the presence of titanium dioxide. J Hazard Mater. 2006;136:85–94.
[7] Yuh-Shan H, El-Khaiary MI. Heavy metals and the environment. CRC Press. 2009.

[8] Strauss SD. Wastewater management. Power. 1986;130:1-6.
[9] Ramar K, Vasanthakumar V, Priyadharsan A, et al. Green synthetic approach of silver nanoparticles from Bauhinia tomentosa Linn. leaves extract for potent photocatalytic and in vitro biological applications. J Mater Sci Mater Electron. 2018;29:11509–11520.
[10] Ong WJ, Tan LL, Ng YH, et al. Graphitic carbon nitride (g-C3N4)-based photocatalysts for artificial photosynthesis and environmental remediation: are we a step closer to achieving sustainability? Chem Rev. 2016;116:7159–7329.
[11] Rajabi HR. Photocatalytic activity of quantum dots. Semicond Photocatal - Mater Mech Appl. 2016. DOI:10.5772/63435
[12] Ishchenko OM, Rogé V, Lamblin G, et al. TiO2- and ZnO-based materials for photocatalysis: material properties, device architecture and emerging concepts. Semicond Photocatal - Mater Mech Appl. 2016. DOI:10.5772/62774
[13] Faraji M, Yousefi M, Yousefzadeh S, et al. Two-dimensional materials in semiconductor photoelectrocatalytic systems for water splitting. Energy Environ Sci. 2019;12:59–95.
[14] Chen S, Huang D, Xu P, et al. Semiconductor-based photocatalysts for photocatalytic and photoelectrochemical water splitting: will we stop with photocorrosion? J Mater Chem A. 2020;8:2286–2322.
[15] Sharma D, Kanchi S, Bisetty K. Biogenic synthesis of nanoparticles: a review. Arab J Chem. 2015. DOI:10.1016/j.arabjc.2015.11.002
[16] Chugh H, Sood D, Chandra I, et al. Role of gold and silver nanoparticles in cancer nano-medicine. Artif Cells Nanomed Biotechnol. 2018;46:1210–1220.
[17] Lee SH, Jun BH. Silver nanoparticles: synthesis and application for nanomedicine. Int J Mol Sci. 2019;20. DOI:10.3390/ijms20040865.
[18] Singh J, Dutta T, Kim KH, et al. "Green" synthesis of metals and their oxide nanoparticles: applications for environmental remediation. J Nanobiotechnology. 2018;16. DOI:10.1186/s12951-018-0408-4.
[19] Das RK, Pachapur VL, Lonappan L, et al. Biological synthesis of metallic nanoparticles: plants, animals and microbial aspects. Nanotechnol Environ Eng. 2017;2. DOI:10.1007/s41204-017-0029-4.
[20] Salem SS, Fouda A. Green synthesis of metallic nanoparticles and their prospective biotechnological applications: an overview. Biol Trace Elem Res. 2020. DOI:10.1007/s12011-020-02138-3
[21] Gahlawat G, Choudhury AR. A review on the biosynthesis of metal and metal salt nanoparticles by microbes. RSC Adv. 2019;9(23):12944–12967.
[22] Vinu R, Madras G. Environmental remediation by photocatalysis. J Indian Inst Sci. 2010;90:189–230.
[23] Wilcoxon JP, Abrams BL. Nanosized photocatalysts in environmental remediation. In: Nanotechnology. Weinheim (Germany):Wiley-VCH Verlag GmbH & Co. KGaA; 2010. DOI:10.1002/9783527628155.nanotech012.
[24] Singh P, Borthakur A, Mishra P, et al. Nano-materials as photocatalysts for degradation of environmental pollutants. Elsevier; 2020. DOI:10.1016/C2018-0-03858-X.
[25] Manikandan V, Jayanthi P, Priyadharsan A, et al. Green synthesis of pH-responsive Al2O3 nanoparticles: application to rapid removal of nitrate ions with enhanced antibacterial activity. J Photochem Photobiol A Chem. 2019;371:205–215.
[26] Ghidan AY, Al-Antary TM, Awwad AM. Green synthesis of copper oxide nanoparticles using Punica granatum peels extract: effect on green peach Aphid. Environ Nanotechnology, Monit Manag. 2016;6:95–98.
[27] Gopinath V, Priyadarshini S, Al-Maleki AR, et al. In vitro toxicity, apoptosis and antimicrobial effects of phyto-mediated copper oxide nanoparticles. RSC Adv. 2016;6(112):110986–110995.
[28] Prakash S, Elavarasan N, Venkatesan A, et al. Green synthesis of copper oxide nanoparticles and its effective applications in Biginelli reaction, BTB photodegradation and antibacterial activity. Adv Powder Technol. 2018;29(12):3315–3326.
[29] Rehana D, Mahendiran D, Kumar RS, et al. Evaluation of antioxidant and anticancer activity of copper oxide nanoparticles synthesized using medicinally important plant extracts. Biomed Pharmacother. 2017;89:1067–1077.
[30] Ali K, Saquib Q, Ahmed B, et al. Bio-functionalized CuO nanoparticles induced apoptotic activities in human breast carcinoma cells and toxicity against Aspergillus flavus: an in vitro approach. Process Biochem. 2020;91:387–397.
[31] Nagaraj E, Karuppannan K, Shanmugam P, et al. Exploration of bio-synthesized copper oxide nanoparticles using pterolobium hexapetalum leaf extract by photocatalytic activity and biological evaluations. J Clust Sci. 2019;30:1157–1168.
[32] Gu X, Cao R, Li Y, et al. Three-component antibacterial membrane of poly(butylene carbonate), poly (lactic acid) and chitosan prepared by electrospinning. Mater Technol. 2019;34:463–470.
[33] Nune KC, Somani MC, Spencer CT, et al. Cellular response of Staphylococcus aureus to nanostructured metallic biomedical devices: surface binding and mechanism of disruption of colonization. Mater Technol. 2017;32:22–31.
[34] Liu H, Li D, Yang X, et al. Fabrication and characterization of Ag 3 PO 4/TiO 2 heterostructure with improved visible-light photocatalytic activity for the degradation of methyl orange and sterilization of E. coli. Mater Technol. 2019;34:192–203.
[35] Depan D, Misra RDK. On the determining role of network structure titania in silicone against bacterial colonization: mechanism and disruption of biofilm. Mater Sci Eng C. 2014;34:221–228.
[36] Misra RDK, Girase B, Depan D, et al. Hybrid nanoscale architecture for enhancement of antimicrobial activity: immobilization of silver nanoparticles on thiol-functionalized polymer crystallized on carbon nanotubes. Adv Eng Mater. 2012;14:B93–B100.
[37] Venkatasubramanian R, Srivastava RS, Misra RDK. Comparative study of antimicrobial and photocatalytic activity in titania encapsulated composite nanoparticles with different dopants. Mater Sci Technol. 2008;24:589–595.
[38] Rawat J, Rana S, Sorensson MM, et al. Anti-microbial activity of doped anatase titania coated nickel ferrite composite nanoparticles. Mater Sci Technol. 2007;23:97–102.
[39] Rana S, Rawat J, Sorensson MM, et al. Antimicrobial function of Nd3+-doped anatase titania-coated nickel ferrite composite nanoparticles: a biomaterial system. Acta Biomater. 2006;2:421–432.

[40] Rawat J, Rana S, Srivastava R, et al. Antimicrobial activity of composite nanoparticles consisting of titania photocatalytic shell and nickel ferrite magnetic core. Mater Sci Eng C. 2007;27:540–545.
[41] Chowdhury R, Khan A, Rashid MH. Green synthesis of CuO nanoparticles using: lantana camara flower extract and their potential catalytic activity towards the aza-Michael reaction. RSC Adv. 2020;10:14374–14385.
[42] Jadhav MS, Kulkarni S, Raikar P, et al. Green biosynthesis of CuO & Ag-CuO nanoparticles from Malus domestica leaf extract and evaluation of antibacterial, antioxidant and DNA cleavage activities. New J Chem. 2018;42:204–213.
[43] Saranya A, Thamer A, Ramar K, et al. Facile one pot microwave-assisted green synthesis of Fe2O3/Ag nanocomposites by phytoreduction: potential application as sunlight-driven photocatalyst, antibacterial and anticancer agent. J Photochem Photobiol B Biol. 2020;207:111885.
[44] Bokuniaeva AO, Vorokh AS. Estimation of particle size using the Debye equation and the Scherrer formula for polyphasic TiO2 powder. J Phys Conf Ser. 2019;1410:012057.
[45] Singaram B, Jeyaram J, Rajendran R, et al. Visible light photocatalytic activity of tungsten and fluorine codoped TiO2 nanoparticle for an efficient dye degradation. Ionics (Kiel). 2019;25:773–784.
[46] Iqbal S, Javed M, Bahadur A, et al. Controlled synthesis of Ag-doped CuO nanoparticles as a core with poly(acrylic acid) microgel shell for efficient removal of methylene blue under visible light. J Mater Sci Mater Electron. 2020;31:8423–8435.
[47] Ahmad R, Mondal PK. Adsorption and photodegradation of methylene blue by using PAni/TiO 2 nanocomposite. J Dispers Sci Technol. 2012;33:380–386.
[48] Sarici-Özdemir Ç, Kiliç F. Kinetics behavior of methylene blue onto agricultural waste. Part Sci Technol. 2018;36:194–201.
[49] Zaleska-Medynska A. Metal oxide-based photocatalysis: fundamentals and prospects for application. Met Oxide-Based Photocatal Fundam Prospect Appl. 2018;1–353. DOI:10.1016/C2016-0-01872-7

Journal of Advanced Scientific Research
Available online through http://www.sciensage.info
ISSN 0976-9595
Research Article

EXTRACTION, ISOLATION, CHARACTERIZATION, *IN-SILICO* MOLECULAR DOCKING AND ANTICANCER EVALUATIONS OF *SOLANOPUBAMINE* FROM *SOLANUM PUBESCENS* WILLD

V. Ayyadurai*[1], C. Neela Mohan[2], V. Nandagopalan[1], K. Ramar[1]
[1]*PG and Research Department of Botany, National College (Autonomous), Tiruchirappalli, Tamilnadu, India.*
[2]*Department of Chemistry, Srimad Andavan Arts and Science College (Autonomous), Tiruchirappalli, Tamilnadu, India*
**Corresponding author: ayyaduraibot@gmail.com*

ABSTRACT
The present work deals with the extraction, isolation, characterization, *in-silico* molecular docking and anticancer activity of Solanopubamine from leaf explants of *Solanum pubescens*. The extraction was carried out by Soxhlet apparatus using DMSO as solvent. The DMSO leaf extracts were purified by column chromatography. The functional groups and structure of the isolated Solanopubamine was confirmed by Fourier transforms infrared spectroscopy (FT-IR), ^{1}H-Nuclear magnetic resonance spectroscopy (^{1}H-NMR) and high performance liquid chromatography (HPLC). The isolated Solanopubamine was separately docked into two different active sites of EGFR and ERα. The docking results by the interaction with EGFR, showed only two hydrogen bond interactions; (i) the side chain hydrogen atom of the negative charged residue of ASP 776 were well interacted with oxygen atom (O-H) of Solanopubamine with bond length of 1.996Å and (ii) hydrogen atom (N-H) of Solanopubamine molecule were well interacted with hydrophobic residue of LEU 694. The results showed that the isolated Solanopubamine compound is potential for anticancer activity. *In-vitro* anticancer activities of the isolated Solanopubamine were screened against human breast cancer cell line (MCF-7) using MTT assay. The IC_{50} values were found to be 86.33µg/ml. The present study reveals that the isolated Solanopubamine shows a significant *in-vitro* anticancer activity against MCF-7 cells and also it is less toxic for human cells.

Keywords: Solanopubamine, Alkaloid, ^{1}H-NMR, HPLC, MCF-7.

1. INTRODUCTION
Solanum pubescens belongs to the family Solanaceae and very closely related to the Turkey berry (*Solanum torvum*). *Solanum pubescens* is commonly known as Kaattusundaikaai in Tamil (Fig.1). It is a medicinally useful plant and is particularly known for its therapeutically bio-active compound; alkaloids. A variety of compounds are reported in *S. pubescens* and some of them are myricetin methyl ethers [1] Solanopubamine [2] and solanopubamides A & B [3]. The tribal people used *S. pubescens* plants for the treatment of liver disorders, diarrhoea and cancer disorders [4], however, there is no report on the anticancer activity research in this plant.

Cancer is a major cause of death and breast cancer is one of the common malignancies leading to death in women around the world. The disease occurs almost entirely in women, but men are also frome to it. Radiation is a well documented risk factor for breast cancer and its exposure induces the formation of free radicals.

As a result, novel ligands for receptors of known structure were designed and their interaction energies were calculated using the scoring functions [5]. The three dimensional structure of the protein ligand composite could be served as a considerable source of understanding the way of interation of proteins with one another and the performance of their biological functions.

Computational homology based on the modeling methods, is the best and fast technique to predict the protein structure from a known 3D template when there is no solved structure [6]. Historically virtual screening of the drug development process is divided into the structure and ligand-based algorithms [7].

The functional groups and structure of the isolated Solanopubamine was confirmed by FT-IR, ^{1}H-NMR and HPLC. The isolated Solanopubamine was separately docked into two different active sites of EGFR and ERα. The docking results showed that the isolated Solanopubamine compound is potential for anticancer activity.

Finally, the *in-vitro* anti-cancer activity of isolated Solanopubamine from *Solanum pubescens* against breast cancer cell line (MCF-7) was confirmed.

Fig. 1: *Solanum pubescens* plant a) Plant habit; b) Fruits and Flower.

2. MATERIAL AND METHODS

2.1. Collection and authentication of plant materials:

The aerial parts of *Solanum pubescens* were collected from Sirumalai hills, a part of the Western Ghats region, Dindigul district, Tamilnadu. The species was authentically identified and compared with herbarium specimen available in BSI, Coimbatore, and Tamilnadu. Specimen No: BSI/SRC/5/23/2017/Tech/2985.

2.2. Preparation of Plant Extracts

The leaves of *S. pubescens* were dried in hot air oven at 40-50°C for a week. The dried plant material was powdered using mixer grinder and subjected to soxhlet extraction with 99% methanol for 24 hours.

2.3. Column chromatography

Solanopubamine was isolated from the condensed extracts using Column chromatography and TLC and structure was confirmed by FT-IR, ^{1}H NMR and HPLC. Separation of Solanopubamine was carried out using petroleum ether as a solvent, as this particular extract showed four different spots. The separation of Solanopubamine from the extracts was carried out by column chromatography [8].

The column was packed by using wet packing technique with silica gel (300 g) as the adsorbent. Slurry was prepared using hexane and the slurry was poured in to the column. Fractions were concentrated and TLC was performed [9].

The R_f value of the spots were calculated using the formula (1)

R_f = Distance traveled by solute/Distance traveled by solvent (1)

2.4. Characterization:

2.4.1. FT-IR

Fourier transforms infrared (FT-IR) spectroscopy (Nicolet 6700 Thermo Fisher) was used to identify the functional groups present in the isolated sample (Solanopubamine) and the scanning rate was recorded in the range of 400-4000 cm^{-1}.

2.4.2. ^{1}H NMR

^{1}H-NMR analysis of the isolated Solanopubamine was performed on Varian Mercury 300 (300 MHz for ^{1}H) and Varian Inova 750 (750 MHz for 1H) instruments (Agilent Technologies, Palo Alto, CA), equipped with a 5 mm probe.

2.4.3. HPLC

Isolated *Solanopubamine* were subjected onto the HPLC analysis using Shimadzu Model LC2010 AHT Auto Sampler (UV-Vis Detector).

2.5. Cell Lines and Cytotoxicity

MCF-7 cells was obtained from the National Centre for Cell Science (NCCS), Pune, India, and grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic-anti-mycotic solution (10000U per ml penicillin, 10,000 µg/mL streptomycin and 25µg/mL amphotericin B in the culture medium). The cells were maintained as monolayer in a 96 well microplate at a density of 4 x 104 cells in 100 mL of culture medium,

and incubated overnight at 37°C under 5% CO_2 atmosphere. The cell lines were maintained at 95% humidity in a CO_2 incubator and the cultures were allowed to grow till 80-90% confluency before drug treatment. Solanopubamine were loaded in each well at a final concentration of 10, 20, 40, 80, 160 and 320 µg/mL. After incubation for 24 and 48 h, unreacted and treated cells were washed with 1% Phosphate-Buffered Saline (PBS). After that, 10 µL of 12 mM MTT stock solution of 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyl-tetrazolium bromide was added to each well and incubated for 3h. Then the medium was carefully removed without disturbing the formazan crystals, and the intracellular purple formazan crystals were dissolved in 100µL of acidified isopropanol. The plates were read for measurement of absorbance at 590 nm.

2.6. Molecular Docking

2.6.1. Preparation of Ligand Structure:

Chemdraw assistance has been taken to sketch the plant isolated molecules (http://www.cambridgesoft.com). The sketched was then prepared by Ligprep 2.7 [10] where 2D structure gets organized into 3D structure and different tautomers were also the result of Ligprep 2.7. Ligands were ionized at a pH range about 7.0 ± 2.0 to preserve the qualities for molecular docking [11].

2.6.2. Qik Prop

QikProp [12] is a quick, accurate, easy-to-use absorption, distribution, metabolism, excretion and Toxicity (ADMET) prediction program designed by Professor William L. Jorgensen. QikProp predicts physically significant descriptors and pharmaceutically relevant properties of organic molecules, either individually or in batches. In addition to predicting molecular properties, QikProp provides ranges for comparing a particular molecule's properties with those of 95% of known drugs.

2.6.3. Selection of target protein EGFR and ERα

The PDB is a key resource in areas of structural biology, such as structural genomics. Most major scientific journals and some funding agencies such as the NIH in the USA, now require scientists to submit their structure data to the PDB. If the contents of the PDB are thought of as primary data, then there are hundreds of derived (*i.e* secondary) databases that categorize the data differently. For example both SCOP and CATH categorize structures according to type of structure and assumed evolutionary relations; GO categorize structures based on genes.

Prior to perform any molecular interaction study, preparing target molecule is significant. The crystal structure of cancer protein EGFR (PDB ID: 1M17) and ERα (PDB ID: 3ERT) were retrieved from protein data bank [13, 14]. Maestro is Schrodinger's powerful united multi-platform Graphical User Interface. Import a ligand/protein co-crystallized structure, typically from the Protein Data Bank, into Maestro. Locate any waters you want to keep then delete all others. Determine whether the protein-ligand complex is a dimer or other multimer containing duplicate binding sites and duplicate chains that are redundant. Adjust the protein, metal ions, and cofactors. If there are bonds to metal ions, delete the bonds, then adjust the formal charges of the atoms that were attached to the metal as well as the metal itself. Set charges and correct atom types for any metal atoms, as needed. Set bond orders and formal charges for any cofactors, as needed.

Finally the protein structure energy was minimized reached until the average root mean square deviation non bonded hydrogen atom 0.30Å [15], the prepared protein was input file for molecular docking. Grid files represent physical properties of a volume of the receptor (specifically the active site) that are searched when attempting to dock a ligand. The complex for this exercise is actually in two files, one containing the receptor and one containing the ligand. In the Van der Waals radii scaling section, we choose Scaling factor default value of 1.00 (no scaling). The grid box X=3.524, Y= 3.241, Z= -1.342 was set for protein.

2.6.4. Examining Glide Data

Glide is employed for the rapid docking of plant isolated molecules into the active site of the target receptor. Grid was prepared for protein with the exact same center and the size of the bounding box set on 30Å the Glide algorithm is operates with a systematic search of positions, orientations, and conformations of the ligand in the receptor binding site using funnel type approach and follows a unique scoring method. Glide score and glide energy was analyzed using XP visualizer [10, 12]. Glide results are examined with an emphasis on visual rather than numerical appraisal. The set of exercises uses the Glide XP Visualizer panel to display information on the terms in the Glide XP scoring function that contribute to the ligand binding. From the Glide Docking window panel we can view docked complex can analyses with its parameter for PI3K and generated conformers with ligands.

3. RESULTS AND DISCUSSION

3.1. Isolation of Solanopubamine

Column chromatography of plant extract sample endowed with number of fraction. The alkaloids isolated from leaf extracts of *Solanum pubescens* were subjected to a detailed evaluation. The chromatographic evaluation indicated that the isolated compound from *Solanum pubescens* was pure as a single spot was obtained in TLC, whereas the compound from *Solanum pubescens* was found to be alkaloids. The range of sample R_f value on 5.8 cm, 4.3 cm and 0.74, have been perfectly correlated with Compound exhibited positive response for Dragendorff's reagent which as indicates that the isolated compound was an alkaloid. The melting point of these compounds was found to be 157-158°C.

3.2. Functional group confirmation by FT-IR spectroscopy

FT-IR spectroscopy is a useful tool for the identification of presence/absence of functional groups in the isolated Solanopubamine and its obtained spectrum is shown in Fig.2. From the results, the sample contained a broad band which appeared at 3370 cm^{-1} indicates the hydroxyl stretching (-OH); the band at 2949 cm^{-1} is attributed to N-H (stretching vibration) group frequency. A band which appeared at 1113.99 cm^{-1} was attributed to C-N stretching vibration. This FT-IR study confirms the presences of functional groups such as hydroxyl, amino and methylene group in the prepared compound. The observed result matched very well with the literature values [16]. The functional groups corresponding to their wavelength were listed in table 1.

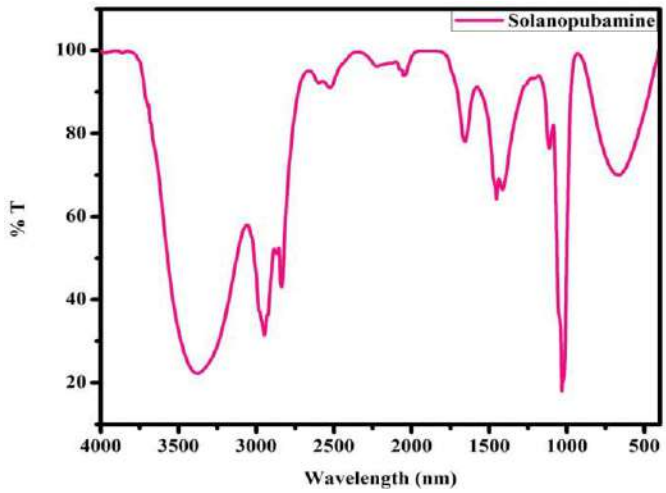


Fig. 2: FT-IR characterization of isolated compound (Solanopubamine)

Table 1: FT-IR characterization of isolated compound

Wavelength in (cm^{-1})	Name of the functional group	Functional group
3370	Alcohol	O-H
2949	Alkane	C-H
2835	Alkane	C-H
2866	Alkane	C-H
2525	Acid	O-H
2221	Alkyne	-C=C-
2025	Alkenes	-C≡C
1665	Alkene	C=C
1453	Aromatic	C=C
1414	Aromatic	C=C
1113	Amine	C-N
1031	Ether	C-O
657	Alkyl halide	C-C

3.3. Structural conformation of Solanopubamine by ^{1}H-NMR

^{1}H-NMR spectra were recorded using DMSO-d6 solvent and the ^{1}NMR spectrum of isolated Solanopubamine presented in Fig.3. According to the literature survey, the proton NMR signal was assigned (ref). Ring A: 1.44 (1H, m, 2-H), 1.54 (1H, m, 2-H), 1.70 (1H, m, 4-H), 1.14 (1H, m, 4-H), 1.70 (1H, m, 1-H), 1.09 (1H, m, 1-H); 3.34 (1H, m, 3-H); Ring B: 1.30 (2H, m, 6-H), 1.91 (1H, m, 7-H), 1.23 (1H, m, 7-H); Ring A/B: 1.26 (1H, m, 5-H); Ring C: 1.54 (1H, m, 11-H), 1.44 (1H, m, 11-H); 1.98 (1H, m, 12-H), 1.63 (1H, m, 12-H); Ring B/C: 0.69 (1H, m, 9-H), 1.57 (1H, m, 8-H);Ring D: 1.95 (2H, m, 15-H); Ring C/D: 1.44 (1H, m, 14-H); Ring E: 2.27 (1H, m, 20-H); Ring D/E: 1.91 (1H, m, 17-H), 2.73 (1H, m, 16-H); Ring F: 2.80 (1H, m, 26-H), 2.22 (1H, dd,J= 3.5 Hz, 7.1 Hz, 26-H), 1.54 (1H, m,24-H), 1.95 (1H, m, 24-H), 4.1 (1H, m, 23-H); Ring E/F: 2.18 (1H, m, 22-H); Methyl: 0.83 (3H, s, 19-H) 0.87 (3H, s, 18-H), 0.98 (3H, d, J= 5.58 Hz, 21-H), 1.23 (3H, d, J= 7.3 Hz, 27-H); the observed ^{1}H-NMR spatral valus were matched with the repots suggested by Adnan *et al,* 2013[17]. The outcome of this reports which us similaer to our report.

Positive HRESI-MS: found for $C_{27}H_{47}N_2O$: 1.39 (1H, m, 11-H); 1.82 (1H, m, 1.61 (1H, m, 8-H); Ring D: 1.84 (2H, m, 15-H); Ring C/D: 1.24 (1H, m, 14-H); Ring E: 2.24 (1H, m, 20-H); Ring D/E: 1.57 (1H, m, 17-H), 2.81 (1H, m, 16-H); Ring F: 2.78 (1H, m, 26- H), 2.06 (1H, dd, J =3.5 Hz, 7.4 Hz, 26-H), 1.61 (1H, m, 24-H), 1.08 (1H, m, 24-H), 3.85 (1H, m, 23-H); Ring

E/F: 1.85 (1H, m, 22-H); Methyl: 0.90 (3H, s, 19-H) 0.97 (3H, s, 18-H), 0.99 (3H, d, J = 6.5 Hz, 21-H), 1.26 (3H, d, J=7.5 Hz, 27-H); and 13C NMR (CDCl3; dC): Ring A: 34.50 (C-2), 37.90 (C-1), 41.10 (C-4), 51.80 (C-3); Ring B: 29.60 (C-6), 32.20 (C-7); Ring A/B: 36.70 (C-10), 46.30 (C-5); Ring C: 22.10 (C-11), 37.80 (C-12); Ring B/C: 36.60 (C-8), 55.60 (C-9); Ring D: 28.0 (C-15); Ring C/D: 42.40 (C-13), 58.60 (C-14); Ring E: 31.90 (C-20); Ring D/E: 63.50 (C-17), 71.0 (C-16); Ring F: 28.60 (C-25), 33.30 (C-24), 59.70 (C-26), 67.40 (C-23); Ring E/F: 79.90 (C-22); Methyl: 12.60 (C-19), 17.40 (C-18), 18.70 (C-21), 22.10(C-27); 12-H), 1.61 (1H, m, 12-H); Ring B/C: 0.76 (1H, m, 9-H), [17].

Proton NMR spectroscopy was used for the confirmation of structure of isolated compound and it shows the presence of 50 hydrogens in the compound [18]. The aromatic protons are observed, and the five methoxy groups were also prominent ^{1}HNMR(300 MHz, DMSOd6) δ[ppm]: 8.02(2H,d,H2',H6');7.16 (2H,d,H3',H5');6.77 (1H,s,H3); 4.02 (3H,s,OCH_3); 3.97-3.78 (12H,m, 4xOCH_3) [19].

3.4. High Performance Liquid Chromatography (HPLC)

The quality control parameter studies clearly indicate the purified Solanopubamine. The total alkaloid fraction was also subjected to HPLC studies and results were presented in Fig.4. The qualitative HPLC profiles were detected at a wavelength of 254 nm due to sharpness of the peaks and proper baseline and recorded its percent area and heights. The Fig.4 shows one major peak at 3.725 retention time. The major peak at 3.725 retention time confirmed the presence of Solanopubamine. However, there is a need to carryout advanced hyphenated spectroscopic studies in order to elucidate the structure of these compounds. Furthermore, this data may be handy in probing of active bio compound of this plant in the future [20].

3.5. Biological Activities

3.5.1. In silico Anticancer analysis

The crystal structure of EGFR (PDB ID: 1M17) and ERα (PDB ID: 3ERT) was recovered from protein data bank. The plant isolated chemical compounds Solanopubamine were separately docked into active site of the EGFR and ERα. The crystal structure of the 3D docking simulation of Solanopubamine with EGFR, the following two residues were mainly involved in hydrogen bond interaction: (i) EGFR ASP 776 with bond length (1.996Å) and (ii) hydrophobic residue of LEU 694. Fig. 5(a) shows that the side-chain protonated oxygen atom (O-H) well interacted with negatively charged residue of ASP 776 and nitrogen atom (N-H) interacted well with hydrophobic residue of LEU 694. Fig. 5(b) shows the 3D docking simulation of Solanopubamine into ERα formed only one hydrogen bond interaction. The side chain hydrogen atom (O-H) interacted well with negative charged residue of ASP 351 with a bond length of 1.994 Å.

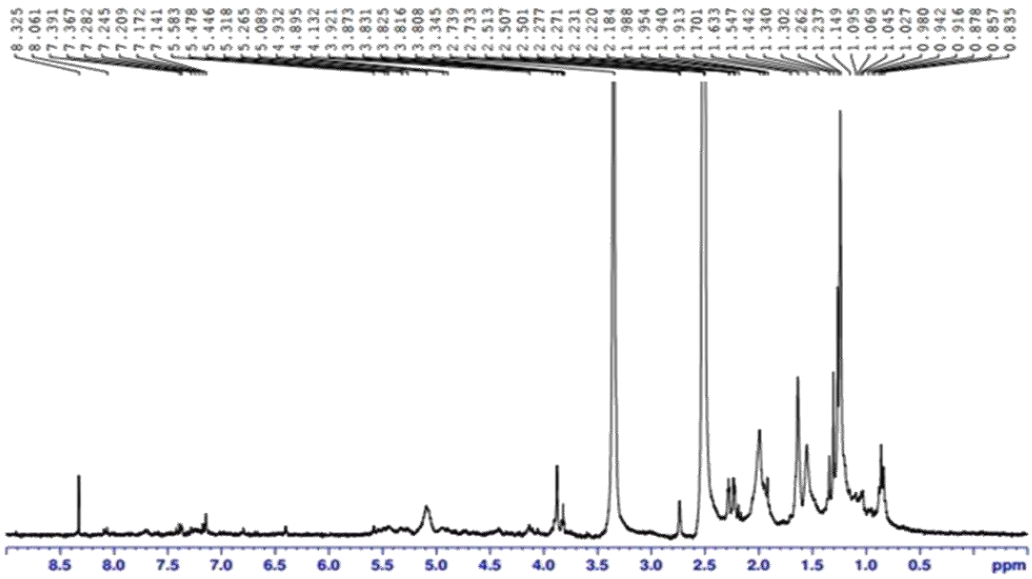


Fig. 3: ^{1}H-NMR Spectra of isolated compound Solanopubamine

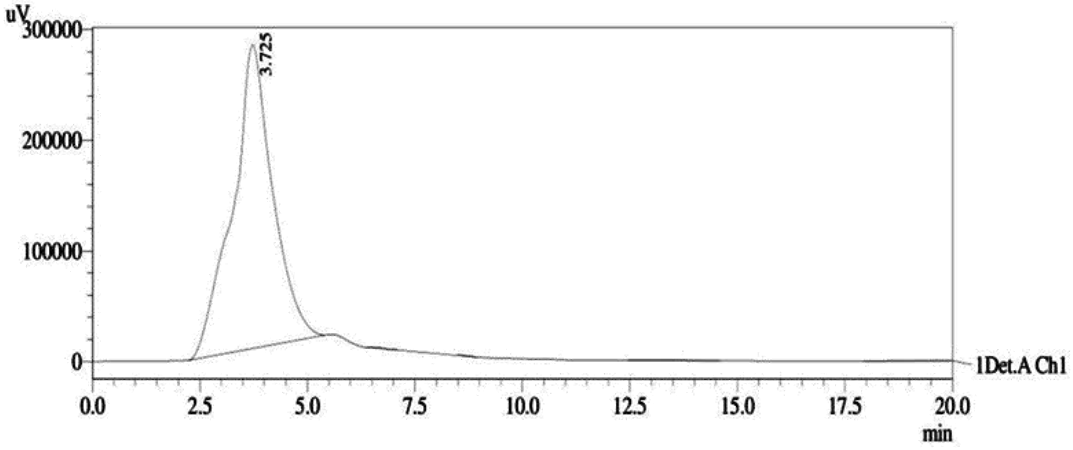


Fig. 4: HPLC- Spectra Conformation of Solanopubamine compound

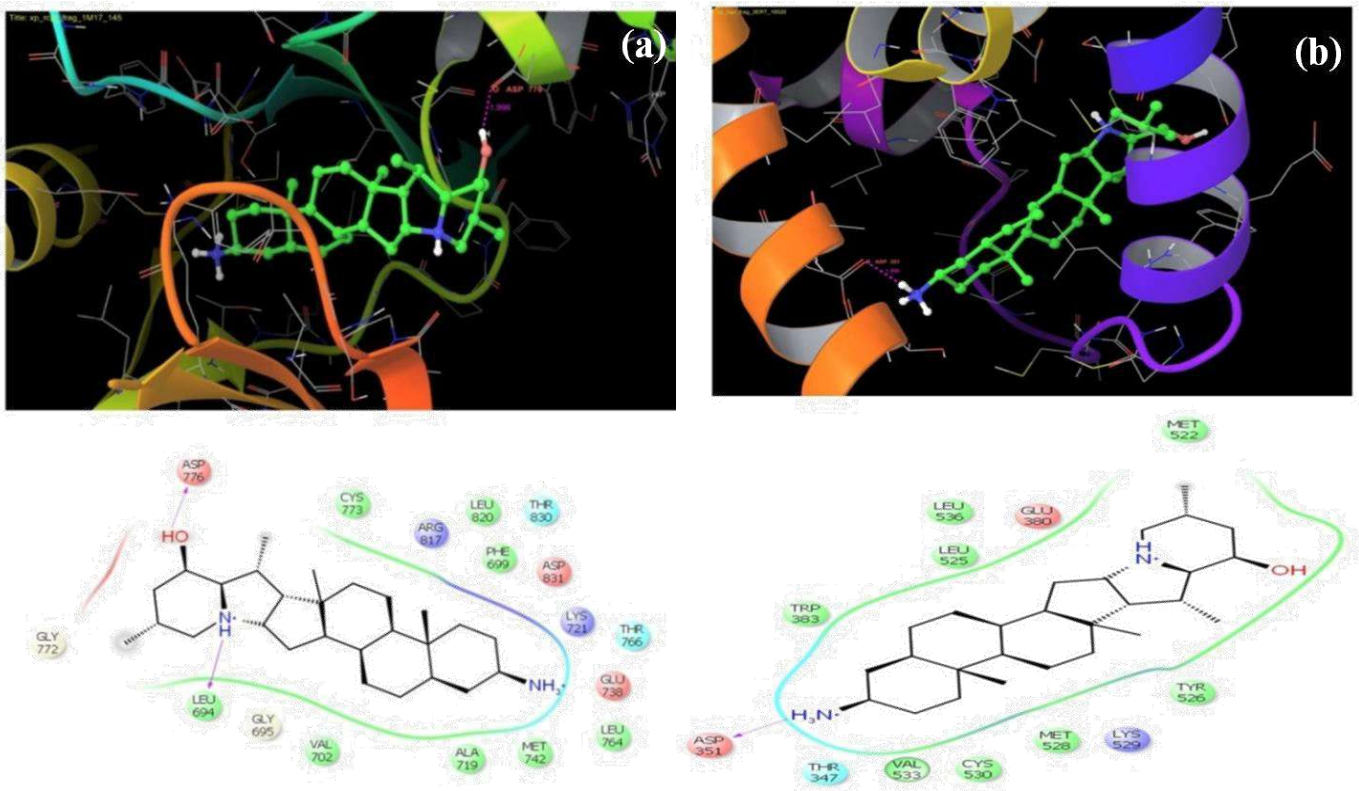


Fig. 5: 3D and 2D Docked structure of Solanopubamine target with (a) EGFR (PDB ID: IM17) protein and (b) ERα (PDB ID: 3ERT) Protein.

Interestingly, the residues TRP 383, LEU 536, MET 522, MET 528, CYS 530.CYS 773, LEU 820, MET 742 were mainly involved in hydrophobic interactions with the active site of the ERα and EGFR. The glide score and glide energy of the compounds were, as reported in Table 2. The glide score of EGFR and ERα are-4.421Kcal/mol, and -5.152Kcal/mol respectively, and the glide energy of EGFR and ERα are -30.13Kcal/mol and -32.61Kcal/mol respectively. The observed docking simulation was matched with the reports suggested by Bari *et al, 2012* [21].

3.5.2. Anticancer activity

In vitro exposures of MCF-7 cells with different concentrations (10, 20, 40, 80, 160 and 320 µg/ml) of Solanopubamine DMSO extracts significantly sup-

pressed MCF-7 cancer cell growth and decrease in cells count was observed with increase in concentration of the extracts tested. A dose dependent increase in cytotoxic activity for all the concentrations was observed. The maximum inhibition of 90.3 % of MCF-7 cells was observed at a concentration of 320 µg/ml of the plant extracted tested. *In-vitro* anticancer activity of isolated Solanopubamine is shown in Fig. 5, 6 and Table 3. The isolated Solanopubamine were shown IC_{50} value of 86.33µg/ml. Anticancer drugs with minimal side effects on normal cells are highly desirable for therapeutic purposes [22] hence; the current study also emphasized that the efficiency of TC DMSO extract-mediated suppression of cell viability on cancer cells. Indeed the concentrations of DMSO extract of TC that was cytotoxic to human breast cancer cells failed to induce apoptosis cell death.

The main aim of analyzing crude plant extracts is either to isolate bioactive agents for direct use as drugs or to identify bioactive compounds that can be used as lead substances in the preparation of semi synthetic drugs [23]. A large number of novel anticancer drugs have been discovered from natural products in the past and new ones are continually being developed thereby improving their efficacy or reducing their toxicity.

Table 2: Docking score of Solanopubamine were retrieved by Glide.

Protein name	PDB ID	Compound Name	Glide Score	Glide Energy	No. of Hydrogen bond interaction	Interacting residue	Distance (Å)
ERα	3ERT	Solanopubamine	-5.152	-32.61	1	ASP 351 (Aspartate)	1.994
EGFR	1M17	Solanopubamine	-4.421	-30.13	2	ASP 776 (Aspartate) LEU 694 (Leucine)	1.996 -

Table 3: Determination of cytotoxicity of *Solanopubamine* on MCF-7 cell line by MTT Assay

Cell line: MCF 7				
Sample	**Conc. (µg/ml)**	**OD @ 590nm**	**%Inhibition**	**IC_{50}**
Control	control	0.69	0.0	
Solanopubamine	10	0.65	6.3	86.33µg/ml
	20	0.60	12.5	
	40	0.50	27.1	
	80	0.30	46.0	
	160	0.18	74.3	
	320	0.07	**90.3**	

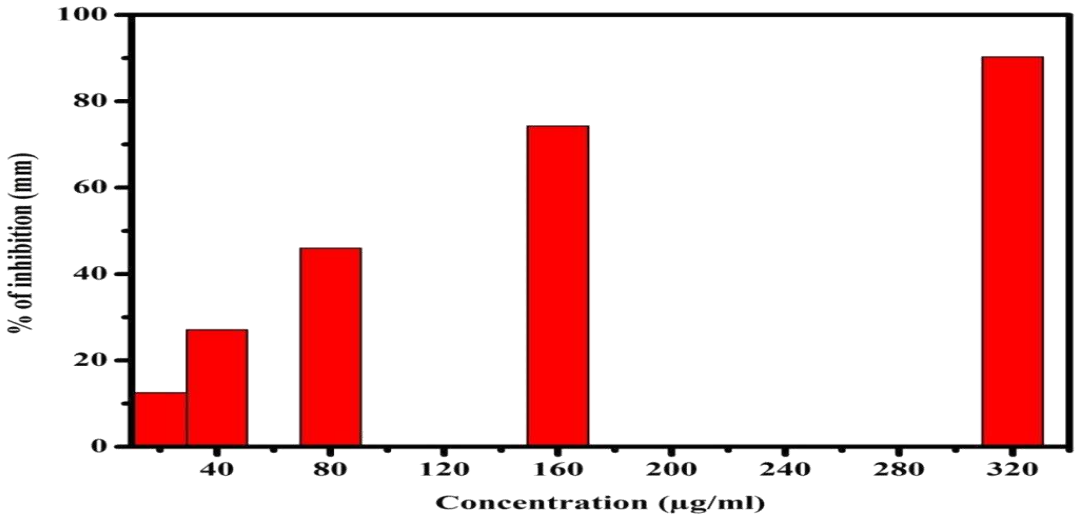


Fig. 6: Percentage (%) of Inhibition activity of isolated Solanopubamine compound with different concentrations (10-320 µg/ml)

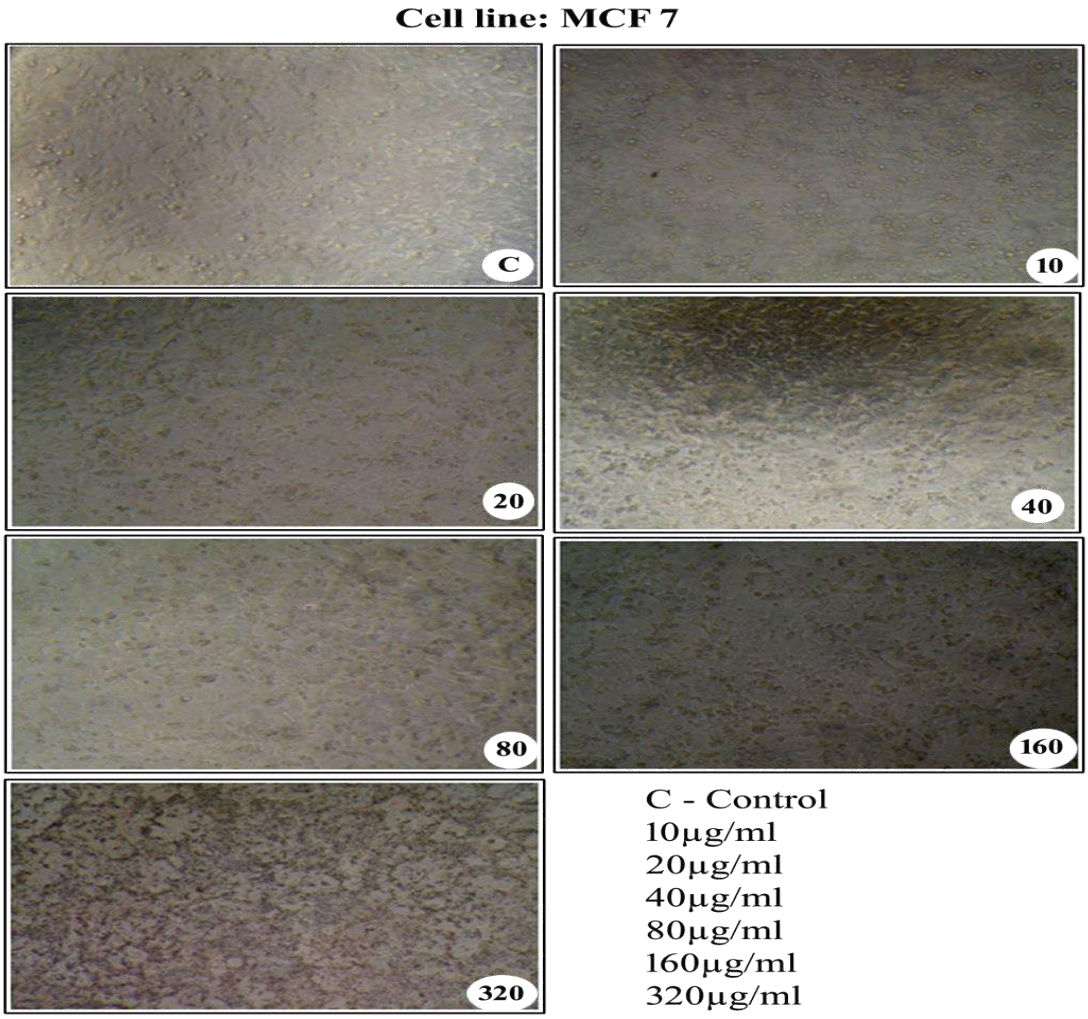


Fig. 7: *In-vitro* anticancer activity of isolated Solanopubamine compound with different concentrations (10-320 µg/ml)

4. CONCLUSION

On the basis of preliminary characterization studies, the isolated Solanopubamine compound has good physical and chemical properties (colour, state, solubility, melting range and R_f value) which are identical to the standard Solanopubamine. Spectral data shows that the isolated compound is mostly similar to standard Solanopubamine. IR peaks of various functional groups of Solanopubamine are found in this isolated compound**.** 50 protons were found in the ^{1}H-NMR spectra of isolated compound. So, it can be concluded that the isolated compound has the molecular formula $C_{27}H_{47}N_2O$ which corresponds to the molecular formula of Solanopubamine. The above results confirmed that the isolated compound is Solanopubamine. The isolated Solanopubamine was separately docked into active site of the EGFR and ERα. Further, the isolated Solanopubamine showed a significant *in-vitro* anti cancer activity against MCF-7 cells.

Conflict of interest

No potential conflict of interest was reported by the authors.

5. ACKNOWLEDGEMENT

The authors are thankful to the management of National College (Autonomous) Tiruchirappalli, Tamil Nadu, India, for providing the necessary facilities.

6. REFERENCES

1. Kumari GNK, Rao LJM, Rao NSP. *Phytochemistry,* 1984; **23(11):**2701-2702.
2. Krishna kumara GN, Rao LJM, Rao KVR, Rao NSP, Kaneko K, Mitsubashi H. *Phytochemistry,* 1985; **24(6):**1369-1371.

3. Kumari GNK, Rao LJM, Rao KVR, Rao NSP, Kaneko K, Tsuhashi HSMT. *Phytochemistry,* 1986; **25(8):**2003-2004.
4. Hemamalini K, Reddy U, Viswaja M, Nagarjuni Y, Sandhya rani V, Vinitha G. *Int. J. Phytopharm. Res.,* 2011; **2(2):**54-57.
5. Irwin JJ, Lorber DM, Mc Govern SL, Wei B, Shoichet BK. *Current Bioinformatics*, 2006; **1:**3-13.
6. Werner T, Morris MB, Dastmalchi S, Church WB, *Adv. Drug Del. Rev*., 2012; **64:**323-343.
7. Mcinnes C. *Curr. Opin. Chem. Biology*, 2007; **11:**494-502.
8. Lawrence T, Gunasekaran S, *International Journal of Innovative Research in Science, Engineering and Technology*, 2013; **2(12):**7581-7590.
9. Choudhury PK, Jadhav S. *Int J Pharm Sci Rev Res., 2013;* **20(2):**101-106.
10. Ligprep, Version 2.7, Schrodinger, LLC, New York, NY.
11. Glide, Version 6.0 (2013) Schrodinger, LLC, New York, NY.
12. Qikprop, Version 3.7, LLC, (2013) New York, NY.
13. Michael JB, Patrick JM, Devitt HZ, Stephen TD. *The Journal of Biological Chemistry*, 2002; **277:**46609-46615.
14. Mohan, Anbuselvam J, Lakshmi Prabha AM, Sumathi S, Murugan E. *Journal of Pharmacy Research,* 2012; **5(8):**4492-4495.
15. Schrodinger LLC. (2013) Protein preparation Wizard Maestro. New York.
16. Alenad AM, Al-Jaber NA, Krishnaswamy S, Yakout MS, Al-Daghri NM, Alokail MS. *J. Med. Plant Res,* 2013; **7:**1561-1567.
17. Al-Rehaily AJ, Ahmad MS, Mustafa J, Al-Oqail MM, Hassan WH, Shabana IK, Ikhlas AK. *J. Saudi Chem. Soc.,* 2013; **17(1):**67-76.
18. Himanshu J, Gyanendra KS, Vikas S, Arya E, Rahul PS. *J. Pharmacogn. Phytochem.,* 2010; **2(1):**145-151.
19. Jasim AR, Hussein AA, Nasser AMAG, *World J. Pharm. Pharm. Sci.*, 2016; **5(5):**109-128.
20. Mansoor A, MehJabeen, Jahan N. *Afr. J. Pharm. Pharmacol.,* 2012; **6(5):**322-329.
21. Bari SB, Adhikari S, Surana SJ. *Journal of PharmaSciTech.,* 2012; **1(2):**36-45.
22. Buolamwini JK. *Curr Opin Chem Biol.,* 1999; **3:**500-509.
23. Svejda B, Moser VA, Sturm S. *Anticancer Res.,* 2010; **30:**5-64.

JNROnline Journal
ISSN: 2320-3358 (e)
ISSN: 0972-5547(p)

Journal of Natural Remedies
Vol. 21, No. 9(1), (2021)

SENA KARUPPAR MINIATURE SACRED GROVE (0.03 HECTARE) IN M. PURAM AS SMALL NATURAL MEDICINAL DISPENSARY

Dhanasekar S[1]., Dr. B. Muthukumar[2].
[1]Department of Botany, Arignar Anna Govt. Arts College, Musiri (Part Time Research Scholar, National College (Autonomous), Tiruchirappalli
[2]Research Supervisor, Associate Professor & Head, Department of Botany, National College (Autonomous), Tiruchirappalli
(1 and 2 – Affiliated to BHARATHIDASAN UNIVERSITY, TIRUCHIRAPPALLI)

ABSTRACT

Floristic study and medicinal uses of plants by local people carried out in the Sena Karuppar miniature sacred grove in 0.03 ha. area present in Mahadhanapuram revenue village belongs to Krishnarayapuram block, Karur District, Tamilnadu, India. In this study area 17plants were recorded and are used as medicine in treating several diseases. Among them genera constitute (16) and families (14). Amaranthaceae family is dominant, recorded with two genera and three species followed by Arecaceae family with two genera and two species. All the plants in this study area have medicinal value and this study area act as small natural medicinal dispensary. The medicinal uses of the plants are collected as information nearly from 20 people that they used as folk medicines. Boerhaviadiffusa, Pedalium murex and Scoparia dulcisused to remove kidney stones and to improve the strength of human body.

Key words: Miniature, Sacred grove, Medicinal dispensary, Folk medicine

INTRODUCTION

Biodiversity is the degree of deviation of life forms within a given ecosystem, biome or entire planet [10]. Biodiversity encompass all species of plants, animals and microorganisms, the ecosystem and ecological processes of which they are parts. It is an umbrella term for the degree of nature's variety, embracing both number and frequency of ecosystems, species or genes in a given assemblage. Biodiversity is defined by Wilson [11] as the variety of organisms pondered at all levels from genetic variants belonging to the same species through arrays of species to arrays of genera, families and still higher taxa.

India is one of the twelve mega-biodiversity countries of the world having rich vegetation with an extensive variety of plants. Biodiversity in India is principally important for its religious, spiritual and other traditional uses. Many plants and animals have significance and considered sacred on account of their association with different deities. In India, conservation of plants and animals by indigenous people is very common. They believe trees are the place of gods. Plant worshiping is one of the earliest religious trends since the time ancient. Numerous sources are available in literatures where plants are treated as to the abode of gods [3]. In the sacred scriptures, these plants are mentioned the Kalpavrisksha and Chaityavrisksha indicating that adoring of trees is an Indian tradition. These plants are repeatedly grown within and along temples and can be respected as sacred plants [1]. Sacred groves are example of this type of conservation. Sacred groves are the locked information sites, about secret of herbs and their medicinal uses by their fore fathers as traditional medicine [9]. Sacred groves are patches of natural vegetation surviving in man modified landscapes. They be grateful their preservation to their distinguished significance to some form of spirituality. The present study aims at flora present within and edges around one such sacred grove and the plants used as medicine by local people.

Materials and Methods

The sacred grove is situated at Mahadhanapuram revenue village of Krishnarayapuram block, Karur district of Tamil Nadu located at latitude N 10° 56' 54" and longitude E 78° 18' 22". The study area is surrounded by agriculture lands where paddy (Oryza sativa), sugarcane (Saccharum

officinarum), banana (Musa paradisiaca), etc. are cultivated and it is nearly 200 m away from Tiruchirappalli to Karur National Highway.

Ficus Bengalese's, Limonin acidosis, Borassus flabellifer and Azedarach indica are keystone species present in the study area.

Floristic study and investigation on medicinal purposes of plants present in this study area were conducted on February, 2020. The plants present in this grove (0.03 ha) are identified with help of local folk medicine practitioners (20 were investigated) from different age groups (40 – 70 age) to know the local name for plants collected by them, parts used as medicine and to know their treat on several health problems for both human beings and cattle are enlisted in the field note book. Unknown plants are identified with the help of Flora of the Presidency of Madras [5] and The Flora of Tamandu Carnatic [7] and Hand Book on Weed Identification [8].

Results and Discussion

The floristic study and medicinal uses of plants to local people of SenaKaruppar sacred grove presented in the tables 1 and 2 respectively. Taxonomically, a total of 17 species belonging to genera (16) and families (14). Amongst genus wise distribution, genus Alternanthera was dominant with two species and other genera were represented by single species each. In order to infer the dominant family in the study area revealed that Amaranthaceae is dominant with species (3) followed by Arecaceae (2 species) and other families (12) recorded single species each (Table – 1).

Table – 1: List of plants recorded in Senna Karuppar Miniature Sacred Grove of M.puram

S.No.	Name of the Species	Local Name	Habit	Family
1.	Achyranthes asper L.	Nayurvi	Herb	Amaranthaceae
2.	Alternanthera pungensKunth	Ottaramul	Herb	Amaranthaceae
3.	Alternanthera sessilis(L.)R.Br. ex DC	Ponnakannikirai	Herb	Amaranthaceae
4.	Azadirachta indica A.Juss	Veppamaram	Tree	Meliaceae
5.	Blepharismadaraspatana(L.) Roth	Nethirapoondu, Nalilainagam	Herb	Acanthaceae
6.	Boerhaviadiffusa L. nom. cons.	Mukkurttaikkodi	Herb	Nyctaginaceae
7.	Borassus flabellifer L.	Panai, Nungumaram	Tree	Arecaceae
8.	Cissus quadrangularis L.	Pirandai	Vine	Vitaceae
9.	Coccinia indica Wight &Arn. (Syn.)	Kovaikodi	Climber	Cucurbitaceae
10.	Cocos nucifera L.	Thenaimaram	Tree	Arecaceae
11.	Cynodondactylon(L.) Pers.	Arugampul	Herb	Poaceae
12.	Ficus benghalensisL.	Alamaram	Tree	Moraceae
13.	Limmoniaacidissima L.	Vilamaram	Tree	Rutaceae
14.	Pedalium murex L.	Anainerunjil	Herb	Pedaliaceae
15.	Prosopis juliflora(Sw.) D.C.	Semaikaruvelam, Seethaimul	Shrub	Fabaceae
16.	Scopalia dulcis L.	Kallurukki	Herb	Plantaginaceae
17.	Tridax procumbens L.	Vettukaipoodu,Kinnarrupasan	Herb	Asteraceae

Amid to habit wise distribution, trees constitute species (4), shrub (1), vine (1), climber (1) and herbs (10) (Figure - 1). All the seventeen species present in the study area are collected by local people for medicinal purposes are given in the table – 2.

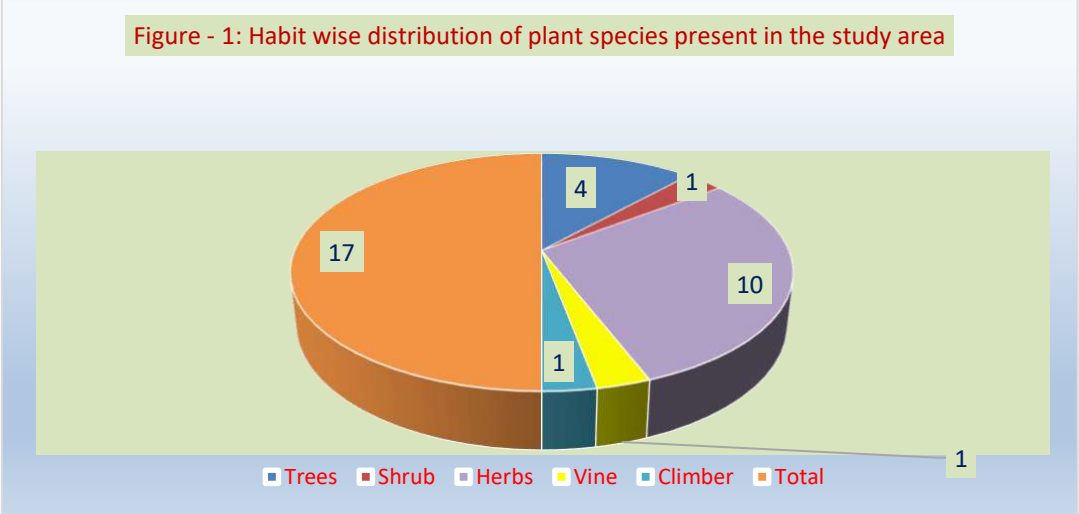


Table – 2: Shows the medicinal uses of plants recorded from the Sena Karruppar Miniature Sacred grove of M.Puram

S. No.	Name of the Species	Useful Parts	Medicinal Uses	Referred by
1.	Achyranthes asper L.	Shadow dried and fresh whole plant	Roots powder or paste to cure cholera and paste with clean water for urine retention, powder of whole plant as paste removes toxicity in blood, cures piles, tooth ache, makes skin shine, seeds paste cures poison stings by applying on bitten site, cures skin diseases, caution to pregnant women.	Information gathered from local people
2.	Alternanthera pungensKunth	Leaves	Diuretic and decoction used to treat gonorrhoea.	Information gathered from local people
3.	Alternanthera sessilis (L.) R.Br. ex DC	Leaves	Topical application of leaf paste is used for drawing foreign object such as spines and stings from the body, also applied externally on snake bites, leaves are applied over eyes for inflammation of one or more sebaceous glands of an eyelid, conjunctivitis and chronic inflammation of the eyelid, juice or soup of fresh leaves increases breast milk and toning of body, asthma, chronic cough and intermittent fever, bleeding piles.	Information gathered from local people

4.	Azadirachta indica A.Juss	Bark, leaves, fruits, seeds and gum	Used in killing worms, cleanse the blood, used against scars and pigmentation, cures tooth ache, antibacterial, antifungal and antiprotozoal, helps in treatment of cancer, eczema, heart diseases, HIV, psoriasis and ulcers, cures muscular pain, herpes, , gum collected from more than 80 years tree increases immunity and strength of body, etc.,	Information gathered from local people
5.	Blepharismadaraspatana (L.) Roth	Leaves	Leaves extract used to treat bone fracture along with wet powder of black gram and sesame oil, crushed leaf juice is cooked with bone of burnt goat leg as soup for fast recover from fracture.	Information gathered from local people
6.	Boerhaviadiffusa L. nom. cons.	Whole plant	Root powder used to enhance sexual health of both men and women, enhance kidney strength, tones body strength, whole plant as decoction provides energy, control diabetes, removes kidney stones etc.	Information gathered from local people
7.	Borassus flabellifer L.	Whole plant	Young roots are diuretic and anthelmintic, decoction of young roots used in respiratory diseases; bark decoction with salt used as mouth wash, charcoal made of bark serve as dentifrice; fruit has anti-inflammatory and antioxidant; jelly part used to cure prickly heat, fruits treat digestive problems; consuming young leaves increases immune system, ash of spadix relieve heartburn, enlarged spleen and liver, sap from the matured tree stalk prized as a tonic, diuretic, stimulant and laxative; Pathaneer or neera (juice) collected by tapping top shoots in early morning has antifungal activity, sprouts (panaikilangu) grown from seeds below surface is highly nutritious.	[5] and Information gathered from local people
8.	Cissus quadrangularis L.	Young Stem/ Slender Stem, Leaves	Relieves pain and inflammation of the body, cures bone fracture, promotes weight loss, reduce the risk of heart disease, lowers blood sugar level, lowers the blood pressure, repairs the skin damage, strengthens the bone etc.	Information gathered from local people
9.	Coccinia indica Wight &Arn. (Syn.)	Leaves, Fruits	Leaves paste applied externally to treat scabies, juice of leaves used in treating diabetes, leprosy, jaundice, asthma and fever; leaves juice along with few ml of mother's milk / goat's milk used as eye	Information gathered from local people

			drops to clear the dusts stuck into eyes, leaves used in treating boils at anus by steaming for two minutes with application of castor oil and kept for an hour and repeated for three to five days till pus appear and get mature and burst and then heal.	
10.	Cocos nucifera L.	Fruits, Kernel of fruits, tender fruit, top shoot of matured tree	Juice of kernel used to treat ulcers and other few problems of digestive system; water of tender coconut used to low blood sugar level, anticancer, stimulates the hormones activity, keeps the body moisture; very young tender fruit (kurumbai) made into paste and applied on pimples to get cure; top shoot with terminal leafy portion are eaten are enriched with minerals.	Information gathered from local people
11.	Cynodondactylon (L.) Pers.	Stem and leaves	Juice of the shoot and leaves is coolant, purify blood, reduces blood sugar, cures gas trouble, helps to relieve tools free etc.	Information gathered from local people
12.	Ficus benghalensis L.	Barks, stem, prop roots, fruits, barks, seeds, leaves etc.,	Infusion of bark is used as powerful tonic, astringent, used in dysentery, diarrhea and treatment of diabetics; leaves are good for ulcers, the young leaves for leprosy; seeds are used as tonic and in cooling; root fiber are used in gonorrhea; the milky juice (latex) is applied externally for pains, rheumatism and lumbago (back pain), by brushing with prop roots clean the teeth and also strengthen the teeth and gums; all the parts of plants are astringent to bowels, ulcers, vomiting, vaginal complaints, fever inflammations.	[2]; [6]; information gathered local people
13.	Limmoniaacidissima L.	Fruits, bark and spines	Ripen fruits used as cooling, tonic, bark and spines used to treat excessive menstruation.	Information gathered from local people
14.	Pedalium murex L.	Leaves, stem	Juice of leaves taken in the morning removes kidney stones, juice of leaves or stem is used as sexual tonic, aphrodisiac and improves sexual performance; decoction of the plant used to treat painful urination, urinary disorders etc.	Information gathered from local people
15.	Prosopis juliflora (Sw.) D.C.	Leaves	Leaves warmed and made into paste used in wound healing, antimicrobial activity, stomachache, colds, fever, as biopesticide also etc.,	Information gathered from local people

16.	Scoparia dulcis L.	Leaves	Juice of fresh leaves used in removing kidney stones in initial stage, skin disorders, dysentery, diabetes etc.	Information gathered from local people
17.	Tridax procumbens L.	Leaves	Leaf extract used in treatment of wound healing, boils, cuts, infectious skin diseases.	Information gathered from local people

CONCLUSION

Altogether 17 plant species prevailed in this study area are collected and used as medicine by local people and folk medicine practioners. Achyranthes aspera, Azadirachta indica, Alternanthera sessilis, Boerhaviadiffusa, Cissus quadrangularis and Ficus benghalensis are used in strengthening of human body. Boerhaviadiffusa, Pedalium murex and Scoparia dulcis are used in removing kidney stones. Boerhaviadiffusa and Pedalium murex are used in preparation of sexual tonic. Cynodondactylon, Borassus flabellifer, Cocos nucifera and Azadirachta indica are used as coolant and blood purifier. Prosopis juliflora and Tridax procumbens are used in wound healing. Thus this sacred grove serve as little natural medicinal dispensary to local communities, such sacred groves are need to be conserved which are remanent of large forests. Government need to take necessary steps to aware people on sustainable use of such plants and to conserve such groves.

BIBLIOGRAPHY

1. Agnihotri, P., Singh, H. and Tariq, H.2012. Sacred Groves. A religious platform for Biodiversity conservation. Enviro News. 18:34–42.
2. Chopra., R.N., Nayar, S.L. and Chopra, J.C. 1996. Glossary of Indian Medicinal Plants.
3. Deshmukh, S., Gogate, M.G. and Gupta, A.K. 1998. Sacred groves and biological diversity: providing new dimensions to conservation issue. In: Ramakrishnan, P.S., Saxena, K.G. and Chandrashekara, U.M. (Eds.). Conserving the Sacred for Biodiversity Management. UNESCO and OXFORD-IBH Publishing. New Delhi. 397–414.
4. Gamble, J. S. 1915 – 1925. Flora of the Presidency of Madras. Neerej Publishing House, Delhi – 110052. Volume I – III (Parts XI). Pp1 – 2017.
5. Jerry.A. (2018). A Comprehensive Review on the Medicinal Properties of Borassus flabellifer. Journal of Academia and Industrial Research (JAIR). 7(7): 93 – 97.
6. Krithikar, K. R. and Basu. B. D. 1995. Indian Medicinal Plants. (4 V0lumes).
7. Mathew, K.M. 1983 & 2010. The Flora of Tamilnadu Carnatic, Publisher – Rapinat Herbarium, St. Joseph's College, Tiruchirappalli, Tamilnadu, India. Original from, University of Chicago. Digitized on 14 September, 2010. Pp. 2154.
8. Naidu, V. S. G. R. 2012. Hand Book on Weed Identification. Directorate of Weed Science Research, Jabalpur, India. Pp. 354.
9. Rajkumar, G., Ravipaul, S., Sivasamy, A., Anuradha, G. and Riyas Mohamed, S. 2014. Floristic Composition and Practices on the selected Sacred Groves of Perambalur District, Tamil Nadu. International Journal of Modern Research and Reviews. 2(11):486–491.
10. Unon, G., Storey, R. and Moore, R. 2001. Principles of Botany. McGraw – Hill.
11. Wilsons, E. O. 1992. The Diversity of Life, Penguin Books.

Plant Archives Vol. 20, Suppliment 1, 2020 pp. 902-906 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

ROLE OF NON-TIMBER FOREST PRODUCTS AND ANALYSIS OF TOTAL IMORTANT VALUE OF WOODY PLANT SPECIES PRESENT IN KOTHAMANGALAPATTI SACRED GROVE, PUDUKKOTTAI, TAMILNADU, INDIA

Dhanasekar S.[1&2*] and B. Muthukumar[3]

[1*]Department of Botany, Arignar Anna Govt. Arts College, Musiri-621 211, Tamilnadu, India.
[2]Department of Botany, National College (Autonomous), Tiruchirappalli-620001, Tamilnadu, India.
[3]Department of Botany, National College (Autonomous), Tiruchirappalli -620001, Tamilnadu, India.

Abstract

Bio – diversity is the totality of genes, species and ecosystem of a region. Sacred groves are a type of in -situ conservation, ideal centre for biodiversity which conserves several living organisms. The present study was carried out in Kothamangalapatti sacred grove, Pudukkottai, Tamilnadu, India. All the woody plant species (38) of this sacred grove contributed non – timber forest products (NTFPs) such as medicine, fire wood (or) fuel, fodder, natural pesticides and as in sacred and cultural activities. It also includes pollution control and air purifier. Total important value (TIV) index was also analysed. *Azadirachta indica.* has contributed highest TIV index (13).

Key words : Biodiversity, sacred groves, NTFPs, air purifier and TIV.

Introduction

Biodiversity is the biological diversity which includes the variety of the whole species present on earth. It includes different plants, animals, microorganisms and their genes, water ecosystems, terrestrial ecosystems and marine ecosystems in which they all are present. Therefore, the term biodiversity refers to the totality of genes, species and ecosystems of a region.

Many parts of the globe are constantly losing organisms through pollution, loss of natural habitats and environmental degradation due to human over population. The conservation of biodiversity is a vast undertaking requiring the globalisation of existing knowledge and new information on the monitoring and management of biodiversity on an unprecedented scale (Smith *et al.,* 1993).

Sacred groves

Many traditional conservation practices of indigenous people in many parts of the world such as protection of small forest patches by dedicating them to the local deity, also contributed to the conservation and protection of biodiversity such forests patches called sacred groves. Sacred groves are tracts of virgin forest harbouring rich biodiversity, protected by the local people based on their indigenous cultural and religious beliefs and taboos.

Such traditional practices have been invariably operating in different parts of India (Anthwal *et al.,* 2006). Sacred groves are the tracts of virgin forest that were left untouched by local inhabitants, sometime women are not allowed enter them, harbour rich biodiversity and are protected by the local people due to their beliefs and taboos that the idols reside in them (Gadgil and Vartak,1975; Khiewtam and Ramakrishnan,1989).

The sacred groves of Tamilnadu are a part of the local folklore and religion. Every village may have a grove, a protected area associate with local folk deities of obscure origin. Amman or the mother goddess enshrined in one of her many synonym tic forms as Kali, Mari, Pidari, Ellaikali, etc. in fulfilment of prayers for a good harvest or good health. The people of the village make votive offerings of terracotta horses, bulls or elephants to Ayyanar, the mythical watchman of the village whose

***Author for correspondence :** E-mail : dhanas39@gmail.com

statue is also consecrated in the groves.

Sacred groves are important, not only because they are sacred, but values of for reaching importance are implicit them. The scientific economical social and spiritual values in them will have to explicit. The vegetation in undisturbed groves is luxuriant and comprises several stories of trees mixed with shrubs, lianas and herbs. The soil is rich in humus and covered with thick litter (Anish Babu *et al.,* 2014). Such vegetation present in the sacred forests produces non – timber forest products (NTFPs) are which help to improve local communities lively hood sustainably. Non-Timber Forest Products (NTFP) have been considered as minor forest products in many countries and it refer to all biological materials which are collected from the natural forests for human use. Ethnobotanical researchers have so far brought on record over 500 plants significantly used by the tribal as food, dyes, tannins, drugs, narcotics, drinks, housing materials weapons, fibres and medicine (Cunningham, 2001). NFTPs play vital role among the tribal people and provide a source of income and subsistence living and are integral part of day-to-day livelihood activities of tribal people (Hedge *et al.,* 1996).

Objectives of the present study

This perspective present study aims of Kothamangalapatti sacred grove

1. To analyse the composition and distribution of woody species in 0.1
2. To enlist the NTFPs and their total important values (TIV) of plants that inhabits in this sacred grove.

Materials and Methods

Study Area

The present study was done in a sacred grove ordained to Ayyanar, the presiding deity. The sacred grove is situated between Kothamangalapatti and Pasumalaipatti villages near the Trichy – Pudukkottai highway. It is located at lat / long of 78 46'53'' E - 10 30'54''N.

The sacred grove is spread over 2-hectare area with the Ayyanar temple in the midst. In spite of the human activity within its precincts, the site is well protected and conserved since it is revered as sacred. The temple is taken care by the local community and a special festival is conducted every year in the month of April in the honour of the deity.

Materials

Four ropes in 12m length with single knot in 10m length at both terminals, Sickle, paper and pen, field note book and rough paper to collect unknown specimens.

Methodology

A one-hectare area was marked and again it was divided into 10, 10 × 10m randomly marked quadrats. Within each quadrat all woody species were identified and entered in the field note book. Unidentified plants were collected, dried using standard herbarium techniques and identified at RHT (Rapinat Herbarium, Trichy). The occurrence of respective species was tabulated with their habit and taxonomic family. The NTFPs of the enlisted woody species were noted by visiting field in different periods from local community for day to day income and their other needs sustainably and valued by giving maximum value 5 and tabulated to evaluate TIV.

Results and Discussion

The data collected during the field sampling are tabulated and consolidated in table 1 and 2. The inventory area (0.1 hectare) in the sacred grove yielded a total of woody species 38 coming under genera 36 and families 22 (Table 1). Of these species 15 were trees, shrubs 15 and straggler, lianas and vines were 8. Considering the number of families for any one hectare in different tropical forests, it shows that its 22 families within the range of 2 to 26 (Johnston and Gillman, 1995). The family *Fabaceae* have contributed species (9) followed by *Rubiaceae* (5 species) and *Euphorbiaceae* (3 species) having similarity to the total of Thirumanikkuzhi on the coromandel coast of south India (Parthasarathy and Karthickeyan, 1997).

Non-timber forest products of woody species present in the study area

The NTFPs or non- wood forest products (NWFPs) collected from all 38 plant species by the local community for their lively hoods. All the plant species have medicinal use except *Reissantia indica (Willd.) N. Halle*. *Azadirachta indica Adr. Juss., Senna auriculata Roxb., Cissus quadrangularis* L., and *Ficus benghalensis* L. have high medicinal value. The whole plant parts of *Azadirachta indica Adr. Juss*. Used as medicine, the alkaloid Margosine is found in the stem bark, the bark is used to relive Kapha and Pittadosha (Ayurvedha) (Kritikar and Basu, 1975). *Cissus quadrangularis* L. contain calcium oxalate 267 mg per 100g of tender stem parts, the juice of the stem is used in irregular menstruation and scurvy, the stem is given internally and applied topically for fracture of bones; beaten into a paste given asthma (Chopra *et al.,* 1996). Such useful medicinal plant parts are collected by the local and nearby village folk medicating persons for their own use and daily income. The fallen dried woods were

Table 1: Woody Plants list present in the Kothamangalapatti Sacred grove showing their Habit and Family.

S.No.	Name of the species	Habit	Family
1	*Acacia caesia* (L.) *Willd.*	Shrub	Fabaceae
2	*Albizia amara (Roxb.) Boivin*	Tree	Fabaceae
3	*Azadirachta indica Adr. Juss.*	Tree	Meliaceae
4	*Benkara malabarica Adanason*	Shrub	Rubiaceae
5	*Canthium parviflorum Lam.*	Shrub	Rubiaceae
6	*Capparis sepiaraia* L.	Shrub	Capparaceae
7	*Cassine glauca (Rottb.) Kuntze*	Tree	Celastraceae
8	*Chloroxylon swietenia D.C.*	Tree	Rutaceae
9	*Cissus quadrangularis* L.	Vine	Vitaceae
10	*Cissus vitigenea* L.	Vine	Vitaceae
11	*Clausena dentata (Willd.) Roxb.*	Shrub	Rutaceae
12	*Cleistanthus collinus Roxb.*	Tree	Euphorbiaceae
13	*Commiphora berryi (Arn.) Engl.*	Shrub	Burseraceae
14	*Cordia obliqua Willd.*	Tree	Cordiaceae
15	*Derris scandens (Roxb.)Benth.*	Liana	Fabaceae
16	*Dichrostachys cinerea* (L.) *Wight & Arn.*	Shrub	Fabaceae
17	*Diospyros ebenum J. Koenig Retz.*	Tree	Ebenaceae
18	*Euphorbia antiquorum* L.	Shrub	Euphorbiaceae
19	*Ficus benghalensis* L.	Tree	Moraceae
20	*Flacourtia indica (Burm. f) Merr.*	Shrub	Flacourtiaceae
21	*Gmelina asiatica* L.	Shrub	Verbenaceae
22	*Holoptelea integrifolia (Roxb.) Planchon*	Tree	Ulmaceae
23	*Jasminum angustifolium Vahl.*	Liana	Oleaceae
24	*Jatropha gossypifolia* L.	Shrub	Euphorbiaceae
25	*Lannea corromandelica (Houtt) Merr.*	Tree	Anacardiaceae
26	*Pavetta indica* L.	Shrub	Rubiaceae
27	*Premna serratifolia* L.	Shrub	Verbenaceae
28	*Prosopis juliflora (Sw.) DC.*	Tree	Fabaceae
29	*Pteralobium haxapetalum (Roth.) Santapau & Wagh*	Straggler	Fabaceae
30	*Randia dumetorum (Retz.) Poinet.*	Shrub	Rubiaceae
31	*Reissantia indica (Willd.) N. Halle*	Liana/Woody climber	Celastraceae
32	*Senna auriculata Roxb.*	Shrub	Fabaceae
33	*Strychnos nuxvomica L.*	Tree	Loganiaceae
34	*Tarena asiatica* L.	Shrub	Rubiaceae
35	*Vachellia horrida* (L.) *Kyal. & Boatwr.*	Tree	Fabaceae
36	*Vachellia leucoploea (Roxb.) Willd.*	Tree	Fabaceae
37	*Wrightia tinctoria (Roxb.) R.Br.*	Tree	Apocynaceae
38	*Ziziphus oenoplia* (L.) *Miller*	Straggler	Rhamnaceae

collected to use as fire wood (FW).

Acacia caesia (L.) *Willd.*, *Albizia amara (Roxb.) Boivin, Prosopis juliflora (Sw.) DC., Pteralobium haxapetalum (Roth.) Santapau & Wagh, Vachellia horrida* (L.) *Kyal. & Boatwr., Vachellia leucoploea (Roxb.) Willd.* And *Ziziphus oenoplia* (L.) *Miller* plant parts such as leaves and pods are collected and used as fodder for cattle and goats. Some plants raw extracts may contain organic chemical compounds that control disease causing microbes, pests, insects etc., such as *Acacia caesia* (L.) *Willd., Azadirachta indica Adr. Juss., Chloroxylon swietenia D.C.* and *Euphorbia antiquorum* L., *Prosopis juliflora (Sw.) DC.* Fruits of *Pteralobium haxapetalum (Roth.) Santapau & Wagh* used as vegetable by olden people.

Azadirachta indica Adr. Juss., Wrightia tinctoria

Table 2: Non-Timber Forest Products obtained from woody plants and their total important value of present study area.

S.No.	Name of the species	MED	FW	FOD	NP	CULT	Total
1	*Acacia caesia* (L.) *Willd.*	2	1	2	1	-	6
2	*Albizia amara (Roxb.) Boivin*	2	2	2	-	1	7
3	*Azadirachta indica Adr. Juss.*	5	2	1	3	2	13
4	*Benkara malabarica Adanason*	1	2	-	-	-	3
5	*Canthium parviflorum Lam.*	2	1	2	-	-	5
6	*Capparis sepiaraia* L.	2	1	-	-	-	3
7	*Cassine glauca (Rottb.) Kuntze*	2	1	-	-	-	3
8	*Chloroxylon swietenia D.C.*	2	3	-	2	-	7
9	*Cissus quadrangularis* L.	3	1	-	1	-	5
10	*Cissus vitigenea* L.	2	1	-	-	-	3
11	*Clausena dentata (Willd.) Roxb.*	1	1	-	-	-	2
12	*Cleistanthus collinus Roxb.*	1	1	-	-	-	2
13	*Commiphora berryi (Arn.) Engl.*	-	1	-	-	-	1
14	*Cordia obliqua Willd.*	2	1	-	1	-	4
15	*Derris scandens (Roxb.) Benth.*	1	2	-	1	-	4
16	*Dichrostachys cinerea* (L.) *Wight & Arn.*	1	1	-	-	-	2
17	*Diospyros ebenum J. Koenig Retz.*	2	1	-	-	-	3
18	*Euphorbia antiquorum* L.	1	-	-	2	-	3
19	*Ficus benghalensis* L.	3	2	1	-	1	7
20	*Flacourtia indica (Burm. f)Merr.*	1	-	-	-	-	1
21	*Gmelina asiatica* L.	1	1	-	-	-	2
22	*Holoptelea integrifolia (Roxb.) Planchon*	1	-	-	-	-	1
23	*Jasminum angustifolium Vahl.*	1	1	-	-	1	3
24	*Jatropha gossypifolia* L.	1	1	-	1	-	3
25	*Lannea corromandelica (Houtt) Merr.*	1	1	-	-	-	2
26	*Paveta indica* L.	1	-	-	-	-	1
27	*Premna serratifolia L.*	1	-	-	-	-	1
28	*Prosopis juliflora (Sw.) DC.*	2	2	1	2	-	7
29	*Pteralobium haxapetalum (Roth.) Santapau & Wagh*	1	1	2	-	-	4
30	*Randia dumetorum (Retz.) Poinet.*	1	1	-	-	-	2
31	*Reissantia indica (Willd.) N.Halle*	-	1	-	-	-	2
32	*Senna auriculata Roxb.*	4	1	1	-	-	6
33	*Strychnos nuxvomica* L.	2	2	-	-	-	4
34	*Tarena asiatica* L.	1	1	-	-	-	2
35	*Vachellia horrida* (L.) *Kyal. & Boatwr.*	1	2	2	-	-	5
36	*Vachellia leucoploea (Roxb.) Willd.*	2	2	2	1	-	7
37	*Wrightia tinctoria (Roxb.) R.Br.*	2	1	-	2	2	7
38	*Ziziphus oenoplia* (L.) *Miller*	1	1	2	1	-	5

Note: MED: Medicine, FW: Fire Wood, FOD: Fodder, NP: Natural Pesticide, CULT: Cultural

(Roxb.) R.Br., *Ficus benghalensis* L. and *Jasminum angustifolium Vahl.,* are used during village functions to decorate and also people believes that they may prevent them from some disease transfer and body odour. Those plant species used in cultural activities during temple functions as sacred of deities. Such plant twigs are collected by the local community during their pooja celebrations and some people collect them in some extent to earn money.

Plants or small and large forest vegetations are natural air purifiers, top soil managers, soil fertile and erosion protectors and ground water level and weather stabilizers.

Total important value analysis (TIV)

Total important value index has been calculated by

adding, up the different uses of plant species *i.e.*, bark, fruit, leaves, etc., (Table 2). *Azadirachta indica Adr. Juss*. (13) has obtained highest value as its parts used for different purposes such as medicine, fuel, fodder, natural pesticide to protect agricultural crops eco- friendly and also a major sacred plant (cultural activities also). Whilst *Albizia amara (Roxb.) Boivin*, *Chloroxylon swietenia D.C.*, *Ficus benghalensis* L., and *Wrightia tinctoria (Roxb.) R.Br.* (each 7) too are considered sacred and have been used for medicine, fire wood, fodder, natural pesticides. Those plant extracts are sprayed along with Panchakavyam.

Conclusion

All the woody plant species (38) have non – timber forest products. The local community known about NTFPs using the sacred grove for collecting parts to improve their daily income by earning money through marketing the plant products such as seeds, shade dried leaves, fruits, roots, barks, etc. TIV index analysis showed that predominant plants that which are used by us in many ways. People must aware of such plants used as medicine, natural pesticide that used by farmers of previous generation to present generation and plants used cultural activities. Urban development in and around the forests and small sacred patches should be restricted by government and its related officials. People also owe to protect such vegetations and even a single tree. Sacred groves also get diminishing due to modernization. Government sectors must take necessary steps to conserve forest resources strictly because they conserve as from pollutions, soil erosions and natural disasters.

References

Anish Babu, V.B., V.T. Antony, B. Thomas and R.N. Haritha (2014). Phytodiversity of sacred grove at Moonam Kadavu, Kasargode District, Kerala, India. *Plant Sciences Feed*., **4(7):** 57-63.

Anthwal, A., R.C. Sharma and A. Sharma (2006). Sacred Groves: Traditional way of conserving plant diversity in garhwal Himalaya, Uttaranchal. *The Journal of American Science*, **2(2):** 35-38.

Chopra, R.N., S.L. Nayar and I.R. Chopra (1996). Glossary of Indian Medicinal Plants. National Institute of Science Communications, CSIR, New Delhi.

Cunningham, A.B. (2007). Applied ethnobotany, people wild plant use and conservation. Earth Sean publishing Ltd. London and Sterling (2001). *Harur Forest office Report*. 2007. Tamil Nadu Forest Department, Harur range, Harur.

Gadgil, M. and V.D. Vartak (1975). Sacred Groves of India a plea of the continuos conservation. *J. Bombay Nat. His. Soc*.**, 72(2):** 313-320.

Hegde, R.S., S. Prakash, L. Achoth and K.S. Bawa (1996). Extraction non timber forest products in the forest of Biligiri Rangan hill, India-I. *Contribution to rural income*, **50:** 243-251.

Johnston, M. and Gillman (1995). Tree population studies in low – diversity forests, Guyana. T. Floristic composition and stand structure. *Biodiversity and Conservation*, **4:** 339-362.

Kheitwan, R.S. and P.S. Ramakrishnan (1989). Socicultural studies of the sacred groves at Cherrapunji and adjoining areas in North Eastern India. *Man in India*, **69(1):** 64 -71.

Krithikar, K.R. and B.D. Basu (1975). Indian Medicinal Plants, 4 volumes. 2nd edition. Jayyed Press, New Delhi.

Parthasarathy, N. and R. Karthickeyan (1997). Plant biodiversity inventory and convention of two tropical dry ever green forests on the coromandel coast, South India. *Biodiversity and Conservation*, **6:** 1063-1083.

Smith, F.D.M, R.M. May, R. Pellew, T.M. Johanson and K.R. Walter (1993). Estimating extinction rates. *J. Nature*, **64:** 494-496.

A-5665
[1-7]

RESEARCH ARTICLE Indian Journal of Agricultural Research, Volume Issue : ()

Whole Genome Sequencing and Genome Annotation of PGPR '*Exiguobacterium sp.* TNDT2' Isolated from Dates Palm Tree Rhizospheric Soil

Thennarasu Sugumar[1], Punithavathi Srinivasan[2], B. Muthukumar[3], E. Natarajan[3]

ABSTRACT

Background: Dates palm is one of the most economically important plant mainly cultivated in Northern Africa, Middle East and South Asia. India is the largest importer of date fruit. In India, Dates palm are cultivated majorly in Gujarat and Rajasthan. Dates farmers facing several problem in India due to lack of scientific resources. Plant growth promoting rhizobacteria (PGPR) are naturally associated with plants and it improves plant growth and yield by providing growth supplements, increasing tolerance to stressful conditions and providing resistance to fungal/bacterial diseases. We have isolated a PGPR belonging to *Exiguobacterium species* TNDT2 from Indian dates palm *Phoenix dactylifera*, in Dindigul region, Tamilnadu, India. The organism's genome was sequenced and identified several potential plant growth promoting (PGP) genes.

Methods: The organisms genome was sequenced using Whole genome shotgun sequencing method in Illumina platform. Sequences are analysed using various bioinformatics tools and assembled using Velvet assembler. Contigs are annotated using RAST server and deposited in NCBI.

Result: The isolated strain revealed various genetic determinants required for plant growth promotion. This study presents the first report of *Exiguobacterium* TNDT2 genome from Dates tree rhizosphere. Whole genome analysis and genome annotation reveals that, its genome consist of a 2,891,840 bp chromosome encoding over 3062 proteins, with a 51.63% GC content. Strain TNDT2 encodes a wide repertoire of proteins for plant growth promotion, heavy metal detoxification (cadmium, arsenic, mercury, copper and tellurite), Multi-drug resistance and stress resistance (Heat, cold and salt). Based on this study, *Exiguobacterium sp.* TNDT2 can be recognized as an important organism with a potential to be incorporated into agricultural practice of Date palm.

Key words: *Exiguobacterium sp. TNDT2, Exiguobacterium*, Extremophiles, Heavy Metal, PGPR, Rhizosphere.

INTRODUCTION

The Date Palm (*Phoenix dactylifera*) is an economically valuable plant grown in Asian and Africa arid and semi-arid zones. It has a wide range of essential nutrients with a lot of dietary

potassium (Lunde 1978). In India, few farmers favor growing Date Palm due to challenges associated with producing higher yield. Limited microbial data available for Dates plant makes designing their growth conditions difficult (Marasco *et al.* 2012). Supplementing plant with Plant Growth Promoting Rhizobacteria (PGPR) has been shown to increases yield by improving plant tolerance to stressful condition (Marasco *et al*. 2012), producing plant hormones (de Zelicourt *et al.* 2013, Garcia-Pichel *et al.* 2003, Barazani and Friedman 1999), promoting biotic and abiotic stress resistance (Ryu *et al.* 2013), promoting tolerance to water shortage (de Zelicourt *et al.* 2013), solublizing inorganic phosphate (Schachtman *et al.* 1998), siderophore production (Tian *et al.* 2009, Sharma *et al.* 2003), producing organic acids (Ndung'u-Magiroi *et al.* 2012), protecting from bacterial and fungal diseases (urnkranz *et al.* 2009), ammonia production (Wani *et al*. 2007). Since Dates trees are cultivated in dry regions, PGPR should withstand all the stressful conditions like high temperature, salinity, water shortage and support the growth of the tree.

[1]Department of Biotechnology, National College, Tiruchirappalli-620 001, Tamil Nadu, India.
[2] Institute for Healthcare Education and Translational Sciences (IHETS), Secunderabad-500 026, Telengana, India.
[3]Department of Botany, National College, Tiruchirappalli-620 002, Tamil Nadu, India.

Corresponding Author: Thennarasu Sugumar; E. Natarajan; Department of Biotechnology, National College, Tiruchirappalli-620 001, Tamil Nadu, India.; Department of Botany, National College, Tiruchirappalli-620 002, Tamil Nadu, India.
Email: thennajay@gmail.com; sking_biotech@yahoo.com

How to cite this article: Sugumar, T., Srinivasan, P., Muthukumar, B. and Natarajan, E. (). Whole Genome Sequencing and Genome Annotation of PGPR *'Exiguobacterium sp.* TNDT2' Isolated from Dates Palm Tree Rhizospheric Soil. Indian Journal of Agricultural Research. ():

Submitted: 04-08-2020 **Accepted:** 02-11-2020 **Online:**

Exiguobacterium is a orange pigmented, facultative anaerobe, motile, Gram positive bacteria with variable morphologies, ranging from small rods to cocci (Collins *et al*. 1983). Member of Genus of this organism isolated from various locations including cold and hot conditions temperature ranging from -12°C to 55°C (Vishnivetskaya

and Kathariou 2005, Vishnivetskaya *et al*. 2007), slightly alkaline and marine environment (Vishnivetskaya *et al*. 2009), plant rhizosphere (Rodrigues *et al*. 2007), biofilms (Carneiro *et al*. 2012), fresh water (Raichand *et al*. 2012), brine shrimp (Lopez-Cortes *et al*. 2006), ice (Chaturvedi and Shivaji 2006) and permafrost (Vishnivetskaya *et al*. 2006). Most members of the genus *Exiguobacterium* are polyextremophiles (Vishnivetskaya *et al*. 2014). These organisms are used in bioremediation and agricultural application. The biotechnological applications are to reduce heavy metals arsenate (Castro-Severyn *et al*. 2017), mercury (Petrova *et al*. 2002), Chromium (Okeke 2008); high catalase activity to remove peroxides in bleaching industry (Takebe *et al*. 2007); to remove pesticide (Lopez *et al*. 2005); to neutralize highly alkaline industry waste water (Kumar *et al*. 2006); and plant growth promotion (Dastager *et al*. 2010).

This study presents the first genomic report of Indian Dates tree PGPR strain *Exiguobacterium*. In the present study we aim to understand genetic determinants of various plant growth promoting activity. We identified many heavy metal resistance, multidrug resistance and stress related genes from this Whole Genome Sequencing (WGS) study.

MATERIALS AND METHODS

Bacterial growth condition and DNA Extraction

Exiguobacterium sp. strain TNDT2 isolated from Dates tree rhizosphere soil from Dindigul of Tamilnadu region, India. Bacteria was grown in LB medium for 48 hrs at 28C. DNA extraction was performed using QIAmp DNA mini kit (Qiagen) following the manufacturer's instruction. DNA quality and quantity was checked spectrophotometrically (OD260/280 ratio).

Genomic DNA library construction and sequencing

Genomic DNA library was prepared using Nextera XT Library preparation kit (Illumina, USA) following the manufacturer's recommendation. The quality and concentration was checked using Bioanalyzer and Qubit. The library sized 75 bases sequenced using NextSeq 500 sequencer (Illumina).

Sequence Quality Control, Assembly and Annotation

The sequences quality was checked using FastQC (Andrews 2010) and all other analysis were carried out using the tools available in GALAXY (Afgan *et al*. 2018). The GALAXY is loaded in Amazon Cloud System and maintained by UC Davis bioinformatics group, was used for the genomic sequence analysis. There are different tools in GALAXY for the analysis and assembly. Adopter trimming was carried out using Scythe(https://github.com/ucdavis-bioinformatics/scythe). Incorrectly called bases in 5' and 3' end regions negatively impact the assembly. Those bad quality bases and reads with <Q30 value are trimmed off in Sickle (Joshi and Fass 2011). Denovo sequence assembly of short sequences were done using Velvet tool (ZerbinoandBirney 2008, Zerbino 2010). Contigs were randomly checked on BLAST (Johnson *et al* 2008, Altschul *et al*. 1990) for quality and identity. Total contigs were annotated on RAST (Aziz *et al*. 2008) server and the contigs are deposited in GenBank. CGView server used to create genome map and check homology with another rhizospheric *Exiguobacterium sp.* MH3 Genbank no. CP006866 (White *et al* 2019, Tang *et al*. 2013, Grant and Stothard 2008). The secondary metabolite biosynthetic gene clusters were predicted using antiSMASH v5.1.2 (Blin *et al*. 2017)

RESULTS AND DISCUSSION

The sequencing reaction generated a total of 4753514 filtered paired-end reads with the average size 76 nucleotides, providing 124- fold coverage of the genome. Different hash lengths tried for the better sequence alignment in Velvet. Hash length 47 provided less number of contigs (66 numbers) with a N50 length of 1,31,572 bp and total length 2,891,840 bp sequences. The GC content of *Exiguobacterium sp.* TNDT2 is 51.63%. RAST identified 3062 coding sequences (CDS) and 345 subsystems (Fig 1) in strain TNDT2.The Whole Genome Shotgun project has been deposited at GenBank (Table 1) under the accession number QLVE01000000, Bioproject number PRJNA476830. In CGViewer contigs are mapped with the help reference

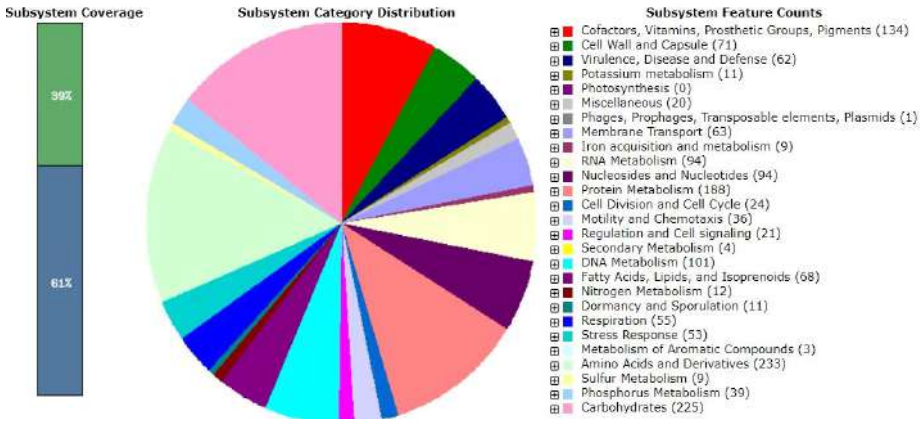


Fig 1: Genes connected to subsystems and their distribution in different categories. Subsystem Coverage: 39% in subsystem, 61% not in subsystem. Total number of subsystems 345.

genome strain MH3 and CDS location identified using BLAST in CGViewer (Fig 2). Using antiSMASH v5.1.2 we have identified terpene biosynthetic gene clusters.

Based on the RAST annotation we found that strain TNDT2 possess several genes (Table 2) encoding protein related to plant growth promotion (Auxin, Catalase, Esterase/ Lipase, Siderophore biosynthesis, Antibiotic biosynthesis and Ammonia production). Siderophore related genes are Siderophore biosynthesis protein monooxygenase, HrtA, HrtB and Hemin uptake protein. The enzyme L-asparaginase involved in ammonia production. We found many genes for Siderophore "Petrobactin" biosynthesis. The gene products are Petrobactin ABC transporter ATP-binding protein, Petrobactin ABC transporter permease protein I and Petrobactin ABC transporter permease protein II. Petrobactin is a bis-catecholate, α-hydroxy acid siderophore (Barbeau et. al. 2002). Siderophores are low molecular weight compounds, which are specific ferric chelating agents and it can promote the mineral dissolution of insoluble phases (Shirvani and Nourbakhsh, 2010). The general mechanism of siderophore-promoted Fe dissolution happens by forming the Fe(III)-siderophore complex at the mineral surface and transferred to the surrounding soil and then available for the uptake of microbes and plants (Kraemer, 2004).

Strain TNDT2 is highly motile organism. It has flagellar genes FlgG, FlgF, FlgB, FlgC,FlgD, FlaA, FliS, FlhS, MotA and MotB. It also has fimbrial assembly genes PilA, PilB and PilC. Fimbria may helps the organism to attach on the host and leads to biofilm formation. In the genome, there is a gene called 'veg' which involves in the biofilm formation (Lei *et al*. 2013). We have also identified genes responsible for Chemotaxis (CheA,CheC CheD and CheV).

Genetic analysis of this organism revealed that it can degrade many potentially dangerous heavy metals- Arsenic, mercury, zinc, Lead, cadmium, copper and tellurite. The proteins identified for the degradation are Arsenate reductase (EC 1.20.4.1); Arsenic, Lead, cadmium, zinc and mercury transporting ATPase (EC 3.6.3.3) (EC 3.6.3.5); Copper-translocating P-type ATPase (EC 3.6.3.4); Cobalt-zinc-cadmium resistance protein CzcA; Mercuric ion reductase (EC 1.16.1.1); Anion permease ArsB/NhaD-like; Cadmium-transporting ATPase (EC 3.6.3.3); Tellurite resistance protein; Camphor resistance CrcB protein and Quaternary ammonium compound-resistance protein SugE. Strain TNDT2 has high potential in treating industrial waste water and reducing heavy metal toxicity in agricultural field.

Table1: GenBank Submission Details.

Subject	Details
Organism name	Exiguobacterium sp. TNDT2
BioSample Number	SAMN09460123
BioProject Number	PRJNA476830
GenBank assembly accession	GCA_003331145.1
RefSeq assembly accession	GCF_003331145.1
WGS Project	QLVE01
Total Sequence Length	2,891,840
Number of contigs	66
Coding sequences	3062
GC content	51.6
N50 Value	1,31,572 bp
Longest Contig	304488

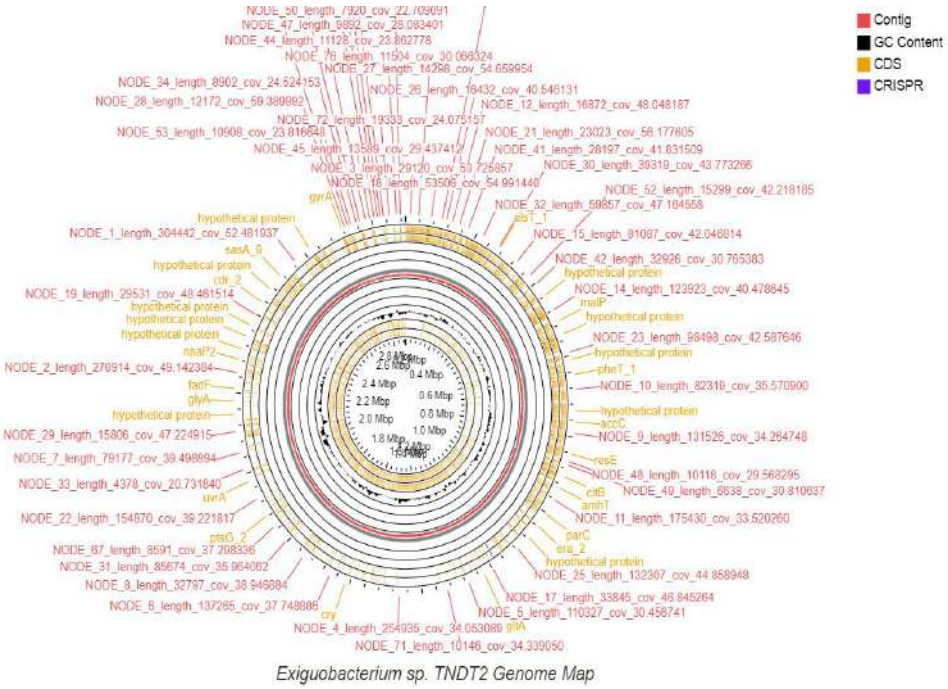


Fig 2: Genome plot of the Strain TNDT2 using CGviewer. Circular genome map made using genome *Exiguobacterium sp.* strain MH3 (GenBank: CP006866) a rhizoshpere bacteria of Lemna minor. Aligned Contigs are in purple. CDS in yellow.

Most of the members of the Genus *Exiguobacterium* are extremophiles (Vishnivetskaya *et al*. 2009). Genome analysis of Strain TNDT2 reveals that it posses many stress related genes for Cold Shock (CspC, CspD), Heat Shock protein 60 family, Heat shock protein 60 family, chaperone GroEL, Heat shock protein HtpX, Carbon starvation protein A, HtrA - prevent heat misfolding of protein, heat shock protein Hsp20, Phosphate starvation-inducible protein PhoH and Alkaline shock protein. We also found genes responsible for capsular biosynthesis CapA, Cap5F. It may help the organisms to withstand high salinity. Normally *Exiguobacterium* species are non-spore former (Chen *et al*. 2017, Collins *et al*. 1983), but we found many proteins related to spore formation - Spore protease, Spore coat protein F, Stage V sporulation protein required for dehydratation of the spore core and assembly of the coat (SpoVS) and Sporulation initiation phosphotransferase (Spo0F). BLASTX search for similar sequences of spore related genes showed that many other *Exiguobacterium* genome also has these genes. Gene SpoVs showed similarity to *Exiguobacterium sp.* Strains AB2, AT1b, S17, SH31, *Exiguobacterium chiriqhucha* RW-2*, Exiguobacterium mexicanum and Exiguobacterium antarcticum* B7 in BLASTX search. Since strain TNDT2 has many spore related proteins, it may be a spore former. Our strain also possess transposase IS30, IS200/IS605 families. The most ancient *Exiguobacterium sp.* strain 255-15 isolated from 2-3 million-year-old Siberian permafrost, has numerous putative transposase sequences, primarily of the IS*200*/ IS*605*, IS*30* and IS*3* families (Tatiana *et al*. 2005). BLASTX of IS200/IS605 family of strain TNDT2 showed similarity to *Exiguobacterium sp.NG55, Exiguobacterium sp. AM39-5BH, Exiguobacterium sibiricum* and many other *Exiguobacterium.* RAST analysis also identified CRISPR elements in our strain TNDT2. CRISPR sequences detect and destroy bacteriophage DNA during subsequent infection hence play a key role in antiviral defence system (Barrangou 2015). These sequences are not present in *Exiguobacterium chiriqhucha* str. N139 (Gutiérrez-Preciado *et. al.* 2017), *Exiguobacterium arabatum* W01(Cong M *et al.* 2017) genomes.

This strain is a mutidrug resistant one. The genome poses resistant genes for drugs vancomycin, tetracycline, chloramphenicol, refampin, Bacitracin, Acriflavin, Streptothricin, Penicillin and Methicillin. The related genes are Vancomycin B-type resistance protein VanW, rifampin ADP-ribosyl transferase, Tetracycline resistance protein, rarD chloramphenicol resistance, Streptothricin acetyltransferase, Multidrug resistance protein B, Bacitracin transport permease protein BCRB, Acriflavin resistance protein, tunicamycin resistance protein, Penicillin-binding protein and Methicillin resistance protein. *Exiguobacterium sp.* strain S3-2 (Jing Yang *et. al.* 2014) is identified in marine fish farms has seven plasmid borne antibiotic resistance genes responsible for 5 antibiotics resistance namely Tetracyclin, Chrlomphenicol, Streptomycin, Erythromycin and Trimethoprim. Genomic study of *Exiguobacterium sp.* AT1b/GX59 (Chen et. al. 2017) showed the presence of antimicrobial resistance genes, including tetracycline resistance genes, macrolide resistance genes, aminoglycoside resistance genes, phenicol resistance genes, cationic antimicrobial peptide, multidrug resistance efflux pumps (abcA and bmrA) and vancomycin resistance modules (vanY, vanW). *Exiguobacterium chiriqhucha* strain RW2 isolated from cold fresh water microbialite in Pavillion Lake possess vancomycin and tetracyclin antibiotic resistance genes and testing revealed

TABLE 2: GENETIC PROPERTIES OF *EXIGUOBACTERIUM SP. TNDT2.*

FUNCTION	GENES/PROTEINS/PRODUCTS
PLANT GROWTH PROMOTION	CATALASE, LIPASE, AUXIN, SIDEROPHORE, PHOSPHATASE, HEAVY METAL DEGRADATION, AMMONIA PRODUCTION
HEAVY METAL DEGRADATION	ARSENIC, MERCURY, CADMIUM, TELLURITE, LEAD, COPPER, COBALT, ZINC
MULTI-DRUG RESISTANCE	VANCOMYCIN, TETRACYCLINE, CHLORAMPHENICOL, REFAMPIN, BACITRACIN, ACRIFLAVIN, STREPTOTHRICIN, PENICILLIN
STRESS-RESPONSE	COLD SHOCK PROTEIN, HEAT SHOCK PROTEIN, OSMATIC STRESS, OXIDATIVE STRESS
FLAGELLAR AND FIMBRIAL GENES	FLGG, FLGF, FLGB, FLGC,FLGD, FLAA, FLIS, FLHS, PILA, PILB, PILC
INDUSTRIAL USEFUL ENZYMES	ALPHA-AMYLASE (EC 3.2.1.1), FRUCTOKINASE (EC 2.7.1.4), NEOPULLULANASE (EC 3.2.1.135), PULLULANASE (EC 3.2.1.41), ESTERASE/ LIPASE, CATALASE (EC 1.11.1.6), AMIDASE, SERINE ALKALINE PROTEASE (SUBTILISIN E), L.ASPARAGINASE (E.C.3.5.1.1), CARBOXYLESTERASE (EC 3.1.1.1) AND ENOLASE (EC 4.2.1.11)
SPORE FORMATION	SPORE PROTEASE, SPORE COAT PROTEIN F, STAGE V SPORULATION PROTEIN REQUIRED FOR DEHYDRATATION OF THE SPORE CORE AND ASSEMBLY OF THE COAT (SPOVS) AND SPORULATION INITIATION PHOSPHOTRANSFERASE (SPO0F)
CAPSULE FORMATION	CAPSULAR BIOSYNTHESIS CAPA, CAP5F

it is sensitive to both antibiotics and resistance to sulfisoxazole (White *et al*. 2019).

Other industrial important enzymes producing genes in strain TNDT2 are Alpha-amylase (EC 3.2.1.1), Fructokinase (EC 2.7.1.4), Neopullulanase (EC 3.2.1.135), Pullulanase (EC 3.2.1.41), Esterase/ Lipase, Catalase (EC 1.11.1.6), amidase, serine alkaline protease (subtilisin E), L-asparaginase (E.C.3.5.1.1), Carboxylesterase (EC 3.1.1.1) and Enolase (EC 4.2.1.11). We also identified some other enzymes Phytoene desaturase, neurosporene or lycopene producing / 4,4'-diapolycopene oxidase and Neurosporene desaturase. These enzymes involves in carotenoid neurosporene or lycopene biosynthesis. This may be responsible for colonies orange pigmentation. The carotenoid, neurosporene is an antioxidant and UV-B radiation protector and mainly used in cosmetics industry (Ramaprasad *et al*. 2013). Lycopene used as a natural colorant for decades and a powerful antioxidant (Ciriminna *et al*. 2016).

CONCLUSION

The above results suggest that *Exiguobacterium strain* TNDT2 can be adopted to wide environments like high/ moderate temperature, high salt and alkaline conditions. Most of the Dates tree grown in hot areas, this organism can be easily adopted to those environment. Since it possess many genes responsible for plant growth promotion auxin, catalase, esterase/lipase, heavy metal degradation, siderophore biosynthesis, antibiotic biosynthesis and ammonia production; it can be used as biofertilizers to increase the yield of Dates tree. This organism can be an excellent candidate for bioremediation application since we predicted many heavy metal degrading genes (Kumar *et al*. 2006, Castro-Severyn *et. al.* 2017). Carotenoid production ability of this strain may have application in natural pigment biosynthesis.

ACKNOWLEDGEMENT

We would like to thank TR and Sons Farm, Dindigal and Valargangai Farm, Vellakoil, Tiruppur, Tamil Nadu, India for providing soil samples and Institute for Healthcare Education and Translational Sciences (IHETS), Telengana, India for comments on this manuscript.

REFERENCES

Afgan, E., Baker, D., Batut, B. *et al.* (2018). The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2018 update. Nucleic Acids Research. 46(W1): W537-W544.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. (1990). Basic local alignment search tool. Journal of Molecular Biology. 215: 403-410.

Andrews, S. (2010). FastQC: a quality control tool for high throughput sequence data. Available online at:http://www. bioinformatics. babraham.ac.uk/projects/fastqc.

Aziz, R.K., Bartels, D., Best, A.A., DeJongh, M., Disz, T. *et al.* (2008). The RAST Server: Rapid Annotations using Subsystems Technology. BMC Genomics. 9: 75.

Barbeau, K., Zhang, G., Live, D.H. and Butler, A. (2002). Petrobactin, a Photoreactive Siderophore Produced by the Oil-Degrading Marine Bacterium *Marinobacter hydrocarbonoclasticus*. Journal of the American Chemical Society. 124 (3): 378-379.

Barazani, O. and Friedman, J. (1999). Is IAA the major root growth factor secreted from plant-growth-mediating bacteria? Journal of Chemical Ecology. 25: 2397-2406.

Barrangou, R. (2015). The roles of CRISPR-Cas systems in adaptive immunity and beyond. Current Opinion in Immunology. 32: 36-41.

Blin, K., Shaw, S., Steinke, K., Villebro, R., Ziemert, N., Lee, S.Y., Medema, M.H. and Weber, T. (2019). antiSMASH 5.0: updates to the secondary metabolite genome mining pipeline. Nucleic Acids Research. 47: W81-W87.

Carneiro, A.R., Ramos, R.T., Dall'Agnol, H., Pinto, A.C., de Castro Soares, S., et al. (2012). Genome sequence of *Exiguobacterium antarcticum* B7, isolated from a biofilm in Ginger Lake, King George Island, Antarctica. Journal of Bacteriology. 194: 6689-6690.

Castro-Severyn, J., Remonsellez, F., Valenzuela, S.L., Salinas, C., Fortt, J., Aguilar, P., Pardo-Esté, C., Dorador, C., Quatrini, R., Molina, F., Aguayo, D., Castro-Nallar, E. and Saavedra, C.P. (2017). Comparative Genomics Analysis of a New *Exiguobacterium* Strain from Salar de Huasco Reveals a Repertoire of Stress-Related Genes and Arsenic Resistance. Frontiers in Microbiology. 8: 456.

Chaturvedi, P. and Shivaji, S. (2006). *Exiguobacterium indicum sp. nov.,* a psychrophilic bacterium from the Hamta glacier of the Himalayan mountain ranges of India. Int. J. Syst. Evol. Microbiol. 56: 2765-2770.

Chen, X., Wang, L., Zhou, J., *et al*. (2017). *Exiguobacterium sp. A1b/GX59* isolated from a patient with community-acquired pneumonia and bacteremia: genomic characterization and literature review. BMC Infect Dis. 17(1):508.

Collins, M., Lund, B., Farrow, J. and Schleifer, K. (1983). Chemotaxonomic study 1226 of an alkalophilic bacterium, Exiguobacterium aurantiacum gen. nov., sp. nov. 1227. J. Gen. Microbiol. 129: 2037-2042.

Cong, M., Jang, Q., Xu, X., Huang, L., Su, Y. and Yan, Q. (2017). The complete genome sequence of *Exiguobacterium arabatum* W-01 reveals potential probiotic functions. Microbiology Open. 6(5): e496.

Ciriminna, R., Fidalgo, A., Meneguzzo, F., Ilharco, L.M. and Pagliaro, M. (2016). Lycopene: Emerging Production Methods and Applications of a Valued Carotenoid. ACS Sustainable Chemistry and Engineering. 4 (3): 643-650.

Dastager, S.G., Kumaran, D.C. and Pandey, A. (2010). Characterization of plant growth-promoting rhizobacterium *Exiguobacterium NII-0906* for its growth promotion of cowpea (*Vigna unguiculata*). Biologia. 65: 197-203.

de Zelicourt, A., Al-Yousif, M. and Hirt, H. (2013). Rhizosphere microbes as essential partners for plant stress tolerance. Molecular Plant. 6: 242-245.

Garcia-Pichel, F., Johnson, S.L., Youngkin, D. and Belnap, J. (2003). Small-scale vertical distribution of bacterial biomass and diversity in biological soil crusts from arid lands in the Colorado Plateau. Microbial Ecology. 46: 312-321.

Grant, J.R. and Stothard, P. (2008). The CGView Server: a comparative

genomics tool for circular genomes. Nucleic Acids Res. 36: W181-W184.

Gutiérrez-Preciado, A., Vargas-Chávez, C., Reyes-Prieto, M., Ordoñez, O.F., Santos-García, D., Rosas-Pérez, T., Valdivia-Anistro, J., Rebollar, E.A., Saralegui, A., Moya, A., Merino, E., Farías, M.E., Latorre, A. and Souza, V. (2017). The genomic sequence of *Exiguobacterium chiriqhucha* str. N139 reveals a species that thrives in cold waters and extreme environmental conditions. PeerJ. 19; 5: e3162.

Jing, Y., Chao, W., Jinyu, W., Li Liu, Gang, Z. and Jie, F. (2014). Characterization of a Multiresistant Mosaic Plasmid from a Fish Farm Sediment *Exiguobacterium sp.* Isolate Reveals Aggregation of Functional Clinic-Associated Antibiotic Resistance Genes. Applied and Environmental Microbiology. 80(4): 1482-1488.

Johnson, M., Zaretskaya, I., Raytselis, Y., Merezhuk, Y., McGinnis, S. and Madden, T.L. (2008). NCBI BLAST: a better web interface. Nucleic Acids Res. 36: W5-W9.

Joshi, N. and Fass, J. (2011). Sickle: A sliding-window, adaptive, quality-based trimming tool for FastQ files (version 1.33), available at https://github.com/najoshi/sickle.

Kraemer, S.M. (2004). Iron oxide dissolution and solubility in the presence of siderophores. Aquat Sci. 66: 3-18.

Kumar, A., Singh, V. and Kumar, R. (2006). Characterization of an alkaliphile, *Exiguobacterium sp*. and it's application in bioremediation", in Proceedings of the International Conference on Extremophiles, Brest-France, 115.

Lei, Y., Oshima, T., Ogasawara, N. and Ishikawa, S. (2013). Functional Analysis of the Protein Veg, Which Stimulates Biofilm Formation in Bacillus subtilis. Journal of Bacteriology. 195(8): 1697-1705.

Lopez, L., Pozo, C., Rodelas, B., Calvo, C., Juarez, B., Martinez-Toledo, M. *et al.* (2005) Identification of bacteria isolated from an oligotrophic lake with pesticide removal capacities. Ecotoxicology. 14: 299-312.

Lopez-Cortes, A., Schumann, P., Pukall, R. and Stackebrandt, E. (2006). *Exiguobacterium mexicanum sp. nov.* and *Exiguobacterium artemiae sp., nov.,* isolated from the brine shrimp Artemia franciscana. Syst. Appl. Microbiol. 29: 183-190.

Lunde, P. (1978). A History of Dates. Saudi Aramco World. 29(2): 176-179.

Marasco, R., Rolli, E., Ettoumi, B. *et al.* (2012). A drought resistance promoting microbiome is selected by root system under desert farming. PLoS ONE. 7: 10.

Ndung'u-Magiroi, K.W., Herrmann, L., Okalebo, J.R., Othieno, C.O., Pypers, P. and Lesueur, D. (2012). Occurrence and genetic diversity of phosphate-solubilizing bacteria in soils of differing chemical characteristics in Kenya. Annals of Microbiology. 62: 897-904.

Okeke, B.C. (2008). Bioremoval of hexavalent chromium from water by a salt tolerant bacterium, Exiguobacterium sp. GS1. J. Ind. Microbiol. Biotechnol. 35: 1571–1579.

Petrova, M., Mindlin, S., Gorlenko, Z., Kalyaeva, E., Soina, V. and Bogdanova, E.S. (2002). Mercury-resistant bacteria from permafrost sediments and prospects for their use in comparative studies of mercury resistance determinants. Russ. J. Genet. 38: 1330-1334.

Ramaprasad, E.V., Sasikala, Ch. and Ramana, Ch.V. (2013). Neurosporene is the major carotenoid accumulated by Rhodobacter viridis JA737. Biotechnol Lett. 35(7): 1093-1097.

Raichand, R., Pareek, S., Singh, N.K. and Mayilraj, S. (2012). *Exiguobacterium aquaticum sp. nov.*, a member of the genus Exiguobacterium. Int. J. Syst. Evol. Microbiol. 62: 2150 -2155.

Rodrigues, D.F. and Tiedje, J.M. (2007). Multi-locus real-time PCR for quantitation of bacteria in the environment reveals *Exiguobacterium* to be prevalent in permafrost. FEMS Microbiol. Ecol. 59: 489-499.

Ryu, C.M., Farag, M.A., Hu, C.H. *et al.* (2013). Bacterial volatiles promote growth in Arabidopsis. Proceedings of the National Academy of Sciences of the United States of America. 100: 4927-4932.

Schachtman, D.P., Reid, R.J. and Ayling, S.M. (1998). Phosphorus uptake by plants: from soil to cell. Plant Physiology. 116: 447-453.

Sharma, A. and Johri, B.N. (2003). Growth promoting influence of siderophore-producing *Pseudomonas strains GRP3A* and PRS9 in maize (*Zea maysL*.) under iron limiting conditions. Microbiological Research. 158: 243-248.

Shirvani. M. and Nourbakhsh, F. (2010). Desferrioxamine-B adsorption to and iron dissolution from palygorskite and sepiolite. Appl. Clay Sci. 48: 393–397.

Takebe, F., Hara. I., Matsuyama, H. and Yumoto, I. (2007). Effect of H2O2 under low- and high-aeration-level conditions on growth and catalase activity in Exiguobacterium oxidotolerans T-2-2T. J. Biosci. Bioeng. 104: 464-469.

Tang, J., Zhang, Y., Meng, H., Xue, Z. and Ma, J. (2013). Complete genome sequence of *Exiguobacterium sp. strain* MH3, isolated from rhizosphere of Lemna minor. Genome Announc. 1(6): e01059-13.

Tatiana, A., Vishnivetskaya and Sophia, K. (2005). Putative Transposases Conserved in *Exiguobacterium* Isolates from Ancient Siberian Permafrost and from Contemporary Surface Habitats. Applied and Environmental Microbiology. 71(11): 6954-6962.

Tian, F., Ding, Y., Zhu, H., Yao, L. and Du, B. (2009). Genetic diversity of siderophore-producing Bacteria of tobacco rhizosphere. Brazilian Journal of Microbiology. 40: 276-284.

urnkranz, M.F., uller, H.M. and Berg, G. (2009). Characterization of plant growth promoting bacteria from crops in Bolivia. Journal of Plant Diseases and Protection. 116: 149-155.

Vishnivetskaya, T.A., Chauhan, A., Layton, A., Pfiffner, S., Huntemann, M., Copeland, A. *et al.* (2014). Draft genome sequences of 10 strains of the genus *Exiguobacterium*. Genome Announc. 2:e01058-14.

Vishnivetskaya, T.A. and Kathariou, S. (2005). Putative transposases conserved in *Exiguobacterium* isolates from ancient Siberian permafrost and from contemporary surface habitats. Appl. Environ. Microbiol. 71: 6954-6962.

Vishnivetskaya, T.A., Kathariou, S. and Tiedje, J.M. (2009). The *Exiguobacterium* genus: biodiversity and biogeography. Extremophiles. 13: 541–555.

Vishnivetskaya, T.A., Petrova, M.A., Urbance, J., Ponder, M., Moyer, C.L., Gilichinsky, D.A. and Tiedje, J.M. (2006). Bacterial community in ancient Siberian permafrost as

characterized by culture and culture-independent methods. Astrobiology. 6:400-414.

Vishnivetskaya, T.A., Siletzky, R., Jefferies, N., Tiedje, J.M. and Kathariou, S. (2007). Effect of low temperature and culture media on the growth and freeze-thawing tolerance of *Exiguobacterium* strains. Cryobiology. 54: 234-240.

Wani, P.A., Khan, M.S. and Zaidi, A. (2007). Synergistic effects of the inoculation with nitrogen-fixing and phosphate-solubilizing rhizobacteria on the performance of field-grown chickpea. Journal of Plant Nutrition and Soil Science. 170: 283-287.

White, R.A.III., Soles, S.A., Gavelis, G., Gosselin, E., Slater, G.F., Lim, D.S.S., Leander, B. and Suttle, C.A. (2019). The Complete Genome and Physiological Analysis of the Eurythermal Firmicute *Exiguobacterium chiriqhucha Strain RW2* Isolated From a Freshwater Microbialite, Widely Adaptable to Broad Thermal, pH and Salinity Ranges. Front. Microbiol. 9: 3189.

Zerbino, D.R. and Birney, E. (2008). Velvet: algorithms for de novo short read assembly using de Bruijn graphs. Genome Res. 18: 821-829.

Zerbino, D.R. (2010). Using the Velvet de novo assembler for short-read sequencing technologies. Curr. Protoc. Bioinformatics. 31: 11.5.1-11.5.12.

JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2022/27275
RESEARCH ARTICLE

Evaluation of the Antioxidant and Antimicrobial Activity of *Thunbergia fragrans* Roxb Leaves Extract

Charankumar Chandrasekaran and Ramar Karuppiah*

Department of Botany, National College (Autonomous), Tiruchirappalli – 620001, Tamil Nadu, India; ramarbot@gmail.com

Abstract

Nowadays plants are the traditional product that has little or no side effects in modern medicine. The *Thunbergia fragrans* belong to the family *Acanthaceae* which contains more biologically active phytoconstituents. The plant leaves were extracted with different solvents including ethanol, aqueous, hexane and ethyl acetate. These extracts have been subjected to phytochemical study. The results of the qualitative phytochemical analysis indicate that the ethanol extracts provided more phytoconstituents. The phytochemical quantitative analysis showed that the ethanol had a higher amount of flavonoid. The ethanol extract of *Thunbergia fragrans* contained antioxidant and antibacterial activity. The GCMS study for the compound identification was studied for all four extracts. The result revealed the presence of various biologically active compounds in the ethanol extract.

Keywords: Antioxidant, Antimicrobial, Ethanolic Extract, *GCMS, Thunbergia fragrans*

1. Introduction

Plants are an important resource for traditional medicine which contains various medicinal properties[1]. *Thunbergia fragrans* is a fast-growing herbaceous vine which is widely grown as an ornamental plant in tropical and subtropical regions of the globe but is widespread in moist disturbed areas as well. Born in India and Ceylon, it is widely cultivated for its attractive white flowers in the tropical and subtropic regions. It belongs to the Acanthaceae family and has some biological function, including antidiabetic, antipyretic, antidote and anti-inflammatory activities. Along with these activities the *T. fragrans* have promising anticancer activity because of the higher flavonoid content in the plant[2,3]. This study was to investigate the chemical constituents of *T. fragrans* leaves extracted with different solvents such as aqueous, ethanol, hexane and ethyl acetate. Standard procedures were used for the qualitative and quantitative determination of chemical constituents. The purpose of the present study was to identify and characterize the phytoconstituents of various *T. fragrans* extracts[4–8]. The qualitative and quantitative phytochemical analysis and the GCMS analysis was performed to identify the compounds present in the extracts[9]. Finally, the antioxidant and antibacterial activity of *T. fragrans* extract was evaluated.

2. Materials and Methods

2.1 Plant Collection

The disease-free healthy leaves of *Thunbergia fragrans* were collected from the botanical garden in Thanjavur, Tamil Nadu, India.

2.2 Chemicals and Glassware

All the analytical grade chemicals and the glasswares were purchased from Nice chemicals and sterilized before use.

*Author for correspondence

Article Received on: 08.03.2021 Revised on: 15.06.2021 Accepted on: 17.10.2021

2.3 Plant Processing

Fresh leaves of *Thunbergia fragrans* were washed thoroughly with distilled water. The leaves were dried in a hot air oven at 45 ºC. These dried leaves were powdered using a mechanical grinder. Then the powder material was extracted in aqueous, ethanol, hexane and ethyl acetate using Soxhlet extractor. The dried extract was stored in an airtight container and maintained at 4 ºC in a refrigerator for further use. The standard methods such as qualitative and quantitative analysis was used to analyze the extract[10–12].

2.4 Qualitative Analysis of Chemical Constituents of *Thunbergia fragrans* Leaves Extracts

2.4.1 Test for Alkaloids

Plant extract (0.2 g) was warmed and filtered into a test tube containing 2 % sulfuric acid (3 mL). Few drops of Dragon dwarf reagent. The appearance of orange-red precipitates confirmed the presence of alkaloids[13,14].

2.4.2 Test for Terpenoids

2 mL of chloroform was added to 0.5 g of the extract in a test tube. The walls of test tube will form a layer when the concentrated sulfuric acid is added. The appearance of a reddish-brown coloured confirmed the presence of terpenoid[13,14].

2.4.3 Test for phenol

To a 5 ml of distilled water, 0.5 ml of plant extracts was added. The mixture was boiled for 10 min and filtered. A few drops of ferric chloride solution were added to 2 ml of filtrate. A greenish blue or violet color appearance indicated the presence of phenolic hydroxyl group[13,14].

2.4.4 Test for Tannins

In a test tube, small quantities of the plant extracts are added into 3 mL of water and placed on a boiling water bath for 5 min. The mixture was filtered and ferric chloride was added. A dark green colour indicated the presence of tannins[13,14].

2.4.5 Test for Quinones

The quinone test was carried out by the addition of 1 mL extract to 1 mL sulfuric acid. Red colour formation revealed the presence of quinines[13,14].

2.4.6 Test for Saponins

In a test tube, approximately 0.2 g of the plant extract and 5 mL of distilled water was added. The above mixture was shaken well and then filtered. The filtrate was boiled. The frothing indicated the presence of saponins[13,14].

2.4.7 Test for Reducing Sugars

2 mL of crude plant extract was added to 5 mL distilled water, shaken well and filtered. The filtrate was boiled for two minutes with 3–4 drops of Fehling solutions, A and B. The formation of orange-red precipitate confirmed the presence of reducing sugars[13,14].

2.4.8 Test for Anthraquinone

To 10 ml of benzene, 0.5 g crude powder and 0.5 ml of 10 % of ammonia was added and filtered. Filtrate were well shaken and the formation of violet colour in the layer phase indicated the presence of anthraquinones[13,14].

2.4.9 Test for Steroids

To 0.5 g of ethanolic extract (plant), 2 mL of acetic anhydride and 2 mL of sulfuric acid was added and mixed well. Violet to blue-green colour formation indicated the presence of steroids[13,14].

2.4.10 Test for Glycoside

A few mL of plant extract was added to water, glacial acetic acid, ferric chloride and concentrated sulphuric acid. The formation of the brown ring at the bottom suggested the presence of cardiac glycosides[13,14].

2.4.11 Test for Flavonoids

A 0.2 g of plant extract and was dissolved in cold diluted sodium hydroxide solution in a test tube. Diluted hydrogen chloride was added to the above solution. The presence of flavonoids was confirmed by the appearance of a colourless yellow solution[13,14].

2.4.12 Test for Protein

A few drops of 4 % sodium hydroxide and 1 % copper sulphate solution were added to 2–3 mL of plant extracts. Violet or pink appearance suggested the presence of proteins[13,14].

2.5 Chemical Constituents of *Thunbergia fragrans* Leaves Extracts

2.5.1 Estimation of Total Alkaloids

Dimethyl sulfoxide and 1 mL of hydrochloric acid (2N) were used to dissolve 1 mg/mL equivalent of the plant sample. The mixture was filtered through the filter medium and transferred to separating funnel. A 5 ml bromo-cresol green solution and a 5 ml of phosphate buffer was added to the filtrate with 1, 2, 3 and 4 ml chloroform. The mixture was vigorously shaking and collected in a 10 ml volumetric bottle and diluted to the $CHCl_3$ amount. At 470 nm with a UV-Vis spectrophotometer, the absorbance for the test and regular solutions is calculated. A series of benchmark atropine solutions were developed as stated previously (20, 40, 60, 80 and 100 µg/ml)[13].

2.5.2 Estimation of Total Flavonoids

The aluminium chloride colorimetric assay measured the total flavonoids. The reaction combination is 1 ml extract and 4 ml of distilled water was subjected to a 5 % sodium nitrite solution of 0.3 ml. 0.3 ml of 10% aluminium chloride is mixed after five minutes. Treatment and dilution of 2 ml of 1 M sodium hydroxide to 10 ml of distilled water were carried out after 5 minutes. At 510 nm with the UV-Vis spectrophotometer, the absorption of test solutions and regular solutions was calculated against the reagent blank. A benchmark quercetin solution package was developed as stated above (20, 40, 60, 80 and 100 µg/ml)[15].

2.5.3 Estimation of Total Saponins

In a conical flask containing 100 ml of 20 % aqueous ethanol, add 20 g of powdered sample. The solution was heated at 55° C for 4 hours. The solution was then filtered and extracted with 20 % ethanol of 200 ml. The two extracts were then combined and the solvent evaporated up to 40 ml of extract volume. Further removing the condensed filtrate with 20 ml diethyl retrieved the aqueous layer, while the ether was discarded. By adding 60 ml of n-butanol, the aqueous extracts were purified. It was then washed with 5% aqueous sodium chloride, twice 10 ml[16].

2.5.4 Estimation of Total Tannins

Folin-Ciocalteu method determined the overall tannins. Around 0.1 ml of this sample had a distilled water content of 7.5 ml and Folin-Ciocalteuphenol (0.5 ml) and a 35 per cent Na_2CO_3 solution of 1 ml and diluted with 10 ml distilled water. The mixture was well shaken and stored for 30 minutes at room temperature. At 725 Nm with UV-Vis spectrophotometer, absorption for testing and standardised solutions was measured against the blank. Gallic acid (20, 40, 60, 80, and 100 µg/ml) was formulated in the same way as the previously mentioned reference standard solutions were developed[17].

2.5.5 Estimation of Total Phenolics

Folin-Ciocalteu assays were used to assess the total phenolics. Approximately 1 ml extract has been added, 1ml Folin-Ciocalteu phenol reagent was shaken with 9 ml distilled water. After 5 minutes, the mixture was treated with 10 ml of 7 per cent of Na_2CO_3 and up to 25 ml in volume. The reagent blank at 550 nm with a UV-Vis spectrophotometer was incubated for 90 min at room temperature, the absorption for testing and regular solution. Some standard Gallic Acid solutions have been developed in the same way as before (20, 40, 40, 60, 80 and 100 µg/ml)[18].

2.6 GC-MS Analysis

Gas chromatography and mass spectrometry QP2010 Plus, Shimadzu, Japan fitted with the RTX–5 MS GC capillary column (5.1 % diphenyl/ 95.0 % dimethyl polysiloxane) of 0.5 µm in diameter and 30 m in length have been evaluated for identifying and quantification of *Thunbergia fragran's* chemical components. Working conditions: the temperature was kept between 40–290° C and progressively increased to 8° C/min. The oven column and the injection were set at 100° C and 270° C. Helium is used as carrier gas (mobile phase) at a rate of 1 ml/min. Injection mode has been set to a fraction

of 20. Working conditions for MS: the temperature of ion sources and interfaces was set to 200°C and 260° C. Cutting time for solvents was set to 4 min and the voltage for detectors was set at 0.1 kV. Conditions for injection: injection 1 µL, injection 10 µL, injecting temperature 240° C; weight range 20–300 m/z[19–21].

2.7 Antioxidant Activity

The radical scavenging activity of *Thunbergia fragrans* extract was calculated by DPPH assay. The stock solution of plant extract was prepared at various concentration. Adding 1 ml of DPPH in each concentration of the extract was incubated at 30 minutes in a dark room. Finally, the absorbance of the plant extract was calculated at 520 nm. Ascorbic acid was used as a standard solution[22]. The scavenging activity percentage was calculated using the formula.

$$\text{Percentage inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample}) \times 100}{\text{Absorbance of control}}$$

2.8 Antibacterial Activity

2.8.1 Disk Diffusion Method

The antibacterial activity of *Thunbergia fragrans* ethanolic leaf extract was studied against two bacterial strains *Escherichia coli* and *Staphyloccocus aureus*. The antibacterial assay was carried out by disk diffusion method as per the study performed by Chan E.,[23]. Finally the zone of inhibition was calculated.

3. Result and Discussion

3.1 Qualitative Determination

Table 1 represents the presence of chemical constituents of *T. fragrans* leaf extract of various solvent (Aqueous, ethanol, hexane, ethyl acetate). The result revealed the presence of various phytoconstituents like alkaloid, flavonoid, cardiac glycoside, saponin, tannin and phenolics. Moreover, ethanol extract showed a high number of secondary metabolites compare with other extracts. Alkaloids, flavonoids and amino acid were present in all the extracts.

3.2 Quantitative Determination

The quantitative phytochemical analysis was performed to various leaves extracts of *T. fragrans* were presented in Table 2. Quantitative evaluation indicates the presence of Alkaloids, flavonoids, saponins, tannins and phenols. Flavonoids and phenols were highly present in the ethanol extract but the tannin content was low. Comparatively ethanol extract showed more number of chemical constituents among all the extract. Further these extracts were taken to the GCMS quantification.

Table 1. Preliminary Phytochemical screening for the various extract of *Thunbergia fragrans* leaves.

S. no	Chemical constituent	Aqueous extract	Ethanol extract	Hexane extract	Ethyl acetate extract
1	Alkaloids	+	++	+	+
2	Flavonoids	+	++	+	+
3	Terpenoids	-	+	-	+
4	Phenol	+	++	++	-
5	Tannins	+	+	-	-
6	Quinones	+	-	+	+
7	Saponins	-	+	-	-
8	Reducing sugar	+	+	-	-
9	Anthraquinones	-	+	-	+
10	Steroids	-	-	+	+
11	Glycoside	-	+	-	-
12	Protein and Free amino acid	+	++	+	+

Note: (++) denotes highly present; (+) denotes present; (-) denotes not detected.

Table 2. Quantitative determination of chemical constituents of *Thunbergia fragrans* Leaf extracts

Extracts	Alkaloids	Flavonoid	Saponins	Tannins	Phenols
Aqueous	2.5±0.25	4.98±0.5	5.40±0.15	0.7±0.6	18.25±0.35
Ethanol	6.25±0.15	22.5±0.85	3.0±0.25	2.5±0.35	15.35±1.5
Hexane	0.7±0.2	10.58±1.8	6.40±0.25	0.8±0.65	7.40±0.65
Ethyl acetate	1.05±0.65	8.40±0.6	2.0±0.15	3.0±0.45	10.40±0.6

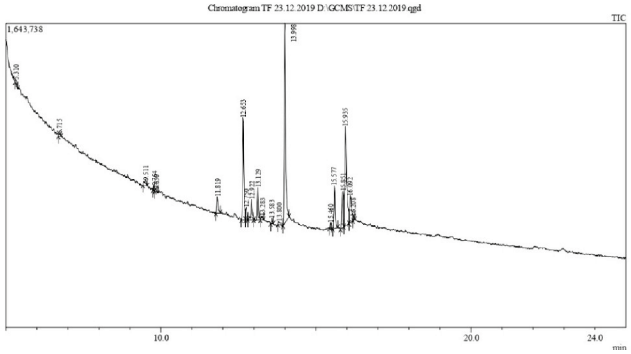


Figure 1. GCMS chromatogram of Ethanolic extract of *Thunbergia fragrans* leaves.

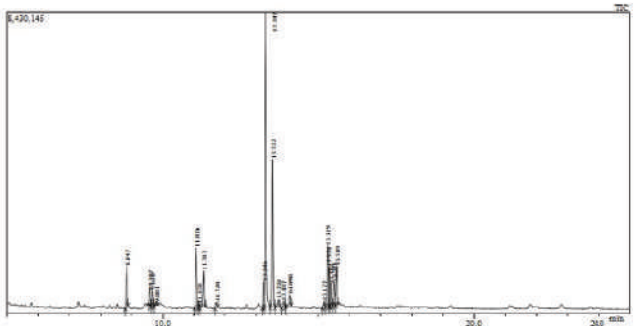


Figure 2. GCMS chromatogram of aqueous extract of *Thunbergia fragrans* leaves.

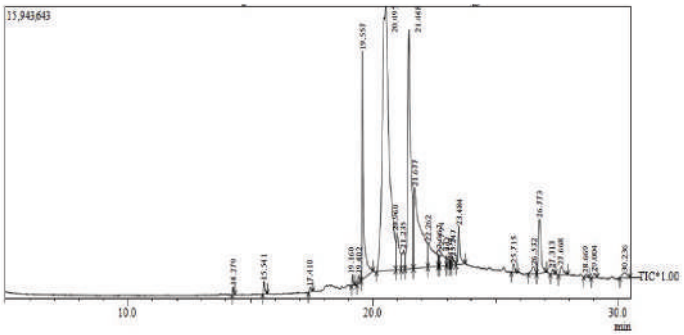


Figure 3. GCMS chromatogram of ethyl acetate extract of *Thunbergia fragrans* leaves.

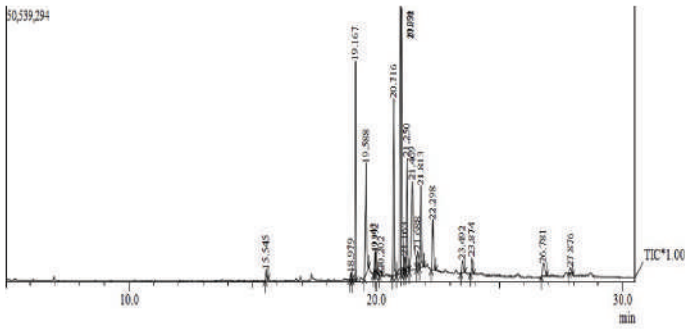


Figure 4. GCMS chromatogram of hexane extract of *Thunbergia fragrans* leaves.

Table 3. Quantitative determination of chemical constituents of *Thunbergia fragrans* Leaf Ethanol extract by Gas chromatography Mass spectrometry

Peak	Retention time	Area %	Name
1	5.310	0.74	3-(3-OXO-3H-BENZO[F]CHROMEN-2-YL)
2	6.715	0.40	[1R*,2R*]-1-acetyl-1,2-dihydroxycyclohex-3-
3	9.511	1.14	4,5-Heptadien-2-0l, 3,3,6-tnmethyl- (CAS)
4	9.764	0.41	Borane, triethyl- (CAS) Triethyl borane
5	9.850	0.47	ARGON, MOL (AR2)
6	11.819	2.56	Hexadecanoic acid (CAS) Palmitic acid
7	12.653	13.42	NEOPHYTADIENE
8	12.750	1.83	2-Heptadecanone (CAS) 2-HEPTADECANO
9	12.922	3.38	2-Hexadecen-1-ol,3,7,11,15-tetramathyl-, [R-
10	13.129	4.22	2-Hexadecen-1-ol,3,7,11,15-tetramethyl-, [R-
11	13.283	0.70	1,9-Nonanediol (CAS) N-NONANE-1,9-DIO
12	13.583	0.66	Methyl 11-(2,3-Dideuterocyclopen

Table 3. (Cont)

Peak	Retention time	Area %	Name
13	13.800	0.47	Bicyclo[4.1.0]heptane, 7-butyl-(CAS) Norcar
14	13.998	28.98	Hexadecanoic acid (CAS) Palmitic acid
15	15.460	0.55	9,12,15-Octadecatrienoic acid, methyl ester (c
16	15.577	5.88	2-Hexadecen-1-OL, 3,7,11,15-Tetrame
17	15.851	6.69	11,14-Eicosadienoic acid, methyl ester (CAS)
18	15.935	21.35	9,12,15-Octadecatrienoic acid, methyl ester (Z)
19	16.092	5.61	Docosanoic acid (CAS) Behenic acid
20	16.208	0.53	7-Ethyl-3-Methyl-2-Nitro-4-Oxo-4,7

Table 4. Quantitative determination of chemical constituents of *Thunbergia fragrans* Leaf Aqueous extract by Gas chromatography Mass spectrometry

Peak	Retention time	Area %	Name
1	8.847	4.18	Phenol, 3,5-bis(1,1-dimethylethyl)- (CAS) 3,5
2	9.587	2.10	1,2-Benzoldicarbonsaeure, Di-(He
3	9.676	1.91	1,2Benzoldicarbonsaeure, Di-(He
4	9.801	0.51	Hexane, 2,4,4-trimethyl- (CAS) 2,4,4-Trimeth
5	11.076	6.98	1-Tetradecanamine, N,N-dimethyl- (CAS) DI-
6	11.158	0.56	Oxaloacetic acid
7	11.313	4.39	Pentanoicacid, 4-methyl-, methyl ester (CAS)
8	11.720	0.71	Hexadecanoic acid (CAS) Palmitic acid
9	13.246	2.75	7,10-Hexadecadienoic acid, methyl ester(CAS)
10	13.307	33.65	Palmitic acid
11	13.532	15.69	Cis aconitic acid
12	13.720	1.12	(3-Tert-Butyl-5-Hydroxymethyl-C
13	13.887	1.13	1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)-
14	14.090	1.60	1,2-Benzenedicarboxylic acid, dibutylester(C
15	15.177	0.82	9-Octadecene, (E)- (CAS)
16	15.319	7.66	Propane, 1,1,3-triethoxy-
17	15.358	3.92	17-Octadecenoic acid, methyl ester (CAS)ME
18	15.408	2.60	9,12,15-Octadecatrienoic acid, methyl ester
19	15.516	3.09	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-
20	15.589	4.64	Methyl Stearate

Table 5. Quantitative determination of chemical constituents of *Thunbergia fragrans* Leaf Ethyl acetate extract by Gas chromatography Mass spectrometry

Peak	Retention time	Area %	Name
1	14.279	0.15	2,6-Difluorobenzoic acid, tridec-2-ynyl ester
2	15.541	0.35	1,2-Benzenedicarboxylic Acid, Die
3	17.410	0.19	Tetradecanoic acid
4	19.160	0.23	Hexadecanoic acid, methyl ester
5	19.402	0.26	cis-9-Hexadecenoic acid
6	19.557	8.20	n-Hexadecanoic acid
7	20.495	42.95	Butyl 9,12-octadecadienoate
8	20.960	2.67	9,12-Octadecadienoic acid (Z,Z)-, methyl este
9	21.235	1.22	Octadecanoic Acid, Methylester

Table 5. (Cont)

Peak	Retention time	Area %	Name
10	21.448	17.52	cis-9-Hexadecenal
11	21.677	12.56	Octadecanoic acid
12	22.262	3.33	Oxirane, [(dodecyloxy)methyl]-
13	22.667	0.32	Eicosanoic acid, 2-hydroxyethyl ester
14	22.794	1.32	Carvacrol
15	23.042	0.38	2-(Dimethylamino)Ethyl 1-Adama
16	23.150	0.18	4,7,10,13,16,19-Docosahexaenoic acid, methy
17	23.247	0.35	3-Cyclopentylpropionic acid, 2-dimethylamin
18	23.484	1.59	Hexadecanoic Acid, 2-Hydroxy-1,3
19	25.715	0.37	2,2-Dimethylpropanoic acid, heptadecyl ester
20	26.532	0.53	2,6,10,14,18-Pentamethyl-2,6,10,14,18-

Table 6. Quantitative determination of chemical constituents of *Thunbergia fragrans* Leaf Hexane extract by Gas chromatography Mass spectrometry.

Peak	Retention time	Area %	Name
1	15.545	0.63	1,2-Benzenedicarboxylic Acid, Die
2	18.979	0.35	9-Hexadecenoic Acid, Methyl Est
3	19.167	8.71	Hexadecanoic Acid, Methylester
4	19.588	7.89	n-Hexadecanoic acid
5	19.943	1.16	Glycidol stearate
6	19.992	1.06	cis-10-Heptadecenoic acid, methyl ester
7	20.202	0.36	Heptadecanoic acid, methyl ester
8	20.716	9.90	Methyl 2-octylcyclopropene-1-heptanoate
9	20.994	20.79	Campesterol
10	21.032	14.24	9-Octadecenoic Acid, Methyleste
11	21.163	1.13	9,12-Octadecadienoic acid (Z,Z)-
12	21.250	6.53	Pentadecanoic acid, 14-methyl-, methyl ester
13	21.469	12.11	cis-13,16-Docasadienoic acid
14	21.688	1.79	Octadecanoic acid
15	21.813	5.38	Ethyl 2-dibromomethyl-6-cyano-7-ethoxy-5 phenyl-1,8-n aphthyridine-3-carboxylate
16	22.298	3.44	cis-10-Nonadecenoic acid, methyl ester
17	23.492	1.14	Ethyl 9,12,15-octadecatrienoate
18	23.874	1.13	Eicosanoic Acid, Methyl Ester
19	26.781	1.80	(R)-(-)-14-Methyl-8-hexadecyn-1-ol
20	27.876	0.47	Docosanoic Acid, Methyl Ester

The above-mentioned values are in percentage by conversion of mg/standardgram equivalent.

Figures 1–4 and Tables 3–6 represents the chromatogram and percentage composition of chemical constituents of ethanol, Aqueous, Ethyl acetate and Hexane leaf extract of *Thunbergia fragrans* respectively. The chromatogram peak compared with the Wiley and NIST database of GCMS Library (spectrum installed). Twenty major compounds were identified and characterized by GCMS. The identified compounds are listed in Tables 3-6 in ascending retention time order. The major compounds were present in the ethanol extract were Neophytadine, Hexadecanoic acid and 9,12,15-Octadecatrienoic acid.

The qualitative and quantitative results revealed ethanol is the most suitable solvent to extract the

Table 7. Antioxidant activity of ethanolic extract of *Thunbergia fragrans* leaf.

Concentration (µg/ml)	Ascorbic acid	Scavenging activity (µg/g)
20	220.35 ± 3.1	190.15±1.5
40	225.65 ± 2.9	206.30±0.5
60	258.55 ± 3.5	229.40±2.5
80	346.85 ± 2.5	260.80±1.5
100	344.55 ± 1.6	275.50±0.5

Values are mean ± S.E.M, n = 3

Figures 5 and 6. Antibacterial activity of *Thunbergia fragrans* ethanolic extract against *Staphyloccocus aureus* and *Escherichia coli*

Table 8. Antibacterial activity of *Thunbergia fragrans* ethanolic leaf extract

Organism Name	Standard (mm)	Sample (Extract) (mm)	Control (mm)
Staphylococcus aureus	11	18	-
Escherichia coli	11	15	-

chemical constituents of *Thunbergia fragrans* leaves. Therefore the ethanol extract is subjected to the DPPH assay for the identification of free radical scavenging activity. Table 7 represents the scavenging activity of *Thunbergia fragrans* at a different concentration such as 20, 40, 60, 80, 100. All concentration had scavenging activity moreover 100(µg/ml) concentration had more scavenging activity (275.50±0.5) when compared with others.

3.3 Antibacterial Activity

The disk size was 8 mm and streptomycin used as a standard. The antibacterial activity of ethanol extract of *Thunbergia fragrans* leaves was found significant against two bacteria, *Escherichia coli, Staphyloccocus aureus* (Table 8, Figures 5, 6). The extract showed very high activity in *S. aureus,* with the zone of inhibition of 18 mm diameter in 100 µg concentration. The standard (10 µg streptomycin) showed zone of inhibition 11 mm in both the *sp.* respectively. Extract showed prominent zone of inhibition.

4. Conclusion

Thunbergia fragrans extracts were quantified for the identification of medicinally important phytochemicals. *T. fragrance* leaves were evaluated by the qualitative and quantitative method. GCMS

results revealed the presence of chemical constituents with the exact composition of different solvents such as ethanol, aqueous, hexane and ethyl acetate. Tables 1 and 2 results revealed ethanol is the most suitable solvent to extract the chemical constituents of *T. fragrance* leaves. Moreover, quantitative result of the ethanolic extract showed a higher amount of flavonoid which had a promising anticancer activity. Tables 7 and 8 revealed *Thunbergia fragrans* ethanol extract had strong antioxidant and antibacterial activity. Further investigation would explore the anticancer activity of *T. fragrance* leaves through *in silico* and *in vitro* studies.

5. Acknowledgement

We thank TanBio R&D solution, Periyar Technology Business Incubator, Vallam, Thanjavur for their kind support in analysing the samples.

6. References

1. Pan SY, Litscher G, Gao SH, Zhou SF, Yu ZL, Chen HQ, *et al*. Historical perspective of traditional indigenous medical practices: the current renaissance and conservation of herbal resources. Evid Based Complement Alternat Med. 2014; 2014:1–20. https://doi.org/10.1155/2014/525340. PMid:25610481 PMCid:PMC4295149
2. PayamalleS, Shinde S, Joseph KS, Mutakekar S, Murgude M, Tawadare R, Murthy HN. Chemical properties and fatty acid composition of *Thunbergia fragrans Roxb*. Seed Oil. J Am Oil Chem Soc. 2015; 93(2):295–7. https://doi.org/10.1007/s11746-015-2767-4
3. Uddin MJ, Alam MN, Biswas K, Rahman MA. In vitro antioxidative and cholinesterase inhibitory properties of *Thunbergia grandiflora* leaf extract. Cogent Food Agric. 2016; 2(1):1–11. https://doi.org/10.1080/23311932.2016.1256929
4. Zhao S, Baik OD, Choi YJ, Kim SM. Pretreatments for the Efficient extraction of bioactive compounds from plant-based biomaterials. Crit Rev Food Sci Nutr. 2014; 54(10):1283–97. https://doi.org/10.1080/10408398.2011.632698. PMid:24564586
5. Nagarasan S, Boominathan M. Perspective pharmacological activities of Leucas aspera: An indigenous plant species. Indo Am J Pharm. 2016; 6(09):6567–72.
6. Magel E. Qualitative and quantitative determination of starch by a colorimetric method. Starch-Stärke. 1991; 43(10):384–7. https://doi.org/10.1002/star.19910431003
7. Fang W, Wu Y, Zhou J, Chen W, Fang Q. Qualitative and quantitative determination of taxol and related compounds in Taxuscuspidata Sieb et Zucc. Phytochem Anal. 1993; 4(3):115–19. https://doi.org/10.1002/pca.2800040307
8. Nagarasan S, Boominathan M. Invitro studies on the primitive pharmacological activities of *Adhatoda vasica*. Int J Life Sci. 2016; 4(3):379–85.
9. Gomathi D, Kalaiselvi M, Ravikumar G, Devaki K, Uma C. GC-MS analysis of bioactive compounds from the whole plant ethanolic extract of *Evolvulus alsinoides* (L.) L. J Food Sci. Technol. 2013; 52(2):1212–17. https://doi.org/10.1007/s13197-013-1105-9. PMid:25694742. PMCid:PMC4325072
10. Sethuraman J, Nehru H, Shanmugam K, Balakrishnanan P. Evaluation of potent phytochemicals and antidiabetic activity of *Ficus racemosa* L. World J Pharm Res. 2017; 6(15):909–20.
11. Balakrishnan P, Kumar GS, Ramalingam PS, Nagarasan S, Murugasan V, Shanmugam K. Distinctive pharmacological activities of *Eclipta alba* and it's coumestan wedolactone. Indo Am J Pharm. 2018; 5(4): 2996-3002. https://doi.org/10.5281/zenodo.1231062.
12. Filip S, Vidović S, Vladić S, Pavlić B, Adamović D, Zeković Z. Chemical composition and antioxidant properties of *Ocimum basilicum* L. extracts obtained by supercritical carbon dioxide extraction: Drug exhausting method. J Supercrit Fluids. 2016; 109:20–5. https://doi.org/10.1016/j.supflu.2015.11.006
13. Ramalingam PS, Sagayaraj M, Ravichandiran P, Balakrishnanan P, Nagarasan S, Shanmugam K. Lipid peroxidation and anti-obesity activity of *Nigella sativa* seeds. World J Pharm Res. 2017; 6(10):882–92. https://doi.org/10.20959/wjpr201710-9389
14. Aruna AP, Balakrishnan P, Shanmugam K, Rajendran K. Biological finger printing of microbial diversity in vermicompost of different raw materials. Indo Am J Pharm. 2017; 7(04):8497–8501.
15. Balakrishnan P, Musafargani TA, Subrahmanyam S, Shanmugam K. A perspective on bioactive compounds from *Solanum trilobatum*. J Chem Pharm. 2015; 7(8):507–12.
16. Soni V, Jha AK, Dwivedi J, Soni P. Qualitative and quantitative determination of phytoconstituents in some antifertility herbs. Indian J Pharm Sci. 2018; 80(1):79–84. https://doi.org/10.4172/pharmaceutical-sciences.1000332
17. Purushothaman B, Srinivasan RP, Suganthi P, Ranganathan B, Gimbun J, Shanmugam KA. Comprehensive Review on *Ocimum basilicum*. J Nat Remedies. 2018; 8(3):41–55. https://doi.org/10.18311/jnr/2018/21324
18. Shanmugam K, Purushothaman B, Suganthi N. Qualitative and quantitative determination of various extracts of *Ocimum basilicum* L. leaves. J Nat Remedies. 2020; 20(1):53–60. https://doi.org/10.18311/jnr/2020/24113
19. Marotti M, Piccaglia, R, Giovanelli E. Differences in essential oil composition of Basil (*Ocimum basilicum* L.) Italian

cultivars related to morphological characteristics. J Agric Food Chem. 1996; 44(12):3926–29. https://doi.org/10.1021/jf9601067

20. Sundaramoorthy M, Prabaharan C, Balakrishnan P, Saravanan TS. Antibacterial and wound healing effects of semi-purified heart proteins from certain selective slaughter house animals. Indo Am J Pharm. 2014; 4(01).
21. Rathnasamy S, Rufus AL, Purusothaman. Comparative studies on isolation and characterization of allinase from garlic and onion using Pegylation- A novel method. Asian J Chem. 2014; 26(12):3733–5. https://doi.org/10.14233/ajchem.2014.17064
22. Mahdi-Pour B, Jothy SL, Latha LY, Chen Y, Sasidharan S. Antioxidant activity of methanol extracts of different parts of *Lantana camara*. Asian Pacific Journal of Tropical Biomedicine. 2012; 2(12):960-965. https://doi.org/10.1016/S2221-1691(13)60007-6
23. Chan E, Tie P, Soh E, Law Y. Antioxidant and antibacterial properties of green, black, and herbal teas of *Camellia sinensis*. Pharmacogn. Res. 2011; 3(4):266. https://doi.org/10.4103/0974-8490.89748. PMid:22224051. PMCid: PMC3249787

Published online in http://ijam.co.in ISSN No: 0976-5921

International Journal of Ayurvedic Medicine, Vol 12 (4), 792-795

Molecular docking studies of potential anticancer agents from *Thunbergia Fragrans* against Colorectal cancer mutant genes through *In silico* study

Research Article

Charankumar Chandrasekaran[1], Ramar K[2]*

1. Research Scholar, 2. Assistant Professor,
Department of Botany, National College (Autonomous), Tiruchirappalli, India.

Abstract

Colorectal cancer (CRC) is one of the deadly diseases which incidence rate will increase every year due to people lifestyle and food habit etc., Moreover, people's required a new therapeutic molecule to resolve this problem. Therefore plant-based chemical constituents are the best option due to the low side effects, easy availability and cost-effective manner. The flowering plant of *Thunbergia fragrans* Roxb belongs to the Acanthaceae family has a vast range of medicinal properties, anticancer activity is one among them. *Thunbergia fragrans* has reported to had chemical constituents of Palmitic acid, Cis-9-Hexadecenal and Campesterol which possess anticancer activity. For the beginning of TF chemical constituents were studied against the Colorectal cancer (CRC) mutant genes such as NRAS (PDB ID: 6ZIZ), Beta-Catenin (PDB ID: 6M93) – Oncogenes; APC (PDB ID: 3NMX), Smad2 (PDB ID: 1KHU) – Tumor Suppressor genes through insilico docking studies. AutoDock 4.2 tool was used to predict the interaction between ligand and receptor, Binding energy and Bond specification in a 3D space. Finally, the results revealed TF chemical constituents showed excellent binding energy against CRC mutant genes such as Palmitic acid against Beta-Catenin (-4.75) and APC (-4.01), Cis-9-Hexadecenal against NRAS (-1.92), Beta-Catenin (-3.96) and APC (-4.41), Campesterol against Beta-Catenin (-8.55) and APC (-8.85) respectively.

Key Words: *AutoDock 4.2, Binding energy, Oncogenes, Tumor suppressor genes, PDB, PubChem*.

Introduction

Colorectal cancer (CRC) is the leading cause of cancer death in both sexes which the incidence rate in men is 3rd and women are 2nd. In Asia, the incidence rate of CRC is increased in well-developed Asian countries such as Japan, Singapore, and South Korea (1). In the recent decade, researchers are focused on the biologically active molecule from natural sources which has a vast range of medicinal properties like Anticancer, Anti-inflammatory, and Antioxidant, etc., Among the natural sources, the plant-derived phytoconstituents are the finest ones due to their low side effects and easy availability (2, 3). *Thunbergia fragrans* Roxb is a flowering plant that belongs to the *Acanthaceae* family, mainly cultivated in tropics and subtropic regions. TF plant contained phytoconstituents are possessed various pharmacological activities such as anti-cancer, Anti-inflammatory, Antimicrobial, and Antidiabetic activity (4, 5). But also the identification, extraction, and purification of the biologically active molecule from a particular plant is the most difficult task. In this manner, computational tools are used to eradicate these problems (6). Among all computational work, docking is one of the best methods because of the identification of active site between Ligand and Receptor in a 3D space which helps to find the biologically active molecule (7). Furthermore, various docking tools are available in the market, AutoDock4.2 is the finest one because of the accuracy and docking score calculation (8, 9). The present investigation was to explore the anti-colon cancer activity of reported compounds of *Thunbergia fragrans* through insilico docking studies.

Aim and Objectives

- Identification of the colon cancer mutant genes (Oncogenes, Tumor suppressor genes)
- Retrieving the data from PubChem and PDB database.
- To reveal the anticancer potential of *Thunbergia fragrans* with insilico manner

Materials and Methods

Retrieval of Protein and file preparation

The 3D structures of Colorectal cancer mutant genes such as NRAS (PDB ID: 6ZIZ), Beta-Catenin (PDB ID: 6M93) – Oncogenes; APC (PDB ID: 3NMX), Smad2 (PDB ID: 1KHU) – Tumor Suppressor genes retrieved from the PDB database showed as Table 1. The excess water molecules and chains are detached from the protein with the help of the Discovery studio visualizer (Visualizing tool). The addition of Polar

*** Corresponding Author:**
Ramar K
Assitant Professor,
Department of Botany, National College (Autonomous), Tiruchirappalli 620001.
Tamilnadu. India.
Email Id: ramarbot@gmail.com

bonds, Kollman charges, and generating the docking parameters in protein structure was performed by the AutoDock 4.2 tool (10).

Retrieval of Ligand and file preparation

The 3D structures of Palmitic acid, Cis-9-Hexadecenal and Campesterol compounds were retrieved from the PubChem database. The setting of the number of torsions (1-6), the addition of aromaticity criteria and the setting of angle cutoff (7.5) was performed by the Auto Dock 4.2 tool. After the above-mentioned process, the docking parameter files are generated (11, 10).

Molecular docking

PDBQT files, Docking parameter files and grid parameter files were generated along with grid box dimensions (50x50x50). 10 runs were set for the default parameters. Lamarckian GA (4.2) genetic algorithm was used for the docking procedure. Cygwin software was used for the molecular docking coding and binding energy calculation (Every run) which also revealed the binding interaction of electrostatic bond, hydrogen bond, van der Waals force and polar bond (12). The interaction between ligand atom and receptor protein was revealed by Binding energy calculation.

Results and Discussion

Table 1: Colon cancer receptor details

S.NO	Receptor Name	Codon	PDB ID
1	NRAS (Oncogene)	Q61R	6ZIZ
2	Beta-Catenin (Oncogene)	S33	6M93
3	APC (Suppressor gene)	Cdc42	3NMX
4	Smad2 (Suppressor gene)	S464L	1KHU

Table 1 indicates colon cancer associated receptor details along with codon and PDB ID.

Table 2: *Thunbergia fragrans* associated ligand details

S.NO	Ligand	PubChem ID
1	Palmitic acid	985
2	Cis-9-Hexadecenal	5364643
3	Campesterol	173183

Table 2 represents the *Thunbergia fragrans* associated ligand details along with PubChem ID.

Table 3: Binding energies of Thunbergia fragrans associated phytoconstituents against colorectal cancer genes.

S.No.	Ligand	Receptor	Binding energy
1	Palmitic acid	NRAS	**9.08**
2		Beta-Catenin	-4.75
3		APC	-4.01
4		Smad2	**0.28**
5	Cis-9-Hexadecenal	NRAS	-1.92
6		Beta-Catenin	-3.96
7		APC	-4.41
8		Smad2	**115.55**
9	Campesterol	NRAS	**523.70**
10		Beta-Catenin	-8.55
11		APC	-8.85
12		Smad2	**126.42**

Table 3 represents the binding energy between ligand and receptor. Campesterol showed highest binding energy against Beta-Catenin (-8.55) and APC (-8.85) compare with Palmitic acid and Cis-9-Hexadecenal. Smad2 receptor showed lowest binding energy against all the three ligands.

Figure 1: The docked complex of Palmitic acid against colorectal cancer mutant genes.

A) Beta-Catenin, **B) APC**

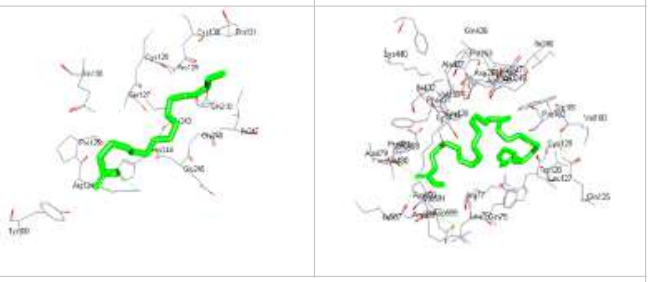


Figure 2: The docked complex of Campesterol against colorectal cancer mutant genes.

A) Beta-Catenin, **B) APC**

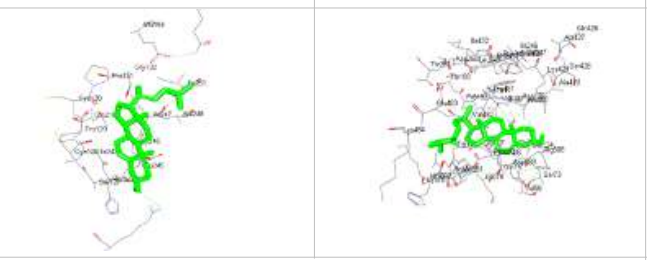


Figure 3: The docked complex of Cis-9-Hexadecenal against colorectal cancer mutant genes.

A) NARS, **B) Beta-Catenin,**

C) APC

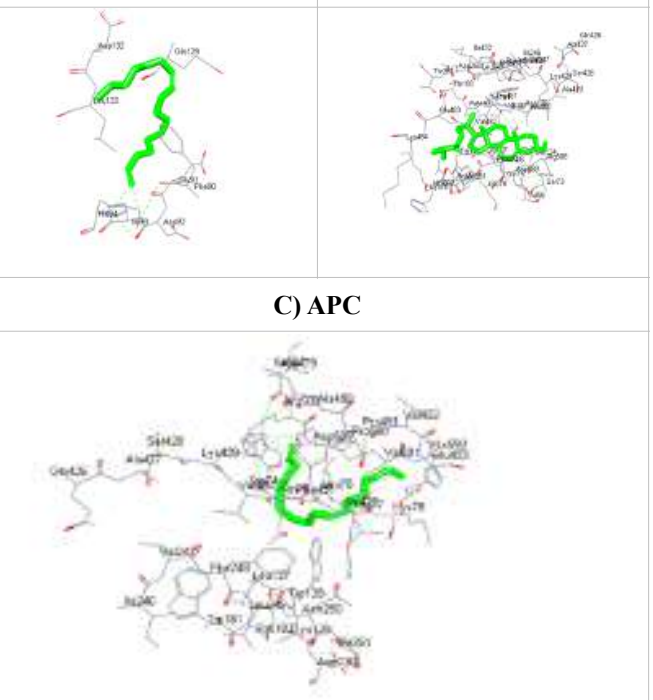


Table 4: Bond specification of Palmitic acid against Beta-Catenin

Receptor Name	Run	Mean binding energy (Kcal/mol)	Bond type	Amino acid Residue	Ligand Atom	Bond length (Å)
Beta-Catenin	10	-4.75	Hydrogen bond	ARG124	O	2.81

Table 4 represents the binding interaction between Palmitic acid (ligand) and Beta-Catenin (S33 codon) receptor. In the 10th run Palmitic acid showed the binding energy of -4.75 (Kcal/mol), bond length 2.81 (Å) between O-atom and ARG124 amino acid residue. Fig 1 (A) illustrate the three-dimensional molecular docking images of the Palmitic acid in the colorectal cancer mutant protein Beta-Catenin (S33 codon).

Table 5: Bond specification of Palmitic acid against APC

Receptor Name	Run	Mean binding energy (Kcal/mol)	Bond type	Amino acid Residue	Ligand Atom	Bond length (Å)
APC	7	-4.01	Hydrogen bond	LYS429	O	2.95

Table 5 represents the binding interaction between Palmitic acid (ligand) and APC (Cdc42 codon) receptor. In the 7th run Palmitic acid showed the binding energy of -4.01 (Kcal/mol), bond length 2.95 (Å) between O-atom and LYS429 amino acid residue. Fig 1(B) illustrate the three-dimensional molecular docking images of the Palmitic acid in the colorectal cancer mutant protein APC (Cdc42 codon).

Table 6: Bond specification of Cis-9-Hexadecenal against NRAS

Receptor Name	Run	Mean binding energy (Kcal/mol)	Bond type	Amino acid Residue	Ligand Atom	Bond length (Å)
NRAS	5	-1.92	Hydrogen bond	HIS94	O	2.89

Table 6 represents the binding interaction between Cis-9-Hexadecenal (ligand) and NRAS (Q61R codon) receptor. In the 5th run, Cis-9-Hexadecenal showed the binding energy of -1.92 (Kcal/mol), bond length 2.89 (Å) between O-atom and HIS94 amino acid residue. Fig 3(A) illustrate the three-dimensional molecular docking images of the Cis-9-Hexadecenal in the colorectal cancer mutant protein NRAS (Q61R codon).

Table 7: Bond specification of Cis-9-Hexadecenal against Beta-Catenin

Receptor Name	Run	Mean binding energy (Kcal/mol)	Bond type	Amino acid Residue	Ligand Atom	Bond length (Å)
Beta-Catenin	4	-3.96	Hydrogen bond	PRO125	O	3.18

Table 7 represents the binding interaction between Cis-9-Hexadecenal (ligand) and Beta-Catenin (S33 codon) receptor. In the 4th run, Cis-9-Hexadecenal showed the binding energy of -3.96 (Kcal/mol), bond length 3.18 (Å) between O-atom and PRO125 amino acid residue. Fig 3(B) illustrate the three-dimensional molecular docking images of the Cis-9-Hexadecenal in the colorectal cancer mutant protein Beta-Catenin (S33 codon).

Table 8: Bond specification of Cis-9-Hexadecenal against APC

Receptor Name	Run	Mean binding energy (Kcal/mol)	Bond type	Amino acid Residue	Ligand Atom	Bond length (Å)
APC	7	-4.41	Hydrogen bond	LYS429	O	2.95

Table 8 represents the binding interaction between Cis-9-Hexadecenal (ligand) and APC (Cdc42 codon) receptor. In the 7th run, Cis-9-Hexadecenal showed the binding energy of -4.41 (Kcal/mol), bond length 2.95 (Å) between O-atom and LYS429 amino acid residue. Fig 3(C) illustrate the three-dimensional molecular docking images of the Cis-9-Hexadecenal in the colorectal cancer mutant protein APC (Cdc42 codon).

Table 9: Bond specification of Campesterol against Beta-Catenin

Receptor Name	Run	Mean binding energy (Kcal/mol)	Bond type	Amino acid Residue	Ligand Atom	Bond length (Å)
Beta-Catenin	3	-8.55	Hydrogen bond	ARG124	O	3.18

Table 9 represents the binding interaction between Campesterol (ligand) and Beta-Catenin (S33 codon) receptor. In the 3rd run, Campesterol showed the binding energy of -8.55 (Kcal/mol), bond length 3.18 (Å) between O-atom and ARG124 amino acid residue. Fig 2(A) illustrate the three-dimensional molecular docking images of the Campesterol in the colorectal cancer mutant protein Beta-Catenin (S33 codon).

Table 10: Bond specification of Campesterol against APC

Receptor Name	Run	Mean binding energy (Kcal/mol)	Bond type	Amino acid Residue	Ligand Atom	Bond length (Å)
APC	1	-8.85	Hydrogen bond	LYS429	O	3.06

Table 10 represents the binding interaction between Campesterol (ligand) and APC (Cdc42 codon) receptor. In the 1st run, Campesterol showed the binding energy of -8.85 (Kcal/mol), bond length 3.06 (Å) between O-atom and LYS429 amino acid residue. Fig 2(B) illustrate the three-dimensional molecular docking images of the Campesterol in the colorectal cancer mutant protein APC (Cdc42 codon).

Conclusion

The present study was to investigate the anti-colon cancer activity *of Thunbergia fragrans* with colon cancer mutant genes through an insilico manner. Binding energy, Electrostatic interaction, Bond length are calculated using Discovery visual studio. The above results revealed Cis-9-Hexadecenal has good binding energy against colorectal cancer mutant genes NRAS (-1.92), Beta-Catenin (-3.96), APC (-4.41) without Smad2. Palmitic acid and Campesterol showed good binding energy against Beta-Catenin (-4.75, -8.55), APC (-4.01, -8.85) respectively without NRAS and Smad2. Therefore *Thunbergia fragrans* have the potential to inhibit the colon cancer mutant genes.

Conflict of interest

The authors declare that they have no conflict of interest for this study.

Funding support

The authors declare that they have no funding support for this study.

References

1. Rawla P, et al, Epidemiology of colorectal cancer: incidence, mortality, survival, and risk factors. Gastroenterology Review, 2019; 14(2): 89–103.
2. Purushothaman B et al, A comprehensive review on *Ocimum basilicum*. Journal of Natural Remedies, 2018; 18(3): 71-85. https://doi.org/10.18311/jnr/2018/21324.
3. Tungmunnithum D, et al, Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview. Medicines. 2018; 5(3): 93.
4. Payamalle S et al, Chemical Properties and Fatty Acid Composition of *Thunbergia fragrans* Roxb. Seed Oil. Journal of the American Oil Chemist's Society, 2015; 93(2): 295–297.
5. Ismail L.D, Iridoid glycosides from *Thunbergia grandiflora*. Phytochemistry, 1996; 42(4): 1223–1225.
6. Waghulde S, et al, In silico docking and drug design of herbal ligands for anticancer property. Journal of Pharmacognosy and Phytochemistry, 2018; 6: 84-91.
7. Glen, R.C, Allen S.C, Ligand-Protein Docking: Cancer Research at the Interface between Biology and Chemistry. Current Medicinal Chemistry, 2003;10(9): 763-77.
8. Morris G.M et al, AutoDock4 and AutoDock Tools 4: Automated docking with selective receptor flexibility. Journal of Computational Chemistry, 2009; 30(16): 2785-91.
9. Norgan A.P. Multilevel Parallelization of AutoDock 4.2. Journal of Cheminformatics, 2011; 3(1): 12.
10. Forli S, Computational protein–ligand docking and virtual drug screening with the AutoDock suite. Nature Protocols, 2016; 11(5): 905–919.
11. Danish Rizvi S.M et al, A simple click by click protocol to perform docking: autodock 4.2 made easy for non-bioinformaticians. EXCLI Journal, 2013; 12: 831-57.
12. Purushothaman B, et al, Molecular Docking Studies of potential anticancer agents from *Ocimum basilicum* L. against human colorectal cancer regulating genes: An insilico approach. Research J. Pharm. and Tech. 2019; 12(7): 3423-3427. https://doi.org/10.5958/0974-360X.2019.00579.1.

*****

Journal of Hazardous Materials Advances 7 (2022) 100123

Contents lists available at ScienceDirect

Journal of Hazardous Materials Advances

journal homepage: www.elsevier.com/locate/hazadv

Rational modification of chitosan biopolymer for remediation of Cr(VI) from water

Anita Shekhawat[a,*], Shashikant Kahu[a], D. Saravanan[b], Sadanand Pandey[c], Ravin Jugade[a]

[a] Department of Chemistry, R.T.M. Nagpur University, Nagpur 440033, India
[b] Department of Chemistry, National College, Tiruchirappalli 620001, India
[c] Department of Chemistry, College of Natural Science, Yeungnam University, 280 Daehak-Ro, Gyeongsan, Gyeongbuk 38541, Republic of Korea

ARTICLE INFO

Keywords:
Chitosan
Ammonium thiocyanate
Irradiation
Adsorption
Regeneration

ABSTRACT

Chitosan, a well-known bioadsorbent used for water treatment. It is treated with ammonium thiocyanate and subjected to microwave irradiation to obtain thiolated chitosan which makes the chitosan more stable and effective for metal adsorption through electrostatic interaction. The thiolated chitosan(TCH) has been thoroughly characterized to ensure the structural changes in the native chitosan. TCH has been efficaciously used for the detoxification of Cr(VI) from water. Batch adsorption methodology has been used to optimize all the parameters for the adsorption process. The Langmuir maximum adsorption capacity for Cr(VI)at 298 K and pH 3.0 was 340.1 mg/g. Adsorption kinetics and thermodynamic were studied for suitable rate model and spontaneity of the adsorption behavior. The adsorption of Cr(VI) onto TC H favors pseudo-second-order rate model. Uptake of Cr(VI) by thiolated chitosan was spontaneous and adsorption was enthalpy driven as obtained through the thermodynamic experimental results. The value of ΔH, ΔG and ΔS were found to be -94.26 kJ/mol, -12.92 kJ/mol and 0.272 kJ/mol.K, respectively. The breakthrough volume and exhaustion volume were found to be 1400 mL and 2100 Ml, respectively during fixed bed adsorption (column studies) of 200 mg/L of Cr(VI) with 1 g of the material proven its applicability for larger volumes of Cr(VI). TCH has greener perspective as it can be easily regenerated 5% (w/v) sodium hydroxide solution.

1. Introduction

Chromium and chromium salts are one of the most prime components in many industries like paints, pigments, glass, ceramic, leather tanning and chrome plating etc. Generally, Cr(VI) exists in the form of chromates like CrO_4^{2-}, $HCrO_4^-$, $Cr_2O_7^{2-}$ whereas Cr(III) forms hydroxides like $Cr(OH)_3$,$Cr(OH)_2^+$. The speciation of chromium in various compounds is based on their pH, redox kinetics, redox reagents and redox potential. Chromium in the form of Cr(III) is beneficial, as it plays an essential role in maintaining the glucose concentration in human body where as in Cr (VI) is a potent respiratory carcinogen in human. Mobility of Cr(VI) ions in water is higher than that of Cr(III) because of higher solubilty of Cr (VI) salts in water as compared to that of Cr(III) . Hence there is a urge to develop a potential and an effective methodology to scavenge Cr (VI) from waste water (Almeida et al., 2019). Adsorption based waste water treatment is considered to be most efficient and economical as it offers flexibility in design and operations (Bayuo, 2021). Double layered hydroxides (Guo et al., 2021) and composites (Cheng, Wang and Ge, 2021) were utilized for the removal of toxicants efficiently. Among various adsorbents, biosorbents like seaweed (Husien, 2019), alginate (Kumar et al., 2018), cellulose (Shao et al., 2021), chitin (Boulaiche et al., 2019), and chitosan (Kahu et al., 2016) have been established as a potential adsorbents in waste water treatment. This is because biosorbents are eco as well as cost viable.

Chitosan is mainly produced by the N-deacetylation of chitin which is second most abundant biopolymer on earth crust after cellulose. The degree of deacetylation has an influence on all the physicochemical properties. Chitosan is a hetero polysaccharide which has hydroxyl and amino functional groups help in chemical and structural modifications of chitosan. However, solubility in acidic medium is the serious drawback of chitosan for metal ion adsorption. To overcome this drawback, chitosan has been structurally modified by means of various chemical reagents (Tandekar and Jugade, 2021), crosslinkers (Yang et al., 2020) etc. We have reported modifications of chitosan for removal of Cr(VI) from water and simulated effluent samples thereby improving the adsorption capacity of the material, retaining its biocompatibility (Kahu et al., 2014; Shekhawat et al., 2015; Kahu et al., 2016a,2016b; Tandekar et al., 2021; Korde et al., 2022). Adsorbents with sulfur in their functional groups like thio groups are endowed chelating, ion exchange or electrostatic interaction (Saha et al., 2016) capability. Thiocyanates

* Corresponding author.
E-mail address: annushekh22@gmail.com (A. Shekhawat).

https://doi.org/10.1016/j.hazadv.2022.100123
Received 12 May 2022; Received in revised form 17 June 2022; Accepted 4 July 2022
2772-4166/© 2022 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)

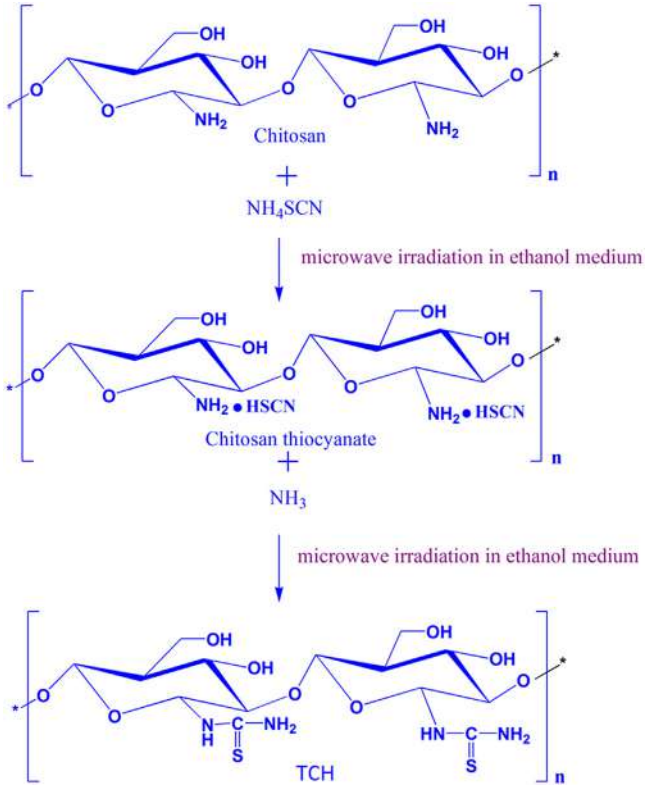


Fig. 1. Schematic presentation of TCH preparation.

can be used to introduce thio functional group for the modification of chitosan (Liu et al., 2021). Microwave assisted synthesis reduces the reaction time and efficient to yield the desired product. WO_3/UiO-66 nanocomposite (Zhang et al., 2021) and Cd/Zr-bimetallic metal–organic frameworks (Cheng, Wang and Ge, 2021) were synthesized by using microwave irradiation for photocatalytic prorerties. This paper deals with the preparation of thiolated-chitosan through microwave irradiation and adsorption studies of thiolated chitosan (TCH) for detoxification of Cr(VI) from water.

2. Materials and methods

2.1. Materials

AR grade chemicals and reagents were used during entire study. Double distilled water was used for the preparation of aqueous solutions. S.D. fine chemicals provided reagents like potassium dichromate, diphenyl carbazide, ammonium thiocyanate, sulfuric acid, hydrochloric acid, sodium hydroxide, ammonia, sodium nitrite etc. Chitosan (85% of degree of deacetylation and molecular weight 120 kD) has been procured from Uniloid Bio-Chemicals India Limited, Hyderabad.

2.2. Preparation of thiolated chitosan(TCH)

5 g of chitosan powder and 3.5 g of ammonium thiocyanate were weighed and taken into a 250 mL conical flask. 100 mL of ethanol was added to the above mixture and subjected to microwave irradiation for 10 min with 2 min of periodic intervals.50 mL of 2% (v/v) ammonia was added to the above precipitate and irradiated for further 2 min. In ammonia solution, thiocyanates were converted to thiourea form, thus the final precipitate obtained was named as thiolated chitosan (TCH). After cooling to room temperature, the thiolated chitosan was repeatedly washed with distilled water and finally dried in oven for 24 h at 50 °C.

The schematic representation of preparation of TCH is given in Fig. 1 (Chen et al., 2005).

2.3. Instrumentation

Structural and functional changes in the modified chitosan were observed through the FT-IR spectra obtained from Bruker *Alpha* spectrometer. Righaku-Miniflex 300 and XPERT-PRO X-ray diffractometer with Cu K_α radiation in the 2θ range (1–100°) were used for recording the XRD data. Thermal stability of TCH observed through thermal analyzer DTG-60. Surface micrographs of chitosan and TCH were recorded SEM model TESCAN VEGA 3 SBH. EDX spectra obtained during SEM observations were recorded by the X- ray analyzer Oxford INCA Energy 250 EDS System. The Brunauer–Emmet–Teller (BET) surface area of the sample was given by Multi Point Surface Area Analyzer Model Quantachrome Nova 2200e. Spectronic 20D+ spectrophotometer was used for the determination of Cr(VI) while pH of the working solutions were adjusted by Equiptronics model EQ-615 pH-meter.

2.4. Batch adsorption experiments

Optimization of various adsorption parameters (contact time, adsorbent dose, initial concentration of Cr(VI), pH) through batch adsorption experiments gives an idea about adsorption mechanism. It helps to predict the best fit adsorption isotherm, kinetics and thermodynamics. The concentration of Cr(VI) in the solution was determined spectrophotometrically by Diphenylcarbazide method (Jeffery et al., 1989). The amount of Cr(VI) uptake by TCH at equilibrium as follows,

$$q_e = \frac{C_0 - C_e}{W} \times V \quad (1)$$

where, q_e is the amount of adsorbed Cr (VI) in mg/g at equilibrium, C_0 and C_e are concentration of Cr(VI) in mg/L initial and equilibrium in solution phase, W is weight of TCH in g and V is the volume of solution in L.

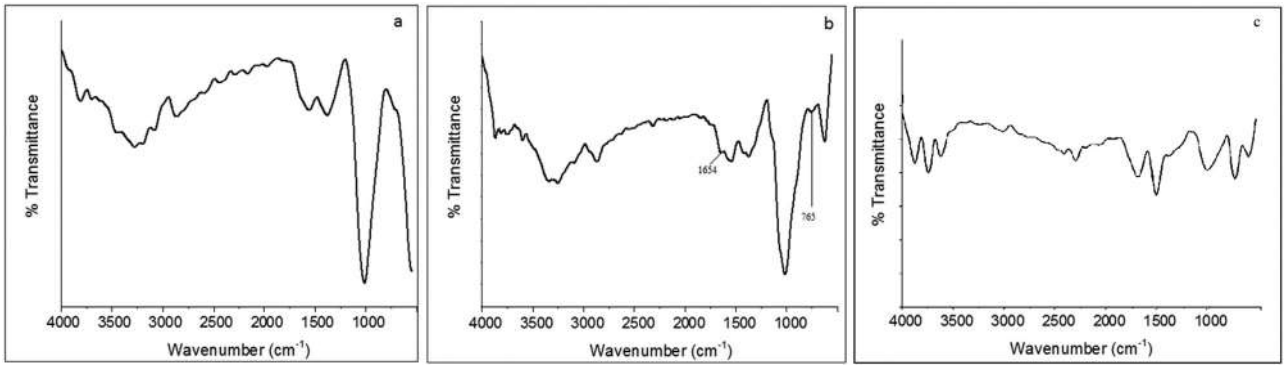


Fig. 2. FT-IR spectra of (a) chitosan (b) TCH and (c) TCH with adsorbed Cr (VI).

Table 1
Surface parameters.

Material	Surface Area (m^2/g)	Pore volume (cm^3/g)
Chitosan	3.47	6.0×10^{-3}
TCH	2.78	4.1×10^{-3}

The percent removal capacity of TCH was given by the equation,

$$\%Removal = \frac{C_e}{C_0} \times 100 \quad (2)$$

3. Results and discussion

3.1. Characterization

In FT-IR spectra (Fig. 2), the peaks observed in the region of 3259to 3500 cm^{-1} were of O-H and N-H stretching vibrations. N-H bending vibration peak obtained at 1554 cm^{-1}. The peak at 2885 cm^{-1} was of C-H stretching. 1021 cm^{-1} peak assigned to the C-O stretching band (Kahu et al. al., 2016). The FT-IR spectra of thiolated chitosan shows two new peaks at 1654$cm^{-1}$and 765cm^{-1}, which are assigned to the characteristic absorbance peaks of thioamide(C=S) asymmetric stretching and S-H bending vibrations respectively (Chen et al., 2005).The changes in FT-IR spectra confirmed the formation of thiolated chitosan. The peaks at 1654 cm^{-1} and 765 cm^{-1} were shifted to 1698 cm^{-1} and 745 cm^{-1}, respectively in case of TCH after adsorption of Cr(VI) (Fig. 2c) due to protonation of sulfur. Along with this peak at 1554 cm^{-1} was shifted to 1513 cm^{-1} due to interaction of hydrogen chromate ion with protonated amine group. For chitosan, the XRD pattern shows peaks at $2\theta = 10.62^{\circ}$ and 20.31° (Fig. 3a). TCH shows new peak at $2\theta = 22.25^{\circ}$ along with peaks of chitosan with slight shifting in 2θ angle (Fig. 3b). The chromium peak observed at $2\theta = 26.23^{0}$ in TCH with adsorbed Cr(VI) (Shekhawat et al., 2016).The shifting of peaks and characteristic peak of chromium in TCH after adsorption shows the feasibility of adsorption process. SEM micrographs (Fig. 4a) of native chitosan shows non uniform particles assembled with slack surfaces whereas morphology of TCH shows the smooth carved pattern (Fig. 4b). After adsorption of Cr(VI) by TCH, the shiny spots were observed on the smooth surface morphology which indicates towards the adsorption of Cr(VI) on the TCH surface as seen in the SEM micrograph (Fig. 4c).In the EDX spectrum of TCH, along with the elemental peaks of carbon, oxygen, nitrogen, new sulfur peak has been clearly indicates the introduction of thio group in the chitosan (Fig. 4b). Peaks of chromium were clearly observed after Cr(VI) adsorption on TCH which confirms the feasibility of the adsorption process. (Fig. 4c).Two-step degradation curve with 71% total weight loss observed in TGA curve of chitosan (Fig. 5a). In case of TCH, 14% weight loss observed between 60 and 200 ^{0}C due to dehydration. TCH is less thermostable than chitosan as second degradation curve was observed at lower temperature as compared to that of chitosan. This degradation corresponds to the breaking of bonds between chitosan and thiourea group. In DTA, strong exothermic peak observed at 288.66 ˚C in chitosan which attributes to the breaking of glucosidic linkages in the chitosan where as in TCH, a strong exothermic peak at 300 ˚C (Fig. 5b) was due to the detachment of thiourea group and rearrangement process.In BET analysis (Table 1), chitosan shows more surface area and pore volume than that of TCH which proves that thio group in TCH creates the obstacle in the diffusion of N_2 gas. In other words, the chitosan surface is more porous than TCH.

3.2. Batch adsorption experiments

Batch adsorption studies were carried out at optimized conditions (Fig. 6), 50 mL of Cr(VI) solutions with varying concentrations were equilibrated with 200 mg of TCH at pH 3.0 for an hour in conical flasks and stirred with magnetic stirrer at room temperature.

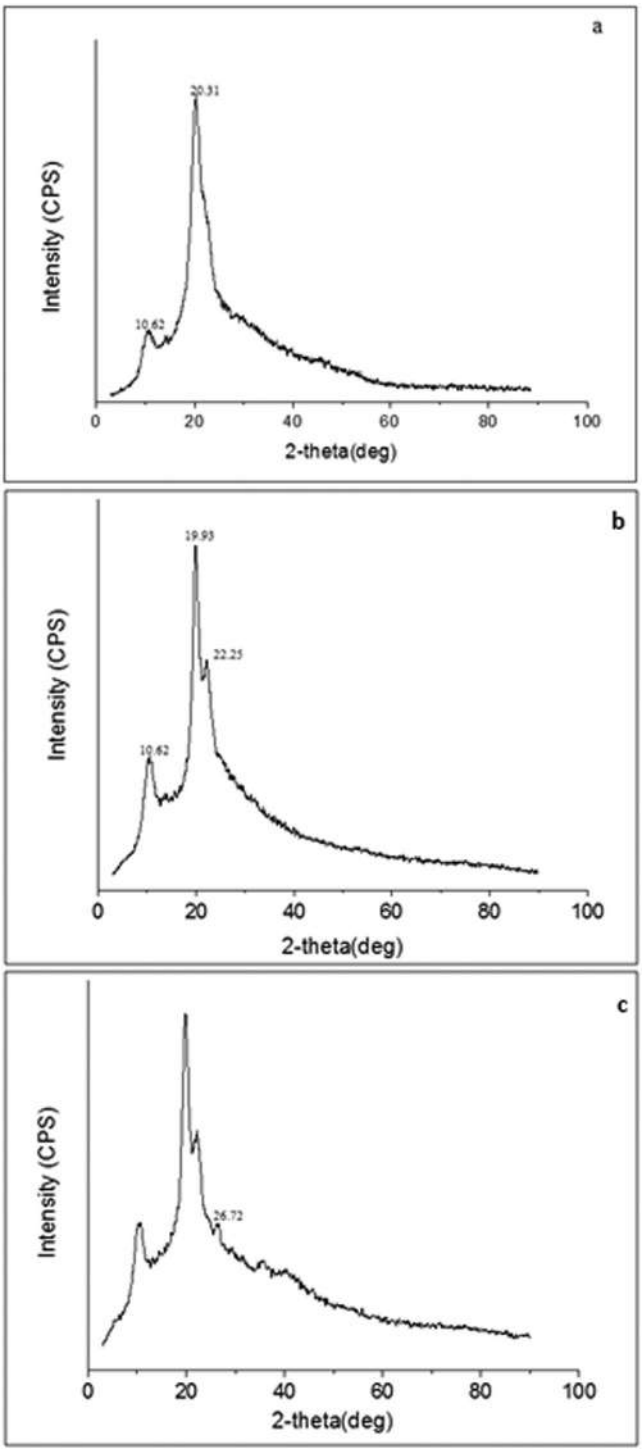


Fig. 3. Powder XRD spectra (a) chitosan, (b) TCH and (c) TCH with Cr(VI).

3.2.1. Effect of contact time

The contact time was varied in the range of 5–120 min for effect of time on% removal of Cr(VI). Due to presence of numerous adsorption sites on TCH, initially the rate of removal was increased with time then, reached to the equilibrium in 60 min with 99.5% adsorption of Cr(VI) on 200 mg of TCH at 298 K. In 60 min, almost all the adsorbent sites were occupied by the adsorbate. Hence, 60 min was fixed as contact time for further adsorption studies.

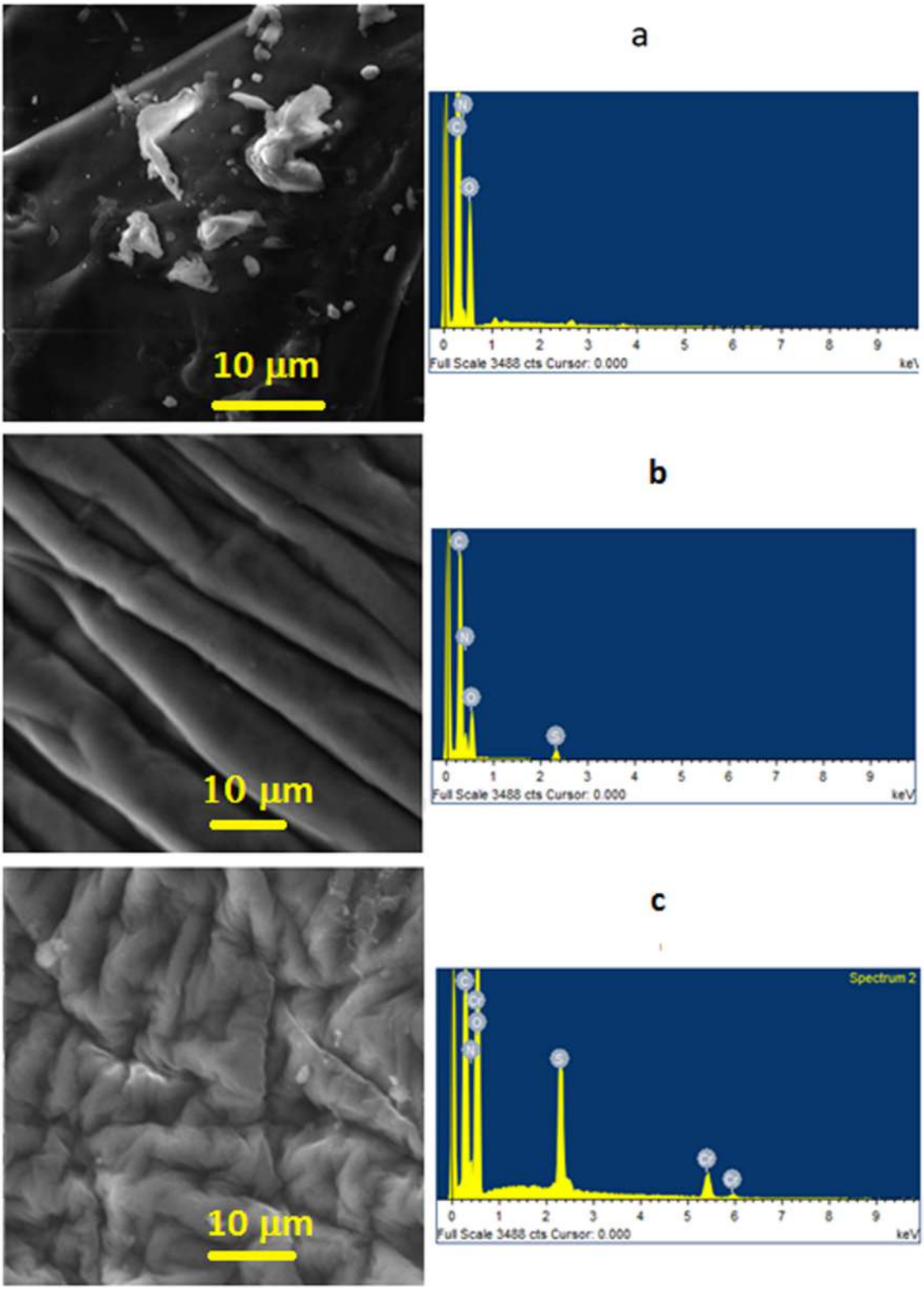


Fig. 4. SEM micrographs and EDX of (a) chitosan, (b) TCH and (c) TCH with Cr(VI).

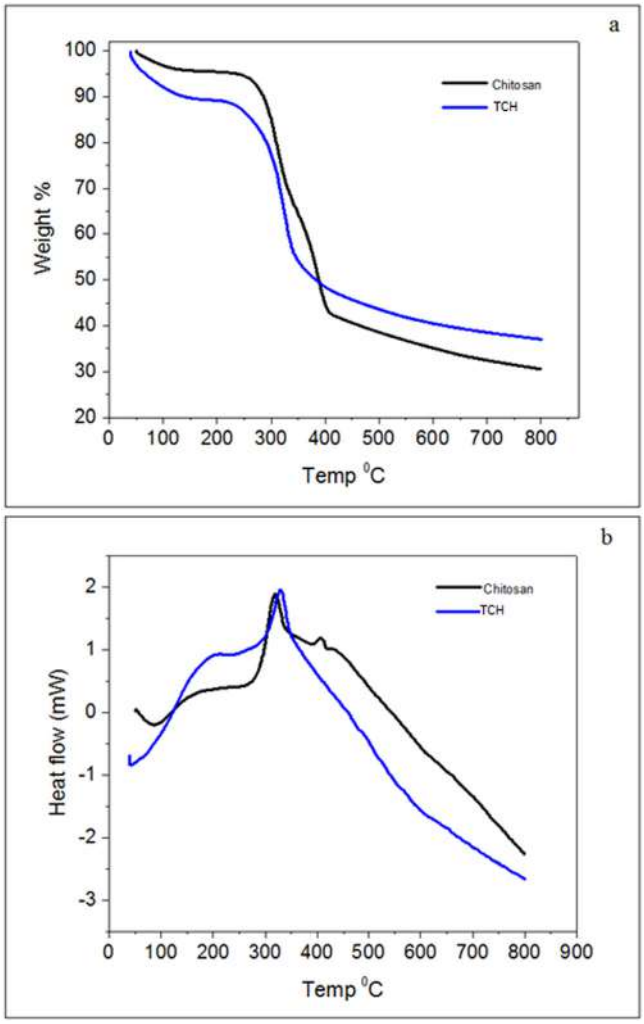


Fig. 5. Thermal Analysis (a) TGA curves and (b) DTA curves.

3.2.2. Effect of adsorbent dose

To understand the effect of adsorbent dose of TCH on% removal of Cr(VI), the amount of TCH varied from 0.05 to 0.30 g and equilibrated for 60 min with initial concentration of Cr(VI). Due to strong interaction of TCH with hydrogen chromate ions, the% removal was increasing continuously with increase in adsorbent dose and beyond 200 mg it remains constant due to unavailability of adsorbate.

3.2.3. Effect of concentration

Different initial Cr(VI) concentrations from 20 to 1000 mg/L were equilibrated with 200 mg of TCH at pH 3. Up to 100 mg/L the chromium solution the percentage removal was almost steady and then it started decreasing due to the saturation of adsorbent. Therefore 100 mg/L Cr(VI) solution was used for further adsorption studies.

3.2.4. Effect of pH

The pH of the solution phase is one of the important parameter to be optimized as speciation of Cr(VI) and TCH varies with different pH values and interactions for adsorption depends on the speciation. The point of zero charge of TCH was found to be 5.4. To optimize the pH of the solutions with initial concentration of 100 mg/L of Cr(VI) solution was varied from 2.0 to 8.0 were stirred with 200 mg of TCH for 60 min (Fig. 6e). The adsorption efficiency was found to be insignificantly changes from 2 to 5pH range. However, the% removal of Cr(VI) was found to be maximum at pH 3 (Fig. 6e). At this pH, Cr(VI) exists as chromate ($HCrO_4^-$) ion which forms electrostatic interaction with the positively charged surface of the TCH. The complete speciation of Cr(VI) depending on pH can be shown as-

$$H_2CrO_4 \leftrightarrow H^+ + HCrO_4^-$$

$$HCrO_4^- \leftrightarrow H^+ + CrO_4^{2-}$$

$$2HCrO_4^- \leftrightarrow Cr_2O_7^{2-} + H_2O$$

Table 2
Adsorption isotherm parameters.

Langmuir isotherm				Freundlich isotherm		
$C_e/q_e = 1/q_0 b + C_e/q_0$ $R_L = \frac{1}{1+bC_0}$				$\log q_e = \log K_F + \frac{1}{n} \log C_e$		
q_0 (mg/g)	B (L/mg)	R_L	r^2	k_F (mg/g/L)	n	r^2
340.1	0.076	0.116	0.966	48.98	2.55	0.991

3.2.5. Adsorption isotherms

Langmuir and Freundlich isotherm models explain the behavior of adsorption on homogeneous and heterogeneous surfaces respectively. Langmuir isotherm gives the maximum adsorption capacity (q_0) and adsorption energy constant (b) from the slope and intercept of the plot of C_e/q_e versus C_e (Fig. 7a). R_L value was found to be 0.12, which refers to the effective interaction (korde et al.,) between the TCH and Cr(VI) (Table 2).

Graph of $\log q_e$ versus $\log C_e$ gives the constants k_F and n for the Freundlich isotherm (Fig. 7b). The value of n indicating strong interaction between Cr(VI) and TCH through favourable adsorption. The freundlich model was found to be more suitable as its correlation coefficient value is highest value close to 1 (Table 2). The same result was obtained through the comparison plot of experimental q_e and q_e obtained from isotherms versus C_e (Fig. 7c). In other words, the adsorption of Cr(VI) on TCH was found to be heterogenous multilayer phenomena.

3.2.6. Kinetics of adsorption

The pseudo-first-order and pseudo-second-order kinetic models were used to understand the contact time effects on the adsorption of Cr(VI) and to correlate the experimental data. For kinetic study, 200 mg of TCH equilibrated with 100 mg/L Cr(VI) solutions of pH 3 for different time intervals from 5 to 60 min at room temperature. The line graphs of log (q_e-q_t) versus t give pseudo-first-order parameters and t/q_t versus t give pseudo-second-order kinetic parameters (Fig. 8). The most suitable kinetic model for adsorption of Cr(VI) on TCH was found to be pseudo-second-order model because of higher value of correlation coefficient.

In the linear plot of q_t verses $t^{1/2}$, the slope gives the intraparticle rate constant k_{int} with the nonzero intercept (Table 3) indicating that the adsorption of Cr(VI) on TCH was controlled by boundary layer and diffusion phenomenon simultaneously.

3.3. Energetics of adsorption

Thermodynamic studies have been performed to understand the energy change occurs during adsorption process. Adsorption of Cr(VI) by TCH was studied at varying temperatures from 298 K –328 K. 50 mL of 100 mg/L Cr(VI) solutions equilibrated with 200 mg of TCH at pH 3.0 for an hour at different temperatures. The ratio of concentration of Cr(VI) adsorbed on TCH to that in the solution gives the equilibrium constant K. The plot of ln K versus 1/T gives the values of ΔH and ΔS obtained from slope and intercept (Fig. 9) respectively. The negative ΔG indicates the spontaneous behavior of adsorption of Cr(VI) on TCH. Negative values of ΔH and ΔS indicate towards the exothermic and decrease in randomness of Cr(VI) in solution phase respectively (Table 4).

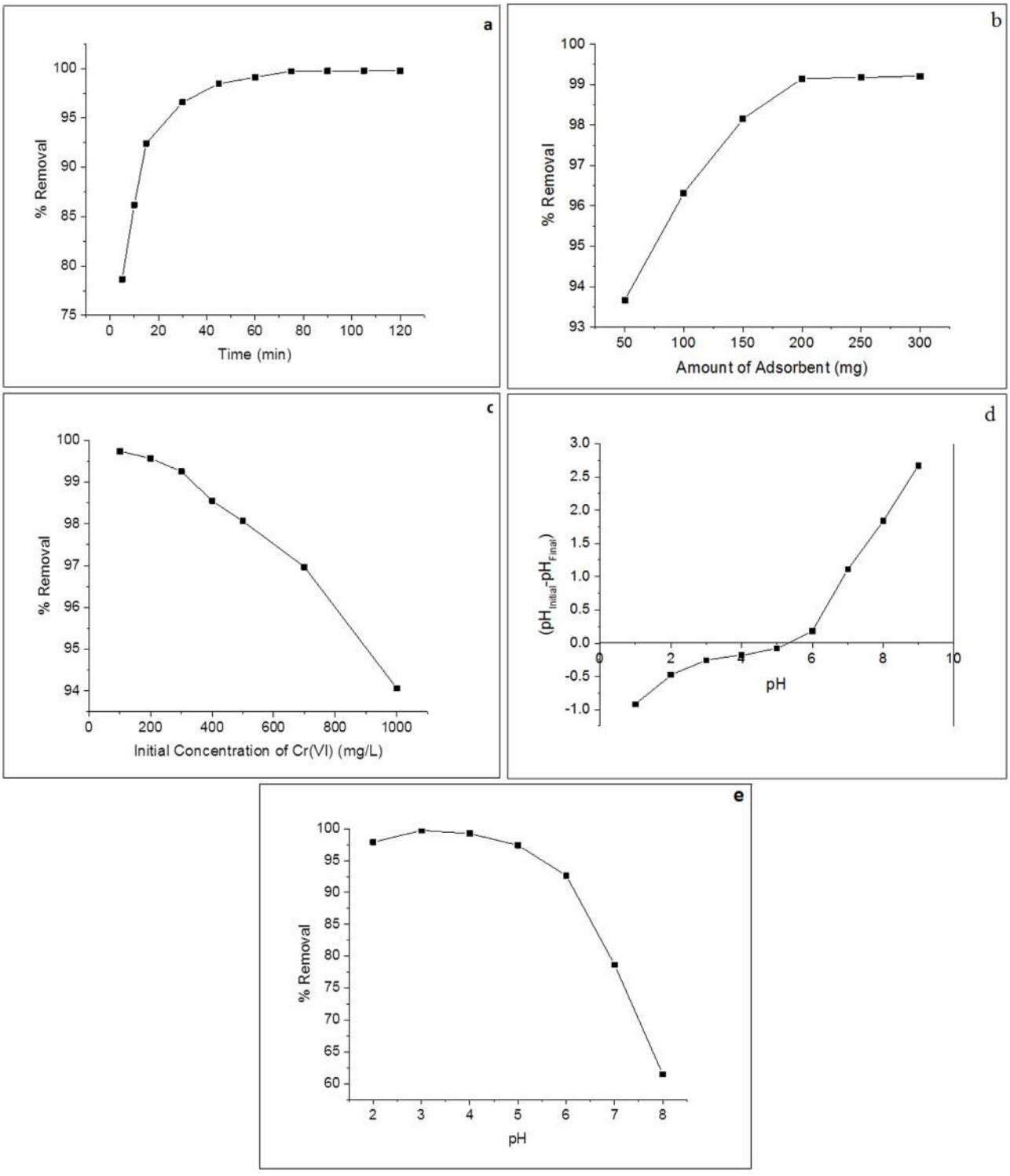


Fig. 6. Optimization of parameters (a) Contact time, (b) Adsorbent dose, (c) Initial concentration (d) point of zero charge and (e) pH on adsorption efficiency.

Table 3
Kinetic parameters.

Pseudo-first-order kinetics		Pseudo-second-order kinetics	Intraparticle diffusion			
$\log(q_e - q_t) = \log q_e - \frac{k_1 t}{2.303}$			$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e}$	$q_t = k_{int}.t^{1/2} + C$		
k_1 (1/min)	r^2		k_2 (g/mg/min)	r^2	k_{int} (mg/g/min$^{1/2}$)	r^2
0.092	0.888		0.026	0.999	0.51	0.716

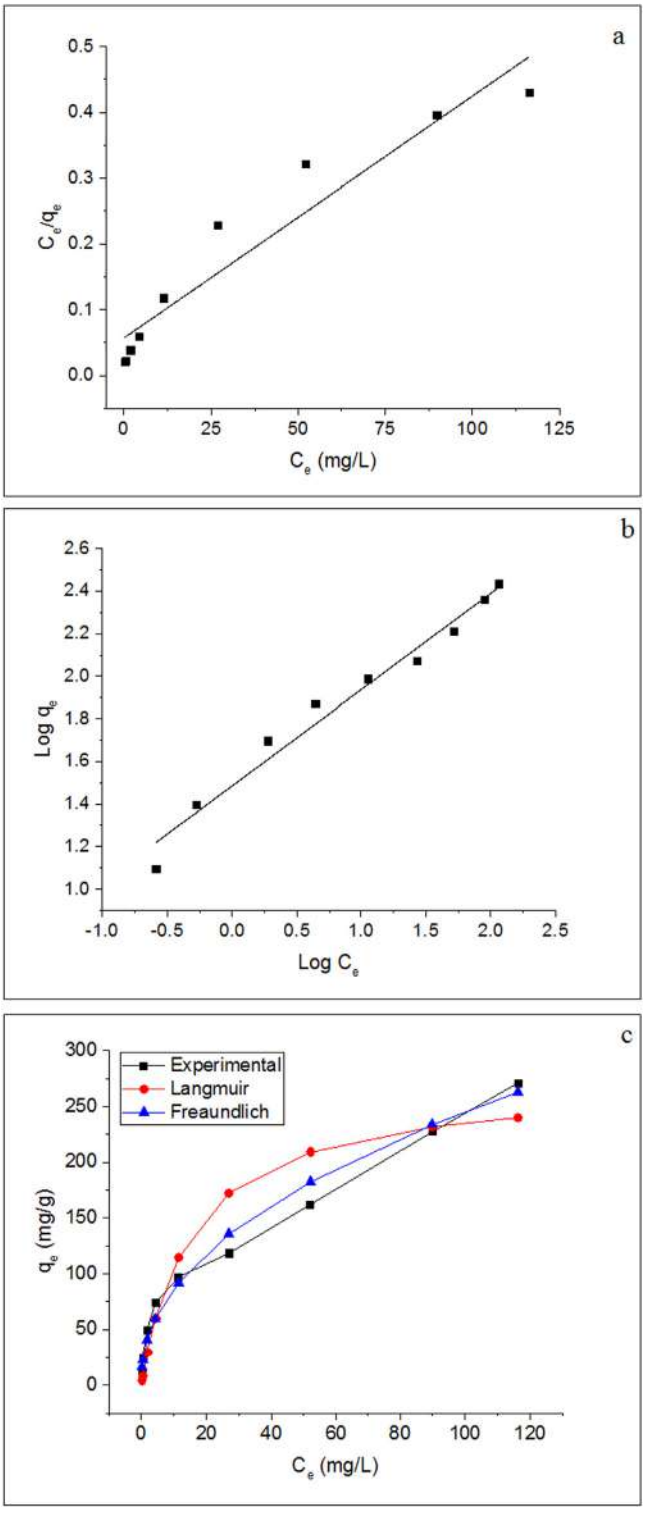


Fig. 7. Adsorption isotherms (a) Langmuir,(b) Freundlich and (c) Plot of q_e versus C_e.

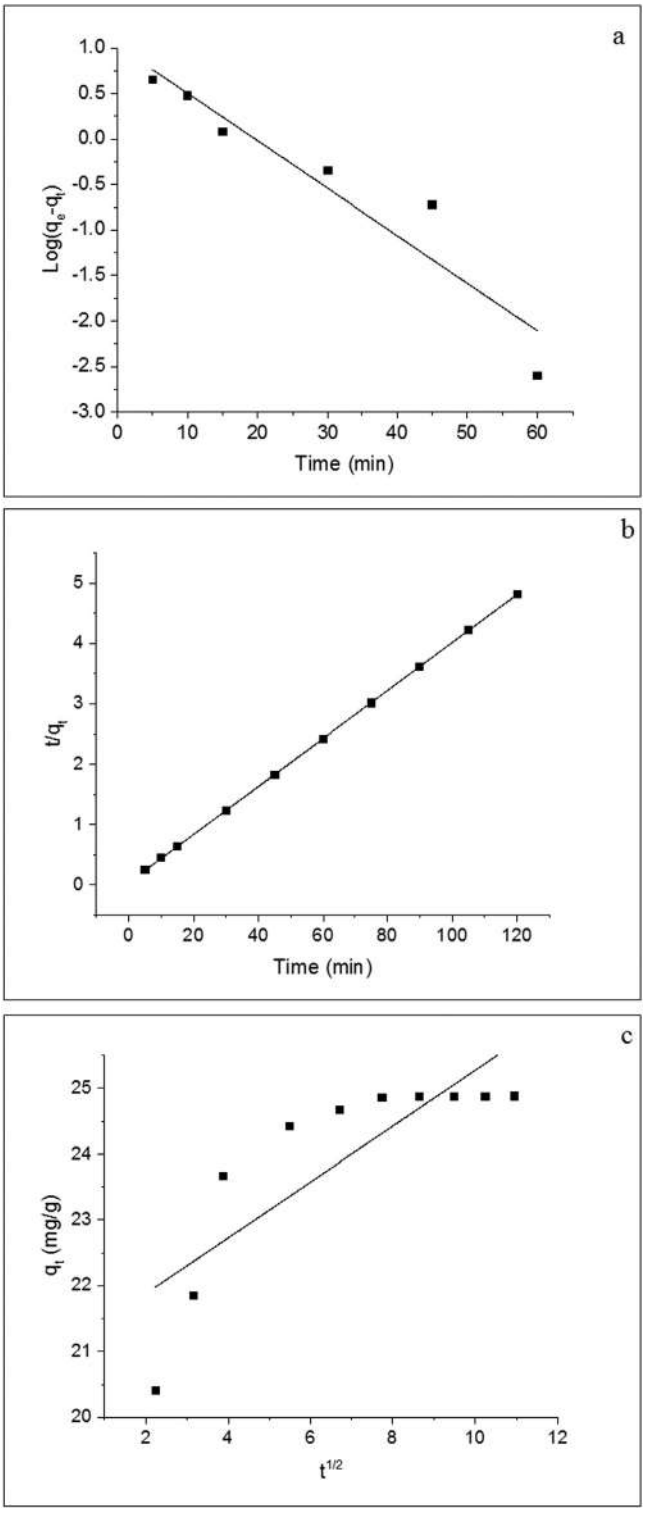


Fig. 8. Kinetic models (a) Pseudo-first-order (b) Pseudo-second-order and (c) Intraparticle diffusion.

Table 4
Thermodynamic parameters.

Temperature	ΔG (kJ/mol)	ΔH (kJ/mol)	ΔS (kJ/mol/K)
298K	−12.92		
303K	−11.93		
308K	−10.26	- 94.26	- 0.272
318K	−7.424		
328K	−4.955		

3.4. Parameters of column studies

200 mg /L solution of Cr(VI) of pH 3 was passed through the column packed with 1 g of TCH (height of 6 cm approximately)with optimized flow rate of 5 mL/min. Efficiency of column was calculated by the amount of eluted Cr(VI) in the solution through spectrophotometric method. Breakthrough curve (Fig. 9) gives the various column pa-

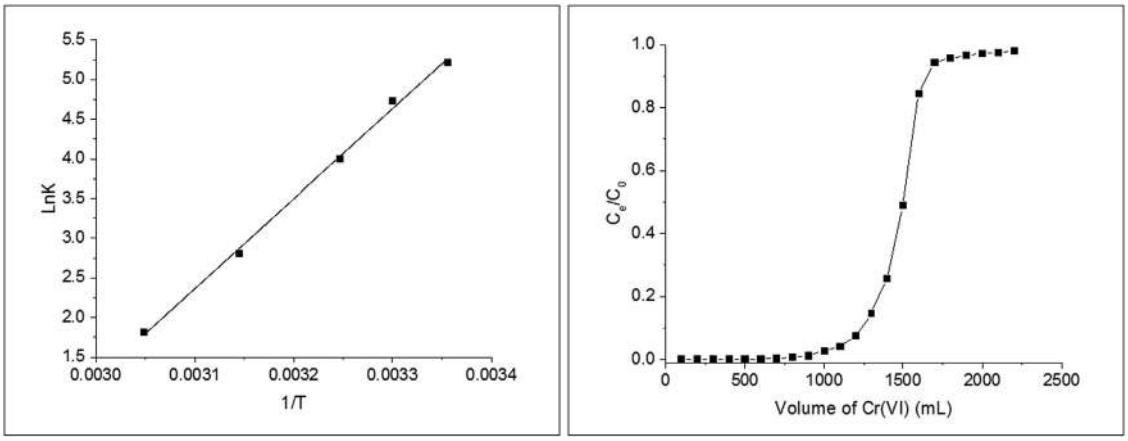


Fig. 9. van't Hoff plot and Breakthrough Curve.

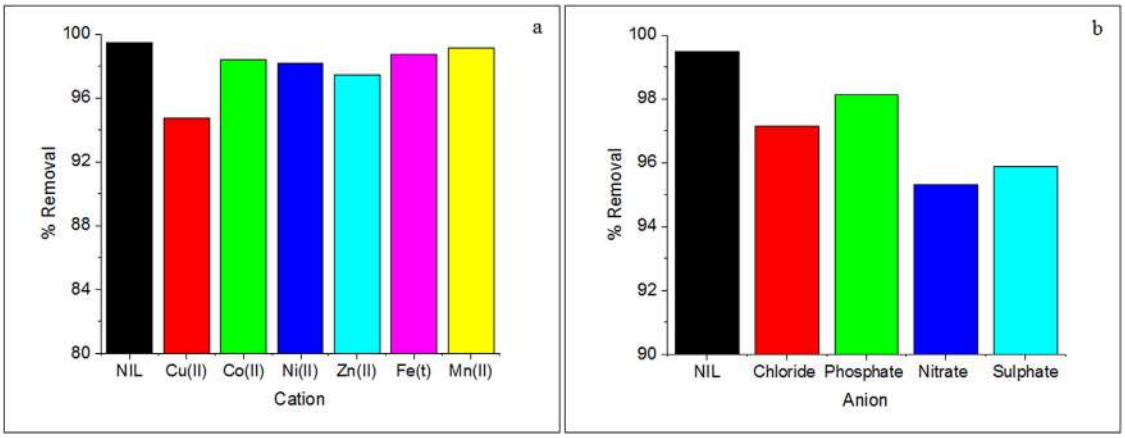


Fig 10. Effect of co-ions.

Table 5
Column study parameters.

Parameter	Result
Inlet Cr(VI) concentration	200 mg/L
Breakthrough volume	1400 mL
Exhaustion volume	2100 mL
Breakthrough Capacity	280 mg/g
Exhaustion Capacity	420 mg/g
Degree of column utilization	66.67%

rameters as mentioned in Table 5. 1400 ml of breakthrough volume and 2100 mL of exhaustion volume of Cr(VI) specify that the column method is more desirable method over batch adsorption. Therefore column methods can be productively used for larger sample volumes containing Cr(VI).

3.5. Effect of interfering ions

The wastewater contains multiple ions which may interfere in the adsorption process of Cr(VI) on TCH as they can compete for the adsorption sites of TCH. To study the effect of co-ions on adsorption efficiency of TCH, 50 mL solution containing 100 mg/L Cr(VI) and 200 mg/L interfering ions (SO_4^{2-}, Cl^-, NO_3^-, PO_4^{3-}, Cu^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+}, Fe^{2+}, Fe^{3+} and Mn^{2+}) were individually studied. From% removal values (Fig. 10), it was observed that the NO_3^{--} anion and Cu^{2+} cation have tendency to decrease the adsorption of Cr(VI) on TCH.

3.6. Regeneration and reusability of TCH

For the desorption of Cr(VI) from TCH, 2 g of TCH with adsorbed Cr(VI) was stirred with 25 mL of 5% reagent solution (sodium hydroxide, ammonia, sodium carbonate, hydrochloric acid and sulphuric acid) individually for 30 min. The treated precipitate was filtered and washed properly with distilled water to give back TCH. The best results were obtained with 5% (w/v) sodium hydroxide solution for the regeneration of TCH (Fig. 11a). The applicability of the regenerated TCH was tested for ten sorption-desorption cycles of Cr(VI). It was found that for initial four cycles there was insignificant change in adsorption efficiency and from fifth cycle it decreases subsequently (Fig. 11b). The regeneration of TCH shows the greener aspect of its application towards Cr(VI) detoxification.

4. Conclusion

TCH has been synthesized by very simple method that is treatment of chitosan with ammonium thiocyanate. Use of microwave technique reduces the time duration for the preparation as compared to the reflux method. The present work reports an effective method for adsorption of Cr(VI) on TCH. The interactions involved in adsorption of Hydrogen chromate ions ($HCrO_4^-$) on the surface of TCH are electrostatic at pH 3 (Fig. 12). The same can be observed from the negative value of entropy change as obtained in thermodynamic study. In order to understand efficacy of TCH, it was compared with other similar reported adsorbents. From Table 6, it is clear that TCH has highest adsorption capacity of 340.1 mg/g. The surface activity is the function of surface area as well

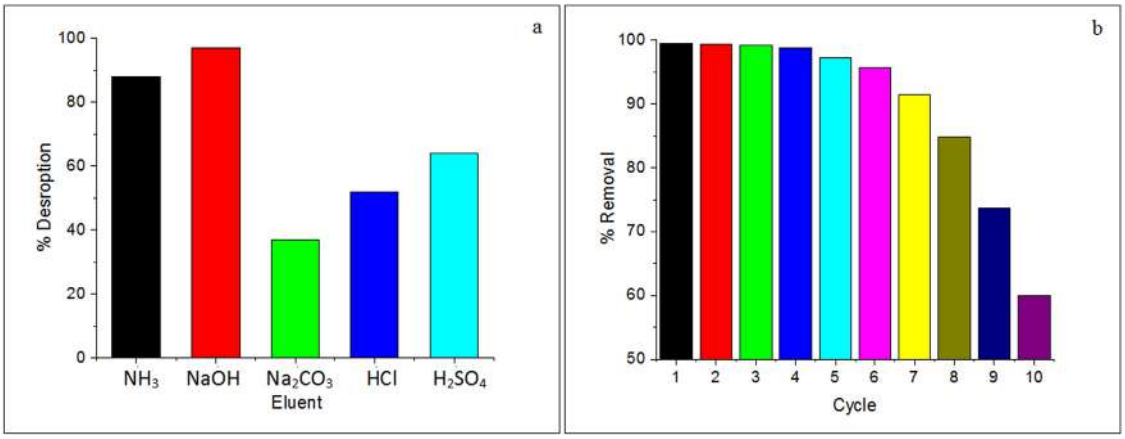


Fig 11. (a)% desorption studies and (b) Efficiency of regenerated TCH in succeeding adsorption cycle.

Table 6
Comparison of adsorption capacities.

Adsorbent	Adsorption capacity (mg/g)	Reference
Chitosan beads	76.92	(Ngah et al., 2006)
Diethylenetriaminepentaetic acid cross linked chitosan	192.31	(Bhatt et al., 2015)
Glulraldehyde crosslinked chitosan beads	28.65	(Watwe and Kulkarni, 2021)
diethylenetriaminepentaacetic acid–thiourea-modified magnetic chitosan (DTCS-Fe_3O_4)	321.3 ± 6.0	(Liu et al., 2021)
Amino terminated hyperbranched dendritic polyamidoamine 3rd generation chitosan beads	224.2	(Ghandhi and Meenakshi, 2013)
Thiolated chitosan (TCH)	340.1	Present study

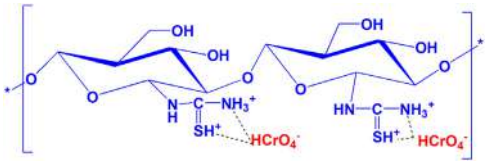


Fig 12. Electrostatic interaction of Cr(VI) and TCH.

as surface composition. It is true that the surface area has decreased in TCH as compared to chitosan. However, due to incorporation of amino and thio groups that get protonated in acidic medium, the surface activity increases enormously. This has been discussed under BET analysis studies. The Freundlich adsorption isotherm is the most suitable for the adsorption process of Cr (VI) which follows the pseudo-second-order kinetics. The negative enthalpy and free energy change indicates the exothermic and spontaneous behavior of the adsorption phenomena respectively. As TCH with adsorbed Cr(VI) can be regenerated and reused with insignificant changes in the same adsorption efficiency, it has a greener perspective.

Declaration of Competing Interest

The authors have no relevant financial or non-financial interests to disclose.

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Anita Shekhawat, Shashikant Kahu and D. Saravanan. The first draft of the manuscript was written by Ravin Jugade and Sadanand Pandey. All authors read and approved the final manuscript.

Acknowledgment

The authors are grateful to CSIR-HRDG, New Delhi, India for providing research associateship (File no.–09/128(0099)/2020-EMR-I) and also thankful to RTM Nagpur University for furnishing all the facilities required for the present work.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

References

Almeida, J.C., Cardoso, C.E.D., Tavares, D.S., et al., 2019. Chromium removal from contaminated waters using nanomaterials – a review. TrAC 118, 277–291. doi:10.1016/j.trac.2019.05.005.

Bayuo, J., 2021. An extensive review on chromium (VI) removal using natural and agricultural wastes materials as alternative biosorbents. J. Environ. Health Sci. Eng. 19, 1193–1207. doi:10.1007/s40201-021-00641-w.

Bhatt, R., Shreedhar, B., Padmaja, P., 2015. Adsorption of chromium from aqueous solutions using crosslinked chitosan–diethylenetriaminepentaacetic acid. Int. J. Biol. Macromol. 74, 458–466. doi:10.1016/j.ijbiomac.2014.12.041.

Boulaiche, W., Hamdi, B., Trari, M., 2019. Removal of heavy metals by chitin: equilibrium, kinetic and thermodynamic studies. Appl. Water Sci. 9, 39. doi:10.1007/s13201-019-0926-8.

Chen, S., Wu, G., Zeng, H., 2005. Preparation of high antimicrobial activity thiourea chitosan–Ag+ complex. Carbohydr. Polym. 60, 33–38. doi:10.1016/j.carbpol.2004.11.020.

Cheng, W., Wang, Y., Ge, S., et al., 2021. One-step microwave hydrothermal preparation of Cd/Zr-bimetallic metal–organic frameworks for enhanced photochemical properties. Adv. Compos. Hybrid Mater. 4, 150–161. doi:10.1007/s42114-020-00199-5.

Gandhi, M.R., Meenakshi, S., 2013. Preparation of amino terminated polyamidoamine functionalized chitosan beads and its Cr(VI) uptake studies. Carbohydr. Polym. 91, 631–637. doi:10.1016/j.carbpol.2012.08.028.

Guo, L., Zhang, Y., Zheng, J., et al., 2021. Synthesis and characterization of ZnNiCr-layered double hydroxides with high adsorption activities for Cr(VI). Adv. Compos. Hybrid Mater. 4, 819–829.

Husien, Sh, Labena, A, El-Belely, E.F, et al., 2019. Adsorption studies of hexavalent chromium [Cr (VI)] on micro-scale biomass of Sargassum dentifolium, seaweed. J. Environ. Chem. Eng. 7, 2213–3437. doi:10.1016/j.jece.2019.103444.

Jeffery, G.H., Bassett, J., Mendham, J., Denney, R.C., 1989. Vogels Text Book of Quantitative Chemical Analysis, 5th ed. ELBS publication.

Kahu, S., Shekhawat, A., Saravanan, D., Jugade, R., 2016a. Ionic solid-impregnated sulphate-crosslinked chitosan for effective adsorption of hexavalent chromium from effluents. Int. J. Environ. Sci. Technol. 13, 2269–2282. doi:10.1007/s13762-016-1059-3.

Kahu, S., Shekhawat, A., Saravanan, D., Jugade, R., 2016b. Two fold modified chitosan for enhanced adsorption of hexavalent chromium from simulated wastewater and industrial effluents. Carbohyd. Polym. 146, 264–273. doi:10.1016/j.carbpol.2016.03.041.

Kahu, S., Saravanan, D., Jugade, R., 2014. Effective detoxification of hexavalent chromium using sulfate-crosslinked chitosan. Water Sci. Techol. 70, 2047–2055. doi:10.2166/wst.2014.455.

Korde, S., Tandekar, S., Jeyaseelan, C., Jugade, R., 2022. Mesoporous magnetic Chitosan-Zirconia-Iron oxide nanocomposite for adsorptive removal of Cr(VI) ions. Mater. Lett. 311. doi:10.1016/j.matlet.2021.131513.

Kumar, R., Kim, S-Joon, K-Ho, Kim, et al., 2018. Removal of hazardous hexavalent chromium from aqueous phase using zirconium oxide-immobilized alginate beads. Appl. Geochem. 88, 113–121. doi:10.1016/j.apgeochem.2017.04.002.

Liu, H., Gao, J., Zhang, L., et al., 2021. Diethylenetriaminepentaacetic acid–thiourea-modified magnetic chitosan for adsorption of hexavalent chromium from aqueous solutions. Carbohydr. Polym. 274, 0144–8617. doi:10.1016/j.carbpol.2021.118555.

Ngah, W.S.W., Kamari, A., Fatinathan, S., et al., 2006. Adsorption of chromium from aqueous solution using chitosan beads. Adsorption 12, 249–257. doi:10.1007/s10450-006-0501-0.

Saha, D., Barakat, S., Van Bramer, S.E., et al., 2016. Noncompetitive and competitive adsorption of heavy metals in sulfur-functionalized ordered mesoporous carbon. ACS Appl. Mater. Interfaces 14, 34132–34142. doi:10.1021/acsami.6b12190.

Shao, Y., Fan, Z., Zhong, M, et al., 2021. Polypyrrole/bacterial cellulose nanofiber composites for hexavalent chromium removal. Cellulose 28, 2229–2240. doi:10.1007/s10570-020-03660-2.

Shekhawat, A., Kahu, S., Saravanan, D., Jugade, R., 2015. Synergistic behaviour of ionic liquid impregnated sulphate-crosslinked chitosan towards adsorption of Cr(VI). Int. J. Biol. Macromol. 80, 615–626. doi:10.1016/j.ijbiomac.2015.07.035.

Tandekar, S., Jugade, R., 2021. Chitosan-zirconia microballs for proficient removal of chromate and phosphate ions from water bodies. J. Chem. Sci. 133, 112. doi:10.1007/s12039-021-01975-6.

Watwe, V., Kulkarni, P., 2021. Evaluation of Cr(VI) adsorption on glutaraldehyde crosslinked chitosan beads using cyclic voltammetry employing gold electrode. J. Anal. Sci. Technol. 12, 37. doi:10.1186/s40543-021-00291-5.

Yang, Y., Chen, G., Murray, P., 2020. Porous chitosan by crosslinking with tricarboxylic acid and tuneable release. SN Appl. Sci. 2, 435. doi:10.1007/s42452-020-2252-z.

Zhang, F., Cheng, W., Yu, Z., et al., 2021. Microwave hydrothermally synthesized WO_3/UiO-66 nanocomposites toward enhanced photocatalytic degradation of rhodamine B. Adv. Compos. Hybrid Mater. 4, 1330–1342. doi:10.1007/s42114-021-00346-6.

Bioresource Technology Reports 18 (2022) 101002

Contents lists available at ScienceDirect

Bioresource Technology Reports

journal homepage: www.sciencedirect.com/journal/bioresource-technology-reports

Gamma degraded oligomeric nanochitosan for adsorptive removal of Cd(II)

Swati Tandekar [a], Sanjiwani Korde [b], D. Saravanan [c], Ravin Jugade [b,*]

[a] Department of Chemistry, Tai Golwalkar Mahavidyalaya, Ramtek 440016, India
[b] Department of Chemistry, R.T.M. University, Nagpur 440033, India
[c] National College, Tiruchirapalli, Tamilnadu, India

ARTICLE INFO

Keywords:
Gamma degradation
Nanochitosan
Adsorption
Cadmium

ABSTRACT

This study is the first ever report of application of gamma degraded nanochitosan biopolymer for the adsorptive removal of Cd(II) from aqueous systems. The viscosity studies revealed that the average molecular weight goes on decreasing with increase in gamma irradiation dose. The degraded products were subjected to characterization and were used for adsorption of Cd(II) in aqueous medium showing optimum adsorption capacity of 47.6 mg g^{-1} at 10 kGy dose in nice accordance with Freundlich model of isotherm and followed pseudo-second order rate expression. The crystallite size of chitosan was found to reduce from 39.15 nm to 7.012 nm with 10 kGy dose.

1. Introduction

Waste water contaminated with heavy metal ions as pollutants need to be treated before their disposal into larger bodies. Various researchers have reported different techniques for the removal of metal toxicants (Bhavani et al., 2016). The use of adsorbents, especially the biopolymers, have attracted various workers in last decade due to their nature sources, abundance in existence and biocompatibility. Chitosan has shown great sorption capacity for numerous toxic metal cations due to presence of hydroxyl and amino groups (da Silva Alves et al., 2021). In general, native chitosan has very high molecular weight and hence a very high viscosity, low solubility in aqueous media limits its use in numerous applications. Oligomeric chitosan has low molecular weight and has shown to have better interaction with metal ions as compared to high molecular weight chitosan (Younis et al., 2019). The decrease in molecular weight can be brought about by various chemical and physical processes. An excellent tool among them is non-chemical gamma irradiation technique. Recently, we have shown application of gamma irradiation on adsorption enhancement of chitosan composite for defluoridation of water (Tandekar et al., 2020).

This paper reports degradation of chitosan through irradiation using ^{60}Co gamma source. Chain breakage led to formation of oligo-nanochitosan will increase with irradiation dosage (Mahmud et al., 2014). Also, the study measured the effect of gamma irradiation on molecular weight drop of chitosan biopolymer and examine the effect of high energy gamma treatment on its adsorption efficiency, Cd(II) was selected as representative pollutant.

2. Materials and methods

All the chemicals and reagents were of Analytical grade. Solid chitosan with DDA 95% was procured from Meck chemicals, Ahmadabad, India while other chemicals were procured from SRL chemicals, India. Gamma irradiation of chitosan was carried out using Gamma Irradiation Chamber GC-1200 (Model: BRIT, India) at an activity of 9.5 kGy h^{-1}.

For functional group analysis, FT-IR spectra were recorded using Bruker Alpha spectrometer working in the range of 500–4000 cm^{-1}, averaging 23 scans, with ZnSe ATR crystal. SEM and EDX analysis simultaneously using TESCAN VEGA 3 SBH and (EDX) Diffractometer model Oxford INCA Energy 250 EDS. The XRD pattern was recorded using Rigaku Miniflex 300 and XPERT-PRO X-ray diffractometer using Cu Kα radiation in the range of 1^0 to 100^0. Thermal studies were carried out using DTG-60 (Shimadzu) thermogravimetric analyser working in nitrogen atmosphere at a flow rate of 100 mL min^{-1} in a temperature range of ambient to 800 °C.

Batch adsorption studies were performed by taking required concentration of cadmium solution (25–500 mg L^{-1}) with variable pH (2.0 to 7.0). It was equilibrated with different dosages of adsorbent (50 to 500 mg) irradiated with different gamma doses (5 to 100 kGy) for different time (10 to 120 min). The solution was filtered and the concentration of Cd(II) was determined using differential pulse polarographic technique. The amount of Cd(II) adsorbed (mg g^{-1}) on the

* Corresponding author.
E-mail address: ravinj2001@yahoo.co.in (R. Jugade).

https://doi.org/10.1016/j.biteb.2022.101002
Received 15 January 2022; Received in revised form 22 February 2022; Accepted 1 March 2022
Available online 4 March 2022
2589-014X/© 2022 Elsevier Ltd. All rights reserved.

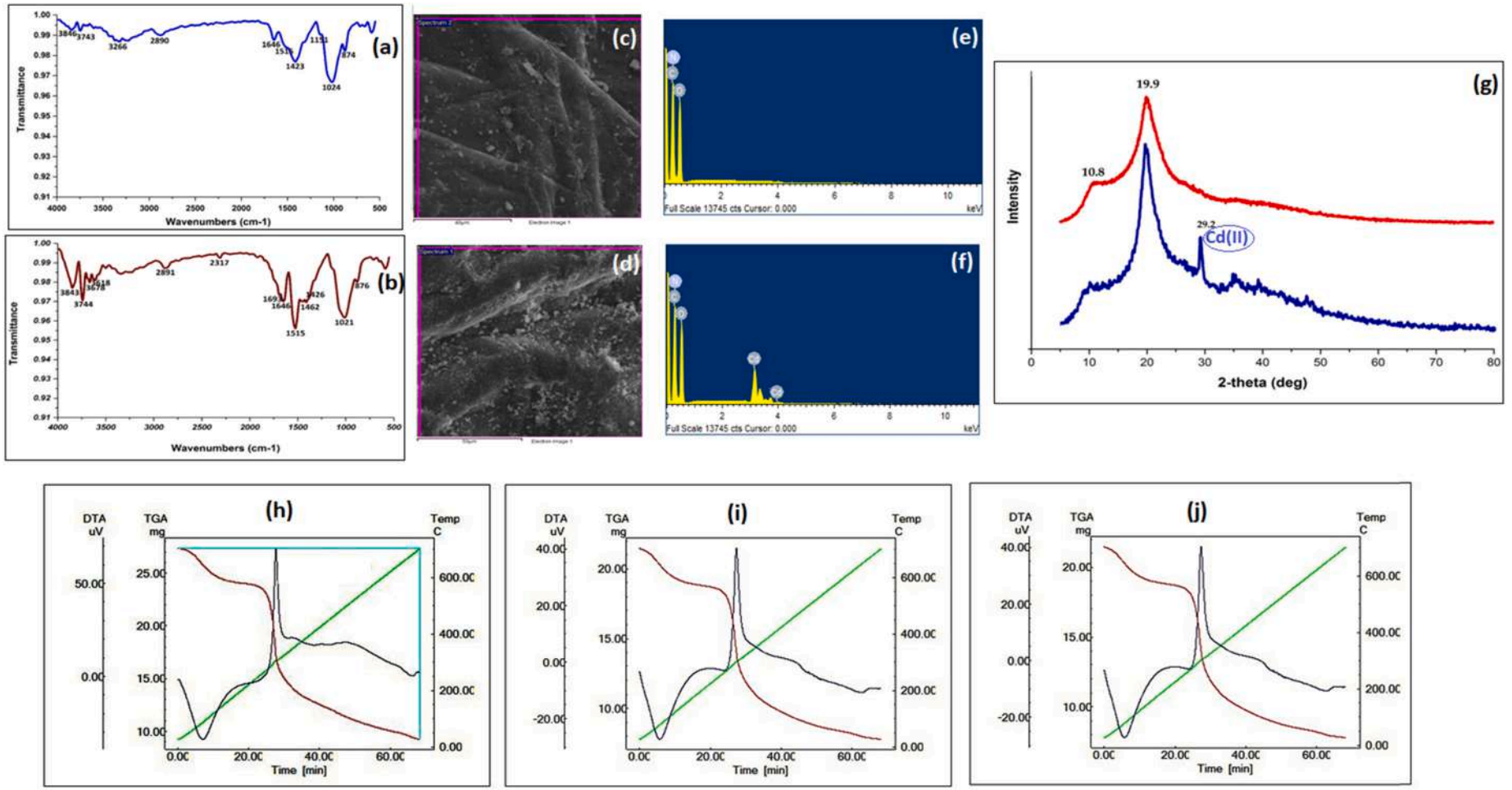


Fig. 1. FTIR images of (a) chitosan and (b) γ-chitosan; SEM images of (c) γ-chitosan and (d) γ-chitosan with adsorbed Cd(II); EDX images of (e) γ-chitosan and (f) γ-chitosan with adsorbed Cd(II); (g) XRD image of γ-chitosan and after Cd(II) adsorption; TGA and DTA of chitosan with (h) 0 kGy (i) 50 kGy and (j) 100 kGy irradiation dosages.

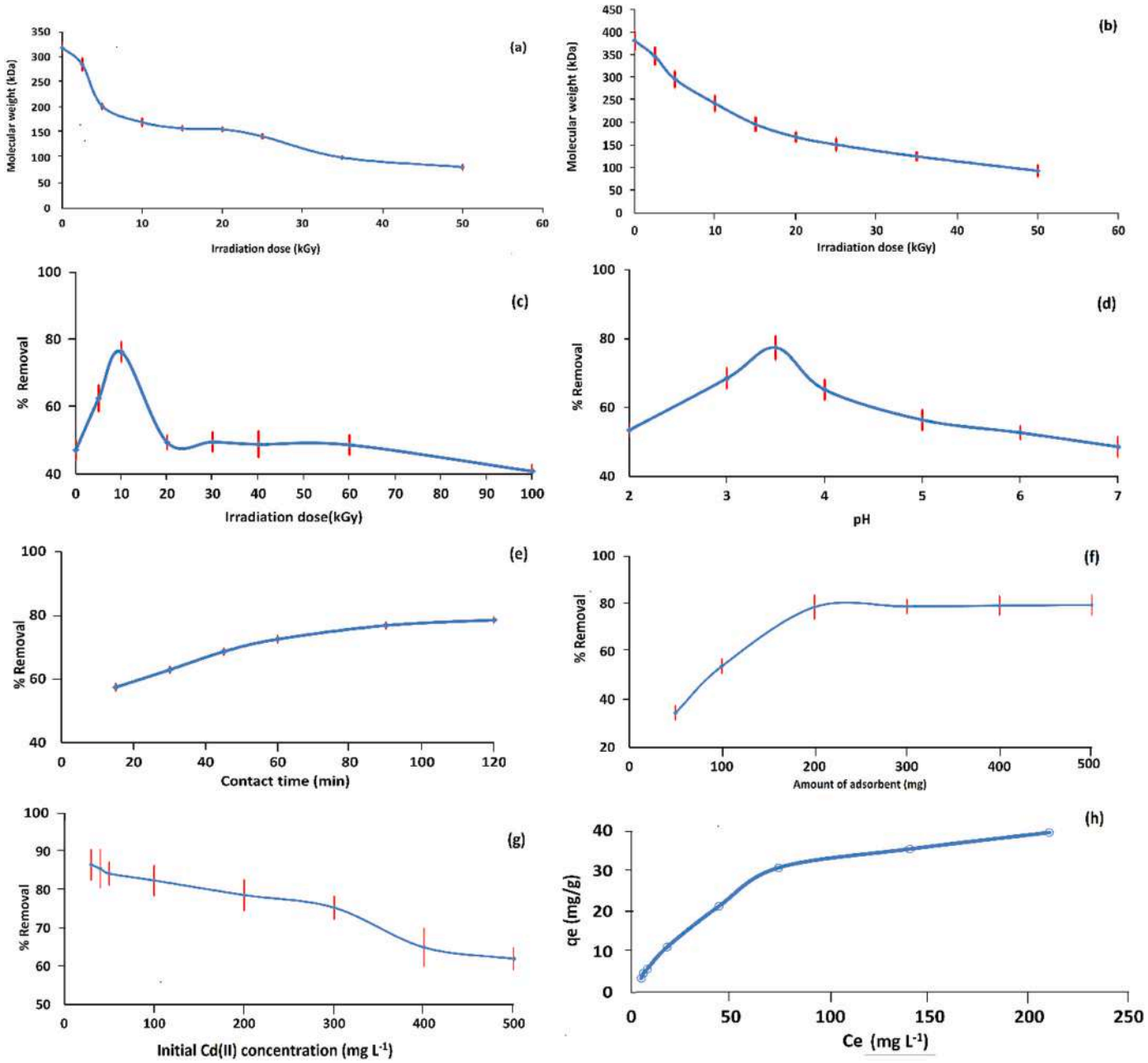


Fig. 2. Plot of Molecular weight (kDa) against Irradiation dose (kGy) using (a) Ostwald viscometer, (b) Ubbelohde viscometer, impact of (c) irradiation dose, (d) pH, (e) contact time, (f) amount of adsorbent, (g) initial Cd(*II*)ion concentration, and (h) adsorption isotherm curve.

irradiated chitosan can be given by the following equation-

$$q_e = \frac{C_0 - C_e}{W} \times V$$

where, C_0 and C_e refer to initial and equilibrium solution phase concentration of Cd(II) ions in mg L^{-1}, V is the volume of solution in L and W is the weight of irradiated chitosan taken for adsorption in g. All the experiments were performed three times to check the reproducibility and the mean of three observations have been reported.

3. Results and discussion

The FTIR analysis of native chitosan (Fig. 1a) indicated characteristic bands at 3846 cm^{-1} and 3743 cm^{-1} that can be assigned to the stretching vibrations of -OH group while the band at 3266 cm^{-1} assigned to $–NH_2$ stretching. The C—H stretching bands in $–CH_2$ and $–CH_3$ groups appeared at 2890 cm^{-1}. The bands at 1515 cm^{-1} and 1423 cm^{-1} have been correlated to N—H and O—H group bending modes respectively. The sharp peak at 1151 cm^{-1} was represents pyranose group of chitosan while a broad and intense peak at 1024 cm^{-1} corresponds to the –CO group stretching frequency of COH, COC and CH_2OH groups (Shekhawat et al., 2022) and a weak peak at 874 cm^{-1} for –NH bending (Zheng et al., 2015). When chitosan was subjected to gamma irradiation, a sequential change was observed with increase in irradiation dose (Fig. 1b). The broad -OH stretching peak was found to get sharper and resulted in a very sharp peat at 3744 cm^{-1}. Another peak at 1693 was found to grow in a sequential manner with gamma dose. This can be ascribed to enhancement in the quantity of C═O groups in the polymer (Muley et al., 2019). Another important observation shows systematic reduction in the peak intensity at 1024 cm^{-1}, showing chain scissoring at C-O-C. This has been supported by reduction in molecular weight irradiation dose (Nandiyanto et al., 2019).

The SEM images clearly reveal that γ-chitosan exhibit irregular morphology due to deformation of chitosan by irradiation due to splintering of β-glycosidic bonds. Bright spots are observed in the matrix after Cd(II) adsorption (Fig. 1c–d). The EDX spectra were recorded along with SEM analysis show distinct peak of cadmium after adsorption confirming the accumulation of Cd(II) ions on the surface (Fig. 1e–f).

XRD patterns reflects no change in the representative peaks of chitosan when compared to chitosan. Broad peaks in the XRD pattern of γ-chitosan at $2\theta = 10.8^0$ (110) and 19.9^0 (020) show that there are no changes in the planes of native chitosan after irradiation. After adsorption of Cd(II) ions, additional peak was observed at $2\theta = 29.2^0$ that has been assigned to cadmium (Fig. 1g) (da Silva Alves et al., 2021). Crystallite size obtained from Scherrer equation was found to decrease from 39.15 nm in chitosan to 7.012 nm in nanochitosan.

The thermograms of chitosan before and after irradiation showed a two-stage breakdown pattern (Fig. 1h–j). The first decomposition for chitosan and γ-degraded chitosan at 50 and 100 kGy doses appeared in the region of 50–180 °C and is assigned to loss of water. The second breakdown above 250 °C with maximum decomposition rate at 350 °C leading to about 64 to 66% weight loss was observed between 280 °C to 360 °C for both native as well as degraded chitosan. This represents the main chain decomposition of chitosan, vaporization and loss of volatile products. The further decomposition of chitosan at higher temperature is depended on its molecular weight, the source of chitosan, and the method employed for its extraction (Kumari et al., 2017; Khapre et al., 2021). The sharpness of the DTA curve was found to get reduced on irradiation and also the heat of decomposition was found to increase from 187.02 J g^{-1} in native chitosan to 199.15 J g^{-1} in 50 kGy irradiated to 234.15 J g^{-1}in 100 kGy degraded chitosan.

Intrinsic viscosity and average molar mass of chitosan were determined using Ostwald and Ubbelohde viscometers. The Mark-Houwink equation was used to obtain probability of the viscosity-average molecular weight of chitosan samples (Kasaai, 2007). A steady decrease in molecular weight was observed with gamma dose of 2.5 kGy to 50 kGy exponentially. Further increase in irradiation dosage of chitosan led to decrease in chitosan average molecular weight, although the rate of degradation was much slower (Fig. 2a–b). It has been reported that, with increase in gamma irradiation dose, the extent of cross-linking of the chitosan chains and its molecular weight reduces owing to the 1–4 β-glycosidic bonds scission the high energy γ-rays (Zheng et al., 2015).

Adsorption studies were performed in batch mode by using required concentration of cadmium solution (50–500 mg L^{-1}) with variable pH (2.0 to 7.0). It was equilibrated with different dosages of adsorbent (50 to 200 mg) irradiated with gamma doses (5 to 100 kGy) for time (10 to 120 min). After filtration, the concentration of Cd(II) was determined in filtrate using differential pulse polarographic technique. It was observed that the adsorption efficiency increased with gamma dose upto 10 kGy and decreased thereafter. A maximum adsorption of Cd(II) occurred with 10 kGy irradiated chitosan (Fig. 2c) which was labelled as γ-chitosan and was used in all further studies. Further irradiation led to decrease the molecular weight of chitosan thereby increasing its solubility in the acidic medium.

In aqueous medium, it was observed that the % removal of Cd(II) was highest at pH 3.5 (Fig. 2d). The adsorption efficiency increased with contact time upto 90 min and then equilibrium was established. It may be concluded that the adsorption sites get saturated with Cd(II) in 90 min (Fig. 2e). The γ-chitosan dose was increased from 50 mg to 500 mg keeping the other parameters constant. It was found that, for 200 mg L^{-1} concentration of Cd(II), the adsorption efficiency increased from 50 mg to 200 mg and a plateau was observed thereafter (Fig. 2f). This is due to establishment of equilibrium at adsorbent-adsorbate interface. The extent of adsorption was found to be about 80% upto 200 mg L^{-1} of Cd (II) and then started decreasing rapidly (Fig. 2g). Hence, further adsorption studies were carried out taking 200 mg L^{-1} concentration of Cd(II). A graph of q_e as a function of C_e has been shown in Fig. 2h that indicates saturation of adsorbent surface in equilibrium with the solution phase concentration of Cd(II) ions.

Table 1
Theoretically derived parameters.

Isotherm model	Freundlich model	K_F ($mg^{1-1/n}$ g^{-1} L^{-1})	1.313
		n	1.510
		R^2	0.971
	Langmuir model	q_m(mg g^{-1})	47.62
		b (L g^{-1})	0.0075
		R^2	0.994
Kinetics of adsorption q_e (exp) = 19.50 mg g^{-1}	PFO	K_1 (min^{-1})	0.0303
		R^2	0.992
		q_e (calculated) (mg g^{-1})	9.34
	PSO	K_2 (g mg^{-1} min^{-1})	1.313
		R^2	0.971
		q_e (calculated) (mg g^{-1})	19.38
Thermodynamic studies Temperature	ΔG (kJ mol^{-1})	ΔH (kJ mol^{-1})	ΔS (J mol^{-1} K^{-1})
298 K	−0.302	19.291	64.39
313 K	−0.627		
323 K	−1.722		
333 K	−2.087		

In order to account for type of interaction at adsorbent-adsorbate interface, the adsorption isotherm, kinetics and thermodynamics studies were carried out. Freundlich isotherm model represents assorted multifaceted adsorption (Freundlich, 1906). A linearized Freundlich isotherm correlating adsorption capacity (K_F) and adsorption intensity (n) in following equation

$$logq_e = logK_F + \frac{1}{n}\, logC_e$$

Higher value of n signifies effective adsorbate-adsorbent interaction.

Langmuir isotherm model (Langmuir, 1918) represents monolayer adsorption on uniform surface. Linearized Langmuir equation is given by

$$C_e/q_e = {}^1/_{q_m b} + C_e/q_0$$

C_e (mg L^{-1}) is the equilibrium heavy metal concentration, q_e(mg g^{-1}) is the equilibrium adsorption capacity, b is the Langmuir isotherm constant which related to the strength of adsorption, qm(mg g^{-1}) is the maximum adsorption capacity. The values of q_m and b were obtained from the slope and intercept of the plot of C_e/q_e against C_e.

The adsorption process was found to follow Langmuir model (R^2 = 0.994) which represents monolayer adsorption on homogenous adsorbent surface with capacity of 47.62 mg g^{-1}. Also, the value of n = 1.51 which is between 1 and 10 indicates favourable adsorption (Table 1).

In kinetics studies, the observations were subjected to pseudo-first-order (PFO) and pseudo-second-order (PSO) models (Ho, 2004). The value of R^2 was found to be closer to 1 for PSO kinetics model. This indicates that the best fitted model is PSO model. Also, the experimental q_e value agrees with the value calculated from PSO model. Thermodynamic parameters evaluation for Cd(II) adsorption was carried out by varying temperatures from 298 K to 353 K maintaining other parameters constant at their respective optimum values. The negative values of ΔG at all temperatures indicate that the adsorption is spontaneous in this temperature range. Positive value of ΔH signposts endothermic nature while positive value of ΔS indicates increase in randomness of adsorbate (Table 1). These values are a clear indication that enthalpy opposes while entropy favours the adsorption process, meaning that, it is an entropy driven process.

Interference of various co-existing ions was studied by mixing 100 mg L^{-1} of Cd(II) solution with same concentration of interfering cation or anion (taken one at a time). It was observed that Cu(II) interferes to maximum extent among cations while chloride shows maximum

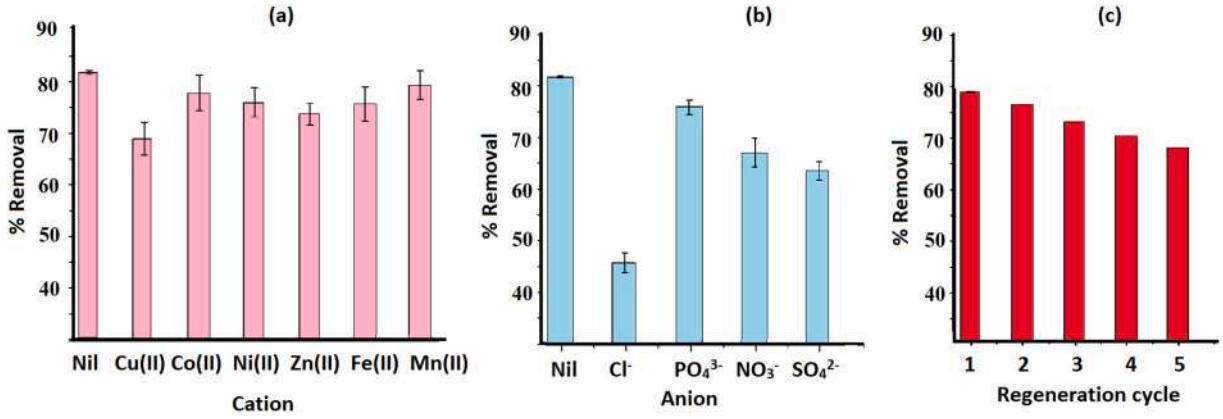


Fig. 3. Effect of coexisting cations (a) and anions (b). Regeneration and reusability study (c).

interference among anions (Fig. 3a–b). The interference of chloride can be attributed to its tendency to form chloro-complex with Cd(II) which is soluble in aqueous medium. This property was used to regenerate the adsorbent by treating with 5% NaCl solution. The material was regenerated and reused for five adsorption-desorption cycles. The adsorption efficiency was found to decrease from 82% in original material to 68% after 5th cycle of regeneration (Fig. 3c).

4. Conclusion

Radiation is known for its safer, environmentally friendly and easier method to modify polymers. Degradation occurs in the chitosan when exposed to irradiation in solid state. The degraded nanochitosan showed improved properties required for better adsorption of Cd(II) leading to 47.62 mg g^{-1} Cd(II) capacity. This short work may lead to further work in the field of degradation of chitosan for betterment of adsorption capacities towards other pollutants.

CRediT authorship contribution statement

Swati Tandekar: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. **Sanjiwani Korde:** Resources, Software, Validation, Visualization. **D. Saravanan:** Investigation. **Ravin Jugade:** Writing – review & editing, Supervision, Project administration.

Declaration of competing interest

No competing interest declared.

Acknowledgements

Acknowledgements are due to Department of Science and Technology for DST-FIST grant; University Grants Commission for SAP funding and RTM Nagpur University for URP scheme.

References

Bhavani, K., Begum, E.R.A., Selvakumar, S., Shenbagarathai, R., 2016. Chitosan– a low cost adsorbent for electroplating waste water treatment. J. Bioremed. Biodeg. 7 (3), 1000346.
da Silva Alves, D.C., Healy, B., Pinto, L.A., Cadaval, T.R.S., Breslin, C.B., 2021. Recent developments in chitosan-based adsorbents for the removal of pollutants from aqueous environments. Molecules 26 (3), 594.
Freundlich, H.M., 1906. Uber die adsorption in losungen. Zeitschrift furPhysikalische Chemie 57, 385–470.
Ho, Y.S., 2004. Lagergren kinetic rate equation. Scientometrics 59, 171–177.
Kasaai, M.R., 2007. Calculation of Mark-Houwink-Sakurada (MHS) equation viscometric constants for chitosan in any solvent-temperature system using experimental reported viscometric constants data. Carbohydr. Polym. 68, 477–488.
Khapre, M., Pandey, S., Jugade, R., 2021. Glutaraldehyde-cross-linked chitosan-alginate composite for organic dyes removal from aqueous solutions. Int. J. Biol. Macromol. 190, 862–875.
Kumari, M., Shukla, S., Pandey, S., Giri, V.P., Bhatia, A., Tripathi, T., 2017. Enhanced cellular internalization: a bactericidal mechanism more relative to biogenic nanoparticles than chemical counterparts. ACS Appl. Mater. Interfaces 9, 4519–4533.
Langmuir, J., 1918. The adsorption of gases on plane surfaces of glass mica and platinum. J. Am. Chem. Soc. 40, 1361–1403.
Mahmud, M., Yacob, I., Talip, N., Abdullah, Z., 2014. Degradation of chitosan by gamma ray with presence of hydrogen peroxide. In: Advancing Nuclear Research and Energy Development, 1584, pp. 136–149.
Muley, A.B., Ladole, M.R., Suprasanna, P., Dalvi, G., 2019. Intensification in biological properties of chitosan after γ-irradiation. Int. J. Biol. Macromol. 131, 435–444.
Nandiyanto, A.D., Oktiani, R., Ragadhita, R., 2019. How to read and interpret FTIR spectroscope of organic material. Indian J. Sci. Technol. 4, 97–118.
Shekhawat, A., Kahu, S., Saravanan, D., Jugade, R., 2022. Bi-functionalized ionic liquid-thiourea chitosan for effective decontamination of Cd(II) and Hg(II) from water bodies. Curr. Res. Green Sustain. Chem. 5, 100246.
Tandekar, S., Saravanan, D., Korde, S., Jugade, R., 2020. Gamma degraded chitosan-Fe (III) beads for defluoridation of water. Mater. Today Proc. 29, 726–732.
Younis, A.M., Aly-Eldeen, M.A., Elkady, E.M., 2019. Effect of different molecular weights of chitosan on the removal efficiencies of heavy metals from contaminated water. Egypt. J. Aquat. Biol. Fish. 23 (4), 149–158.
Zheng, X., Yin, Y., Jiang, W., Xing, L., Pu, J., 2015. Synthesis and characterization of low molecular weight chitosan. BioResearch 10 (2), 2338–2349.

ISSN 1063-7745, Crystallography Reports, 2021, Vol. 66, No. 7, pp. 1258–1261. © Pleiades Publishing, Inc., 2021.

STRUCTURE OF ORGANIC COMPOUNDS

Crystal Structure of 1-(4-(5,6-Dihydropyridazin-1(4*H*)-yl)Phenyl)-*N*-Methylmethanesulfonamide

S. Ramesh[a], A. Amuthavalli[b], M. Balaguru[c], and D. Saravanan[a,*]

[a] *Department of Chemistry, National College, Tiruchirappalli, 620001 India*

[b] *Department of Chemistry, Kongunadu Arts and Science College, Coimbatore, 641029 India*

[c] *Department of Chemistry, Banasthali University, Vanasthali, 304022 India*

***** *e-mail: dsaravananchem@nct.ac.in*

Received May 21, 2020; revised December 28, 2020; accepted December 28, 2020

Abstract—The title compound, 1-(4-(5,6-dihydropyridazin-1(4*H*)-yl)phenyl)-*N*-methylmethanesulfonamide ($C_{12}H_{17}N_3O_2S$), was isolated as an impurity during the preparation of sumatriptan succinate at an enhanced level and analyzed by X-ray diffraction. This compound crystallizes in a monoclinic system with space group *Pc* $a = 12.684$ (4) Å, $b = 5.5864$ (17) Å, $c = 9.323$ (3) Å, $V = 645.1$ (3) Å^3, $Z = 2$. The molecules are linked by hydrogen bonds N–H···O between S=O and amino groups, forming a zigzag chain along the *a*-axis.

DOI: 10.1134/S1063774521070130

INTRODUCTION

Sumatriptan succinate (Sumatriptan, Imitrex, Treximet, Imigrane) is a synthetic drug belonging to the triptan class, which is widely used for the treatment of migraine headaches [1, 2]. Structurally, it is an analog of the naturally occurring serotonin receptor family (5-hydroxytriptamine, 5-HT). Sumatriptan, the first selective 5-HTID agonist, has been shown to be a selective vasoconstrictor of cranial blood vessels and has been shown to be effective in the treatment of acute migraine attacks. Tryptamine derivatives have a common feature –the presence of an amide function or a small-ring heterocyclic attached to the C_5 atom of the indole nucleus through a methylene spacer. Sumatriptan succinate is chemically known as 1-[3-(2-dimethylaminoethyl)-1*H*-indol-5yl]-*N*-methylmethanesulphonamide succinate.

Indoles are heterocyclic compounds that are widely distributed in nature, and their synthesis has attracted great attention. Numerous methods are available for the synthesis of indoles and their derivatives [3–6]; on the whole, acid-mediated Fischer indolization of phenyl hydrazones was first reported as a method more than 120 years ago [7–11] and is the most frequently used procedure. Migraine drugs contain indole moiety, such as sumatriptan, rizatriptan, almotriptan and zolmitriptan, prepared by this method. During the development of the synthesis of sumatriptan, the formation of a cyclized by-product 1-(4-(5,6-dihydropyridazin-1(4*H*)-yl)phenyl)-*N*-methylmethanesulfonamide (Fig. 1) was revealed at an enhanced level, which was isolated and characterised using an advanced analytical tool [12].

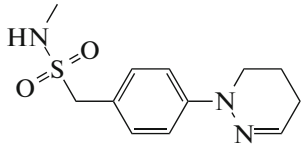


Fig. 1. Chemical structure of 1-(4-(5,6-dihydropyridazin-1(4*H*)-yl)phenyl)-*N*-methylmethanesulphonamide.

EXPERIMENTAL

Isolation and Growth of Crystals of 1-(4-(5,6-Dihydropyridazin-1(4H)-yl)Phenyl)-N-Methyl Methane Sulphonamide

4-Chlorobutyraldehyde diethyl acetal (7.6 g, 0.042 mol) and disodium hydrogen phosphate (5.6 g, 0.04 mol) were successively added to a solution of 4-hydrazino-*N*-methylbenzene methane sulphonamide hydrochloride (10 g, 0.04 mol) in a mixture water/methanol (120 mL) in a ratio of 1 : 2, and the mixture was boiled under reflux for 5 h. After the mixture was cooled to room temperature, the solution was filtered, and the filtrate was acidified with 50% aqueous HCl and extracted with dichloromethane. The solvent was evaporated and the residue was purified by column chromatography on silica gel using ethyl acetate–hexane (1 : 9) as eluent. The yield was 3.0 g (28%, yellow solid). Single crystals of the title compound suitable for X-ray diffraction analysis were obtained by slow evaporation using ethanol as solvent in a sealed tube at room temperature for five days.

Table 1. Crystallographic characteristics, details of X-ray data collection and structure refinement parameters for title compound

Chemical formula	$C_{12}H_{17}N_3O_2S$
M, g/mol	266.35
System, sp. gr., Z	Monoclinic, Pc, 2
a, b, c, Å	12.684(4), 5.5864(17), 9.323(3)
β, deg	102.439(10)
V, Å^3	645.1(3)
D_x, Mg/m^3	1.371
Radiation; λ, Å	MoK_α, 0.71073
μ, mm^{-1}	0.25
T, K	293(2)
Crystal sizes, mm	0.30 × 0.20 × 0.20
Diffractometer	Bruker AXS Kappa Apex2 CCD
Absorption corrections	Multi-scan SADABS [13]
T_{min}, T_{max}	0.872, 0.911
θ_{max}, deg	26.0
Collection range	$-15 \le h \le 15$, $-6 \le k \le 6$, $-11 \le l \le 11$
Number of reflections: measured/unique ($N1$), R_{int}/with $I > 2\sigma(I)$ ($N2$)	11 351/2514, 0.054/2099
Refinement technique	Full matrix least squares method based on F^2
Number of refined parameters	214
$R1/wR2$ based on $N1$	0.0508/0.0899
$R1/wR2$ based on $N2$	0.0360/0.0796
S	0.99
$\Delta\rho_{min}/\Delta\rho_{max}$, e/A^3	−0.22/0.16
Programs	SIR92 [14]], SHELXL2014 [15]

Spectroscopic and X-ray Studies

^{1}H and ^{13}C NMR spectra were recorded on a Bruker spectrometer (400 and 100 MHz) using tetramethylsilane as an internal reference in $CDCl_3$ (chemical shifts δ in ppm). Single crystal X-ray diffraction data were collected on a Bruker AXS Kappa Apex2 CCD diffractometer. The compound was purified by column chromatography.

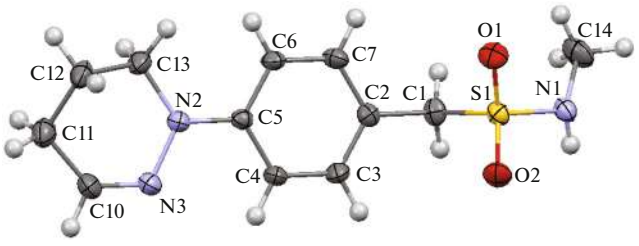


Fig. 2. Molecular structure of the title compound, displacement ellipsoids are drawn at a 30% probability level.

Crystal Structure Determination

The single crystal was carefully selected under a polarizing microscope in order to perform its structural analysis by X-ray diffraction. Crystal data, experimental parameters and structure refinement parameters are summarized in Table 1. Anisotropic thermal parameters were used for all atoms except hydrogen. The C-bonded H atoms were placed in idealized positions at the C–H distances of 0.93 and 0.97 Å for aromatic C–H and pyridazine CH_2 groups, respectively, and refined using a riding model. The crystal data are deposited with the Cambridge Crystallographic Database (deposit CCDC no. 1421874). These data are free and available at www.ccdc.cam.ac.uk.

RESULT AND DISCUSSION

^{1}H NMR Spectrum

^{1}H NMR spectrum of 1-[4-(5,6-dihydropyridazin-1(4*H*)-yl)phenyl]-*N*-methylmethanesulphonamide showed two doublets at δ = 7.15 ppm (2H, J = 5.6 Hz) and 7.27 ppm (2H, J = 5.6 Hz) for aromatic C_3, C_4 and C_6, C_7 protons, respectively. The proton of the pyridazine ring (C_{10}) appears as a doublet at δ = 6.80 (1H) ppm due to the adjacent imine bond, while the multiplets at δ = 2.23 (2H) and 2.04 (2H) ppm are assigned to protons of (C_{11}, C_{12})–CH_2–. In addition, two protons of carbon C_{13} appear as a triplet at δ = 3.58 ppm (2H, J = 4 Hz). The protons of the methyl group (C_{14}) appear as a doublet at δ = 2.68 ppm (3H, J = 3.6 Hz). The proton of the N–H group appears as a doublet at δ = 3.97 ppm (1H, J = 2.8 Hz). The benzyl protons (C_1) appear as singlet at δ = 4.19 ppm (2H).

^{13}C NMR Spectrum

^{13}C NMR spectrum of 1-[4-(5, 6-dihydropyridazin-1(4*H*)-yl) phenyl]-*N*-methyl methane sulphonamide shows 12 carbon signals. The signal of the *N*-methyl carbon atom (C_{14}) appears at δ = 18.23 ppm. The corresponding carbon atoms in the pyridazine ring (C_{10}, C_{11}, C_{12}, and C_{13}) give signals at 22.86, 29.98, 43.25, and 57.21 ppm. The signals at 113.72, 119.93, 131.30, 137.44, 148.26 ppm are assigned to the carbon atoms of the benzene ring (C_2, C_3, C_4, C_5, C_6, and C_7). The benzyl carbon atom (C_1) appears at δ = 77.22 ppm.

Description of the Structure

The isolated compound crystallizes in a monoclinic system with space group *Pc*, has cell parameters a = 12.684(4) Å, b = 5.5864(17) Å, c = 9.323(3) Å, β = 102.439(10)° and the cell volume is 645.1(3) Å^3, Z = 2. The crystal consists of pyridazine ring and phenyl sulphonamide moieties. The molecular structure of the title compound is shown in Fig. 2. The packing consists of two symmetrically related molecules of 1-[4-

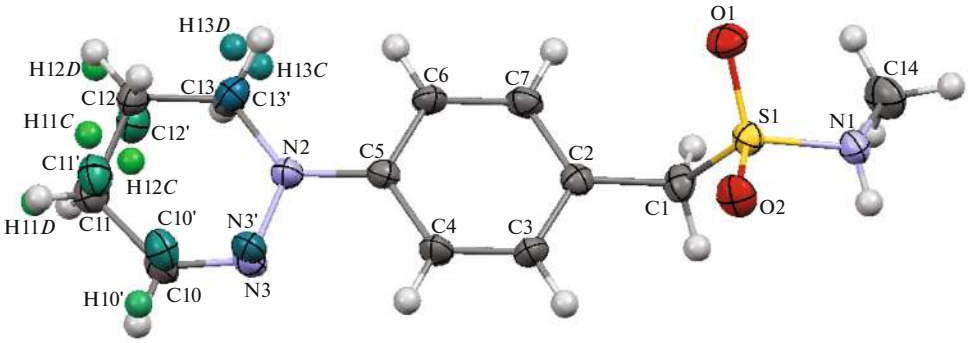


Fig. 3. Disordered spatial arrangement of the pyridazine ring.

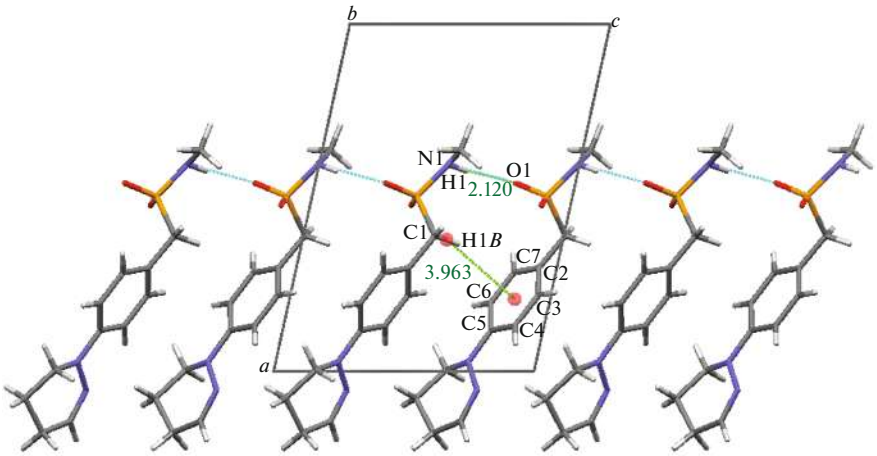


Fig. 4. 1D chain extended along the *c* axis the through N(1)–H(1)···O(1) hydrogen bonding and the C–H-π contact.

(5,6-dihydropyridazin-1(4*H*)-yl)phenyl]-*N*-methylmethanesulphonamide. The mean C–C bond lengths in the phenyl and pyridazine rings are 1.379(6) and 1.510(13) Å, respectively. The main bond lengths and angles are listed in Table 2.

The pyridazine ring has a slightly distorted envelope conformation. Part of the pyridazine ring is spatially disordered and has two positions N3, C10, C11, C12, C13 and N3', C10, C11', C12', C13' with occupancies of 0.5210 and 0.4790, respectively. The geometrical centres of the two positions coincide, and the angle between their planes is ~11° (Fig. 3).

The main planes of the molecules are connected by hydrogen bonds N(1)–H(1)···O(1) (Table 3). In the packing diagram along the *b* axis, the 1D chain is extended along the *c* axis through the N(1)–H(1)···O(1) hydrogen bond (Fig. 4). This links the molecules held together by these strong to medium interactions into a zigzag chain (Fig. 5). Neighbouring chains are weakly connected via van der Waals interactions and C1–H1*B*···π interactions involving the ben-

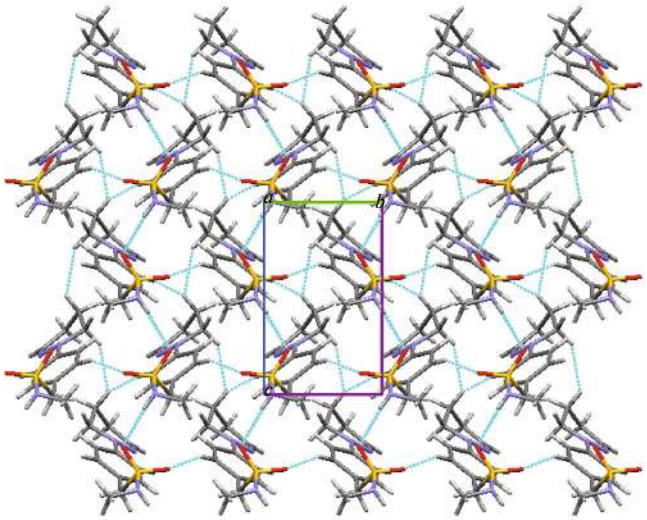


Fig. 5. Zigzag molecular packing of the title compound as viewed along the *a*-axis. Dashed lines indicate hydrogen bonds.

Table 2. Selected bond lengths *d* and angles ω of the title compound

Bond	*d*, Å	Angle	ω, deg
C(1)–S(1)	1.766(4)	C(2)–C(1)–S(1)	113.0(3)
C(5)–N(2)	1.394(5)	S(1)–C(1)–H(1*A*)	109.0
C(14)–N(1)	1.461(6)	S(1)–C(1)–H(1*B*)	109.0
N(2)–N(3')	1.32(3)	C(6)–C(5)–N(2)	121.2(4)
N(2)–N(3)	1.41(2)	C(4)–C(5)–N(2)	120.8(3)
N(2)–C(13')	1.441(16)	N(1)–C(14)–H(14*A*)	109.5
N(2)–C(13)	1.456(15)	N(1)–C(14)–H(14*B*)	109.5
N(3)–C(10)	1.267(15)	N(1)–C(14)–H(14*C*)	109.5
N(3')–C(10')	1.268(16)	N(3')–N(2)–C(5)	117.4(12)
N(1)–S(1)	1.601(4)	C(5)–N(2)–N(3)	113.8(10)
N(1)–H(1)	0.82(4)	N(3')–N(2)–C(13')	121.7(14)
O(1)–S(1)	1.432(3)	C(5)–N(2)–C(13')	120.9(8)
O(2)–S(1)	1.419(3)	C(5)–N(2)–C(13)	121.5(8)
		N(3)–N(2)–C(13)	122.0(14)
		C(10)–N(3)–N(2)	116(2)
		N(3)–C(10)–C(11)	129.4(18)
		N(3)–C(10)–H(10)	115.3
		N(2)–C(13)–C(12)	111.5(14)
		N(2)–C(13)–H(13*A*)	109.3
		N(2)–C(13)–H(13*B*)	109.3
		C(10')–N(3')–N(2)	121(3)
		N(3')–C(10')–C(11')	126(2)
		N(3')–C(10')–H(10')	116.9
		N(2)–C(13')–C(12')	110.8(15)
		N(2)–C(13')–H(13*C*)	109.5
		N(2)–C(13')–H(13*D*)	109.5
		C(14)–N(1)–S(1)	118.8(4)
		C(14)–N(1)–H(1)	113(3)
		S(1)–N(1)–H(1)	109(3)
		O(2)–S(1)–O(1)	118.3(2)
		O(2)–S(1)–N(1)	106.8(2)
		O(1)–S(1)–N(1)	107.5(2)
		O(2)–S(1)–C(1)	109.0(2)
		O(1)–S(1)–C(1)	107.7(2)
		N(1)–S(1)–C(1)	107.0(2)

Table 3. Hydrogen bond geometry

D–H···*A*, Å	*d*(*D*–H), Å	*d*(H···*A*), Å	*d*(*D*···*A*), Å	∠(*D*H*A*), deg
N(1)–H(1)···O(1)$^{(i)}$	0.82(4)	2.12(5)	2.936(5)	172(4)
C(7)–H(7)···O(2)$^{(ii)}$	0.93	2.59	3.457	155

Symmetry code: (i) $x, -y, z + 1/2$; (ii) $x, -y, z$.

zene (C_2, C_3, C_4, C_5, C_6, and C_7) ring system with a distance 3.9 Å between the planes, as well as through weak interaction between pyridazine and the benzene rings. Thus, the crystal structure is built up by a combination of hydrogen bonds and C–H···π interactions.

CONCLUSIONS

1-[4-(5,6-Dihydropyridazin-1(4*H*)-yl)phenyl]-*N*-methylmethanesulphonamide was synthesized. The atomic structure was determined by ^{1}H and ^{13}C NMR spectroscopy, confirmed by X-ray diffraction. The crystal structure was found to be zigzag chains of molecules built by a combination of hydrogen bonds and C–H···π interactions.

ACKNOWLEDGMENTS

S. Ramesh thanks Mr. K Sivamuthuraman, Department of Biotechnology, IIT Madras, for the support of single crystal X-ray diffraction analysis.

REFERENCES

1. The Sumatriptan Cluster Headache Study Group, N. Engl., J. Med. **325**, 322 (1991).
2. J. L. Brandes, D. Kudrow, S. R Stark, et al., JAMA **297**, 1443 (2007).
3. G. R. Humphrey and J. T. Kuethe, Chem. Rev. **106**, 2875 (2006).
4. S. Cacchi and G. Fabrizi, Chem. Rev. **105**, 2873 (2005).
5. G. W. Gribble, Org. Synth. **1**, 145 (1994).
6. G. W. Gribble, J. Chem. Soc. Perkin Trans. **1**, 1045 (2000).
7. E. Fischer and F. Jourdan, Ber. Dtsch. Chem. Ges. **16**, 2241 (1883).
8. E. Fischer and O. Hess, Ber. Dtsch. Chem. Ges. **17**, 559 (1884).
9. B. Robinson, Chem. Rev. **63**, 373 (1963).
10. B. Robinson, Chem. Rev. **69**, 227 (1969).
11. B. Robinson, *Fischer Indole Synthesis* (Wiley-Inter Science, New York, 1982).
12. S. Ramesh, G. Shanmugam, and D. Saravanan, Int. J. Adv. Sci. Eng. Technol. **5**, 63 (2017).
13. Bruker, *SADABS* (Bruker AXS, Madison, Wisconsin, USA, 1999).
14. A. Altomare, G. Cascarano, C. Giacovazzo, and A. Guagliardi, J. Appl. Crystallogr. **26**, 343 (1993).
15. G. M. Sheldrick, *SHELXL97. Program for Crystal Structure Refinement* (University of Göttingen, Göttingen, Germany 1997).

Current Research in Green and Sustainable Chemistry 5 (2022) 100246

Contents lists available at ScienceDirect

Current Research in Green and Sustainable Chemistry

journal homepage: www.elsevier.com/journals/current-research-in-green-and-sustainable-chemistry/2666-0865

Bi-functionalized Ionic Liquid-Thiourea Chitosan for effective decontamination of Cd(II) and Hg(II) from water bodies

Anita Shekhawat [a], Shashikant Kahu [a], D. Saravanan [b], Ravin Jugade [a,*]

[a] Department of Chemistry, R.T.M. Nagpur University, Nagpur, 440033, India
[b] Department of Chemistry, National College, Tiruchirappalli, 620001, India

ARTICLE INFO

Keywords:
Chitosan
Adsorption
Water treatment
Cadmium
Mercury

ABSTRACT

Chitosan biopolymer was converted to thiourea-chitosan followed by impregnation with ionic liquid Aliquat-336 using microwave irradiation method. The obtained material Ionic Liquid-Thiourea Chitosan (ILTC) was effectively used for decontamination of Cd(II) and Hg(II) from water bodies. FT-IR, SEM-EDX, XRD and TGA-DTA gives insight of ILTC adsorbent. Choro-complexes of Cd(II) and Hg(II) showed strong adsorption on ILTC as compared native Cd(II) and Hg(II) ions hence the adsorption was carried out in the form of chloro complexes. The adsorption behavior of Cd (II) and Hg (II) ions onto ILTC was investigated through batch method and various batch parameters viz. Contact time, adsorbent dose, initial concentration of adsorbate, pH and point of zero charge were optimized. The maximum adsorption capacities for Cd (II) and Hg (II) at 298 K were found to be 442.5 mg/g and 203.2 mg/g respectively at pH 3.0. Kinetic and thermodynamic parameters of the adsorption process were obtained. The results indicated that adsorption process was spontaneous, exothermic and followed pseudo-second-order kinetics model. Column studies were carried out in order to access the applicability of the material for bulk samples at larger volumes. ILTC could be easily regenerated and efficiently reused.

1. Introduction

Cd (II) and Hg (II) are the heavy metals that exhibit the greatest potential hazard to human health and various ecosystems [1]. The challenge for the removal of these heavy metals from wastewater is that they are usually in low concentrations and exist in complex mixtures. There are a number of variables like solution composition, the pH, organic matter on which removal of heavy metals depends. For a few decades, different methodologies have been employed for the water treatment. The most common techniques are precipitation, solvent extraction, ion-exchange, filtration, electrochemical techniques, adsorption etc. [2] Among these methodologies, the adsorption process offers flexibility in design and operation and in many cases will produce high-quality treated effluent [3].

Chitosan a well-known biosorbent and its properties like nontoxic, biocompatible and biodegradable nature make it acceptable in water treatment applications as compared to inorganic adsorbents [4]. The chemical modifications of chitosan have been performed under several strategies aiming to improve its properties and to expand its range of applications. The amino and hydroxyl groups of chitosan are generally targeted for the modifications. Chemical functionalization of chitosan can be done by microwave heating to accelerate chemical reactions, to increase reaction yield, to enhance product purity and material properties as compared to conventional experiments in which heating by convection or conduction is used. Preparation of amphiphilic derivatives of chitosan using microwave irradiation has been investigated [5] and it was found that it is possible to obtain modified chitosan at lower reaction time without any degradation of the polymer chain. The synthesis of chitosan polyacrylamide by using microwave irradiation leading to higher reaction yield was achieved in rather shorter time as compared to the reaction carried out under conventional heating [6]. The study of the microwave-assisted synthesis of quaternized chitosan has also been reported [7]. Thiourea compounds are very efficient at chelating noble metals and are widely used for extracting metals. In order to introduce thiourea group into chitosan main chain, thiourea grafting chitosan using glutaraldehyde as cross-linking agent was prepared and applied to platinum recovery [8]. Adsorption of mercury from synthetic wastewater by thiourea modified chitosan derivative has also been reported [9]. Porous hydrogels based on chitosan functionalized with thiourea groups (CHITU) were reported for the sorption of metal ions [10]. We have reported dual modification of chitosan using crosslinking and impregnation [11,12]. By considering the advantages in improving the adsorption

* Corresponding author.
E-mail address: ravinj2001@yahoo.co.in (R. Jugade).

https://doi.org/10.1016/j.crgsc.2021.100246
Received 27 May 2021; Received in revised form 26 November 2021; Accepted 22 December 2021
Available online 28 December 2021
2666-0865/© 2021 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

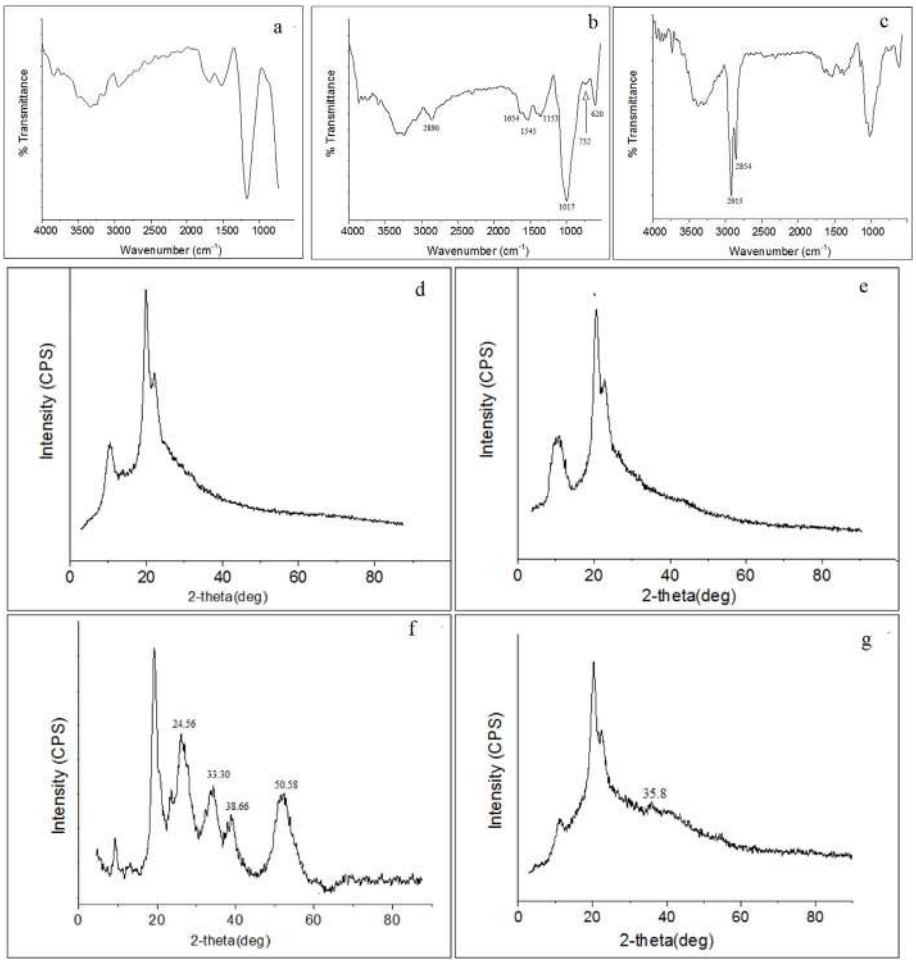


Fig. 1. FT-IR spectra of (a) chitosan (b) TUCH and (c) ILTC; XRD spectra of (d) chitosan (e) ILTC (f) ILTC with adsorbed Cd(II) and (g) ILTC with adsorbed Hg(II).

efficiency of the material on modification and functionalization, taking advantage of microwave assisted synthesis, this work involves preparation of thiourea chitosan and impregnation of Aliquat-336 for high adsorption capacity towards Cd(II) and Hg(II) from water bodies.

2. Materials and methods

2.1. Materials

Analytical grade chemicals and reagents were used throughout the experimental procedures. The aqueous solutions were prepared in double distilled water. Aliquat-336 (Trioctylmethylammonium chloride) was purchased from Sigma-Aldrich. Uniloid Bio-Chemicals India Limited, Hyderabad supplied chitosan having 85% of degree of deacetylation with molecular weight 120 kDa. All the reagents were used without further purification.

2.2. Synthesis of ILTC

Thiourea chitosan was prepared as reported earlier [13] with improvement of microwave irradiation which accelerates the chemical reaction. About 3 g chitosan powder, 2 g ammonium thiocyanate and 50 ml of ethanol were taken in a conical flask and subjected to microwave irradiation for 10 min with intermittent time of 2 min. After cooling to room temperature, the precipitate was collected, repeatedly washed with distilled water and finally air dried to get thiourea chitosan (TUCH).

2.5 g of TUCH prepared in above step was taken in a conical flask followed by the addition of 30 mL of 0.05 M Aliquat-336 in dichloromethane and subjected to irradiation in domestic microwave oven (LG, India model MS2049) at 2450 MHz. The reaction mixture was irradiated for 5 min in small instalments of 30 s, followed by cooling and irradiation. The resulting residue (ILTC) was filtered, washed with double distilled water, dried at room temperature and used for further adsorption studies. Preparation scheme has been shown in supplementary Fig. 1.

2.3. Preparation of metal-chloro complexes

Since Cd(II) and Hg(II) cations have low adsorption tendency on chitosan-based adsorbents, they were adsorbed in the form of their chloro complexes [14]. This was achieved by mixing Metal (II) chloride and sodium chloride solutions in molar ratios of 1:2 leading to formation of $[MCl_4]^{2-}$ type complexes of both Cd(II) and Hg(II). The above solutions were used for adsorption experiments with subsequent dilutions by maintaining the pH 3.0.

2.4. Characterization of ILTC

Fourier Transform-Infra Red (FT-IR) spectrum of the ILTC was recorded using Bruker *Alpha* spectrometer (Bruker India Scientific Pvt Ltd) with ZnSe ATR crystal in the wavelength range 500–4000 cm^{-1}. For FT-IR analysis, the powdered sample was directly placed on analyzing surface and spectrum was recorded averaging 23 scans. Powder X-ray diffraction (XRD) analysis of samples was carried out on Rigaku-Miniflex 300 X-ray diffractometer (Rigaku, Japan) system using Cu K_α radiation in the 2θ range of 1 to 100°. Thermogravimetric (TG) and Differential

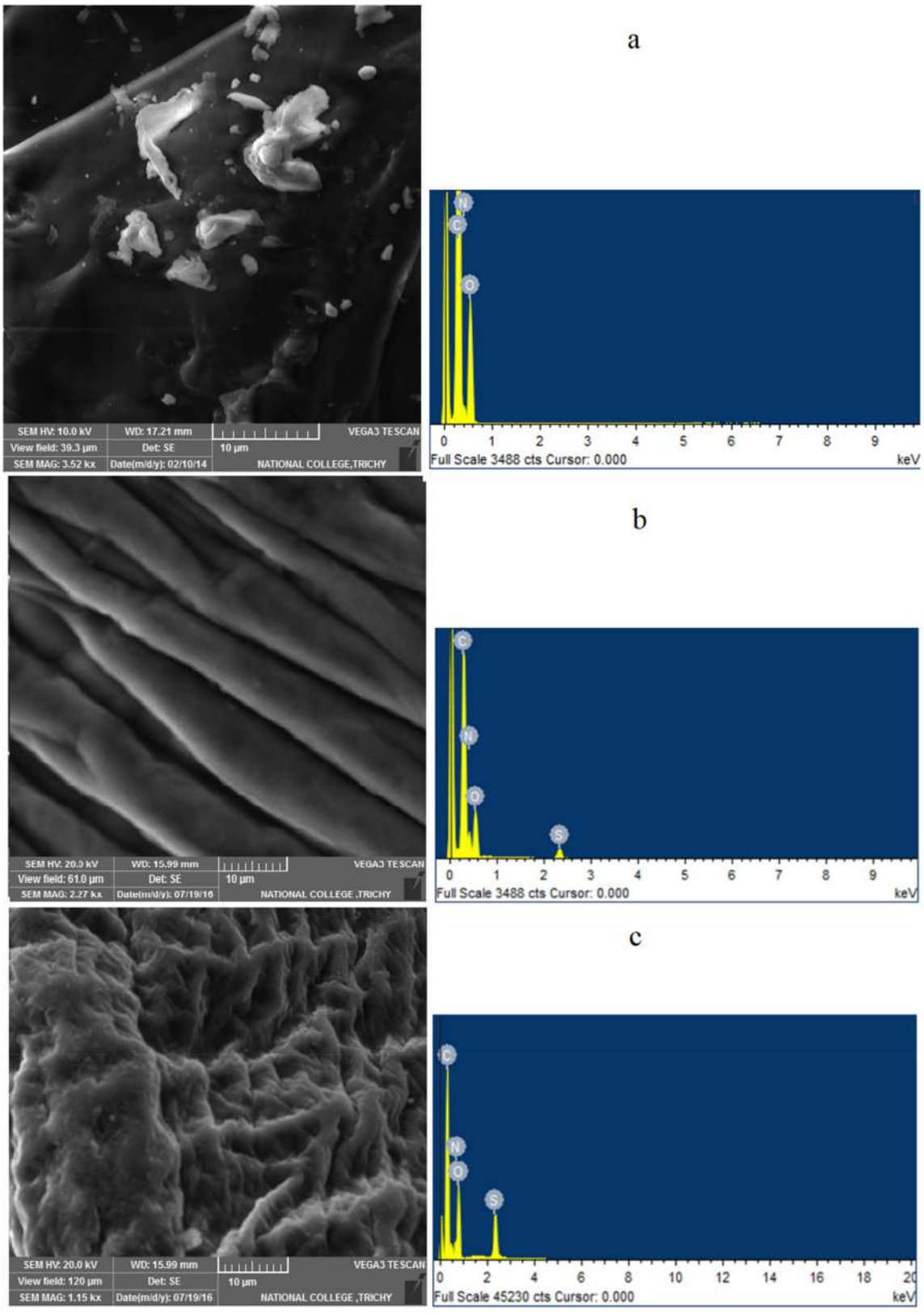


Fig. 2. SEM micrographs and EDX spectra of pattern of (a) chitosan (b) TUCH, (c) ILTC.

thermal analysis (DTA) were recorded on HITACHI TG-DTA 7200 instrument in air medium at a temperature scan rate of 20 °C min^{-1}. The temperature was raised from ambient to 800 °C. Surface morphology of adsorbent prior and consequent to the adsorption was studied using Scanning Electron Microscopy (SEM) model TESCAN VEGA 3 SBH manufactured by TESCAN, Czech Republic. Energy dispersive X-ray (EDX) spectra were recorded on X-ray analyzer Oxford INCA Energy 250 EDS System during SEM observations. BET analysis of the material was estimated by nitrogen adsorption–desorption method on Single Point Surface Area Analyzer Model Smart sorb 92/93.

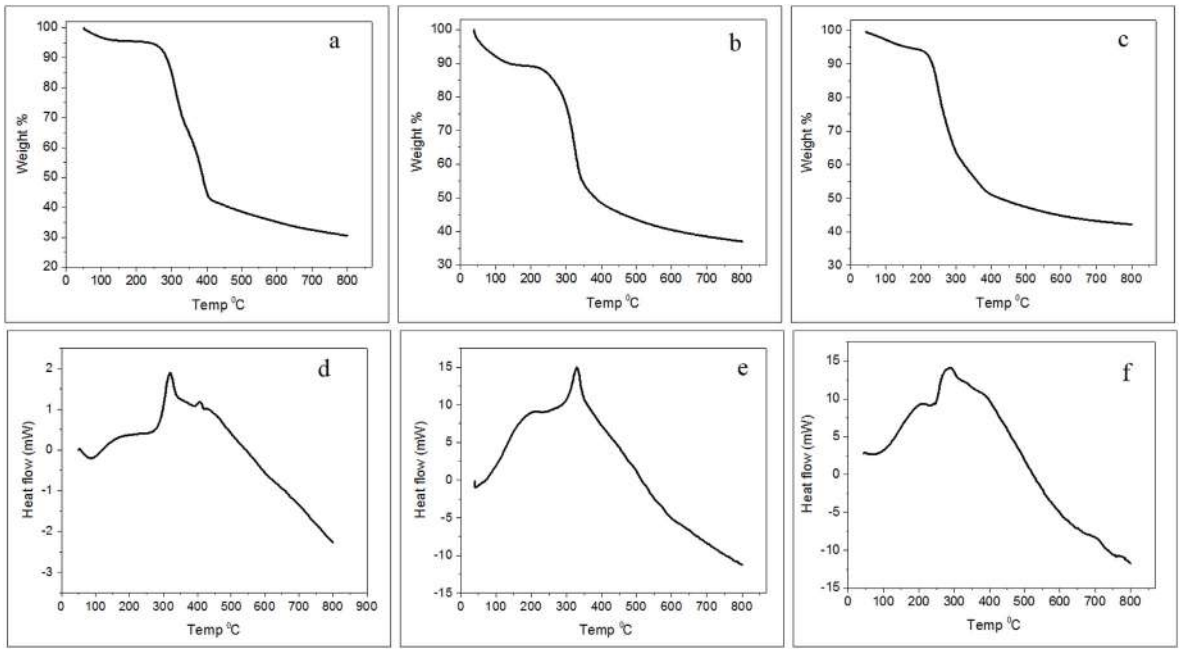


Fig. 3. TGA curves of (a) chitosan (b)TUCH (c) ILTC and DTA curves of (d) chitosan (e) TUCH and (f) ILTC.

2.5. Analysis of Cd(II) and Hg(II)

The concentration of cadmium was determined using ICP-AES. For analysis of cadmium the solutions were diluted with 5% HNO_3. The solution phase concentration of Hg(II) was estimated by the standard Cold vapour-atomic absorption spectrophotometric (CV-AAS) technique. The mercury vapours were analyzed by Mercury Analyzer Model MA-5840 supplied by Electronics Corporation of India Ltd. Hyderabad, India.

2.6. Adsorption experiments

Absorption studies were performed by using varying concentrations of Cd(II) from 100 mg/L to 1000 mg/L and Hg(II) from 50 mg/L to 1000 mg/L. The required pH was maintained and the solutions were stirred with optimized adsorbent dose in a stoppered conical flask for 60 min at 298 K. The amount of metal ions adsorbed (mg/g) on ILTC at equilibrium (q_e) can be given by

$$q_e = \frac{C_0 - C_e}{W} \times V \tag{1}$$

where C_0 and C_e specify the initial and equilibrium liquid phase concentrations in mg/L of metal ions, V is the volume of metal ions solution in liters and W is the weight of adsorbent in gram used for adsorption process.

2.6.1. Adsorption isotherms

In order to understand the association between the adsorbent and the targeted metal ions, Langmuir, Freundlich, and Temkin isotherm models [15] were studied.

2.6.2. Langmuir isotherm model

This isotherm model represents monolayer adsorption on uniform surface [16]. Linearized Langmuir equation is given by

$$C_e/q_e = {}^1/_{q_m b} + C_e/q_0 \tag{2}$$

C_e (mg/L) is the equilibrium heavy metal concentration, q_e(mg/g) is the equilibrium adsorption capacity, b is the Langmuir isotherm constant which related to the strength of adsorption, q_m(mg/g) is the maximum adsorption capacity. The values of q_m and b were obtained from the slope and intercept of the plot of C_e/q_e against C_e. Effectiveness of adsorption process can be evaluated in terms of separation factor R_L given by the following equation

$$R_L = \frac{1}{1 + bC_0} \tag{3}$$

$R_L < 1$ proves effective adsorbent-adsorbate interaction.

2.6.3. Freundlich isotherm

The model represents assorted multifaceted adsorption [17]. A linearized Freundlich isotherm correlating adsorption capacity (k_F) and adsorption intensity (n) in following equation

$$\log q_e = \log K_F + \frac{1}{n} \log C_e \tag{4}$$

Higher value of n signifies effective adsorbate-adsorbent interaction.

2.6.4. Temkin isotherm

Temkin isotherm assumes that adsorption declines linearly with increasing adsorbent coverage [18]. A linearized Temkin isotherm equation is given as

$$q_e = \frac{RT}{b} \ln A + \frac{RT}{b} \ln C_e \tag{6}$$

where A is Temkin isotherm constant and b is the binding energy.

2.6.5. Adsorption kinetics

Kinetics of adsorption of heavy metals on to ILTC was studied in order to evaluate the time dependency that controls the adsorption process. Pseudo-first-order and pseudo-second-order kinetic models were studied to correlate the kinetic data with the adsorption mechanism [19,20]. The studies were carried out using 50 mL of 100 mg/L of Cd(II) and 50 mg/L of Hg(II) solutions at pH 3.0. It was equilibrated with 200 mg of ILTC adsorbent for different time intervals of 5–60 min.

The pseudo-first-order kinetics is given by the equation

$$\log(q_e - q_t) = \log q_e - \frac{k_1 t}{2.303} \tag{7}$$

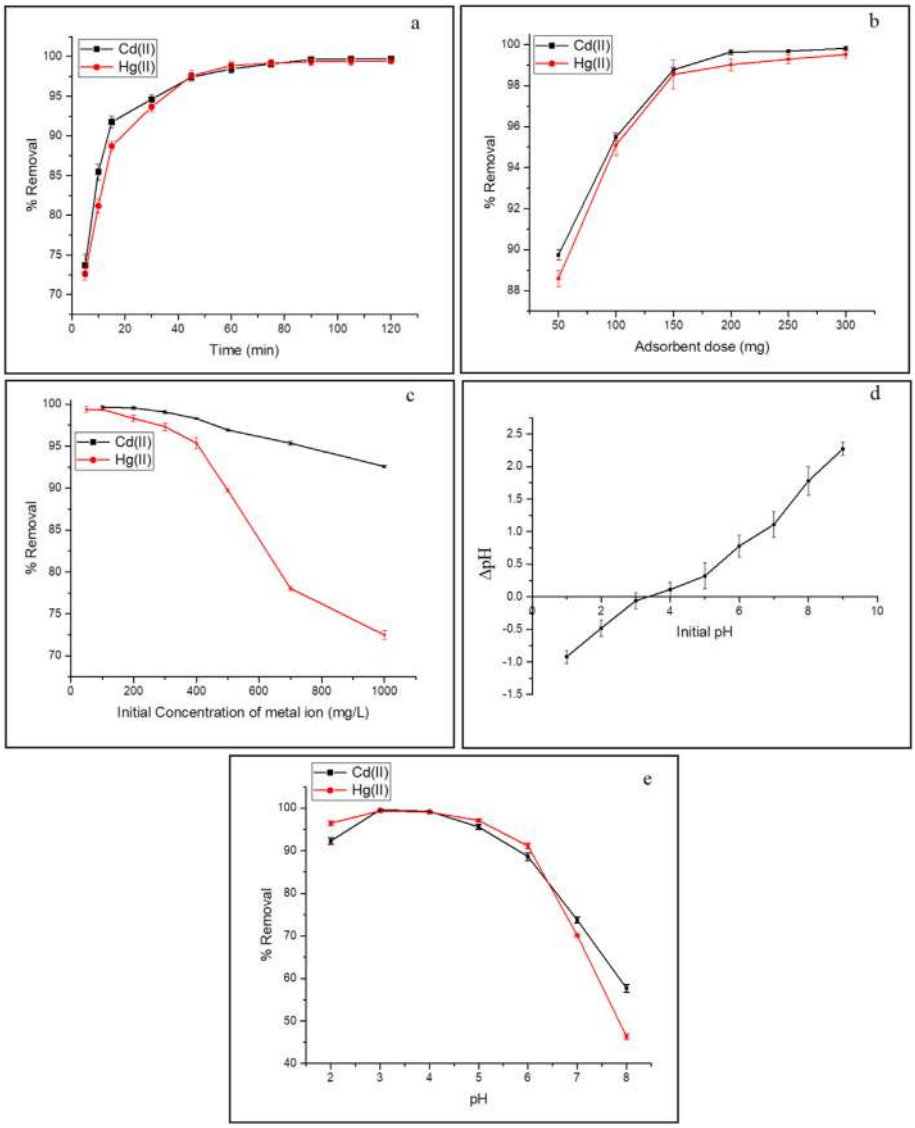


Fig. 4. Effect of (a) Contact time (dose 200 mg, conc. 100 mg/L, pH 3.0), (b) Adsorbent dose (contact time 60 min, conc. 100 mg/L, pH 3.0), (c) Initial concentration (dose 200 mg, contact time 60 min, pH 3.0) (d) pH point of zero charge and (e) pH on adsorption efficiency of Cd(II) and Hg(II) (dose 200 mg, conc. 100 mg/L, contact time 60 min).

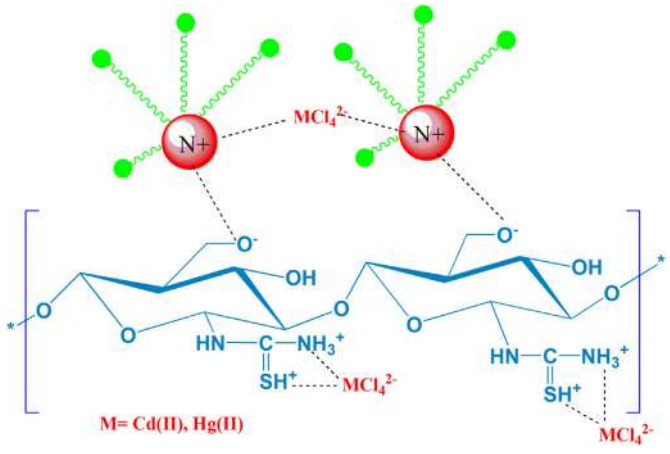


Fig. 5. Adsorption mechanism for heavy metals on ILTC.

The pseudo-second-order equation is given as

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \tag{8}$$

Weber Morris intraparticle diffusion equation is expresses as

$$q_t = k_{int}.t^{½} + C \tag{9}$$

The linear plot of q_t versus $t^{1/2}$ gives the intraparticle rate constant k_{int} through its slope.

2.7. Column studies

For column adsorption studies, a glass column (30 cm length, 1 cm inner diameter) was packed with glass wool at the bottom. 1 g of ILTC powder was loosely filled in column which attained height of 6–7 cm. Metal solutions were maintained at pH 3.0 and passed through the column at a flow rate of 5 mL/min. The eluent was collected after every 10 min interval and it was analyzed for metals content. The various column parameters were calculated using following formulas.

$$\text{Breakthrough Capacity}(\text{mg/g}) = \frac{\text{Breakthrough Volume}(\text{L}) \times \text{Inlet concentration}(\text{mg/L})}{\text{Wt. of adsorbent (g)}} \quad (10)$$

$$\text{Exhaussion Capacity} = \frac{\text{Exhaustion Volume}(\text{L}) \times \text{Inlet concentration}(\text{mg/L})}{\text{Wt. of adsorbent (g)}} \quad (11)$$

$$\text{Degree of column utilization (\%)} = \frac{\text{Breakthrough Volume}}{\text{Exhaustion Volume}} \times 100 \quad (12)$$

2.8. Preparation of synthetic effluents

In order to confirm the applicability of ILTC for removal of cadmium and mercury, 1 L stock solution of two different synthetic effluents containing Sulphate (1155 mg/L), Chloride (626 mg/L), Nitrate (200 mg/L), Manganese(II) (200 mg/L), Iron(II) (200 mg/L), Zinc(II) (200 mg/L), Cadmium(II) (200 mg/L) in first while Sulphate (577 mg/L), Chloride (235 mg/L), Nitrate (100 mg/L), Manganese(II) (100 mg/L), Iron(II) (100 mg/L), Zinc(II) (100 mg/L), Mercury(II) (100 mg/L) in second were prepared having similar composition to that of the real effluents.

3. Results and discussion

3.1. Characterization of ILTC

FT-IR spectra of chitosan, TUCH and ILTC were recorded in their powdered form using 23 scans in the range of 500–4000 cm^{-1}. FT-IR spectra of chitosan (Fig. 1a) revealed characteristic O–H and N–H stretching broad peak in the region 3300 cm^{-1} and 3500 cm^{-1} respectively. The N–H bending vibration in chitosan was observed around 1545 cm^{-1}, the C–H and the C–O stretching bands were obtained around 2890 cm^{-1} and 1017 cm^{-1} respectively. Thiourea group of TUCH (Fig. 1b) shows characteristic peaks at 1654 cm^{-1}, 732 cm^{-1} and 620 cm^{-1} [13]. A peak of N–C═S group in TUCH can be seen at 1153 cm^{-1}. In case of ILTC (Fig. 1c), two new peaks at 2915 and 2854 cm^{-1} have been assigned to $-CH_2$ groups of aliphatic chains confirming the impregnation of Aliquat-336 on TUCH [11].

The powder X-ray diffraction patterns of TUCH, ILTC, ILTC with adsorbed Cd(II) and Hg(II) (Fig. 1) were recorded to understand the

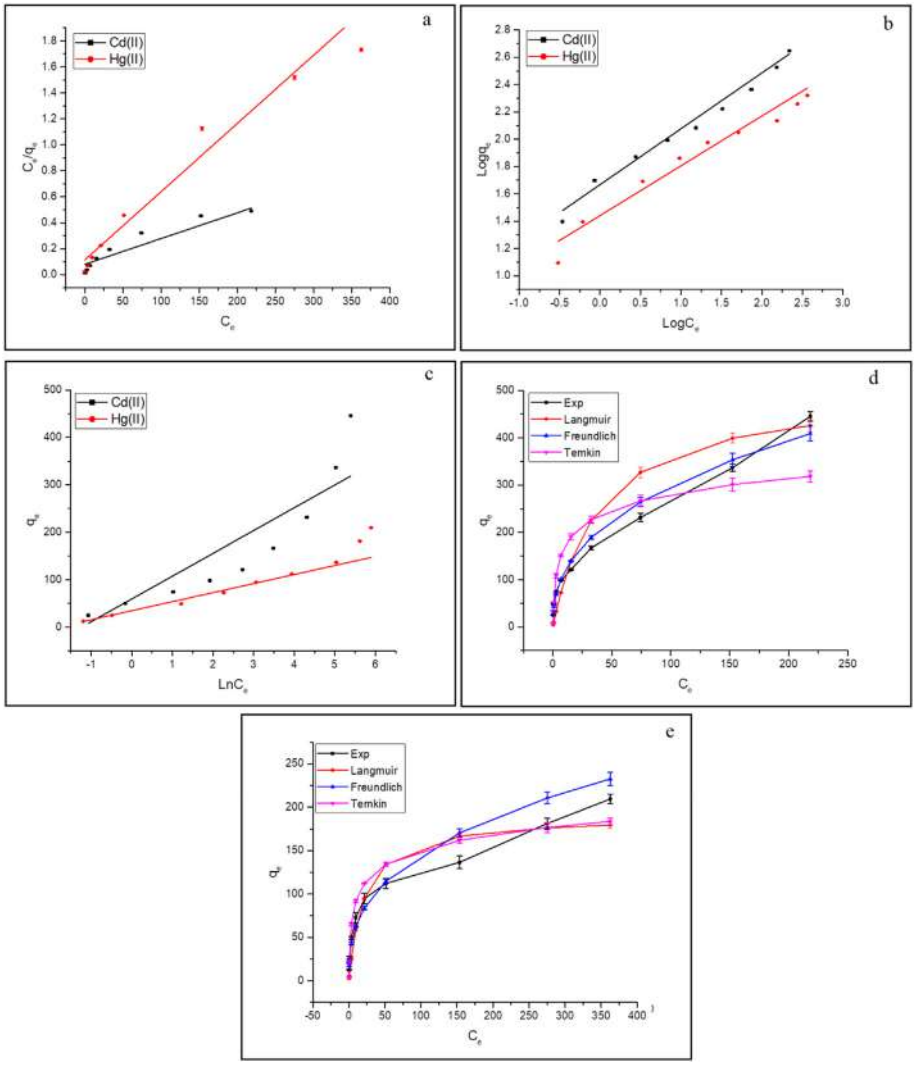


Fig. 6. (a) Langmuir isotherms, (b)Freundlich isotherm, (c) Temkin isotherm, (d) comparison of experimental q_e with other isotherms for Cd(II) and (e) for Hg(II).

feasibility of adsorption. Characteristic peaks of TUCH were observed at $2\theta = 10.62^{\circ}$, 19.93° and 22.25°. ILTC shows marginal shifting of peaks to $2\theta = 10.96^{\circ}$, 20.20° and 22.45° probably due to interaction of ionic solid with TUCH causing decrease in crystalinity of the adsorbent [21]. After adsorption of Cd(II) and Hg(II) on ILTC, additional peaks were observed at $2\theta = 24.56^{\circ}$, 33.30°, 38.66° and 50.58° which belong to Cd(II) [22] and peak at $2\theta = 35.8^{0}$ assigned to Hg(II).

SEM micrographs reveals that surface of chitosan had smooth morphology (Fig. 2a). On treatment with thiourea, the surface gets linear folding (Fig. 2b) while further impregnation with aliquat-336 it leads to random folded patter. This can be attributed to the enhanced ionic interactions leading to surface folding of the material. The EDX spectrum of native chitosan showed peaks due to C, N and O as expected. In the EDX spectrum of TUCH, the peaks for elements such as carbon (K_{α} – 0.277 keV), oxygen (K_{α} – 0.525 keV), nitrogen (K_{α} – 0.392 keV) and sulphur (K_{α} – 2.307 keV) could be seen (Fig. 2a). After introducing Aliquat-336, intensity of carbon and nitrogen peaks were increased this confirms the impregnation (Fig. 2b).

The TGA curve (Fig. 3) of TUCH showed initial degradation between 60 to 200 °C with weight loss of 14% which corresponds to dehydration. The second degradation temperature of ILTC is lower than that of TUCH indicating that ILTC is less thermostable than TUCH. This shift in the degradation temperature could be result of decomposition due to thermo-labile bonds or functional groups in ILTC [23]. The same was observed in DTA curve of ILTC as there is shifting of exothermic peak.

BET surface area and pore volume of chitosan and ILTC were measured by nitrogen adsorption-desorption method at 77 K it is observed that the surface area (3.5 m^2/g) and pore volume (6.0×10^{-3} cm^3/g) of chitosan are higher than that of ILTC. The decrease in surface area (2.2 m^2/g) and pore volume (3.8×10^{-3} cm^3/g) of ILTC is attributed to incorporation of thiourea functional group and impregnation of Aliquat-336 on chitosan which is obstacle in the diffusion of N_2 gas. However, surface area is not the only criteria governing adsorption capacity. Stronger adsorbate-adsorbent interaction is the more important criteria that leads to enhancement of adsorption capacity even if surface area is reduced [24].

3.2. Batch adsorption studies

Batch adsorption studies were carried out using 50 mL of Cd(II) and Hg(II) solutions with varying concentrations at pH 3.0 ± 0.1. The solutions were equilibrated with 200 mg of ILTC in stoppered conical flasks and stirred with magnetic stirrer at 298 K for 60 min. All the experiments were repeated in triplicate and mean ± SD have been shown in all the figures and tables.

3.2.1. Effect of contact time

The effect of contact time on the adsorption of heavy metals was studied by varying the contact time in the range 5–120 min with initial concentration of 100 mg/L Cd(II) and 50 mg/L Hg(II) (Fig. 4a) at 298 K respectively. It was observed that the rate of adsorption of these metals is higher at the beginning and leads to saturation with time. Adsorption equilibrium was attained in about 60 min. Therefore, 60 min adsorption period was selected for further studies.

3.2.2. Effect of adsorbent dosage

The effect of the ILTC dose was studied by varying its amount from 50 to 300 mg at room temperature (298 K), the initial concentration, pH and contact time for heavy metals were kept constant. It was observed that, with increase in adsorbent amount, the percentage adsorption goes on increasing (Fig. 4b). This can be attributed to availability of surface and adsorption sites for the adsorbate. More than 98% adsorption was observed for both Cd(II) and Hg(II) with an adsorbent dose of 200 mg with negligible increase above this dose. Hence, the adsorbent dose of 200 mg was fixed in all the further studies.

Table 1
Adsorption isotherms and kinetic parameters.

Sr. No.	Model	Parameters	Cd(II)	Hg(II)
1.	Langmuir isotherm	q_0 (mg/g)	442.5 ± 1.1	203.2 ± 3.6
		b(L/mg)	0.0349 ± 0.0007	0.0449 ± 0.0001
		R_L	0.223 ± 0.003	0.1821 ± 0.0004
		r^2	0.899	0.962
2.	Freundlich isotherm	K_F ($mg^{1-1/n}/g/L$)	44.18 ± 0.89	26.77 ± 0.12
		n	2.467 ± 0.040	2.802 ± 0.009
		r^2	0.982	0.977
3.	Temkin isotherm	B	56.800 ± 1.713	25.408 ± 0.307
		A (L/mg)	1.662 ± 0.179	2.960 ± 0.121
		b (J/mol)	43.619 ± 1.351	97.511 ± 1.196
		r^2	0.803	0.925
4	Pseudo-first-order kinetics	K_1 (1/min)	0.073 ± 0.010	0.078 ± 0.013
		r^2	0.845	0.972
5	Pseudo-second order kinetics	k_2 (g/mg/min)	0.022 ± 0.001	0.019 ± 0.001
		r^2	0.999	0.999
6	Intraparticle diffusion	k_{int} ($mg/g/min^{1/2}$)	0.592 ± 0.016	0.676 ± 0.013
		r^2	0.709	0.762

3.2.3. Effect of initial metal concentration

Adsorption experiments were performed at different initial concentrations ranging from 100 to 1000 mg/L for Cd(II) and 50–1000 mg/L for Hg(II) (Fig. 4c). At 100 mg/L concentration of Cd(II) and 50 mg/L concentration of Hg(II), the percentage removal was maximum and then it started decreasing due to sorbent saturation. Thus 100 mg/L of Cd(II) solution and 50 mg/L for Hg(II) solution were used for further adsorption studies.

3.2.4. Effect of pH

pH of the metal ion solution affects the overall adsorption process as it strongly affects the adsorption capacity, degree of ionization, the speciation of adsorbate and surface charge of the adsorbent. pH point of zero charge (pH_{pzc}) of the material was determined by taking different initial pH of 25 ml solutions from 1.0 to 9.0 [25]. To each solution, 100 mg of adsorbent was added and stirred for 24 h. The final pH of each system was determined. A graph was plotted between change in pH as a function of initial pH. The point of intersection with X-axis gives the pH_{pzc}. Point of zero charge of ILTC was found to be 3.3 (Fig. 4d) indicating that the material surface is positively charged below pH 3.3 and negatively charged above this pH. The maximum adsorption capacity was found at pH 3.0 ± 0.1 (Fig. 4e).

This may be attributed to the protonation of nitrogen and sulphur atoms of ILTC suitable for electrostatic interaction with the chloro complex of cadmium and mercury. Positive surface charge of ILTC also favours the adsorption of cadmium and mercury in the form of anionic complexes at pH 3.0 [26]. The mechanism can be represented as shown in Fig. 5.

3.3. Adsorption isotherms

Langmuir, Freundlich and Temkin isotherm were studied to have insight of adsorption mechanism (Fig. 6). According to Langmuir isotherm, maximum adsorption capacity (q_m) of 442.5 mg/g and 203.2 mg/g were found for Cd(II) and Hg(II) respectively. R_L values were found to be more than zero and less than one (Table 1) which indicates that both metal ions get effortlessly adsorbed on ILTC. In the Freundlich

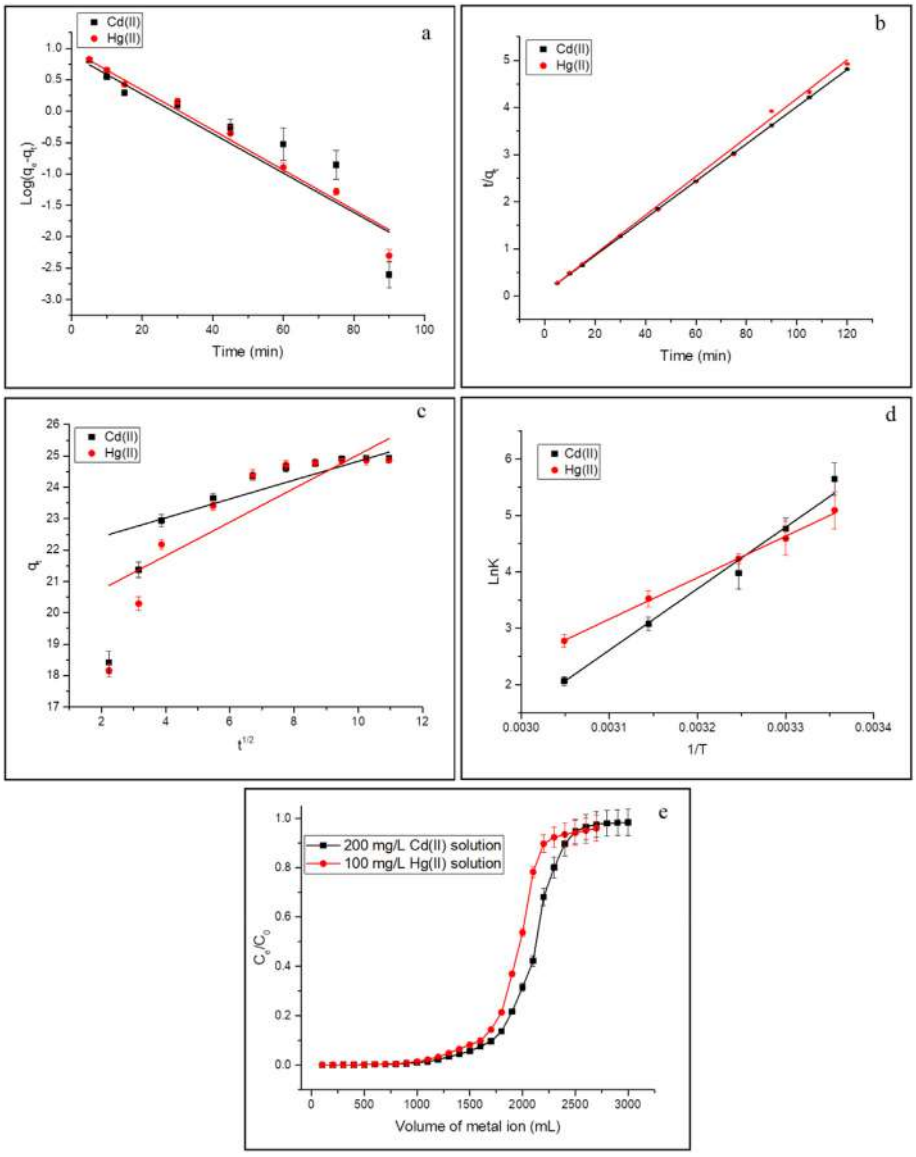


Fig. 7. Kinetic study, Weber Morris plot, van't Hoff plot and Breakthrough Curve for Cd(II) and Hg(II).

isotherm, lower values of 1/n, 0.405 and 0.357 for Cd(II) and Hg(II) respectively indicates an effective interaction between adsorbate and adsorbent. Lower values of binding energy (b) obtained in Temkin isotherm indicate that there is an electrostatic interaction between metal ions and ILTC [27].

It was observed that the value of r^2 is close to 1 in Freundlich isotherm model indicating that it is the best fit model. Also, it can be seen in Fig. 6d–e that the experimental values are close to those predicted by Freundlich model. This model indicates multilayer adsorption on heterogeneous surface of adsorbent. The positive values of b in Temkin model indicate exothermic nature of adsorption process (Fig. 6) which also follows the observations obtained in the thermodynamic studies.

3.4. Adsorption kinetics

It is clear from the kinetics data Table 1 that for adsorption of Cd(II) and Hg(II) ions on ILTC, pseudo second order kinetics is the best fit model as can be seen from r^2 values close to 1. The non-zero intercept of Weber Morris model shows that adsorption process consists of both diffusion and boundary layer phenomena for the adsorption of Cd(II) and Hg(II) (Fig. 6).

3.5. Thermodynamics of adsorption

Effect of temperature on adsorption of heavy metals by ILTC was studied to obtain relevant thermodynamic parameters at 298 K, 308 K, 318K and 328 K. The free energy change of adsorption (ΔG^0) is given by

$$\Delta G^0 = -RT\ln K \tag{14}$$

van't Hoff equation which relates entropy (ΔS^0) and enthalpy (ΔH^0) changes is given by

$$\ln K = \frac{\Delta S^0}{R} - \frac{\Delta H^0}{RT} \tag{15}$$

where R is the gas constant (8.314 J mol/K). The value of equilibrium constant K has been evaluated from the ratio of concentration of heavy metals adsorbed to that in the solution phase. The values ΔH and ΔS were obtained from slope and intercept of the plot of lnK against 1/T respectively (Fig. 7).

The negative values of ΔH (−49.23 kJ/mol for Cd(II) and −60.60 kJ/mol for Hg(II)) indicates the exothermic nature of adsorption process while negative values of ΔS (−0.14 kJ/mol/K for Cd(II) and −0.16 kJ/

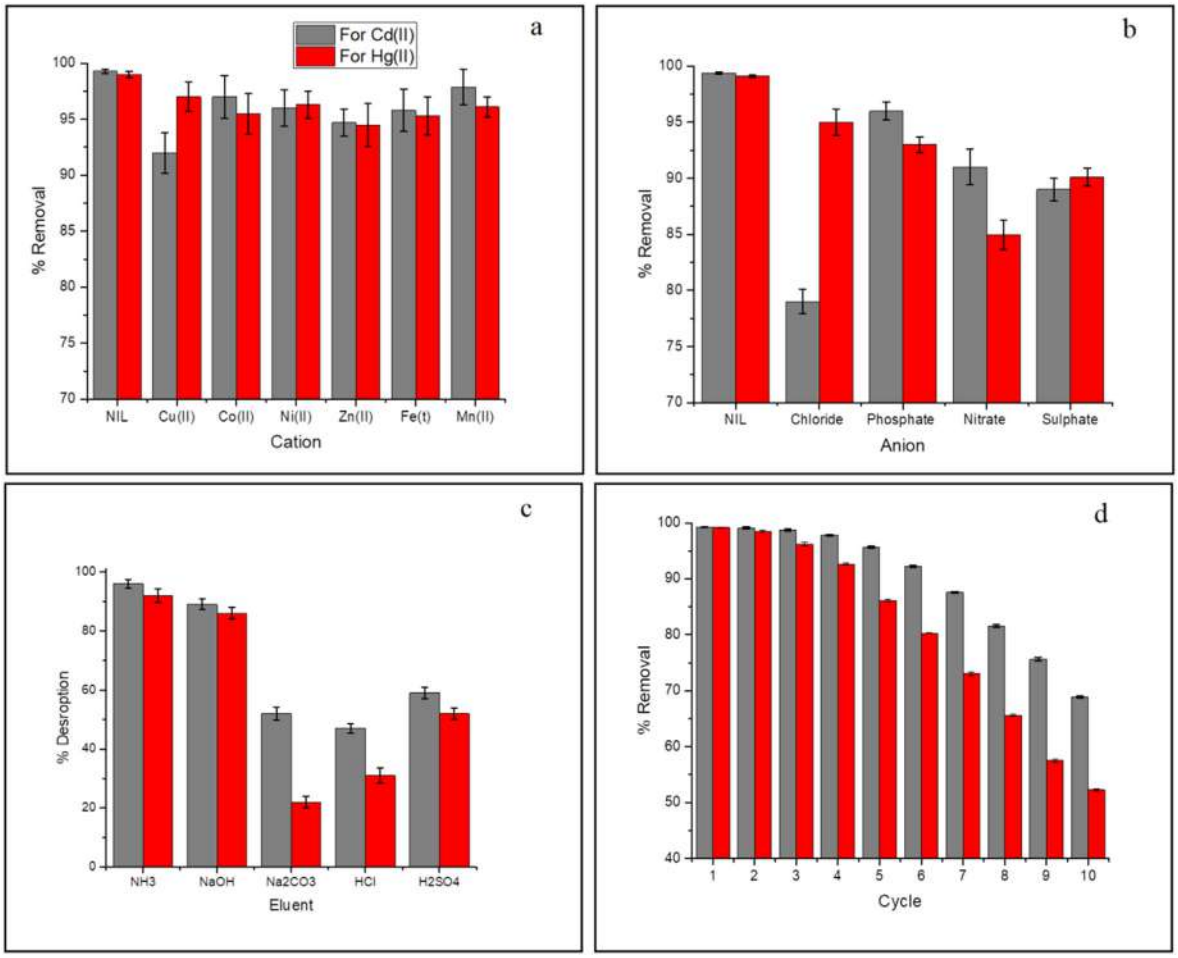


Fig. 8. (a) Effect of cations (b) Effect of anions (c) Desorption studies and (d) Regeneration cycles.

Table 2
Comparison of ILTC with reported adsorbents.

Sorbents	Adsorption capacity (mg/g)		References
	Cd(II)	Hg(II)	
Chitosan	9.9	–	[28]
Chitosan phenyl thiourea resin	120.3	135.5	[29]
Chitosan dithiocarbamate	71.9	25.1	[30]
Chitosan xanthate beads	357.1	–	[31]
Ionic solid impregnated chitosan	341.3	43.4	[32]
Potato Starch Phosphate Polymer	91.8	51.4	[33]
Dopamine-Modified Magnetic Nano-Adsorbent	21.58	–	[34]
Activated carbon and acid-processed montmorillonite clay	0.63	7.97	[35]
Thiourea chitosan	–	6.29 mmol/g	[9]
Thiourea grafted chitosan hydrogel	134	–	[10]
ILTC	442.5	203.2	Present study

mol/K for Hg(II)) indicates the decrease in randomness of metal ions as it passes from solution to adsorbed state. The negative free energy change indicates the spontaneous nature of the adsorption process.

3.6. Breakthrough curve analysis

A column studies were carried out to obtain breakthrough curve (Fig. 8) and results have been depicted in Table 3. The column is eluted with 200 mg/L Cd(II) and 100 mg/L of Hg(II) till a Breakthrough volume of 1800 mL for Cd(II) and 1700 mL for Hg(II), Exhaustion volume of 2400 mL for Cd(II) and 2200 mL for Hg(II). Cd(II) and Hg(II) had Breakthrough Capacity of 360 mg/L and 170 mg/L respectively while ILTC had Exhaustion Capacity of 480 mg/L for Cd(II) and 220 mg/L for Hg(II) with 75% degree of column utilization for Cd(II) and 83.3% for Cd(II). These results clearly indicate that larger sample volumes containing these metal ions can be effectively treated using column method.

Table 3
Column parameters.

Parameter	Result	
	Cd(II)	Hg(II)
Inlet concentration(mg/L)	200	100
Breakthrough volume (mL)	1800	1700
Exhaustion volume (mL)	2400	2200
Breakthrough Capacity (mg/g)	360	170
Exhaustion Capacity (mg/g)	480	220
Degree of column utilization (%)	75.0	83.3

3.7. Effect of co-ions

200 mg/L of cations such as Cu(II), Ni(II), Fe(II), Mn(II), Zn(II) and Co(II) and anions such as Chloride, Sulphate, Nitrate and Phosphate were individually studied for their effect on adsorption of 50 mL 100 mg/L Cd(II) solution equilibrated with 200 mg of ILTC at pH 3. For Hg(II) adsorption efficiency, 50 mL of 50 mg/L Hg(II) was mixed individually

with 50 mL 100 mg/L concentrations of the same ions used above for Cd(II) and stirred with 200 mg of ILTC. From Fig. 8a and b, it is clear that, the removal of Hg(II) is affected to greatest extent by the presence of Zn(II) and nitrate ions. While percentage removal of Cd(II) was reduced by Cu(II) and chloride ions.

3.8. Application to synthetic effluents

50 mL aliquots of synthetic effluent 1 and 2 were taken in a stoppered conical flask along with 200 mg of ILTC and stirred for 1hr. After adsorption final concentration of Cd(II) and Hg(II) were find out. % Removal for Cd(II) was found to be 81.2 ± 0.6% and that for Hg(II) was 76.8 ± 4.0%, which proves applicability of ILTC for effective treatment of real effluents containing Cd(II) and Hg(II) (Supplementary Table 1). As the removal efficiency was found to reduce in the presence of interfering ions, it can be concluded that multiple extractions will be required to achieve desired efficiency. Another way of achieving higher removal efficiency is the fixed bed treatment like column extraction.

3.9. Regeneration and reusability of adsorbent

The regeneration of adsorbent material is very important for the potent practical application and reusing. Various acidic and alkaline reagents such as sodium hydroxide, ammonia, sodium carbonate, hydrochloric acid and sulphuric acid were examined in desorption study for ILTC. Maximum desorption was shown by ammonia solution and NaOH solution. It was found that 5% (v/v) solution of ammonia desorbs more than 90% metal ions. This is in accordance with the fact that maximum adsorption is observed in acidic medium and hence maximum desorption resulted in strongly basic medium. Under strongly basic medium, these two ions are known to form hydroxides and hydroxo complexes. From Fig. 8c it can be seen that ammonia solution effectively leach cadmium and mercury adsorbed on ILTC. The absorption efficiency which is about 99.0 ± 0.3% for the first cycle decreases feebly with subsequent regeneration cycles (Fig. 8d). Reduction of adsorption efficiency was found to be more for Hg(II) as compared to Cd(II). The removal efficiency was reduced to 68% for Cd(II) and 55% for Hg(II) after 10 cycles. This shows a reasonable degree of reusability of the material.

3.10. Comparison with other related adsorbents

A comparison of ILTC with other chitosan based adsorbents, most of them containing the thio group has been shown in Table 2. It can be seen that native chitosan has poor adsorption tendency towards Cd(II) and negligible adsorption of Hg(II). Structural and functional modifications have enhanced the capacities enormously. The introduction of thiourea as reported earlier [9,10] have improved the adsorption capacities quite remarkably. However, they do not report removal of both Cd(II) and Hg(II) while this paper reports both of them with excellent adsorption capacities.

4. Conclusion

The present study focuses on use of doubly functionalized efficient adsorbent ILTC for the removal of cadmium and mercury from aqueous solutions. Adsorption of these metal ions is found to be effective at pH 3. Equilibrium isotherm data reveals that the adsorption of Cd(II) and Hg(II) were through both the chemisorption and physiosorption process. The best fit isotherm model was Freundlich model indicating multilayer adsorption on heterogeneous surface. The adsorption of cadmium and mercury on ILTC was feasible, spontaneous and exothermic process obtained from the thermodynamic parameters ΔG°, ΔH° and ΔS°. The mechanism for chemisorption includes mainly ionic interactions between chloro-complexes of metal ions and ILTC. The adsorption–desorption cycle results demonstrated that the regeneration and subsequent use the ILTC would enhance the economics of practical applications for the removal of cadmium and mercury from the effluents.

Date availability statement

The raw data required to reproduce these findings cannot be shared at this time as the data also forms part of an ongoing study.

CRediT authorship contribution statement

Anita Shekhawat: Data curation, Formal analysis. **Shashikant Kahu:** Conceptualization, Writing – original draft, Project administration. **D. Saravanan:** Investigation, Methodology. **Ravin Jugade:** Supervision, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The authors are thankful to CSIR, HRDG, New Delhi for providing Research Associateship File. No. 09/120(0099)/2020 EMR-1. Also, acknowledge RTM Nagpur University for support under URS project Dev/2117, DST-FIST and UGC-SAP programs for funding under various schemes.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crgsc.2021.100246.

References

[1] M. Rana, J. Tangpong, Md Rahman, Toxicodynamics of Lead, Cadmium, Mercury and Arsenic- induced kidney toxicity and treatment strategy: a mini review, Toxicol. Rep. 5 (2018) 704–713.
[2] F. Fu, Q. Wang, Removal of heavy metal ions from wastewaters: a review, J. Environ. Manag. 92 (2011) 407–411.
[3] S. De Gisi, G. Lofrano, M. Grassi, M. Notarnicola, Characteristics and adsorption capacities of low-cost sorbents for wastewater treatment: a review, Sustain. Mater. Techno. 9 (2015) 10–40.
[4] I. Anastopoulos, A. Bhatnagar, D. Bikiaris, G. Kyzas, Chitin adsorbents for toxic metals: a review, Int. J. Mol. Sci. 18 (2017) 114.
[5] C. Petit, S. Reynaud, J. Desbrieres, Amphiphilic derivatives of chitosan using microwave irradiation. Toward an eco-friendly process to chitosan derivatives, Carbohydr. Polym. 116 (2015) 26–33.
[6] V. Singh, A. Tiwari, D. Tripathi, R. Sanghi, Microwave enhanced synthesis of chitosan-graft-polyacrylamide, Polymer 47 (2006) 254–260.
[7] D. Martins dos Santos, A. de Lacerda Bukzem, S. Campana-Filho, Response surface methodology applied to the study of the microwave-assisted synthesis of quaternized chitosan, Carbohydr. Polym. 138 (2016) 317–326.
[8] E. Guibal, T. Vincent, R. Navarro Mendoza, Synthesis and characterization of a thiourea-derivative of chitosan for platinum recovery, J. Appl. Polym. Sci. 75 (2000) 119–134.
[9] L. Wang, R. Xing, S. Liu, S. Cai, H. Yu, J. Feng, R. Li, P. Li, Synthesis and evaluation of a thiourea-modified chitosan derivative applied for adsorption of Hg(II) from synthetic wastewater, Int. J. Biol. Macromol. 46 (2010) 524–528.
[10] C.A. Ghiorghita, K.B.L. Borchert, A.L. Vasiliu, M.M. Zaharia, D. Schwarz, M. Mihai, Porous thiourea-grafted-chitosan hydrogels: synthesis and sorption of toxic metal ions from contaminated waters, Colloids. Surf., A 607 (2020) 125504.
[11] A. Shekhawat, S. Kahu, D. Saravanan, R. Jugade, Synergistic behaviour of ionic liquid impregnated sulphate-crosslinked chitosan towards adsorption of Cr(VI), Int. J. Biol. Macromol. 80 (2015) 615–626.
[12] S. Kahu, A. Shekhawat, D. Saravanan, R. Jugade, Ionic solid-impregnated sulphate-crosslinked chitosan for effective adsorption of hexavalent chromium from effluents, Int. J. Environ. Sci. Technol. 13 (2016) 2269–2282.
[13] S. Chen, G. Wu, H. Zeng, Preparation of high antimicrobial activity thiourea chitosan–Ag^+ complex, Carbohydr. Polym. 60 (2005) 33–38.
[14] H.C. Hahne, W. Kroontje, Significance of pH and chloride concentration on behavior of heavy metal pollutants: mercury(II), cadmium(II), zinc(II), and lead(II), J. Environ. Qual. 2 (1973) 444–450.
[15] H.K. Boparai, M. Joseph, D.M. O'Carroll, Kinetics and thermodynamics of cadmium ion removal by adsorption onto nano zerovalent iron particles, J. Hazard Mater. 186 (2011) 458–465.
[16] J. Langmuir, The adsorption of gases on plane surfaces of glass mica and platinum, J. Am. Chem. Soc. 40 (1918) 1361–1403.

[17] H.M. Freundlich, Uber die adsorption in losungen, Z. Phys. Chem. 57 (1906) 385–470.
[18] M.I. Temkin, Adsorption equilibrium and the kinetics of processes on non-homogeneous surfaces and in the interaction between adsorbed molecules, Zh. Fiz. Chim. 15 (1941) 296–332.
[19] B. Royer, N.F. Cardoso, E.C. Lima, J.C.P. Vaghetti, N.M. Simon, T. Calvete, R.C. Veses, Applications of Brazilian-pine fruit shell in natural and carbonized forms as adsorbents to removal of methylene blue from aqueous solutions—kinetic and equilibrium study, J. Hazard Mater. 164 (2009) 1213–1222.
[20] Y.S. Ho, Selection of optimum sorption isotherm, Carbon 42 (2004) 2113–2130.
[21] P. Cairns, M.J. Miles, V.J. Morris, M.J. Ridout, G.J. Brownsey, V.T. Winter, X-ray fibre diffraction studies of chitosan and chitosan gels, Carbohydr. Res. 235 (1992) 23–28.
[22] A.K. Mishra, A.K. Sharma, Synthesis of γ-cyclodextrin/chitosan composites for the efficient removal of Cd(II) from aqueous solution, Int. J. Biol. Macromol. 49 (2011) 504–512.
[23] W.M. Khairul, A.I. Daud, N. Ismail, Understanding the properties of chitosan aryl substituted thioureas in their role and potential as antibacterial agents, AIP Conf. Proc. 1933 (2018), 20002.
[24] A. Shekhawat, S. Kahu, D. Saravanan, R. Jugade, Tin(IV) cross-linked chitosan for the removal of As(III), Carbohydr. Polym. 172 (2017) 205–212.
[25] S. Korde, S. Tandekar R. Jugade, Novel mesoporous chitosan-zirconia-ferrosoferric oxide as magnetic composite for defluoridation of water, J. Environ. Chem. Eng. 8 (2020) 104360.
[26] A.S.K. Kumar, S. Sharma, R.S. Reddy, M. Barathi, N. Rajesh, Comprehending the interaction between chitosan and ionic liquid for the adsorption of palladium, Int. J. Biol. Macromol. 72 (2014) 633–639.
[27] A.S.K. Kumar, N. Rajesh, Exploring the interesting interaction between graphene oxide, Aliquat-336 (a room temperature ionic liquid) and chromium(VI) for wastewater treatment, RSC Adv. 3 (2013) 2697–2709.
[28] R. Bassi, S.O. Prasher, B.K. Simpson, Removal of selected metal ions from aqueous solutions using chitosan flakes, Separ. Sci. Technol. 35 (2000) 547–560.
[29] M. Monier, D.A. Abdel-Latif, Preparation of cross-linked magnetic chitosan-phenylthiourea resin for adsorption of Hg(II), Cd(II) and Zn(II) ions from aqueous solutions, J. Hazard Mater. 209–210 (2012) 240–249.
[30] R.A.A. Muzzarelli, F. Tanfani, S. Mariotti, M. Emanuelli, Preparation and characteristic properties of dithiocarbamate chitosan, a chelating polymer, Carbohydr. Res. 104 (1982) 235–243.
[31] N. Sankararamakrishnan, A.K. Sharma, R. Sanghi, Novel chitosan derivative for the removal of cadmium in the presence of cyanide from electroplating wastewater, J. Hazard Mater. 148 (2007) 353–359.
[32] A. Shekhawat, S. Kahu, D. Saravanan, R. Jugade, Removal of Cd(II) and Hg(II) from effluents by ionic solid impregnated chitosan, Int. J. Biol. Macromol. 104 (2017) 1556–1568.
[33] A. Bashir, T. Manzoor, L.A. Malik, A. Qureashi, A.H. Pandith, Enhanced and selective adsorption of Zn(II), Pb(II), Cd(II), and Hg(II) ions by a dumbbell- and flower-shaped potato starch phosphate polymer: a combined experimental and dft calculation study, ACS Omega 5 (2020) 4853–4867.
[34] T. Lei, S.J. Li, F. Jiang, Z. Ren, L. Wang, L. Tang, S. Wang, Adsorption of cadmium ions from an aqueous solution on a highly stable dopamine-modified magnetic nano-adsorbent, Nanoscale Res. Lett. 14 (2019) 352.
[35] M. Wang, G. Bera, K. Mitra, T. Wade, A. Knap, T. Phillips, Tight sorption of arsenic, cadmium, mercury, and lead by edible activated carbon and acid-processed montmorillonite clay, Environ. Sci. Pollut. Res. 28 (2021) 6758–6770.

Journal of Molecular Structure 1225 (2021) 129153

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: www.elsevier.com/locate/molstr

Pyrene based Schiff bases: Synthesis, crystal structure, antibacterial and BSA binding studies

Venkatesan Srinivasan[a,1], Themmila Khamrang[b,1], Chairman Ponraj[c], Dhandayutham Saravanan[c,*], Rekha Yamini[d], Soumen Bera[d], Mariadoss Asha Jhonsi[a,*]

[a] Department of Chemistry, B. S. Abdur Rahman Crescent Institute of Science and Technology, Chennai, Tamil Nadu, India
[b] Department of Chemistry, C. I. College, Bishnupur, Manipur, 795126, India
[c] Department of Chemistry, National College (Affiliated to Bharathidasan University), Tiruchirappalli 620 001, India
[d] School of Life Sciences, B. S. Abdur Rahman Crescent Institute of Science and Technology, Chennai, Tamil Nadu, India

ARTICLE INFO

Article history:
Received 5 May 2020
Revised 22 August 2020
Accepted 26 August 2020
Available online 27 August 2020

Keywords:
Pyrene Schiff bases
π-spacer
Crystal structure
Antibacterial activity
BSA binding studies

ABSTRACT

Two novel pyrene-based Schiff base compounds 4-[(5-*pyren*-1-*yl*-*thiophen*-2-*ylmethylene*)-*amino*]-phenol (KSB-1) and 4-[(4-*pyren*-1-*yl*-*benzylidene*)-*amino*]-phenol (KSB-2) were designed and synthesized by a condensation reaction with a diminutive modification of π-spacers. The KSB-1 and KSB-2 molecules are thoroughly characterized by pivotal spectroscopic techniques. The prepared products were found to yield excellent quantity, and crystallographic data was also analyzed for KSB-1. Moreover, the antibacterial activities of the synthesized KSB-1 and KSB-2 were investigated against the two strains of *Pseudomonas aeruginosa*. BSA binding interaction with these pyrene-based Schiff bases was investigated by fluorescence quenching method, and the quenching of BSA followed a static type. The binding constant and number of binding modes were calculated.

© 2020 Elsevier B.V. All rights reserved.

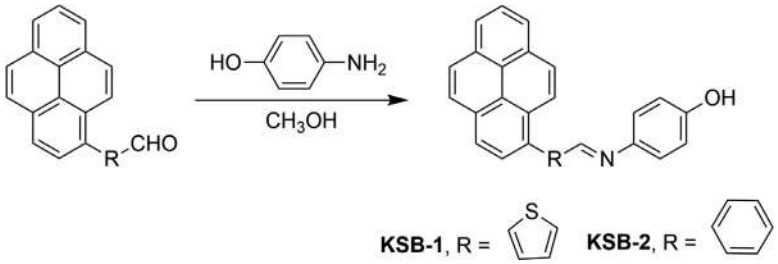
1. Introduction

Schiff bases are an essential category of organic compounds, and it could be prepared from the condensation of molecules contain aldehyde or ketone functional groups with an amine [1]. Schiff bases are named after Hugo Schiff [2], also known as imine or azomethine and have played an important role in the enrichment of chemical sciences. Intriguingly, the imine or azomethines group are found to be a versatile pharmacophore for design and development of various bioactive compounds and also could be tuned by varying the substituents. The Schiff bases posses with heterocyclic rings containing nitrogen, oxygen and sulfur donor atoms show the different pharmacological activities such as antibacterial, antifungal, antimalarial, anti-inflammatory and antipyretic [3–12]. On the other hand, the Schiff bases are effectively used to develop various chemosensors since the imine group acts as a strong binder with metal ions [13]. As a result, the Schiff base compounds utilized for diverse applications in various research fields [14]. For instance, Mesbah and co-workers developed different Schiff bases from heterocyclic moiety, and the compounds are investigated for their antimicrobial and antifungal activities against some pathogenic strains [15]. Raja et al. reported the Schiff base of isatin derivatives [16] for antibacterial activity. Antimicrobial activity of the derivatives was assessed by minimum inhibitory concentration (MIC) in comparison with standard antimicrobial drugs (ciprofloxacin and ketoconazole). Results show that the electron-donating groups present in the isatin derivatives plays a vital role in improving the antimicrobial activity. Other small molecules such as amino acids, coumarins, sulphonamides, 2-aminophenol and triazoles for the preparation of Schiff bases for antibacterial activities [17]. Previously, we have also reported the pyrene-based Schiff base with AIE property as a prospective biomaterial for in vitro bioimaging and BSA-binding studies [18,19]. Thus, the potent biological activity of pyrene based Schiff bases propels to develop new class of pyrene derivatives and to investigate the binding interaction with albumins. In this connection, bovine serum albumin (BSA) is considered by the researchers due to its availability and structural homology with human serum albumin (HSA) [20].

The design of the Schiff bases KSB-1 and KSB-2 are such that it can be advantageously used as a dual functional probe consisting of a hydrophobic pyrene group with phenyl and thiophene substituents and hydrophilic hydroxyl groups [21]. Hence it can be expected to show a high affinity for hydrophobic and hydrophilic sites of BSA protein. Since, π-conjugated pyrene derivatives

* Corresponding authors.
E-mail addresses: dsaravananchem@nct.ac.in (D. Saravanan), jhonsiasha@gmail.com (M.A. Jhonsi).
[1] Authors are equally contributed.

https://doi.org/10.1016/j.molstruc.2020.129153
0022-2860/© 2020 Elsevier B.V. All rights reserved.



Scheme 1. Synthetic scheme of KSB-1 and KSB-2.

have been successfully used as fluorophores [22], imaging [23], cytotoxicity [24], sensing [25] and antimicrobial activities [26]. Meng et al. investigated the interaction of pyrene with BSA under physiological condition [27]. Besides, Ali et al. had also reported phenanthrene-pyrene based fluorescent probe with BSA [28]. Here, we report the pyrene-based Schiff bases for biological applications. The 4-aminophenol is used for producing the Schiff base due to the presence of hydroxyl group. The hydroxyl group at para position has high electron releasing properties and it activates aromatic ring thereby increasing the biological activity of the compound. Hence, 4-aminophenol is used for synthesis of Schiff base as it increases the biological activity thereby upon treatment it reduces the bacterial growth by affecting its motility. On the other hand, the developed Schiff bases are only differing in π-spacers, such as thiophene and benzene. These molecules are characterized by ^{1}H NMR, ^{13}C NMR and mass spectroscopic techniques. In this manuscript, we targeted to explore the role of π-spacers on the BSA binding and antimicrobial studies. Therefore, the antibacterial activities of KSB-1 and KSB-2 compounds were tested against multi-drug resistant strains of *Pseudomonas aeruginosa.*

2. Experimental section

2.1. Materials and methods

4-aminophenol and bovine serum albumin were purchased from Sigma-Aldrich. Methanol, glacial acetic acid, petroleum ether, dichloromethane and dimethyl sulphoxide were purchased from Sisco Research Laboratories (SRL), India, and solvents of spectrometric grade were used without any further purification. The starting materials 5-(pyren-1-yl)thiophene-2-carbaldehyde, 4-(pyren-1-yl)benzaldehyde were prepared as reported previously [29]. PA9027 cells were obtained from the Microbial Type Culture Collection, and Gene Bank (MTCC), India, LB broth was obtained from Himedia, India.

2.2. Instrumentation

Nuclear magnetic resonance (NMR) spectra were recorded on a BRUKER AVANCE II spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C and collected at ambient probe temperature with spectra calibrated to either an internal tetramethyl silane (TMS) standard or a residual protio-solvent. Electrospray ionization mass spectra (ESI-MS) were collected on a Thermo Finnigan (San Jose, CA, USA) LCQTM Advantage MAX quadrupole ion trap instrument, by infusing samples directly into the source at 20 mL min^{-1} using a syringe pump. Absorption spectra were recorded by using Perkin Elmer Lamda 25 UV–visible spectrophotometer. The BSA quenching studies were carried out using a Hitachi F-4500 fluorescence spectrometer. Crystal data were collected at ambient temperature using an Oxford diffraction X-Calibur Eos Gemini diffractometer with graphite-monochromated Mo-Kα radiation (λ = 0.7107 Å). The data were solved using direct methods with SHELXS and refined SHELEXL-2013. The figures were generated using the ORTEP 3.07 generation package. The hydrogen atoms bound to the carbon were placed in geometrically constrained positions and refined with isotropic temperature factors, generally 1.2 Ueq of their parent atoms.

2.3. Synthesis

The pyrene containing Schiff base compounds were prepared by the following general procedure (Scheme 1).

2.3.1. *4-[(5-*pyren-*1-*yl-thiophen-*2-*ylmethylene*)-*amino*]-phenol (KSB-1)*

To a hot methanolic solution (20 mL) of 5-pyren-1-yl-thiophene-2-carbaldehyde (0.156 g, 0.5 mmol), 4-aminophenol (0.06 g, 0.55 mmol) dissolved in 10 mL of methanol was added drop wise and catalytic amount of glacial acetic acid were added and the mixture was refluxed for 1 h. The yellow precipitate obtained after cooling to the ambient temperature was filtered, washed several times with cold methanol and dried yielding yellow solid (0.143 g, 71%). Single crystals of KSB-1, suitable for X-ray diffraction were obtained by slow evaporation of a DCM/hexane solution. ^{1}H NMR (DMSO–d_6, 500 MHz) δ ppm: 9.57 (s, 1H), 8.86 (s, 1H), 8.53–8.51 (d, 1H, J = 10 Hz), 8.38–8.34 (m, 3H), 8.30–8.20 (m, 4H), 8.14–8.11 (t, 1H, J = 7.5 Hz), 7.79–7.78 (d, 1H, J = 5 Hz), 7.57–7.56 (d, 1H, J = 5 Hz), 7.25–7.23 (t, 2H, J = 5 Hz), 6.82–6.80 (m, 2H); ^{13}C NMR (DMSO–d_6, 500 MHz) δ ppm: 156.8, 150.8, 145.4, 144.3, 142.4, 133.5, 131.4, 131.3, 130.8, 129.7, 129.1, 129.0, 128.6, 128.4, 127.7, 127.1, 126.3, 125.9, 125.5, 124.6, 124.5, 124.3, 123.0, 116.2; ESI-MS: *m/z* calcd for $C_{27}H_{17}NOS$ (403.49), found 403.49. (Fig. S1-S3)

2.3.2. *4-[(4-*pyren-*1-*yl-benzylidene*)-*amino*]-phenol (KSB-2)*

Pale yellow solid (0.127 g, 65%); ^{1}H NMR (DMSO–d_6, 500 MHz) δ ppm: 9.57 (s, 1H), 8.76 (s, 1H), 8.39–8.38 (d, 1H, J = 5 Hz), 8.35–8.33 (d, 1H, J = 10 Hz), 8.31–8.29 (d, 1H, J = 10 Hz), 8.24 (s, 2H), 8.20–8.18 (d, 1H, J = 10 Hz), 8.15–8.0 (m, 5H), 7.77–7.75 (d, 2H, J = 10 Hz), 7.29–7.27 (d, 2H, J = 10 Hz), 6.85–6.84 (d, 2H, J = 5 Hz); ^{13}C NMR (DMSO–d_6, 500 MHz) δ ppm: 157.1, 156.8, 143.1, 143.0, 136.8, 136.0, 131.4, 131.2, 130.8, 128.9, 128.4, 128.1, 128.0, 127.8, 126.9, 125.9, 125.6, 125.4, 124.9, 124.6, 124.4, 123.0, 116.2; ESI-MS: *m/z* calcd for $C_{27}H_{17}NOS$ (397.46), found 397.46. (Fig. S4-S6)

2.4. Molecular docking

We have retrieved the three-dimensional structure of bovine serum albumin (PDB ID: 4F5S) from the protein data bank (PDB) database. The binding site of bovine serum albumin was identified by PrankWeb (https://academic.oup.com/nar/article/47/W1/W345/5494740) web server. The predicted binding region consists of the following residues positions Tyr400, Asn404, Phe501, Phe506,

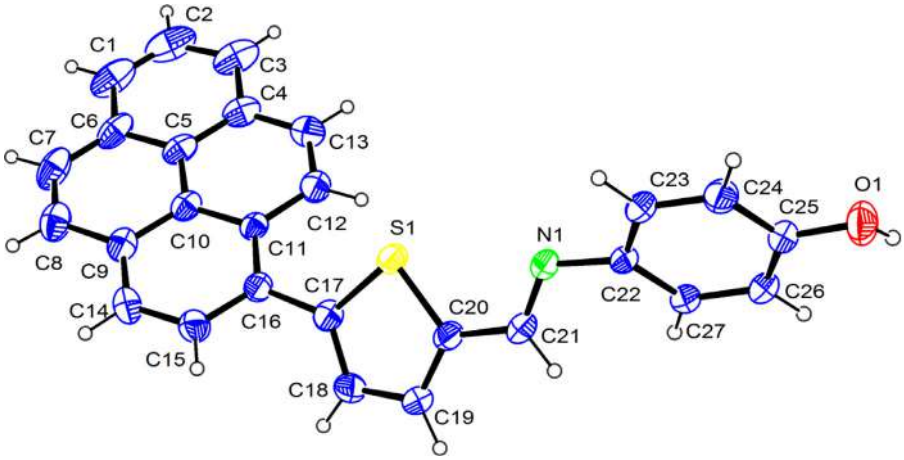


Fig. 1. Molecular structure of KSB-1 with crystallographic labeling (30% probability displacement ellipsoids).

Phe508, Lys524, Gln525, Ala527, Leu528, Leu531, His534, Val546, Met547, Phe550, Val551, Leu574, Val575, Thr578, Gln579 in the A chain. The semi-flexible docking was performed with the default parameters by using the AutoDock program (https://pubmed.ncbi.nlm.nih.gov/19085980/). During the docking process, Autodock uses the Lamarckian genetic algorithm for searching the free energy minimum of the BSA-pyrene derivatives. The interaction between BSA and pyrene derivatives was analyzed and visualized using LIGPLOT program (https://pubmed.ncbi.nlm.nih.gov/7630882/).

3. Results and discussion

3.1. Crystal structure of KSB-1

Single crystal of KSB-1 was grown from a mixture of petroleum ether and dichloromethane (2:1) mixture at room temperature. The crystallographic data and structural refinement details are given in Table 1. Additionally, the selected bond distances and bond angles are depicted in Table S1. The compound KSB-1 crystallizes in the triclinic system with P-1 space group. Fig. 1 shows the ORTEP diagram of the KSB-1 with the atomic numbering scheme. The structure of KSB-1 is stabilized by intermolecular C–H…S, C–H…O, C–H…π(pyrene) and C15-H…π(ph) hydrogen bonding, responsible for producing a three-dimensional structure (Fig. 2). In KSB-1, the pyrene ring (atoms C1–C16) and the thiophene ring (S1/C17–C20) are twisted with respect to each other making a dihedral angle of 135.04°; the benzene ring (C22–C27) is nearly perpendicular to the thiophene ring (S1/C17–C20), with a dihedral angle of 174.15°. In this structure, the pyrene rings are widely separated with the closest centroid distance (C9-C11, C14-C16) of 4.626 Å, suggesting weak π-π interactions exists between them.

Table 1
Crystallographic data and structure refinement parameters for KSB-1.

CCDC Number	2000499
Formula	$C_{28}H_{21}NO_2S$
Formula weight	435.52
Crystal system	triclinic
Space group	P-1
Temperature (K)	293(2)
Wavelength (Å)	0.71073
a (Å)	8.8290(13)
b (Å)	11.1388(16)
c (Å)	12.3805(17)
α (deg)	75.415(12)
β (deg)	69.948(13)
γ (deg)	79.462(12)
V (Å^3)	1100.6(3)
Z	2
Radiation type	Mo $K\alpha$
μ (mm^{-1})	0.173
Goodness-of-fit on F^2	1.098
R_{int}	0.030
ρ_{calc} mg/mm^3	1.314
final R indices	0.0969
	0.2581
R_1[a]	0.1405
wR_2[b]	0.2855

[a] $R_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$.
[b] $_wR_2 = \{\Sigma w[(F_o^2 - F_c^2)^2]/\Sigma w[(F_o^2)^2]\}_{1/2}$.

3.2. Optical properties

UV-Visible absorption spectra of KSB-1 and KSB-2 in DMSO are shown in Fig. 3. The KSB-1 and KSB-2 dyes show strong absorption bands at 388 nm and 358 nm, respectively. By comparing two dyes, KSB-1 absorbs at a longer wavelength with high molar absorption coefficient (9.5×10^4 M^{-1}cm^{-1}) than KSB-2 (8.7×10^4 M^{-1}cm^{-1}), which is due to thiophene ring present in the KSB-1 molecule, which increases the effective delocalization electron over the entire molecule (pyrene unit to the acceptor via thiophene π –bridge) [29]. Because, the insertion of different aromatic ring systems as a π-spacer is intended to improve the charge transfer character of the dye since benzene and thiophene can provide effective conjugation and reduce the charge-transfer energy transition due to the reduced resonance energy (thiophene, (29 kcal mol^{-1}) and benzene (36 kcal mol^{-1})) [30].

3.3. Antibacterial activity

The KSB-1 and KSB-2 were screened for their antibacterial activity against the two strains of *Pseudomonas aeruginosa* ATCC 9027 and ATCC 27853, which are nosocomial pathogens as they are known to cause hospital-borne infections. *P. aeruginosa* cells were grown overnight in shaking at 37 °C in LB broth. From the overnight culture, an inoculum size of OD_{600nm} ~ 0.01 was used in each of the experiments. KSB-1 and KSB-2 were dissolved in DMSO to treat the cells for 24 hrs at 37 °C in a shaking incubator. In PA 9027 cells, KSB-1 treatment has shown to have minimum effect on the cell viability at concentration range from 100 to 500µg/ml. Only at higher concentration of KSB-1 i.e. 1000µg/ml was found to be cytotoxic where almost 70% cell growth inhibition was evident. PA9027 cells were also found to be

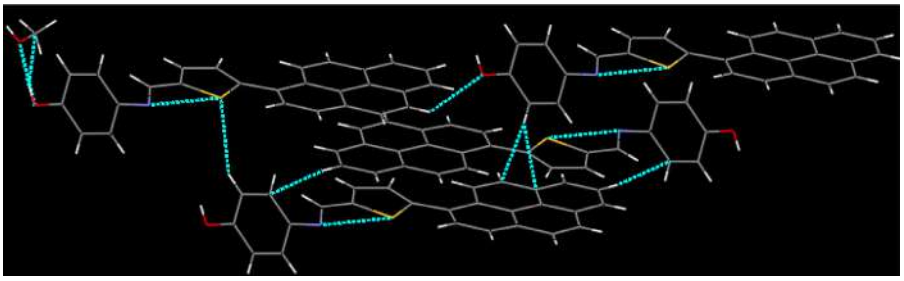


Fig. 2. Hydrogen-bonding network of KSB-1 with hydrogen bonds indicated by dashed lines.

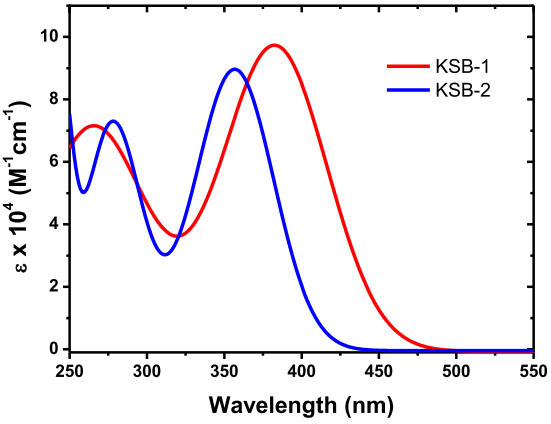


Fig. 3. Absorption spectra of KSB-1 and KSB-2 molecule in DMSO solvent.

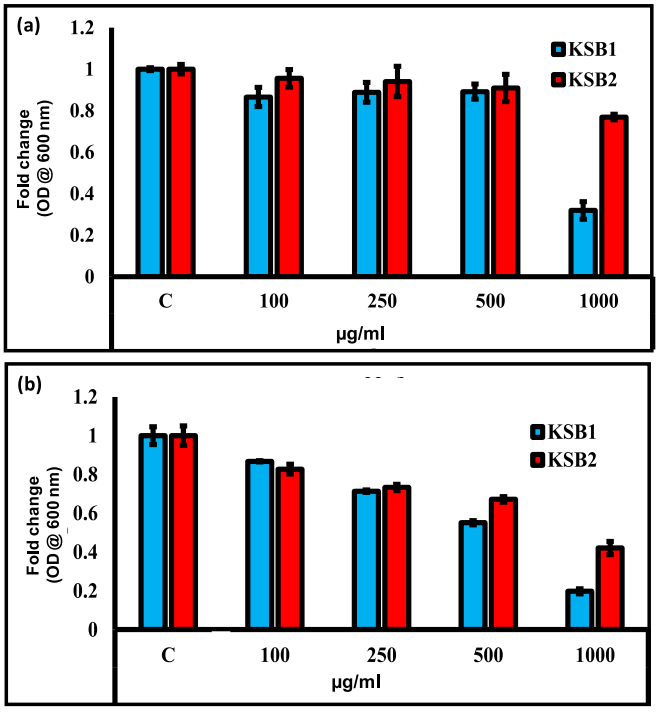


Fig. 4. Mic Broth dilution method effect of pyrene derivatives in DMSO (a) on PA 9027 planktonic cells and (b) on PA 27853 planktonic cells.

insensitive to KSB-2 with all the dosages used (100- 1000µg/ml) (Fig. 4a). On the contrary, the PA27853 cells were sensitive to both the KSB-1 and KSB-2 treatments and the effects were dose-dependent while KSB-1 is more effective in comparison to the KSB-2 (Fig. 4b). Schiff base developed from heterocyclic compounds containing nitrogen, oxygen and sulfur atoms generally shows momentous antimicrobial activity [31–41].

3.4. BSA binding studies

Proteins are the most copious macromolecules in cells and are critical to maintaining healthy cell functions. Bovine serum albumin (BSA), one of the vital components in plasma protein, plays a vital role in transporting and metabolizing of many endogenous and exogenous compounds in metabolism [42]. In this work, BSA was selected as a target protein molecule for studying the interaction because of its medically significant, unusual ligand-binding properties, availability, and structural homology with human serum albumin (HSA) [43].

Fig. 5a & b shows the fluorescence spectra of BSA with increasing concentrations of pyrene derivatives. The results showed that the fluorescence intensity of BSA quenched in a regular pattern with increasing concentrations of KSB-1. The quenching results indicated that the occurrence of interactions between BSA and KSB-1. Moreover, no significant shift in the wavelength of BSA was observed, which indicates that KSB-1 did not make any conformational changes in the structure of albumin. A similar type of spectral behavior was observed for KSB-2.

In order to understand the quenching mechanism, the fluorescence quenching data were analyzed by the Stern–Volmer Eqs. (1) & (2):

$$I_0/I = 1 + K_{SV}[Q] \quad (1)$$

$$K_{SV} = k_q \cdot \tau_0 \quad (2)$$

where I_0 and I are the fluorescence intensities of BSA in the absence and presence of quencher, respectively, k_q is the quenching rate constant, K_{SV} is the quenching constant, τ_0 is the lifetime (τ_0) of BSA without the quencher (6 ns) and [Q] is the concentration of the quencher. According to Eq. (1), the plots were constructed between I_0/I vs [Q] and shown in Fig. 6a. Both plots were linear indicates that the quenching may follow a dynamic type. However, in some cases, static type also shows such a type of linear plot. Hence, quenching rate constants were calculated using the slope of the linear plot and lifetime of BSA. The quenching constant and quenching rate constants values are shown in Table 2. Typically, the maximum limit of collisional (dynamic) quenching rate constant (k_q) in the water medium is 2.0×10^{10} $M^{-1}s^{-1}$ [44]. However, the rate constant (~10^{13} $M^{-1}s^{-1}$) we have calculated is much higher than the limited one. Thus, the higher k_q supports

Table 2
The parameters K_{SV}, k_q, K_b and n of BSA with the pyrene derivatives.

Molecules	K_{SV}($\times 10^5$ M^{-1})	k_q($\times 10^{13}$ M^{-1} s^{-1})	K_b($\times 10^5$ M^{-1})	n
KSB-1	1.64	2.73	3.81	0.78
KSB-2	1.04	1.73	4.53	0.93

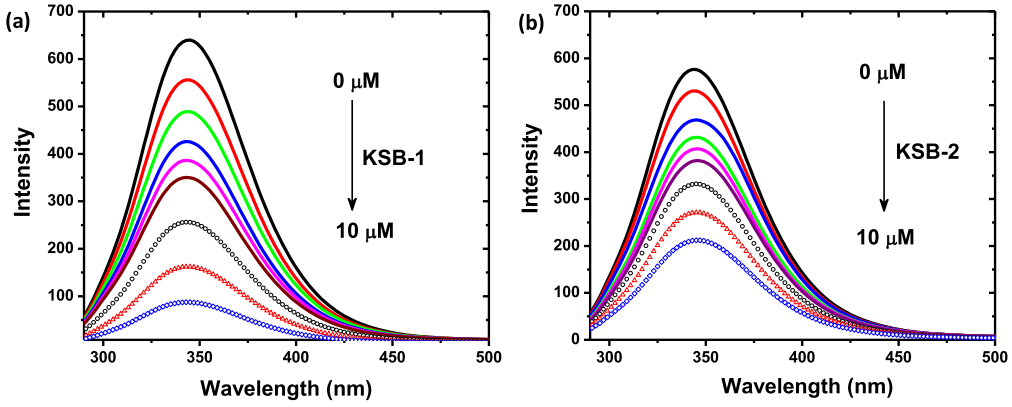


Fig. 5. (a) Fluorescence quenching of BSA with various concentrations of (a) KSB-1 and (b) KSB-2.

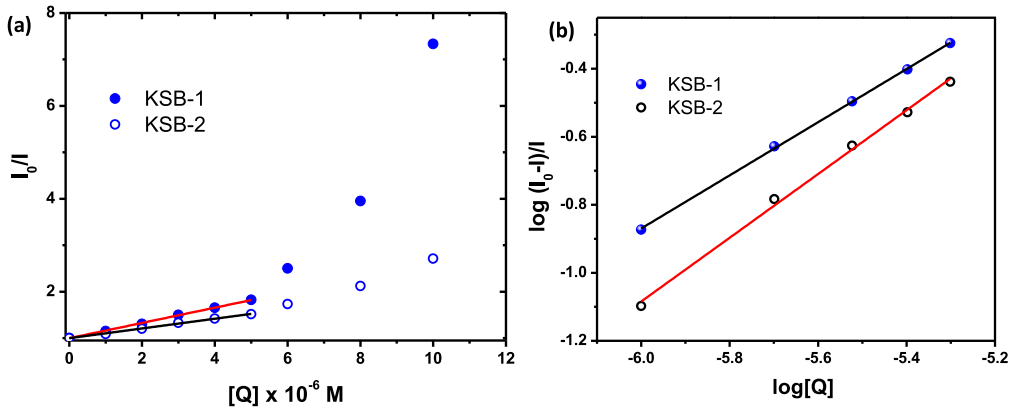


Fig. 6. (a) Stern-Volmer plot and (b) double-logarithmic plot of the fluorescence quenching of BSA by KSB-1 and KSB-2.

the quenching pattern followed by the static type [45–50] rather than the dynamic type.

As the quenching process is static, the binding constant (K_b) and the number of binding sites (n) can be calculated using the double-logarithmic Eq. (3)

$$\log (I_0 - I)/I = \log K_b + n \log [Q] \quad (3)$$

The values of K_b and n can be calculated from the intercept and the slope of the linear plot (Fig. 6b), respectively. Table 2 shows the calculated K_b and n values. The higher K_b value exposes the strong interaction between BSA and pyrene derivatives. On the other hand, the value of n was determined to be nearly 1, suggesting a 1:1 binding type of BSA with pyrene derivatives.

3.5. Free energy calculations

The standard Gibb's free energy change (ΔG_o) can be evaluated from the binding constant and using the following equation to understand the spontaneity of the reaction:

$$\Delta G_o = -RT \ln K_b \quad (4)$$

where R and T refers to the gas constant and the room temperature, respectively. The calculated ΔG_o values for the interaction of BSA with pyrene derivatives were −31.83 kcal/mol and −32.27 kcal/mol for KSB-1 and KSB-2, respectively. The obtained negative values were clearly highlighting that the binding between BSA and pyrene derivatives is highly favourable and spontaneous [51]. Intriguingly, the ΔG_o value is higher for KSB-1 and it is nicely corroborate with antimicrobial results.

3.6. Molecular docking analysis

To understand the nature of molecular interactions between BSA and pyrene derivatives, molecular docking investigations were carried out. The two dimensional docking results were compiled in Fig. 7. As shown in this Fig. 7, the hydrophobic interactions are existing between pyrene derivatives and surround amino acids of BSA. In addition, the hydrogen bonding interactions also exist between hydroxyl group of pyrene derivatives and BSA. Intriguingly, both pyrene derivatives pose the hydrogen binding interactions with similar amino acid residues such as Gln579 and Val575. Although, both the hydrophobic and electrostatic interactions are existing, but the hydrophobic interactions are more vital for the interaction between pyrene derivatives and BSA. The binding free energy for KSB-1 and KSB-2 are −9.03 kcal/mol and −8.39 kcal/mol, respectively. The obtained negative value indicating that the interaction between pyrene derivatives and BSA is energetically favorable.

4. Conclusion

In this work, two new pyrene-based Schiff bases were synthesized by a diminutive modification of π-spacers and their structures were thoroughly characterized by NMR, mass and UV–Vis spectroscopic techniques. Absorption spectral studies indicated that the introduction of thiophene π-spacer ring increased the effective delocalization of electron over the entire molecule compared to benzene π-spacer. Besides, the synthesized KSB-1 and KSB-2 compounds were verified for their antibacterial activities against the drug sensitive and resistant strains

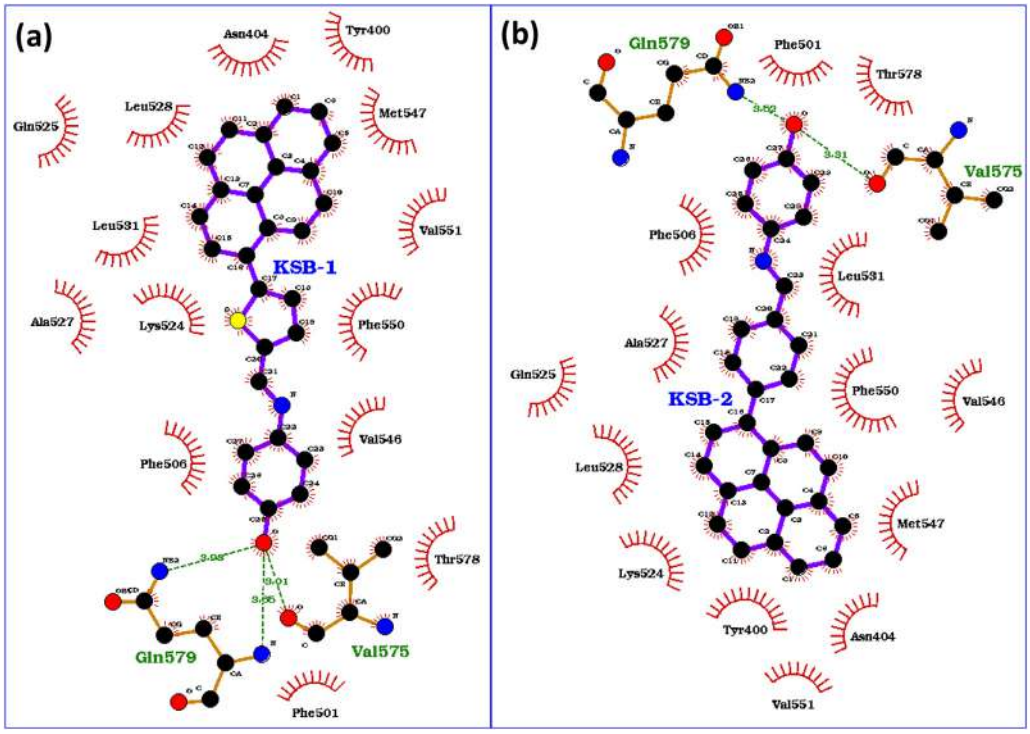


Fig. 7. The 2D schematic representation onnature of interactions between pyrene derivatives and BSA. Residues involved in the hydrophobic interactions are represents in red color sweeps. Hydrogen bond interacting residues are shown with their complete structure. color of the atoms: black – carbon, blue – nitrogen, yellow – sulfur and red – oxygen.

Pseudomonas aeruginosa ATCC 9027 and ATCC 27853. The results indicate that the KSB-1 was more active when compared with KSB-2, because of the introduction of a thiophene ring which alters the chemical structure and effectively affects the biologic activity. The KSB-1 and KSB-2 compounds showed excellent binding interaction to BSA, giving relatively high binding constants. Also, the fluorescence quenching results show that both the compounds were a potent quencher and higher quenching rate constant supports the quenching pattern followed by the static type rather than the dynamic type. From the molecular docking results, the hydrophobic interactions are played major role for the interaction between pyrene derivatives and BSA. Therefore, due to these properties, the synthesized pyrene-based Schiff bases KSB-1 and KSB-2 can further the used in therapeutic applications.

Declaration of Competing Interest

The authors declare that they have no known competing financialinterestsor personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Venkatesan Srinivasan: Methodology, Data curation, Writing - original draft. **Themmila Khamrang:** Methodology, Data curation, Writing - original draft. **Chairman Ponraj:** Methodology, Data curation, Writing - original draft. **Dhandayutham Saravanan:** Conceptualization, Validation, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing, Visualization, Funding acquisition, Supervision, Project administration, Investigation, Writing - review & editing, Funding acquisition. **Rekha Yamini:** Methodology, Formal analysis. **Soumen Bera:** Methodology, Formal analysis. **Mariadoss Asha Jhonsi:** Conceptualization, Validation, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing, Visualization, Funding acquisition, Supervision, Project administration, Investigation, Writing - review & editing, Funding acquisition.

Acknowledgements

V.S. thanks to the Department of Chemistry and BSACIST for the Senior Research Fellowship. MAJ thanks to SERB TARE project (TAR/2018/000458, Dated: 05.11.2018) MAJ also acknowledges IIT, SAIF for NMR and ESI-MS measurements. MAJ very much thankful to Dr. M. Jaccob and Mr. A. Kulandai Samy for assistance in molecular docking analysis.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2020.129153.

References

[1] H. Puchtler, S. Meloan, On Schiff's bases and aldehyde-Fuchsin: a review from H. Schiff to R. D. Lillie, Histochemistry 72 (1981) 321–332.
[2] H. Schiff, Mittheilungen aus dem Universitätslaboratorium in Pisa: eine neue Reihe organischer Basen, Justus Liebigs Ann. Chem. 131 (1864) 118–119.
[3] K.S. Kumar, S. Ganguly, R. Veerasamy, E.D. Clercq, Synthesis, antiviral activity and cytotoxicity evaluation of Schiff bases of some 2-phenyl quinazoline-4 (3) H-ones, Eur. J. Med. Chem. 45 (11) (2010) 5474–5479.
[4] O. Güngör, P. Gürkan, Synthesis and characterization of higher amino acid Schiff bases, as monosodium salts and neutral forms. Investigation of the intramolecular hydrogen bonding in all Schiff bases, antibacterial and antifungal activities of neutral forms, J. Mol. Struct. 1074 (2014) 62–70.
[5] A.A. Shanty, J.E. Philip, E.J. Sneha, M.R.P. Kurup, S. Balachandran, P.V. Mohanan, Synthesis, characterization and biological studies of Schiff bases derived from heterocyclic moiety, Bioorg. Chem. 70 (2017) 67–73.
[6] E. Pontiki, D. Hadjipavlou-Litina, A. Chaviara, Evaluation of anti-inflammatory and antioxidant activities of copper (II) Schiff mono-base and copper (II) Schiff base coordination compounds of dien with heterocyclic aldehydes and 2-amino-5-methyl-thiazole, J. Enzyme Inhib. Med. Chem. 23 (6) (2008) 1011–1017.

[7] S. Amer, N. El-Wakiel, H. El-Ghamry, Synthesis, spectral, antitumor and antimicrobial studies on Cu (II) complexes of purine and triazole Schiff base derivatives, J. Mol. Struct. 1049 (2013) 326–335.

[8] N. El-wakiel, M. El-keiy, M. Gaber, Synthesis, spectral, antitumor, antioxidant and antimicrobial studies on Cu(II), Ni(II) and Co(II) complexes of 4-[(1*H*-*Benzoimidazol*-2-*ylimino*)-*methyl*]-benzene-1,3-diol, Spectrochim. Acta A 147 (2015) 117–123.

[9] S.M. Bensaber, H. Allafe, N.B. Ermeli, S.B. Mohamed, A.A. Zetrini, S.G. Alsabri, M. Erhuma, A. Hermann, M.I. Jaeda, A.M. Gbaj, Chemical synthesis, molecular modelling, and evaluation of anticancer activity of some pyrazol-3-one Schiff base derivatives, Med. Chem. Res. 23 (2014) 5120–5134.

[10] A. Sinha, K. Banerjee, A. Banerjee, S. Das, S.K. Choudhuri, Synthesis, characterization and biological evaluation of a novel vanadium complex as a possible anticancer agent, J. Organomet. Chem. 772 (2014) 34–41.

[11] H.A. Pramanik, D. Das, P.C. Paul, P. Mondal, C.R. Bhattacharjee, Newer mixed ligand Schiff base complexes from aquo-N-(20-hydroxy acetophenone) glycinato copper(II) as synthon: DFT, antimicrobial activity and molecular docking study, J. Mol. Struct. 1059 (2014) 309–319.

[12] C.M. da Silva, D.L. da Silva, L.V. Modolo, R.B. Alves, M.A. de Resende, C.V.B. Martins, Â. de Fátima, Schiff bases: a short review of their antimicrobial activities, J. Adv. Res. 2 (2011) 1–8.

[13] C. Sasaki, K. Nakajima, M. Kojima, Preparation and characterization of optically active quadridentate Schiff base-titanium(IV) complexes and the catalytic properties of these complexes on asymmetric oxidation of methyl phenyl sulfide with organic hydroperoxides, Bull. Chem. Soc. Jpn. 64 (1991) 1318–1324.

[14] A. Rani, M. Kumar, R. Khare, H.S. Tuli, Schiff bases as an antimicrobial agent: a review, J. Bio. Chem. Sci. 2 (2015) 62–91.

[15] M. Mesbah, T. Douadi, F. Sahli, S. Issaadi, S. Boukazoula, S. Chafaa, Synthesis, characterization, spectroscopic studies and antimicrobial activity of three new Schiff bases derived from Heterocyclic moiety, J. Mol. Struct. 1151 (2018) 41–48.

[16] C.R. Prakash, S. Raja, Synthesis, characterization and in vitro antimicrobial activity of some novel 5-substituted Schiff and Mannich base of isatin derivatives, J. Saudi Chem. Soc. 17 (2013) 337–344.

[17] C.M. Da Silva, D.L. da Silva, L.V. Modolo, R.B. Alves, M.A. de Resende, C.V. Martins, Â. de Fátima, Schiff bases: a short review of their antimicrobial activities, J. Adv. Res. 2 (2011) 1–8.

[18] A. Kathiravan, K. Sundaravel, M. Jaccob, G. Dhinagaran, A. Rameshkumar, D. Arul Ananth, T. Sivasudha, Pyrene Schiff base: photophysics, aggregation induced emission, and antimicrobial properties, J. Phys. Chem. B 118 (2014) 13573–13581.

[19] V. Srinivasan, M.A. Jhonsi, N. Dhenadhayalan, K.-C. Lin, D.A. Ananth, T. Sivasudha, R. Narayanaswamy, A. Kathiravan, Pyrene-based prospective biomaterial: in vitro bioimaging, protein binding studies and detection of bilirubin and Fe^{3+}, Spectrochim. Acta A 221 (2019) 117150.

[20] X.L. Han, P. Mei, Y. Liu, Q. Xiao, F.L. Jiang, R. Li, Binding interaction of quinclorac with bovine serum albumin: a biophysical study, Spectrochim. Acta A 74 (2009) 781–787.

[21] A. Kathiravan, V. Srinivasan, T. Khamrang, M. Velusamy, N. Pavithra, S. Anandan, K. Velappan, M. Jaccob, Pyrene based D-p-A architectures: synthesis, density functional theory, photophysics and electron transfer dynamics, Phys. Chem. Chem. Phys. 19 (2017) 3125–3135.

[22] A.S.M. Islam, M. Sasmal, D. Maiti, A. Dutta, B. Show, M. Ali, Design of a Pyrene scaffold multifunctional material: real-time turn-on chemosensor for nitric oxide, AIEE behavior, and detection of TNP explosive, ACS Omega 3 (2018) 10306–10316.

[23] C.S. Abeywickrama, K.J. Wijesinghe, R.V. Stahelin, Y. Pang, Red-emitting pyrene–benzothiazolium: unexpected selectivity to lysosomes for real-time cell imaging without alkalinizing effect, Chem. Commun. 55 (2019) 3469–3472.

[24] (a) K. Karami, A. Ramezanpour, M. Zakariazadeh, A. Shahpiri, M. Kharaziha, A. Kazeminasab, Luminescent palladacycles containing a pyrene chromophor; synthesis, biological and computational studies of the interaction with DNA and BSA, Chem. Sel. 4 (2019) 5126–5137; (b) J. Lewkowski, M.R. Moya, A. Wrona-Piotrowicz, J. Zakrzewski, R. Kontek, G. Gajek, Synthesis, fluorescence properties and the promising cytotoxicity of pyrene-derived amino phosphonates, Beilstein J. Org. Chem. 12 (2016) 1229–1235; (c) L. Li, J. Lu, C. Xu, H. Li, X. Yang, Studies on the interaction mechanism of pyrene derivatives with human tumor-related DNA, Molecules 17 (2012) 14159–14173.

[25] M. Shellaiah, T. Simon, V. Srinivasadesikan, C.-M. Lin, K.W. Sun, F.-H. Ko, M.-C. Lin, H.-C. Lin, Novel monomeric and dimeric pyrene comprising supramolecular AIEE active nano-probes utilized in selective “off-on” trivalent metal and highly acidic pH sensing with live cell applications, J. Mater. Chem. C 4 (2016) 2056–2071.

[26] J. Lasri, S.M. Soliman, S.E. Elsilk, M. Haukka, A. El-Faham, Synthesis, crystal structure, DFT and biological activity of *E*-pyrene-1-carbaldehyde oxime and E-2-naphthaldehyde oxime, J. Mol. Struct. 1207 (2020) 127848.

[27] C. Xu, J. Gu, X. Ma, T. Dong, X. Meng, Investigation on the interaction of pyrene with bovine serum albumin using spectroscopic methods, Spectrochim. Acta A 125 (2014) 391–395.

[28] M. Sasmal, R. Bhowmick, A.S.M. Islam, S. Bhuiya, S. Das, M. Ali, Domain-specific association of a phenanthrene-pyrene-based synthetic fluorescent probe with bovine serum albumin: spectroscopic and molecular docking analysis, ACS Omega 3 (2018) 6293–6304.

[29] M. Panneerselvam, A. Kathiravan, R.V. Solomon, M. Jaccob, The role of p-linkers in tuning the optoelectronic properties of triphenylamine derivatives for solar cell applications – A DFT/TDDFT study, Phys. Chem. Chem. Phys. 19 (2017) 6153–6163.

[30] J.T. Lin, P.C. Chen, Y.S. Yen, Y.C. Hsu, H.H. Chou, M.C.P. Yeh, Organic dyes containing furan moiety for high-performance dye-sensitized solar cells, Org. Lett. 11 (2009) 97–100.

[31] Y. Li, C.-P. Zhao, H.-P. Ma, M.-Y. Zhao, Y.-R. Xue, X.-M. Wang, H.-L. Zhu, Design, synthesis and antimicrobial activities evaluation of Schiff base derived from secnidazole derivatives as potential FabH inhibitors, Bioorg. Med. Chem. 21 (11) (2013) 3120–3126.

[32] K.M. Vyasa, R.G. Joshib, R.N. Jadejaa, C.R. Prabhab, V.K. Gupta, Synthesis, spectroscopic characterization and DNA nuclease activity of Cu(II) complexes derived from pyrazolone based NSO-donor Schiff base ligands, Spectrochim. Acta A. 84 (1) (2011) 256–268.

[33] E. Pontiki, D.H. Litina, A.T. Chaviara, Evaluation of anti-inflammatory and antioxidant activities of copper (II) Schiff mono-base and copper(II) Schiff base coordination compounds of dien with heterocyclic aldehydes and 2-amino-5-methyl-thiazole, J. Enzyme Inhib. Med. Chem. 23 (6) (2008) 1011–1017.

[34] A. Pandey, D.S. Verma, A. Mishra, R.D. Dubey, Synthesis of Schiff bases of 2-amino-5-aryl-1, 3,4-thiadiazole and its analgesic, antiinflammatory, anti-bacterial and antitubercular activity, Int. J. Chem. Tech. Res. 3 (2011) 178–184.

[35] C. Chandramouli, M.R. Shivanand, T.B. Nayanbhai, B. Bheemachari, R.H. Udupi, Synthesis and biological screening of certain new triazole schiff bases and their derivatives bearing substituted benzothiazole moiety, J. Chem. Pharm. Res. 4 (2012) 151–1159.

[36] R.P. Chinnasamy, R. Sundararajan, S. Govindaraj, Synthesis, characterization, and analgesic activity of novel schiff base of isatin derivatives, J. Adv. Pharm. Technol. Res. 1 (3) (2010) 342–347.

[37] A.A. Shanty, J.E. Philip, E.J. Sneha, M.R.P. Kurup, S. Balachandran, P.V. Mohanan, Synthesis, characterization and Biological studies of Schiff bases derived from Heterocyclic moiety, Bioor. Chem., Bioorg. Chem. 70 (2017) 67–73.

[38] M.S. Nair, D.A. Johnson, Synthesis, characterization and biological studies on some metal complexes with Schiff base ligand containing pyrazolone moiety, J. Saudi Chem. Soc. 20 (1) (2016) S591–S598.

[39] R.P. Saini, V. Kumar, A.K. Gupta, G.K. Gupta, Synthesis, characterization, and antibacterial activity of a novel heterocyclic Schiff's base and its metal complexes of first transition series, Med. Chem. Res. 23 (2014) 690–698.

[40] M. Mesbaha, T. Douadia, F. Sahlib, S. Soraya, S. Boukazoulaa, Chafaa, synthesis, characterization, spectroscopic studies and antimicrobial activity of three new Schiff bases derived from Heterocyclic moiety, J. Mol. Struct. 1151 (2018) 41–48.

[41] A.-N.M.A. Alaghaza, M.E. Zayed, S.A. Alharbi, R.A.A. Ammarc, A. Chinnathambi, Synthesis, spectroscopic identification, thermal, potentiometric and saudi antibacterial activity studies of 4-amino-5-mercapto-S-triazole Schiff's base complexes, J. Mol. Struct. 1087 (2015) 60–67.

[42] D.C. Carter, J.X. Ho, Structure of serum albumin, Adv. Protein Chem. 45 (1994) 153–203.

[43] X.L. Han, P. Mei, Y. Liu, Q. Xiao, F.L. Jiang, R. Li, Binding interaction of quinclorac with bovine serum albumin: a biophysical study, Spectrochim. Acta A. 74 (2009) 781–787.

[44] C.P. Ponce, R.P. Steer, M.F. Paige, Photophysics and halide quenching of a cationic metalloporphyrin in water, Photochem. Photobiol. Sci. 12 (2013) 1079–1085.

[45] K. Douadi, S. Chafaa, T. Douadi, M. Noaimi, I. Kaabi, Azoimine quinoline derivatives: synthesis, classical and electrochemical evaluation of antioxidant, anti-inflammatory, antimicrobial activities and the DNA / BSA binding, J. Mol. Struct. 1217 (2020) 128305.

[46] N. Ugin Inba Raj, Synthesis, single crystal XRD and CT DNA/BSA binding studies of new paracetamol derivatives, J. Mol. Struct. 1208 (2020) 127911.

[47] A. Kathiravan, G. Paramaguru, R. Renganathan, Study on the binding of colloidal zinc oxide nanoparticles with bovine serum albumin, J. Mol. Struct. 934 (2009) 129–137.

[48] M. Asha Jhonsi, A. Kathiravan, R. Renganathan, Spectroscopic studies on the interaction of colloidal capped CdS nanoparticles with bovine serum albumin, Colloids Surf. B 72 (2009) 167–172.

[49] A. Kathiravan, M. Chandramohan, R. Renganathan, S. Sekar, Spectroscopic studies on the interaction between phycocyanin and bovine serum albumin, J. Mol. Struct. 919 (2009) 210–214.

[50] A. Kathiravan, R. Renganathan, Interaction of colloidal TiO_2 with bovine serum albumin: a fluorescence quenching study, Colloids Surf. A 324 (2008) 176–180.

[51] P. Manivel, S. Anandakumar, M. Ilanchelian, Exploring the interaction of the photodynamic therapeutic agent thionine with bovine serum albumin: multispectroscopic and molecular docking studies, J. Lumin. 30 (2015) 729–739.

Journal of Molecular Structure 1238 (2021) 130442

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: www.elsevier.com/locate/molstr

Y-shaped fluorophore: Synthesis, crystal structure and picric acid detection

Themmila Khamrang[a], Arunkumar Kathiravan[b,*], Chairman Ponraj[c], Dhandayutham Saravanan[c,*]

[a] Department of Chemistry, C. I. College, Bishnupur 795126, Manipur, India
[b] Department of Chemistry, Vel Tech Rangarajan Dr Sagunthala R & D Institute of Science and Technology, Avadi, Chennai 600 062, Tamil Nadu, India
[c] Department of Chemistry, National College (Affiliated to Bharathidasan University), Tiruchirappalli 620 001, India

ARTICLE INFO

Article history:
Received 27 December 2020
Revised 21 March 2021
Accepted 2 April 2021
Available online 10 April 2021

Keywords:
Sensors, Fluorescence
Inner filter effect
Phenothiazine
Picric acid

ABSTRACT

Design and synthesis of a novel fluorescent probe for the detection of picric acid is a topic of an incessant research interest. In this context, we have developed a novel 'Y' shaped D-A-D type chromophore (TP171). Phenothiazine and methoxy phenyl groups are acting as donor whilst N-substituted imidazole as acceptor unit. As synthesized TP171 show an excellent fluorescent property with a quantum yield of 24%. Intriguingly, the TP171 fluorescent probe instantaneously responded towards the detection of picric acid with a concentration of 12 μM. The detection limit is found to be 7.95 nM.

© 2021 Elsevier B.V. All rights reserved.

1. Introduction

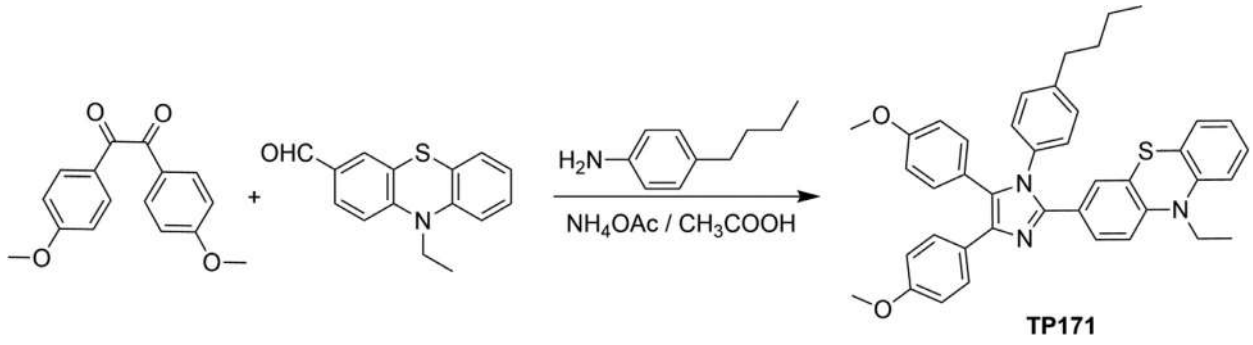
Detection of explosives mainly nitroaromatic compounds is one of the pivotal research topics due to their toxicity and fiery nature [1,2]. Generally, picric acid, 2,4,6-trinitrotoluene and dinitrotoluene are consumed as prime constituents of many explosive compositions [3,4]. The currently available explosive detection methods rely on a wide variety of instrument techniques and the real-time uses of these methods is being limited due to requirement of pre-treatment of samples, are vulnerable to interference and suffer from low sensitivity [5]. To address these issues, the attention has been directed in the recent years towards the design of a novel and efficient turn-off fluorescence probes for explosive detection [6]. The fluorescence quenching method [7] is preferred for sensing due to the non-fluorescent nature of explosives. Theoretically, the alteration of fluorescence intensity or lifetime and wavelength shift (either bathochromic or hypsochromic) upon expose to the explosives was considered to be a sensing method. There are varieties of nanomaterials, inorganic complexes and organic molecules were reported as fluorescent probes for explosive sensing [8–12] and however the searching of a new and efficient fluorescent probe is not exhausted yet. In this juncture, a 'Y' shaped donor–acceptor–donor (D–A–D) type fluorophore based on N1-substituted imidazole as acceptor unit, (C2) phenothiazine and (C4 and C5) methoxy phenyl groups act as donor groups namely 3-[1-(4-butyl-phenyl)-4,5-bis-(4-methoxy-phenyl)-1*H*-imidazol-2-yl]-10-ethyl-10*H*-phenothiazine (TP171) was successfully synthesized and meticulously characterized. The targeted fluorophore was synthesized by four component cyclo-condensation reaction. The condensation occurs between 1,2-diketone, aryl amine and aryl aldehyde in the presence of ammonium acetate and glacial acetic acid. The targeted fluorescent molecule was successfully characterized and contented results were obtained. The crystal structure is also presented to attest the 'Y' shaped structure. Further, the fully characterized fluorescent probe is successfully employed for picric acid detection through fluorescence quenching method.

2. Experimental section

The starting materials 10-ethyl-10*H*-phenothiazine-3-carbaldehyde was prepared as reported in the literature [13]. The ^{1}H and ^{13}C NMR spectra of TP171 were measured in 400 MHz and 100 MHz respectively. The NMR and mass spectra were depicted in the supporting information (Figure S1, S2 and S3). Absorption and fluorescence spectra of TP171 were recorded in a Shimadzu UV-1800 spectrophotometer and Hitachi make fluorescence spectrofluorimeter (Model: F-4500), respectively. X-ray diffraction data for TP171 was collected by using an Oxford Diffraction Xcalibur Eos Gemini diffractometer. Crystal data were

* Corresponding authors.
E-mail addresses: akathir23@gmail.com (A. Kathiravan), dsaravananchem@nct.ac.in (D. Saravanan).

https://doi.org/10.1016/j.molstruc.2021.130442
0022-2860/© 2021 Elsevier B.V. All rights reserved.



Scheme 1. Synthesis and structure of TP171.

collected at ambient temperature using graphite-monochromated Mo-Kα radiation (λ = 0.7107 Å).

3. Results and discussion

The title compound 3-[1-(4-butyl-phenyl)-4,5-bis-(4-methoxy-phenyl)-1*H*-imidazol-2-yl]-10-ethyl-10*H*-phenothiazine (TP171) was prepared by the Debus-Radziszewski multi- component reaction as illustrated in Scheme 1.

*3.1. Synthesis of 3-[1-(4-butyl-phenyl)-4,5-bis-(4-methoxy-phenyl)-1*H*-imidazol-2-yl]-10-ethyl-10*H*-phenothiazine (TP171)*

To the stirring mixture of 4,4′-dimethoxybenzil (0.97 g, 3.6 mmol), 10-ethyl-10*H*-phenothiazine-3-carbaldehyde (1.02 g, 4 mmol), ammonium acetate (3.6 g, 86.4 mmol) in 40 ml of glacial acetic acid, 4-n-butylaniline (1.07 g, 7.2 mmol) was added dropwise. The resulting mixture was placed under reflux for 12h in the inert atmosphere. On completion of reaction, the mixture was cooled to room temperature. Further, the mixture was poured into ice cold water and extracted from dichloromethane solvent. The organic layer was washed with brine and the volatile solvent was removed to obtain crude product. The residue was then purified on column chromatography using silica gel and ethyl acetate: hexane (1:3) yielding greenish yellow solid. Yield 76 %; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.54-7.52 (d, 2H, J = 8 Hz), 7.14-7.12 (d, 2H, J = 8 Hz), 7.10-7.08 (d, 3H, J = 8 Hz), 7.06-7.01 (m, 3H), 6.95-6.93 (d, 3H, J = 8 Hz), 6.82-6.80 (d, 3H, J = 8 Hz), 6.76-6.74 (d, 3H, J = 8 Hz), 3.89-3.84 (m, 2H), 3.80 (s, 3H), 3.78 (s, 3H), 2.62-2.59 (t, 2H, J = 6 Hz), 1.63-1.55 (m, 2H), 1.40-1.36 (t, 3H, J = 8 Hz), 1.33-1.26 (m, 2H), 0.95-0.91 (t, 3H, J = 8 Hz); ^{13}C NMR (400 MHz, $CDCl_3$) δ: 159.16, 158.48, 143.24, 137.59, 134.81,132.41, 129.21, 128.60, 128.27, 127.93, 127.47, 127.25, 122.97, 122.36, 115.02, 114.36, 113.79, 113.60, 55.25, 55.18, 41.82, 35.23, 33.34, 22.14, 14.11, 12.88. Element. Anal.: Calc. for $C_{41}H_{39}N_3O_2S$: C, 77.21; H, 6.16; N, 6.59; found: C, 77.17; H, 6.22; N, 6.62. ESI-MS: *m/z* Calc. for ($C_{41}H_{39}N_3O_2S$): 637.28; Found [M+H] 638.30.

3.2. Crystal structure description of TP171

An appropriate crystal of TP171 for crystallographic study was obtained from the mixture of petroleum ether and DCM (1:1) at room temperature. The crystallographic data and structural refinement details are given in Table 1 and selected bond distances and bond angles are given in **table S1**. The molecule crystallized in monoclinic system with P21/n space group. Fig. 1 shows the ORTEP diagram of the TP171 with the atomic numbering scheme and Fig. 2 shows the packing diagram of the compound along the *c*-axis. The phenothiazine, *p*-butylbenzene and two *p*-anisole moieties are connected to the middle imidazole ring with non-planar

Table 1
Crystallographic data and structure refinement parameters for TP171.

Formula	$C_{41}H_{39}N_3O_2S$
crystal system	Monoclinic
space group	P 21/n
a (Å)	17.2506(18)
b (Å)	9.5049(9)
c (Å)	22.073(2)
α (deg)	90
β (deg)°	104.135(10)
γ (deg)	90
V (Å^3)	3509.7(6)
Temperature (K)	293(2)
Wavelength (Å)	0.71073
Radiation type	Mo *K*α
Z	4
μ (mm^{-1})	0.131
ρ_{calc} mg/mm^3	1.207
Goodness-of-fit on F^2	1.016
final R indices	R_1 = 0.0649, wR_2 = 0.1407
R_1[a]	0.1123
wR_2[b]	0.1666
CCDC Number	2031794

[a] $R_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$. [b] $_wR_2 = \{\Sigma w[(F_o^2 - F_c^2)^2]/\Sigma w[(F_o^2)^2]\}_{1/}$

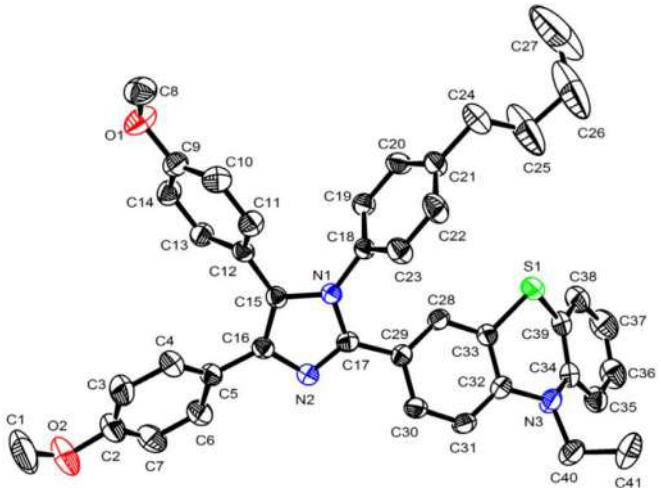


Fig. 1. ORTEP diagram of compound T171. Hydrogen atoms are omitted for clarity.

conformation. The two *p*-anisole rings bonded to the imidazole ring at the position of C15 and C16 are inclined to the imidazole ring at the dihedral angle 151.0(3), 121.8(3) respectively. The 4-butylbenzene ring (atoms C18–C23) and the imidazole ring (C15–C17/N1/N2) are twisted with respect to each other making a dihedral angle of 106.37°. However, the central six-membered ring

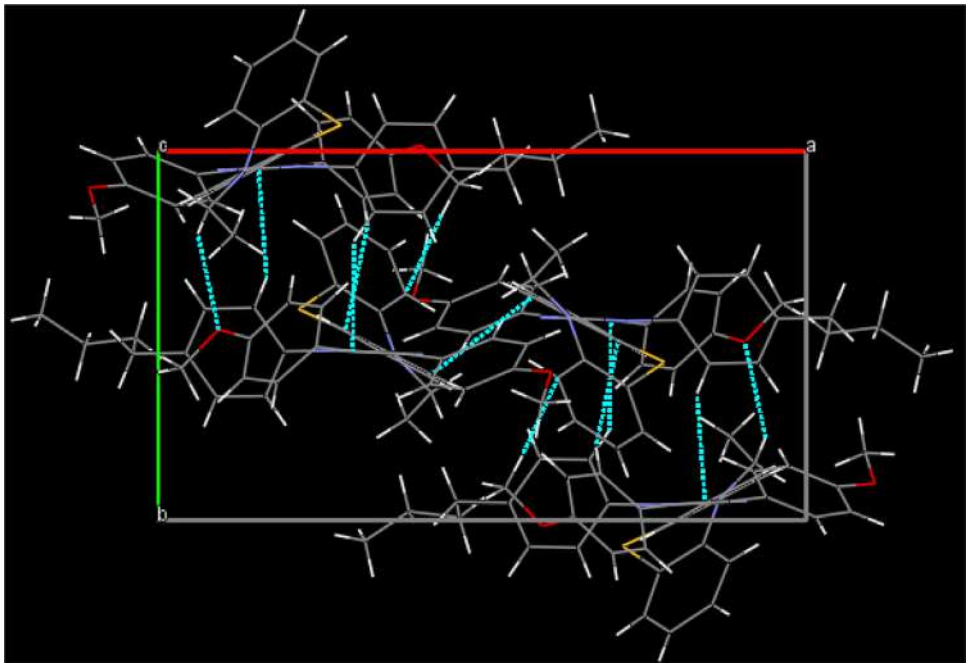


Fig. 2. Packing diagram of TP171 along the *c* axis.

(N3/C32/C33/S1/C34/C39) adopts a boat conformation. The structure of TP171 is stabilized by intermolecular C–H…O and C–H…π hydrogen bondings and are responsible for producing a three dimensional structure (Fig. 2).

3.3. Thermal properties

The thermal character of TP171 was examined by thermogravimetric (TGA) and differential scanning calorimetry (DSC) measurements under nitrogen atmosphere at a heating rate of 10 °C/min. The TGA curve of TP171 is presented in **figure S4a**. As shown in this figure, the first and second inflection temperatures of TP171 are observed at ca. 380 °C and 520 °C, respectively. This result clearly demonstrates that TP171 has good thermal stability. The DSC curve (**Figure S4b**) displays a glass transition temperature (T_g) is around 173 °C. The high T_g of TP171 designates the good morphological stability and it could be used for assorted applications.

3.4. Fluorescence quantum yield and lifetime

Under UV light illumination the TP171 exhibits bright fluorescence in both solution and powder forms (**Figure S5**). Therefore, the fluorescence quantum yield (ϕ_F) measurements are conducted in THF solvent. The value of ϕ_F is calculated using the following Eq. (1)

$$\phi_F/\phi_R = (I_S/I_R) \times (1 - 10^{-AR}/1 - 10^{-AS}) \times (\eta_S/\eta_R)^2 \quad (1)$$

where, the S and R refers to the sample (TP171) and the reference (Quinine sulphate), respectively. *A* is the fraction of light absorbed at the excitation wavelength (366 nm), *I* is the integrated area of the fluorescence spectrum, and η is refractive index of the solvent. Quinine sulphate ($\phi_R = 0.54$) was used as a standard for the TP171. Intriguingly, the measured quantum yield is of 24% and this propels us to employ the TP171 as fluorescent probe for sensing applications.

The fluorescence decay of TP171 in THF solvent is measured using TCSPC technique. The excitation and emission wavelengths are 375 nm and 450 nm, respectively. The measured fluorescence decay of TP171 is fitted with single exponential function (Fig. 3).

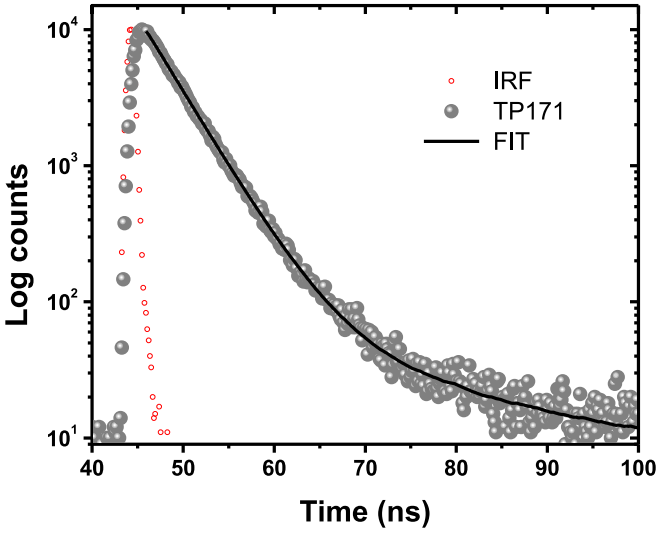


Fig. 3. Time resolved fluorescence decay of TP171 in THF solvent.

The calculated fluorescence lifetime of TP171 is 3.9 ns. Further, to verify the fluorescence properties of TP171 in solution, the radiative [$k_r = \phi_F/\tau$] and the non-radiative [$k_{nr} = (1 - \phi_F)/\tau$] rate constants were calculated using the fluorescence lifetime and the quantum yield. The calculated k_r and k_{nr} values are 6.15×10^7 s^{-1} and 1.94×10^8 s^{-1}, respectively. The k_{nr} value was higher than k_r value. The obtained higher k_{nr} value is the major reason for the lower quantum yield in solution.

3.5. Detection of picric acid

To investigate the photoinduced interaction between TP171 and PA, the UV-visible absorption and fluorescence spectroscopic technique have been employed. Fig. 4**a** shows the absorption spectrum of TP171 with various concentrations of PA. Upon titration of TP171 with increasing concentrations of PA, the absorption spectrum of

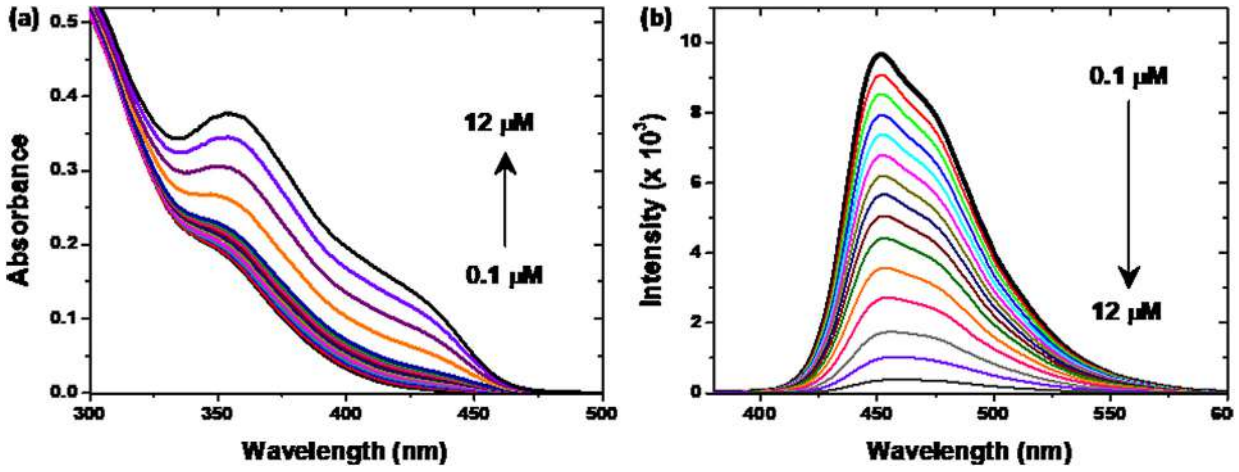


Fig. 4. (a) UV-visible absorption (b) fluorescence quenching spectra of TP171 with varying concentration of PA.

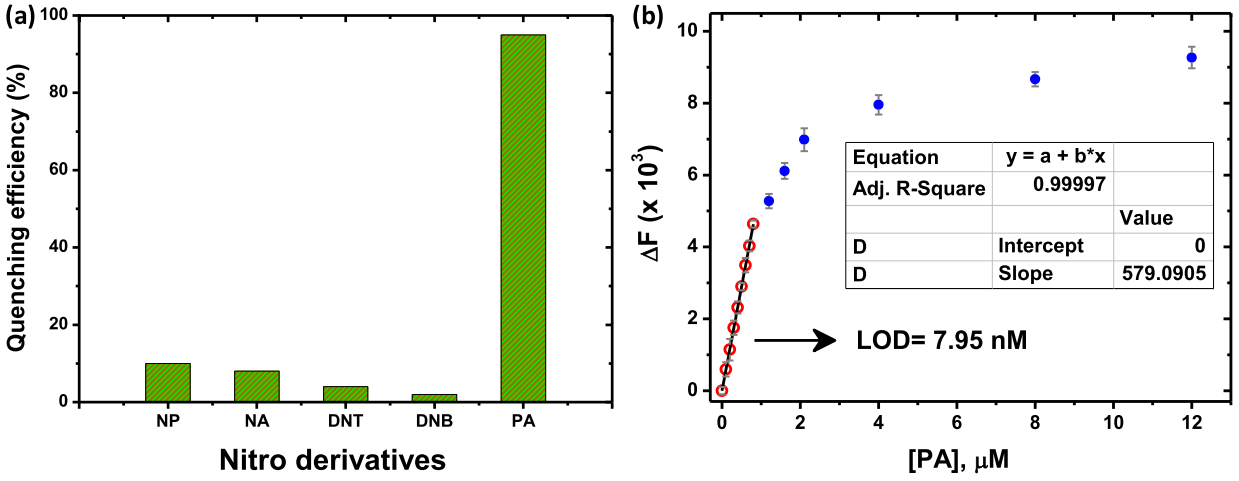


Fig. 5. (a) Selectivity and (b) fluorescence intensity responses of TP171 with varying concentration of PA.

TP171 is regularly increased without any noticeable spectral shift. This result clearly infers that there is no ground state charge transfer complex formation between TP171 and PA. With this setting, the fluorescence titration measurements also conducted to understand excited state interactions. Fig. 4**b** shows the fluorescence quenching results of TP171 exposed with various concentrations of PA.

The fluorescence of TP171 is quenched with regular intervals and completely quenched with the PA concentration of 12 μM. On the other hand, the other relevant interferences such as 4-nitrophenol (NP), 4-nitroaniline (NA), 2,4-dinitrotoluene (DNT) and dinitrobenzene (DNB), have did not interfere the fluorescence of TP171 (Fig. 5**a**). To evaluate the interference of other analytes, fluorescence intensity titration measurements of TP171 with PA were investigated in the presence of metal ions, anions and organic acids. No interference from external analytes was perceived (**Figure S6a-c**). These results unambiguously demonstrate that the PA selectively quenches the fluorescence of TP171. From the quenching results the detection limit (LOD) could be calculated using 3σ/k relationship. The plot between changes in fluorescence intensity and PA concentration yielded an upward curvature. From the linear portion of the plot, the LOD was calculated and is found to be 7.95 nM (Fig. 5**b**). When compared to relevant fluorescent probes [14–19], the obtained LOD values are consistent to the reported values as shown in Table 2**.** The obtained higher sensitivity of TP171 fluorescent probe infers that this could be a prospective candidate to detect PA even at very low concentrations. The mechanism behind the quenching could be operated by many photophysical channels such as photoinduced electron transfer [20], energy transfer and inner filter effect (IFE). However, here we have considered IFE quenching mechanism [21] which is clearly evident from the spectral overlap between absorption spectra of PA and TP171 (**Figure S6**). Further, to provide evidence for the role of IFE mechanism, the observed fluorescence intensity was corrected by using the following Eq. (2):

Table 2
Comparison of LOD values for PA detection with various fluorescent probes.

Fluorescent probes	LOD (nM)	Ref.
Imidazole derivative	170	[14]
Benzimidazole derivative	21	[15]
Coumaryl linked imidazolium salts	87	[16]
Pyrene-appended imidazolium probe	10	[17]
Imidazole-appended anthracene	20	[18]
Y-shaped D–π–A compounds	61	[19]
Y-shaped Fluorophore	7.95	This work

$$Fcor = Fobse^{(A_{ex}+A_{em})/2} \quad (2)$$

where, F_{cor} is the corrected fluorescence intensity of TP171 and F_{obs} is the observed fluorescence intensities of TP171 in the presence of PA, A_{ex} and A_{em} are the absorbance at the excitation and the emission wavelengths (λ), respectively. According to the

Table 3
Recovery of PA spiked with different real water samples by TP171.

Sample	Added (μM)	Found (μM)	Recovery (%)	RSD (%)
	0.5	0.4856	97	1.89
Lake water	1	0.9975	99	1.36
	2	2.0004	100	0.27
	4	4.0802	102	1.29
	0.5	0.0782	16	3.09
Sea water	1	0.8082	81	2.01
	2	1.8827	94	1.36
	4	4.0050	100	1.88
	0.5	0.4826	96	1.46
Pond water	1	0.9732	97	1.36
	2	1.9877	99	1.17
	4	4.0302	101	1.43
	0.5	0.4954	99	1.40
Tap water	1	0.9847	98	1.25
	2	1.9952	99	1.08
	4	4.0102	100	1.08
	0.5	0.4989	99	1.04
Distilled water	1	0.9947	99	1.24
	2	2.0047	100	1.17
	4	4.0052	100	1.03

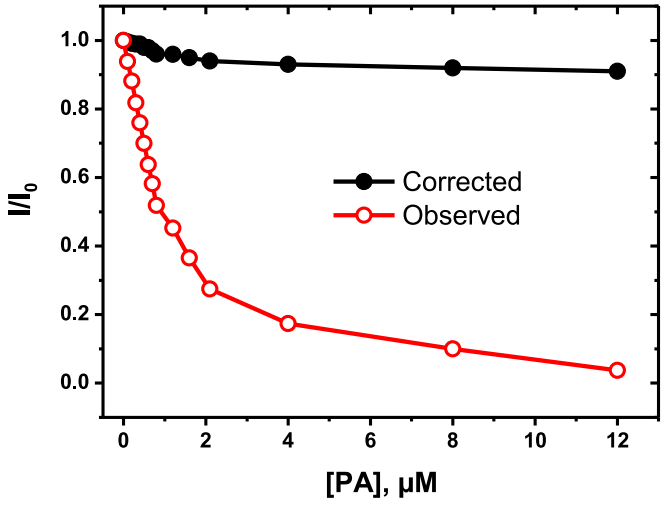


Fig. 6. A plot of observed and corrected fluorescence intensities with various PA concentrations.

Eq. (2), the fluorescence intensities were corrected and plotted with respect to the various PA concentrations (Fig. 6). The obtained plot unambiguously validated the fluorescence quenching mainly originated from IFE mechanism only. Similar type of IFE based sensors have been reported for the detection of analytes [22–24].

3.6. Detection in aqueous environment

To investigate the sensitivity of TP171 in aqueous environment, the detection of PA in various water samples was also conducted. The water samples were collected from the nearby lake, sea, pond and the tap water and double distilled water were used from the source of our laboratory. The solution of TP171 was prepared in the above said water samples followed by spiking of PA with different concentrations (0.5, 1, 2 and 4 μM) and the fluorescence intensity measurements were performed. Table 3 depicts the average recovery of PA and relative standard deviations (RSD) for three replicates of quenching experiments. Except sea water, the percentage of recovery at low level PA concentration (1 μM) was nearly 100% indicating the reliability of the TP171 for PA determination in various water samples.

4. Conclusion

A novel Y-shaped fluorescent probe was synthesized and an adequate characterization results were obtained. The fluorescent probe showed a high selectivity and sensitivity toward picric acid over other relevant nitroaromatics. The prominent fluorescence quenching upon interaction with picric acid was observed and achieved a detection limit of 7.95 nM. The inner filter effect is the main photophysical channel for detection of picric acid. Intriguingly, the fluorescent probe is successfully applied for the on-site detection of PA in real water samples. Thus, the synthesized Y-shaped fluorescent probe is a prospective material for the detection picric acid in the local environment. Further, the deployment of this material for on-site analysis is under progress with the help of 3D printed portable device.

Author statement

All the authors have participated sufficiently in the design, synthesis, and spectroscopic studies along with writing of the manuscript.

Declaration of Competing Interest

There are no conflicts of interest to declare.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2021.130442.

References

[1] M.E. Germain, M.J. Knapp, Optical explosives detection: from color changes to fluorescence turn-on, Chem. Soc. Rev. 38 (2009) 2543–2555.
[2] K. Maiti, A.K. Mahapatra, A. Gangopadhyay, R. Maji, S. Mondal, S.S. Ali, S. Das, R. Sarkar, P. Datta, D. Mandal, Simple Bisthiocarbonohydrazone as a Sensitive, Selective, Colorimetric, and Ratiometric Fluorescent Chemosensor for Picric Acids, ACS Omega 2 (2017) 1583–1593.
[3] M. Chhatwal, R. Mittal, R.D. Gupta, S.K. Awasthi, Sensing ensembles for nitroaromatics, J. Mater. Chem. C 6 (2018) 12142–12158.
[4] K.L. Dieh, E.V. Anslyn, Array sensing using optical methods for detection of chemical and biological hazards, Chem. Soc. Rev. 42 (2013) 8596–8611.
[5] D.S. Moore, Instrumentation for trace detection of high explosives, Rev. Sci. Instrum. 75 (2004) 2499–2512.
[6] X. Sun, Y. Wang, Y. Lei, Fluorescence based explosive detection: from mechanisms to sensory materials, Chem. Soc. Rev. 44 (2015) 8019–8061.

[7] M.E. Germain, M.J. Knapp, Optical explosives detection: from color changes to fluorescence turn-on, Chem. Soc. Rev. 38 (2009) 2543–2555.
[8] E.V. Verbitskiy, G.L. Rusinov, O.N. Chupakhin, V.N. Charushin, Design of fluorescent sensors based on azaheterocyclic push-pull systems towards nitroaromatic explosives and related compounds: A review, Dyes Pigment 180 (2020) 108414.
[9] V. Srinivasan, M. Asha Jhonsi, M. Kathiresan, A. Kathiravan, Nanostructured graphene oxide dots: synthesis, characterization, photoinduced electron transfer studies, and detection of explosives/biomolecules, ACS Omega 3 (2018) 9096–9104.
[10] A. Kathiravan, A. Gowri, T. Khamrang, M.D. Kumar, N. Dhenadhayalan, K.C. Lin, M. Velusamy, M. Jaccob, Pyrene-Based Chemosensor for Picric Acid—Fundamentals to Smartphone Device Design, Anal. Chem. 91 (2019) 13244–13250.
[11] Z.W. Zhai, S.H. Yang, M. Cao, L.K. Li, C.X. Du, S.Q. Zang, Rational design of three two-fold interpenetrated metal–organic frameworks: luminescent Zn/Cd-Metal–organic frameworks for detection of 2,4,6-trinitrophenol and nitrofurazone in the aqueous phase, Cryst. Growth Des. 18 (2018) 7173–7182.
[12] H. Li, B. Fu, W. Yang, L. Ding, Y. Yang, J. Dong, F. Wang, Q. Pan, A recyclable fluorescent probe for picric acid detection in water samples based on inner filter effect, Spectrochim. Acta A 226 (2020) 117575.
[13] H. Tian, X. Yang, R. Chen, Y. Pan, L. Li, A. Hagfeldt, L. Sun, Phenothiazine derivatives for efficient organic dye-sensitized solar cells, Chem. Commun. (2007) 3741–3743.
[14] H.L. Ding, L.D. Chen, N. Wang, K. Li, Y. An, C.W. Lü, Two highly selective and sensitive fluorescent imidazole derivatives design and application for 2,4,6-trinitrophenol detection, Talanta 195 (2019) 345–353.
[15] K. Jiang, S.H. Luo, C.M. Pang, B.W. Wang, H.Q. Wu, Z.Y. Wang, A functionalized fluorochrome based on quinoline-benzimidazole conjugate: From facile design to highly sensitive and selective sensing for picric acid, Dyes Pigment 162 (2019) 367–376.
[16] S. Kumari, S. Joshi, T.C. Cordova-Sintjagoc, D.D. Pant, R. Sakhuja, Highly sensitive fluorescent imidazolium-based sensors for nanomolar detection of explosive picric acid in aqueous medium, Sens. Actuators B 229 (2016) 599–608.
[17] A. Kumar, A. Pandith, H.S. Kim, Pyrene-appended imidazolium probe for 2,4,6-trinitrophenol in water, Sens. Actuators B 231 (2016) 293–301.
[18] A. Pandith, A. Kumar, H.S. Kim, Imidazole-appended 9,10-anthracenedicarboxamide probe for sensing nitrophenols and selective determination of 2,4,6-trinitrophenol in an EtOH–water medium, RSC Adv. 6 (2016) 68627–68637.
[19] S.H. Chen, K. Jiang, J.Y. Lin, K. Yang, X.Y. Cao, X.Y. Luo, Z.Y. Wang, Rational design and synthesis of Y-shaped fluorophores with multifarious emission properties and their application in the sensitive detection of PA, J. Mater. Chem. C 8 (2020) 8257–8267.
[20] D. Xi, Y. Xu, R. Xu, Z. Wang, D. Liu, Q. Shen, L. Yue, D. Dang, L. Meng, A facilely synthesized dual-state emission platform for picric acid detection and latent fingerprint visualization, Chem. Eur. J. 26 (2020) 2741–2748.
[21] A. Gowri, R. Vignesh, A. Kathiravan, Anthracene based AIEgen for picric acid detection in real water samples, Spectrochim. Acta Part A 220 (2019) 117144.
[22] L.R. Adil, P. Gopikrishna, P.K. Iyer, Receptor-free detection of picric acid: a new structural approach for designing aggregation-induced emission probes, ACS Appl. Mater. Interfaces 10 (2018) 27260–27268.
[23] S.K. Panigrahi, A.K. Mishra, Inner filter effect in fluorescence spectroscopy: as a problem and as a solution, J. Photochem. Photobiol. C 41 (2019) 100318.
[24] A.S. Tanwar, S. Hussain, A.H. Malik, M.A. Afroz, P.K. Iyer, Inner filter effect based selective detection of nitroexplosive-picric acid in aqueous solution and solid support using conjugated polymer, ACS Sens. 1 (2016) 1070–1077.

Materials Advances

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal | View Issue

Check for updates

Cite this: *Mater. Adv.*, 2022, 3, 3278

Mesoporous Fe–Al-doped cellulose for the efficient removal of reactive dyes†

Maithili Khapre,[a] Anita Shekhawat,[a] D. Saravanan,[b] Sadanand Pandey[c] and Ravin Jugade *[a]

Received 9th February 2022,
Accepted 24th February 2022

DOI: 10.1039/d2ma00146b

rsc.li/materials-advances

A highly efficient mesoporous bimetallic cellulose composite has been prepared by the simultaneous precipitation of Fe and Al metal solutions in a cellulose matrix. This Fe–Al doped cellulose (FADC) was characterized using XRD, FT-IR, SEM, EDAX, BET, and pH_{PZC}. It was used for the removal of two reactive dyes namely Reactive red 21 (RR21) and Reactive blue 19 (RB19) by a batch adsorption technique. The batch experiments were carried out to optimize the various operational parameters. The adsorption isotherm study showed that the data fitted to the Langmuir isotherm with a monolayer adsorption capacity of 117.86 mg g^{-1} for RR21 dye and 95.62 mg g^{-1} for RB19 dye. The pseudo-second-order model was fitted well while the mechanism of adsorption was deduced using the Weber–Morris model of intraparticle diffusion. Adsorption thermodynamics calculations revealed that the mechanism of adsorption is impulsive and driven by negative enthalpy change.

Introduction

Biosorption is the most convenient method to remove industrial pollutants from natural water bodies. The various advantages offered by this technique are simple design, low cost and target specificity.[1] Biosorption studies using a natural polymer in its native or modified state are gaining more importance nowadays. A number of naturally occurring polymers that are abundantly available in nature and upon proper treatment have been reported as effective adsorbents are cellulose,[2–4] chitin[5,6] and chitosan,[7–11] alginates,[12,13] clays,[14] magnetic ferrites[15,16] and resins.[17,18] They are applied for the removal of various water pollutants such as heavy metals,[19,20] dyes in single[21,22] and binary systems,[23] pesticides, paints *etc.* Formation of composites[13,14] is another useful method that combines the essential properties of two or more polymers or a blend of polymer with transition metals leading to high adsorption capacity. Among all, cellulose is the most abundant natural polymer resource with an annual yield of nearly 1.5 trillion tones which makes it an almost inexhaustible source of raw material on planet earth. Cellulose is obtained from plant, algal cell walls and also from some bacteria.[24] Cellulose is a linear chain of repeating β-D-glucopyranose units which are covalently linked through acetal functions (β-1,4-glucan).[25]

Reactive dyes are the dyes that get covalently bonded with –OH or $–NH_2$ groups of fibres thereby adhering very strongly to the fibre polymer. They are commonly used for dyeing cellulose fibres in the dye industry. Inhalation of reactive dyes may lead to respiratory problems including asthma. Many workers have reported removal of reactive dyes from water bodies using various biopolymers as well as their composites. However, most of the reported materials suffer from the limitation of very low adsorption capacities, which make them unsuitable for use as efficient adsorbents.

Doping of biopolymers with metals and metal oxides lead to enhanced porosity in their structures leading to improvement in the adsorption capacities. Many workers have reported similar kinds of composites for the efficient removal of fluoride ions.[26–28] Our research group has reported various mesoporous composites for the adsorptive removal of fluoride,[5,9] dyes,[10–13] arsenic[8] and Cr(VI).[14] In the present study, a mesoporous composite of cellulose has been prepared by co-precipitation of iron and aluminium in the cellulose matrix. This doping resulted in the enhancement of adsorption sites as well as the porosity of the material. Two reactive dyes, Reactive red 21 (RR21) and Reactive blue 19 (RB19) (Fig. 1), have been chosen as representatives of anionic dyes in this study.

Materials and method

Chemicals

All chemicals and reagents used were of analytical grade. Cellulose microcrystalline powder was obtained from Molychem India

[a] *Department of Chemistry, R. T. M. Nagpur University, Nagpur – 440010, Maharashtra, India. E-mail: ravinj2001@yahoo.co.in*
[b] *Department of Chemistry, National College, Tiruchirapalli – 620001, India*
[c] *Department of Chemistry, College of Natural Science, Yeungnam University, 280 Daehak-Ro, Gyeongsan, Gyeongbuk, 38541, Republic of Korea*

† Electronic supplementary information (ESI) available. See DOI: 10.1039/d2ma00146b

Open Access Article. Published on 24 February 2022. Downloaded on 6/29/2022 11:54:44 AM.
This article is licensed under a Creative Commons Attribution-NonCommercial 3.0 Unported Licence.

© 2022 The Author(s). Published by the Royal Society of Chemistry

Open Access Article. Published on 24 February 2022. Downloaded on 6/29/2022 11:54:44 AM.
This article is licensed under a Creative Commons Attribution-NonCommercial 3.0 Unported Licence.

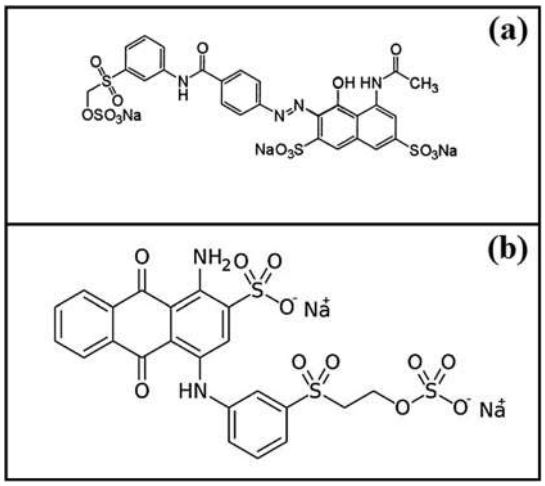


Fig. 1 Chemical structures of (a) RR21 and (b) RB19 dyes.

Pvt. Ltd. Urea, thiourea, sodium hydroxide (NaOH) pellets, ferric chloride ($FeCl_3$) anhydrous, ferrous sulfate ($FeSO_4{\cdot}7H_2O$), aluminium chloride ($AlCl_3$) anhydrous and hydrochloric acid (HCl) were obtained from S D Fine Chemicals Ltd. Mumbai. Reactive red 21 and Reactive blue 19 dyes were acquired from Loba chemicals, Mumbai. Deionized–distilled water was used for preparing all the solutions.

Instrumentation

The synthesized material FADC was characterized using various techniques. The structural and functional details could be explained on the basis of the FT-IR spectra recorded using a BrukerAlphaE spectrometer in the range 500–4000 cm^{-1}. The XRD pattern was recorded using an X-ray diffractometer system Rigaku-Miniflex 300. Surface morphology was studied using a scanning electron microscope (SEM) model TESCAN VEGA 3 SBH. Energy dispersive spectroscopy (EDAX) analysis was performed for elemental composition using an X-ray analyzer Oxford INCA Energy 250 EDS system during SEM observations. Thermal analysis was carried out using DTG-60 simultaneous DTA/TG instrument (Shimadzu) in nitrogen medium. The Brunauer–Emmett–Teller surface area was estimated by the nitrogen adsorption–desorption method on a Quantachrome Nova 2200e analyzer. The pH_{PZC} was performed using Equiptronics EQ-615. The λ_{max} and absorbance of the dyes were recorded using Equiptronics EQ-824 while Equiptronics magnetic stirrer model EQ-770 was used for batch adsorption stirring.

Preparation of adsorbent

Cellulose solution was prepared by dissolving 2.5 g of microcrystalline cellulose powder in a mixture of NaOH/thiourea/urea/H_2O in the ratio of 8 : 6.5 : 8 : 77.5 by weight with continuous stirring for about an hour. A 0.1 M $FeCl_3$ solution and 0.1 M $AlCl_3$ solution were prepared in 100 mL of 0.01M HCl respectively and heated to 60 °C. The two metal solutions were mixed in a 500 mL beaker and the cellulose solution was dripped drop-wise to neutral pH. This solution was stirred for an hour at 1000 rpm for homogenous dispersion of the Fe–Al precipitate in the cellulose matrix and then aged for 48 hours. The precipitate was washed using de-ionized water and the supernatant liquid was removed using the decantation method until it tested negative for chloride ions. The resultant precipitate, Fe–Al doped cellulose (FADC) was kept in a hot air oven for 4 hours at 50 °C followed by sieving through a -100 micron mesh to get uniform grain size.

Batch adsorption tests

Batch adsorption experiments were performed by taking 25 mL dye solution of fixed concentration administered with a known weight of FADC in a 50 mL conical flask. Adsorption systems were stirred in a magnetic stirrer equipped with magnetic needle for the desired interval of time at room temperature. The systems were centrifuged and the absorbance value of the supernatant was recorded. Triplicate readings were taken and averaged out to check reproducibility. The equilibrium adsorption efficiency in mg g^{-1} can be calculated using eqn (1).

$$q_e = (C_o - C_e) \times V/W \quad (1)$$

where C_o and C_e are the initial and equilibrium liquid phase dye concentration in mg L^{-1}, V is the dye solution volume in L, and W is the weight of adsorbent in g.

pH point of zero charge

The pH point of zero charge (pH_{PZC}) is the pH value at which there is no net charge on the surface of the adsorbent (Jeyaseelan 2018). Below this pH, the net charge on the adsorbent surface is positive while above this pH, it is negative. Hence pH_{PZC} can be used for presuming the adsorbent–adsorbate interaction. For determination of pH_{PZC} of the adsorbent, 25 mL of 0.01M sodium chloride solution was taken in a conical flask. The initial pH values were adjusted in the range 3.0 to 11.0 using dilute solutions of HCl and NaOH. To each of the flasks, 0.1g of the adsorbent was added and kept in contact for 24 hours. After that, the solutions were centrifuged and the solution pH was recorded again. The plot of change in pH as a function of initial pH crosses the X-axis at pH_{pzc}.

Adsorption isotherms

The adsorption isotherms were studied using the Langmuir, Freundlich and Temkin models. Adsorption isotherm models help in predicting various parameters such as adsorbate–adsorbent interaction, surface coverage, enthalpy of adsorption and nature of adsorption. The isotherms were studied by varying the concentration of the adsorbing systems (keeping other parameters at optimized levels) in the concentration range of 25 to 500 mg L^{-1}. The details regarding each of the isotherm models studied along with the defining variables are summarized in Table S1, ESI.†

Adsorption kinetics

For kinetics studies, pseudo first-order, pseudo second-order and intraparticle diffusion models were applied. For studying

© 2022 The Author(s). Published by the Royal Society of Chemistry

Open Access Article. Published on 24 February 2022. Downloaded on 6/29/2022 11:54:44 AM.
This article is licensed under a Creative Commons Attribution-NonCommercial 3.0 Unported Licence.

kinetics, adsorption systems were kept in contact for a time range of 10 to 90 minutes. Calculations leading to R^2 values were used to obtain the appropriate model fitting the experimental data. The detailed information regarding these three models along with equations and variables is described in the table (S2, ESI†).

Adsorption thermodynamics

Three important parameters namely ΔG, ΔH and ΔS were evaluated using the van't Hoff plot. For this purpose, adsorption systems (with optimized values of other operational parameters) were run at four different temperatures. The details of the van't Hoff plot are described in Table S3, ESI.†

Results and discussion

Characterization of FADC

FT-IR spectrum. The FT-IR spectra show typical peaks for microcrystalline cellulose (Fig. 2a) in two adsorption regions particularly in the wavelength region of 3660 cm^{-1} to 2900 cm^{-1} and 1630 cm^{-1} to 900 cm^{-1} respectively.[29] The peak at 3331 cm^{-1} is typical of the stretching vibration of the hydroxyl group in the carbohydrate polymer and also includes the intra- as well as intermolecular hydrogen bonding in polysaccharides. The band at 2895.88 cm^{-1} corresponds to the C–H stretching vibrations of all hydrocarbon constituents in cellulose. The peak at 1636.87 cm^{-1} indicates the presence of moisture in the cellulose polymer. Other absorption bands at 1429.15 cm^{-1}, 1318.89 cm^{-1} and 1043.35 cm^{-1} are due to stretching and bending vibrations of $-CH_2$, –CH, –OH and –CO bonds. The FT-IR spectra of FADC (Fig. 2b) show typical peaks of cellulose along with sharp peaks in the region below 900 cm^{-1}.[30] The peaks at 695 cm^{-1}, 610 cm^{-1} and 542 cm^{-1} correspond to Fe–O and Al–O stretching vibrations. The peaks obtained in the region 700 to 500 cm^{-1} are relatively sharp as compared to that of cellulose and show intense interaction of metals with the hydroxyl group of the cellulose polymer thus confirming the formation of the composite.

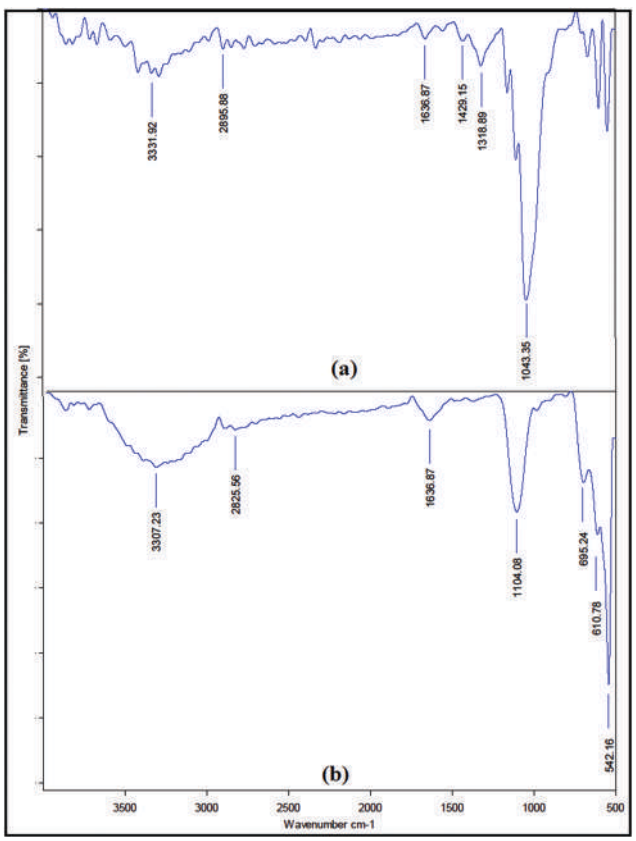


Fig. 2 FT-IR analysis of (a) cellulose and (b) FADC composite.

XRD pattern

The characteristic peaks for cellulose (Fig. 3a) were obtained at 2θ = 16.52° and 22.61° which accounts for its crystalline behaviour.[31,32] These two peaks have been attributed to (110) and (200) planes of cellulose. The XRD pattern of FADC (Fig. 3b) shows additional peaks at 2θ = 36.99° and 44.62° corresponding to the interaction between (110) planes of Fe_2O_3 and Al_2O_3 respectively.[33]

SEM and EDAX studies

The SEM micrographs of cellulose and FADC (Fig. 4) were obtained at three different resolutions as shown in Fig. 3.

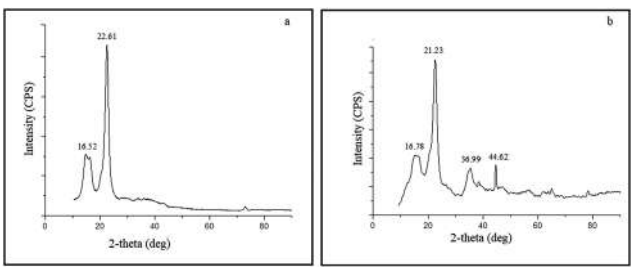


Fig. 3 X-ray diffractograms of (a) cellulose and (b) FADC.

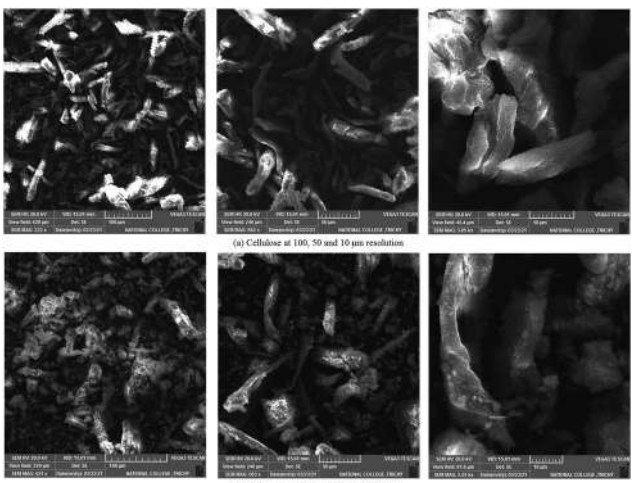


Fig. 4 SEM micrographs of (a) cellulose and (b) FADC at 100, 50 and 10 μm resolution.

© 2022 The Author(s). Published by the Royal Society of Chemistry

Open Access Article. Published on 24 February 2022. Downloaded on 6/29/2022 11:54:44 AM.
This article is licensed under a Creative Commons Attribution-NonCommercial 3.0 Unported Licence.

Cellulose shows smooth fibrous morphology that has been modified to a rough granular form on interaction with Fe and Al metal. This granular morphology is also responsible for the high surface area value obtained using the BET technique. SEM micrographs give a clear picture of transformation of cellulose having a fibrous nature into the FADC composite with a mesoporous nature.[28]

The EDAX studies of native cellulose shows the important peaks for carbon and oxygen, which are among the most abundant elements of polysaccharides (Fig. 5). The EDAX of FADC gives the compositional data. It shows the peaks for Fe, Al along with C and O. Small peaks are also obtained for S, Si and Cl which are present in a minute amount.

TGA-DTA analysis

The TGA curve of cellulose (Fig. 6a) shows a small weight loss of about 8% at 100 °C corresponding to loss of moisture and second major weight loss of about 80–85% between 220 and 400 °C, which indicate complete degradation.[34] The TGA curve of FADC shows moisture loss of about 10–15% around 150 °C and a sharp degradation curve between 300 and 400 °C. Also, the weight loss in FADC is much less as compared to that in native cellulose due to the presence of metals that remain as residue and stable at very high temperature.

The DTA curve of cellulose (Fig. 6b) shows two endothermic peaks, the first corresponding to moisture loss and the second corresponding to complete degradation. On the other hand, the DTA curve of FADC shows a sharp exothermic peak around 350 °C. This change in nature from endothermic to exothermic can be attributed to the formation of the composite with strong forces of attraction that undergo exothermic degradation on heating.

BET surface area analysis

The pore size distribution of the adsorbent material including the available surface area for adsorption and pore volume availability are important factors for obtaining good adsorption capacity. In order to determine the exact values of these factors, BET analysis was carried out. The BET surface area analysis is based on the adsorption–desorption of nitrogen gas on the surface of the adsorbent while the BJH method which is based on pore filling pressure is used to calculate the pore volume (Fig. 7).[9]

The surface area of microcrystalline cellulose powder was found to be 0.092 $m^2\ g^{-1}$. After formation of FADC composite, it increased to 205.988 $m^2\ g^{-1}$ and the pore volume was 0.3561 $cm^3\ g^{-1}$. This huge enhancement in surface area is suggestive of the porous nature of the adsorbent surface. The adsorption–desorption curve is of Type IV as per IUPAC nomination.[35,36] The average pore radius was 3.414 nm indicating mesoporous nature as per IUPAC classification. The adsorption–desorption isotherm shows a small hysteresis loop as the H_4 type. Such a loop is observed if the material is highly complex showing microporosity as well as mesoporosity. This observation is consistent with the granular appearance of the surface of the material as obtained in SEM micrographs. The isotherm shows desorption curve shifted to lower values than adsorption which can be attributed to cavitation persuaded desertion.[37] The shape of the micro- and mesopores is the deciding factor for the lower limit of the hysteresis loop.[38] Such an isotherm suggests that the micropores are initially filled with the inert gas and later multilayer physisorption and capillary condensation occurs. Also, the '*C*' value in the BET equation was evaluated as 551.036 which show strong adsorbate–adsorbent interaction.[39] The high adsorption capacity of FADC composite towards the two reactive dyes, RR21 and RB19

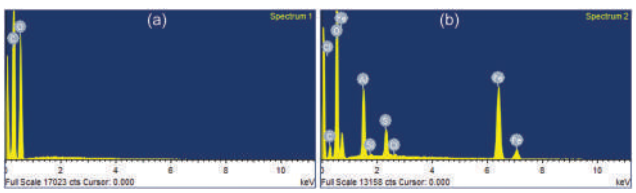


Fig. 5 EDAX elemental composition of (a) cellulose and (b) FADC.

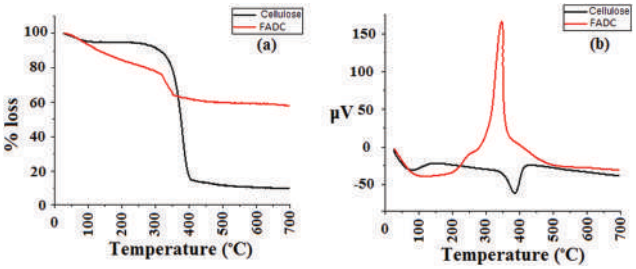


Fig. 6 TGA (a) and DTA (b) curves of cellulose and FADC.

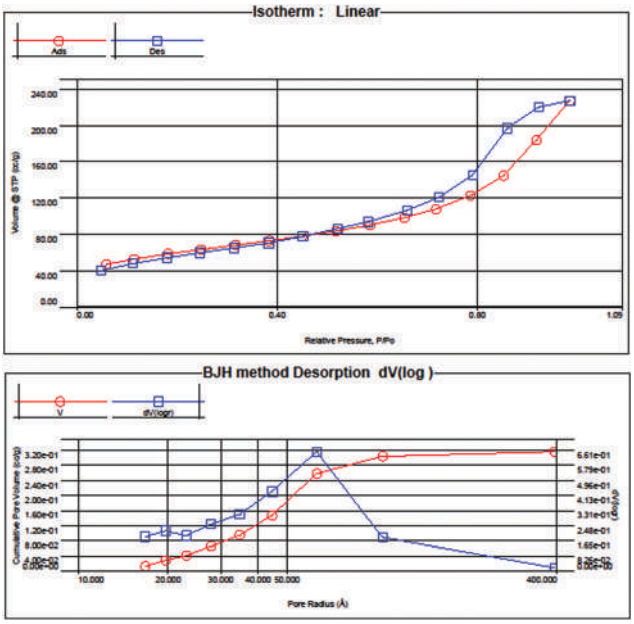


Fig. 7 BET curves and pore volume distribution.

Open Access Article. Published on 24 February 2022. Downloaded on 6/29/2022 11:54:44 AM.
This article is licensed under a Creative Commons Attribution-NonCommercial 3.0 Unported Licence.

can be safely attributed to the high surface area, large pore volume and mesoporous nature of the adsorbent.

Optimization of operational parameters

The pH_{PZC} of adsorbent and effect of dye solution pH. The pH_{PZC} of FADC was found to be 7.5 which indicates that the surface has a positive charge at pH greater than 7.5 and negative charge at pH lower than 7.5. The pH of the dye solution plays an important role in the adsorption process. For studying the effect of pH, a range of dye solutions (having a known concentration of 50 mg L^{-1}) were prepared and their pH was varied from 3.0 to 10.0 for both the dyes. To each of the systems, 100 mg of adsorbent was added and stirred for 60 minutes. After that, the systems were centrifuged until a clear solution was obtained and absorbance of the filtrate was recorded. It was observed that the maximum removal efficiency was obtained at pH 8.0 for both the dyes. (Fig. 8a and b).

Effect of contact time

The adsorbate–adsorbent systems having initial dye concentration of 50 mg L^{-1}, pH 8.0 and 100 mg of adsorbent were kept in contact for a time range of 10 to 120 minutes with constant stirring. It was observed that with increase in the contact period, the removal efficiency increased regularly up to 45 min. But after 45 minutes, there was negligible hike in removal efficiency and so the contact time of 45 minutes was optimized for both the dyes (Fig. 8c).

Effect of adsorbent dose

The effect of adsorbent dose on the process of adsorption was studied by varying the dose between 25 and 500 mg. The adsorbate–adsorbent systems (50 mg L^{-1}) having optimized values of pH and contact time was taken and varying dose of the adsorbent was administered. It was observed that with increase in dose, the removal efficiency increases. This can be

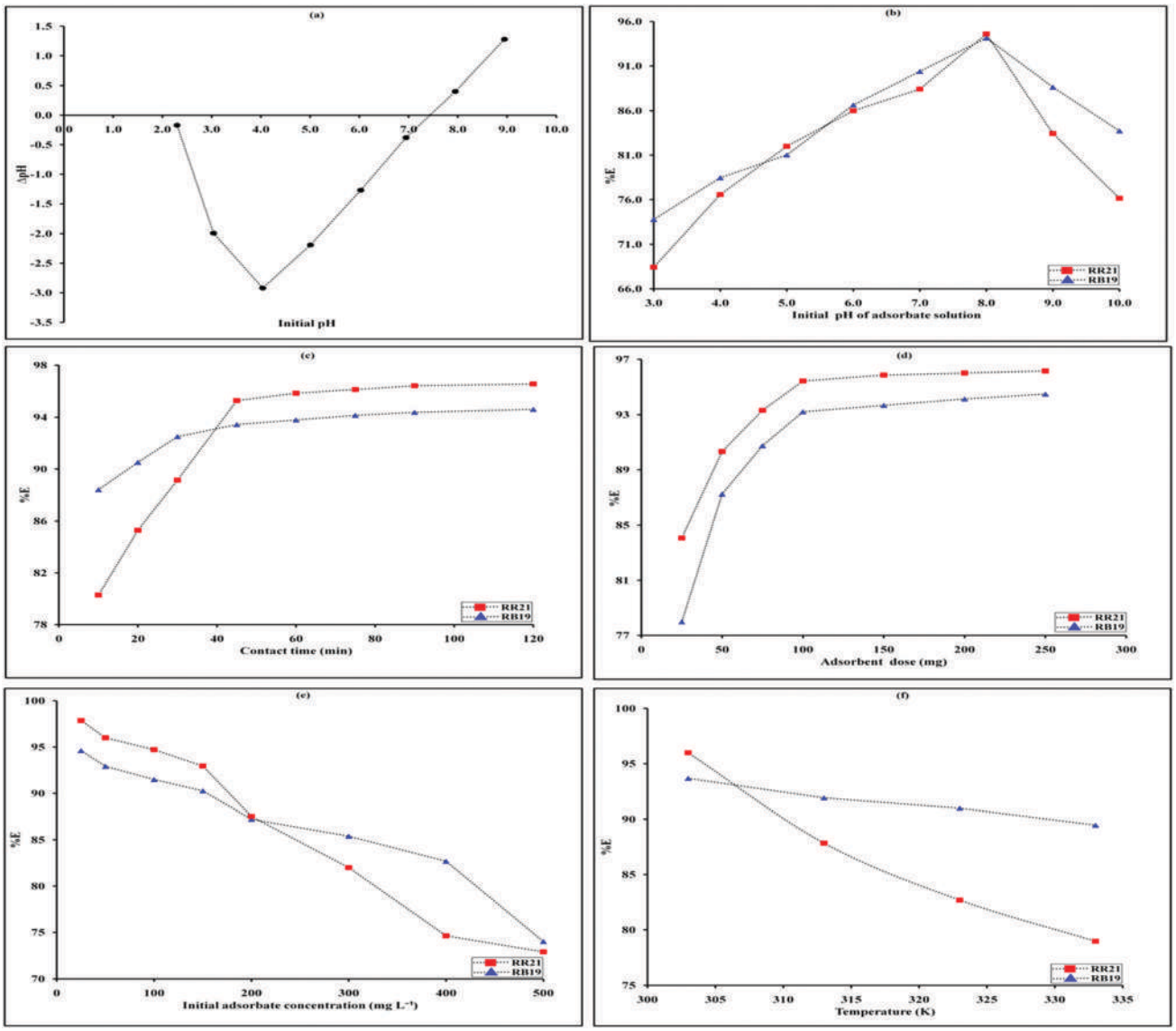


Fig. 8 Variation of dye removal efficiency for two dyes with respect to the operating condition.

© 2022 The Author(s). Published by the Royal Society of Chemistry

Open Access Article. Published on 24 February 2022. Downloaded on 6/29/2022 11:54:44 AM.
This article is licensed under a Creative Commons Attribution-NonCommercial 3.0 Unported Licence.

attributed to the availability of a large number of active adsorption sites as compared to the adsorbing dye molecules (Fig. 8d). But after a certain dose, there seems to be negligible increase in the removal efficiency as almost 95% of both the dyes have been adsorbed. This indicates establishment of equilibrium in the adsorbent–adsorbate interface. An adsorbent dose of 100 mg was optimized for both the dyes as after this there was only slight increase in the removal efficiency.

Effect of initial adsorbate concentration

The initial dye concentration was varied from 25 to 500 mg L^{-1}. Increase in dye concentration led to percentage removal decrease (Fig. 8e). This observation is consistent with the availability of the active adsorption sites on the surface of the adsorbent. As the number of incoming dye molecules increase, the availability of active adsorption sites decreases thus lowering the removal efficiency. With this context, an initial dye concentration of 50 mg L^{-1} was fixed for further studies.

Effect of temperature

To study the effect of temperature, the temperature was varied from 303 to 333 K. It was observed that the maximum removal efficiency was obtained at 303 K and later on it decreased (Fig. 8f). This observation can be correlated with the usual observation that as the temperature increases, adsorption decreases. With increase in temperature, the kinetic energy of the molecules increases and hence their binding to the surface of the adsorbent decreases.

Adsorption isotherms

On subjecting the data obtained in batch adsorption experiments to the Langmuir, Freundlich, Temkin and Dubinin–Radushkevich (D–R) isotherm models (Fig. S1, ESI†), it was observed that the Langmuir model is best fit model for both the dyes. This was concluded from the high values of correlation coefficient, $R2$. Also the RL values lie between 0 and 1 which are indicative of the favourability of the adsorption process. The maximum monolayer adsorption capacity (q_{max}) was found to be 117.86 mg g^{-1} for RR21 and 95.62 mg g^{-1} for Rb19 dye respectively. Furthermore, the values of $n > 1$ obtained from the Freundlich isotherm suggest strong interaction between the FADC and dye molecules. The values of enthalpy of adsorption (b) more than 120 J mol^{-1} given by the Temkin isotherm suggest that some extent of chemisorption is also involved during uptake of dyes. The average free energy of adsorption in excess of 100 kJ mol^{-1} obtained from D–R model also supports chemisorption phenomenon. The values of different parameters evaluated for the four isotherm models studied are summarized in Table 1.

Adsorption kinetics

On studying the three kinetic models, it was observed that the pseudo second-order is the best fit kinetic model (Fig. S2, ESI†). This was concluded on the basis of following two reasons. First, the values of correlation coefficient, $R2$ were found to be unity and second, the values of calculated q_e obtained were found to be in good agreement with the values of experimental q_e for both the dyes under study. The details of the variables evaluated using the kinetic models are summarized in Table 2.

Table 1 Isotherm studies for RR21 and RB19

Parameters	RR21	RB19
Langmuir isotherm model		
K_L (L g^{-1})	1.346	1.225
q_m (mg g^{-1})	117.86	95.62
R_L	0.035	0.009
R^2	0.990	0.990
Freundlich isotherm model		
K_F ($mg^{1-1/n}$ g^{-1} L^{-1})	5.189	8.566
$1/n$	0.643	0.497
n (g L^{-1})	1.556	2.013
R^2	0.979	0.973
Temkin isotherm model		
K_T (L g^{-1})	0.496	1.098
b (J mol^{-1})	120.45	159.19
R^2	0.942	0.949
D–R isotherm model		
q_m (mg g^{-1})	47.48	46.13
E (kJ mol^{-1})	566	937
R^2	0.697	0.700

Table 2 Summary of adsorption kinetic models studied for RR21 and RB19 dyes

Dyes	RR21	RB19
Experimental q_e	11.61	12.00
Pseudo first order		
k_1 (min^{-1})	0.124	0.099
q_e (calculated)	1.45	2.54
R^2	0.927	0.953
Pseudo second order		
k_2 (mg g^{-1} min^{-1})	18.353	14.590
q_e (calculated)	11.92	12.39
R^2	1.000	1.000
Intraparticle diffusion		
i_{int} (mg g^{-1} $min^{-1/2}$)	0.095	0.268
R^2	0.840	0.817

Adsorption thermodynamics

Adsorption thermodynamics shows that the process is spontaneous with negative values of ΔG at all the four temperatures were studied (Fig. S3, ESI†). The values of adsorption enthalpy and entropy are also found to be negative suggesting an exothermic process with decrease in the randomness of the mobile dye molecules due to adsorption on the surface of

Table 3 Adsorption thermodynamics of RR21 and RB19

Temperature	303 K	313 K	323 K	333 K
RR21				
ΔG (kJ mol^{-1})	−7.25	−6.51	−5.77	−5.03
ΔH (kJ mol^{-1})	−29.673			
ΔS (kJ mol^{-1} K^{-1})	−0.074			
RB19				
ΔG (kJ mol^{-1})	−6.61	−5.52	−4.43	−3.34
ΔH (kJ mol^{-1})	−39.637			
ΔS (kJ mol^{-1} K^{-1})	−0.109			

© 2022 The Author(s). Published by the Royal Society of Chemistry

Open Access Article. Published on 24 February 2022. Downloaded on 6/29/2022 11:54:44 AM.
This article is licensed under a Creative Commons Attribution-NonCommercial 3.0 Unported Licence.

Table 4 Comparison with reported work on reactive dyes

Reactive dye	Adsorbent	Maximum adsorption capacity (q_m)	Ref.
RB19	Co-electrospun nanofibres	61.2 mg g^{-1}	40
	Lemna minor	9.45 mg g^{-1}	41
	Fly ash (FA)	47-86 mg g^{-1}	42
	Metal hydroxide waste sludge	91.0 mg g^{-1}	43
	Fe–Al doped cellulose (FADC)	95.62 mg g^{-1}	Present work
RR133	Aluminium residue	6.88 mg g^{-1}	44
RR 120	Sugarcane bagasse powder	94.62%	45
RR 228	Fe_3O_4-aminopropyltriethylsilane (Fe_3O_4/APTES)	51.4 mg g^{-1}	46
RR 195	Pinus sylvestris linneo	6.69 mg g^{-1}	47
RR 21	Fe–Al doped cellulose (FADC)	117.86 mg g^{-1}	Present work

FADC. The values of ΔH are largely negative as compared to $T\Delta S$ which indicates that the process is enthalpy driven in nature (Table 3).

Conclusions

An Fe–Al doped cellulose (FADC) adsorbent for adsorptive removal of RR21 and RB19 has been fabricated successfully by using the simultaneous co-precipitation method. FADC was characterized using various techniques which confirmed the formation of a composite having Fe and Al as intrinsic constituents. SEM micrographs and EDAX analysis showed the morphological transformation and composition of FADC respectively. BET surface area analysis using the nitrogen adsorption–desorption method revealed good surface area and pore volume and also confirmed the mesoporous nature of the adsorbent. Furthermore, the value of pH_{PZC} was found to be 7.5 which favoured the adsorption of RR21 and RB19 at slightly basic pH. The adsorption isotherm was fitted to the Langmuir model with an adsorption capacity of 117.86 mg g^{-1} for RR21 and 95.62 mg g^{-1} for the RB19 dye respectively. Kinetics followed the second-order model while the thermodynamics of adsorption revealed spontaneous adsorption phenomena with negative values of enthalpy and entropy which indicated an exothermic nature and an ordered manner of process respectively. Various materials reported in the literature for the adsorptive removal of reactive dyes have been summarized and compared with FADC in Table 4.

Author contributions

MK and AS: methodology, validation, investigation, writing first draft. DS: formal analysis, resources. SP: review and editing. RJ: conceptualization, writing: review and editing, supervision.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The authors are thankful to R. T. M. Nagpur University for URPS No. Dev. 2117, DST, New Delhi for DST-FIST grant and UGC, New Delhi, for UGC-SAP grant. MK acknowledges DST – INSPIRE while AS acknowledges CSIR-RA funding.

References

1 M. H. Rahaman, M. A. Islam, M. M. Islam, M. A. Rahman and S. M. Nur Alam, *Curr. Res. Green Sustainable Chem.*, 2021, **4**, 100119.
2 B. Baghaei and M. Skrifvars, *Molecules*, 2020, **25**, 2836.
3 Y. Tian, M. Wu, R. Liu, D. Wang, X. Lin, W. Liu, L. Ma, Y. Li and Y. Huang, *J. Hazard. Mater.*, 2011, **185**, 93.
4 M. Kaiser, A. Mudasir, A. Suhail and I. Saiqa, *RSC Adv.*, 2019, **9**, 7890.
5 A. Shekhawat, S. Kahu, D. Saravanan and R. Jugade, *RSC Adv.*, 2016, **6**, 18936.
6 X. Yang, J. Liu, Y. Pei, X. Zheng and K. Tang, *Energy, Environ. Mater.*, 2020, **3**, 492.
7 S. A. Qamar, M. Ashiq, M. Jahangeer, A. Riasat and M. Bilal, *Case Stud. Chem. Environ. Eng.*, 2020, **2**, 100021.
8 A. Shekhawat, S. Kahu, D. Saravanan and R. Jugade, *Carbohydr. Polym.*, 2017, **172**, 205.
9 S. Korde, S. Tandekar and R. M. Jugade, *J. Environ. Chem. Eng.*, 2020, **8**, 104360.
10 S. Korde, S. Deshmukh, S. Tandekar and R. Jugade, *Carbohydr. Polym. Technol. Appl.*, 2021, **2**, 100081.
11 M. A. Khapre and R. M. Jugade, *Water Sci. Technol.*, 2020, **82**, 715.
12 S. H. Vithalkar and R. M. Jugade, *Mater. Today: Proc.*, 2020, **29**, 1025.
13 M. A. Khapre, S. Pandey and R. M. Jugade, *Int. J. Biol. Macromol.*, 2021, **190**, 862.
14 S. Korde, S. Tandekar, C. Jeyaseelan, D. Saravanan and R. Jugade, *Mater. Lett.*, 2022, **311**, 131513.
15 M. Kumar, H. S. Dosanjh and H. Singh, *Fibers Polym.*, 2019, **20**, 739.
16 M. Kumar and H. Singh, *Environ. Sci.: Water Res. Technol.*, 2020, **6**, 491.
17 S. Popa, M. E. Radulescu-Grad and A. Perdivara, *Sci. Rep.*, 2021, **11**, 5889.
18 X. Feng, W. Fugang, C. Shuai, J. Shengui and X. Weihong, *J. Chem. Eng. Data*, 2017, **62**, 4266.
19 H. A. Alalwan, M. A. Kadhom and A. H. Alminshid, *J. Water Supply: Res. Technol. – AQUA*, 2020, **69**, 99.

© 2022 The Author(s). Published by the Royal Society of Chemistry

Open Access Article. Published on 24 February 2022. Downloaded on 6/29/2022 11:54:44 AM.
This article is licensed under a Creative Commons Attribution-NonCommercial 3.0 Unported Licence.

20 N. A. A. Qasem, R. H. Mohammed and D. U. Lawal, *npj Clean Water*, 2021, **4**, 36.
21 V. Gupta and K. Suhas, *J. Environ. Manage.*, 2009, **90**, 2313.
22 E. Forgacs, T. Cserhati and G. Oros, *Environ. Int.*, 2004, **30**, 953.
23 M. Kumar, H. S. Dosanjh and H. Singh, *J. Inorg. Organomet. Polym. Mater.*, 2018, **28**, 880.
24 L. M. J. Kroon-Batenburg and J. Kroon, *Glycoconjugate J.*, 1997, **14**, 677.
25 D. W. O'Connell, C. Birkinshaw and T. F. O. Dwyer, *Bioresour. Technol.*, 2008, **99**, 6709.
26 S. Raghav, S. Nehra and D. Kumar, *Process Saf. Environ. Prot.*, 2019, **127**, 211.
27 A. Vázquez-Guerrero, R. Cortés-Martínez, R. Alfaro-Cuevas-Villanueva, E. M. Rivera-Muñoz and R. Huirache-Acuña, *Water*, 2021, **13**, 89.
28 N. Sankararamakrishnan, I. Srivastava and S. Mishra, *Int. J. Biol. Macromol.*, 2019, **128**, 556.
29 V. Hospodarova, E. Singovszka and N. Stevulova, *Am. J. Anal. Chem.*, 2018, **9**, 303.
30 M. Chaudhary, S. Rawat, N. Jain, A. Bhatnagar and A. Maiti, *Carbohydr. Polym.*, 2019, **216**, 140.
31 L. Du, J. Wang, Y. Zhang, C. Qi, M. P. Wolcott and Z. Yu, *Nanomaterials*, 2017, **7**, 51.
32 Y. Guo, X. Xing, Y. Shang, B. Gao, L. Zhang, Q. Yue and Z. Wang, *J. Hazard. Mater.*, 2019, **379**, 120634.
33 J. Zhao, Z. Lu, X. He, X. Zhang, Q. Li, T. Xia and C. Lu, *ACS Sustainable Chem. Eng.*, 2017, **5**, 7723.
34 G. F. Leal, L. A. Ramos, D. H. Barrett, A. A. S. Curvelo and C. B. Rodella, *Thermochim. Acta*, 2015, **616**, 9.
35 K. Sing and R. Williams, *Adsorpt. Sci. Technol.*, 2004, **22**, 773.
36 M. Thommes, K. Kaneko, A. Neimark, J. Olivier, F. Rodriguez-Reinoso, J. Rouquerol and K. Sing, *Pure Appl. Chem.*, 2015, **87**, 1051.
37 S. Ahmed and A. Iqbal, *Global Challenges*, 2018, **1800056**, 1.
38 M. Kruk, M. Jaroniec, T. Kim and R. Ryoo, *Chem. Mater.*, 2003, **15**, 2815.
39 M. Thommes, B. Smarsly, M. Groenewolt, P. Ravikovitch and A. Neimark, *Langmuir*, 2006, **22**, 756.
40 R. A. Hakro, M. Mehdi, R. F. Qureshi, R. B. Mahar, M. Khatri, F. Ahmed, Z. Khatri and I. S. Kim, *Mater. Res. Express*, 2021, **8**, 055502.
41 D. Balarak, Y. Mahdavi and A. Joghataeic, *Archieves. Hyg. Sci.*, 2015, **4**, 199.
42 N. Dizge, C. Aydiner, E. Demirbas, M. Kobya and S. Kara, *J. Hazard. Mater.*, 2008, **150**, 737.
43 S. C. Santos, V. J. Vílar and R. A. Boaventura, *J. Hazard. Mater.*, 2008, **153**, 999.
44 T. C. Silva, T. J. M. Fraga, M. Carvalho, V. L. Silva and M. A. Da Motta, *Lat. Am. Appl. Res.*, 2018, **48**, 101.
45 S. Ahmed, Y. C. Wong and K. V. Veloo, *IOP Conf. Ser.: Earth Environ. Sci.*, 2018, **140**, 012027.
46 T. G. Yan and L. J. Wang, *Adsorpt. C.I. Water Sci. Technol.*, 2014, **69**, 612.
47 O. Aksakal and H. Ucun, *Equilib. J. Hazard. Mater.*, 2010, **181**, 666.

© 2022 The Author(s). Published by the Royal Society of Chemistry

Materials Advances

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal

Check for updates

Cite this: DOI: 10.1039/d2ma00508e

Chitosan entrapped microporous activated carbon composite as a supersorbent for remazol brilliant blue R

Pradip M. Nandanwar,[a] D. Saravanan,[b] Pankaj Bakshe[a] and Ravin M. Jugade *[a]

Received 5th May 2022,
Accepted 24th May 2022

DOI: 10.1039/d2ma00508e

rsc.li/materials-advances

In this work, we synthesized a chitosan-activated carbon composite (Cs–C) using sodium tripolyphosphate (STTP) as a crosslinker. The Cs–C was characterized through Fourier transform infrared, X-ray diffraction, scanning electron microscopy, energy dispersive X-ray spectroscopy, and the pH point of zero charge for the physicochemical, structural, and morphological analyses. The material was subjected to the adsorption of Remazol Brilliant Blue R (RBBR) dye. The effects of the solution pH, adsorbent dose, contact time, temperature, and dye concentration were examined. The equilibrium data was described well by the Langmuir isotherm model with a coefficient of determination (R^2) value of 0.9994 and chi-square value of 6.94. At near-neutral pH, with a contact time of only 60 min at room temperature, the material showed an extremely high adsorption capacity of 540.3 mg g^{-1} which is much higher in contrast to all the previously reported materials. The kinetics of uptake was well-described by the pseudo-second-order model chemisorption model. Evaluation of the thermodynamic parameters reflected the spontaneous, endothermic and entropy-driven nature of the adsorption.

Open Access Article. Published on 26 May 2022. Downloaded on 6/29/2022 11:53:27 AM.
This article is licensed under a Creative Commons Attribution-NonCommercial 3.0 Unported Licence.

Introduction

Being one of the essential aspect of all lives on earth, water defines the characteristic of human beings. With a very limited accessibility of pure water on earth, unfortunately the rapid increase in water pollution is posing a challenge due to industrial and anthropogenic contamination in water bodies.[1] The main toxicants include dyes,[2] pesticides,[3] colouring matter,[4] and oils.[5] Among the various concerns, textile industries are the major contributors towards the environmental pollution due to the discharge of various dyes in water bodies.[6] The dyeing industry waste is one of the major concerns of the textile industry, including coloured waste water, which causes hazardous effects on the environment. Majority of these dyes have a complex aromatic molecular structure, and are water soluble, non-biodegradable, and toxic in nature; therefore, it has become highly important to remove these dyes from water effluents to make the environment safe and clean in terms of protecting the livelihoods of all creatures.[7]

Reactive dyes are used for dyeing cellulosic fibres in textile industries to a large extent, and these are usually characterized by azo bonds. The colour of azo dyes is due to a chromophoric effect of the azo group. First, the dyes are treated with cellulose and then are made to interact with the fibrous material of the cellulose.[8] The reaction occurs by the formation of a covalent bond between the dye molecule and the fibre. Adsorption, electrochemical oxidation, biological treatment, coagulation, physicochemical flocculation, precipitation, ozonation, and other comprehensive technologies have been applied and critically reviewed recently.[9–13] Conventional wastewater treatment methods have a low removal efficiency.[14] The adsorption process was found to be one of the effective techniques among several chemical, bacteriological, catalytic, and physical methods that have been successfully employed for the removal of colours from waste water due to its low cost, ease of operation, greater efficiency, and non-generation of toxic materials.[15]

Several adsorbents have been tested for their applicability in dye adsorption from effluents, such as activated sludge,[16] alum,[17] coal ash,[18] silicates,[19] acetyl acetone,[20] and graphene-based nanomaterials.[21] The ability of chitosan to remove contaminants from effluents is highly appreciated due to its diverse functionality, including amino and hydroxyl functional groups in the molecule for forming various interactions, including hydrogen bonding, electrostatic interaction, and van der Waals forces of attraction.[22] However, the insufficient strength and stability in acidic medium hinder the greater application of chitosan.[23] In order to resolve these issues and to improve the physicochemical properties of chitosan, its modifications using various crosslinkers as well as by composite formation with mechanically and thermally stable materials are the most obvious

[a] Department of Chemistry, RTM Nagpur University, Nagpur, 440033, India. E-mail: ravinj2001@yahoo.co.in
[b] National College, Tiruchirapalli, Tamilnadu, India

© 2022 The Author(s). Published by the Royal Society of Chemistry

Open Access Article. Published on 26 May 2022. Downloaded on 6/29/2022 11:53:27 AM.
This article is licensed under a Creative Commons Attribution-NonCommercial 3.0 Unported Licence.

pathways. Composites of chitosan with activated charcoal can be applied to improve the surface structure, morphology, and surface-assimilative property of chitosan biopolymers.[24] Adding a crosslinking agent enhances the chemical stability and leads to microstructure improvement for its improved adsorption performance.[25] Several studies have been carried out on the adsorption of water pollutants using chitosan-activated charcoal composites involving the removal of dyes in the recent years.[26–28]

Recently, we reported a composite of cellulose fabricated by the co-precipitation of Fe and Al in the cellulose material for the detoxification of various reactive dyes with high adsorption capacities.[29] In this study, we used a hybrid approach involving crosslinking chitosan with sodium tripolyphosphate (STTP) for imprioved mechanical strength and for entrapping activated carbon through the enhanced surface area and porosity, leading to a supersorbent with excellent confiscation capability for RBBR dye.

Experimental

Reagents

All the chemicals and reagents used in the experiments were of analytical grade. Chitosan with a degree of deacetylation of >90% was purchased from Sisco Research Laboratory, India. Acetic acid and 25% ammonia solution were obtained from SD Fine Chemicals Ltd (India). Activated charcoal, STTP, and Remazol brilliant blue R dye were acquired from Loba Chemie (India). All the chemicals were used without further purification and deionized distilled water was used throughout the studies.

Instruments

FT-IR spectra were obtained using a Bruker AlphaE spectrometer (USA) for an average of 23 scans. A Rigaku Miniflex 300 system (Tokyo, Japan) was used for the XRD analysis. The surface morphology was studied by a scanning electron microscopy (SEM) system model TESCAN VEGA 3 SBH (Czech Republic), while EDX analysis was performed by an X-ray analyzer on an Oxford INCA Energy 250 EDS system during the SEM observations. Thermal analysis was carried out using a DTG-60 simultaneous DTA/TG instrument (Shimadzu, Japan) at a scan rate of 20 °C min^{-1} and in a nitrogen flow rate of 100 mL min^{-1}. The Brunauer–Emmett–Teller surface area was estimated by the nitrogen adsorption–desorption method on a Quantachrome Nova 2200e analyzer (Florida). The estimation of dye concentration was carried out using an Equiptronics EQ-824 instrument (India) at the dye absorption maxima of 594 nm.

Synthesis of the adsorbent

Chitosan solution was prepared using a previously reported method[30] by dissolving 5 g chitosan in 500 mL of 2% acetic acid. To dissolve chitosan in acetic acid, it was added gradually by keeping it under magnetic stirring for 60 min. After the complete dissolution of chitosan in acetic acid, 2.5 g activated charcoal was added step by step and the solution was kept on gentle stirring for 30 min. The resultant solution was dripped into a beaker containing 1000 mL of 6% ammonia solution with the help of a syringe, leading to the formation of spherical beads. The fresh beads were rinsed with distilled water several times for removal of all traces of ammonia. Next, 250 mL of 1% solution of STTP crosslinker was added to the beads and with very gentle stirring on a magnetic stirrer at 40 °C for 2 h. The resulting Cs–C beads were washed with distilled water several times and dried overnight in a hot air oven at 50 °C. The beads were crushed by a pestle and mortar and sieved to −100 micron mesh before being used in the adsorption experiments.

Batch adsorption tests

In each experiment, 25 mL dye solution of a predecided concentration along with a known weight of Cs–C, was stirred on a magnetic stirrer for a predetermined time. It was then filtered and the residual concentration of the solution was evaluated spectrophotometrically. Triplicate observations were obtained and the mean values were reported. The equilibrium adsorption efficiency in mg g^{-1} was calculated as-[31]

$$q_e = \frac{(C_0 - C_e)V}{W}$$

where Co (mg L^{-1}) and Ce (mg L^{-1}) are the dye concentration initially and at equilibrium, respectively, while W (g) is the weight of adsorbent used in the respective study.

Trial runs were performed to compare the adsorption efficiencies of unmodified and sequentially modified adsorbents for the RBBR dye. For this, 50 mg L^{-1} dye solution was equilibrated for 60 min with 100 mg of unmodified chitosan, activated charcoal, STTP crosslinked chitosan, and Cs–C composite in different flasks. The solution phase concentrations in each flask were determined after filtration and the adsorption efficiency was calculated for each of them.

The pH_{PZC} of Cs–C was determined by a previously reported method[32] in order to establish the surface charge on the adsorbent. For this, 50 mL 0.1 M NaCl solutions with varying initial pH from 2.0 to 9.0 were taken in a series of conical flasks. These solutions were stirred with 100 mg of Cs–C for 24 h. The final pH of the supernatant solutions were measured. A graph was plotted of the change in pH as a function of the initial pH, with the point it intersects the *x*-axis called the pH point of zero charge.

For studying the effect of the initial solution pH on the adsorption efficiency, a series of dye solutions of 100 mg L^{-1} were prepared and their pH was varied from 4.0 to 9.0. To each of the systems, 100 mg of Cs–C adsorbent was administered and equilibrated for 30 min. After that, the systems were filtered and the absorbance values were obtained.

The kinetics of adsorption was studied by equilibrating 100 mg L^{-1} dye solution with 25, 50, and 100 mg of Cs–C from 5 to 150 min. The residual solution phase dye concentration was determined after filtration.

In order to study the effect of the initial dye concentration, various dye concentrations from 20 to 400 mg L^{-1} were equilibrated with 25, 50, and 100 mg Cs–C for 60 min and then the dye concentration in the solution was determined.

© 2022 The Author(s). Published by the Royal Society of Chemistry

Open Access Article. Published on 26 May 2022. Downloaded on 6/29/2022 11:53:27 AM.
This article is licensed under a Creative Commons Attribution-NonCommercial 3.0 Unported Licence.

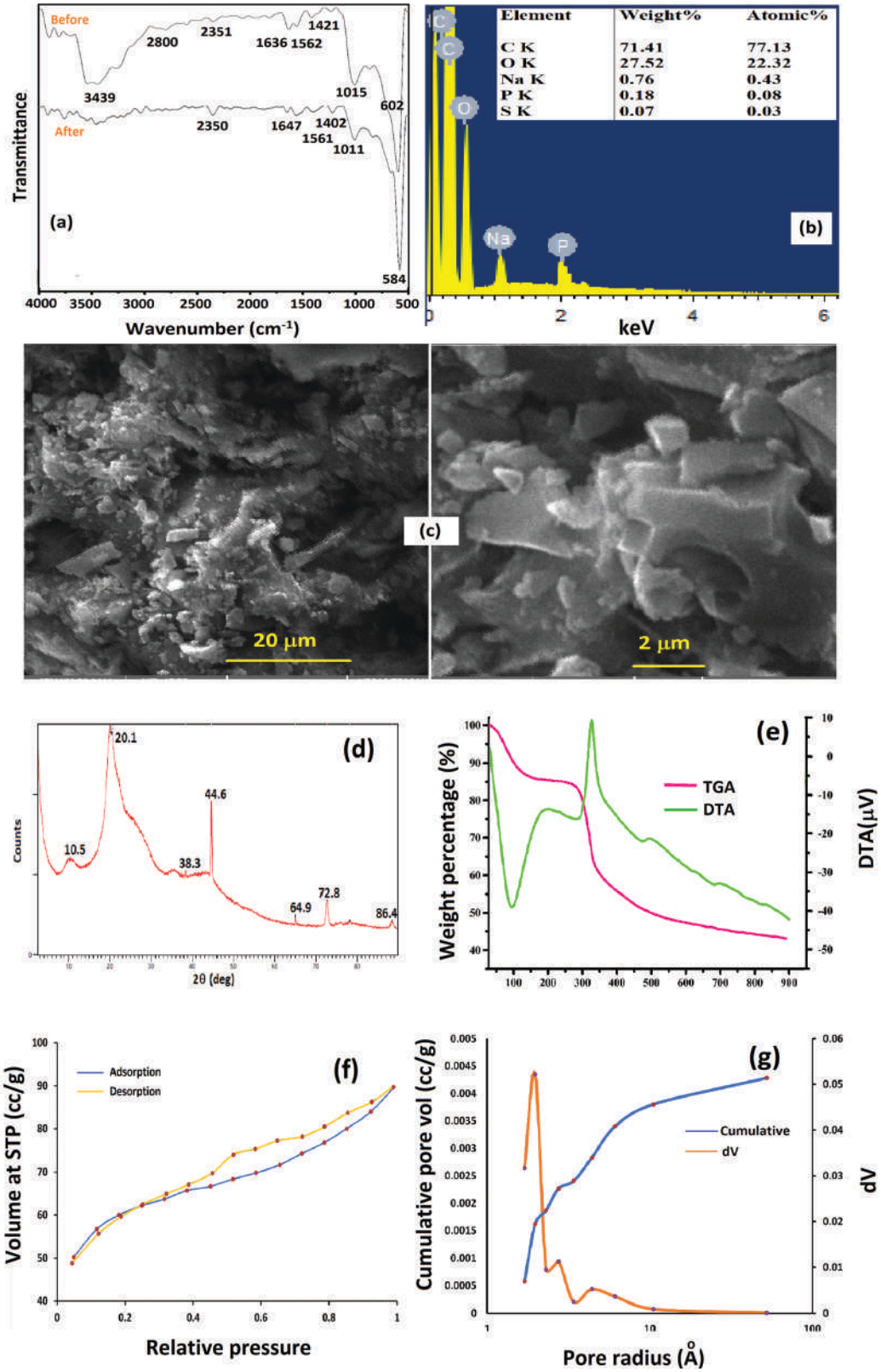


Fig. 1 (a) FT-IR spectra of Cs–C before and after the adsorption of RBBR. (b) EDX spectrum and elemental composition of Cs–C and (c) SEM micrographs of Cs–C at two different resolutions. (d) XRD spectrum, (e) TGA–DTA curve, (f) N_2 adsorption–desorption curves, and (g) pore-size distribution curves of Cs–C.

© 2022 The Author(s). Published by the Royal Society of Chemistry

Open Access Article. Published on 26 May 2022. Downloaded on 6/29/2022 11:53:27 AM.
This article is licensed under a Creative Commons Attribution-NonCommercial 3.0 Unported Licence.

The Cs–C dose was increased from 25 to 300 mg. Four dye concentrations of 50, 100, 150, and 200 mg L^{-1} were stirred with various doses of Cs–C for the optimized contact time of 60 min and then their final concentrations were determined spectrophotometrically.

To study the effect of temperature as part of the evaluation of the thermodynamics parameters, the temperature was varied from 298 to 333 K. The quantity of adsorption of RBBR upon Cs–C was investigated at an initial RBBR concentration of 100 mg L^{-1} using a 25 mL volume and adsorbent dose of 100 mg.

Results and discussion

Characterization of Cs–C

Fig. 1a depicts the FT-IR spectra of Cs–C and Cs–C after RBBR dye adsorption. The Cs–C spectrum showed a characteristic broad peak around 3440 cm^{-1} corresponding to the stretching vibrations of –NH and –OH bonds in chitosan along with a skeletal vibration peak of C–O–C at 1015 cm^{-1}.[33] A highly intense peak at 602 cm^{-1} corresponding to O–P–O bending of the STTP group confirmed the crosslinking of chitosan with STTP. Peaks observed at 1630 and 1562 cm^{-1} could be attributed to the linkage between the phosphoric and ammonium ions. These results were attributed to the crosslinking between the amino groups of chitosan and phosphate groups of STPP.[34] After adsorption of the dye on the Cs–C surface, the important peaks were found to shift from their positions, indicating an interaction between the adsorbate and adsorbent molecules. The reduction in the intensity of the peak around 3440 cm^{-1} was an indication of the strong interaction between –OH and $-NH_2$ groups of chitosan with the RBBR molecules, consistent with the reported literature.[35]

The crosslinking could be further confirmed by EDX studies of Cs–C (Fig. 1b). The EDX spectrum showed the important peaks for phosphorous and sodium along with those of carbon and oxygen, indicating the incorporation of tripolyphosphate moieties in the chitosan framework.

The SEM micrographs of Cs–C (Fig. 1c) revealed that the surface was non-uniform and heterogenous. This heterogeneity led to an overall enhancement in the surface area and hence in the adsorption efficiency.

XRD analysis was carried out to determine the crystalline and/or amorphous nature of Cs–C, as shown in Fig. 1d.[36] The diffractogram clearly showed the characteristic peaks of chitosan at 2θ = 10.5° and 20.1°, corresponding to the (020) and (110) planes. The slightly crystalline nature of the chitosan matrix could be attributed to the intermolecular and intramolecular hydrogen bonding. The XRD pattern of Cs–C showed additional peaks at 2θ = 44.5° and 64.9°, which were typical for the tripolyphosphate crosslinked chitosan.[37,38]

The TGA curve of Cs–C. (Fig. 1e) showed a small weight loss of about 15% up to 150 °C associated with an endothermic crest in the DTA curve corresponding to the loss of moisture. A second major weight loss of about 50–55% occurred between 330 °C and 500 °C and was associated with an exothermic peak in the DTA curve indicating the degradation of the polymeric organic framework.[39]

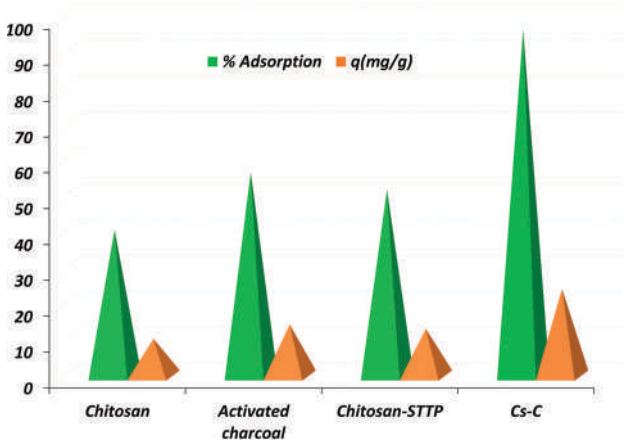


Fig. 2 Comparison of the adsorption efficiencies of various materials.

The pore dimensions and surface area of Cs–C were determined by N_2 adsorption–desorption isotherms, as depicted in Fig. 1f, while the pore-size distribution is shown in Fig. 1g. The surface area of the unmodified chitosan was found to be 0.65 $m^2\ g^{-1}$ which was found to be enhanced to 190 $m^2\ g^{-1}$ in the Cs–C composite. These values were obtained by BET isotherm. As Cs–C was a porous material, its pore properties were established through the BJH method using the nitrogen adsorption–desorption curve. The pore volume was found to be 1.388 $m^3\ g^{-1}$, showing the highly porous nature of the material in contrast to native chitosan, which is completely non-porous in nature. The mean pore radius of 1.459 nm was an indication of the microporous nature of the composite. As shown in Fig. 1f, the N_2 physisorption isotherm could be assigned as a type IV hysteresis on the basis of IUPAC classification. This indicated the presence of micropores in the Cs–C structure.[40]

Activated charcoal (AC) has a large surface area and microporous structure. The incorporation of AC in the chitosan matrix led to an enhancement of the adsorption of anionic RBBR dye molecules on the Cs–C surface because of the high diffusion of RBBR dye molecules through the micropores of Cs–C.[41] The hysteresis loop (Fig. 1g) was clear evidence of the presence of micro- and mesopores in the composite material. This observation was consistent with the flake-like appearance of the surface of the material as obtained in the SEM micrographs. The shift in the desorption curve towards the left was due to the cavitation-driven desertion.[42,43] The high adsorption capacity of the Cs–C composite towards RBBR could be prominently attributed to the high surface area, large pore volume, and micro- and mesoporous nature of the adsorbent.

Batch adsorption studies

During the trial experiments with unmodified chitosan, pure activated charcoal, chitosan crosslinked with STTP, and the final composite Cs–C, each of the materials adsorbed RBBR dye to a different extent. It was observed that Cs–C adsorbed 96.4% of the dye with an adsorption efficiency of 24 mg g^{-1} in just

© 2022 The Author(s). Published by the Royal Society of Chemistry

Open Access Article. Published on 26 May 2022. Downloaded on 6/29/2022 11:53:27 AM.
This article is licensed under a Creative Commons Attribution-NonCommercial 3.0 Unported Licence.

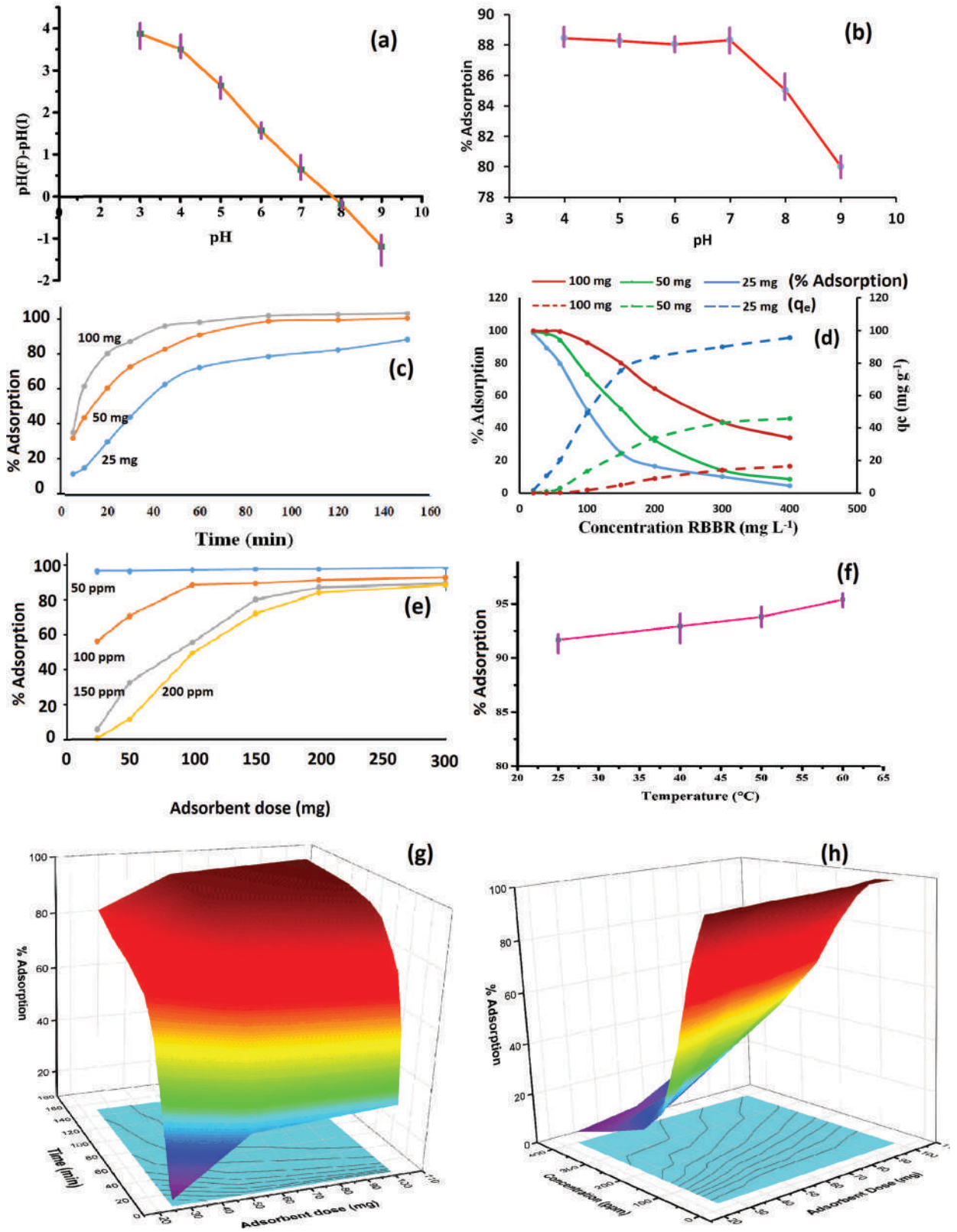


Fig. 3 (a) pHpzc of Cs–C. Effect of: (b) the solution pH, (c) contact time, (d) initial dye concentration, (e) adsorbent dose, and (f) temperature on the adsorption efficiency. (g) Dose-time dependence and (h) dose-concentration dependence.

© 2022 The Author(s). Published by the Royal Society of Chemistry

Open Access Article. Published on 26 May 2022. Downloaded on 6/29/2022 11:53:27 AM.
This article is licensed under a Creative Commons Attribution-NonCommercial 3.0 Unported Licence.

60 min, and so it was established to be the best material compared to any of the precursors (Fig. 2). Both chitosan as well as activated charcoal had a tendency to adsorb RBBR dye to some extent. However, the combination of the two led to a much effective adsorption owing to the enhanced surface area of charcoal and the effective molecular interactions of chitosan. Similar results were reported by Khapre ***et al.*** (2020) for successively modified materials towards alizarin red S dye.

The effects of various variables in the adsorption experiments are presented in Fig. 3. The pH at which the surface charge on Cs–C was zero, *i.e.* the pHpzc was found to be 7.8, representing that the material exhibited a near-neutral pHpzc (Fig. 3a), meaning that the Cs–C surface would be distinctly positive below pH 7.8 and negative above pH 7.8. It was observed that there was no significant effect of pH on the removal efficiency of dye from pH 4 to 7 (Fig. 3b). In this pH range, the surface charge on Cs–C was positive, indicating a strong attractive interaction with the anionic dye molecules. In acidic pH, the $-NH_2$ group of chitosan was protonated as $-NH_3^+$ while the RBBR dye has an $-SO_3^-$ group, which would be responsible for the interaction in the acidic range.[44] Strong electrostatic forces between the adsorbent and adsorbate were responsible for the high adsorption capacity of Cs–C towards RBBR dye. However, with the increase in pH above 7.8, the adsorption efficiency was found to be reduced at pH 8 and 9. This was quite obvious and could be related to the surface charge of the adsorbent. Hence, the original solution with pH 6.0 was used in all the studies.

The increase in the adsorption duration from 5 min to 150 min showed a rapid increase in adsorption in the beginning. This could be attributed to the available surface for the adsorbent molecules in the beginning. As the surface got covered with the dye, the percentage adsorption reached almost saturation in 60 min. After 60 min, there was a negligible hike in removal efficiency, and so the contact time of 60 min was considered optimum for RBBR dye (Fig. 3c). This was quite obvious as the maximum number of sites were available on the Cs–C surface in the beginning, but over time the sites get occupied by dye molecules, and equilibrium is reached in about 60 min.[45]

The increase in RBBR concentration led to a reduction in the percentage adsorption but increase in the value of q_e (Fig. 3d). As the concentration of incoming RBBR molecules increased, the relative availability of active adsorption sites decreased, thus lowering the removal percentage. However, the gathering of the excess dye on the adsorbent surface enhanced its adsorption capacity in terms of mg of dye adsorbed per gram of adsorbate material. As the adsorbent dose was increased, the availability of the surface increased and so the accumulation per unit mass (q_e) went on decreasing with the increase in adsorbent dose for the same concentration. With this context, an initial dye concentration of 100 mg L^{-1} was fixed for the further studies.

As the Cs–C amount was increased from 25 mg to 300 mg, the availability of a greater number of active adsorption sites led to an enhancement in the adsorption percentage (Fig. 3e). For 100 mg L^{-1} solution, an adsorbent dose of 100 mg was found to give more than 90% adsorption, and so this dose was selected as the optimum dose.

Table 1 RBBR dye adsorption on Cs–C in terms of the kinetics, isotherm, and thermodynamics parameters

Studies	Model	Parameter	Observation
Kinetics studies	PFO	K_1 (min^{-1})	0.0456
		q_e cal (mg g^{-1})	62.01
		q_e exp (mg g^{-1})	24.67
		R^2	0.965
	PSO	K_2 (mg g^{-1} min^{-1})	0.0050
		q_e cal (mg g^{-1})	26.41
		q_e exp (mg g^{-1})	24.67
		R^2	0.999
	Intraparticle diffusion	K_{int} (mg g^{-1} min^{-1}/2)	1.351
		Intercept (C)	11.03
		R^2	0.772
Isotherm models	Langmuir	q_m (mg g^{-1})	540.3
		b (L g^{-1})	0.478
		R_L	0.040
		R^2	0.9994
		χ^2	6.94
	Freundlich	K_F (mg L^{-1}/n g^{-1} L^{-1})	10.418
		n	5.216
		R^2	0.9140
		χ^2	69.37

Thermodynamic parameters	Temperature	ΔG (kJ mol^{-1})	ΔH (kJ mol^{-1})	ΔS (J mol^{-1} K^{-1})
	298 K	−5.932	14.186	67.125
	313 K	−6.697		
	323 K	−7.290		
	333 K	−8.383		

It was observed that the adsorption was favoured by rise in temperature, indicating the endothermic nature of the adsorption process (Fig. 3f). The simultaneous effect of two parameters is depicted in Fig. 3g and h. These graphs clearly indicate that a longer adsorption period, higher adsorbent dose, and lower dye concentration led to a greater percentage adsorption.

Isotherms, kinetics, and thermodynamics of the adsorption process

Adsorption isotherms were applied as mathematical models that could be used to predict the interaction between RBBR and the Cs–C adsorbent.[46] The two isotherm models of Langmuir[47] and Freundlich[48] were applied to investigate the equilibrium isotherms of the adsorption and for calculating the q_{max}. The Langmuir isotherm equation in linearized form is given by:

$$\frac{C_e}{q_e} = \frac{1}{bq_m} + \frac{C_e}{q_m}$$

where q_m (mg g^{-1}) and b (L mg^{-1}) are Langmuir isotherm coefficients, and q_m represents the maximum adsorption capacity.

$$\ln q_e = \frac{1}{n}\ln C_e + \ln K_F$$

© 2022 The Author(s). Published by the Royal Society of Chemistry

Open Access Article. Published on 26 May 2022. Downloaded on 6/29/2022 11:53:27 AM.
This article is licensed under a Creative Commons Attribution-NonCommercial 3.0 Unported Licence.

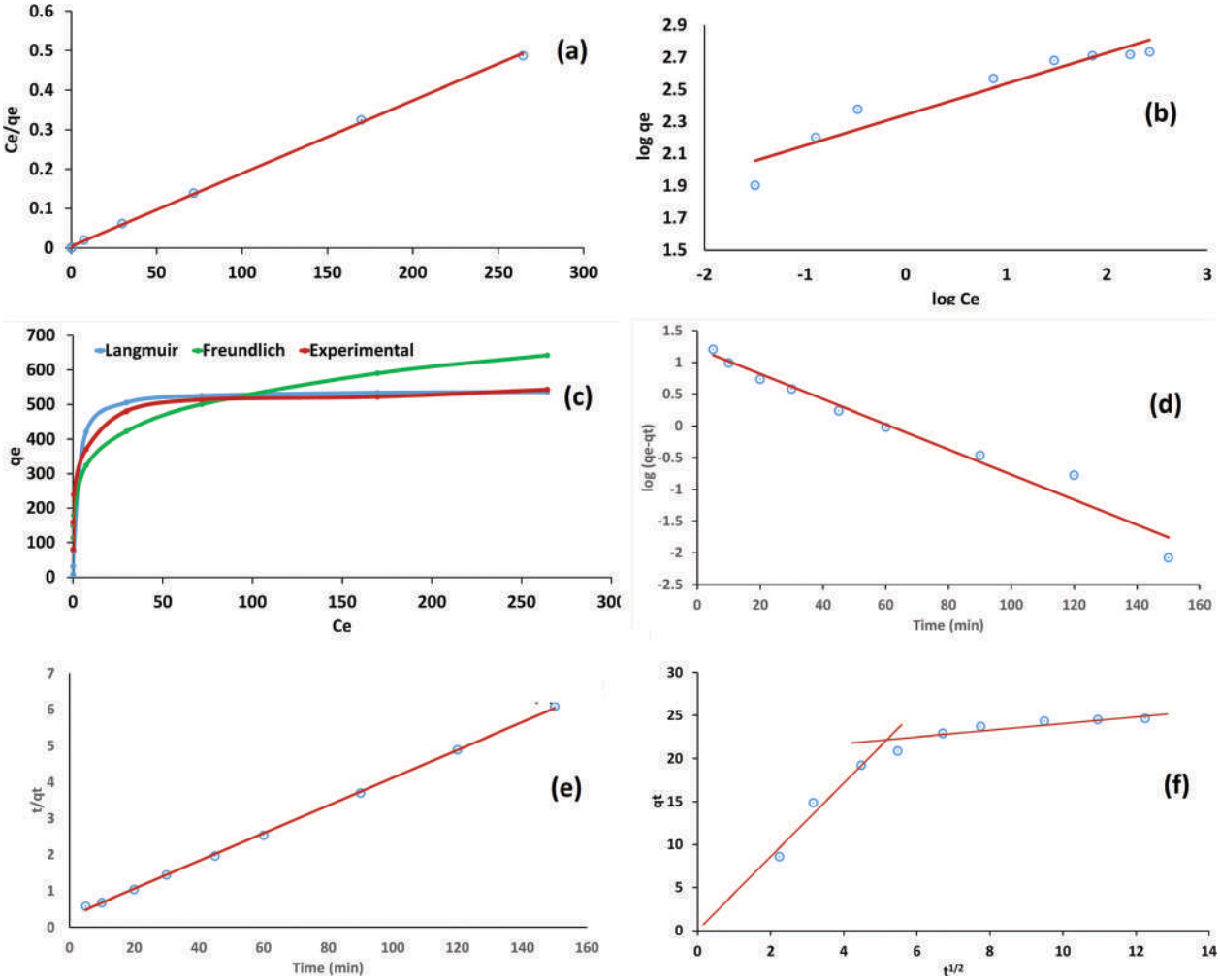


Fig. 4 (a) Langmuir, (b) Freundlich, (c) C_e–q_e plot, (d) PFO, (e) PSO, and (f) intraparticle diffusion.

where K_F and n are Freundlich constants that indicate the adsorption capacity and adsorption intensity, respectively. A value of n between 1 and 10 indicates favourable adsorption.

It can be clearly seen from Table 1 and Fig. 4 that the Langmuir model fitted best with the experimental data, with a coefficient of determination value very close to 1 and a smaller value of χ^2, indicating monolayer adsorption on the homogeneous surface of the adsorbent. The maximum monolayer adsorption capacity obtained from the Langmuir model was found to be 540.3 mg g^{-1}, which showed the excellent efficiency of the material. $R_L < 1$ and $1 < n < 10$ were indications of the feasible adsorption of the dye on the Cs–C surface.

Pseudo-first-order (PFO), pseudo-second-order (PSO), and intraparticle diffusion models were applied under optimized operating conditions. The models were applied in a time-dependent study. As can be observed form Table 1 and Fig. 4, the PSO model was the best fitted model for the kinetics of adsorption. This was clear from the correlation coefficient (R^2) values. The calculated q_e values from the PSO model were in agreement with experimental value of q_e, thereby suggesting that the adsorption of RBBR dye on the Cs–C surface involved chemical interactions, such as electrostatic attraction between the negative charge of RBBR dye and positive charge available on the Cs–C surface.[49] The Weber Morris intraparticle diffusion model showed that the adsorption process was diffusion controlled in the beginning with a zero intercept and linear relation between q_t and $t^{1/2}$. However, with time, the process led to saturation and the boundary layer played an important role in the non-zero intercept of the graph. The overall intercept value of 11.03 indicated that the overall process was not just controlled by the diffusion.[46]

The equilibrium constant K was established at different temperatures from 298 to 333 K taking the ratio of concentration of dye in the adsorbed phase to that in the solution phase. Using the equation $\Delta G^\circ = -RT\ln K$, the corresponding values of ΔG were calculated, while the values of ΔH and ΔS were calculated from the intercept and slope of the vant Hoff plot of lnK as a function of $1/T$. It was interesting to note that the adsorption process was endothermic in nature, but spontaneous over the entire temperature range and resulted in an increase in randomness. This showed the entropy-driven nature of the process (Table 1).

© 2022 The Author(s). Published by the Royal Society of Chemistry

Open Access Article. Published on 26 May 2022. Downloaded on 6/29/2022 11:53:27 AM.
This article is licensed under a Creative Commons Attribution-NonCommercial 3.0 Unported Licence.

Table 2 Comparison of the adsorption capacities of RBBR dye by various adsorbents

Adsorbent	q_m (mg g^{-1})	Ref.
(DTMA) bromide modified bentonite	206.58	50
Magnesium oxide	250	51
Lignocellulosic waste	75.19	52
Activated clay	400	53
Co-electrospun nanofibres	61.2	54
Lemna minor	9.45	55
Fly Ash	47.86	56
Metal hydroxide waste sludge	91	57
Mesoporous activated carbon	35.5	58
Fe–Al doped cellulose (FADC)	95.62	59
Activated charcoal from leaves of Thuja orientalis	170	60
Fomes fomentarius	90	61
Sewage sludge biochar	126.59	62
Yarrowia lipolytica Biomass	103	63
Cs–C	**540.3**	**Present work**

Probable mechanism

The activated charcoal provided the surface for the chitosan molecules to interact with the dye ions with an anionic sulphate group. Chitosan has protonated amine groups that have a strong electrostatic interaction with the anionic dye molecules. This was in accordance with the observation that increasing the pH in the basic range reduced the adsorption capacity of Cs–C and also as the $-NH_2$ peak was suppressed after adsorption of the dye molecules.

Conclusion

A microporous crosslinked Cs–C composite was successfully synthesized and then analyzed by various characterizations. The composite was utilized to remove RBBR dye (anionic dye) from aqueous medium. Cs–C was characterized using various techniques, which confirmed the formation of a composite having STTP crosslinked well to the chitosan. SEM micrographs and EDAX analysis showed the morphological transformation and composition of Cs–C, respectively. BET surface area analysis using the nitrogen adsorption–desorption method revealed a good surface area and pore volume and also confirmed the microporous nature of the adsorbent. The amount of dye adsorption was dependent on the adsorbent dosage, temperature, and contact time. The maximum qt of Cs–C was obtained from the Langmuir model as 540.3 mg g^{-1}, which was quite high compared to most existing biosorbents. The thermodynamics results indicated that the adsorption process was spontaneous and endothermic in nature and was entropy driven. The adsorption mechanism of the RBBR dye on the Cs–C surface could be assigned to various types of interactions, such as electrostatic attraction, H-bonding interaction, and π–π interaction. The adsorption results indicated that Cs–C can be considered as a feasible and promising biosorbent for the removal of anionic dyes from an aqueous environment. A comparison with the reported materials, as shown in Table 2, highlights the advantage of this material over the previously reported materials for the adsorption of RBBR dye.

Author contributions

P. N.: methodology, validation, investigation, writing first draft. D. S.: formal analysis, resources. P. B.: conceptualization, R. J.: writing: review and editing, supervision.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

Thanks are due to DST and UGC for funding to Department of Chemistry under various schemes. Thanks to RTM Nagpur University for URPS.

References

1 S. Rojas and P. Horcajada, *Chem. Rev.*, 2020, **120**, 8378–8415.
2 M. Naushad, G. Sharma and Z. A. Alothman, *J. Cleaner Prod.*, 2019, **241**, 118263.
3 I. Ali, O. M. L. Alharbi, Z. A. Alothman, A. Al-Mohaimeed and A. Alwarthan, *Environ. Res.*, 2019, **170**, 389–397.
4 S. M. Wabaidur, M. Khan, M. Siddiqui, M. Otero, B. Jeon, Z. Alothman and A. Hakami, *J. Mol. Liq.*, 2020, **317**, 113916.
5 M. Khan, A. Alqadami, S. Wabaidur, M. Siddiqui, B. Jeon, S. Alshareef, Z. Alothman and A. Hamedelniel, *J. Hazard. Mater.*, 2020, **400**, 123247.
6 H. Tang, W. Zhou and L. Zhang, *J. Hazard. Mater.*, 2012, **209–210**, 218–225.
7 G. San Miguel, S. D. Lambert and N. J. D. Graham, *J. Chem. Technol. Biotechnol.*, 2006, **81**, 1685–1696.
8 Y. Al-Degs, M. A. M. Khraisheh, S. J. Allen and M. N. Ahmad, *Water Res.*, 2000, **34**, 927–935.
9 M. Beheraa, J. Nayak, S. Banerjee, S. Chakrabortty and S. K. Tripathy, *J. Environ. Chem. Eng.*, 2021, **9**, 105277.
10 A. Mittal, M. Naushad, G. Sharma, Z. A. ALothman, S. M. Wabaidur and M. Alam, *Desalin. Water Treat.*, 2016, **57**, 21863–21869.

© 2022 The Author(s). Published by the Royal Society of Chemistry

Open Access Article. Published on 26 May 2022. Downloaded on 6/29/2022 11:53:27 AM. This article is licensed under a Creative Commons Attribution-NonCommercial 3.0 Unported Licence.

11 A. Azhar, Y. Yamauchi, A. Allah, Z. A. Alothman, A. Badjah, M. Naushad, M. Habila, S. Wabaidur, J. Wang and M. Zakaria, *Nanomaterials*, 2019, **9**, 776.
12 A. Alqadami, M. Khan, M. Siddiqui and Z. A. Alothman, *Microporous Mesoporous Mater.*, 2018, **261**, 198–206.
13 I. Ali, O. Alharbi, Z. A. ALOthman, A. Al-Mohaimeed and A. Alwarthan, *Environ. Res.*, 2019, **170**, 389–397.
14 T. Robinson, G. McMullan, R. Marchant and P. Nigam, *Bioresour. Technol.*, 2001, **77**, 247–255.
15 Z. Li, A. Gómez-Avilés, L. Sellaoui, J. Bedia, A. Bonilla-Petriciolet and C. Belver, *Chem. Eng. J.*, 2019, **371**, 868–875.
16 A. Pala and E. Tokat, *Water Res.*, 2002, **36**, 2920–2925.
17 J. Dwyer, P. Griffiths and P. Lant, *Water Res.*, 2009, **43**, 553–561.
18 M. T. Butt, N. Imtiaz, S. Ahmed, F. Arif and S. R. Khan, *Pak. J. Sci. Ind. Res., Ser. B*, 2010, **53**, 81–84.
19 Z. Sun, Z. Sun, X. Duan, C. Srinivasakannan and J. Liang, *RSC Adv.*, 2018, **8**(16), 8625.
20 F. Yang, B. Sheng, Z. Wang, Y. Xue, J. Liu, T. Ma, R. Bush, H. Kušić and Y. Zhou, *J. Hazard. Mater.*, 2021, **406**, 124774.
21 M. D. Faysal Hossain, N. Akther and Y. Zhou, *Chin. Chem. Lett.*, 2020, **31**, 2525–2538.
22 Y. Zhou, J. Lu, Y. Zhou and Y. Liu, *Environ. Pollut.*, 2019, **252**, 352–365.
23 M. Z. Elsabee, R. E. Morsi and A. M. Al-Sabagh, *Colloids Surf., B*, 2009, **74**, 1–16.
24 M. Guo, J. Wang, C. Wang, P. J. Strong, P. Jiang, Y. S. Ok and H. Wang, *Sci. Total Environ.*, 2019, **682**, 340–347.
25 J. Yang, Y. Han, Z. Sun, X. Zhao, F. Chen, T. Wu and Y. Jiang, *ACS Omega*, 2021, **6**, 15885–15891.
26 S. Korde, S. Deshmukh, S. Tandekar and R. Jugade, *Carbohydr. Polym. Technol. Appl.*, 2021, **2**, 100081.
27 M. A. Khapre and R. M. Jugade, *Water Sci. Technol.*, 2020, **82**, 715–731.
28 S. H. Vithalkar and R. M. Jugade, *Mater. Today: Proc.*, 2020, **29**, 1025–1032.
29 M. A. Khapre, S. Pandey and R. M. Jugade, *Int. J. Biol. Macromol.*, 2021, **190**, 862–875.
30 A. H. Jawad, A. S. Abdulhameed and M. S. Mastuli, *J. Polym. Environ.*, 2020, **28**, 1095–1105.
31 E. R. Kenawy, A. A. Ghfar, S. M. Wabaidur, M. A. Khan, M. R. Siddiqui and Z. A. Alothman, *J. Environ. Manage.*, 2018, **219**, 285–293.
32 S. Kahu, A. Shekhawat, D. Saravanan and R. Jugade, *Int. J. Environ. Sci. Technol.*, 2016, **13**, 2269–2282.
33 S. Tandekar, S. Korde and R. Jugade, *Carbohydr. Polym. Technol. Appl.*, 2021, **2**, 100128.
34 D. R. Bhumkar and V. B. Pokharkar, *AAPS PharmSciTech*, 2006, **7**, E138–E143.
35 C. Theerakarunwong and D. Boontong, *Results Chem.*, 2020, **2**, 100024.
36 Z. A. Alothman, A. Bahkali, M. Khiyami, S. Alfadul, S. Wabaidur, M. Alam and B. Alfarhan, *Sep. Sci. Technol.*, 2020, **55**, 1766–1775.
37 X. Qi, L. Lin, L. Shen, Z. Li, T. Qin, Y. Qian, X. Wu, X. Wei, Q. Gong and J. Shen, *ACS Sustainable Chem. Eng.*, 2019, **7**, 11014–11023.
38 S. P. Facchi, D. B. Scariot, P. V. A. Bueno, P. R. Souza, L. C. Figueiredo, H. D. M. Follmann, C. S. Nunes, J. P. Monteiro, E. G. Bonafe, C. V. Nakamura, E. C. Muniz and A. F. Martins, *Int. J. Biol. Macromol.*, 2016, **87**, 237–245.
39 G. F. Leal, L. A. Ramos, D. H. Barrett, A. A. S. Curvelo and C. B. Rodella, *Thermochim. Acta*, 2015, **616**, 9–13.
40 S. Korde, S. Tandekar, D. Saravanan and R. Jugade, *J. Environ. Chem. Eng.*, 2020, **8**, 104360.
41 M. J. Ahmed, P. U. Okoye, E. H. Hummadi and B. H. Hameed, *Bioresour. Technol.*, 2019, **278**, 159–164.
42 S. Ahmed and A. Iqbal, *Global Challenges*, 2018, **2**, 1800056.
43 K. S. W. Sing, *Pure Appl. Chem.*, 1985, **57**, 603–619.
44 G. Ozturk and H. Silah, *Environ. Processes*, 2020, **7**, 479–492.
45 T. Khan, M. Nouman, D. Dua, S. Khan and S. Alharthi, *J. Saudi Chem. Soc.*, 2022, **26**, 101417.
46 X. Qi, M. Chen, Y. Qian, M. Liu, Z. Li, L. Shen, T. Qin, S. Zhao, Q. Zeng and J. Shen, *Int. J. Biol. Macromol.*, 2019, **132**, 429–438.
47 I. Langmuir, *J. Am. Chem. Soc.*, 1918, **40**, 1361–1403.
48 H. M. Freundlich, *Z. Phys. Chem.*, 1906, **57**, 385–470.
49 M. V. Subbaiah and D.-S. Kim, *Ecotoxicol. Environ. Saf.*, 2016, **128**, 109–117.
50 A. Ozcan, C. Omeroglu, Y. Erdogan and A. S. Ozcan, *J. Hazard. Mater.*, 2007, **140**, 173–179.
51 N. K. Nga, P. T. T. Hong, T. Dai Lam and T. Q. Huy, *J. Colloid Interface Sci.*, 2013, **398**, 210–216.
52 M. Asgher and H. N. Bhatti, *Can. J. Chem. Eng.*, 2012, **90**, 412–419.
53 R. S. Juang, F. C. Wu and R. L. Tseng, *Environ. Technol.*, 1997, **18**, 525–531.
54 R. A. Hakro, M. Mehdi, R. F. Qureshi, R. B. Mahar, M. Khatri, F. Ahmed, Z. Khatri and I. S. Kim, *Mater. Res. Express*, 2021, **8**, 055502.
55 D. Balarak, Y. Mahdavi and A. Joghataei, *Arch. Hyg. Sci.*, 2015, **4**, 199–207.
56 N. Dizge, C. Aydiner, E. Demirbas, M. Kobya and S. Kara, *J. Hazard. Mater.*, 2008, **150**, 737–746.
57 S. C. R. Santos, V. J. P. Vilar and R. A. R. Boaventura, *J. Hazard. Mater.*, 2008, **153**, 999–1008.
58 T. Silva, A. Ronix, O. Pezoti, L. Souza, P. Leandro, K. Bedin, K. Beltrame, A. Cazetta and V. Almeida, *Chem. Eng. J.*, 2016, **303**, 467–476.
59 M. Khapre, A. Shekhawat, D. Saravanan, S. Pandey and R. Jugade, *Mater. Adv.*, 2022, **3**, 3278–3285.
60 M. Arya, P. Bafila and D. Mishra, *SN Appl. Sci.*, 2020, **2**, 265.
61 C. Arslant, I. M'barek, M. Saleh, Z. Isik, S. Ozdemir, A. Dundar and N. Dizge, *Water Pract. Technol.*, 2022, **17**, 749–762.
62 A. Raj, A. Yadav, A. Rawat, A. Singh, S. Kumar, A. Pandey, R. Sirohi and A. Pandey, *Environ. Technol. Innovation*, 2021, **23**, 101556.
63 Y. D. Aracagok, *Arch. Microbiol.*, 2022, **204**, 128.

© 2022 The Author(s). Published by the Royal Society of Chemistry

Adoption of Disruptive Technologies by the Farmers: Evidence from India

G. Yoganandan
Project Director, ICSSR-IMPRESS (3615), ICSSR, New Delhi, & Associate Professor,
Department of Management Studies, Periyar University, Salem, Tamil Nadu, India.
g_yoganandan@yahoo.com

M. Dinesh Kumar
Research Assistant, ICSSR-IMPRESS (3615) & M.Phil Scholar, Department of Management Studies, Periyar University, Salem, Tamil Nadu, India.
dineshtmmech@gmail.com

M.Vasan
Assistant Professor of Commerce,
National College (Autonomous), Tiruchirappalli, Tamil Nadu, India. (Corresponding author)
m.vasan@yahoo.com

Doris Padmini Selvaratnam
Associate Professor, Faculty of Economics and Management, Universiti Kebangsaan Malaysia, Malaysia.
pegasus@ukm.edu.my

2022 8th International Conference on Advanced Computing and Communication Systems (ICACCS) | 978-1-6654-0816-5/22/$31.00 ©2022 IEEE | DOI: 10.1109/ICACCS54159.2022.9785219

***Abstract* - This paper investigates the perception and attitude of farmers towards adopting disruptive technologies. The data were collected from 220 jasmine farmers using a survey instrument (questionnaire). The study results showed that the farmers have a positive intention to use disruptive technologies. It is also observed that perceived usefulness, perceived ease of use and attitude of confidence influenced the attitude to use disruptive technologies. The study also indicated that the farmers' attitude to use is emerging as a critical factor in determining jasmine farmers' behavioural intention. The study recommended that the government and other relevant bodies like universities focus on providing hands-on training to jasmine farmers to positively change the farmers' attitude to use disruptive technologies. Findings from this study may be helpful to policymakers to enhance the sustainability of the flower farming system.**

Keywords: Behavioral attitude, Behavioral intention, Disruptive farming technologies, Structural equation modelling, Technology acceptance model.

I. INTRODUCTION

Traditional agriculture with low mad-made inputs in the form of fertilizers and pesticides was not sufficient for the growing population of India. So, the government of India had to introduce Green Revolution in the 1960s, which focused on increasing yield through fertilizers, pesticides and other industrial outputs [1]. Recently, there has been a change in the perspective of both farmers and the government. There is a realization that the Green Revolution model is no longer sustainable [2]. This realization paves the way for technology adoption in farming. Newer technologies aim at enhancing the yield while maintaining the level inputs and limiting the negative impact of external environmental factors [3].

Though the government pushed technology adoption in the last decade, it has not gained enough traction among farmers due to smaller farms [4]. India will gain only if the technology adoption in agriculture becomes an inclusive agenda that brings small-scale farmers into the fold. There is an urgent need to implement modern technology, or otherwise the Precision Agriculture, which is the emerging trend in agriculture across the globe, focusing on the application of sophisticated technology in agriculture. An Indian agriculture-focused initiative should consider the cost of technology, pollution-free productivity, and a small landholding pattern [5].

Why technology adoption and precision agriculture are required in Jasmine farming? Traditional farming practices are susceptible to the impacts of extreme weather conditions, which can logically be attributed to climate change [6]. Excess rainfall and higher temperature also play a spoilsport on jasmine cultivation [7]. So, to address these issues, the floriculture industry is fast adopting new innovative cultivation methods. Though the cost of technology impedes flower farmers with smaller farm sizes, the continuous downward trend in the cost of technology finds some traction among small farmers [8]. However, not all farmers perceive the need for precision agriculture in the same way [9]. Janani et al. [10] identified that only one-third of jasmine farmers had adopted better technology to manage their flower farming. Rani et al. [11] argued that technology adoption could solve problems like low productivity, non-availability of skilled labour, pest control, and vagaries in the climate.

Jasmine has been cultivated across India, and there is a historical background for floriculture. Jasmine was brought to India during the mid-sixteenth century, and they were originally from the tropical and subtropical areas. It is cultivated throughout India in an area of 8,000 hectares. There are more than 75 varieties of jasmine species, but it has to be noted that only three

978-1-6654-0816-5/22/$31.00 ©2022 IEEE

Authorized licensed use limited to: SASTRA. Downloaded on July 12,2022 at 03:43:51 UTC from IEEE Xplore. Restrictions apply.

varieties have gained prominence and importance in commercial cultivation [12]. Hence, this flower plays a unique role in every celebration, be it festivals of all religious, cultural, social, and political events [13]. When compared to field crops and plantation crops in India, floriculture has boomed as an income-generating sector with a high potential for returns and profits. Only recently, flower farming has blossomed vibrantly as a commercial business start-up [14]. It has created massive employment opportunities in urban, rural and suburban areas [15]. Besides, integrated farming helps in sustainable production, income generation, and the development of hitherto underdeveloped backward regions of the state [16].

India is in a tremendous position to reach a leadership position in the world of floriculture trade. Partly, this could be attributed to the incentives, schemes and subsidies offered by both central and state governments of various states for floriculture development [17]. The demand in the domestic market for jasmine is very encouraging. The demand is growing every year at an average rate of 25 percent due to its wide usage in perfumery, cosmetics, and medicinal values to relieve anxiety, stress, depression, and nervous tension [18]. Also, jasmine has been exported after value addition [19]. India exports jasmine flowers to countries like Malaysia, Singapore, Japan, USA, UK and Gulf countries [11]. Sustaining the competitive edge in the global market could only be achieved through precision agriculture. In this context, this study aims to investigate the factors influencing jasmine farmers' attitudes and behavioural intention to adopt disruptive farming technologies.

II. Materials and Methods

This study investigates farmers' perceptions and attitudes towards adopting disruptive technologies by the jasmine farmers in the Salem district of Tamilnadu. The total sample size for the study is 220 Jasmine farmers. A well-structured survey instrument was employed to gather qualitative data from the farmers for measuring their adoption of disruptive technologies. The Technology Acceptance Model (TAM) developed by Davis [20] was used to measure the perceptions and attitudes towards adopting disruptive technologies. This model is considered a well-acclaimed model for measuring technology adoption [21]. In addition, the variables relating to the attitude of confidence and attitude to use were adopted from the studies conducted by Adrian et al. [22] and Compeau and Higgins [23]. The study constructs were measured using 15 variables with the help of a five-point agreeability scaling technique.

A pilot study was conducted prior to the main study to gauge the validity of the data collection tool. To reduce sampling error, Roscoe [24] recommends a pilot trial sample size of at least 30. As a result, the questionnaire was distributed to 40 jasmine farmers in the study area. The reliability of the data collection tool was checked by using the Cronbach $\propto$ test. The survey instrument is highly suitable for data collection since its $\propto$ value (0.912) surpassed the minimum threshold of 0.70 [25].

The SPSS was used to analyze the qualitative collected data from the jasmine farmers, and this measurement model was developed using SPSS-AMOS. The exploratory factor analysis (EFA) facilitated the extraction of factors that influence the adoption of disruptive technologies. Further, the causal effect among the study variables was assessed by the structural equation modelling (SEM).

III. Results and Discussion

The outcome of the Factor analysis is exhibited in Table 1. The KMO value stood at 0.734. It was more than its threshold of 0.5. So, the application of EFA was apt for the variables encompassed in the study. Moreover, a high level of inter-relationship was found among the scale variables since Bartlett's $\chi 2$ value was significant at a 5% level ($p=<0.05$). So, the study variables were suitable for the application of PCA. The EFA under PCA with varimax rotation had identified five Eigenvalues with greater than 1. It showed that there were five significant factors extracted with 69.901% of the variation. It depicted that five factors predominantly influenced the adoption of disruptive technologies by the farmers.

The first, second and third set of variable loadings consists of each three variables with 17.380%, 16.424% and 14.075% of the variances. These were suitably labelled as 'perceived usefulness', 'perceived ease of use' and 'attitude of confidence', respectively. The fourth variable loading contains four variables that account for 12.638% of the variance. This group of variables was properly termed as 'attitude to use.' The fifth set of variable loading included two variables that accounted for 9.834% of the variation. This element was appropriately termed as 'behavioural intention'.

III.1 Measurement model

In this study, the causal relationship among the study variables was gauged by the structural equation modelling (SEM) using SPSS-AMOS. The standardized regression coefficient results in Table - 2 divulged how far the study variables were inter-related. The regression values were ranged between 0.515 and 0.724. This result showed that study variables were adequately interrelated with each other.

The various indices relating to model fit have presented in Table 3. The path analysis dimension as highlighted by the SEM revealed that the model has a good fit to the data since, χ^2/df = 2.67; GFI = 0.90; AGFI = 0.89; CFI = 0.92; TLI = 0.86; IFI = 0.93, NFI = 0.92 and RMSEA = 0.05. Based on these results, all the model fit indices were within the acceptable fit level. It is evident that the model was sufficient for the relationship among the study variables.

Authorized licensed use limited to: SASTRA. Downloaded on July 12,2022 at 03:43:51 UTC from IEEE Xplore. Restrictions apply.

Table - 1: FACTORS INFLUENCING DISRUPTIVE FARMING TECHNOLOGY ADOPTION

Constructs	Factor Loadings	Eigen Value	% of Variance
Perceived Usefulness (PU)		2.781	17.380
Disruptive technologies support to raise productivity	.834		
I feel a smartphone helpful in agriculture	.781		
Using disruptive technologies in agriculture is helpful to do my other duties quickly	.726		
Perceived Ease of Use (PEU)		2.628	16.424
Using disruptive technologies can enhance farming operations	.861		
Using disruptive technologies improve farmers efficiency	.847		
I would find disruptive technologies is comfortable to use	.687		
Attitude of Confidence (AC)		2.252	14.075
Disruptive technologies facilitate farming processes	.847		
Familiar with disruptive technologies is essential for all farmers	.592		
Usage of disruptive technologies in flower farming is highly suitable	.518		
Attitude to Use (AU)		2.022	12.638
If disruptive technologies are affordable, I will actually use of it	.822		
I will encourage my employees to adopt disruptive technologies	.747		
If disruptive technologies are unavailable, I will ask supervisors to find ways to obtain them.	.722		
Due to the disruptive advantages of technologies, I will use them.	.679		
Behavioural Intention (BI)		1.501	9.384
I will suggest the use of disruptive technologies to other farmers	.951		
I will regularly find new disruptive technologies relating to my field.	.920		

Table - 2: STANDARDIZED REGRESSION WEIGHTS

Constructs			Estimate
AU	<---	AC	.649
AU	<---	PU	.515
AU	<---	PEU	.661
BI	<---	AU	.724

Table - 3: ABSOLUTE FIT INDICES OF MODEL

Indices	Actual Value	Acceptable Range
χ^2/df	2.67	≤3
NFI	0.92	≥.90
GFI	0.90	≥.90
RMSEA	0.05	≤0.8
AGFI	0.89	≥.90
CFI	0.92	≥.90
IFI	0.93	≥.90
TLI	0.86	≥.90

The regression weights were diagrammatically represented in Fig. 1. The path diagram portrayed that perceived usefulness (γ = 0.52), perceived ease of use (γ = 0.66) and attitude of confidence (γ = 0.65) influenced the attitude to use disruptive technologies. This result is in accordance with the prior study conducted by Liu et al. [26] and Wu and Wang [27]. The study also indicated that attitude to use strongly affected behavioural intention (γ =0.72). It is observed that attitude to use was the most effective variable, which ultimately influenced the behavioural intention to use disruptive technologies. Similar results were reported by Karahanna et al. [28], Malhotra & Galletta [29] and Phillips et al. [30]. Moreover, based on the results, the variables included in the study present a suitable model for measuring the behavioural attitude and behavioural intention towards disruptive technologies.

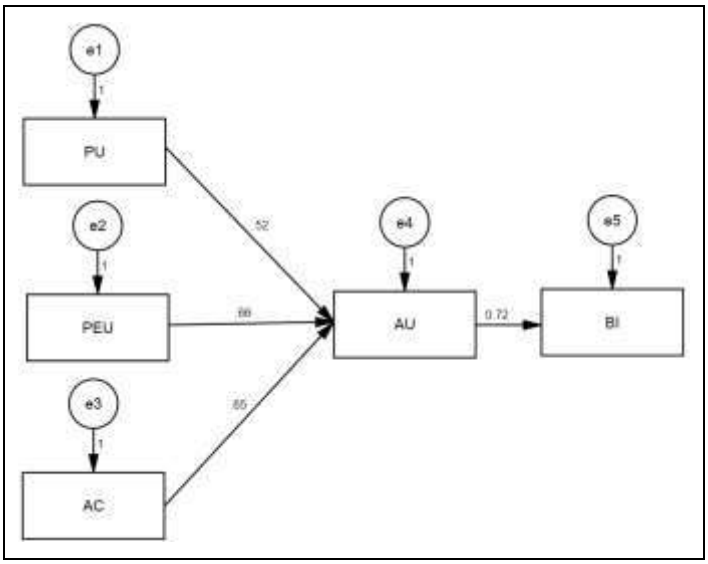


Fig. 1. Measurement Model

IV. CONCLUSION

Technology adoption in Indian agriculture is an answer to the problems of the previous Green Revolution model, which focused only on increasing yield. The policymakers in India need to consider the lessons learned from the previous agricultural model and have to come out with a sustainable model [31]. The disruptive agricultural technology adoption could significantly affect yield while protecting the natural ecosystem and rural poverty reduction [32]. This study was conducted to find out the jasmine farmers' wiliness to adopt newer technology in farming. The study outcome throws light on the farmers' intention to use disruptive technologies. Thus, perceived usefulness, perceived ease of use and attitude of confidence had influenced on attitude to use of disruptive technologies. The study also indicated that the farmers' attitude to use is emerging as a critical factor in determining jasmine farmers' behavioural intention. Based on the observations made in this study, we recommend that the government and other relevant bodies like universities focus on providing hands-on training to jasmine farmers to positively change the farmers' attitude to use newer technologies [33, 34]. The adoption of Artificial Intelligence in Supply Chain Management, Greenhouse climate control, Storage, irrigation management, fertilizer management and pesticide management will drastically improve the profitability of jasmine farmers [35]. Farmers lead approach to technology adoption will find more acceptances among jasmine farmers [36, 37]. The study findings

Authorized licensed use limited to: SASTRA. Downloaded on July 12,2022 at 03:43:51 UTC from IEEE Xplore. Restrictions apply.

will be of social, economic and business implications and may be helpful to policymakers to enhance the sustainability of the flower farming system.

Acknowledgement

The authors thank ICSSR, New Delhi for supporting this research under IMPRESS Scheme.

REFERENCES

[1] A. Eliazer Nelson, K. Ravichandran and U. Antony, "The impact of the Green Revolution on indigenous crops of India", *Journal of Ethnic Foods*, vol. 6, no. 8, 2019. 10.1186/s42779-019-0011-9.

[2] B.A. Newman and Bitter, "Harvest: Farmer suicide and the unforeseen social, environmental and economic impacts of the Green Revolution in Punjab, India". 2007; https://foodfirst.org/wp-content/uploads/2013/12/DR15-A-Bitter-Harvest.pdf

[3] P. Pingali, A. Aiyar, M. Abraham and A. Rahman, "Agricultural Technology for Increasing Competitiveness of Small Holders", *Transforming Food Systems for a Rising India*, pp. 215-240, 2019. https://doi.org/10.1007/978-3-030-14409-8_9.

[4] Singh, R., Singh, K. and Abhay, K., 2014. *A Study on Adoption of Modern Agricultural Technologies at Farm Level in Bihar*. [online] Mpra.ub.uni-muenchen.de. Available at: <https://mpra.ub.uni-muenchen.de/73831/1/MPRA_paper_73831.pdf> [Accessed 26 January 2022].

[5] P. Mondal and M. Basu, "Adoption of precision agriculture technologies in India and in some developing countries: Scope, present status and strategies", *Progress in Natural Science*, vol. 19, no. 6, pp. 659-666, 2009. https://doi.org/10.1016/j.pnsc.2008.07.020

[6] N. Chhogyel and L. Kumar, "Climate change and potential impacts on agriculture in Bhutan: a discussion of pertinent issues", *Agriculture & Food Security*, vol. 7, no. 79, 2018. https://doi.org/10.1186/s40066-018-0229-6.

[7] M. Rahman, S. Kang, N. Nagabhatla and R. Macnee, "Impacts of temperature and rainfall variation on rice productivity in major ecosystems of Bangladesh", *Agriculture & Food Security*, vol. 6, no. 10, 2017. https://doi.org/10.1186/s40066-017-0089-5.

[8] R. Mishra, P. Mahesh and S. Kolluru, "Contribution of Organized Retailing to Agricultural Distribution System: Prospects and Challenges", *India Studies in Business and Economics*, pp. 37-58, 2015. https://doi.org/10.1007/978-81-322-2476-1_3.

[9] T. Yamano, S. Rajendran and M. Malabayabas, "Farmers' self-perception toward agricultural technology adoption: evidence on adoption of submergence-tolerant rice in Eastern India", *Journal of Social and Economic Development*, vol. 17, no. 2, pp. 260-274, 2015. https://doi.org/10.1007/s40847-015-0008-1

[10] P. Bagya Janani, R. Premavathi and D. Puthira Prathap, "Technology Adoption Behaviour of Jasmine Growers - A Critical Analysis", *Journal of Extension Education*, vol. 28, no. 1, pp. 5607-5613, 2016. https://doi.org/10.26725/JEE.2016.1.28.5607-5613

[11] A. Rani and P. Murugan, "Yield Gap and Constraints in Jasmine Cultivation and Suggestions to Increase the Production in Tamil Nadu, India, *International Journal of Current Microbiology and Applied Sciences*, vol. 9, no. 3, pp. 1373-1381, 2020. https://doi.org/10.20546/ijcmas.2020.903.159

[12] R. Thulasiram and P. Sivaraj, "An economic analysis of production and marketing of jasmine in Madurai District of Tamil Nadu", *International Journal of Farm Sciences*, vol. 10, no. 2, pp. 60-67, 2020. 10.5958/2250-0499.2020.00035.x

[13] H. Ray, S. Majumdar, S. Parua Biswas, A. Das, T. Ghosh, A. Ghosh, "Characterization of the Volatile Aroma Compounds from the Concrete and Jasmine Flowers Grown in India", *Chemical Engineering Transactions*, vol. 40, pp. 265-270, 2014. https://doi.org/10.3303/CET1440045

[14] L. De and D. Singh, "Floriculture Industries, Opportunities and Challenges in Indian Hills", *International Journal of Horticulture*, vol. 6, no. 13, pp. 1-9, 2016. 10.5376/ijh.2016.06.0013

[15] D. Satterthwaite, G. McGranahan and C. Tacoli, "Urbanization and its implications for food and farming", *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 365, no. 1554, pp. 2809-2820, 2010. 10.1098/rstb.2010.0136

[16] M. Nasrollahi, M. Fathi and N. Hassani, "Eco-innovation and cleaner production as sustainable competitive advantage antecedents: the mediating role of green performance", *International Journal of Business Innovation and Research*, vol. 22, no. 3, pp. 388-407, 2020. https://dx.doi.org/10.1504/ijbir.2020.107978

[17] M. Saripalle, "Jasmine cultivation in Tamil Nadu: Market structure and pricing", *World Development Perspectives*, vol. 1, pp. 12-14, 2016. https://doi.org/10.1016/j.wdp.2016.05.004

[18] J. Abey and R. Velmurugan, "Obstacles mentioned by the rural entrepreneurs in central districts of Tamilnadu". *Journal of Critical Reviews*, vol. 7, no. 3, pp.230-232, 2020. http://dx.doi.org/10.31838/jcr.07.03.42.

[19] H. Ray, S. Majumdar, A. Das, N. Bhattacharyya, S. Kumar and R. Bhattacharjya, "Aroma quality evaluation of Jasmine flower", *Proceedings of the 2014 International Conference on Control, Instrumentation, Energy and Communication (CIEC)*, 2014. 10.1109/ciec.2014.6959159.

[20] F. Davis, R. Bagozzi and P. Warshaw, "User Acceptance of Computer Technology: A Comparison of Two Theoretical Models", *Management Science*, vol. 35, no. 8, pp. 982-1003, 1989. https://doi.org/10.1287/mnsc.35.8.982.

[21] M. Salimi, R. Pourdarbani and B. Nouri, "Factors Affecting the Adoption of Agricultural Automation Using Davis's Acceptance Model (Case Study: Ardabil)", *Acta Technologica Agriculturae*, vol. 23, no. 1, pp. 30-39, 2020. https://doi.org/10.2478/ata-2020-0006

[22] A. Adrian, S. Norwood and P. Mask, "Producers' perceptions and attitudes toward precision agriculture technologies", *Computers and Electronics in Agriculture*, vol. 48, no. 3, pp. 256-271, 2005. https://doi.org/10.1016/j.compag.2005.04.004

[23] D. Compeau and C. Higgins, "Computer Self-Efficacy: Development of a Measure and Initial Test", *MIS Quarterly*, vol. 19, no. 2, pp. 189-211, 1995. https://doi.org/10.2307/249688.

[24] J.T. Roscoe, Fundamentals Research Statistics for Behavioural Sciences. New York: Holt, Rinehart and Winston; 1975.

[25] J.F. Hair, W.C. Black, B.J. Babin and R.E. Anderson, Multivariate Data Analysis, Pearson, 2010.

[26] S. Liu, H. Liao, and C. Peng, Applying the technology acceptance model and flow theory to on-line e-learning users acceptance behaviour". *Issues Information System*, vol. 6, no. 2, pp. 175-181. https://doi.org/10.48009/2_iis_2005_175-181

[27] J. Wu and S. Wang, "What drives mobile commerce?", *Information & Management*, vol. 42, no. 5, pp. 719-729, 2005. https://doi.org/10.1016/j.im.2004.07.001

[28] E. Karahanna, D.W. Straub and N. Chervany, "Information Technology Adoption Across Time: A Cross-Sectional Comparison of Pre-Adoption and Post-Adoption Beliefs", *MIS Quarterly*, vol. 23, no. 2, pp. 183-213, 1999. https://doi.org/10.2307/249751.

[29] Y. Malhotra, and D.F. Galletta, "Extending the technology acceptance model to account for social influence: Theoretical bases and empirical validation". In: Proceedings of the 32nd Hawaii International Conference on System Sciences, pp. 1-14, 1999.

[30] L. Phillips, R. Calantone and M. Lee, "International Technology Adoption", *Journal of Business & Industrial Marketing*, vol. 9, no. 2, pp. 16-28, 1994. 10.1108/08858629410059762

Authorized licensed use limited to: SASTRA. Downloaded on July 12,2022 at 03:43:51 UTC from IEEE Xplore. Restrictions apply.

[31] S. Tohidyan Far and K. Rezaei-Moghaddam, "Determinants of Iranian agricultural consultants' intentions toward precision agriculture: Integrating innovativeness to the technology acceptance model", *Journal of the Saudi Society of Agricultural Sciences*, vol. 16, no. 3, pp. 280-286, 2017. https://doi.org/10.1016/j.jssas.2015.09.003.

[32] K. Mottaleb, "Perception and adoption of a new agricultural technology: Evidence from a developing country", *Technology in Society*, vol. 55, pp. 126-135, 2018. https://doi.org/10.1016/j.techsoc.2018.07.007.

[33] M. Vasan, "Moderating effect of demographic and business characteristics on performance of women owned small enterprises: Empirical evidence from India", *Serbian Journal of Management*, vol. 15, no. 2, pp. 319-333, 2020. https://doi.org/10.5937/sjm15-21922

[34] G. Yoganandan and M. Dinesh Kumar, "Qualities of successful entrepreneurs – A review of relevant literature", *Materials Today: Proceedings*, 2021. https://doi.org/10.1016/j.matpr.2021.03.022.

[35] M. Vasan, "Impact of promotional marketing using Web 2.0 tools on purchase decision of Gen Z", *Materials Today: Proceedings*, 2021. https://doi.org/10.1016/j.matpr.2021.03.188

[36] S. Naspetti et al., "Determinants of the Acceptance of Sustainable Production Strategies among Dairy Farmers: Development and Testing of a Modified Technology Acceptance Model", *Sustainability*, vol. 9, no. 10, p. 1805, 2017. https://doi.org/10.3390/su9101805

[37] G. Yoganandan, A.A.A. Rahman, M. Vasan and A. Meero, "Evaluating agripreneurs' satisfaction: exploring the effect of demographics and emporographics", *Journal of Innovation and Entrepreneurship*, vol. 11, no. 1, 2022. https://doi.org/10.1186/s13731-022-00193-9

Authorized licensed use limited to: SASTRA. Downloaded on July 12,2022 at 03:43:51 UTC from IEEE Xplore. Restrictions apply.

Quality & Quantity
https://doi.org/10.1007/s11135-021-01254-z

Evaluating the quality of scientific research papers in entrepreneurship

Yoganandan G.[1] · **Vasan M.**[2]

Accepted: 30 September 2021
© The Author(s), under exclusive licence to Springer Nature B.V. 2021

Abstract
The study aims to find the quality of research papers published in the domain of entrepreneurship in India. This study covers 100 research papers. A standardized measurement tool developed by the earlier researchers was used to evaluate the research quality. The data compiled using the measurement tool were analyzed with the support of the SPSS. The statistical tools such as descriptive statistics, Friedman's test, factor analysis, two-sample 't' test, and ANOVA are applied to analyze the data. The study findings reported that the quality of research papers published in the field of entrepreneurship is not up to the quality standards. The quality of multiple-author papers is better than single-author papers. Similarly, the quality of papers published by foreign authors is comparatively better than Indian authors. Further, the quality of papers published with the combination of foreign and Indian authors is substantially good. The quality of papers published in foreign journals is higher as compared with Indian journals. Further, the standard of papers published under the qualitative approach was comparatively better than the quantitative approach. The authors developed a Conceptual Model of Process and Product of Research (YOVA model). This model clearly shows that the whole research process yields six levels of research products. The study recommended that the researchers need to go for international collaborations to improve the quality of the publication. The funding agencies, higher learning institutions and research institutions should focus on enhancing research infrastructure. The study examined the validity of research articles searched by novice researchers in India in Google by using keywords related to entrepreneurship and, as such this non-focused approach is a big impediment to quality research.

Keywords Entrepreneurship · Quality criteria · Research collaboration · Research quality · YOVA model

✉ Vasan M.
m.vasan@yahoo.com

Yoganandan G.
yoganandan@periyaruniversity.ac.in

[1] Department of Management Studies, Periyar University, Salem, Tamilnadu 636 011, India

[2] Department of Commerce, National College (Autonomous), Tiruchirappalli, Tamilnadu 620 001, India

Published online: 15 October 2021

1 Introduction

After the invention of internet communication, globalization has become possible in every sphere of economic activity and the educational sector is no exception to it. The world has become more interconnected, interdependent and integrated. As a result, a plethora of educational institutions operates among themselves in the cut-throat competitive environment. The competition extends into three areas of their operation, namely, academics, research and consultancy/ extension activities. The educational institutions hitherto focused on academics want to move up by orienting themselves with more research activities. This has resulted in the growth of academic research outputs in the form of scholarly research papers, seminar reports, conference papers, book chapters, books, reservoirs/ databases and other presentation materials, making the assessment of their quality a very challenging task (Roghayeh et al. 2019). The research articles not only assist the educational institutions but also benefits the individual researcher or professor. In many top-ranked academic institutions, pay, fellowship, research grants, promotion, tenure and other rewards and recognition are dependent on the individual teacher's contribution and the perceived quality of the scholarly research output (Michael et al. 2016; James and Vincent 1974). Now, before us, there are three questions namely What, Why and How is Quality Scientific Research? (Pär Mårtensson et al. 2015). In this study, the focus is predominantly on research papers published in the domain of entrepreneurship. In fact, there is a greater agreement among the research community that the research measurement in social sciences is more complex and influenced by many uncontrolled variables (Andrea et al. 2015).

2 What is research?

Research is a term, very often, used interchangeably with inquiry, investigation and study. But, a closer look at the usage of these words clearly pinpoints the differences in their meaning. Research can be defined as conducting an inquiry in a structured, logical and systematic manner. In other words, research is the practice of working in a manner supported by scientific rules, guidelines and principles, and the outcome of this work is science (Pär Mårtensson et al. 2015). Yet, another way to understand the research is that human inquiry wants to examine, explain, and detail things and phenomena. The research by scientific method provides it logic and justification (Sheikh, 2011).

3 What is quality research?

Quality is understood and defined by many in different ways depending upon the context and circumstances. Quality of research denoted its ability to meet the stated research objectives. Can we conclude if the research leads us to an unexpected outcome, is a low-quality of research? In hindsight, it may look like that is not quality research. X-rays were discovered by Professor Wilhelm Roentgen in 1895, in an experiment carried out by him with an objective to analyze the properties of cathode rays on the glass. So, this result clearly proves that if the research can meet the unstated needs, then it should be called Quality Research. Like beauty, the quality, in a certain angle can be said, lies in the eyes of the beholder. Quality can be, in cases of processes, perceived quality; here, the quality is based

on the perception of the reader or evaluator. Quality can also be defined as the absence of defects or a degree of excellence (Shewfelt 1999). Quality Research is that research with all the essential features expected by its users (Sheikh 2011). Some authors argue that the term Quality research refers to the scientific process encompassing every dimension of the research design (NCDDR, 2002). One of the vital requirements of Quality Research in Entrepreneurship is that it should have industrial, social, academic and (future) research utilities (Sheikh 2011). The basic impediment for coming out with one definition for Quality Research is the subject area, domain, or field of study is entirely different. Therefore, under these circumstances, it is construed that the Domain or Field is the real determiner of Quality Research (Michael et al. 2016). Also, there are various types of studies viz., qualitative, quantitative and experimental studies, and the understanding of what constitutes quality in research varies to a greater extent (Gürol and Hakan 2019).

4 Why is quality research?

Quality research has multiple benefits to all stakeholders, including the individual researcher, educational institution, business organizations, governments and society at large.

The following is the list of major benefits of quality research.

- Getting published in top-ranked journals
- Getting research grants from government and non-governmental organizations.
- Higher rank for the institution in National /International Ranking
- Higher salary, promotion and other career-related benefits.
- Recognition by peers, institutions, business organizations and government.
- Recognition in the form of both monetary and non-monetary awards
- New products, services or business models for business organizations.
- Cost, time, effort and space utilities for consumers/society
- Contributing to the nation's science and innovation (Pär Mårtensson et al. 2015).

It is clearly visible that developed countries are more competitive in the international market due to their superior quality of research activities. Therefore, it is pertinent to note that the developing countries also need to enhance their research output in terms of quantity and quality by increasing collaboration with national and international researchers and research organizations (Ritu et al. 2014).

5 How is quality research?

The quality of research should be ensured in the entire sequence of the research process. The authors of this study present a Conceptual Model of Process and Product of Research depicting this complete chain of process and product of Quality Research (Fig. 1). This model may be called as YOVA model of Process and Product of Research. The application of the scientific method enables measuring the quality of the entire sequence of the research process including the data collection and analysis (Sanne et al. 2006). The process of research, as per the proposed YOVA model, can be broadly categorized into three stages

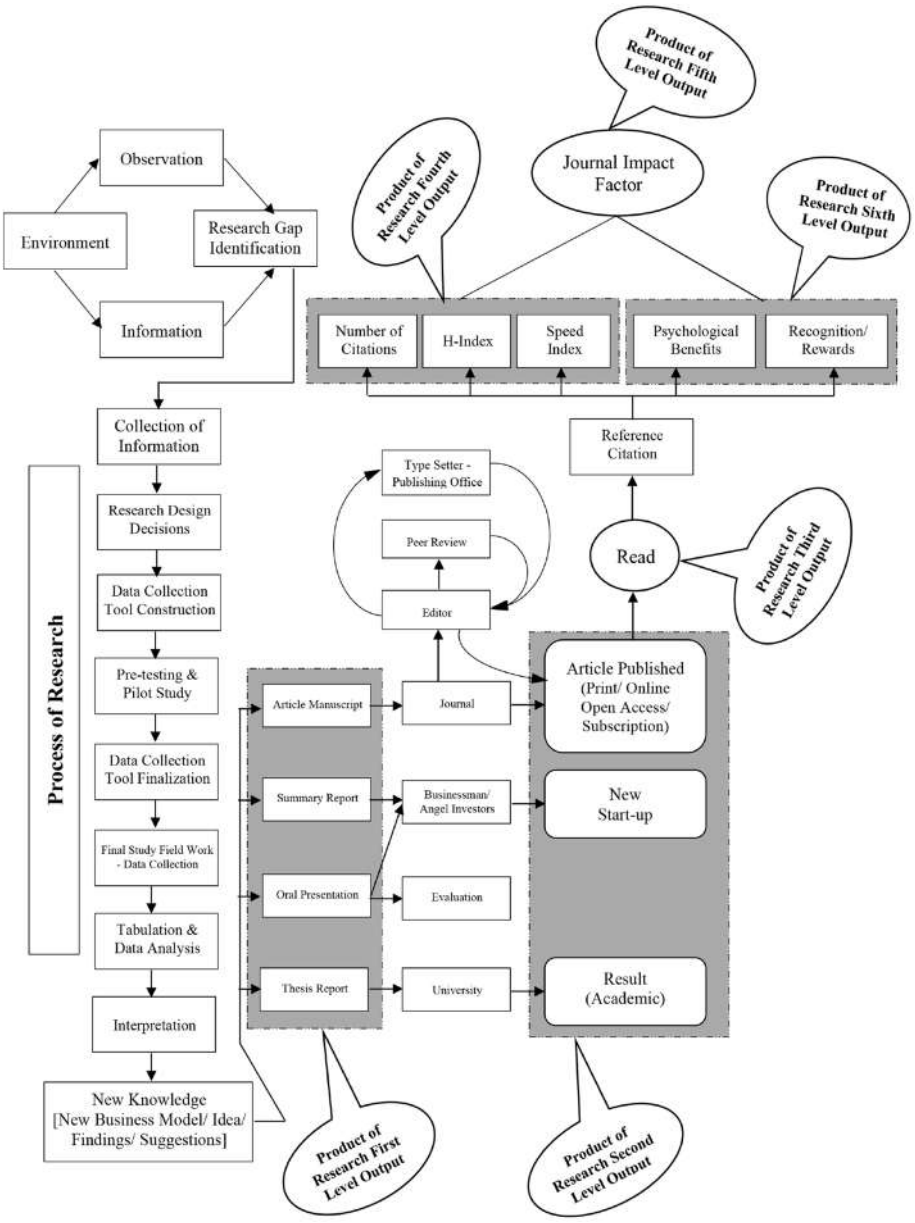


Fig. 1 YOVA Model of Process and Product of Research Source: Developed by Authors

namely, i) pre-research stage or research planning stage, ii) execution or implementation stage and iii) post-research stage.

Many authors look at their research work leading to article publication as a two-step activity in which conducting research is considered as a process-oriented activity and research report writing is considered as a product-oriented activity (Ortrun and Margaret

2007). But, the YOVA model, the Conceptual model of Process and Product of Research clearly shows that the whole research process yields six levels of research products.

The Quality focus starts from Research Planning and permeates into every stage, including writing an effective research thesis, oral presentation and articles (Ortrun and Margaret 2007). Needless to say, a well-defined problem is half-solved. So, the research problem must be clearly formulated to either ensure quality or at least the research problem is dealt with effectively (RAND, 2010). There are studies that highlight how the overall research design, objectives, research methods, findings influence the type and quality of research output produced (NCDDR, 2002). It is worth noticing that if there are no apparent formal methodologies to apply, the analytical research method should always be recommended, and it should be defended (RAND, 2010). Another aspect of Quality Research is that if research does not have validation procedures as one part of the research, it is challenging to assess the quality of the research. In such cases, there is a concern that the researchers may not pay adequate attention to maintain the quality of their research (Sanne et al. 2006). The discussion on quality in the broader stages of the research process is curtailed at this juncture as this study aims to discuss only the finer dimensions of the quality of research articles.

6 Quality research article

Many learners and researchers assume that research is solely a professional activity. In fact, the research activity, publication of their articles, and citation of the publications by co-workers and peers in the academic and research field are all social activities (Lutz et al. 2012)**.** Another interesting aspect of Research Articles is the availability of fewer scientific literature on research quality (Pär Mårtensson et al. 2015).

A quality research article should be strongly supported by a validated, demanding research design, reliable data, application of most suitable statistical tools, properly cited (plagiarism-free) article with finding and suggestions having utility for all stakeholders. There is a misconception about the articles published in top-ranking journals. It is evident that all the articles that get published in top-ranked, middle-ranked or lower-ranked journals are not of the same quality (Sendhilkumar et al. 2013).

7 Measuring the quality of research article

There are many ways and means available to assess the quality of the research article. The following is the list of (bibliometric and non-bibliometric) methods available to gauge the quality of a research paper.

Non-bibliometric methods.

- Style of writing and Clarity in communication
- Use of appropriate technical terms
- Language and Grammar mistakes
- Alignment
- Quality of the content/ result

Non-bibliometric methods are mainly used at the time of article publication process by editor, peer reviewers and publishers' office to evaluate the quality of the research article. Once the research is completed his/ her article, it is sent to a journal for publication. The editor of the journals is the first person to evaluate the quality of the manuscript submitted for publication, thereby contributing to further improvement in the quality of the manuscript, which is also a significant contribution to that specific area of research (Lutz et al. 2012). The editor makes two kinds of decisions; one is based on his assessment of the quality of the research article. The other decision is based on the peer reviewers' comments and suggestions. The rejection of an article by the editor does not necessarily mean that the article is of poor quality. Those articles that do not fall within the journal's scope are also got rejected as per the journal's policy. The articles which are in line with the subject area of the journal are then sent to the peer reviewers.

The peer-review process is a very age-old practice to evaluate the quality of manuscripts, and the traces of such practice can be seen since the times of the Second World War (Moyses, 2006). The peer-review process is one of the Consensus methods among the various other consensus methods like conferences, position statements and systemic review, considered by the research community as the most important means of assessing the quality of research articles (NCDDR 2002). The role of reviewers is critical in ensuring the quality of the research article as they address several quality dimensions of the manuscript under review and their recommendation plays a key role in the acceptance decision of the editor (Abdel, 2004). A recent study points out that the top reasons for rejection by peer reviewers are 'absence of message', lack of originality and inadequate methods (Piyush et al. 2006). Even then, the peer review process is not a foolproof and perfect mechanism for control of quality in scientific research article publication (Lutz et al. 2012) and, the assessment of quality remains elusive (Moyses 2006). Though editors wish to accept the best manuscripts, they rely on the reviewers' recommendations as they are constrained to act only on the basis of the pool of manuscripts submitted for publication (Michael et al. 2016). At times, evaluators get to carry forward by applying subjective assessment methods such as the qualification (academic degrees) of the researcher, grants received from national & international organizations, grants from the government, number of Ph.D. scholars supervised, number of post-doctoral fellows mentored, prominence in the national and international academic & science networks of researchers, to judge the quality of the research manuscript under review (Pär Mårtensson et al. 2015). In a nutshell, the non-bibliometric methods have too many pitfalls than bibliometric methods.

Bibliometric methods.

- Read
- Download
- Subscriptions
- Citations
- Journal Ranking
- h-index
- Sentiment analysis
- Other indexes

The research community considered the bibliometric assessment mechanism expressed through various indicators, offers a better degree of comparison between quality and non-quality research articles (Valérie and Pierre 2010) as it is essential to assess the quality of papers after they are published (Moyses, 2006). It is purely published article-based

measurements that pinpoint the emerging trends in a particular area of research (Shri Ram, 2016). The substantial growth in the internet-enabled noticeable increase in the publication of scientific research outcomes being published in the form of abstracts and articles online by journals while print journals (printed version) are losing their distinction (Valérie, & Pierre, 2010). Many online open access journals and online research databases like ResearchGate etc., just provide the full paper through a web link on the title of the research paper. The readers need to click on (open) the web link to read the full paper. Every click on the title is counted as one Read. Thus, the number of Reads is assumed as the indicator of other researchers in the articles, but this is not widely accepted as it offers greater scope for easy manipulation. Some online journals provide the articles in the form of freely downloadable PDF files, and every download is counted. The number of downloads signifies a higher level of interest than the Read indicator but, similar to the Read indicator, the Download indicator is also not a better evaluation method. Some closed-access journals, usually top-ranking journals allow fee-based or subscription-based access to the research articles echoing a much higher level of other researchers' interest in the research article indirectly denoting the higher quality of the said research article. Once again, this subscription-based method also cannot be completely relied upon since, in this method, the quality of an article is measure in terms of not only the willingness but also the purchasing power of the other interested researchers. After the article is published in the journal, many researchers have accessed it not only to read but also to refer for their studies (Valérie and Pierre 2010).

Citations are the ways and means through which the source is acknowledged and, thereby ensures the genuineness of the author who refers to that published research article (Sendhilkumar et al. 2013), (Lutz et al. 2012). Evaluators of research articles, in general, agree that the citation(s) of a publication is an acceptable measure of the quality of a research article as it demonstrates the recognition by peers (Roghayeh et al. 2019). It is pertinent to point out that not every researcher attempts to cite or adequately cite the referred article leading to unethical and regulatory issues like plagiarism and theft of intellectual property rights. Journals are ranked by the journal impact factor which shows the frequency with which the concerned journal's articles are cited in the research articles of other researchers (Roghayeh et al. 2019)**.** Another method for increasing the citations by journals are accepting more literature review article than results of single empirical studies (Moyses, 2006).

Self-citations play spoilsport in the genuine assessment to evaluate the quality of both the researcher and journal. There is a growing trend among researchers to collaborate and produce articles jointly to increase their chances of enhancing the article quality and getting acceptance for publication in top-ranked journals. The studies show that there is a positive correlation between the number of authors per article, the proportion of high-quality research articles and acceptance of the article by higher-ranked journals (Bryan et al. 2016). and this is substantiated by the collaborative index which is a measure of quality in the aggregate (Lawani, 1986).

Presumably, higher-ranked journals follow the rigorous peer-review process and publication process and attract a higher percentage of quality articles than lower-ranked journals (Michael et al. 2016 and Roghayeh et al. 2019). But, these so-called ranked journals are just a tiny proportion in the massive pool of scientific journals and even then, specific subject areas/ disciplines dominate the ranking. So, the subject bias is a limitation that relates to the journal ranking with article quality. To overcome the drawback in the citation method, J.E Hirsch introduced the h-index in 2005 (Roghayeh et al. 2019)**.** The h-index is an author-oriented measurement unit that measures both the productivity and total citations

of the publications of a researcher. It is calculated by matching the number of citations with the number of publications (Lutz et al. 2011). The citation speed index was introduced by Schubert and Glänzel in 1986 as an improved bibliometric measure for the quality of research articles. This index is calculated on the basis of those publications in a publication set whose first citation was made a certain minimum time ago (Lutz 2010).

Sentiment analysis of citations is, yet another, a novel method of assessing the quality of research articles that focuses on analyzing the attitude of the authors by way of positive credits and negative criticisms and performed on scientific texts and other genres (Sendhilkumar et al. 2013). The application of machine learning methods makes it convenient to automatically retrieve high-quality, specific theme-oriented articles using inclusion or citation by a particular database (Yindalon et al. 2005). SJR is an essential introduction to the toolkit of bibliometric analysis. It measures the prestige or influence of a scientific journal by considering the citations of three years, which is comparatively a better option than the journal impact factor which considers only two years of citations (Roghayeh et al. 2019). There are many other methods used in evaluating the quality of research like the Boolean queries which is a decision tree method and whatever may be the measurement methods they come with their share of strengths and limitations (Valérie and Pierre 2010).

8 Materials and methods

This paper is intended to gauge the quality of research on entrepreneurship in India. It is based on the research papers published in the journals which are amassed via Google Search engine with keywords 'Entrepreneurship in India', 'Research on Entrepreneurship', 'Study on Entrepreneurship' and 'Trends in Entrepreneurship Research in India'. Research papers published by researchers in the first 50 links for each keyword were considered. The logic behind using keywords and Google Search Engine is to mimic the search behaviour of novice and young researchers who may not have better information about the databases of various reputed publishers and lack digital libraries in their research centres. As such the research design of this study is bibliometric analysis or quantitative analysis of the quality of published articles. At this juncture, it is relevant to ask this question, how would young and novice researchers in India, after collecting these articles to learn, understand and conduct quality research? So, this study diligently analyzed the quality of research on entrepreneurship based on papers collected through the Google Search engine.

This study covers 100 papers relating to research on entrepreneurship in India for a period of 12 years starts from 2008 to 2020. Many studies consider this sample size is good enough for bibliometric studies (Mays and Pope, 2000; Timmer et al. 2003). For assessing the quality of research papers, a checklist has been prepared based on the tool developed by Cho and Bero (1994) and Timmer et al. (2003) and the guidelines recommended by Mays and Pope (2000) and Popay et al. (1998). This modified assessment tool is suitable for qualitative as well as quantitative studies. The five-point scale starts from Excellent [5] to Very poor [1], was used to measure the research quality. Hence, measurement tools include general details relating to sample research papers such as the number of authors, place of the institution, location of publisher and research approach. Hence, these variables were considered as the independent variable for the study. The data compiled through a measurement tool were analyzed by using the SPSS. The statistical tools such as descriptive statistics, Friedman's test, factor analysis, two-sample 't' test, and ANOVA were applied to analyze the data and to test the following hypotheses.

- H_1: Number of authors has not influenced the quality of research.
- H_2: Place of institution has not influenced the quality of research.
- H_3: Domicile of journal publisher has not influenced the quality of research.
- H_4: Research approach has not influenced the quality of research.

9 Analysis/ Results

This segment analyses the data collected through a research quality measurement tool. The discussion aims to elucidate the quality of research papers in entrepreneurship. The first part delves into the quality of research papers. The second part delineates a composite appraisal of journal paper quality. Next, the score of quality of papers is compared with the general details relating to sample research papers such as the number of authors, place of the institution, location of publisher and research approach. An interesting feature of the study is so far no researchers in India have analyzed the quality of research on entrepreneurship.

A plethora of research papers has been published in the sphere of entrepreneurship. The set of research papers considered for the study had varied sub-themes but focused on the themes like the status of entrepreneurship in India, MSMEs, business performance, entrepreneurial skills and their work-life balance, social entrepreneurship, youth entrepreneurship, women entrepreneurship, rural entrepreneurial, problems and prospects, innovation, demography of entrepreneurs, leadership qualities, the impact of entrepreneurial development of programmes, students' perception on entrepreneurship and government policies and initiatives.

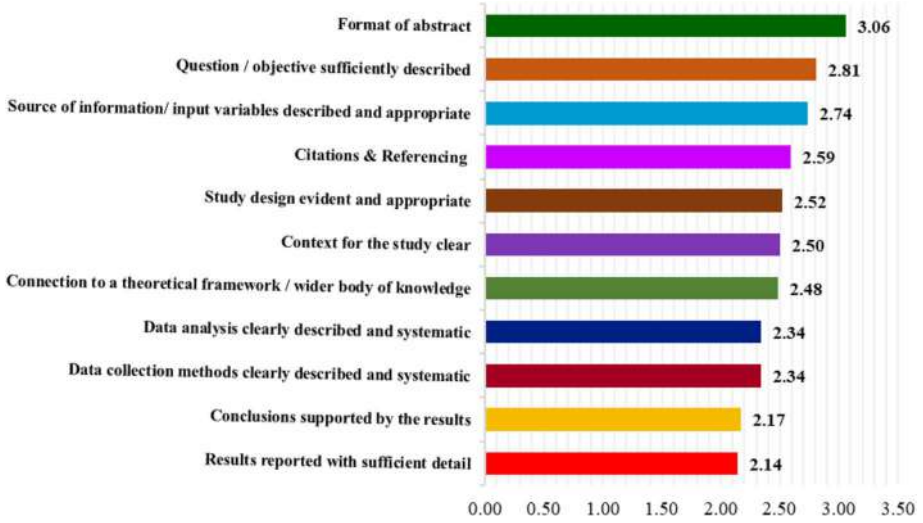


Fig. 2 Mean Score for Quality of Research Papers in Entrepreneurship

9.1 Indicators of quality of research papers

The mean values for each quality indicators were grouped into their rank order (Fig. 2). Means for 11 items using a 5-point Likert scale relating to quality indicators were in the range of 2.14 to 3.06. These values for quality indicators ranged between low/ moderate quality. It indicated that the overall quality of research papers published in the domain of entrepreneurship in India was not up to the mark.

The χ^2 test statistic was 187.376. It was significant at a 5% level ($p = < 0.05$). It insinuated that there was a substantial variance in the quality indicators of journal papers. The mean rank showed that the highest quality was recorded for abstract format followed by sources of information/ input variables, question/ objective sufficiently described. Also, the lowest mean scores were obtained for variables Conclusions were supported by the results and Results reported with sufficient details. This indicates that the authors did not pay adequate attention to the systematic process which needs to be followed while preparing a research paper (Table 1).

9.2 Developing and assessing a composite appraisal of journal paper quality

The result of Cronbach's Alpha is 0.979 which is higher than that of a threshold of 0.70 (Hair et al. 2009). It implied that the reliability of scale for quality indicators was high. Factor analysis was applied for the 11 indicators of quality of journal papers in the field of entrepreneurship. This analysis extracted a single factor solution with 83.19% of the total variance which is more than that of 60% of the total variance suggested by Hair et al. (2010).

The highest factor loadings for the seven quality indicators such as results reported with sufficient details, the context for the study clear, data analysis clearly described and systematic, study design evident and appropriate, conclusions supported by the results, connection to a theoretical framework/ wider body of knowledge and source of information/ input variables described and appropriate. This result proved that these seven key areas are highly important and should be strengthened to improve the quality of research papers (Table 2).

Table 1 Ranking of Indicators of Quality of Research Papers in Entrepreneurship

Variables	Mean Rank	Rank
Format of abstract	8.04	1
Question / objective sufficiently described	7.06	3
Study design evident and appropriate	5.93	5
Context for the study clear	5.82	6
Source of information/ input variables described and appropriate	7.24	2
Connection to a theoretical framework / wider body of knowledge	5.80	7
Data collection methods clearly described and systematic	5.43	8
Data analysis clearly described and systematic	5.30	9
Results reported with sufficient details	4.35	11
Conclusions supported by the results	4.55	10
Referencing	6.51	4
$\chi^2 = 187.376$; d.f. = 10;Sig. = .000		

Table 2 Factor Loadings of Quality Indicators of Research Papers

Indicators	Factor Loadings
Results reported with sufficient details	.948
Context for the study clear	.943
Data analysis clearly described and systematic	.940
Study design evident and appropriate	.937
Conclusions supported by the results	.928
Connection to a theoretical framework / wider body of knowledge	.927
Source of information/ input variables described and appropriate	.920
Data collection methods clearly described and systematic	.895
Question / objective sufficiently described	.888
Referencing	.886
Format of abstract	.813
Variance Explained	83.19%
Alpha Reliability	.979

Table 3 Results of ANOVA

Description	Variables	N	$\bar{x}$	σ	F	Sig
Number of Authors Vs Quality of Paper	1	38	22.526	11.413	5.484	.000
	2	43	27.465	13.041		
	3	13	39.615	15.359		
	4	3	23.000	10.536		
	5	1	55.000	8.140		
	7	2	46.500	3.536		
	Total	100	27.690	14.003		
Place of Institution Vs Quality of Paper	India	87	25.862	13.317	7.544	.001
	Foreign	9	36.333	12.520		
	Both	4	48.000	9.899		
	Total	100	27.690	14.003		

Table 4 Results of 't' Test

Description	Variables	N	$\bar{x}$	σ	t	Sig
Domicile of Publisher Vs. Quality of Paper	India	71	22.423	10.845	7.263	.000
	Foreign	29	40.586	12.514		
Research Approach Vs Quality of Paper	Qualitative	30	34.067	13.308	3.027	.000
	Quantitative	68	25.103	13.596		

9.3 Results of ANOVA and two-sample '*t*' Test

In this part, variation in the quality of research papers (Dependent Variable) with regard to the number of authors, place of the institution, location of publisher and research approach

(Independent Variables) were analyzed. In this regard, ANOVA was used to find variation among the two more variables and the 't' test is applied for dichotomous variables.

According to ANOVA results, the quality of papers significantly varied with the number of authors ($F = 5.484$; $p = < 0.05$) and the place of the institution ($F = 7.544$; $p = < 0.05$). So, hypotheses H_1 and H_2 were not supported. Mean values indicate that the quality of multiple-author papers was better than single-author papers. It implied that joint and collaborative works were comparatively good. Similarly, the quality of papers published by foreign authors was comparatively better than Indian authors. Further, the quality of papers published with the combination of foreign and Indian authors was substantially good (Table 3).

The results of two-sample 't' test proved that the quality of papers significantly varied with the domicile of the publisher and research approach. So, hypotheses H_3 and H_4 were not supported. Mean values indicate that the quality of papers published in foreign journals was higher as compared with Indian journals. Further, the standard of papers published under the qualitative approach was comparatively better than the quantitative approach (Table 4).

10 Discussion

This study investigated the quality of research papers published in the domain of entrepreneurship. Based on the empirical evidence, the researchers note that the research papers published in the domain of entrepreneurship failed to furnish certain relevant details and also did not maintain quality standards in the areas of abstract, research questions/ objectives, data sources, context of the study, theoretical framework, data collection methods, data analysis, reporting of results, linkage of conclusion with results and citations and referencing. These anomalies created structural defects and severely affected the rudimentary quality of a research paper. This indicates that the researchers did not give due importance to all the facets of a research paper while preparing a research paper for publication in the journals. Moreover, it is also observed that many of the journals do not have a robust double-blind peer review system, and as a result, the reviews are not done rigorously. This finding is in line with the studies conducted by Negin (2017) and Seglen (1997).

The study found that the quality of multiple-author papers was better than single-author papers. This finding reiterates akin to the previous studies conducted by various researchers like Mattsson et al. (2008) and Wasko and Faraj (2005). It indicates the joint and collaborative work facilitates skill-sharing among the authors which has ultimately improved the quality of a research paper (John 1996, Li et al. 2013, Stokols et al. 2005 and Walter et al. 2007). Further, the research papers published by the authors of foreign countries were substantially better than Indian authors. It is due to inadequate research infrastructure in the higher learning institutions and insufficient financial aids available at the disposal for research. This result corroborates previous studies done by Djulbegovic et al. (2000), Inayatullah & Fitzgerald (1996) and Seglen (1997). So, the need of the hour is funding support from the government to compete for emerging economies like India with established economies at the global level (Adams, 2013).

The results proved that the quality of papers published in foreign journals was better than in Indian journals. The same result was reported by Nanjundaiah and Dinesh (2016) and Rashmi et al. (2020). The quality of papers published under the qualitative approach was comparatively better than the quantitative approach. This result reflected the research

done by Catherine and Rebecca (2017). Based on the above evidence, this study recommends the need for proper citations and referencing as it tremendously enhances the quality of research publications. The study also recommends international collaborations to improve publication quality and publish papers in high-impact factor journals.

11 Direction for future research

This study has covered only one subject area namely Entrepreneurship in the social sciences. So, future studies can be directed to appraise the research quality in various other domain areas within the broader social sciences domain. Also, the authors, in this research paper, have presented a comprehensive six-level research output model; the Conceptual Model of Process and Product of Research (YOVA model) for other researchers to take up. Future studies can be conducted on this model to either prove/ complement, disprove, extend or shorten the model.

12 Conclusion

This paper has systematically examined the quality of research papers published in journals in the domain of entrepreneurship. The study results proved that the quality of research papers published in the Indian journals did not fulfill the rudimentary quality parameters. It is due to the fact that researchers have not followed systematic steps while preparing a research paper. Based on the study results, the authors recommend that the researchers should focus on linkage of the theoretical framework with study area, clarity in study context, study design, an appropriate description for input variables, systematic data analysis, results reported with sufficient details and conclusions supported by the results. The authors also strongly recommend the need for proper citations and referencing as it tremendously enhances the quality of research publications.

The study recommends international collaborations to improve publication quality and publish papers in the high impact factor journals (Low et al. 2014). It will enrich the visibility of the research paper and also get good citation counts. The funding agencies and leaders of higher learning and research institutions should focus on enhancing research infrastructures like the subscription to leading scientific databases and journals. Further, providing sufficient publication and research grants for quality works will facilitate to climb up on the research ladder in the arena of entrepreneurship.

Funding No funding received.

Declarations

Conflict of interest The authors declare that there is no conflict of interest.

References

Abdel Moneim Aly: Quality of medical journals with special reference to the Eastern Mediterranean health journal. Saudi Med. J. **25**(1), 18–20 (2004)

Adams, J.: The fourth age of research. Nature **497**, 557–560 (2013)

Akkerman, S., Admiraal, W., Brekelmans, M., Oost, H.: Auditing quality of research in social sciences. Qual. Quant. **42**(2), 257–274 (2006)

Aphinyanaphongs, Y., Tsamardinos, I., Statnikov, A., Hardin, D., Aliferis, C.F.: Text categorization models for high-quality article retrieval in internal medicine. J. Am. Med. Inform. Assoc. **12**(2), 207–217 (2005)

Bonaccorsi, A., Cicero, T., Ferrara, A., Malgarini. M., (2015). Journal Ratings as Predictors of Articles Quality in Arts, Humanities and Social Sciences: An Analysis based on the Italian Research Evaluation Exercise. *F1000Research*, 4, 196.

Bornmann, L., Schier, H., Marx, W., Daniel, H.-D.: Does the h index for assessing single publications really work? a case study on papers published in chemistry". Scientometrics **89**, 835–843 (2011)

Bornmann, L., Schier, H., Marx, W., Daniel, H.-D.: What factors determine citation counts of publications in chemistry besides their quality? J. Informet. **6**, 11–18 (2012)

Bornmanna, L., Daniel, H.-D.: The Citation speed index: a useful bibliometric indicator to add to the h index. J. Informet. **4**, 444–446 (2010)

Chawla, R., Gupta, M., Anand, N.: Ranking of Indian journals with popular international journals: a comparative study. Int. J. Sci. Technol. Res. **9**(3), 72–81 (2020)

Cho, M.K., Bero, L.A.: Instruments for assessing the quality of drug studies published in the medical literature. J. Am. Med. Assoc. **272**, 101–104 (1994)

Djulbegovic, B., Lacevic, M., Cantor, A.: The uncertainty principle and industry-sponsored research. Lancet **356**, 635–638 (2000)

Durieux, V., Gevenois, P.A.: Bibliometric indicators: quality measurements of scientific publication. Radiology **255**(2), 342–351 (2010)

Ghazavi, R., Taheri, B., Ashrafi-rizi, H.: Article quality indicator: proposing a new indicator for measuring article quality in scopus and web of science. J. Sci. Res. **8**(1), 9–17 (2019)

Gupta, P., Kaur, G., Sharma, B., Shah, D., Choudhury, P.: What is submitted and what gets accepted in Indian Pediatrics: analysis of submissions, review process, decision making, and criteria for rejection. Indian Pediatr. **43**(6), 479–489 (2006)

Gupta, R., Tiwari, R., Mueen Ammed, K.K.: Dengue research in India: a scientometric analysis of publications - 2003–12. Int. J. Med. Public Health **4**(1), 1–9 (2014)

Hair, J.F., Black, W.C., Babin, B.J., Anderson, R.E.: Multivariate Data Analysis, 7th edn. Pearson Education, U.K (2010)

Hair, H.H., Jr, Black, W.C., Babin, B.J., Anderson, R.E., & Tatham, R.L., (2009). *Multivariate Data Analysis*. London, U.K: Pearson Education.

Hudson, J.: Trends in multi-authored papers in economics. J. Econom. Perspect. **10**(3), 153–158 (1996)

Inayatullah, S., Fitzgerald, J.: Gene Discourses: politics, culture, law, and futures. Technol. Forecast. Soc. Chang. **52**(2–3), 161–183 (1996)

James, J.B., Vincent, C.B.: Perception of journal quality. Account. Rev. **49**(2), 360–362 (1974)

Lawani, S.M.: Some bibliometric correlates of quality in scientific research. Scientometrics **9**, 13–25 (1986)

Li, E.Y., Liao, C.H., Yen, H.R.: Co-authorship networks and research impact: a social capital perspective. Res. Policy **42**(9), 1515–1530 (2013)

Low, W.Y., Ng, K.H., Kabir, M.A., et al.: Trend and impact of international collaboration in clinical medicine papers published in Malaysia. Scientometrics **98**, 1521–1533 (2014)

Mårtensson, P., Fors, U., Wallin, S.-B., Zander, U., Nilsson, G.H.: Evaluating research: a multidisciplinary approach to assessing research practice and quality. Res. Policy **45**, 593–603 (2015)

Mattsson, P., Laget, P., Nilsson, A., & Sundberg, C.J. (2008). Intra-EU Vs. Extra-EU Scientific Co-publication Patterns in EU. *Scientometrics*, 75(3), 555–574.

Mays, N., & Pope, C. (2000). Quality in Qualitative Health Research. In: Mays, N., and Pope, C. *Qualitative Research in Health Care*. 2nd ed. London: BMJ Books.

Michael, J. Cuellar, Duane, P. Truex, & Hirotoshi Takeda. (2016). Can we trust journal ranking to assess article quality? *Proceedings of the Twenty-second Americas Conference on Information Systems*, San Diego. 1–11.

Nanjundaiah & Dinesh, K.S. : Ranking and comparison of journals published in india with special reference to humanities and social sciences. Int. J. Inf. Dissem. Technol. **6**(4), 251–257 (2016)

National Center for the Dissemination of Disability Research - NCDDR. (2002). *A Technical Brief: What Are the Standards for Quality Research?* Southwest Educational Development Laboratory.

Popay, R., Rogers, A., Williams, G.: Rationale and standards for the systematic review of qualitative literature in health services research. Qual Health Research **8**, 341–351 (1998)
Ram, S.: A quantitative assessment of "chikungunya" research publications, 2004–2013. Trop. J. Med. Res. **19**, 52–60 (2016)
RAND. (2010). *Technical Report: Standards for High-Quality Research and Analysis*. RAND Corporation.
Salimi, N.: Quality assessment of scientific outputs using the BWM. Scientometrics **112**, 195–213 (2017)
Seglen, P.O.: Why the impact factor of journals should not be used for evaluating research? BMJ **314**(7079), 497 (1997)
Sendhilkumar, S., Elakkiya, E., Mahalakshmi, G.S.: Citation semantic based approaches to identify article quality. Comp Sci Inform Technol **1**, 411–420 (2013)
Sheikh Tariq Mahmood: Factors affecting the quality of research in education: student's perceptions. J. Educ. Pract. **2**(11 & 12), 34–39 (2011)
Shewfelt, R.L.: What is quality? Postharvest Biol. Technol. **15**, 197–200 (1999)
Stokols, D., Harvey, R., Gress, J., Fuqua, J., Phillips, K.: In vivo studies of transdisciplinary scientific collaboration: lessons learned and implications for active living research. Am. J. Prev. Med. **28**(2), 202–213 (2005)
Moyses Szklo. (2006). Quality of Scientific Articles. *Rev Saúde Pública*, 40 (N Esp), 30–35.
Timmer, A., Sutherland, L.R., Hilsden, R.J.: Development and evaluation of a quality score for abstracts. BMC Med Res Methodol **3**, 2 (2003)
Victor, B.G., Hodge, D.R., Perron, B.E., Vaughn, M.G., Salas-Wright, C.P.: The rise of co-authorship in social work scholarship: a longitudinal study of collaboration and article quality, 1989–2013. Br. J. Soc. Work. **47**(8), 1–16 (2016)
Walter, J., Lechner, C., Kellermanns, F.W.: Knowledge transfer between and within alliance partners: private versus collective benefits of social capital. J. Bus. Res. **60**(7), 698–710 (2007)
Wasko, M.M., Faraj, S.: Why should I share? examining social capital and knowledge contribution in electronic networks of practice. MIS Q. **29**(1), 35–57 (2005)
Welch, C., Piekkari, R.: How should we (not) judge the 'quality' of qualitative research? a re-assessment of current evaluative criteria in international business. J. World Bus. **52**(5), 714–725 (2017)
Yokuş, G., Akdağı, H.: Identifying quality criteria of a scientific research adopted by academic community: a case study. Int. J. Eurasia Soc. Sci. **10**(36), 516–527 (2019)
Zuber-Skerritt, O., Fletcher, M.: The quality of an action research thesis in the social sciences. Qual. Assur. Educ. **15**(4), 413–436 (2007)

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Yoganandan *et al.*
Journal of Innovation and Entrepreneurship (2022) 11:2
https://doi.org/10.1186/s13731-022-00193-9

Journal of Innovation and Entrepreneurship

RESEARCH Open Access

Evaluating agripreneurs' satisfaction: exploring the effect of demographics and emporographics

G. Yoganandan[1], Abdul Aziz Abdul Rahman[2*], M. Vasan[3] and Abdelrhman Meero[2]

*Correspondence:
a.abdulrahman@ku.edu.bh
[2] Kingdom University, Riffa, Bahrain
Full list of author information is available at the end of the article

Abstract

This paper attempts to gauge the satisfaction of agripreneurs and seeks to explore the effect of demographics and emporographics on the agripreneurs' satisfaction. This study proposes a seven-dimension survey instrument, called *AprenSAT*, for measuring agripreneurs' satisfaction. Responses from 784 agripreneurs are analyzed by applying exploratory and confirmatory factor analysis and multiple linear regression. The extraction of seven factors confirms that agripreneurs' satisfaction is influenced by material availability, government support, farm growth, farm income, market performance, cultivation & production and perceived farm image. The linear regression result delineates that demographic factors such as age, education level and farming experience significantly influence the agripreneurs' satisfaction. Similarly, variables of emporographics such as farm age, farm size, annual income, land ownership, sources of funds, and intercropping have a substantial influence on agripreneurs' satisfaction. We recommend information dissemination, hands-on training, the creation of adequate infrastructure and technology adoption to enhance agripreneurs' satisfaction and rural development.

Keywords: Agricultural entrepreneurship, Agripreneurs, Agripreneurship, Agribusiness, Demographics, Emporographics, Rural Farmers

Introduction

Entrepreneurship is widely recognized as one of the key drivers of economic growth and development of emerging economies in the globe (Spring, 2009). It is best described as the pursuit of economic affluence through the individual's innovative ideas while functioning in an uncertain environment with limited resources (Austin et al., 2006). Entrepreneurs launch new business ventures to lift their wealth and elevate the prosperity of their country through faster economic growth (Smit, 2004). In emerging economies, a plethora of research works primarily focused on entrepreneurship and entrepreneurial behaviour among small business units (Adom et al., 2018; Obeng et al., 2014). Although mainstream entrepreneurship research had previously ignored agriculture and its allied sectors, this scenario has changed in recent years with new studies on diverse phenomena in many sectors across the world (Afreh et al., 2019; Boer, 2013). Still, those studies are inadequate to address the burning issues of entrepreneurs in agriculture and allied

Springer Open

© The Author(s) 2022. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

sectors. We observe a marked difference among urban and rural populations in which poverty incidence is higher among rural poor in developing nations, especially in India. Obviously, there is an expectation that entrepreneurship development in the agricultural sector would play a critical role in the mitigation of poverty and wealth generation (Smit, 2004).

At the time of independence, the economy of India was predominantly an agrarian economy. During 1950–51, the contribution of agriculture, in terms of real gross value added, to the real total gross value addition was 53.7%. Since then, the contribution of agriculture to the GDP has been slowly drifting lower every year and in terms of percentage, the values stand at 33% and 19.9% for the year 1990–91 and 2020–21, respectively (GOI, 2021). At the same time, captivatingly, the number of people employed in agriculture and allied sectors declined only moderately from 70% during the 1950s to around 58% during 2020 (IBEF, 2021). This paradoxical phenomenon provides the answer to two pertinent questions. One answer is that agriculture is slowly losing out to other sectors such as industry and services in their contribution to GDP. Another point of explanation that worth attention is that, still, more people completely rely on agriculture for their livelihood. This translates into more people sharing lesser income from agriculture which ultimately resulted in disguised unemployment, a lower standard of living and, poverty for those dependent on agriculture. India being the second most populated nation having 17.5% of the world population has only 2.4% of world land to feed its people (World Bank, 2016).

Even though agriculture is seen as a low-tech industry, it is also considered as a critical sector contributing hugely to the industry and service sector in the form of raw material and other inputs (Bairwa et al., 2014; Lans et al., 2013). The changing political, economic, social and natural environmental factors necessitate the need to revisit the agriculture sector with new vigour for the sustainable development of other sectors and the survival of humankind. At this juncture, it is highly desired that the farmers are donned with entrepreneurial skill sets. Originally, during the eighteenth century, the term entrepreneurship was associated with agriculture by French physiocrats, but apparently, it is used the least to relate an agricultural activity (Anand Singh & Krishna, 1994; Hazarika & Goswami, 2018; Singh, 2013). Agricultural entrepreneurship refers to the establishment of an innovative economic enterprise for the purpose of growth or gains under situations of risk and uncertainty in agriculture (Pindado & Sánchez, 2017). The term agricultural entrepreneur is synonymously used with many evolving terms, such as agropreneur, agripreneur, flowerpreneur, farmerpreneur, horti-preneurs, apipreneur, fishpreneurs, and aquapreneurs. We propose to define *Agripreneurs as entrepreneurial people in agriculture and allied sector who either create or run either formal or informal agriventures.*

Farmers and those who perform agri-related activities are entrepreneurs in that they run businesses (McElwee, 2008). There is a general trend suggesting farmers need to become entrepreneurs (Frans et al., 2011). The ever-changing environmental factors invite the farmer entrepreneurs to acquire new sets of relevant entrepreneurial skills that need not be the same as industrial and service sector entrepreneurs (Pyysiäinen et al., 2006; Subagyo & Ernestivita, 2020). The globalization and economic reforms around the globe have compelled agripreneurs to take up higher responsibility to run their

farm-oriented businesses (Alex, 2011). Agripreneurs have to find ways and means of carrying out their farm businesses innovatively yet, profitably. As agriculture in India is the largest employer, innovation and creativity in the agriculture sector by agripreneurs will provide new impetus to the decaying farming sector and, consequently, create more high-income jobs (Ashoka et al., 2017; Noltea & Ostermeierab, 2017). There is a slow but steady trend of enterprising agripreneurs going all out for technology adoption in their farming and related activities. Application of artificial intelligence and smart technologies in cultivation, crop management, harvesting to post-harvest management, usage of agribots, precision farming, greenhouse farming and organic farming are some of the emerging themes preferred by modern tech-savvy agripreneurs (Ajili et al., 2012; Caro & Cavallo, 2019).

As ingenious agripreneurs in India start to look beyond their domestic market to market their produce, technology adoption and innovation are the means through which they can ensure world-class quality while increasing both farm productivity and profitability. Highly educated youth will be inclined to agripreneurship as a career option once; it becomes socially acceptable and highly profitable. Carefully crafted agripreneurship programmes will mend youth's ways in favour of either agripreneurship or managerial workforce to serve the agricultural sector across the globe (Bairwa et al., 2014). Based on these assessments, this study seeks to gauge the satisfaction of agripreneurs and explore the effect of demographic and emporographics on agripreneurs' satisfaction by taking farmer entrepreneurs in India as the respondent unit.

Literature review

Agripreneurs' satisfaction

Assessing the satisfaction of agripreneurs who are in the business of cultivation and marketing agricultural products is essential for developing suitable policy initiatives that will address the needs and demands of agripreneurs (Kassem et al., 2021). Many factors that determine the overall satisfaction of farming entrepreneurs include agricultural credit usage (Gunes et al., 2016), the supports provided by the government for maintaining the financial structure and livestock (Demirtas, 2021) and cognitive and emotional factors (Higuchi et al., 2020). Sharma (2014) revealed that satisfaction was greatly influenced by information sources and services among the farmer entrepreneurs as reliable sources of information were relatively inaccessible to them. Agripreneurs expect the availability of timely information, since most of their produce is perishable in nature. Ao et al. (2017) found that the rural infrastructure, including access roads and uninterrupted electricity supply, plays an undiminished role in the agripreneurs' satisfaction. Relationship marketing, logistics (Acar, 2020), and communication technology play a vital role in enhancing farmers' satisfaction (Elias et al., 2017).

Demographic characteristics and agripreneurs' satisfaction

Demographic factors describe the characteristics of study participants. It is essential to determine whether the sample respondents in a study are a representative sample of the target population for the generalization of results. In general, they are considered as independent variables, because they cannot be changed. Demographic factors include age, gender, education, marital status, family type, professional experience,

etc. They play a considerable role in the satisfaction of agripreneurs (Ali et al., 2018). Lauwere (2005) found that personal characteristics had negatively affected agripreneurs.

Age is a vital demographic variable. Bradley and Roberts (2004) and Gazioglu and Tansel (2006) identified a U-shaped connection between age and satisfaction. Indeed, young entrepreneurs have a high level of confidence than older (Forbes, 2005). Age has a substantial influence on the agripreneurs' satisfaction (Dias et al., 2019a; Ovharhe et al., 2020). Mubeena et al. (2020) discovered that the interest in agipreneurship was high among youth. Abdullah and Sulaiman (2013) found that when compared to old farmers, young agripreneurs have the willingness to adopt new technology.

Previous studies have yielded mixed results on confirming the relationship between gender and satisfaction with firm performance. Selvendran (2017) and Wayne et al. (2014) noted that gender was correlated with agripreneurs' satisfaction. Women entrepreneurs were more satisfied with business than men (Cooper & Artz, 1995). However, the result of studies conducted by Madhumitha and Karthikeyan (2020), Kuada (2009) and Sinyolo et al. (2017) identified that gender was not related to the satisfaction of agripreneurs.

Education is a vital component that provides the required knowledge, skills, ability, self-confidence and motivation (Cooper et al., 1994; Pliakoura et al. (2020); ; ; ; ; ; observed that farmers who pursue entrepreneurship have a low level of relevant entrepreneurship education. Bannor et al. (2021), Sinyolo et al. (2017) and Wayne et al. (2014) noted that the education level of agripreneurs significantly influenced their satisfaction.

A life partner gives moral support and sharing business responsibilities as well as problem. Several studies confirmed that marital status is positively related to the satisfaction of agripreneurs (Addo, 2018; Ovharhe et al., 2020). El Shoubaki and Meike (2018) found that support of life-partner had increased the satisfaction of agripreneurs with their entrepreneurial activities.

The knowledge and skills needed to run an agri-business can be gained by observing how family members perform their tasks on their farms. The children of the agricultural family can easily understand business nuances (Kim et al., 2006). Madhumitha and Karthikeyan (2020) claimed that family support was considered as the key factor in motivating women to become successful agripreneur as young women in many societies rely on family members' opinions on their business and personal decisions. Dias et al. (2019a) and Ovharhe et al. (2020) proved that family type had a substantial impact on farmers' satisfaction.

Like education, experience in agribusiness is also one of the crucial factors which support acquiring business skills. An experienced entrepreneur can realize their limitations than young entrepreneurs (Wright et al., 1997). According to Hayward et al. (2006), experienced entrepreneurs may be overconfident when the nature of an enterprise varies from core areas. Fraser and Greene (2006) noted that experienced entrepreneurs were apparently more satisfied. Similarly, Dias et al. (2019b), Ovharhe et al. (2020) and Selvendran (2017) showed a positive correlation between farming experience and satisfaction of agripreneurs. However, Bradley and Roberts (2004) found that entrepreneurial experience did not affect entrepreneurs' satisfaction.

Emporographics and agripreneurs' satisfaction

Emporographics are known as farm characteristics which play a vibrant role in the segmentation of the agricultural business. Several prior research works disclose the satisfaction of agripreneurs influenced by the set of essential emporographics variables viz., farm age, farm size, annual income, land ownership, sources of funds and intercropping.

Age of the farm notably connected with the gaining professional experience of the agripreneurs. Seasoned farmers perhaps, could learn more entrepreneurial skills from their routine business operations. The longevity of business operations ultimately determines the business growth and performance (Sleuwaegen & Goedhuys, 2002). Bannor et al. (2021) revealed that farm age has positively influenced the satisfaction of agripreneurs. In addition, many studies identified that farms' age is considerably related to the satisfaction of agripreneurs (Antoncic, 2009; Lucas, 2017).

A farm size denotes an area of land devoted to agricultural processes to produce food and other crops that determine the capacity of production and volume of business operations of the agripreneurs. It is an indication of the farm's potential for profit-making. Thus, it serves as a surrogate for the entrepreneur's prestige, success and eliciting satisfaction (Weaver, 1977). It ultimately influenced the satisfaction of agripreneurs (Konyar & Osborn, 1990; Ovharhe et al., 2020). Wayne et al. (2014) identified the strong relationship between farm size, extension service offered, and agripreneurs' satisfaction. Addo (2018) and Bannor et al. (2021) claimed that agripreneurs' satisfaction is related to farm size.

Dobryagina (2020) revealed that the quantum of monetary rewards directly influences individuals choosing agricultural entrepreneurship as their career option. Some studies found a positive correlation between income and agripreneurs' satisfaction (Ao et al., 2017; Reddy et al., 2020). Handy et al. (2011) identified that agripreneurs under normal circumstances have an assured income derived from cultivation. Studies by Fan and Luo (2009), Ovharhe et al. (2020) and Singh et al. (2019) divulged that the income from agripreneurship has a positive impact on personal growth, and thereby, the satisfaction of agriculture entrepreneurs.

The pattern of land ownership is a crucial factor that ultimately determines the freedom of doing agripreneurial activities. It determines the livelihood capabilities of agricultural entrepreneurs and, indirectly, their income level. The agripreneurs who have their land can do business better than those who cultivate in leased lands. Morgan et al. (2010) and Ovharhe et al. (2020) discovered that agripreneurs who cultivate in their land are more satisfied than those who cultivate in leased land. Yet, another study by Rao et al. (2016) identified that the farmers who cultivate in the contract land are highly satisfied.

In general, there is literature evidence that shows the critical role of sources of funds in the degree of agripreneurial operations. According to McMahon (2001), increased reliance on external finance was associated with better business growth. Kristiansen et al. (2003) showed that entrepreneurs who mobilized funds from family and third-party investment had a higher business success rate. Most of the agripreneurs are reluctant to approach organized financial institutions for their financial requirements. Access to unorganized sector credit has plumped the economic status of the agripreneurs because of usurious private lenders who charge exorbitant rates of interest. Mubeena et al. (2020) stated that those agripreneurs who availed financial support with nil interest from

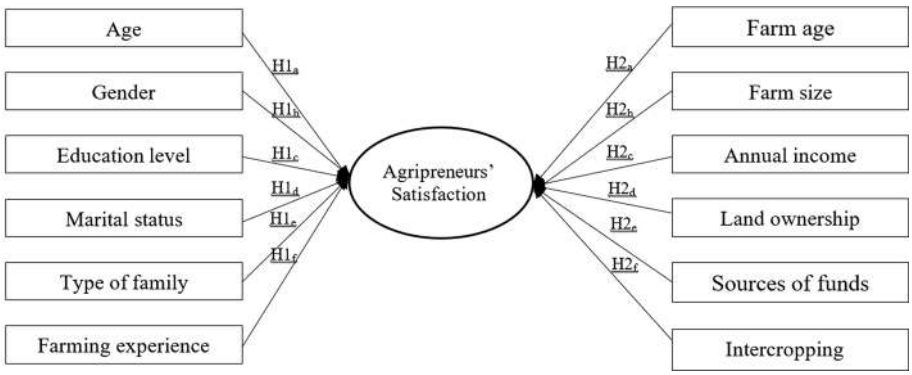


Fig. 1 Hypothesized model

families are more satisfied with agricultural entrepreneurship, which is in contradiction to those agripreneurs who availed financial support from banks as they are more dissatisfied due to the high cost of loans. Dias et al. (2019a), Bannor et al. (2021) and Chatterjee et al. (2020) found that sources of funds had a significant influence on the satisfaction of agripreneurs.

The intercropping practice facilitates a higher yield and additional income from the same piece of land. Handy et al. (2011) and Dai et al. (2017) identified that intercropping has positively influenced the income of small landholders. Jaganathan and Nagaraja (2015) found that agripreneurs derived additional income from intercrops, generate more employment and also, increase soil fertility.

Even though our review of the literature is not extensive, it clearly highlights the gaps in existing research. Based on the analysis of available literature, we seek to explore the role of demographics and emporographics on agripreneurs' satisfaction. In this regard, the following two hypotheses are framed and tested. Based on prior studies, we hypothesize that demographic characteristics such as age (H1a), gender (H1b), education level (H1c), marital status (H1d), type of family (H1e) and farming experience (H1f) significantly influence the agripreneurs' satisfaction. Likewise, emporographics factors such as farm age, (H2a) farm size (H2b), annual income (H2c), land ownership (H2d), sources of funds (H2e) and intercropping (H2f) significantly influence the agripreneurs' satisfaction. Figure 1 depicts the hypothesized relationship between the dependent (agripreneurs' satisfaction) and independent variables (demographics and emporographics).

Materials and methods

The current study aims at gauging the satisfaction of agripreneurs and seeks to explore the effect of demographics and emporographics on agripreneurs' satisfaction. The agripreneurs who had been involved in the cultivation and marketing of agricultural products in the rural area were the target population. The Salem district was purposively selected for the study, since relatively a great number of people have rigorously engaged in agripreneurial activities than their counterparts in other districts of Tamilnadu. A field survey was conducted to gather data from the agripreneurs. In all, 800 questionnaires were distributed, 16 of the questionnaires were incomplete. Thus, 784 questionnaires were used for analysis.

In this study, carefully crafted a questionnaire was used to amass responses from the agripreneurs. Using a questionnaire for data collection is considered an appropriate method to obtain correct responses (Chisnall, 2001).

After the extensive literature survey, we amassed scales and items related to agripreneurs satisfaction from earlier studies. These were reworded to suit agricultural entreprenerus. Moreover, we found that there is no comprehensive standardized instrument for measuring agripreneurs' satisfaction. Therefore, we propose a new survey instrument developed by us that effectively measures the agripreneurs' satisfaction. We would prefer to call this instrument *AprenSAT*. This instrument covers seven dimensions, namely, material availability, government support, farm growth, farm income, market performance, cultivation and production and perceived farm image. The pilot study, frequent discussions made with agripreneurs and in-depth interactions with subject experts provided the necessary input and direction in defining, crafting, and refining the AprenSAT Questionnaire. Furthermore, the content validity was checked by a panel of experts consisting of two representations from each category, namely, subject expert, doctoral scholar and agripreneur. To check the comprehensibility of the AprenSAT instrument, a pilot study was conducted with the original pre-final version of the drafted questionnaire that comprised 34 attributes for measuring the agriprenerus' satisfaction. After the pilot study, we observed that only 29 attributes are consistent with the seven dimensions of AprenSAT. Eventually, the final version of the questionnaire has seven dimensions of AprenSAT covering only 29 attributes out of the original 34 attributes (Table 1). This novel AprenSAT Questionnaire could effectively be used not only in India but across the globe to measure the satisfaction of agripreneurs.

Assessment of validity and reliability is one of the most fundamental aspects of instrument development. Therefore, in this study, the validity and reliability of our AprenSAT instrument were ascertained by Pearson's correlation and Cronbach Alpha ($\propto$) test, respectively. Pearson's correlation was applied between the total score of seven dimensions of AprenSAT. The correlation values range between 0.56 and 0.63 for all the seven dimensions of AprenSAT. In general, correlation in the range of 0.40 to 0.70 denotes strong validity of construct (Post, 2016). Hence, this result indicates that the newly developed questionnaire is a valid measurement tool. The Cronbach Alpha ($\propto$) test was used to verify the reliability and its test value stands at 0.907, which is more than that of a minimum threshold of 0.70 suggested by Hair et al. (2010), which denotes this questionnaire is highly reliable.

The survey questionnaire contains demographic characteristics, emporographics, and agripreneurs' satisfaction. Among the various types of scales, we preferred the widely accepted Likert's scale containing five points as it offers better clarity to respondents (Malhotra & Birks, 2003) by assigning 5 points to high satisfaction at one end of the scale and assigning 1 point to high dissatisfaction at the other end of the scale, while the remaining values are orderly fixed in between these extreme value and, named accordingly as satisfaction, neither satisfaction nor dissatisfaction and dissatisfaction.

The data collected from the agripreneurs were analyzed using the SPSS and SPSS-AMOS. The construct validity was measured through the Exploratory Factor Analysis (EFA) and Confirmatory Factor Analysis (CFA). The discriminant validity was assessed using Construct Reliability (CR) and Average Variance Extracted (AVE). Furthermore,

Table 1 Seven dimensions of AprenSAT and attributes of the study

Constructs	No	Code	Items
Materials availability (Ao, et al., 2017; Singh & Kalra, 2019)	1	MA-1	Availability of quality saplings
	2	MA-2	Availability of farm equipment
	3	MA-3	Availability of round the clock uninterrupted power
	4	MA-4	Availability of enough water for irrigation and related purposes
	5	MA-5	Availability of fertilizers and manures
	6	MA-6	Availability of Pesticides and weedicides
	7	MA-7	Cost of equipment and fertilizers
Government support (Kakkar et al., 2014; Mazibuko & Antwi, 2018; Mwamakimbula, 2014; Ncube, 2017; Selvaraj, 2015; Zawojska, 2010)	8	GS-1	Conduction of Education/ awareness programmes by government
	9	GS-2	Availability of tailor-made insurance schemes offered by the government
	10	GS-3	Subsidies provided for the purchase of farm equipment, fertilizers etc
	11	GS-4	Relief measures during drought, flood and other natural disasters
	12	GS-5	Technical assistance from the government (such as soil report, weather report, market data etc.)
Farm growth (Lingesiya, 2017; Singh et al., 2019)	13	FG-1	Increase in cultivation area
	14	FG-2	Increase in production Turnover/ sales from the agri-business
	15	FG-3	Increase in usage of modern equipment
	16	FG-4	Growth in the number of labourers employed
Farm income (Ayranci, 2011; Chakraborty et al., 2019; Lingesiya, 2017)	17	FI-1	Support of income generated from agriventure to maintain the desired level of standard of living
	18	FI-2	Income derived from allied agricultural activity
	19	FI-3	Return on Investment (ROI) from the agripreneurship
Market performance (Lingesiya, 2017; Singh & Kalra, 2019)	20	MP-1	Reception of farm produces in the markets
	21	MP-2	The market price for produces
	22	MP-3	Increase in the customer base as compared with the commencement of business
	23	MP-4	Growth in market coverage
Cultivation and production (Lingesiya, 2017; Singh & Kalra, 2019)	24	CP-1	Cost of crop maintenance
	25	CP-2	Quantity of produce from the farm
	26	CP-3	The ability of a farm to cultivation new crops based on market demand
Perceived farm image (Ayranci, 2011; Chakraborty et al., 2019)	27	PFI-1	Image of farm compared with neighbouring farms
	28	PFI-2	Recognition to farm in the society
	29	PFI-3	Individual recognition/ identify created by agriventure

multiple linear regression was employed to identify the effect of demographic and emporographics on agripreneurs' satisfaction.

Results

This section presents the demography of agripreneurs, emporographics of agri-ventures, agripreneurs' satisfaction, and the effect of demographic and emporographics on agripreneurs' satisfaction.

Demographics and emporographics of agripreneurs

The demography of the agripreneurs as exhibited in Table 2 reveals that out of 784 agripreneurs, 44.6% were in the age group of below 35 years. It indicates that young farmers as agriculture entrepreneurs constitute the major chunk of the study population, while the middle-aged and old-aged farmers groups constitute the remaining study population in the study area. Another interesting inference that can be made from this descriptive analysis is that the majority (73.2%) of the agripreneurs are male. This denotes the entry of a noticeable number of women agripreneurs in the study area. 39.1 and 25.2% of the agripreneurs hold higher secondary and college-level education, respectively. Most of the agripreneurs (87.8%) are married and belonged to the joint family system. 44.8% of the respondents have 11–15 years of experience in agripreneurship.

The emporographics as presented in Table 3 divulges that 49.7% of the farmers run 6–10 years old enterprise. The majority, 57.3% of the respondents cultivated crops in less than 1 hectare. 47.3% of the agripreneurs earned ₹50,001–1,00,000 per annum from cultivation. The majority of the agripreneurs cultivated on their own land (81.1%). Most of the agripreneurs (44.6%) have availed of loans for cultivation purposes, while 32.1% of farmers have borrowed money without interest from relatives and friends. 57.7% of the agripreneurs have cultivated multiple crops in their farmland along with the main crop.

Results of exploratory factor analysis for satisfaction of agripreneurs

The result of EFA presented in Table 4. As the value of KMO stood at 0.644, the application of factor analysis was highly appropriate for the variables included in this study (Hair et al., 2010). Bartlett's χ^2 value was 23,721.048, and it was significant ($p \leq 0.05$) at a 5% level. It is noted that a high level of inter-relationship was found among the scale variables. Therefore, these variables were adequate for the PCA. The factor analysis by PCA with varimax rotation identified seven Eigenvalues, which were greater than 1. These

Table 2 Demographic characteristics of agripreneurs

Category		Frequency($N = 784$)	%
Age (in years)	Upto 35	350	44.6
	36–55	158	20.1
	Above 55	277	35.3
Gender	Male	574	73.2
	Female	210	26.8
Education level	Primary School	150	19.1
	Secondary School	130	16.6
	Higher Secondary	307	39.1
	College	198	25.2
Marital status	Married	688	87.8
	Unmarried	96	12.2
Type of family	Nuclear	170	21.7
	Joint family	614	78.3
Farming experience (in years)	Up to 10	80	10.2
	11–15	351	44.8
	16–20	250	31.9
	Above 20	103	13.1

Table 3 Emporographics

Category		Frequency ($N = 784$)	%
Farm age (in years)	Upto 5	237	30.2
	6–10	390	49.7
	Above 10	158	20.1
Farm size (in hectare)	Below 1	449	57.3
	2–4	267	34.0
	Above 4	68	8.7
Annual income (in INR)	Below ₹50,000	127	16.2
	₹50,001–1,00,000	371	47.3
	₹1,00,001–1,50,000	191	24.4
	Above ₹1,50,000	95	12.1
Land ownership	Own	636	81.1
	Lease	148	18.9
Sources of funds	Relatives/ Friends	252	32.1
	Banks	183	23.3
	Moneylenders	350	44.6
Intercropping	Cultivated	452	57.7
	Not cultivated	332	42.3

seven extracted factors explained 78.77% of the total variation depicting the presence of seven factors that have predominantly influence the satisfaction of agripreneurs.

The first set of variable loading has seven variables with 16.20% of the variance. This component was suitably named as 'Materials Availability'. Thus, these variables have highly influenced the satisfaction of agripreneurs. The second component consists of five variables with 11.31% of the variance. These variables were aptly named as 'Government Support'. The third set of variable loading has four variables with 11.31% of the variance. This component was appropriately named as 'Farm Growth'. The fourth variable loading consists of three variables with 11.08% of the variance. Hence, this factor was named as 'Farm Income'. The fifth variable loading includes four variables with 10.79% of the variance. Hence, this factor was called as 'Market Performance'. The sixth variable loading comprises three variables with 8.75% of the variance. Hence, this factor was noted as 'Cultivation and Production'. The seventh variable loading comprises three variables with 6.73% of the variance. Hence, this factor was suitably named as 'Perceived Farm Image'. In a nutshell, the material availability, government support, farm growth, farm income, market performance, cultivation and production and perceived farm image were highly influenced in the agripreneurs' satisfaction (Fig. 2).

Results of confirmatory factor analysis (cfa) and discriminant validity

Table 5 exhibits the CFA and measurement properties. The composite reliability (C.R.) value for each factor ranged between 0.758 and 0.875, clearly surpasses the minimum threshold of 0.70 (Hair et al., 2010) and obviously, indicates good internal consistency among all the constructs. The goodness of fit indices for CFA yielded an acceptable level of fit (GFI = 0.906; CFI = 0.912; TLI = 0.901; χ^2/df = 3.16). The value of the RMSEA was 0.049, which denotes that the model fit was good as RMSEA value less than 0.08 is the gold standard for a strong fit of the model (Browne & Cudeck, 1993). This goodness of fit

Table 4 Results of exploratory factor analysis

Constructs/factors	Factor loadings	Eigen value	% of Variance explained
Factor—1: Materials availability		4.698	16.202
MA-1	0.824		
MA-2	0.821		
MA-3	0.745		
MA-4	0.740		
MA-5	0.714		
MA-6	0.696		
MA-7	0.651		
Factor—2: Government support		4.027	13.885
GS-1	0.822		
GS-2	0.787		
GS-3	0.783		
GS-4	0.694		
GS-5	0.598		
Factor—3: Farm growth		3.283	11.319
FG-1	0.850		
FG-2	0.791		
FG-3	0.715		
FG-4	0.559		
Factor—4: Farm income		3.214	11.084
FI-1	0.829		
FI-2	0.717		
FI-3	0.709		
Factor—5: Market performance		3.131	10.797
MP-1	0.875		
MP-2	0.700		
MP-3	0.693		
MP-4	0.677		
Factor—6: Cultivation and production		2.538	8.752
CP-1	0.803		
CP-2	0.653		
CP-3	0.646		
Factor—7: Perceived farm image		1.954	6.739
PFI-1	0.773		
PFI-2	0.593		
PFI-3	0.589		

index for the seven-factor model shows the confirmation of construct distinctiveness for materials availability, government support, farm growth, farm income, market performance, cultivation and production and perceived farm image.

Moreover, we checked for discriminant validity by comparing the variance-extracted estimates of the measures with the squared correlation between constructs, as described by Fornell and Larcker (1981) and Netemeyer et al. (1990). The average variance-extracted (AVE) for all variables in this investigation was more than the suggested value of 0.50 (Malhotra & Dash, 2011). Since the value of variance extracted was more than the squared correlation, the measures have discriminant validity. In

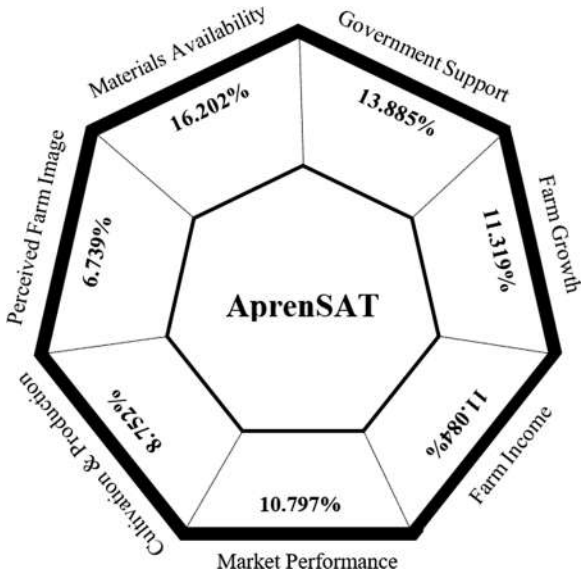


Fig. 2 Seven dimension heptagon model of AprenSAT

this study, the estimates of variance extracted for cultivation and production and perceived farm image were 0.563 and 0.546, respectively, and both variables were more than the squared correlation between them. Likewise, the squared correlation between government support and farm growth was more than the variance extracted. Based on these statistics along with the CFA results, the study establishes discriminant validity between these seven variables.

Table 6 reports mean ($\bar{x}$), standard deviation (σ) and correlation. The mean values indicate that agripreneurs were highly satisfied with the market performance followed by farm growth, perceived farm image, farm income, materials availability, government support and cultivation and production. The study observed a strong positive correlation (0.594) between farm growth and government support, a moderate positive correlation (0.473) between materials availability and cultivation and production and a weak positive correlation (0.385) between materials availability and perceived farm image. Furthermore, government support was significantly related to farm growth, farm income, market performance, cultivation and production and perceived farm image.

Effect of demographic and emporographics on agripreneurs' satisfaction

Multiple regression was employed to find out the effect of independent variables, namely, demographic factors and emporographics on the dependent variable, namely, satisfaction of agripreneurs.

The effect of demographics and emporographics on the agripreneurs' satisfaction as presented in Table 7 reveals that the satisfaction of agripreneurs was substantially influenced by the demographic characteristics included in the model, since Adj.R^2 value stood at 0.427. It denotes that 42.7% of the difference in the agripreneurs' satisfaction was influenced by the set of demographic variables, which confirms that the

Table 5 Results of confirmatory factor analysis

Constructs	C.R	Standardized loadings (λy_i)	Reliability ($\lambda^2 y_i$)	Variance (Var(ε_i))	AVE $\Sigma(\lambda^2 y_i)/[(\lambda^2 y_i)+/(\text{Var}(\varepsilon_i))]$
Materials availability	0.875				0.503
MA-1		0.806	0.650	0.350	
MA-2		0.772	0.596	0.404	
MA-3		0.673	0.453	0.547	
MA-4		0.735	0.540	0.460	
MA-5		0.742	0.551	0.449	
MA-6		0.636	0.404	0.596	
MA-7		0.569	0.324	0.676	
Government support	0.869				0.574
GS-1		0.901	0.812	0.188	
GS-2		0.638	0.407	0.593	
GS-3		0.806	0.650	0.350	
GS-4		0.704	0.496	0.504	
GS-5		0.713	0.508	0.492	
Farm growth	0.836				0.568
FG-1		0.626	0.392	0.608	
FG-2		0.869	0.755	0.245	
FG-3		0.888	0.789	0.211	
FG-4		0.580	0.336	0.664	
Farm income	0.758				0.516
FI-1		0.613	0.376	0.624	
FI-2		0.679	0.461	0.539	
FI-3		0.843	0.711	0.289	
Market performance	0.806				0.512
MP-1		0.728	0.530	0.470	
MP-2		0.778	0.605	0.395	
MP-3		0.731	0.534	0.466	
MP-4		0.614	0.377	0.623	
Cultivation and production	0.790				0.563
CP-1		0.858	0.736	0.264	
CP-2		0.787	0.619	0.381	
CP-3		0.578	0.334	0.666	
Perceived farm image	0.782				0.546
PFI-1		0.640	0.410	0.590	
PFI-2		0.754	0.569	0.431	
PFI-3		0.813	0.661	0.339	

Table 6 Descriptives and correlation

	$\bar{x}$	σ	1	2	3	4	5	6	7
1. Materials availability	3.690	0.719	1						
2. Government support	3.585	0.960	0.025	1					
3. Farm growth	4.014	0.702	0.047	0.594**	1				
4. Farm income	3.844	0.724	0.078*	0.389**	0.379**	1			
5. Market performance	4.056	0.551	0.257**	0.429**	0.314**	0.283**	1		
6. Cultivation and production	3.319	0.781	0.473**	0.273**	0.015	0.060	0.108**	1	
7. Perceived farm image	3.916	0.545	0.385**	0.286**	0.167**	0.300**	0.043	0.649**	1

Note: ** denotes 1% and * denotes 5% levels of significance

Table 7 Regression model summary

Variables	Constructs	β	't'	P	Model summary				
					R	R^2	Adj.R^2	F	P
Demographics	Constant	18.365	27.829	0.000	0.687	0.442	0.427	18.692	0.000
	Age	− 0.328	− 1.702	0.000					
	Gender	0.017	0.275	0.123					
	Education level	0.160	1.163	0.001					
	Marital status	0.041	0.634	0.153					
	Type of family	0.025	0.415	0.421					
	Farming experience	0.371	4.447	0.000					
Emporographics	Constant	19.147	29.487	0.000	0.704	0.496	0.462	24.071	0.000
	Farm age	0.532	1.053	0.000					
	Farm size	0.419	2.285	0.003					
	Annual income	0.241	2.360	0.000					
	Land ownership	− 0.128	− 0.756	0.002					
	Sources of funds	− 0.122	− 0.523	0.004					
	Intercropping	0.438	1.576	0.000					

proposed model was a strong predictor. There was a strong association between agripreneurs' satisfaction and demographic characteristics, since the 'R' value was 0.687. The F statistic (18.692) was significant at a 5% level ($p \leq 0.05$) points out that the overall model fit was significant.

The regression coefficient shows that the demographic characteristics of agripreneurs such as age ($\beta = -0.328$; $t = .- 1.702$, $p \leq 0.05$), education level ($\beta = 0.160$; $t = 1.163$, $p \leq 0.05$) and farming experience ($\beta = 0.371$; $t = 4.447$; $p \leq 0.05$) substantially influence the agripreneurs' satisfaction. It could be ascertained that that age has a substantial negative influence on agripreneurs' satisfaction. Furthermore, gender, marital status and type of family did not significantly ($p > 0.05$) influence the agripreneurs' satisfaction. Therefore, the study hypotheses $H1_a$, $H1_{c,}$ and $H1_f$ are proven correct, whereas the study failed to prove the remaining hypothesis, such as $H1_b$, $H1_{d,}$ and $H1_{e.}$

The satisfaction of agripreneurs was substantially influenced by the emporographics included in the model, since Adj.R^2 value stood at 0.462. It implied that 46.2% of the difference in the agripreneurs' satisfaction was determined by the emporographic variables, which hint at the strong predictable nature of the proposed model. There was a strong association between agripreneurs' satisfaction and emporographics, since the 'R' value was 0.704. The F statistic (24.071) was significant at a 5per cent level ($p \leq 0.05$). Therefore, the overall model fit was significant.

The emporographics factors such as farm age ($\beta = 0.532$; $t = 1.053$, $p \leq 0.05$), farm size ($\beta = 0.419$; $t = 2.285$, $p \leq 0.05$), annual income ($\beta = 0.241$; $t = 2.360$, $p \leq 0.05$), land ownership ($\beta = -0.128$; $t = -0.756$, $p \leq 0.05$), sources of funds ($\beta = 0.122$; $t = -0.523$, $p \leq 0.05$) and intercropping ($\beta = 0.438$; $t = 1.576$, $p \leq 0.05$) were significantly related to the agripreneurs' satisfaction. This study observes that land ownership had a negative effect on the agripreneurs' satisfaction. Therefore, the hypotheses $H2_a$, $H2_b$, $H2_c$, $H2_d$, $H2_e$, and $H2_f$ are proved correct (Fig. 3).

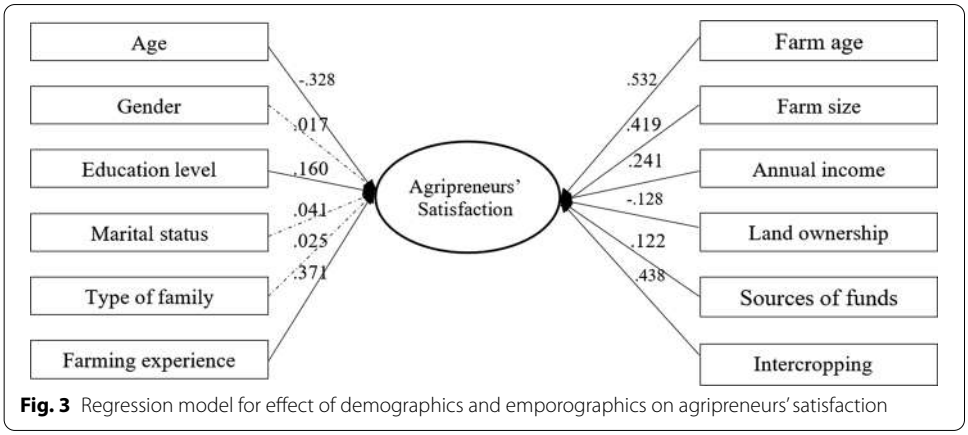


Fig. 3 Regression model for effect of demographics and emporographics on agripreneurs' satisfaction

Discussion

This study observes that agripreneurs' satisfaction was highly influenced by factors, such as market performance, farm growth, perceived farm image, farm income, materials availability, government support and cultivation and production. There was a marked linkage between cultivation and production and farm image. There exists a moderate positive correlation between materials availability and cultivation and production. Moreover, government support was significantly related to farm growth, farm income, market performance, cultivation and production and perceived farm image. In addition, the study identifies that age has a negative influence on the agripreneurs' satisfaction. It denotes that as the farmers' age and their satisfaction towards agripreneurship drift downwards. In other words, the young agripreneurs are more satisfied with the cultivation and marketing of agricultural products. This result is in line with the studies of Ovharhe et al. (2020), Sinyolo et al. (2017), and Wayne et al. (2014).

This study finds that gender does not influence the agripreneurs' satisfaction. This result confirms the results of earlier studies of Madhumitha and Karthikeyan (2020), Kuada (2009), and Sinyolo et al. (2017). The education level of agripreneurs positively impacts the agripreneurs' satisfaction, which denotes that as the farmers' education level increases, there is an increase in their satisfaction towards agripreneurship. This finding is in harmony with the findings of Ovharhe et al. (2020) and Bannor et al. (2021), while this contradicts the findings of Singh et al. (2019) and Sinyolo et al. (2017).

As marital status does not influence the agripreneurs' satisfaction, their agriventure operations are not hindered by marital and family commitments as observed by Selvendran (2017) and El Shoubaki and Meike (2018). This study discovered that type of family has no influence on the agripreneurs' satisfaction which corroborates with the findings of Madhumitha and Karthikeyan (2020) and contradicted the findings of Kuada (2009) and Bannor et al. (2021). This study results portrayed that the farming experience has a positive influence on the agripreneurs' satisfaction. This result is supported by several earlier studies, including those by Dias et al. (2019a), Ovharhe et al. (2020) and Wayne et al. (2014). However, this result contradicted the observation of Bradley and Roberts (2004).

The study found that farm age has positively influenced the agripreneurs' satisfaction. Similar results were reported by Antoncic (2009) and Lucas (2017). It is also observed that the farm size has positively influenced the agripreneurs' satisfaction echoing the findings of Addo (2018), Bannor et al. (2021) and Vasan (2020). In this study, marginal-level farmers make up most of the study population. It is inferred that Indian farmers are marginal and small farmers. Cultivation and marketing of agricultural products are mostly preferred by these agripreneurs as it is an intensive crop that requires a constant high level of attention.

The success of any business operation is highly dependent on the income generated by it. This holds well in the case of agripreneurship too. In this study, it is found that the annual income has a positive influence on the agripreneurs' satisfaction. It indicates that as the level of income increases, the satisfaction level of the agripreneurs also increases. This resonates well with the studies of Bannor et al. (2020), Fan and Luo (2009) and Chatterjee et al. (2020).

Land ownership has decreased the agripreneurs' satisfaction. It implies that agripreneurs who cultivate in their land are more satisfied than their counterparts who got engaged in agriculture on leased land. This finding corroborates with the finding of Morgan et al. (2010) and Ovharhe et al. (2020). Sources of funds have a strong impact on the agripreneurs' satisfaction. It signifies that those agripreneurs who received funds from family and friends, also termed 'love money', are more satisfied than those who invest the money borrowed from banks and other moneylenders. It is observed that most of the agripreneurs in the study area borrows from private moneylenders for their immediate financial requirements connected with farming and agriventure operation. Moneylenders charge an exorbitant rate of interest which increases the cost of cultivation, leading to a decrease in profitability. Ultimately, this lower profitability leads to dissatisfaction among the agripreneurs. This result is consistent with several prior studies (Bannor et al., 2021; Chatterjee et al., 2020; Mubeena et al., 2020).

In the study area, the farmers follow intercropping practices, which facilitate getting a higher return from the same piece of land. Thus, the intercropping pattern has a positive influence on the satisfaction level of the agripreneurs in the study area. This result conforms with the findings of prior research works done by Dai et al. (2017) and Jaganathan and Nagaraja (2015).

Recommendations

Among agripreneurial operations, cash crop cultivation is considered to be the most profitable income-generating business in the Indian economy. The agripreneurs will be highly motivated when the government comes out with supportive policies and implement suitable and actionable marketing strategies. Adequate information about the market, marketing strategies and export opportunities must be communicated to the agripreneurs through proper channels and facilitated by the fast-growing digital economy. Besides, capitalizing on IoT during the production phase will also help in data mining and open doors to new economic activities in the blockchain industry. The fourth industrial revolution is not just for the heavy or technological-based industries but also agriculture and, in this case, the cultivation, production, promotion and marketing, distribution and sales of the agricultural products and agro-based value-added

products. Cultivation and production of agricultural products are seasonal. Hence the agripreneurs need to engage in other income-generating activities to supplement their livelihood. Scheduling the production and harvesting times, the agripreneurs and farm managers may plan with the cultivation of intercrops or be engaged in other sectors to generate additional cash flow. This will ensure that they have continuous income for daily and monthly expenses. The agripreneurs may also be assisted with financial support during the seasonal demand to compensate for the unexpected loss encountered. On the other hand, the government can take necessary steps to provide an incentive for research and development (R&D) to diversify the use of agricultural products.

Training programmes can be conducted for the agripreneurs so that they can learn new techniques and technologies in cultivation, which in turn takes them to the next level in cultivation. Capacity-building initiatives are crucial for the empowerment of the farmers' community, facilitating the creation of an effective value chain for agricultural products and value-added products. This will increase the demand for agricultural and agro-based value-added products domestically and internationally, which will boost the growth of the economy in the long run.

The cost of production or the yield per acre is significantly high. The farmers can meet the expenses only when the product reaches the markets on time. Besides having a good access road, transportation facilities and adequate storage facilities must be provided. The entrepreneurs who see this as a business opportunity may be provided financial and technical support for the creation of transportation and storage facilities. These facilities in turn will create more jobs. Most importantly, these measures will ensure that the agricultural products reach the clients in various markets be it domestic or international in pristine conditions and on time; otherwise, most of the agricultural produce would lose its value and may only be sold below the market value or just be disposed of due to its perishable nature. The barriers and obstacles related to the communication process between wholesalers, retailers and commission agents must be rectified. An integrated system that allows artificial intelligence to record the trend of production will help the buyers and clients be updated on the availability of the agricultural products based on the origin of locality, the quantity and the quality of the produce. This will also help the clients be prepared for the arrival times for collection on various occasions, celebrations, and events. In a nutshell, only a coordinated set of activities that include government support, training, institutional support, and technology modernization in every stage of production to supply chain can positively enhance the livelihood and satisfaction of agripreneurs. Moreover, agripreneurship activities significantly support the economic growth and development of India.

Conclusion

The current study investigates satisfaction of agripreneurs and seeks to explore the effect of demographics and emporographics on agripreneurs' satisfaction. We propose a new survey instrument, called *AprenSAT*, for effective measurement agripreneurs' satisfaction cutting across regions. The extraction of seven factors confirms the presence of seven dimensions of agripreneurs' satisfaction. The *heptagon model of AprenSAT* reveals the seven factors as market performance, farm growth, perceived farm image, farm income, materials availability, government support and cultivation and production. The

regression result proves that demographic factors such as age, education level and farming experience have a significant influence on the agripreneurs' satisfaction. In addition, emporographics such as farm age, farm size, annual income, land ownership, sources of funds, and intercropping have a substantial influence on agripreneurs' satisfaction. The results derived from the current study are relevant and valuable in numerous ways. First, this study helps to understand the level of agripreneurs' satisfaction towards their agri-business. Second, the proposed survey instrument, the *AprenSAT* is a valid, reliable and effective tool for measuring the agripreneurs' satisfaction. Third, the proposed *seven-dimension heptagon model of AprenSAT* has remarkable utility to all stakeholders starting from researchers, academicians, etc., to policymakers. Fourth, this study makes an immense contribution to the existing body of knowledge. Fifth, since we recommend to policymakers the coordinated set of activities including training, institutional support and technology modernization in every stage of production to supply chain to enhance the livelihood and satisfaction of agripreneurs, our recommendation will have a ripple effect on the well-being of not only agripreneurs but also, on the rural, industry and service sector.

Abbreviations
AprenSAT: : Agripreneurs satisfaction; GOI: : Government of India; IBEF: : India Brand Equity Foundation.

Acknowledgements
Not applicable

Authors' contributions
All authors have equally contributed to this study from proposal development to manuscript writing stages. All authors read and approved the final manuscript.

Funding
No funding has been received by the authors for this research.

Availability of data and materials
Not applicable.

Declarations

Ethics approval and consent to participate
During data collection, respondents gave verbal consent for the data collectors and showed their willingness to fill the dispatched questionnaires.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Department of Management Studies, Periyar University, Salem 636 011, Tamilnadu, India. [2]Kingdom University, Riffa, Bahrain. [3]Department of Commerce, National College (Autonomous), Tiruchirappalli 620 001, Tamilnadu, India.

Received: 5 December 2021 Accepted: 9 January 2022
Published online: 21 January 2022

References

Abdullah, A. A., & Sulaiman, N. N. (2013). Factors that influence the interest of youths in agricultural entrepreneurship. *International Journal of Business and Social Science, 4*(3), 288–302. https://doi.org/10.30845/ijbss

Acar, Z. (2020). The mediating role of value innovation between market orientation and business performance: Evidence from the logistics industry. *International Journal of Business Innovation and Research, 21*(4), 540–563. https://doi.org/10.1504/ijbir.2020.106012

Addo, L. K. (2018). Factors influencing agripreneurship and their role in agripreneurship performance among young graduate agripreneurs. *International Journal of Environment, Agriculture and Biotechnology, 3*(6), 2051–2066. https://doi.org/10.22161/ijeab/3.6.14

Adom, K., Asare-Yeboa, I. T., Quaye, D. M., & Ampomah, A. O. (2018). A critical assessment of work and family life of female entrepreneurs in sub-Saharan Africa: Some fresh evidence from Ghana. *Journal of Small Business and Enterprise Development, 25*(3), 405–427. https://doi.org/10.1108/jsbed-02-2017-0063

Afreh, B., Rodgers, P., Vershinina, N., & Williams, C. C. (2019). Varieties of context and Informal Entrepreneurship: Entrepreneurial activities of migrant youths in rural Ghana. *International Journal of Entrepreneurial Behaviour and Research, 25*(5), 996–1013. https://doi.org/10.1108/ijebr-02-2018-0109

Ajili, A., Salehi, S., Rezaei-moghaddam, K., Hayati, D., & Karbalaee, F. (2012). Estimating the model of investigating attitude and intention in the usage of variable rate irrigation technology. *Journal of Experimental Agriculture International, 2*(3), 542–556. https://doi.org/10.9734/ajea/2012/1267

Ali, S., Kashem, M. A., & Aziz, M. A. (2018). An Overview of socio-economic and farming systems status of farmers in the Haor Area under Sunamganj District. *Asian Journal of Advances in Agricultural Research, 7*(3), 1–9. https://doi.org/10.9734/ajaar/2018/41655

Anand Singh, K., & Krishna, K. V. S. M. (1994). Agricultural entrepreneurship: The concept and evidence. *The Journal of Entrepreneurship, 3*(1), 97–111. https://doi.org/10.1177/097135579400300106

Antoncic, B. (2009). The Entrepreneur's general personality traits and technological developments. *World Academy of Science, Engineering and Technology, 53*, 236–241.

Ao, Y., Li, J., Wang, Y., Liu, C., & Shuhong, Xu. (2017). Farmers' satisfaction of rural facilities and its influencing indicators: A case study of Sichuan, China. *Mathematical Problems in Engineering*. https://doi.org/10.1155/2017/1908520

Ashoka, N., Shrinivasulu, G. B., Anupama, G., Harshavardhan, M., & Kattimani, K. N. (2017). Economic analysis of production and marketing of jasmine in Hyderabad, Karnataka Region: A case in Koppal District, India. *International Journal of Current Microbiology and Applied Sciences, 6*(9), 1702–1711. https://doi.org/10.20546/ijcmas.2017.609.210

Austin, J., Stevenson, H., & Wei-Skillern, J. (2006). Social and commercial entrepreneurship: Same, different, or both? *Entrepreneurship Theory and Practice, 30*(1), 1–22. https://doi.org/10.1111/j.1540-6520.2009.00354.x

Ayranci, E. (2011). A study on the factors of job satisfaction among owners of small and medium-sized turkish Businesses. *International Journal of Business and Social Science, 2*(5), 87–100. https://doi.org/10.13140/RG.2.1.1538.2882

Bairwa, S. L., Lakra, K., Kushwaha, S., Meena, L. K., & Kumar, P. (2014). Agripreneurship development as a tool to upliftment of agriculture. *International Journal of Scientific and Research Publications, 4*(3), 1–4.

Bannor, R. K., Kumar, G. A. K., Oppong-Kyeremeh, H., & Wongnaa, C. A. (2020). Adoption and impact of modern rice varieties on poverty in eastern India. *Rice Science, 27*(1), 56–66. https://doi.org/10.1016/j.rsci.2019.12.006

Bannor, R. K., Ros-Tonen, M. A. F., Mensah, P. O., Derkyi, M., & Nassah, V. F. (2021). Entrepreneurial behaviour among non-timber forest product-growing farmers in Ghana: An analysis in support of a reforestation policy. *Forest Policy and Economics, 122*, 102331. https://doi.org/10.1016/j.forpol.2020.102331

Boer, T. (2013). Farmer business schools to strengthen entrepreneurship of potato farmers in Rwanda. Preparatory Analysis for Developing a Farmer Business School Approach for the Rwandan Potato Sector, Wageningen University.

Bradley, D. E., & Roberts, J. A. (2004). Self-employment and job satisfaction: Investigating the role of self-efficacy, depression, and seniority. *Journal of Small Business Management, 42*(1), 37–58. https://doi.org/10.1111/j.1540-627X.2004.00096.x

Browne, M. W., & Cudeck, R. (1993). Alternative Ways of Assessing Model Fit. In K. A. Bollen & J. S. Long (Eds.), *Testing of structural equation models* (pp. 136–162). Sage Publications.

Caro, F., & Cavallo, E. (2019). The effects of individual variables, farming system characteristics and perceived barriers on actual use of smart farming technologies: Evidence from the Piedmont Region, Northwestern Italy. *Agriculture, 9*(5), 111. https://doi.org/10.3390/agriculture9050111

Chakraborty, T., Ganguly, M., & Natarajan, A. (2019). Predicting entrepreneurial satisfaction: The role of non-financial incentive factors and quality of life among women digital entrepreneurs. *Journal for Global Business Advancement, 12*(3), 328–355. https://doi.org/10.1504/JGBA.2019.101388

Chatterjee, S., Chakraborty, R., & Banerjee, H. (2020). Economic impact assessment of conservation agriculture on small and marginal farm households in eastern India. *Agricultural Economics Research Review, 33*, 1–6. https://doi.org/10.22004/ag.econ.310344

Chisnall, P. (2001). *Marketing research*. McGraw-Hill Education.

Cooper, A. C., & Artz, K. W. (1995). Determinants of satisfaction for entrepreneurs. *Journal of Business Venturing, 10*, 439–457. https://doi.org/10.1016/0883-9026(95)00083-K

Cooper, A. C., Gimeno-Gascon, F. J., & Woo, C. Y. (1994). Initial human and financial capital as predictors of new venture performance. *Journal of Business Venturing, 9*(5), 371–395. https://doi.org/10.1016/0883-9026(94)90013-2

Dai, X., Pu, L., & Rao, F. (2017). Assessing the effect of a crop-tree intercropping program on smallholders' incomes in rural Xinjiang, China. *Sustainability*. https://doi.org/10.3390/su9091542

De Lauwere, C. C. (2005). The role of agricultural entrepreneurship in Dutch agriculture of today. *Agricultural Economics, 33*, 229–238. https://doi.org/10.1111/j.1574-0862.2005.00373.x

Demirtas, B. (2021). Agricultural supports and farmers' satisfaction: A latent variable application in Turkey. *Revista De La Facultad De Agronomía, 38*, 151–175. https://doi.org/10.47280/RevFacAgron(LUZ).v38.n1.08

Dias, C. S. L., Rodrigues, R. G., & Ferreira, J. J. (2019a). What's new in the research on agricultural entrepreneurship? *Journal of Rural Studies, 65*, 99–115. https://doi.org/10.1016/j.jrurstud.2018.11.003

Dias, C. S. L., Rodrigues, R. G., & Ferreira, J. J. (2019b). Agricultural entrepreneurship: Going back to the basics. *Journal of Rural Studies, 70*, 125–138. https://doi.org/10.1016/j.jrurstud.2019.06.001

Dobryagina, N. (2020). Agricultural entrepreneurship fostering from behavioral decision theory perspective Celebrity Branding Impact on Financial and Non-Financial Motivation. *The European Journal of Finance*. https://doi.org/10.1080/1351847X.2020.1841663

El Shoubaki, A., & Meike, S. (2018). The life partner and the life satisfaction of the entrepreneur. *Central European Business Review, 7*, 26–41. https://doi.org/10.18267/j.cebr.201

Elias, A., Nohmi, M., & Yasunobu, K. (2017). Cost-benefit analysis of cultivating three major crops and its implication to agricultural extension service: a case study in North-West Ethiopia. *Japanese Journal of Agricultural Economics*, *19*, 31–36. https://doi.org/10.18480/jjae.19.0_31.

Fan, L., & Luo, Y. (2009). Influencing factors of farmers' satisfaction to rural infrastructure based on the structural equation model analysis of 670 questionnaires. *Agricultural Economic Problems, 9*, 51–59.

Forbes, D. P. (2005). Are some entrepreneurs more overconfident than others? *Journal of Business Venturing, 20*, 623–640. https://doi.org/10.1016/j.jbusvent.2004.05.001

Fornell, C., & Larcker, D. (1981). Evaluating structural equation models with unobservable variables and measurement error. *Journal of Marketing Research, 18*, 39–50. https://doi.org/10.1177/002224378101800104

Fraser, S., & Greene, F. J. (2006). The effects of experience on entrepreneurial optimism and uncertainty. *Economica, 73*, 169–192. https://doi.org/10.1111/j.1468-0335.2006.00488.x

Gazioglu, S., & Tansel, A. (2006). Job satisfaction in Britain: Individual and job related factors. *Applied Economics, 38*, 1163–1171. https://doi.org/10.1080/00036840500392987

GOI. 2021. 'Ministry of Finance, Economic Survey 2020–21 Statistical appendix', https://www.indiabudget.gov.in/economicsurvey/doc/Statistical-Appendix-in-English.pdf Accessed on 24 May 2021.

Gunes, E., OrkanOzer, O., & Movassaghi, H. (2016). Factors affecting Turkish farmers' satisfaction with agricultural credit. *International Journal of Research Studies in Agricultural Sciences, 2*(6), 33–44. https://doi.org/10.20431/2454-6224.0206005

Hair, J. F., Black, W. C., Babin, B. J., & Anderson, R. E. (2010). *Multivariate Data Analysis*. Pearson Education.

Handy, F., Cnaan, R. A., Bhat, G., & Meijs, L. C. (2011). Jasmine growers of coastal Karnataka: Grassroots sustainable community-based enterprise in India. *Entrepreneurship & Regional Development, 23*(5–6), 405–417. https://doi.org/10.1080/08985626.2011.580166

Hayward, M. L. A., Shepherd, D. A., & Griffin, D. (2006). A hubris theory of entrepreneurship. *Management Science, 52*(2), 160–172. https://doi.org/10.1287/mnsc.1050.0483

Hazarika, B., & Goswami, K. (2018). Micro-entrepreneurship Development in the Handloom Industry: An Empirical Analysis among the Tribal Women in Assam. *International Journal of Rural Management, 14*(1), 22–38. https://doi.org/10.1177/0973005218754437

Higuchi, A., Coq-Huelva, D., Arias-Gutierrezd, R., & Alfalla-Luquee, R. (2020). Farmer satisfaction and cocoa cooperative performance: Evidence from Tocache Peru. *International Food and Agribusiness Management Review*. https://doi.org/10.22434/ifamr2019.0166

IBEF. 2021. 'Agriculture in India: Information about Indian Agriculture & its Importance'. Retrieved 26 May, 2021 from, https://www.ibef.org/industry/agriculture-india.aspx

Jaganathan, D., & Nagaraja, N. R. (2015). Perception of farmers about arecanut based multispecies cropping system. *Indian Research Journal of Extension Education, 15*(2), 49–54.

Josephine, R. M., Jayasudha, D., & Sri Rashmy, M. (2014). Cut flowers harvesting and marketing potential of Nilgiris. *International Journal of Current Microbiology and Applied Sciences, 3*(11), 953–956.

Kakkar, N., Kaur, P., & Dhaliwal, R. K. (2014). Satisfaction of Farmers Regarding Subsidies Provided Under National Food Security Mission. *Journal of Community Mobilization and Sustainable Development., 9*(2), 107–109.

Kassem, H. S., Alotaibi, B. A., Muddassir, M., & Herab, A. (2021). Factors influencing farmers' satisfaction with the quality of agricultural extension services. *Evaluation and Program Planning, 85*, 1–10. https://doi.org/10.1016/j.evalprogplan.2021.101912

Kim, P., Aldrich, H., & Keister, L. (2006). Access (not) denied: The impact of financial, human, and cultural capital on entrepreneurial entry in the United States. *Small Business Economics, 27*(1), 5–22. https://doi.org/10.1007/s11187-006-0007-x

Konyar, K., & Osborn, C. T. (1990). A national-level economic analysis of conservation reserve program participation: A discrete choice approach. *Journal of Agricultural Economics Research, 42*(2), 5–12. https://doi.org/10.22004/ag.econ.139017

Kristiansen, S., Furuholt, B., & Wahid, F. (2003). Internet cafe entrepreneurs: Pioneers in information dissemination in Indonesia. *The International Journal of Entrepreneurship and Innovation, 4*(4), 251–263. https://doi.org/10.5367/000000003129574315

Kuada, J. (2009). Gender, social networks, and entrepreneurship in Ghana. *Journal of African Business, 10*(1), 85–103. https://doi.org/10.1080/15228910802701445

Lans, T., Seuneke, P., & Klerkx, L. (2013). Agricultural entrepreneurship. In E. G. Carayannis (Ed.), *Encyclopedia of creativity, invention, innovation and entrepreneurship*. Springer. 10.1007/978-1-4614-3858-8_496.

Lauto, G., Pittino, D., & Visintin, F. (2020). Satisfaction of entrepreneurs: A comparison between founders and family business successors. *Journal of Small Business Management, 58*(3), 474–510. https://doi.org/10.1080/00472778.2019.1660937

Lingesiya, Y. (2017). Identifying Factors to Indicate the Business Performance of Small Scale Industries: Evidence from Sri Lanka. *Global Journal of Management and Business Research, 12*(21), 75–82.

Lucas, S. (2017). The impact of demographic and social factors on firm performance in Kenya. *Journal of Business and Economic Development, 2*(4), 255–261. https://doi.org/10.11648/j.jbed.20170204.18

Madhumitha, S., & Karthikeyan, C. (2020). Motivating factors for women to become agripreneurs. *Madras Agricultural Journal, 107*(7–9), 333–337. https://doi.org/10.29321/maj.2020.000387

Malhotra, N. K., & Birks, D. F. (2003). *Marketing research: An applied approach*. U.S.Prentice Hall.

Malhotra, N. K., & Dash, S. (2011). *Marketing research: An applied orientation*. Pearson Publishing.

Mazibuko, N. V. E., & Antwi, M. A. (2018). Socio-Economic factors influencing smallholder farmers agricultural infrastructure availability, accessibility and satisfaction: A case on North West Province in South Africa. *OIDA International Journal of Sustainable Development, 12*(5), 11–26.

McElwee, G. (2008). A taxonomy of entrepreneurial farmers. *International Journal of Entrepreneurship and Small Business, 6*(3), 465–478. https://doi.org/10.1504/ijesb.2008.019139

McMahon, R. G. P. (2001). Growth and performance of manufacturing SMEs: The influence of financial management characteristics. *International Small Business Journal, 19*(3), 10–28. https://doi.org/10.1177/0266242601193001
Morgan, S. L., Marsden, T., Miele, M., & Morley, A. (2010). Agricultural multifunctionality and farmers' entrepreneurial skills: A study of Tuscan and welsh farmers. *Journal of Rural Studies, 26*(2), 116–129. https://doi.org/10.1016/j.jrurstud.2009.09.002
Mubeena, Md., Lakshmi, T., Praveena, P. L. R. J., Nagavani, A. V., & Ramana Murthy, B. (2020). Profile characteristics of rural youth agripreneurs of Andhra Pradesh. *The Pharma Innovation Journal, 9*(6), 314–319.
Mwamakimbula Alunas Maxwell. 2014. 'Assessment of the factors impacting agricultural extension training programs in Tanzania: A descriptive study'. Graduate Theses and Dissertations. 14227. https://lib.dr.iastate.edu/etd/14227.
Ncube, B. L. (2017). Institutional support systems for small-scale farmers at new forest Irrigation Scheme in Mpumalanga, South Africa: constraints and opportunities. *South African Journal of Agricultural Extension, 45*(2), 1–13. https://doi.org/10.17159/2413-3221/2017/v45n2a395
Netemeyer, R. G., Johnston, M. W., & Burton, S. (1990). Analysis of role conflict and role ambiguity in a structural equation framework. *Journal of Applied Psychology, 75*, 148–157. https://doi.org/10.1037/0021-9010.75.2.148
Noltea, K., & Ostermeierab, M. (2017). Labour market effects of large-scale agricultural investment: Conceptual considerations and estimated employment effects. *World Development, 98*, 430–446. https://doi.org/10.1016/j.worlddev.2017.05.012
Obeng, B. A., Robson, P., & Haugh, H. (2014). Strategic entrepreneurship and small firm growth in Ghana. *International Small Business Journal, 32*(5), 501–524. https://doi.org/10.1177/0266242612463946
Ovharhe, O. J., Emaziye, P., & Okwuokenye, G. F. (2020). Farmers' satisfaction with agricultural extension services in Delta State, Nigeria. *International Journal of Agricultural Technology, 16*(6), 463–1474.
Pindado, E., & Sánchez, M. (2017). Researching the entrepreneurial behaviour of new and existing ventures in European Agriculture. *Small Business Economics, 49*(2), 421–444. https://doi.org/10.1007/s11187-017-9837-y
Pliakoura, A., Beligiannis, G., & Kontogeorgos, A. (2020). Education in agricultural entrepreneurship: Training needs and learning practices. *Education Training, 62*(7/8), 723–739. https://doi.org/10.1108/ET-04-2020-0095
Post, M. W. (2016). What to do with "Moderate" reliability and validity coefficients? *Archives of Physical Medicine and Rehabilitation, 97*(7), 1051–1052. https://doi.org/10.1016/j.apmr.2016.04.001
Pyysiäinen, J., Anderson, A., McElwee, G., & Vesala, K. (2006). Developing the entrepreneurial skills of farmers: Some myths explored. *International Journal of Entrepreneurial Behavior & Research, 12*(1), 21–39. https://doi.org/10.1108/13552550610644463
Rao, F., Spoorb, M., Maa, X., & Shia, X. (2016). Land tenure (in) security and intercropping in rural Xinjiang, China. *Land Use Policy, 50*, 102–114. https://doi.org/10.1016/j.landusepol.2015.09.001
Reddy, G. K., Govardhan, M., Kumari, C. P., Pasha, M. L., Baba, M. A., & Rani, B. (2020). Integrated farming system a promising farmer and eco friendly approach for doubling the farm income in India—A review. *International Journal of Current Microbiology and Applied Sciences, 9*(1), 2243–2252. https://doi.org/10.20546/ijcmas.2020.901.254
Selvaraj, A. (2015). Crop Insurance: A study with farmers' awareness and satisfaction. *International Journal of Current Research, 7*(7), 18680–18687.
Selvendran, S. (2017). Impact of farmer's social status in the cultivation of jasmine flowers: Special reference to Dindigul district. *International Journal of Interdisciplinary Research in Arts and Humanities, 2*(2), 2456–3145. https://doi.org/10.5281/zenodo.1038393
Sharma, A. K. (2014). Farmer's satisfaction with information sources and services: A study on farmer's opinion. *International Journal of Information Research, 3*(4), 246–259.
Singh, D., & Kalra, R. K. (2019). Level of satisfaction of farmers from the services provided by agricultural technology and information centre (ATIC) run by Punjab Agricultural University. *International Journal of Bio-Resource and Stress Management, 10*(5), 575–579. https://doi.org/10.23910/ijbsm/2019.10.5.2032
Singh, P., Farhan, M., Saajan, S., & Singh, P. (2019). A study to access factors affecting agripreneurship in diversified utilization of potato crop in state of Punjab and Himachal Pradesh. *Plant Archives, 19*(2), 2335–2342.
Singh, S. (2013). Dynamics of agricultural marginalization in emergent rural economy: a study in South Bihar. *International Journal of Rural Management, 9*(1), 71–96. https://doi.org/10.1177/0973005213479208
Sinyolo, S., Mudhara, M., & Wale, E. (2017). The impact of social grant-dependency on agricultural entrepreneurship among rural households in Kwazulu-Natal, South Africa. *The Journal of Developing Areas, 51*(3), 63–76. https://doi.org/10.1353/jda.2017.0061
Sleuwaegen, L., & Goedhuys, M. (2002). Growth of firms in developing countries, evidence from Cote D'Ivoire. *Journal of Development Economics, 68*(1), 117–135. https://doi.org/10.1016/S0304-3878(02)00008-1
Smit, A. B. (2004). Changing external conditions require high levels of entrepreneurship in agriculture. *Acta Horticulturae, 655*(1), 167–173. https://doi.org/10.17660/ActaHortic.2004.655.21
Spring, A. (2009). African women in the entrepreneurial landscape: Reconsidering the formal and informal sectors. *Journal of African Business, 10*(1), 11–30. https://doi.org/10.1080/15228910802701296
Subagyo, V. K., & Ernestivita, G. (2020). Entrepreneurial parameters and performance of MSMEs in East Java province of Indonesia. *International Journal of Business Innovation and Research, 23*(2), 267–282. https://doi.org/10.1504/IJBIR.2020.110102
Vasan, M. (2020). Moderating effect of demographic and business characteristics on performance of women owned small enterprises: Empirical evidence from India. *Serbian Journal of Management, 15*(2), 319–333. https://doi.org/10.5937/sjm15-21922
Verhees, F. J., Kuipers, A., & Klopcic, M. (2011). Entrepreneurial proclivity and farm performance: The cases of Dutch and Slovenian farmers. *The International Journal of Entrepreneurship and Innovation, 12*(3), 169–177. https://doi.org/10.5367/ijei.2011.0039
Wayne, G. G., Webster, N., & Lendel, K. N. (2014). Farmers' satisfaction with extension services in the organization of Eastern Caribbean States. *Journal of International Agricultural and Extension Education, 21*(3), 49–62. https://doi.org/10.5191/jiaee.2014.21304

Weaver, C. N. (1977). Relationships among pay, race, sex, occupational prestige, supervision, work autonomy, and job satisfaction in a national sample. *Personnel Psychology, 30*(3), 437–445. https://doi.org/10.1111/peps.1977.30.issue-3
World Bank. 2016. World Development Indicators [Database]. Retrieved January 11, 2016, from http://databank.worldbank.org/data/reports.aspx?source=world-development-indicators&preview=on
Wright, M., Robbie, K., & Ennew, C. (1997). Venture capitalists and serial entrepreneurs. *Journal of Business Venturing, 12*, 227–249. https://doi.org/10.1016/S0883-9026(96)06115-0
Zawojska, A. (2010). Determinants of farmers' trust in government agricultural agencies in Poland. *Agricultural Economics, 56*(6), 266–283. https://doi.org/10.17221/103/2009-agricecon

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Submit your manuscript to a SpringerOpen journal and benefit from:

- ► Convenient online submission
- ► Rigorous peer review
- ► Open access: articles freely available online
- ► High visibility within the field
- ► Retaining the copyright to your article

Submit your next manuscript at ► springeropen.com

Design Engineering

ISSN: 0011-9342 | Year 2021
Issue: 9 | Pages: 689 - 701

Comparative Study on Sentiment Analytics Using Deep Learning Techniques

[*]Dr M Anusha[1], R Leelavathi[2]

[1]Assistant Professor, PG & Research Department of Computer Science, National College (Autonomous), Bharathidasan University, Tamilnadu, India

[1]Email id – anusha260505@gmail.com

[2] Research Scholar, PG & Research Department of Computer Science, National College (Autonomous), Bharathidasan University, Tamilnadu, India

[2]Email id – leelavathi4794@gmail.com

* Corresponding Author – R. Leelavathi

ABSTRACT-Sentiment analytics is the process of applying natural language processing and methods for text-based information to define and extract subjective knowledge of the text. Natural language processing and text classifications can deal with limited corpus data and more attention has been gained by semantic texts and word embedding methods. Deep learning is a powerful method that learns different layers of representations or qualities of information and produces state-of-the-art prediction results. In different applications of sentiment analytics, deep learning methods are used at the sentence, document, and aspect levels. This review paper is based on the main difficulties in the sentiment assessment stage that significantly affect sentiment score, pooling, and polarity detection. The most popular deep learning methods are a Convolution Neural Network, Recurrent Neural Network and Long Short Term Memory. Finally, a comparative study is made with a vast literature survey using deep learning models.

Keywords: Sentiment Analytics (SA), Natural Language Processing (NLP), Deep Learning (DL), Recurrent Neural Network (RNN), Convolution Neural Network (CNN), Long Short Term Memory (LSTM).

ISSN: 0011-9342 | Year 2021
Issue: 9 | Pages: 689 - 701

IINTRODUCTİON

Data is a prevalent source of social media communication. Data in the form of sentences and sentiments, individuals share their views [1]. Web 2.0 has contributed to the spread of blogs, forums, and social networks online, allowing people to discuss any topic and express their ideas. A social network for instance peeps or Facebook has become a common way to learn about user views and has a broad range of applications [2]. The way to recognize the individual polarity of the text is sentiment. It decides that positive, negative, or neutral is the given document. Sentiment analytics is the process of using techniques for natural language processing to extract individual knowledge from the text [3]. Sentiment analytics can be classified at various levels. Sentiments are classified into various forms, namely feature level, sentence level, and document level [4]. A sense dependent upon the position polarity for each element is defined by the identification of the character level. On the other hand, based on their intensity of meaning about certain topics, categorization at the level of the sentence in each word. It is mutual opposition of this text created in the class aside document level. The entire file is labeled as positive or negative and neutral [5]. In order to acquire the natural language processing (NLP) concerned with the processing and interpretation of human languages in computational terms. Natural Language Processing using linguistic techniques is sentence segmentation, word tokenization, removal of stop words, part-of-speech tagging stemming. The current present-day sentiment analytics has become more mainstream as a meaningful step forward in deep learning in the field of common language preparation [6]. The key regions of fundamental issues are language demonstrating, the measurement associations between words that naturally occur morphological handling, the segmentation of meaningful words, and recognize the real piece of word stream, grammar preparing, or parsing that create sentence structures as potential catalysts of semantic process. The fields of application include topics such as extracting useful knowledge, translating text within and between languages, comprehending written works, and answering questions automatically by referencing answers and classifying documents [7]. For sentiment analytics of texts are important, and Bag of Words (BoW), Word Embedding is a widely-used model. In this sequel, artificial intelligence is introduced to design a model that could perform. Sentiment analytics using automatic feature extraction. The feature extraction can make systemized with deep learning techniques. [8]. It is the subset of machine learning. This system learns many layers of data interpretations or features and delivers a predictive output that is state-of-the-

ISSN: 0011-9342 | Year 2021
Issue: 9 | Pages: 689 - 701

art. The neural network model of sentiment examination is arranged into three classes, in particular CNN, RNN, LSTM and hybrid models. Also the achievement of deep learning in numerous application fields and has also been used in sentiment analytics [9]. This review paper focuses on sentiment analytics that combines natural language processing and deep learning methods. The sentiment rank is based on the entities, pooling, sentiment polarity, and themes categories within a sentence or phrase. This research uses deep learning models such as recurrent neural networks and convolution neural networks to help solve the issue of sentiment analytics [1]. This paper is organized as follows section 2 briefly introduces the related work section 3 comparative study section 4 is extended with results and the discussion ends with a conclusion.

II LİTERATURE SURVEY

Kleenankandy, J. et al. [10] showed an improved LSTM architecture was implemented, called this relationship, which can show the connection between a sequence of two data sources utilizing a control input. These models are called Typed Dependency Tree that uses both forms of the term dependency parse and type of encoding the meaning of phrase into a dense vector. The DT-LSTM model was produced Precision of 88.4%, Recall of 84.8%, F1–score of 86.6%, and accuracy of 86.6%. Basiri, M.E. et al. [11] Attention-focused CNN-RNN Deep bidirectional model for temporal data flow in both information ABCDM can derive both past and future context with the use of two different models LSTM and GRU layers. The consideration meaning is often applied to outputs of functioning in two directionshave more or less effect on individual terms. It incorporates complexity and pool mechanisms to minimize the dimensionality of characteristics and extract stance essential features. The efficacy is measured in determining the polarity of sentiment, which is the most common and important role of sentiment analytics. The proposed ABCDM model was performed Recall of 99.08%, Precision of 99.04%, F1-score of 96.94% and, the accuracy of 96.87%. Huang, M. et al. [12] showed that a neural network of context-dependent, lexicon-based convolution is used to successfully separate the attributes and intensity of focus from words and text. To create this convolution neural network, the model is prepared to utilize the polarization for each word with a co-event example of words and marks. The algorithm has produced sentiment strength of document for six different datasets accuracy of 83%, 77%, 65%,74%,

ISSN: 0011-9342 | Year 2021
Issue: 9 | Pages: 689 - 701

70%, 98%. Shuang, K. et al. [13] explored a function distillation network for noise reduction and an aspect-relevant view of the sentiment highlights for filtering. The aim of the Novel double gate mechanism is to designed includes the points of view and their respective contexts in fine granularity. In front of the double-gate mechanism add a relevant nonlinear prediction layer to deliver aspect-specific word representation, and helps the twofold entryway component to accurately separate between feeling qualities of a similar context word corresponding to the numerous viewpoints. The FDN model has produced three different gating mechanisms on ATSA accuracy of 76.8%, 82.3%, 73.7%, and macro F1 score of 72.5%, 75%, 72.2%. Senthil Kumar, N. K. et al. [14] proposed an enhanced richer presentation, not only binary and ternary classification it goes deeper in classifying the collection of text from the social network. To verify the multiple sentiment prediction classes checked for the short text in sentiment data based on polarity. The algorithm has produced an accuracy of 98.6%. Sharma, A.K et al. [15] the proposed model has cleaned the information and creates significance from the Word2Vec model used for the convolution layer to extract highlights for short sentences. The proposed model was built on training accuracy of 99.07% and testing accuracy of 82.19 %. Abid, F. et al. [16] projected a Convolutional Neural Network combined with a recurrent neural network based on weighted attentive pooling. The regular pooling activities contain a few layers that are just fit for capturing proper highlights. To enable sentiment analytics such as Word2vec, Fast Text, and Glove to produce a dense powerful linked representations following the long-term dependencies on a single RNN layer obtained explicitly by part of speech tagging with verbs, adverbs, and noun. The model has produced an accuracy of 89.67%. Zhou, M. et al. [17] proposed a text classification model based on the double word embedding techniques are Glove and Word2vec. Models combined with the content form a combinatory information double convolution neural network channel. The initial word vector is consistently trained and modified the classification model with a single input vector representation, based on the word vector fine-tuning technique. The glove and word2vec model has classified the accuracy of 94.8%. Samat, N.A. et al. [18] extract features from sentiment analytics used for the CNN algorithm. This algorithm used for three different pooling functions reduced the noisy data during the training process. Finally to capturing the semantic features. The three different pooling functions are produced the better accuracy of 97.73%, 93.05%, and 95.58%. Naderalvojoud, B. et al. [19] proposed two approaches for opinion to learn embedding techniques. The first approach encodes the word

ISSN: 0011-9342 | Year 2021
Issue: 9 | Pages: 689 - 701

sentiment knowledge set up pre-trained importance of words, and the second approach semantic context creates synthetic sentiment context for inserting models. On the various sentiment classification tasks used for Skip-gram and Glove models. The proposed model based on two approaches has produced an accuracy of (87%,47.4%) and Macro F1 score of (91.3%, 69.4%). Jang, B. et al. [20] noted that Bi-LSTM+CNN hybrid models supporting an attention function to additional enhance accuracy and decrease the number of learnable parameters. It is used to extract specific characteristics in a sentence from various locations with reducing the number of input features. To remove the context-oriented information from the highlights acquired from the layer of convoluted. The length of the attention mechanism is improved the distribution process. The proposed hybrid model was built maximum accuracy of 91.41%, average accuracy of 90.26%, F1 score of 90.18%, Recall of 90.57%, and precision of 80.97%. Xu, J. et al. [21] planned that text characterization using CNN based on the neural network called DE-CNN. To coordinate with context-relevant ideas into a convolution neural network. First and foremost used for two layers are extracting their concepts and meaning individually. That point of short text classification is implemented into a text representation. The DE-CNN model was provided three different datasets have classified the accuracy of 94.6%, 84.6%, 88.9%. Hassan, A. et al. [25] Deep learning network pre-trained parameters were trained in word embedding then to activating the model. The new framework mixes accessible information with different feature maps are learned by a convolution layer, and the long-term dependencies learned by long-flitting memory at the last level. This technique achieves excellent results with minor hyperparameter tuning for static vectors using multiple sentiment analytics benchmarks dataset. The algorithm has performed the three different datasets, and the accuracy of 93.3%, 48.8%, and 89.2%. Liao, W. et al. [26] presented a new model for sequence-to-sequence learning for multi-label classification. The two unmistakable neural network modules are called encoder and decoder individually. Using the convolution neural network, the encoder removes the high-level local sequential semantics to joined with the word vector using the recurrent neural network and the attention mechanism creates the last content representation. Then totally initialized, the decoder used for the association layer randomly connecting the two labels. The algorithm has been produced a hamming loss of 78%, a micro-Recall of 85.1%, and a Micro-F1 score of 87.3%. Ray, P. et al. [27] proposed a novel deep convolutional neural network model is used for labeling every sentence. Deep learning approaches combined with previous rule-based

ISSN: 0011-9342 | Year 2021
Issue: 9 | Pages: 689 - 701

techniques for improving the feature extraction based on sentiment scoring technique. The proposed method was performed with an overall accuracy of 87%. Chen, C. et al. [28] projected a gated sentiment relationship recurrent neural network model to get the sentiment information from the effects of text and their modifier. The inputs are updated multiplicatively by the existing encoded sentiment modifier proposed the sentiment polarity. The two subsets are dividing the sentiment relation model was performed the overall accuracy of 94.6% and 95.2%. Guo, X. et al. [4] designed have been analyzed three levels of granularity: text, sentence, and aspect level. An advanced att-RCNN model that joins the recurrent neural network and convolution neural network. Both models are extracting the features for word-level and sentence-level, improving the significant terms and functionality promoting the performance of the proposed model. The Att-RCNN model was built on the F1 Score of 86.6%. Umer, M. et al. [23] projected the dimensionality reduction techniques to classified the feature vectors. The principal component analysis and chi-square are taken care of to nonlinear characteristics offering more contextual features to detect fake news. The proposed CNN-LSTM with PCA performed an accuracy of 97.8%, precision of 97.4%, Recall of 98.2%, and f-score of 97.8%.Cekik, R. et al. [24] a novel filter feature selection tool, uses the rough set for a territorial distinction according to the term of a value set to classify reports that have a class. By multiplying a coefficient called documents that may belong to a class are penalized. Also, using a rough set, the impact of sparsity in the term vector space is determined. The current filter attribute selection techniques such as Gini index, data gain, feature selector distinguishing, max-min ratio methods of normalized difference calculation. The proposed model perform was six different datasets are F1 score 80.20%, 72.91%, 80.25%, 80.17%, 79.84%, and 81.84%.

III COMPARATİVE STUDY

In this section comparative Study on deep learning models about datasets, merits, and demerits, to discuss below for the sentiment analytics process.

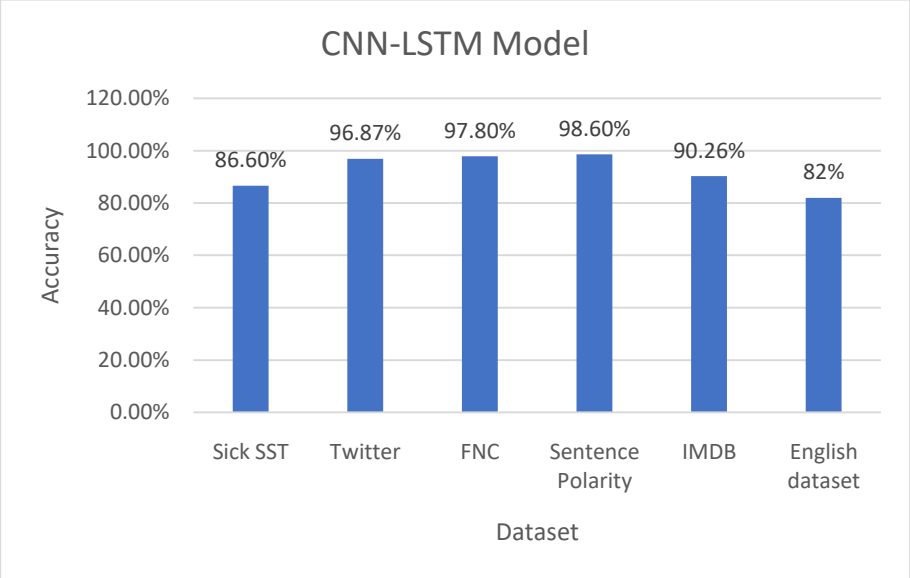
Design Engineering

ISSN: 0011-9342 | Year 2021
Issue: 9 | Pages: 689 - 701

TABLE I. Comparative Study with Convolutional Neural Network Model and Long Short Term Memory

AUTHOR/ YEAR	MODEL	DATASET	MERIT	DEMERIT
Kleenankandy, J. et al.[10] / (2021)	CNN LSTM	Sick SST	Sentence representation	Sentence Dependency Parse tree
Basiri, M.E., et al.[11]/ (2021)	CNN LSTM	Twitter Dataset	Decrease the dimensionality	Rating prediction
Umer, M. et al. [23] / (2020)	CNN LSTM	FNC	Dimensionality reduction of the feature vector Reduce overfitting	Noise data Inconsistency
Senthil kumar, N.K., et al.[14] / (2020)	CNN LSTM	Sentence Polarity	Part-of-speech (PoS) tagging	Sarcasm class Sentiment classification
Jang, B., et al. [20] / (2020)	CNN LSTM	IMDB	Text classification Sentence classification	LongTerm dependency.
Shuang, K. et al .[22] / (2020)	CNN LSTM	English dataset	The fuses context and task specific information	Polysemous unaware Task –unaware

Fig.1 Overall Accuracy of CNN-LSTM

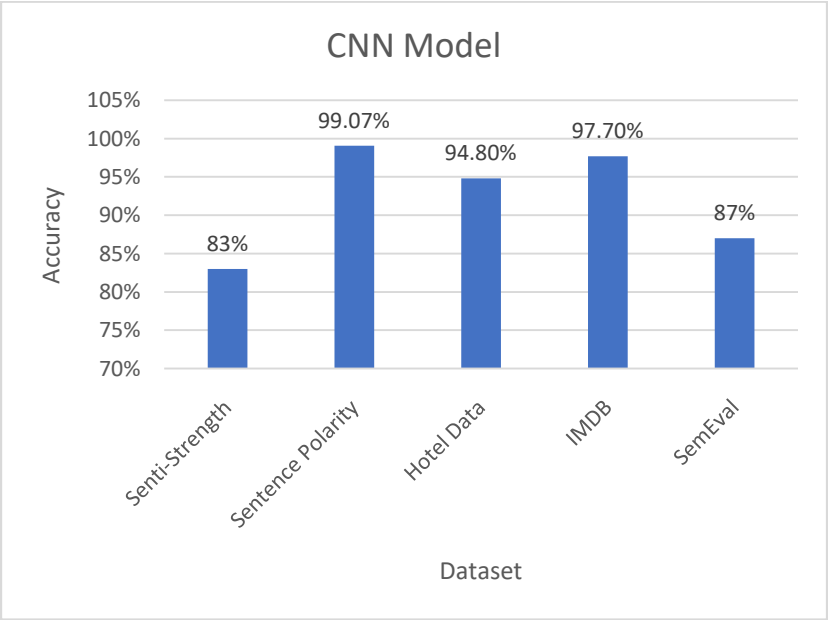


ISSN: 0011-9342 | Year 2021
Issue: 9 | Pages: 689 - 701

TABLE II. Comparative Study with Convolutional Neural Network Model

AUTHOR/ YEAR	MODEL	DATASET	MERIT	DEMERIT
Huang, M. et al .[12] / (2020)	CNN	Senti-Strength	Sentiment Strength	Context-dependent sentiment lexicon
Sharma, A.K., et al .[15] / (2020)	CNN	Sentence Polarity	Word embedding -text classification	Long sentence categorization
Zhou, M. et al. [17] / (2020)	CNN	Hotel Data	Single-channel representation	Non-static Word Vector
Samat, N.A. et al.[18] / (2020)	CNN	IMDB	Pooling Operation	Overfitting on the training set
Ray, P. et al.[27] / (2019))	CNN	SemEval	Sentence Score	Word Embedding

Fig.2 Overall accuracy of CNN

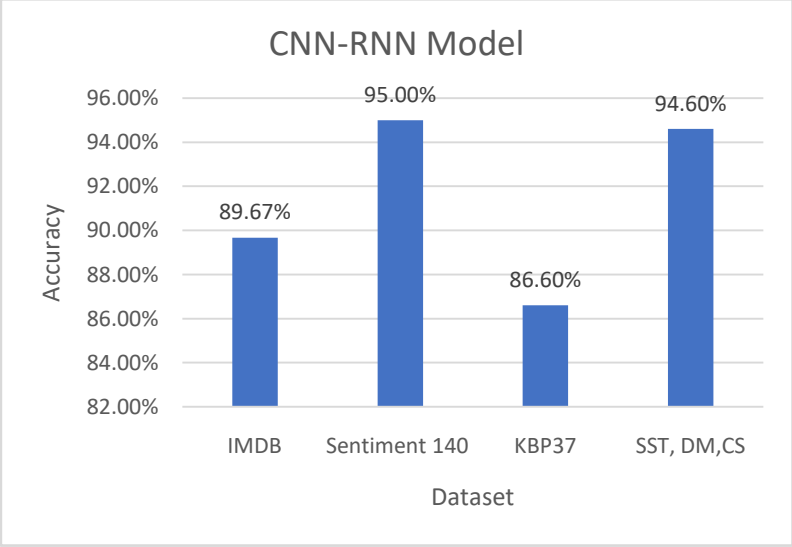


ISSN: 0011-9342 | Year 2021
Issue: 9 | Pages: 689 - 701

TABLE III. Comparative Study with Convolutional Neural Network and Recurrent Neural Network Model

AUTHOR/ YEAR	MODEL	DATASET	MERIT	DEMERIT
Abid, F. et al. [16] / (2020)	CNN RNN	IMDB	Word weighted Mechanism	Overfitting
Dang, N.C. et al.[2] / (2020)	CNN RNN	Sentiment 140	CPU runtime. Reduce the computational cost	Automatic Sentiment Analytics Sentiment polarity
Guo, X. et al .[4] / (2019)	CNN RNN	KBP37	Attention Mechanisms	Noise data based on SDP- word
Chen,C. et al. [28] / (2019)	RNN	SST, DM, CS	Sentiment modifier sentiment relations	Sentiment context Content word and Shifting negation

Fig.3 Overall accuracy of CNN-RNN



ISSN: 0011-9342 | Year 2021
Issue: 9 | Pages: 689 - 701

IVRESULT AND DİSCUSSİON

In this survey, it is explored the applications of natural language processing and deep learning techniques. It is inferred that most of the research work is carried out in a feature extraction phase. The enormous literature survey related to large datasets, overfitting, number of classifiers, pooling, and dimensionality reduction were reviewed and compared with some viewpoints, such as datasets, methodology, merits, demerits, and overall accuracy of the existing models. With the help of comparative analysis, the core research is identified along with the related techniques. The datasets are chosen as unstructured datasets that have an impact on social media sectors. Hence, by selecting the social media dataset the research may move towards the problem in social media related identified the streaming news. Based on the comparative study, a research gap on feature extraction is noted for sentiment analytics.

V CONCLUSİON

The major revelation of the survey is based on real time problem available in social meadia which has more impacts on sentiment analytics. It is the kind of streamed text research that systematically determines the successful state of subjective knowledge at the sentence level by extracting quantifies on polarity detection. This paper addresses the problem of detecting sentiment polarity, pooling, Sentiment ranking, and sentiment extraction. Deep learning methods have been established to address the major obstacles of sentiment analytics. The merits and demerits are thoroughly identified. The limitations of the research work are aspect and word level. Hence, with the help of a study on sentiment analytics using deep learning, it is motivated to proceed with the research work is noise reduction, overfitting, dimensionality reduction and pooling operation using deep learning models are CNN, LSTM and RNN for sentiment analytics.

REFERENCES

[1] Chakraborty, K., Bhattacharyya, S., Senior Member, IEEE, and Bag, R.: A Survey of Sentiment Analytics from Social Media Data. Compute. Social. Syst. 1-15 IEEE Trans (2020)

ISSN: 0011-9342 | Year 2021
Issue: 9 | Pages: 689 - 701

[2] Dang, N.C., María, N., Moreno-Garcia and Prieta, F.D.: Sentiment analytics Based on Deep Learning: A Comparative Study. *Electronics.* 483. 9, 2-29 MDPI (2020)

[3] Mohey, D.E1, Hussein, D. M.: A survey on sentiment analytics challenges. 30. 4, 330-338 Elsevier (2018).

[4] Guo, X., Zhang, H., Yang, H., Xu, L.,and Zhiwen.: A single Attention-based combination of CNN and RNN for Relation Classification. Proc. Comput. Science. 7.131, 12467-12475 IEEE Access (2019).

[5] Habimana1, O., Li, L., Li, R., Gu X., & Yu, G.: Sentiment analytics using deep learning approaches: an overview," Info. sci. 63, Springer (2020)

[6] Daniel W. O., Julian R. Medina, and Jugal K. Kalita.: A Survey of the Usages of Deep Learning for Natural Language Processing. Neural. Netws. Learning. Syst., IEEE. Trans (2019)

[7] Wafaa S. EI-Kassas, Cherif R. Salama, Ahmed A. Rafea, Hoda K. Mohamed, " Automatic text Summarization: A comprehensive survey," Expert. Syst. Applns. 165, 113679 Elsevier (2021)

[8] Liu, R., Shi, Y., Ji, C., and Jia, M.: A Survey of Sentiment Analytics Based on Transfer Learning. IEEE Access (2019).

[9] Liu, L. Z. W. B.: Deep learning for sentiment analytics: A survey. Springer (2020)

[10] Kleenankandy, J., Nazeer, K.A.: An enhanced Tree-LSTM architecture for sentence semantic modelling using typed dependencies. Info. Pro. Management 57(6), 102362 ACM(2020)

[11] Basiri, M, E., Nemati, S., Adar, M., Cambria, E.: ABCDM: An Attention-based Bidirectional CNN-RNN Deep Model for sentiment analytics. Future Gen. Compute. Syst. 279-294 Elsevier (2021)

[12] Huang, M., Xie, H., Jingrong, Y.R., Fen,Wang, F.L.: Sentiment strength detection with a context-dependent lexicon-based convolution neural network. Info. Science. 520,389-399 Elsevier(2020)

[13] Shuang, K., Yanga, Q., Loo, J., Li, R., Gua, M., "Feature distillation network for aspect-based sentiment analytics," Info. Fusion, vol. 61, pp: 13-23, Elsevier, 2020.

ISSN: 0011-9342 | Year 2021
Issue: 9 | Pages: 689 - 701

[14] Senthil Kumar, N, K., Malarvizhi,N.: "Bi-directional LSTM-CNN Combined method for sentiment analytics in Part of Speech Tagging (POS)," Int. Journal. Of. Speech. Tech., Springer, 2020.

[15] Sharma, A.K., Chaurasia, S., Srivastava, D. K.: Sentiment Short Sentences Classification by Using CNN Deep Learning Model with Fine Tuned Word2Vec. Proc. Compute. Science. 167, 1139-1147 Elsevier (2020)

[16] Abid, F., Li, C., Alam, M.: Multi-source social media data sentiment analytics using bidirectional recurrent convolution neural networks, Compute. Communes. 157, 102-115 Elsevier (2020)

[17] Zhou, M., Liu, D., Zheng, Y., Zhu, Q., Guo,P.: A text sentiment classification model using double word embedding methods, Multmed. Tools. Appln. Springer (2020)

[18] Samat, N.A., Mohd, N.S., Ali, H.: The Comparison of pooling functions in a conventional neural network for sentiment analytics task. Research Gate (2020).

[19] Naderalvoijoud, B., Sezer, E.A.: Sentiment Aware Word Embedding using Refinement and Senti-Contextualized Learning Approach. Neuro. Comput, 405, 149-160 Elsevier (2020).

[20] Jang, B., Kim, M., Harerimana, G., Sang-ug K., and Kim, J.W.: Bi-LSTM Model to Increase Accuracy in Text Classification: Combining Word2Vec CNN and Attention Mechanism, Appl. Sci, 10. 5841. 1-14 MDPI (2020).

[21] Xu, J., Cai, Y., Wu, X., Lei, X., Huang, Q., Ho-Fung L, Li, Q.: Incorporating Context-Relevant Concepts into Convolution Neural Networks for short Text Classification," Neuro Comput., 386, 42-53 Elsevier (2020)

[22] Shuang, K., Zhang, Z., Loo,J., Sen Su.: Convolution-deconvolution word embedding: An end-to-end multi-prototype fusion embedding method for natural language processing," Info.Fusion. 53. 112-122, Elsevier (2020)

[23] Umer, M., Imtiaz, Z., Ulla, S., Mehmood, A., Choi G.S, and Byung-Won On.: "Fake News Stance Detection Using Deep Learning Architecture (CNN-LSTM). 8, 156695 – 156706 IEEE Access (2020)

[24] Cekik, R., Uysal, A.K.: A novel filter feature selection method using a rough set for short text data," Expert. Syst. Applns. 160, 113691 Elsevier (2020)

ISSN: 0011-9342 | Year 2021
Issue: 9 | Pages: 689 - 701

[25] Hassan, A., Mahmood, A, A.: Convolutional Recurrent Deep Learning Model for Sentence Classification," MultiDisci. Rapid. View. 6, 13949 - 13957 IEEE Access, (2018)

[26] Liao, W., Wang, Y., Yin, Y., Zhang, X., Pan M.: Improved sequence generation model for multi-label classification via CNN and initialized fully connection. Neuro. Comput. Elsevier (2019)

[27] Ray, P., Chakrabarti, A.: A Mixed approach of Deep Learning method and Rule-Based method to improve Aspect Level Sentiment analytics. Appl. Compute. Info. Elsevier (2019)

[28] Chen, C., Zhuo, R., Ren,J.: Gated recurrent neural network with sentiment relations for sentiment classification. Info. Science. 502, 268-278 Elsevier(2020).

I am R. Leelavathi Ph.D Full-Time Research Scholar in Department of Computer Science, National College, Trichiruappalli. Currently doing my research topic is Sentiment Analytics. Research interests include data analytics, natural language processing and deep learning.

Analysis on Sentiment Analytics Using Deep Learning Techniques

1st M.Anusha
PG & Research Department of Computer Science
National College (Affiliated to Bharathidasan Univsity)
Tiruchirappalli, India
anusha260505@gmail.com

2nd R. Leelavathi
PG & Research Department of Computer Science
National College (Affiliated to Bharathidasan University)
Tiruchirappalli, India
leelavathi4794@gmail.com

Abstract*---Sentiment analytics is the process of applying natural language processing and methods for text-based information to define and extract subjective knowledge of the text. Natural language processing and text classifications can deal with limited corpus data and more attention has been gained by semantic texts and word embedding methods. Deep learning is a powerful method that learns different layers of representations or qualities of information and produces state-of-the-art prediction results. In different applications of sentiment analytics, deep learning methods are used at the sentence, document, and aspect levels. This review paper is based on the main difficulties in the sentiment assessment stage that significantly affect sentiment score, pooling, and polarity detection. The most popular deep learning methods are a Convolution Neural Network and Recurrent Neural Network. Finally, a comparative study is made with a vast literature survey using deep learning models.

Keywords*----Sentiment Analytics (SA), Natural Language Processing (NLP), Deep Learning (DL), Recurrent Neural Network (RNN), Convolution Neural Network (CNN).

I. INTRODUCTION

Data is a prevalent source of social media communication. Data in the form of sentences and sentiments, individuals share their views [1]. Web 2.0 has contributed to the spread of blogs, forums, and social networks online, allowing people to discuss any topic and express their ideas. A social network for instance peeps or Facebook has become a common way to learn about user views and has a broad range of applications [2]. The way to recognize the individual polarity of the text is sentiment. It decides that positive, negative, or neutral is the given document. Sentiment analytics is the process of using techniques for natural language processing to extract individual knowledge from the text [3]. Sentiment analytics can be classified at various levels. Sentiments are classified into various forms, namely feature level, sentence level, and document level [4]. A sense dependent upon the position polarity for each element is defined by the identification of the character level. On the other hand, based on their intensity of meaning about certain topics, categorization at the level of the sentence in each word. It is mutual opposition of this text created in the class aside document level. The entire file is labeled as positive or negative and neutral [5]. In order to acquire the natural language processing (NLP) concerned with the processing and interpretation of human languages in computational terms. Natural Language Processing using linguistic techniques is sentence segmentation, word tokenization, removal of stop words, part-of-speech tagging stemming. The current present-day sentiment analytics has become more mainstream as a meaningful step forward in deep learning in the field of common language preparation [6]. The key regions of fundamental issues are language demonstrating, the measurement associations between words that naturally occur morphological handling, the segmentation of meaningful words, and recognize the real piece of word stream, grammar preparing, or parsing that create sentence structures as potential catalysts of semantic process. The fields of application include topics such as extracting useful knowledge, translating text within and between languages, comprehending written works, and answering questions automatically by referencing answers and classifying documents [7]. For sentiment analytics of texts are important, and Bag of Words (BoW), Word Embedding is a widely-used model. In this sequel, artificial intelligence is introduced to design a model that could perform. Sentiment analytics using automatic feature extraction. The feature extraction

can make systemized with deep learning techniques. [8]. It is the subset of machine learning. This system learns many layers of data interpretations or features and delivers a predictive output that is state-of-the-art. The neural network model of sentiment examination is arranged into three classes, in particular CNN, RNN, and hybrid models. Also the achievement of deep learning in numerous application fields and has also been used in sentiment analytics [9]. This review paper focuses on sentiment analytics that combines natural language processing and deep learning methods. The sentiment rank is based on the entities, pooling, sentiment polarity, and themes categories within a sentence or phrase. This research uses deep learning models such as recurrent neural networks and convolution neural networks to help solve the issue of sentiment analytics [1]. This paper is organized as follows section 2 briefly introduces the related work section 3 comparative study section 4 is extended with results and the discussion ends with a conclusion.

II. Literature Survey

Kleenankandy, J. et al. [10] showed an improved LSTM architecture was implemented, called this relationship, which can show the connection between a sequence of two data sources utilizing a control input. These models are called Typed Dependency Tree that uses both forms of the term dependency parse and type of encoding the meaning of phrase into a dense vector. The DT-LSTM model was produced Precision of 88.4%, Recall of 84.8%, F1–score of 86.6%, and accuracy of 86.6%. Basiri, M.E. et al. [11] Attention-focused CNN-RNN Deep bidirectional model for temporal data flow in both information ABCDM can derive both past and future context with the use of two different models LSTM and GRU layers. The consideration meaning is often applied to outputs of functioning in two directions have more or less effect on individual terms. It incorporates complexity and pool mechanisms to minimize the dimensionality of characteristics and extract stance essential features. The efficacy is measured in determining the polarity of sentiment, which is the most common and important role of sentiment analytics. The proposed ABCDM model was performed Recall of 99.08%, Precision of 99.04%, F1-score of 96.94% and, the accuracy of 96.87%. Huang, M. et al. [12] showed that a neural network of context-dependent, lexicon-based convolution is used to successfully separate the attributes and intensity of focus from words and text. To create this convolution neural network, the model is prepared to utilize the polarization for each word with a co-event example of words and marks. The algorithm has produced sentiment strength of document for six different datasets accuracy of 83%, 77%, 65%,74%, 70%, 98%. Shuang, K. et al. [13] explored a function distillation network for noise reduction and an aspect-relevant view of the sentiment highlights for filtering. The aim of the Novel double gate mechanism is to designed includes the points of view and their respective contexts in fine granularity. In front of the double-gate mechanism add a relevant nonlinear prediction layer to deliver aspect-specific word representation, and helps the twofold entryway component to accurately separate between feeling qualities of a similar context word corresponding to the numerous viewpoints. The FDN model has produced three different gating mechanisms on ATSA accuracy of 76.8%, 82.3%, 73.7%, and macro F1 score of 72.5%, 75%, 72.2%. Senthil Kumar, N. K. et al. [14] proposed an enhanced richer presentation, not only binary and ternary classification it goes deeper in classifying the collection of text from the social network. To verify the multiple sentiment prediction classes checked for the short text in sentiment data based on polarity. The algorithm has produced an accuracy of 98.6%. Sharma, A.K et al. [15] the proposed model has cleaned the information and creates significance from the Word2Vec model used for the convolution layer to extract highlights for short sentences. The proposed model was built on training accuracy of 99.07% and testing accuracy of 82.19 %. Abid, F. et al. [16] projected a Convolutional Neural Network combined with a recurrent neural network based on weighted attentive pooling. The regular pooling activities contain a few layers that are just fit for capturing proper highlights. To enable sentiment analytics such as Word2vec, Fast Text, and Glove to produce a dense powerful linked representations following the long-term dependencies on a single RNN layer obtained explicitly by part of speech tagging with verbs, adverbs, and noun. The model has produced an accuracy of 89.67%. Zhou, M. et al. [17] proposed a text classification model based on the double word embedding techniques are Glove and Word2vec. Models combined with the content form a combinatory information double convolution neural

network channel. The initial word vector is consistently trained and modified the classification model with a single input vector representation, based on the word vector fine-tuning technique. The glove and word2vec model has classified the accuracy of 94.8%. Samat, N.A. et al. [18] extract features from sentiment analytics used for the CNN algorithm. This algorithm used for three different pooling functions reduced the noisy data during the training process. Finally to capturing the semantic features. The three different pooling functions are produced the better accuracy of 97.73%, 93.05%, and 95.58%. Naderalvojoud, B. et al. [19] proposed two approaches for opinion to learn embedding techniques. The first approach encodes the word sentiment knowledge set up pre-trained importance of words, and the second approach semantic context creates synthetic sentiment context for inserting models. On the various sentiment classification tasks used for Skip-gram and Glove models. The proposed model based on two approaches has produced an accuracy of (87%,47.4%) and Macro F1 score of (91.3%, 69.4%). Jang, B. et al. [20] noted that Bi-LSTM+CNN hybrid models supporting an attention function to additional enhance accuracy and decrease the number of learnable parameters. It is used to extract specific characteristics in a sentence from various locations with reducing the number of input features. To remove the context-oriented information from the highlights acquired from the layer of convoluted. The length of the attention mechanism is improved the distribution process. The proposed hybrid model was built maximum accuracy of 91.41%, average accuracy of 90.26%, F1 score of 90.18%, Recall of 90.57%, and precision of 80.97%. Xu, J. et al. [21] planned that text characterization using CNN based on the neural network called DE-CNN. To coordinate with context-relevant ideas into a convolution neural network. First and foremost used for two layers are extracting their concepts and meaning individually. That point of short text classification is implemented into a text representation. The DE-CNN model was provided three different datasets have classified the accuracy of 94.6%, 84.6%, 88.9%. Hassan, A. et al. [25] Deep learning network pre-trained parameters were trained in word embedding then to activating the model. The new framework mixes accessible information with different feature maps are learned by a convolution layer, and the long-term dependencies learned by long-flitting memory at the last level. This technique achieves excellent results with minor hyperparameter tuning for static vectors using multiple sentiment analytics benchmarks dataset. The algorithm has performed the three different datasets, and the accuracy of 93.3%, 48.8%, and 89.2%. Liao, W. et al. [26] presented a new model for sequence-to-sequence learning for multi-label classification. The two unmistakable neural network modules are called encoder and decoder individually. Using the convolution neural network, the encoder removes the high-level local sequential semantics to joined with the word vector using the recurrent neural network and the attention mechanism creates the last content representation. Then totally initialized, the decoder used for the association layer randomly connecting the two labels. The algorithm has been produced a hamming loss of 78%, a micro-Recall of 85.1%, and a Micro-F1 score of 87.3%. Ray, P. et al. [27] proposed a novel deep convolutional neural network model is used for labeling every sentence. Deep learning approaches combined with previous rule-based techniques for improving the feature extraction based on sentiment scoring technique. The proposed method was performed with an overall accuracy of 87%. Chen, C. et al. [28] projected a gated sentiment relationship recurrent neural network model to get the sentiment information from the effects of text and their modifier. The inputs are updated multiplicatively by the existing encoded sentiment modifier proposed the sentiment polarity. The two subsets are dividing the sentiment relation model was performed the overall accuracy of 94.6% and 95.2%. Guo, X. et al. [4] designed have been analyzed three levels of granularity: text, sentence, and aspect level. An advanced att-RCNN model that joins the recurrent neural network and convolution neural network. Both models are extracting the features for word-level and sentence-level, improving the significant terms and functionality promoting the performance of the proposed model. The Att-RCNN model was built on the F1 Score of 86.6%. Umer, M. et al. [23] projected the dimensionality reduction techniques to classified the feature vectors. The principal component analysis and chi-square are taken care of to nonlinear characteristics offering more contextual features to detect fake news. The proposed CNN-LSTM with PCA performed an accuracy of 97.8%, precision of 97.4%, Recall of 98.2%, and f-score of 97.8%. Cekik,

R. et al. [24] a novel filter feature selection tool, uses the rough set for a territorial distinction according to the term of a value set to classify reports that have a class. By multiplying a coefficient called documents that may belong to a class are penalized. Also, using a rough set, the impact of sparsity in the term vector space is determined. The current filter attribute selection techniques such as Gini index, data gain, feature selector distinguishing, max-min ratio methods of normalized difference calculation. The proposed model perform was six different datasets are F1 score 80.20%, 72.91%, 80.25%, 80.17%, 79.84%, and 81.84%.

III.COMPARATIVESTUDY

In this section comparative study on deep learning models about datasets, merits, and demerits, to discuss below for the sentiment analytics process.

TABLE I. Comparative Study with Convolutional Neural Network Model and Long Short Term Memory

AUTHOR/ YEAR	MODEL	DATASET	MERIT	DEMERIT
Kleenankandy, J. et al.[10] / (2021)	CNN LSTM	Sick SST	Sentence representation	Sentence Dependency Parse tree
Basiri, M.E., et al.[11]/ (2021)	CNN LSTM	Twitter Dataset	Decrease the dimensionality	Rating prediction
Umer, M. et al. [23] / (2020)	CNN LSTM	FNC	Dimensionality reduction of the feature vector Reduce overfitting	Noise data Inconsistency
Senthil kumar, N.K., et al.[14] / (2020)	CNN LSTM	Sentence Polarity	Part-of-speech (PoS) tagging	Sarcasm class Sentiment classification
Jang, B., et al. [20] / (2020)	CNN LSTM	IMDB	Text classification Sentence classification	LongTerm dependency.
Shuang, K. et al .[22] / (2020)	CNN LSTM	English dataset	The fuses context and task specific information	Polysemous unaware Task –unaware

Fig.1 Overall Accuracy of CNN-LSTM

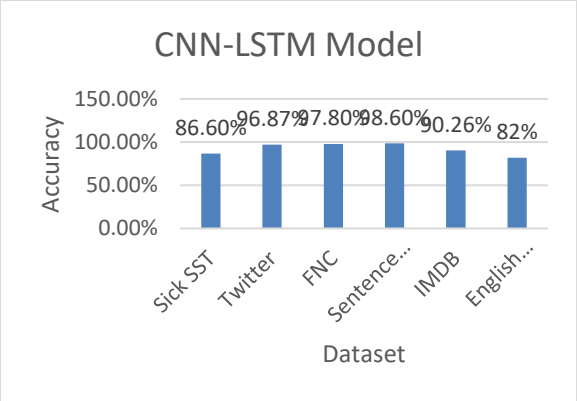


TABLE II. Comparative Study with ConvolutionalNeural Network Model

AUTHOR/ YEAR	MODEL	DATASET	MERIT	DEMERIT
Huang, M. et al .[12] / (2020)	CNN	Senti-Strength	Sentiment Strength	Context-dependent sentiment lexicon
Sharma, A.K., et al .[15] / (2020)	CNN	Sentence Polarity	Word embedding -text classification	Long sentence categorization
Zhou, M. et al. [17] / (2020)	CNN	Hotel Data	Single-channel representation	Non-static Word Vector
Samat, N.A. et al.[18] / (2020)	CNN	IMDB	Pooling Operation	Overfitting on the training set
Ray, P. et al.[27] / (2019))	CNN	SemEval	Sentence Score	Word Embedding

Fig.2. Overall accuracy of CNN

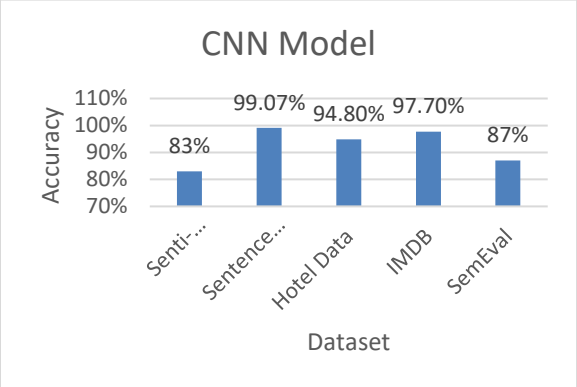
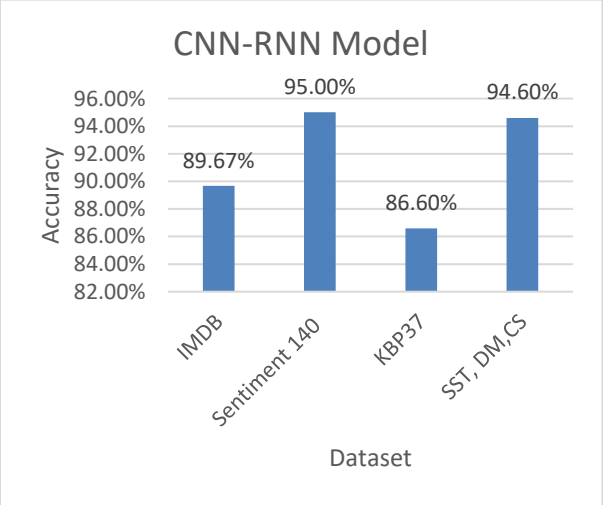


TABLE III. Comparative Study with Convolutional Neural Network and Recurrent Neural Network

AUTHOR/ YEAR	MODEL	DATASET	MERIT	DEMERIT
Abid, F. et al. [16] / (2020)	CNN RNN	IMDB	Word weighted Mechanism	Overfitting
Dang, N.C. et al.[2] / (2020)	CNN RNN	Sentiment 140	CPU runtime. Reduce the computational cost	Automatic Sentiment Analytics Sentiment polarity
Guo, X. et al .[4] / (2019)	CNN RNN	KBP37	Attention Mechanisms	Noise data based on SDP- word
Chen,C. et al. [28] / (2019)	RNN	SST, DM, CS	Sentiment modifier sentiment relations	Sentiment context Content word and Shifting negation

Fig.3 Overall accuracy of CNN-RNN



IV. Result and Discussion

In this survey, it is explored the applications of natural language processing and deep learning techniques. It is inferred that most of the research work is carried out in a feature extraction phase. The enormous literature survey related to large datasets, overfitting, number of classifiers, pooling, and dimensionality reduction were reviewed and compared with some viewpoints, such as datasets, methodology, merits, demerits, and overall accuracy of the existing models. With the help of comparative study, the core research is identified along with the related techniques. The datasets are chosen as unstructured datasets that have an impact on social media sectors. Hence, by selecting the social media dataset the research may move towards the problem in social media related identified the streaming news. Based on the comparative study, a research gap on feature extraction is noted for sentiment analytics.

V. Conclusion

The major revelation of the survey is based on real time problem available in social meadia which has more impacts on sentiment analytics. It is the kind of streamed text research that systematically determines the successful state of subjective knowledge at the sentence level by extracting quantifies on polarity detection. This paper addresses the problem of detecting sentiment polarity, pooling, Sentiment ranking, and sentiment extraction. Deep learning methods have been established to address the major obstacles of sentiment analytics. The merits and demerits are thoroughly identified. The limitations of the research work are aspect and word level. Hence, with the help of a study on sentiment analytics using deep learning, it is motivated to proceed with the research work is noise reduction, overfitting, dimensionality reduction and pooling operation using deep learning models are LSTM, CNN, and RNN for sentiment analytics.

References

[1] Chakraborty, K., Bhattacharyya, S., Senior Member, IEEE, and Bag, R.: A Survey of Sentiment Analytics from Social Media Data. Compute. Social. Syst. 1-15 IEEE Trans (2020)

[2] Dang, N.C., María, N., Moreno-Garcia and Prieta, F.D.: Sentiment analytics Based on Deep Learning: A Comparative Study. *Electronics*. 483. 9, 2-29 MDPI (2020)

[3] Mohey, D.E1, Hussein, D. M.: A survey on sentiment analytics challenges. 30. 4, 330-338 Elsevier (2018).

[4] Guo, X., Zhang, H., Yang, H., Xu, L., and Zhiwen.: A single Attention-based combination of CNN and RNN for Relation

Classification. Proc. Comput. Science. 7.131, 12467-12475 IEEE Access (2019).

[5] Habimana1, O., Li, L., Li, R., Gu X., & Yu, G.: Sentiment analytics using deep learning approaches: an overview," Info. sci. 63, Springer (2020)

[6] Daniel W. O., Julian R. Medina, and Jugal K. Kalita.: A Survey of the Usages of Deep Learning for Natural Language Processing. Neural. Netws. Learning. Syst., IEEE. Trans (2019)

[7] Wafaa S. EI-Kassas, Cherif R. Salama, Ahmed A. Rafea, Hoda K. Mohamed, " Automatic text Summarization: A comprehensive survey," Expert. Syst. Applns. 165, 113679 Elsevier (2021)

[8] Liu, R., Shi, Y., Ji, C., and Jia, M.: A Survey of Sentiment Analytics Based on Transfer Learning. IEEE Access (2019).

[9] Liu, L. Z. W. B.: Deep learning for sentiment analytics: A survey. Springer (2020)

[10] Kleenankandy, J., Nazeer, K.A.: An enhanced Tree-LSTM architecture for sentence semantic modelling using typed dependencies. Info. Pro. Management 57(6), 102362 ACM(2020)

[11] Basiri, M, E., Nemati, S., Adar, M., Cambria, E.: ABCDM: An Attention-based Bidirectional CNN-RNN Deep Model for sentiment analytics. Future Gen. Compute. Syst. 279-294 Elsevier (2021)

[12] Huang, M., Xie, H., Jingrong, Y.R., Fen, Wang, F.L.: Sentiment strength detection with a context-dependent lexicon-based convolution neural network. Info. Science. 520,389-399 Elsevier(2020)

[13] Shuang, K., Yanga, Q., Loo, J., Li, R., Gua, M., "Feature distillation network for aspect-based sentiment analytics," Info. Fusion, vol. 61, pp: 13-23, Elsevier, 2020.

[14] Senthil Kumar, N, K., Malarvizhi, N.: "Bi-directional LSTM-CNN Combined method for sentiment analytics in Part of Speech Tagging (POS)," Int. Journal. Of. Speech. Tech., Springer, 2020.

[15] Sharma, A.K., Chaurasia, S., Srivastava, D. K.: Sentiment Short Sentences Classification by Using CNN Deep Learning Model with Fine Tuned Word2Vec. Proc. Compute. Science. 167, 1139-1147 Elsevier (2020)

[16] Abid, F., Li, C., Alam, M.: Multi-source social media data sentiment analytics using bidirectional recurrent convolution neural networks, Compute. Communes. 157, 102-115 Elsevier (2020)

[17] Zhou, M., Liu, D., Zheng, Y., Zhu, Q., Guo, P.: A text sentiment classification model using double word embedding methods, Multmed. Tools. Appln. Springer (2020)

[18] Samat, N.A., Mohd, N.S., Ali, H.: The Comparison of pooling functions in a conventional neural network for sentiment analytics task. Research Gate (2020).

[19] Naderalvoijoud, B., Sezer, E.A.: Sentiment Aware Word Embedding using Refinement and Senti-Contextualized Learning Approach. Neuro. Comput, 405, 149-160 Elsevier (2020).

[20] Jang, B., Kim, M., Harerimana, G., Sang-ug K., and Kim, J.W.: Bi-LSTM Model to Increase Accuracy in Text Classification: Combining Word2Vec CNN and Attention Mechanism, Appl. Sci, 10. 5841. 1-14 MDPI (2020).

[21] Xu, J., Cai, Y., Wu, X., Lei, X., Huang, Q., Ho-Fung L, Li, Q.: Incorporating Context-Relevant Concepts into Convolution Neural Networks for short Text Classification," Neuro Comput., 386, 42-53 Elsevier (2020)

[22] Shuang, K., Zhang, Z., Loo, J., Sen Su.: Convolution-deconvolution word embedding: An end-to-end multi-prototype fusion embedding method for natural language processing," Info.Fusion. 53. 112-122, Elsevier (2020)

[23] Umer, M., Imtiaz, Z., Ulla, S., Mehmood, A., Choi G.S, and Byung-Won On.: "Fake News Stance Detection Using Deep Learning Architecture (CNN-LSTM). 8, 156695 – 156706 IEEE Access (2020)

[24] Cekik, R., Uysal, A.K.: A novel filter feature selection method using a rough set for short text data," Expert. Syst. Applns. 160, 113691 Elsevier (2020)

[25] Hassan, A., Mahmood, A, A.: Convolutional Recurrent Deep Learning Model for Sentence Classification," MultiDisci. Rapid. View. 6, 13949 - 13957 IEEE Access, (2018)

[26] Liao, W., Wang, Y., Yin, Y., Zhang, X., Pan M.: Improved sequence generation model for multi-label classification via CNN and initialized fully connection. Neuro. Comput. Elsevier (2019)

[27] Ray, P., Chakrabarti, A.: A Mixed approach of Deep Learning method and Rule-Based method to improve Aspect Level Sentiment analytics. Appl. Compute. Info. Elsevier (2019)

[28] Chen, C., Zhuo, R., Ren, J.: Gated recurrent neural network with sentiment relations for sentiment classification. Info. Science. 502, 268-278 Elsevier(2020).

Springer

International Conference on Ubiquitous Computing and Intelligent Information Systems

ICUIS 2021 | 16-17, April 2021

Proceedings

ICUIS

Organised by

Shree Venkateshwara Hi-Tech Engineering College

Erode, India

A Comparative Study on Augmented Analytics using Deep Learning Techniques

*Dr M Anusha [1], P Kiruthika [2]

[1] Assistant Professor, PG & Research Department of Computer Science, National College (Autonomous), Bharathidasan University, Tamilnadu, India

[1*] Email id – anusha260505@gmail.com

[2] Ph.D Research Scholar, PG & Research Department of Computer Science, National College (Autonomous), Bharathidasan University, Tamilnadu, India

[2] Email id – kiruthikashyne@gmail.com

* P. Kiruthika

Abstract. Image augmentation is the most recognized type of data augmentation and intrinsic development for transforming image diversities in the training dataset that belong to a similar class as the novel image. In the field of image augmentation handling a collection of operations, namely shifting, flipping, zooming, cropping, rotation, and transformation in color space. A wide range of applications frequently used the aspects of deep learning are industry, science, and government domain, namely adaptive testing, image classification, computer vision, object detection, and face recognition has achieved substantial development and accomplishment of deep learning. This study concentrates on the most important challenges present in the image estimation level that have a significant effect on dimension reduction, pooling, and edge detection. The deep learning methods involved here, areConvolution Neural Network (CNN), Generative Adversarial Network (GAN), and Deep Convolution Neural Network (DCNN). Finally, a comparative study has performed a massive literature survey on various deep learning models.

Keywords: Image Augmentation (IA), Deep Learning (DL), Convolution Neural Network (CNN), Generative Adversarial Network (GAN), Deep Convolution Neural Network (DCNN).

1 Introduction

Image data are a pictorial representation of data, which includes two categories of data one is balanced data and another one is imbalanced data. The number of positive and negative values will be approximately same in balanced data and the number of positive and negative values will be highly different in imbalanced data. Depending on the size of the data, it is not very important to train the model and derive useful training data features depending on the size and consistency of the data. The model needs a lot of input data to provide a better solution. To overcome this challenge image augmentation technique is used [27]. Image augmentation is considered as one of the types of data augmentation techniques.

The data augmentation technique helps generate a novel data from the remaining data expand artificially, where the data augmentation methods are helping to train and show a strong generalization ability of deep learning models [1]. The basic image augmentation techniques are Geometric transformation (Flipping, Rotation, Translation, Cropping, and Scaling), Color space transformation (Color casting, Varying brightness, and Noise injection), Kernel filters, images mixing, Erase Randomly, Augmentation in Feature space, Adversarial training, and augmentation based on GAN, Transferring an image style, and self-regulated learning. The image augmentation technique discussed in this study is geometric transformation. Geometric transformation provides a better solution for training the data, such as shifting, flipping, zooming, cropping, rotation, and color space in transformations. A major cause for geometric transformation is horizontal flipping, which is far more common than vertical flipping. The color channel space is one of the techniques used in augmentation, which is quite realistic to implement a relatively modest color augmentation, which includes dividing an atomic color channel and contrast between randomly cropping. Further, the image data translation method has been used by cropping to reduce the dimensions of the input data, then translations maintain spatial dimensions of the image. The rotation augmentation is accomplished by rotating the input [2]. The rapid development of deep learning covers machine learning, which is created on neural networks with the knowledge representation. Deep Learning helps to solve complex issues for machine learning. The neural network comprises several layers used to describe abstraction data to construct a computational model for deep learning [3]. The knowledge representation is supervised, semi-supervised and unsupervised. Supervised learning techniques used for labeled input data to predict the desired output data. Semi-supervised learning is another learning technique used for labeled data is small amount and the unlabeled data is large amount during the training process. Finally, Unsupervised technique is used for unlabeled data to extract the generative features [28]. Deep learning architectures are Deep Neural Network, Convolutional Neural Network, Recurrent Neural Network, Long Short-Term memory, Gated Recurrent Units, Deep Belief Network, Generative Adversarial Network, and Auto-Encoder is included. This survey concentrates on the generative adversarial network, convolutional neural network, and the deep convolutional neural network have been applied in the domain of picture classification, computer visualization, object detection, and face recognition [4]. A generative adversarial network is an influential tool for performing unsupervised learning representation to generate new image data from the existing data to fool the discrimination. GAN to overcome these problems are picture to picture translation, image to text synthesis, and picture resolution. The convolutional neural network is most commonly used for picture wise classification, and CNN concentrates on many parameters that reach the enlarged size of networks as well as lacking training data with generalization abilities [5]. A deep convolutional neural network is an effective approach for computer visualization, pedestrian detection, and image segmentation. DCNN retrieve the knowledgeable feature extraction of the training model [6]. This study concentrates on the image level estimation that has a significant effect on dimension reduction, pooling, and edge

detection. The rest of the paper is organized as follows section 2 briefly introduces the related work, section 3 comparative study, to end section 4 is extended with the result and the discussion ends with a conclusion.

2 Literature Survey

Feng X. et al. [7] suggested a method for data augmentation by using decoding weights. Due to the collection of training data, the autoencoder is trained and the decoding weights are enabled, and the weights are compared with the samples obtained to produce the augmented samples. The different networks used for this proposed method and their accuracy of VGG-16 50.9%, ResNet-50 52.1%, DenseNet-121 used for two datasets and their outcome is 50.2% and 75.8% of image weight. Chen F. et al. [8] A novel algorithm for individual re-identification based on self-monitored data increase. Our approach is informed by the recently enacted part-based algorithms, which have accomplished impressive outcomes on the re-identification of the individual. It is important to realize that the intention is not to use split in terms of learning more biased functionality. The algorithm was produced an identification rate and average precision of 93.88% and 84.45%, 87.52% and 75.68%, 71.27%, and 65.91% in three different types. Liu S. et al. [9] designed a lightweight transfer learning approach based on ResNet architecture, and it is used for a number of layers that are fused their feature and then take a benefit of the confined layer in various attributes fused them for SoftMax regression detection. The FTOTLM method was produced with augmentation techniques for six different datasets accuracy of 99.87%, 99.45%, 97.45%, 97.38%, 94.05%, and 85.21%. Umer S. et al. [10] To fix this issue, an innovative approach of data increase technique for extracting features and tasks performed in recognition owed to retina and region around the retina is merged, then to the enhanced performance of the indicator depending on various conditions, that picture comes with data redundancy to generate the data irregularly or the recognition process is difficult. The proposed system was performed in the iris accuracy of (CASIA-dist-99.64%, UBIRIS.v2-98.76%) and particular accuracy of (CASIA-dist-99.64%, UBIRIS.v2-98.76%) for image recognition. Fu Y. et al. [11] Built a novel adversarial generative network is called Fine-grained Conditional GAN, to solve the issue of fine-grained depending upon classes in image generation. Fine-grained Conditional GAN produces first class-dependent low-resolution images. The consequence is that high-resolution class-dependent images are developed by the generator. Every generator is accompanied by a discriminator. To gain valuable fine-grained data, we create two finer resolution in the Fine-grained Conditional GAN. The proposed work used one or more augmentation techniques with a classification accuracy of dataset1 is 65.95% and 71.15%, dataset2 is 75.69% and 79.65%, dataset3 is 73.39% and 76.16% for high-resolution images. Kaur S. et al. [12] handled two approaches for these problems, one of the approaches is suggested a paradigm focused on the transition of learning based on the pre-trained AlexNet architecture. Another viewpoint that reflects on the new data augmentation technique is based on the Generative Adversarial Network. Even after the two approaches performed the Parkinson

classification method has increased accuracy. The algorithm was classified with an average accuracy of 89.23% for disease classification. Saini M. et al. [13] The class imbalanced distribution results in a deterioration in the efficiency of the classification models owing to a bias in the category toward this dominant class to tackle this issue. The author has suggested a different learning technique includes a deep transfer network in conjunction with the Deep Convolution Generative Adversarial Network. The algorithm was performed in four different magnification factors, accuracy of 96.5%, 94%, 95.5%, and 93%. Moon W.K. et al. [14] Note that an accurate and quick computer-aided detection (CADe) method located on a three-dimensional convolutional neural network (CNN) introduced as another reader for physicians to minimize the duration of the examination and frequency of misdetection. The proposed algorithm has produced a sensitivity of 95.3% for misdetection. Karakanis S. et al. [15] Proposed an innovative method for coronavirus detection in a chest x-ray image dataset used for conditional generative adversarial networks. The author creates a large amount of data using augmentation techniques to overcome the limited dataset. It suggested two models in deep learning by using the availability of the dataset. The proposed binary model has achieved an accuracy of 98.7%, sensitivity of 100%, and specificity of 98.3%, and the three-class model achieved an accuracy of 98.3%, a sensitivity of 99.3%, and specificity of 98.1% for detecting covid-19. Rai H.M. et al. [16] Presented the novel Deep Neural Network model with minimum layers and fewer complex to build on U-Net for detecting tumors. This work includes categorizing the brain MR images in regular or irregular classes of 253 high pixel images. The proposed LU-net model was performed recall 1.00%, precision 97%, f-score 98%, specificity 95%, and the accuracy of 98% for detecting tumors. Ibrahim Saad Aly Abdelhalim I.S.A. et al. [17] proposed the self-attention mechanism to detect skin melanoma images used for convolutional neural networks based on dimensionality reduction and to achieve better results. The proposed self-attention model was performed macro recall 64.7%, AUC 79.3%, macro precision 50.1%, macro f-score 53.4%, average training time in minutes is 16.3 and the accuracy of this model is 66.1% for dimensionality reduction. Hosny K.M. et al. [18] a novel deep convolutional neural network approach is exhausting the skin melanoma images to be classified. To overcome the insufficient dataset using augmented techniques developed a large dataset. The proposed method was improved the classification accuracy MED-NODE, DermIS & DermQuest, and ISIC 2017 for 99.29%, 99.15%, and 98.14%. Mzoughi H. et al. [19] A new classification approach was used for brain tumor MRI images established on a deep convolutional neural network model aimed at low-grade glioma and high-grade glioma. To combine both gliomas information then to reduce weight-based on three-dimensional convolutional neural network. The classification approach has produced an accuracy of 96.49% of this dataset. Pasyar P. et al. [20] The author aims to develop a new hybrid classifier, then verify the liver level based on the liver image dataset handling of a deep convolutional neural network. Clarify the weighted possibility of every class, including its majority voting process. The proposed ResNet50 model has produced an accuracy of 86.4% the sensitivity and specificity of the first group classified 90.9% and 86.4% and the last group classified the

sensitivity of 90.9% and specificity of 81.8%. Loey M. et al. [21] proposed different deep convolutional neural network models to be identified the infected coronavirus patients using chest CT scan images. The author collects possible CT scan images used for traditional augmentation techniques to create a large image dataset. The outcome is classified as covid or non-covid. The ResNet50 model was performed in traditional augmentation techniques and their accuracy of testing is 82.91%, sensitivity of 77.66%, and specificity of 87.62% found covid-19. Alzubaidi L. et al. [22] proposed a new deep convolutional neural network model built on a new 754-ft image dataset to detect automatically whether it is healthy or not. The proposed model was performed to an F1-score of 94.5%. Jain G. et al. [23] Another problem-solving tool for detecting covid-19 in chest X-ray images used for deep learning techniques. The deep learning techniques enlarged the clarity of image detection. The proposed method was produced an average accuracy of 98.93%, a sensitivity of 98.93%, a specificity of 98.66%, a precision of 96.39%, and an F1-score of 98.15% for covid-19 detection. Deepak Nayak R. et al. [24] diagnosing the brain irregularities in brain MRI datasets utilizing a deep convolutional neural network focused on the automatic approach reduced the dimension reduction and achieved better classification. The proposed approach was performed with a classification accuracy of 100.00% and 97.50% for dimensionality reduction. Abrishami M.S. et al. [25] improved the generalization ability is based on the augmentation techniques used for deep convolutional neural networks to reduce the computation process. The pre-trained network model for base and target network based on dataset CIFAR-100 and CIFAR-10 (71.47%, 74.48% 78.20%) and (64.44% 78.87% 83.98%) for computation process. Wang J. et al. [26] improved the human identification process by consuming a deep convolutional neural network constructed on augmented CPAF images. The proposed CPAFNet model was producing an accuracy of 92.63% of the image identification process.

3 Comparative Study

In this section comparative study of deep learning models about datasets, merits, and demerits, to discuss below for the image augmentation process.

Table 1. Comparative Study with Convolutional Neural Network Model

AUTHOR/ YEAR	MODEL	DATASET	MERIT	DEMERIT
Mzoughi .H et al. [19] / (2020)	CNN	Clinical dataset	Kernel size reducing the image weight	Capsule networks for image enhancement
Moon W.K et al. [14] / (2020)	CNN	3-D ABUS	Decrease training time and fault rate	Insufficient 3D images for accurate detection
Rai H.M et al. [16] /	CNN	MRI	Minimum layer and Less complexity	Minimum input Layer for

(2020)				classification
Alzubaidi L et al. [22] / (2019)	CNN	754-ft images	Minimum computational cost	Algorithm failed for detection
Jain G et al. [23] / (2020)	CNN	COVID19 X-ray images	Less requirements for computational process	Sensitivity for detection

Table 2. Comparative Study with Deep Convolutional Neural Network Model

AUTHOR/ YEAR	MODEL	DATASET	MERIT	DEMERIT
Hosny K.M et al. [18] / (2020)	DeepCNN	BraTS-2018	Inception Module Loss 3-classifier	Ensemble Model for image identification
Pasyar P et al. [20] / (2020)	Deep CNN	ILSVRC	Weighted probability	Algorithm failed to work in Three-class classifier
Abrishami M.S et al. [25] / (2020)	DeepCNN	CIFAR100 CIFAR10	Cost reduction while transfer learning	Embedding space for more complexity
Nayak D.R et al. [24] / (2020)	DeepCNN	MD-1,MD-2	Promote automatic feature learning-Sequence of hidden layers	Inadequate 3D images for detection
Wang J et al. [26] / (2020)	DeepCNN	CPAF	Training time	Kernel Size for classification

Table 3. Comparative Study with Generative Adversarial Network Model

AUTHOR/ YEAR	MODEL	DATASET	MERIT	DEMERIT
Abdelhalim I.S.A et al. [17] / (2020)	GAN	HAM10000	Attention mechanism for image classification	Convincing on 600*450 resolution of the image
Loey M et al. [21] / (2020)	CGAN	COVID-19 CT scan	Two class classification	Neutrosophic approach
Karakanis S et al. [15] / (2020)	CGAN	COVID-19 CXR	Biased model	Pre-trained weights
Kaur S et al., [12] / (2020)	GAN	PPMI dataset	Bilateral filter for noise reduction	Overfitting
Saini M et al., [13] /	DCGAN	BreakHis dataset	Global Average Pooling	Failed in sub-optimal

4 Result and Discussion

Image augmentation is the kind of image handling research that systematically determines the successful states of subjective knowledge at transformations by extracting quantities on image detection. In this comparative survey, it is explored about image augmentation using deep learning techniques. A vast literature survey related to a large dataset, overfitting, number of classifiers, edge detection, pooling, and dimensionality reduction were reviewed and compared with some viewpoints, such as datasets, methodology, merits, and demerits of the existing models. This paper identifies the core deep learning models and related techniques that have been applied to the image data that is unstructured. Based on the comparative study, a research gap on feature extraction is noted for the image augmentation techniques.

Conclusion

At present, extracting knowledge from complex medical image data is more crucial. It is important to use sophisticated analytics techniques in this data-rich age to generate useful knowledge and information about massive, complex data sets. In this survey, a massive image augmented analytics using deep learning techniques, research papers are reviewed for identifying the research gap. The research merits and demerits are thoroughly identified. This survey does not concentrate on image segmentation and image processing. Hence, with the help of the study, it is motivated to proceed with the research work is dimensionality reduction, pooling, noise reduction, large dataset, object detection and overfitting using deep learning models are GAN, CNN, and DCNN for image augmentation.

References

1. Zadeh, H.E., Koutini, K., Primus P., Haunschmid V., Lewandowski M., Zellinger W., Moser B.A., Widmer G.: On Data Augmentation and Adversarial Risk: An Empirical Analysis. arXiv:2007.02650v1 [cs. LG], (2020)
2. Shorten, C., Khoshgoftaar, T.M.: A survey on Image Data Augmentation for Deep Learning. J. of Big. 6:60, Springer (2019)
3. Pathak, A.R., Pandey, M., Rautaray S.: Application of Deep Learning for Object Detection. Pro. Com. Sci. 132, 1706–1717 Elsevier (2018)
4. Pouyanfar, S., Sadiq, S., Yan, Y., Tian, H., Tao, Y., Reyas, M.P., Shyu, M., Chen, S.C., Iyengar, S.S.: A Survey on Deep Learning: Algorithms, Techniques, and Applications. ACM. Com. Surv. 51, 1–36 (2018)
5. Mikolajczyk, A., Grochowski, M.: Data augmentation for improving deep learning in image classification problem. IIPhDW. 1–6 (2018)

6. Qayyuma, A., Anwar, S.M., Awais, M., Majida, M.: Medical image retrieval using deep convolutional neural network. Neu. Comp. 266, 8–20 Elsevier (2017)
7. Fenga, X., Wu, Q.M.J., Yang, Y., Cao, L.: An Auto encoder-based Data Augmentation Strategy for Generalization Improvement of DCNNs. Neu. Comp. 402, 283–297 Elsevier (2020)
8. Chen, F., Wang, N., Tang, J., Liang, D., Feng, H.: Self-supervised data augmentation for person re-identification. Neu. Comp. 415, 48–59 Elsevier (2020)
9. Liu, S., Tian, G., Xu, Y.: A novel scene classification model combining ResNet based transfer learning and data augmentation with a filter. Neu. Comp. 338, 191–206 Elsevier (2019)
10. Umer, S., Sardar, A., Dhara, B.C., Raout, R.K., Pandey, H.M.: Person identification using fusion of iris and periocular deep features. Neu. Net. 122, 407–419 Elsevier (2020)
11. Fua, Y., Li, X., Yea, Y.: A multi-task learning model with adversarial data augmentation for classification of fine-grained images. Neu. Comp. 337, 122–129 Elsevier (2020)
12. Kaur, S., Aggarwal, H., Rani, R.: Diagnosis of Parkinson's disease using deep CNN with transfer learning and data augmentation. MulMed. Too. Appl. 1–27 Springer (2020)
13. Saini, M., Susan, S.: Deep transfer with minority data augmentation for imbalanced breast cancer dataset. App. Sof. Comp. 97, 1–44 Elsevier (2020)
14. Moon, W.K., Huang, Y.S., Hsu, C. H., Chein, T.Y.C., Chang, J.M., Lee, S.H., Huang, C.S., Chang, R.F: Computer-aided tumor detection in automated breast ultrasound using a 3-D convolutional neural network. Comp. Met. an. Pro. İn Biom. 190, 1–9 Elsevier (2020)
15. Karakanis, S., Leontidis, G.: Lightweight deep learning models for detecting COVID-19 from chest X-ray images. Comp. in Bio. an. Medi. 130, 1–9 Elsevier (2021)
16. Rai, H.M., Chatterjee, K.: Detection of brain abnormality by a novel Lu-Net deep neural CNN model from MR images. Mac. Lear. wit. Appl. 2, 1–10 Elsevier (2020)
17. Abdelhalim, I.S.A., Mohamed, M.F., Mahdy, Y.B.: Data augmentation for skin lesion using self-attention based progressive generative adversarial network. Exp. Sys. wit. Appl. 165, 1–13 Elsevier (2021)
18. Hosny, K.M., Kassem, M.A., Foaud, M.M.: Skin melanoma classification using ROI and data augmentation with deep convolutional neural networks. Mulmed. Tol. Appl. 24029–24055 Springer (2020)
19. Mzoughi, H., Njeh, I., Wali, A., Slima, M.B., Hamida, A.B., Mhiri, C., Mahfoudhe, K.B.: Deep Multi-Scale 3D Convolutional Neural Network (CNN) for MRI Gliomas Brain Tumor Classification. J. of Dig. Imag. 903–915 Springer (2020)
20. Pasyar, P., Mahmoudi, T., Kouzehkanan, S.Z.M., Ahmadian, A., Arabalibeik, H., Soltanian, N., Radmard, A.R.: Hybrid Classification of Diffuse Liver Diseases in Ultrasound Images Using Deep Convolutional Neural Networks. Info. İn Med. Unlo. 22, 1–27 Elsevier (2020)
21. Loey, M., Manogaran, G., Khalifa, N.E.M.: A deep transfer learning model with classical data augmentation and CGAN to detect COVID-19 from chest CT radiography digital images. Neu. Comp. and Appl. 1–13 Springer (2020)
22. Alzubaidi, L., Fadhel, M.A., Oleiwi, S.R., Shamma, O.A., Zhang, J.: DFU_QUTNet: diabetic foot ulcer classification using novel deep convolutional neural network. Mulmed. Tol. Appl. 15655–215677 Springer (2019)
23. Gifani, P., Shalbaf, A., Vafaeezadeh, M.: Automated detection of COVID-19 using ensemble of transfer learning with deep convolutional neural network based on CT scans. J. Comp. Ass. Radi. Surg. 1–9 Springer (2020)

24. Nayak, D.R., Dashb, R., Majhi, B.: Automated diagnosis of multi-class brain abnormalities using MRI images: A deep convolutional neural network based method. Pat. Reco. Let. 138, 385–391 Elsevier (2020)
25. Abrishami, M.S., Eshratifar, A.E., Eigen, D., Wang, Y., Nazarian, S.: Efficient Training of Deep Convolutional Neural Networks by Augmentation in Embedding Space. arXiv:2002.04776v1 [cs.CV] (2020)
26. Wang, J., Li, Y., Feng, H., Ren, L., Du, X., Wu, J.: Common pests image recognition based on deep convolutional neural network. Comp. and Elec. in Agri. 179, 1–9 Elsevier (2020)
27. Ding, J., Li, X., Kang, X., Gudivada V.N.: A Case Study of the Augmentation and Evaluation of Training Data for Deep Learning. J. Dat. and Info. qual. 11, 1–22 ACM (2019)
28. Alom, M.Z., Taha, T.M., Yakopcic, C., Westberg, S., Sidike, P., Nasrin, M.S., Hasan, M., Essen, B.C.V., Awwal, A.A.S., Asari, V.K.: A State-of-the-Art Survey on Deep Learning Theory and Architectures. Elect. 1–66 MDPI (2019)

Investigation on Malware Detection Using Deep Learning Methods for Sustainable development

1st M.Anusha
PG & Research Department of Computer Science
National College (Affiliated to Bharathidasan Univsity)
Tiruchirappalli, India
anusha260505@gmail.com

2nd M.Karthika
PG & Research Department of Computer Science
National College (Affiliated to Bharathidasan University)
Tiruchirappalli, India
karthikaparthi2009@gmail.com

Abstract - The world of today is interconnected with computer systems, is more liable to cyber-attacks. Cyber security was basically designed to help developers in understanding the modern information, system protection technology and methods using machine learning techniques is not a novelty to solve computer security challenges, and the progressive technology of Deep Learning has a significant thirst for knowledge in the community of cyber security for green environment. Deep Learning was basically designed to detect and deal with cybersecurity issues like intrusion detection & prevention system, dealing with malware, spam and social implementation detection, network traffic and user behavior analytics. This survey is a complete audit of different Deep Learning techniques for detecting malware. The aftereffect of this paper is an answer to an insufficient and complete beginning to malware recognition by utilizing deep learning by investigating most recent exploration papers in the field of deep learning to malware detection, the impact of datasets, algorithms, merit and demerits.

Keywords - Cyber Security, Cyber-attack, Deep Learning (DL), Malware detection

I. INTRODUCTION

Cyber security is a prime issue in computer and network security [1]. It incorporates strategy, method, innovation, and interaction that are consolidated to save the confidentiality, integrity, and availability of registering system resources, organizations, programming projects, and the information from attacks in Fig. 1. People and associations are exposed to cyber threats on instant access to the global network [2]. Digital safeguard systems exist at the application, organization, host, and information level by methods for different techniques as firewalls and antivirus programming have been being utilized to secure both client's protection and delicate information.

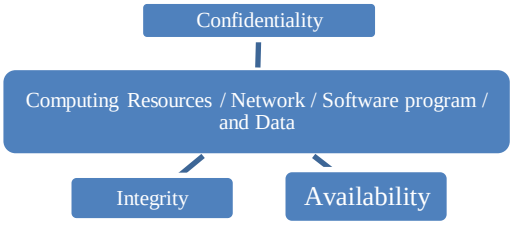


Fig. 1. Resources of Cyber Security

Cyber security influences to incur more cost to ensure security all over the world. Even the US Government estimates a huge amount in malicious cyber activities protection. Malware is a great challenge to cyber security. This urges to detect Malware by advance techniques and great concern in computer security [3].A huge numbers of researchers have analyzed different techniques for detecting and classifying malware. A traditional predictive tool depends on signature-based method [4]. Due to its limitation many malware can easily run away from signature-based detection. Hence, Machine learning for malware detection has been used as a tool to displace the signature-based anti-malware systems [5]. Malware detection approaches are classified as static analysis, dynamic analysis, and hybrid analysis approach. A static approach takes up Source code and verifies the code without implementation to detect malware. Dynamic type monitors the interactions of

the executed code to address the malware, whereas hybrid type takes the advantages of both static and dynamic. Traditional Machine Learning (ML) algorithms like SVM, Bayesian Networks, Logistic Regression, and MLP have been used for malware detection and categorization [6-9]. Machine learning algorithms need lot of domain expertise, human intervention, and its shallow architectures make them not to scale well with large datasets. The fileless malware is harmful to any network due to its persistence and power to get away from any filtering solutions [15]. Hence, Deep Learning (DL) models are a fortunate way to overcome these limitations to malware detection and analysis mainly based on PC and Android for both file and file less malware [10-13]. This survey aims to provide a significant contribution based on cyber-attack and detection of malware using deep learning and delighted to precede this research work. This survey is followed as Section II provides a brief history of different cyber security malware attacks and the various deep learning methods for cyber security is focused. Next Section III and IV is the literature review on malware detection and an investigation study encountered with the platform used, datasets, and learning algorithms for cyber security using Deep Learning. Finally, provided with an analysis and discussion and a conclusion based on deep learning.

II. CYBER SECURITY MALWARE ATTACKS AND DEEP LEARNING APPROCHES

Malicious software (Malware), hackers injects the malicious code to infect and obtain the access permission to the computer without the consent of users by means of file or fileless software is termed as malware attack in cyber security. The malware are of different types like spyware, ransomware, viruses, adware, worms, Trojan Horses, etc. There are many reasons for the hackers to set going with injecting malware on any target system. The attackers explore the vulnerability of the already installed software or OS on the machine as a factor to inject the malicious code. Malware attacks are of different kinds, namely Trojan horse, Virus, Adware, Bot, Ransomware, Rootkit, and Spyware. Adware is an attacking software, used for advertising takes the loop through pop ups on websites and ads. In Botnets attack, hackers assume responsibility for numerous frameworks and convey pernicious exercises like malicious activity. Targeted computers, used for malicious activities like distributed denial of service attacks, identifying theft, phishing, spoofing, and spamming [28]. Ransomware is malicious software for criminal money making by infecting the targeted computer and displays messages demanding a payment to continue work, unlock the encrypted file, and improve the performance via the links through a mail, popup message or website. A rootkit is a malignant programming, permits an unapproved admittance to the PC and to its confined zones in the product. Spyware is a malicious program expected to get to douse the figuring device by taking the web usage data and fragile information or damage your PC, as often as possible without your understanding. A Trojan horse or Trojan is a kind of malware goes about as a genuine programming and gain secondary passage admittance to your framework to empower digital lawbreaker's exercises. A virus is replicating https://en.wikipedia.org/wiki/Computer_programsoftware to infect the target machine by injecting its code in another software coding. Deep Learning allows machines to solve cyber security problems. Since, it's a part of machine learning and artificial intelligence. CNN (Convolutional Neural Network), RNN (Recurrent Neural Network), LSTM (Long Short Term Memory Network), DBN (Deep Belief Network), AE (Auto Encoder) is some popular kinds of Deep Learning models which are utilized in cyber security for identifying malware [16-17]. CNN is a deep neural network, with different layers and are chiefly utilized for image recognition and object detection. CNN can process and extract features from data with the elements of multiple layers [2] like convolutional, Rectified Linear (ReLU), Polling, and Fully connected layers. RNN is a feed-forward neural network, which has a significant obstacle gradient exploding or vanishing for training the data [1] in Fig. 2. RNN has been extended with numerous memory units as variants, including LSTM and Gated Recurrent Unit to overcome exploding gradients and vanishing [8] in Fig. 3. LSTM is a type of RNN that can learn and memorize long-term dependencies to resolve the vanishing gradient problem [5] in Fig. 4. The Boltzmann machine is a probabilistic and unsupervised neural network, consists of binary values as hidden layer to decide about the activation

but brings about passive learning. RBM is a stochastic network model to solve the complexity issue of Boltzmann machine by using two visible and hidden units. A deep belief network (DBN) is made out of stacked RBMs, which are prepared in an insatiable layered style to communicate with one another [8]. DBN overcomes the problem of not optimal solutions for dimensionality reduction [1] in Fig. 5. An Auto Encoder can be designed for dimensionality reduction and to have a better data representation including layers and feature vectors by consolidating an encoder and decoder to train them together with back propagation to extract and learn the raw features and reconstruct the original features [8] in Fig. 6.

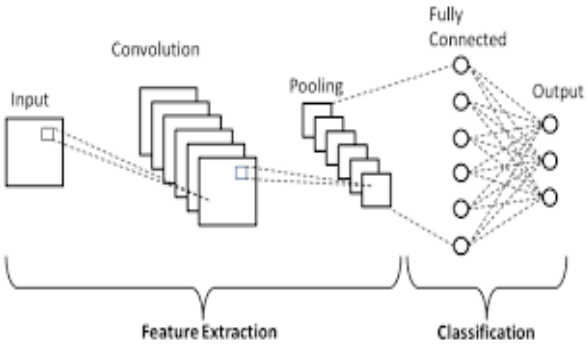


Fig. 2. Convolutional Neural Network

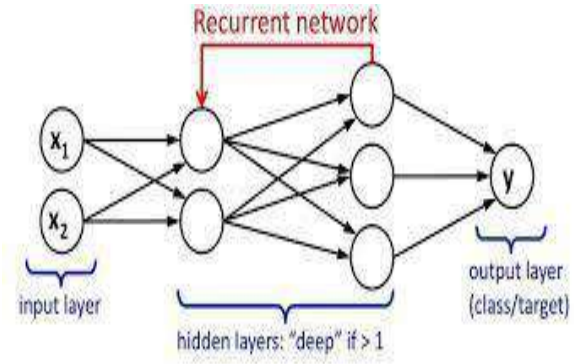


Fig. 3. Recurrent Neural Network

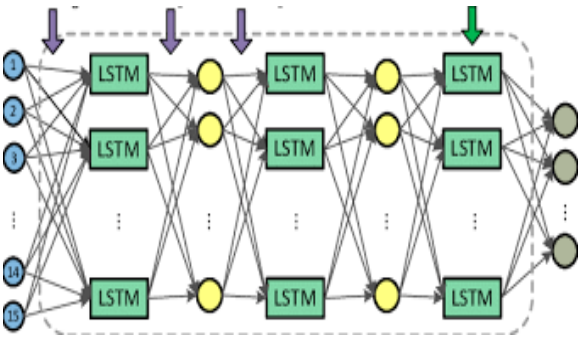


Fig. 4 . Long Term Short Memory

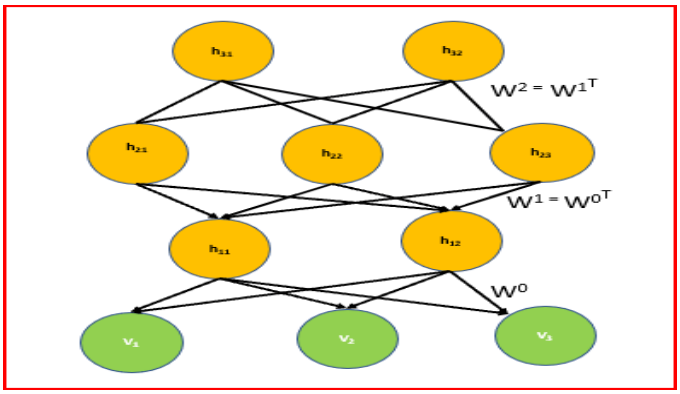


Fig. 5. Deep Belief Network

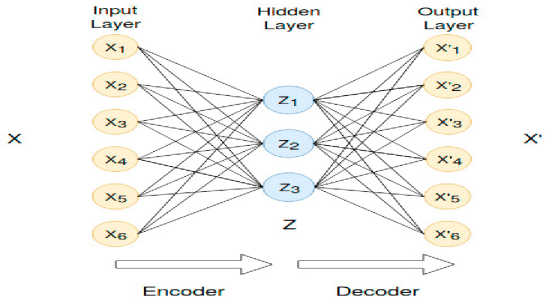


Figure 6: AutoEncoder

III. LITERATURE REVIEW ON DEEP LEARNING BASED MALWARE DETECTION

Zhiqiang et al. [21] proposed an adaptable system dependent on DNN by applying distinctive feature reduction techniques, activation functions to get the best outcome by testing the model in ISCX 2012 and CICIDS 2017 datasets to detect zero-day

attacks. It is observed with an Precision value of 99.79% and 99.78%, Recall value of 99.97% and 99.36%, FAR value as 0.21 and 0.22, F1-Score as 99.79% and 99.78%, AUC as 99.12% and 99.91%, and the overall accuracy of 99.76% and 99.3% using the two datasets. Ahmed et al. [27] suggested a model to detect zeroday botnet attacks using deep learning and the model is trained and tested with various neural network and hidden layers with CTU-13 dataset. The botnet detection accuracy 99.6% is reached in this model. Srinivasan et al. [30] had arranged his recognition to catch a few kinds of data in URL to identify variations of malicious URLs and named it as DeepURL Detect approach. It is computationally modest when contrasted with different important cutting edge deep learning-based character level embedding techniques and produces a Precision of 97.4%, Recall of 96.4%, F1-score of 96.9%, TPR value of 87.6%, FPR of 0.116, AUC as 0.9964 and the detection accuracy as 97.2%. Pei et al. [22] build a novel framework (AMalNet) using reliable deep learning methods like Graph Convolutional Networks (GCNs) for displaying undeniable level graphical semantics, which naturally recognizes and learns the semantic and consecutive examples, and utilizations different benchmark datasets with Independently Recurrent Neural Network (IndRNN) to decode the deep semantic data, utilizing far off ward data between hubs to freely extricate highlights for Android malware identification and to distinguish its family attribution. The Proposed model results with Precision of 99.52%, Recall of 99.4%, F1 Score of 99.46% and Accuracy of 99.47%. Cui et al. [23] proposed an attack distinguishing proof model LeNet-4 convolutional neural network by disposing of the first pooling layer and the last completely association layer of the first LeNet-5 network. LeNet-4 decreases the computational load and network complexity in the model, and the network selflearning capacity was improved by assessing CICIDS2017 dataset by its design as two convolutional layers and one pooling layer. The recursive feature elimination algorithm in LeNet-4 has enhanced the accuracy rate of 97.8% and 98.5% on multiclass and binary attack identification while diminishing the time cost. Zhang et al. [26] projected a new feature engineering model to detect malware by using hashing and gated approach and extracted a test performance report as Recall of 95.33%, AUC of 98.71% and an Accuracy of 95.33%. Darabian et al. [28] used LSTN and CNN strategies to advance the analysis of cryptomining malware by analyzing it in cuckoo sandbox and achieves an Accuracy rate of 95% and 99% in static and dynamic analysis. Amin et al. [31] investigated the use of deep learning with fully connected NN (Neural Network), CNN, AE, DBN and RNN to detect static malware in Android based smartphones by interrogating the nomenclature of a large-scale byte-code dataset and prove that it beats the state-of-the-art. The contributed model has achieved an F1-score of 0.999, AUC of 0.988 and accuracy of 0.999. Imtiaz et al. [29] manages another scope of difficulties for the vendors of the devices, software developers, cybersecurity professionals and comes out with novel approach DeepAMD and is assessed by utilizing the current CICAndMal2019 dataset for distinguishing and recognizing Android malware on the Static as well as Dynamic layer. DeepAMD model results with an Precision of 93.5% , Recall of 93.4% and achieves a Accuracy rate of 93.4% in binary classification. Gibert et al. [19] have introduced a multimodal deep learning novel malware characterization system by joining both handengineered features and end-to-end components in a modular architecture to detect and classify malware by using CNN, DeepConv, Malconv models. HYDRA model has a macro F1-score of 99.54% and Accuracy of 99.75%. Jha et al. [18] proposed an efficient method for malware detection using RNN and Kaggle dataset. This RNN model has been measured with various word embedding feature vectors and brings out with an AUC value of 91.69, F1-score of 91.76 and Accuracy of 91.91%. Tian et al. [24] dealt with over fitting, low classification accuracy, and high false positive rate issues in the existing intrusion detection systems and by using improved deep belief network (DBN) various propagation tests are performed on the with public dataset like NSL-KSS and UNSW-NB15 and provides FPR score of 1.46% and 5.56% with an accuracy rate of 96.17% and 86.49%. Lu et al. [25] recommended an innovation with new static features with solid antiobfuscation capacities are added, and the dynamic features of the application programming at runtime are separated to enhance the Android malware include set by building a cross breed deep learning model dependent on DBN, RNN and Gate

Recurrent Unit (GRU) by utilizing different dataset to identify Android malware. This hybrid model was performed with both benign and malware samples and attains a Precision of 95.79%, Recall of 97.62%, and Accuracy of 96.82% for malware samples. Sun et al. [32] built up a deep learning based intrusion detection system based interruption recognition framework by utilizing the crossover network like CNN and LSTM to separate the spatial features and temporal features of network traffic to recognize the malware and interruption. A dataset which includes all the common, updated intrusions and cyber-attacks is CICIDS2017 tested for DL-IDS to explore an accurate detection rate of 98.67% and F1-score of 93.32 %. Kim et al.[20] analyzed the universal use of smartphones and the exponential increase of number of malware. To dispense this issue a novel Android malware detection framework using MNN approach and evaluated the model with Virus Share, Google Play App store datasets and produces a Precision of 0.98, Recall of 0.99, F-measure of 0.99 and the accuracy of detection as 98%.

IV. INVESTIGATION STUDY

A relative investigation is led to audit the various attacks experienced based on model, datasets, merits and demerits in the area of cyber security using deep learning is listed below in TABLE. I.

TABLE. I. Comparative study on Malware Detection

AUTHOR/ YEAR	MODEL	DATASET	MERIT	DEMERIT
Zhiqiang et al. [21]/ 2021	DNN	ISCX 2012 CICIDS2017	Binary Classification for Zeroday malware detection	Dynamic Classification for detecting attacks
Ahmed et al. [27]/ 2020	DNN	CTU-13	Neural Network identifies the random noise instead of relationships	Detects only Botnet attack
Gibert et al. [19]/ 2020	MultimodalDNN	Kaggle	Modality Fusion for malware classification	More Data modalities needed for analysis
Kim et al .[20]/ 2018	Multimodal DNN	Android APK	Multiple feature types are encompassed to detect malware	Dynamic Feature Extraction for malware detection
Cui et al. [23]/ 2020	CNN	CICIDS2017	Recursive Feature elimination Algorithm to reduce the dimensionality	Individual attack recognition for classification
Srinivasan et al. [30]/ 2021	CNN LSTM	URL	Character level Keras embedding for URL malware detection	Auxillary Modules for Robust Solution
Sun et al. [32]/ 2020	CNN LSTM	CICIDS2017	Feature fusion for Intrusion detection	LSTM unit Quantity, Training Packet length, Per-Flow packet quantity,

				Batch size and weight effects the performance
Zhang et al. [26] / 2020	Gated-CNN Bi-LSTM	Real-World	Hashing trick to process heterogeneous information Gated-CNN to transform high dimensional hash feature for malware detection	Algorithm failed to work on Virtual addres
Darabian et al. [28]/ 2020	CNN ATT-LSTM	Cuckoo Sandbox	Attention Network for calculating the word weight	Multi-view Learning for classification
Amin et al. [31]/ 2020	Bi-LSTM	Android Malware Dataset	Automatic and Feature engineering to detect malware in low power and memory-limited devices	Time consuming for training
Jha et al. [18]/ 2020	RNN	Kaggle	Negative sampling technique to reduce the computational cost	Specialized Feature extraction method to detect malware
Pei et al. [22]/ 2020	GCN IndRNN	DREBIN, AMD Lab-built, AndroZoo, Praguard	Multiple Embedded for Android Malware detection	Deep Hybrid Exploration for malware detection
Imtiaz et al. [29]/ 2020	DeepANN	CICInvesAndMal2019	Identify Android malware on Static and Dynamic layer	Online malware detection Service
Tian et al. [24]/ 2020	DBN	NSL-KDD UNSW-NB15	Sparcity Penality to avoid overfitting	Customization of parameter selection
Lu et al. [25]/ 2020	GRU DBN	Google Play, APKpure VirusShare, PRAGuard	Anti-Obfuscication ablity to extract malware feature	Limited Comuational Resources for malware detection

V. ANALYSIS AND DISCUSSION

This survey was analyzed and discussed on cyber security attacks and an exhaustive investigation of deep learning strategies to detect malware in Personal computer, Android smartphones, URL, and network traffic. The overwhelming deep learning techniques are compared with the traditional machine learning in the field of network protection with selected papers interrelated to this review topic. As for various deep learning techniques is concerned cyber security issues takes place in various real world issues like Zero-day attack, Malicious URL attack, botnet attack, malicious Android application attack, Crytomining Malware attack, real-world android malware attack, and Intrusion in network traffic data attack with the means of intruders and hackers. Based on the above literature review, it is identified that deep learning approach is giving prominent solution for malware detection and the various detection

accuracy rate in Fig. 7. It is considered with conventional datasets and real world datasets for identifying malware for a green environment.

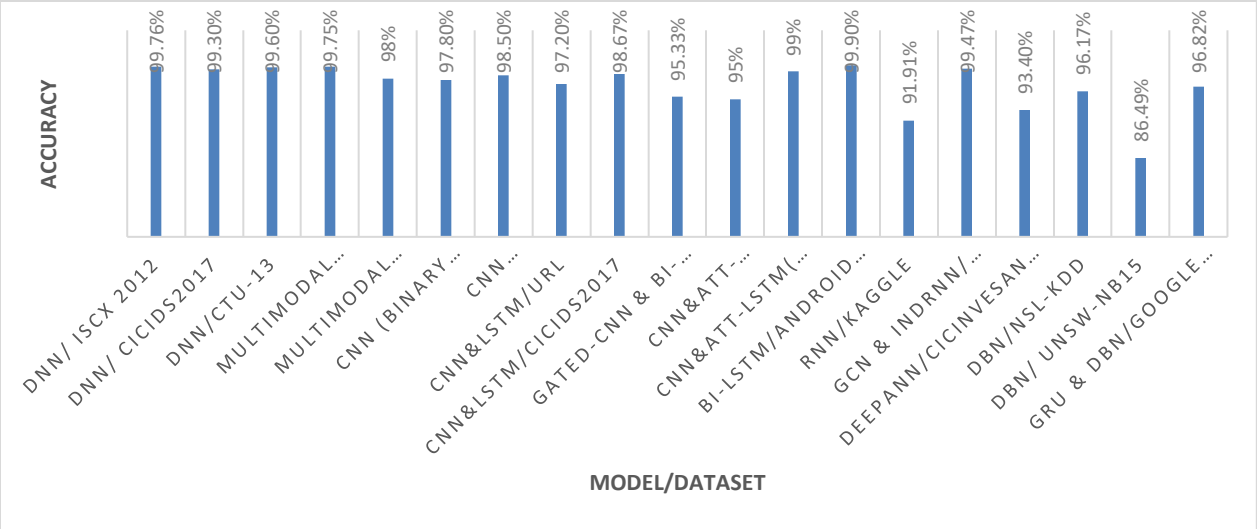


Fig. 7: Investigation Report of detection accuracy on malware

CONCLUSION

In this paper, a relative investigation review of deep learning approaches for malware detection by means of static and dynamic features. Special analysis about deep learning techniques includes Convolutional Neural Network, Recurrent Neural Network, Long Short Term memory Network, Deep Belief Network, and Auto Encoder. In this way, it is noted that Deep Learning techniques play a vital role in analyzing the feature extraction for identifying malware in Android, URL, botnet, crytomining, and intrusion in network traffic with a large datasets like CICIDS2017, CICInvesAndMal2019, Android ADK, Cuckoo Sandbox and NSL-KDD for the detection of malware. The limitation of this work is to include a concentration on other malware attacks like DoS, phishing, and messaging attack. Hence, this review aims to provide a significant contribution to cyber-attack based on the algorithms merits and demerits it is motivated and attracted to proceed with the research using hybrid approaches of deep learning for malware detection.

REFERENCE

[1] Drewek-Ossowicka, A., Pietrołaj, M. and Rumiński, J., 2020. A survey of neural networks usage for intrusion detection systems. *Journal of Ambient Intelligence and Humanized Computing*.

[2] Berman, D.S., Buczak, A.L., Chavis, J.S. and Corbett, C.L., 2019. A survey of deep learning methods for cyber security. *Information*, *10*(4), p.122.

[3] Wang, Z., Liu, Q. and Chi, Y., 2020. Review of android malware detection based on deep learning. *IEEE Access*, *8*, pp.181102-181126.

[4] Yan, J., Qi, Y. and Rao, Q., 2018. Detecting malware with an ensemble method based on deep

neural network. *Security and Communication Networks*, *2018*.

[5] Mahdavifar, S. and Ghorbani, A.A., 2019. Application of deep learning to cybersecurity: A survey. *Neurocomputing*, *347*, pp.149-176.

[6] Alabadi, M. and Celik, Y., 2020, June. Anomaly Detection for Cyber-Security Based on Convolution Neural Network: A survey. In *2020 International Congress on Human-Computer Interaction, Optimization and Robotic Applications (HORA)* (pp. 1-14). IEEE.

[7] Sohn, I., 2020. Deep Belief Network based Intrusion Detection Techniques: A Survey. *Expert Systems with Applications*, p.114170.

[8] Aldweesh, A., Derhab, A. and Emam, A.Z., 2020. Deep learning approaches for anomaly-based intrusion detection systems: A survey, taxonomy, and open issues. *Knowledge-Based Systems*, *189*, p.105124.

[9] Ferrag, M.A., Maglaras, L., Moschoyiannis, S. and Janicke, H., 2020. Deep learning for cyber security intrusion detection: Approaches, datasets, and comparative study. *Journal of Information Security and Applications*, *50*, p.102419.

[10] Mercaldo, F. and Santone, A., 2020. Deep learning for image-based mobile malware detection. *Journal of Computer Virology and Hacking Techniques*, pp.1-15.

[11] Dixit, P. and Silakari, S., Deep Learning Algorithms for Cybersecurity Applications: A Technological and Status Review. *Computer Science Review*, *39*, p.100317.

[12] Vinayakumar, R., Barathi Ganesh, H.B., Poornachandran, P ., Anand Kumar, M. and Soman, K.P, 2018. Deep-Net: Deep Neural Network for Cyber Security Use Cases. *arXiv preprint arXiv:1812.03519*

[13] Zhang, Z., Qi, P. and Wang, W., 2020, April. Dynamic Malware Analysis with Feature Engineering and Feature Learning. In *Proceedings of the AAAI Conference on Artificial Intelligence* (Vol. 34, No. 01, pp. 1210-1217).

[14] Maalem Lahcen, R.A., Caulkins, B., Mohapatra, R. and Kumar, M., 2020. Review and insight on the behavioral aspects of cybersecurity. *Cybersecurity*, *3*, pp.1-18.

[15] Kumar, S., 2020. An emerging threat Fileless malware: a survey and research challenges. *Cybersecurity*, *3*(1), pp.1-12.

[16] Vinayakumar, R., Soman, K.P. and Alazab, M., 2020. A Comprehensive Tutorial and Survey of Applications of Deep Learning for Cyber Security.

[17] Choi, Y.H., Liu, P., Shang, Z., Wang, H., Wang, Z., Zhang, L., Zhou, J. and Zou, Q., 2020. Using deep learning to solve computer security challenges: a survey. *Cybersecurity*, *3*(1), pp.1-32.

[18] Jha, S., Prashar, D., Long, H.V. and Taniar, D., 2020. Recurrent neural network for detecting malware. *Computers & Security*, *99*, p.102037.

[19] Gibert, D., Mateu, C. and Planes, J., 2020. HYDRA: A Multimodal Deep Learning Framework for Malware Classification. *Computers & Security*, p.101873.

[20] Kim, T., Kang, B., Rho, M., Sezer, S. and Im, E.G., 2018. A multimodal deep learning method for android malware detection using various features. *IEEE Transactions on Information Forensics and Security*, *14*(3), pp.773-788.

[21] Zhiqiang, L., Zhijun, L., Ting, G. and Yucheng, S., 2021. A Three-Layer Architecture for Intelligent Intrusion Detection Using Deep Learning. In *Proceedings of Fifth International Congress on Information and Communication Technology* (pp. 245-255). Springer, Singapore.

[22] Pei, X., Yu, L. and Tian, S., 2020. AMalNet: A deep learning framework based on graph convolutional networks for malware detection. *Computers & Security*, p.101792.

[23] Cui, W., Lu, Q., Qureshi, A.M., Li, W. and Wu, K., 2021. An adaptive LeNet-5 model for anomaly detection. *Information Security Journal: A Global Perspective*, *30*(1), pp.19-29.

[24] Tian, Q., Han, D., Li, K.C., Liu, X., Duan, L. and Castiglione, A., 2020. An intrusion detection approach based on improved deep belief network. *APPLIED INTELLIGENCE*.

[25] Lu, T., Du, Y., Ouyang, L., Chen, Q. and Wang, X., 2020. Android malware detection based on a hybrid deep learning model. *Security and Communication Networks*, *2020*.

[26] Zhang, Z., Qi, P. and Wang, W., 2020, April. Dynamic Malware Analysis with Feature Engineering and Feature Learning. In *Proceedings of the AAAI Conference on Artificial Intelligence* (Vol. 34, No. 01, pp. 1210-1217).

[27] Ahmed, A.A., Jabbar, W.A., Sadiq, A.S. and Patel, H., 2020. Deep learning-based classification model for botnet attack detection. *Journal of Ambient Intelligence and Humanized Computing*, pp.1-10.

[28] Darabian, H., Homayounoot, S., Dehghantanha, A., Hashemi, S., Karimipour, H., Parizi, R.M. and Choo, K.K.R., 2020. Detecting Cryptomining Malware: a Deep Learning Approach for Static and Dynamic Analysis. *Journal of Grid Computing*, pp.1-11.

[29] Imtiaz, S.I., ur Rehman, S., Javed, A.R., Jalil, Z., Liu, X. and Alnumay, W.S., 2020. DeepAMD: Detection and identification of Android malware using high-efficient Deep Artificial Neural Network. *Future Generation Computer Systems*, *115*, pp.844-856.

[30] Srinivasan, S., Vinayakumar, R., Arunachalam, A., Alazab, M. and Soman, K.P., 2021. DURLD: Malicious URL Detection Using Deep Learning-Based Character Level Representations. In *Malware Analysis Using Artificial Intelligence and Deep Learning* (pp. 535-554). Springer, Cham.

[31] Amin, M., Tanveer, T.A., Tehseen, M., Khan, M., Khan, F.A. and Anwar, S., 2020. Static malware detection and attribution in android byte-code through an end-to-end deep system. *Future Generation Computer Systems*, *102*, pp.112-126.

[32]Sun, P., Liu, P., Li, Q., Liu, C., Lu, X., Hao, R. and Chen, J., 2020. DL-IDS: Extracting features using CNN-LSTM hybrid network for intrusion detection system. *Security and Communication Networks*, *2020*.

शोध–प्रभा (ISSN : 0974-8946)
(A REFERRED & PEER- REVIEWED QUARTERLY RESEARCH JOURNAL)

THE INVOLVEMENT OF SOCIAL MEDIA FOR THE ENLIGHTENMENT OF WOMEN'S WORLD

Ancymol Joy
Research Scholar, Department of English, National College (Autonomous), Affiliated to Bharathidasan University, Tiruchirappalli,
srancymj123@gmail.com

Vasanthan R
Assistant Professor, Department of English, National College (Autonomous), Affiliated to Bharathidasan University, Tiruchirappalli,
vasanthleut@gmail.com

Abstract
The great influence of the media plays an unavoidable role in modern times to secure women's rights and liberate their status in society. The use of media to portray a new wave of women's empowerment summarizes the opportunities for women to speak and leverage. In today's modern culture, social media is reflected as a powerful weapon for building a thriving world. Social media plays a major role in spreading the strong image of women. Social media can have a profound effect on women's public remarks about oppression and sexual abuse, closing the door to the dominance of patriarchal societies. This is a platform for women to publicly show their ability to claim their rights. It is a way to make people aware of the difficult situations of women in the dominant hands of men-dominated societies. It provides generous support for women to lead a bright future to the top of the world and to realize their evergreen dreams to ensure gender equality. The emergence and quick growth of social media, as part of the modern technological world, has aided women's empowerment and liberation. The impact of social media has largely perceived women's cries for empowerment over the world. Social media empowers women to protest the social and political violence that is destroying the peaceful atmosphere of women's world. Social media is calling on all women in modern society to share their demands and views through this platform of benefits. The social media space expands the territory of the women's world by offering many opportunities to create images of women in the new world. This paper is an attempt to analyze the impact of social media on the liberation and empowerment of modern women in current scenarios.

Keywords: Women, Social media, Empowerment, Women liberation, Case study

Introduction

The world of modern technology is undergoing a revolutionary change, absorbing the voice of women and advancing into a world of complete freedom and complete liberation. This reflects women's protests to create a unique identity in modern society. The media has expressed a desire and a dream to empower modern women to play a role in the community. Social media has become a tool for changing the standards of women in a growing cultural society. Social media, an efficient lever of women's voice, has demonstrated its power to raise awareness and accountability for women's rights, as well as to challenge discrimination and stereotypes. The rise of social media and the unparalleled implementation

of new technology by women involve important opportunities to bring gender equality and women's rights to the frontline of policymaking and media coverage. The emergence of social media represents an explosive change that plays a remarkable role in building an independent and dynamic world of women and shaping the traditional image of women. Social media promotes equal rights and equal opportunities to share their voices, which was not common in the past centuries or in traditional patriarchal societies.

Social media as an effective platform for empowering women

The question of women's exclusion from mainstream societies was answered, especially with the advent of the media in the second half of the 20th century. The media has changed the face of the women's world through the unique identity of women, the problems of the modern women's world, and the depiction of the hidden productivity of women's nature in the past. The world of modern technology welcomes women to compete with men. This is not allowed in traditionally male-dominated societies. Social media has changed the landscape of global information sharing and the relationship between citizens and governments (Shirky, 2011). Social media is a growing trend and a potent tool for drawing attention to women's rights concerns in the domestic and international society, as well as motivating and encouraging governments and policymakers to work toward gender equality (Kumari, 2020, p. 626).

The media reflects the social situation and makes a significant contribution to making women aware of equality. The media is reaching out to drive reforms for women today and create healthy literacy among men in society. In Postfeminism: Cultural Texts and Theories, Stephane Genz and Benjamin A. Brabon (2009) state that “cyberspace becomes a location where the distinction between the subject and the object, the self and the other, dissolves” (147). The upsurge in the flourishing has witnessed an integral merge of the women’s world with the global attention that nurtures the interaction of women with a wider public. The social media outlets pave the way for encouraging the empowerment of women from the subjugation of the male structure. In their study, Narayana and Ahamad (2016) found that while media has a significant possibility for women’s empowerment through women’s collective uses it is quite low.

In addition, Khan and Moin (2013) stated that women are using the internet for a variety of purposes now that they have access to it at home. It has made it possible for women to engage in essential daily state concerns such as home chores, education, health, and government. Opportunities for women introduced by the world of technology challenges the world of women to emphasize their illustrious public presence. This is a true means of celebrating women's social, political, and personal achievements, which are lacking in the traditionally patriarchal world. The media plays a remarkable role in uncovering social issues related to gender inequality reflected in patriarchal societies. Madhu Kumari describes social media in her article "Social Media and Women's Empowerment" as follows: Social media such as Facebook, Twitter, and Instagram help women reach out to reveal their glorious victories and the oppressive nature of patriarchal societies. Social media is a better medium for raising a woman's voice to show her physical beauty and spiritual vitality in front of the human world (626).

The influence of social media is pointed out by Jennifer Anyan in the essay, “Boredom and Reinvention for the Female Gaze within Personal Fashion Blogs” is that “The rapid development of hardware, software and the Web 2.0(with social media performs being a part of this) has facilitated a new, democratic channel for women to use in shaping and communicating identity constructs for themselves in the twenty-first century” (70-72). In modern culture, feminist writers and activists obscure the distinction between gender rights. Women's advertising on social media is opening up new horizons

for women's empowerment. Michelle M. Lazar in "The Right to be Beautiful: Postfeminist Identity and Consumer Beauty Advertising" reinforces this idea by saying that "An 'emancipated femininity is constructed in the ads as liberation and freedom from women's self-restrictions" (39).

Social media provides women with ample opportunity to wipe out the concept of the female body in patriarchal societies and fight for freedom of expression. The media plays an important role in exposing and responding to violence against women's communities. The media is helping all women bring their views and ideas to the forefront of society by dismantling sexism, the most devastating factor for women's liberation. Social media paves the way for women to accelerate their healthy confidence in building a world-leading role on an equal footing with men. Social media warns modern women about the negative portrayals of women in the patriarchal world. The impact of social media focuses on the psychological approval and self-realization of women in modern society. Social media has a faster impact on humanity and faster and other women bend their heads to achieve their goals.

The dissemination of modern cultural factors and the knowledge of women's private and public interests are aimed at freeing women from the shackles of a power-structured society. The advent of social media has shaken the gender separation that limits the status of women in the human world. The media is responsible for eradicating all kinds of prejudice and discrimination against women and is helping women to liberate themselves in the public community. The media are the most effective media for learning the wider situation of women around the world, and they play a major role in shaping the physical and psychological awareness of their role in the world. Ranjay Vardhan has an opinion about the relevance of social media in his article "Social Media and Women Empowerment: A Sociological Analysis" "Social media is posing questions and answering questions that could raise and suggests solutions for every search of hers. It can really turn out to be a friend indeed that gives her both intellectual and emotional company without having to lose her identity" (119).

Social media paves the way for increasing educational and intellectual levels and increasing the productivity of women's communities. Similarly, social media provides support to protect the privacy and freely express its effectiveness. Facebook, Whatsapp, Instagram, Twitter, etc. are just some of the tools you can use to share your desire to get out of the room. With the advent of social media, freedom of speech and expression has increased. Self-expression and self-expression in the women's world are promoted through social media gateways and encourage women to share their dreams, opinions, aspirations, and abilities. Women`s goals to exhibit their beauty are widely accepted through the platform of advertisements. The freedom of the expression of women comes to reality mainly based on the emergence of advertisements. One of the major privileges of social media is to build up widespread relationships and social contact with the human world.

Social media can colour women's dreams with great potential and expose them to social scrutiny and reflection. The advent of social media has paved the way for women's awakening in the social and economic arena. They guide women to maintain their identities and distinguish themselves through achievements in societies where patriarchy still exists. It encourages women to reveal their experiences and make other women aware of society's perception of the world of women. Shey Welch in her book A Theory of Freedom: Feminism and the Social Construct has the opinion that "the social sphere is where individuals develop and express their individuality" (22).

The use of social media plays an important role in brightening women's daily lives by deepening their connection with the outside world. It also helps women to focus on activities that are involved in

their success. Therefore, it helps increase women's participation in women's uplifting. In a sense, women's participation and increased access to self-expression and decision-making through the media and new communication technologies are empowering women. (Azad Pratap Singh 50) The impact of the media, which is always focused on sexual harassment and violations of women's rights, has drawn international attention to women's issues.

The desire of women to venture beyond the confines of their environment has found expression in the public sphere thanks to technological advancements. The impact of social media encourages women's empowerment by giving a wide range of information about education, lifestyle, health, and other topics that help them fit into society and maximize their contribution.

The Triumph of Social Media to Uplift Modern Women: An Overview

To understand how social media takes initiatives for widening the liberation of the female world, the study creates a platform for women to share their viewpoints to conclude the study. The participants in this study based on the questionnaire are a hundred women. The questionnaire encloses the significance and trends of social media including favourite social media, the reason to use social media, the significance, and the role of social media to liberate women in society. The questionnaire has been sent to the participants and collected through e-mail. According to the survey the distribution and analysis of the questions are the following:

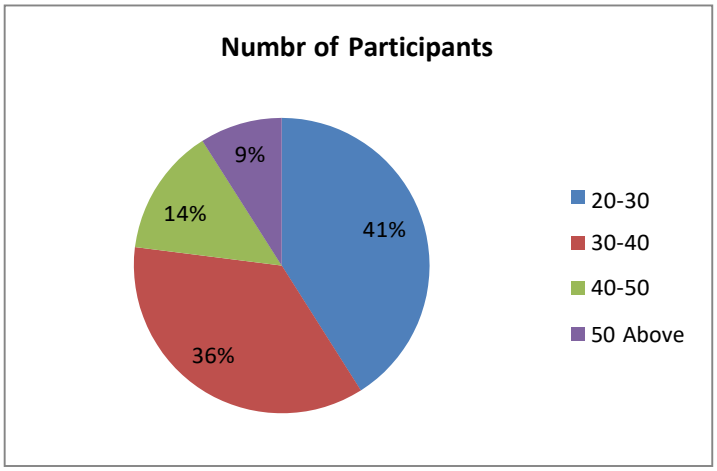
Data Analysis and Discussion

Table 1: Distribution of Participants' Age

Age	Number of participants	Percentage
20-30	41	41
30-40	36	36
40-50	14	14
Above 50	9	9
Total	100	100

Source: Primary data

Figure 1: Percentage Distribution of the Age of the Participants



A clear overview of Table 1 suggests that out of a hundred participants, 41% of women are from the age group 20-30. 36% of respondents belong to the 30-40age group while 14% are from 40-50. There are only 9% of participants are above 50 years.

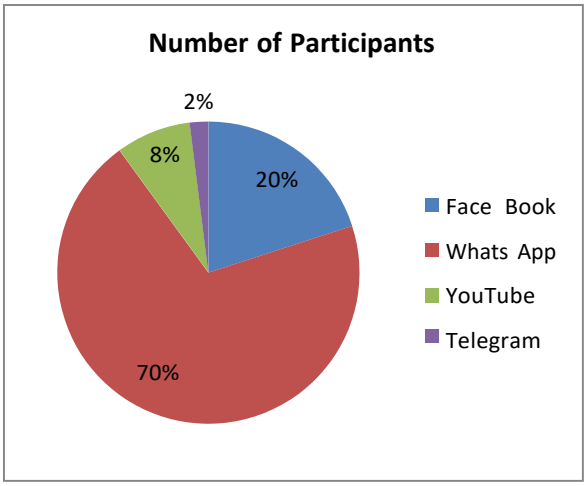
Table 2: Distribution of the Favorite Social Media

Favourite Social Media	Number of participants	Percentage
FaceBook	20	20

Whats App	70	70
YouTube	8	8
Telegram	2	2
Total	100	100

Source: Primary data

Figure 2: Percentage Distribution of the Favourite Social Media



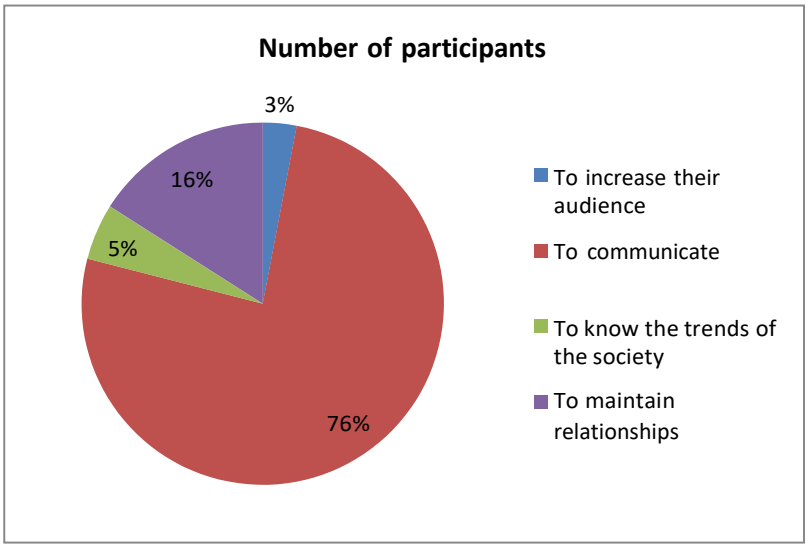
From Table 2, the details indicate that 20%of women suggest Facebook as the medium of their communication whereas 70% use Whatsapp as their favorite mode. Only 8% and 2% reveal that they use YouTube and Telegram as their favorite social media application. This analysis suggests that Whatsapp is the most preferred social media among the suggested participants.

Table 3: The Reason to Use Social Media

The Reason to Use Social Media	Number of participants	Percentage
To increase their audience	3	3
To communicate	76	76
To know the trends of the society	5	5
To maintain relationships	16	16
Total	100	100

Source: Primary data

Figure 3: Percentage Distribution of the Reason to Use Social Media



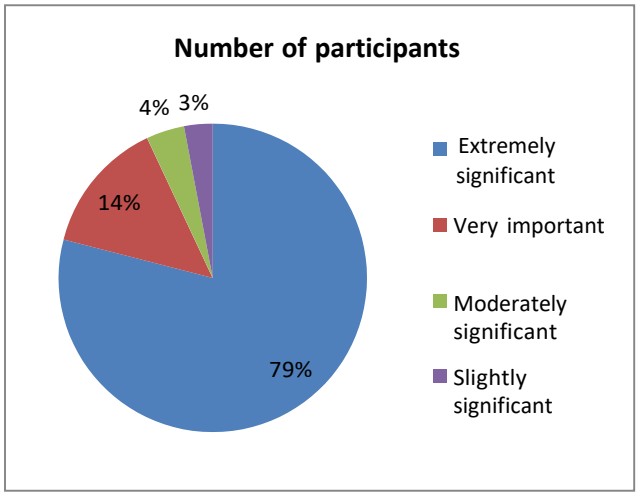
The examination of the graph explains that for 76% of the participants, social media is for communication while 16% of them use it to maintain their relationships. Only 5% of the respondents seek its use to know the trends of the world whereas 3% of women accept social media to increase their audience.

Table 4: The Significance of Social Media

The Significance of Social Media	Number of participants	Percentage
Extremely significant	79	79
Very important	14	14
Moderately significant	4	4
Slightly significant	3	7
Not at all	-	-
Total	100	100

Source: Primary data

Figure 4: Percentage Distribution of the Significance of Social Media



From Figure 4, the opinion of the participants regarding the significance of social media explains

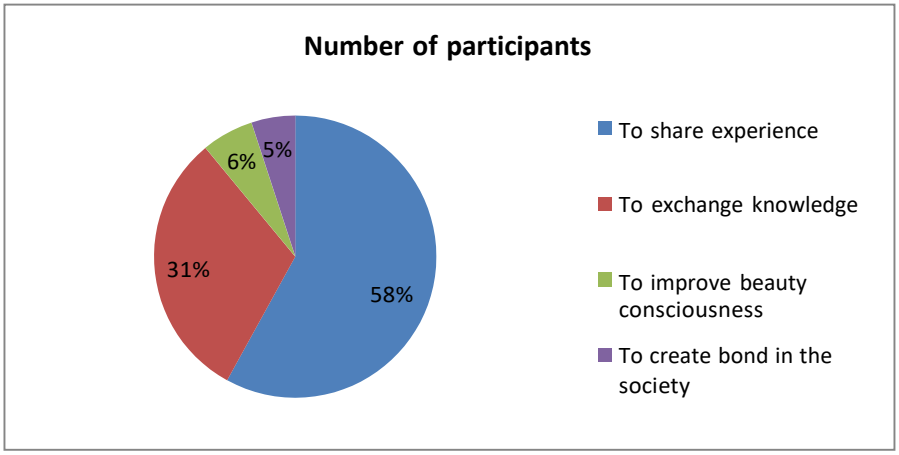
that 79% have the opinion that the use of social media is extremely significant in the modern world. When 14% suggest that social media is very important, only 4% reveal that the use of social media is moderately significant. Whereas 3% pinpoint that the impact of social media has slight significance none of them stated that it doesn't have any significance.

Table 5: The Liberation of Women through Social Media

The Liberation of Women through Social Media	Number of participants	Percentage
To share experience	58	58
To exchange knowledge	31	31
To improve beauty consciousness	6	6
To create bond in the society	5	5
Total	100	100

Source: Primary data

Figure 5: Percentage Distribution of the Liberation of Women through Social Media

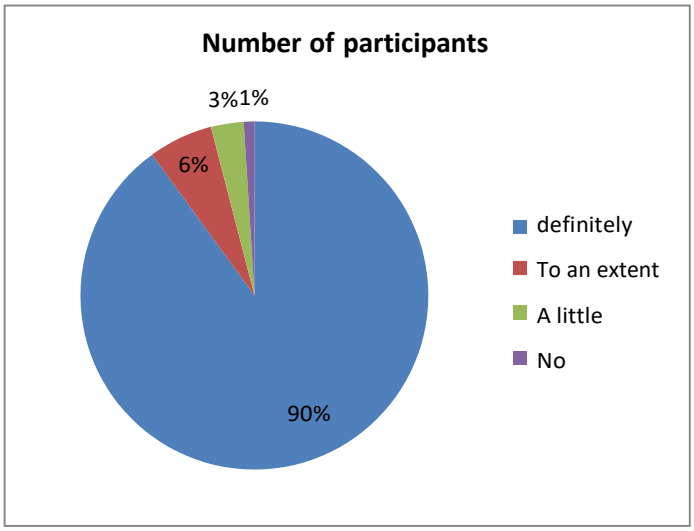


Graph 5 indicates the factors that enhance the liberation of women through social media. Among the participants, 58% support that social media helps to share the experiences of women whereas 31% of participants suggest that it promotes the exchange of knowledge in a wide range. Only 6% and 5% support that it ensures improved beauty consciousness among women and creates a bond in the society respectively.

Table 6: The Standard of Women through Social Media

The Standard of Women through Social Media	Number of participants	Percentage
definitely	90	90
To an extent	6	6
A little	3	3
No	1	1
Total	100	100

Source: Primary data



Graph 6 expresses the suggestions of the participants regarding the relationship between the standard of women and the use of social media. The graph analyses that 90% of participants strongly support that social media increase the standard of women whereas 6% agree that to an extent it supports women to make a stand in society. Only 3% slightly support the statement whereas one participant does not agree with the part of social media in the liberation of women.

Major Findings of the Study

In the age bracket of 20-30, the majority of the participants (41%) use social media. Though 70% of participants use Whatsapp, apart from this social media, 20% have FaceBook, 8% prefer YouTube, and 2% use Telegram. According to the result of the study, the major proportion of individuals recommends adopting social media for interaction while 16 per cent use it to establish a link with others. Women prefer social media to expand their audience and keep up with societal trends, as evidenced by the low percentage of women who use it.

As per the participants' suggestions and opinions on the importance and popularity of social media platforms, 79% strongly agree that it is extremely significant in today's world. Only 4% of those polled believed that social media is relevant, while 14% consider that it is essential. While 3% of respondents think that social media has a minor impact, none of them believes that it has no impact. The analysis of the factors that promote the liberation of women through social media that a majority of participants reported that social media helps to share women's experiences; while 31% suggested that it promotes the exchange of knowledge across a wide range of topics. Only 6% and 5% indicated the factors of beauty consciousness and creation of bonds respectively. In the study, a majority of participants strongly believe that social media elevates women's standards, while 6% agree that to some extent it does so. Only 3% slightly support the statement, while one participant does not believe social media plays a role in women's status. The study highlights that social media is a helping hand for women to demand their rights in public and to enjoy equal freedom in society.

Conclusion

In the present day, social media plays a critical role in accelerating women's liberation at a rapid pace. Women's place in society is elevated by social media, which raises awareness of their competitiveness and highlights their talents. Women's empowerment from a position of powerlessness

to self-sufficiency is achieved by sharing their struggles in the public sphere. It is laudable that social media has pushed the bounds of old societal ideas that have been damaging to women's growth.

References

- Anyan, J. (2015). Boredom and reinvention for the female gaze within personal fashion blogs. In C. Nally, & A. Smith (Eds.), Twenty-first century feminism: Forming and performing femininity (pp. 66-92). Palgrave Macmillan.
- Genz, S., & Benjamin, A. B., (2009). Postfeminism: Cultural texts and theories. Edinburg University Press.
- Khan, E. A. & Moin, A. (2013). Women empowerment: Role of new media. Excellence international journal of education and research, 1(3), 206-216.
- Kumari, M. (2020). Social media and women empowerment. International journal of scientific & technology research , 9(3), 626-629.
- Kumari, M. (2020). Social media and women empowerment. International journal of scientific and technology research,9(3), 626-629.
- Lazar, M. M. (2011). The right to be beautiful: Postfeminist identity and consumer beauty advertising. In R. Gill, & C. Scharff(Eds.), New femininities: Postfeminism, neoliberalism and subjectivity (pp. 37-51). Palgrave Macmillan.
- Narayana, A. & Ahamad, T. (2016). Role of media in accelerating women empowerment. International journal of advanced education and research, 1(1), 16-19.
- Shirky, C.(2011). The political power of social media: Technology, the politic sphere, and political change. Foreign affirs, 28-41.
- Singh, A. P. (2017). Role of media in women empowerment. International journal of advanced educational research, 2(5), 50-53.
- Vardhan, R. (2017). Social media and women empowerment: A sociological analysis. EPRA international journal of economic and business review, 5(8), 117-121.
- Welch, S. (2012). A theory of freedom: Feminism and the social construct. Palgrave Macmillan.

Available online at www.jlls.org

JOURNAL OF LANGUAGE AND LINGUISTIC STUDIES

ISSN: 1305-578X

Journal of Language and Linguistic Studies, 17(4), 2884-2890; 2021

The Interconnection between Feminism and Education for Women Empowerment in Laura Bates' *Girl Up*

Ancymol Joy [a1], Vasanthan R[b]

[a]*Research Scholar, Department of English, National College (Autonomous), Affiliated to Bharathidasan University, Tiruchirappalli- 620001, India.*

[b]*Assistant Professor, Department of English, National College (Autonomous), Affiliated to Bharathidasan University, Tiruchirappalli-620001, India.*

APA Citation:

Ancymol Joy., & Vasanthan, R. (2021). The Interconnection between Feminism and Education for Women Empowerment in Laura Bates' *Girl Up*. *Journal of Language and Linguistic Studies, 17*(4), 2884-2890

Submission Date:10/10/2021

Acceptance Date:24/12/2021

Abstract

Feminism fosters the erasing of the social discrepancy between the expectation of the patriarchy and the real standard of women to make their own choices to uphold gender equality. As the central concept of the development of humanity, women's education has been an ultimate goal for years and decades of human history. The sharp eyes of feminism concentrate on the benefits of women's education and capture the attention of the global community to spread the implications of women education and empowerment. Education has a pivotal role to mould both the woman society and the human world to craft a perfect realm where men and women have equal rights and freedom.Women empowerment and education of women are inextricably linked to augment the standard of women in humanity. As an agent of the distinctive and flourishing society, education stretches its hands to boost the social role of women and builds up a platform where the women community can mark their unique and courageous performance in the human world. Laura Bates is a leading figure of the contemporary feminism to throw light on the role of modern education to uphold the liberated women society. This paper is an endeavour to explain the role of feminism to hold up women education in the light of Laura Bates' *Girl Up*.

Keywords: Feminism, education, gender equality, women empowerment, gender inequality.

1. Introduction

Women education is the foundation stone of gender equality and the rapid expansion of the social, political and economic dimensions of the human world. The rights and facilities of education without discrimination build up a world of ambitious women. Equal right of education without gender inequality has a supreme role to build a perfect human world with self respect and self confidence. Women education is a medium to endorse the rising status of women in the patriarchal society. Through women education feminism takes a role to wipe away the sexual harassment and domestic oppression and to inculcate the needs of equal rights and the individual freedom from the walls of their

[1] Corresponding author.
E-mail address: srancymj123@gmail.com

© 2021 JLLS and the Authors - Published by JLLS.

domestic world. Feminism has a distinct role to eradicate the social evils such as dowry system, unequal payment and lack of job opportunity for women and to mould an educated family as well as an educated society.The reverberation of feminism on the educational level is a relevant theme in modern society. Education is a leading force which annihilates the patriarchal concept which brings women to the feet of the patriarchal system. As a driving force of the reinforcement of women's society, women's education boosts the aspiration of women to secure equal prestige with men. Feminism breaks down the disparity among men-women relationship through the inception of education and upgrades the standard of women by improving women's education. In the traditional male dominated society women are undervalued in the academic level as well as in the social interaction.

2. Literature Review

Role of Education in the field of women Empowerment

Education is the key to the economic growth and the single sharp bolt of the women's world against the household and public oppression of the patriarchal world. There has been a momentous transformation in the educational pattern and a steady and undeniable advancement in women education in the modern era. Education system is the cradle of women's achievement and empowerment of the society in which boys and girls can acquire equal benefits and advantages. The handcuffs of the traditional patriarchal society resist women to pursue their higher education and to enjoy equal political and legal rights in the male dominated society. As Robin Truth Goodman(2005, p. 5) in *World, Class, Women: Global Literature, Education, and Feminism*suggests, "feminism began as a radical discourse about education, particularly because discussing education required thinking about civic access on which rests the very notion of political freedom for women". Domestic violence and the discrimination against the equal legal rights close the door against the empowerment of women through the improper education system. Education not only boosts the intellectual level of women but also makes them doers of the society (Aiston 2010).

Rouf Ahmad Bhat (2015, p.189) in "Role of Education in the Empowerment of Women in India" expresses his opinion as,"Education is milestone of women empowerment because it enables them to respond to the challenges, to confront their traditional role and change their life". He also emphasises the empowerment of humanity through education as "empowerment means a psychological sense of personal control or influence and a concern with actual social influence, political power and legal rights. It is a multilevel construct referring to individuals, organizations and community" (Bhat 2015, p.189)

3. Methods

The method applied in this research is descriptive and interpretive study. The study explores the background of the emergence of feminism through the field of education. In order to support the argument, the study analyses and interprets Laura Bates' *Girl Up* in the context of the interconnection between education in relation with feminism and women empowerment throughout the centuries. The paper upholds a close study of the interpretations and views of eminent critics on feminism and role of education in women empowerment. The study primarily is an interpretation on the role of education and feminism to wipe away the control of the patriarchal structure and to support women liberation in the society.

4. Result And Discussion

Feminist movement has a wide and salutary influence on the modern society especially on high access on women education, on the right of women to take personal and individual decisions and domestic and social freedom to come forward in the main stream of the society. Women education fosters the idea regarding the unequal payment in the work field and the social situation where women

© 2021 JLLS and the Authors - Published by JLLS.

are identified as the sexual objects in the hands of men. This section describes the role of education and feminism to support the liberation of women in the light of Laura Bates' *Girl Up*.

Liberation of Women in the First Wave of Feminism

In the past centuries, women were kept away from all sources of knowledge and educational systems to cease them against questioning the rules and the oppressive nature of the patriarchal world. The lack of education puts a stop to the possibilities of women to come into the front row of the society and to obtain the leadership quality in the male dominated society. The explicit barriers of women to acquire education are a prominent problem and the theme of the first wave of feminism.

The disparity in the education system of the patriarchal world makes women slaves in the male dominated society where they are underestimated and subjugated. First wave feminists fight against the unequal treatment of women through the inferior nature of educational policy. They envisage the idea that education plays a lion part role to exercise reason and the capacity of women to compete with the male centred society. This deep rooted disparity is obviously evident in the writings of the first wave writers such as Mary Wollstonecraft and Virginia Woolf. First wave feminism contributed a strong and world wide support to ensure the educational rights of women in the late 18^{th} and early 19^{th} century. The improvement of women status results in wide-reaching effects on the total welfare of the society and a developed social system.

Virginia Woolf forms a substantial and concrete foundation which supports women to gain their role in the education dimension. She pinpoints her viewpoints about the unequal access to education and elevates the value of equal status of women through equal education throughout her work, *A Room of One's Own* (1929). She investigates the glory of women education and advocates for the equal education of the woman community. The concern of education equality is reflected in her works such as *The Voyage Out* (1915). *The Room of One's Own* explores the subject matter of self actualization and empowerment of women through equal education which men achieve in the society.

Virginia Woolf draws the picture of women with highly educated and self-esteem in her literary creations. She questions the exclusion of women from the quality education system which destroys the whole human world in her *Three Guineas* (1938). She takes her pen to dismantle the prevailing education system and inspires a proper channel to educate and to provide knowledge to women also. Virginia Woolf strongly opposes the discrepancy between the education system of men and women and upholds a dream of a novel world where both sexes enjoy equal rights of education. The writings of Mary Wollstonecraft revolve around the basic concepts that "education was the key to personal independence and empowerment" (Pedersen, 2010, p.27). In *The Second Sex*,Simone de Beauvoir (1949, p.725) provides an indication of the equal education for men and women where she declares to be"a complete individual, equal to man, woman has to have access to the male world".

The Role of Education in the Modern Feminism

The repercussions of gender equality in education and the limited opportunities for job and education are explicitly expressed by modern feminism. The unequal treatment directly intercepts the physical and psychological growth of the whole of humanity. Contemporary feminism fights for gender equality and prevents the social oppression of the patriarchal society. Modern feminism guarantees a world regardless of gender inequality and social status. It designs the empowerment of the modern young women through sex education and liberal principles. Diane Elam (1994, p.43) in *Feminism and Deconstruction* says that "liberation arrives when all women have come to authoritative consciousness of their own, identical, sexual identity".

Liberal feminism announces the idea that women should be able to exercise their individual freedom and also exhorts women to fight for the legal rights, property rights and equal job opportunity in the society. Feminism advocates for the overall development of women through equal education

© 2021 JLLS and the Authors - Published by JLLS.

policy and the profile of women empowerment has an indefinable and vital change in comparison with the condition of women in the past.

Modern feminism also takes its arms to fight for women's education and a total liberation of women in the technological world and pushes them to fit for the existing nature of human society. Modern education system is a challenging revolution that charges women with confidence and capacities to encounter contemporary social issues. Modern women writers and activists put emphasis on the concept that in order to flourish the ideas and contributions of women to the patriarchal world women should be free and come forward to mark their fortunate and truly enviable position in the history of humanity. Feminism is a catalyst to indoctrinate and to impel women to make use of their chances through liberal and thorough education which makes them fit for the contemporary existing world. The self actualization and the liberation of women lead to a frivolous and hopeless dream without a proper and equal education system. Sara Ahmed (2010, p.54) in *The Promise of Happiness* says that "Education provides a way of getting the would-be subject to face the right way such that they can receive the right impressions".

In modern feminism, women empowerment is boosted by the advent of technology. Mobile phone technology and the internet pave the way to extract and to exchange information among women. In the article "Role of Media in Women Empowerment" Azad Pratap Singh (2017, p.50) reveals that "The increase in the participation and access of women to self-expression and decision-making through the media and new technologies of communication is in a way empowering women". In the present era, women are more productive to engage in the area of social media "to express themselves creatively and communicate with others" (Kearney, 2006, p. 2). The availability of the online materials which prompt them to acquire knowledge leads the young women generation to create a path for their future uplift. The comparison between the traditional way of women education and the modern one underlines the same concept:

> In contrast to previous generations of female youth, whose forms of communication and creativity were largely restricted to writing and the domestic arts, girls today are using virtually every medium currently available, including film, music, periodicals, and the Internet, to express themselves, explore their identities and connect with others. (Kearney, 2006, p. 3)

The advent of the media culture of the present century meets the needs of contemporary women's education to undergo a sea change to strengthen their knowledge and career path. Feminism creates a straight way to lead the modern women generation to broaden their vision regarding their role in humanity along with men in an effective manner. The transformation of women through technology and media makes them tighten their hold to satisfy their demands in the modern cultural society. The involvement of modern women in the technological world as a result of the contributions of contemporary feminism produces an in-depth analysis of the capabilities of women to support and build up the society. The contemporary approaches of feminism to elevate the academic productivity through social media is a blow to the contemporary patriarchal norms that prevent the "productive cultural practices" (Kearney, 2006, p. 4) of women.

Cyber feminism is a catalyst which accelerates the widespread education and the substantial and credible information among the women community. As Agrawal and Dutta (2013, p. 112) in *Feminism in Modern English Literature* says, " The Internet promises women a network of lines on which to chatter, natter, work and play; virtuality brings a fluidity to identities which once had to be fixed; and multimedia provides a tactile environment in which women artists can find their space….".

Feminism suggests social media as a form for the young generation to reinforce their activity to mould them as an independent individual and to construct a bold relationship with other women around them. Kearney's (2006, p. 5) words support the transformation of the modern female world as, "Contemporary girls who make media are therefore evidence of a notable transformation in gender

© 2021 JLLS and the Authors - Published by JLLS.

and generational politics". Along with social media women magazines and periodicals encourage the young modern generation "to adopt an ideology of self-improvement and to practice self-surveillance" (Kearney, 2006, p. 10). The self-improvement through improving the physical appearance and positive attitude towards their physical beauty make women to construct their own stand in the male dominated society.

The modern female youth takes privilege to acquire a position in society with the association of the technological world and breaks the strong bond of the patriarchy that holds women's world from independence and individual liberation. Through the acceptance of social media and technology, the modern female youth challenges the patriarchal conventional notions which consider women education and women liberation are taboos of humanity. Through the sincere involvement of the modern women in the world of social media, they achieve a standard place in the history of the human world and expand their realm to the horizons of the entire world. The visible resistance of the modern young women against the patriarchal viewpoints through social media builds up a strong exposure and equal status of women in the society.

Women Empowerment through Education in Laura Bates' *Girl Up*

Laura Bates is a strong proponent of women liberation through a proper educational system in her well known work *Girl Up*(2016). The acquisition of the modern knowledge through technology and social media is highlighted in her work that acts as a milestone in the way of individual woman freedom. Through *Girl Up,* Bates navigates the young women generation to the anchor of a brighter future that supports them to mark their individual contribution in the history of human beings. She frankly addresses the young women generation of the modern era to hold the control of their lives and to emerge from the shackles of the male powered society.

As a guideline to the teenage girls, she transforms the educational system of the past centuries and provides a new face to the modern educational system. She introduces sex education, information about technology and social media and the assaults against women in the modern cultural society. *Girl Up* performs as a testimony to inform the young generation against the challenges and competence of the modern technological world where women are excluded from the mainstream of the society.

Laura Bates highlights her instructions to beware social media which traps the young generation in the present social situation. She (2016, p. 5) says, "The easiest way to protect yourself online is to think of the online space as if it were a real space – think of your social media accounts like your home". This book can be considered as a guide for modern women youth as a collection of suggestions to ensure the safety of women in the age of social media, that is to treat "the online world like the real one" (2016, p. 6). Her book plays a vital role in ensuring the safety of women in various walks of life in the modern world such as social media, female body, mental health of women, pornography and sexuality, and also paves the way for imparting knowledge on all aspects of life.

Bates (2016, p. 307) instructs the women world to construct a feminist society to promote proper education system as her words suggest that "Encourage school or university authorities to engage with you by approaching them with concerns and areas you could work on together". She (2016, p. 306) encourages building up a feminist society to support and uplift the youth generation by challenging that "If there isn't one in your school or university, why not start your own?" and which ensure mutual support and exposure. Laura Bates is an emerging proponent of the novel educational system where women achieve equal rights with men to obtain knowledge.

The flourishing influence of social media and technology amplifies the satisfactions and needs of the young girls to handle individual knowledge acquisition equally with the boys and to engage in the liberation of the female world in the twenty first century. Laura Bates concentrates on the overall development of the modern women generation both in academic level and social environment. She also encourages girls to fight for an equal external environment along with boys in the educational

© 2021 JLLS and the Authors - Published by JLLS.

institutions. She (2016, p. 59) argues, "If you're planning to challenge authority, whether with your parents or at school or university, make sure you arm yourself with information".

Bates' instructional mode of writing holds together the young girls to protest against the traditional way of thinking that subjugates them under the feet of men's society. She instructs them to come out from the confinement of the society and to fight for the empowerment through proper educational system:

> But if girls throughout history had always accepted the rules and restrictions that were placed on them because it was 'just the way things are' or 'because I said so' or 'because you're a girl'. We might still be sitting at home knitting socks for the men, with no money or property of our own, no right to work and really, *really* unfulfilling sex lives. (2016, p. 60)

Bates' brilliant explanation to take proper arms that can effectively protest the gender discrimination that still exists in the modern cultural society. She invites the young generation to embrace social media to proclaim their rights in the society. As a writer of fourth wave feminism, Bates welcomes online platforms to protest against the injustice of contemporary society. She (2016, p. 72) says, "Sometimes it might work to campaign different kinds of protest-like an online campaign supported by a rally, or a petition which uses a hashtag and social media account to raise awareness of what is going on". As an ardent proponent of fourth wave feminism, her work leads the young generation to a novel platform where they can build up a liberated society and a fast transmission of information and knowledge.

Bates introduces the healthy relationship between social media and the contemporary young women to empower themselves to acquire equal treatment and gender equality in the modern world. She clearly illuminates the young girls about the inevitable role of technology to escalate the social role of women to mould the humanity in the present century. She suggests utilising social media for acquiring novel information and to introduce modern strategies to fight for the rights in a successful manner.

The twenty first century feminism upholds the percept that the modern education system should transfer the knowledge without the discrimination of class and gender and women should be independent in all walks of their lives. The creation of a society where women are brave and courageous is the ceaseless and strong desire of modern feminism. The explicit barriers of women education in the past has been thrown away by the feminist movement. The digital skills of the modern women, in fact, divert their war-path to a state of freedom and independence. The struggle for gender equality in the education field has been succeeded partly with the support of the feminist movement. The disproportional division of education disrupts the liberation of women in the male dominated structure. Feminist writings are the milestones which exhibit the achievement of women in the public realm and to encourage women study as a discipline. In the present age women education is the framework through which women acquire self respect and self esteem and a medium which instructs the women's world to pop out from the oppressive nature of the patriarchal world as well as from the traditional concept that women should be confined in the domestic world. Feminism can play a considerable role in ensuring poverty alleviation, child health and economic security in the modern world.

5. Conclusion

Women studies create a new world in the field of research and higher studies which extends its area to the horizons of humanity. The predominant themes of the modern feminism such as dalit studies, queer theories and transgender issues introduce the crucial problems of the contemporary world. Women education and the feminist movement is the answer to the outcry of the society for gender equality and socio political justice of the women society. In the contemporary age, the realm of

© 2021 JLLS and the Authors - Published by JLLS.

education opens its windows to satisfy the needs of education of women and to achieve equal rights of education. In modern society the marginalization of women has been wiped away by the educational system of the society which ensures equal education chances for both men and women. Feminism calls the attention of humanity to break the traditional male dominated subjects of the education system and suggests a curriculum which promotes equal status of men and women in the contemporary world. Feminism disseminates the essential views of women education which generates highly qualified women in modern humanity. The inevitable role of education gives birth to a woman's world with highly aspiration and glorious victories throughout the past decades.

References

Agrawal, R., & Divyarani, D. (2013). *Feminism in Modern English Literature*. Crescent.

Ahmed, S. (2010). *The Promise of Happiness*. Duke University Press.

Aiston, S. J. (2010). Women, Education, and Agency, 1600–2000: An Historical Perspective. In J. Spence, S. J. Aiston, & M. M. Meikle (Eds.), *Women, Education, and Agency, 1600–2000* (pp. 1–8). Routledge.

Bates, L. (2016). *Girl Up*. Simon & Schuster.

Beauvoir, S. D. (1953). *The Second Sex*. Knopf.

Bhat, R. A. (2015). Role of Education in the Empowerment of Women in India. *Journal of Education and Practice*, 6(10), 188-291.

Elam, D. (1994). *Feminism and Deconstruction*. Routledge.

Goodman, R. T. (2005). *World, Class, Women: Global Literature, Education, and Feminism.* RoutledgeFalmer.

Kearney, M. C. (2006). *Girls Make Media*. Routledge.

Pederson, J. S. (2010). Women and Agency: The Education Legacy of Mary Wollstonecraft.In J. Spence, S. J. Aiston, & M. M. Meikle (Eds.), *Women, Education, and Agency, 1600–2000* (pp. 27–48). Routledge.

Singh, A. P. (2017). Role of Media in Women Empowerment. *International Journal of Advanced Educational Research*, 2(5), 50-53.

© 2021 JLLS and the Authors - Published by JLLS.

Journal for Educators, Teachers and Trainers
The PROFESIOLab electronic, peer-reviewed, open-access Magazine

ISSN 1989 – 9572

DOI: 10.47750/jett.2022.13.01.007

Literature for Gender Sensitivity: An Overview

Vasanthan R[1]

Ancymol Joy[2]

Journal for Educators, Teachers and Trainers, Vol. 13 (1)

https://jett.labosfor.com/

Date of reception: 20 Oct 2021

Date of revision: 11 Dec 2021

Date of acceptance: 16 Dec 2021

Vasanthan R, Ancymol Joy (2022). Literature for Gender Sensitivity: An Overview *Journal forEducators, Teachers and Trainers,* Vol. 13(1). 61 – 69.

---

[1]Assistant Professor, Department of English, National College (Autonomous), Affiliated to Bharathidasan University, Tiruchirappalli,
[2].Research Scholar, Department of English, National College (Autonomous), Affiliated to Bharathidasan University, Tiruchirappalli,

Journal for Educators, Teachers and Trainers
The LabOSfor electronic, peer-reviewed, open-access Magazine

Journal for Educators, Teachers and Trainers, Vol. 13 (1)
ISSN 1989 – 9572

https://jett.labosfor.com/

Literature for Gender Sensitivity: An Overview

Vasanthan R[1], Ancymol Joy[2]
[1].Assistant Professor, Department of English, National College (Autonomous), Affiliated to Bharathidasan University, Tiruchirappalli,
[2].Research Scholar, Department of English, National College (Autonomous), Affiliated to Bharathidasan University, Tiruchirappalli,
Email: vasanthleut@gmail.com, srancym@gmail.com

ABSTRACT
Literature has always been a key component in the development of children's linguistic skills as well as their moral awareness. This study will look at how literature can be used to teach gender awareness as well as other aspects of learning to literature students. A survey was conducted using a series of questions to learn about literature students' perceptions about the use of literature for emotional refinement. Based on the replies, a thorough evaluation has been conducted, and suggestions for classroom discussion, as well as the possibilities for further research, have been provided.

Keywords: Literature, Gender equality, Gender Sensitivity, Education

1. INTRODUCTION

Literature teaching is being introduced at the school level, right from primary education for teaching grammar and language. Literature has long been a subject of study at the secondary or tertiary level in many nations. Still, it has received little attention concerning the positive effects on learners that it is capable. For instance, one of the pressing issues that we have today is gender equality and gender sensitivity, and this awareness can be better achieved through the support of appropriate literature. It is generally believed that it can be achieved through various sensitization drives, training, workshops, programs, etc. Gender sensitivity refers to the understanding of people concerning the role of the sex according to the society that he or she belongs. Ozumba (2005) states that gender sensitivity indicates the level of consciousness and the assessment of the need to sustain a reasonable level of gender difference between men and women. Sandra Harding in The Science Question in Feminism pinpoints how society treats the concept of gender from the beginning of human history. She explains the notion of gender that "Gender is an ancient symbol-system, cultures use to understand and to organize both human behavior and non-human entities" (Harding, 1986). As her words indicate, gender is an age-old concept that society has exercised to organize both men and women's role in human society. Nandia Dutta and Sumitral Jha (2014) articulate the concept of gender as that gender as a long-standing symbol system that societies have applied to interpret and organize both human and nonhuman behaviours. Sally Engle Merry (2009) considers "gender as a way of describing identities that are socially constructed" and rooted in culture (Aaltio and Mills, 2002). The world is progressing in technology and advancing almost in all fields. However, there is still a necessity to make people come out of the social setup that is gender-based where women meet inequality compared to men (Hamberg 2008). Kate Millet in her work, Sexual Politics provides a detailed explanation of the gender concept that "Patriarchal ideology exaggerates biological differences between men and women, making certain that men always have the dominant, or 'musculine,' roles and that women always have the subordinate, or 'feminine ones'". Thus, the traditional patriarchal structure emphasizes the dominant responsibilities of the masculine world and the subservient responsibilities of the female society throughout the ages.

As one of the primary goals of education is to liberate individuals from irrational practices and attitudes, educational content needs to focus on the importance of helping the students understand the essentials of gender equality. Weitzman et al. (1972) suggest that by the time they enter kindergarten, the youngster can distinguish between the sex role preferences. It is also obvious that literature has the best content in the best form to learn about things in general and society in particular. However, dealing with literature in the classroom is often overshadowed by the story narration part of it then affecting the character of an individual or helping towards rational and unbiased thinking. Lizbath Goodman and Alison Smith (1996) believe that the stories and principles that are unravelled through children's literature, frame the viewpoints of children from their early childhood. They explain it as "The stories told and the morals which unfold help to shape our view as we learn

to read and take meaning from what we read". Therefore, the teaching of literature and literary texts could be further trimmed to positively influence the children from the start. This paper reviews the possible ways of choosing literary content and taking them to the students to positively affect their minds.

The first and foremost thing about teaching literature and creating awareness on sensitivity among the children is choosing the right content and avoiding the types of content which will indirectly promote the social construct of gender significance. Dr. Montessori (1969) emphasizes the importance of education that disrupts the barriers of social violence related to gender issues as, "an education from birth that brings about a revolution that eliminates every violence". Literature here has to be scanned for the stereotype roles played by males and females, which would go according to society's way. The second essential thing is handling the content effectively at the time of classroom discussion. Implementing gender stereotype reflections into classrooms empowers students to question misconceptions in school and society (Taylor &Ligozat, 2019). People belonging to several generations over the years usually enjoy literary content prescribed for children. It is essential to note here that along with the development of the human mind, our understanding about ourselves and society also changes. In this context, it is essential to upgrade the literary contents based on the present social set up or with a futuristic perspective. For example, the typical portrayal of women who are always in need of support from men and a man as someone who helps women presented to be in need has to be reworked as it is not how the world is at present. When discussing the content, the teacher needs to ensure that, even unconsciously, they do not go back to the normal understanding of gender roles.

Though the term literature is usually given to works that have a universal appeal, it is not always the same when it comes to portraying gender roles. Nor can it be ignored just because of not giving equal importance to the characterisation of men and women in the work of art. Readers of any particular time need to travel time if required to the past are to the future as per the requirement. It is because beautiful literary works are meant to outlive time. It will only be a sensible approach if we begin to analyse the characterisation of men and women in the works, as one of the main aims of any literary creation is to satisfy the needs of the contemporary readers. So, better reading of the literary works in the light of the context, cultural elements involved and emotional attributes will help reveal the characterisation of men and women as the representatives of a particular age with limitations in their roles. In this way, students will be able to visualise the characters as the creations with liberties and limitations of a particular age then as the ideal ones for all ages. This will also lead to a biased understanding of the characters influenced by the cultural and emotional attributes of the reader. The following paragraphs will analyse the essentials of dealing with a literary work in the classrooms to understand gender pictures in literature and come out of gender bias.

1.1 Research Questions

1. Is there any general awareness about the use of literature?
2. Do students learn about gender sensitivity in the class rooms?
3. Is there any special importance given for gender understanding in the class rooms?

2. Methodology

The study was conducted with the responses of 50 students. In order to support the study, a questionnaire has been prepared with six questions related to the objective of the study and the demographic details of the respondents including their name, Email ID, and age. A Goole Form has been created and distributed among the students. Instead of giving the multiple choices questions, the respondents were asked to supply their responses in the form of short answers to indirectly understand their perceptions about the topic and to get a clear design to draw the conclusion of the study. Data analysis has been done with the help of the graphical representation of the responses given by the participants. The responses are categorised according to the understanding and responses of the respondents.

2.1 Participants

The participants of the survey were 50 in number: Degree and M. Phil students of the English Department of National College (Autonomous), Tiruchirappalli in Tamil Nadu. The participants of the study come under three categories of age such as 17-20, 21-25 and above 25.

2.2 Data Collection

A questionnaire has been prepared through Google Form to understand about the students' knowledge about gender sensitivity and the class room practices regarding the importance of the topic. The responses were categorised and analysed with the help of graphical representation.

2.3 Data Analysis

The questions given to analyse the study are given below.

- Why do you think literature is important?

- What are the benefits of learning literature?
- What is it that you believed to have gained through literature?
- Do you think literature will change one's personality?
- Do you feel that you can understand about the emotions of people because of literature?
- Is there anything you learnt about gender sensitivity in your class?

The analysis and interpretation of the study based on the graphical representation are given below.

1. Why do you think literature is important?

Table 1: The reason of the importance of literature

Importance of literature	No. of respondents	Percentage (%)
Mirror of life	25	50
To understand people	10	20
To identify emotion	8	16
To understand language	7	14

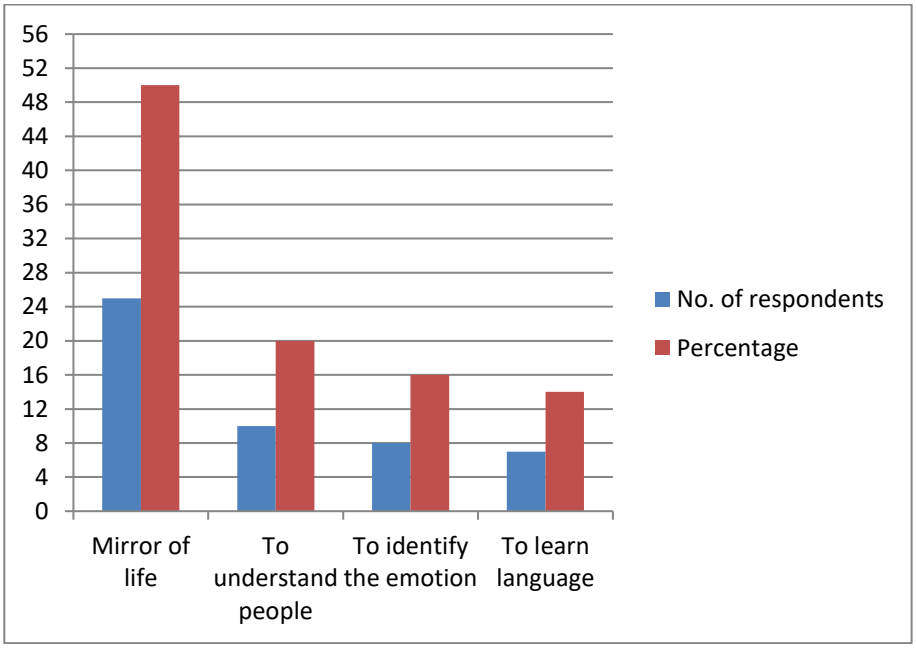


Fig.1: Percentage distribution of the reason of the importance of literature

According to the above graph, 50% of students agreed that literature is important because it is the mirror of life whereas 20% of respondent have the opinion that literature is helpful to understand people. 16% and 14% of the students provided their suggestion that literature is helpful to identify the emotions and to understand language respectively.

2. What are the benefits of learning literature?

Table 2: The benefits of learning literature

Benefits of literature	No. of respondents	Percentage (%)
Creativity	15	30
To understand the emotion of others	7	14
To understand the personality of others	22	44
Language acquisition	6	12

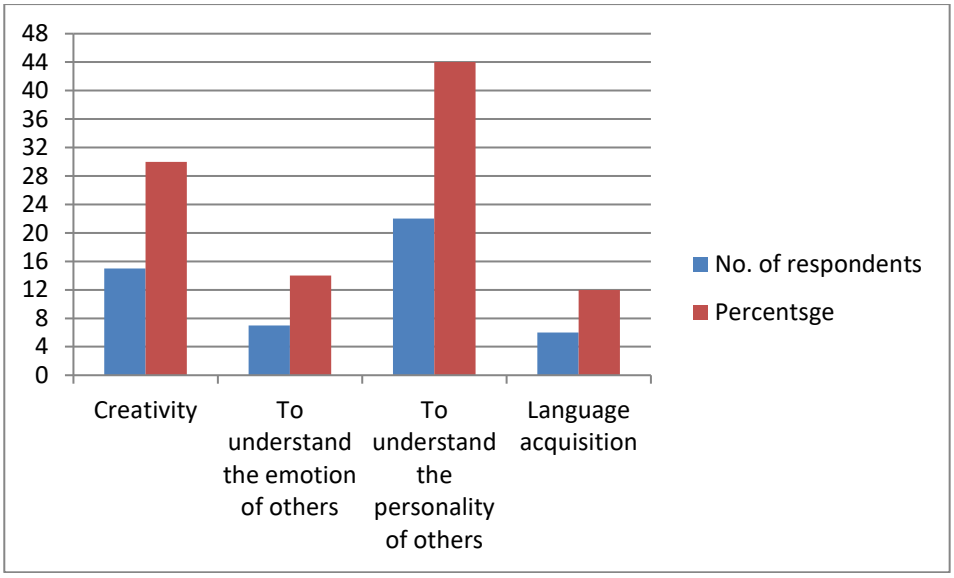


Fig.2: Percentage distribution of the benefits of learning literature

From the graph it is clear that 44% of the students agreed about the benefits of learning literature so as to understand the personality of others. For 30%, literature is to build up their creativity whereas 14% and 12% of students provided their opinion as literature helps to understand the personality of others and for the language acquisition respectively.

3. What is it that you believed to have gained through literature?

Table 3: The profit through literature

Profit through literature knowledge	No. of respondents	Percentage (%)
Empathy	18	36
Entertainment	7	14
Getting information	4	8
Understanding of life	21	42

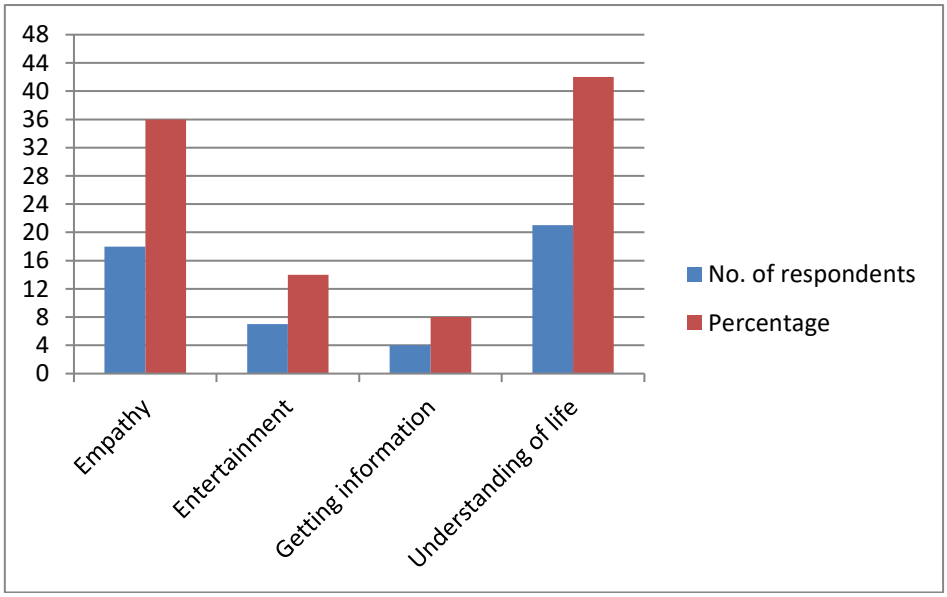


Fig.3: Percentage distribution of the profit of literature

As the graph shows, literature knowledge helps 42% students to understand life of others, but 36% get empathy through literature. For 14% and 8% of students, literature is for entertainment and for getting information respectively.

4. Do you think literature will change one's personality?

Table 4: Relationship between literature and personality

Relationship between literature and personality	No. of respondents	Percentage (%)
Yes	38	76
No	8	16
Can't say	4	8

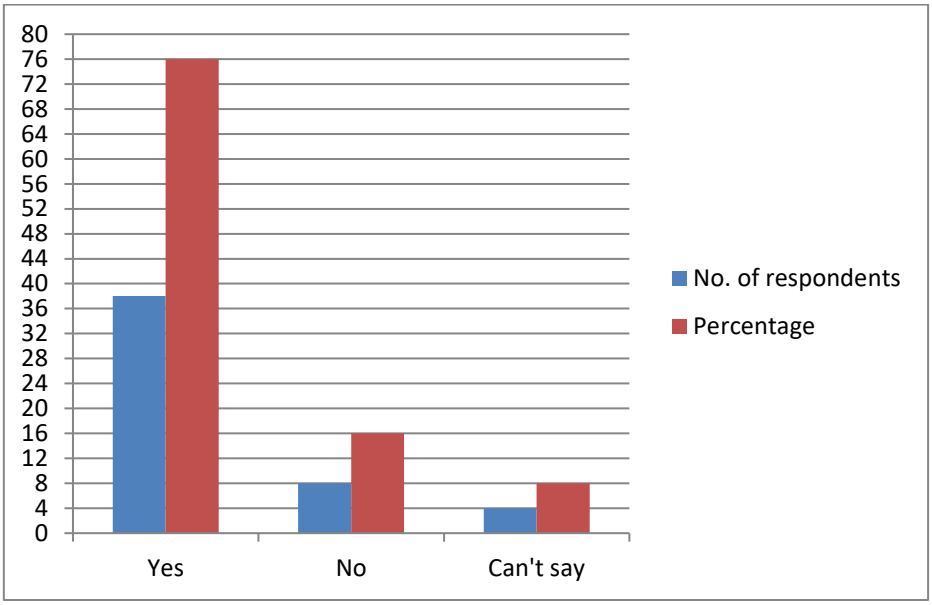


Fig.4: Percentage distribution of the relationship between literature and personality

According to the opinion of majority (76%), literature has the potentiality to change the personality, but 16% rejected the idea.

5. Do you feel that you can understand about the emotions of people because of literature?

Table 5: The understanding about the emotions of people because of literature

Emotions of people and literature	No. of respondents	Percentage (%)
Yes	34	68
No	10	20
Can't say	6	16

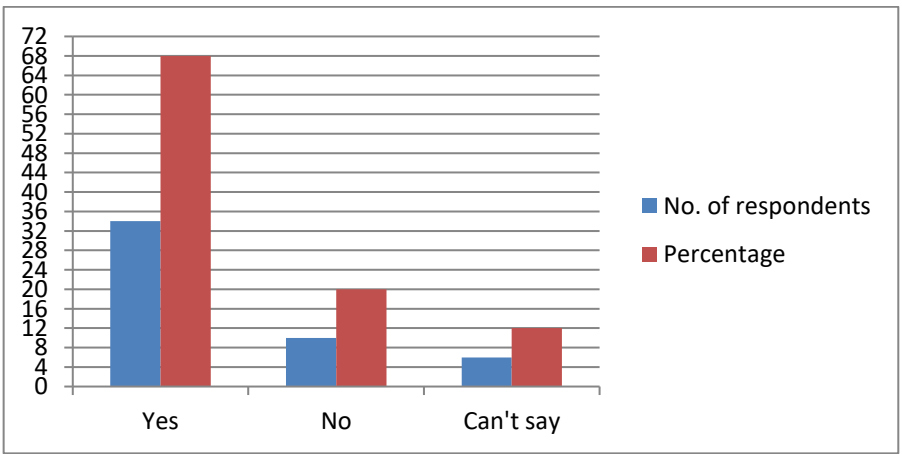


Fig.5: Percentage distribution of the understanding about the emotions of people because of literature

The above graph shows that majority of students (68%) have the attitude that literature is a medium to understand the emotions of people, whereas 20 % disagreed to this opinion. 6% respondents didn't give a proper feedback to this question.

6. Is there anything you learnt about gender sensitivity in your class?

Table 6: The teaching of gender sensitivity in the class rooms

Teaching of gender sensitivity in the class rooms.	No. of respondents	Percentage (%)
Yes	39	78
No	11	22

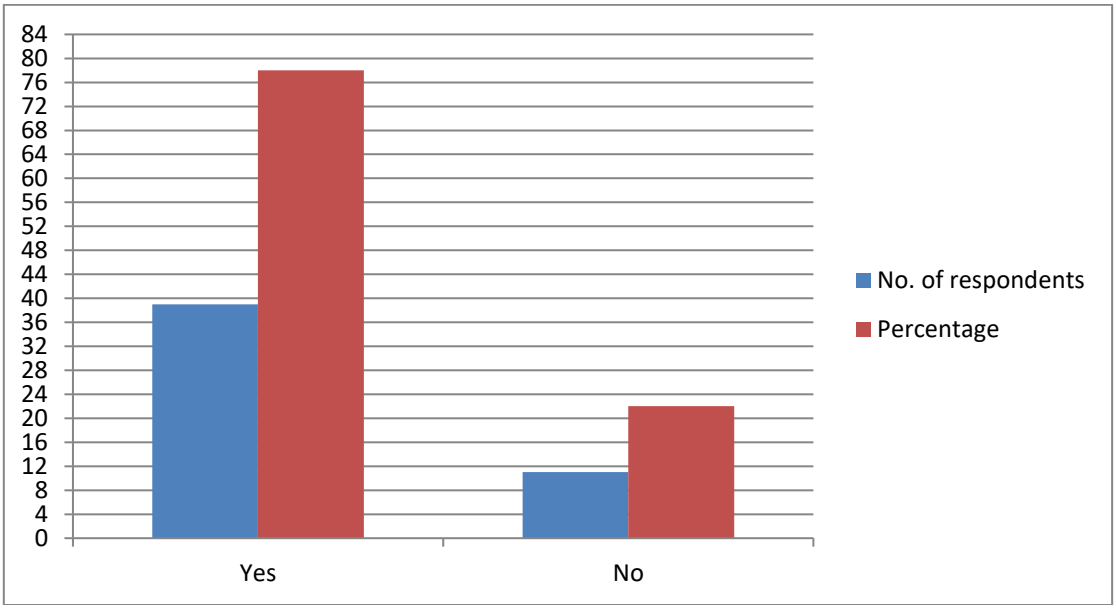


Fig.6: Percentage distribution of the teaching of gender sensitivity in the class rooms.

As the graph suggests, 78% of the respondents support that they can acquire some knowledge about gender sensitivity from the class room, but 22% of the students are against the concept.

3.RESULTS

The study, based on survey, tries to give answer to the research questions with ample suggestions. From the survey, it is observed that the respondents have different opinions about the importance of literature and the key role of literature to create awareness about gender sensitivity among students in the class rooms. The detailed analysis provides the information that literature links various dimensions of life of people and stretches its hands to build up skills and emotions from the childhood level. In order to construct personality development and gender sensitivity, the class room activities and learning styles have pivotal roles in the academic level. People believe that literature could help to understand the key issues of both personal and private realms of the society. Class room is a platform to raise consciousness about gender sensitivity among the youngsters.

4.DISCUSSION

Literature has the potential to engage people in emotional contexts and help them drive better rational conclusions. It is essential to plan a lecture and involve learners with the target emotional refinement. In this way, gender sensitivity needs a structural approach for the involvement of the students and for probing deeply into gender related literary contexts. The possible ways have been presented in the following paragraphs.

4.1 Contextualisation of Literature

Author of a literary text presents his literary work with situations conceived of the past, present, or even future. The author also determines the roles of the characters based on the historical pieces of evidence or at times, they could determine themselves, the roles as per the requirement of the work conceived, revealing them beforehand to the readers. It is an agreement established with the readers, and the rest is determined by it. The duties and responsibilities of the characters may very well be as per the historical pieces of evidence or as per the author's

characterisation. While dealing with literary works and characters, it is essential for a teacher to help the students understand the factors that determine the characterisation and the roles of the characters as male or female. The enlightenment of individuals through education enhances the perceptions of the needs of society "there is also a high regard for the personality, responsibility and autonomy of individual" (Kraus, 2019,). These characters based on the past or the present could be fictional and based on historical evidence. Characters could also be conceived because of the author's vision about the future. In this way, literary characters need to be analysed beyond the context, keeping in mind that their roles may be real, fictional, or even futuristic. This type of contextual analysis will help the students be aware of the facts involved in characterisation and not give too much importance to it and be biased.

4.2. Culturalising the Content

Priorities in the lives of human beings vary according to the influence of the culture, which, "includes all means of human survival" (Abraham, 1973), that they belong to. It is also possible that a reader is culturalised while reading and at times they pick up only what is culturally relevant to them. Therefore, the teacher needs to help the students be aware of their cultural priorities that they carry along. It is also essential for students to learn to keep the cultural elements completely away while reading a text, especially while learning about gender roles and keeping personal prejudices away. Another fact is that presentation of the elements of culture in the text is so complex that the authenticity is very hard to determine and it is, in fact, created by the author more often than not. In this way, aligning with the roles of gender because of the cultural similarities and dissimilarities can be made known to the students. The students can thus be facilitated to handle the text and evolve as potential scholars.

4.3. Moving away from Emotion to Reason

Passion is the base for any literary creation, and it requires a lot of emotional investment in the creation of literary content. The character created by the author and the plots appeal to the emotions of the people. While reading or watching a drama, individuals undergo related emotional experiences in their imagination. It is also possible for the readers to get emotionally attracted to the characters especially, gender roles, mostly because of their personal experiences and expectations. Any kind of role other than that of the normal practice is enjoyed as something additional for people's amusement. At times this extra characterization gives pleasure to the readers because of the variety. While teaching literature, it is essential to make this fact known by the students, which will help them know about the emotions at work in literary content. In other words, students should be made to experience and learn that they could emotionally take sides with the characters and roles. It is important for them to reason out as individuals capable of keeping away from emotions for better judgment.

5. CONCLUSION

The role of education plays a pivotal role in promoting gender equality among youngsters and demolishing gender-based discrimination in society. Literature offers the best platform to disuses the issues related to gender among the students. Taking the literary content appropriate to students and following it up with thought proving discussions, as mentioned above, will re-establish the role of literature among the youth and help them grow with a broad outlook over the society in particular and the world in general. Conclusions related to the study throws light on the area of class room where students can attain the ground information about gender sensitivity through literature. Apart from the knowledge acquisition, literature study widens the path of students to grasp the knowledge of personality development, understanding the life and emotions of others and skill development from the childhood stage. Literature prompts children to interrogate long-held impartial attitude and unequal treatment of human beings. Thus, education is a crucial determinant and institutional support that can effectively inculcate gender sensitivity that will guide a wide range of profits in human society. The present study is a leading one to impart modern academic levels to uphold the remedies of social issues and gender inequalities. As a local and international issue, gender sensitivity should be brought into public observation through proper education system and teaching methods. Literature study will help students to visualise gender impartiality through better reasoning and deliberation.

REFERENCES

1. Abraham, M. F. (1973). Contemporary Sociology: An Introduction to Concepts and Theories. Oxford University Press.p.64
2. Aaltio, I. And Mills, A. J. (2002). Organisational Culture and Gendered Identities in Context. In Aaltio, I. and Mills. A. J. (Eds.), Gender Identity and the Culture of Organisation, Routledge, London, pp. 3-18.
3. Dutta, N.,&Sumitral, J. (2014). Women and Gender Inequality. New Delhi: Pacific Books International. p. 18

4. Goodman, L., & Smith, A. (2008). Literature and Gender. In L. Goodman (Ed.), Literature and Gender (pp. 1-39). Routledge. p.16
5. Hamberg, K. (2008). Gender Bias in Medicine. Women's Health, pp. 237-243.
6. Harding, S. (1986). The Science Question in Feminism. Ithaca: Cornwell Publishing University Press. p. 17
7. Ercan-Demİrel, E., Ulaş-Taraf, H.A remedial course design on teaching teens and young adults in FLTE programmes(2021) Pegem Egitim ve Ogretim Dergisi, 11 (4), pp. 53-66. https://www.scopus.com/inward/record.uri?eid=2s2.085117959943&doi=10.47750%2fpegegog.11.04.06&partnerID=40&md5=28e6c184a1776488851d48be7b6897fa
8. Kraus, A. (2019). Gender, the postmodern paradigm shift and pedagogical Anthropology. In C. A. Taylor, C. Amede-Escot& A. Abbas (Eds.), Gender in Learning and Teaching: Feminist Dialogues across International Boundaries (pp. 54-67). Routledge. p. 56
9. Merry, S. E. (2009). Gender Violence: A Cultural Perspective. Wiley-Blackwell. p.84,186
10. Millet, K. (1970). Sexual Politics. Doubleday.
11. Montessori, M., & Claremont, C. A. (1969). The Absorbent Mind. New York: Dell Pub. Co.
12. Ozumba, G. O. (2005). Gender-sensitivity in Igbo culture: A Philosophical Reappraisal. Quidlibet Journal, 7(2). p 63
13. Taylor, C.A., &Ligozat, F. (2019). In Conversation: Debating gender and feminism in learning, teaching and didactics. In C. A. Taylor, C. Amede-Escot& A. Abbas (Eds.), Gender in Learning and Teaching: Feminist Dialogues across International Boundaries (pp. 172- 180). Routledge. p. 178
14. Weitzman, L. J., Eifler, D., Hokada, E., & Ross, C. (1972). Sex-role socialization in picture books for preschool children. American Journal of Sociology, 77(6), 1125-1150.

THE PORTRAYAL OF DISABLED CHARACTERS IN THE SHORT STORIES: *THE BLIND MAN* BY GUY DE MAUPASSANT AND *THE LAME SHALL ENTER FIRST* BY FLANNERY O'CONNOR.

M. Salma Banu Research Scholar National College Trichy
Dr. D.E Benet Research Guide, Associate Professor, National College Trichy Email.Id: noordheensalma@gmail.com

'Kindness is the language which the deaf can hear and the blind can see' says Mark Twain. Showing respect for each other and honoring one another is what really demonstrates wisdom. In many ways, human beings connect themselves with other creatures in the universe. The Ancient Greek philosopher Plotinus, systematized the concept of 'the Great Chain of Being' which had its major premise in the form of the hierarchical order of every creature of the universe. The chain begins with God and ends with minerals that are of great use to human beings. In the chain, humans occupy a unique position. Humans were thought to possess divine powers such as love, reason, and imagination. Like angels, human beings are knotted with a physical body. When the physical body is affected by impairment, which makes it more difficult for a person to do normal physical activities they are marginalized as disabled people. At some point, a clear distinction is often made between 'mind' and 'body'. But when considering mental and physical health, these two should not be considered separate entities. Moving the needle to disabled characters in literature, they are portrayed as villains in many works and they are used to create fear and hatred, or at another point, they are introduced to create pity in the minds of readers. This paper aims to show how the disabled characters are portrayed in the selected short stories.

The Portrayal of Disabled Characters in the Short Stories: *The Blind Man* by Guy de Maupassant and *The Lame Shall Enter First* by Flannery O'Connor.

"Attacking people with disabilities is the lowest display of power I can think of" says Morgan Freeman. The word 'Disability' hints at something either physically, mentally, or psychologically impaired. In the past, people with disability is to be marginalized and they should be excluded from the community without involving them in society The World Health Organization (WHO) has defined disability as an "umbrella term, covering impairments, activity limitations, and participation restrictions. Impairment is a problem in body function or structure; an activity limitation is a difficulty encountered by an individual in executing a task or action". The disability not only includes impairment with visual, hearing, speech, and locomotive disabilities but also mental retardation and mental illness. While some people have a congenital disability, some become disabled later in life due to accidents, falls, and assaults. The notion of TAB which is Temporarily Abled Body says that every individual will become disabled at a certain point in life.

Disability studies emerged out of a civil rights movement in the 1960s when a group of disabled people bursts out for their rights and duties in the United States. Much like the minority groups, disabled Americans were often segregated and denied certain rights to take part in social events. People with disabilities were denied entering the United States. The Public stigma changed after world war the second when many returned as disabled people. Like the Great Vowel Shift that happened in the 15th century in England, the United States took many revolutionary steps for the welfare of disabled people. Many theorists emerged during these times and gave their views on what constitutes a disability. Garland Thomson Rosemarie in her essay, "Integrating Disability, Transforming Feminist Theory" (2002) says, 'Disability is best understood as a sign system that, by differentiating and marking bodies and minds, produces dis/abled bodies and maintains the idea of the inherently stable non-disabled body or mind'. Robert McRuer and Abby Lynn Wilkerson in their book, *Desiring Disability: Queer Theory Meets Disability Studies* (2002), say 'Disabled people are infantilized, constructed as helpless and viewed as asexual'. 'Disability is understood as an act of exclusion: people

are disabled by contemporary society' says Carol Thomas in her book, *Sociologies of Disability and Illness: Contested Ideas in Disability Studies and Medical Sociology* (2007). Tom Shakespeare in his work, *Disability Rights and Wrongs Revisited* (2014) defines disabled people as 'Alive people with impairments are ignored, pitied, patronized, objectified, and fetishized. Their very presence raises questions about the Rights to Die and Assisted Suicide'.

These definitions of disability and views on disabled people give us insight into different models of disability such as the medical model, social model, cultural model, economic model, and charity model of disability. The Medical Model of disability talks about an individual's physical, mental and psychological disorder amounting to inability and debility. The Social Model of disability explains that disability is not only the debility or deformity of an individual but it is a social construct. The difference between the social model and the medical model of disability-led us to talk about the differences that 'impairment' and 'disability'. Impairment is regarded as a physical and mental deformity while disability is regarded as a social barrier that denies the opportunity for disabled people to take part in the normal life. The Cultural Model of disability defines disability as a secluded culture. The Economic Model of disability talks about the economic inability of disabled people to find work or livelihood. The Charity Model of disability is otherwise called as 'tragedy model' because it touches the integrity of a disabled person by showing pity or sympathy towards them.

In the past, several attempts have been made to categorize the negative aspects of disabled people. When the sin, and punishment in disabled people are taken as a component of psychodynamics psychology, the result will be an aversive reaction towards them. Several unconscious psychological processes have been attributed by the non-disabled to the disabled. They are:

1) Disability as a punishment for sin.
2) The individual with a disability is a dangerous person.
3) Vicarious self-punishment offered by the punished disabled person.

Edith Wharton says that 'every literature, in its main lines, reflects the chief characteristics of the people for whom, and about whom, it is written'. Disability studies as an interdisciplinary area of study talk about the existence of disabled people and then about their experiences as disabled people and the major theme in disability studies is how the non-disabled people treat disabled people. It views disability in the social, political, and cultural context rather than giving importance to the medical model of disability. The approaches and the methodologies for this particular area of study include the themes of disability in literature, language (metaphors) used to point out disabled characters, the history of disabled people and disability studies, and the most important element in disability literature is the portrayal of disability experience and how the human society gaze and treat people with disability, the psychology of disabled people and the point when their disability becomes their identity and considered disability as their pride.

Moving the needle to the American literature, the disabled characters are often portrayed as the 'other' and their presence is used to create fear, and pity and to show horror by describing their physical appearance. The authors used the disabled characters to be villains, who will undoubtedly face a tragic situation created by a hero or else even die at the end in the hands of a hero. Portraying disabled people with negative stereotypes is the main concern on one hand whereas on the other they are treated as less than human beings. The disabled character, Captain Ahab in Herman Melville's *Moby-Dick* (1851) is the captain of the ship Pequod and he wears a prosthetic leg made out of whalebone dies at the end as the whale drags him to the sea. This character had a strong will to catch the whale but at last, death is given as a gift for his determination and Captain Ahab is a disabled character who died without attaining success. Then in the 20th century, *The Sound and the Fury* (1929) by William Faulkner introduced Benjy, a 33-year-old character dependent upon Caddy due to his severe intellectual disability and the character whose thoughts are not heard but he is the one who understands every character's inner conflict and the one who moans the death of his father and understands the situation of caddy. In this novel, Faulkner portrays the disabled character as a curse or punishment to their families. Then the character, Laura in the play, *The Glass Menagerie* (1944) by Tennesse Williams is

a person who walks with a limp and wears a leg brace after being crippled by pleurosis and she is portrayed as sensitive both emotionally and as well as physically. This character is also secluded from the play but as a reader, we can learn from Laura the importance of self-acceptance and on critically analyzing the character we will know that she is the one to pacify his brother and mother when they engage in a heated argument. In John Steinbeck's, *Of Mice and Men* (1937) Lennie Small is a mentally retarded character and is often compared to animals, he is portrayed as a 'shapeless face, with large, pale eyes, with wide sloping shoulders, and he walked heavily dragging his feet a little, the way a bear drags his paws' (2). And in the poem, *The Applicant* (1963) by Sylvia Plath, the salesman interviews a person who has applied to buy a wife and it is a satirical poem that condemns the gender stereotypes but the poem starts with these lines

> First, are you our sort of person?
> Do you wear
> A glass eye, false teeth or a crutch,
> A brace or a hook,
> Rubber breasts or a rubber crotch,
> Stitches to show something's missing?

This shows how disabled people will be denied marital life. So almost all the genres of American literature such as novels, plays, poetries portray disabled characters.

The history of disability in France dates back to the French revolution and especially after the First World War when the people with disabilities began to protest for their rights and development after Second World War, schools and inclusive education were given importance. French literature also has characters with disabilities and in this literature also disabled characters are portrayed as evil, sometimes used to create horror and most of the time to create sympathy. Victor Hugo was a French poet, novelist, and dramatist of the Romantic movement. He touched on all the genres of writing such as novels, lyrics, history, critical essays, drama, prose, etc. In his novel, *The Hunchback of Notre Dame* (1831) has the character, Quasimodo a 19-year-old hunchback and half-blind and fully deaf who confines himself to the cathedral because the citizens of Paris make fun of him for his appearance. In the 20th century, a writer like Henri Rene Albert Guy de Maupassant who is considered a veteran short story writer wrote nearly 300 short stories, three travel books, and one volume of verse. He has written short stories which had characters with disability and he has shown them how society treated those people and how they are used for entertainment purposes in the short story, *The Blind Man* by Guy de Maupassant.

The short story, *The Blind Man* by Guy de Maupassant represents a blind man who is a peasant and the son of a Norman farmer. As long as his father and mother lived, he is more or less taken care of but when they were gone, everybody in the village treated him as a beggar who is eating the bread of the strangers. His brother-in-law had taken all the possession of his portion of the inheritance and he was called a drone, a clown and he is used as a laughing stock in the village.

'Sometimes they placed before his plate, when he was beginning to eat his soup, some cat or dog. The animal instinctively perceived the man's infirmity, and soft approaching, commenced eating noiselessly, lapping up the soup daintily, and when they lapped the food rather noisily, rousing the poor fellow's attention, they would prudently scamper away to avoid the blow of the spoon directed at random by the blind man' (70).

The blind man suffered a lot because of the appalling brutality and he is treated as the worst creature by the so-called normal human beings. He is beaten by lashes and the brother-in-law cuffed him incessantly and there came a new pleasure for them that is the pleasure of smacking his face. And in the end, he was forced to beg. The cruel treatment by the people around him ends in the death of the person. Literature reflects life and thus so this short story reflects how disabled people were treated in the past. 'The following week these gloomy birds were still there and a young fellow went to see what they are doing and discovered the body of the blind man, already half devoured, mangled. His wan

eyes had disappeared, pecked out by the long, voracious beaks' (70). And even after the death of the blind man nobody cared to bury his body and it is left as prey for the birds.

The disability in American literature also treated people with disability very cruelly and also the disabled people were used as an alibi. The best example of this kind of treatment toward disabled character is shown in the short story, *The Lame Shall Enter First* (1965) by Flannery O'Connor. This short story first appeared in The Sewanee Review in 1962 and was published in 1965 in her short story collection, *Everything That Rises Must Converge*. The story, *The Lame Shall Enter First (*1965) is about a fourteen-year-old boy with a clubfoot named Johnson Rufus who strongly believes in God and was raised by his cruel grandfather. Sheppard who is an atheist and a middle-aged widower supported him and showed pity and concern for him to get the best out of him. Rufus was raised by his 'grandfather in a shack without water or electricity and the old man beat him every day' (447). When Sheppard met Rufus for the first time, he was trying to get something to eat out of a garbage can. This shows how people with disability are not cared for and they were denied food, shelter, and education. As Sheppard was a City Recreational Director, he worked at the reformatory as a counselor and by that time Sheppard happens to read the record of the boy which says 'senseless destruction, windows smashed, city trash boxes set fire, tires slashed but the IQ of Rufus Johnson was 140. A small boy with an IQ of nearly 140 which is considered a high or genius-level IQ is denied education and he doesn't have a piece of bread to eat. Sheppard bought Johnson into his home and the first thing Sheppard wants to do buy for Johnson is a new orthopedic shoe. Then the days passed with heated arguments between Johnson and Sheppard about Bible and God. One day when Johnson was about to leave the house, Sheppard expressed the real notion of bringing Johnson to his house. Sheppard says

'We need another boy in the house. Norton never had to divide anything in his life. He doesn't know what it means to share. And I need somebody to teach him' (457). So the character is used as a tool to correct the behavior of his son Norton. While Norton, Sheppard, and Johnson were discussing about the astronauts and the hell, Johnson said, 'I ain't going to the moon and get there alive and he said, when I die I'm going to hell' (461). These words shocked Norton and Norton asked about his mother, 'Is she there?' (461). The opening up of the truth about Norton's mother created a rage in the mind of Sheppard who behaved violently towards Johnson and as a result, Johnson left home. When Johnson was out, he was falsely accused of having broken the dishes in a house and furniture turned upside down. When Sheppard came out searching for Johnson, he saw police accusing Johnson but he is not ready to save Johnson.

'This was crucial. The boy would have to learn that he could not be protected when he was guilty. "You'll have to go with him, Rufus," he said' (465). This incident shows how a disabled person was used as an alibi and he was charged and accused of theft in which he was not even slightly involved. And again the police freed him and again when Sheppard took Rufus to his house and offered him the new orthopedic shoe, Johnson refused it completely by saying, 'The Lame Shall Enter First'. This shows how disabled people refuse and really hate to treat them as disabled people and they completely avoid the assistive technology which will remind them as disabled people and which seclude them from the society. Again the policemen entered the house and arrested Johnson and this time it was Johnson want to get caught himself deliberately moving out from the place where his pride in his disability gets wounded. Finally, Johnson's voice is heard as he muffled inside the car. The story ends with the hanging of Norton as he frequently gets the thoughts of his dead mother. This story shows Johnson, a disabled person by birth had to suffer a lot to live in this society and he is taken as an alibi and he is accused as the murderer of Norton.

In discussing the portrayal of the disabled characters in French and American pieces of literature, they are shown as supporting characters, sometimes pitied, most of the times hatred, ignored, and presented as victims and villains to make the storyline. Like a puppet in the hands of a puppeteer, the disabled characters are puppets in the hands of so-called normal human beings and the disabled characters are used as tools to create horror, pity in the minds of readers in literature and it will be

wrong to say that disabled characters are not portrayed in literature. They are portrayed with negative stereotypes.

References

Maupassant, Guy de. *Original Short Stories.* Volume II. Translated by Albert M.C. McMaster, B.A., A.E Henderson, B.A., NME.Quesada. 2018. www.gutenberg.org

O'Connor, Flannery. *Everything That Rises Must Converge*. Farrar, Straus and Giroux, 1964.

Davis, Lennard J. (ed.). *The Disability Studies Reader*. Newyork: Routledge, 2006.

Goodley, Dan. *Disability Studies: An Interdisciplinary Introduction.* SAGE publications, 2011.

Shakespeare, Tom. *Disability Rights and Wrongs Revisited*. Newyork: Routledge, 2013.

Thomas, Carol. *Sociologies of Disability and Illness: Contested Ideas in Disability Studies and Medical Sociology*. United Kingdom: Palgrave MacMillan, 2007.

Available online at www.jlls.org

JOURNAL OF LANGUAGE
AND LINGUISTIC STUDIES

ISSN: 1305-578X

Journal of Language and Linguistic Studies, 17(4), 2356-2359; 2021

CHETAN BHAGAT'S *2 STATES:* A PSYCHOANALYTIC READING

Ms. K. Prabha [a1], Dr. R. Soundararajan [b]

[a] *Research Scholar (Ph.D), PG and Research Department of English, National College (Autonomous), (Affiliated to Bharathidasan University), Tiruchirapalli, Tamilnadu.*
prabhaeng@nct.ac.in

[b] *Associate Professor & Research Advisor, PG and Research Department of English, National College (Autonomous),(Affiliated to Bharathidasan University), Tiruchirapalli.*
soundararajaneng@nct.ac.in

APA Citation:

Ms. K. Prabha , Dr. R. Soundararajan (2021). CHETAN BHAGAT'S *2 STATES:* A PSYCHOANALYTIC READING, *Journal of Language and Linguistic Studies, 17*(4), *2356-2359*

Submission Date: 22/08/2021
Acceptance Date: 18/11/2021

ABSTRACT:

Chetan Bhagat's *2 States*: *The story of My Marriage* deals with the hardships of the two protagonists Krish and Ananya. To rebel or fight is easy but to convince the parents is tough. The differences in State, Language, Culture and Tradition between them creates chaos and confusions in the story. The couple undergo a tough battle to fulfil their dream wedding. Bhagat, on the other hand portrays the emotional conflicts between Krish and his father. Krish's hatred with him can be taken as an act of repression. This paper is an attempt to study on the psychoanalytical concepts in the novel.

Keywords: unconscious mind, repression, emotions, depression, anxiety, psychopath

Introduction:

Sigmund Freud's contributions to the development of psychoanalytical theories are innumerable. His theories, therapeutic methods, to analyse the functions of both conscious and unconscious levels of mind is a boon to the field of psychology. In literature, one may psychoanalyze a particular character in any literary work using the theories of Freud. Psychoanalytic criticism interpret texts in various ways to analyse the desires, emotions, psyche of the characters projected by the author M.H.Abrams in his *A Handbook of Literary Terms States* the importance of Freudian concepts to analyse literature as,

Freud proposes that literature and the other arts, like dreams and neurotic symptoms, consist of the imagined or fantasied, fulfilment of wishes that are either denied by reality or are prohibited by the social standards of mortality and propriety (248)

2 States the story of my marriage:

Chetan Bhagat deals with various issues like monotonous academic system, corruption and scandals in politics, caste discrimination, gender bias etc in India in almost all of his novels. The novel chosen for the study is quite unique, as it focuses on the difference in culture, language, food and identity in two important states in India – Punjab and Tamilnadu respectively. Both Krish and Ananya hails

[1] Corresponding author.
E-mail address: prabhaeng@nct.ac.in

© 2021 Cognizance Research Associates - Published by JLLS.

from the above mentioned states, meets in Indian Institute of Management, Ahmedabad where they pursue their master's degree. They become friends in the beginning later on they fall in love with each other unminding the bias in their culture and language.

The love between them grows stronger and they decided to get married with the consent of the elders in the family. The young protagonists are ready to accept the new changes in the family and society. But the elders are very strict in tradition, rituals, beliefs and conventions. Neither Krishs' mother nor Ananya's family is ready to approve the marriage. The young lovers are very strong in the decision, not to elope but to get the consent of the elders to have a happy wedding life. They took many efforts to bring harmony between the two families. They arrange special meetings among the elders in various occasions to let them share their opinions.

Chetan Bhagat's *2 States* portrays the emotional struggle of a father and a son. From the beginning of the novel, Krish mentions this towards the strict father. To implement discipline in the family, he strongly opposes gathering of relatives, watching TV etc. After retired from the Armed Forces, he joins hand with friends to start business. His mess with higher authorities in the army shows his uncontrollable anger.

There is no one-on-one conversation between the father and the son for many years. The only consoling soul in the family to uphold the spirit of Krish is his mother Kavitha. Being a single child in the family, Krish needs to maintain the balance in this bond. Kavitha's role as a quiet mother shows her concern for both the father and son. Her tough battles with Mr.Malhotra after her wedding is a typical representation of male chauvinism. Krish admits the fact of denying his matriarchal relatives due to the strict orders of his father. Kavitha finds opportunities to invite her siblings during the absence of Malhotra, which Krish admits as,

'my father didn't come home until late so my mother had taken the risk and invited her sister home for lunch. Some Indian men cannot stand any happiness in their wives' lives, which includes her meeting her siblings' (*2 States* 67).

Lack of Parental care:

Love and care of parents plays an important role in the psyche of a child. Krish never sees that in his lifetime. On the other hand, he receives abundant love from Kavitha, his mother. From biscuits to bride, Kavitha interferes in the choices made by Krish. When the love between Krish and Ananya is revealed to Malhotra, the whole family is under severe emotional trauma. Krish spends sleepless nights thinking about the quarrel. He finds emotional comfort. Freud in his *Interpretation of Dreams* (1895) coined 'Oedipus complex' which refers to the child's unconscious sexual desire for the opposite sex parent and hatred for the same-sex parent. In the novel, a letter from Krish's father disturbs him a lot. His mind is conceived with hatred towards his father. Family issues, problems in love life, pressures in workplace stimulates the level of anxiety within him. In the late night, he visits his mother to have a peaceful sleep. He says, I came back to my bed and tossed the letter in the bin. I felt light after speaking to my mother and drifted off to sleep in five minutes. What would the world be without mothers? (*2 States 159)*.

Bhagat celebrates 'motherhood' in the novel and equally a kind of bitterness towards the attitude of his father is mentioned in some chapters. Human mind is a complex process which can express love and hatred at the same time. Elizabeth Auchincloss' *The Psychoanalytic Model of the Mind'* refers to negative Oedipus complex as 'hatred for the opposite-sex-parent (110). This hatred is beyond the limit when the father and son deal with the problem in sharing the same house. Krish's extreme revulsion is revealed when his father says,

'I hope you leave home soon.'

I hope you leave this world soon, I responded mentally as I took my plate and left the room. (*2 States* 59)

Repression:

Sigmund Freud's theory of repression throws light on his urging the patients to remember the past in a conscious state. The past experiences in one's life eventually mixes up with the present time. To decide a healthy future, one needs to hold positive approach towards the life. Human beings bury the past incidents within the mind and voluntarily prevents them to mess in the present. This act of repression is also a defence mechanism to never permit the bitter past to mix up with the present happiness. Freud in his *Five Lectures on Psycho Analysis* mentions the struggles of his patients to recall the past memories as:

© 2021 Cognizance Research Associates - Published by JLLS.

…there was some force that prevented them from becoming conscious and compelled them to remain unconscious… pushed the pathogenetic experiences in question out of consciousness. I gave the name of repression to this hypothetical process (28-29).

The conversations between Krish and Guruji in Pondicherry Ashram reveals the repression in Krish's mind. He struggles a lot to recollect the quarrel between his father and him. His pain come out as emotional outburst during his recollection. His father mocks Krish for crying like a weak man. He can't bear the disgusted glances and gave his father a slap.

Krish's repressed memories are recollected during the therapy session with Guruji. By revealing it to him, Krish tries to minimise the feelings of guilt and anxiety. Holding a baggage of anger, pain and loss is heavy for him. To stop the never-ending inner sufferings of Krish, Guruji offers 'Forgiveness' as a solution. He says, for you, forgiving doesn't make the person who hurt you feel better, it makes you feel better' (*2 States* 168). All counselling, meditations and therapy sessions offers lightness in his conscious mind. He feels light and decides to surrender to God.

To convince the parents is an unending task for both Krish and Ananya in which they fail often. The reconciliation between the two families in Goa fails and results in a kind of disgrace to Ananya's parents. Ananya's sudden breakup in relationship puts Krish in severe emotional turmoil. Bala, his friend takes his to a psychiatrist Dr. Iyer. During the counselling session, Krish exercises wide range of emotions like frustrations, anxieties, depressions and sudden unpredictable emotions. His sleepless nights and longings for love disturbs him a lot. Krish understands the tight situation in his house. This restricted communication results in a serious mental disorder known as 'split personality' (*Dictionary of Psychopathology* 57) within him. Krish's encounter between him and his freaked-out me goes on like this:

Calm down, Krish this is going to pass. I told myself. This was the sensible me talking. No baby, this time you are so fucked. This is how you will feel for the rest of your life, the freaked-out me said. That's nonsense. Whatever crap happens in life, one gets used to it … The world's most sensible person and the biggest idiot both stay within us. The worst past is, you can't ever tell who is who (242-243).

This alternative personalities in one's mind will create disorder. To get a better recognition and smooth life, every individual is striving hard. A kind of split personality will occur when violation in social, cultural or psychological norms happen.

To live a normal life in this complicated world is a dream. The primary instinct of any individual to lead a life with tolerance and liberty needs energy to balance the emotions. The world offers different emotions, different experiences to all of us. The basic understanding of all these variations is necessary for a peaceful life. Krish and Ananya tries to pacify the differences between the families. Mr.Malhotra, the father plays a significant role in convincing the elders. To resolve the problems, he makes secret moves for his son. The final chapters show a major twist in the relationship between Krish and his father Malhotra. In the contemporary world, all are playing crucial roles in multiple ways. To settle as a normal is a probability, which Krish admits as:

why does it have to be a choice? Why can't mom get her relatives and you come as well? Why can't we be a normal family for once? I thought. I guess there are no normal families in the world. Everyone is a psycho and the average of all psychos is what we call normal (247).

Bhagat's portrayal of female characters in the novel shows his concern for the opposite sex. Mrs. Kavitha is a silent sufferer, whose only hope is her son. As a responsible mother, she tolerates with the elders in the family to maintain the balance in the family. Familial relationships in the story adds more flavour in the course of life. Radha Swaminathan, Ananya's mother, on the other hand achieves her desire without violating the traditional beliefs. Her interest in constant learning promotes her success to the next level. Mr.Swaminathan and others are astonished by her hidden talents. The younger women in the story like Anaya and Minti are steady in their passion. With a balanced mind, Ananya thinks a lot before she dives into action. Her potential to convince the elders is executed carefully without hurting the beliefs of them. On the whole, women characters in the novel stand strong as pillar of support to the men, which is inevitable for peaceful existence.

Conclusion:

The writer Chetan Bhagat is a renowned columnist, motivational speaker and excellent screenplay writer. He has got many opportunities to meet the young students in and around India. He is travelling

© 2021 Cognizance Research Associates - Published by JLLS.

to many educational institutions to deliver motivational lectures. His chance to meet the youth of today has helped him to analyse closely the problems and aspirations of them. His interpretations with them provides him an idea about the pulse of the younger generation, which he depicts well in his novels.

Bhagat's *2 States* satisfies the readers by providing the importance of maintaining cordial relationship with others. To meet the outside world isn't easy. Every character in the novel is characterised with such demands. The psychoanalytic reading of the novel helps the readers to understand the inner psyche of the characters. The ego, unconscious mind, repressions and dreams are interpreted and analysed carefully. More than a love story, the novel projects a close reading of the conscious mind of the characters which is essential in the present day.

References:

- Abram's M.H. & Geoffrey Galt Harpham.,editors. *A Handbook of Literary Terms*. India: Wadsworth, a part of Cengage Learning. 2009
- Auchincloss, Elizabeth. L. *The Psychoanalytic Model of the Mind.* Washington, DC American Psychiatric Publishing., 2015.
- Bhagat, Chetan. *2 States: The Story of my Marriage.* New Delhi: Rupa Publications India Private. Ltd., 2009
- Freud, Sigmund. *Fire Lectures of Psychoanalysis*. Penguin. 1995.
- Kellerman, Henry. *Dictionary of Psychopathology.* Columbia University Press. 2009.
- Nagera, Humberto.,editor. *"Oedipus complex" Basic psychoanalytic concepts on the Libido Theory.* London: Karnak Books. 2012.

© 2021 Cognizance Research Associates - Published by JLLS.

Available online at www.jlls.org

JOURNAL OF LANGUAGE AND LINGUISTIC STUDIES

ISSN: 1305-578X

Journal of Language and Linguistic Studies, 17(3), 2013-2020; 2021

High School Students Attitude Towards Speaking And Reading Skills In English

S. Jayasudhaa[a] , Dr. V. Srividhya[b]

[a]*Full- time PhD Research scholar, National College (Autonomous) (Affiliated to Bharathidasan University), Tiruchirappalli – 620 018.*

[b]*Assistant Professor (SS) & Research Supervisor, PG & Research Department of English National College (Autonomous) (Affiliated to Bharathidasan University), Tiruchirappalli - 620 018.*

APA Citation:

Jayasudhaa, S., & Srividhya, V. (2021). High School Students Attitude towards Speaking and Reading Skills in English, *Journal of Language and Linguistic Studies, 17*(3), 2013-2020

Submission Date:08/10/2021

Acceptance Date:22/12/2021

Abstract

When it comes to language development, growth or degradation, repair or destruction, attitudes are critical. Language learning is made up of four skills: listening, speaking, reading, and writing. Though each skill is significant in and of itself, reading and speaking abilities are more vital because language is used to communicate or express one's ideas and emotions. The purpose of this survey was to find out how high school pupils felt about their English speaking and reading abilities. The research included 94 high school students from the Tiruchirappalli district. The tool created by the investigator was employed. The attitudes of high school pupils about English speaking and reading abilities were evaluated in connection to a number of demographic parameters. The results demonstrated that parental education had an impact on pupils' attitudes regarding their English speaking and reading abilities. These study results should be considered by syllabi designers and instructors in order to offer students with world-class language learning.

Keywords*: Attitude, Reading Skill, Speaking Skill, and High School Students*.

1. Introduction

The English language plays an important role in expanding work prospects, and efforts should be made to educate and develop students' English language abilities at all stages of school, resulting in world-class education and training. It changes the nation by preparing the country's young for a bright future. Reading is a mental activity or mental process that involves digesting and comprehending information from written materials (Windra,2017). Reading ability is one of the most powerful tools people have in both their scholastic and social lives. It also comes before all of the other abilities. The act of recognising, understanding, and comprehending written or printed content is known as reading (Binti and Binti,2021). Reading habit is an attitude that should be considered while interpreting life's reality, and one of the greatest methods to instil a good attitude in children is for instructors and teacher candidates to have the same attitudes. Listening, Speaking, Reading, and Writing (LSRW) are the four language abilities that are regarded to be the most essential and sought after by individuals from all walks of life (Ali, Shamsan, Guduru & Yemmela,2019). Attitude is a taught propensity to react evaluatively to people, ideas, and situations. Attitudes are made up of beliefs, sentiments, and proclivities to behave (Muchtar n.d). Attitudes have a critical role in the development or deterioration

© 2021 JLLS and the Authors - Published by JLLS.

of a language, as well as its repair or annihilation. Internal states that determine what learners are likely to do are known as attitudes. The internal state is a response to an item that is either good or negative, or favourable or unfavourable (Zeinivand et al.,2015). Attitude is not something that is passed down from generation to generation, and it is influenced by knowledge and dependability. These components work together to determine a person's credibility. Language attitude is an important issue because it affects how people learn and teach languages. One of the most important determinants of English communication success is one's attitude toward the language (Toomnan and Intaraprasert,2015).

2. Literature Review

The role of emotional elements on learning to read has been addressed in recent literacy studies (Nootens et. Al,2019). Parents, students, and the general public are becoming more aware of the need of learning English for many vocational and employment goals. Students may use English to communicate and showcase their culture to the rest of the world (Ali, Shamsan, Guduru and Yemmela,2019).

Assignments on reading habits should be offered to pupils to assist them establish a good attitude toward reading (Kirmizi et al,2014).

Learners' attitudes about language acquisition are regarded one of the most important variables in encouraging learners to learn that language (Zeinivand et al,2015).

For coping with communication failures, students with good views reported utilising substantially more often than students with negative attitudes (Toomnan and Intaraprasert,2015). Because of its relevance to reading behaviour and the refining of reading abilities, reading attitude is an essential educational outcome and evaluating criterion (Muchtar,n.d).

Invalid examinations, a bad examination system for language courses, or a lack of systematic evaluation for speaking abilities may be to blame for underachievement in speaking skills (Kurapati,2016).

Students who had a favourable attitude toward speaking English used strategies more often and in a greater variety than those who had a negative attitude (Rosmalasari , Sutapa, and Susilawati,n.d).

Today, there is a pressing need to instil in kids the habit of lifelong learning. It not only improves their reading attitudes, but it also enhances their comprehension of reading (Binti and Binti,2021).

Understanding the impact of readers' attitudes is crucial since attitudes may influence a student's final level of ability by influencing aspects like engagement and practise (Akbari et. al,2017).

Some findings also show how important individual student and household circumstances are in improving pupils' reading attitudes (Nonte,Hartwich and. Willems,2018).

According to Elkilic (2016), the most crucial skill is speaking, and the participants were satisfied with speaking classes despite the fact that they were not skilled enough to talk. Crivilare investigated the association between various environmental conditions and reading, as well as the relationship between reading habits and college preparedness (2019).

3. Objectives of the Study

- To assess the level of attitude of high school students towards reading and speaking skills in English.
- To identify the level of attitude of high school students towards reading and speaking skills in English in relation to certain demographic factors.

© 2021 JLLS and the Authors - Published by JLLS.

4. Objectives of the Study

- There is no significant difference between high school students in their attitude towards speaking and reading skills in English with respect to Gender.
- There is no significant difference between high school students in their attitude towards speaking and reading skills in English with respect to Locality.
- There is no significant difference between high school students in their attitude towards speaking and reading skills in English with respect to Type of Management.
- There is no significant difference between high school students in their attitude towards speaking and reading skills in English with respect to Type of School.
- There is no significant difference between high school students in their attitude towards speaking and reading skills in English with respect to Type of Stay.
- There is no significant difference between high school students in their attitude towards speaking and reading skills in English with respect to Type of Family.
- There is no significant difference between high school students in their attitude towards speaking and reading skills in English with respect to Parents Educational Status.
- There is no significant difference between high school students in their attitude towards speaking and reading skills in English with respect to Parents Annual Income.
- There is no significant difference between high school students in their attitude towards speaking and reading skills in English with respect to Parent Occupation.
- There is no significant difference between high school students in their attitude towards speaking and reading skills in English with respect to Type of Board.

5. Method

The current research is descriptive and used a survey approach to collect data. The perspectives of high school pupils about their English speaking and reading abilities were investigated. The research used stratified random sampling. The study's participants are high school students from Tamilnadu Tiruchirappalli district. The kids were divided into two groups depending on whether they attended rural or urban schools. The investigator then chose high school pupils at random from several schools. The research included a sample of 94 high school pupils. They were 14 and 15 years old and in the 9th and 10th grades, respectively.

6. Instruments

The Attitude Scale Towards Speaking and Reading Skills in English (ASTSRE), which was designed by the investigator, was employed. The questionnaire has 40 questions with likert answers such as strongly agree, agree, uncertain, disagree, and strongly disagree. For both positive and negative items, an appropriate scoring system was used. Five of the forty items were negatively phrased.

The survey was given to 94 high school students, and the results were gathered. The content validity of the question items was reviewed by three experts in order to enhance simplicity of administration and assure improved accuracy of results. More significantly, the participants were informed before to the administration of the questionnaires to ensure that they were aware of the study's purpose and significance. The investigator said that there is no right or incorrect response and asked the participants to provide honest replies. The investigator also expressed gratitude and assured secrecy. The scale's Split half dependability score was determined to be 0.712. For the study of the collected raw data, statistical tests such as Mean, Standard Deviation, "t" test, and ANOVA were computed.

7. Results and Findings

Hypothesis 1: In terms of gender, there is no substantial difference in high school pupils' attitudes on English speaking and reading ability.

© 2021 JLLS and the Authors - Published by JLLS.

Table 1 Mean, Standard Deviations and 't' value of Male and Female of high school students						
Variable	Gender	N	Mean	Std. Deviation	"t" value	Level of Significance
Gender	Male	46	152.24	15.805	0.359	Not Significant at 0.05%
	Female	47	153.43	16.077		

The 't'-value of mean score of male and female high school students is 0.359, which is not significant at the 0.05 level, as shown in table 1. It suggests that attitudes regarding speaking and reading abilities do not change considerably across male and female pupils. Furthermore, the mean results suggest that male students have a score of (152.24) and female students have a score of (152.24). (153.43) As a result, the null hypothesis "There is no substantial gender difference in high school students' attitudes on speaking and reading abilities in English" is accepted.

Hypothesis 2: In terms of attitude toward speaking and reading abilities in English, there is no substantial difference amongst high school pupil.

Table 2 Mean, Standard Deviations and 't' value of Rural and Urban of high school students						
Variable	Locality	N	Mean	Std. Deviation	"t" value	Level of Significance
Locality	Rural	43	151.98	19.532	0.484	Not Significant at 0.05%
	Urban	50	153.58	12.021		

The 't'-value of the mean score of Rural and Urban high school students is 0.484, which is not significant at the 0.05 level, as shown in table 2. It shows that attitudes regarding speaking and reading abilities in English are not substantially different between rural and urban pupils. Furthermore, the mean scores suggest that rural students have a score of (151.98) and urban students have a score of (151.98). (153.58). As a result, the null hypothesis is accepted: "There is no substantial difference in high school students' attitudes on speaking and reading abilities in English with regard to locality."

Hypothesis 3: In terms of Type of Management, there is no substantial difference in high school students' attitudes about speaking and reading abilities in English.

Table 3 Summary of ANOVA showing the significance difference in the Type of Management

ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	31.335	2	15.667	.061	.941
Within Groups	23131.246	90	257.014		

It can be observed from table (3) that the ANOVA comparisons do not demonstrate a significant difference. The computed 'F' value is (.061), which is not statistically significant at the 0.05 level when using df (2,90). It shows that the degree of attitude toward speaking and reading abilities in English does not alter considerably depending on the kind of management. As a result, the null hypothesis is accepted: "There is no significant difference between high school students in their attitude on speaking and reading abilities in English with respect to Type of Management."

Hypothesis 4: With regard to Type of School, there is no substantial variation in high school students' attitudes regarding speaking and reading abilities in English.

© 2021 JLLS and the Authors - Published by JLLS.

Table 4 Summary of ANOVA showing the significance difference in the Type of School

ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	75.479	2	37.739	.147	.863
Within Groups	23087.102	90	256.523		

It can be observed from table (4) that the ANOVA comparisons do not demonstrate a significant difference. The computed 'F' value is (.147), which is not statistically significant at the 0.05 level when using df (2,90). It implies that the students' attitudes on speaking and reading abilities in English are unaffected by the kind of school they attend. As a result, the null hypothesis 'There is no substantial difference in high school students' attitudes on speaking and reading abilities in English as a function of school type' is accepted.

Hypothesis 5: There is no significant difference between high school students in their attitude towards speaking and reading skills in English with respect to Type of Stay.

Table 5 Mean, Standard Deviations and 't' value of high school students based on their type of Stay						
Variable	Type of Stay	N	Mean	Std. Deviation	"t" value	Level of Significance
Type of Stay	Hosteller	12	153.08	20.434	0.057	Not Significant at 0.05%
	Day Scholar	81	152.80	15.235		

The 't'-value of Hosteller and Day Scholar high school students is.057, which is not significant at the 0.05 level, as shown in table 5. Based on Type of Stay, it seems that attitudes regarding speaking and reading abilities in English do not vary considerably between Hosteller and Day Scholar students. Furthermore, Hosteller students have a mean score of (153.08) and Day Scholar students have a mean score of (152.80). As a result, the null hypothesis is accepted: "There is no significant difference in high school students' attitudes on speaking and reading abilities in English with regard to Type of Stay."

Hypothesis 6: In terms of Type of Family, there is no substantial variation in high school students' attitudes regarding speaking and reading abilities in English.

Table 6 Mean, Standard Deviations and 't' value of high school students based on their type of family						
Variable	Type of Family	N	Mean	Std. Deviation	"t" value	Level of Significance
Type of Family	Nuclear	63	152.48	14.460	0.318	Significant at 0.05%
	Joint	30	153.60	18.730		

The 't'-value of nuclear and joint family high school pupils is 0.318, which is not significant at the 0.05 level, as shown in table 6. It shows that attitudes regarding speaking and reading abilities in English do not change substantially between kids from nuclear and joint families in high school. Furthermore, the mean results for Nuclear students (152.48) and Joint students (152.48) reveal that (153.60). As a result, the null hypothesis "There is no substantial difference in high school students' attitudes on speaking and reading abilities in English based on family type" is accepted.

Hypothesis 7: With regard to parents' educational status, there is no substantial variation in high school pupils' attitudes regarding speaking and reading abilities in English.

© 2021 JLLS and the Authors - Published by JLLS.

Table 7 Mean, Standard Deviations and 't' value of high school students based on their Parents Educational Status						
Variable	Parents Educational Status	N	Mean	Std. Deviation	"t" value	Level of Significance
Parents Educational Status	Graduate	42	156.26	16.088	1.965	Significant at 0.05%
	Non-Graduate	51	150.02	15.266		

The 't'-value of Graduate and Non-Graduate high school students is 1.965, which is significant at the 0.05 level, as shown in table 7. It shows that attitudes on speaking and reading abilities in English vary substantially between Graduate and Non-Graduate students. Furthermore, Graduate students have a mean score of (156.26), whereas Non-Graduate students have a mean score of (150.02). As a result, the null hypothesis "There is no substantial difference in high school students' attitudes on speaking and reading abilities in English as a function of their parents' educational status" is rejected. "There is a considerable difference in high school students' attitudes about speaking and reading abilities in English in relation to their parents' educational status," it might be rephrased.

Hypothesis 8 : In terms of parents' annual income, there is no substantial difference in high school students' attitudes regarding speaking and reading abilities in English.

Table 8 Mean, Standard Deviations and 't' value of high school students based on their Parents Annual Income						
Variable	Parents Annual Income	N	Mean	Std. Deviation	"t" value	Level of Significance
Parents Annual Income	Below 1 Lakh	49	151.45	17.023	0.645	Not Significant at 0.05%
	From 1 Lakh to 5 Lakhs	36	153.72	14.603		

Table 8 shows that the 't'-value of high school pupils whose parental income is less than one lakh and between one lakh and five lakhs is 0.645, which is not significant at the 0.05 level. It shows that high school kids are not considerably different depending on their parents' annual income. Furthermore, the mean scores of pupils with less than one lakh students are (151.45), while those with one lakh to five lakhs students are (153.72). As a result, the null hypothesis is accepted: "There is no substantial difference in high school students' attitudes about speaking and reading abilities in English in relation to their parents' annual income."

Hypothesis 9: With regard to Parent Occupation, there is no significant variation in high school kids' attitudes regarding speaking and reading abilities in English.

Table 9 Summary of ANOVA showing the significance difference in the Parents Occupation

ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	690.428	2	345.214	1.383	.256
Within Groups	22472.153	90	249.691		

The ANOVA comparisons do not demonstrate a significant difference in the preceding table

(9) as can be observed. (1.383) is the computed 'F' value, which is not significant at the 0.05 level for df (2,90). It shows that high school students' attitudes regarding speaking and reading abilities in English do not change substantially dependent on their parents' occupation. As a result, the null hypothesis

© 2021 JLLS and the Authors - Published by JLLS.

"There is no substantial difference in high school students' attitudes about speaking and reading abilities in English as a function of parent occupation" is accepted.

Hypothesis 10: With regard to Type of Board, there is no substantial variation in high school students' attitudes regarding speaking and reading abilities in English.

Table 10 Summary of ANOVA showing the significance difference in the Type of Board

ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	81.784	2	40.892	.158	.854
Within Groups	22976.423	89	258.162		

The ANOVA comparisons do not demonstrate a significant difference, as shown in the table (10) above. The estimated 'F' value is (0.158), which at the 0.05 level with df is not significant (2,89). It shows that high school students' attitudes regarding speaking and reading abilities in English do not alter much depending on the kind of board. As a result, the null hypothesis "There is no substantial difference in high school students' attitudes about speaking and reading abilities in English as a function of type of board" is accepted.

8. Conclusion

The attitude toward reading and speaking English is linked to parental education. It's worth noting that parents have an important influence in language acquisition. Parents are supposed to help and promote their children's language acquisition. When parents are educated, their children are also educated. As a result, the data show that teaching a language may be passed down across generations. When we teach a youngster, we are also educating future generations. This has ramifications for curricular design, since the syllabus must be framed correctly as it is passed down down the generations. The study's results may be used as a springboard for discussing some educational implications.

Furthermore, the study's findings can assist language developers, syllabus designers, and decision makers in developing programmes, designing syllabi, and creating engaging textbooks and programmes that encourage students to speak, as well as employing foreign exchange programmes to help students meet and converse with more and varied people, understand and appreciate their way of life, and interact and communicate more easily with speakers of other languages in general and English, in particular.

References

Agustiani, I. W. D. (2017). The Correlation Between Students Reading Attitude and Their Reading Comprehension Achievement. *English Community Journal*, *1*(2), 75–85. https://doi.org/10.32502/ecj.v1i2.764.

Akbari, H., Ghonsooly, B., Ghazanfari, M., & Shahriari, H. (2017). Attitude Toward Reading: L1 or L2 or Both. *SAGE Open*, 1–10. https://doi.org/10.1177/2158244017717303.

Al-Amleh, M. J., & Shehadeh, A. (2015). [Attitudes of English Teachers' and Students' Towards Oral Communication in Palestinian Classrooms], (21316107). Retrieved from https://www.academia.edu/17619086/Attitudes_of_English_Teachers_and_Students_Towards_Oral_Communication_in_Palestinian_Classrooms

Binti Ahmad, N. A., & Binti Yamat, H. (2021). Students' Attitudes and Perception of Achievement in Reading Comprehension. *Journal of English Language Teaching and Applied Linguistics*, 3(4), 47–56. https://doi.org/10.32996/jeltal.2021.3.4.7

Borja, C. (2016). Attitude in English and Competence of Students at Integrated Refinery Petro-Chemical Complex (IRPCT) Technological College, Rayong, Thailand, (May).

© 2021 JLLS and the Authors - Published by JLLS.

Retrieved from https://files.eric.ed.gov/fulltext/ED577112.pdf.
Crivilare, H. G. (2019). Exploring High School Students' Attitudes Towards Reading, 1–47. Retrieved from http://thekeep.eiu.edu/cgi/viewcontent.cgi?article=5407&context=theses.
Elkilic, G. (2016). Attitudes of Kafkas University EFL Students Towards Speaking Skill and Classes. *International Journal of Humanities and Social Science Invention*, *5*(12), 62–67. Retrieved from http://www.ijhssi.org/papers/v5(12)/version-4/G0512046267.pdf
Hagan, E. (2013). Students Reading Attitudes in Relation to the Instructional Approach, 1–10. Retrieved from http://www.nwmissouri.edu/library/researchpapers/2013/HaganElizabeth.pdf
Kaid Mohammed Ali, J., Abdulhalim Shamsan, M., Guduru, R., & Yemmela, N. (2019). Attitudes of Saudi EFL Learners Towards Speaking Skills. *Arab World English Journal*, *10*(2), 353–364. https://doi.org/10.24093/awej/vol10no2.27.
Kırmızı, F. S., Akkaya, N., Bıçak, E., & İşçi, C. (2014). Teacher Candidates' Attitudes Towards Reading Habit (Case of Dokuz Eylul and Pamukkale Universities). *Procedia – Social and Behavioral Sciences, 127–131. https://doi.org/10.1016/j.sbspro.2014.01.179*
Kurapati, S. (2016). Attitudes Towards Testing Speaking Skills: A case for Concideration *International Journal of English Language, Literature and Translation Studies (IJELR)*, *3*(2), 694–700. Retrieved from http://www.ijelr.in/3.2.16B/694-700 SURESH KURAPATI.pdf
Miecha, B. (2017). Adama Science and Technology University School of Humanities and Law Department of Geography and Environmental Studies. Retrieved from http://213.55.101.23/bitstream/handle/123456789/749/Tagu Ankala.pdf?sequence=1&isAllowed=y
Muchtar, N. (n.d.). Attitude of Students in Reading Comprehension, 8. Retrieved from https://ejournal.iainpalopo.ac.id/index.php/ideas/article/download/141/127
Nonte, S., Hartwich, L., & Willems, A. S. (2018). Promoting Reading Attitudes of Girls and Boys: a NewCchallenge for Educational Policy? Multi-group Analyses Across Four European Countries. *Large-Scale Assessments in Education*, 1–22. https://doi.org/10.1186/s40536-018-0057-y.

© 2021 JLLS and the Authors - Published by JLLS.

Regional Studies in Marine Science 44 (2021) 101776

Contents lists available at ScienceDirect

Regional Studies in Marine Science

journal homepage: www.elsevier.com/locate/rsma

Paleoproductivity shifts since the last 130 ka off Lakshadweep, Southeastern Arabian Sea

Kannaiyan Neelavannan [a,b,c,*], S.M. Hussain [a], N. Mohammed Nishath [a], Hema Achyuthan [b], S. Veerasingam [d], Muthusamy Prakasam [e], Pankaj Kumar [f], Pramod Singh [g], P. John Kurian [h]

[a] Department of Geology, University of Madras, Chennai 600005, India
[b] Institute for Ocean Management, Anna University, Chennai 600025, India
[c] Department of Earth Sciences, Indian Institute of Technology Kanpur, UP 208016, India
[d] Environmental Science Center, Qatar University, Doha 2731, Qatar
[e] Wadia Institute of Himalayan Geology, 33, GMS Road, Dehradun 248001, India
[f] Inter-University Accelerator Centre, New Delhi 110067, India
[g] Department of Earth Science, Pondicherry University, Puducherry 605014, India
[h] National Centre for Polar and Ocean Research, Goa 403804, India

ARTICLE INFO

Article history:
Received 30 June 2020
Received in revised form 24 February 2021
Accepted 8 April 2021
Available online 20 April 2021

Keywords:
Calcium carbonate
Organic carbon
Paleoproductivity
SW monsoon
Arabian Sea

ABSTRACT

Marine sediment deposited on the ocean floor and near coastal areas, the western coastal regions of India provide records of monsoonal shifts and productivity. To understand the paleoproductivity in the northern Indian Ocean, we analyzed carbon, hydrogen, nitrogen, sulfur, total organic carbon (TOC) and calcium carbonate ($CaCO_3$) in a deep-sea sediment core collected using gravity corer (GC-01) off Lakshadweep, Southeastern Arabian Sea. The results were then compared with the previous data generated from the surrounding area sediment cores to understand the productivity variations since the last 130 ka. The $CaCO_3$ content in the sediment core varies from 40.82% to 62.48% (with a mean value of 51.96%) and it is noted that these values were lower during the glacial episodes (Marine Isotope Stages-2 and 4) than the interglacial episodes (MIS-1, 3 and 5). The C/N ratio varied from 0.14 to 34.25, but was less than 9, since ~74 ka to recent, suggesting a marine origin for the organic carbon. The C/N ratio fluctuated significantly during MIS-5, and relatively the highest C/N ratio was observed at 5e ~127, 5d ~110 and 5b ~85 ka, corresponding to stadials 5b and 5d, (except 5e) indicating terrestrial OC from C_3 plants. The low C/N ratios during ~128, ~102, ~76 and ~32 ka match with the interstadials especially during MIS 5 (5a, 5c and 5e), correspondingly, and are marine OC in the source. This suggests that the MIS-5 stadial was interrupted via land source signifying higher productivity owing to the strong southwest monsoon during these periods. Further, high productivity was also observed during the Last Glacial Maximum (LGM) and Holocene in the Southeastern Arabian Sea since the 130 ka.

© 2021 Elsevier B.V. All rights reserved.

1. Introduction

The Arabian Sea is well known for its high productivity due to the summer monsoon precipitation during the southwest (SW) monsoon (August to September). The cloud bearing winds blow from the Arabian Sea towards the Indian subcontinent and bring strong rain to India and neighboring countries (Barber et al., 2001). Terrigenous matter supply and biological productivity in the Arabian Sea is based on the monsoon intensity (Gupta and Anderson, 2005). Intense SW wind occur during the summer monsoon cause upwelling along the Somalian coast, Oman and along SW coast of India by which transport nutrient rich subsurface waters to the photic zone arise in increased productivity (Banse, 1987). High concentration surface productivity was reported towards the end of the SW (August–September) and northeast (February) monsoon (Marra and Barber, 2005). Different parts of the Arabian Sea, though, react in a different way to the seasonal wind forcing, most important to a massive regional difference in productivity during different seasons (Wiggert et al., 2005). Also regional variations in surface productivity has been inferred in the oligotrophic eastern and mesotrophic northern eastern Arabian Sea, and relatively weaker variations in productivity between interglacial and glacial intervals are noticed in the

* Corresponding author at: Department of Earth Sciences, Indian Institute of Technology Kanpur, UP 208016, India.
E-mail addresses: k.neelavannan@gmail.com, neela@iitk.ac.in (K. Neelavannan).

https://doi.org/10.1016/j.rsma.2021.101776
2352-4855/© 2021 Elsevier B.V. All rights reserved.

Table 1
List of research work done from present study region.

Authors	Area	Proxy used	Significances
Nagoji and Tiwari (2017)	Southeastern Arabian Sea	Multiple geochemical records	South Asian Summer Monsoon variability since mid-Holocene to the present past 4772 yr BP
Naik et al. (2017)	Southeastern Arabian Sea	Planktic foraminifera records	Last glacial–interglacial productivity past 32 ka
Avinash et al. (2016)	Eastern Arabian Sea	Environmental magnetism, geochemistry and clay mineralogy	Sedimentary sources and processes (surficial sediment samples used).
Das et al. (2013)	Southeastern Arabian Sea	Clay mineral assemblages	Paleoenvironmental significance last 30 ka
Farooqui et al. (2014)	Southeastern Arabian Sea	Palynological record	Rainforest vegetation and sea level fluctuations last 140 ka
Kessarkar et al. (2010)	Southeastern Arabian Sea	Sedimentological and stable isotopic	Fluctuations in productivity and denitrification last 70 ka
Pattan and Pearce (2009)	Southeastern Arabian Sea	Redox-sensitive elements record	Bottom water oxygenation history past 140 ka
Pattan et al. (2003)	Southeastern Arabian Sea	Multiple geochemical record	Productivity Fluctuations history past 140 ka

eutrophic upwelling region off Oman in the western Arabian Sea (Ivanova et al., 2003). Based on the modeling studies forecast a huge variations in monsoon patterns in a warming world, suggesting a probable deference in productivity in the Arabian Sea (Christensen et al., 2007). Variations in productivity through a longer time periods also the Holocene and interglacial–last glacial transition have been reconstructed from various regions of the Arabian Sea (Agnihotri et al., 2003; Singh et al., 2011).

The monsoon variability has been recorded in the marine sediments, lake sediments, speleothems, and tree rings in south Asian monsoonal regime. Previous studies found that the sediment cores from the Arabian Sea are widely used to study the past climate variability and its forcing factors with continuous, high resolution and longer records (Kroon et al., 1991; Clift et al., 2008; Gupta et al., 2015). However, understanding the mechanisms controlling the amplitude and frequency of climate changes requires the comparison of various proxies in many well-dated sediment records from this region.

Numerous studies have been carried out in the eastern Arabian Sea (Table 1) (Singh et al., 2011; Ishfaq et al., 2013; Naik et al., 2017), northwestern Arabian Sea (Kroon et al., 1991; Gupta et al., 2015) northeastern (Clift et al., 2008), and southeastern Arabian Sea (Agnihotri et al., 2003; Pattan and Pearce, 2009; Kessarkar et al., 2010; Saravanan et al., 2019, 2020) to understand the Late Quaternary terrigenous input and paleoproductivity shifts in relation with the SW monsoon strength (Sirocko and Sarnthein, 1989; Shimmield et al., 1990; Clemens et al., 1991; Anderson and Prell, 1993; Sirocko et al., 1993; Naidu and Malmgren, 1996). In the western continental margin of India, research work was carried out with a primary focus on the OC and $CaCO_3$ variations (Naidu, 1991; Paropkari et al., 1991; Thamban et al., 1997; Sarkar et al., 2000; Bhushan et al., 2001; Thamban and Rao, 2000; Pattan et al., 2003; Govil and Naidu, 2010). However, paleoproductivity variations related to the intensity of SW monsoon in the southeastern Arabian Sea covering the Late Quaternary period using multiproxy records is still lagging. Therefore, the preset study is aimed to investigate the paleoproductivity changes and linkage with the SW monsoon variations from the southeastern Arabian Sea sediment core.

2. Oceanography settings and sediment flux

The Arabian Sea is bounded by deserts such as Thar (India)-Makran (Iran) in the northern region, arid lands such as Arabian Peninsula and Africa in the western region, and eastern region covered by coastal highlands of the west coast, India (Nair et al., 1989). It is geographically surrounded by Chagos Laccadive Ridge (Carlsberg Ridge) in the southern region, and the Murray Ridge (Owen Ridge) in the western region (Avinash et al., 2016). Eastern Arabian Sea covers mountain ranges of Western Ghats of India,

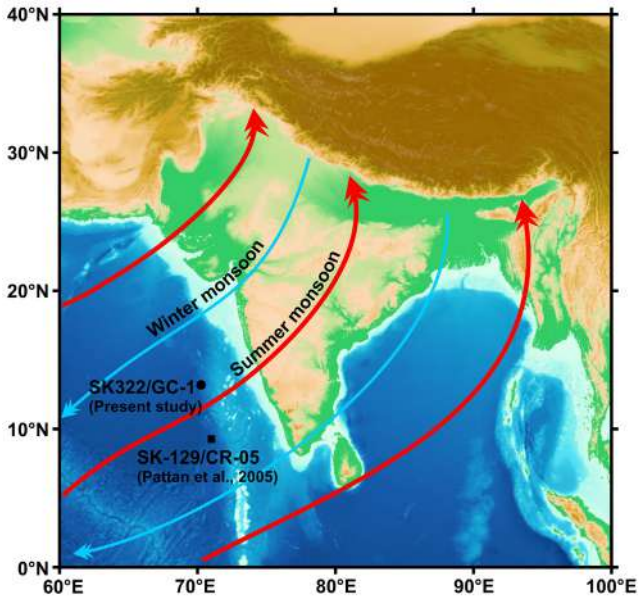


Fig. 1. Indian monsoon system surface wind pattern: red color show summer monsoon and blue color show winter monsoon. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

present all along the coast in the western peninsular. A few medium and small rivers drain these regions into the Arabian Sea (Rao and Wagle, 1997). The Arabian Sea is influenced by two monsoon seasons (Fig. 1): the dominant by the SW or the summer monsoon (June to September) and moderately by the northeast (December to February) (Olsen, 1990; Schott et al., 1990; Tindale and Pease, 1999; Avinash et al., 2016). The west India coastal current brings comparatively huge saline water towards southeastern Arabian Sea equatorward from the northern Arabian Sea during SW monsoon, while winter monsoon current transports poleward the low salinity water from the western Bay of Bengal into the southeastern Arabian Sea during boreal winder (Prasanna Kumar et al., 2004). During the pre-summer monsoon season in southeastern Arabian Sea the sea surface temperature increases and form as a mini warm pool that peaks in April. The mini warm pool dissipates starts the summer monsoon rain, as a result of upwelling (Shenoi et al., 1999). The upwelling causes a numerous double increase in productivity during summer monsoon period in the southeastern Arabian Sea (Lévy et al., 2007). The southeastern Arabian Sea marked by perennial intermediate water depth oxygen minimum zone which is ranging from ~150 m to ~1200 m (Naqvi et al., 2003).

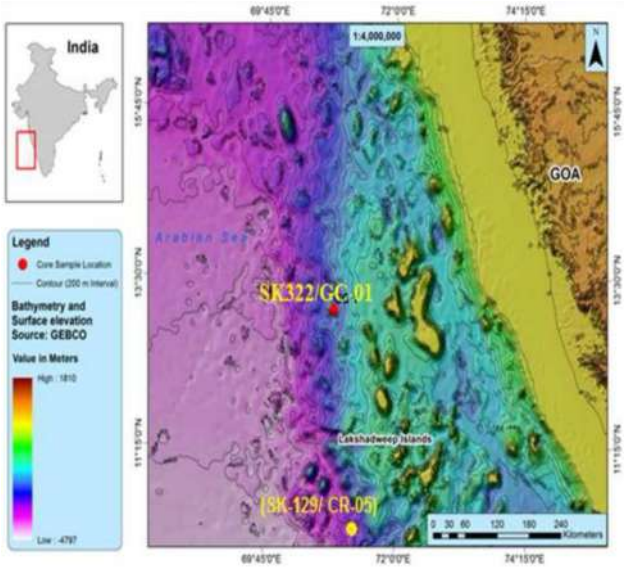


Fig. 2. Location and bathymetry map of GC-01 in the Southeastern Arabian Sea.

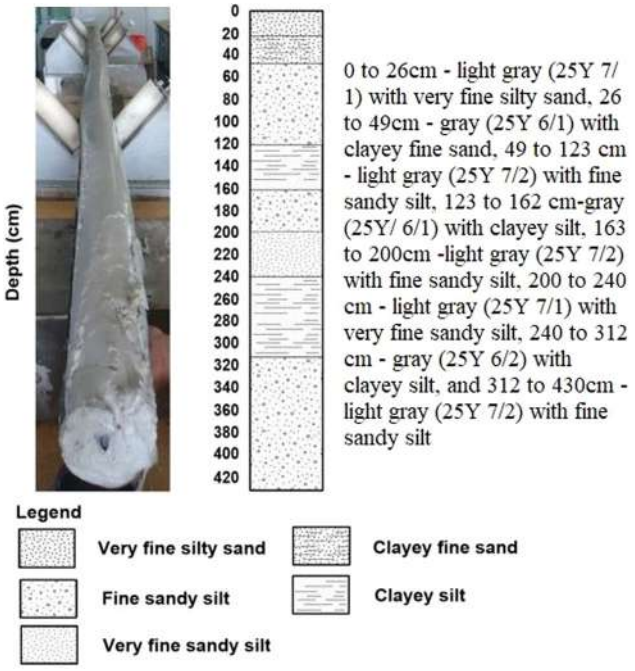


Fig. 3. Lithology of the studied core GC-01, SE Arabian Sea.

Sediment and dust influx into the Arabian Sea is received via several sources like the aeolian dust deposited from the Arabian Peninsula, Northern India (mainly from the Thar Desert), Pakistan and Eastern Africa (Dahl and Oppo, 2006). The major fluvial sediment sources are from the Indus, Narmada and Tapti Rivers and the Makran margin. Indus River has the largest drainage area in the NW Himalaya (2900 km length, basin area: ~966,000 km^2) and carry a huge amount of sediments to the Arabian Sea (Haq and Milliman, 1984) and drains several thousand tonnes of sediment flux from the Himalayas and forms the second largest deep-sea fan in the world (Prins et al., 2000). The Sabarmati River flows from the Aravali mountain range and through the Quaternary formations before reaching the Gulf (Sharma et al., 1994). The Narmada River with its elongated drainage basin area around 1300 km long (basin area: ~93,000 km^3) flows through a complex lithology comprising the Precambrian gneisses, Vindhyans sedimentary formation, and Deccan basalts in the upper and middle reaches, and the Quaternary alluvium in the lower reaches. The Western Ghats due to its orographic effect receives a maximum annual rainfall of ~3000 mm and are drains several small and short flowing rivers that transport sediments into the Arabian Sea coastal regions. Extensive lateralization of the parent rocks is a characteristic feature of the Western Ghats. Common rocks found in the coastal Kachchh and Saurashtra regions are bauxite and laterite and their derived sedimentary facies (Valeton and Wilke, 1993; Kessarkar et al., 2003). The Deccan trap basalts are present in the northern part of Goa, Charnokites, Precambrian gneisses and schists are the main rock type in between Goa and Cochin (Thamban et al., 2002).

3. Materials and methods

In this study, a 4.3 m long sediment gravity core (GC-1) was retrieved from off the Lakshadweep islands (Latitude: 13°04'5.50" N, Longitude: 70°56'10.21" E) using a gravity corer by R/V Sagar Kanya-322 expedition (National Center for Polar and Ocean Research, Goa, India) during August 2015. The water depth of the sampling location was 2057 m (Fig. 2). The sediment core was later sub-sampled at every 2 cm interval from top to 2 m, and 5 cm interval for the remaining portion of the core until the depth of 4.3 m.

3.1. Radiocarbon dating

^{14}C dating was performed by using the Accelerator Mass Spectrometry (AMS) facility at Inter-University Accelerator Center (IUAC), New Delhi. For this purpose, two bulk sediment samples were selected from different depths for the AMS ^{14}C dating. The selected sediment samples were dried and examined under the microscope for careful removal of microfossils, shells and other impurities. Thus, cleaned sediments were pretreated using the ultrapure acid (HCl), base (NaOH), and acid (HCl) protocol. Furthermore, the sediment samples were neutralized by frequent washing and then freeze-dried for graphitization. For graphitization, the samples were combusted and thus CO_2 produced was reduced to graphite powder form using Automated Graphitization Equipment (AGE) (Wacker et al., 2010; Sharma et al., 2019). The graphitized samples were measured for different isotopes of carbon 12, 13 and 14 with a 500 kV tandem ion accelerator (Kumar et al., 2015; Rajendran et al., 2018; Raja et al., 2019). The ^{14}C ages obtained from AMS measurement were corrected with Arabian Sea reservoir age (400 years) on the basis of findings of Southon et al. (2002) in the Indian Ocean and finally calibrated by using Calib 6.0 software (Stuiver and Reimer, 1993; Stuiver et al., 2010).

3.2. CHNS (Carbon, Hydrogen, Nitrogen, and Sulfur) analysis

The sub-sampled sediments were analyzed for Total Carbon (TC), Total Nitrogen (TN), Total Hydrogen (TH) and Total Sulfur (TS) were analyzed using an Elemental analyzer (Vario EL cube; Make Elementary) at Pondicherry University, Puducherry. In this method, the powdered samples were weighed (~10 mg) in the aluminum tin sample boats and placed in an auto-sampler. The accuracy of the measurements was determined in triplicate by sulfanilamide test standard, and the error was observed to be <1%.

3.3. Total Inorganic Carbon (TIC) and $CaCO_3$

Total Inorganic Carbon (TIC) was determined by using a coulometer (UIC model 5030) at the National Center for Polar

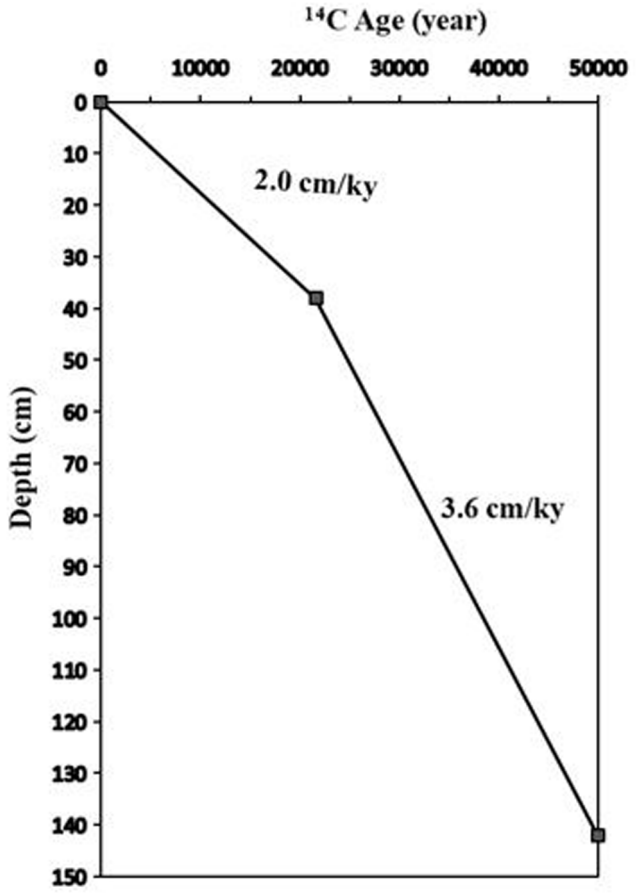


Fig. 4. Chronology and sedimentation rate of the marine sediment core GC-01, SE Arabian Sea.

and Ocean Research (NCPOR), Goa, India. 10 mg of homogenized sediment samples were treated with 5 mL of 4N HCl acid at 50 °C (Bhushan et al., 2001). Pure and dried $CaCO_3$ (supra pure grade Merck) was used as a standard for calibration. The precision of $CaCO_3$ analysis was found to be <3% based on repeated standard and sample analysis. The percentage of $CaCO_3$ was calculated using the formula of O'Connell et al. (1996).

$$CaCO_3(wt\%) = \%C_{inorg.} \times 8.334$$

3.4. Trace elements

The sediment samples were analyzed for trace element contents using Jarvis and Jarvis (1985) method. A 0.2 g of the homogenized sample was transferred to cleaned Teflon beakers with a mixture of hydrofluoric acid: perchloric acid: nitric acid in the ratio of 7:3:1. The digested solutions were then analyzed for significant element as Cd using an Atomic Absorption Spectrophotometer (AAS) (Perkin Elmer, AA 800 model). During the analysis, blank corrections were applied, wherever needed. The average recoveries obtained were 90%–97%. The accuracy and precision were monitored by standard reference material MESS-2 and triplicate analysis of the selective sediment samples within the batches.

3.5. Data analysis

Past software (V#3.2.6b) has been used for principal component analysis (PCA) and spectral analysis (Hammer et al., 2001) of all the proxies for the multivariate study to get organized

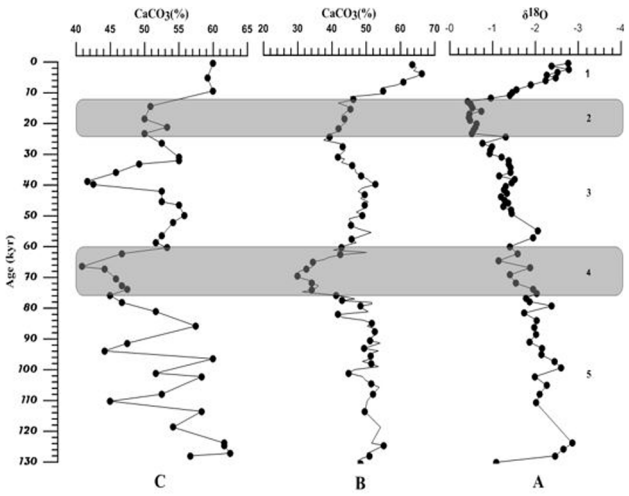


Fig. 5. A, Oxygen isotope composition of *Globigerinoides ruber* in a sediment core (SK-129/GC-05) versus age (ka). Numbers 1–6 indicate marine isotope stages and shaded blocks are glacial (Pattan et al., 2003). B, Distribution of calcium carbonate content in a sediment core (Pattan et al., 2005). C, Present core SK322/GC-01 calcium carbonate distribution.

covariations between a collection of variables. REDFIT spectral analysis (Schulz and Mudelsee, 2002) of unevenly spaced time series were conducted on the $CaCO_3$ data of Site SK322/GC-1 to examine the cyclicity of summer monsoonal records and its potential driving forces during the last 130 ka. For prefoming spectral analysis, we have used a Hanning window of 2 segments and an oversampling value of 3 with the Monte Carlo method. Significance lines of 90%, and 80% levels of chi-square test applied.

4. Results and discussion

4.1. Lithology of the sediment core

The sediment core exhibited sediment layers in shades of light gray and the sediment lithology was classified based on sediment color following Munsell color chart, sedimentary structures and grain texture (Fig. 3).

4.2. Chronology of the core

Based on reservoir corrected age, the average sediment age for sub-samples of 38 and 142 cm were calculated as 21583 ± 87 and 49953 ± 827 years, respectively. The sedimentation rate varied from 2.0 to 3.6 cm/kyr at the depth of 38 to 142 cm, respectively (Fig. 4). Below 142 cm depth, interpolation of ages was calculated by considering the sedimentation rates between the obtained calibrated radiocarbon ages. Distribution of $CaCO_3$ values was compared with the nearby site SK-129/GC-05 (Latitude 9°21' N, Longitude 71°59' E) oxygen isotope and $CaCO_3$ proxy studied (Pattan et al., 2003, 2005). Oxygen isotope values of *Globigerinoides ruber* and $CaCO_3$ distribution were found to be matching well with the present core (Fig. 5). Further, the highest cadmium and sulfur contents noticed ~75ka BP (Fig. 6) was inferred as the result of the Youngest Toba Tuff effusion (Pattan et al., 2003). Traces of the Toba eruption have been found in deep-sea cores of the northeastern Indian Ocean, the Bay of Bengal, and the Andaman Sea. Similar deposits have also been reported from the Arabian Sea, the South China Sea (Song et al., 2000), the Indian subcontinent and the Central Indian Basin (Pattan et al., 1999).

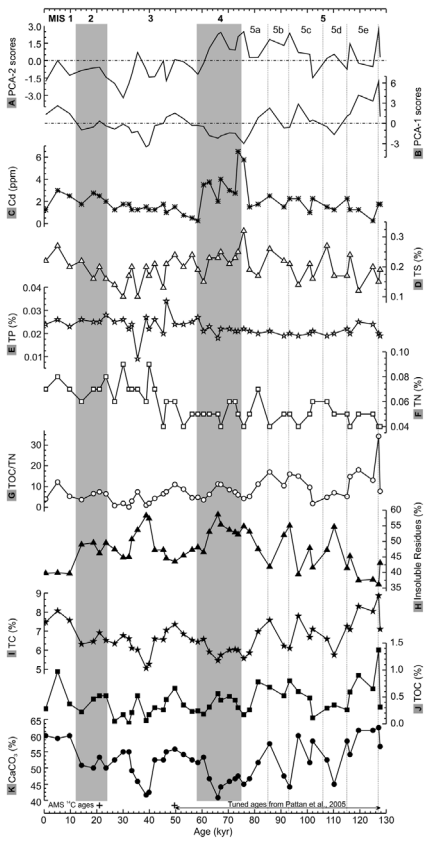


Fig. 6. Down core variations of organic and inorganic proxies in the studied core.

4.3. Calcium Carbonate

The $CaCO_3$ content in the sediment core ranged from 40.82 to 62.48% (mean 51.96%) (Fig. 6). The carbonate content was observed to be higher during MIS-5e, c, a 3, and 1, and lower during MIS-5d, b, 4 and 2. During the MIS 4 and 2 glacial periods, values of $CaCO_3$ were lower (40.82%) than the interglacial periods, MIS-5 (62.48%) (Fig. 6), which could be due to the rate of dissolution of carbonates and dilution by the non-carbonate portion in the area. During the SW monsoon, more terrestrial input and higher biological productivity were noticed by sediment trap studies (Haake et al., 1993). Earlier, Gupta and Anderson (2005) found that the terrigenous matter and biological productivity supplied by the effect of monsoon during Late Quaternary into the Arabian Sea.

Many researchers observed that $CaCO_3$ and the OC contents contribute to the productivity in the marine sediment (Jaccard et al., 2013; Cartapanis et al., 2018). It was also noticed that the persevered $CaCO_3$ is generally affected by terrigenous dilution in the Arabian Sea (Pattan et al., 2003). During the summer monsoon, there are fluctuations in the lithogenic terrigenous flux derived from the Somalian peninsula and the Arabian Sea. The $CaCO_3$ dilution was mainly due to the terrigenous fluctuation derived from the SW coast of India or due to the detrital contribution, or both (Sarkar et al., 1993; Pattan et al., 2003). Nagoji and Tiwari (2017) reported that Chagos-Laccadive Ridge and the shelf sediments are characterized by a high $CaCO_3$ content when compared to deep-sea sediments.

4.4. Total Phosphorus (TP) and total Sulfur (TS)

The phosphorus content varied from 0.01 to 0.03% (Fig. 6). During MIS-5e, 3, 2, and 1, a higher value (0.034%) was noticed and the lower value (0.018%) was found during MIS-5d, c, b, a, and 4. Based on phosphorus concentration it can be noted that high value indicated higher productivity and the low value indicated lower productivity. Sulfur content in sediment core ranged between 0.10 and 0.32% (Fig. 6), and relatively higher values (0.32%) were noticed during the glacial than the interglacial period. Except for MIS-1, the highest TS noticed at sharp ~75 ka (MIS-4) (0.32%) might be due to the effect of the Youngest Toba Tuff origin.

4.5. Organic Carbon and total Nitrogen

In the marine environment, the primary sources of OC are terrestrial as well as marine origins. The OC content in the marine sediments was strongly associated with surface water productivity. Hence, variations of the OC in marine sediments were used as a productivity proxy (Ausín et al., 2019). Total organic carbon (TOC) content in sediment core varied from 0.01 to 1.37% (mean 0.42%) (Fig. 6), whereas the Total Nitrogen (TN) content in the core varied from 0.04 to 0.09% (mean 0.06%). TN values in MIS-1, 2, and 3 showed the same trend to those of TOC and with higher values compared to the sediments below MIS-3 (only in the case of TN). The distribution pattern of the TN and OC in the sediment core was almost similar, which could be the plankton incorporates in both nitrogen and carbon in a defined molar ratio (Paulsen et al., 2018).

The lower average TOC content (0.31%) was found in MIS-1, whereas those values in MIS-2 increased gradually (0.43%). Within MIS-2, a higher OC value (0.52%) was noticed during the Last Glacial Maximum (LGM, Fig. 6). Higher OC was noticed during MIS-1 than LGM. The obtained results in this study were similar to some previously published research articles (Sarkar et al., 1993; Pattan et al., 2003) and inferred that more suboxic

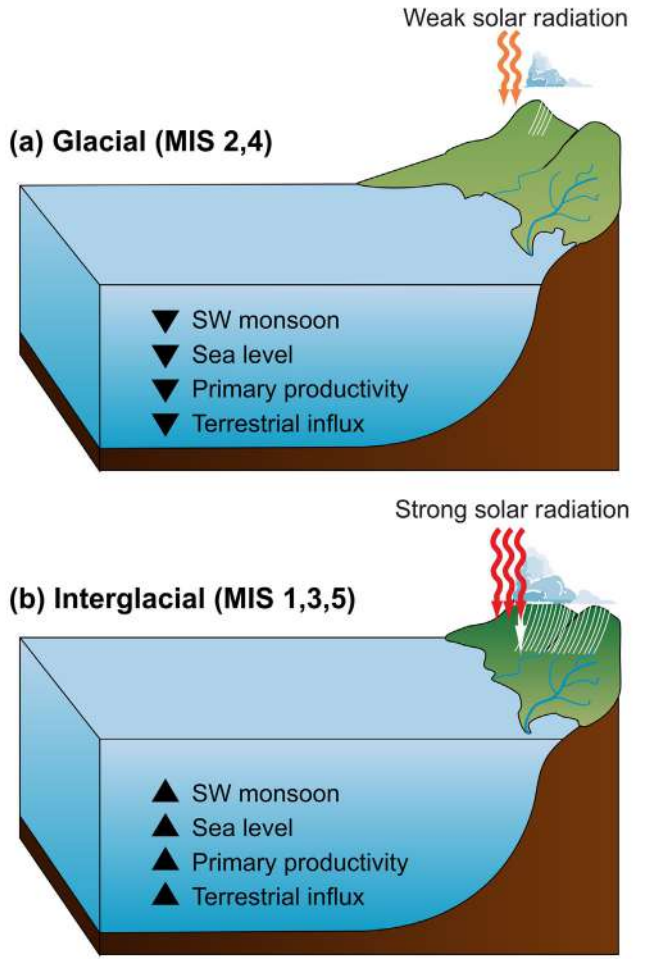


Fig. 7. A conceptual model representing glacial and inder glacial depositional conditions.

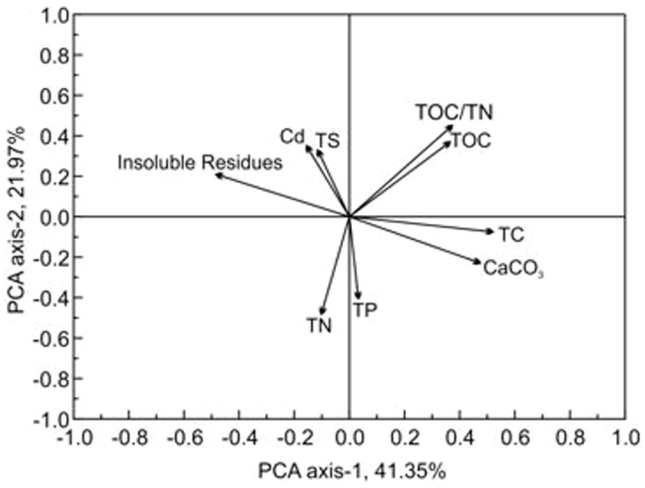


Fig. 8. Biplot of the principal component analysis (PCA) of productivity proxies.

conditions existed in MIS-2 compared to MIS-1. Likewise, higher OC was observed in other parts of the world during the LGM, for example, the Argentine Basin (Stevenson and Cheng, 1972) and the Pacific Ocean (Pedersen, 1983). The higher TOC observed during the interglacial (5a and 5e) of MIS-5 and in the stadials (5b and 5d), which might be due to the result of suboxic conditions. An oxic environment existed in MIS-1 (Holocene) due to oxidation of OC, and the resulting sediments contained less OC contents (Fig. 6). During MIS-5 higher TOC was observed during stadials 5b and 5d (93 and 111 ka) and similar results were observed by Pattan et al. (2003), Pattan and Pearce (2009). The interstadials and stadials of the OC ranged from low (∼0.5%) to high (∼1.5%). Carbon sources were influenced by both marine and land originated OC by (C/N) ratios of ∼7 and 25% (Pattan et al., 2003).

The low values of TOC content fluctuated from ∼24 to 58 ka; however, from ∼62 to ∼75 ka it gradually increased and again decreased. Significant fluctuation in the OC (0.17 to 1.37%) was observed during MIS-5. The TOC reached a minimum of 0.17%, 0.11%, and 0.31% at ∼76, ∼102 and ∼128 ka, respectively which may correspond to interstitials 5.a, 5.c, and 5.e, whereas the carbon content was high 0.8% and 0.35% at ∼93 and ∼110 ka, may correspond to stadials 5.b and 5.d, respectively (Fig. 6). A higher TOC (0.46%) was noted during the LGM (∼18 ka) in the sediment core, supporting suboxic bottom water conditions (Pattan et al., 2003; Pattan and Pearce, 2009). OC variation has not shown any trend regarding core depth and the glacial and interglacial periods. A similar observation was noticed from the Oman margin of the Arabian Sea (ODP site 724) (Zahn and Pedersen, 1991). Further, compared to the glacial period the Holocene had higher OC, which can be attributed to the higher productivity during Holocene in the northeastern, western, and northern Arabian Sea, and the southern continental margin of India (Sirocko et al., 2000). This suggests that the OC preservation pattern in the southeastern Arabian Sea could be different from these areas.

In marine sediments, carbon/nitrogen (C/N) ratio has been widely used to trace the source of OC. In core GC-01, the C/N ratio differed from 0.14 to 34.25. However, C/N values less than 9 were observed from ∼74 ka to recent. This suggested that organic carbon has derived from the marine origin (Fig. 6). In the marine sources, TOC is originated via phytoplankton, whereas that in terrestrial is originated from plant remains and dissolved humic substances through the river (Emerson and Hedges, 1988). The C/N ratio fluctuated significantly during MIS-5, and relatively highest C/N ratio was noticed at 5e (127 ka), 5d (110 ka) and 5b (85 ka), which may correspond to stadials 5.b and 5.d, (except 5e), suggesting a land originated OC from C_3 continental plants (Meyers, 1994). The low C/N ratios at (∼32) at ∼76, ∼102 and ∼128 ka matched to interstitials 5.a, 5.c and 5.e within MIS-5, respectively, and the OC sources were from in situ marine origin (Fig. 7). This suggests that the MIS-5 stadials were interrupted via land-based OC. Land originated OC decreased the contribution as the distance from the coast to the deep ocean increased (Fontugne and Duplessy, 1986). Even though the studied core is around 450 km away from the coast, land originated OC found during ∼110 and ∼85 ka, possibly indicated that the Indian subcontinent had abundant rainfall and more vegetation during these periods. The land originated OC was brought through rivers; later it got transported to the southeastern Arabian Sea by the bottom water current. During MIS-5, corresponding to the stadials and interstadials with the variation of the C/N ratio, and the TOC needs to be confirmed by studying long sediment cores closer to the coastal region of the southeastern Arabian Sea (see Fig. 6).

4.6. Computational analysis

The principal component analysis was carried out based on eight geochemical parameters, having two components PC1 (41.35%) and PC2 (21.97%) with 63.32% of the total variation (Fig. 8). $CaCO_3$, TOC/TN, TC, TOC, and TP were dominated in the positive side of the PC1, whereas TN was located on the negative side. In PC2, TOC/TN, TOC, insoluble residues, Cd and TS were

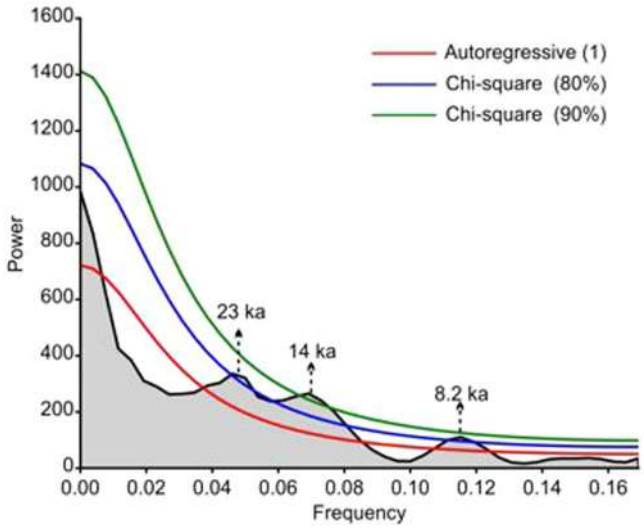


Fig. 9. Spectral analysis of calcium carbonate percentage during the last 130 ka.

located on the positive side and TN was located on the negative side. MIS-1, 3 and 5 had higher PC1 scores, corresponding to the interglacial period with higher productivity, which could be due to the deposition of higher $CaCO_3$ and OC in the sediments (Pattan et al., 2003).

Spectral analyses shows periodicities at 23 kyr, 14 kyr and 8.2 kyr (Fig. 9). The occurrence of 23 and 14 kyr periodicities in the $CaCO_3$ record confirms the dynamic connection of the primary productivity changes with the summer monsoon to glacial–interglacial and orbital forcings. These periodicity has been studied by Clemens et al. (2010) in the Indian summer monsoon regime. The 23 kyr and 14 kyr cycles are linked with the precession of the Earth's orbit (with a phase of −125°) inferred from the Summer Monsoon proxies of the northern Indian Ocean (Clemens and Prell, 1991; Wang et al., 2005).

The observed 8.2 kyr spectral frequency is close to the 6 kyr cycle, which is probably associated with the Heinrich rhythm cycles (~6 kyr). This cycle was documented in the North Atlantic sediments and Greenland ice core (Bond et al., 1993; Heinrich, 1988). It illustrates the teleconnection between the Indian summer monsoon and the North Atlantic Sea surface temperature changes. These periodicities suggesting that the low latitude summer insolation and Atlantic teleconnection may play a major role in shaping the Indian summer monsoon variability and primary productivity shift in this region.

4.7. The last 130 ka paleoproductivity shifts

The paleoproductivity has fluctuated since the past geological record as it is defined by the monsoon in the southeastern Arabian Sea. Rostek et al. (1997), based on alkenones and coccoliths reconstructed the paleoproductivity changes. The primary productivity during the periods increased and decreased during the beginning of interglacial periods. The higher productivity at glacial periods had been credited due to the strong NE monsoon wind pressure, and this was accountable for bringing nutrient-rich water to break the water column stratification into the photic zone, inducing higher productivity. According to Thamban et al. (2001), during the early Holocene period productivity was low. Sarkar et al. (1993) showed maximum productivity during the (LGM) period using the productivity indicator of $CaCO_3$ content of sediments. Fontugne and Duplessy (1986) reported

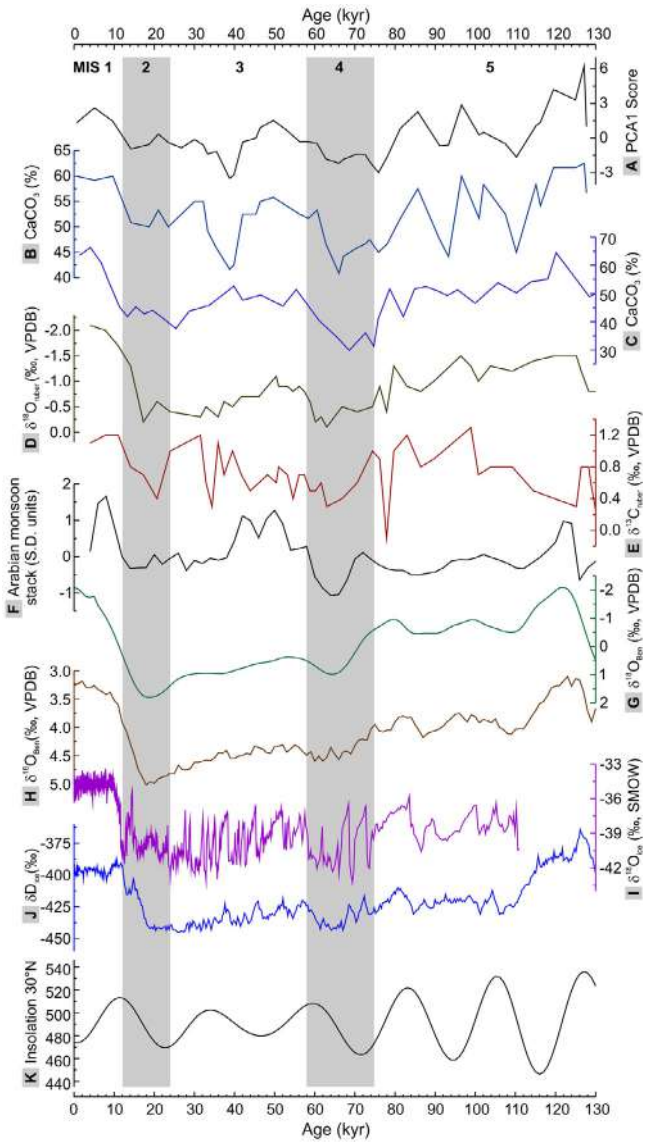


Fig. 10. Paleoclimatic and paleoproductivity changes during the last 130 ka. (A) PCA1 Score (present study), (B) Percentage of $CaCO_3$ (Present study), (C) Percentage of $CaCO_3$ from SE Arabian Sea (Pattan and Pearce, 2009), (D) & (E) oxygen and carbon isotope record from SE Arabian Sea (Ivanova et al., 2003), (F) Arabian Monsoon Stack (Clemens and Prell, 2003), (G) SPECMAP (Imbrie et al., 1984), (H) LR04 global benthic foraminifera isotope stack (Lisiecki and Raymo, 2005), (I) GISP2 ice core data from Greenland (Grootes et al., 1993), (J) EPICA ice core data from Antarctic (Augustin et al., 2004) and (K) Insolution 30°N (June) (Berger and Loutre, 1991).

higher primary productivity during the glacial periods than the interglacials. A similar observation was reported in the Arabian Sea paleoproductivity based on various geochemical productivity proxies such as biogenic barium, calcium carbonate, and biogenic opal on the sediments suggested that higher productivity was observed during major interglacials and low during the glacial periods (Pattan et al., 2003; Prabhu et al., 2004; Banakar et al., 2005; Prabhu and Shankar, 2005; Ishfaq et al., 2013; Naik et al., 2017). A few more researchers also made similar observations from northwestern, eastern, and northern parts of the Arabian Sea, Atlantic Ocean, and Antarctica regions (Shimmield et al., 1990; Clemens and Prell, 1991; Paropkari et al., 1991; Howard and Prell, 1994; Shimmield et al., 1994; Frank et al., 1995; Bonn et al., 1998; Reichart et al., 1998).

4.8. Monsoon link with global Climate: Glacial-interglacial episodes

Long-term changes in solar insolation 30 °N seem to overlay the millennial-scale calcium carbonate fluctuations during 130 ka in the southern Arabian Sea (Fig. 10). The present data were compared with other regional and global data such as LR04 Global isotope data (Lisiecki and Raymo, 2005), SPECMAP (Imbrie et al., 1984), Arabian Monsoon Stack (Clemens and Prell, 2003), and oxygen isotope record of (δ^{18}O) SE Arabian Sea (Ivanova et al., 2003), and percentage of $CaCO_3$ from SE Arabian Sea (Pattan and Pearce, 2009). The Quaternary climate phase showed huge latitudinal changes, and incoming solar radiation was the main energy source to the earth. The solar insolation changes are cyclic with a periodicity of 100, 41, and 23 ka and are jointly defined as Milankovitch cycles (Hays et al., 1976). Efforts have been made to understand the effect of Milankovitch equilibrium changes in solar radiation on the intensity of the monsoon. Based on terrestrial and marine proxies, monsoon records had inferred a strong summer monsoon on obliquity maxima and 8 ka after precession minima (Clemens et al., 2010). As long-standing recodes imply during the interglacial–glacial period, the variability of monsoon was distinct during the Pleistocene (Clemens and Prell, 1991). During the late interglacial–glacial transition as reconstructed widely and easily using gravity cores (< 6 m length) from the lakes as well as continental shelf regions, out of numerous interglacial–glacial cycles (Banakar et al., 2010). Various proxy records from both Bay of Bengal and the Arabian Sea suggest that the summer monsoon is weakening accompanied with a probable intensification of winter monsoon at the glacial period (Jain and Tandon, 2003; Banakar et al., 2010; Govil and Naidu, 2011; Saraswat et al., 2013). The weedy monsoon interval period was interrupted by a short-lived phase of intense monsoon, which was marked during the last deglaciation (Saraswat et al., 2013). Throughout the Bølling–Allerød period, the summer monsoon precipitation was high compared to the last glacial and Younger Dryas, probably in reaction to changes in North Atlantic climate (Sinha et al., 2005; Rashid et al., 2007).

5. Conclusions

In this study, $CaCO_3$, TOC, trace metals, Carbon, Hydrogen, Nitrogen, and Sulfur in the present core GC-1 Southern Arabian Sea were used as a paleoproductivity proxy and these results were compared with previous studies carried out in this region. $CaCO_3$ content during Marine Isotope Stages (MIS) 1, 3, and 5a, c, and d were higher compared to that during MIS-2, 4, 5b, and d, which could be due to higher interglacial productivity than in the glacial. Likewise, OC also showed increased productivity during MIS-1, 3, and 5a, c, and d. The C/N ratio revealed that the source of OC is marine-based from ~62 ka to recent. However, the C/N ratio has fluctuated considerably during MIS-5, which could be due to the OC derived from both marine and fluvial origins. The SW monsoon also plays a significant role in controlling the primary productivity changes in this region.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors are thankful to the Director, National Center for Polar and Ocean Research (NCPOR), Goa, for giving the opportunity to participate in the oceanographic cruise program to collect the sediment core samples. Authors are thankful to the Director, IUAC for extending ^{14}C AMS facility funded by Ministry of Earth Science (MoES), Govt. of India with reference number MoES/16/07/11(i)-RDEAS and MoES/P.O.(Seismic)8(09)-Geochron/2012. The authors are thankful to Dr Rahul Mohan, Scientist E, NCAOR, Goa, for providing laboratory facilities Ms Kalpana Dhiman, for helping during the Coulometer analysis, and Dr G. N. Nayak, Professor and Dr Ms Shabnam Choudhary, Department of Marine Science, Goa University, for providing laboratory facilities to carry out the phosphorus analysis.

All authors have contributed for the preparation of the manuscript.

Funding sources

The author K.N is thankful to the Department of Science and Technology, Promotion of University Research and Scientific Excellence (DST-PURSE), for providing Junior Research Fellowship (JRF).

References

Agnihotri, R., Sarin, M.M., Somayajulu, B.L.K., Jull, A.T., Burr, G.S., 2003. Late-Quaternary biogenic productivity and organic carbon deposition in the eastern Arabian Sea. Palaeogeogr. Palaeoclimatol. Palaeoecol. 197 (1–2), 43–60.

Anderson, D.M., Prell, W.L., 1993. A 300 kyr record of upwelling off Oman during the late Quaternary: evidence of the Asian southwest monsoon. Paleoceanography 8 (2), 193–208.

Augustin, L., Barbante, C., Barnes, P.R., Barnola, J.M., Bigler, M., Castellano, E., Cattani, O., Chappellaz, J., Dahl-Jensen, D., Delmonte, B., Dreyfus, G., 2004. Eight glacial cycles from an Antarctic ice core. Nature 429, 623–628.

Ausín, B., Magill, C., Haghipour, N., Fernández, Á., Wacker, L., Hodell, D., Baumann, K.H., Eglinton, T.I., 2019. (In) coherent multiproxy signals in marine sediments: Implications for high-resolution paleoclimate reconstruction. Earth Planet. Sci. Lett. 515, 38–46.

Avinash, K., Kurian, P.J., Warrier, A.K., Shankar, R., Vineesh, T.C., Ravindra, R., 2016. Sedimentary sources and processes in the eastern Arabian Sea: Insights from environmental magnetism, geochemistry and clay mineralogy. Geosci. Front. 7 (2), 253–264.

Banakar, V.K., Mahesh, B.S., Burr, G., Chodankar, A.R., 2010. Climatology of the Eastern Arabian Sea during the last glacial cycle reconstructed from paired measurement of foraminiferal δ 18 O and Mg/Ca. Quat. Res. 73 (3), 535–540.

Banakar, V.K., Oba, T., Chodankar, A.R., Kuramoto, T., Yamamoto, M., Minagawa, M., 2005. Monsoon related changes in sea surface productivity and water column denitrification in the Eastern Arabian Sea during the last glacial cycle. Mar. Geol. 219 (2–3), 99–108.

Banse, K., 1987. Seasonality of phytoplankton chlorophyll in the central and northern Arabian Sea. Deep-Sea Res. 34, 713–723.

Barber, R.T., Marra, J., Bidigare, R.C., Codispoti, L.A., Halpern, D., Johnson, Z., Latasa, M., Goericke, R., Smith, S.L., 2001. Primary productivity and its regulation in the Arabian Sea during 1995. Deep-Sea Res. II 48 (6–7), 1127–1172.

Berger, A., Loutre, M.F., 1991. Insolation values for the climate of the last 10 million years. Quat. Sci. Rev. 10 (4), 297–317.

Bhushan, R., Dutta, K., Somayajulu, B.L.K., 2001. Concentrations and burial fluxes of organic and inorganic carbon on the eastern margins of the Arabian Sea. Mar. Geol. 178 (1–4), 95–113.

Bond, G., Broecker, W., Johnsen, S., McManus, J., Labeyrie, L., Jouzel, J., Bonani, G., 1993. Correlations between climate records from north atlantic sediments and greenland ice. Nature 365 (6442), 143–147.

Bonn, W.J., Gingele, F.X., Grobe, H., Mackensen, A., Fütterer, D.K., 1998. Palaeoproductivity at the Antarctic continental margin: opal and barium records for the last 400 ka. Palaeogeogr. Palaeoclimatol. Palaeoecol. 139 (3–4), 195–211.

Cartapanis, O., Galbraith, E.D., Bianchi, D., Jaccard, S., 2018. Carbon burial in deep-sea sediment and implications for oceanic inventories of carbon and alkalinity over the last glacial cycle. Clim. Past 14 (11), 1819–1850.

Christensen, J.H., Hewitson, B., Busuioc, A., Chen, A., Gao, X., Held, I., Jones, R., Kolli, R.K., Kwon, W.-T., Laprise, R., Rueda, V., Magaña Mearns, L., Menéndez, C.G., Räisänen, J., Rinke, A., Sarr, A., Whetton, P., 2007. Regional climate projections. In: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M., Miller, H.L. (Eds.), Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Clemens, S.C., Prell, W.L., 1991. One million year record of summer monsoon winds and continental aridity from the Owen Ridge (Site 722), northwest Arabian Sea. In: Proceedings of the Ocean Drilling Program. In: Scientific Results, vol. 117, Ocean Drilling Program College Station, 365-388.

Clemens, S.C., Prell, W.L., 2003. A 350,000 year summer-monsoon multi-proxy stack from the owen ridge. Northern Arabian Sea. Mar. Geol 201 (1-3), 35–51.

Clemens, S., Prell, W., Murray, D., Shimmield, G., Weedon, G., 1991. Forcing mechanisms of the Indian Ocean monsoon. Nature 353 (6346), 720–725.

Clemens, S.C., Prell, W.L., Sun, Y., 2010. Orbital-scale timing and mechanisms driving Late Pleistocene Indo-Asian summer monsoons: Reinterpreting cave speleothem δ18O. Paleoceanography 25 (4).

Clift, P.D., Hodges, K.V., Heslop, D., Hanningan, R., Van Long, H., Calves, G., 2008. Correlation of himalayan exhumation rates and asian monsoon intensity. Nat. Geosci 1, 875–880.

Dahl, K.A., Oppo, D.W., 2006. Sea surface temperature pattern reconstructions in the Arabian Sea. Paleoceanography 21 (1).

Das, S.S., Rai, A.K., Akaram, V., Verma, D., Pandey, A.C., Dutta, K., Prasad, G.R., 2013. Paleoenvironmental significance of clay mineral assemblages in the southeastern arabian sea during last 30 kyr. J. Earth Syst. Sci 122 (1), 173–185.

Emerson, S., Hedges, J.I., 1988. Processes controlling the organic carbon content of open ocean sediments. Paleoceanography 3 (5), 621–634.

Farooqui, A., Pattan, J.N., Parthiban, G., Srivastava, J., 2014. Palynological record of tropical rain forest vegetation and sea level fluctuations since 140 ka from sediment core, south-eastern Arabian Sea. Palaeogeogr. Palaeoclimatol. Palaeoecol. 411, 95–109.

Fontugne, M.R., Duplessy, J.C., 1986. Variations of the monsoon regime during the upper Quaternary: evidence from carbon isotopic record of organic matter in North Indian Ocean sediment cores. Palaeogeogr. Palaeoclimatol. Palaeoecol. 56 (1–2), 69–88.

Frank, M., Eisenhauer, A., Bonn, W.J., Walter, P., Grobe, H., Kubik, P.W., Dittrich-Hannen, B., Mangini, A., 1995. Sediment redistribution versus paleoproductivity change: Weddell Sea margin sediment stratigraphy and biogenic particle flux of the last 250,000 years deduced from ^{230}Thex,^{10}Be and biogenic barium profiles. Earth Planet. Sci. Lett. 136 (3–4), 559–573.

Govil, P., Naidu, P.D., 2010. Evaporation-precipitation changes in the eastern Arabian Sea for the last 68 ka: Implications on monsoon variability. Paleoceanography 25 (1).

Govil, P., Naidu, P.D., 2011. Variations of Indian monsoon precipitation during the last 32 kyr reflected in the surface hydrography of the Western Bay of Bengal. Quat. Sci. Rev. 30 (27–28), 3871–3879.

Grootes, P.M., Stuiver, M., White, J.W.C., Johnsen, S., Jouzel, J., 1993. Comparison of oxygen isotope records from the GISP2 and GRIP Greenland ice cores. Nature 366 (6455), 552–554.

Gupta, A.K., Anderson, D.M., 2005. Mysteries of the Indian Ocean monsoon system. J. Geol. Soc. India 65, 54–60.

Gupta, A.K., Yuvaraja, A., Prakasam, M., Clemens, S.C., Velu, A., 2015. Evolution of the south asian monsoon wind system since the late middle miocene. Palaeogeogr. Palaeoclimatol. Palaeoecol 438, 160–167.

Haake, B., Ittekot, V., Rixen, T., Ramasamy, V., Nair, R.R., Curry, W.B., 1993. Seasonality and internal variability of particle fluxes to the deep Arabian Sea. Deep-Sea Res. 40, 1323–1344.

Hammer, Ø., Harper, D.A., Ryan, P.D., 2001. Past: paleontological statistics software package for education and data analysis. Palaeontol. Electron. 4 (1), 9.

Haq, B.U., Milliman, J.D., 1984. Marine Geology and Oceanography of Arabian Sea and Coastal Pakistan. VanNostrund Reinhold, New York, p. 382.

Hays, J.D., Imbrie, J., Shackleton, N.J., 1976. Variations in the Earth's orbit: pacemaker of the ice ages. Science 194 (4270), 1121–1132.

Heinrich, H., 1988. Origin and consequences of cyclic ice rafting in the northeast atlantic ocean during the past 130,000 years. Quat. Res 29 (2), 142–152.

Howard, W.R., Prell, W.L., 1994. Late Quaternary $CaCO_3$ production and preservation in the Southern Ocean: Implications for oceanic and atmospheric carbon cycling. Paleoceanography 9 (3), 453–482.

Imbrie, J., Hays, J.D., Martinson, D.G., McIntyre, A., Mix, A.C., Morley, J.J., Pisias, N.G., Prell, W.L., Shackleton, N.J., 1984. The orbital theory of pleistocene climate: support from a revised chronology of the marine d18o record. In: Berger, A., Imbrie, J., Hays, J., Kukla, G., Saltzman, B. (Eds.), Milankovitch and Climate (Pt. 1), NATO ASI Ser. C, Math Phys. Sci., 126. 269–305.

Ishfaq, A.M., Pattan, J.N., Matta, V.M., Banakar, V.K., 2013. Variation of paleoproductivity and terrigenous input in the eastern Arabian Sea during the past 100 ka. Geol. Soc. India 81 (5), 647–654.

Ivanova, N., Sorokin, A., Anderson, I., Galleron, N., Candelon, B., Kapatral, V., Bhattacharyya, A., Reznik, G., Mikhailova, N., Lapidus, A., Chu, L., 2003. Genome sequence of *Bacillus cereus* and comparative analysis with *Bacillus anthracis*. Nature 423 (6935), 87–91.

Jaccard, S.L., Hayes, C.T., Martinez-Garcia, A., Hodell, D.A., Anderson, R.F., Sigman, D.M., Haug, G.H., 2013. Two modes of change in Southern Ocean productivity over the past million years. Science 339 (6126), 1419–1423.

Jain, M., Tandon, S.K., 2003. Fluvial response to Late Quaternary climate changes, western India. Quat. Sci. Rev. 22 (20), 2223–2235.

Jarvis, I., Jarvis, K.E., 1985. Rare-earth element geochemistry of standard sediments: a study using inductively coupled plasma spectrometry. Chem. Geol. 53 (3–4), 335–344.

Kessarkar, P.M., Rao, V.P., Ahmad, S.M., Babu, G.A., 2003. Clay minerals and Sr–Nd isotopes of the sediments along the western margin of India and their implication for sediment provenance. Mar. Geol. 202 (1–2), 55–69.

Kessarkar, P.M., Rao, V.P., Shynu, R., Mehra, P., Viegas, B.E., 2010. The nature and distribution of particulate matter in the Mandovi estuary, central west coast of India. Estuar. Coast. 33 (1), 30–44.

Kroon, D., Steens, T., Troelstra, S.R., 1991. Onset of monsoonal related upwelling in the western arabian sea as revealed by planktonic foraminifers. In: Prell, W.L., Niitsuma, N., et al. (Eds.), Proceedings of the Ocean Drilling Program, Scientific results, vol. 117. Ocean Drilling Program, College Station, Texas. 257–263.

Kumar, P., Chopra, S., Pattanaik, J.K., Ojha, S., Gargari, S., Joshi, R., Kanjilal, D., 2015. A new AMS facility at Inter University Accelerator Centre, New Delhi. Nucl. Instrum. Methods Phys. Res. B 361, 115–119.

Lévy, M., Shankar, D., André, J.-M., Shenoi, S.S.C., Durand, F., de Boyer Montégut, C., 2007. Basin-wide seasonal evolution of the Indian Ocean's phytoplankton blooms. J. Geophys. Res. 112, C12014.

Lisiecki, L.E., Raymo, M.E., 2005. A Pliocene-Pleistocene stack of 57 globally distributed benthic δ18O records. Paleoceanography 20 (1).

Marra, J., Barber, R.T., 2005. Primary productivity in the Arabian Sea: a synthesis of JGOFS data. Prog. Oceanogr. 65, 159–175.

Meyers, P.A., 1994. Preservation of elemental and isotopic source identification of sedimentary organic matter. Chem. Geol. 114 (3–4), 289–302.

Nagoji, S.S., Tiwari, M., 2017. Organic carbon preservation in Southeastern Arabian Sea sediments since mid-Holocene: I mplications to South Asian Summer Monsoon variability. Geochem. Geophys. Geosyst. 18 (9), 3438–3451.

Naidu, P.D., 1991. Glacial to interglacial contrasts in the calcium carbonate content and influence of Indus discharge in two eastern Arabian Sea cores. Palaeogeogr. Palaeoclimatol. Palaeoecol. 86 (3–4), 255–263.

Naidu, P.D., Malmgren, B.A., 1996. A high-resolution record of late Quaternary upwelling along the Oman Margin, Arabian Sea based on planktonic foraminifera. Paleoceanography 11 (1), 129–140.

Naik, D.K., Saraswat, R., Lea, D.W., Kurtarkar, S.R., Mackensen, A., 2017. Last glacial-interglacial productivity and associated changes in the eastern Arabian Sea. Palaeogeogr. Palaeoclimatol. Palaeoecol. 483, 147–156.

Nair, R.R., Ittekkot, V., Manganini, S.J., Ramaswamy, V., Haake, B., Degens, E.T., Desai, B.T., Honjo, S., 1989. Increased particle flux to the deep ocean related to monsoons. Nature 338 (6218), 749–751.

Naqvi, S.W.A., Naik, H., Narvekar, P.V., 2003. The Arabian Sea. In: Black, K., Shimmield, G.B. (Eds.), Biogeochemistry of Marine Systems. Oxford, UK, 157–207.

O'Connell, S., Wolf-Welling, T.W., Cremer, M., Stein, R., 1996. Neogene paleoceanography and paleoclimate history from Fram Strait: changes in accumulation rates. In: Proc. ODP. In: Sci. Results, vol. 151, 569–582.

Olsen, P.E., 1990. Tectonic, climatic, and Biotic modulation of lacustrine ecosystems–examples from newark supergroup of eastern north America: Chapter 13. In: Katz, B. (Ed.), Lacustrine Basin Exploration: Case Studies and Modern Analogis, Vol. 50. Am. Assoc. Pet. Geol. Mem., 209-224.

Paropkari, A.L., Iyer, S.D., Chauhan, O.S., Babu, C.P., 1991. Depositional environments inferred from variations of calcium carbonate, organic carbon, and sulfide sulfur: a core from southeastern Arabian Sea. Geo-Mar. Lett. 11 (2), 96–102.

Pattan, J.N., Masuzawa, T., Naidu, P.D., Parthiban, G., Yamamoto, M., 2003. Productivity fluctuations in the southeastern Arabian Sea during the last 140 ka. Palaeogeogr. Palaeoclimatol. Palaeoecol. 193 (3–4), 575–590.

Pattan, J.N., Masuzawa, T., Yamamoto, M., 2005. Variations in terrigenous sediment discharge in a sediment core from southeastern Arabian Sea during the last 140 ka. Curr. Sci. 1421–1425.

Pattan, J.N., Pearce, N.J.G., 2009. Bottom water oxygenation history in southeastern Arabian Sea during the past 140 ka: results from redox-sensitive elements. Palaeogeogr. Palaeoclimatol. Palaeoecol. 280 (3–4), 396–405.

Pattan, J.N., Shane, P., Banakar, V.K., 1999. New occurrence of Youngest Toba Tuff in abyssal sediments of the Central Indian Basin. Mar. Geol. 155 (3–4), 243–248.

Paulsen, M.L., Seuthe, L., Reigstad, M., Larsen, A., Cape, M.R., Vernet, M., 2018. Asynchronous accumulation of organic carbon and nitrogen in the Atlantic Gateway to the Arctic Ocean. Front. Mar. Sci. 5, 416.

Pedersen, T.F., 1983. Increased productivity in the eastern equatorial Pacific during the last glacial maximum (19,000 to 14,000 yr BP). Geology 11 (1), 16–19.
Prabhu, C.N., Shankar, R., 2005. Palaeoproductivity of the eastern Arabian Sea during the past 200 ka: A multi-proxy investigation. Deep-Sea Res. II 52 (14–15), 1994–2002.
Prabhu, C.N., Shankar, R., Anupama, K., Taieb, M., Bonnefille, R., Vidal, L., Prasad, S., 2004. A 200-ka pollen and oxygen-isotopic record from two sediment cores from the eastern Arabian Sea. Palaeogeogr. Palaeoclimatol. Palaeoecol. 214 (4), 309–321.
Prasanna Kumar, S., Narvekar, J., Kumar, A., Shaji, C., Anand, P., Sabu, P., Rejomon, G., Jacob, J., Jayaraj, K.A., Radhika, A., Nair, K.K.C., 2004. Intrusion of the Bay of Bengal water into the Arabian Sea during winter monsoon and associated chemical and biological response. Geophys. Res. Lett. 31, L15304.
Prins, M.A., Postma, G., Weltje, G.J., 2000. Controls on terrigenous sediment supply to the Arabian Sea during the late Quaternary: the Makran continental slope. Mar. Geol. 169 (3–4), 351–371.
Raja, P., Achyuthan, H., Farooqui, A., Ramesh, R., Kumar, P., Chopra, S., 2019. Tropical rainforest dynamics and palaeoclimate implications since the late Pleistocene, Nilgiris, India. Quat. Res. 91 (1), 367–382.
Rajendran, C.P., Sanwal, J., John, B., Anandasabari, K., Rajendran, K., Kumar, P., Jaiswal, M., Chopra, S., 2018. Footprints of an elusive mid-14th century earthquake in the central Himalaya: Consilience of evidence from Nepal and India. Geol. J. 54 (5), 2829–2846.
Rao, P., Wagle, B.G., 1997. Geomorphology and surficial geology of the western continental shelf and slope of India: A review. Curr. Sci. 73 (4), 330–350.
Rashid, H., Flower, B.P., Poore, R.Z., Quinn, T.M., 2007. A ∼25 ka Indian Ocean monsoon variability record from the Andaman Sea. Quat. Sci. Rev. 26 (19–21), 2586–2597.
Reichart, G.J., Lourens, L.J., Zachariasse, W.J., 1998. Temporal variability in the northern Arabian Sea oxygen minimum zone (OMZ) during the last 225,000 years. Paleoceanography 13 (6), 607–621.
Rostek, F., Bard, E., Beaufort, L., Sonzogni, C., Ganssen, G., 1997. Sea surface temperature and productivity records for the past 240 kyr in the Arabian Sea. Deep-Sea Res. II 44 (6–7), 1461–1480.
Saraswat, R., Lea, D.W., Nigam, R., Mackensen, A., Naik, D.K., 2013. Deglaciation in the tropical Indian Ocean driven by interplay between the regional monsoon and global teleconnections. Earth Planet. Sci. Lett. 375, 166–175.
Saravanan, P., Gupta, A.K., Zheng, H., Panigrahi, M.K., Prakasam, M., 2019. Late Holocene long arid phase in the Indian subcontinent as seen in shallow sediments of the eastern Arabian Sea. J. Asian Earth Sci. 181, 103915.
Saravanan, P., Gupta, A.K., Zheng, H., Panigrahi, M.K., Tiwari, S.K., Rai, S.K., Prakasam, M., 2020. Response of shallow-sea benthic foraminifera to environmental changes off the coast of Goa, eastern Arabian Sea, during the last ∼ 6100 cal yr BP. Geol. Mag. 157 (3), 497–505.
Sarkar, N.K., Mukherjee, M., Basu, M.A., Ghosh, A.S., 1993. Resonance in positron-hydrogen scattering at medium energy. J. Phys. B: At. Mol. Opt. Phys. 26 (14), L427.
Sarkar, A., Ramesh, R., Somayajulu, B.L.K., Agnihotri, R., Jull, A.T., Burr, G.S., 2000. High resolution Holocene monsoon record from the eastern Arabian Sea. Earth Planet. Sci. Lett. 177 (3–4), 209–218.
Schott, F., Swallow, J.C., Fieux, M., 1990. The Somali Current at the equator: annual cycle of currents and transports in the upper 1000 m and connection to neighbouring latitudes. Deep-Sea Res. I 37 (12), 1825–1848.
Schulz, M., Mudelsee, M., 2002. Redfit: estimating red-noise spectra directly from unevenly spaced paleoclimatic time series. Comput. Geosci 28 (3), 421–426.
Sharma, P., Borole, D.V., Zingde, M.D., 1994. ^{210}Pb based trace element fluxes in the nearshore and estuarine sediments off Bombay, India. Mar. Chem. 47 (3–4), 227–241.
Sharma, R., Umapathy, G.R., Kumar, P., Ojha, S., Gargari, S., Joshi, R., Chopra, S., Kanjilal, D., 2019. AMS and upcoming geochronology facility at Inter University Accelerator Centre (IUAC), New Delhi, India. Nucl. Instrum. Methods Phys. Res. B 438, 124–130.
Shenoi, S.S.C., Shankar, D., Shetye, S.R., 1999. The sea surface temperature high in the Lakshadweep Sea before the onset of the southwest monsoon. J. Geophys. Res. 104, 703–712.
Shimmield, G., Derrick, S., Mackensen, A., Grobe, H., Pudsey, C., 1994. The history of barium, biogenic silica and organic carbon accumulation in the Weddell Sea and Antarctic Ocean over the last 150,000 years. In: Carbon Cycling in the Glacial Ocean: Constraints on the Ocean's Role in Global Change. Springer, Berlin, Heidelberg, 555-574.
Shimmield, G.B., Mowbray, S.R., Weedon, G.P., 1990. A 350 ka history of the Indian Southwest Monsoon—evidence from deep-sea cores, northwest Arabian Sea. Earth Environ. Sci. Trans. R. Soc. Edinb. 81 (4), 289–299.
Singh, A.D., Jung, S.J., Darling, K., Ganeshram, R., Ivanochko, T., Kroon, D., 2011. Productivity collapses in the Arabian Sea during glacial cold phases. Paleoceanography 26 (3).
Sinha, A., Cannariato, K.G., Stott, L.D., Li, H.C., You, C.F., Cheng, H., Edwards, R.L., Singh, I.B., 2005. Variability of Southwest Indian summer monsoon precipitation during the Bølling–Allerød. Geology 33 (10), 813–816.
Sirocko, F., Garbe-Schönberg, D., Devey, C., 2000. Processes controlling trace element geochemistry of Arabian Sea sediments during the last 25,000 years. Glob. Planet. Change 26 (1–3), 217–303.
Sirocko, F., Sarnthein, M., 1989. Wind-borne deposits in the northwestern Indian Ocean: Record of Holocene sediments versus modern satellite data. In: Paleoclimatology and Paleometeorology: Modern and Past Patterns of Global Atmospheric Transport. Springer, Dordrecht, 401-433.
Sirocko, F., Sarnthein, M., Erlenkeuser, H., Lange, H., Arnold, M., Duplessy, J.C., 1993. Century-scale events in monsoonal climate over the past 24,000 years. Nature 364 (6435), 322–324.
Song, S.R., Chen, C.H., Lee, M.Y., Yang, T.F., Iizuka, Y., Wei, K.Y., 2000. Newly discovered eastern dispersal of the youngest Toba Tuff. Mar. Geol. 167 (3–4), 303–312.
Southon, J., Kashgarian, M., Fontugne, M., Metivier, B., Yim, W.W., 2002. Marine reservoir corrections for the indian ocean and southeast asia. Radiocarbon 44, 167–180.
Stevenson, F.J., Cheng, C.N., 1972. Organic geochemistry of the Argentine Basin sediments: carbon-nitrogen relationships and Quaternary correlations. Geochim. Cosmochim. Acta 36 (6), 653–671.
Stuiver, M., Reimer, P.J., 1993. Extended 14 C data base and revised CALIB 3.0 14 C age calibration program. Radiocarbon 35 (1), 215–230.
Stuiver, M., Reimer, P.J., Reimer, R.W., 2010. CALIB6.0 Available online at http://calib.qub.ac.uk/calib.
Thamban, M., Rao, V.P., 2000. Distribution and composition of verdine and glaucony facies from the sediments of the western continental margin of India. In: Glenn, C.R., Prevot-Lucas, L., Lucas, J. (Eds.), Marine Authigenesis: From Global to Microbial, 66. SEPM Spec. Publ., 233–244.
Thamban, M., Rao, V.P., Raju, S.V., 1997. Controls on organic carbon distribution in sediments from the eastern Arabian Sea margin. Geo-Mar. Lett. 17 (3), 220–227.
Thamban, M., Rao, V.P., Schneider, R.R., 2002. Reconstruction of late Quaternary monsoon oscillations based on clay mineral proxies using sediment cores from the western margin of India. Mar. Geol. 186 (3–4), 527–539.
Thamban, M., Rao, V.P., Schneider, R.R., Grootes, P.M., 2001. Glacial to Holocene fluctuations in hydrography and productivity along the southwestern continental margin of India. Palaeogeogr. Palaeoclimatol. Palaeoecol. 165 (1–2), 113–127.
Tindale, N.W., Pease, P.P., 1999. Aerosols over the Arabian Sea: Atmospheric transport pathways and concentrations of dust and sea salt. Deep-Sea Res. II 46 (8–9), 1577–1595.
Valeton, I., Wilke, F., 1993. Tertiary bauxites in subsidence areas and associated laterite-derived sediments in northwestern India. Contrib. Sedimentol. 18, 104.
Wacker, L., Němec, M., Bourquin, J., 2010. A revolutionary graphitisation system: fully automated, compact and simple. Nucl. Instum. Methods Phys. Res. 268 (7–8), 931–934.
Wang, P., Clemens, S., Beaufort, L., Ganssen, G., Jian, Z., Kershaw, P., Sarnthein, M., 2005. Evolution and variability of the asian monsoon system: state of the art and outstanding issues. Quant. Sci. Rev. 24 (5–6), 595–629.
Wiggert, J.D., Hood, R.R., Banse, K., Kindle, J.C., 2005. Monsoon-driven biogeochemical processes in the Arabian Sea. Prog. Oceanogr. 65, 176–213.
Zahn, R., Pedersen, T.F., 1991. A Pleistocene evolution of surface and mid-depth hydrography at the Oman margin: planktonic and benthic isotope records at site 724. In: Prell, W.L., Niitsuma, N., et al. (Eds.), Proc. Ocean Drilling Programme. In: Scientific Results, vol. 117. College Station, TX, 291–303.

Arabian Journal of Geosciences (2021) 14:2646
https://doi.org/10.1007/s12517-021-08882-7

ORIGINAL PAPER

Quantitative stream network analysis for assessing form and hydrological processes of the watersheds of Kolli hills, Tamil Nadu, India

N. Jawahar Raj[1] · A. Prabhakaran[1] · A. Muthukrishnan[2]

Received: 12 December 2020 / Accepted: 8 November 2021
© Saudi Society for Geosciences 2021

Abstract
The present study attempts to bring out information relating to the form and processes of the major watersheds of the Kolli hills of southern India. For this purpose, various parameters which are routinely considered for stream network analysis such as stream ordering, numbering, stream length, bifurcation ratio, length ratio, watershed length, area, drainage density, stream frequency, form factor, length of overland flow, relief, slope, gradient, ruggedness number, relative relief, and relief ratio were quantitatively estimated for each watershed using ArcGIS software. These estimations of the watersheds were analysed to infer form and processes such as watersheds' shape, size, elongation, slope steepness and length, degree of development of stream network, degree of branching of streams, degree of terrain dissection, infiltration potential, mean annual runoff, rainfall intensity, relative potential energy, relative denudation rate, relative discharge rates, mean and peak discharge, lag time, intensity of erosion, relative amount of sediment transported, and flash flood proneness. The understanding of these forms and processes of the watersheds could serve as useful inputs for the planners in the formulation of strategies for the sustainable development of the Kolli hills.

Keywords Kolli hills · Drainage morphometry · Watersheds

Introduction

The term watershed is a finite area, whose runoff is channelled through a single outlet. It embraces all its natural and man-made features, which include its surface and subsurface features, climate and weather patterns, geologic and topographic settings, soils and vegetation characteristics, and land use. Increasingly watersheds have been used as the natural planning unit especially for managing natural resources. In India, the watershed has been used as the planning unit especially in hill areas since the seventh Five-Year Plan (1985–1990). Understanding of the form and processes is a vital input in watershed-based planning, for which watershed-based quantitative analysis of stream network is indispensable. In the early days, the analysis and description of stream network within a watershed were purely qualitative. Gradually, this transformed into a rigorous quantitative science capable of providing numerical data of practical value. Horton's works (1932, 1945) which are still quoted widely in literature laid a strong foundation for this quantitative approach. These pioneering works were supplemented by Langbein (1947) and were later developed in detail by Strahler (1952), Miller (1953), Leopold and Miller (1956), Schumm (1956), Melton (1957), Morisawa (1959), Shreve (1966), Scheidegger (1967), Doornkamp (1968), Chorley (1969), Gregory and Walling (1973), Gardiner (1975), Zavoianu (1985), and others.

In the past, topographic maps and data obtained from fieldwork were the common data sources for quantitative analysis of stream networks. However, since the last two decades, aerial photographs and satellite images have been increasingly used as the data source. The availability of high-resolution satellite data and advancements made in the field of GIS has provided the opportunity for accurately delineating stream networks of smaller watersheds/large river basins.

Responsible Editor: Stefan Grab

✉ N. Jawahar Raj
njrtrichy@gmail.com

[1] Department of Geology, National College, Tiruchirappalli-1, India

[2] Department of Geography, University of Madras, Chennai, India

Published online: 26 November 2021

Delineation of stream networks along with their boundaries, on a global scale, which is widely known as global river network programme has been attempted by several pioneering agencies. The first such attempt was undertaken by the United States Geological Survey (USGS) and the United Nations Environmental Programme Global Resources Information Database (UNEP/GRID) in the year 1996. In this attempt, they generated Global River Network and Watershed Boundary Data Set (HRDRO 1 K), from the USGS' 30 arc-second DEM data (USGS 2001). These Global River Networking programmes have been periodically updated with the increasing availability of high-resolution satellite data. During the period 2006–2008, the World Wildlife Fund, the USGS, the International Centre for Tropical Agriculture (CIAT), the Nature Conservancy (TNC), and the Kassel University of Germany have collaborated to generate global hydrological data and maps (HydroSHEDS), at multiple scales from the 90-m resolution Shuttle Radar Topographic Mission (SRTM) satellite data (Lehner et al. 2008). This was followed by the development of a new global high-resolution hydrologic derivative database, entitled Hydrologic Derivatives for Modeling and Analysis (HDMA)13, based on HydroSHEDS, GMTED2010 (Global Multi-resolution Terrain Elevation Data 2010), and SRTM data by the USGS (Verdin 2017). In recent years, attempts have been made to improve the accuracy of the data, especially in plain areas, such as the one carried out by Yan et al. (2019). They have combined SRTM DEM data and Advanced Spaceborne and Thermal Emission and Reflection Radiometer (ASTER) GDEM V2 data and adopted the "stream burning method" to improve the accuracy of the data. These developments, along with growing knowledge on the inter-relationship of various measures of stream network with that of the form and process, have led to the spurt in such studies using remote sensing and GIS techniques (Reddy et al. 2004; Gelaberta et al. 2005; Ng 2006; Altın and Altin 2011; Al-Rowaily et al. 2012; Ghosh et al. 2014; Sangireddy et al. 2016; Taha et al. 2017; Argyrioua et al. 2017; Bisht et al. 2018; Arabameri et al. 2020).

The present study attempts to understand the form and processes of the watersheds of the Kolli hills, located in the interior part of Tamil Nadu State of southern India. This has been done through quantitative analysis of the stream network of watersheds spread over the hills. The area, chosen for the present study, the Kolli hills, is biodiversity-rich, and it serves as a major catchment area for the important rivers of the region such as Sweta Nadhi, Aiyar, Thirumanimuthar, and Karaipottanar. The inhabitants of the Kolli hills (about 42,000 persons, most of whom are tribals) and those of the adjoining plains depend upon the water flow in the streams of the hills for carrying out agricultural activities and catering to their other water needs. In the last few decades, unscientific exploitation for its rich resources has led to the degradation of these hills, resulting in increased incidences of flash flood and mass movement, increased rates of soil erosion, reduction of perennial streams to ephemeral streams, drying up of springs, etc. To reverse this trend, suitable strategies should be evolved based on scientific investigations on varied themes, including the form and processes of the watersheds. So far, investigations relating to the form and processes of the watersheds of the study area have not been undertaken. The present study aims to provide these vital inputs for the planners. The objectives considered for the study are to estimate the linear, areal, and relief aspects of the stream network and to bring out information relating to the form and processes of the watersheds of the study area from their analysis.

The study area

Kolli hills, the study area, is spread between north latitudes 11°12′36″–11°29′40″ and east longitudes 78°17′51″–78°29′11″ (Fig. 1) and is located in Tamil Nadu State of southern India. Almost the entire study area lies in the Namakkal District of Tamil Nadu State; a few small pockets on the eastern margins of the study area lie in Tiruchirappalli District. Most of the streams of the hills are confined to eight independent watersheds demarcated, and the present study is confined to these eight watersheds (Fig. 2). The areal extent of the study area is 380.07 km^2. The climate of the study area varies with the altitude. In general, the outer and the lower slopes of the hills receive relatively lesser rainfall (ranging from 787 to 910 mm) and are characterised by relatively higher temperatures (ranging from 20 to 40 °C). In the higher slopes and the plateau portion of the hills, rainfall is substantially higher (ranging from 1189 to 1333 mm) and temperatures are lesser ranging from 10 to 30 °C. The southwest monsoon is considerably more active in the western part while the northeast monsoon is more active in the eastern part of the hills. Three types of soils, viz., clayey, gravelly-clay, and gravelly-loamy soils are predominant in the study area. Among these, clayey soils occupy most of the undulating and moderately sloping higher plateau portion of the hills and are relatively deep. Gravelly-loamy soils, typically shallow, are associated with the steeper slopes of the hills, while gravelly-clay soils are found associated with the gentler slopes. Charnockite, the predominating rock, occupies 93.40% of the study area. The remaining 6.6% of the study area is shared by ten other rocks which include hornblende biotite gneiss, quartzo-feldspathic gneiss, pyroxene granulite, basic dyke, banded magnetite quartzite, laterite, ultramafics, granite, pegmatite, and quartz veins (Fig. 3). Interestingly, five of these minor rocks of the study area such as hornblende biotite gneiss, quartzo-feldspathic gneiss, ultramafics, granite, and quartz veins

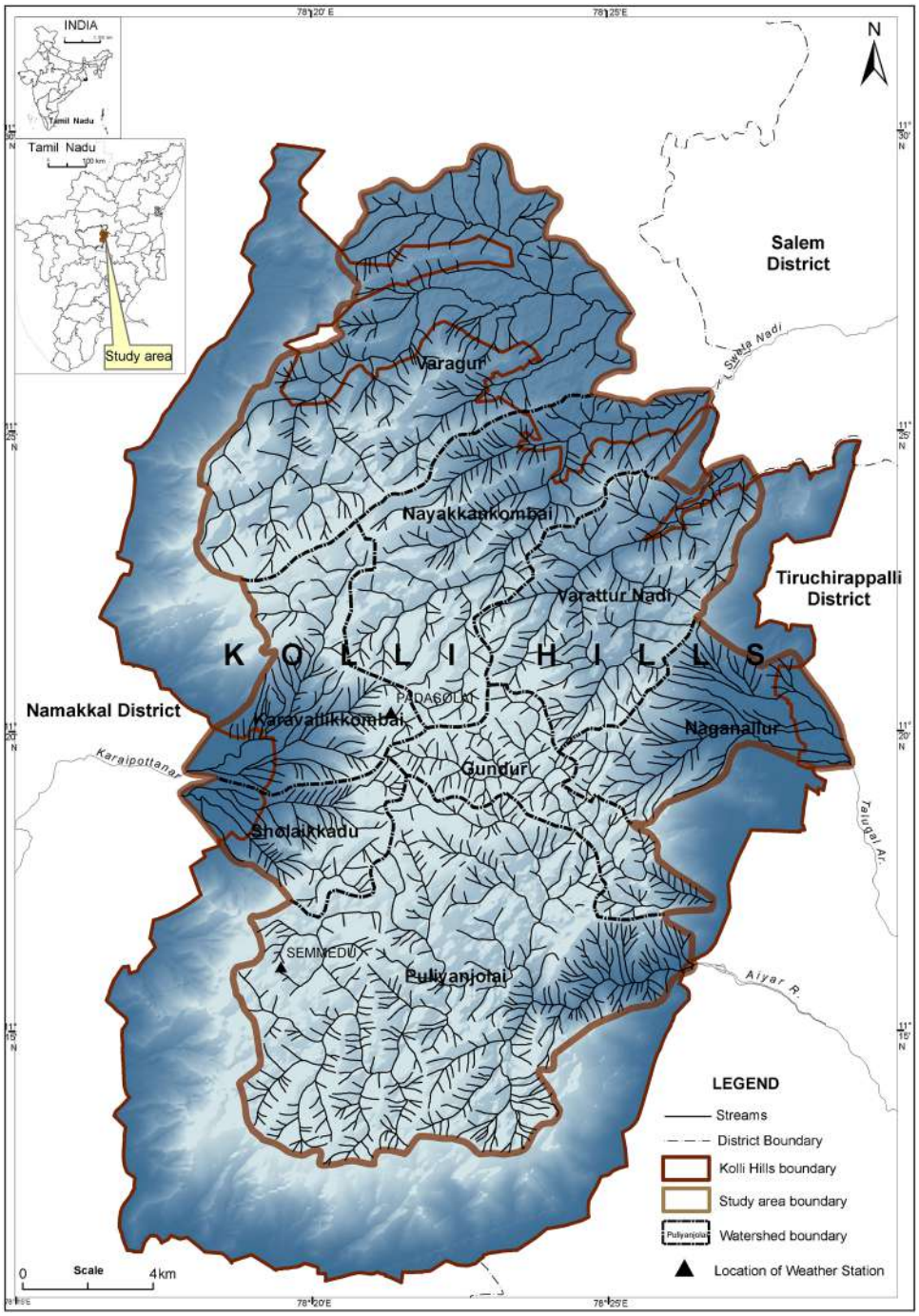


Fig. 1 Study area

are restricted to the north-western part of the study area. The geological age of these rocks ranges from Archaean to Tertiary, while charnockite, pyroxene granulite, banded magnetite quartzite, and ultramafics are of Archaean age, and hornblende biotite gneiss, quartzo-feldspathic gneiss, and basic dykes range in age from Archaean to Proterozoic. Granites, pegmatites, and quartz veins are of the Proterozoic age, while the laterites are of the Tertiary age. The study area forms part of the Southern Granulitic Terrain (SGT), a geologically important region that has experienced multiple episodes of crustal deformation (Drury and Holt 1980; Chetty and Bhaskar Rao 2006) and has been metamorphosed to granulitic facies. A major NE-SW trending regional fault, designated as "Attur Fault" (Narula et al. 2000), passes through the south-eastern part of the Kolli hills.

The analysis of the areal extent of various elevation zones of the study area deduced from the digital elevation model (DEM) generated from SRTM (Shuttle Radar Topographic Mission) satellite data (Fig. 4) reveals that in about 42% of the study area, elevation exceeds 1000 m. Such high elevated

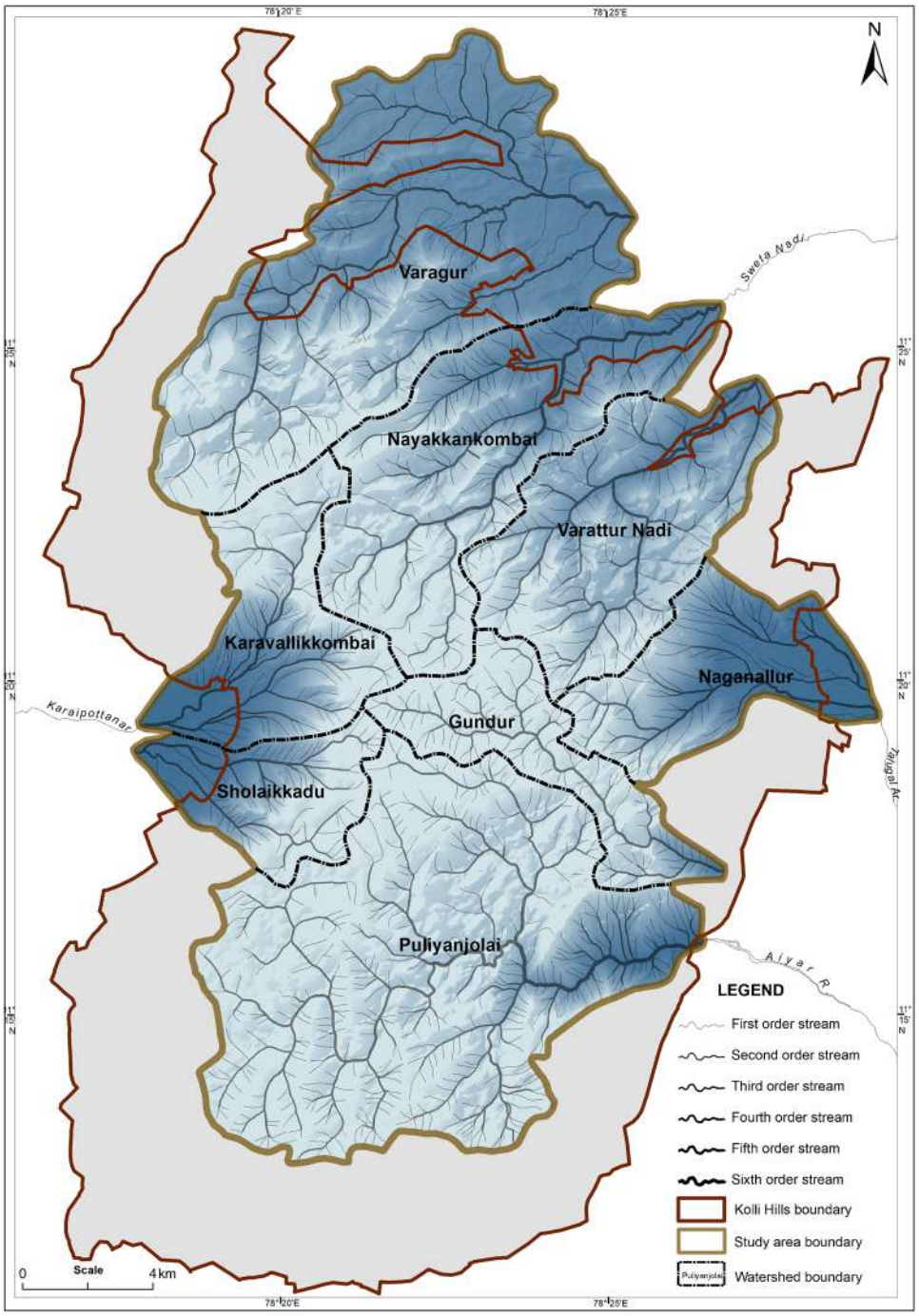


Fig. 2 Watersheds

areas are confined to the higher undulating plateau portion. In 21% of the study area, the elevation ranges from 751 to 1000 m, and this elevation zone is confined to the higher portion of the outer slopes. Areas with elevation, ranging from 501 to 750 m, occupy about 16% of the study area, and this elevation zone is confined to the middle portion of the outer slopes. In 19% of the study area, the elevation ranges from 251 to 500 m, and this elevation zone is restricted to the lower portion of the outer slopes. Areas, where elevation is less than 250 m, are meagre in areal extent and are confined to the foothills. Information relating to the slope (Fig. 5) deduced from the DEM generated from SRTM satellite data reveals that in most of the study area, the slope is either moderate (5 to 15°) or strong (15 to 25°). These slope classes occupy together about 64% of the study area with their proportion individually being 36% and 28%, respectively. Gentle (1 to 5°) and steep (25 to 35°) slopes are next in importance, and they occupy about 17% and 14%, respectively. In about 2% of the study area, the terrain is nearly level.

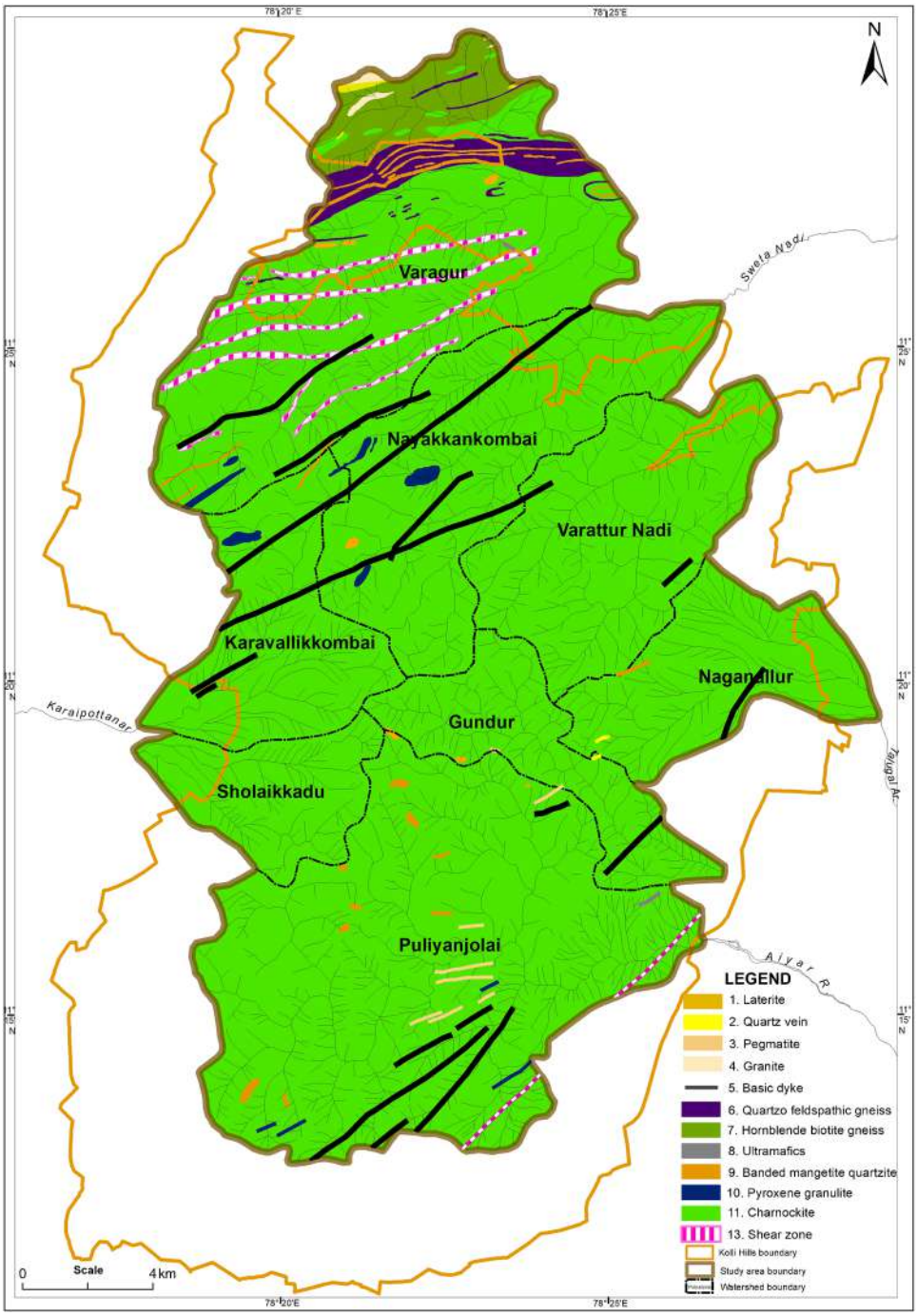


Fig. 3 Geology

Methodology

DEM generated from the SRTM satellite data, Indian Remote Sensing Satellite (IRS) P6's LISS IV, and CARTOSAT sensors' merged data are the data sources utilised for the present study. The spatial analyst toolset, of the ArcGIS software and its various tools, was made use of in the present study. In this toolset, tools such as fill, flow accumulation, and flow direction were made use of to delineate streams of the study area. Stream to feature tool was made use of for smoothening the delineated streams. Snap pour point tool was made use to delineate watersheds of the study area. Thus, the stream network within each watershed of the study area was generated. Altogether, eight watersheds were delineated along with their stream network. Each of these watersheds was given distinct names. The names were assigned based either on the name of the trunk stream or the name of the major settlement/reserved forests within the watershed. This was followed by the ordering of streams within each watershed. For this

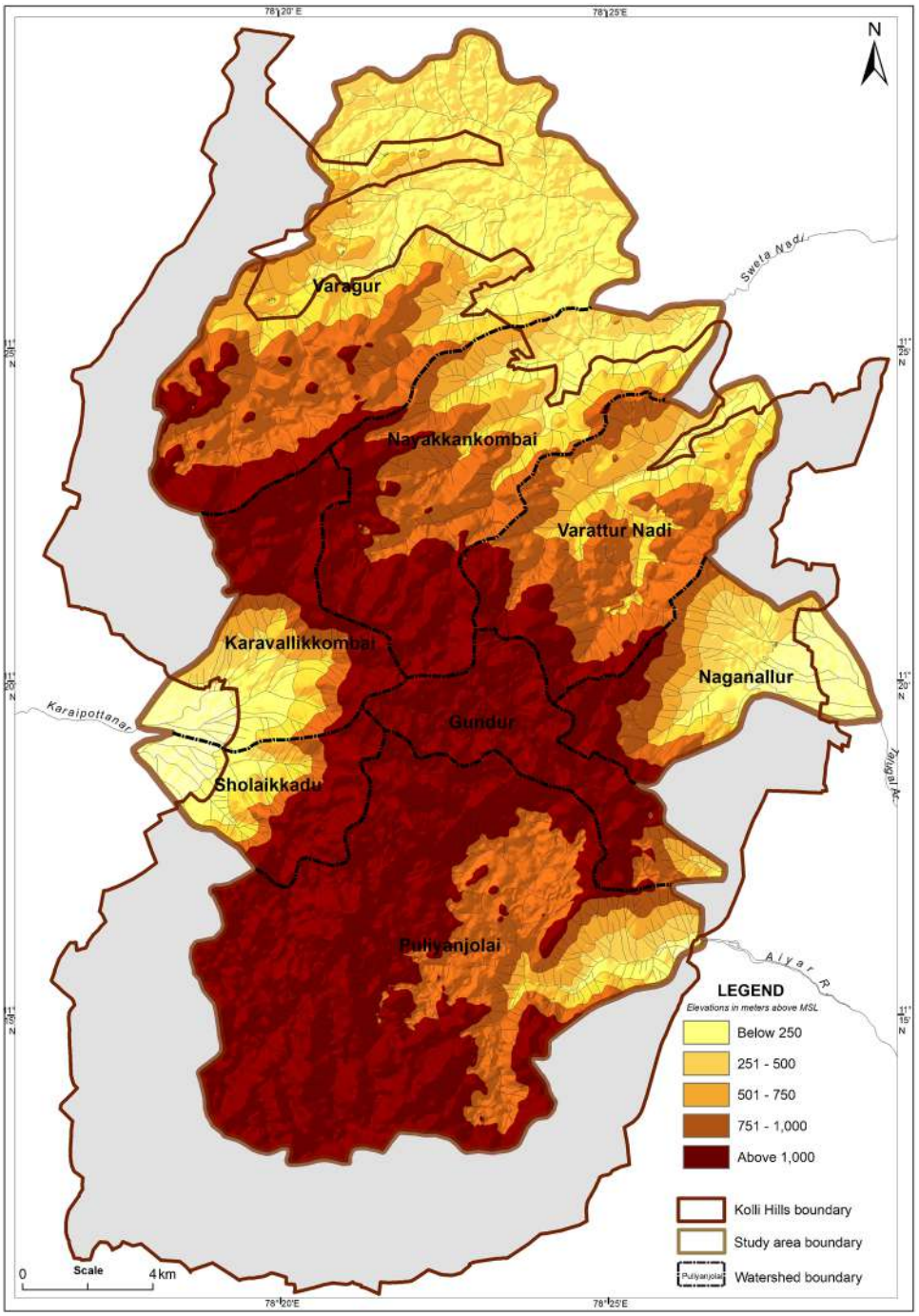


Fig. 4 Elevations zones

purpose, stream ordering tool of the spatial analyst toolset was made use of. The streams were ordered by adopting Strahler's method, which is the most widely adopted method. Later, the raster output of watersheds and their streams of the study area were converted into vector output. This was done to facilitate quantitative determination of parameters such as watershed area, watershed length, and watershed perimeter, which form the basic input in the calculation of various linear, areal, and relief aspects. The linear aspects considered for the present study are bifurcation ratio and length ratio. The areal aspects considered are drainage density, stream frequency, form factor, length of overland flow, and infiltration number. In the case of relief aspects, parameters such as relief, relief ratio, relative relief, and ruggedness number were considered. Based on the established relationships between these parameters with that of the form and processes, an attempt has been made in the present study to understand watershed-wise form and hydrological processes.

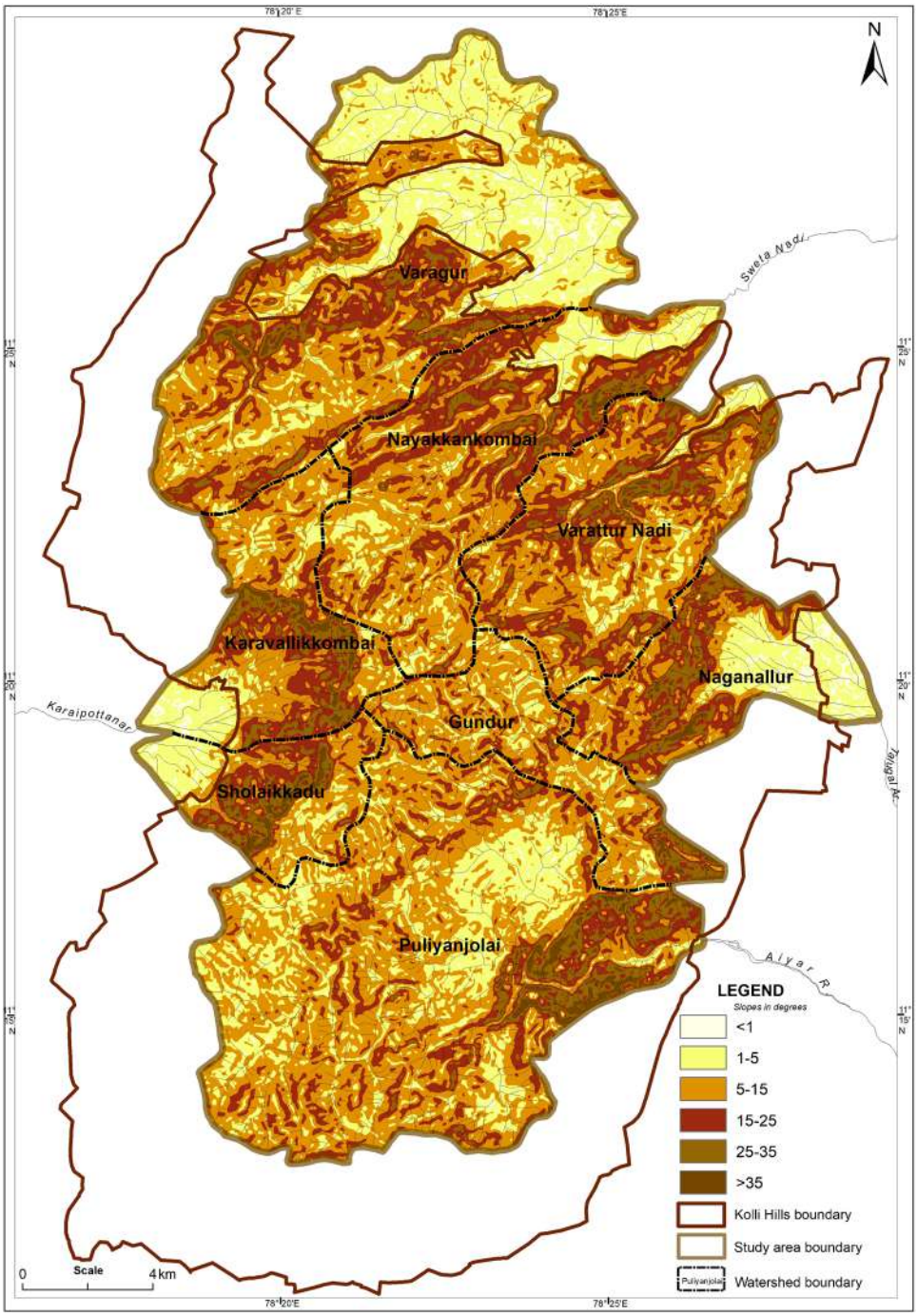


Fig. 5 Slopes zones

Results and discussion

In the following section, the procedure adopted for the estimation of various measures relating to the linear, areal, and relief aspects has been described. The well-established inter-relationships between these measures with that of the form and processes of the watersheds, put forth by experts in the field, have also been presented. Furthermore, based on the range of estimated values of each parameter, the watersheds of the study area have been grouped to facilitate inferring the form and processes.

Linear aspects

The study of linear aspects of a watershed is related to the study of channel patterns of the stream network, wherein the topological characteristics of the stream segments in terms of open links of the network system (streams) are analysed. This includes the analysis of stream order, stream number,

bifurcation ratio, stream lengths, and length ratio. Watershed-wise estimations of these parameters are shown in Table 1a and b and have been consolidated (Table 2). These estimations have been analysed to draw inferences regarding the form and hydrological processes of the watersheds of the study area.

Stream order

Stream ordering is the hierarchical ranking of a stream within a drainage basin or watershed. In quantitative stream network analysis, it is customary to make stream order-wise estimations of several parameters. This implies that the ordering of streams within each watershed is the fundamental step. Besides facilitating hierarchical ranking of streams within a watershed, ordering of streams paves the way to designate the trunk stream of a watershed (as either headwater stream or medium stream or river) or the watershed itself. This would help to facilitate the comparison of watersheds in an area. Ordering of streams of the eight watersheds of the study area shows that the order of trunk stream is the least for Sholaikkadu watershed (fourth-order) and is the maximum for Puliyanjolai watershed (sixth-order). In general, trunk streams are designated as headwater streams if it is third-order or less. If it ranges from fourth- to sixth-order, it is designated as medium streams, and if it is greater than sixth order, it is designated as a river. Analysis of the order of the trunk streams of the watersheds of the study area shows that they range from fourth to sixth order implying that the trunk streams of the watersheds are medium streams. Apart from facilitating designation of trunk streams, stream ordering can also be helpful in designating the watershed itself, based on the order of their trunk streams (as third-order or fourth-order or fifth-order watershed, etc.). It is observed that in six of the eight watersheds, the trunk stream is of fifth-order; this implies that most of the watersheds of the study area are fifth-order watersheds. Exception to this are Sholaikkadu and Puliyanjolai watersheds, where the trunk stream is fourth- and sixth-order, respectively, and hence, they are fourth-order and sixth-order watersheds, respectively.

Total stream number

This refers to the sum of stream segments within a watershed. This measure is an important input towards directly estimating stream order-wise bifurcation ratio, mean stream length, length ratio, stream frequency, and infiltration number. Estimation of the number of stream segments in each order shows that the number of streams decreases with increasing stream order in all the watersheds (Table 1a and b). Furthermore, it is observed that the proportion of lower-order streams, which includes both first- and second-order streams, constitute the bulk of the streams in all the watersheds (Table 2), ranging from 92% (in Naganallur watershed) to 96.19% (in Nayakkankombai watershed). Such a very high proportion of lower-order streams in all the watersheds of the study area reflect the rugged terrain of the study area, typical of hilly watersheds wherein the slopes will be steeper with quicker water flow in their streams. It is observed that in Nayakkankombai and Gundur watersheds, which are exclusively confined to the hilly portion, the proportion of lower-order streams is relatively greater, while in watersheds that extend up to foothills and adjoining plains (Varagur and Naganallur watersheds), the proportion of lower-order streams is relatively lesser.

Bifurcation ratio

The bifurcation ratio (R_b) is the ratio of the number of streams of a given order to that of the next higher order. It is a measure of the branching of streams (Doornkamp and King 1971) and the degree of development of stream network (Strahler 1957) within a watershed. It is also an index of the shape of a watershed (Chow 1964; Zavoianu 1985). The estimated stream order-wise bifurcation ratio in each watershed (Table 2) shows that it ranges narrowly (between 2 and 5), which reflects the overall uniformity in the branching streams. Exceptions to this general observation are the Nayakkankombai, Gundur, and Sholaikkadu watersheds, where the bifurcation ratio between certain stream orders is higher. In the case of the Nayakkankombai watershed, the bifurcation ratio is higher between I and II orders (where $R_b = 5.31$), and II and III orders (where $R_b = 6.40$), and in the case of Gundur and Sholaikkadu watersheds, it is higher between I and II orders, where it is 5.75 and 5.67, respectively. These variations reflect the distinct differences in local physiography and geologic conditions in these watersheds, especially in the areas drained by the first and second orders.

Numerous studies conducted in a wide variety of environments have shown that the mean bifurcation ratio of watersheds/drainage basins ranges from 3 to 5 (Strahler 1964; Giusti and Schneider 1965; Shreve 1966; Shulits 1968; Verstappen 1983). As the estimated mean bifurcation ratio of the watersheds of the study area varies from 3.02 (in Naganallur watershed) to 4.22 (in Sholaikkadu watershed), it can be concluded that the bifurcation ratio of the watersheds of the study area are within the normal range. Investigations by Strahler (1957) have shown that in watersheds with well-developed stream network, the mean bifurcation ratio ranges from 3 to 5. As the estimated mean bifurcation ratio for the watersheds of the study is within this range, it can be inferred that the stream network in all the watersheds of the study area is well developed. Studies conducted by Horton (1945) have shown that the mean bifurcation ratio of hilly watersheds typically ranges between 3 and 4. The estimated mean bifurcation values of the watersheds of the study area

Table 1 Linear aspects of individual watersheds

Stream order	Stream number	Stream number (in %)	Bifurcation ratio	Stream length (in km)	Stream length (in %)	Mean stream length (in km)	Length ratio
Varagur Watershed							
I	199	76.83		118.71	59.98	0.60	
II	43	16.60	4.63	35.66	18.02	0.83	0.72
III	13	5.02	3.31	20.58	10.40	1.58	0.52
IV	3	1.16	4.33	7.00	3.54	2.33	0.68
V	1	0.39	3.00	15.95	8.06	15.95	0.15
Total/mean	259	100.00	3.82	197.90	100.00		0.52
Nayakkankombai Watershed							
I	170	80.95		87.94	62.85	0.52	
II	32	15.24	5.31	26.67	19.06	0.83	0.62
III	5	2.38	6.40	12.59	9.00	2.52	0.33
IV	2	0.95	2.50	7.39	5.28	3.70	0.68
V	1	0.48	2.00	5.34	3.82	5.34	0.69
Total/mean	210	100.00	4.05	139.93	100.00		0.58
Varattur Nadi Watershed							
I	134	78.36		69.77	60.63	0.52	
II	27	15.79	4.96	21.30	18.51	0.79	0.66
III	7	4.09	3.86	11.07	9.62	1.58	0.50
IV	2	1.17	3.50	7.08	6.15	3.54	0.45
V	1	0.58	2.00	5.85	5.08	5.85	0.61
Total/mean	171	100.00	3.58	115.07	100.00		0.55
Naganallur Watershed							
I	71	71.00		50.38	59.37	0.71	
II	21	21.00	3.38	16.78	19.77	0.80	0.89
III	5	5.00	4.20	9.83	11.58	1.97	0.41
IV	2	2.00	2.50	7.57	8.92	3.79	0.52
V	1	1.00	2.00	0.30	0.35	0.30	12.62
Total/mean	100	100.00	3.02	84.86	100.00		3.61
Stream order	Stream number	Stream number (in %)	Bifurcation ratio	Stream length (in km)	Stream length (in %)	Mean stream length (in km)	Length ratio
Gundur Watershed							
I	115	80.99		46.66	63.62	0.41	
II	20	14.08	5.75	11.44	15.60	0.57	0.71
III	4	2.82	5.00	6.12	8.34	1.53	0.37
IV	2	1.41	2.00	8.04	10.96	4.02	0.38
V	1	0.70	2.00	1.08	1.47	1.08	3.72
Total/mean	142	100.00	3.69	73.34	100.00		1.30
Puliyanjolai Watershed							
I	402	78.21		192.6	62.44	0.48	
II	85	16.54	4.73	62.94	20.40	0.74	0.65
III	19	3.70	4.47	24.73	8.02	1.30	0.57
IV	5	0.97	3.80	16.37	5.31	3.27	0.40
V	2	0.39	2.50	4.84	1.57	2.42	1.35
VI	1	0.19	2.00	7.00	2.27	7.00	0.35
Total/mean	514	100.00	3.50	308.48	100.00		0.66
Sholaikkadu Watershed							
I	68	80.00		36.76	61.91	0.54	
II	12	14.12	5.67	8.95	15.07	0.75	0.72
III	4	4.71	3.00	8.61	14.50	2.15	0.35

Table 1 (continued)

Stream order	Stream number	Stream number (in %)	Bifurcation ratio	Stream length (in km)	Stream length (in %)	Mean stream length (in km)	Length ratio
IV	1	1.18	4.00	5.06	8.52	5.06	0.43
Total/mean	85	100.00	4.22	59.38	100.00		0.50
Karavallikkombai Watershed							
I	84	76.36		53.98	61.45	0.64	
II	19	17.27	4.42	17.93	20.41	0.94	0.68
III	4	3.64	4.75	7.69	8.75	1.92	0.49
IV	2	1.82	2.00	7.58	8.63	3.79	0.51
V	1	0.91	2.00	0.66	0.75	0.66	5.74
Total/mean	110	100.00	3.29	87.84	100.00	1.59	1.86

Table 2 Linear aspects of the watersheds (consolidated)

Name of the watershed	Maximum stream order	Total stream number	First-order streams (%)	Second-order streams (%)	Lower-order streams (%)	Total stream length (km)	Mean R_b	Mean R_l
Varagur	V	259	76.83	16.60	93.43	197.90	3.82	0.52
Nayakkankkombai	V	210	80.95	15.24	96.19	139.93	4.05	0.58
Varattur Nadi	V	171	78.36	15.79	94.15	115.07	3.58	0.55
Naganallur	V	100	71.00	21.00	92.00	84.86	3.02	3.61
Gundur	V	142	80.99	14.08	95.07	73.34	3.69	1.30
Puliyanjolai	VI	514	78.21	16.54	94.75	308.48	3.50	0.66
Sholaikkadu	IV	85	80.00	14.12	94.12	59.38	4.22	0.50
Karavallikkombai	V	110	76.36	17.27	93.63	87.84	3.29	1.86

R_b bifurcation ratio, R_l length ratio

range from 3.02 to 4.22, which conforms to the findings of Horton.

Stream length

Stream length is the distance measured along the stream channel from the source to a given point or the outlet. This measure is found to be directly related to mean annual runoff (Morisawa 1962) and, furthermore, is an essential input for computing various other morphometric parameters such as mean stream length, drainage density, and length of overland flow. Stream length was estimated for all stream orders within each watershed of the study area (Table 1a and b), and they were added together to find out the total stream length of watersheds (Table 2). The total stream length estimated for the watersheds of the study area varies from 59.38 km (Sholaikkadu watershed) to 308.48 km (Puliyanjolai watershed). Among the watersheds of the study area, the total stream length is higher (> 150 km) in the larger watersheds such as Puliyanjolai and Varagur, while it is lower (< 100 km) in Sholaikkadu, Gundur, Naganallur, and Karavallikkombai watersheds. In the rest of the other watersheds, it is moderate (101 to 150 km). Furthermore, the analysis of the estimated total stream length of the watersheds shows that in the study area, the total stream length is directly related to the size of the watershed, i.e., the larger the watershed, the greater the total stream length.

Mean stream length

The mean stream length is a dimensional property, revealing the characteristic size of the components of a drainage network and its contributing basin surface (Strahler 1964). Stream order-wise mean length in each watershed was obtained by dividing the total length of streams of a particular order by the total number of stream segments of the order. In general, it is observed that the mean stream length increases with the increasing stream order in all the watersheds (Table 1a and b).

Length ratio

It is the ratio of the mean length of streams of an order to that of the next higher order. Several studies, conducted in a variety of environments (Horton 1945; Melton 1957; Morisawa 1962; Smart 1972), have shown that length ratio,

like that of bifurcation ratio, is remarkably stable (Abrahams 1984). Among the watersheds of the study area, it is found to range from 0.50 to 3.61. The ratio is low (≤ 0.60) in Sholaikkadu, Varagur, Varattur Nadi, and Nayakkankombai watersheds and high (> 1.80) in Naganallur and Karavallikombai watersheds. In the rest of the other two watersheds, viz., Puliyanjolai and Gundur watersheds, it is moderate (0.60 to 1.80). Minor differences in length ratio values for the watersheds of the study area can be attributed mainly due to differences in slope and topographic conditions.

Areal aspects

Areal aspects express the overall plan form and dimensions of watersheds. The areal aspects considered for the present study include watersheds' areal extent, length, shape, drainage density, stream frequency, length of overland flow, and infiltration number. The estimation of these parameters for the watersheds of the study area is given in Table 3.

Watershed area

The areal extent of watersheds (or) drainage basins is perhaps the most frequently employed morphometric variable used in the estimation of stream-flow characteristics (Patton and Baker 1976). Its significance has long been recognised and has always featured itself in most prediction models (Peh 1986). Its distinct relationship with several hydrological parameters such as mean discharge, mean annual runoff, sediment yield, and maximum flood discharge per unit area is well documented. Also, studies have shown that it has a direct relationship with the mean discharge of the watershed (Leopold and Maddock 1953; Strahler 1964; Dunne and Leopold 1978; Zavoianu 1985) and mean annual runoff (Strahler 1957; Morisawa 1962), and it has an inverse relationship with sediment yield (Strahler 1957) and maximum flood discharge per unit area (More et al. 1967 as quoted in Chorley 1969). The estimated areal extent of the watersheds of the study area shows that it varies distinctly from 18.21 km^2 (Sholaikkadu watershed) to 103.15 km^2 (Puliyanjolai watershed). In the study area, Puliyanjolai and Varagur watersheds are larger (> 70 km^2), whereas Sholaikkadu, Gundur, Naganallur, and Karavallikkombai watersheds are smaller (≤ 30 km^2). The other two watersheds, viz., Nayakkankombai and Varattur Nadi watersheds, are moderate-sized (30 to 70 km^2).

Watershed length

Watershed length is the longest length of the watershed, from the catchment to the point of confluence (Gregory and Walling 1973), and this measure is essential towards the estimation of other morphometric parameters such as form factor and relative relief of the watersheds. In the study area, the watershed length varies from 7 km (Sholaikkadu) to 14.27 km (Puliyanjolai watershed). Puliyanjolai, Varagur, and Nayakkankombai watersheds are longer (> 13 km), whereas Sholaikkadu and Naganallur are shorter in length (≤ 9 km). In Varattur Nadi and Gundur watersheds, the length is moderate (9–13 km).

Form factor

It is one of the commonly used indices to decipher the shape of the watersheds and is defined as the ratio of the area of the watershed to the square of the length of the watershed (Horton 1945). The shape of the watershed is the direct outcome of its development of a stream network. The shape is pear-shaped in the early stages, and as the erosion cycle advances, the shape becomes more elongated. Though several shape indices such as form factor (Horton 1945), elongation ratio (Schumm 1956), circularity ratio (Miller 1953), lemniscate loop (Chorley 1957), and compactness coefficient (Luchisheva 1950) are in use to quantitatively describe the shape of the watershed, in the present analysis, Horton's (1945) form factor (*F*), the most widely used method, has

Table 3 Areal aspects of the watersheds

Name of the watershed	Area (in km^2)	W.L (in km)	P (in km)	Drainage density (km/km^2)	S.F (km/km^2)	LOF (in km)	Form factor	I. no
Varagur	87.22	14.24	45.92	2.27	2.97	0.22	0.43	6.74
Nayakkankkombai	51.10	13.92	38.04	2.74	4.11	0.18	0.26	11.25
Varattur Nadi	41.91	9.67	29.76	2.75	4.08	0.18	0.45	11.20
Naganallur	26.66	8.99	26.35	3.18	3.75	0.16	0.33	11.94
Gundur	22.27	10.60	30.12	3.29	6.38	0.15	0.20	21.00
Puliyanjolai	103.15	14.27	49.51	2.99	4.98	0.17	0.51	14.90
Sholaikkadu	18.21	7.00	20.40	3.26	4.67	0.15	0.37	15.22
Karavallikkombai	29.55	9.06	28.27	2.97	3.72	0.17	0.36	11.07

W.L watershed length, *P* perimeter, *S.F* stream frequency, *LOF* length of overland flow, *I. no.* infiltration no

been made use. According to Horton (1945), the value of F varies from 0 (for basins with the highly elongated shape) to 1 (for a basin with the perfect circular shape). In other words, the smaller the form factor value, the more the elongation of the watershed. The estimated form factor values range from 0.20 (Gundur watershed) to 0.51 (Puliyanjolai watershed) for the watersheds of the study area (Table 3).

Drainage density

Drainage density refers to the length of streams per unit area in a watershed (Horton 1932). It is a measure of how well or how poorly a watershed is drained by stream channels. It is estimated by dividing the total length of all streams in a watershed by the total watershed area. It is one of the most sensitive and variable drainage morphometric parameters; it has a direct relationship with rainfall intensity (Chorley 1957; Chorley and Morgan 1962), runoff intensity (Abrahams 1972), and resistance to rock weathering (Zavoianu 1985; Gardiner et al. 1996; Sangireddy et al. 2016) and an inverse relationship with infiltration capacity (Horton 1945; Melton 1957)/permeability (Strahler 1956; Zavoianu 1985; Peh 1986), lag time (Chorley 1969), vegetation cover (Chorley 1957; Abrahams 1972), and texture of landscape dissection and spacing of streams (Chorley 1969). The estimated drainage density values for the watersheds of the study area (Table 3) show that it ranges from 2.27 (Varagur watershed) to 3.29 km/km^2 (Gundur watershed). It is observed that drainage density is high (>3.25 km/km^2) in Gundur and Sholaikkadu watersheds; low (≤ 2.75 km/sq.km) in Varagur, Nayakkankombai, and Varattur Nadi watersheds; and moderate (2.75 to 3.25 km/sq.km) in rest of the three watersheds.

Stream frequency

Stream frequency is a measure of topographic texture. It is expressed as the ratio of the number of streams in a watershed to the area of the watershed. It is directly related to the degree of terrain dissection and runoff. The analysis of the estimated stream frequency of the watersheds of the study area (Table 3) shows that it is high (>4.75 streams/km^2) in Gundur and Puliyanjolai watersheds, while it is low (≤ 3.75 streams/sq.km) in Varagur, Naganallur, and Karavallikkombai watersheds. In other watersheds such as Nayakkankombai, Varattur Nadi, and Sholaikkadu, it is moderate (3.76 to 4.25 streams/km^2).

Length of overland flow

The length of overland flow (LOF) refers to the length of the flow of water over the ground before it becomes concentrated in definite stream channels. It is expressed as half the reciprocal of drainage density (Horton 1945). In watersheds with shorter LOF values, lesser rainfall is sufficient to contribute a significant volume of surface runoff to stream discharge, as rainwater will enter the stream relatively quickly in such watersheds. This factor has an important control over lag time (Chorley 1969), since the mean velocity of the unconcentrated overland flow is less than concentrated channel flow. Smaller LOF values can cause flooding in days of heavy rain, due to the reduced possibility of water infiltration into the soil (Olszevski et al. 2011). The estimated LOF values of the watersheds of the study area (Table 3) show that it is shorter (≤ 0.15 km) in Gundur and Sholaikkadu watersheds, longer (>0.20 km) in Varagur watershed, and in other watersheds, it is moderate (0.16–0.20 km).

Infiltration number

The infiltration number is the product of drainage density and stream frequency, and it is a measure of the infiltration potential of a watershed. Estimation of infiltration number for the watersheds of the study area (Table 3) shows that it is higher (>18) for Gundur and Sholaikkadu watersheds and lower (≤ 8) for Varagur, Karavallikkombai, Varattur Nadi, and Nayakkankombai watersheds. In the rest of the other watersheds, it is moderate (9 to 18).

Relief aspects

Relief aspects are related to the study of the three-dimensional features of watersheds involving area, volume, and attitude of vertical dimension of landforms. The relief aspects considered for the present study include basin relief, relief ratio, relative relief, and ruggedness number. The procedure adopted for the estimation of these parameters and their inter-relationship with the form and processes of the watersheds have been discussed in the following section, and the estimations are given in Table 4.

Relief

Relief of the watershed is the elevation difference between the mouth and the highest point on the watershed perimeter and is expressed in meters. Relief possesses a direct relationship with potential energy (Strahler 1968), denudation rate (Ahnert 1970), amount of sediment that can be transported (Hadley and Schumm 1961), and discharge rate (Mociornita 1964; Diaconu 1966) of a watershed. However, other relief parameters conceived later have taken into account other relevant factors such as watershed length (relief ratio) and watershed perimeter (relative relief) besides the elevation difference and have been reported to be better indices in assessing the above-mentioned hydrological parameters. Hence, the estimated relief of watersheds, which considers only the elevation difference between the mouth and the

Table 4 Relief aspects of the watersheds

Name of the watershed	Maximum elevation (in m.)	Minimum elevation (in m.)	Relief (in m.)	Relative relief	Relief ratio	Ruggedness number
Varagur	1415	355	1060	23.08	74.44	0.46
Nayakkankkombai	1361	340	1021	26.84	73.35	0.53
Varattur Nadi	1287	340	947	31.28	97.93	0.49
Naganallur	1311	210	1101	41.78	122.47	0.66
Gundur	1362	280	1082	35.92	102.08	0.67
Puliyanjolai	1361	201	1160	23.43	81.29	0.66
Sholaikkadu	1361	210	1151	56.42	164.43	0.71
Karavallikkombai	1415	210	1205	42.62	133.00	0.68

highest point on the watershed, was not directly used for the assessment of these hydrological parameters, and their use in the present study is limited towards the estimation of other parameters of relief aspects such as relief ratio, relative relief, and ruggedness number of the watersheds.

Relief ratio

The relief ratio is the ratio between the total relief of the basin and its longest dimension parallel to the principal drainage line. It is an indicator of the steepness (Schumm 1956), the intensity of erosion process (Chopra et al. 2005; Javed et al. 2009; Ajibade et al. 2010), and also a measure of potential energy available to move water and sediment downslope (Sarkar and Gundekar 2007), peak discharge (Morisawa 1962), and is directly related with all these processes. For the watersheds of the study area, the estimated relief ratio values vary from 73.35 (Nayyakkankombai watershed) to 164.43 (Sholaikkadu watershed). It is found to be high (> 130) for Sholaikkadu and Karavallikombai watersheds, low (≤ 80) for Nayakkankombai and Varagur watersheds, and moderate (81 to 130) for the other watersheds of the study area.

Relative relief

Relative relief, introduced by Melton (1957), is the ratio between relief and perimeter of the watershed. It has the advantage over the relief ratio in that it is not dependent on the basin length which is a questionable parameter in oddly shaped watersheds. Relative relief is considered to be the most reliable potential predictor of denudation rates (Ahnert 1970; Summerfield and Hulton 1994; and Gunnel 1998). Relative relief values estimated for the watersheds of the study area show that it ranges from 23.08 (Varagur watershed) to 56.42 (Sholaikkadu watershed). Among the watersheds of the study area, relative relief is high (> 45) in the Sholaikkadu watershed, low (≤ 25) in Varagur and Puliyanjolai watersheds, and moderate (26 to 45) in the rest of the other watersheds.

Ruggedness number

Ruggedness number, the product of the watershed's relief and drainage density, is an index that reflects slope steepness and length. It was estimated using the relationship, Ruggedness Number = ((Drainage Density × Relief) / 5280), as suggested by Hart (1986). Extremely high values of the ruggedness number occur when slopes are not only steep but long as well (Chow 1964). Furthermore, it is found to be directly proportional to relative peak discharge (Patton et al. 1988). Among the watersheds of the study area, the estimated ruggedness number is high (> 0.70) for the Sholaikkadu watershed and low (≤ 50) in Varagur and Varattur Nadi watersheds. In other watersheds, it is moderate.

Form and hydrological processes of the watersheds

The established relationships between the various stream network measures and the form and processes described in the previous section have been made use of to infer the form and processes of the watersheds of the study area. It is to be noted that inferences about most of the form and hydrological process considered such as terrain dissection, slope steepness, slope length, vegetation cover, rainfall intensity, resistance to rock weathering, runoff intensity, infiltration capacity, mean discharge, mean annual runoff, the quickness with which the rainwater enters into the stream, sediment yield, and peak discharge were made from their respective well-established drainage morphometric parameter. However, for inferring a few other hydrological parameters such as mean annual runoff, potential energy, denudation rate, lag time, and flash flood susceptibility of watersheds, a review of relevant literature reveals the absence of unanimity among

the workers towards the selection of the appropriate drainage morphometric parameter. In the present study, in such cases (for inferring mean annual runoff, potential energy, denudation rate), the most widely used drainage morphometric parameter was selected to make inferences. Inferences regarding lag time and flash flood susceptibility were made by adopting ranking methodology which has been elaborated in the relevant section.

Degree of dissection

For evaluating the degree of dissection of the terrain, the estimated stream frequency values have been made use of. Because of the direct relationship between the two factors, it can be inferred that the degree of dissection of the terrain would be greater in watersheds of higher stream frequency such as Gundur and Puliyanjolai, while it would be lesser in watersheds of lower stream frequency such as Varagur, Naganallur, and Karavallikkombai watersheds. It would be moderate in other watersheds. In general, it is observed that the degree of terrain dissection is higher in watersheds located in the southern and south-central portion of the hills.

Steepness and length of slopes

The steepness of the slopes of the watersheds of the study area varies distinctly on account of the rugged nature of the terrain, typical of hilly areas. To understand the steepness of the slope of the watersheds, the relief ratio, the commonly used parameter, has been made use of. Schumm (1956) has established the close relationship between the two parameters and are found to be directly related. This implies that in watersheds of high relief ratio such as Sholaikkadu and Karavallikkombai, the slopes would be steeper, while in watersheds of low relief ratio such as Nayakkankombai and Varagur, the slopes would be relatively less steep. In the rest of the other watersheds, the slope steepness would be moderate. Ruggedness number is another parameter that can throw light on the nature of the overall slope of a watershed; extremely high values of the ruggedness number occur when slopes are not only steep but also long (Chow 1964). This implies that in the Sholaikkadu watershed, for which the estimated ruggedness number is extremely high, slopes would not only be steep but long as well.

Shape of the watersheds

In the present study, the shape of the watersheds was inferred from their estimated form their factor values. According to Horton (1945), the form factor value varies from 0 (for basins with the highly elongated shape) to 1 (for a basin with a perfect circular shape). Except for the Puliyanjolai watershed, the estimated form factor value for the watersheds of the study area is closer to zero than one, which reflects their elongated shape. Puliyanjolai watershed is the only watershed in the study area, which is relatively rotund because of its form factor value being closer to one than zero. Furthermore, based on form factor values of the elongated watersheds, the variation in elongation among them can be deciphered. Watersheds with low form factor values (≤ 0.3) such as Gundur and Nayakkankombai watersheds are highly elongated, while those with relatively higher values (0.4 to 0.5) such as Varagur and Varattur Nadi watersheds are less elongated. The other three watersheds, viz., Naganallur, Sholaikkadu, and Karavallikkombai, are moderately elongated.

Rainfall intensity, resistance to weathering, runoff intensity, and vegetation cover

Variations in rainfall intensity, resistance to weathering, runoff intensity, and vegetation cover among the watersheds were inferred from the analysis of drainage density values of the watersheds. Among these parameters, drainage density has a direct relationship with rainfall intensity (Chorley 1957; Chorley and Morgan 1962), runoff intensity (Abrahams 1972), resistance to rock weathering (Zavoianu 1985; Gardiner et al. 1996; Sangireddy et al. 2016), and an inverse relationship with vegetation cover (Chorley 1957; Abrahams 1972). This implies that in the Gundur and Sholaikkadu watersheds, where drainage density is high, rainfall intensity, runoff intensity, and resistance to rock weathering would be higher, whereas in Varagur, Nayakkankombai, and Varattur Nadi watersheds, where drainage density is low, rainfall intensity, runoff intensity, and resistance to rock weathering would be relatively low. In the rest of the other watersheds of the study area, these parameters would be moderate, on account of their moderate drainage densities. These results indicate that rainfall intensity, resistance to weathering, and runoff intensity would be higher in the watersheds located in the western part and would be lower in those located in the northern part of the Kolli hills. Furthermore, from the inverse relationship between drainage density and vegetation cover brought out by Chorley (1957) and Abrahams (1972), it is inferred that in watersheds with low drainage density such as Varagur, Nayakkankombai, and Varattur Nadi, vegetation cover would be greater, and in watersheds with a high density such as Gundur and Sholaikkadu, vegetation cover would be lesser. In general, it is inferred that vegetation cover is higher in the watersheds located in the northern part of the hills.

Infiltration capacity and runoff

Infiltration capacity and runoff potential of the watersheds of the study area were assessed from the analysis of the

estimated infiltration number of the watersheds. Infiltration number has a direct relationship with runoff potential and an indirect relationship with infiltration potential (Faniran 1968; Das and Mukherjee 2005; Joji et al. 2013; Elewa et al. 2016). These relationships imply that for Gundur and Sholaikkadu watersheds, for which the infiltration number is higher, the infiltration potential would be lower and runoff would be higher. On the other hand in Varagur, Karavallikkombai, Varattur Nadi, and Nayakkankombai watersheds, for which the estimated infiltration number is low, the infiltration potential would be higher, and runoff would be lower. In other watersheds, both infiltration and runoff potentials would be moderate, on account of their moderate infiltration capacity.

Quickness with which the rainwater enters into the streams

In watersheds with a shorter length of overland flow (LOF) values, lesser rainfall is sufficient to contribute a significant volume of surface runoff to stream discharge as rainwater will enter the stream relatively quickly in such watersheds. This implies that in watersheds with shorter LOF (≤ 0.15 km) such as Gundur and Sholaikkadu, rainwater will enter the stream relatively quickly, and lesser rainfall is sufficient to contribute a significant volume of surface runoff to stream discharge. On the other hand, in the Varagur watershed where the length of overland flow is longer (> 0.20 km), rainwater will take relatively more time to enter into the stream, and hence only in case of higher rainfall, there would be a significant volume of surface runoff to stream discharge.

Sediment yield

Investigations by Strahler (1957) have shown that sediment yield and the areal extent of watersheds/basins are inversely related. This established relationship between these two parameters has been made of in the present study to bring out the variation in sediment yield among the watersheds of the study area. From the relationship between these two parameters, it can be inferred that sediment yield would be relatively greater in smaller watersheds of the study area such as Sholaikkadu, Gundur, Naganallur, and Karavallikkombai while it would be lesser in larger watersheds such as Puliyanjolai and Varagur; in the rest of the other watersheds, it would be moderate. Analysis of sediment yield variations among the watersheds of the study area shows that in general sediment yield would be greater in the watersheds of the western and eastern part of the Kolli hills.

Relative peak discharge

Relative peak discharge has a close relationship with ruggedness number, and both the factors are directly related (Patton et al. 1988). This implies that the peak discharge would be greater in the Sholaikkadu watershed for which the estimated ruggedness number is relatively high, while it would be low in Varagur and Varattur Nadi watersheds for which the estimated ruggedness number is low. In the rest of the other watersheds, it would be moderate. In general, it is observed that the peak discharge is lower in the watersheds located in the northern part of the hills.

Mean annual runoff

For assessing mean annual runoff, variations among watersheds, either watershed area (Strahler 1957; Morisawa 1962) or total stream length (Morisawa 1962), have been the commonly used indices. Among the watersheds of the study area, the total stream length of a watershed is found to be directly related to the size of the watershed; the larger the watershed, the greater the total stream length and vice versa. This implies that the results would be very much the same if the variation is assessed using both these parameters individually. Hence, it is felt that assessment based on any one of these parameters would be sufficient. In the present study, watershed area alone has been used to assess mean annual runoff. The direct relationship between watershed's areal and mean annual runoff (Strahler 1957; Morisawa 1962) shows that the mean annual runoff would be greater in larger watersheds such as Puliyanjolai and Varagur, while it would be relatively lesser in the smaller watersheds such as Sholaikkadu, Gundur, Naganallur, and Karavallikkombai watersheds. In the rest of the other watersheds, it would be moderate on account of their moderate areal extent. Furthermore, it is interesting to note that the mean annual runoff would be lesser in the watersheds of the western and eastern part of the Kolli hills.

Relative mean discharge

For assessing mean discharge from a watershed, the areal extent of the watershed has been the widely used parameter. Mean discharge of the watershed is found to be directly related to its areal extent (Leopold and Maddock 1953; Strahler 1964; Dunne and Leopold 1978; Zavoianu 1985). This implies larger watersheds would have greater mean discharge and vice versa. Hence, the mean discharge would be greater in the larger watersheds of the study area such as Puliyanjolai and Varagur, while it would be lesser in the smaller watersheds such as Sholaikkadu, Gundur, Naganallur, and Karavallikkombai watersheds. In other watersheds, it would be moderate on account of their moderate areal

extent. From the above discussion, it is interesting to note that, in general, the mean discharge would be lesser in the watersheds of the western and eastern parts of the Kolli hills.

Potential energy and denudation rate

For assessing potential energy, several measures such as relief, relief ratio, and relative relief have been used as indices. Unlike the parameter relief, which considers only elevation differences, the other two parameters, relief ratio and relative relief, consider other measures such as watershed length and watershed perimeter for their estimation respectively besides elevation difference. Hence, it is felt that both relief ratio and relative relief are better indices than relief for assessing the potential energy of watersheds. But of these two parameters, relative relief was preferred for it is quoted in the literature as the most reliable potential predictor of denudation rate (Ahnert 1970; Summerfield and Hulton 1994; and Gunnel 1998), which indirectly holds good for potential energy also. On account of the direct relationship between relative relief and the other two parameters, viz., potential energy and denudation rates, it can be inferred that the potential energy and denudation rate would be greater in the Sholaikkadu watershed, where the relative relief value is high and they would be lower in Varagur and Puliyanjolai watersheds, for which the estimated relative relief values are low. It would be moderate in the rest of the other watersheds on account of their moderate relative relief.

Lag time

Lag time is found to have a close relationship with drainage density (Chorley 1969), length of overland flow (Chorley 1969), shape (Zavoianu 1985; Waugh 1995; Bhagwat et al. 2011), and size of the watersheds. In the present study, the three parameters, viz., length of overland flow, shape (inferred from form factor), and size of the watersheds have been chosen for assessing the relative lag time while the other factor, viz., drainage density, was not considered to avoid duplication of results since the length of overland flow indirectly reflects drainage density. The length of overland flow was chosen because of its strong control over lag time, because the mean velocity of the unconcentrated overland flow is less than concentrated flow (Chorley 1969). This implies that the smaller length of overland flow values would result in a shorter lag time. The shape of the watershed was chosen because of its direct influence over lag time, since in a circular watershed, runoff from various parts of the watershed would reach the outlet at the same time resulting in a shorter lag time (Zavoianu 1985; Waugh 1995; Bhagwat et al. 2011), whereas in elongated watersheds, runoff from tributaries flow into the main stream at greater intervals of time resulting in longer lag time. The other parameter, viz., size of the watershed, was considered for the assessment because most of the intense storms are usually of the smallest size (More et al. 1967). Considering the strong influence of these three parameters with that of lag time, they were chosen to assess the relative lag time among the watersheds of the study area.

Variations in lag time among the watersheds were assessed based on the ranking procedure (Table 5). Based on the estimated length of overland flow values, the eight watersheds of the study area were ranked from one to eight; rank 1 was assigned to the watershed with the shortest length of overland flow (which implies the shortest lag time) because the mean velocity of the concentrated channel flow would be greater than the unconcentrated overland flow. Watershed with the next shortest overland flow was ranked two and so on, and the last rank was assigned to the watershed with the longest length of overland flow. A similar rank was assigned if the value of the parameter is the same.

Table 5 Ranking of watersheds for lag time

Name of the watershed	Form factor	Length of overland flow (km)	Area (km^2)	Ranking for lag time			C.V ($A+B+C$)	Ranking of watersheds for lag time based on C.V
				Based on form factor (A)	Based on length of overland flow (B)	Based on area (C)		
Varagur	0.43	0.22	87.22	3	5	7	15	5
Nayakkankkombai	0.26	0.18	51.10	8	4	6	18	6
Varattur Nadi	0.45	0.18	41.91	2	4	5	11	3
Naganallur	0.33	0.16	26.66	6	2	3	11	3
Gundur	0.20	0.15	22.27	7	1	2	10	2
Puliyanjolai	0.51	0.17	103.15	1	3	8	12	4
Sholaikkadu	0.37	0.15	18.21	4	1	1	6	1
Karavallikkombai	0.36	0.17	29.55	5	3	4	12	4

C.V compound value

Table 6 Ranking of watersheds for flash flood susceptibility

Name of the watershed	Peak discharge (inferred from RN)	Ranking for flash flood		C.V ($A+B$)	Ranking of watersheds for flash flood susceptibility based on C.V
		(A)	(B)		
Varagur	0.46	7	5	12	7
Nayakkankkombai	0.53	5	6	11	6
Varattur Nadi	0.49	6	3	9	5
Naganallur	0.66	4	3	7	3
Gundur	0.67	3	2	7	3
Puliyanjolai	0.66	4	4	8	4
Sholaikkadu	0.71	1	1	2	1
Karavallikkombai	0.68	2	4	6	2

RN ruggedness number, *A* ranking based on RN, *B* ranking based on lag time from Table 5

Similarly, watersheds were ranked based on form factor values of the watersheds, which is an index of the shape. Watershed with the highest form factor value (0.51), for Puliyanjolai watershed which reflects its relatively rotund shape), was assigned rank one, because in rotund watersheds, runoff from various parts of the watershed would reach the outlet at the same time resulting in shorter lag time (Zavoianu 1985; Waugh 1995; Bhagwat et al. 2011). Watershed with the next highest form factor value was ranked two and so on, and the last rank was assigned to the watershed with the least form factor value (0.20 for Gundur watershed which reflects its highly elongated shape).

Furthermore, watersheds were also ranked based on the third parameter considered, viz., size. The smallest watershed was assigned rank one because most of the intense storms are usually of the smallest size (More et al. 1967). The next smallest watershed was assigned the next rank and so on, and the last rank was assigned to the largest watershed.

These rankings made based on the estimated length of overland flow, form factor, and areal extent for each watershed were summed up, to obtain a compound value for each watershed, which represents the collective impact of these important parameters on lag time in each watershed. Watersheds were again ranked based on their compound value. Watershed with the least compound value was assigned rank one, and it represents the watershed with the shortest lag time. The next rank was assigned to the watershed with the next least compound value. The ranking was progressively increased with the increase in the compound value, and the watershed with the highest compound value represents the one with the longest lag time. The results of the analysis show that lag time will be the shortest in the Sholaikkadu watershed followed by Gundur, Naganallur, etc., while it would be the longest for the Nayakkankkombai watershed.

Flash flood

For assessing watersheds susceptible to flash floods, the two parameters, viz., lag time and peak discharge, are important. While lag time reflects the quickness of peak discharge at the outlet of the watershed, peak discharge would reflect the volume of water flow in unit time. The ranking procedure similar to the one described earlier has been adopted to assess the susceptibility of the watersheds for flash floods (Table 6). Based on the estimated ruggedness number values (which reflect peak discharge), the eight watersheds of the study area were ranked from one to eight; rank 1 was assigned to the watershed for which the estimated ruggedness number is the highest (which represents watershed with highest peak discharge). Watershed with the next highest ruggedness number was ranked two and so on, and the last rank was assigned to the watershed with the least ruggedness number value (which represents watershed with lowest peak discharge). Watersheds with the same ruggedness number value were assigned the same rank. As lag time was also considered for assessing flash flood susceptibility, the ranking of watersheds that has already been made (Table 6) was made use of for further analysis. These rankings made based on lag time and peak discharge were added together to obtain a compound value for each watershed, which represents the collective impact of the two important parameters on flash flood susceptibility in each watershed. Watersheds were again ranked based on their compound value. Watershed with the least compound value was assigned rank 1, and it represents the watershed with the highest susceptibility for flash floods. The next rank was assigned to the watershed with the next least compound value. The ranking was progressively increased with the increase in the compound value, and the watershed with the highest compound value represents the watershed with the least susceptibility for flash floods. The results of the analysis show that among

the watersheds of the study area, the Sholaikkadu watershed is the most susceptible for flash flood followed by Karavallikkombai, Gundur, Naganallur, etc., while Varagur, Varattur Nadi, and Nayakkankkombai watersheds are relatively less susceptible. In general, it is observed that watersheds, located on the western and eastern part of the study area, are more susceptible to flash floods while those watersheds located on the northern and north-western part of the hills are less susceptible to flash floods.

Conclusions

The results of the present study have provided a wealth of information relating to the form and processes and their variations among the watersheds of the study area. Information relating to the form of the watersheds that were brought out includes shape, size, elongation, slope steepness and length, degree of development of stream network, degree of branching of streams, and the degree of terrain dissection. Information relating to hydrological processes of the watersheds that were brought out from the study includes infiltration potential, mean annual runoff, rainfall intensity, relative potential energy, relative denudation rate, relative discharge rates, mean and peak discharge, lag time, the intensity of erosion, the relative amount of sediment transported, and flash flood proneness. This watershed-based information can be highly helpful for the resource planners in formulating strategies towards the development and management of the resources of this hill.

Declarations

Conflict of interest The authors declare no competing interests.

References

Abrahams AD (1972) Drainage densities and sediment yields in Eastern Australia. Aus Geograph Studies 10:19–41

Abrahams AD (1984) Channel networks: a geomorphological perspective. Water Resour Research 20(2):161–168

Ahnert F (1970) Functional relationships between denudation, relief and uplift in large mid-latitude drainage basins. Am J Sci 268:243–263

Ajibade LT, Ifabiyi IP, Iroye KA, Ogunteru S (2010) Morphometric analysis of Ogunpa and Ogbere drainage basins, Ibadan, Nigeria. Ethi J Envil Stud and Manag 3(1):13–19

Al-Rowaily SA, El-Bana M, Al-Dujain FAR (2012) Changes in vegetation composition and diversity in relation to morphometry, soil and grazing on a hyper-arid watershed in the central Saudi Arabia. Catena: https://doi.org/10.1016/j.catena.2012.05.004.

Altın TB, Altin BN (2011) Development and morphometry of drainage network in volcanic terrain, Central Anatolia, Turkey. Geomorph. https://doi.org/10.1016/j.geomorphol.2010.09.023

Arabameri A, Tiefenbacher JP, Blaschke T, Pradhan B, Bui DT (2020). Morphometric analysis for soil erosion susceptibility mapping using novel GIS-based ensemble model. Remote Sens 12:874. https://doi.org/10.3390/rs12050874.

Argyrioua AV, Teeuwa RM, Soupiosb P, Sarrisc A (2017) Neotectonic control on drainage systems: GIS-based geomorphometric and morphotectonic assessment for Crete, Greece. J Struc Geol. https://doi.org/10.1016/j.jsg.2017.10.002.

Bhagwat TN, Shetty A, Hegde VS (2011) Spatial variation in drainage characteristics and geomorphic instantaneous unit hydrograph (GIUH); implications for watershed management - a case study of the Varada River basin, Northern Karnataka. CATENA 87:52–59

Bisht S, Chaudhry S, Sharma S, Soni S (2018) Assessment of flash flood vulnerability zonation through geospatial technique in high altitude Himalayan watershed, Himachal Pradesh India. Remote Sens Appl Soc Envi. https://doi.org/10.1016/j.rsase.2018.09.001.

Chetty TRK, Bhaskar Rao YJ (2006) Constrictive deformation in transpressional regime, field evidence from the Cauvery Shear Zone, Southern Granulite Terrain, India. J Struc. Geol 28:713–720

Chopra R, Dhiman RD, Sharma PK (2005) Morphometric analysis of sub-watersheds in Gurdaspur District, Punjab using remote sensing and GIS techniques. J Indian Soc Remote Sens 33(4):531–539

Chorley RJ (1957) Climate and morphometry. J Geol 65:628–638

Chorley RJ (1969) The drainage basin as the fundamental geomorphic unit. In: Chorley RJ (ed) Introduction to fluvial processes. Methuen Co., Ltd, pp 30–52

Chorley RJ, Morgan MA (1962) Comparison of morphometric features, Unaka Mountains, Tennessee and North Carolina, and Dartmoor. England Geol Soc Amer Bull 73:17–34

Chow VT (1964). Handbook of hydrology, McGraw-Hill Book Co. Inc. New York.

Das AK, Mukherjee S (2005) Drainage morphometry using satellite data and GIS in Raigad District, Maharashtra. J Geol Soc India 65:577–586

Diaconu C (1966) Altitude, one of the basic criteria for organizing hydro meteorological networks in mountainous regions. Studii De Hidrologie 18:41–55 (in Romanian)

Doornkamp J (1968) The analysis of morphometric properties of drainage basins by the Spearman's Rank correlation technique. In: Slaymaker HO (ed.), Morphometric analysis of maps, British Geomorphol Res Group Occasional Paper 4:31–40.

Doornkamp JC, King CAM (1971) Numerical analysis in geomorphology – an introduction. Macmillan and Co. Ltd. London, 372p.

Drury SA, Holt RW (1980) The tectonic framework of South Indian Craton: a reconnaissance involving Landsat imagery. Tectonophysics 65:1–15

Dunne T, Leopold LB (1978) Water in environmental planning. W.H. Freeman Co., San Francisco, p 818p

Elewa HH, Ramadan EM, Nosair AM (2016) Spatial-based hydromorphometric watershed modeling for the assessment of flooding potentialities. Envi Ear Sci 75:906–927. https://doi.org/10.1007/s12665-016-5692-4

Faniran A (1968) The index of drainage intensity - a provisional new drainage factor. Aus J Sci 31:328–330

Gardiner V (1975). Drainage basin morphometry. British Geomorphological Research Group, Tech Bull 14:48 p.

Gardiner V (1996) Channel networks: progress in the study of spatial and temporal variations of drainage density. In: Gurnell A, Petts GE (eds) Changing river channels. Wiley, New York, pp 65–85

Gelaberta B, Forno´sa JJ, Pardob JE, Rossello VM, Segura F (2005) Structurally controlled drainage basin development in the south of Menorca (Western Mediterranean, Spain). Geomorphol 65:139-155. https://doi.org/10.1016/j.geomorphol.2004.08.005

Ghosh P, Sinha S, Misra A (2014) Morphometric properties of the Trans-Himalayan river catchments: clues towards a relative chronology of orogen-wide drainage integration. Geomorphol 233:127–141. https://doi.org/10.1016/j.geomorph.2014.10.035

Giusti EV, Schneider WJ (1965) The distribution of branches in river networks. U.S.G.S Prof. Pap. 422-G.

Gregory KJ, Walling DE (1973) Drainage basin form and process – a geomorphological approach. Edward Arnold Pub. Ltd., London, p 321p

Gunnel Y (1998) Present, past and potential denudation rates: Is there a link? Tentative evidence from fission-track data, river sediment loads and terrain analysis in the South Indian shield. Geomorp 25:135–153

Hadley RF, Schumm SA (1961) Sediment sources and drainage basin characteristics in Upper Cheyenne river basin. U.S.G.S Water-Supp 1531-B, 198p.

Hart MG (1986) Geomorphology - pure and applied. Allen and Unwin Pub. Ltd., London, p 211p

Horton RE (1932) Drainage basin characteristics. Trans Amer Geophy Uni 13:350–361

Horton RE (1945) Erosional development of streams and their drainage basins: hydrophysical approach to quantitative morphology. Bull Geol Soc of Amer 56:275–370

Javed A, Khanday MY, Ahmed R (2009) Prioritisation of sub-watersheds based on morphometric and land use analysis using remote sensing and GIS techniques. J Ind Soc Remote Sens 37:261–274

Joji VS, Nair ASK, Baiju KV (2013) Drainage basin delineation and quantitative analysis of Panamaram watershed of Kabani river basin, Kerala using remote sensing and GIS. J Geol Soc India 82:368–378

Langbein WB (1947) Topographic characteristics of drainage basins. U.S.G.S Water Supp 968-C:125–158.

Lehner B, Verdin K, Jarvis A (2008) New global hydrography derived from spaceborne elevation data. EOS Trans Am Geophys Union 89:93–94

Leopold LB, Miller JP (1956) Ephemeral streams: hydraulic factors and their relation to the drainage network. U.S.G.S Profess Paper 282 A, Washington, DC. 37p.

Leopold LB, Maddock T (1953) The hydraulic geometry of stream channels and some physiographic implications. USGS Profess Paper 252:57p

Luchisheva AA (1950) Practical hydrology. Gidrometeoizdat, Leningrad

Melton MA (1957) An analysis of the relations among elements of climate, surface, properties and geomorphology. Project NR 389–042 Technical Report 11, Columbia University, Department of Geology, ONR, Geography Branch, New York.

Miller VC (1953) A quantitative geomorphic study of drainage basin characteristics in the Clinch mountain area, Virginia and Tennessee, Dept. of Geology, Columbia University, Contract N6 ONR 271–30, Technical Report 3, 30p.

Mociornita C (1964) Some results for maximum rainfall discharge by Romania's rivers. Studii De Hidrologie 11:35–48 ((**in Romanian**))

More RJ (1967) Hydrological models and geography. In: Chorley RJ, Hagget P (eds) Models in geography. Methuen publishers, London, pp 45–85

Morisawa ME (1959) Relation of quantitative geomorphology of stream flow in representative watersheds of the Appalachian Plateau Province, Columbia University, Office of Naval Research, Project NR 389–042, Technical Report 20.

Morisawa (1962) Quantitative geomorphology of some watersheds in the Appalachian Plateau, Geol Soc Ame Bull 73:1025-1046.

Narula PL, Acharyya SK, Banerjee J (2000) Seismotectonic atlas of India and its environs. Published by the Director General, Geol Sur Ind Calcutta, p 87p

Ng KY (2006) Landslide locations and drainage network development: a case study of Hong Kong. Geomorphol 76:229–239. https://doi.org/10.1016/j.geomorph.2005.10.008

Olszevski N, Filho EIF, Costa LMDA, Schaefer CEGR, Souza EDE, Costa ODV (2011) Morphology and hydrological aspects of Black River basin, division of State of Rio de Janeiro and Minas Gerais. Braz J for Sci 35:485–492

Patton PC (1988) Drainage basin morphometry and floods. In: Baker VR, Kochel RC, Patton PC (eds) Flood geomorphology. Wiley Interscience Publishers, New York, p 503p

Patton PC, Baker VR (1976) Morphometry and floods in small drainage basins subject to diverse hydrogeomorphic controls. Water Resour Res 12(5):941–952

Peh CH (1986) Catchment geomorphology and its relationship with stream flow - a case study of selected drainage basins in Peninsular Malaysia. GEOSEA V Proceedings Vol. II, Geol Soc Malaysia Bull 20: 803-815.

Reddy GPO, Maji AM, Gajbhiye KS (2004) Drainage morphometry and its influence on landform characteristics in a basaltic terrain, Central India – a remote sensing and GIS approach. Int J App Earth Obser and Geoinfo 6:1–16. https://doi.org/10.1016/j.jag.2004.06.003

Sangireddy H, Carothers RA, Stark CP, Passalacqua P (2016) Controls of climate, topography, vegetation, and lithology on drainage density extracted from high resolution topography data. J Hydrol 537:271–282

Sarkar S, Gundekar HG, (2007) Geomorphological parameters: are they indicators for installation of a hydropower site? Proc. International conference on small hydropower - Sri Lanka, 22–24.

Scheidegger AE (1967) On the topology of river nets. Water Resour Res 3(1):103–106

Schumm SA (1956) The evolution of drainage systems and slopes in badlands at Perth Amboy, New Jersey. Geol Soc Amer Bull 67:597–646

Shreve RL (1966) Statistical law of stream numbers. J Geol 74(1):17–37

Shulits S (1968) Quantitative formulation of stream and watershed morphology. Bull Int Asso Sci Hydrology 3:201–208

Smart JS (1972) Channel networks. Adv Hydrosci 8:305–346

Strahler AN (1952) Dynamic basis of geomorphology. Geol Soc Amer Bull 63: 923-938.

Strahler AN (1957) Quantitative analysis of watershed geomorphology. Transactions of American Geophysical Union 38(6):913–920

Strahler AN (1964) Quantitative geomorphology of drainage basins and channel networks. In: Chow V (ed) Handbook of applied hydrology. McGraw Hill, New York, pp 439–476

Strahler AN (1968) Quantitative geomorphology. In: Fairbridge, RW (ed) The encyclopedia of geomorphology, Reinhold Book Corp, New York.

Strahler (1956) Quantitative slope analysis, Bull Geol Soc America, 67: 671-696

Summerfield MA, Hulton NJ (1994) Natural controls of fluvial denudation rates in major world drainage basins. J Geophys Res 99:13871–13883

Taha MNM. Elbarbary SM, Naguib DM, El-Shamy I (2017) Flash flood hazard zonation based on basin morphometry using remote sensing and GIS techniques: a case study of WadiQena basin, Eastern Desert, Egypt. Remote Sens App Soc Envi 8:157-167. https://doi.org/10.1016/j.rsase.2017.08.007

Verdin, KL (2017) Hydrologic derivatives for modeling and analysis—a new global high-resolution database. USGS publications.

Verstappen H (1983) The applied geomorphology, International Institute for Aerial Survey and Earth Science (ITC). Enschede.
Waugh D (1995) Geography: an integrated approach. Chapter-3: morphometry of drainage basins. 2nd edn. Nelson Pub, New York.
Zavoianu I (1985) Morphometry of drainage basins. Elsevier Science Pub, Amsterdam, p 238p
Yan D, Wang K, Quin T, Weng B, Wang H, Bi W, Li X, Li M, Ly Z, Liu F, He S, Ma J, Shen Z, Wang J, Bai H, Man Z, Sun C, Shi X, Jing L, Sun R, Cao S, Hao C, Wang L, Pei M, Dorjsuren B, Gedefaw M, Girma A, Abiyu A (2019) A data set of global river networks and corresponding water resources zones divisions. Sci Data 6:219. https://doi.org/10.1038/s41597-019-0243-y

Applied Ecology and Environmental Sciences, 2021, Vol. 9, No. 9, 786-794
Available online at http://pubs.sciepub.com/aees/9/9/1
Published by Science and Education Publishing
DOI:10.12691/aees-9-9-1

Influence of Bottom Water Parameters on the Distribution of Benthic Foraminifera from the Gulf of Mannar, Off Tiruchendur, Southeast Coast of India

R. Vijayan[1,*], V. Kumar[1], K. Sivakumar[1], J. Christinal[1], S. Selvaraj[1], T. Gangaimani[2]

[1]Department of Geology, National College (Autonomous), (Bharathidasan University), Tiruchirappalli, Tamilnadu - 620001
[2]Indian Geological Consulting Services (IGC), Tiruchirappalli, Tamil Nadu - 620 018
*Corresponding author: geoviji86@gmail.com

Received July 23, 2021; Revised August 29, 2021; Accepted September 09, 2021

Abstract Foraminifera are found in all marine environments, but they are very particular about the congenial environment for their reproduction, diversity and abundance. To understand the influence of bottom water parameters on the distribution of foraminifera from the shallow shelf region of Bay of Bengal, off Tiruchendur, Southeast Coast of Tamil Nadu, sediment and bottom water samples were collected at 27 stations, along three traverses viz. from off Veerapandianpattinam, off Tiruchendur and from off Alandurai, ranging in depths from 1.6m to 18.5m. The samples were collected during three seasons representing, summer (May 2016), pre monsoon (October 2016) and winter (January 2017) and thus the total samples amounted to 81. A total of 105 species of foraminifera have been identified from 47 genera, 32 families, 16 superfamilies and 4suborders. Among these 105 foraminiferal species, five were found to be widespread and abundant viz. Ammonia beccarii, A. tepida, Asterorotalia inflata, Nonioniodes boueanum and Quinqueloculina seminulam. Various bottom water parameters such as temperature, salinity, dissolved oxygen and pH were determined and correlated with the foraminiferal population. Temporally, higher temperature, salinity, dissolved oxygen and pH of bottom water observed during summer (May 2016) were congenial for higher population in the study area. Optimum conditions for the higher population and abundance of foraminifera are: Temperature: > 32.0°C, Dissolved oxygen: >6.0 mg/l, Salinity: >32 ppt and pH>8.1.

Keywords: *benthic foraminifera, inner shelf, bottom water characteristics, Gulf of Mannar, off Tiruchendur*

Cite This Article: R. Vijayan, V. Kumar, K. Sivakumar, J. Christinal, S. Selvaraj, and T. Gangaimani, "Influence of Bottom Water Parameters on the Distribution of Benthic Foraminifera from the Gulf of Mannar, Off Tiruchendur, Southeast Coast of India." *Applied Ecology and Environmental Sciences*, vol. 9, no. 9 (2021): 786-794. doi: 10.12691/aees-9-9-1.

1. Introduction

The shallow marine shelf regions of the east coast of India have provided a congenial environment for thriving of foraminifera. They nurtured with a broad ecological adaptability and their distribution and abundance are influenced by environmental factors such as substrate characteristics, depth and bottom water parameters. The growth and reproduction are constant as long as the environmental factors are favourable but as these depart from optimal values, rate of growth and frequency of reproduction decline. There are many studies relating to foraminifera and their relation to many ecological factors. Foraminifera are considered as environmental indicators owing to their rapid response to various pollution interferences [1,2,3]. Foraminiferal abundance and diversity are controlled by many factors like nutrition, dissolved oxygen conditions, pH, salinity, substrate and temperature [4,5,6,7,8]. High numbers of aberrant tests were attributed to high salinity conditions rather than anthropogenic stresses [9].

Benthic foraminifera are excellent indicators of water quality which influence their distribution and abundance [10]. Numerous studies have been made on the ecology and distribution of recent benthic foraminifera from the southeast and west coasts of India [11-16]. Higher temperature, salinity, pH and dissolved oxygen content noticed during April favoured higher reproduction and abundance of foraminiferal population [17]. The present study elucidates the influence of bottom water parameters such as temperature, salinity, dissolved oxygen and pH on the abundance and distribution of the widespread foraminiferal species in the shallow shelf region of Bay of Bengal, off Tiruchendur and adjoining coasts Southeast Coast of Tamil Nadu.

2. Study Area

The area under study is situated in the Gulf of Mannar. It is the region with 130 -275 km width, 160 km length

covering an area of about 10,000 sq. km. that lies in between south eastern India and western Sri Lanka. In India, it extends from Rameshwaram to Kanyakumari districts of Tamil Nadu state. It has one of the important bioreserve popularly known as the Gulf of Mannar Biosphere Reserve that was declared as the first marine biosphere reserve of India in 1989.

The area under investigation covers the inner shelf from off the coast of Veerapandianpattinam in the north to from off Alandurai in the south and geographically situated between the latitudes $8^{o}27'$- $8^{o}33'$ N and longitudes $78^{o}06'$-$78^{o}14'$ E (Figure 1).

The study area practices generally a tropical climate and experiences two types of monsoons namely, Southwest and Northeast. Southwest monsoon which extends from June to September, adds only scanty amount to the annual rainfall. On the other hand, Northeast monsoon, from October to December is often accompanied by cyclones gives ample amount to the annual rainfall. Summer is experienced from March to May while winter from December to February.

The coastal tract, belongs to the study area is geologically dominated by recent sediments comprising red soil, coastal sand, river alluvium, laterite, red teri, shell limestone, calcareous sandstone and patches of garnet and ilmenite sand. (Source: District survey report, Dept. of Geology and Mining, Govt. of Tamil Nadu).

3. Objective

In order to appraise the influence of bottom water parameters on the distribution of foraminifera from the inner shelf sediment of Gulf of Mannar, off Tiruchendur the following objectives were addressed: i. to ascertain the composition and to discover the distribution of living and total (living + dead) population of the fauna, ii. to correlate foraminiferal population distribution with observed environmental parameters and iii. to decipher the influence of bottom water parameters on distribution of foraminiferal species.

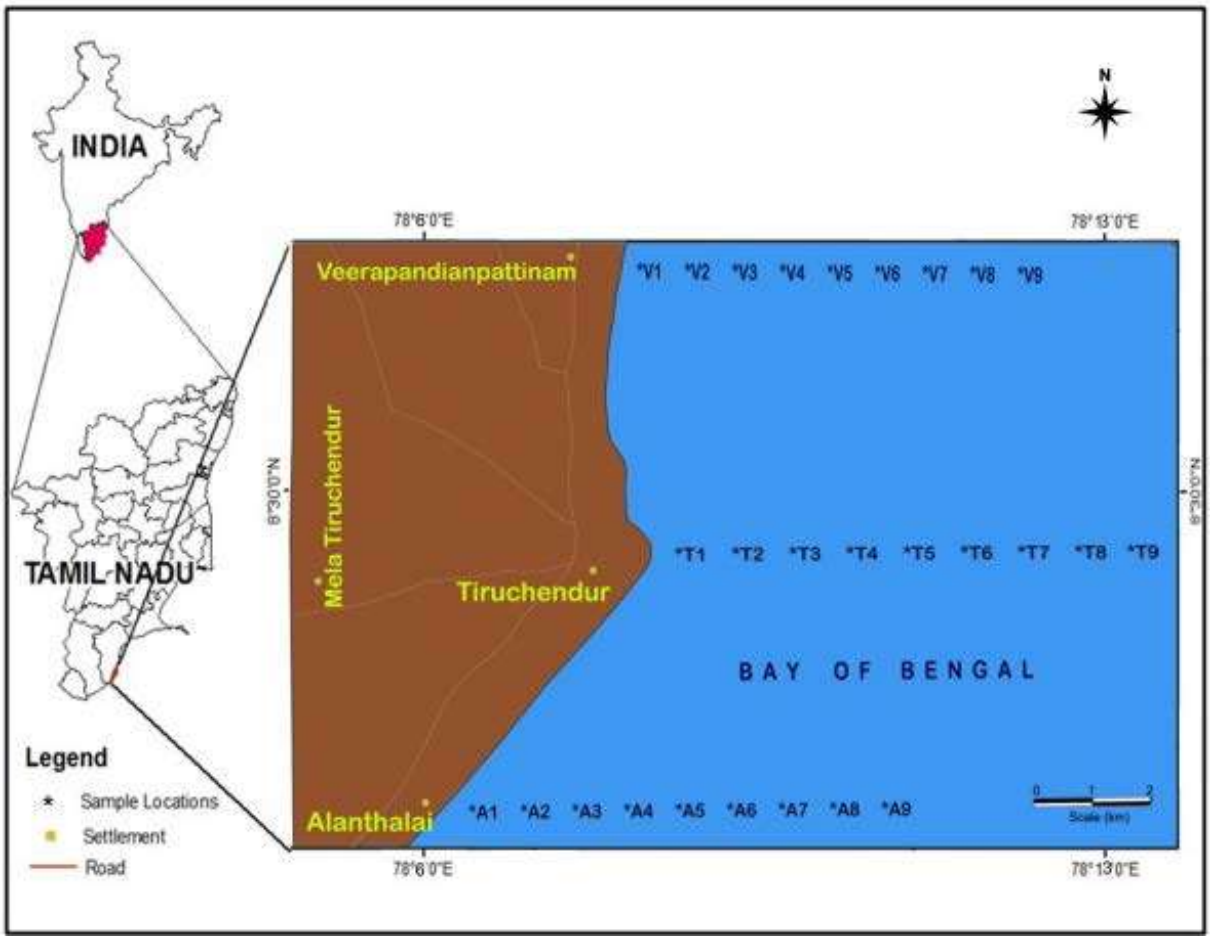


Figure 1. Location map of the area

4. Materials and Methods

4.1. Field Work

For the present study, sediment and bottom water samples were collected at 27 stations, along three traverses viz. from off Veerapandianpattinam, off Tiruchendur and from off Alandurai, ranging in depths from 1.6m to 18.5m. The samples were collected during the months of May 2016, October 2016 and January 2017 representing summer, pre monsoon and winter and thus the collection amounted to a total of 81.

The sediment samples were collected taking necessary precautions using Van Veen grab. A unit volume of 50 ml. wet sediment was taken from each sample and was preserved in a 10 % solution of neutralized formaldehyde

for foraminiferal study. Small quantity of sodium carbonate was added to the samples to maintain the alkaline condition and all the samples were indexed. Respective depths of the water column above the sampling sites were also recorded.

Samples of water from the sediment water interface were collected using a Nansen reversible water sampler. Temperature of the bottom water was recorded from the built-in thermometer for all the samples collected. Dissolved oxygen content of the bottom water was measured by using dissolved oxygen analyser (Make: Hanna model A113), immediately after the collection. Salinity is a measure of total salt content of water and measured using portable salinometer (Make: ATC 23907). The values of pH of the water sample were observed by a pH meter (Make: Hanna, Model 101E) after making necessary precautions in sampling and standardization.

4.2. Laboratory Work

For the study of living foraminifera Walton's [18] staining technique with rose Bengal has been used. The foraminiferal tests were separated by floatation method using carbon tetrachloride [19]. The faunal specimens thus obtained were picked and mounted using a stereo binocular microscope and different genera and species were identified by following the classification proposed by Loeblich and Tappan [20]. The living and dead population were counted separately for all the samples collected.

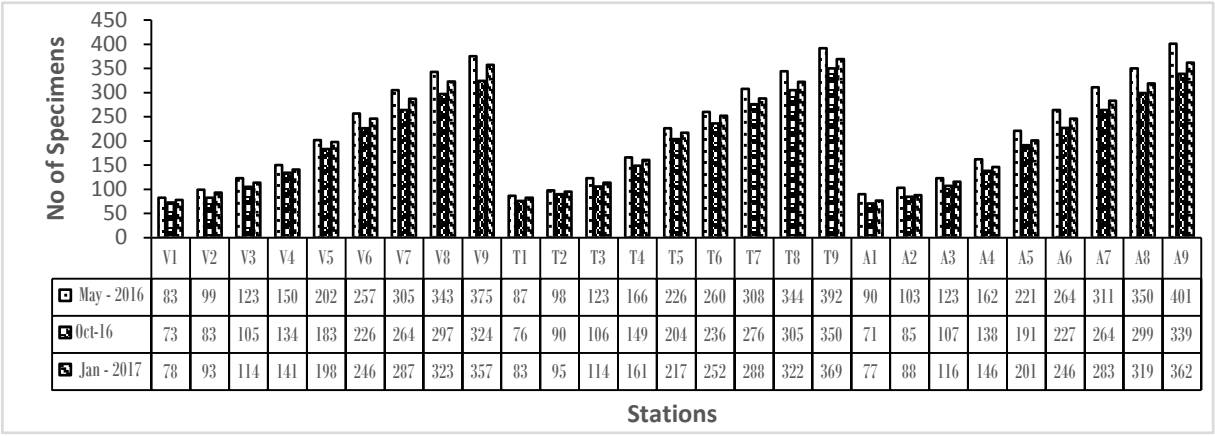
5. Results and Discussion

5.1. Foraminifera

The qualitative analysis of the sediment samples from the area led to the recognition of 105 benthic species of foraminifera belong to 42 genera, 28 families, 15 super families and 4 orders. All the 105 benthic foraminiferal species identified in the study area are found both as living and dead. Among these 105 foraminiferal species, five were considered as widespread and abundant viz. *Ammonia beccarii*, *A. tepida*, *Asterorotalia inflata*, *Nonioniodes boueanum* and *Quinqueloculina seminulam*, as they were found in all the 81 samples as living and the living foraminiferal number for a season is greater 210 specimens per 50 ml of wet sediment.

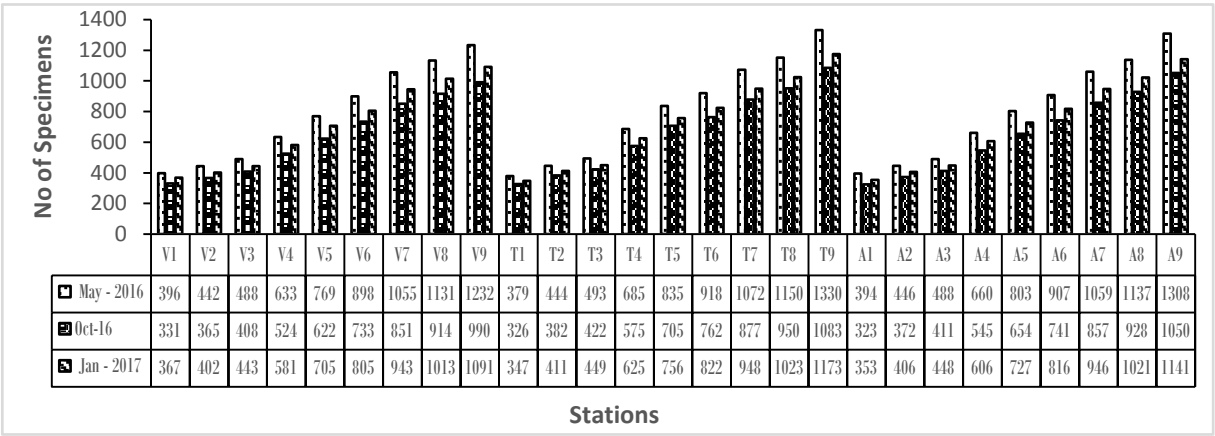
5.1.1. Living Foraminiferal Number

Living foraminiferal number (LFN) per 50 ml of wet sediment varies from 71 (Station A1, October) to 401 (Station A9, May) (Figure 2). LFN is more in samples collected from off Veerapandianpattinam than the other two areas. Spatially, maximum LFN is noticed in stations V9, T9 and A9 (farthest stations from the shore) and is very less in stations V1, T1 and A1 (near shore stations). In general, the LFN is gradually increasing from the shore station to the farthest station of each traverse. Seasonally, the LFN is found to be minimum (5202) in October and is maximum (5966) in May.



	V1	V2	V3	V4	V5	V6	V7	V8	V9	T1	T2	T3	T4	T5	T6	T7	T8	T9	A1	A2	A3	A4	A5	A6	A7	A8	A9
May - 2016	83	99	123	150	202	257	305	343	375	87	98	123	166	226	260	308	344	392	90	103	123	162	221	264	311	350	401
Oct-16	73	83	105	134	183	226	264	297	324	76	90	106	149	204	236	276	305	350	71	85	107	138	191	227	264	299	339
Jan - 2017	78	93	114	141	198	246	287	323	357	83	95	114	161	217	252	288	322	369	77	88	116	146	201	246	283	319	362

Figure 2. Living Foraminiferal Number (per 50 ml. of wet sediments)



	V1	V2	V3	V4	V5	V6	V7	V8	V9	T1	T2	T3	T4	T5	T6	T7	T8	T9	A1	A2	A3	A4	A5	A6	A7	A8	A9
May - 2016	396	442	488	633	769	898	1055	1131	1232	379	444	493	685	835	918	1072	1150	1330	394	446	488	660	803	907	1059	1137	1308
Oct-16	331	365	408	524	622	733	851	914	990	326	382	422	575	705	762	877	950	1083	323	372	411	545	654	741	857	928	1050
Jan - 2017	367	402	443	581	705	805	943	1013	1091	347	411	449	625	756	822	948	1023	1173	353	406	448	606	727	816	946	1021	1141

Figure 3. Total (Live + Dead) Foraminiferal Number (per 50 ml of wet sediment)

5.1.2. Total Foraminiferal Number

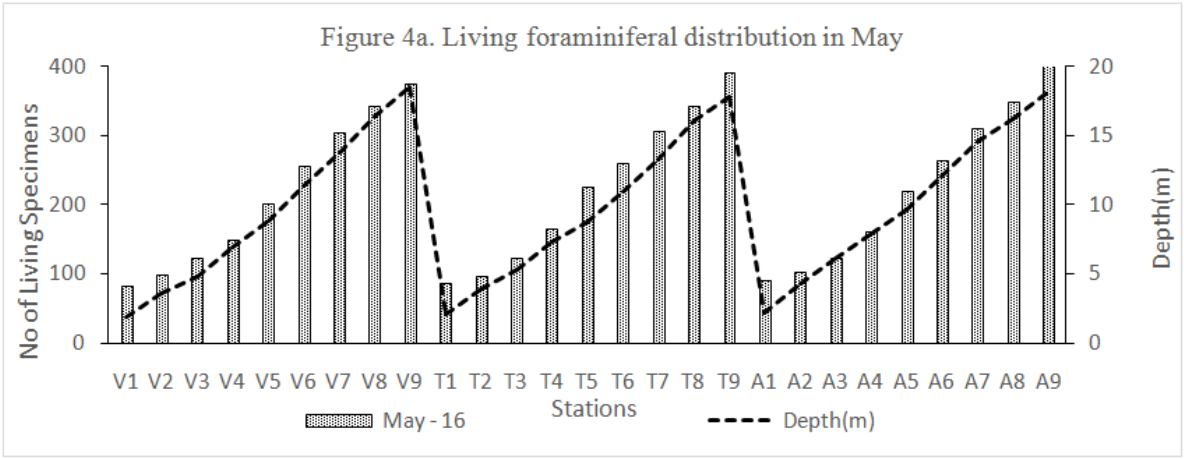
Total foraminiferal number (TFN) per 50 ml. of wet sediment varies from 323 (Station A1, October) to 1330 (Station T9, May) (Figure 3). Spatially, maximum TFN is noticed in stations V9, T9 and A9 (farthest stations from the shore) and is very less in stations V1, T1 and A1 (near shore stations). In general the TFN is gradually increasing from the shore station to the farthest station of each traverse. Seasonally, the TFN is found to be is minimum in October (17701) and is maximum in May (21552).

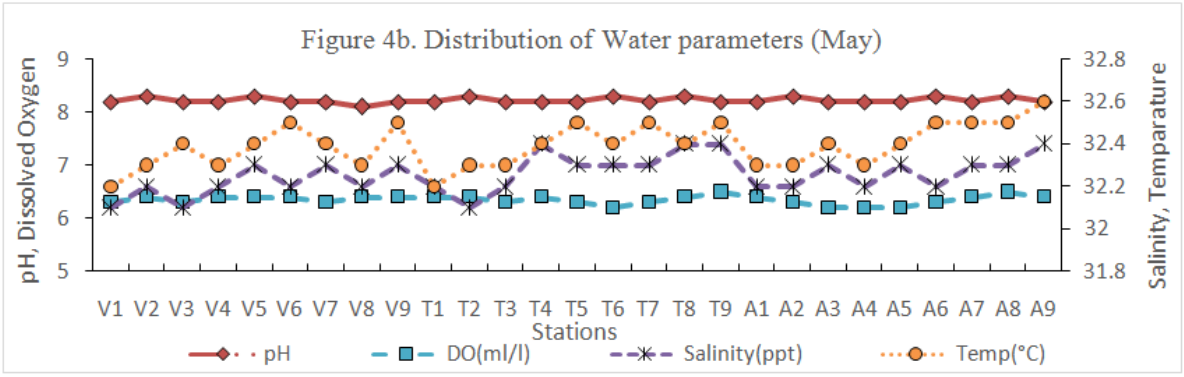
5.2. Bottom Water Parameters

5.2.1. Temperature

The relative abundance of both the living and dead calcareous benthic foraminifera is negatively correlated with depth and positively correlated with temperature [21]. The foraminiferal response to temperature variation is species-specific; the shell size of several benthic foraminifera increased with decreasing temperature but a few other benthic foraminifera show a decrease in shell size with decreasing temperature [22]. Temperature was seen to be positively correlated with population size. Collections during May recorded a high mean temperature and favoured a higher population [23]. Temperature was found to be the significant stress factor than salinity and critical temperature thresholds were observed at 30 and 35°C. [24].

In the present shelf region, temperature varies between 32.2°C and 32.6°C at different depths during May, 30.3°C and 30.9°C during October and between 29.8°C and 30.1°C during January (Figure 4a - 4f). Spatially, there was no appreciable variation of temperature between stations of a collection and hence it had no specific effect on LFN.





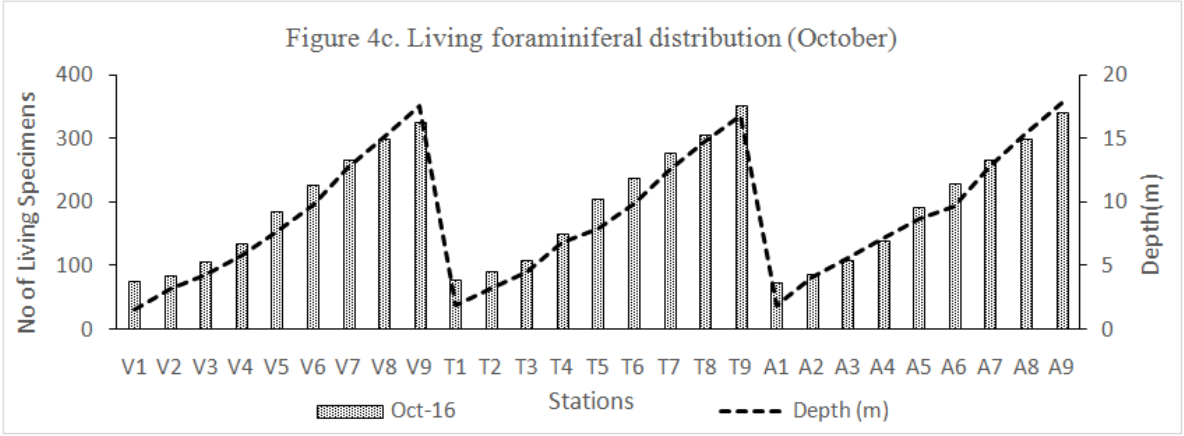


Figure 4 a-c. Distribution of water parameters and the corresponding LFN

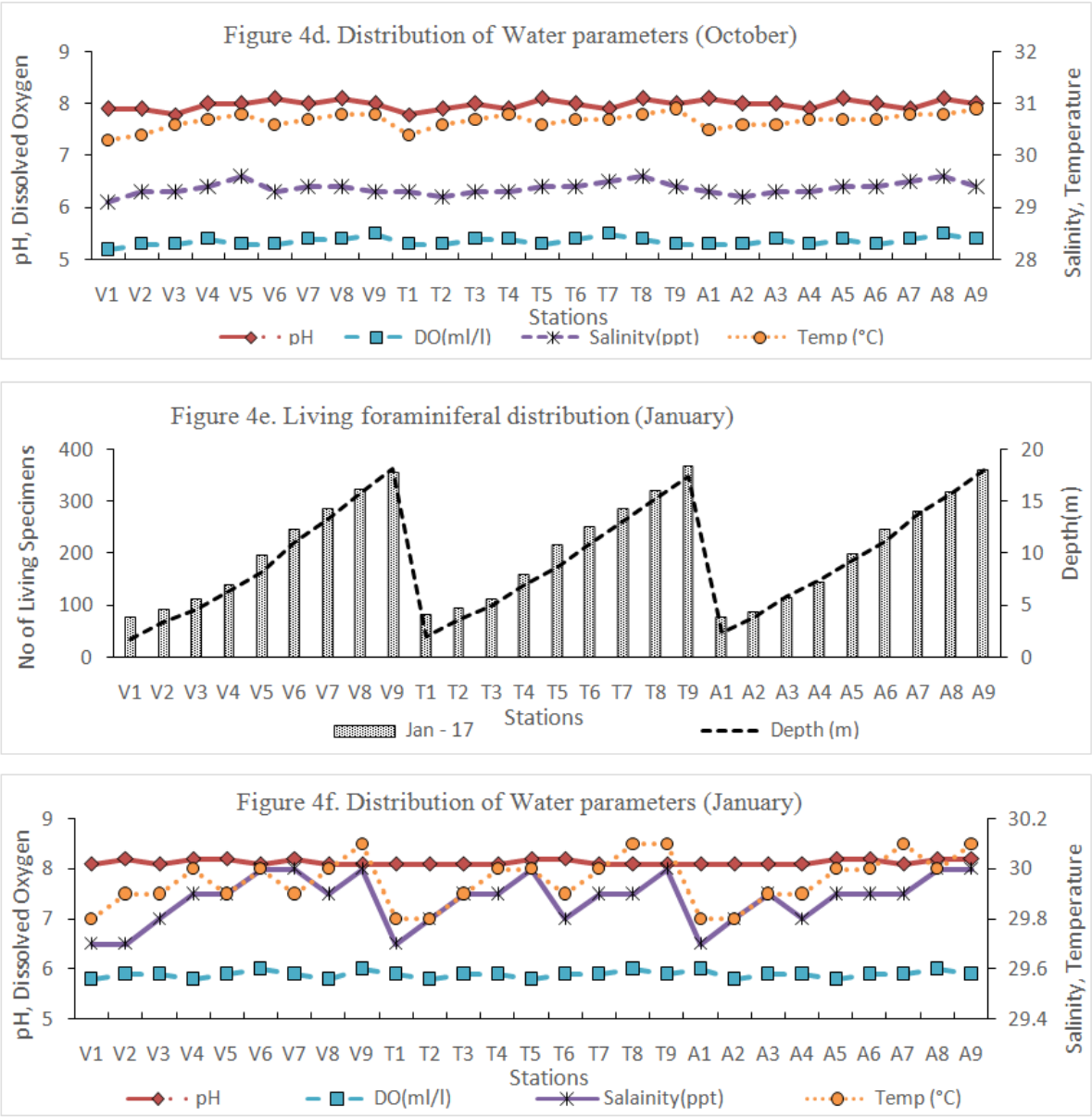


Figure 4 e-f. Distribution of water parameters and the corresponding LFN

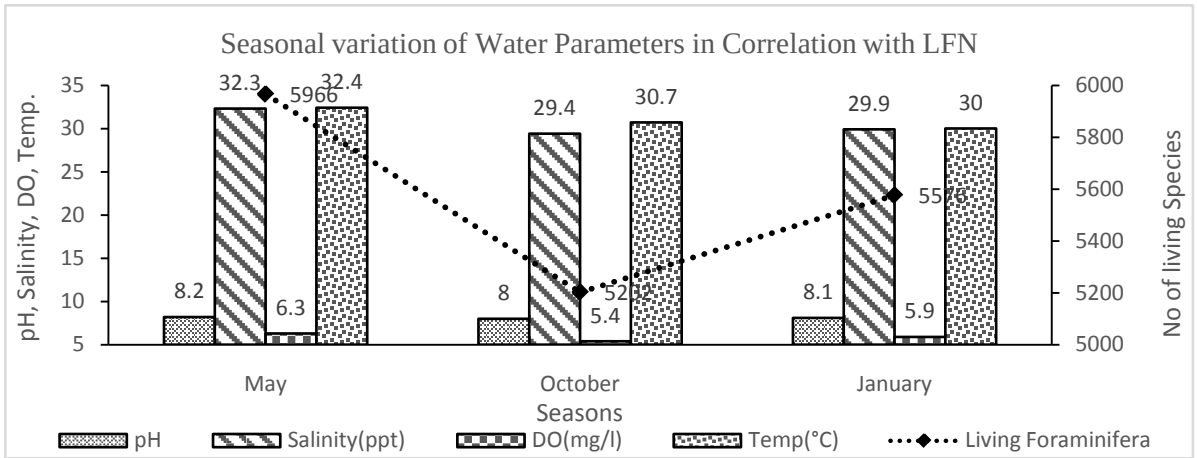


Figure 5. Seasonal Variation of Water parameters in Correlation with LFN

There is a marked seasonal variation, which can be noticed by the mean temperature values being higher during May (32.4°C) and minimum during January (30.0°C) (Figure 5). Hence, collections during summer (May) recorded a higher population. Increasing temperature was seen to be positively correlated with population size.

5.2.2. Salinity

Musco et al. [25] concluded after their study in the intertidal zone of the Yellow zone that foraminiferal abundance and species richness have a positive relation to salinity. Sreenivasalu et al. [26] through their study in the Beypore estuary reconfirmed this and also suggested that salinity lower than normal value causes a reduction in the test size of foraminiferal species.

Each benthic foraminiferal species has specific limits of tolerance to salinity for their survival, growth and reproduction, supported by other environmental factors [27,28]. Higher salinities are considered to be favourable for the growth and abundance of living foraminiferal population [23,24].

In the present study, the bottom water salinity value ranges between 32.1 ‰ and 32.4 ‰ during May, 29.1 ‰ and 29.6 ‰ during October and 29.7‰ and 30.0 ‰ during January (Figures. 4a - 4f). The salinity values do not exhibit conspicuous variations among the stations of a season; but temporally they exhibit appreciable variation. During summer (May) mean salinity value is higher (32.4 ‰) and minimum (30.0 ‰) during winter (January) (Figure 5). The relatively lower values recorded during October may be attributed to heavy inflow of freshwater into the sea during this part of the season. The LFN had a positive relationship with salinity values and seasonally, summer accounts for higher LFN.

5.2.3. Dissolved Oxygen

A number of studies have suggested that bottom water dissolved oxygen concentration is a key factor controlling the distribution of benthic foraminifera [29,30,31,32]. Dissolved oxygen has been considered as one of the parameters influencing the standing crop of foraminifera [17,23,33]. The species abundance is mainly controlled by the bottom water dissolved oxygen content of the region [34]. Dissolved oxygen content (5.7 to 6 mg/l) existed during summer months are considered to be favourable along with other congenial factors for higher foraminiferal population [24].

In the current work, the dissolved oxygen content ranges between 6.2 (mg/l) and 6.5 (mg/l) during May, 5.2 (mg/l) and 5.5 (mg/l) during October and 5.8 (mg/l) and 6.0 (mg/l) during January (Figures. 4a - 4f). But there is considerable variation in DO is observed between seasons, which can be observed from the mean values, maximum (6.3 mg/l) during May followed in January (5.9 mg/l) and is minimum (5.4 mg/l) during October (Figure 5). Though spatially, it has no influence over LFN, its impact on LFN is considerable temporally. In general, dissolved oxygen content of bottom water has a positive relationship with LFN. Higher dissolved oxygen content prevailed during summer season (May) accounts for higher LFN in the present study.

5.2.4. pH

The hydrogen ion concentration of approximately less than 7 is expected to adversely affect the production of calcareous tests [35]. The reduced pH is generally associated with low abundance and diversity of foraminifera [36,37]. The water has maintained alkalinity during all the three seasons, well within the limits of the tolerance of the fauna.

Saraswat et al. [38] after the experimental study of salinity induced pH confirmed that a pH below 7.5 can severely disrupt the calcite secretion and reproduction ability of benthic foraminifera.

In the present area, the pH values oscillate between 8.1 and 8.3 in May, 7.9 and 8.1 in October and 8.1 and 8.2 during January (Figure 4a-4f).Seasonally the mean values varied between 8.0 (October) and 8.2 (May) (Figure 5). The pH values of the bottom water show only a negligible variation both spatially and seasonally and hence its control over LFN is negligible.

5.2.5. Depth

Water depth is an important factor in determining the foraminiferal distribution. The other ecological variables which determine the abundance and diversity of foraminifera are directly associated with changes in depth [23,24,34]. It may be ascribed that increasing in depth changes the nature of substratum to more finer sand and turbidity maintain favourable conditions for the abundance of population [38].

Benthic foraminifera showed much stronger correlation to environmental gradients like water depth, percent carbonate mud, percent gravel, organic carbon flux, temperature and salinity rather than microbiotic community [39]. Rethikala et al. [17] observed that the foraminiferal population shows an increase with respect to depth. The response of foraminifera to phyto- detritus found to be varied with the depth at which the species were found in ocean. Thus, the depth had a great influence on foraminiferal responses to food and plays an important role in the changes in diversity and abundance of foraminifera observed during various seasons and in different geographical regions.

In the present study area comprising 27 stations, depth ranges from 1.6 m (station V1, October) to 18.5 m (station V9, May). Stations V1, T1 and A1 are the nearest stations with depth ranges from 1.6 m –2.4 m and stations V9, T9 and A9 are the farthest with depth ranges from 16.8 m –18.5m (Figure 4a, 4c, 4e). It is observed that in all seasons the diversity and the foraminiferal number (living and total) were found to be minimum at near shore stations and maximum at the farthest stations (V9, T9 and A9). It may be concluded that the depth along with other parameters played an important role in controlling the population and diversity of foraminifera in the present study.

5.3. Distribution and Ecology of Abundant and Widespread Foraminifera

The following five species viz. *Ammonia beccarii, Ammonia tepida, Asterorotalia inflata, Nonioniodes boueanum,* and *Quinqueloculina seminulam* are considered to be widespread and abundantly occurring since they are found as living in all the samples collected and studied and their LFN for a season is greater than other species of the present area. The living foraminiferal number of these five abundant and widespread living foraminifera in actual number of specimens for all the samples collected during May, October and January is given in Figure 6, Figure 7 and Figure 8 respectively while the temporal distribution is clearly exhibited in Figure 9.

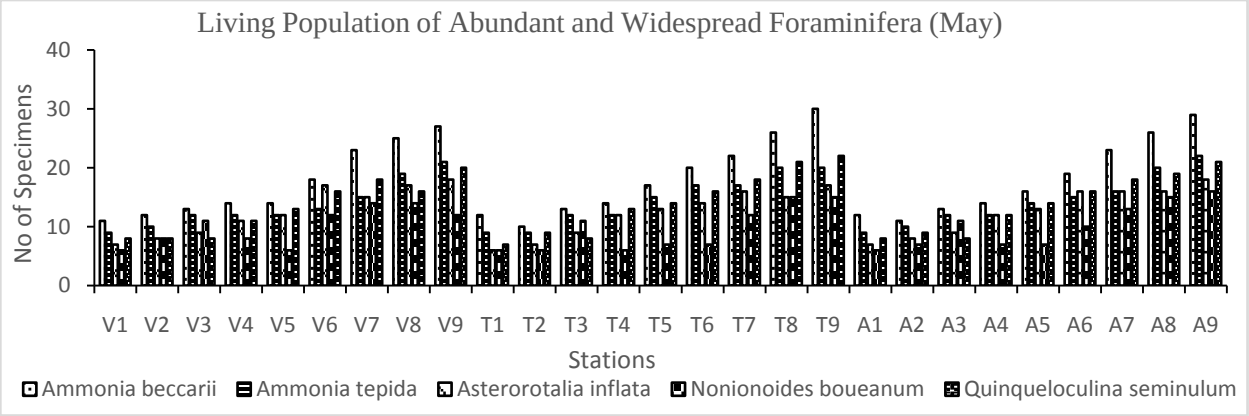


Figure 6. LFN of Abundant and Widespread Foraminifera (May 2016)

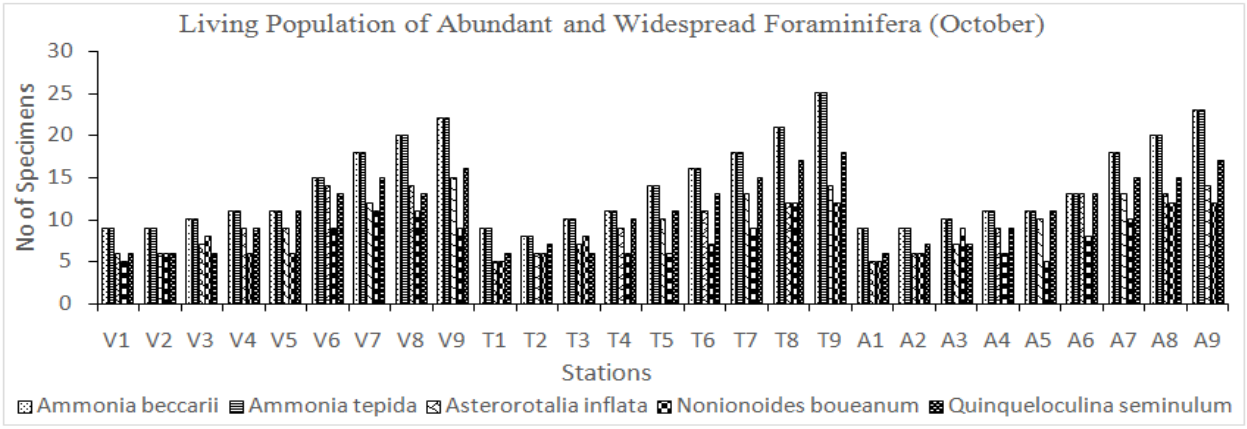


Figure 7. LFN of Abundant and Widespread Foraminifera (October 2016)

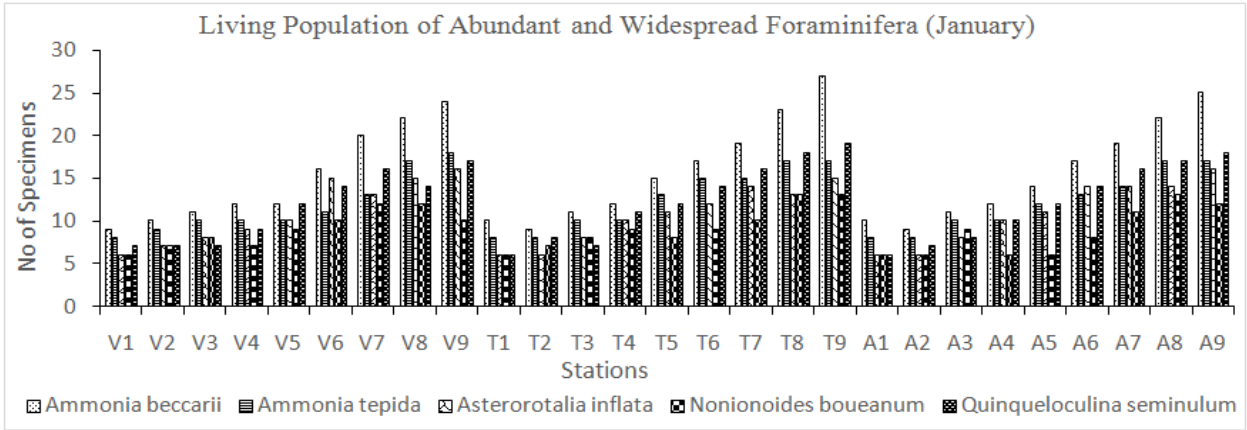


Figure 8. LFN of Abundant and Widespread Foraminifera (January 2017)

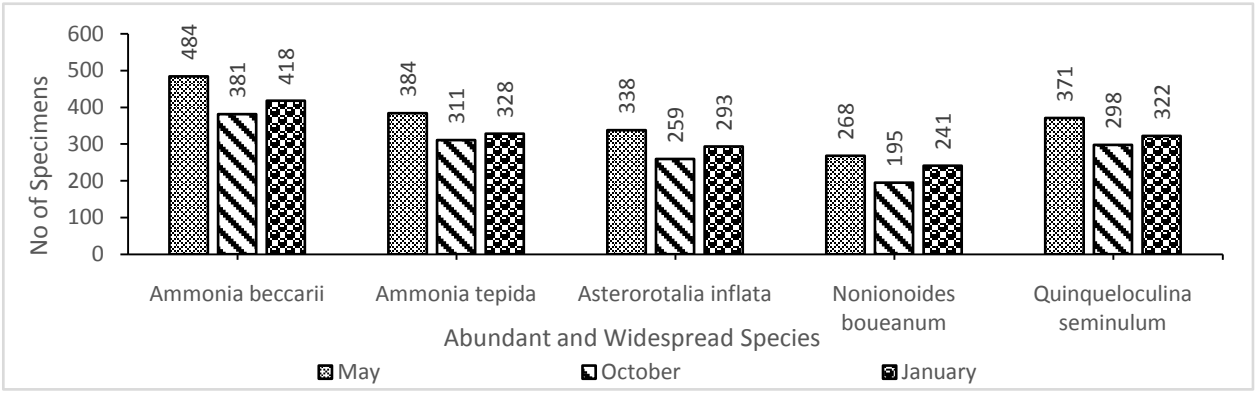


Figure 9. Temporal Distribution of Abundant and widespread Species

Kumar and Manivannan [33] recorded eight abundant and widespread species of foraminifera in the Palk Bay, off Rameshwaram and concluded that the increase in temperature, salinity and dissolved oxygen content are the causative factors for the abundance of the living population. According to Twinkle Jacob and Kumar [23] the distribution of benthic foraminifera in the shelf part of Bay of Bengal, east coast of India is chiefly controlled by nature of sediments and bathymetry coupled with hydrodynamic condition, salinity and dissolved oxygen.

Ecological and distribution study of the abundant and wide spread foraminiferal species of the study area revealed the following:

- The LFN of all the five abundant and wide spread fauna were found to be maximum during May followed by January and minimum during October.
- The lesser LFN encountered during October and January was mainly because of the bay depression and monsoon rains and the resultant modifications of the bottom water parameters.
- Spatially, the LFN of all these abundant species were less in stations nearer to the shore and gradually increasing in stations away from the shore. Lesser population noticed in near shore stations were mainly due to wave agitation.
- The environmental conditions that prevailed during May (summer) were congenial for higher reproduction and higher LFN.
- In general, the living foraminiferal number increases with increasing temperature, salinity and dissolved oxygen content of bottom water.
- The LFN was positively correlated with depth and found to be more in samples collected in comparatively deeper waters.

6. Summary and Conclusion

Influence of bottom water parameters on the distribution of recent benthic foraminifera from the shallow shelf sediments of the Palk Bay, off the coast of Tiruchendur have been studied in detail and the conclusion are summarised below:

For the study, sediment and bottom water samples have been collected at 27 stations, along three traverses viz. From off Veerapandianpattinam, off Tiruchendur and from off Alandurai, during May 2016, October 2016 and January 2017 representing the seasons summer, pre monsoon and winter respectively. A total of 105 benthic foraminiferal species belong to 47 genera, 32 families, 16 super families and 4 suborders have been identified. Among these 105 species, the following five species viz. *Ammonia beccarii*, *A. tepida*, *Asterorotalia inflata*, *Nonioniodes boueanum* and *Quinqueloculina seminulam* were found to be abundant and widespread. Various bottom water parameters like temperature, salinity, dissolved oxygen content and pH were determined and correlated with the distribution of LFN and the distribution of wide spread foraminiferal species. Bottom water parameters showed negligible variations between stations of a season but exhibited a distinct variation among seasons. It was observed that higher temperature, salinity and dissolved oxygen content of the bottom waters influence the proliferation of the living population during summer (May 2016). Optimum conditions for the higher population and abundance of foraminifera are: Temperature: > 32.0^{o}C, Dissolved oxygen: > 6.0 mg/l, Salinity: >32 ppt and pH >8.1. The population is found to be more in samples collected in comparatively deeper waters as the other ecological variables which determine the abundance and diversity of foraminifera are directly associated with changes in depth.

Acknowledgements

The authors are greatly indebted to the authorities of the National College, Tiruchirappalli for granting permission to utilise the laboratory and library facilities. The first author acknowledges with gratitude the financial assistance provided by the Rajiv Gandhi National Fellowship Programme (UGC) for carrying out the research work.

References

[1] Alve, E.: Benthic froeminiferal responses to estuarine pollution: A review, *Jour. Foram. Res*., (1995), v.25, no.3, pp. 190-203.

[2] Frontalini, F. and Coccioni, R. : Benthic foraminifera for heavy metal pollution monitoring: A case study from the central Adriatic Sea Coast of Italy, *Estuarine coastal and shelf science*, (2007), v.20, pp. 1-14.

[3] Elshanawany R., Ibrahim, M. I., Frihy, O, and Abodia, M.: Foraminiferal evidence of anthropogenic pollution along the Nile Delta coast, *Environmental Earth Sciences*, (2018), v. 77(12), pp. 1-33.

[4] Murray, J.W.: The living foraminifera of Christ Curch Harbour, Engaland, *Micropal*., (1968), v. 14, pp. 83-96.

[5] Alve, E., Nagy, J.: Estuarine foraminiferal distribution in Sandebukta, a branch of the Oslo Fjord, Jour. *Foram. Res.* (1986), v. 16, pp. 261- 284.

[6] Boltovskoy. E., Scott, D.B. and Medioli, F.S.: Morphological variations of Benthic foraminiferal test in response to changes in ecological parameters, *Jour. Paleontol*., (1991), v .5, pp 175-185.

[7] Barbosa, C. F., Suguio, K.: Biosedimentary facies of a subtropical micro-tidal estuary-an example from southern Brazil, Jour. *sediment. Res.*, (1999), v. 69, pp. 576-587.

[8] Debenay, J. P., Guiral, D. and Parra, M.: Ecological factors acting on the microfauna in mangrove swamps. The case of foraminiferal assemblages in French Guiana, Estuarine Coast, Shelf Sci., (2002), v 55, pp. 509-533.

[9] Debenay, J., Geslin, E., Eichler, B., and Duleba W.: Foraminiferal assemblages in a hypersaline lagoon, Araruama (RJ), Brazil, *Jour. Foram., Res.*, (2001), v.31(2), pp. 133-151.

[10] Uthicke, S. and Nobes, K. : Benthic foraminifera as ecological indicators for water quality on the Great Barrier Reef, *Estuarine coastal and shelf science*, (2008), v.78, pp. 763-773.

[11] Kumar, V., Manivannan, V., and Ragothaman, V. : Spatial and temporal variation in foraminifera abundance and their relation to Substrate Characteristics in the Palk Bay off Rameswaram, Tamil Nadu, *Proc. XV ICMS, Dehredun*, (1996), pp. 367-379.

[12] Elakkiya, P., and Manivannan, V. : Recent benthic foraminifera from off the coast of Arkattuthurai (near Nagapattinam), south east coast of India, *Indian Journal of Geo-Marine Sciences*, (2013), v. 42 (7), pp. 877-887.

[13] Kumar, V., Sivakumar, K., and Jeevanandam S. : Sediment - Benthic foraminifera relationship of the inner shelf sediments of Bay of Bengal, Off Chennai, Southeast coast of India, *Jour. Indian Association of Sedimentologists*, (2004), v. 23, nos. 1&2, pp. 87-93.

[14] Suresh Gandhi, M., Kasilingam. K, Arumugam, T., Lalthansangi and Rajeshwara Rao, N. : Distribution of benthic foraminifera, sediment characteristics and its environmental condition in and

around Manalmelkudi Spit, Palk Strait, Tamil Nadu, East coast of India, *Indian Jour. Geo Marine Sciences*, (2017), v. 46 (05), pp. 893-906.

[15] Nigam R., and Khare N.: Spatial and temporal distribution of foraminifera in sediments off the central west coast of India and use of their test morphologies for the reconstruction of palaeomonsoonal precipitation, Micropaleontology, (1999), v.45, pp. 1-15.

[16] Nigam, R. and Rao, A.S.: Proloculus size variation in Recent benthic foraminifera: Implications for paleoclimatic studies, *Estuar. Coastal Shelf Sci.*, (1987), v. 24, pp. 649 -655.

[17] Rethikala, K.R., Kumar, V. and Sathish, B. : Inner Shelf Characteristics and Their Impact on the Distribution of Recent Foraminifera from the Southern Part of Gulf of Mannar, Southeast Coast of India, *Applied Ecology and Environmental Science*, (2021), v.1, pp.30-41.

[18] Walton, W.R.: Techniques for recognition of living foraminifera, Contr. Cushman Found. Foram. Res. (1952), v.3, pp. 56-60.

[19] Cushman, J.A.: Foraminifera, Their classification and Economic use, 4th Edition, with an illustrated key to the Genera, *Hardward University Press, Massachusetts*, (1952), 588p.

[20] Leobilich, A. R., Jr., and Tappan, H.: Foraminiferal genera and their classification, *Van Nostrand Reinhold Company, New York*, (1988), v. 1 & 2, 970+212p. 846 pls.

[21] Saalim, S.M., Saraswat, R., Thejasino Suokhrie and Nigam. R.: Assessing the ecological preferences of agglutinated benthic foraminiferal morpho groups from the western Bay of Bengal, *Deep-Sea Research Part II, Topical Studies in Oceanography* (2018), v.161, pp.38-51

[22] Kurtarkar, S.R., Saraswat, R., Kaithwar, A, and Nigam, R. : How will Benthic Foraminifera Respond to Warming and Changes in productive? A Laboratory Culture Study on Cymbaloporetta plana, *Acta Geologica Sinica*, (2019), v. 93, (1), pp.175-182.

[23] Twinkle Jacob. and Kumar, V.: Bottom Water Characteristics and Their Influence on Temporal Distribution of Benthic Foraminifera from off Manapad, Gulf of Mannar, South East Coast of India, *Applied Ecology and Environmental Sciences*, (2019), v. 7, no. 6, pp.245-254.

[24] Christinal J., Kumar, V., Selvaraj, S., and Gangaimani, T. : Inner Shelf Environments and Their Impact on the Distribution of Benthic Foraminifera from the Gulf of Mannar, off Kayalpattinam, Southeast Coast of India, *Applied Ecology and Environmental Sciences*, (2021), v 9(3), pp. 396-409.

[25] Musco, M., Cuttitta, A., Bicchi, E., Quinci, E.M., Sprovieri, M., Tranehida, G., Giaramita, L., Traina, A., Salvagio Manta, D., Gherardi, S., Mercurio, P., Siragusa, A. and Mazzola, S.: Benthic foraminifera as bio-indicators of anthropogenic impacts in coastal environments: Acqua Dei Corsari area case study (Paleomo, Italy), *Mar. Pollut. Bull.*, (2017), v. 117, pp. 75-87.

[26] Sreenivasulu G., Praseetha B. S., Daudb N. R., Varghese T. I, Prakash T.N. and Jayaraju N.: Benthic foraminifera as potential ecological proxies for environmental monitoring in coastal regions: A study on the Beypore estuary, Southwest coast of India, *Marine Pollution Bulletin*, (2019), v.138, pp. 341-351.

[27] Lei Y., Li T., Jian Z. and Nigam R.: Taxonomy and distribution of benthic foraminifera in an intertidal zone of the Yellow Sea, PR China: Correlations with sediment temperature and salinity, *Marine Micropaleontology*, (2017), v. pp.133, 1-20.

[28] Lee Y. G., Kim S., Kim Y. W., Jeong D. U., Sick Lee J. S., Woo H. J. and Shin H. C.: Benthic foraminifera as bioindicators of salinity variation in Lake Shihwa, South Korea, *Jour. Foram. Res.*, (2015), v. 45, no. 3, pp. 235–249.

[29] Ramadan H. and Abu-Zied et al. Benthic foraminiferal response to changes in bottom-water oxygenation and organic carbon flux in the eastern Mediterranean during LGM to Recent times, *Marine Micropaleontology* , (2008), v. 67, pp.46–68.

[30] Hess, S., and Jorissen, F.J.: Distribution patterns of living benthic foraminifera from Cap Breton canyon, Bay of Biscay: Faunal response to sediment instability, *Deep Sea Research*, (2009), v.56, pp.1555-1578.

[31] Amrata Kaithwar, D.P. and Saraswat, R.: A highly diverse living benthic foraminiferal assemblage in the oxygen deficient zone of the south-eastern Arabian Sea, *Biodiversity and Conservation*, (2020), v.29, pp.3925-3958.

[32] Martins M. V. A., Yamashita C., Sousa S. H. M., Koutsoukos E. A. M., Disaró S. T., Debenay J. P and Duleba W. : Response of Benthic Foraminifera to Environmental Variability: Importance of Benthic Foraminifera in Monitoring Studies, (2019), Monitoring of Marine Pollution, Houma Bachari Fouzia, Intech Open.

[33] Kumar, V., and Manivannan V.: Benthic foraminifera responses to bottom water characteristics in the Palk Bay, off Rameshwaram, Southeast Coast of India, *Ind. Jour. Mar. Sci.*, (2001), v. 30, pp. 173-179.

[34] Kumar, V., Sivakumar, K., Gangaimani, T. and Anand, K.J.: Morphological abnormalities of benthic foraminifera from the Palk Bay, off Rameswaram, Tamilnadu: A tool for environmental monitoring, *Pollution Research*, (2006), v. 25(1), pp. 35-42.

[35] Anbuselvan, N, and Senthil Nathan. D,: Benthic foraminiferal distribution and biofacies in the shelf part of Bay of Bengal, east coast of India, *Marine Biodiversity*, (2018), v. 49(2), pp. 697-706..

[36] Kasilingam, K., Suresh Gandhi, M., Rajeshwara Rao, N.: Near- shore foraminifera along the Palk strait, Southeast Coast of India, Tamilnadu, *Jour. Pal. Soc. of India*, (2020), v.65 (1), pp. 90-106.

[37] Saraswat, R., Kouthanker, M., Kirtankar, S., Nigam R., and Linshy, V.N.: "Effect of salinity induced pH/ alkalinity changes on benthic foraminifera: a laboratory culture experiment, *Estuarine, Coastal and Shelf Science*, (2015), v. 153, pp. 96-107.

[38] Suresh Gandhi, M., and Sonia Nathan, A.: "Benthic foraminifera and geochemical studies with influence on pollution studies along the coast of Cuddalore, Tamil Nadu-ITS, *India Arab Jour. Geosci.*, (2014), v.7, pp. 917-925.

[39] Post, A.L., Sbaffi, L., Passlow, V., and Collins, D.C.: Benthic foraminifera as environmental indicators in Torres Strait–Gulf of Papua, in Todd, B.J., and Greene, H.G., eds., Mapping the Seafloor for Habitat Characterization, *Geological Association of Canada, Special Paper*, (2007), v.47, pp. 329-347.

© The Author(s) 2021. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).

Applied Ecology and Environmental Sciences, 2021, Vol. 9, No. 3, 396-409
Available online at http://pubs.sciepub.com/aees/9/3/10
Published by Science and Education Publishing
DOI:10.12691/aees-9-3-10

Inner Shelf Environments and Their Impact on the Distribution of Benthic Foraminifera from the Gulf of Mannar, off Kayalpattinam, Southeast Coast of India

Christinal J.[1,*], V. Kumar[1], S. Selvaraj[1], T. Gangaimani[2]

[1]Department of Geology, National College (Autonomous), Tiruchirapalli, Tamilnadu - 620001
[2]Department of Civil Engineering, M. A. M. College of Engineering, Siruganur, Tamilnadu -621105
*Corresponding author: kumarvgeol@gmail.com

Received February 19, 2021; Revised March 26, 2021; Accepted April 06, 2021

Abstract Identification of change in foraminiferal assemblage with respect to environmental modifications will be a positive contribution to know varied marine micro ecosystem. The occurrence of benthic foraminiferal species is controlled to a great extent by physical factors such as depth, temperature, salinity, and dissolved oxygen content of bottom water, character of the bottom sediments, and availability of biological factors such as food supply, presence of symbiotic organisms and predators. In order to observe the impact of inner shelf environments on the distribution of benthic foraminifera, sediment and bottom water samples were collected from the inner shelf off Kayalpattinam, once in four months for a year, at 18 locations with a depth range between 1.76 m and 14.89 m., during the months of May 2016, September 2016 and January 2017 representing summer, pre-monsoon and winter/post monsoon respectively. In the present study, 81 foraminiferal species belonging to 42 genera, 30 families, 16 super families of the 4 sub orders (Textulariina, Miliolina, Lagenina and Rotaliina) have been identified. The spatial and temporal distribution of the foraminiferal fauna has been evaluated and correlated with observed environmental parameters like CaCO3 content, organic matter content, sand-silt-clay ratio of the substrate and temperature, salinity, dissolved oxygen content and pH of bottom water. Higher calcium carbonate content (>26%), organic matter content (0.9 - 1.03%) higher temperature (32.7 -33.3°C), higher salinity (32.6 to 33.3ppt) and dissolved oxygen (5.7 to 6 ml/l) prevailed during summer months are considered to be favourable for higher foraminiferal population in the present study. Depth is also considered as one of the effective factors controlling the population of foraminifera along with the other congenial parameters. The favourable niche for population abundance is found to be silty sand.

Keywords: *inner shelf, benthic foraminifera, substrate characters, bottom water characters, spatial and temporal distribution, Kayalpattinam, southeast coast of India*

Cite This Article: Christinal J., V.Kumar, S. Selvaraj, and T. Gangaimani, "Inner Shelf Environments and Their Impact on the Distribution of Benthic Foraminifera from the Gulf of Mannar, off Kayalpattinam, Southeast Coast of India." *Applied Ecology and Environmental Sciences*, vol. 9, no. 3 (2021): 396-409. doi: 10.12691/aees-9-3-10.

1. Introduction

Foraminifers are unicellular microorganisms widely distributed in marine and brackish water environments [1]. Many studies relating foraminiferal population distribution with factors like nutrition, dissolved oxygen, salinity, temperature and substrate have been carried out. Benthic foraminiferal species grouped by changing depth and latitudinal variation [2]; change in environmental factors have an impact on the morphology of foraminiferal test [3], salinity controlled the distribution of foraminifera [4], sediment type and organic matter content have an impact on living benthic foraminifera[5], oxygen depleted environments lead to diverse aspects of foraminiferal adaptation [6], distribution of brackish water benthic foraminifera can be used to understand bio geography and paleoenvironmental assessment [7], typical paralic sub-environments were characterized by typical foraminiferal species assemblages [8], strong and highly significant relationship of foraminifera assemblages have corelated with elevation and environment [9].

Industrial waste mainly with trace elements, agricultural and domestic waste affected the density and diversity of foraminiferal assemblages [10,11,12], environmental stress by anthropogenic and natural elements can result in test deformation [13]. Distribution of inner shelf benthic foraminifera with respect to the changes in substrate and bottom water characters have been studied from the Bay of Bengal, off Porto Novo [14], from the Palk Bay, off Rameswaram [15,16] and from the Palk strait from off Manamelkudi [17].

Foraminifera adapted to various niches on the sea floor viz., estuaries, salt marshes, marine marginal water bodies etc., are very sensitive to the environmental modifications and pollutional interferences and hence can effectively be used as an indicator for understanding the inner shelf environments of Gulf of Mannar, off Kayalpattinam.

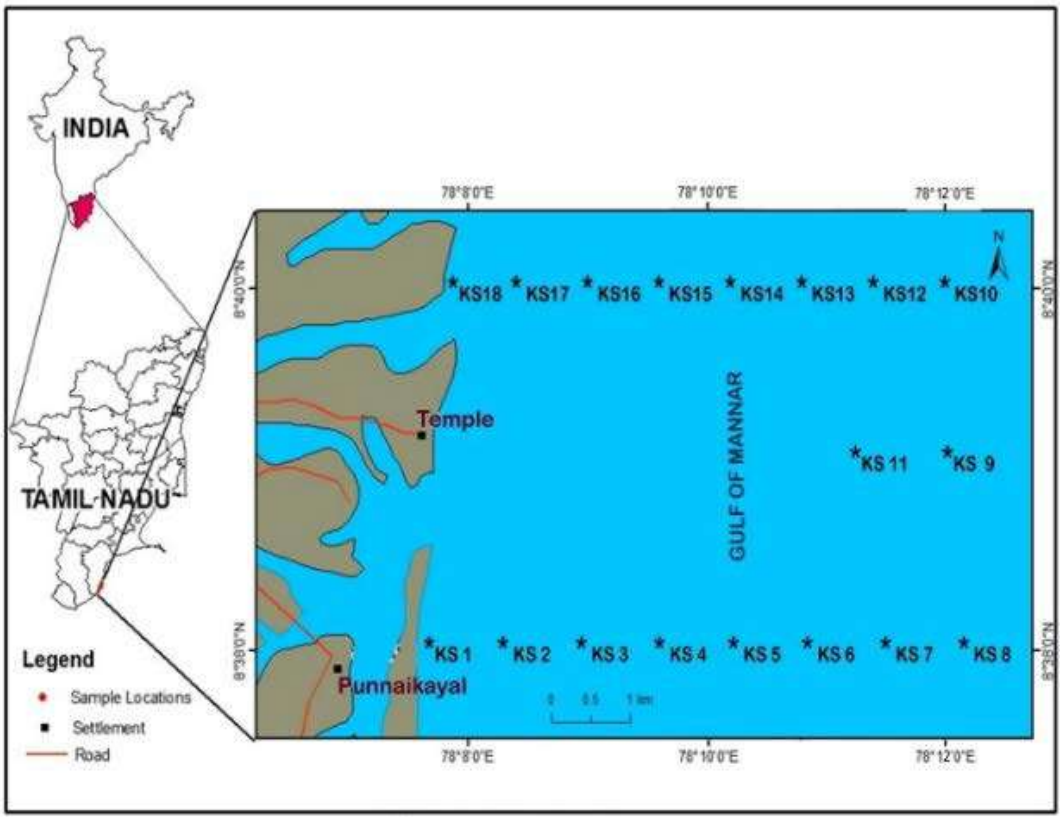


Figure 1. Location map of the study area

2. Study Area

The study area comprising the southern part of Gulf of Mannar, East coast of India (Figure 1), lies in the geographic coordinates N 8°38' to N 8°40'15" to E 78°07'30" to E 78°12'30" and forms a part of toposheet numbers 58 L/1 and 58 L/5 of the Survey of India.

The region forms the southern part of the South Indian Granulite terrain (SGT), which is a high-grade metamorphicterrain under granulite facies. The Gulf of Mannar receives riverine input through a number of rivers, of which the Thamirabaraniis the major source, debouches in the study area. As there are few check dams constructed recently in the estuarine portion the sedimentation influx into the shore is reduced considerably. Generally, the area is an undulating topography with general slope towards the east.

The area experiences generally a tropical climate and witnessing two types of monsoons namely, Southwest and Northeast. Southwest monsoon is from June to September, accounts for scanty to moderate rainfall while during Northeast monsoon, from October to December, often accompanied by cyclones andgives ample amount of annual rainfall. The annual average temperature varies from 26.0°C to 37.0°C with an average humidity of ~65%. Generally, summer is experienced during months March, April and May while winter from December to February.

3. Objective

In order to assess the environmental impact on foraminifera from the inner shelf sediments of Gulf of Mannar, off Kayalpattinam the following objectives are taken up:

- to inventory the foraminifera fauna and their quantitative composition
- to ascertain the inner shelf environmental parameters
- to discover the effect of environmental parameters on the seasonal and spatial variation of benthic foraminiferal fauna.

4. Materials and Methods

4.1. Field Work

The sediment and bottom water samples were collected at 18 stations from the inner shelf sediments of Gulf of Mannar, off Kayalpattinam ranging in depths from 1.76 to 14.89 meters, during the months of May 2016, September 2016 and January 2017 representing summer, pre-monsoon and winter/post monsoon respectively and the total samples amounted to 54. All the sediment samples were collected by taking necessary precautions, making use of Petersen mud grab and an unit volume of 100 ml wet sediment taken from the top layer of each sample was preserved immediately in a 10% neutralized formaldehyde solution for foraminiferal study. At each station, samples of water from the sediment - water interface were collected with the help of a Nansen reversible water sampler and the temperature of the bottom water was noted using built in thermometer. Dissolved oxygen content of each bottom water sample was observed by using portable A113 dissolved oxygen analyser, salinity

values by using Kerro salinometer and pH with the aid of H19828 pH meter after making necessary precautions in sampling and standardisation.

4.2. Laboratory Work

For identifying living foraminifera, Walton's [18] rose Bengal staining technique have been used.Foraminiferal tests were then separated from the residue by floatation method using carbon tetrachloride.In the laboratory, sand-silt-clay ratio in the sediment samples was estimated using standard sieving and pipette method of Krumbein and Pettijohn [19]. Textural nomenclature of Trefethen [20] has been used todescribe the sediment types.

Calcium carbonate was determined by following titration method of Piper [21] while Organic matter was estimated by the chromic acid method of Walkley and Black as detailed out by Jackson [22]. Benthic foraminifera tests were picked and mounted using a stereo binocular microscope and their taxa were identified by following the classification of Loeblich and Tappan [23].

5. Results and Discussion

5.1. Foraminifera

In the present study, 81 foraminiferal species belonging to 42 genera, 30 families, 16 super families of the 4 sub orders (Textulariina, Miliolina, Lagenina and Rotaliina) have been identified (Table 1). Among these, nine species belong to Textulariina, 28 belong to Miliolina, eight species belong to Lagenina and the rest 36 species belong to Rotaliina.

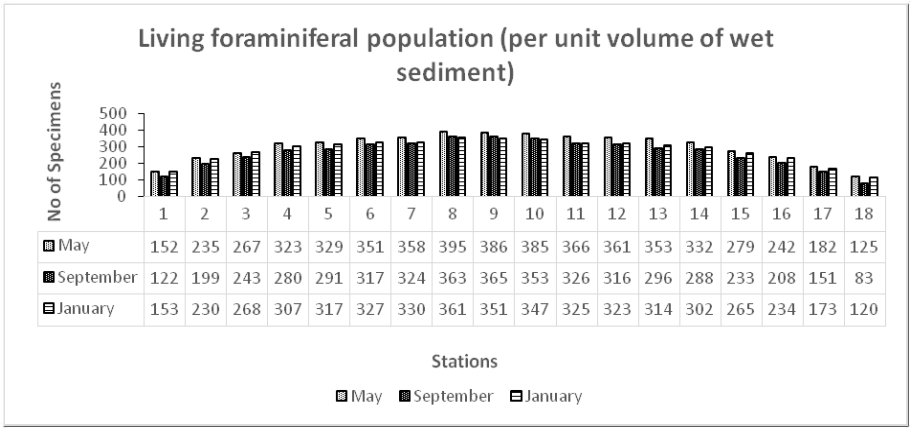
5.1.1. Living Foraminifera Population

In the current study, living foraminiferal species were observed in all the 54 samples collected and studied. Spatially, the living population varies from 83 (station 18, September) to 395 (station 8, May) specimens per 100ml. of wet sediment. The maximum living population was noticed in the farthest stations from the shore (stations 8, 9, 10) and the minimum foraminiferal population was found in near shore stations (stations 1 and 18).

Spatially, living foraminiferal population gradually increases from the shore to farthest stations. Seasonally, the living population size is found to be maximum (5392 specimens) in summer (May), minimum (4676 specimens) in pre-monsoon (September) (Figure 2).

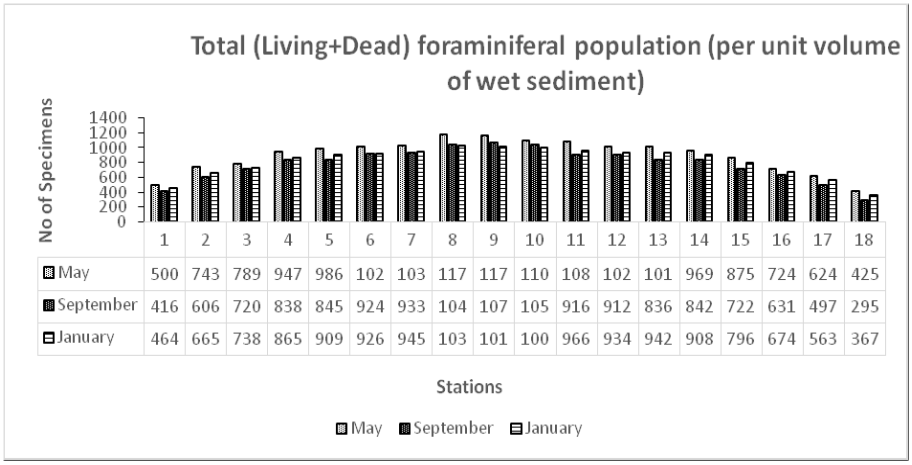
5.1.2. Total Foraminiferal Population

In the study area, the total (living + dead) population size ranges from 295 (station 18, September) to 1177 (station 8, May) specimens per 100 ml of wet sediments. Temporally, the total population is found to be maximum (16097 specimens) during summer (May) and minimum (13910 specimens) in pre-monsoon (September) (Figure 3).



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
May	152	235	267	323	329	351	358	395	386	385	366	361	353	332	279	242	182	125
September	122	199	243	280	291	317	324	363	365	353	326	316	296	288	233	208	151	83
January	153	230	268	307	317	327	330	361	351	347	325	323	314	302	265	234	173	120

Figure 2. Living foraminifera population



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
May	500	743	789	947	986	102	103	117	117	110	108	102	101	969	875	724	624	425
September	416	606	720	838	845	924	933	104	107	105	916	912	836	842	722	631	497	295
January	464	665	738	865	909	926	945	103	101	100	966	934	942	908	796	674	563	367

Figure 3. Total foraminifera population

5.2. Substrate Characters

5.2.1. Sand-Sily-Clay Ratio

The correlation between texture of sediment and foraminiferal population was understood from several studies. The most favorable accommodative substrate for the foraminiferal abundance is silty sand [16,24,25,26,27,28]. Elakkiya and Manivannan [29] after their study on recent benthic foraminifera from off the coast of Arkattuthurai (near Nagapattinam), south east coast of India concluded that the important ecological factors that controls the distribution of foraminifera are organic matter content, calcium carbonate content and nature of the substrate. Kurbjeweit et. al [30] described the positive relation of benthic foraminiferal abundance with oxygen content, pore volume, carbon content and chloroplastic pigment equivalents of the sediment while distributional patterns related to sand fraction, dissolved oxygen, calcium carbonate and organic carbon content of the sediment of the Arabian sea.

Table 1. Taxonomy Chart of 81 Benthic Foraminiferal Species from the study Area

SUPERFAMILY	FAMILY	SUBFAMAILY	GENUS	SPECIES
LITUOLACEA	LITUOLIDAE	AMMOMARGINULININAE	Ammobaculites	*Ammobaculitesdilatatus*
				A. exiguous
TROCHAMMINACEA	TROCHAMMINIDAE	TROCHAMMININAE	Trochammina	*Trochamminainflate*
TEXTULARIACEA	EGGERELLIDAE	EGGERELLINAE	Eggerella	*Eggerellaadvena*
	TEXTULARIIDAE	TEXTULARIINAE	Textularia	*Textulariaagglutinans*
				T. aura
				T. conica
				T. foliacea
				T. saggitulaarata
CORNUSPIRACEA	FISCHERINIDAE	NODOBACULARILLINAE	Vertebralina	*Vertebralina striata*
MILIOLACEA	SPIROLOCULINIDAE		Adelosina	*Adelosina laevigata*
			Spiroloculina	*Spiroloculinaangulate*
				S. communis
				S. costifera
	HAUERINIDAE	HAUERININAE	Hauerina	*Hauerinabradyi*
				H. fragilissma
			Quinqueloculina	*Quinqueloculinaagglutinans*
				Q. bicostata
				Q. echinata
				Q. lamarckiana
				Q. polygona
				Q. seminulum
				Q. sulcata
		MILIOLINELLINAE	Miliolinella	*Miliolinella circularis*
				M. labiosa
			Pseudomassilina	*Pseudomassilina australis*
				P. macilenta
			Pyrgo	*Pyrgoelongate*
			Triloculina	*Triloculina oblonga*
				T. schrieberiana
				T. terquemiana
				T. tricarinata
				T. trigonula
	MILIOLIDAE	MILIOLINAE	Rupertianella	*Rupertianellarupertiana*
SORITACEA	PENEROPLIDAE		Peneroplis	*Peneroplisplanatus*
			Spirolina	*Spirolinaaritinus*
	SORITIDAE	SORITINAE	Sorites	*Sorites orbiculus*
NODOSARIACEA	LEGENIDAE		Lagena	*Lagena gracillima*
				L. laevis
				L. semistriata
				L. striata
	POLYMORPHINIDAE	POLYMORPHININAE	Globulina	*Globulinagibba*
	ELLIPSOLAGENIDAE	OOLININAE	Oolina	*Oolinaovoidea*
		ELLIPSOLAGENINAE	Fissurina	*Fissurina laevigata*
				F. marginata

SUPERFAMILY	FAMILY	SUBFAMAILY	GENUS	SPECIES
BOLIVINACEA	BOLIVINIDAE		Bolivina	*Bolivinadoniezi*
				B. nobilis
			Brizalina	*Brizalinastriatula*
LOXOSTOMATACEA	LOXOSTOMATIDAE		Loxostomum	*Loxostomumlimbatum*
BULIMINACEA	SIPHOGENERINOIDIDAE	SIPHOGENERINOIDINAE	Rectobolivina	*Rectobolivinaraphanus*
	BULIMINIDAE		Bulumina	*Buliminellamilletti*
	BULIMINELLIDAE		Buliminella	*Buluminamaginata*
	UVIGERINIDAE	UVIGERININAE	Uvigerina	*Uvigerinaproboscidea*
	REUSSELLIDAE		Reussella	*Reusella spinulosa*
FURSENKOINACEA	FURSENKOINIDAE		Sigmavirgulina	*Sigmavirgulinatortuosa*
DISCORBACEA	BAGGINIDAE	BAGGININAE	Cancris	*Cancrisauriculus*
				C. oblonga
	EPONIDIDAE	EPONIDINAE	Poroeponoides	*Poroeponides lateralis*
	ROSALINIDAE		Rosalina	*Rosalina globularis*
PLANORBULINACEA	CIBICIDIDAE	CIBICIDINAE	Cibicides	*Cibicidesdispars*
				C. lobatulus
				C.refulgens
	CYMBALLOPORIDAE	CYMBALLOPORININAE	Cymbaloporetta	*Cymbaloporettabradyi*
NONIONACEA	NONIONIDEA	NONIONINAE	Nonionella	*Nonionellaauris*
			Nonionoides	*Nonionoidesboueanum*
				N. grateloupi
				N. labradoricum
ROTALIACEA	ROTALIIDAE	PARAROTALIINAE	Pararotalina	*Pararotalinanipponica*
		AMMONIINAE	Ammonia	*Ammonia beccarii*
				A. dentate
				A. tepida
			Asterorotalia	*Asterorotaliainflate*
				A. trispinosa
	ELPHIDIIDAE	ELIPHIDIINAE	Elphidium	*Elphidiumadvenum*
				E. crispum
				E. excavatum
				E. hispidulum
				E. incertum
				E. macellum
				E. norwangii
NUMMULITACEA	NUMMULITIDAE		Operculina	*Operculinaammonoides*

Table 2. Sediment types of varied stations, off Kayalpattinam.

Month	Sand	Silty sand
May 2016	1,2,17,18	3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15& 16
Sep. 2016	1, 2, 16,17, 18	3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14& 15
Jan. 2017	1,2,17,18	3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15& 16

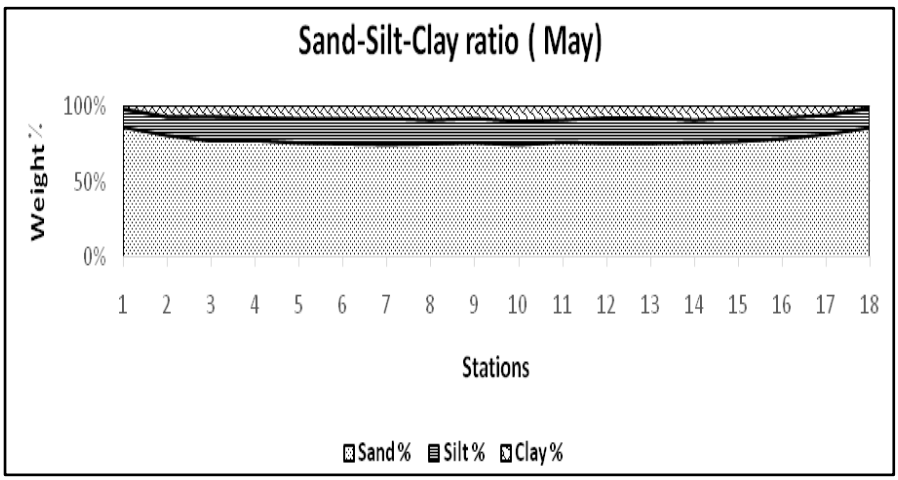


Figure 4. Relative abundance of Sand -Silt -Clay (May 2016)

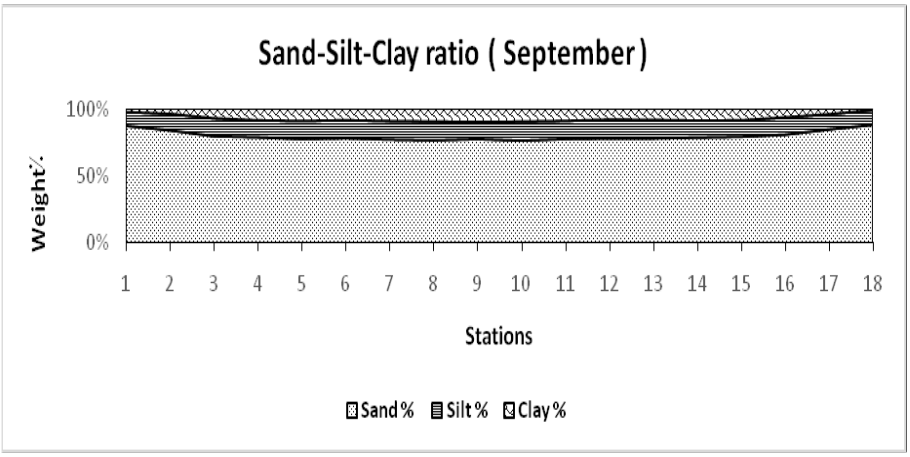


Figure 5. Relative abundance of Sand-Silt-Clay (September 2016)

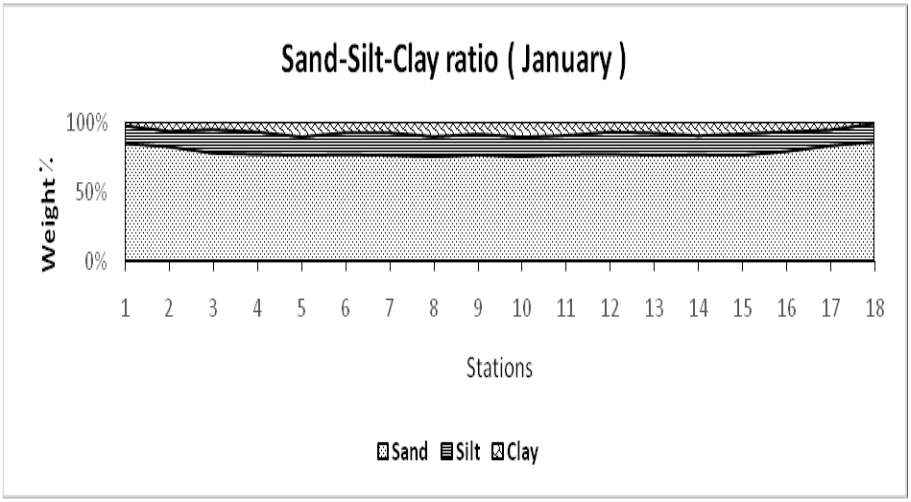


Figure 6. Relative abundance of Sand-Silt-Clay (January 2017)

In the present study, among the twelve possible sediment types of Trefethen's [20] textural nomenclature, the sediments taken from the study area fell only in two sediment types viz. sand and silty sand (Table 2). The foraminiferal population is relatively very poor in stations 1, 2, 16, 17 and 18 where the substrate is sandy and the population is more in stations 3-15 where the substrate is siltysand in all the three seasons (Figure 2). In general, larger foraminiferal populations are associated with low energy area and comparatively deeper part of the collection while the population is less in samples near the high-energy environment (near shore stations). The data related to sand silt clay percentages of all the samples collected are in relative abundance chart (Figure 4, Figure 5 and Figure 6). Thus, it is evident that the silty sand substrate offers a favourable condition for the foraminiferal abundance of the area.

5.2.2. Calcium Carbonate

The variation in calcium carbonate content of the sediments had a bearing on the abundance of foraminifera and showed a positive relation [25,29,30,31,32]. The calcium carbonate content, in the deeper segment of the traverse, coinciding with the alignment of coral reefs, is found to be higher that favours higher population [33]. Post et.al [34] exhibited the relationship between benthic foraminifera and whole micro biotic community with changes in environment and found that benthic foraminifera showed much stronger correlation to calcium carbonate content rather than other micro biotic community. The higher calcium carbonate content in beach sands of Kovalam favors maximum distribution of foraminifera [35]. Temporally, the mean values CaCO3 content was higher during May (summer) and spatially along the deeper part of all the collections which was positively correlated with foraminiferal population [27]. Temporally, higher percentage of CaCO3 prevailed during April was one of the congenial factors for higher population during that period [36].

In the present study the percentage of calcium carbonate content is generally moderate ranging from 17.9% (station 18, September) to 26.6% (station 8, May). Pearl and chunk beds are the main source for the higher CaCO3 content of sediments in stations 7-12. Relatively, the calcium carbonate content was found to be higher in stations 8, 9 and 10 in all the seasons and positively correlated with higher population. Lower values are recorded in station 1 followed by 18. Seasonally, higher percentage of calcium carbonate observed during May (Table 3) and was one of the controlling factors for the seasonal abundance of population in that period. In general, calcium carbonate content is considered to be the main influencing factor for abundance of living population in the study area (Figure 7, Figure 8, Figure 9, Figure 10, Figure 11 and Figure 12).

Table 3. CaCo3 content range of varied stations, off Kayalpattinam

Month	$CaCO_3$ content range %	Station numbers
May-16	> 25	4,5,6,7,8,9,10,11,12,13,14 &15
	23 - 25	3 & 16
	20 - <23	2 &17
	< 20	1 & 18
Sep-16	> 25	8,9,10
	23 - 25	3,4,5,6,7,11,12,13,14
	20 - <23	2,15,16,17
	< 20	1 & 18
Jan-17	> 25	5,6,7,8,9,10,11,12,13,14
	23 - 25	4 & 15
	20 - <23	2,3,16,17
	< 20	1 & 18

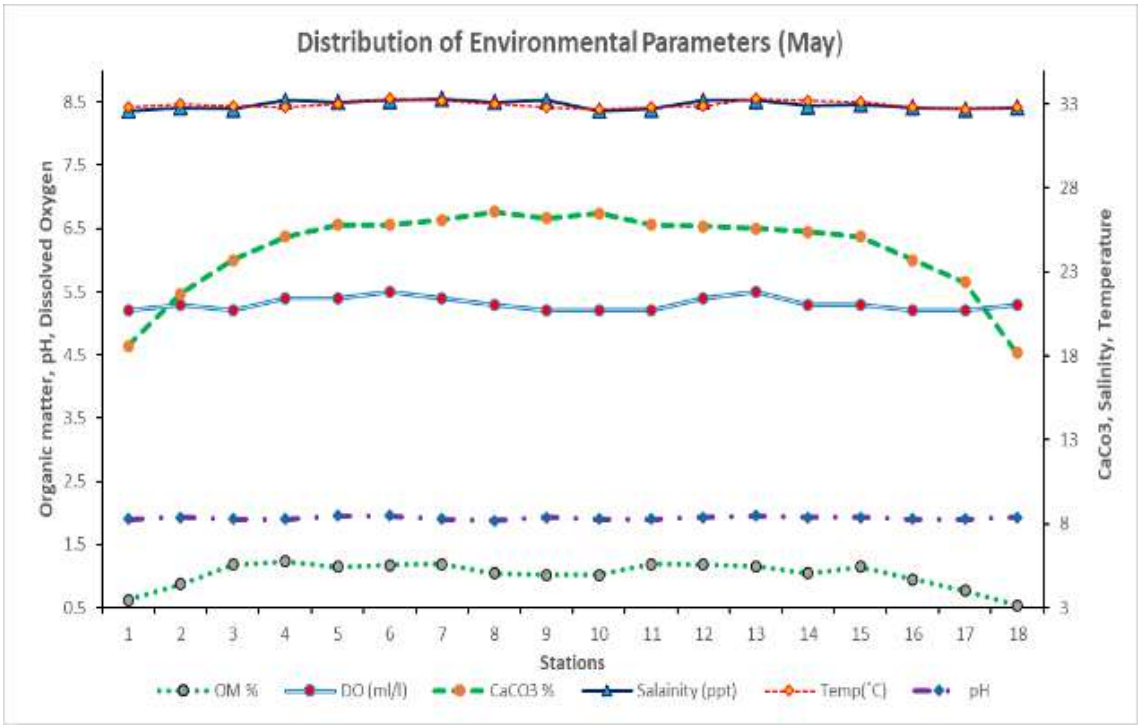


Figure 7. Distribution of Environmental parameter (May-2016)

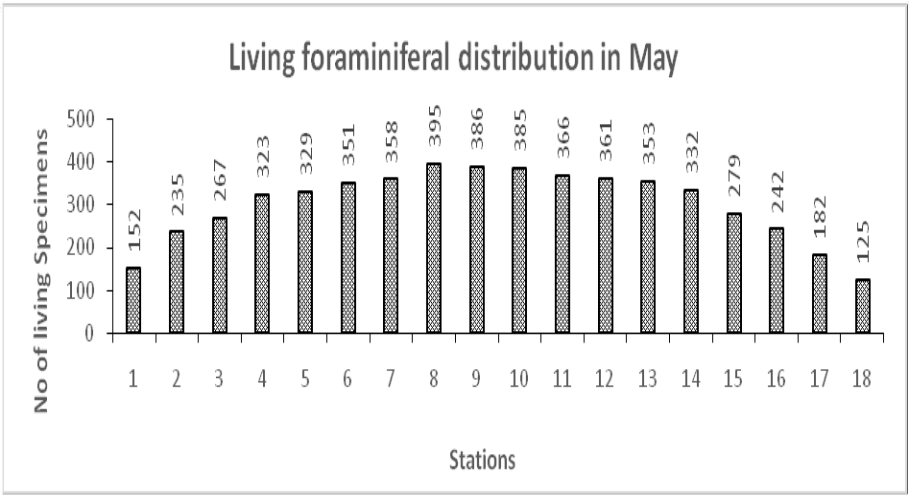


Figure 8. Living foraminifera distribution in May-2016

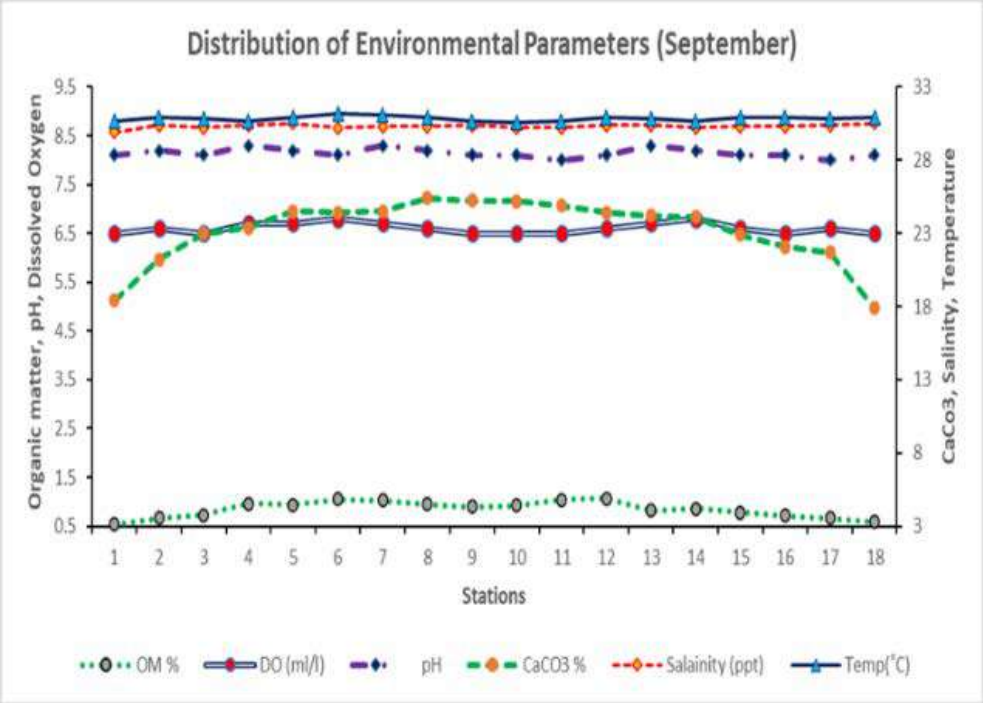


Figure 9. Distribution of Environmental parameters September-2016

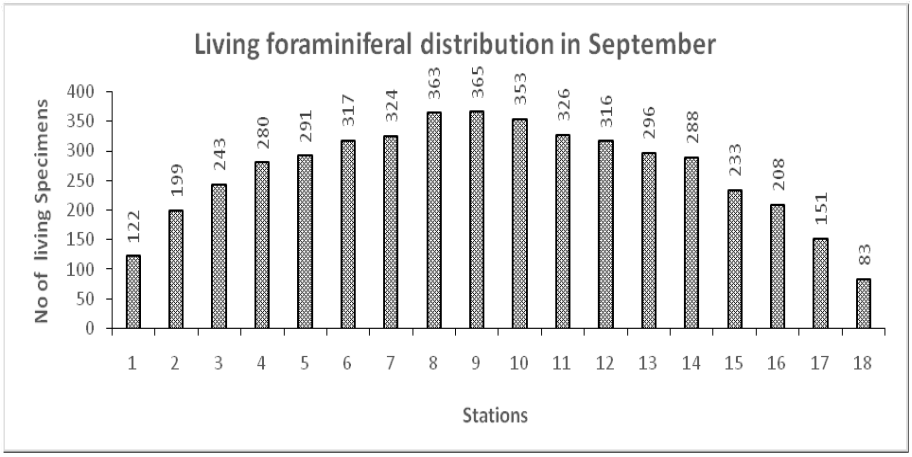


Figure 10. Living foraminifera distribution in September -2016

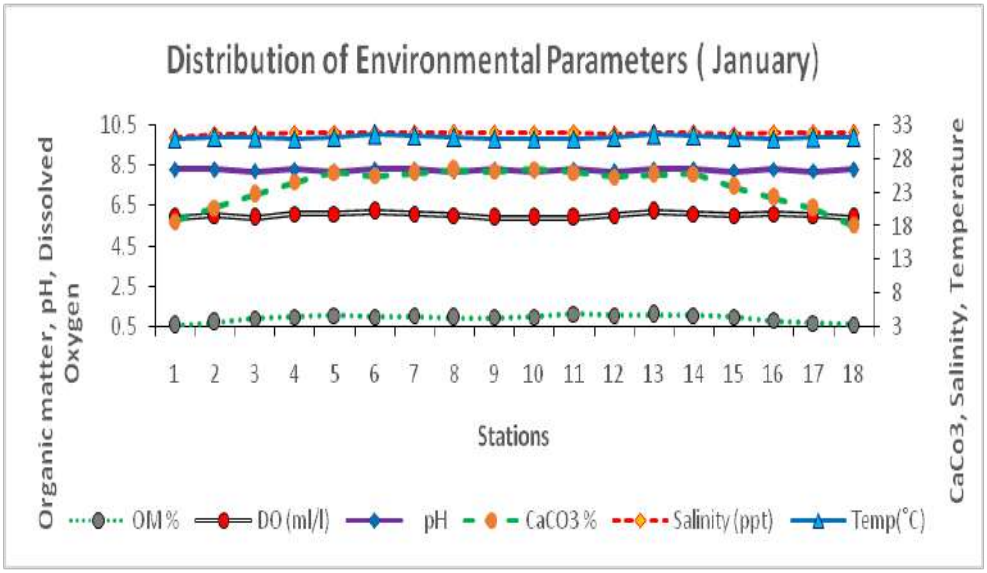


Figure 11. Distribution of Environmental parameters January - 2017

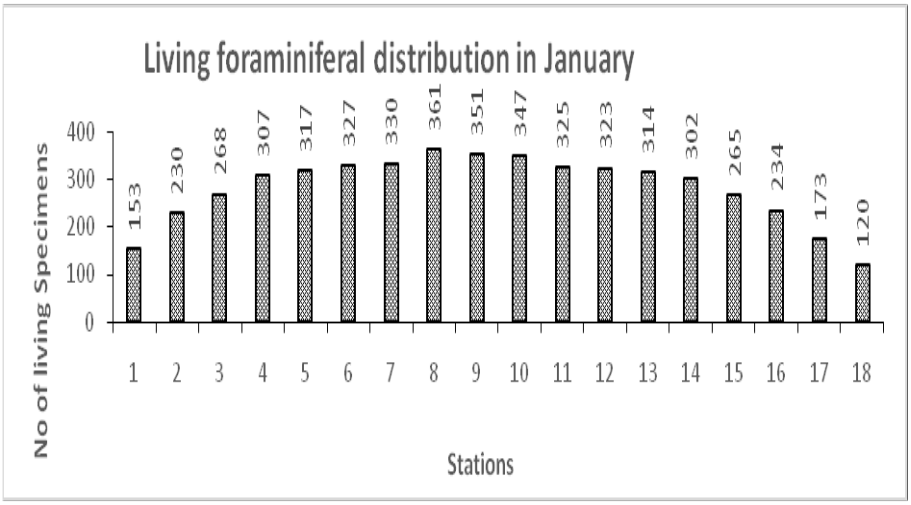


Figure 12. Living foraminifera distribution in January -2017

5.2.3. Organic Matter

High sedimentation, greater input, slow decay and humid condition may be considered to be the reason for the high values of organic matter [32]. Organic matter content is not an effective factor that controls the population abundance, but along with other parameters like CaCO3 content, depth and water parameters like salinity, dissolved oxygen content and temperature plays an important role [27]. The organic matter content was found higher and favoured higher foraminiferal population, when the other parameters were congenial [29,30,36,37]. Eric Armynot et. al [5] confirmed that foraminifera density is low and species richness is high in sediments with a low organic matter content (<2%) and foraminifera density is high and species richness at an intermediate value in sediments with very high organic matter content. Martins et. al [38] revealed that opportunistic species populate in areas where the substrate is composed of fine-grained sediments enriched in organic matter. The lower organic matter content in sandy sediments and higher organic matter content in the fine sediments are observed in inner shelf sediments [27,36].

Seasonally, the mean values organic matter content was higher during May (summer) and spatially, in stations collected in the deeper part of every collection was positively correlated with foraminiferal population [27]. Though, organic matter content has no specific relationship with foraminiferal population, with its optimum amount (0.70 - 0.93%) along with other parameters plays an important role [36].

Hess and Jorissen [39] concluded that the distribution pattern of living benthic foraminifera in the Cap Breton canyon, Bay of Biscay, depend on organic matter content and oxygen concentration in the sedimentary column.

In the present study, the percentage of organic matter content is generally low ranging from 0.535% (station 1, September) to 1.234% (station 4, May) and directly proportional to the sediment fines. Active freshwater influx, turbidity of the coast because of monsoonal weather attributed to lesser sediment fines and the resultant lower organic matter content during September. Higher calcareous dust subsequently sediment fines accounts for higher organic matter along greater depths. Spatially, the organic matter content was found to be more between stations 5 to 14 in all the seasons. Lowest values are recorded in station 1 followed by 18, where the substrate is always sandy (Table 4). Organic matter content was relatively higher in stations 5, 6, 7, 11 and 12 as the clay percentage was comparatively higher than other stations (Figure 7, Figure 8, Figure 9, Figure 10, Figure 11 and Figure 12). Seasonally, organic matter 0.9 to 1.03 % in the sediments observed during May (summer) are suggested for an abundance of living foraminiferal population in the present study.

Table 4. Organic matter content range of varied stations,off Kayalpattinam

Month and year	Organic matter content range (%)	Station Numbers
May-16	1 - 1.3	3, 4,5,6,7,8, 9, 10, 11, 12, 13,14,15.
	0.5 - <1	1, 2, 16 ,17 & 18
	< 0.5	Nil
Sept -16	1 - 1.3	Nil
	0.5 - <1	1,2,3,4,5,6,7,8, 9, 10, 11, 12, 13,14,15,16,17 & 18
	< 0.5	Nil
Jan -17	1-1.3	5,7,8,9,10,14
	0.5 - <1	1,2,3,4,6,11,12,13,15,16,17,18
	< 0.5	Nil

5.3. Bottom Water Characteristics

5.3.1. Salinity

Many studies revealed that salinity play a vital role in the diversity and abundance of foraminiferal population. An increase or decrease in salinity values was found to influence abundance of certain species and complete absence of others. The salinity shows positive correlation with foraminiferal population [27,40,41]. Lei et.al [42] found out that foraminiferal abundance and species richness have a positive relation to salinity. Benthic species undergone partial deformation after short term exposure to low salinity, can regain the form, still holding impressions of the hyposaline conditions [43]. Kasilingam et.al [28] concluded that the general trend in modern shallow water foraminiferal assemblage is increasing spatial diversity with increasing salinity

gradients and environmental stability. Scott et.al [44] revealed that the low diversity and high abundance of foraminifera species in the Nemuro Bay wetland areas of Japan where salinity values were low. Hayward et.al [45] concluded that with increasing salinity there is an increase in calcareous forams and decrease in agglutinated forams for the brackish foraminifera of New Zealand. Yanko et. al [46] confirmed that low salinity or high heavy metal contamination results in the predominance of deformed living foraminiferal species. Moghaddasi et.al [47] after their study of benthic foraminiferal abundance and distribution in the Northern Oman Sea (Iranian side) continental shelf sediments explained the dominance of suborder ROTALIINA in the northern region and LAGENINA in the stations with higher depth. Important environmental factors controlling distribution of species were found to be water depth, salinity and substrate. Jean-Perre Debanay [48] observed that, in the hypersaline Araruama Lagoon of Brazil, morphological abnormalities were attributed to variations in salinity conditions rather than anthropogenic impact and was responsible for the predominance of Ammonia tepida in the more affected northern part of the area.

In the study area, salinity variation is very meagre between stations of a season and hence have a limited influence on foraminiferal population. Temporally, salinity values show considerable variation which is ascertained by the average salinity values of different seasons; higher values during May (32.95ppt) followed by January (31.68ppt) while the minimum is observed during September (30.3 ppt) (Figure 13). Higher salinity values (32.6 to 33.3ppt) observed during May is mainly due to the scanty flow of fresh water from the river and higher evaporation rate and are positively correlated with the foraminiferal population, while the minimum during September is mainly caused by the bay depression and higher input of fresh water through rivers (Figure 7, Figure 8, Figure 9, Figure 10, Figure 11 and Figure 12).

5.3.2. Temperature

Temperature is an important parameter that governs the growth and reproduction of foraminifera in coastal areas [43,49]. Kurtarkar et.al [50] discovered that out of all specimens fed with the same amount of food, both the growth was more rapid and the reproduction was more frequent in specimens kept in comparatively warmer water. Temporally, the temperature variation exhibited an effect on both living and total population and was seen to be positively correlated with population size [40]. Kurtarkar et.al [51] suggested that the foraminiferal response to temperature variation is species-specific; the shell size of several benthic foraminifera increased with decreasing temperature but a few other benthic foraminifera shows a decrease in shell size with decreasing temperature.

Subba Rao et.al [52] after observing the distribution pattern and population density of foraminiferal species from shelf sediments of Visakhapatnam concluded that these were influenced by bottom water temperature and currents, rather than sediment attributes and water salinity.

In the study area, spatially, no appreciable variation in temperature is noticed but there is a considerable variation between seasons. Temporally, mean temperature is relatively higher (32.95°C) during summer followed by January (31.17°C). The minimum temperature is observed during September (30.84°C) (Figure 13). In general, increasing temperature is positively correlated with population of foraminifera (Figure 7, Figure 8, Figure 9, Figure 10, Figure 11 and Figure 12).

5.3.3. Dissolved Oxygen

The dissolved oxygen concentration is higher compared to other stations due to the dense and extensive patches of benthic algae in the shallow environment [34]. The abundance of population of foraminifera is positively correlated with an increase of dissolved oxygen content [30,40]. Dissolved oxygen has repeatedly been shown to be a limiting factor for benthic foraminifera [53] and to have an influence on morphology in terms of shell and pore size [54]. Some low oxygen benthic foraminifera have been observed to migrate to habitats with low oxygen conditions [55,56,57,58,59]. Rasheed and Ragothaman [14] stated that dissolved oxygen is one of the factors contributing to the abundance of living population in the Bay of Bengal, off Porto Novo. In benthic foraminifera, species with smaller shells, larger pores and higher pore densities have been linked with low oxygen [31,60,61]. Ramanathan [62] stated that dissolved oxygen was one of the factors favouring the growth of foraminifera.

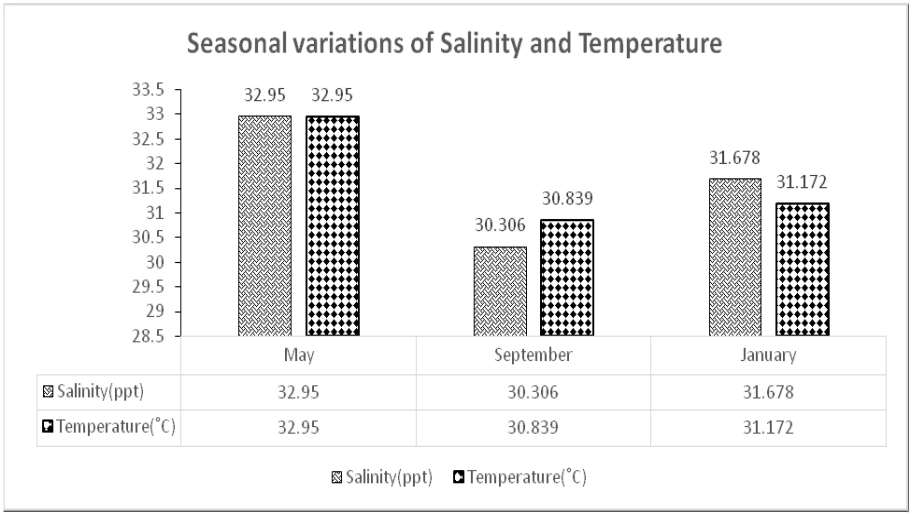


Figure 13. Seasonal variations of Salinity and Temperature

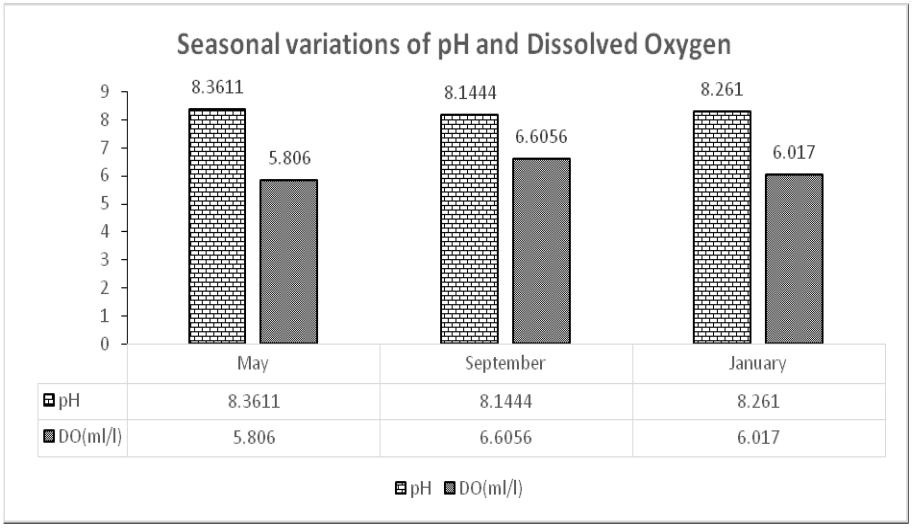


Figure 14. Seasonal variations of pH and Dissolved Oxygen

In the current study, distribution of dissolved oxygen shows a clear seasonal variation and no appreciable spatial variation. Temporally, maximum mean volume (6.6 ml/l) is observed in September and followed by January. The seasonal average of dissolved oxygen content is May 5.81 ml/l, Sep 6.61 ml/l, and January 6.02 ml/l (Figure 14). Higher dissolved oxygen content observed during September may be due to lower temperature and salinity values. Warm water holds less dissolved oxygen than cold water because the molecules are moving faster than in cold water thereby allow oxygen to escape from the water (Figure 7, Figure 8, Figure 9, Figure 10, Figure 11 and Figure 12). Dissolved oxygen content (5.7 to 6 ml/l) existed during summer months are considered to be favourable along with other congenial factors for higher foraminiferal population in the present study.

5.3.4. pH (Hydrogen Ion Concentration)

The reduced pH is commonly connected with low abundance and diversity of foraminifera [63]. Low fluctuation in pH values maintained the alkaline medium within the limit of faunal indulgence [13]. The values of pH did not vary much during different seasons and between stations, in the inner shelf environments [40,64]. Kurtarker et.al [43] noted that the dissolution of calcareous tests also take place in alkaline seawater than that was noted before. Influx of fresh water during rainy season can decrease salinity that can directly result in a low pH, altogether causing dissolution of foraminiferal tests [65]. If dissolution of calcareous tests is high, it can cause predominance of non-calcareous agglutinated forms than calcareous forms [66].

Saraswat R. et. al. [67] after the experimental study of salinity induced pH confirmed that a pH below 7.5 can severely disrupt the calcite secretion and reproduction ability of benthic foraminifera. An experimental study to understand influence of increased atmospheric CO2 that may happen in the near future revealed that high CO2 can end up in ocean acidification declining foraminiferal survival rate and calcification [68]. The reduced pH is generally associated with low abundance and diversity of foraminifera [69].

In the present study, the pH values of the bottom waters show only a negligible variation both spatially and temporally (Figure 14). Hence its impact on the distribution of foraminifera is not for any accurate determination (Figure 7, Figure 8, Figure 9, Figure 10, Figure 11 and Figure 12).

5.3.5. Depth

Water depth is an important factor in determining the foraminiferal distribution. The abundance and diversity of foraminifera are directly associated with changes in depth [36]. It may be ascribed that increasing in depth changes the nature of substratum to more finer sand and turbidity maintain favourable conditions [70]. The relative abundance of benthic foraminifera is positively correlated with depth [71]. Moghaddasi et.al [47] contemplate water depth as a preferable factor in controlling the foraminiferal distribution, in their detailed study on continental shelf sediments along with factors like salinity and substrate type. They proposed that suborder Lagenina shows an increase in abundance towards stations with greater depth in the continental shelf sediments of Northern Oman Sea.

Living specimens have linked with depth distribution in certain foraminifera (Uvigerina peregrina) to macro fauna -influenced microhabitat such as zones containing biotic structures [72,73]. Martins et.al [38] emphasized that in the continental shelf of Campos basin, living foraminiferal composition varies with respect to bathymetry along with sediment characteristics andorganic matter supply. Denne et.al [74], from an investigation in the North-western Gulf ofMexico, demonstrated that the depth distribution of benthic foraminifera is strongly correlated with the limits of water masses. Kumar et.al [27,64] observed that the foraminiferal population shows an increase with respect to depth.

In the study between living and dead foraminiferal assemblages done on Aveiro Continental Shelf of Portugal, it was confirmed that with an increase in depth, dead species shows an increase in diversity with limited changes in heterogeneity while living ones exhibits a decrease in diversity with an increase in heterogeneity [75]. The deep-sea foraminifera are adapted to limited or no supply of food materials for long periods [76]. Stations near to the shore, (< 1m depth) in the Pak bay, contain negligible living populationKumar et.al [77].

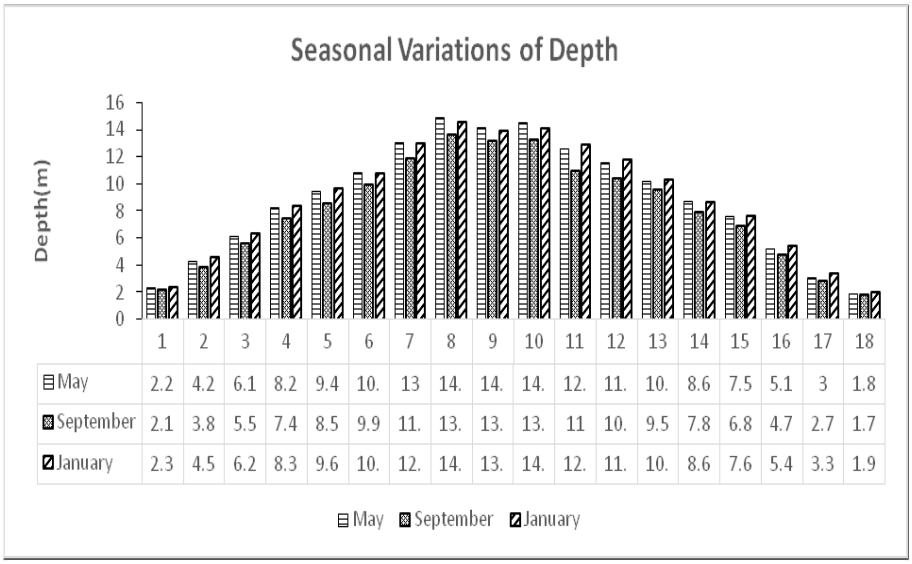


Figure 15. Seasonal variations of Depth(m)

In study area, the depth ranges between 1.76 m and 14.89 m (Figure 15). Sediment fines like silt and clay content and organic matter percentage of the substrate is directly proportional to the depth. Pearl and chunk beds are associated with depths above 10 m and account for higher CaCO3. It may be concluded that the depth is generally considered to be important if the other variables are directly associated with changes in depth.

5.4. Summary and Conclusion

Impact of inner shelf environments on the distribution of Benthic foraminifera from the Gulf of Mannar, off Kayalpattinamreveals the following:

Sediment and bottom water samples were collected from 18 stations ranging in depth between 1.76 and 14.89 meters for the months of May 2016, September 2016 and January 2017 representing Summer, Pre-monsoon and winter/post monsoon respectively. In the present study, 81 foraminifera species belonging to 42 genera have been identified and among them are 10 are arenaceous agglutinated, 24 calcareous imperforate and the rest 47 are calcareous perforate.

The fauna is found in living condition in all the 54 samples collected and studied. The living and the total (living+dead) population size are found to be higher in summer (May).Spatially, the population size is more in stations collected along deeper part of the traverse (stations 8-10) during the all seasons.

Sediment parameters like organic matter content, calcium carbonate content and sediment fines show a considerable variation both temporally and spatially. Depth is considered to be important with respect to the distribution of sediment parameters.Bottom water parameters like temperature, salinity, dissolved oxygen content and pH showed negligible variations among stations of a season but showed a distinct variation between seasons. Calcium carbonate content is considered to be the main influencing factor for abundance of living population both spatially and seasonally. Higher calcium carbonate content (>26%) and organic matter content (0.9 - 1.03%) along with other congenial parameters favours higher foraminiferal population in the present study area. Higher temperature (32.7 -33.3°C), higher salinity (32.6 to 33.3 ppt) and dissolved oxygen (5.7 to 6 ml/l) prevailed during summer months are considered to be favourable for higher foraminiferal population in the present study. Depth is also considered as one of the effective factors controlling the population of foraminifera along with the other congenial parameters.

Acknowledgements

The authors are very much thankful to Mr. R. Raghunathan, Secretary, National College (Autonomous), Dr. R. Sundararaman, Principal, National College (Autonomous), Tiruchirappalli for providing the lab and other infra structural facilities. The first author is grateful to Mr. R. Balaji, Secretary & Correspondent, SBIOA Educational Trust, Mr. R. Ganapathy Subramaniyan, Correspondent, SBIOA Schools, Tiruchirapalli and Mrs. Shakudala Sundaram, Principal, SBIOA Senior Secondary School, Trichirapalli-07 for giving permission to pursue the research.

References

[1] J. W. Murray, “Ecology and Palaeoecology of Benthic Foraminifera”, Logman Scientific & Technical, London, (1991), 1-397.

[2] S. J. Culver, & M. A. Buzas, “Distribution of Recent foraminifera off the North American Pacific coast from California to Baja”, Smithsonian Contributions to the Marine Sciences, (1986), (28), 1-634.

[3] E. Boltovskoy, D. B. Scott, & F. S. Medioli, “Morphological variations of benthic foraminiferal tests in response to changes in ecological parameters: a review”. Journal of Paleontology, 65(02) (1991). 175-185.

[4] S. De Rijk, “Salinity control on the distribution of salt marsh foraminifera (Great Marshes, Massachusetts)”, The Journal of Foraminiferal Research, 25(2), (1995), 156-166.

[5] Eric Armynot du Chatelet, Viviane Bour-Roumazeilles, Armelle Riboulleau and Alain Trentesaux, “Sediment (grain size and clay mineralogy) and organic matter quality control on living benthic foraminifera”, Revue de micropaleontologie, V. 52, (2009), pp.75-84.

[6] B. K. Sen Gupta, "Foraminifera in marginal marine environments", Modern Foraminifera, (1999), pp.141-159.

[7] B. W. Hayward, H. R. Grenfell, C. M. Reid and K.A. Hayward, "Recent New Zealand shallow-water benthic foraminifera: Taxonomy, ecologic distribution, biogeography, and use in paleoenvironmental assessment. New Zealand Geological Survey", GNS Science Monograph (1999), 21: Lower Hutt, NZ.

[8] J. P. Debenay, & J. J. Guillou, "Ecological transitions indicated by foraminiferal assemblages in paralic environments", Estuaries, 25(6), (2002), pp. 1107-1120.

[9] B. P. Horton,P. Larcombe,S. A. Woodroffe, J. E. Whittaker,M. R. Wright & C. Wynn,"Contemporary foraminiferal distributions of a mangrove environment, Great Barrier Reef coastline, Australia: implications for sea-level reconstructions" Marine Geology, 198(3-4),(2003), pp. 225-243.

[10] F. Frontalini, C. Buosi,S. D. Pelo, R. Coccioni,A. Cherchi and C. Bucci, "Benthic foraminifera as bio-indicators of trace element pollution in the heavily contaminated Santa Gilla lagoon (Cagliari, Italy)", Marine Pollution Bulletin, vol.58,(2009), pp.858-877.

[11] V. Yanko, Joel Kronfeld, and Akiva Flexer "Response of benthic foraminifera to various pollution sources: Implications for pollution monitoring", Journ. Foram. Res., vol.24, no.1, (1994), pp.1-17.

[12] A. M. Samir and A. B. El-Din, "Benthic foraminifera assemblages and morphological abnormalities as pollution proxies in two Egyptian bays", Marine Micropaleontology, vol. 41, (2001), pp. 193-227.

[13] E. Alve," Benthic foraminiferal distribution and recolonization of formerly anoxic environments in Drammensfjord, southern Norway", Marine Micropaleontology, 25(2-3), (1995), pp. 169-186.

[14] D. A. Rasheed and V. Ragothaman, "Ecology and distribution of Recent foraminifera from the Bay of Bengal off Porto Novo, Tamil Nadu state, India", Proc VII Indian Coll Micropal Strat, (1978), pp. 263-298.

[15] V. Ragothaman, V. Kumar, "Recent foraminifera off the coast of Rameshwaram, Palk Bay, Tamil Nadu", Bull Geol Min Met Soc India 52: (1985), pp. 97-121.

[16] V. Kumar V. Manivannan V and V. Ragothaman, "Spatial and temporal variations in foraminiferal abundance and their relation to substrate characteristics in the Palk Bay, off Rameshwaram, Tamil Nadu", Proc XV Indian Coll Micropal Strati, (1996), pp. 393-402.

[17] M. Suresh Gandhi, K. Kasilingam, T. Arumugam, Lalthansangi, N. Rajeswara Rao, "Distribution of Benthic foraminifera, sediment characteristics, and environmental conditions in and around Manalmelkudi Spit, Palk Strait, Tamil Nadu, East coast of India", (2017).

[18] W. R. Walton, "Techniques for recognition of living foraminifera. Contr. Cushman Found", Journ. Foram. Res., vol.3, (1952), pp.5660.

[19] W. C. Krumbein and F. J. Pettijohn, "Manual of sedimentary petrography", D.Appleton Century Co., Inc. New York, (1938), pp. 549.

[20] J. M. Trefethen, (1950). Classification of sediments. Amer. Jour. Sci., vol.248, pp.55-62.

[21] Piper, C.S. (1947). Soil and plant analysis. University of Adelaide Press, Adelaide, 368p.

[22] Jackson, M.L, "Soil chemical analysis", Prentice Hall of India Pvt. Ltd., New Delhi, (1967), pp. 498.

[23] A. R. Loeblich and H. Tappan, "Foraminiferal genera and their classification", Van Nostrand Reinhold Company, New York, vols.1&2, (1988), pp. 212.

[24] V. Kumar, V. Manivannan and R. Priya, "Epiphtyic foraminifera and its relation to algae in the Palk Bay off Rameshwaram", Proc XVI Indian Coll Micropal Strati. NIO, Goa, (1998), Abstract 74.

[25] V. Kumar, T. Gangaimani, K. Sivakumar, S. Jeevanandam and S. Mohan, "Foraminifera from the Gulf of Mannar, Off Tuticorin, Southeast of India: Implications to delineate substrate Characteristics", Gondwana Geological Magazine, Vol.25(1), pp. 23-32.

[26] P. Jeshma, M. Suresh Gandhi, N. Rajeshwara Rao, "Benthic foraminifera and geochemical assessment of Puravadaiyanar and Vettar Estuaries, Karaikal, South east cost of India-Implication for pollution monitoring studies", Regional studies in Marine Science 9, (2016), pp.76-88.

[27] V. Kumar and Twinkle Jacob, "Distribution of Benthic Foraminifera with Reference to Sediment Characters from off the Coast of Manapad, South East Coast of India. Applied Ecology and Environmental Science, 2019, Vol. 7, No.3, 110-116.

[28] K. Kasilingam, M. S. Gandhi and N. R. Rao, "Textural characteristics and ecology of near shore benthic foraminifera from Kottaipattinam to Kodiyakarai, Palk Strait, Southeast Coast of India", Regional Studies in Marine Science, (2019), pp.29.

[29] P. Elakkiya, V. Manivannan "Recent benthic foraminifera from off the coast of Arkattuthurai (near Nagapattinam), south east coast of India", Indian Journal of Geo Marine Sciences, Vol. 42(7): (2013), pp.877-887

[30] F. Kurbjeweit, P. Schafer, "Distribution, biomass and diversity of benthic foraminifera in relation to sediment geochemistry in the Arabian Sea", Deep Sea Research Part II: Topical Studies in Oceanography. Vol.47, (2000), pp. 2913-2955.

[31] V. Manivannan, V. Kumar, V. Ragothaman and S. K.Md. Hussain, "Calcium carbonate a major factor in controlling foraminiferal populations in Gulf of Mannar, off Tuticorin, Tamil Nadu", Proceeding of Contribution XV India. Colloquium Micropalaeontology and Stratigraphy., Dehra Dun. (1996), pp. 381-385.

[32] V. Kumar and K. R. Rethikala, "Calcium carbonate and its control over the distribution of Benthic foraminifera, from the Inner shelf sediments of Southern Gulf of Mannar, Southeast coast of India". The International journal of analytical and experimental modal analysis, (2019).

[33] V. Kumar and K. Siva Kumar, "Influence of estuarine environment on the benthic foraminifera. A case study from the Uppanar river estuary of Tamil Nadu", Journal of Environment and Pollution 3, (2001), pp. 277-283.

[34] A. L. Post, L. Sbaffi, V. Passlow and D. C. Collins, "Benthic foraminifera as Environmental indicators in Torres Strait-Gulf of Papua, in Todd, B.J., and Greene, H.G., eds., mapping the Seafloor for Habitat Characterization: Geological Association of Canada, Special Paper 47, (2007), pp. 329-347.

[35] S. M. Hussain, Merin Maria Joy, A. Rajkumar, N. Mohammed Nishath and T. F. Shabhangi, "Distribution of calcareous microfauna (foraminifera and Ostracoda) from the beach sands of Kovalam, Thiruvananthapuram, Kerala, Southwest coast of India", Journal of the Palaeontological Society of India, 61:(2016), pp.267-272.

[36] K. R. Rethikala, V. Kumar and B. Sathish, "Inner Shelf Characteristics and Their Impact on the Distribution of Recent Foraminifera from the Southern Part of Gulf of Mannar, Southeast Coast of India". Applied Ecology and Environmental Science, (2021), Vol. No.1, pp. 30-41.

[37] M. Mojtahid, F. Jorissen, B. Lansard, C. Fontanier, B. Bombled and C. Rabouille, "Spatial distribution of live benthic foraminifera in the Rhône prodelta: Faunal response to a continental-marine organic matter gradient", Marine Micropaleontology, Vol.70, (2009), pp. 177-200.

[38] M. V. A. Martins, C. Yamashita, S. H. M. Sousa, E. A. M. Koutsoukos, S. T. Disaró, J. P. Debenay and W. Duleba, "Response of Benthic Foraminifera to Environmental Variability: Importance of Benthic Foraminifera in Monitoring Studies, Monitoring of Marine Pollution, Houma Bachari Fouzia", IntechOpen, (2019).

[39] S. Hess and F. J. Jorissen, "Distribution pattern of living benthic foraminifera from Cape Breton canyon, Bay of Biscay: Faunal response to sediment instability", Deep Sea Research, Vol.56, (2009), pp.1555-1578.

[40] Twinkle Jacob and V. Kumar,"Bottom Water Characteristics and Their Influence on Temporal Distribution of Benthic Foraminifera from off Manapad, Gulf of Mannar, South East Coast of India." Applied Ecology and Environmental Sciences, vol. 7, no. 6 (2019), pp. 245-254.

[41] V. N. Linshy, S. S. Rana, S. Kurtarkar,R. Sarawat and R. Nigam, "Appraisal of laboratory culture experiments on benthic foraminifera to assess/develop paleoceanographic proxies", (2007).

[42] Lei Y, Li T, Jian Z, R. Nigam, "Taxonomy and distribution of benthic foraminifera in an intertidal zone of the Yellow Sea, PR China: Correlations with sediment temperature and salinity", Marine Micropaleontology, 133, (2017), pp. 1-20.

[43] S. R. Kurtarkar, R. Nigam, R. Saraswat and V. N. Linshy, "Regeneration and abnormality in benthic foraminifera Rosalina leei: implications in reconstructing past salinity changes", Rivista Italiana di Paleontologia e Stratigrafia, v. 117, (2011), pp. 189-196.

[44] D. B. Scott, J. R. Suter and E. C. Kosters, "Marsh foraminifera andarcellaceans of the lower Mississippi Delta: Controls on spatial distributions", Micropal., Vol. 37, (1991), pp. 373-392.

[45] B. W. Hayward and C. J. Hollis, "brackish Foraminifera in New Zealand: A taxonomic and Ecologic review", Micropalaeontology, Vol.40(3), (1994), pp.185-222.

[46] V. Yanko, M. Ahmed and M. Kaminski, "Morphological deformities of benthic foraminiferal tests in response to pollution by heavy metals: Implications for pollution monitoring", Jour. For. Res., Vol. 28, (1998), pp.177-200.

[47] B. Moghaddasi, S. M. B. Nabavi, G. Vosoughi, S. M. R. Fatemi and S. Jamili, "Abundance and distribution of benthic foraminifera in the Northern Oman sea (Iranian side) continental shelf sediments", Research journal of Environmental sciences. Vol 3(2), (2009), pp.210-217.

[48] Jean-Perre Debanay, Emmanuella Geslin, Beatriz Beck Eichlier, Wania Duleba, Florence Sylvestre and Patricia Eichler, "Foraminiferal assemblages in a Hyper saline Lagoon, Araruma (R.J), Brazil, Jour. Foram. Res., Vol.31, No.2, (2001), pp.133-151.

[49] R. Saraswath, R. Nigam and S. Pachkhande, ''Difference in optimum temperature for growth and reproduction in benthic foraminifera Rosalina globularis:Implications for paleonclimatic studies", Journal of Experimental Marine Biology and Ecology, 405. (2011), pp.105-110.

[50] S. R. Kurtarkar, V. N. A. Linshy,R. Saraswat and R. Nigam, "Experimental studies on benthic foraminifera: and update on the changing trends and challenges over the past decade", Micropaleontology and its applications. Scientific Publishers (India).(2017), pp. 311-330.

[51] Kurtarkar, S.R., Saraswat, R., Kaithwar, A, and Nigam, R, ''How will Benthic Foraminifera Respond to Warming and Changes in productive? A Laboratory Culture Study on Cymbaloporetta plana," Journal: Acta Geologica Sinca, volume 93, (1). 175-182.

[52] M. Subba Rao, D. Vedanatam and S. Nageswara Rao, "Distribution and ecology of benthonic foraminifera in the sediments of the Visakhapatnam Shelf, East Coast of India", Palaeogeography, Palaeoclimatology, Palaeoecology, 27.(1979), pp. 349-369.

[53] B. K. S. Gupta and M. L. Machain,"Benthic foraminifera in oxygen-poor habitats", Marine Micropaleontology, 20(3-4), (1993), pp. 183-201.

[54] J. J. Helley and L. A. Levin, "Global distribution of Naturally occurring marine hypoxia on continental margins", Deep- Sea Research, 515(9), (2004), pp. 1159-1168.

[55] L. Moodley, G. J. Van der Zwaan, G. M. W. Rutten, R. C. E. Boom and A. J. Kempers, "Subsurface activity of benthic foraminifera in relation to porewater oxygen content: laboratory experiments", Marine Micropaleontology, 34(1-2).(1998), pp. 91-106.

[56] Gross, Onno, "Influence of temperature, oxygen and food availability on the migrational activity of bathyal benthic foraminifera: evidence by microcosm experiments". Life at interfaces and under extreme conditions. Springer, Dordrecht. (2000), pp. 123-137.

[57] E. Gesline, J. P. Debenay, W. Duleba, C. Bonetti, "Morphological abnormalities of foraminiferal tests in Brazilian environments: Comparison between polluted and non-polluted areas", Mar. Micropaleontol, 45, (2002), pp. 151-168.

[58] C. Fontanier, A. Mackensen, F. J. Jorissen, I. Anschutz,p, C. Griveadud, "Stable oxygen and carbon isotopes of live benthic foraminifera from the Bay of Biscay ; micrhabitat impact and seasonal variability", Mar. Micropalentol.58(3), (2006), pp. 159-183.

[59] L. L. Perez-Cruz, Machain, M. L. Castillo, "Benthic foraminifera of the oxygen minimum zone, continental shelf of the Gulf of Tehuantepec, Mexico", Foraminifera. Res. 20(4), (1990), pp. 312-325.

[60] T. Kuhnt, O. Friedrich, G. Schmiedl, Y. Milker, A. Mackensen, A. Luckge, "Relationship between pore density in benthic foraminifera and bottom water oxygen content", Deep-sea Research, 176, (2013), pp. 85-95.

[61] T. Kuhut, R. Schiebel, G. Schmiedl, Y. Milker, A. Mackensen, O. Friedrich, "Automated and manual analyses of the port density to oxygen relationship in Globobulimina turgida", Foraminifer. Res. 44(1), (2014), PP. 5-16.

[62] R. M. Ramanathan, "Quantitative differences in the living benthonic foraminifera of Vellar estuary, Tamil Nadu", Jour. Geol. Soc. India, vol.11, no.2, (1970), pp.127 141.

[63] E. Boltovskoy, R. Wright, "Recent Foraminifera", The Hague, Dr. W. Junk B.V. Publishers, (1976), pp. 515.

[64] V. Kumar and V. Manivannan, "Benthic foraminifera responses to bottom water characteristics in the Palk Bay, off Rameswaram, Southeast Coast of India", Ind. Journ. Mar. Sci., vol.30, (2001 b), pp.173-179.

[65] Y. G. Lee, S. Kim, Y. W. Kim, D. U. Jeong, J. S. Sick Lee, H. J. Woo and H. C. Shin, "Benthic foraminifera as bioindicators of salinity variation in Lake Shihwa, South Korea", Journal of Foraminiferal Research, v. 45, no. 3, p. 235-249, July 2015.

[66] J. W. Murray, E. Alve, "Natural dissolution of modern shallow water benthic foraminifera: taphonomic effects on the palaeoecological record", Palaeogeography, Palaeoclimatology, Palaeoecology, 146, (1999), pp. 195-209.

[67] R. Saraswat, M. Kouthanker, S. Kirtankar, R. Nigam and V. N. Linshy, "Effect of salinity induced pH/ alkalinity changes on benthic foraminifera: a laboratory culture experiment", Estuarine, Coastal and Shelf Science, v. 153, (2015), pp. 96-107.

[68] F. Guama´n-Guevara, H. Austin, N. Hicks,R. Streeter, W. E. Austin, "Impacts of ocean acidification on intertidal benthic foraminiferal growth and calcification", PLoS ONE, 14(8), (2019).

[69] K. Kasilingam, M. Suresh Gandhi, Rajeshwara Rao, "Near- shore foraminifera along the Palk strait, Southeast Coast of India, Tamilnadu", Journal of the Palaeontological Society of India volume 65 (1), (2020), pp. 90-106.

[70] M. Suresh Gandhi, A. Sonia Nathan, "Benthic foraminifera and geochemical studies with influence on pollution studies along the coast of Cuddalore, Tamil Nadu-ITS", India Arab J. Geosci. 7, (2014), pp. 917-925.

[71] S. M. Saalim, R. Saraswat, R. Thejasino Suokhrie and R. Nigam, "Assessing the ecological preferences of agglutinated benthic foraminiferal morphogroups from the western Bay of Bengal", Deep-Sea Research Part II, (2018).

[72] P. Loubere, P. Meyers and A. Gary, "Benthic foraminiferal microhabitat selection, carbon isotope values, and association with larger animals; a test with Uvigerina peregrina", The Journal of Foraminiferal Research, 25(1), (1995), pp. 83-95.

[73] C. V. Davis, S. E. Myhre and T. M. Hill,"Benthic foraminiferal shell weight: Deglacial species-specific responses from the Santa Barbara Basin", Marine Micropaleontology, 124, (2016), pp.45-53.

[74] R. A. Denne and B. K. Sen Gupta, "Matching of benthic foraminiferal depth limits and water-mass boundaries in the northwestern Gulf of Mexico; an investigation of species occurrences". The Journal of Foraminiferal Research, 23(2), (1993), pp.108-117.

[75] M. V. A. Martins, J. Honegger, F. Frontalini, J. M. A. Dias, M. C. Geraldes, F. Rocha, "Dissimilarity between living and dead benthic foraminiferal assemblages in the Aveiro Continental Shelf (Portugal)", PLoS ONE, 14(1), (2019).

[76] P. Linke, "Metabolic adaptations of deep-sea benthic foraminifera to seasonally varying food input", Marine Ecology Progress Series, 81. (1992), pp. 51-63.

[77] V. Kumar, K. Sivakumar, T. Gangaimani and K. J. Anand, "Morphological abnormalities of Benthic foraminifera from the Palk Bay, Off Rameswaram, Tamil Nadu: A tool for environmental monitoring", Pollution Research, 25(1), (2006), pp.35-42.

© The Author(s) 2021. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).

Journal of Applied Geochemistry
Vol. 23, No. 4 (2021), pp. 225-236

PETROLOGY AND MINERAL CHEMISTRY OF THE ULTRAMAFIC ROCKS IN P.METTUPALAIYAM AREA, TAMIL NADU, INDIA WITH SPECIAL REFERENCE TO THE OCCURRENCE OF Cu, Ni, Cr AND PGE

*S.B.Vijay Kumar and S. Selvaraj
Department of Geology, National College (Autonomous), Tiruchirappalli, TamilNadu, India
E-mail: vijay4mcdm@gmail.com

Abstract

The ultramafic rocks of P.Mettupalaiyam area, forming a part of Mettupalaiyam Ultramafic Belt, Tamil Nadu, India was studied for petrographic characterization, mineral chemistry and geochemistry of Cu, Ni, Cr and Platinum group of Elements (PGE). A total of 37rock chip samples were collected and analysed for the trace elements Cu, Ni and Cr and PGE using HR – ICP – MS, and mineral chemistry was estimated with an automated wave-length dispersive CAMECA SX 100 electron microprobe. The petrological studies reveal the presence of hypersthene and diopside as the major constituents of pyroxenite and magensio-hornblende in amphibolite. The ΣPGE concentration varies between 19 and 1798 ppb with an average of 212 ppb in the 37 samples. A total of 5 samples collected from the pyroxenite have yielded more than 500ppb while 11 samples including 2 samples from amphibolite which have yielded ΣPGE between 100 and 500 ppb, which indicates a notable PGE mineralisation. The trace element analysis reveals Cu: 3-284ppm; Ni: 146-553ppm and Cr: 610-4625ppm. The presence of chromite accounts for the higher concentration of Cr, while nickeliferous pyrite and pentlandite account for Ni.Pd/Ir ratio which indicates that the ultramafic rocks are enriched in Platinum group PGE (PPGE) over the Iridium group PGE (IPGE). PGE concentrations normalized with primitive mantle values indicate that PPGE (Pt, Pd) are enriched over IPGE (Ir, Rh, Ro) with a distinct positive Pd-anomaly, which may be inferred as a result of partial melting of the upper mantle aided by ultra-high temperature metasomatism. Ni/Cu vs Pd/Ir and Ni/Cu vs Pd/Pt depicts ultramafic rocks in komatiite, ophiolite and layered intrusion fields, suggesting an Mg rich parental magma generated by the partial melting of upper mantle and emplacement into the crustal rocks through deep seated weak planes.

Keywords: Mettupalaiyam Ultramafic Belt, pyroxenite, magnesio-hornblende, ophiolite, pentlandite.

1. Introduction

The main tool to study the earth's deep crust and mantle is through the materials derived from a deep seated geological environment (Rollinson, 2008). The ultramafic rocks originating at greater depths act as a record of thermal gradients associated with partial melting of rocks, cooling of magma, fractional crystallization of minerals, magmatic differentiation and emplacement, which are useful in reconstructing tectonic histories (Windley and Smith,1976; Windley et al., 1994). Apart from the significance of mantle characterization, the mafic and ultramafic rocks are also known for the association of Ni, Cu and Pt-group elements (PGE) deposits (Barnes and Maier, 1999).The Platinum Group of Elements (PGE) represent a family of six, greyish to silver white metals, viz. Platinum (Pt), Palladium (Pd), Rhodium (Rh), Iridium (Ir), Osmium (Os) and Ruthenium (Ru), which can be further classified as Platinum group PGE (Pt, Pd) and Iridium group IPGE (Ir, Rh, Ro) based on the similarity in geochemical affinity and distinct genetic nature. These metals are highly refractory with high melting points, high specific gravity, chemically inert nature and resistance to corrosion. PGE are siderophilic and incompatible in nature and have a strong affinity towards chalcophile elements / metals like arsenic, antimony, bismuth, selenium, sulphur and tellurium, and are generally associated with ultramafic rocks of plutonic origin.

PGE mineralisation occurs in varied rock types like chromitite, pyroxenite, gabbro and troctolite, and at various stratigraphic levels in mafic-ultramafic layered complexes. According to Godel et al., (2008), most of the economic PGE reefs are found in the lower part of any large ultramafic-mafic layered intrusions. In India, four major PGE prospects has been identified namely, i) Baula – Nausahi Ultramafic Complex, Odisha ii) Hanumalpura Ultramafic-mafic Complex, Karnataka iii) Sittampundi Ultramafic-Anorthosite Complex, Tamil Nadu iv) Mettupalaiyam Ultramafic-mafic Complex, Tamil Nadu. Sittampundi and Mettupalaiyam complexes represent the layered mafic-ultramafic sequence of rocks emplaced

within the hornblende-biotite gneiss of Archaean age.The present study area (P.Mettupalaiyam) falls within the Mettupalaiyam Ultramafic Belt. In the present study an attempt has been made to study the petrological characters and mineral chemistry of the ultramafic rocks with special reference to the geochemistry of trace elements and PGE.This is the first baseline attempt to assess the PGE potentiality of the ultramafic rocks in P. Mettupalaiyam area.

2. Regional geological setup and tectonic frame work

The Southern Granulite Terrane (SGT), occurring in the southern part of India comprising the states of Tamil Nadu, Karnataka, and Kerala, hosts varied lithology of high grade rocks like charnockites, khondalites and other granulite facies metamorphic rocks with younger granitic intrusions. It is one of few terranes in the world that has preserved Archaean and Proterozoic crust represented by extensive high-grade granulite facies of rocks (Gopalakrishnan, 1993). Two major deep seated lineaments, the Moyar–Bhavani – Attur Lineament (MOBL) and the Palghat–Cauvery Lineament (PCL), divide the entire region into three main blocks: the northern, the central and the southern blocks characterized by distinct set of lithological assemblage, metamorphism and structure. The region between the MOBL and PCL referred to as the central block (Fig. 1) is popularly called the Cauvery Shear Zone (CSZ), which comprises of high grade granulite facies metamorphic rocks with enclaves of two major mafic-ultramafic layered igneous complexes called the Sittampundi Anorthosite Complex (SAC) and Mettupalaiyam Ultramafic Complex (MUC) (Gopalakrishnan, 1993).

3. Mettupalaiyam Ultramafic Belt (MUB)

The lithological assemblages in Cauvery Shear Zone are marked by their complexity. Highly contorted and fissile hornblende-biotite gneiss and its variants - the Bhavani Gneissic Complex form the country rock in CSZ and show a series of near parallel to subparallel deep seated shear zones marked by mylonite bands. A suite of mafic-ultramafic rocks comprising of peridotite - pyroxenite - amphibolite, gabbroic-anorthosite, anorthositic-gabbro and anorthosite occur as intrusive along these weak zones in the form of parallel to subparallel bands/ lenses. This series of mafic to ultramafic rocks, designated as Mettupalaiyam Ultramafic Complex (MUC), occur as a linear belt trending NE/SW for 150km with 5-7km width (Fig. 1) and collectively termed as the Mettupalaiyam Ultramafic Belt (MUB), which acts as a potential zone for PGE mineralisation (Rajan et al, 1993).

Selvan and Basu (1976) reported two types of migmatitic gneisses occupying the CSZ–the older pyroxene gneiss carrying lenses of pyroxenite and its migmatitic variant seen as garnetiferous-quartzo-felspathic gneiss and a younger hornblende-biotite gneiss/hornblende-gneiss, intrudedby layered igneous

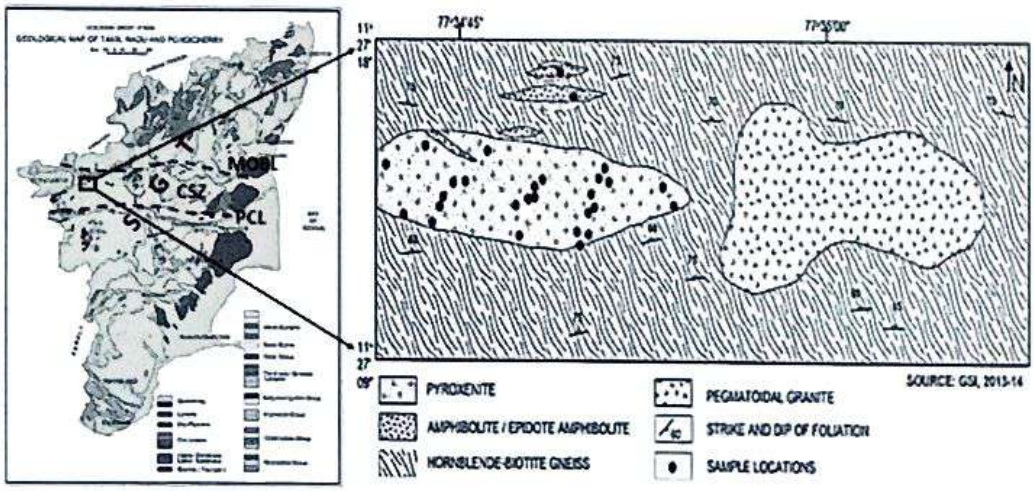


Fig. 1. Location and Geological map of P.Mettupalaiyam area, MUB.

complex comprising of peridotite, pyroxenite, gabbro, leuco-gabbro (anorthositic-gabbro, gabbroic-anorthosite), hornblende-anorthosite, and corundum bearing anorthosite. They also opined that these ultramafic rocks are the hidden ore bodies for Cu, Ni, Cr, Co, V, Ti and Au. Selvan (1978) reported that the ultramafic rocks show a gradational variation in the lithological units from lower ultramafites, garnetiferous-gabbro to upper leuco-gabbro with relict igneous features, such as rhythmic layering, and amineral assemblage of hornblende-hypersthene-plagioclase-diopside-perthite-quartz reflecting the upper amphibolite facies of metamorphism.

Systematic exploration for PGE in the ultramafic rocks occurring in the Mettupalaiyam Ultramafic Belt has been carried out by GSI for a long time. However, report on detailed rock characterization of the ultramafic rocks with reference to their genesis is sparse. The G-4 stage exploration for PGE in the ultramafic rocks occurring in regions of Toposheet (s) 58E/7&11 was carried out by Geological Survey of India (Vijay Kumar et.al., 2014), based on which the present work is aimed at studying the petrological characteristics and mineral chemistry of the ultramafic rocks occurring in the P.Mettupalaiyam area, with special reference to geochemistry of the trace elements (Cu, Ni & Cr) and PGE, and their significance in understanding their petrogenesis.

4. Methodology

In P. Mettupalaiyam area, the ultramafic rocks represented by pyroxenite occur as a lensoidal body trending E/W with a 300 m strike length and 80m width, along with 5-10 m thin amphibolite bands, as enclaves within hornblende-biotite gneiss (Fig-1). A total of 35 rock chip samples from pyroxenite and 2 samples from amphibolite were collected from three sample lines at an interval of 50m along the strike and with an average sample interval of 25 m across the strike. The rock chip samples weighing 500gm were collected using chisel and powdered to –200 mesh (metallic sieve) by using steel mortar and pestle. The pulverized samples were thoroughly homogenized and reduced to 100gm by repeated coning and quartering. The trace element analysis of the powdered samples was carried out using HR-ICP-MS, dissolved in reagent grade HF:HNO3 acid mixture in Savillex screwtop vessels, and PGE analysis was carried out by nickel-sulfide fire assay and ICP-MSat the Regional Chemical Laboratory, Geological Survey of India, Hyderabad. JB-2 standard was used for checking the precision and accuracy of analysis. The overall accuracy (%RSD) for trace elements is <20. The mineral chemistry of pyroxenes and amphiboles present in the ultramafic rocks was estimated with an automated wave-length dispersive CAMECA SX 100 electron microprobe and associated software at the Regional Petrological Laboratory, SR, Geological Survey of India, Hyderabad. Analyses were performed with a 15 kV accelerating voltage, 12 nA current, 1-micron beam size. All natural mineral standards were used except for Mn and Ti for which synthetic standards were used. The precision of analyses is within the error limit of ± 3%. The cation calculation for pyroxene and amphibolite was carried out based on 6 and 23 oxygen atoms respectively.

5. Results

5.1 Ultramafics of P. Mettupalaiyam area

The general trend of foliation as exhibited by hornblende-biotite gneiss (country rock) varies between ENE/WSW to E/W with a 60°-80° northerly dip. These ultramafic bodies vary compositionally from pyroxenite to amphibolite/ epidote-amphibolite based on the relative abundance of pyroxene and amphibole group of minerals.The pyroxenite is fine to medium grained,green in colour (Fig-2a) with short prismatic crystals of pyroxenes exhibiting two sets of cleavage intersecting at right angles and limonitised at places. The amphiboliteis black to dark green, medium to coarse grained with hornblende grains showing distinct two sets of cleavages intersecting at 56° -124°. The coarse grained nature of the rock indicates deep seated origin under slow cooling conditions. Though the ultramafics of P. Mettupalaiyam area form a part of Mettupalaiyam Ultramafic belt, they have been classified as a layered ultramafic sequence, in which the lithological variants other than pyroxenite and amphibolite are not exposed except for the medium grained white anorthosite occurring as stringers or thin bands (<1cm to 5cm) within pyroxenite and amphibolite (Fig-2b). The pyroxenite and amphibolite mark a gradational contact due to progressive compositional variation (Fig-2c). Epidotisation of amphibolite is noticed at places along the contact with hornblende-biotite gneiss (Bhavani

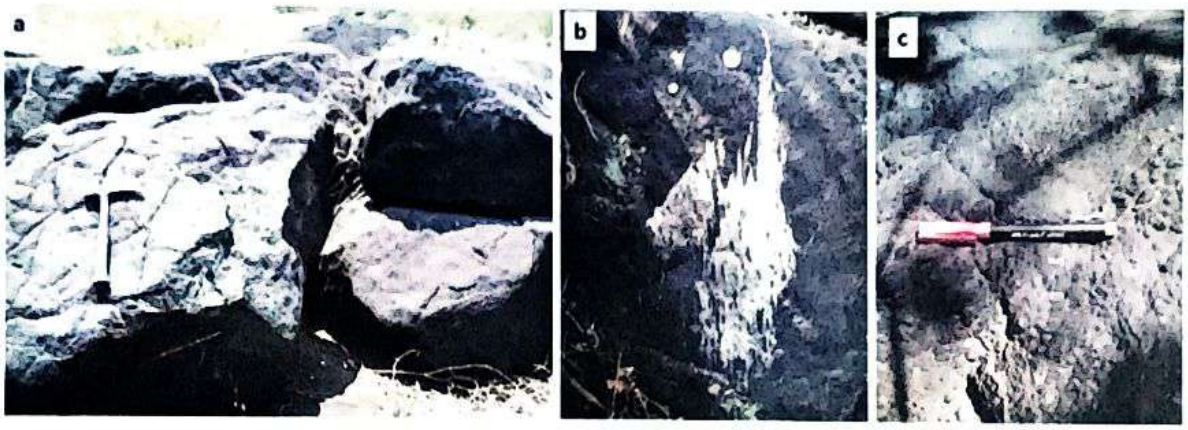


Fig. 2. Field photographs showing a) bouldery exposure of medium to fine grained pyroxenite, b) pyroxenite with stringers of anorthosite (leuco) and c) gradational contact of coarse amphibolite with fine grained pyroxenite.

gneiss) indicating retrograde metamorphism of the rocks by the influx of aqueous solutions (Vijay Kumar, et.al., 2014). Thin pegmatite and quartz veins of unmappable dimensions traverse all these rock types. Disseminated occurrence of sulfides is present at places.

5.2 Petrography

The petrographical studies show the pyroxenite and amphibolite exhibiting hypiodiomorphic texture with almost equal degree of granularity and comprising of euhedral to subhedral pyroxene and amphibole grains. The mineral grains possess interlocking texture of igneous origin with diffused and shattered grain boundaries at places, indicating the impact of deformation (Fig. 3a). The pyroxenite contains almost equal amount of CPX and OPX grains. Coarse grains of augite (CPX) showing higher order interference colour and 40^0 - 45^0extinction angle occur in almost equal proportion to hypersthene (OPX) identified by the distinct pleochroism and straight extinction. Twinning of augite grains is noticed at places(Fig. 3b). The amphibolite has a predominance of medium to coarse hornblende, identified by the typical pale to dark green dichroism, high relief and intersection of two sets of cleavage planes at nearly 54^0 and 126^0 (Fig. 3c). The primary igneous zoning inOPX and CPX is noticed occasionally (Fig.3d). Accessory minerals including biotite and opaques are present in both pyroxenite and amphibolite. The intensity of deformation is witnessed by the bending of cleavage planes, corroded grain boundaries and intersection of micro shear planes.

5.3 Mineral chemistry

The EPMA analytical data along with cation calculation and end member determination for the orthopyroxenes (OPX), clinopyroxenes (CPX), amphiboles and sulphide phases present in the pyroxenite and amphibolite of P.Mettupalaiyam is provided in Tables-1, 2 & 3. In pyroxenite, the OPX and CPX grains compositionally belong to hypersthene and diopside variety respectively. The amphibolite consists majorly of magensio-hornblende variety of amphiboles with a few disintegrated grains of relict pyroxene of diopside composition (Fig.4a).

Native copper with 76.67 wt% of Cu is identified occasionally (Fig. 4b). The oxide includes ilmenite, sphene and chromite occurring in the interstitial spaces of silicates (Fig. 4c). The sulphides occurring as minute droplets / blebs as intercumulus and along the grain boundary and/or along the cleavage planes of silicates include nickeliferous pyrite and pentlandite (Figs. 4d, f). The exsolution of ilmenite and chromite indicate the differential partition coefficient and immiscible nature of the two end members (Fig. 4e).

5.4 PGE and Trace element

The chemical analysis of the rock chip samples collected from pyroxenite and amphibolite reveals total PGE ranging between 19 and 1798 ppb with an average of 212 ppb (Table-4). The individual elemental result shows

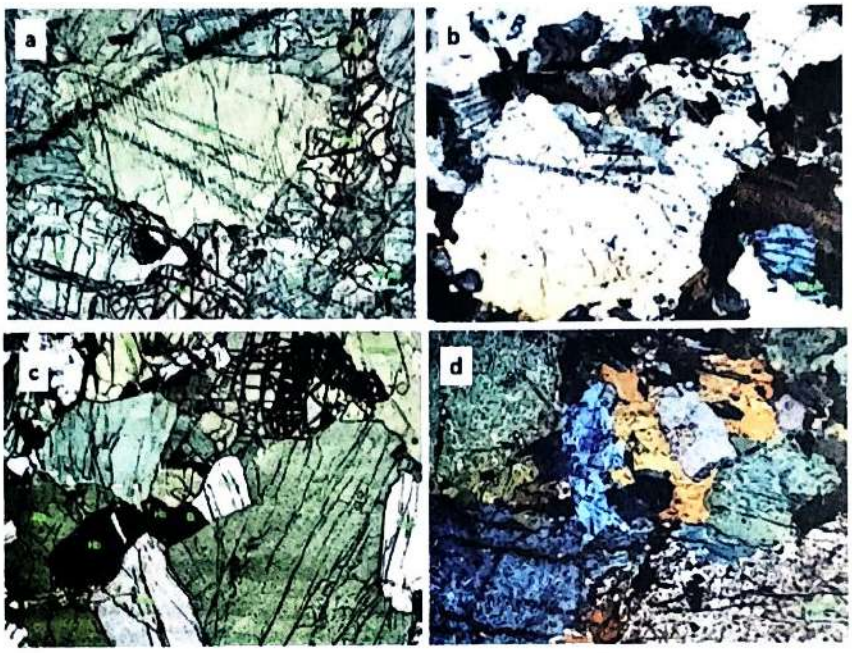


Fig. 3. Photomicrograph showing a) pyroxenite - augite grain displaying the intersection of two generation of micro shear planes; b) pyroxenite - twinned augite; c) amphibolite - coarse grained hornblende and medium grained augite and d) primary zoning of OPX and CPX in pyroxenite.

variation of Pt: 3- 486 ppb, Pd: 2 -1263 ppb; Ir: 2 - 21ppb; Ru; 2-59 ppb and Rh: 2-30 ppb. The trace element analysis reveals Cu:3-284 ppm; Ni: 146-553 ppm and Cr: 610-4625 ppm. The metal ratios of Pd-Pt ranges between 0.04 – 6.76, Pd-Ir between 1.00 - 139.11 and Ni – Cu between 1-111.

6. Discussion

The orthopyroxene in the ultramafics of P.Mettupalaiyam has a limited compositional range of hypersthene with Mg# (100Mg/ Mg+Fe^{2+}) at 65-74, as shown in the pyroxene ternary diagram (Fig. 5a, after Morimoto, 1988), while, clinopyroxene shows diopside compositions with relatively high Mg# of 78–82 (Table-1). The cation recalculation of amphibole analysis based on 23 oxygen atoms, with Fe^{2+}/Fe^{3+} estimation assuming "13 cations, indicates the amphiboles to be magnesio-hornblende (Fig. 5b, after Hawthorne et al., 2007) of ultramafic magnesium variety following the nomenclature scheme of Leake et al. (1997), with Mg# varying between 85 and 96 (Table-2). The Ti vs. Al (total) plot of clinopyroxenes (CPX) (after Leterrier et al., 1982) indicates the ultramafic rocks of P.Mettupalaiyam area to be under tholeiitic magma series (Fig. 7a).

Total PGE values in the ultramafic rocks of P.Mettupalaiyam area range between 19 and 1798 ppb with an average of 212 ppb (Table-4). Five samples collected from the pyroxenite have shown more than 500ppb, while eleven samples including two samples from amphibolite have yielded total PGE between 100 and 500 ppb, which is a notable PGE mineralization from the economic point of view. The average Cu, Ni and Cr concentrations in ultramafic rocks are 26 ppm, 240 ppm and 1328 ppm, respectively. The presence of chromite accounts for the higher concentration of chromium. Cr/ Ni ratio indicates the fifth order abundance of Cr over Ni. The Ni bearing phases as determined by micro probe analysis include nickeliferous pyrite and pentlandite, while copper occurs in native form (Fig. 5). Ni/Cu ratio in ultramafic rocks of P.Mettupalaiyam area, indicates the dominance of Ni enrichment over Cu.

Pd/Ir ratio indicates that the ultramafic rocks of P.Mettupalaiyam area are enriched in PPGE (Pt,Pd, Ru)

Table 1. EPMA analytical data of OPX and CPX in pyroxenite, P. Mettupalaiyam area, MUB.

Analysis	CPx-1	CPx-2	CPx-3	CPx-4	CPx-5	OPx-1	OPx-2	OPx-3	OPx-4	OPx-5
SiO_2	49.79	49.873	49.354	48.719	48.94	49.517	48.154	46.812	47.695	48.812
TiO_2	0.047	0.036	0.302	0.198	0.004	0.086	0.029	0	0.091	0.055
Al_2O_3	1.182	1.298	4.888	3.753	2.065	3.04	3.317	2.934	3.595	3.514
Cr_2O_3	0.05	0.066	0.163	0.187	0.083	0.089	0.063	0.064	0.014	0.04
FeO	6.2	5.874	6.603	5.29	5.23	18.694	16.977	20.519	18.16	15.846
MnO	0.27	0.208	0.171	0.133	0.185	0.474	0.38	0.7	0.395	0.292
MgO	14.122	13.928	12.869	13.764	13.902	23.54	23.264	21.168	23.629	24.917
CaO	21.071	21.67	20.102	21.339	22.042	0.176	0.423	0.196	0.219	0.209
Na_2O	0.71	0.723	1.258	0.945	0.64	0	0.112	0	0.019	0.009
K_2O	0	0.011	0.04	0.01	0.014	0	0	0.006	0.009	0.002
Recalculation of pyroxene analysis based on 6 oxygen atoms (all Fe as FeO) and solving for Fe^{+3}										
Si	1.9715	1.9693	1.9027	1.9051	1.9429	1.9050	1.9001	1.8925	1.8716	1.8914
Ti	0.0014	0.0011	0.0088	0.0058	0.0001	0.0025	0.0009	0.0000	0.0027	0.0016
Al	0.0552	0.0604	0.2221	0.1730	0.0966	0.1378	0.1543	0.1398	0.1663	0.1605
Cr	0.0016	0.0021	0.0050	0.0058	0.0026	0.0027	0.0020	0.0020	0.0004	0.0012
Fe	0.2053	0.1940	0.2129	0.1730	0.1736	0.6015	0.5603	0.6938	0.5960	0.5135
Mn	0.0091	0.0070	0.0056	0.0044	0.0062	0.0154	0.0127	0.0240	0.0131	0.0096
Mg	0.8336	0.8199	0.7396	0.8024	0.8228	1.3501	1.3685	1.2758	1.3822	1.4393
Ca	0.8939	0.9168	0.8304	0.8941	0.9376	0.0073	0.0179	0.0085	0.0092	0.0087
Na	0.0545	0.0554	0.0940	0.0716	0.0493	0.0000	0.0086	0.0000	0.0014	0.0007
K	0.0000	0.0006	0.0020	0.0005	0.0007	0.0000	0.0000	0.0003	0.0005	0.0001
Total	4.0260	4.0264	4.0230	4.0357	4.0324	4.0223	4.0252	4.0367	4.0434	4.0266
Mg#	80	81	78	82	83	69	71	65	70	74

over the IPGE (Ir, Rh). Pd/Pt ratio suggests that the majority of samples show dominance of Pd over Pt, excepting for two samples with higher Pt concentration. The abundance of PGE in magmas depends on the nature and abundance of sulfides, oxides, and alloys that help in retaining these elements (Arndt et.al., 2005). The ΣPGE vs Ni/Cu plot and ΣPGE vs Cr/Ni (Fig-6a,b) indicates that the samples with higher concentration of PGE (>100ppb) have a positive correlation with the Cu and Cr respectively, corroborating the observation of Keays (1982, 1995), that the PPGE group of PGE metals in sub-solidus ultramafics are hosted mainly by Cu-rich sulfides.

The metal ratios and discrimination diagrams like Ni/Cu vs Pd/Ir and Ni/Cu vs Pd/Pt (Fig-7c, d) indicate that the ultramafic rocks of P.Mettupalaiyam area belong to the komatiite, ophiolite and layered intrusion fields (Barnes et al. 1985), suggesting a Mg rich parent magma generated by partial melting of upper mantle and subsequent emplacement into crustal rocks through deep seated fractures. According to Arndt et al., (2005), the high temperature and low viscosity komatiites and tholeiitic picrites have a high capability of forming Ni-Cu-(PGE) deposits, while, lower temperature, volatile-rich alkali picrites and basalts have less potential. Barnes, (1990) had suggested that PGE has a greater affinity for Fe-alloy than Ni and Cu, due to depletion of these metals in the mantle and crust and there by forming ore deposits due to partial melting of the mantle.

PGE concentrations in ultramafic rocks normalized to primitive mantle indicate that PPGE (Pt, Pd) are enriched over IPGE (Ir, Rh, Ro) with a distinct positive Pd-anomaly (Fig-7b, after Sun and McDonough., 1989),

Table 2. EPMA analytical data of amphiboles in amphibolites, P. Mettupalaiyam area, MUB.

Analysis	Amph-1	Amph-2	Amph-3	Amph-4	Amph-5
SiO_2	49.632	46.93	46.556	48.744	48.084
TiO_2	0.224	0.406	0.355	0.146	0.306
Al_2O_3	5.872	8.031	8.989	3.076	5.964
FeO	8.445	8.976	8.769	7.673	6.909
MnO	0.138	0.135	0.191	0.198	0.142
MgO	16.566	15.71	15.649	17.868	17.295
CaO	11.476	10.693	10.968	11.061	11.133
Na2O	1.122	1.505	1.765	0.708	1.168
K_2O	0.169	0.207	0.137	0.086	0.041
Cr_2O_3	0.062	0.002	0.119	0.009	0.112
No. of oxygens	23	23	23	23	23
Recalculation of amphibole analysis based on 23 oxygen atomswith Fe^{2+}/Fe^{3+} estimation assuming 13 cations					
Si	7.279	6.953	6.851	7.393	7.176
Al iv	0.721	1.047	1.149	0.550	0.824
Al vi	0.294	0.356	0.411	0.000	0.225
Ti	0.025	0.045	0.039	0.017	0.034
Cr	0.007	0.000	0.014	0.001	0.013
Fe^{3+}	0.414	0.734	0.657	0.809	0.612
Fe^{2+}	0.621	0.378	0.422	0.164	0.250
Mn	0.017	0.017	0.024	0.025	0.018
Mg	3.622	3.470	3.433	4.040	3.848
Ca	1.803	1.697	1.729	1.798	1.780
Na	0.319	0.432	0.504	0.208	0.338
K	0.032	0.039	0.026	0.017	0.008
OH*	2.000	2.000	2.000	2.000	2.000
Total	17.154	17.169	17.259	17.022	17.126
(Ca+Na) (B)	2.000	2.000	2.000	2.000	2.000
Na (B)	0.197	0.303	0.271	0.202	0.220
(Na+K) (A)	0.154	0.169	0.259	0.022	0.126
$Mg/Mg+Fe^{2+}$	0.854	0.902	0.891	0.961	0.939
$Fe^{3+}/Fe^{3+}+Al$	0.585	0.674	0.616	1.000	0.731
Mg#	85	90	89	96	94
End member	Mg-hb	Mg-hb	Mg-hb	Mg-hb	Mg-hb
Mg-hb = magnesio-hornblende					

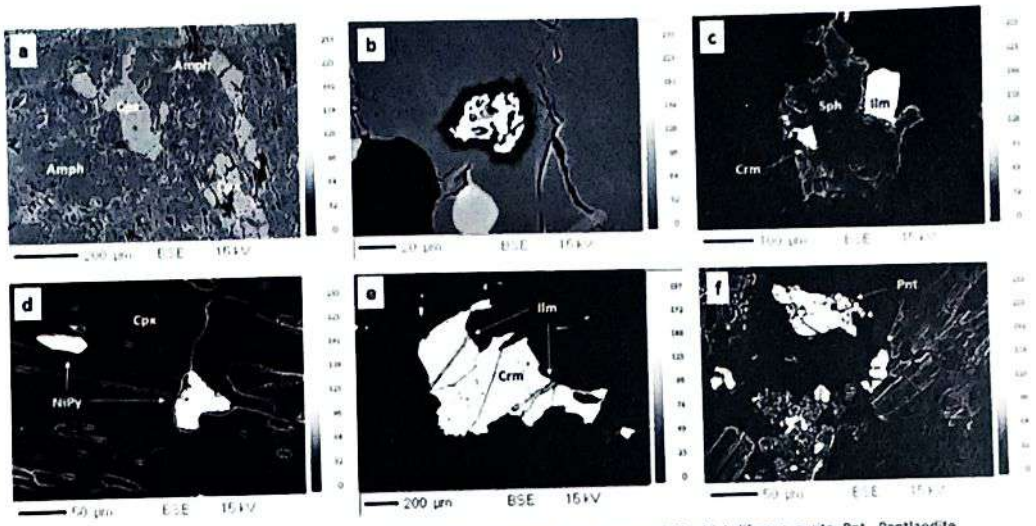
Table 3. Elemental concentration in sulfides in Weight% (by probe analysis).

	Sul-1	Sul-2	Sul-3	Sul-4	Sul-5	Sul-6
Zn	0.06	0.09	0	0.04	0	0.12
Fe	54.42	54.02	20.05	56.05	56.46	53.45
S	32.91	26.46	27.16	37.19	37.93	35.51
Co	0.02	0.01	0.47	0.04	0	0
Cu	0.04	0	0	0.11	0	0.15
Bi	0.06	0.09	0.16	0.24	0.20	0.09
Ni	1.3	1.70	37.82	1.33	1.23	1.41
As	0.03	0	0	0.01	0.03	0.02
	Ny.Py	Ny.Py	Pnt	Ny.Py	Ny.Py	Ny.Py
Ny.Py: Nickeliferous Pyrite; Pnt: Pentlandite						

which may be inferred to be due to the partial melting of the upper mantle under ultra-high temperature metasomatic conditions. According to Arndt et al., (2005), the magmatic Fe-Ni-Cu ± platinum-group element (PGE) bearing sulfide deposits form when mantle-derived mafic and ultramafic magmas become saturated with sulfides and segregate as immiscible sulfide rich liquids, commonly following interaction with crustal rocks.The exsolution of nickeliferous pyrite and pentlandite,ilmenite in chromite reflects the immiscible nature of incompatible elements, late phase fractionation and remobilization. The assimilation of crustal rocks by the upwelling komatiitic magma leading to contamination and sulphur saturation may result in ore formation.Thus,the ultramafic rocks of P.Mettupalaiyam area falling under komatiite, ophiolite and layered intrusion type fields showing higher concentration of sulphide minerals, mainly nickeliferous pyrite and pentlandite, are inferred to have resulted from crustal contamination and thereby enrichment of PGE. Though the notable PGE values as recorded in chemical analysis (ΣPGE max of 1798ppb), PGM phases could not be identified during the EPMA analysis and hence the phase determination of PGM phases would help in a better understanding of the genesis of PGE in the ultramafic rocks of P.Mettupalaiyam area.

7. Conclusion

The detached mafic-ultramafic bodies occurring as lensoidal bodies within hornblende-biotite gneiss in P.Mettupalaiyam area, compositionally belong to pyroxenite consisting of hypersthene and diopside, and amphibolite consisting amphiboles of magensio-hornblende variety. The oxide phase represented by chromite and ilmenite along with sulphides like



Amph - Amphibole; CPx – Clino pyroxene; Sph – Sphene; Crm – Chromite; Ilm – Ilmenite; NiPy- Nickeliferous pyrite; Pnt - Pentlandite

Fig. 4. BSE images showing a) silicate phases in amphibolite (coarse grained hornblende and medium grained augite); b) native copper with 76.67 wt% of Cu; c) Oxide phases like chromite and ilmenite associated with sphene; d) Nickeliferous pyrite occurring as interspatial / intergarnular grains in pyroxenite; e) Exsolution of illmenite in chromite and f) Pentlandite occurring in the interstitial spaces in pyroxenite.

Table 4. PGE and Trace element values in ultramafic rocks of P.Mettupalaiyam area, MUB.

Sample.No	Lithology	PGE Elements (ppb)						Trace elements (ppm)			Pd/Pt	Pd/Ir	Ni/Cu	Cr/Ni
		PPGE			IPGE		ΣPGE							
		Pt	Pd	Ru	Ir	Rh		Ni	Cr	Cu				
MBT-15/1	Pyroxenite	111	91	52	21	14	289	251	1138	65	0.8	4.3	3.9	4.5
MBT-15/2	Pyroxenite	486	1263	9	9	30	1798	310	1337	40	2.6	139.1	7.8	4.3
MBT-15/3	Pyroxenite	55	59	54	12	7	187	304	1388	60	1.1	4.9	5.1	4.6
MBT-15/4	Pyroxenite	48	53	37	12	6	157	314	1485	40	1.1	4.4	7.9	4.7
MBT-16/1	Pyroxenite	3	17	2	2	2	24	304	1073	260	6.8	11.3	1.2	3.5
MBT-16/2	Pyroxenite	12	29	10	4	2	56	321	1249	284	2.4	7.4	1.1	3.9
MBT-16/3	Pyroxenite	42	1.5	1.5	1.5	5	52	553	4625	5	0.03	1.0	110.6	8.4
MBT-16/4	Pyroxenite	3	12	2	2	2	19	281	850	44	4.9	8.2	6.4	3.0
MBT-17/1	Pyroxenite	263	469	42	16	20	810	160	1046	9	1.8	30.1	17.8	6.5
MBT-17/2	Pyroxenite	15	35	2	2	2	54	181	1524	11	2.3	23.0	16.5	8.4
MBT-17/3	Pyroxenite	35	42	53	12	8	149	192	1440	8	1.2	3.6	24.0	7.5
MBT-17/4	Pyroxenite	104	170	5	2	3	284	211	816	6	1.6	113.3	35.2	3.9
MBT-17/5	Pyroxenite	169	261	59	12	9	510	207	610	5	1.5	21.4	41.4	2.9
MBT-17/6	Pyroxenite	12	20	3	2	2	38	213	1153	7	1.7	13.4	30.4	5.4
MBT-17/7	Pyroxenite	41	41	54	17	12	165	222	1217	5	1.0	2.4	44.4	5.5
MBT-17/8	Pyroxenite	24	32	8	3	3	69	288	1362	5	1.3	12.0	57.6	4.7
MBT-17/9	Pyroxenite	9	23	2	2	2	36	244	1173	5	2.5	15.0	48.8	4.8
MBT-17/10	Pyroxenite	25	24	2	2	2	53	213	1153	7	1.0	15.9	30.4	5.4
MBT-17/11	Pyroxenite	23	25	3	2	2	54	222	1217	5	1.1	16.6	44.4	5.5
MBT-17/12	Pyroxenite	359	498	18	4	5	884	288	1362	5	1.4	117.2	57.6	4.7

Table 4. Contd.....

Sample.No	Lithology	PGE Elements (ppb)						Trace elements (ppm)			Pd/Pt	Pd/Ir	Ni/Cu	Cr/Ni
		PPGE			IPGE		ΣPGE							
		Pt	Pd	Ru	Ir	Rh		Ni	Cr	Cu				
MBT-17/13	Pyroxenite	99	127	2	2	2	231	244	1173	5	1.3	84.9	48.8	4.8
MBT-17/14	Pyroxenite	120	148	2	2	2	273	184	673	6	1.2	98.9	30.7	3.7
MBT-18/1	Pyroxenite	13	26	12	4	3	59	230	1817	5	2.0	6.1	46.0	7.9
MBT-18/2	Pyroxenite	143	377	5	21	12	557	237	1765	5	2.6	18.3	47.4	7.4
MBT-18/3	Pyroxenite	15	35	26	7	3	86	262	1774	5	2.4	5.3	52.4	6.8
MBT-18/4	Pyroxenite	20	51	7	3	3	86	265	1880	5	2.5	16.6	53.0	7.1
MBT-18/5	Pyroxenite	26	26	2	2	2	56	268	1758	5	1.0	17.0	53.6	6.6
MBT-18/6	Pyroxenite	31	34	16	5	3	90	258	1420	5	1.1	6.5	51.6	5.5
MBT-18/7	Pyroxenite	21	37	23	5	4	91	207	1029	5	1.8	7.2	41.4	5.0
MBT-18/8	Pyroxenite	15	46	2	2	2	66	196	1029	13	3.0	30.7	15.1	5.3
MBT-18/9	Pyroxenite	25	51	2	2	2	80	230	1289	3	2.1	33.9	76.7	5.6
MBT-18/10	Pyroxenite	36	40	14	5	4	100	152	1649	3	1.1	7.4	50.7	10.8
MBT-18/12	Amphibolite	40	56	6	3	4	109	152	795	5	1.4	19.9	30.4	5.2
MBT-18/13	Amphibolite	40	53	8	4	4	108	139	699	5	1.3	13.0	27.8	5.0
MBT-18/15	Pyroxenite	21	24	2	2	2	49	178	1502	5	1.1	15.8	35.6	8.4
MBT-18/17	Pyroxenite	10	16	2	2	2	30	262	991	10	1.6	10.3	26.2	3.8
MBT-18/18	Pyroxenite	36	54	2	2	2	95	146	668	5	1.5	36.3	29.2	4.6

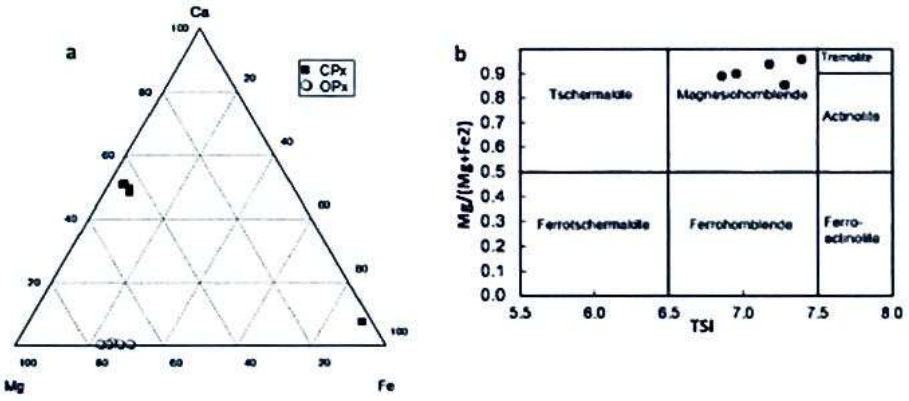


Fig. 5. a) pyroxene composition based on the classification of Morimoto (1988); b) amphibole composition based on classification of Hawthorne and Oberti (2007).

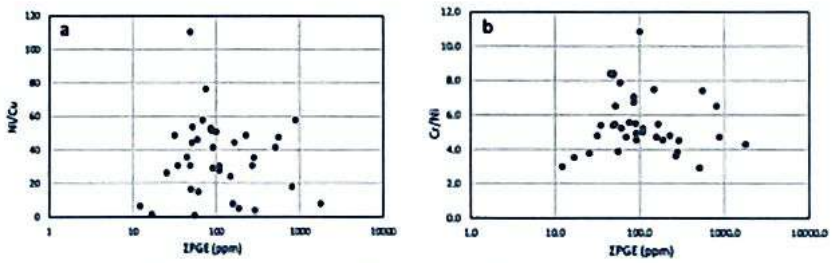


Fig. 6. Metal ratio plots: a) ΣPGE vs Ni/Cu and b) ΣPGE vs Cr/Ni.

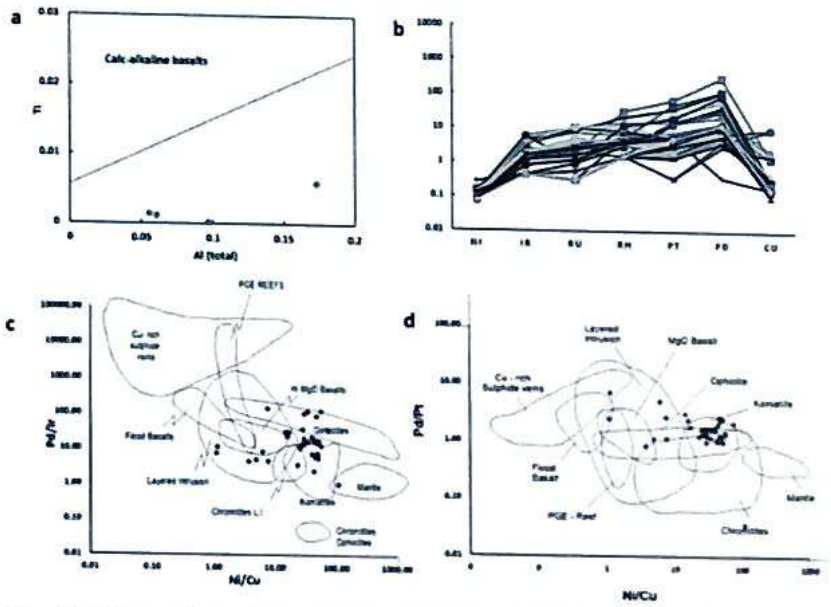


Fig. 7 a) Ti vs. Al (total) plot of clinopyroxene (CPX) in ultramafic rocks of P.Mettupalaiyam area (Fields after Leterrier et al., 1982); b) Spider diagram of TE and PGE normalised to Primitive mantle (after Sun and McDonough., 1989); c) Classification diagram of Ni/Cu vs. Pd/Ir; d) Classification diagram of Ni/Cu vs. Pd/Pt (after Barnes et al. 1985).

nickeliferous pyrite and pentlandite occur in the interstitial spaces of silicates. The presence of granular aggregates of chromites, Ni sulphides and PGMs in theinterstitial spaces of olivine cumulates, and alongthe cleavages in pyroxenes indicates the prevalence of S-saturation during cooling conditions (Balaram., et.al., 2013).The Ti vs. Al (total) plot of clinopyroxene (CPX) suggests thatthe ultramafic rocks of P.Mettupalaiyam area fall under tholeiitic magma series. The Pd/Ir ratio indicates that the ultramafic rocks area are enriched in PPGE (Pt,Pd, Ru) over the IPGE (Ir, Rh). Pd/Pt ratio indicates that the majority of samples show dominance of Pd over Pt. The metal ratio of PGE and trace elements indicates that the ultramafic rocks of P.Mettuapaliyam area fall in the komatiite, ophiolite and layered intrusion fields, suggesting a Mg rich parent magma generated by the partial melting of upper mantle and emplacement into crustal rocks through deep seated weak planes leading to contamination and sulphur saturation and resulting in ore formation. The exsolution of nickeliferous pyrite and pentlandite, ilmenite in chromite reflects the immiscibility of incompatible elements, late phase fractionation and remobilization.

Acknowledgement

The authors are grateful to the authorities of Geological Survey of India for permitting to carry out the research work. Sincere gratitude to Shri. (Late) Sundaravanan.D, Director (Retd), GSI, Chennai for support and encouragement. The authors express thanks to Dr. Jeena G.B, Senior Geologist, GSI, Chennai for support rendered in field work. The Chemical Division, and the Petrological Division, GSI, SR, Hyderabad are highly acknowledged for carrying out the chemical analysis and petrological studies including EPMA.

References

Arndt, N.T., Lesher, C.M. and Czamanske, G.K., (2005). Mantle-Derived Magmas and Magmatic Ni-Cu-(PGE) Deposits, Society of Economic Geologists, Inc. Economic Geology 100th Anniversary Volume, pp. 5–23.

Balaram, V., Singh, S.P., Satyanarayanan, M. and Anjaiah, K. V., (2013). Platinum group elements geochemistry of ultramafic and associated rocks from Pindar in Madawara Igneous Complex, Bundelkhand massif, central India, J. Earth Syst. Sci. 122, No. 1, pp. 79–91.

Barnes, S. J., Naldrett, A. J. and Gorton, M. P., (1985). The origin of the fractionation of the platinum-group elements in terrestrial magmas, Chem. Geol. v. 53, pp. 303–323.

Barnes, S. J., (1990). The use of metal ratios in prospecting for a platinum-group element deposit, J. Explor. Geochem. v. 37, pp. 91–99.

Barnes, S.J. and Maier., W.D., (1999). The fractionation of Ni, Cu and the noble metals in silicate and sulfide liquids. In: Lesher, Lightfoot, and Farrow, (eds) Dynamic Processes in Magmatic Ore Deposits and their application in mineral exploration, Case, Geological Association of Canada, Short Course v. 13, pp. 69-106.

Godel, Belinda & Barnes, Sarah-Jane., (2008). Platinum-group elements in sulfide minerals and the whole rocks of the JM Reef (Stillwater Complex): Implication for the formation of the reef. Chemical Geology. v. 248. pp. 272-294.

Gopalakrishnan, K., (1993). Geology of parts of Gopichettipalaiyam and Avinashi Taluks, Coimbatore district, Tamilnadu, GSI Progress Report (unpublished) for the Field Season 1990-92.

Hawthorne, F. C. and Oberti, R., (2007). Classification of amphiboles, Reviews in Mineralogy & Geochemistry, v. 67, pp. 55-88.

Keays, R.R., (1982). Palladium and iridium in komatiites and associated rocks: Application to petrogenetic problems. In:Arndt, N.T. and Nisbet, E.G. (eds.), Komatiites: London, George Allen and Unwin, pp. 435–458.

Keays, R.R., (1995). The role of komatiite and picritic magmatism and S-saturation in the formation of ore deposits: Lithos, v. 34, pp. 1–18.

Leake, B.E., Woolley, A.R., Arps, C.E.S., Birch, W.D., Gilbert, M.C., Grice, J.D., Hawthorne, F.C., Kato, A., Kisch, H.J., Krivovichev, V.G, Linthout ,K., Laird, J., Mandarino, J.A., Maresch, W.V., Nickel, E.H., Rock, N.M.S., Schumacher, J.C., Smith, D.C., Stephenson, N.C.N., Ungaretti, L., Whittaker, E.J.W., Guo, Y., (1997). Nomenclature of amphiboles: Report of the subcommittee on amphiboles of the International Mineralogical Association, Commission on New Minerals and Mineral Names. Can Mineral v. 35, pp. 219-246.

Leterrier, J., Maury, R.C., Thonon, P., Girard, D., Marchal, M., (1982). Clinopyroxene composition as a method of identification of the magmatic affinities of paleo-volcanic series. Earth and Planet Science Letters v. 59, pp. 139–154.

Morimoto, N., (1988). Nomenclature of pyroxenes. Mineralogy and Petrology, v. 39, pp. 55–76.

Rajan, K.T., Muthu, V.T., Radhakrishnan, K., Suthanandam, P. and Sunderrajan, P., (1993). Report on the Geochemical Survey for Ni-Platinoid mineralization in Satyamangalam Belt, Coimbatore District, GSI Progress Report for the Field season: 1991-92 (unpublished).

Rollinson, H. (2008). The geochemistry of mantle chromitites from the northern part of the Oman ophiolite: inferred parental melt compositions, Contrib Mineral Petrol, v. 156, pp. 273–288.

Selvan, T.A. and Basu., (1976). Ultamafic and associated anorthosite in Bhavanisagar- Sirumugai-Punjai Puliampatti area, Coimbatore District, GSI Progress Report (unpublished) for the Field Season: 1974-75.

Selvan, T.A., (1978). Study of Ultramafic-Mafic-Anorthosite Complex of Kasipalaiyam – Kugalur - Dasapalaiyam areas, Coimbatore District, GSI Progress Report (unpublished) for the Field Season: 1976-77.

Sun, S. and McDonough, W.F., (1989). Chemical and isotopic systematic of oceanic basalts: implications for mantle composition and processes. In: A.D.Saunders and M.J.Norry

(Eds.), Magmatism in the ocean basins, Geol. Soc., London, Sp. Pub. 42, pp. 313-345.

Vijay Kumar, S. B., Geena, G B. and Bhoopathi, D., (2014). Preliminary investigation for platinum group elements in Karatadipalayam–Gopichettipalayan – Dasamplaiyam sector, Mettupalaiyam Ultramafic Belt, Erode district, Tamil Nadu. GSI Progress Report (unpublished) for the Field Season: 2012-14.

Windley, B.F. and Smith, J.V., (1976). Archaean high grade complexes and modern continental margins. Nature, v. 260, pp. 671-675.

Windley, B.F., Razafiniparany, A., Razakamanana, T., and Ackermand, D., (1994). Tectonic framework of the Precambrian of Madagascar and its Gondwana connections: a review and reappraisal, Geol. Rundsch., v. 83, pp. 642-659.

Received on 22-06-21; Revised Ms accepted on 28-08-21

Scientometric Portrait of Prof. Mohammad Abdulkader Akbarsha from Bharathidasan University, Tamilnadu

Surulinathi, M.
Assistant Professor, Department of Library and Information Science
Bharathidasan University, Tiruchirappalli-620 024, India
Corresponding author: surulinathi@gmail.com

Sureshkumar, T.
Librarian, National College (Autonomous), Tiruchirappalli-620001, India
sureshkumart@nct.ac.in

Abstract

The research publications of Prof. Mohammad Abdulkader, Akbarsha, a Scientist of Life Sciences, were quantitatively and qualitatively analyzed in this study. His first paper was published in 1992 when he was working in the School of Life Sciences, Bharathidasan University. His 184 research papers cover research topics in Biochemistry, Genetics and Molecular Biology (82); Chemistry (46); Pharmacology, Toxicology and Pharmaceutics (40); Agricultural and Biological Sciences (39); Medicine (25); Health Professions (15); Chemical Engineering (14); Material Science (12); Multidisciplinary (10); Physics and Astronomy (9); Environmental Science (6); Immunology and Microbiology (5); and so on. Many of his research publications are done in collaboration with scientists from abroad, Saudi Arabia (42); United States (16); France and Singapore (6); Switzerland (5); Finland, Germany, Italy, Spain and Taiwan (3); Hong Kong and South Korea (2); 6 countries each (1). He collaborated with 331 researchers and recorded 4368 Citations (5639 Cited References), (H-Index-34), (4734 citations in Google Scholar). His research productivity and quality are of an order that can inspire the young researchers/scientists and he can be considered as a "role model scientist to emulate" in the field of Life Sciences.

Keywords: Scientometric Portrait; Scientometrics; Citations; Highly Cited Papers

INTRODUCTION

Most Scientific and technological activities in India are funded directly and/or indirectly, from the public money, and virtually all scientists and technologists today are salaried people or hold fellowships. This calls for a continues assessment of performance both of the individual scientists, technologists and of the institutions, thus ensuring that neither the scientist, the technologist nor the administrative structure get stratified and therefore, fossilized.

Scientometrics provides an understating of the structure of the scientific

activity, subject disciplines being researched, institutions involved, strengths in the scientific groups and their communication/outlet channels. Scientometrics is an application of quantitative methods to the history of science/scientists (Kalyane, 1995). In recent years, we have used scientometric tools in order to assess the quantitative analysis of many scientific research fields such as pheromone biology (Rajagopal et al., 2013), yoga research (Poornima and Surulinathi, 2019), Indian contribution to drugs discovery (Sankaralingam et al., 2020), global research output of COVID-19 (Surulinathi et al., 2020) etc. It is one of the techniques for documenting and collating works of eminent scientists and researchers. In this paper, we present a concise sketch of Prof. Mohammad Abdulkader Akbarsha, (Scopus Author ID: 7003760724) stressing on his scientific achievements. His research has had a great impact in the fields dealing with Biochemistry, Genetics and Molecular Biology, Chemistry, Pharmacology, Toxicology and Pharmaceutics, Agricultural and Biological Sciences, Medicine, Chemical Engineering, Material Science, Health Professions, Physics and Astronomy, Environmental Science, Immunology and Microbiology, Engineering, Energy, Mathematics, Nursing, Computer Science and Veterinary.

M. A. Akbarsha – Scientist of Modern Life Science:

His independent research, though started at Jamal Mohamed College, Tiruchirappalli, has been mostly at Bharathidasan University which he moved into in 1989, where he was working in the School of Life Sciences. He started University career as a Reader, then Professor of Animal Science and Biomedical Science, Co-ordinator of the School, with 7 departments, and then Dean-Faculty of Science. He superannuated in June 2008, and immediately became Emeritus Professor of Life Sciences. Within a year he was invited by Doerenkamp-Zbinden Foundation, with the support of a huge grant, to work for popularizing alternatives to animal experiments in India, which he started in 2009 and the tenure was over in 2017. Thereafter, he is working as Honorary Research Coordinator in National College, Tiruchirappalli. His

research endeavor started in 1974, and continues to date. Areas his of research include Endocrinology, Reproductive Biology, Phytotherapy, Cancer Biology, Obesity, *In Vivo* & *In Vitro* Toxicology, Alternatives to Animal Experiments, with great emphasis on histological, ultrastructural and molecular biology tools. More than 280 research/review publications in peer-reviewed journals, out of which 130 are listed in the NCBI-PubMed; and more than 180 in Scopus. A few appeared in RSC and ACS Journals. Full coverage could be found in Science Direct, Research Gate, etc. He authored more than 15 review chapters in international edited publications. He is Editor-in-Chief of *Journal of Endocrinology and Reproduction since 2006*, the official organ of the Society for Reproductive Biology and Comparative Endocrinology, India, the Society for which he was President for 2 years, 2008-2010. He Co-authored of two books on *Indian Medicinal Plants* and has two international patents and quite a few gene bank submissions. He attended more than 300 seminars / symposia / conferences and presented papers / chaired sessions / delivered key-note addresses. Twenty three candidates have been awarded Ph.D., under his direct guidance, and he has been co-guide to many more candidates. He has operated several sponsored projects (UGC, DST, CSIR, ICMR) and has been awarded grants for international collaborative studies, including from NIH-USA and Koman Breast Foundation, USA and Doerenkamp-Zbinden Foundation, Switzerland. He was conferred Peranaidu Govindarajulu Gold Medal Oration award of Society for Reproductive Biology and Comparative Endocrinology, in 2016. He has also been conferred several other recognitions and awards including more than 20 travel awards to attend international conferences. Thus, he has visited countries such as Australia, Norway, UK, USA, Germany, Italy, France, Ethiopia, Saudi Arabia, Sri Lanka, Canada, Czech Republic, Japan, Brazil and Finland, some of these countries twice or more. He is best known for research collaborations, within India and abroad.

Service Organizations, University Grants Commission (UGC), Ministry of Environment and Forests (MoEF), and Medical & Pharmacy Councils, had raised concerns about the use of animals in learning and research. In this

backdrop, Prof. M. A. Akbarsha, established the Mahatma Gandhi – Doerenkamp Center (MGDC) for Alternatives to Use of Animals in Life Science Education in 2009 at Bharathidasan University, Tiruchirappalli, Tamil Nadu, for which he received a grant from Doerenkamp-Zbinden Foundation (DZF), Switzerland. He was Director of the Center and also Gandhi-Gruber-Doerenkamp Chair, an endowed Chair of the DZF. The center played a pivotal role in changing the attitude of students, teachers and research community towards the alternatives to use of animals in academics and research. The standalone facility of MGDC specialized in three domains viz., in vitro, in silico and alternative model organisms, each domain conducting research, training and outreach programs to overall achieve the vision of making India as a leader in modern tools of learning and research. The tenure of the grant was over in 2016, when in order to continue the brilliance of the center, the university proposed to expand the activity of MGDC by bringing the expertise of faculties from various departments to enrich its multi-disciplinary activities. The idea of establishment of a national facility named National Centre for Alternatives to Animal Experiments (NCAAE) was approved and funded by the UGC under CPEPA (Centre with Potential for Excellence in Particular Area) scheme. The main aim of this Centre is to promote excellence in education by launching modern tools in the field of alternatives to animal experiments. The primary focus of NCAAE is to establish the country as a leader on par with International Institutes in Human Science by adopting alternative methods to animal experiments. Since the tenure of DZF fund was over Prof. Akbarsha had to leave the University, and he joined the National College as Research Co-ordinator, on invitation, which assignment he still continues. In this role he helps the faculty in conducting quality research, writing effective proposals for availing grants from funding agencies, writing technically sound research articles for publication in reputed journals. He also continues his own research collaborations and engaes in research and publication.

OBJECTIVES

- To analyze the Author Productivity;
- To identify the Domain-wise contribution;
- To identify the Channels of publications;
- To analyze the Research collaboration of Author, Institutions and Country;
- To identify the Funding Sponsors for his research;
- To analyze the Citations and Publications Impact;
- To identify the highly Cited Papers and
- To identify the year-wise distribution of publications.

METHODOLOGY

A complete bibliography of his research publications from 1992–2020 has been catalogued and standard bibliometric fields was analyzed by normal count procedure for various domains such as author Citations, Collaboration Institutions, Countries, Authors, Funding Source and Subject domains. The data was downloaded from Scopus database, with the search string "Mohammad Abdulkader Akbarsha" with author field.

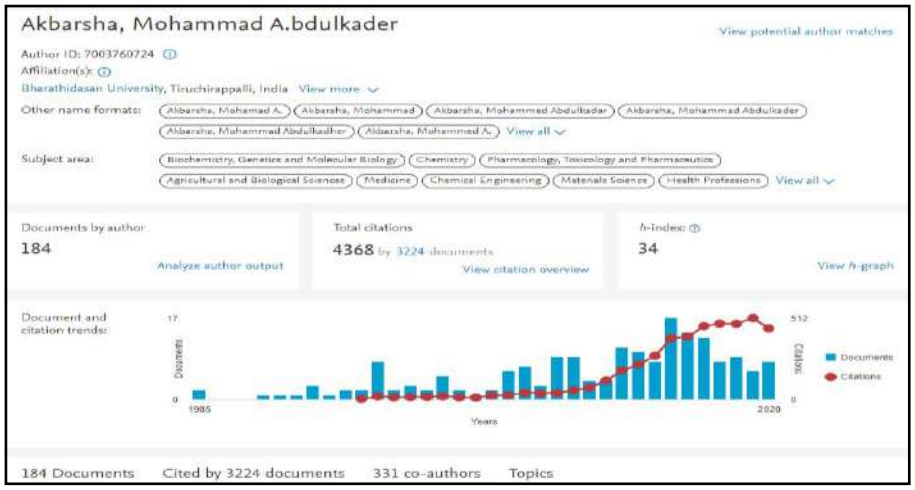


Figure: 1. Citation Profile of Prof. M.A. Akbarsha

RESULTS AND DISCUSSION

During the period, Professor published 184 research publications. Details of International Collaboration, domain-wise contribution, Channels of communication, Author and Institutional collaboration, Funding sponsors, and highly cited papers are highlighted quantitatively.

International collaboration:

The publications cover a total of 18 countries by way of international collaborations. The most productive countries of collaboration are Saudi Arabia with 42, USA with 16, France and Singapore with 6, Switzerland with 5, followed by 13 Countries which are listed in the below table 1.

Table: 1. International collaboration of Prof. Akbarsha

Country	Publications		Country	Publications
Saudi Arabia	42		Taiwan	3
United States	16		Hong Kong	2
France	6		South Korea	2
Singapore	6		Austria	1
Switzerland	5		China	1
Finland	3		Netherlands	1
Germany	3		Sri Lanka	1
Italy	3		Turkey	1
Spain	3		United Kingdom	1

Author Collaboration:

Figure 2 shows that among 184 investigated documents, 29 documents was collaborated with Dr. A. Riyasdeen (Prince Sultan Military Medical City, Riyadh, Saudi Arabia), 23 documents with Dr. M. Palaniandavar (School of Chemistry, Bharathidasan University), 20 documents with Dr. O.V. Oommen (University of Kerala, Thiruvananthapuram, India), and 18 documents with Dr. V. S. Periasamy (King Saud University, Riyadh, Saudi Arabia). Majority of the co-authors are from various, Schools, Centres and Departments of Bharathidasan University.

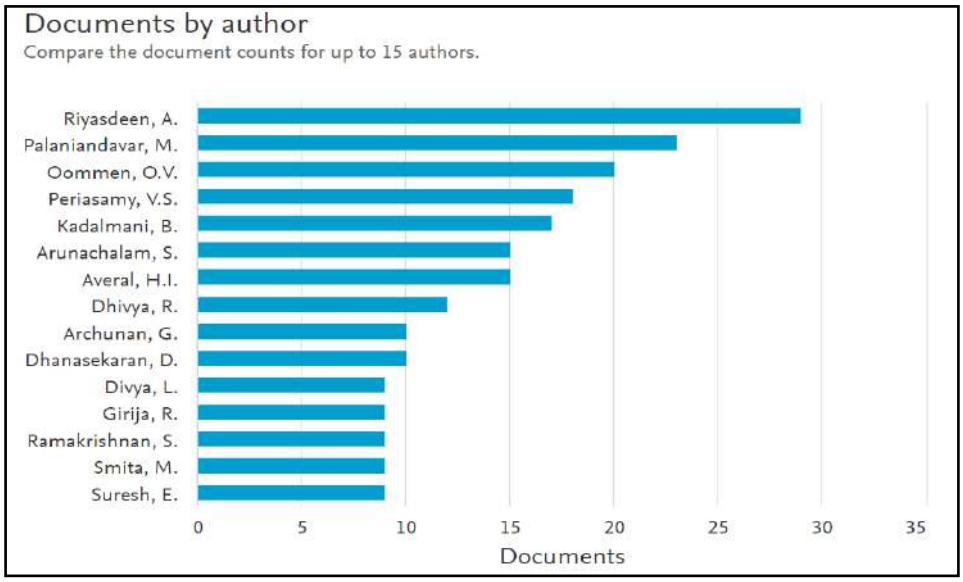


Figure: 2. Co-authored document

Collaborator Institutions:

Professor Akbarsha got the gain in impact due to collaborations both at national and international level Institutions (Table 2). The results depict that top institutions, which do more collaboration with institutions, get higher and positive impact out of his collaborations; overall, there is a positive impact on collaboration. With respect to collaborations, King Saud University, to which he was Visiting Professor for two years (41) stands at the first, National College, Tiruchirappalli (20), is standing at the second position, University of Kerala (20) is standing at third position, followed by Central University of Tamil Nadu (10), Central Salt and Marine Chemicals Research Institute India (9), Jamal Mohamed College, Tiruchirappalli (6), and Holy Cross College, Tiruchirappalli (6). The study found that the collaborations count (142) with National and International Institutions have been raised rapidly in terms of publications and Citations.

Table: 2. Institution-wise collaboration

Institution	Rec.	Institution	Rec.
King Saud University, Riyad, Saudi Arabia	41	Institute for Risk Assessment Sciences, Utrecht University, Utrecht, The Netherlands	1
National College, Tiruchirappalli, India	20	Departement Dier in Wetenschap en maatschappij, Universiteit Utrecht, The Netherlands	1
University of Kerala, Trivandrum, India	20	Faculteit Diergeneeskunde, Universiteit Utrecht, The Netherlands	1
Central University of Tamil Nadu, Tiruvarur, India	10	Institute for In Vitro Sciences, Inc., Gaithersburg, MD, USA	1
Central Salt and Marine Chemicals Research Institute, Bhavnagar, India	9	IRSEA Research Institute in Semiochemistry and Applied Ethology, France	1
Jamal Mohamed College, Tiruchirappalli, India	6	EnvisBE Solutions Pvt. Ltd, Mumbai, India	1
Holy Cross College, Tiruchirappalli, India	6	People for Ethical Treatment of Animals (PeTA) India, New Delhi, India	1
Raghunathpur College, Puralia. West Bengal, India	5	Konkuk University, Seoul, S. Korea.	1
National Centre for Biological Sciences, Bangalore, India	5	Emory University, Atlanta, Georgia, USA	1
Nanyang Technological University, Singapore	5	Universitat de Barcelona, Spain	1
University of Madras, Chennai, India	5	Universite de Neuchatel, Swirzerland	1
Tata Institute of Fundamental Research, Mumbai	5	University of Valencia, Spain	1
Lee Kong Chian School of Medicine, Singapore	5	Harvard Medical School, Boston, MA, USA	1
Indian Institute of Technology, Mumbai, India	4	Universidad de la Laguna, Spain	1
University of Kentucky, Lexington, Kentucky, USA	4	Marshall University, West Virginia, USA	1
Annamalai University, Annamalai Nagar, India	4	National Taiwan University, Taipei, Taiwan	1
Dr A.L.M Postgraduate Institute of Basic Medical Sciences, University of Madras, Chennai, India	4	National Institute of Technology, Tiruchirappalli, India	1
Shibpur Dinobundhoo Institution, Howra, WB, India	4	Jawaharlal Institute of Postgraduate Medical Education and Research, Aligarh, India	1
SRM Institute of Science and Technology, Kattankulathur, Chennai, India	3	Johns Hopkins Bloomberg School of Public Health, MD, USA	1
Abo Akademi University, University of Turuku, Finland	3	Mahatma Gandhi Medical College and Research Institute, Pondicharry, India	4
Texas A & M University, Texas, USA	3	Agharkar Research Institute, Pune, India	3
Ecole Polytechnique Fédérale de Lausanne, Switzerland	3	Bishop Heber College, Tiruchirappalli, India	1
Aligarh Muslim University, Aligarh, India	3	Louisiana State University, Baton, Rughe, USA	1
Central University of Kerala, Kasargod, Kerala, India	3	University of Kentucky College of Medicine, Lexington, KY, USA	1
Bharath College of Science and Management, Chennai, India	2	Utrecht University, Utrecht, The Netherlands	1
In Vitro ADMET Laboratories LLC, Columbia, MD, USA.	2	Medizinische Universitat Innsbruck, Austria	1

Episkin Academy, L'Oréal Research and Innovation, Linz, France	2	Pusan National University, South Korea	1
Kerala State Biodiversity Board, Trivandrum, India	2	University of Kalyani, Kalyani, WB, India	1
Winro Science Research Foundation, Tiruchirappalli, India	2	Gebze Teknik Üniversitesi, Turkey	1
Fisk University, Nashville, Tennessee, USA	2	City University of Hong Kong, Hong Kong	1
King Saud University College of Science, Riyadh, Saudi Arabia	2	Oxford Brookes University, Oxford, England	1
National Dairy Research Institute, Karnal, Haryana, India	2	National Cheng Kung University, Taipei, Taiwan	1
Bharathiar University, Coimbatore, India	2	Alagappa University, Karaikudi, India	1
Indian Institute of Science, Bengaluru, India	2	National University of Singapore, Singapore	1
Central Leather Research Institute, Chennai, India	2	Rajiv Gandhi Centre for Biotechnology, Trivandrum, India	1
Jamia Hamdard, New Delhi, India	2	Jadavpur University, Jadavpur, Kolkatta, WB, India	1
Bhabha Atomic Research Centre,	2	North-Eastern Hill University, Shillong, India	1
Homi Bhabha National Institute, Mumbai, India	2	South China Seas Institute of Oceanography, Chinese Academy of Sciences, Beijing, China	1
Kalasalingam Academy of Research and Education, Krishnankoil, Srivilliputtur, India	2	University of Calcutta, Kolkatta, WB, India	1
Vivekananda Mahavidyalaya, Burdwan, WB, India.	2	Madras Christian College, Tambaram, Chennai, India	1
National Institute of Technology, Durgapur, India	2	Universität Konstanz, Konstanz, Germany	1
Indian Institute of Science Education and Research, Mohali, India	2	Veterinary College and Research Institute, Namakkal, India	1
Urumu Dhanalakshmi College, Tiruchirappalli, India	2	Turun Yliopistollinen Keskussairaala, Turuku, Finland	1
Kanchi Mamunivar Centre for Post-Graduate Studies, Pondicherry, India	1	Università degli Studi di Camerino, Italy	1
Mother Theresa Post Graduate and Research Institute of Health Sciences, Pondicherry, India	1	Université de Genève Faculté de Médecine, Switzerland	1
Ramsaday College, Amta, WB, India	1	Loyola College, Chennai, India	1
Bhatath College of Science and Management, Chennai, India	1	Institute of Science, Mumbai, India	1
Raja Serfoji Government College, Thanjavur, India	1	Turku Centre for Biotechnology, Finland	1
Doerenkamp-Zbinden Foundation, Zurich, Switzerland	1	Hindustan Unilever Limited, Bangalore, India	1
Manokaryon Bioproducts and Services, Salem, India	1	University of Oklahoma Health Sciences Center, Oklahama, USA	1
Government College, Chittur, AP, India	1	Università degli Studi di Milano, Italy	1
Devaswom Board Pampa College, Parumala, Kerala	1	Children's Hospital, Boston, USA	1
Center for Alternatives to Animal Testing CAAT-Europe, Konstanz, Germany	1	Vector Control Research Centre India, Pondicherry, India	1
National Facility for Biopharmaceuticals, Mumbai, India	1	Università degli Studi della Tuscia Viterbo, Italy	1

National Yang-Ming University, Taiwan	1	University of Colombo, Sri Lanka	1
Universite de Neuchatel, Institut de chimie, Switzerland	1	University of Colombo Faculty of Medicine, Colombo, Sri Lanka	1
Mithibai College of Arts, Chauhan Institute of Science & Amrutben Jivanlal College of Commerce and Economics, Mumbai, India	1	A.V.V.M Sri Pushpam College, Poondi, Thanjavur, TN, India	1
ICAR - Central Institute of Brackishwater Aquaculture, Chennai, India	1	Narsee Monjee Institute of Management Studies, Mumbai, India	1
Anna University of Technology, Tiruchirappalli, TN, India	1	Jawaharlal Nehru Medical College, Aligarh, UP, India	1
Jawaharlal Nehru Institute of Advanced Studies, Hyderabad, India	1		

Subject domain-wise distribution of Publications:

Among the subject-wise distribution of publications, the largest share (82) of publications comes from Biochemistry, Genetics and Molecular Biology, followed by Chemistry (46), Pharmacology, Toxicology and Pharmaceutics (40), Agricultural and Biological Sciences (39), Medicine (25), Health Professions (15), Chemical Engineering (12), Multidisciplinary (10), etc., during 1992-2020 (Fig. 3). The study found that the Professor touched several disciplines of science subjects.

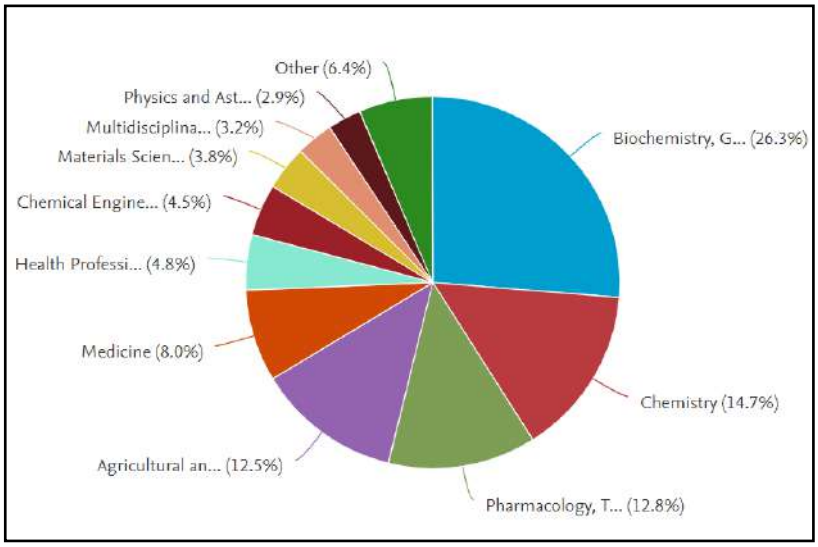


Figure: 3. Subject area-wise distribution of Publications

Bibliographical form-wise distribution of Publications:

In terms of document types, 90.76% (167) appeared as Journal Articles, 5.43% (10) as Conference papers, 1.09% (2) in Book Chapters, Erratum and Review respectively, and only one publication in Editorial publication (Fig. 4).

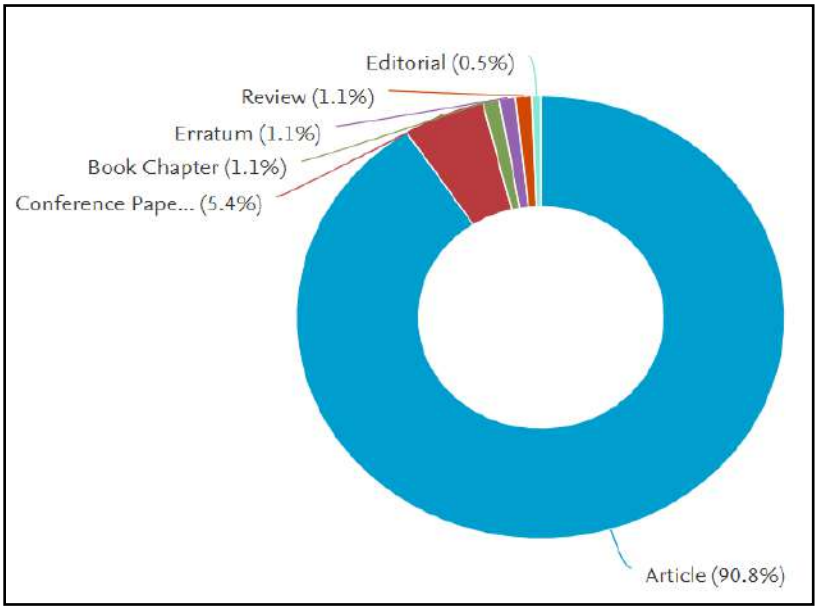


Figure: 4. Document type-wise distribution of Publications

Source title-wise distribution of Publications:

In terms of sources, 91 titles were preferred by Professor Akbarsha for his publication at National and International levels (Table 3). 12 publications appeared in Altex (Cite Score-8.4, SNIP-1.529, SJR-1.270 and Impact Factor: 4.56) and Journal Of Morphology (Cite Score-2.7; SNIP-0.800; SJR-0.681 and Impact Factor:1.56) stand at first position, Biomedical Letters (SNIP-0.339, SJR-0.108 and Impact Factor-1.25)) and Indian Journal Of Experimental Biology (Cite Score-3.0; SNIP-0.512; SJR-0.227 and IF-1.25) stand at second position with 10 publications. The list of 10 most productive source titles along with their output is shown in the table below. According to Cite Score Human Reproduction registered with 10.1 (SNIP-2.256, SJR-2.491 and IF-5.72) is standing at first position followed by Fertility and Sterility with 9.8 Cite Score (SNIP-2.015, SJR-2.31 and IF-5.21) is standing at second position, Archives of Toxicology with 9.6 Cite Score (SNIP-1.550, SJR-1.379 and IF-5.66) is sanding at third position.

Table: 3. Source title-wise distribution of Publications

Source Title	Publications	Cite Score	SNIP	SJR
Altex	12	8.4	1.529	1.270
Journal Of Morphology	12	2.7	0.800	0.681
Biomedical Letters	10	-	0.339	0.108
Indian Journal Of Experimental Biology	10	3.0	0.512	0.227
Scientific Reports	8	7.2	1.365	1.341
Dalton Transactions	7	6.8	0.938	1.048
Journal Of Inorganic Biochemistry	7	6.0	0.926	0.63
RSC Advances	6	6.5	0.827	0.736
Food And Chemical Toxicology	4	6.7	1.353	0.902
Inorganic Chemistry	4	7.9	1.164	1.349
Cytobios	3	-	0.505	0.171
New Journal Of Chemistry	3	4.7	0.775	0.712
Phytotherapy Research	3	6.7	1.423	0.905
Polyhedron	3	3.9	0.663	0.451
Reproduction	3	5.9	1.148	1.201
Reproductive Toxicology	3	5.6	0.894	0.816
Acta Zoologica	2	2.4	0.668	0.445
Biological Structures And Morphogenesis	2	-	-	-
Fish Physiology And Biochemistry	2	3.4	0.999	0.676
Journal Of Biological Sciences	2	1.1	1.031	0.191
Journal Of Cellular Biochemistry	2	3.9	0.907	0.971
Journal Of Chemical Sciences	2	2.6	0.538	0.321
Journal Of Pharmacology And Pharmacotherapeutics	2	2.0	0.495	0.269
Journal Of Photochemistry And Photobiology B Biology	2	8.1	1.289	0.835
Reproduction In Domestic Animals	2	2.3	0.856	0.553
Zeitschrift Fur Mikroskopisch Anatomische Forschung Abteilung 2	2	-	-	-
Zoology	2	-	-	-
ACS Omega	1	2.7	0.728	0.767
Acta Physiologica Hungarica	1	-	-	-
Advanced Science Letters	1	0.4	0.316	0.126
Animal Reproduction New Research Developments	1	-	-	-
Annals Of Applied Biology	1	3.5	0.954	0.713
Anticancer Research	1	3.3	0.633	0.716
Apoptosis	1	8.0	1.115	1.127
Applied Biochemistry And Biotechnology	1	4.2	0.828	0.562
Applied Organometallic Chemistry	1	3.6	0.711	0.508

Applied Surface Science	1	8.7	1.439	1.230
Archiv Der Pharmazie	1	3.8	0.670	0.366
Archives Of Toxicology	1	9.6	1.550	1.379
Australian Journal Of Chemistry	1	2.3	0.296	0.278
Biocatalysis And Agricultural Biotechnology	1	2.8	0.986	0.506
Biochemical Pharmacology	1	7.5	1.217	1.509
Biochemistry And Biophysics Reports	1	3.6	0.853	0.635
Biology Of Reproduction	1	5.2	1.025	1.248
Biomass And Bioenergy	1	6.6	1.415	1.110
Biometals	1	4.7	0.877	0.650
Biotechnology Letters	1	4.3	0.727	0.595
Cancer Chemotherapy And Pharmacology	1	5.6	1.040	1.239
Cell And Tissue Research	1	7.1	1.106	1.402
Cell Biochemistry And Biophysics	1	3.9	0.703	0.599
Chemico Biological Interactions	1	5.9	1.181	0.896
Colloids And Surfaces B Biointerfaces	1	7.1	1.095	0.929
Comparative Biochemistry And Physiology Part C Toxicology And Pharmacology	1	4.4	0.940	0.718
Domestic Animal Endocrinology	1	4.4	0.941	0.683
Environmental Pollution	1	9.3	1.805	1.968
European Journal Of Inorganic Chemistry	1	4.5	0.676	0.693
European Journal Of Medicinal Chemistry	1	8.3	1.540	1.144
Evidence Based Complementary And Alternative Medicine	1	2.9	0.809	0.510
Fertility And Sterility	1	9.8	2.015	2.311
Future Microbiology	1	3.9	0.779	1.017
Human Reproduction	1	10.1	2.256	2.491
Indian Journal Of Chemistry Section A Inorganic Physical Theoretical And Analytical Chemistry	1	1.2	0.278	0.178
Inorganica Chimica Acta	1	3.9	0.690	0.447
Journal Of Antibiotics	1	4.3	0.862	0.559
Journal Of Basic Microbiology	1	3.4	0.809	0.611
Journal Of Biomolecular Structure And Dynamics	1	4.5	0.980	0.504
Journal Of Coordination Chemistry	1	2.7	0.500	0.291
Journal Of Ethnopharmacology	1	6.3	1.599	0.898
Journal Of Nutrition And Metabolism	1	3.1	1.172	0.637
Journal Of Reproduction And Fertility	1	-	1.687	0.676
Journal Of Zoology	1	-	-	-
Materials Letters	1	5.5	0.941	0.753
Medicinal Chemistry Research	1	3.2	0.796	0.357
Microscopy Research And Technique	1	2.8	0.903	0.469
Molecular And Cellular Biochemistry	1	5.1	0.842	0.836
Molecular Biosystems	1	6.8	1.048	0.988

Particle And Particle Systems Characterization	1	5.5	0.538	0.909
Pharmacognosy Magazine	1	2.1	0.878	0.345
Phytomedicine	1	5.7	1.502	0.958
Planta	1	5.4	1.269	1.259
Rare Animals Of India	1	-	-	
Reproductive Biology	1	3.0	0.745	0.622
Saudi Journal Of Biological Sciences	1	4.8	1.734	0.649
Science Asia	1	0.7	0.419	0.160
Scientia Pharmaceutica	1	2.9	1.071	0.384
Spectrochimica Acta Part A Molecular And Biomolecular Spectroscopy	1	5.1	1.088	0.550
Springer Plus	1	3.4	1.476	0.409
Toxicology In Vitro	1	5.0	0.905	0.799
Zoological Science	1	1.7	0.565	0.441
Zygote	1	2.6	0.600	0.435

Most frequently occurred Keywords:

A set of main keywords and related keywords along with their research output have been identified, which throw light on the type of work undertaken in different keywords. Among the 159 main keywords, the largest number of publications (129) on Animals, followed by Human with 102, Controlled Study 54, Nonhuman 53, Male 49, Apoptosis and Metabolism with 43, Chemistry with 37, animal Experiment and DNA with 35, respectively (Table 4).

Table: 4. Keyword-wise distribution of Publications

Keywords	Occurs	Keywords	Occurs	Keywords	Occurs
Animals	129	DNA Binding	13	Circular Dichroism	7
Human	102	Animal Model	12	DNA Fragmentation	7
Controlled Study	54	Phenanthrolines	12	Diseases	7
Non-human	53	Reproductive Toxicity	12	Dose Response	7
Male	49	Cobalt	11	Dose-Response Relationship, Drug	7
Apoptosis	43	Mouse	11	Drug Synthesis	7
Metabolism	43	Tumor Cell Line	11	Epithelium Cell	7
Chemistry	37	X Ray Crystallography	11	Histopathology	7
Animal Experiment	35	Animal Testing Alternative	10	Imine	7
DNA	35	Binding Energy	10	Imines	7

Cytotoxicity	33	Caecilia	10	In Vivo Study	7
Human Cell	32	Conference Paper	10	Leydig Cell	7
Drug Effect	31	Hydrophobicity	10	Mitochondria	7
Female	30	MCF-7 Cells	10	Mitochondrial Membrane Potential	7
Rat	27	Protein Binding	10	Plant Extract	7
Unclassified Drug	27	Spermatozoon Motility	10	Rattus	7
Priority Journal	25	Toxicity Testing	10	Reactive Oxygen Metabolite	7
Cell Death	23	Calf Thymus DNA	9	Reactive Oxygen Species	7
Testis	22	Caspase 3	9	Sperm	7
Copper	21	Chemical Structure	9	Spermatozoon	7
Transmission Electron Microscopy	21	Complexation	9	Toxicity	7
Antineoplastic Agent	20	Drug Cytotoxicity	9	Antioxidants	6
Antineoplastic Agents	20	Mice	9	Binding Affinity	6
Epididymis	20	Mitochondrion	9	Breast Neoplasms	6
Cell Line, Tumor	19	Molecular Docking	9	Caecilian	6
Ligands	19	Oxidative Stress	9	Cancer Cell Culture	6
Rats	19	Pathology	9	Carbendazim	6
Ultrastructure	19	Phenanthroline Derivative	9	Cell Cycle Arrest	6
Animal Tissue	18	Sertoli Cell	9	Cell Structure	6
In Vitro Study	18	1,10-phenanthroline	8	Cells	6
Physiology	18	Caecilians	8	Chlorine Compounds	6
Spermatogenesis	18	Cancer Cell	8	Cisplatin	6
Animal Cell	17	Cattle	8	Cobalt Compounds	6
Cell Survival	17	Cell Proliferation	8	Concentration Response	6
Drug Effects	17	Cytoplasm	8	Copper Complex	6
Histology	17	Drug Screening	8	Crystal Structure	6
Ligand	17	Gene Expression Regulation	8	Drug Screening Assays, Antitumor	6
Cell Viability	16	IC50	8	Enzyme Activity	6
Comet Assay	16	Ichthyophis Tricolor	8	Gene Expression	6
DNA Cleavage	16	India	8	Genetics	6
Amphibia	15	Lizard	8	Hydrogen Bond	6
Antineoplastic Activity	15	MCF 7 Cell Line	8	IC 50	6
Cell Culture	15	Microscopy	8	Models, Molecular	6
DNA Damage	15	Platinum Compounds	8	Molecular Docking Simulation	6
Microscopy, Electron, Transmission	15	Procedures	8	Organometallic Compounds	6
Coordination Compound	14	Protein Bcl 2	8	Ruthenium	6

				Compounds	
Gymnophiona	14	Protein Expression	8	Scanning Electron Microscopy	6
Rats, Wistar	14	Spermatozoa	8	Sperm Motility	6
Synthesis	14	Absorption Spectroscopy	7	Synthesis (chemical)	6
Vincristine	14	Animalia	7		
Cell Nucleus	13	Antiproliferative Activity	7		
Coordination Complexes	13	Binding Site	7		
Cytology	13	Uraeotyphlus narayani	6		

Funding Sponsors:

Collaboration in research can improve project outcomes and communication. 32 research funding agencies have supported National and International collaborations (Table 5). While research funds greatly determine the success of scientists, it is not always easy to find sources of funding. To help researchers in the search for project grants, we have made a list of more than 30 funding agencies that provide grants for international collaboration. It is evident from the below table that University Grants Commission 32 is emerging as top funding agency, followed by Department of Science and Technology, Ministry of Science and Technology, India for 19 research activities, Department of Science and Technology, Government of Kerala for 15 publications. Bangladesh Council of Scientific and Industrial Research (11). It can be concluded with below analysis that UGC and DST supported most of his research.

Table: 5. Funding agency wise distribution of Publications

Funding Agency	**Rec.**	**Funding Agency**	**Rec.**
University Grants Commission	32	Ministry of Education - Singapore	2
Department of Science and Technology, Ministry of Science and Technology, India	19	Academy of Finland	1
Department of Science and Technology, Government of Kerala	15	Biotechnology and Biological Sciences Research Council	1
Bangladesh Council of Scientific and Industrial Research	11	CLRI Chennai	1
SAP North America	5	Chonbuk National University	1
Science and Engineering Research Board	5	Connecticut State Emergency Response Commission	1

Lee Kong Chian School of Medicine, Nanyang Technological University	4	Council of Scientific and Industrial Research	1
CSIR, India	3	Deutsche Forschungsgemeinschaft	1
DBT, Government of West Bengal	3	Fondation de CoopÃ©ration Scientifique Campus Paris-Saclay	1
Indian National Science Academy	3	Ministerio de Ciencia y TecnologÃa	1
King Saud University	3	National Research Foundation of Korea	1
Bhabha Atomic Research Centre	2	Sigrid JusÃ©liuksen SÃ¤Ã¤tiÃ	1
Indian Council of Agricultural Research	3	University of Madras	1
Indian Council of Medical Research	2	Ã…bo Akademi	1
Kerala State Council for Science, Technology and Environment	2		

Highly Cited papers:

The 24 top high cited papers (listed in the table 6) have received citations 4366 (since their publication till 2020). These 7 papers together have received 1313 citations, registered an average citation per paper of 187.54. 24 papers have received 2484 citations and registered an average citation per paper 103.5. 24 publications are registered with more than 50 Citations at Global level. With respect to Citations count, paper entitled "Mixed-ligand copper(II)-phenolate complexes: Effect of coligand on enhanced DNA and protein binding, DNA cleavage, and anticancer activity, Inorganic Chemistry, Volume 46, Issue 20, 1 October 2007, Pages 8208-8221" stands at the first with 447 Citations, Internationally collaborated (Institut de Chimie, Université de Neuchâtel, Avenue de Bellevaux 51, CH-2007 Neuchâtel, Switzerland), Nationally (National Centre for Biological Science, Tata Institute for Fundamental Research, Bangalore 560 065, India) collaborated paper. Citation benchmarking-97th percentile shows how citations received by this document compare with the average for similar documents and Field-Weighted Citation Impact is 5.29 *sh*ows how well this document is cited when compared to similar documents. A value greater than 1.00 means the document is more cited than expected. *Followed by paper entitle "*Induction of cell death by ternary copper(II) complexes of l-tyrosine and diimines: Role of coligands on DNA binding and cleavage and anticancer activity, Inorganic Chemistry, Volume 48, Issue 4, 16 February 2009, Pages 1309-1322" stands at the second position with 201

citations. Citation benchmarking-97th percentile and Field-Weighted Citation Impact is 5.22.

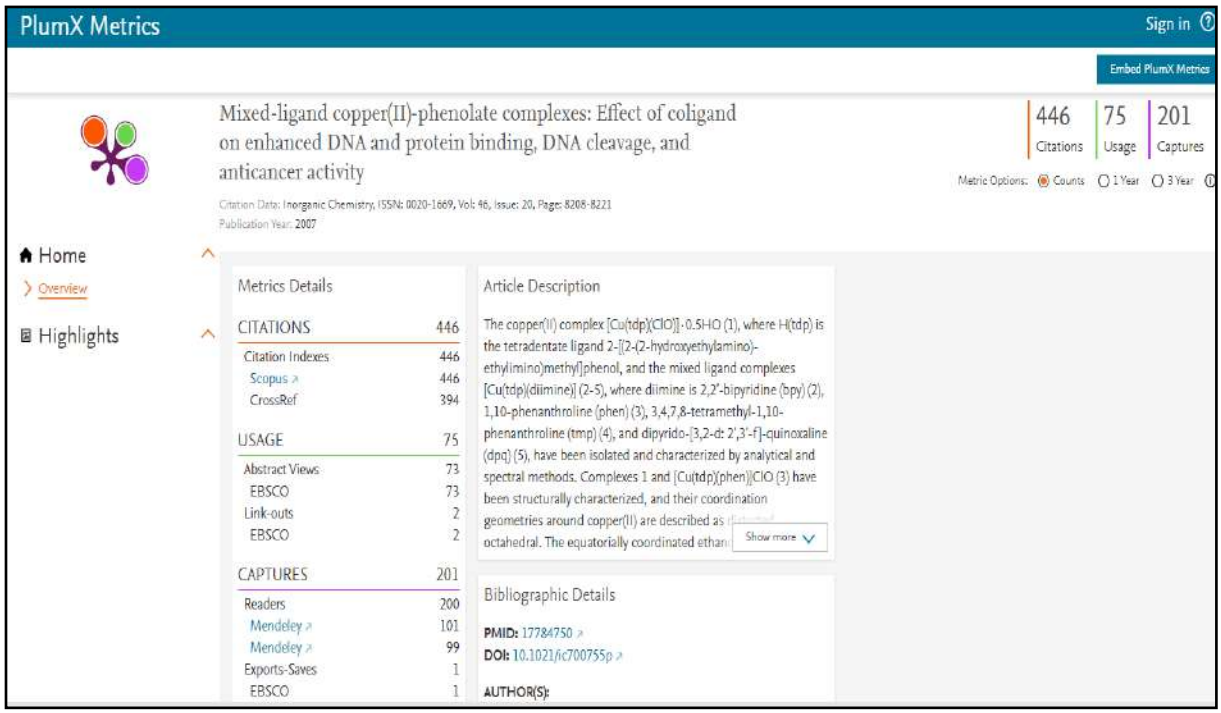


Figure: 5. PlumX Metrics analysis used to evaluate the highly cited paper

Further, PlumX analytics shows the top highly cited paper (446) of Rajendiran et al (2007) had been published in Inorganic Chemistry (IF: 4.825) (Fig. 5). This indicates that publishing article in the high impact factor (IF) journal is one of the major factors with respect to get more citation. PlumX metrics is providing insights into the scholarly significance with individual pieces of research output.

Table: 6. The highly cited papers

S.No	Documents	Citations	<2016	2016	2017	2018	2019	2020	Sub total	>2020	Total
1	Mixed-ligand copper(II)-phenolate complexes: Effect of colig...	2007	243	46	42	38	47	31	204		**446**
2	Induction of cell death by ternary copper(II) complexes of l...	2009	109	20	25	17	22	8	92		**201**
3	Mixed ligand copper(II) complexes of N, N-bis(benzimidazol-2...	2012	59	20	18	19	20	18	95	1	**155**
4	Ternary dinuclear copper(II) complexes of a hydroxybenzamide...	2011	62	14	13	20	18	17	82		**144**
5	Non-covalent DNA binding and cytotoxicity of certain mixed-l...	2008	80	13	13	9	12	4	51	1	**132**
6	New ruthenium(II) arene complexes of anthracenyl-appended di...	2014	15	26	20	19	22	15	102		**117**
7	Influence of nutrient deprivations on lipid accumulation in ...	2012	46	16	12	14	14	14	70	1	**117**
8	Efficient DNA cleavage mediated by mononuclear mixed ligand ...	2012	46	12	11	9	7	7	46		**92**
9	Anticancer mechanism of plumbagin, a natural compound, on no...	2008	50	12	11	9	6	3	41		**91**
10	Chronic chromium exposure-induced changes in testicular hist...	2005	50	9	6	7	3	5	30		**80**
11	Surfactant-cobalt(III) complexes: Synthesis, critical micell...	2009	46	11	6	4	5	5	31		**77**
12	Copper(ii) complexes with 2NO and 3N donor ligands: Synthesi...	2013	27	10	8	12	13	5	48		**75**
13	Interaction of mixed ligand copper(II) complexes with CT DNA...	2013	21	17	10	8	8	7	50	1	**72**
14	A trinuclear zinc-Schiff base complex: Biocatalytic activity...	2014	8	13	19	12	10	7	61		**69**
15	Interaction of rac-[M(diimine)<inf>3</inf>]²⁺ (M ...	2011	32	5	12	6	7	7	37		**69**
16	Antifertility effect of Andrographis paniculata (Nees) in ma...	1990	61	1	2	1	1	1	6		**67**
17	Mixed ligand copper(II) complexes of 1,10-phenanthroline wit...	2014	10	7	11	12	15	11	56		**66**
18	Spermatotoxic effect of aflatoxin B<inf>1</inf> in the albin...	2003	44	3	2	4	8	5	22		**66**
19	Mixed ligand μ-phenoxo-bridged dinuclear copper(ii) complexe...	2014	12	11	9	10	11	10	51		**63**
20	Aspects of the male reproductive toxicity/male antifertility...	2000	43	4	6	2	4	3	19		**62**
21	Synthesis and characterization of nano-hydroxyapatite at amb...	2009	37	2	5	7	3	5	22		**59**
22	DNA binding, prominent DNA cleavage and efficient anticancer...	2011	34	6	4	9	2	3	24		**58**
23	Hepatotoxic effect of ochratoxin A and citrinin, alone and i...	2015	2	8	10	8	14	11	51		**53**
24	Mixed ligand copper(II) complexes of 2,9-dimethyl-1,10-phena...	2014	9	13	5	6	8	11	43		**52**
	Total		**1986**	**461**	**477**	**476**	**512**	**445**	**2371**	**9**	**4366**

FINDINGS AND CONCLUSION

Professor Akbarsha has contributed 184 publications in Life Sciences and other subject domains during 1992-2020, registered 4366 citations and average Citation per Article is 23.73. The most productive countries of collaboration are Saudi Arabia (42), USA with 16, France and Singapore with 6 each, Switzerland with 5, followed by 13 Countries. According to Cite Score Human Reproduction registered with 10.1 (SNIP-2.256, SJR-2.491 and IF-5.72)

is standing at first position followed by Fertility and Sterility with 9.8 Cite Score (SNIP-2.015, SJR-2.31 and IF-5.21) is standing at second position, Archives of Toxicology with 9.6 Cite Score (SNIP-1.550, SJR-1.379 and IF-5.66) is sanding at third position. The study found that the collaborations count (142) with National and International Institutions have raised rapidly in terms of publications and citations.

We conclude that scientometrics plays an important role in the dissemination of a particular scientist whose interest lies in the number of important papers he or she have published. In the above case study, Prof. Mohammad Abdulkader Akbarsha has truly advanced our understanding in subjects dealing with Life Sciences. Citing the number of publications, citations and collaboration details reveal the enormous interest the author has taken to reach his goal as of now. There is no dearth of ideal role model scientists in India. What we lack is a systematic and continuous study on such scientists. Hence, the comment "Most of the developing countries lack local "role models' to motivate other scientists does not hold good at least for India.

REFERENCES

Kalyane, V.L. (1995). Scientometric portrait of P.M. Bhargava. *Lucknow Librarian*, 27(1-4), 42-70.

Poornima, A.; Surulinathi, M. (2019). A Scientometric Study on Yoga Research during 1989-2018. *Asian Journal of Information Science & Technology,* 9(2), 17-22.

Surulinathi, M., **Balasubramani, R., and Amsaveni, N. (2020).** COVID-19 research output in 2020: The Global Perspective using Scientometric Study. *Library Philosophy and Practice* (e-journal), https://digitalcommons.unl.edu/libphilprac/4196.

Sankaralingam, R., Surulinathi, M. and Srinivasaragavan, S. (2020). Indian contribution to Drugs Discovery: A Scientometric Mapping of Publications. *Gedrag and Organisatie Review*, 33(3), 712-734.

Rajagopal, T., Archunan, G., Surulinathi, M., & Ponmanickam, P. (2013). Research output in pheromone biology: a case study of India. *Scientometrics*, 94(2), 711-719.

Advances and Applications in Mathematical Sciences
Volume 20, Issue 5, March 2021, Pages 815-822
© 2021 Mili Publications

ON THE GAUSSIAN INTEGER SOLUTIONS FOR AN ELLIPTIC DIOPHANTINE EQUATION

MANJU SOMANATH[1], K. RAJA[2], J. KANNAN[3] and V. SANGEETHA[4]

[1,2,4]Department of Mathematics
National College
(Autonomous, affiliated to Bharathidasan University)
Trichy 620001, India

[3]Department of Mathematics
Ayya Nadar Janaki Ammal College
(Autonomous, affiliated to Madurai Kamaraj University)
Sivakasi-626124, India
E-mail: jayram.kannan@gmail.com

Abstract

The quadratic Diophantine equation with two unknowns represented by an elliptic curve $DE: 65J^2 + 225K^2 - 230JK = 1600$ is analyzed for its non-zero separate solutions in $Z[i]$. We also gain a few formulae and reappearance relations on the Gaussian integer solutions (J_n, K_n) of DE.

1. Introduction

The Diophantine equation $x^4 \pm y^4 = z^2$ where x, y and z being Gaussian integer were examined by Hilbert. It was proved that there exist only inconsequential solutions in $Z[i]$. Elliptic curves have also been used in [3] to prove that the Diophantine equation $x^3 + y^3 = z^3$ has only trivial solutions in Gaussian integers. These outcomes have motivated us to find non-zero distinct Gaussian integer solutions to a homogenous quadratic Diophantine equation in three variables. Convinced Diophantine problems

2010 Mathematics Subject Classification: 11D09, 11D99.
Keywords: continued fraction expansion, Diophantine equation, Gaussian integer solutions, Pell's equation.
Received January 13, 2020; Accepted May 13, 2020

move towards beginning problems or immediate arithmetical generalizations and others come from geometry in a variety of ways. Swayed Diophantine problems are neither inconsequential nor complicated to analyze [1, 2, 4, 5, 6].

In this manuscript, we look into Gaussian integer solutions of the Diophantine equation $65J^2 + 225K^2 - 230JK = 1600$ which is malformed into a Pell's equation and is solved by a variety of methods.

2. The Diophantine Equation

Think about the Diophantine equation

$$DE : 65J^2 + 225K^2 - 230JK = 1600 \tag{1}$$

to be solved over $Z[i]$. It is not trouble-free to work out and discover the nature and properties of the solutions of (1). So we concern a linear conversion Trs to (1) to shift to a simpler form for which we can find out the integral solutions.

Let

$$Trs : \begin{cases} J = ha + ib \\ K = a + ikb \end{cases} \tag{2}$$

be the shift where $a, b, h, k \in Z$.

Applying to Trs to DE, we get

$$Trs(DE) = D\tilde{E} : 65(ha + ib)^2 + 225(a + ikb)^2$$

$$-230((ha + ib)(a + ikb)) = 1600. \tag{3}$$

Equating the imaginary part to zero and the coefficient of a^2 and b^2 to the smallest amount numeral, we get $h = 2$ and $k = 3$.

Hence for $J = 2a + ib$ and $K = a + i3b$, we have the Diophantine equation

$$\tilde{D}E : a^2 - 56b^2 = 64 \tag{4}$$

which is a Pell equation. Now we try to find all integer solutions (a_n, b_n) of

$\tilde{D}$ and then we can retransfer all outcome as of $\tilde{D}$ to by using the converse of *Trs*.

Theorem 2.1. *Let* $\tilde{D}E$ *be the Diophantine equation in* (4). *Then*

(i) *The continued fraction expansion of* $\sqrt{56}$ *is*

$$\sqrt{56} = \left[7; \overline{2, 14}\right]$$

(ii) *The primary result of* $a^2 - 56b^2 = 1$ *is* $(u_1, v_1) = (15, 2)$

(iii) *For* $n \geq 4$,

$$u_n = 31(u_{n-1} - u_{n-2}) + u_{n-3}$$

$$v_n = 31(v_{n-1} - v_{n-2}) + v_{n-3}$$

Proof.

(i) The continued fraction expansion of $\sqrt{56} = 7 + (\sqrt{56} - 7)$

$$= 7 + \frac{1}{\frac{1}{\sqrt{56} - 7}}$$

$$= 7 + \frac{1}{\frac{\sqrt{56} + 7}{7}}$$

$$= 7 + \frac{1}{2 + \frac{\sqrt{56} - 7}{7}}$$

$$= 7 + \frac{1}{2 + \frac{1}{\sqrt{56} + 7}}$$

$$= 7 + \frac{1}{2 + \frac{1}{14 + (\sqrt{56} - 7)}}.$$

Therefore the continued fraction expansion of $\sqrt{56}$ is

$$\left[7; \overline{2, 14}\right]$$

(ii) Note that by (3), if $(u_1, v_1) = (15, 2)$ is the primary result of $a^2 - 56b^2 = 1$, then the supplementary solutions (u_n, v_n) of $a^2 - 56b^2 = 1$ can be consequently employing the equalities

$(u_n + v_n\sqrt{56}) = (u_1 + \sqrt{56}v_1)^n$ for $n \geq 2$, in supplementary expressions,

$$\begin{pmatrix} u_n \\ v_n \end{pmatrix} = \begin{pmatrix} u_1 & 56v_1 \\ 2 & u_1 \end{pmatrix}^n \begin{pmatrix} 1 \\ 0 \end{pmatrix}.$$

For $n \geq 2$.

Hence it can be given away by generation on n that

$$u_n = 31(u_{n-1} - u_{n-2}) + u_{n-3}$$

and also

$$v_n = 31(v_{n-1} - v_{n-2}) + v_{n-3}$$

for $n \geq 4$.

Now we think about the problem

$$a^2 - 56b^2 = 64.$$

Make a note of that we stand for the integer solutions of $a^2 - 56b^2 = 64$ by (a_n, b_n), and represent the integer solutions of $a^2 - 56b^2 = 1$ by (u_n, v_n). Then we have the subsequent theorem.

Theorem 2.2. *Describe a progression* $\{(a_n, b_n)\}$ *of positive integers by* $(a_1, b_1) = (120, 16)$ *and*

$$a_n = 120u_{n-1} + 896v_{n-1}$$

$$b_n = 16u_{n-1} + 120v_{n-1}.$$

Somewhere $\{(u_n, v_n)\}$ *is a progression of constructive solutions of* $a^2 - 56b^2 = 1$. *Then*

(1) (a_n, b_n) *is a solution of* $a^2 - 56b^2 = 64$ *for any integer* $n \geq 1$.

(2) *For* $n \geq 2$,

$$a_{n+1} = 15a_n + 112b_n$$

$$b_{n+1} = 2a_n + 15b_n.$$

(3) *For* $n \geq 4$

$$a_n = 31(a_{n-1} - a_{n-2}) + a_{n-3}$$

$$b_n = 31(b_{n-1} - b_{n-2}) + b_{n-3}.$$

Proof.

(1) It is without a doubt seen to

$$(a_1, b_1) = (120, 16)$$

is a way out of $a^2 - 56b^2 = 64$ since

$$a_1^2 - 56b_1^2 = (120)^2 - 56(16)^2$$

$$= 64(15^2 - 56(2^2)) = 64(1)$$

$$= 64.$$

A reminder that by description (u_{n-1}, v_{n-1}) is a method out $a^2 - 56b^2 = 1$, of that is,

$$u_{n-1}^2 - 56v_{n-1}^2 = 1. \tag{5}$$

Moreover, we observe as greater than that (a_1, b_1) is a resolution of $a^2 - 42b^2 = 16,$ to is,

$$a_1^2 - 56b_1^2 = 64. \tag{6}$$

Applying (5) and (6), we get

$$a_n^2 - 56b_n^2 = (120u_{n-1} + 896v_{n-1})^2 - 56(16u_{n-1} + 120v_{n-1})^2$$

$$= u_{n-1}^2(2^6) - v_{n-1}^2(2^6(56))$$

$$= 2^6(u_{n-1}^2 - 56v_{n-1}^2)$$

$$= 2^6.$$

Consequently (a_n, b_n) is a way out of $a^2 - 56b^2 = 2^6$.

(2) Bear in mind that

$$a_{n+1} + b_{n+1}\sqrt{d} = (u_1 + v_1\sqrt{d})(a_n + b_n\sqrt{d})$$

Consequently $a_{n+1} = u_1 a_n + v_1 b_n d$ and $b_{n+1} = v_1 a_n + u_1 b_n$.

So

$$a_{n+1} = 15a_n + 112b_n \text{ and } b_{n+1} = 2a_n + 15b_n. \tag{7}$$

Since $u_1 = 15$ and $v_1 = 2$.

(3) Applying the equalities

$$a_n = 2^2(15)u_{n-1} + 2^3(56)v_{n-1} \text{ and } a_{n+1} = 15a_n + 112b_n.$$

We find by generation on n that

$$a_n = 31(a_{n-1} - a_{n-2}) + a_{n-3}$$

for $n \geq 4$. In the same way, it knows how to be given away that

$$b_n = 31(b_{n-1} - b_{n-2}) + b_{n-3}.$$

We saw as on top of that the Diophantine equation DE could be malformed into the $D\widetilde{E}$ via the conversion *Trs*. Also, we showed that $J = 2a + ib$ and $K = a + i3b$. So we can retransfer all results from $\widetilde{DE}$ to by using the converse of *Trs*. Thus we can give the subsequent major theorem.

Theorem 2.3. *Let DE be the Diophantine equation in* (1). *Subsequently*

(1) *The primary solution of DE is* $(J_1, K_1) = (240 + 16i, 120 + 48i)$.

(2) *Describe the progression* $\{(J_n, K_n)\}_{n\geq 1} = \{(2a_n + ib_n, a_n + i3b_n)\}$, *wherever* $\{(a_n, b_n)\}$ *defined in* (7).

Then (J_n, K_n) *is a solution of DE.*

(3) *The explanation* (J_n, K_n) *convince*

$$J_n = 2(15a_{n-1} + 112b_{n-1}) + i(2a_{n-1} + 15b_{n-1})$$

$$K_n = (15a_{n-1} + 112b_{n-1}) + i3(2a_{n-1} + 15b_{n-1}).$$

(4) *The solutions* (J_n, K_n) *satisfy the reappearance relations*

$$J_n = (62(a_{n-1} - a_{n-1}) + 2a_{n-3}) + i(31(b_{n-1} - b_{n-2}) + b_{n-3})$$

$$K_n = (31(a_{n-1} - a_{n-1}) + a_{n-3}) + i(93(b_{n-1} - b_{n-2}) + 3b_{n-3}).$$

Conclusion

Diophantine equations are prosperous in diversity. There is no general method for discovering the entire possible Gaussian integer solutions (if it exists) for Diophantine equations one possibly will explore for additional choices of Diophantine equations to find their equivalent Gaussian integer solutions.

References

[1] C. Baltus, Continued fraction and the Pell equations: The work of Euler and Lagrange, Comm. Anal. Theory Continued Fraction 3 (1994), 4-31.

[2] D. Hensley, Continued Fractions, World Scientific Publishing, Hackensack, N.J., 2006.

[3] D. Hilbert, In Gaussian integer has trivial solutions a new approach, Integer 8 (2008) A32.

[4] E. Barbeau, Pell's equation, Springer Verlag, 2003.

[5] H. P. Edward, Fermat's Last Theorem, Graduate Texts in Mathematics, 50. Springer Verlag, New York, 1996.

[6] H. W. Lenstra, Solving The Pell's equation, Notice of the AMS 49(2) (2002), 182-192.

[7] K. Mathews, The Diophantine equation Expositiones Math. J 1-7.

[8] Manju Somanath and J. Kannan, On A Class of Solutions for a Diophantine equation of Second Degree, International Journal of Pure and Applied Mathematics (IJPAM), 117(12) (2017), 55-62.

[9] Manju Somanath, J. Kannan and K. Raja, Gaussian Integer Solutions of an Infinite Elliptic Cone, International Journal of Science and Research (IJSR), 6(5) (2017).

[10] P. Kaplan and K. S. Williams, Pell's equations and Continued Fractions, Journal of Number Theory 23 (1986), 169-182.

[11] S. P. Arya, On the Brahmagupta-Bhaskara equation, Math. Ed. 8(1) (1991), 23-27.

[12] J. Kannan, Manju Somanath and K. Raja, Solutions of negative Pell equation involving twin prime, JP Journal of Algebra, Number Theory and Applications 40(5) (2018), 869 - 874.

[13] J. Kannan, Manju Somanath and K. Raja, On a class of solutions for a hyperbolic Diophantine equation, International Journal of Applied Mathematics 32(3) (2019), 443-449.

Advances and Applications in Mathematical Sciences
Volume 21, Issue 8, June 2022, Pages 4863-4870
© 2022 Mili Publications, India

SOLUTIONS OF PELL'S EQUATION USING EISENSTEIN PRIMES

MANJU SOMANATH[1], RADHIKA DAS[2] and V. A. BINDU[3]

[1]Assistant Professor
Department of Mathematics
National College, Autonomous
Affiliated to Bharathidasan University, Trichy

[2,3]Assistant Professor
Department of Mathematics
Rajagiri School of Engineering
and Technology, Autonomous, Kochi, India

Abstract

Pell's equation is any Diophantine equation of the form $x^2 - dy^2 = \pm N$, where d is a positive non-square integer and N is any fixed positive integer. In this paper, we search for solutions to the equation $x^2 - 53y^2 = -17^t$, $\forall\ t \in \mathbb{N}$. Here we choose d and N to be Eisenstein primes 53 and 17 respectively and search for the solutions to the equation for different choices of t given by (i) $t = 1$, (ii) $t = 3$, (iii) $t = 5$, (iv) $t = 2k$, (v) $t = 2k + 5$, $\forall\ k \in \mathbb{N}$. Finally the recurrence relations on the solutions are obtained.

1. Introduction

The Pell's equation is any Diophantine equation of the form $x^2 - dy^2 = 1$, where d is a given positive non-square integer and integer solutions are sought for x and y. Pell's equation is named after the Mathematician John Pell. Pell's equation has infinitely many distinct integer solutions.

The Pell's equation discussed here is a negative Pell's equation given as

2020 Mathematics Subject Classification: 11D09.

Keywords: Pell equation, Integer Solutions, Diophantine equations, Eisenstein integers, Eisenstein Primes, Recurrence relation.

Received February 23, 2021; Accepted March 4, 2022

$x^2 - dy^2 = -N$, to be solved in positive integers x and y. In this paper, we have chosen the Eisenstein primes as coefficients in negative Pell's equation to find the positive integer solutions. An Eisenstein prime is an Eisenstein integer of the form $z = a + bw$, where $w = e^{\frac{2\pi i}{3}}$ that is irreducible. An Eisenstein integer is said to be an Eisenstein prime if it satisfies one of the following conditions:

(i) $b = 0$ and $a = p$, where p is a prime with $p \equiv 2 (\mathrm{mod}\, 3)$.

(ii) $a = 0$, $b = p$, where p is a prime with $p \equiv 2 (\mathrm{mod}\, 3)$.

(iii) $N(a + bw) = a^2 - ab + b^2 = p$, where p is a prime such that $p = 3$ or $p \equiv 1 (\mathrm{mod}\, 3)$.

In other words, a prime number p is an Eisenstein prime if it is equal to a natural prime of the form $3p - 1$. In the Pell's equation $x^2 = 53y^2 - 17^t$, $t \in \mathbb{N}$; we use Eisenstein primes 53 and 17 and 2 search for its non-trivial integer solutions. In order to get the solutions, we have approached it with the choices of t given by (i) $t = 1$ (ii) $t = 3$ (iii) $t = 5$ (iv) $t = 2k$ and (v) $t = 2k + 5$.

Using Brahma Gupta lemma, we obtain the sequence of non-zero distinct integer solutions. Also we obtain recurrence relations on the solution.

2. Preliminaries

Theorem 2.1 [2]. *If* (x_1, y_1) *is the fundamental solution of* $x^2 - dy^2 = 1$. *Then every positive solutions of the equation is given by* (x_n, y_n) *where* y_n *and* x_n *are the integers determined from*

$$x_n + y_n\sqrt{d} = (x_1 + y_1\sqrt{d})^n, \text{ for } n = 1, 2, 3, \ldots$$

2.1 [12] Testing the solubility of the negative Pell equation

Suppose D is a positive integer, not a perfect square. Then the negative Pell equation $x^2 - Dy^2 = 1$. is soluble if and only if D is expressible as

$D = a^2 + b^2$, $\gcd(a, b) = 1$, a and b positive, b is odd and the Diophantine equation $bV^2 + 2aVW + bW^2 = 1$ has a solution. (The case of solubility occurs for exactly one such (a, b)).

The Algorithm

(i) Find all expressions of D as a sum of two relatively-prime squares using Cornacchia's method. If none, exist - the negative Pell equation is not solvable.

(ii) For each representation $D = a^2 + b^2$, $\gcd(a, b) = 1$, a and b positive, b odd, test the solubility of $-bV^2 + 2aVW + bW^2 = 1$ using the Lagrange-Matthews algorithm. If soluble, exist - the negative Pell equation is solvable.

(iii) If each representation yields no solution, then the negative Pell equation is insolvable.

Theorem 2.2 [6]**.** *Let p be a prime. The negative Pell's equation $x^2 - py^2 = -1$ is solvable if and only if $p = 2$ or $p \equiv 1 (\mathrm{mod}\, 4)$.*

This paper deals with a negative Pell equation

$$x^2 = 53y^2 - 17^t,\ t \in \mathbb{N}$$

For this particular equation, we consider the prime $p = 53$, which satisfies the conditions of Theorem 2.2. Therefore, we can substantiate the proof that the negative Pell's equation $x^2 = 53y^2 - 17^t$, $t \in \mathbb{N}$ is solvable in integers.

Using the Algorithm as in 2.1 and testing $(a, b) = (2, 7)$:

$-bV^2 + 2aVW + bW^2 = 1$ has a solution $(V, W) = (-3, 4)$, so $x^2 - 53y^2 = -1$ is soluble.

3. Method of Analysis

3.1. Choice 1. $t = 1$.

The Pell equation is

$$x^2 = 53y^2 - 17 \tag{1}$$

Let (x_0, y_0) be the initial solution of (1) given by

$$x_0 = 6; y_0 = 1$$

To find the other solutions of (1), consider the more general Pell equation

$$x^2 = 53y^2 + 1 \tag{2}$$

whose initial solution is (66249,9100) and the general solution (x_n, y_n) is given by theorem 2.1 as

$$x_n = \frac{1}{2} f_n$$

$$y_n = \frac{1}{2\sqrt{73}} g_n$$

where $f_n = (66249 + 9100\sqrt{53})^{n+1} + (66249 + 9100\sqrt{53})^{n+1}$

$g_n = (66249 + 9100\sqrt{53})^{n+1} + (66249 + 9100\sqrt{53})^{n+1}$, $n = 0, 1, 2\ldots$

Applying Brahma Gupta lemma between (x_0, y_0) and (x_n, y_n) the sequence of non-zero distinct integer solutions to (1) are obtained as

$$x_{n+1} = x_0 x_n + d y_0 y_n, y_{n+1} = x_0 y_n + y_0 x_n \tag{3}$$

$$x_{n+1} = \frac{1}{2}[6f_n + \sqrt{53} g_n]$$

$$y_{n+1} = \frac{1}{2\sqrt{53}}[\sqrt{53} f_n + 6f_n] \tag{4}$$

The recurrence relations satisfied by the solutions of (1) are given by

$$x_{n+2} - 132498 x_{n+1} + x_n = 0$$

$$y_{n+2} - 132498 y_{n+1} + y_n = 0 \tag{5}$$

3.2 Choice 2. $t = 3$

The Pell equation is

$$x^2 = 53y^2 - 17^3 \tag{6}$$

Let (x_0, y_0) be the initial solution of (6) given by

$$x_0 = 102; y_0 = 17$$

Applying Brahma Gupta lemma between (x_0, y_0) and (x_n, y_n) the sequence of non-zero distinct integer solutions to (6) are obtained by equation (3) as

$$x_{n+1} = \frac{1}{2}[102f_n + 17\sqrt{53}g_n]$$

$$y_{n+1} = \frac{1}{2}[17\sqrt{53}f_n + 102g_n] \tag{7}$$

The recurrence relations satisfied by the solutions of (6) are given by

$$x_{n+2} = 132498x_{n+1} + x_n = 0$$

$$y_{n+2} = 132498y_{n+1} + y_n = 0 \tag{8}$$

3.3 Choice 3. $t = 5$

The Pell equation is

$$x^2 = 53y^2 - 17^5 \tag{9}$$

Let (x_0, y_0) be the initial solution of (9) given by

$$x_0 = 1734; y_0 = 289$$

Applying Brahma Gupta lemma between (x_0, y_0) and (x_n, y_n) the sequence of non-zero distinct integer solutions to (9) are obtained by equation (3) as

$$x_{n+1} = \frac{1}{2}[1734f_n + 289\sqrt{53}g_n]$$

$$y_{n+1} = \frac{1}{\sqrt{53}}[289\sqrt{53}f_n + 1734g_n] \tag{10}$$

The recurrence relations satisfied by the solutions of (9) are given by

$$x_{n+2} - 132498x_{n+1} + x_n = 0$$

$$y_{n+2} - 132498y_{n+1} + y_n = 0 \tag{11}$$

3.4 Choices 4. $t = 2k,\ k \in \mathbb{N}$

The Pell equation is

$$x^2 = 53y^2 - 17^{2k},\ k \in \mathbb{N} \tag{12}$$

Let $(x_0,\ y_0)$ be the initial solution of (12) given

$$x_0 = 182(17)^k;\ y_0 = 25(17)^k$$

Applying Brahma Gupta lemma between $(x_0,\ y_0)$ and (x_n, y_n) the sequence of non-zero distinct integer solutions to (12) are obtained by equation (3) as

$$x_{n+1} = \frac{17^k}{2}\left[182f_n + 25\sqrt{53}g_n\right]$$

$$y_{n+1} = \frac{17^k}{2\sqrt{53}}\left[25\sqrt{53}f_n + 182g_n\right] \tag{13}$$

The recurrence relations satisfied by the solutions of (12) are given by

$$x_{n+2} - 132498x_{n+1} + x_n = 0$$

$$y_{n+2} - 132498y_{n+1} + y_n = 0 \tag{14}$$

3.5 Choices 5. $t = 2k,\ 5,\ k \in \mathbb{N}$

The Pell equation is

$$x^2 = 53y^2 - 17^{2k+5},\ k \in \mathbb{N} \tag{15}$$

Let $(x_0,\ y_0)$ be the initial solution of (15) given by

$$x_0 = 29478(17)^{k-1};\ y_0 = 4913(17)^{k-1}$$

Applying Brahma Gupta lemma between $(x_0,\ y_0)$ and (x_n, y_n) the sequence of non-zero distinct integer solutions to (15) are obtained by

equation (3) as

$$x_{n+1} = \frac{17^{k-1}}{2}[2947f_n + 4913\sqrt{53}g_n]$$

$$y_{n+1} = \frac{17^{k-1}}{2\sqrt{53}}[4913\sqrt{53}f_n + 29478g_n] \quad (16)$$

The recurrence relations satisfied by the solutions of (15) are given by

$$x_{n+2} - 132498x_{n+1} + x_n = 0$$

$$y_{n+2} - 132498y_{n+1} + y_n = 0 \quad (17)$$

4. Conclusion

Solving a negative Pell's equation involving the Eisenstein primes has provided a powerful tool for finding solutions of equations of similar types. It is possible to determine the solvability of Pell-like equation using current methods.

References

[1] Ivan Niven, H. S. Zuckerman and H. L. Montgomery, An introduction to The Theory of Numbers, Fifth Edition, John Wiley and Sons, Inc, New York, 1991.

[2] David M. Burton, Elementary Number Theory, Tata McGraw-Hill Edition, 2012.

[3] Ahmet Tekcan, Betul Gezer and Osman Bizin, On the integer solutions of the Pell equation $x^2 - dy^2 = 2^t$, World Academy Science Engineering and Technology 1 (2007), 522-526.

[4] V. Sangeetha, M. A. Gopalan and Manju Somanath, On the integer solutions of the Pell equation $x^2 = 13y^2 - 3^t$, International Journal of Applied Mathematical Research 3(1) (2014), 58-61.

[5] K. Matthews, The Diophantine equations $x^2 = Dy^2 - N$, $D > 0$, Expositiones Math. 18 (2000), 363-369.

[6] Titu Andreescu, Dorin Andrica and Ion Cucurezeanu, An Introduction to Diophantine Equations, A problem based Approach, Birkahasuser Verlag, New York, 2010.

[7] Andre Weil, Number theory, An approach through history, From Hammurapito Legendre Boston (Birkahasuser Boston), 1984.

[8] Tituandreescu, Dorin Andrica, An introduction to Diophantine equations, Springer Publishing House, 2002.

[9] L. Euler, Elements of Algebra, Springer, New York, 1984.

[10] L. U. Mordell, Diophantine Equations, Academic Press, New York, 1969.

[11] H. W. Lenstra Jr., Solving the Pell equation, Notices of the American Mathematical Society 49(2) (2002), 182-192.

[12] Kenneth Hardy, Kenneth S. Williams, Pacific Journal of Mathematics 124 (1986).

IJIRSET
International Journal of Innovative Research in
SCIENCE | ENGINEERING | TECHNOLOGY

e-ISSN: 2319-8753 | p-ISSN: 2320-6710

INTERNATIONAL JOURNAL OF INNOVATIVE RESEARCH

IN SCIENCE | ENGINEERING | TECHNOLOGY

Volume 11, Issue 5, May 2022

INTERNATIONAL STANDARD SERIAL NUMBER INDIA

Impact Factor: 8.118

9940 572 462 | 6381 907 438 | ijirset@gmail.com | www.ijirset.com

International Journal of Innovative Research in Science, Engineering and Technology (IJIRSET)

| e-ISSN: 2319-8753, p-ISSN: 2320-6710| www.ijirset.com | Impact Factor: 8.118|

||Volume 11, Issue 5, May 2022||

| DOI:10.15680/IJIRSET.2022.1105165 |

Lattice Points for the Quadratic Diophantine Equation $21(x^2+y^2)-19xy=84z^2$

Manju Somanath [1], T.Anupreethi [2], V.Sangeetha [3]

Assistant Professor, Department of Mathematics, National College, Trichy, Tamil Nadu, India.[1,3]

Research Scholar, Department of Mathematics, National College, Trichy, Tamil Nadu, India.[2]

ABSTRACT: The ternary quadratic diophantine equation $21(x^2+y^2)-19xy=84z^2$ is analysed for its non-zero integral solutions. Different patterns of solutions are obtained by employing the methods of factorization, cross multiplication and the concept of double equations.

KEYWORDS: Diophantine equation, integral solutions, lattice points.

I. INTRODUCTION

The Diophantine equations are equations involving only sums, products and powers in which all the constants are integers and the only solutions of interest are integers. For example,
$3x+7y=1$ *or* $x^2-y^2=z^2$ where x, y *and* z are integers. Named in honour of the 3rd century Greek Mathematician Diophantus of Alexandria, these equations were first systematically solved by Indian Mathematicians beginning with Aryabhata. Diophantine equations fall into three classes: those with no solutions, those with only finitely many solutions and those with infinitely many solutions. Many methods are available to solve these types of equations but there is no universal method to determine whether the solutions to diophantine equation exists or not.
In this paper, an interesting quadratic diophantine equation with three unknowns
$21(x^2+y^2)-19xy=84z^2$ is discussed and solved for its non-zero integral solutions. Different patterns of solutions are obtained by employing various methods.

II. METHOD OF ANALYSIS

The ternary quadratic diophantine equation to be solved for its non-zero distinct integral solution is $21(x^2+y^2)-19xy=84z^2$……(1). The introduction of the linear transformations
$x=u+v,\ y=u-v, u\neq 0, v\neq 0$ …….. (2) reduces (1) to $23u^2+61v^2=84z^2$ …….. (3). Different patterns of solutions of (1) are presented below.

| e-ISSN: 2319-8753, p-ISSN: 2320-6710| www.ijirset.com | Impact Factor: 8.118|

||Volume 11, Issue 5, May 2022||

| DOI:10.15680/IJIRSET.2022.1105165 |

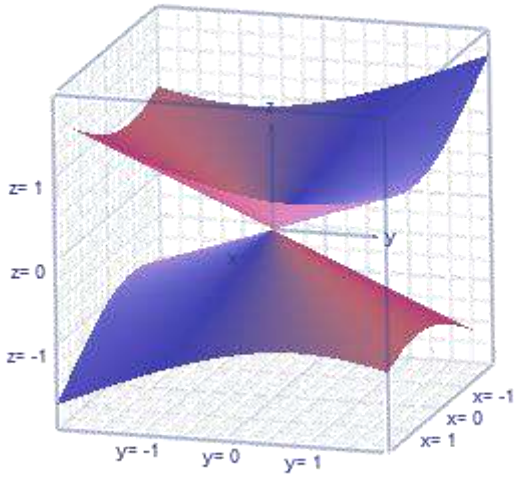


Figure 1: Pictorial representation of the equation

Choice I: The complex number factorization of 84 is employed to obtain two patterns of solutions.

Pattern-1:

Consider 84 as 84 $= \left(\sqrt{23} + i\sqrt{61}\right)\left(\sqrt{23} - i\sqrt{61}\right)$……..(4) and assuming the value of z as

$z = 23a^2 + 61b^2$ ……..(5) for the non-zero integers a and b.

The substitution of (4) and (5) in (3) yields

$\sqrt{23}u+i\sqrt{61}v = \left(\sqrt{23} + i\sqrt{61}\right)\left(\sqrt{23}a + i\sqrt{61}b\right)^2$ ……. (6)

On equating the real and imaginary parts of (6) we get

$u = 23a^2 - 61b^2 - 122ab$ and $v = 23a^2 - 61b^2 + 46ab$

From these values, we can find the non-zero integral solutions of (1) as

$x = 46a^2 - 122b^2 - 76ab;\ y = -168ab;\ z = 23a^2 + 61b^2.$

Pattern-2:

The choice of 84 as 84=$\left(-\sqrt{23} + i\sqrt{61}\right)\left(-\sqrt{23} - i\sqrt{61}\right)$ and $z = 23a^2 + 61b^2$

in (3) and following the method as above, we get the integral solutions of (1) as

$x = -168ab;\ y = -46a^2 + 122b^2 - 76ab;\ z = 23a^2 + 61b^2.$

Choice II: Two different patterns of solutions are derived from the irrational number factorization of 61 and 23.

Pattern-3:

Consider 61 as 61 $= \left(\sqrt{84} + \sqrt{23}\right)\left(\sqrt{84} - \sqrt{23}\right)$ …….. (7) and assuming the value of v *as*

| e-ISSN: 2319-8753, p-ISSN: 2320-6710| www.ijirset.com | Impact Factor: 8.118|

||Volume 11, Issue 5, May 2022||

| DOI:10.15680/IJIRSET.2022.1105165 |

$v = 84a^2 - 23b^2$ …….. (8) for the non-zero integers a and b.

The substitution of (7) and (8) in (3) yields

$\sqrt{84}z + \sqrt{23}u = \left(\sqrt{84} + \sqrt{23}\right)\left(\sqrt{84}a + \sqrt{23}b\right)^2$ …….. (9)

On equating the irrational parts of (9), we get

$z = 84a^2 + 23b^2 + 46ab$ and $u = 84a^2 + 23b^2 + 168ab$

From these values, we can find the non-zero integral solutions of (1) as

$x = 168a^2 + 168ab$; $y = 46b^2 + 168ab$; $z = 84a^2 + 23b^2 + 46ab$.

Pattern-4:

The choice of 23 as $23 = \left(\sqrt{84} + \sqrt{61}\right)\left(\sqrt{84} - \sqrt{61}\right)$ and assuming $u = 84a^2 - 61b^2$ in (3) and following the method as above, we get the integral solutions of (1) as $x = 168a^2 + 168ab$; $y = -122b^2 - 168ab$; $z = 84a^2 + 61b^2 + 122ab$.

Choice III: Employing the method of cross multiplication, we have computed the integral solutions of (1) in 6 different patterns.

Pattern-5:

Consider (3) as $23(u^2 - z^2) = 61(z^2 - v^2)$ …….(10) which can be written in the ratio form as

$\frac{23(u+z)}{61(z+v)} = \frac{(z-v)}{(u-z)} = \frac{\alpha}{\beta}$, $\beta \neq 0$. which is equivalent to the following two equations

$-\alpha u - \beta v + z(\alpha + \beta) = 0$; $23u\beta - 61\alpha v + z(23\beta - 61\alpha) = 0$

Solving the above equations, we get

$u = 61\alpha^2 - 23\beta^2 + 122\alpha\beta$; $v = -61\alpha^2 + 23\beta^2 + 46\alpha\beta$; $z = 61\alpha^2 + 23\beta^2$

From these values, we can find non-zero integral solutions to (1) as

$x = 168\alpha\beta$; $y = 122\alpha^2 - 46\beta^2 + 76\alpha\beta$; $z = 61\alpha^2 + 23\beta^2$.

Following the above process, we get the different patterns of integral solutions to (1) and are presented below.

Table: 1 System of equations and its solutions

Pattern	System Of Equations	Solutions
6	$23\beta u - \alpha v + z(23\beta - \alpha) = 0$ $-\alpha u - 61\beta v + z(61\beta + \alpha) = 0$	$x = -168\alpha\beta$ $y = -2\alpha^2 + 2806\beta^2 - 76\alpha\beta$ $z = -\alpha^2 - 1403\beta^2$

| e-ISSN: 2319-8753, p-ISSN: 2320-6710| www.ijirset.com | Impact Factor: 8.118|

||Volume 11, Issue 5, May 2022||

| DOI:10.15680/IJIRSET.2022.1105165 |

7	$23u\beta - \alpha v - z(23\beta + \alpha) = 0$ $-\alpha u - 61\beta v + z(61\beta - \alpha) = 0$	$x = 2\alpha^2 - 2806\beta^2 - 76\alpha\beta$ $y = -168\alpha\beta$ $z = -\alpha^2 - 1403\beta^2$
8	$23\beta u - 61\alpha v - z(23\beta + 61\alpha) = 0$ $-\alpha u - v\beta + z(\beta - \alpha) = 0$	$x = 122\alpha^2 - 46\beta^2 - 76\alpha\beta$ $y = -168\alpha\beta$ $z = -61\alpha^2 - 23\beta^2$
9	$\beta u - \alpha v - z(\beta + \alpha) = 0$ $-23\alpha u - 61\beta v + z(61\beta - 23\alpha) = 0$	$x = 46\alpha^2 - 122\beta^2 - 76\alpha\beta$ $y = -168\alpha\beta$ $z = -23\alpha^2 - 61\beta^2$
10	$\beta u - 61\alpha v - z(61\alpha + \beta) = 0$ $-23\alpha u - v\beta + z(\beta - 23\alpha) = 0$	$x = 2806\alpha^2 - 2\beta^2 - 76\alpha\beta$ $y = -168\alpha\beta$ $z = -1403\alpha^2 - \beta^2$

Choice IV: Applying linear transformations and solving the corresponding equation in the concept of double equations, we have yet another patterns of integral solutions to (1).

Pattern-11:

Consider $z = X + 61T,\ v = X + 84T$……..(11) reduces (3) to $X^2 = u^2 + 5124T^2$ …….. (12)

which is satisfied by $T = 2mn;\ X = m^2 + 5124n^2;\ u = m^2 - 5124n^2$ …….. (13)

The substitution of (13) in (11) yields, $z = m^2 + 5124n^2 + 122mn;\ u = m^2 - 5124n^2;$

$v = m^2 + 5124n^2 + 168mn$. From these values, we can find non-zero integral solutions to (1)

as $x = 2m^2 + 168mn;\ y = -10248n^2 - 168mn;\ z = m^2 + 5124n^2 + 122mn.$

Equation (12) can be expressed as a system of double equations in which the factorization employed is given in the following table.

Table: 2 Factorization for double equations

System	$X + u$	$X - u$
1	$7T^2$	732
2	732	$7T^2$
3	732T	7T
4	7T	732T
5	$3T^2$	1708
6	1708	$3T^2$
7	1708T	3T
8	3T	1708T

| e-ISSN: 2319-8753, p-ISSN: 2320-6710| www.ijirset.com | Impact Factor: 8.118|

||Volume 11, Issue 5, May 2022||

| DOI:10.15680/IJIRSET.2022.1105165 |

9	4	$1281T^2$
10	$1281T^2$	4
11	1281T	4T
12	4T	1281T
13	$21T^2$	244
14	244	$21T^2$
15	244T	21T
16	21T	244T
17	$427T^2$	12
18	427T	12T
19	12T	427T
20	28T	183T
21	183T	28T
22	28	$183T^2$
23	$61T^2$	84
24	$42T^2$	122
25	122T	42T
26	122	$42T^2$
27	42	$122T^2$
28	5124T	T

Solving each of the system of double equations and using (2) and (11), the corresponding integral solutions to (1) are found and they are presented below.

System:1 $x = 168k + 28k^2; y = -168k - 732; z = 366 + 122k + 14k^2$.

System:2 $x = 168k + 732; y = -168k - 28k^2; z = 366 + 122k + 14k^2$.

System:3 $x = 1632k; y = -182k; z = 861k$.

System:4 $x = 182k; y = -1632k; z = 861k$.

System:5 $x = 168k + 12k^2; y = -1708 - 168k; z = 854 + 122k + 6k^2$.

System:6 $x = 1708 + 168k; y = -168k - 12k^2; z = 854 + 122k + 6k^2$.

System:7 $x = 3584k; y = -174k; z = 1833k$.

System:8 $x = 174k; y = -3584k; z = 1833k$.

System:9 $x = 4 + 168k; y = -168k - 5124k^2; z = 2 + 122k + 2562k^2$.

System:10 $x = 5124k^2 + 168k; y = -4 - 168k; z = 2 + 122k + 2562k^2$.

System:11 $x = 5460k; y = -352k; z = 2814k$.

System:12 $x = 176k; y = -2730k; z = 1407k$.

System:13 $x = 168k + 84k^2; y = -244 - 168k; z = 122 + 122k + 42k^2$.

System:14 $x = 336k + 244; y = -336k - 336k^2; z = 122 + 244k + 168k^2$.

| e-ISSN: 2319-8753, p-ISSN: 2320-6710| www.ijirset.com | Impact Factor: 8.118|

||Volume 11, Issue 5, May 2022||

| DOI:10.15680/IJIRSET.2022.1105165 |

System:15 $x = 656k; y = -210k; z = 387k$.

System:16 $x = 420k; y = -1312k; z = 774k$.

System:17 $x = 336k + 6832k^2; y = -12 - 336k; z = 6 + 244k + 3416k^2$.

System:18 $x = 1022k; y = -192k; z = 561k$.

System:19 $x = 384k; y = -2044k; z = 1122k$.

System:20 $x = 224k; y = -534k; z = 333k$.

System:21 $x = 1068k; y = -448k; z = 666k$.

System:22 $x = 336k + 28; y = -336k - 2928k^2; z = 14 + 244k + 1464k^2$.

System:23 $x = 168k + 244k^2; y = -168k - 84; z = 42 + 122k + 122k^2$.

System:24 $x = 672k^2 + 336k; y = -122 - 336k; z = 366k^2 + 244k + 61$.

System:25 $x = 824k; y = -504k; z = 572k$.

System:26 $x = 168k + 122; y = -168k^2 - 168k; z = 61 + 122k + 84k^2$.

System:27 $x = 42 + 336k; y = -336k - 1952k^2; z = 21 + 244k + 976k^2$.

System:28 $x = 10416k; y = -170k; z = 5247k$.

Pattern-12:

Consider $z = X + 23T; u = X + 84T$,reduces (3) to $X^2 = v^2 + 1932T^2$……..(14)

and following the method as above, we get the integral solutions of (1) as

$x = 2m^2 + 168mn;\ \ y = 3864n^2 + 168mn;\ \ z = m^2 + 1932n^2 + 46mn.$

Equation (14) can be expressed as a system of double equations in which the factorization employed is given in the following table.

Table: 3 Factorization for double equations

System	$X + v$	$X - v$
1	$23T^2$	84
2	84	$23T^2$
3	23T	84T
4	84T	23T
5	$161T^2$	12
6	12	$161T^2$
7	12T	161T
8	161T	12T
9	92	$21T^2$
10	21T	92T
11	92T	21T

| e-ISSN: 2319-8753, p-ISSN: 2320-6710| www.ijirset.com | Impact Factor: 8.118|

||Volume 11, Issue 5, May 2022||

| DOI:10.15680/IJIRSET.2022.1105165 |

12	$69T^2$	28
13	28	$69T^2$
14	28T	69T
15	69T	28T
16	$483T^2$	4
17	4	$483T^2$
18	276T	7T
19	644T	3T
20	T^2	1932
21	1932T	T

Solving each of the system of double equations, the corresponding integral solutions to (1) are given below.

System:1 $x = 92k^2 + 168k; y = 84 + 168k; z = 46k^2 + 46k + 42$.

System:2 $x = 84 + 336k; y = 368k^2 + 336k; z = 184k^2 + 92k + 42.$

System:3 $x = 214k;\ y = 336k;\ z = 153k$.

System:4 $x = 672k; y = 428k; z = 306k$.

System:5 $x = 644k^2 + 168k; y = 12 + 168k; z = 322k^2 + 46k + 6.$

System:6 $x = 12 + 336k; y = 2576k^2 + 336k; z = 1288k^2 + 92k + 6.$

System:7 $x = 192k; y = 490k; z = 219k.$

System:8 $x = 980k;\ y = 384k; z = 438k.$

System:9 $x = 92 + 168k; y = 84k^2 + 168k; z = 42k^2 + 46k + 46.$

System:10 $x = 210k; y = 352k; z = 159k.$

System:11 $x = 704k; y = 420k; z = 318k.$

System:12 $x = 276k^2 + 168k; y = 28 + 168k; z = 138k^2 + 46k + 14.$

System:13 $x = 28 + 336k; y = 1104k^2 + 336k; z = 552k^2 + 92k + 14.$

System:14 $x = 224k; y = 306k; z = 143k.$

System:15 $x = 612k; y = 448k; z = 286k.$

System:16 $x = 1932k^2 + 168k; y = 4 + 168k; z = 966k^2 + 46k + 2.$

System:17 $x = 4 + 336k; y = 7728k^2 + 336k; z = 3864k^2 + 92k + 2.$

System:18 $x = 720k; y = 182k; z = 329k.$

System:19 $x = 1456k; y = 174k; z = 693k.$

System:20 $x = 16k^2 + 336k; y = 1932 + 336k; z = 966 + 92k + 8k^2.$

| e-ISSN: 2319-8753, p-ISSN: 2320-6710| www.ijirset.com | Impact Factor: 8.118|

||Volume 11, Issue 5, May 2022||

| DOI:10.15680/IJIRSET.2022.1105165 |

System:21 $x = 4032k; y = 170k; z = 1979k$.

III. CONCLUSION

Quadratic diophantine equations in two, three or four unknowns is very important in the study of diophantine equations. Various methods are available to solve diophantine equations. This paper emphasizes infinite number of solutions to a particular diophantine equation in three unknowns.

REFERENCES

[1] Andre Weil, Number Theory: An Approach through History, From Hammurapito to Legendre, Bikahsuser, Boston, 1987.
[2] Bibhotibhusan Batta and Avadhesh Narayanan Singh, History of Hindu Mathematics, Asia Publishing House, 1983.
[3] Boyer. C.B., History of Mathematics, John Wiley & sons lnc., New York, 1968.
[4] L.E. Dickson, History of Theory of Numbers, Vol.2, Chelsea Publishing Company, New York, 1952.
[5] L.J.Mordel, Diophantine Equations, Academic Press, New York, (1969).
[6] Tituandreescu, Dorin Andrica, "An introduction to Diophantine equations" Springer Publishing House, 2002.
[7] Manju Somanath, J. Kannan, K. Raja, "Integral solutions of an infinite elliptic cone", International Journal of Innovative Research in science, Engineering and Technology, 5(10) (2016), 17549-17557.
[8] Manju Somanath, J. Kannan and K. Raja, "On Polynomial Solutions of Quadratic Equation", International Journal of Mathematics and its Applications (IJMAA), 5(4) (2017), 839-844.
[9] Manju Somanath, J. Kannan and K. Raja, "On A Class of Solutions for a Diophantine Equation of Second Degree", International Journal of Pure and Applied Mathematics, 117 (12) (2017), 55-62.
[10] J. Kannan, Manju Somanath and K. Raja, "Solutions of Negative Pell Equation Involving Twin Prime", JP Journal of Algebra, Number Theory and Applications, 40 (5) (2018), 869-874.
[11] Manju Somanath, K. Raja, J. Kannan and M. Mahalakshmi, " On A Class Of Solutions For A Quadratic Diophantine Equation", Advances and Applications in Mathematical Sciences, 19 (11) (2020), 1097-1103.
[12] M.A. Gopalan, J. Kannan, Manju Somanath and K. Raja , "Integral Solutions of an Infinite Elliptic Cone", International Journal of Innovative Research in Science, Engineering and Technology, (IJIRSET), 5(10) (2016), 17551-17557.
[13] Manju Somanath, K. Raja, J. Kanna and B. Jeyashree, "Non Trivial Integral Solutions Of Ternary Quadratic Diophantine Equation", Advances and Applications in Mathematical Sciences, 19(11) (2020), 1105-1112.
[14] J. Kannan, Manju Somanath and K. Raja, "On a Class of Solutions for the Hyperbolic Diophantine Equation", International Journal of Applied Mathematics, 32(03) (2019), 443-449.
[15] Manju Somanath, J. Kannan and K. Raja, "On the Positive Integer Solutions for a Diophantine Equation", Journal of Mathematics and Informatics, 10 (2017), 173-177.
[16] Manju Somanath and J. Kannan, "Congruum Problem", International Journal of Pure and Applied Mathematical Sciences (IJPAMS), 9(2) (2016), 123-131.

Impact Factor: 8.118

INTERNATIONAL JOURNAL OF INNOVATIVE RESEARCH

IN SCIENCE | ENGINEERING | TECHNOLOGY

9940 572 462 6381 907 438 ijirset@gmail.com

www.ijirset.com

Scan to save the contact details

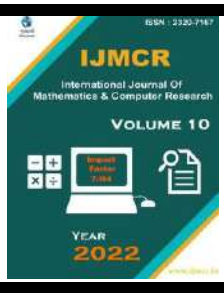
International Journal of Mathematics and Computer Research

ISSN: 2320-7167

Volume 10 Issue 02 February 2022, Page no. – 2555-2558

Index Copernicus ICV: 57.55, Impact Factor: 7.184

DOI: 10.47191/ijmcr/v10i2.01



Remodeled RSA Algorithm for Messages of Length Two Employing G- Primes

J. Kannan[1], Manju Somanath[2], M. Mahalakshmi[3], K. Raja[4]

[1,3]Department of Mathematics, Ayya Nadar Janaki Ammal College (Autonomous, affiliated to Madurai Kamaraj University, Madurai), Sivakasi – 626 124, Tamil Nadu, India.

[2,4]Department of Mathematics, National College (Autonomous, affiliated to Bharathidasan University, Trichy), Trichy- 620001.

ARTICLE INFO

01 February 2022

Corresponding Author:
J. Kannan

ABSTRACT

Now - a- days, the exchange of sensitive information, such as credit card numbers, over the internet is common practice. Protecting data and electronic systems is crucial to our way of living. Cryptography deals with the study of communication over a channel may not be secure and problems related with them. One such well known algorithm is RSA algorithm. This paper displays the classical RSA algorithm and modifies the work in generation of keys. There will be two assignments for the alphabets. Here the only interest is on messages with two letters.

KEYWORDS: RSA Algorithm; Remodeled RSA algorithm; Encryption decryption algorithm; Gaussian primes; Cryptography.

I. INTRODUCTION

Cryptography is a concept of protecting information and conversations which are transmitted through a public source, so that the intended persons only read and process it. There are several encryption and decryption algorithm which involves mathematical concepts to provide more security to the text which has to be shared through a medium.

RSA algorithm is a well- known asymmetric cryptography algorithm which uses public and private keys. Public key is visible to all but private key is used by intended persons only. The idea of this algorithm depends on the fact that factorization of large integers into primes is hard. The larger the chosen primes, the more is the security. Initially, an example dealing RSA algorithm was provided.

Already there are some works regarding the Gaussian integer application on RSA algorithm [1,2,3]. Motivated by them, this work was built. The main idea in this paper is to just remodel the classical RSA algorithm by using Gaussian primes. Making use of Gaussian primes with large real and imaginary parts will leads to a difficulty in factorizing. Also for the process of key generation, assignment based on Gaussian primes is employed whereas for encryption, usual alphabet assignment is used. Furthermore, the differences between both the algorithms were provided. This paper was developed mainly for sharing two lettered messages.

II. PRELIMINARIES

Euler ϕ function on $\mathbb{N}$**:** Euler phi function $\varphi(n)$ is defined on natural numbers which counts the numbers which are less than n and prime to it.

1. $\varphi(n) = |\{k \in \mathbb{N}: k < n \text{ and } (k,n) = 1\}|$.
2. For a prime p, $\varphi(p) = p - 1$.
3. If $n = pq$ and $(p,q) = 1$, then $\varphi(n) = (p-1)(q-1)$.

Gaussian integers:

Gaussian integers are complex numbers $z = a + ib$ where a, b are integers and it is denoted by $\mathbb{Z}[i]$. The norm of $z = a + ib$ is given by $N(a + ib) = a^2 + b^2$.

Gaussian primes (G- primes):

$z = a + ib$ is a Gaussian prime if one of the following holds:

1. If $a \neq 0, b \neq 0$, then $a + ib$ is a Gaussian prime if and only if $N(a + ib) = a^2 + b^2$ is an integer prime.
2. If $a \neq 0$, then bi is a Gaussian prime if $|b|$ is an integer prime and $|b| \equiv 3(mod\ 4)$.
3. If $b \neq 0$, then a is a Gaussian prime if $|a|$ is an integer prime and $|a| \equiv 3(mod\ 4)$.

Note: If p is an integer prime such that $p \equiv 1(mod\ 4)$, then $p = x^2 + y^2$. Thus $x + iy, x < y$ is a Gaussian prime corresponding to p.

A. RSA Algorithm

Generating public key:

1. Select two integer primes p and q.
2. Find n such that $n = pq$ and $\varphi(n)$.
3. Choose e such that $1 < e < \varphi(n)$ and $\gcd(e, \varphi(n)) = 1$.
4. The public key is given by (n, e)

Generating private key:

The private key is d which is found from $ed \equiv 1(mod\ \varphi(n))$.

Encryption:

1. Consider the two lettered message which have to be sent. Convert the letters to numbers by assigning $1 - 26$ for $A - Z$ and take it as m.
2. Solve $c \equiv m^e(mod\ n)$for c. This c is the encrypted data.

Decryption:

1. Solve $m \equiv c^d(mod\ n)$ for m which is the decrypted data.
2. Convert the digits to alphabets.

All paragraphs must be indented as well as justified, i.e. both left-justified and right-justified.

Example 1:

Consider the message "HI".

Generating public key**:**

1. Let $p = 61$ and $q = 71$.
2. $n = pq = 4331$, $\varphi(n) = 4200$.
3. Choose $e = 11$.

The public key is (4200,11).

Generating private key:

$$ed \equiv 1\big(mod\ \varphi(n)\big) \Longrightarrow 11d \equiv 1(mod\ 4200)$$
$$\Longrightarrow d = 2291.$$

Encryption:

1. The message to be sent is "*HI*". From its positions, one can see that $m = 89$.
2. $c \equiv m^e(mod\ n) \Longrightarrow c \equiv 89^{11}(mod\ 4331)$
$$\Longrightarrow c = 1802.$$

Decryption:

1. $m \equiv c^d(mod\ n) \Longrightarrow m \equiv 1802^{2291}(mod\ 4331)$
$\Longrightarrow m = 89$.
2. Converting it to alphabets one can get "*HI*".

III. REMODELED RSA ALGORITHM FOR TWO LETTERED MESSAGES

Here is a little modified RSA algorithm which employs Gaussian primes. Some places of RSA algorithm is modified and this algorithm is created to improve the secrecy of messages. The discussion is made only for messages of length 2. Before entering into the algorithm, the differences of both algorithms are presented.

S. No	RSA Algorithm	Modified RSA Algorithm
1.	For a message to be sent, there is only one assignment of digits which is made only on encryption phase.	There are two assignments for messages. One is made when generating public key and other happens on encryption phase.
2.	e is chosen such that it is relatively prime to $\varphi(n)$	$e$is chosen such that it is relatively prime to $\varphi\big(N(n)\big)$ where N is the norm of Gaussian integer.
3.	Modulo n is used on both encryption and decryption.	Modulo $N(n)$, ie., norm of n is used on encryption and decryption.

B. Assignments:

Assignment – 1:

This is used in public key generation. Let $\{p_n\}$ be the sequence of all integer primes in ascending order. Make a short- term assignment by fixing any 26 elements in $\{p_n\}$to $A - Z$. If the assigned prime p is of the form $4k + 3$, the alphabet is given the name $p + 0i$. If the assigned prime p is of the form $4k + 1$, then $p = x^2 + y^2$. So name the alphabet as $x + iy$ where $x < y$.

Assignment – 2:

This is used on encryption phase. It is the usual assignment for $A\ to\ Z$ as $1\ to\ 26$.

C. Modified RSA Algorithm:

Generating public key:

1. Assign positions of alphabets by Assignment – 1.
2. Consider two G- primes p and q such that p and q are the positions of alphabets on the message to be sent based on Assignment – 1.
3. Find n such that $n = pq$and N(n). Also find $\varphi\big(N(n)\big)$.
4. Choose e such that $1 < e < \varphi\big(N(n)\big)$ and gcd $\Big(e, \varphi\big(N(n)\big)\Big) = 1$.

The public key is given by (n, e).

Generating private key:

The private key is d which is found from $ed \equiv 1\Big(mod\ \varphi\big(N(n)\big)\Big)$.

Encryption

1. Consider the two lettered message which have to be sent. Convert the letters to numbers based on Assignment - 2and take it as m.
2. Solve $c \equiv m^e(mod\ N(n))$for c. This c is the encrypted data.

Decryption

1. Solve $m \equiv c^d (\text{mod } N(n))$ for m which is the decrypted data.
2. Convert the digits to alphabets based on Assignment – 2.

Example 2:

Consider the message "*HI*"

Generating public key:

1. Suppose the short- term assignment is taken as

2	3	5	7	11	13	17	19	23	29	31	37	41
A	B	C	D	E	F	G	H	I	J	K	L	M
N	O	P	Q	R	S	T	U	V	W	X	Y	Z
43	47	53	59	61	67	71	73	79	83	89	97	101

This gives Assignment 1.

2.

A	**B**	**C**	**D**	**E**
$1+i$	$3+0i$	$1+2i$	$7+0i$	$11+0i$
F	**G**	**H**	**I**	**J**
$2+3i$	$1+4i$	$19+0i$	$23+0i$	$2+5i$
K	**L**	**M**	**N**	**O**
$31+0i$	$1+6i$	$4+5i$	$43+0i$	$47+0i$
P	**Q**	**R**	**S**	**T**
$2+7i$	$59+0i$	$5+6i$	$67+0i$	$71+0i$
U	**V**	**W**	**X**	**Y**
$3+8i$	$79+0i$	$83+0i$	$5+8i$	$4+9i$
Z				
$1+0i$				

Choose $p = 19 + 0i$ and $q = 23 + 0i$.

3. $n = pq = 437 + 0i$, $N(n) = 437^2 = 190969$.
   $\varphi(N(n)) = \varphi(190969) = 173052$.
4. Choose $e = 7$.

The public key is $(n, e) = (437 + 0i, 7)$.

Generating private key:

$$ed \equiv 1 \left(mod\ \varphi(N(n))\right) \Rightarrow 7d \equiv 1(mod\ 73052)$$
$$\Rightarrow d = 98887$$

Encryption:

1. The message to be sent is "*HI*". From its positions (Assignment-2), one can see that $m = 89$.
2. $c \equiv m^e (mod\ N(n)) \Rightarrow c \equiv 89^7 (mod\ 190969)$
   $\Rightarrow c = 117620$

Decryption:

1. $m \equiv c^d (mod\ N(n))$
   $\Rightarrow m \equiv 117620^{98887} (mod\ 190969) \Rightarrow m = 89$
2. Converting it to alphabets one can get "*HI*"

Example 3:

Consider the message "*TN*".

Generating public key:

1. Suppose the short- term assignment is taken as

3	7	13	19	29	37	43	53	61	71
A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
K	**L**	**M**	**N**	**O**	**P**	**Q**	**R**	**S**	**T**
79	89	101	107	113	131	139	151	163	173
191	197	2	7	11	17				
U	**V**	**W**	**X**	**Y**	**Z**				

2. Assignment -1 as

A	**B**	**C**	**D**
$3+0i$	$\mathbf{7+0i}$	$\mathbf{2+3i}$	$\mathbf{19+0i}$
D	**F**	**G**	**H**
$2+5i$	$1+6i$	$43+0i$	$2+7i$
I	**J**	**K**	**L**
$5+6i$	$71+0i$	$79+0i$	$5+8i$
M	**N**	**O**	**P**
$1+10i$	$107+0i$	$163+0i$	$2+13i$
Q	**R**	**S**	**T**
$191+0i$	$1+14i$	$1+i$	$1+2i$
U	**V**	**W**	**X**
$11+0i$	$1+4i$		
Y	**Z**		

Choose $p = 2 + i13$ and $q = 107 + 0i$.

3. $n = pq = 214 + 139i, N(n) = 65117$.
   $\varphi(N(n)) = \varphi(65117) = 60096$.
4. Choose $e = 5$.

The public key is $(214 + 139i, 5)$.

Generating private key:

$$ed \equiv 1 \left(mod\ \varphi(N(n))\right) \Rightarrow 5d \equiv 1(mod\ 60096)$$
$$\Rightarrow d = 48077$$

Encryption:

1. The message to be sent is "*TN*". From its positions (AS-2), one can see that $m = 2014$.

2. $c \equiv m^e (mod\ N(n)) \Rightarrow c \equiv 2014^5 (mod\ 65117)$
   $\Rightarrow c = 11751$

Decryption:

1. $m \equiv c^d (mod\ N(n)) \Rightarrow m \equiv 11751^{48077} (mod\ 65117)$
   $\Rightarrow m = 2014$.

2. Converting it to alphabets one can get "*TN*".

IV. CONCLUSION

In this paper, the classical RSA algorithm was displayed along with an illustration. Then its remodeled form is

provided with the same example which was dealt previously. Decryption is difficult in large cases since the prime factorization is hard. Here the concentration is only on messages of length two. One can initiate to extend this algorithm for messages of any finite length.

REFERENCES

1. Elkamchouchi, H., Elshenawy, K., &Shaban, H. 2002, Extended RSA cryptosystem and digital signature schemes in the domain of Gaussian integers. In The 8th International Conference on Communication Systems, 2002, 91-95.
2. El-Kassar, A. N., Haraty, R. A., Awad, Y. A., & Debnath, N. C. 2005, Modified RSA in the Domains of Gaussian Integers and Polynomials Over Finite Fields. In CAINE 298-303.
3. Manju Somanath, J. Kannan and K. Raja, 2017 "On a Class of Solutions for a Diophantine Equation of Second Degree", International Journal of Pure and Applied Mathematics, 117(12), 55 – 62.
4. Manju Somanath and J. Kannan, 2016 "Congruum Problem", International Journal of Pure and Applied Mathematical Sciences (IJPAMS), 9(2), 123-131.
5. Manju Somanath, K. Raja, J. Kannan and M. Mahalakshmi, "On A Class of Solutions for A Quadratic Diophantine Equation", Advances and Applications in Mathematical Sciences, 19(11), 1097 – 1103.
6. Manju Somanath, J. Kannan and K. Raja, 2016 "Lattice Points of an Infinite Cone $x^2 + y^2 = (\alpha^{2n} + \beta^{2n})z^2$", International Journal of Mathematical Trends and Technology, 38(2), 95 - 98.
7. Manju Somanath, K. Raja, J. Kannan and V. Sangeetha, "On the Gaussian Integer Solutions for an Elliptic Diophantine Equation", Advances and Applications in Mathematical Sciences, 20(5), 2021, 815 – 822.
8. Manju Somanath, K. Raja, J. Kannan and B. Jeyashree, "Non Trivial Integral Solutions of Ternary Quadratic Diophantine Equation", Advances and Applications in Mathematical Sciences, 19(11), 2020, 1105 – 1112.
9. Manju Somanath, J. Kannan and K. Raja, 2017 "On Polynomial Solutions of Quadratic Equation", International Journal of Mathematics and its Applications (IJMAA), 5(4 – F), 839 – 844.
10. Pradhan, S., & Sharma, B. K. 2014, A modified variant of RSA algorithm for Gaussian integers. In Proceedings of the Second International Conference on Soft Computing for Problem Solving (SocProS 2012), December 28-30, 2012 (pp. 183-187). Springer, New Delhi.
11. Trappe, W., & Washington, L. C. 2006, Introduction to Cryptography. Prentice Hall.

iJRASET
International Journal For Research in Applied Science and Engineering Technology

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 10 **Issue:** I **Month of publication:** January 2022

DOI: https://doi.org/10.22214/ijraset.2022.40138

www.ijraset.com

Call: 08813907089 | E-mail ID: ijraset@gmail.com

International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.538
Volume 10 Issue I Jan 2022- Available at www.ijraset.com

Cryptographic Algorithm Based on Prime Assignment

M. Mahalakshmi[1], J. Kannan[2], Manju Somanath[3], K. Raja[4], K. Kaleeswari[5]
[1, 2, 5]*Department of Mathematics, Ayya Nadar Janaki Ammal College (Autonomous), Sivakasi, India*
[3, 4]*Department of Mathematics, National College (Autonomous, affiliated to Bharathidasan University), Trichy, India*

Abstract: ***Cryptography is a concept of protecting information and conversations which are transmitted through a public source, so that the send and receiver only read and process it. There are several encryption and decryption algorithm which involves mathematical concepts to provide more security to the text which has to be shared through a medium. In this paper, an algorithm is provided for both coding and decoding using cyclic symmetric matrices. Also Euler totient function, prime numbers are employed here. Furthermore, algorithm using prime number in integers is extended to prime numbers in Gaussian integers. This concept increases the security of the text.***
Keywords: Cryptography; encryption- decryption algorithms; Gaussian primes; cyclic symmetric matrix. 2010 MSC Subject Classifications - 11T71, 11C20, 11A25, 11R04.

I. INTRODUCTION

For centuries, people have sent secret messages by various means. But some messages were not maintained secretly as there was no proper security. In order to maintain secrecy, cryptography was developed. It is the process of converting ordinary plain text (message to be sent) into some unintelligible text and vice versa. It helps to transmit data in a particular form so that the intended persons can read and process it. It is also useful for user authentication.

In olden days, an algorithm in cryptography was based on concepts which are well- known by all. But now- a- days, it is mainly based on mathematical theory and computer applications. Especially, number theory is playing a vital role in it, which employs the concepts such as congruence, Euler's theorem.

In modern days, one can make use of any mathematical concepts to make their algorithm. As much as mathematics imposed, as much as security increases. Motivated by [3], this work aims to propose an algorithm to improve the security based on things such as prime numbers, Euler phi function, cyclic symmetric matrices. By modifying the assignments of alphabets in [3], this work is developed.

This paper involves two algorithms. First one uses integer prime assignment whereas second one uses Gaussian prime assignment. For the second case, Euler phi function on Gaussian integers $\varphi_{\mathbb{Z}[i]}$ is employed.

Common notations and definitions:

1) $W_i = i^{th}$ word in the message to be sent

2) $\eta(W_i) =$ no. of letters in W_i

3) $E(\eta(W_i)) = \begin{cases} k_i = \frac{\eta(W_i)+1}{2}, if\ \eta(W_i)\ is\ odd \\ k_i = \frac{\eta(W_i)}{2}, \quad if\ \eta(W_i) is\ even \end{cases}$

4) $I_i =$ Identity matrix of order i

5) $A_i = k_i^{th}$ row of cyclic matrix M_i

6) $D(A_i) =$ diagonal matrix with values in A_i

II. ALGORITHM BASED ON INTEGER PRIMES

A. *Euler phi Function on* $\mathbb{N}$

1. Euler phi function $\varphi(n)$ is defined on natural numbers which counts the numbers which are less than n and prime to it. ie.,$\varphi(n) = |\{k \in \mathbb{N}: k < n \text{ and } (k,n) = 1\}|$
2. For a prime p, $\varphi(p) = p - 1$

B. *Algorithm for Encryption*

Assign the first 26 prime numbers to the alphabets. i.e.,

2	3	5	7	11	13	17	19	23	29	31	37	41
A	B	C	D	E	F	G	H	I	J	K	L	M
N	O	P	Q	R	S	T	U	V	W	X	Y	Z
43	47	53	59	61	67	71	73	79	83	89	97	101

The algorithm is defined as follows:

1) Let the message to be encrypted be $W_1, W_2, \dots, W_n$ (separated as words and omitting spaces).
2) For all $1 \le i \le n$, assign each letters in W_i with the above defined table.
3) Apply φ for each assigned values in W_i.
4) Construct a cyclic symmetric matrix for each W_i separately.
5) Calculate $E(\eta(W_i))$, for all $1 \le i \le n$
6) Find the k_i^{th} row from the matrix and construct $A_i, 1 \le i \le n$
7) Evaluate $D(A_i) - \eta(W_i)I_{\eta(W_i)}, 1 \le i \le n$, which is the encrypted key. (For each word it is separated by commas)

C. *Algorithm for Decryption*

1) Let $B_i = D(A_i) - \eta(W_i)I_{\eta(W_i)}, 1 \le i \le n$
2) Calculate $C_i = D(B_i) + \eta(B_i)I_{\eta(B_i)}, 1 \le i \le n$
3) Find $E(\eta(B_i)) = k_i, 1 \le i \le n$

This gives that first digit of C_i is the k_i^{th} letter of the i^{th} word.
Now, the second digit of C_i is the $(k_i + 1)^{th}$ letter of i^{th} word and so on (This works on cyclic order).

4) Rewrite C_i as in the above order.
5) For every digit in C_i, add 1.
6) Convert the digits into alphabets.

a) Example 1: Let us work on the word "HELLO"

- *Encryption*

➢ Let W_1 be the word $HELLO$
➢ Assign the positions of the letters.

H	E	L	L	O
19	11	37	37	47

➢ Apply φ

H	E	L	L	O
18	10	36	36	46

➢ The cyclic symmetric matrix is

$$M = \begin{pmatrix} 18 & 10 & 36 & 36 & 46 \\ 10 & 36 & 36 & 46 & 18 \\ 36 & 36 & 46 & 18 & 10 \\ 36 & 46 & 18 & 10 & 36 \\ 46 & 18 & 10 & 36 & 36 \end{pmatrix}$$

- Here $\eta(W_1) = 5$, which is odd.
  Thus, $E(\eta(W_1)) = k_1 = \frac{5+1}{2} = 3$
- The 3^{rd} row of M forms A_1.
  i.e.,$A_1 = (36\ 36\ 46\ 18\ 10)$
- $$D(A_1) - \eta(W_1)I_{\eta(W_1)} = \begin{pmatrix} 36 & 0 & 0 & 0 & 0 \\ 0 & 36 & 0 & 0 & 0 \\ 0 & 0 & 46 & 0 & 0 \\ 0 & 0 & 0 & 18 & 0 \\ 0 & 0 & 0 & 0 & 10 \end{pmatrix} - \begin{pmatrix} 5 & 0 & 0 & 0 & 0 \\ 0 & 5 & 0 & 0 & 0 \\ 0 & 0 & 5 & 0 & 0 \\ 0 & 0 & 0 & 5 & 0 \\ 0 & 0 & 0 & 0 & 5 \end{pmatrix}$$
  $$= \begin{pmatrix} 31 & 0 & 0 & 0 & 0 \\ 0 & 31 & 0 & 0 & 0 \\ 0 & 0 & 41 & 0 & 0 \\ 0 & 0 & 0 & 13 & 0 \\ 0 & 0 & 0 & 0 & 5 \end{pmatrix}$$
  The encrypted key is 31 31 41 13 5.

- *Decryption*
- $B_1 = D(A_1) - \eta(W_1)I_{\eta(W_1)}$
  $= (31\ 31\ 41\ 13\ 5)$
- $C_1 = D(B_1) + \eta(B_1)I_{\eta(B_1)}$
  $= (36\ 36\ 46\ 18\ 10)$
- $E(\eta(B_1)) = \frac{5+1}{2} = 3$
  So first digit of C_1 is the 3^{rd} letter in W_1.
  The order is

36	36	46	18	10
3^{rd}	4^{th}	5^{th}	1^{st}	2^{nd}

- Thus the rewritten form is (18 10 36 36 46)
- Adding 1, one can get (19 11 37 37 47), which corresponds to the word "HELLO".

b) Example 2: Let us consider "WORK HARD"

- *Encryption*
- Let W_1 be the word $WORK$ and W_2 be $HARD$
- Assign the positions of the letters.

W	O	R	K
83	47	61	31

H	A	R	D
19	2	61	7

- Apply φ

W	O	R	K
82	46	60	30

H	A	R	D
18	1	60	6

- The cyclic symmetric matrix is

$$M_1 = \begin{pmatrix} 82 & 46 & 60 & 30 \\ 46 & 60 & 30 & 82 \\ 60 & 30 & 82 & 46 \\ 30 & 82 & 46 & 60 \end{pmatrix} \text{and} M_2 = \begin{pmatrix} 18 & 1 & 60 & 6 \\ 1 & 60 & 6 & 18 \\ 60 & 6 & 18 & 1 \\ 6 & 18 & 1 & 60 \end{pmatrix}$$

- Here $\eta(W_1) = \eta(W_2) = 4$, which is even.

  Thus, $E(\eta(W_1)) = E(\eta(W_2)) = k_1 = k_2 = \frac{4}{2} = 2$

- The 2^{nd} row of M_1 forms A_1 and 2^{nd} row of M_2forms A_2.

  i.e., $A_1 = (46\ 60\ 30\ 82)$ and $A_2 = (1\ 60\ 6\ 18)$

- $$D(A_1) - \eta(W_1)I_{\eta(W_1)} = \begin{pmatrix} 46 & 0 & 0 & 0 \\ 0 & 60 & 0 & 0 \\ 0 & 0 & 30 & 0 \\ 0 & 0 & 0 & 82 \end{pmatrix} - \begin{pmatrix} 4 & 0 & 0 & 0 \\ 0 & 4 & 0 & 0 \\ 0 & 0 & 4 & 0 \\ 0 & 0 & 0 & 4 \end{pmatrix}$$

$$= \begin{pmatrix} 42 & 0 & 0 & 0 \\ 0 & 56 & 0 & 0 \\ 0 & 0 & 26 & 0 \\ 0 & 0 & 0 & 78 \end{pmatrix}$$

$$D(A_2) - \eta(W_2)I_{\eta(W_2)} = \begin{pmatrix} 1 & 0 & 0 & 0 \\ 0 & 60 & 0 & 0 \\ 0 & 0 & 6 & 0 \\ 0 & 0 & 0 & 18 \end{pmatrix} - \begin{pmatrix} 4 & 0 & 0 & 0 \\ 0 & 4 & 0 & 0 \\ 0 & 0 & 4 & 0 \\ 0 & 0 & 0 & 4 \end{pmatrix}$$

$$= \begin{pmatrix} -3 & 0 & 0 & 0 \\ 0 & 56 & 0 & 0 \\ 0 & 0 & 2 & 0 \\ 0 & 0 & 0 & 14 \end{pmatrix}$$

The encrypted key is $42\ 56\ 26\ 78, -3\ 56\ 2\ 14$.

- *Decryption*
- $B_1 = D(A_1) - \eta(W_1)I_{\eta(W_1)}$

$$= (42\ 56\ 26\ 78)$$

$$B_2 = D(A_2) - \eta(W_2)I_{\eta(W_2)} = (-3\ 56\ 2\ 14)$$

- $C_1 = D(B_1) + \eta(B_1)I_{\eta(B_1)}$

$$= (46\ 60\ 30\ 82)$$

$$C_2 = D(B_2) + \eta(B_2)I_{\eta(B_2)}$$

$$= (1\ 60\ 6\ 18)$$

- $E(\eta(B_1)) = E(\eta(B_2)) = \frac{4}{2} = 2$

  So first digit of C_1 is the 2^{nd} letter in W_1 and first digit of C_2 is 2^{nd} letter in W_2.

  The order is

46	60	30	82
2^{nd}	3^{rd}	4^{th}	1^{st}

1	60	6	18
2^{nd}	3^{rd}	4^{th}	1^{st}

- Thus the rewritten form is $(82\ 46\ 60\ 30)$ and $(18\ 1\ 60\ 6)$
- Adding 1, one can get $(83\ 47\ 61\ 31)$ and $(19\ 2\ 61\ 7)$, which corresponds to the words "WORK" and "HARD"

III. ALGORITHM BASED ON GAUSSIAN PRIMES

A. Gaussian Integers

Gaussian integers are complex numbers $z = a + ib$ where a, b are integers and it is denoted by $\mathbb{Z}[i]$. The norm of $z = a + ib$ is given by $N(a + ib) = a^2 + b^2$.

B. Gaussian Primes

$z = a + ib$is a Gaussian prime if one of the following holds:

- If $a \neq 0, b \neq 0$, then $a + ib$ is a Gaussian prime if and only if $N(a + ib) = a^2 + b^2$ is an integer prime.
- If $a \neq 0$, then bi is a Gaussian prime if $|b|$ is an integer prime and $|b| \equiv 3(mod\ 4)$.
- If $b \neq 0$, then a is a Gaussian prime if $|a|$ is an integer prime and $|a| \equiv 3(mod\ 4)$.

Note

If p is an integer prime such that $p \equiv 1(mod\ 4)$, then $p = x^2 + y^2$. Thus $x + iy, x < y$ is a Gaussian prime corresponding to p.

Euler phi function on $\mathbb{Z}[i]$

- In [1], $\varphi_{\mathbb{Z}[i]}(a + ib)$ is defined as the number of Gaussian integers which are invertible modulo $a + ib$.
- If $p + qi$ is a Gaussian prime, then $\varphi_{\mathbb{Z}[i]}(p + qi) = N(p + qi) - 1 = p^2 + q^2 - 1$

Algorithm for encryption.

Assign the Gaussian prime numbers to the alphabets. i.e.,

$1 + i$	$3 + 0i$	$1 + 2i$	$7 + 0i$	$11 + 0i$	$2 + 3i$
A	B	C	D	E	F
$1 + 4i$	$19 + 0i$	$23 + 0i$	$2 + 5i$	$31 + 0i$	$1 + 6i$
G	H	I	J	K	L
$4 + 5i$	$43 + 0i$	$47 + 0i$	$2 + 7i$	$59 + 0i$	$5 + 6i$
M	N	O	P	Q	R
$67 + 0i$	$71 + 0i$	$3 + 8i$	$79 + 0i$	$83 + 0i$	$5 + 8i$
S	T	U	V	W	X
$4 + 9i$	$1 + 10i$				
Y	Z				

The algorithm is defined as follows:

1) Let the message to be encrypted be $W_1, W_2, \dots, W_n$ (separated as words and omitting spaces).

2) For all $1 \leq i \leq n$, assign each letters in W_i with the above defined table.

3) Apply $\varphi_{\mathbb{Z}[i]}$ for each assigned values in W_i.

4) Construct a cyclic symmetric matrix for each W_i separately.

5) Calculate $E(\eta(W_i))$, for all $1 \leq i \leq n$

6) Find the k_i^{th} row from the matrix and construct $A_i, 1 \leq i \leq n$

7) Evaluate $D(A_i) - \eta(W_i)I_{\eta(W_i)}, 1 \leq i \leq n$, which is the encrypted key. (For each word it is separated by commas)

Algorithm for decryption

a) Let $B_i = D(A_i) - \eta(W_i)I_{\eta(W_i)}, 1 \leq i \leq n$

b) Calculate $C_i = D(B_i) + \eta(B_i)I_{\eta(B_i)}, 1 \leq i \leq n$

c) Find $E(\eta(B_i)) = k_i, 1 \leq i \leq n$

This gives that first digit of C_i is the k_i^{th} letter of the i^{th} word.

Now, the second digit of C_i is the $(k_i + 1)^{th}$ letter of i^{th} word and so on (This works on cyclic order).

d) Rewrite C_i as in the above order.

e) For every digit in C_i, add1 and then observethat the remaining is in the form $x^2 + y^2$ so write it as $x + iy$ where $0 < x < y$ and if $y = 0$, write it as $x + 0i$.

f) Convert the digits into alphabets.

Example 3

Let us consider "WORK HARD"

- *Encryption*
- Let W_1 be the word $WORK$ and W_2 be $HARD$
- Assign the positions of the letters.

W	O	R	K
$83 + 0i$	$47 + 0i$	$5 + 6i$	$31 + 0i$

H	A	R	D
$19 + 0i$	$1 + i$	$5 + 6i$	$7 + 0i$

- Apply $\varphi_{\mathbb{Z}[i]}$

W	O	R	K
6888	2208	60	960

H	A	R	D
360	1	60	48

- The cyclic symmetric matrix is

$$M_1 = \begin{pmatrix} 6888 & 2208 & 60 & 960 \\ 2208 & 60 & 960 & 6888 \\ 60 & 960 & 6888 & 2208 \\ 960 & 6888 & 2208 & 60 \end{pmatrix} \text{and} M_2 = \begin{pmatrix} 360 & 1 & 60 & 48 \\ 1 & 60 & 48 & 360 \\ 60 & 48 & 360 & 1 \\ 48 & 360 & 1 & 60 \end{pmatrix}$$

- Here $\eta(W_1) = \eta(W_2) = 4$, which is even.

  Thus, $E(\eta(W_1)) = E(\eta(W_2)) = k_1 = k_2 = \frac{4}{2} = 2$

- The 2^{nd} row of M_1 forms A_1 and 2^{nd} row of M_2forms A_2.

  i.e., $A_1 = (2208 \quad 60 \quad 960 \quad 6888)$ and $A_2 = (1 \quad 60 \quad 48 \quad 360)$

- $$D(A_1) - \eta(W_1) I_{\eta(W_1)} = \begin{pmatrix} 2208 & 0 & 0 & 0 \\ 0 & 60 & 0 & 0 \\ 0 & 0 & 960 & 0 \\ 0 & 0 & 0 & 6888 \end{pmatrix} - \begin{pmatrix} 4 & 0 & 0 & 0 \\ 0 & 4 & 0 & 0 \\ 0 & 0 & 4 & 0 \\ 0 & 0 & 0 & 4 \end{pmatrix}$$

$$= \begin{pmatrix} 2204 & 0 & 0 & 0 \\ 0 & 56 & 0 & 0 \\ 0 & 0 & 956 & 0 \\ 0 & 0 & 0 & 6884 \end{pmatrix}$$

$$D(A_2) - \eta(W_2) I_{\eta(W_2)} = \begin{pmatrix} 1 & 0 & 0 & 0 \\ 0 & 60 & 0 & 0 \\ 0 & 0 & 48 & 0 \\ 0 & 0 & 0 & 360 \end{pmatrix} - \begin{pmatrix} 4 & 0 & 0 & 0 \\ 0 & 4 & 0 & 0 \\ 0 & 0 & 4 & 0 \\ 0 & 0 & 0 & 4 \end{pmatrix}$$

$$= \begin{pmatrix} -3 & 0 & 0 & 0 \\ 0 & 56 & 0 & 0 \\ 0 & 0 & 44 & 0 \\ 0 & 0 & 0 & 356 \end{pmatrix}$$

The encrypted key is $2204 \;\; 56 \;\; 956 \;\; 6884, -3 \;\; 56 \;\; 44 \;\; 356$

- *Decryption*

➢ $B_1 = D(A_1) - \eta(W_1)I_{\eta(W_1)}$

$= (2204\ 56\ 956\ 6884)$

$B_2 = D(A_2) - \eta(W_2)I_{\eta(W_2)}$

$= (-3\ 56\ 44\ 356)$

➢ $C_1 = D(B_1) + \eta(B_1)I_{\eta(B_1)}$

$= (2208\ 60\ 960\ 6888)$

$C_2 = D(B_2) + \eta(B_2)I_{\eta(B_2)}$

$= (1\ 60\ 48\ 360)$

➢ $E(\eta(B_1)) = E(\eta(B_2)) = \frac{4}{2} = 2$

So first digit of C_1 is the 2^{nd} letter in W_1 and first digit of C_2 is 2^{nd} letter in W_2.

The order is

2208	60	960	6888
2^{nd}	3^{rd}	4^{th}	1^{st}

1	60	48	360
2^{nd}	3^{rd}	4^{th}	1^{st}

➢ Thus the rewritten form is $(6888\ 2208\ 60\ 960)$ and $(360\ 1\ 60\ 48)$.

➢ Adding 1, one can get $(6889\ 2209\ 61\ 961)$and $(361\ 2\ 61\ 49\ )$, which can be written as $(83^2 47^2 5^2 + 6^2 31^2)$ and $(1^2 + 1^2 5^2 + 6^2 7^2 19^2)$. Hence one can get $(83 + 0i\ \ 47 + 0i\ \ 5 + 6i\ \ 31 + 0i)$ and $(19 + 0i\ \ 1 + i\ \ 5 + 6i\ \ 7 + 0i\ )$ which corresponds to the words "WORK" and "HARD".

IV. CONCLUSION

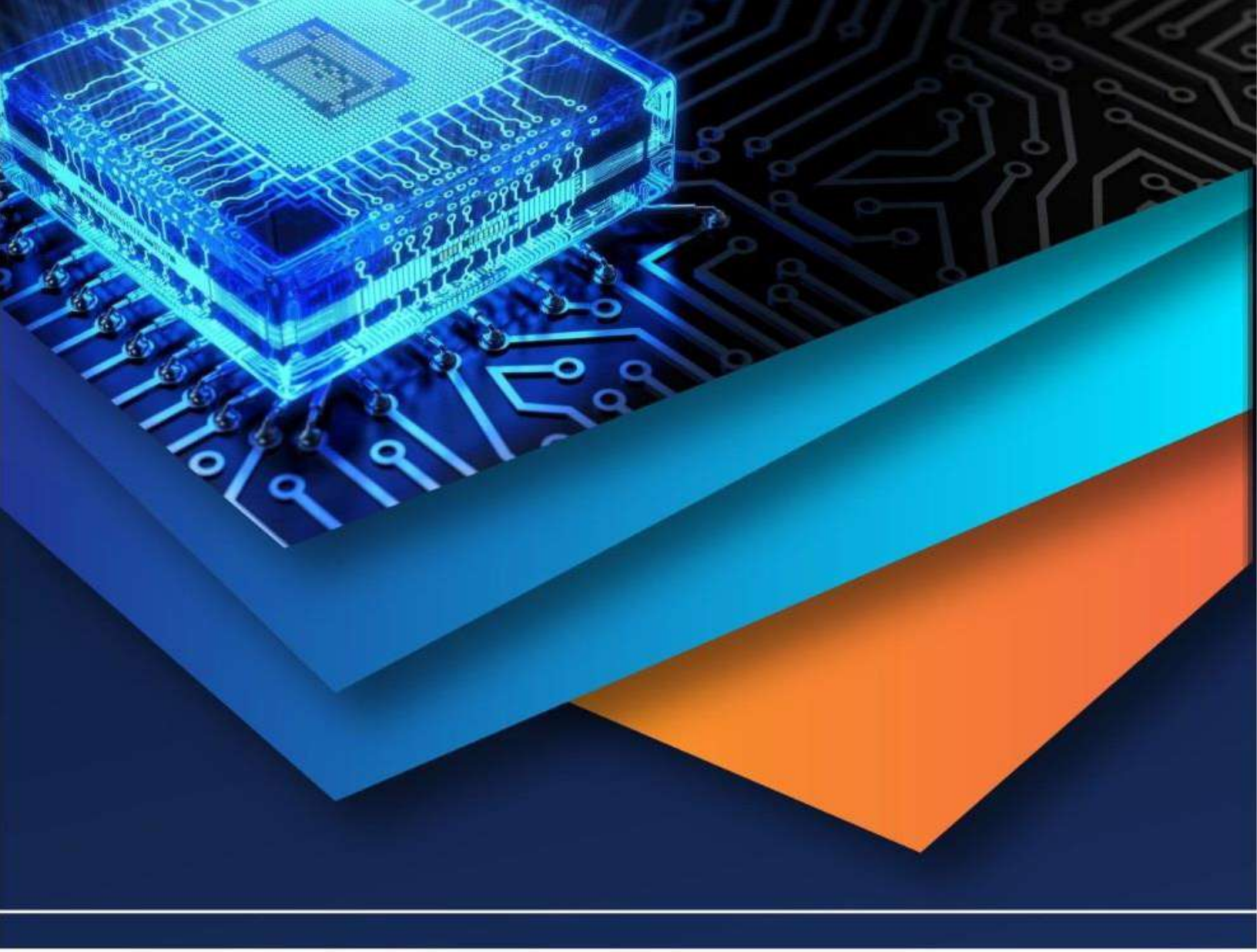
In this paper, there are two algorithms. One involving integer primes and the other uses Gaussian primes. One can maintain comparatively more secrecy in second one than that of first one. To improve much more security, one can modify the assignment by taking large primes as well as Gaussian primes.

REFERENCES

[1] Apostol, T. M., Introduction to Analytic Number Theory (1st ed. 1976. Corr. 5th printing 1998 ed.). Springer, 1976.
[2] May, C. A., Application of the Euler Phi Function in the Set of Gaussian Integers, 2015.
[3] Thiagarajan, K., Balasubramanian, P., Nagaraj, J., &Padmashree, J., Encryption and decryption algorithm using algebraic matrix approach. In *Journal of Physics: Conference Series* (Vol. 1000, No. 1, p. 012148). IOP Publishing, 2018.
[4] Trappe, W., & Washington, L. C., Introduction to Cryptography. Prentice Hall, 2006.
[5] Andre weil, Number Theory : An Approach through History, From Hammurapito to Legendre, Bikahsuser, Boston, 1987.
[6] Bibhotibhusan Batta and Avadhesh Narayanan Singh, History of Hindu Mathematics, Asia Publishing House, 1983.
[7] Boyer. C. B., History of mathematics, John Wiley & sons lnc., New York, 1968.
[8] L.E. Dickson, History of Theory of Numbers, Vol.2, Chelsea Publishing Company, New York, 1952.
[9] Davenport, Harold, The higher Arithmetic: An introduction to the Theory of Numbers (7^{th} ed.) Cambridge University Press, 1999.
[10] James Matteson, M.D. "A Collection of Diophantine problems with solutions" Washington, ArtemasMartin, 1888.
[11] Tituandreescu, DorinAndrica, "An introduction to Diophantine equations" Springer Publishing House, 2002.
[12] Manju Somanath, J. Kannan, K.Raja, "Gaussian integer solutions to space Pythagorean Equation $x^2 + y^2 + z^2 = w^2$", International Journal of Modern Trends in Engineering and Research, Volume 3, Issue 4, April 2016, pp . 287 - 289.
[13] Manju Somanath, J. Kannan, K.Raja , "Gaussian Pythagorean Triples", International Journal of Engineering Research and Management (IJERM), Volume 03, Issue 04, April 2016, pp . 131 - 132.
[14] Manju Somanath, J. Kannan, "Congruum Problem", International Journal of Pure and Applied Mathematical Sciences (IJPAMS), Volume 9, Number 2 (2016), pp. 123-131.
[15] M. A. Gopalan, J. Kannan, Manju Somanath, K.Raja, "Integral Solutions of an Infinite Elliptic Cone $x^2 = 4y^2 + 5z^2$", IJIRSET, Volume 5, Issue10, October 2016, pp .17551 - 17557.

[16] Manju Somanath, J. Kannan, K.Raja, "Lattice Points of an Infinite Cone $x^2 + y^2 = 85z^2$", International Journal of Recent Innovation in Engineering and Research, Vol. 1 Issue. 5, September 2016, pp. 14 -17.
[17] Manju Somanath, J. Kannan, K.Raja, "Lattice Points of an Infinite Cone $x^2 + y^2 = (\alpha^{2n} + \beta^{2n})z^2$", International Journal of Mathematical Trends and Technology, Vol. 38 No. 2, October 2016, pp(95 - 98).
[18] Manju Somanath, K. Raja, J. Kannan and V. Sangeetha, "On the Gaussian Integer Solutions for an Elliptic Diophantine Equation", Advances and Applications in Mathematical Sciences, Vol. 20, No. 05, March, 2021, pp. 815 – 822.
[19] Manju Somanath, K. Raja, J. Kannan and K. Kaleeswari, "Solutions Of Negative Pell Equation Involving Chen Prime", Advances and Applications in Mathematical Sciences, Vol. 19, No. 11, Sep. 2020, pp. 1089 – 1095
[20] Manju Somanath, K. Raja, J. Kannan and M. Mahalakshmi, "On A Class of Solutions for A Quadratic Diophantine Equation", Advances and Applications in Mathematical Sciences, Vol. 19, No. 11, Sep. 2020, pp. 1097 – 1103.
[21] Manju Somanath, K. Raja, J. Kannan and B. Jeyashree, "Non Trivial Integral Solutions of Ternary Quadratic Diophantine Equation", Advances and Applications in Mathematical Sciences, Vol. 19, No. 11, Sep. 2020, pp. 1105 – 1112.
[22] Manju Somanath, K. Raja, J. Kannan and S. Nivetha, "Exponential Diophantine Equation in Three Unknowns", Advances and Applications in Mathematical Sciences, Vol. 19, No. 11, Sep. 2020, pp. 1113 – 1118.
[23] Manju Somanath, K. Raja, J. Kannan and A. Akila, "Integral Solutions of An Infinite Elliptic Cone $x^2 = 9y^2 + 11z^2$ ", Advances and Applications in Mathematical Sciences, Vol. 19, No. 11, Sep. 2020, pp. 1119 – 1124.
[24] J. Kannan, "Ternary Exponential Diophantine Equation", Adalya Journal, Vol. 08, No. 09, September 2019, pp. 1283-1286.
[25] J. Kannan, "On the Diophantine Equation $47^x + 48^y = z^2$", Infokara Research, Vol.:08, No. 09, September 2019, pp. 778-781.
[26] J. Kannan, "On Sequences of special Diophantine Triples generated through Linear Polynomials", A Journal of Composition Theory, Vol.:12, No. 09, September 2019, pp. 703-706.
[27] J. Kannan, Manju Somanath and K. Raja, "On a Class of Solutions for the Hyperbolic Diophantine Equation", International Journal of Applied Mathematics, Vol.: 32, No. 03, July 2019, pp. 443- 449. [Scopus Indexed].
[28] J. Kannan, Manju Somanath and K. Raja, "Solutions of Negative Pell Equation Involving Twin Prime", JP Journal of Algebra, Number Theory and Applications, Vol.: 40, No.: 5, October 2018, pp. 869-874.

©IJRASET: All Rights are Reserved | SJ Impact Factor 7.538 | ISRA Journal Impact Factor 7.894 |



10.22214/IJRASET

45.98

IMPACT FACTOR: 7.129

IMPACT FACTOR: 7.429

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Call : 08813907089 (24*7 Support on Whatsapp)

International Journal of Multidisciplinary Research and Growth Evaluation.

On the ternary quadratic equation $7(x^2+y^2) - 13xy = 27z^2$

A Vijayasankar [1], V Krithika [2], MA Gopalan [3]
[1-2] Assistant Professor, Department of Mathematics, National College, Trichy, Tamil Nadu, India
[3] Professor, Department of Mathematics, SIGC, Trichy, Tamil Nadu, India

Corresponding Author: **Nenshi Iorvekaa David**

Article Info

ISSN (online): 2582-7138
Volume: 03
Issue: 01
January-February 2022
Received: 08-12-2021
Accepted: 26-12-2021
Page No: 97-102

Abstract
We obtain infinitely many non-zero integer triples $(x, y, z,)$ satisfying the homogenous quadratic equation with three unknowns. Various interesting properties among the values of $x, y,$ and z are presented. Some relations between the solutions and special numbers are exhibited.

Keywords: Ternary quadratic, Homogeneous quadratic, integral solutions, 2010 Mathematics subject classification, 11D09

Introduction

There is a great interest for mathematicians since ambiguity in homogeneous and non-homogeneous Quadratic Diophantine Equations [1-3]. In this context, one may refer [4-15] for varieties of problems on the quadratic Diophantine equations with three, four and five variables. In this paper, quadratic equation with three variables given by $7(x^2 + y^2)-13xy = 27z^2$ is analyzed for its non-zero distinct integer solutions. A few interesting relations between the solutions and special numbers are exhibited.

Method of analysis

The Diophantine equation representing the ternary quadratic equation to be solved for its non-zero distinct integral solution is

$$7(x^2 + y^2)-13xy = 27z^2 \quad (1)$$

Substituting the linear transformations

$$x = 3u + v, y = 3u - v \text{ , } u \neq v \neq 0 \quad (2)$$

In (1), it leads to

$$u^2 + 3v^2 = 3z^2 \quad (3)$$

(3) Can be solved through different methods and we obtain different sets of integer solutions to (1).

Set 1

Assume $z = a^2 + 3b^2$ (4)

Where a and b are non-zero distinct integers.

Write 3 as $3 = \left(i\sqrt{3}\right)\left(-i\sqrt{3}\right)$ (5)

Substituting (4) and (5) in (3) and applying the method of factorization, define

$$u + i\sqrt{3}v = \left(i\sqrt{3}\right)\left(a + i\sqrt{3}b\right)^2$$

Equating the real and imaginary parts, we have

$$\left.\begin{aligned} u &= -6ab \\ v &= a^2 - 3b^2 \end{aligned}\right\} \quad (6)$$

From (2), the non-zero distinct integer solutions to (1) are found to be

$$x = -18ab + \left(a^2 - 3b^2\right)$$
$$y = -18ab - \left(a^2 - 3b^2\right)$$
$$z = a^2 + 3b^2$$

Properties

- $y\left(2^b, b\right) + z\left(2^b, b\right) + 18\left(W_b - 1\right)$ is a nasty number.
- $2\left(2t_{4,a} - x(a, a) - z(a,a)\right)$ *is a perfect square.*
- $x(a,a) + y(a,a) + 36t_{4,a} = 0.$

Note 1

Instead of (5), write 3 as

$$3 = \frac{\left(3 + i\sqrt{3}\right)\left(3 - i\sqrt{3}\right)}{4}$$

Following the procedure as in set 1, the corresponding non-zero distinct integer solutions to (1) are obtained as

$$x = 5a^2 - 15b^2 - 6ab$$
$$y = 4a^2 - 12b^2 - 12ab$$
$$z = a^2 + 3b^2$$

Note 2

Instead of (5), write 3 as

$$3 = \frac{\left(12 + i\sqrt{3}\right)\left(12 - i\sqrt{3}\right)}{49}$$

By applying the same process as derived in set 1, we have the corresponding non-zero distinct integer solutions to (1) are obtained as

$$x = 259A^2 - 777B^2 + 42AB$$
$$y = 245A^2 - 735B^2 - 294AB$$
$$z = 49\left(A^2 + 3B^2\right)$$

Set 2
One may write (3) as

$$u^2 + 3v^2 = 3z^2 * 1 \tag{7}$$

Write 1 as

$$1 = \frac{(1+i\sqrt{3})(1-i\sqrt{3})}{4} \tag{8}$$

Using (4) , (5) and (8) in (7) and applying the method of factorization, define

$$\left(u + i\sqrt{3}\,v\right) = \left(i\sqrt{3}\right)\frac{\left(1+i\sqrt{3}\right)}{2}\left(a + i\sqrt{3}\,b\right)^2 \tag{9}$$

Equating the real and imaginary parts of (9), we have

$$u = \frac{1}{2}\left\{-3a^2 + 9b^2 - 6ab\right\}$$
$$v = \frac{1}{2}\left\{a^2 - 3b^2 - 6ab\right\}$$

In view of (2), the integer solutions of (1) are given by

$$x = -4a^2 + 12b^2 - 12ab$$
$$y = -5a^2 + 15b^2 - 6ab$$
$$z = a^2 + 3b^2$$

Note 3

- Instead of (11), '1' can also be considered as $1 = \frac{(1+i4\sqrt{3})(1-i4\sqrt{3})}{49}$, and the corresponding non-zero solutions are as follows:

$$x = -196A^2 + 588B^2 - 3528AB$$
$$y = -560A^2 + 1680B^2 - 3024AB$$
$$z = 196\left(A^2 + 3B^2\right)$$

- It is worth to mention that, '1' maybe considered in general as

$$1 = \frac{\left\{\left(3r^2 - s^2\right) + i\sqrt{3}\,rs\right\}\left\{\left(3r^2 - s^2\right) - i\sqrt{3}\,rs\right\}}{\left(3r^2 + s^2\right)^2}$$

Properties

- $3\left\{4z(a, a+1) - x(a, a+1) - 12\,\mathrm{Pr}_a\right\}$ is a nasty number.

- $5z(1,b)+y(1,b)-10t_{4,b}-S_b+1=0.$
- $z(a,a)-x(a,a)+2y(a,a)$ is a perfect square.

Set 3

Equation (3), can also be written as $3z^2-u^2=3v^2$ (10)

Assume $v=3a^2-b^2$ (11)

Write 3 as $3=\left(2\sqrt{3}+3\right)\left(2\sqrt{3}-3\right)$ (12)

Substituting (11) and (12) in (10) and applying the method of factorization, define

$$\sqrt{3}z+u=\left(2\sqrt{3}+3\right)\left(\sqrt{3}a+b\right)^2$$

Equating the rational and irrational roots, we get

$$\left.\begin{aligned}u&=9a^2+3b^2+12ab\\z&=6a^2+2b^2+6ab\end{aligned}\right\} \quad (13)$$

From (2), the non-zero distinct integer solutions to (1) are found to be

$$x=30a^2+8b^2+36ab$$
$$y=24a^2+10b^2+36ab$$
$$z=6a^2+2b^2+6ab$$

Properties

- $x(a+1,a)-5z(a+1,a)+2*t_{4,a}=6*\mathrm{Pr}_a.$
- $6z\left(2^a+1,1\right)-y\left(2^a+1,1\right)-12Ky_a-26=0.$
- $6\left[x(a,a)-y(a,a)\right]$ is a nasty number.

Note 4

In equation (12), we can choose '3' as $3=\left(7\sqrt{3}+12\right)\left(7\sqrt{3}-12\right).$
For this choice, we obtain the non-zero distinct integer solution to (1) are given by

$$x=111a^2+35b^2+126ab$$
$$y=105a^2+37b^2+126ab$$
$$z=21a^2+7b^2+24ab$$

Set 4
Now we are going to introduce another set of transformations as

$$u=X+3T\ ,\ v=X-T\ ,\ z=2W \quad (14)$$

In (3), we obtain

$$X^2+3T^2=3W^2 \quad (15)$$

For this, the solutions are said to be

$$\left.\begin{aligned} W &= a^2 + 3b^2 \\ X &= -6ab \\ T &= a^2 - 3b^2 \end{aligned}\right\} \qquad (16)$$

By using (16) in (14), we get

$$u = 3a^2 - 9b^2 - 6ab$$
$$v = -a^2 + 3b^2 - 6ab$$
$$z = 2a^2 + 6b^2$$

In view of (2), the non-zero distinct integer solutions to (1) are found to be

$$x = 8a^2 - 24b^2 - 24ab$$
$$y = 10a^2 - 30b^2 - 12ab$$
$$z = 2a^2 + 6b^2$$

Properties

- $x(a,a) - 2y(a,a)$ is a nasty number.
- $x\left(1,\sqrt{2^b}\right) - 2y\left(1,\sqrt{2^b}\right) - 12Tk_b = 0.$
- $\frac{-1}{8}\left[\, x(a,a) + 4z(a,a) \,\right]$ is a perfect square.

Notations Used

- Regular Polygonal Number of rank n with sides m: $t_{m,n} = n\left[1 + \frac{(n-1)(m-2)}{2}\right]$
- Pronic Number of rank n : $\Pr_n = n(n+1)$
- Woodall Number of rank n: $W_n = n(2^n) - 1$
- Star Number of rank n : $S_n = 6n(n-1) + 1$
- Thabit-ibn-Kurrah Number of rank n: $TK_n = 3(2^n) - 1$
- Kynea Number of rank n: $Ky_n = (2^n + 1)^2 - 2$

Conclusion

In this paper, we have presented different patterns of integer solutions to the ternary quadratic equation $7(x^2 + y^2) - 13xy = 27z^2$. As the diophantine equations are rich in variety, one may attempt to find integer solutions to other choices of equations of degree two with three or more unknowns.

References

1. LE Dickson. History of Theory of Numbers, vol 2, Chelsea publishing company, New York, 1952.
2. LJ Mordell. Diophantine Equations, Academic press, London, 1969.
3. RD Carmichael. The theory of numbers and Diophantine analysis, New York, Dover, 1959.
4. MA Gopalan, S Vidhyalakshmi, A Kavitha, D Marymadona. On the Ternary Quadratic Diophantine equation $3(x^2 + y^2) - 2xy = 4z^2$, International Journal of Engineering science and Management. 2015; 5(2):11-18.
5. K Meena. S Vidhyalakshmi. E Bhuvaneshwari, R Presenna. On ternary quadratic Diophantine equation $5(X^2 + Y^2) - 6XY = 20Z^2$, International Journal of Advanced Scientific Research. 2016; 1(2):59-61.
6. S Devibala, MA Gopalan. On the ternary quadratic Diophantine equation $7x^2 + y^2 = z^2$, International Journal of Emerging Technologies in Engineering Research. 2016; 4:9.
7. N Bharathi S. Vidhyalakshmi, Observation on the Non-Homogeneous Ternary Quadratic Equation $x^2 - xy + y^2 + 2(x+y) + 4 = 12z^2$, Journal of mathematics and informatics, 2017, vol.10, 135-140.

8. A. Priya, S. Vidhyalakshmi, On the Non-Homogeneous Ternary Quadratic Equation $2(x^2+y^2)-3xy+(x+y)+1=z^2$, Journal of mathematics and informatics, 2017, vol.10,49-55.
9. M.A. Gopalan, S. Vidhyalakshmi and U.K. Rajalakshmi, On ternary quadratic Diophantine equation $5(X^2+Y^2)-6XY=196Z^2$, Journal of mathematics,2017 , 3(5),1-10.
10. M.A. Gopalan, S. Vidhyalakshmi and S. Aarthy Thangam, On ternary quadratic equation $X(X+Y)=Z+20$, IJIRSET, 2017, 6(8), 15739-15741.
11. M.A. Gopalan and Sharadha Kumar, "On the Hyperbola $2x^2-3y^2=23$", Journal of Mathematics and Informatics, Dec 2017, vol-10, 1-9.
12. T.R. Usha Rani and K. Ambika, Observation on the Non-Homogeneous Binary Quadratic Diophantine Equation $5x^2-6y^2=5$, Journal of Mathematics and Informatics, Dec 2017 vol-10, 67-74.
13. S. Vidhyalakshmi, A. Sathya, S. Nivetha, "On the pellian like Equation $5x^2-7y^2=-8$ ", IRJET ,2019, 6(3) , 979-984 .
14. T.R. Usha Rani, V. Bahavathi, S. Sridevi, "Observations on the Non-homogeneous binary Quadratic Equation $8x^2-3y^2=20$ ", IRJET, 2019, 6(3) , 2375-2382.
15. S. Vidhyalakshmi, T. Mahalakshmi, "A Study On The Homogeneous Cone $x^2+7y^2=23z^2$", International Research Journal of Engineering and Technology (IRJET) , March 2019, 6(3), 5000-5007

Advances and Applications in Mathematical Sciences
Volume 21, Issue 8, June 2022, Pages 4615-4623
© 2022 Mili Publications, India

MEAN SQUARE DEVIATION OF TIME TO HIRING IN A SINGLE GRADE WORKFORCE STRUCTURE WITH CLUMP OF EGRESSES AND A RANDOM THRESHOLD

G. RAVICHANDRAN[1] and K. SRIVIDHYA[2]

[1]Assistant Professor in Mathematics
Saranathan College of Engineering (SCE)
Panjappur, Tiruchirappalli-620 012, Tamil Nadu, India
E-mail: mathgravi@gmail.com

[2]Assistant Professor in Mathematics
National College (Autonomous)
Tiruchirappalli-620 001, Tamil Nadu, India
E-mail: srividhyamath@nct.ac.in

Abstract

A single grade workforce structure in which egress of employees occurs in clumps at random epochs is considered. Two stochastic exemplars are constructed utilizing CUM and MAX strategies of hiring. Analytical result for mean square deviation of time to hiring is determined under the conditions (i) cumulative count for egresses is a compound Poisson process (ii) deprivation of workforce in each clump of egresses are independent exponential random variables and (iii) threshold is an exponential random variable. A quantitative illustration for analytical results with relevant findings and conclusion are presented.

1. Introduction

As a pioneer of modelling workforce designing, the author in [1] and [2] has discussed the statistical approach and renewal theory exemplars. Several stochastic exemplars utilizing CUM strategy of hiring for the problem of time to hiring in a single grade workforce structure are constructed by many researchers. Assuming that egress occurs as the result of strategic decision

2020 Mathematics Subject Classification: 60H30, 60K05, 90B70, 91D35.

Keywords: Single grade workforce structure, Clump of egresses, Compound Poisson Process, random threshold, CUM and MAX strategies of hiring, mean square deviation of time to hiring and average aggregate deprivation of workforce up to hiring time.

Received March 24, 2022; Accepted April 10, 2022

taken in the workforce structure, mean square deviation of time to hiring is determined under different conditions on deprivation of workforce, thresholds and egress times. For details, one may refer to [11], [15], [10], [17], [14], [12], [16], [7], [9], [3] and [6]. In [4], mean square deviation for time to hiring is determined for the workforce structure with egresses in clumps when the process of total number of egresses is a compound Poisson process with independent and identically distributed decapitated geometric random variables as summands and the threshold is a positive integer valued random variable. The problem of time to hiring for the workforce structure is also analyzed by several authors utilizing another strategy of hiring referred as MAX strategy of hiring in the literature. In this context, one may refer to [11], [15], [17], [13], [8], [3] and [6]. Recently, in [5] the authors have analyzed the problem in [4] utilizing the MAX strategy of hiring. Present paper is an extension of [4] and [5] when the egress in the workforce structure occurs in clumps and deprivation of workforce is continuous, by composing two exemplars. In exemplar-1, the partial sum of independent exponential deprivation of workforce is randomly indexed by a homogeneous Poisson process; threshold is an exponential random variable. Mean square deviation of time to hiring is determined utilizing CUM strategy of hiring for this exemplar. In exemplar-2, the partial sum and CUM strategy of hiring of exemplar-1 are replaced by maximum and MAX strategy of hiring. A remark on the suitability of the exemplars is given with justification.

2. Description of Exemplar-1

Consider a workforce structure consisting of a single grade in which egress of employees occurs in clumps at random epochs in $(0, \infty)$. Let $\{B(x), x \geq 0\}$ be a Poisson process with degree 'b', $b > 0$, where $B(x)$ is the count for clumps in $(0, x]$. Let U_i be the deprivation of workforce due to egresses in the i^{th} clump, $i = 1, 2, 3, \ldots$. It is presumed that U_i's are independent and $U_i \sim \exp(\theta_1)$, $\theta_1 > 0$, $i = 1, 2, 3, \ldots$. Let $f_m(\cdot)$ be probability density of $\sum_{i=1}^{m} U_i$ with Laplace transform $\overline{f_m}(\cdot)$. Let $C(x)$ be the total deprivation of workforce due to $B(x)$ clumps of egresses. Let Y be the threshold level for the cumulative deprivation of workforce due to clumps of egresses. It is presumed that $Y \sim \exp(\theta_2)$, $\theta_2 > 0$. Y, $B(x)$ and U_i, $x \geq 0$,

$i \geq 1$ are statistically independent. The CUM strategy of hiring states that hiring is done when the total deprivation of workforce due to clumps of egresses exceeds the threshold. Let R_{CUM} be the time to hiring with expectation $M(R_{CUM})$, mean square deviation $V(R_{CUM})$, and average aggregate deprivation of work force due to clump of egresses in the interval of time to hiring $M(C(R_{CUM}))$.

3. Main Result for Examplar-1

By CUM strategy of hiring

$$P(R_{CUM} > x) = P(C(x) \leq Y)$$

Since, $C(x) = \sum_{i=1}^{B(x)} U_i$, by conditioning upon $B(x)$ and using the independence of $B(x)$ over U_i and Y for all $x \geq 0$, $i = 1, 2, 3, \ldots$ we get

$$P(R_{CUM} > x) = \sum\nolimits_{m=0}^{\infty} P(\sum\nolimits_{i=1}^{m} U_i \leq Y)\, P(B(x) = m) \tag{1}$$

Note that $\sum_{i=1}^{0} U_i \leq Y$ is an empty set. Therefore without loss of generality, m can vary from 0 to ∞.

Since U_i's are independent of Y, by conditioning upon $\sum_{i=1}^{0} U_i$ and using the assumption that Y is an exponential random variable with parameter $\theta_2 (\theta_2 > 0)$, $P(\sum_{i=1}^{m} U_i \leq Y)$ in (1) can be written as

$$P(\sum\nolimits_{i=1}^{m} U_i \leq Y) = \overline{f_m}(\theta_2) \tag{2}$$

By hypothesis, $f_m(\cdot)$ is a gamma density function with scale parameter θ_1 and shape parameter m. Applying convolution theorem for Laplace transform we get

$$\overline{f_m}(\theta_2) = \left(\frac{\theta_1}{\theta_1 + \theta_2}\right)^m \tag{3}$$

From (2) and (3) we get

$$P\left(\sum_{i=1}^{m} U_i \leq Y\right) = \left(\frac{\theta_1}{\theta_1 + \theta_2}\right)^m \tag{4}$$

From (1) and (4) we get

$$P(R_{CUM} > x) = \sum_{m=0}^{\infty} \left(\frac{\theta_1}{\theta_1 + \theta_2}\right)^m P(B(x) = m)$$

which is the probability generating function of the Poisson process $B(x)$ evaluated at $\left(\frac{\theta_1}{\theta_1 + \theta_2}\right)$.

Therefore

$$P(R_{CUM} > x) = e^{-\left(\frac{b\theta_2}{\theta_1+\theta_2}\right)x} \tag{5}$$

From (5) we find that the distribution of R_{CUM} is exponential with parameter $\frac{b\theta_2}{\theta_1 + \theta_2}$.

Therefore

$$M(R_{CUM}) = \left(\frac{\theta_1 + \theta_2}{b\theta_2}\right) \tag{6}$$

and

$$V(R_{CUM}) = \left(\frac{\theta_1 + \theta_2}{b\theta_2}\right)^2 \tag{7}$$

(6) and (7) give the expectation and mean square deviation of time to hiring.

Result for $M(C(R_{CUM}))$ is derived below:

Since $C(R_{CUM}) = \sum_{i=1}^{B(R_{CUM})} U_i$, by Wald's lemma, the average aggregate deprivation of workforce up to time to hiring is given by

$$M(C(R_{CUM})) = M(U_i)M(B(R_{CUM})), 1, 2, 3, \ldots, \quad (8)$$

Since R_{CUM} is independent of $B(x)$ and $M[B(x)] = bx$, by conditioning upon R_{CUM} it can be shown that

$$M(B(R_{CUM})) = bM(R_{CUM}) \quad (9)$$

From (6), (8) and (9) we get

$$M(C(R_{CUM})) = \left(\frac{\theta_1 + \theta_2}{\theta_1\theta_2}\right) \quad (10)$$

(10) gives the average aggregate deprivation of workforce up to hiring time.

4. Description of Exemplar-2

In this section, we study the work in exemplar-1 utilizing the MAX strategy of hiring. The MAX strategy of hiring states that hiring is done when the maximum deprivation of workforce due to clumps of egresses exceeds the threshold.

5. Main Result of Exemplar-2

By MAX strategy of hiring

$$P(R_{MAX} > x) = P(\max_{1\le i\le B(x)} U_i \le Y)$$

By conditioning upon $B(x)$ and using the independence of $B(x)$ over U_i and Y for all $x \ge 0$, $i = 1, 2, 3, \ldots$ we get

$$P(R_{MAX} > x) = \sum\nolimits_{m=0}^{\infty} P(\max_{1\le i\le m} U_i \le Y)P(B(x) = m) \quad (11)$$

By conditioning upon Y and using the independence of U_i over Y, $i = 1, 2, 3, \ldots$ we get

$$P(\max_{1\le i\le m} U_i \le Y) = \int_0^{\infty} P(\max_{1\le i\le m} U_i \le y)\, dP(Y \le y)$$

i.e. $P(\max_{1\le i\le m} U_i \le Y) = \theta_2 \int_0^\infty [F(y)]^m e^{-\theta_2 y} dy$ (12)

where $F(y) = P[U_i \le y] = 1 - e^{-\theta_2 y}$, 1, 2, 3, …, by hypothesis.

Since $\sum_{m=0}^\infty P(B(x) = m)s^m = e^{-bx[1-s]}$, by hypothesis, from (11) and (12) we get

$$P(R_{MAX} > x) = \theta_2 \int_0^\infty e^{-\theta_2 y} e^{-bx(e^{-\theta_1 y})} dy. \quad (13)$$

We now determine $M(R_{MAX})$ and $V(R_{MAX})$ from (13).

We know that

$$M(R_{MAX}^n) = n \int_0^\infty x^{n-1} P(R_{MAX} > x) dx,\ n = 1, 2, 3, \ldots \quad (14)$$

From (13) and (14) it can be shown that

$$M(R_{MAX}) = \frac{\theta_2}{b(\theta_2 - \theta_1)},\ \text{if } \theta_2 > \theta_1. \quad (15)$$

and

$$M(R_{MAX}^2) = \frac{2\theta_2}{b^2(\theta_2 - 2\theta_1)},\ \text{if } \theta_2 > 2\theta_1. \quad (16)$$

Since $(2\theta_1^2 + \theta_2^2 - 2\theta_1\theta_2) = [\theta_1^2 + (\theta_2 - \theta_1)^2] > 0$ for all values θ_1 and θ_2, from (15) and (16) it can be shown that

$$V(R_{MAX}) = \frac{\theta_2(2\theta_1^2 + \theta_2^2 - 2\theta_1\theta_2)}{b^2(\theta_2 + 2\theta_1)(\theta_2 - \theta_1)^2},\ \text{if } \theta_2 > 2\theta_1. \quad (17)$$

(15) and (17) give the expectation and mean square deviation of time to hiring for this Exemplar.

6. Quantitative Illustration and Findings for Exemplars-1 and 2

b, θ_1 and θ_2 are the parameters for expectation and mean square

deviation. It is palpable that (i) $M(R_{CUM})$ increases with $'\theta_1'$ keeping $'\theta_2'$ and $'b'$ fixed for exemplar-1 (ii) $M(R_{CUM})$ decreases when $'\theta_2'$ increases, keeping $'\theta_1'$ and $'b'$ fixed for exemplar-1 and (iii) Expectation and mean square deviation decrease when 'b' alone increases for both the exemplars.

For exemplar-1, table-1 given below indicates the impact on $M(R_{CUM})$ and $V(R_{CUM})$ when b = 0.1 and θ_1 and θ_2 vary together.

Table 1.

θ_1	θ_2	$M(R_{CUM})$	$V(R_{CUM})$
5	16	13.125	172.266
6	17	13.529	183.045
7	18	13.889	192.901
8	19	14.211	201.939

For exemplar-2, table-2 given below indicates the effect of θ_1 and θ_2 on $M(R_{CUM})$ and $V(R_{CUM})$ when b = 0.1.

Table 2.

θ_1	θ_2	$M(R_{MAX})$	$V(R_{MAX})$
4	15	13.636	242.621
5	15	15.000	375.000
4	16	13.333	222.222
4	17	13.077	206.772
5	16	14.545	321.763
6	17	15.455	441.157
7	18	16.364	632.231

Findings

(1) From Table-1, it is observed that $M(R_{CUM})$ and $V(R_{CUM})$ both decrease when θ_1 and θ_2 decrease simultaneously.

(2) From Table-2, it is observed that $M(R_{MAX})$ and $V(R_{MAX})$ both increase when (i) θ_1 increases and θ_2 fixed and (ii) θ_1 and θ_2 increase simultaneously.

(3) From Table-2, it is observed that $M(R_{MAX})$ and $V(R_{MAX})$ both decrease when θ_2 increases and θ_1 fixed.

Remark. Since U_i is positive for at least one i, $\sum_{i=1}^{m} U_i$ is always greater than $\max_{1\le i\le m} U_i$, the time to hiring is advanced in exemplar-1, but it is delayed in exemplar-2. This justifies the following observation on the choice of the suitability of these exemplars.

(i) Exemplar-1 is more suitable than exemplar-2 if the plans in the work force structure is to prepone the time to hiring.

(ii) Exemplar-2 is suitable than exemplar-1 if the plans in the work force structure is to postpone the time to hiring.

7. Conclusion

The present work contributes to the existing literature in the sense that the exemplars-1 and 2 discussed when egress of employees occurs in clumps in the workforce structure and threshold is an exponential random variable, utilizing CUM and MAX strategies of hiring. Consistency of results on averages with logical inference is an important finding.

Acknowledgement

The authors express heartfelt gratitude to their respective college management for its continuous encouragement towards research.

References

[1] D. J. Bartholomew, The statistical approach to manpower planning, statistician 20 (1971), 3-26.

[2] D. J. Bartholomew, Renewal theory models in manpower planning symposium proceedings series No.8, The Institute of Mathematics and Its Applications, (1976), 57-73.

[3] A. Devi, A probabilistic analysis on time to recruitment for a single grade manpower system with different epochs for decisions and exits, Ph.D. Thesis, Bharathidasan

University, 2019.

[4] S. Dhivya, K. Elangovan, G. Ravichandran and K. Srividhya, A stochastic model on time to recruitment in a single grade manpower system with cluster of exits, International Journal of Aquatic Science 12(2) (2021), 2747-2752.

[5] S. Dhivya and K. Elangovan, A stochastic model on time to recruitment in a single grade manpower system with cluster of exits using MAX policy of recruitment, Communicated to International Journal of Applied Mathematics, 2021.

[6] K. Elangovan, Contributions to the study on stochastic manpower models in a single grade system, Ph.D. Thesis, Bharathidasan University, 2019.

[7] G. Iswarya, Stochastic models on the time to recruitment in a single and two graded manpower system with two thresholds for the organization, M.Phil, Dissertation, Bharathidasan University, 2011.

[8] G. Jayalakshmi and A. Srinivasan, Probabilistic analysis on time to recruitment for a single grade manpower system when the breakdown threshold has two components using a different policy of recruitment, Indian Journal of Applied Research 5(7) 2015(a), 551-553.

[9] G. Jayalakshmi and A. Srinivasan, Probabilistic analysis on time to recruitment for a single grade manpower system when the breakdown threshold has two components, International Journal of Scientific Engineering and Applied Science 1(4) 2015(b), 180-183.

[10] K. Kasturri, Mean time for recruitment and cost analysis on some univariate policies of recruitment in manpower models, Ph.D. thesis, Bharathidasan University, 2007.

[11] P. Mariappan, A study of some manpower models and their analysis, Ph.D. thesis, Bharathidasan University, 2002.

[12] A. Muthaiyan, A study on stochastic models in manpower planning, Ph.D. thesis, Bharathidasan University, 2010.

[13] N. Muthulakshmi, K. Kasturi and A. Srinivasan, Mean and variance of the time for recruitment in a single graded manpower system with an univariate policy of recruitment involving geometric threshold, Acta Ciencia Indica 34M(4) (2008), 1637-1641.

[14] S. N. Poorni Periyanayaki and A. Srinivasan, Mean and variance of time to recruitment in a single graded manpower system having correlated inter-decision times associated with an univariate policy of recruitment when the threshold is continuous, Acta Ciencia Indica 34M(1) (2008), 65-68.

[15] V. Saavithri, Cost analysis on some policies of recruitment in manpower models, Ph.D. Thesis, Bharathidasan University, 2003.

[16] K. P. Uma, A study on manpower models with univariate and bivariate polices of recruitment, Ph.D. Thesis, Avinashilingam University, 2010.

[17] B. Venkatesh, Mean and variance of time to recruitment in a single graded manpower system associated with an univariate policy of recruitment, M.Phil, Dissertation, Bharathidasan University, 2007.

ISBN: 978-93-82606-09-3

IMPACT OF THERABAND TRAINING, PILATES TRAINING AND PLYOMETRICS TRAINING ON SELECTED PHYSICAL FITNESS VARIABLES AMONG STATE LEVEL MEN ARTISTIC GYMNASTS

P S Poukinreiyang Kamei*
Ph.D. Research Scholar, Department of Physical Education and Sports
National College, Trichirapalli-01, Tamilnadu
Dr.D.Boopathy**
Assistant Professor, Department of Physical Education,
National College, Tiruchirapalli, Tamilnadu,India
Dr. D. Gunaseelan***
Assistant Professor & Research Advisor,
Department of Hotel Management,
Jamal Mohamed College (Autonomous) Trichy

Abstract:

Sports play a very prominent role in the modern society. It is important to individuals, a group, a nation and indeed the world. Throughout the world, sports have popular appeal among people of all ages and both sexes. Much of the attraction of sports comes from the wide variety of experience and feeling that result from participation such as success, failure, exhaustion pain, relief and feeling of belonging. Sport can bring money, glory, status and goodwill. However, sport can also bring tragedy, grief and even death (Coakley, jay J.,1998)

The objective of this research was to assess the selected motor fitness variables of state level men artistic gymnastics (speed, abdominal strength, flexibility) the adjusted mean on speed on plyometrics training group was 7.08, pilates training group was 7.11, theraband training group was 7.13 and control group was 7.32, the adjusted mean on abdominal strength on pilates training group was 38.26, plyometric training group was 38.22, thera band training group was 40.09 and control group was 36.43, the adjusted mean on Flexibility on thera band group was 24.07, plyometrics training group was 23.82, pilates training group was 24.05 and control group was 21.51.

Key Words: Gymnastics, Speed, Abdominal Strength, Flexibility, thera band training, plyometrics training, pilates training.

Introduction:

Artistic gymnastic is one of the few sports which can be practiced from a very young age, and which develops different components of physical fitness. Pupils learn basic elements which are of great significance, especially for children's orientation in space, such as jump and leaps, hanging, rotating, crawling, rolling, etc. (Pajek, Cuk, Kovac, & Jakese, 2010). Motor fitness (speed, agility and coordination), and cardirespiratory fitness (ALPHA, 2009; Artero et al.,2011; Ruize et al., 2010). There are different fitness test batteries, such as Alpha-fit, Eurofit, Fitnessgram, Etc., which are applied around the world in order to assess physical fitness in children and adolescents (Kolimechkov, 2017).

Sports plays a important role in the modern society. It is important to individuals, a group, a nation and indeed the world. Throughout the world, sports have a popular appeal among people of all ages and both sexes. Much of the attraction of sport comes from the wide verity of experience and feeling that result from participation as success, failure, exhaustion pain, relief and feeling of belonging. Sports can bring money, glory, status and goodwill. However, sport can also bring tragedy, grief and even death (Coakley, Jay J., 1998).

Preparation of an athlete today for achievement is a complex dynamic matter, characterized b a high level of physical and physiological efficiency and the degree of perfection of necessary skills, knowledge proper teaching and tactics. An athlete arrives at this state only, as a result of corresponding training sports activity directed at steadily enhancing the preparation of an athlete and grooming him for a higher-level achievement. Fitness is a state which often characterizes the degree to which a person is able to function. Ability to function depends upon the physical, mental, emotional, Social and spiritual components of fitness. While fitness is the maximal, economical and efficient functioning of the body, health is referred to as optimal homeostatic functioning of the body.

Objectives of the study:

The objective of the research was to assess the selected physical fitness variables of 12 to 14 years state level gymnasts, to find out the effect of varied training methods, such as Thera band trainng, Plyometric Trainng and pilates training on selected physical variables. The effect of Thera band trainng, Plyometric Trainng and pilates trainingon gymnasts on each of the physical fitness variables was compared with these effects with control group to determine whether these training produce significant changes in the selected variables. If so, which of the training method is better than the other one? Thus, the current research focuses on the effects of varied training packages such as Thera band trainng, Plyometric Trainng and pilates training on selected physical fitness variables.

Methodology:

Physical Fitness Variables:
1. Speed 2. Abdominal Strength 3. Flexiblity
Independent Variables
1. Twelve-week Thera band Training
2. Twelve weeks Plyometrics Training
3. Twelve weeks Pilates Training

Selection of subjects:

To achieve the purpose of this study sixty state level men artistic gymnasts from different clubs in trichy were selected. The selected subject's age group was ranging from twelve to fourteen years. group one acted as experimental group one and group two acted as experimental group two and group three acted as experimental group three and group four acted as control group. Group- four underwent routine activities and care was taken that they should not involve in special exercise programmes. Before starting the study, all subjects were informed about it and signed an informed consent form.

Warm up and cool down exercise:

All subjects started every training session with 5 minutes of walking and jogging. After that, they performed gymnastics stretching exercise including upper and lower body extremities for 10 minutes. Subjects performed 5 minutes jogging for a cool down at the end of each training session.

Flexibility test:

Sit and reach test were used to obtain subjects flexibility scores. Subjects performed two trails within 1-minute rest period between the trails for flexibility test. The best score was recorded. The subject was asked to wait at least 2 seconds at the maximum reached distance. The trail was not considered successful if the subject a) failed to maintain the contact with the target, b) failed to maintain position at least 2 seconds at maximum reached distance.

Speed Test: 30mts Dash

The test involves running a single maximum sprint over 30 meters, with the time recorded. A through warm up should be given, including some practice starts and accelerations. Start form a stationary position, with one foot in front of the other. The front foot must be on or behind the starting line. This starting position should be held for 2seconds prior to starting, and no rocking movements are allowed. Two trails are allowed and the best time is recorded to the nearest 2 decimal places. The timing starts from the first movement (if using a stopwatch) or when the timing system is triggered, and finishes when the chest crosses the finish line the finishing trimming gate is triggered.

Plank Test: Abdominal Strength:

The aim of this test is to hold an elevated position for as long as possible. Start with upper body supported off the ground by the elbows and forearms, and the legs straight with the weight taken by the toes. The hip is lifted off the floor creating a straight line from head to toe. As soon as the subject is in the correct position, the stopwatch is started. The head should be facing towards the ground and not looking forwards. The test is over when the subject is unable to hold the back straight and the hip is lowered. The score is the total time completed.

Significance of the study:

The study is significant in the following ways:

1. This study is unique in suggesting varied training schedules for the physical fitness variables of state level men artistic gymnasts.

2. The findings of this study would suggest which training schedule is better in improving and developing which of the specific physical fitness variables of men gymnasts.

3. The findings would be helpful for the coaches and administrators to modify their existing training schedules for the preparation of gymnast's probable.

4. This study may help the sports scientists to suggest ways and means to improve better standard in sports through suggesting suitable training methods.

5. This study may help the coaches and physical educators to train the gymnasts to improve

Analysis of Data

Computation of analysis of covariance due to thera band training.plyometric training and pilates training and control group on speed

	Source of variance	Sum of squares	Df	Mean Squares	Obtained F
Pretest Mean	Between	0.17	3	0.06	0.47*
	Within	9.30	76	0.12	
Post-test mean	Between	1.16	3	0.39	4.55*
	Within	6.44	76	0.08	
Adjusted Post -test Mean	Between	6.71	3	0.24	22.76
	Within	6.79	75	0.01	

Required F(0.05), (df3,75) = 2.77

**Significant at 0.05 level of confidence*

As shown in Table 4.2, the obtained F- Raito of 0.47 on preOtest means of the groups was not significant at 0.05 level as the obtained F- value was less than the required table F-Value of 2.77 to be significant at 0.05 level. This shows that there was no significant difference in means of the groups at initial stage. The results presented in table 4.2, the obtained F-ratio of 4.55 on post- test means of the group was significant at 0.05 levels as the obtained F-value was grater then the required table F-value of 2.77 to be significant at 0.05 levels. This shows that there was a significant difference in means of the groups at initial stage. Taking into consideration of the pre-test mans and post-test means, adjusted postOtest means were determined and analysis of covariance was done. The obtained F-value on adjusted means was 22.76. The obtained F-value was greater than the required value of 2.77 and,

ISBN: 978-93-82606-09-3

hence it was accepted that there were significant differences among adjusted means on the speed of the subjects.

Computation of analysis of covariance due to thera band training.plyometric training and pilates training and control group on abdomianl strength

	Source of variance	Sum of squares	Df	Mean Squares	Obtained F
Pre-test Mean	Between	115.04	3	38.35	0.96
	Within	3025.85	76	39.81	
Post-test mean	Between	137.70	3	45.90	1.58
	Within	2213.30	76	29.12	
Adjusted Post -test Mean	Between	132.71	3	44.24	84.97*
	Within	39.05	75	0.52	

Required F(0.05), (df3,75) = 2.77

**Significant at 0.05 level of confidence*

As shown in Table 4.5, the obtained F- Raito of 0.96 on pre-test means of the groups was not significant at 0.05 level as the obtained F- value was less than the required table F-Value of 2.77 to be significant at 0.05 level. This shows that there was no significant difference in means of the groups at initial stage. The results presented in table 4.5, the obtained F-ratio of 1.58 on post- test means of the group was significant at 0.05 levels as the obtained F-value was grater then the required table F-value of 2.77 to be significant at 0.05 levels. This shows that there was a significant difference in means of the groups at initial stage. Taking into consideration of the pre-test mans and post-test means, adjusted post0test means were determined and analysis of covariance was done. The obtained F-value on adjusted means was 84.97. The obtained F-value was greater than the required value of 2.77 and, hence it was accepted that there were significant differences among adjusted means on the speed of the subjects.

Computation of analysis of covariance due to thera band training. plyometric training and pilates training and control group on flexibility

	Source of variance	Sum of squares	Df	Mean Squares	Obtained F
Pre-test Mean	Between	3.74	3	1.25	0.22
	Within	438.65	76	5.77	
Post-test mean	Between	79.64	3	26.55	5.38*
	Within	374.85	76	4.93	
Adjusted Post -test Mean	Between	92.27	3	30.76	18.81*
	Within	122.66	75	1.64	

*Required F(0.05), (df3,75) = 2.77 *Significant at 0.05 level of confidence*

As shown in Table 4.8, the obtained F- Raito of 0.22 on pre-test means of the groups was not significant at 0.05 level as the obtained F- value was less than the required table F-Value of 2.77 to be significant at 0.05 level. This shows that there was no significant difference in means of the groups at initial stage. The results presented in table 4.5, the obtained F-ratio of 5.38 on post- test means of the group was significant at 0.05 levels as the obtained F-value was grater then the required table F-value of 2.77 to be significant at 0.05 levels. This shows that there was a significant difference in means of the groups at initial stage. Taking into consideration of the pre-test mans and post-test means, adjusted post0test means were determined and analysis of covariance was done. The obtained F-value on adjusted means was 18.81. The obtained F-value was greater than the required value of 2.77 and, hence it was accepted that there were significant differences among adjusted means on the speed of the subjects.

Results & Conclussions

1. It was concluded that varied packages of training, namely plyometric training, thera band training and pilates training significantly improved physical fitness variable, such as speed among state level gymnasts compared to control group. It was also found that there was no significant difference among treatment groups in altering speed.

2. 1. It was concluded that varied packages of training, namely plyometric training, thera band training and pilates training significantly improved physical fitness variable, such as abdominal strength among state level gymnasts compared to control group. It was also found that there was no significant difference among treatment groups in altering abdominal strength.

3. It was concluded that varied packages of training, namely plyometric training, thera band training and pilates training significantly improved physical fitness variable, such as flexibility among state level gymnasts compared to control group. It was also found that there was no significant difference among treatment groups in altering flexibility.

REFERENCES:

5 1.Borresen, J., & Ian Lambert, M. (2009). The Quantification of TrainingLoad, the Training Response and theEffect on Performance. Sports Medicine,39(9), 779–795.

6 2.Harriss, D. J., MacSween, A., &Atkinson, G. (2019). Ethical Standards in Sport and Exercise Science Research: 2020 Update. International Journal of Sports Medicine, 40(13), 813–817.

7 3.Foster, C., Florhaug, J. A., Franklin, J., Gottschall, L., Hrovatin, L. A., Parker,S., … Dodge, C. (2001). A New Approachto Monitoring Exercise Training. Journalof Strength and Conditioning Research,15(1), 109–115.

8 4.Alcaraz, P.E., Palao, J.M &Elvira, J.L. (2009). Determining the optimal load for resisted sprint training with sled showing. J Strength Cond Res. 23(2):480-5.

9 5.Hoeger, W. W., & Hoeger, S. A. (2013). Principles and labs for physical fitness: Cengage Learning.

10 6.Hosmer, D., Lemeshow, S., & Sturdivant, R. (2000). Applied Logistic Regression. New York, NY: A WileyInterscience Publication. In: John Wiley & Sons Inc.

11 7.Creswell, J. W. (2012). Educational research: planning, conducting, and evaluating quantitative and qualitative research. Boston: Pearson.

12 8.Hoeger, W. W., & Hoeger, S. A. (2013). Principles and labs for physical fitness: Cengage Learning.Hosmer, D., Lemeshow, S., & Sturdivant, R. (2000). Applied Logistic Regression. New York, NY: A WileyInterscience Publication. In: John Wiley & Sons Inc.

ISBN: 978-93-82606-09-3

EFFECTS OF PLYOMETRIC TRAINING ON SOCCER RELATED PHYSICAL FITNESS VARIABLES OF ANNA UNIVERSITY INTERCOLLEGIATE FEMALE SOCCER PLAYERS

Dr.D.Boopathy*
Assistant Professor, Department of Physical Education, National College, Tiruchirapalli, Tamilnadu,India
Soraisham Sunilkumar Singh**
Ph.D. Research Scholar, Department of Physical Education and Sports National College, Trichirapalli-01, Tamilnadu
Dr.D.PrasannaBalaji***
Head and Director, Department of Physical Education National college, Triuchirtapalli, India.

Abstract

Plyometric training is an important training program in improving physical fitness and soccer skills of players. The study was conducted to find out the effects of plyometric training on soccer related physical fitness variables of Anna University intercollegiate female soccer players. For this study twenty female soccer players were selected through purposive sampling. The participants were engaged in a supervised plyometric training program 3 days/week for 12 weeks. The physical fitness and soccer skill variables selected for the study were: Speed, Explosive power, Agility, Dribbling, Kicking Right and Left Feet. Tests were taken three times at pre training, during training and post training. Data were analysed by using SPSS software version 20. Comparison of mean was done by paired T-test. The results obtained in this study indicated that there was significant improvement in selected physical fitness and soccer skill variables due to the effects of plyometric training. After 12 weeks of plyometric training participant's speed (MD= 0.78), agility (MD= 2.64), and explosive power (MD= 7.85) were changed significantly ($p < 0.05$). Participant's dribbling soccer skill (MD= 1.92), kicking right foot for distance (MD= 2.19) and kicking left foot for distance (MD= 2.91) were significantly improved through plyometric training. This study proved that plyometric training was significantly better in improving the physical fitness variables and soccer skills ($p < 0.05$) of female soccer players.

Key words: intercollegiate, physical fitness variables, plyometric training, soccer skills.

Introduction

Soccer (also known as football) is the world's most popular form of sport, being played in every nation without exception. Soccer has a rich history though it was formalized as we know it today by the establishment of the Football Association in 1863. The game soon spread to continental European countries and later to South America and the other continents. The world's governing body, Federation International de Football Association (FIFA), was set up in 1904 (Thomas, 2003).

Plyometric are training techniques used by players in all types of sports to increase strength and explosiveness (Chu, 1998). Plyometric consists of a rapid stretching of a muscle (eccentric action) immediately followed by a concentric or shortening action of the same muscle and connective tissue (Baechle and Earle, 2000). The stored elastic energy within the muscle is used to produce more force than can be provided by a concentric action alone 2 (Pfeiffer, 1999; Miller et al., 2002). Researchers have shown that plyometric training when used with a periodized strength-training program, can contribute to improvements in vertical jump performance, acceleration, leg strength, muscular power, increased joint awareness and overall pro-perception (Adams et al., 1992; Paasuke et al., 2001; Miller et al., 2002).

Strength, speed, and explosiveness are all crucial for success in soccer. All three of these factors are enhanced with a progressive resistance training program, but greater increases in speed and explosiveness are thought to be possible with plyometric exercise. The objective is to generate the greatest amount of force in the shortest time. A solid strength base is necessary before attempting plyometric exercises. Plyometric training is popular in sports that require powerful movements, such as basketball, soccer, volleyball, sprinting, jumping, and gymnastics. A typical plyometric exercise involves jumping off and back onto a box, attempting to rebound as quickly as possible on each jump. Box heights are increased progressively from about 12 to 22 inches. The bounding action attempts to take advantage of the stretch-recoil and stretch reflex characteristics of muscle. The rapid stretch applied to the muscle during contact with the ground is thought to augment muscle contraction, leading to more explosiveness (Hoeger, 2011).

Therefore, keeping in mind the study focused on answering the following questions:

- Is there any effect of plyometric training on soccer related physical fitness variables of Anna University intercollegiate female soccer players?
- What is the effect of plyometric training exercises on soccer skills like dribbling and kicking of Anna University intercollegiate female soccer players?

- What are the effects of plyometric training that contributes for the improvement of soccer related skills to our country's female soccer players?

Statement of the Problem

Plyometric training has many advantages for the improvement of athletes overall performance in various sport events including soccer. Plyometric training exercise improve explosive power, muscular strength, speed and quickness, agility, neuromuscular coordination, vertical jump performance, leg strength, muscular power, increase joint awareness and enhance soccer skill performances of the athletes. Haramaya University female intercollegiate soccer players were easily susceptible to many injuries in previous intercollegiate competition that plyometric training exercises can reduce it. Plyometric training is widely used in conditioning, power training and in prevention and rehabilitation of injuries in some sports (RoopchandMartin and Lue-Chin, 2010). Thus, this scientific study had provided practical information that can be used by all soccer players to develop physical fitness and soccer skill performance with simple and easy plyometric exercise training.

Materials and Methods

The study was focused on experimental study within 12 weeks of plyometric training exercises to examine soccer related physical fitness variables and soccer skills ability of the participants. For this study informal design was used. The study had only one group which consists of 20 participants in total. The training program was given to the participants for 12 weeks 3 days per week for 40 – 60 minutes per session. The pre-test was given to them to check their initial level. At the middle of the training period (six weeks) during test and after twelve weeks the post-test were taken to observe the changes.

Results and Discussion

This chapter deals with the analysis of data collected from the samples under study. The purpose of this study was to find out the effects of plyometric training on performance enhancement of selected physical fitness and soccer skills variables. To achieve the purpose of the study 20 players were purposely selected as subjects. The variables selected for this study were: speed, explosive power, agility, dribbling, kicking with right and left feet. Pre, during and post tests were conducted for all the 20 subjects on selected physical fitness and soccer skills variables and the scores were recorded. The collected data were analysed by paired 'T'test. The results for each fitness variable are discussed below.

Effects of plyometric training on physical fitness variables

Table 1. Mean values of Speed (sec), Agility (sec) and Vertical Jump (cm) for twenty Anna University intercollegiate female soccer players

Mean ± SD			
	PT	DT	PoT
SP	5.72±0.22	5.26 ± 0.20	4.94±0.15
AG	21.48±0.76	19.90±1.04	18.84±0.90
VJ	24.75±2.14	28.40±2.3	32.60±2.74

Note: values are mean ± SD, SP = Speed, AG = Agility, VJ =Vertical Jump. PT = Pre Training, DU = During Training, PoT = Post Training

Table 1 showed that, the speed, agility and explosive power of participants changed after post training. The mean difference showed that there was an improvement in speed after plyometric training. According to the above data the speed of participants was 5.72 before training and this was improved to 4.94 after progressive endurance training with a mean value difference of 0.78.

Effects of Plyometric Training on Soccer Skill Variables

Mean ± SD			
	PT	DT	PoT
DRB	19.96±1.45	19.13±1.17	18.04±0.90
KRF	20.76±3.40	21.81±3.16	22.95±3.05
KLF	15.44±3.89	16.95±3.48	18.35±3.11

Note: values are mean ± SD, DRB = Dribbling, KRF = Kicking Right Foot, KLF =Kicking Left Foot. PT = Pre Training, DU = During Training, PoT = Post Training

Table 2 showed that, soccer skills of dribbling, kicking right foot and kicking left foot were changed significantly after post training. The mean difference showed that there was an improvement in dribbling after plyometric training. Before the intervention, the mean difference value of dribbling as shown in the above table was 19.96 and improved to 18.04 after the intervention of plyometric training. This showed that the mean value difference of 26 1.92 was measured. Therefore, plyometric training significantly improved the dribbling skill of female soccer players.

The mean difference values and significant level of Anna University intercollegiate female soccer players.

Dependent Variable	Parametersl	Param-eterll	MD	Sig.
Speed (Sec)	PoT	PT DT	0.78 0.31	0.000 0.000
Agility (Sec)	PoT	PT DT	2.64 1.06	0.000 0.000
Vertical jump (cm)	PoT	PT DT	7.85 4.20	0.000 0.000
Dribbling (Sec)	PoT	PT DT	1.91 1.08	0.000 0.000
Kicking Right Foot (m)	PoT	PT DT	2.19 1.14	0.000 0.000
Kicking Left Foot (m)	PoT	PT DT	2.90 1.39	0.000 0.000

MD= mean difference, Sig= significance, PT= pre training test, DT= during training test, PoT= post training test. Sec=second, m= meter, cm= centimetre.

Table 3 showed the overall result of each test. It includes the mean difference from pre-test to post test and during test to post test and also the significance of post-tests in reference to pretest. As indicated in the above table, in all parameters remarkable and

ISBN: 978-93-82606-09-3

significant changes were observed. This was due to the plyometric training exercise they were engaged in.

Generally, the above table showed that there was visible effect of plyometric training on female participant's performance.

Conclusions

- Plyometric training program have shown significant improvement on speed performance of Haramaya University intercollegiate soccer players by reducing the time to cover the distance.
- Plyometric training greatly evoked the University female intercollegiate soccer player's kicking skill performances by increasing their kicking abilities for distance.
- The program also improved agility and explosive power of the University female soccer players
- At the end of the study period the dribbling skill of the players in relation to time showed reduction when compared to the pre training test. Therefore, it is possible to conclude that plyometric training improved the dribbling skill of intercollegiate female soccer players.
- The program has also shown significant change in improving the selected variables of physical fitness components and soccer skill variables.

REFERENCES:

1. Adams, K., P. O' Shea, L. O'Shea, M. Climstein, 1992. The effects of six weeks of squat, plyometric and squat plyometric training on power production. Journal of Applied Sports Science Research, 6: 34-41.
2. Agel, J., A. Arendt, B. Bershadsky, 2005. Anterior cruciate ligament injury in national collegiate athletic association basketball and soccer: a 13-year review. Am J Sports Med., 33: 524–530.
3. Alavi, H., 2006. Assessment and Measurement in Physical Education, Hafiz Publication, Tehran. Pp. 305.
4. Ashok, C., 2008. Test Your Physical Fitness: Kalpaz Publications; New Delhi, India. Pp. 359.
5. Astrand, P., K. Rodahl, H. Dahl, 2003. Textbook of Work Physiology, 4th ed. Champaign, IL: Human Kinetics; UK. Pp 186-195.
6. Baechle, R., W. Earle, 2000. Essentials of strength training and conditioning. 2nd edition. Taylor & Francis Group; London. Pp 274-283.
7. Bangsbo, J., M. Mohr, P. Krustrup, 2006. Physical and metabolic demands of training and match-play in the elite football player. J Sports Sci., 24: 665–674.
8. Barrow, H., R. McGee, 1979. A practical approach to measurement in physical education. Henry Kimpton; London. Pp. 280-282.
9. Bilal, M., 2003. Effect of the Plyometric Electric Training on the Development of the Explosive Power of the Legs and Its Relationship with the Performance of the Players to Some Basic Principles in Basketball. Ph.D. Thesis. Faculty of Physical Education, Alexandria University, Egypt. Pp: 70.
10. Billot, M., A. Martin, C. Paizis, C. Cometti, N. Babault, 2010. Effects of an electrostimulation training program on strength, jumping and kicking capacities in soccer players. J Strength Cond Res., 24:1407-1413.
11. Chelly, S., M. Fathloun, N. Cherif, M. Ben Amar, Z. Tabka, E. Van Praagh, 2009. Effects of a back squat training program on leg power, jump- and sprint performances in junior soccer players. J Strength Cond Res., 23: 2241–2249.
12. Chimera, J., A. Swanik, B. Swanik, J. Straub, 2004. Effects of plyometric training on muscleactivation strategies and performance in female athletes. J Athl Training., 39: 24-31.

ISBN: 978-93-82606-09-3

EVALUATING ACADEMIC PERFORMANCE OF SCHOOL CHILDRENS THROUGH PHYSICAL EDUCATION INTEGRATED MACHINE LEARNING (ML) APPROACH

[1]ANBIL MAHESH POYYAMOZHI, [2]PRASANNA BALAJI
[1,2]Department of Physical Education
National College (Autonomous)
Tiruchirappalli-01
Email: prasanna@nct.ac.in

Abstract:

In recent scenario every element of modern life, including education is rapidly being impacted by artificial intelligence (AI). Through predictions of academic sustainability or termination, AI can also offer learners extra support. Even though AI research is still in its infancy, it is important to monitor how it develops and realizes its promise over time. We can improve AI's potential for usage in sports applications and change the nature of physical education (PE), its visualization, and its repeatability. This present study analyses the principles and uses of AI in physical education and provides a targeted, in-depth research experimental design and systematic review of the areas of PE technology where AI could be used, including personalized PE programmes, knowledge provision, learner evaluation, and learner counseling techniques. Our findings shows how skilled AI techniques could be implemented to PE teachers, this study discusses how AI innovations are affecting all areas of life, including physical education. It also emphasizes the relevance of AI applications to PE technology based on existing research, which also suggests that the implications of AI for PE may apply to other educational domains.

Key Words: Physical Education, Artificial Intelligence, Children's Capability

1. INTRODUCTION

The present investigation aims to determine possibility to develop and enrich the best use of physical education programmes for strengthening and monitoring particular connected abilities together with sporting talents for the development. The study faces a number of realistic difficulties, including disputes among participants, poor communication, poor lifestyle choices made early in life, etc. These problems will be solved with the aid of painstakingly planned strategies, like machine learning and related skills, which would improve children's lives and also promote them as players and all-around effective human beings. We are all prepared to dive into the dynamic alteration of children's lives in the future, but as the studies are still in its early stages. There is evidence that supports increased physical activity and physical fitness may enhance academic achievement, and that allocating time to physical education classes, recess, and in-class physical activity may also help academic success.

The research that is currently available indicates that reading and mathematics are the academic subjects mostly influenced by physical activity. Executive function, which has been connected to physical exercise and physical health, is essential to these themes. The foundation of academic performance is executive function and brain health. Learning is facilitated by fundamental cognitive processes related to attention and memory, which are improved by exercise and greater cardiovascular fitness. Physical activity both in short bursts and over time enhances mental function and brain health. youngsters who take part in physical trainings. The network-based remote education and education system has made significant development and progress due to the rising maturity of Internet technology, computer technology, and information technology. Furthermore, teaching tasks are increasingly carried out in network environments due to the full integration of network communication technology with multimedia technology at this point, which has fundamentally altered the teaching mode. Regarding the drawbacks of conventional models, which are constrained by physical and temporal constraints, the network teaching model has the qualities of autonomy and interaction, which facilitate and improve communication between students as well as between students and professors. The network teaching model also addresses the classic single-assisted teaching paradigm's weaknesses, which include its inability to provide timely feedback on a variety of inputs.

The sports video image analysis system can collect and analyze the actual movements of the human body, improve the technical essentials of athletes, and find problems and find gaps. At present, there is no perfect sports video analysis software, which makes it difficult to correct sports movement errors. Therefore, the field of sports analysis has important application value in sports training. Motion analysis software is widely used in the daily training of sports associations around the world. The use of these systems can greatly facilitate the training of athletes. Moreover, it is an effective tool to provide in-depth analysis and comparison of the completion of technical actions after training. Sports digital training system can collect and analyze training data and simulation training results on a large scale, promote training experience to theory, and verify whether some training methods are

reasonable. With the support of computer-assisted training mode, sports training will be organically combined with information science such as statistical analysis, video processing, simulation technology, and functional technology to provide powerful tools for the development of sports training and expand the application space for information science'

The sports professional test requires that the specialty students reach a certain level in strength, speed, endurance, flexibility, and agility. This requires schools to have a complete and scientific training system for the training of high school sports students and requires physical education teachers not only to rely on the experience to train, but also to fully consider the individual differences of the students. If we hope that we can quickly improve all aspects of our specialty students in a short period of time to achieve a good level in all aspects of physical fitness, strength, sports technology, and athletic qualities, we must use different management methods and adopt different methods and methods to carry out effective sports specialty training. To improve the performance of specialty students, scientific management, scientific training and scientific selection of talents are indispensable. Then, to realize the scientific and intelligent management, training and selection is the only way. At present, information technology has become the backbone of the continuous progress of human society, and has had a huge impact on people's production and lifestyle. At the same time, the development of sports in our country is also deeply affected. Therefore, for the training of high school sports students, it is very necessary to rely on modern methods to create a convenient, fast, reasonable and practical training management system.

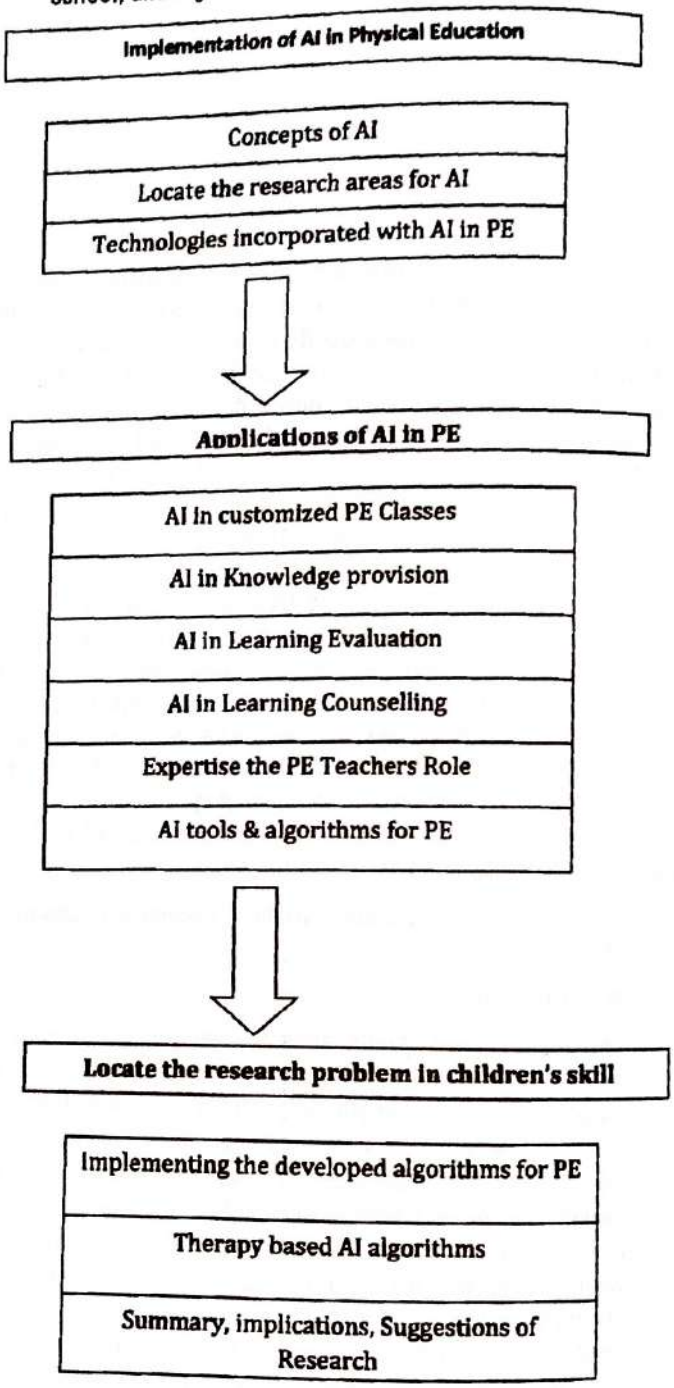
2.

3. DETAILS EXPERIMENTAL

2.1. Materials and Procedures

Methodology

As the researcher mentions that the study is at the initial stage, the researcher could explain the methodology to be undertaken for the due course. The study will commence with the observation of physical education activities in general. In the continuation of that, the infrastructure, learning and teaching resources available in the schools will be taken into consideration. A curriculum is to be designed based on a popular game in which the students of the region would love to involve and a mechanism to monitor the associated skills of Communication, Leadership, Collaborative learning, Tolerance, Interpersonal, Intra-personal, Problem Solving and Crisis Management along with sporting skill will be worked out. A government school, a private school and a government- aided school will be taken for the study simultaneously and the proposed method will be experimented in the vernacular region. Given that the study is in its early stages, the researcher should outline the approach that will be used in due time. Starting with a general observation of physical education activities, the study will proceed. The infrastructure, educational and instructional resources offered by the schools will be taken into account in the following section. A mechanism to track the associated skills of Communication, Leadership, Collaborative Learning, Tolerance, Interpersonal, Intra-personal, Problem Solving and Crisis Management along with sporting ability will be developed. The curriculum will be based on a well-liked game that the students of the area would love to participate in. The test will be administered in a government school, a private school, and a government-sponsored school.

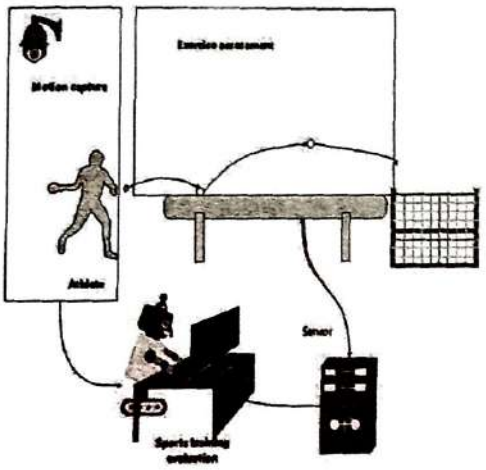


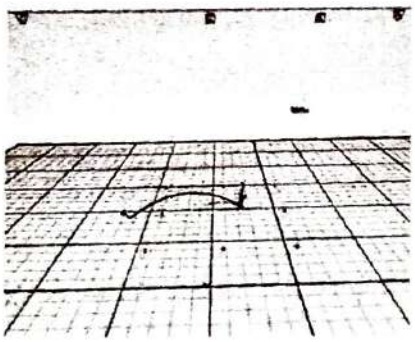
RESULTS AND DISCUSSION

3.1. As the title goes, Physical Activities for Skill Development among School Children using Machine Learning Technology, several skills would be proliferated and monitored, assessed with the aid of Physical Education. On the other hand Inter disciplinary learning could be achieved in classroom activities by incorporating

ISBN: 978-93-82606-09-3

the virtues attained from physical activities. This study would enable the potential to unearth the importance of inculcating regular physical education activities much more productive and vibrant by nurturing entire related skills mentioned in the study. It would encompass the teachers of other subjects to offer integrated instructions through physical activities. Predominantly this research focuses on to deduce the boredom and maximise learning through activity based teaching and learning process. The sports digital training system is of great significance to the development of sports. The sports video image analysis system can collect and analyze the actual movements of the human body, improve the technical essentials of athletes, and find problems and find gaps. At present, there is no perfect sports video analysis software, which makes it difficult to correct the movement error. Therefore, it has important application value in the field of sports analysis in sports training. This study uses the Internet of Things technology to achieve long-term uninterrupted data collection. After certain processing of the data, an extreme learning machine is used to predict the situation of sports training. In order to solve some shortcomings of the original extreme learning machine, gradual optimization is carried out from two aspects of hidden layer mapping and parameter optimization to further improve the accuracy of prediction. Moreover, this research constructs a physical education training system based on actual needs to optimize the physical education and training process. In addition, this paper designs experiments to verify the performance of the system constructed in this paper. From the experimental research, it can be seen that the system constructed in this paper can basically meet the actual needs of physical education and training.





CONCLUSIONS

This study positively elevates the dynamic ideologies of profound transformation in the learning curriculum of children.

Previous research and academic evidences suggests that increasing physical activity and physical fitness may improve academic performance and that time in the school day dedicated to recess, physical education class, and physical activity in the classroom may also facilitate academic performance.

Available evidence suggests that mathematics and reading are the academic topics that are most influenced by physical activity. These topics depend on efficient and effective executive function, which has been linked to physical activity and physical fitness.

Executive function and brain health underlie academic performance. Basic cognitive functions related to attention and memory facilitate learning, and these functions are enhanced by physical activity and higher aerobic fitness.

Single sessions of and long-term participation in physical activity improve cognitive performance and brain health. Children who participate in vigorous- or moderate-intensity physical activity benefit the most.

Given the importance of time on task to learning, students should be provided with frequent physical activity breaks that are developmentally appropriate.

Although presently understudied, physically active lessons offered in the classroom may increase time on task and attention to task in the classroom setting.

6. ACKNOWLEDGMENTS

I would like to express my special thanks of gratitude to **K Raghunathan, Secretary, National College (Autonomous), Tiruchirappalli** as well as our principal who gave me the golden opportunity to do this wonderful project on the topic Importance of Physical Fitness to Elevate the Academic Skills in School Children's, which also helped me in doing a lot of Research.

7. REFERENCES

1. Rein R, Memmert D (2016) Big data and tactical analysis in elite soccer: future challenges and opportunities for sports science[J]. Springerplus 5(1):1–13
2. Ridderinkhof KR, Brass M (2015) How kinesthetic motor imagery works: a predictive-processing theory of visualization in sports and motor expertise[J]. J Physiol-Paris 109(1–3):53–63
3. Mulligan D, Lohse KR, Hodges NJ (2016) An action-incongruent secondary task modulates prediction accuracy in experienced performers: evidence for motor simulation[J]. Psychol Res 80(4):496–509
4. Khudolii OM, Ivashchenko OV, Iermakov SS et al (2016) Computer simulation of junior gymnasts' training process[J]. Sci Gymnast J 8(3):215–228

5. Tivener KA, Gloe DS (2015) The effect of high-fidelity cardiopulmonary resuscitation (CPR) simulation on athletic training student knowledge, confidence, emotions, and experiences[J]. Athl Train Educ J 10(2):103–112

6. Ivashchenko OV, Kapkan OO (2015) Simulation of process of 14–15 years old girls' training of light athletic and gymnastic exercises[J]. Pedag, Psychol, Med-Biol Probl Phys Train Sports 8:32–40

7. Owen PD, King N (2015) Competitive balance measures in sports leagues: the effects of variation in season length[J]. Econ Inq 53(1):731–744

8. Yang J (2019) The Simulation of table tennis during the course of sports[J]. Carib J Sci 52(4):1561–1564

9. Bulat M, Can NK, Arslan YZ et al (2019) Musculoskeletal simulation tools for understanding mechanisms of lower-limb sports injuries[J]. Curr Sports Med Rep 18(6):210–216

10. Payne T, Mitchell S, Bibb R et al (2015) The evaluation of new multi-material human soft tissue simulants for sports impact surrogates[J]. J Mech Behav Biomed Mater 41(5):336–356

11. Juan Pablo ngel-Lo´pez, Nelson Arzola de la Pen´a. (2015) Kinematic hand analysis using motion capture technology[J]. IFMBE proceedings, 49(6):257-260.

12. Kim MK, Kim TY, Lyou J (2015) Performance improvement of an AHRS for motion capture[J]. J Institute Control 21(12):1167–1172

 ISBN: 978-93-82606-09-3

THE IMPORTANCE OF HEALTH, FITNESS AND WELLNESS

Dr.Rn Woreithing
Assistant Professor, Department of Physical Education,
National College, Tiruchirapalli, Tamilnadu,India
P S Poukinreiyang Kamei,
Ph.D. Research Scholar, Department of Physical Education and Sports
National College, Trichirapalli-01, Tamilnadu
Soraisham Sunilkumar Singh
Ph.D. Research Scholar, Department of Physical Education and Sports
National College, Trichirapalli-01, Tamilnadu

Abstract

In the present of world of space age and automation era, all human being appear to be living in a more and more inactive life, they ride instead of walk, sit instead of stand and watch instead of participating. Such type of inactivity or sedentary life is detrimental to mental and physical health, Thus there is a great need for physical education as a part of balanced living.

Physical activity ought to become as a routine of every living human being for achieve their goal of life. The sports such as cycling, swimming, jogging, walking, aerobics, and soccer played regularly, are not only for fun but could promote healthy life. Physical activity can be healthful in the every way which you could do with the proper ways and through guidance. Physical activities also reduce the level of fatigue, distress anxiety and other type of uncomforted and maintain proper health, weight which is very useful to keep strong from the diseases and give us a peacefulmind to live a tremendous life through a physical activities.

Primary components of fitness

Cardio respiratory capacity

Muscular Capacity
Flexibility
Body Composition

Secondary Components of Fitness

Balance
Coordination
Agility
Reaction time
Speed
Power
Mental Capability
Health and wellness
Social health
Mental health
Emotional health
Spiritual health

The Importance of Health, Fitness and wellness

In the present of world of space age and automation era, all human being appear to be living in a more and more inactive life, they ride instead of walk, sit instead of stand and watch instead of participating. Such type of inactivity or sedentary life is detrimental to mental and physical health, Thus there is a great need for physical education as a part of balanced living.

Primary components of fitness

The four primary components (also known as the components of health related fitness) that are important to improved physical health are as follows.

Cardio respiratory capacityis the ability of the body to take in oxygen (respiration), and used it at the cellular level to create energy (Bioenergetics) for physical work (activity). In fitness, we also refer to cardio respiratory capacity as aerobic capacity. This capacity includes aerobic endurance (how long) aerobicpower (how fast). Some of the long term adaptations of cardio respiratory training are: decreased resting heart rate, decreased risk of cardio vascular disease, improve endurance, increase stroke volume and cardiac output.

Muscular Capacity refers to the spectrum of muscular capability, this includes muscular endurance (i.e. the ability to apply force over a long period of time or to compete repeated muscle contraction); Muscular strength (i.e. the ability to generate force, or the maximum amount of force that a muscle can exert in a single contraction); and muscular power) i.e., the ability to generate strength In an explosive way) some of the long term adaptations of improving muscular capacity are increased strength, improved muscular endurance, increased basal metabolic rate, improved joint strength and overall posture.

Flexibilityis the range of movement or amount of motion that a joint 1 capable of performing, each joint has a different amount of flexibility, some of the long term adaptations of improved flexibility are decreased risk o injury, improved range of motion, improved bodily movements and improved posture.

Body composition

Is the proportion of fat- free mass (muscle, bone, blood, organs and fluids) to fat mass(adipose tissues deposited under the skin and

around organs) Some of the long term adaptations of improving body composition are decreased risk of cardiovascular diseases improved basal metabolic rate, improved bodily function and improved **BMI**

Secondary Components of fitness

The secondary components of fitness (also known as the components of performance based fitness) are involved in all physical activity and are necessary for the daily functioning athletes experience different levels of success depending on how well these secondary fitness components are developed. Although the primary components of fitness are thought to be the most important, we should not ignore the secondary components because of their importance in the completion of daily tasks. The secondary components include the following.

Balance is the ability to maintain a specific body position in either a stationery or dynamic (moving) situation.

Coordination is the ability to use all body parts together to produce smooth and fluid motion.

Agility is the ability to change direction quickly

Reaction time is the time required to respond to a specific stimulus.

Speed is the ability is to move rapidly, speed is also known as explosive strength.

Power is the product of strength and speed, power is also known explosive strength.

Mental capability is the ability to concentrate during exercise to improve training effects as well as the ability to relax and enjoy the psychological benefits of activity (endorphins).

Health and wellness

Health is a dynamic process because it is always changing. We all have times of good health, times of sickness, and may be even times of serious illness as our lifestyle change so does our level of health.

Those of us who, participate in regular physical activity do so partly to improve the current and future level of our health. We strive toward on optimal state of well-being. As our lifestyle improves and we experience less disease and sickness, when most people are asked what it means to be healthy, they normally respond with the four components of fitness mentioned earlier.(cardio respiratory ability, muscular ability, flexibility and body composition).Although these components are a critical part of being healthy, they are not the only contributing factors. Physical health is only one aspect of our overall health.

The other components of health (Greenberg,2004, p.7) that are just as important as physical health include the following.

Social health – The ability to interact well with people and the environment and to have satisfying personal relationships.

Mental health – The ability to learn and grow intellectually. Life experience as well as more formal structures enhances mental health.

Emotional health- The ability to control emotions so that you feel comfortable expressing them and can express them appropriately.

Spiritual health- A belief in some unifying force. It varies from person to person but has the concept of faith at its core. Wellness is the search for enhanced quality of life, personal growth and potential through positive lifestyle behaviors and attitudes. If we take responsibility for your own state of wellness including nutrition physical activity, stress- coping methods, good relationship and career success.

Each day we work toward maximizing our level of health and wellness to live long full and healthy lives. The pursuits of health, personal growth and improve quality of life relies on living a balanced life to achieve balance; we need to care for our mind body and spirit.

If any one of these areas is consistently lacking or forgotten about, we will not be at our optimal level of health, we are constantly challenged with balancing each of these three areas throughout life.

As fitness professional we have responsibilities to guide and motivate others to improve their level of health and wellness. We can promote a holistic approach to health (mind, body and spirit) not just encourage physical activity. As good role models, we should demonstrate positive health behaviours that assist in improving our own health and the health of others. If our focus is strictly on the physical benefits of exercise we are doing a disservice two hour clients and we are not fulfilling our professional obligation.

Benefit of physical activity

As fitness professional we spend a greater deal of time inspiring and assisting others in their pursuit of improving health. Education is important aspects of these we must promote be benefits of regular activity and help people understand why they should be active.

Activity guideline

Health Canada introduced Canada's physical activity guide to healthy active living to help Canadians make wise choice about physical activity as a way to improve health. Scientist say you should accumulate 60 minutes wise choice about physical activity everyday to stay healthy or improve health. The recommendations in the physical activity guide are as follows.

- Endurance on 4 to 7 days a week, perform continuous activity for your heart, lungs and circulatory system. Time required for improvements depends on effort.
- Flexibility on 4 to 7 days a week, perform gentle reaching, bending and stretching to keep muscles relaxed and joints mobile.
- Strength on 2 to 4 performs resistance exercise to strengthen muscles and bones and improve posture. The American college of sports medicine (ACSM) has also developed activity guidelines for improving health.
- Perform 30 minutes or more for moderate intensity physical activity on most days of the week for cardiovascular health, the 30 minutes need and endurance.

ISBN: 978-93-82606-09-3

- Performing 1 set of 8 to 12 repetitions of resistance training for the entire body is necessary to maintain and develop muscular strength and endurance.
- Flexibility training should be performed daily. Including stretches for all major muscles groups in order to maintain mobility.

Benefits	Effects on health
Reduce the risk of pre mature death	Active people live longer with a better quality of life
Reduce the risk of cardio vascular disease	Strengthens the heart, cardio vascular system and respiratory help fight cardio vascular disease
Decrease resting heart rate	Reduce the daily wear and tear on the cardio vascular system
Keeps resting blood pressure normal	Improve the ability to for form daily activities at higher integral greater ease
Improves heart efficiency	Reduce the risk of mature life-threatening diseases, such as cardio vascular diseases and diabetes.
Decrease body far	Develops strong abdominals and back muscles easier and safer when the body has better control
Promotes joints stability	All movements in daily life become easier and safer when the body has better control
Increases muscular strength	An improved outlook on life makes all activities more enjoyable
Strengthen bones	Everyday life becomes more enjoyable because the person has a positive way to deal with stress

Conclusion:

Physical fitness is one of the most important elements of leading a healthy life style. Physical education promotes the importance of inclusion of a regular fitness activity in the routine. This helps the students to maintain their fitness, develops their muscular strength, increase their stamina and thus stretch their physical abilities and optimal level.

REFERENCE:

1. **Bauman A. E. (2004).** Updating the evidence that physical activity is good for health: an epidemiological review 2000-2004. J sci med sports;7(1supp1): pp. 6-19.
2. **Penedo F.J., Dahn J.R. (2005).** Exercise and wellbeing: a review of mental and physical health benefits associated with physical activity. Curropin psychiatry; 18(2): pp. 189-93.
3. **Littman A.J., Forsberg C.W., Koepsell T.D. (2009).** Physical activity in national sampleof veterans. Med sci. sports exerc; 41(5): pp. 1006-13.
4. http://bodyfitnesshealth.com/benefits-and-importance-of-physical-education
   www.humankinetics.com/exeperts/exeperts/the-importance-of-health-fitness-and wellness.

Journal of Electronic Materials
https://doi.org/10.1007/s11664-022-09546-2

ORIGINAL RESEARCH ARTICLE

Crystal Growth of L-Alanine Oxalic Acid Crystal and its Spectral, NLO, Mechanical, Thermal, and Impedance Properties

S. Thangavel[1] · V. Kathiravan[2] · R. Ashok Kumar[1] · S. Eniya[2] · G. Satheesh Kumar[3] · P. Selvarajan[4] · M. Kumaresavanji[3]

Received: 18 December 2021 / Accepted: 22 February 2022
© The Minerals, Metals & Materials Society 2022

Abstract
Single crystals of L-alanine oxalic acid (LAO) were grown by adopting the slow evaporation technique at room temperature. Single-crystal x-ray diffraction was used to evaluate the crystal structure of the as-grown LAO. The functional groups of LAO were identified by FT-IR spectroscopy. UV-Visible-NIR transmittance study was carried out to determine the bandgap energy. A fluorescence study was performed to obtain the emission spectrum of the LAO crystal. Vickers microhardness study was carried out to evaluate the mechanical properties of the LAO crystal. Thermogravimetric and differential thermal analysis were also conducted to investigate the thermal properties of the LAO crystal. The efficiency of the title crystal's second harmonic generation (SHG) was investigated. The electrical properties were estimated by impedance study. A laser damage threshold (LDT) study revealed that the LAO crystal has a high laser damage threshold.

Keywords X-ray diffraction · FTIR · UV · fluorescence · thermal analysis · impedance · SHG

Introduction

Organic materials are gaining research prominence due to their fascinating nonlinear optical properties in applications such as optical communication, optical computing, optical amplifiers, optical parametric oscillators, second harmonic devices, and other electro-optical devices.[1] Organic materials are immensely appealing from a technical standpoint because of their inherent nonlinearity, synthetic flexibility, high laser damage threshold, and ability to modify their characteristics via functional replacements.[2,3] Amino acids are chemical compounds composed of a proton donor carboxylic acid (COOH) and a proton acceptor amine (NH_2) group. They are utilized to synthesize novel derivatives and as a guide for the discovery of new second- and third-order NLO [nonlinear optical] materials.[4–7]

L-Alanine is the smallest chiral amino acid known in nature. It has a non-reactive hydrophobic methyl group (CH_3) as a side chain. Over a broad pH spectrum, L-alanine has the zwitterionic form (NH_3^+ and COO^-) both in crystal and in an aqueous solution. The family's simplest acentric member, L-alanine, was crystallized for the first time by Bernal and assigned to the $P2_12_12_1$ space group.[8–10] Earlier in the literature, the development and characterization of complexes of L-alanine single crystals were described.[11] Improved nonlinear optical (NLO) characteristics are expected when L-alanine is mixed with a variety of organic and inorganic acids to form new materials.[12,13]

A comparative study was performed by Urit et al.[14] on L-alaninium maleate (LAM) single crystal grown by the slow evaporation technique and by the Sankaranarayanan-Ramasamy (SR) method. An organic nonlinear optical material LAM was synthesized by Balasubramanian et al.[15] A new nonlinear optical organic single-crystal *l*-alaninium fumarate (LAF) belonging to the amino acid group was

✉ R. Ashok Kumar
rajamaniashokkumar@gmail.com

1 PG and Research Department of Physics, Thiruvalluvar Government Arts College, Periyar University, Rasipuram, Tamilnadu 637401, India

2 Crystal Growth Laboratory, PG and Research, Department of Physics, Government Arts College (Autonomous), Bharathidasan University, Karur, Tamilnadu 639005, India

3 Crystal Growth Laboratory, PG and Research, Department of Physics, National College (Autonomous), Bharathidasan University, Tiruchirappalli, Tamilnadu 620001, India

4 Department of Physics, Aditanar College of Arts and Science, Manonmaniam Sundaranar University, Tiruchendur, Tamilnadu 628216, India

Published online: 16 March 2022

grown from aqueous solution by employing a slow evaporation solution technique (SEST) by Ramachandra Raja et al.[16] Good-optical-quality bulk single crystals of NLO material L-alanine formate (L-ALF) were grown successfully by a modified SR method and characterized by Justin Raj et al.[17] Wojciechowski et al. have studied the photoinduced effects in L-alanine crystals.[18] Novel nonlinear single crystals with large second harmonic generation and efficient nonlinear optical properties have been studied, and these crystals could be used for optoelectronic and photonic applications.[19–27] Keeping various applications in mind, L-alanine was combined with oxalic acid using the slow evaporation technique to create L-alanine oxalic acid (LAO) crystals. Single-crystal x-ray diffraction (XRD), UV-visible transmission, Fourier transform infrared (FT-IR) spectral analysis, thermal analysis, microhardness measurement, and impedance analysis were performed on the generated crystal. The Kurtz–Perry powder test was also used to study the developed LAO crystal's NLO characteristics.

Experimental Procedure

Preparation and Growth

L-Alanine (LOBA, 99% purity) and oxalic acid (LOBA, 99% purity) were taken in a stoichiometric ratio of 1:1 and dissolved in double-distilled water. Saturated solution was prepared, and continuous stirring was performed at room temperature for 5 hours. This solution was then filtered in a vessel using Whatman filter paper. It took about 5 days to change the saturated solution into a supersaturated solution due to slow evaporation. In the supersaturated solution, minute crystals were initially formed after a period of 10 days. These minute crystals were used as the seed crystals for the further growth. Some good-quality seed crystals were immersed into the supersaturated solution in the growth vessel. After a growth period of 26 days, optically excellent crystals of LAO were extracted from the mother solution using the slow evaporation technique. The photograph of grown crystal of LAO is shown in Fig. 1. The crystal of LAO has high optical transparency and is colourless.

Results and Discussion

Single-crystal X-ray Diffraction (XRD) Analysis

Using a Bruker Apex-II single-crystal x-ray diffractometer, the lattice parameters of the grown crystal were determined using single-crystal XRD analysis. This study shows that the grown crystal was an orthorhombic system with space group $P2_12_12_1$. This space group is a non-centrosymmetric space group, and hence LAO crystal has both second-order and third-order NLO properties. The values of lattice parameters are $a = 5.605$ Å, $b = 7.304$ Å, and $c = 19.465$ Å, the angles are $\alpha = 90°$, $\beta = 90°$, and $\gamma = 90°$, and the volume is $V = 798.87$ (Å^3). The lattice parameter values are given in Table I.

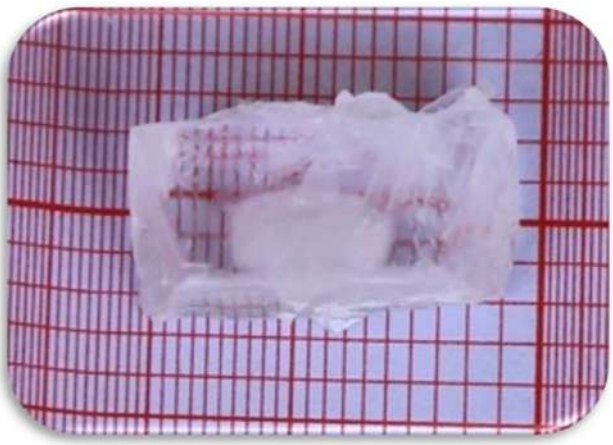


Fig. 1 As-grown crystal of LAO.

Table I Lattice parameter values of LAO crystal

Cell parameter (Å)			Volume (Å^3)	System
a	*b*	*c*	798.87	Orthorhombic
5.605	7.304	19.465		

Fourier Transform Infrared (FT-IR) Spectral Study

A Perkin Elmer spectrometer was used to record the FT-IR spectrum in the range 4000–500 cm^{-1} at room temperature using the KBr pellet technique. Figure 2 shows the LAO crystal's recorded FT-IR spectrum.

O–H stretching causes the absorption peak to appear at 3210 cm^{-1}. C–H stretching causes the vibration peak at 2917 cm^{-1}. NH stretching is responsible for the peak at 2379 cm^{-1}. C=O symmetric stretching is responsible for the peak at 1715 cm^{-1}. The transmission peaks observed at 1581 and 1506 cm^{-1} correspond to the ammonium group (NH_3^+ bending). In the crystal, the carboxylic group appears as COOH, and an ionized carboxylic group is clearly observed in the region of 1444–1111 cm^{-1}.

The vibration peaks observed at 969 and 915 cm^{-1} are attributed to the overtone of the torsional oscillation of NH. The C–N deformation and C=O deformation mode of the vibration peak at 820 cm^{-1} are due to ring deformation. Absorption peaks characterizing different functional groups are shown in Table II.

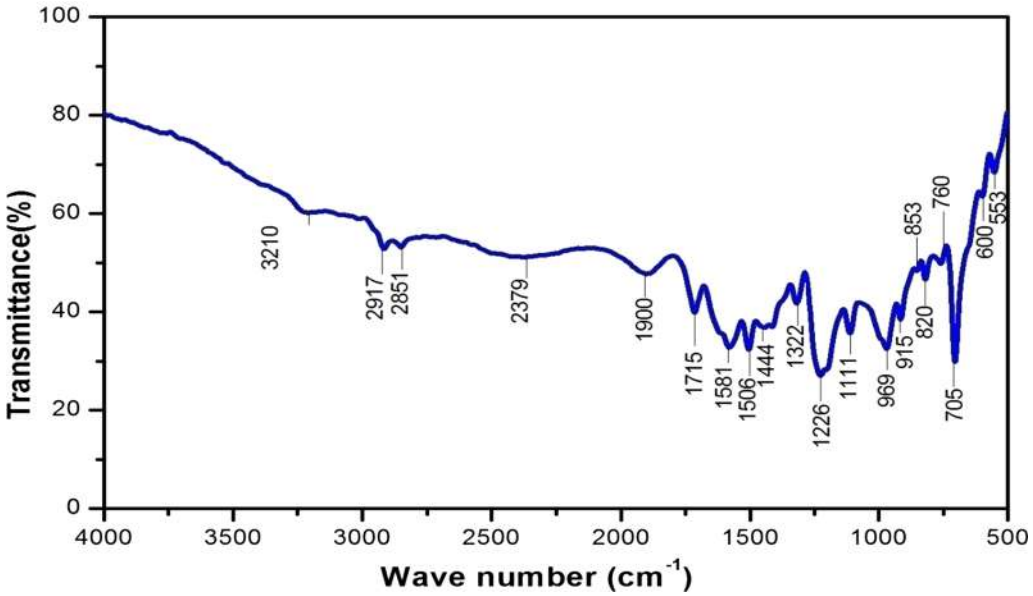


Fig. 2 FT-IR spectrum of LAO crystal.

Table II FTIR peaks and their assignments for LAO crystal

Wavenumber (cm^{-1})	Assignment
3210	O–H stretching vibrations
2917	C-H stretching
2379	NH stretching
1715	C=O symmetric stretching
1581,1506	NH bending
1444–1111	COO^- symmetric stretching
969,915	Overtone torsional oscillation of NH
853	C–C–N stretching
820	Ring deformation
760	O–C–O deformation
705	C=O deformation
600	C–N deformation
553	COO^- rocking

Optical Studies

UV-Vis-NIR Spectral Analysis

The optical transmission spectra of a well-grown LAO crystal were measured in the range 190–1100 nm using a PerkinElmer Lambda 35 spectrophotometer. The spectrum was recorded using an as-grown LAO crystal with a thickness of 3 mm. Figure 3 illustrates the UV-Vis-near-infrared (NIR) transmittance spectrum of the as-grown LAO crystal. It is observed that the transparency of the crystal is high in the visible region. Because this NLO crystal is mainly utilized in optical applications, it is critical to determine the UV transmission and lower cut-off wavelength. The LAO crystal's lower cut-off wavelength is 295 nm, and the bandgap energy was determined to be 4.2 eV using the formula $E_g = hc/\lambda$ eV. Due to its absence of absorption in the visible region, this crystal is obviously suitable for use as window material in optical equipment.

Nonlinear Optical Study

The second harmonic generation (SHG) performance of the LAO was estimated using the Kurtz and Perry Powder method. The fundamental beam was generated by a Q-Switched Nd:YAG laser with a wavelength of 1064 nm, a pulse duration of 10 ns, and a frequency repetition rate of 10 Hz.[28] It was transmitted through the powder sample. The laser beam's output, which emitted a brilliant green light (λ = 532 nm), verified the SHG behaviour. With 29 mJ/pulse input energy, the LAO crystal generated a 4.65 mV second harmonic signal, whereas the conventional potassium dihydrogen phosphate (KDP) crystal generated an 8.9 mV SHG. Consequently, the grown LAO crystal's SHG efficiency is 0.52 times that of the standard KDP crystal.

Luminescence Studies

When the electronic energy levels of solids are excited by light of a particular energy and then released as light, the phenomenon of fluorescence occurs. The fluorescence spectrum of the crystal was examined to verify the emission spectrum for that particular emitted state of the system and for decaying the components using a

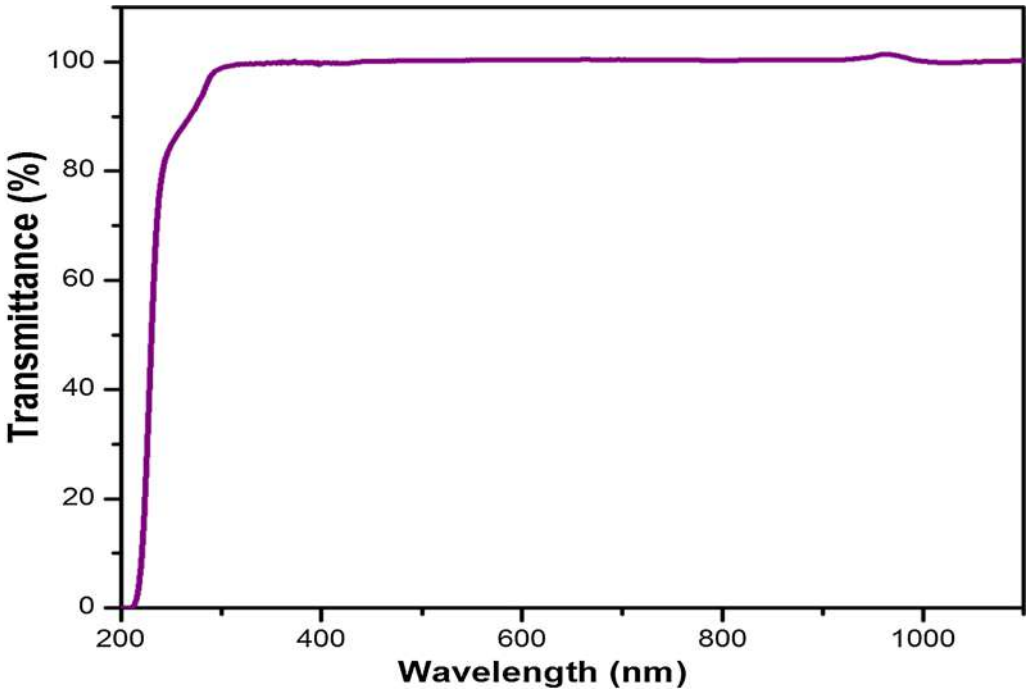


Fig. 3 UV-Vis-NIR spectrum of LAO crystal.

Perkin Elmer fluorescence spectrofluorometer, as seen in Fig. 4.[29] UV light at a wavelength of 240 nm was used as the excitation wavelength. An intense emission peak was observed around 532 nm, which is in the visible region of the electromagnetic spectrum. The result indicates that the LAO crystal has a green fluorescence emission. The title compound's powerful fluorescence emission implies that it may be a successful candidate for optoelectronic applications.[30]

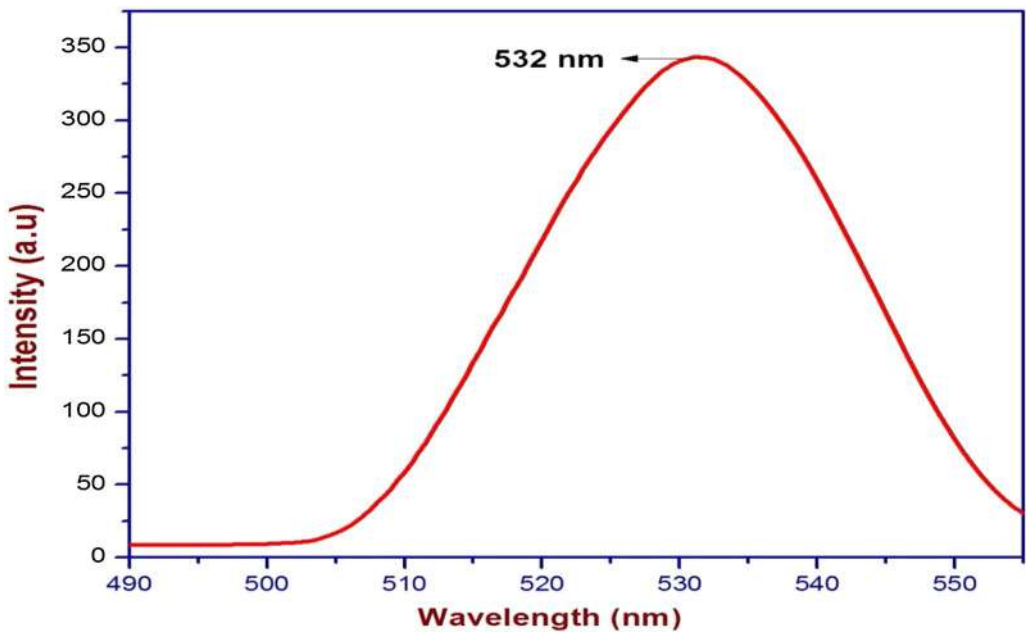


Fig. 4 Fluorescence emission spectrum of LAO crystal.

Mechanical studies

At room temperature, microhardness measurements were conducted on the flat polished face of the grown LAO crystal using a Shimadzu HMV-2T tester equipped with a Vickers diamond indenter. Using the following equation, the Vickers microhardness number (H_v) was calculated.

$$H_v = \frac{1.8544p}{d^2}\ \mathrm{kg/mm^2} \tag{1}$$

where p denotes the indenter load in kilograms and d represents the impression's diagonal length in millimetres.[31] Figure 5 illustrates the estimated load dependence of Vicker's hardness number. According to the graph, the hardness value increases as the load increases, and cracks appeared on the smooth surface of the LAO crystal above 55 g due to the release of internal stresses produced locally by indentation. The increasing trend of hardness with increase of applied load is due to reverse indentation size effect. The well-known Meyer's law $P = ad^n$, where a and d are constants that vary depending on the content, gives the relationship between load and indentation size.

The plot of log p versus log d for the LAO crystal in Fig. 6 is a straight line, and the work hardening coefficient n was determined to be 3.701. Onitsch and Hanneman previously demonstrated that the value of n is between 1 and 1.6 for hard materials and greater than 1.6 for soft materials.[32] The estimated value of $n = 3.701$ indicates that the grown LAO is soft and displays a reverse indentation size effect [RISE] (Figs. 7, 8).[33]

The yield strength (σ_y) has also been calculated from the hardness value using the relationship (for $n > 2$).[34]

$$\sigma_y = H_v/3\ \mathrm{N/m^2} \tag{2}$$

The elastic stiffness constant (C_{11}) was determined empirically using Wooster's law.[35]

$$C_{11} = \left(H_v\right)^{7/4}\ \mathrm{N/m^2} \tag{3}$$

The fracture toughness (K_c) value reflects how well the fracture resists fracture when subjected to continuous stress. The following relationship is used to determine the fracture toughness:

$$K_c = \frac{P}{\beta C^{3/2}}\mathrm{kg\ mm^{-3/2}} \tag{4}$$

where C is the crack length estimated from the middle of the indentation mark to the crack tip, and β is the indenter constant equal to 7 for the Vickers indenter.[34]

Brittleness is an inborn property that influences a material's mechanical behaviour and decides whether or not it can fracture without significant deformation. The brittleness index (B_i) value is determined using the following formula:

$$B_i = \frac{H_v}{K_c} \tag{5}$$

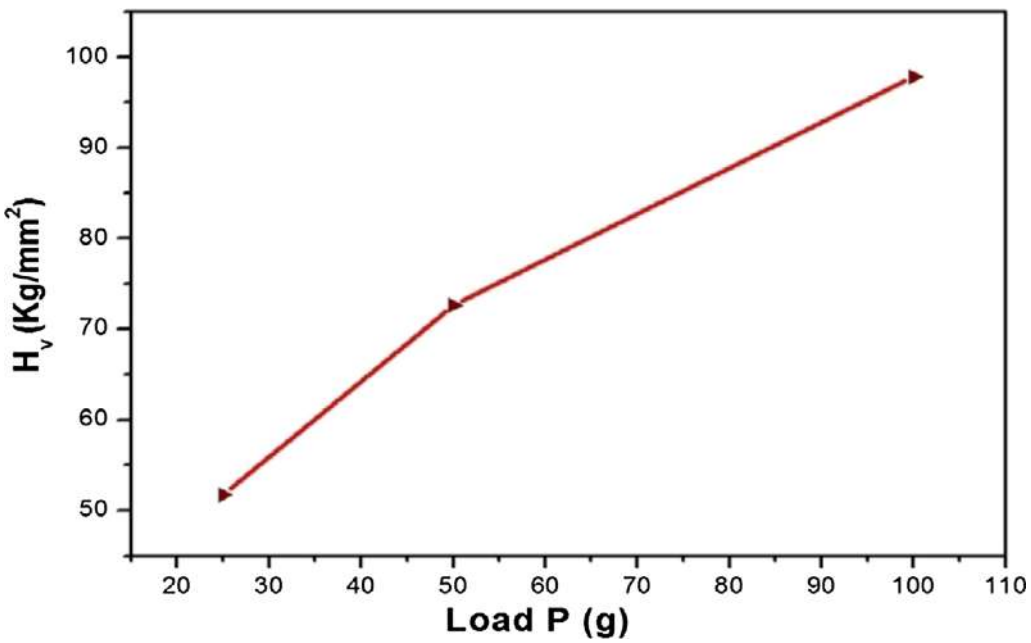


Fig. 5 Variation of Vickers hardness number with applied load for LAO crystal.

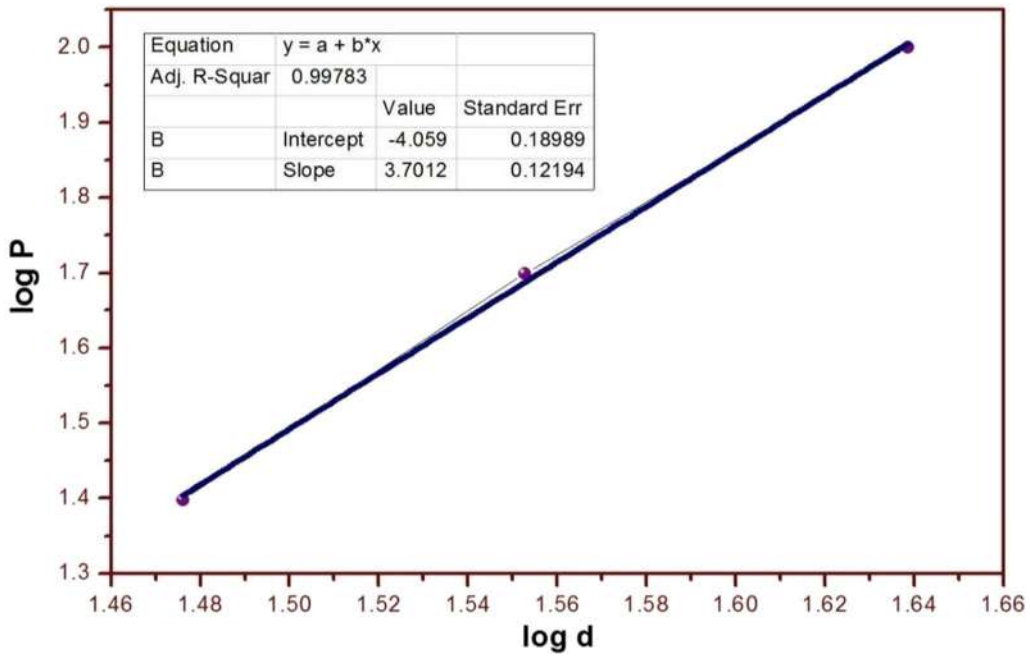


Fig. 6 Plot of log p versus log d for LAO crystal.

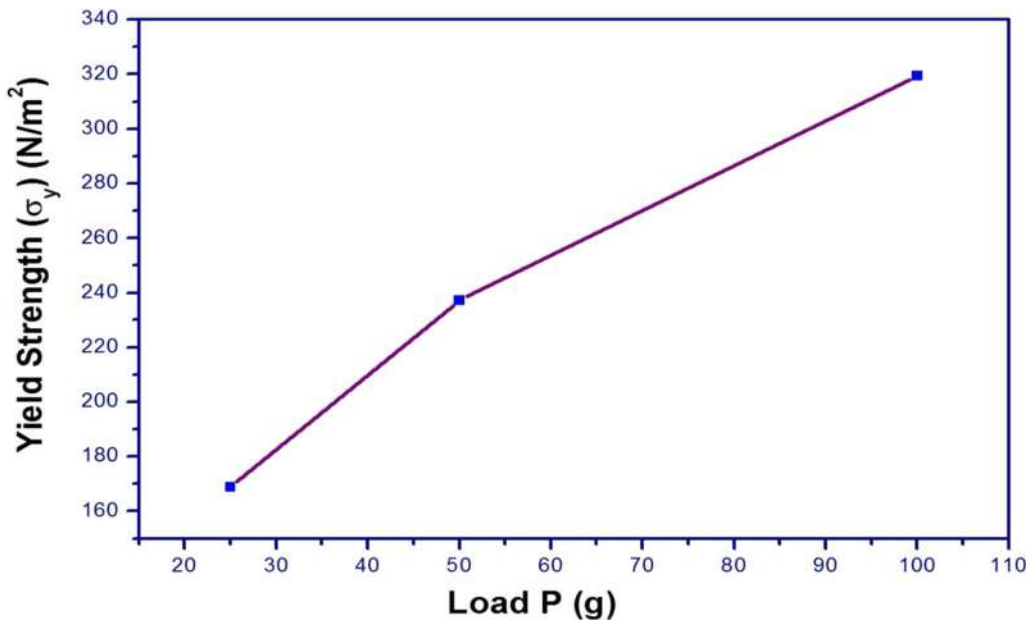


Fig. 7 Plot of yield strength versus load for LAO crystal.

The mechanical parameters like Vicker's hardness number (H_v), stiffness constant (C_{11}), yield strength (σ_y), fracture toughness (K_c) and brittleness index (B_i) are detailed in Table III for various loads (P).

Thermal Analysis—TG/DTA

Thermal analysis is a very helpful approach for characterizing a crystal's thermal stability and determining its thermal conductivity.[36] The NETZCHSTA449F3 simultaneous thermogravimetric/differential thermal analysis analyser was utilized in a nitrogen environment to conduct

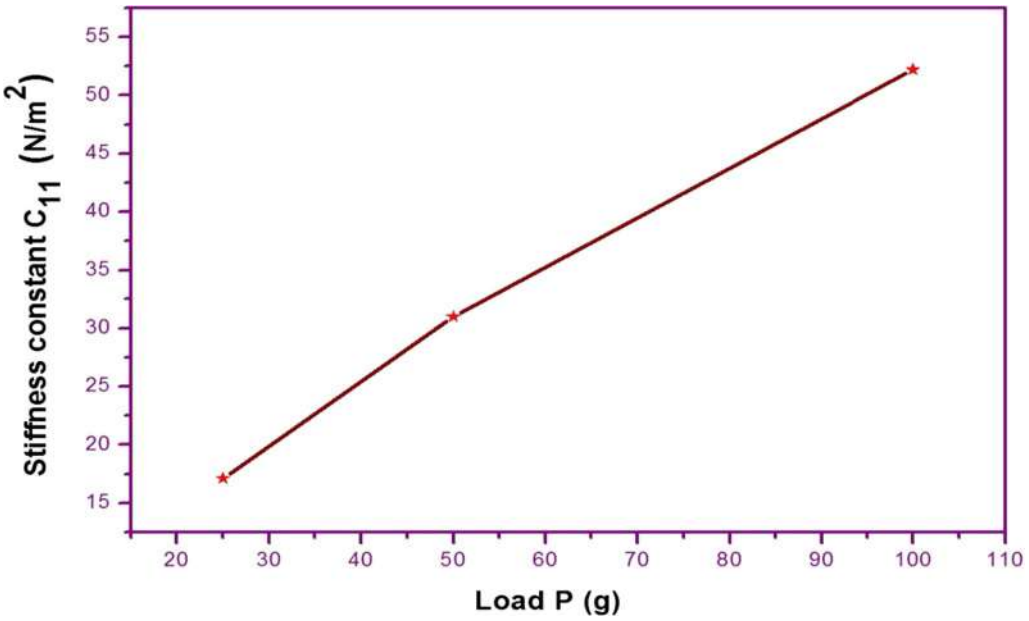


Fig. 8 Plot of stiffness constant versus load for LAO crystal.

Table III Yield strength (σ_y), stiffness constant (C_{11}), fracture toughness (k_c) and brittleness index (B_i) of LAO crystal

Load (g)	$\sigma_y \times 10^6$ N/m^2	$C_{11} \times 10^{14}$ N/m^2	K_c (kg m$^{-3/2}$)	$B_i \times 10^{-4}$
25	168.88	1.7110	1.2584	4.10
50	237.16	3.099	2.5169	2.88
100	319.48	5.2208	5.0339	1.94

thermogravimetric (TG) and differential thermal analysis (DTA) measurements in the temperature range of 27 to 1200°C at a heating rate of 10 K/min. The sample was 6.354 milligrams in weight. Figure 9 depicts the thermogram of LAO material. The TG results indicate that the loss begins at 171°C. The point of breakdown is caused by dehydration. However, no weight loss is observed between 0 and 171°C. The absence of weight loss up to 100°C verified the crystallization of LAO crystals without the presence of a water molecule. As a result, the title chemical is stable up to 171°C. Two endothermic peaks are observed on the DTA curve at 257°C and 292°C. The sharpness of these endothermic peaks indicates that the LAO crystal is a crystalline material.[37]

Impedance Analysis

The complex impedance measurement is a strong technique for determining the bulk resistance and the electrical response of a crystalline material.[38,39] The impedance spectroscopy was undertaken in order to collect the electrical features of the grown LAO crystal.[40]

Complex impedance measurement of the pellet sample was performed using a Versa STAT MC model LCR meter in the frequency range of 1 Hz to 1 MHz with the pellets held between two silver electrodes. At room temperature, it was accomplished by changing the frequency. Figure 10 illustrates the Nyquist diagram of the LAO crystal. The DC conductivity of the sample was determined using the relation

$$\sigma_{dc} = t/AR_b\ \Omega^{-1}m^{-1} \quad (6)$$

where t denotes the crystal thickness, A denotes the electrolyte's area, and R_b denotes the LAO crystal's bulk resistance. The calculated value of DC conductivity for the LAO crystal is $\sigma_{DC} = 6.536 \times 10^{-6}\ \Omega^{-1}m^{-1}$. DC conductivity values are low because charge carrier mobility decreases as ionic size decreases. The well-resolved semicircle at high frequency implies ionic conduction and the parallel combination of bulk capacitance and resistance.[41,42]

Laser Damage Threshold Study

A laser damage threshold (LDT) study was undertaken for the grown LAO crystal using a Nd:YAG laser with a wavelength of 1064 nm and 18 ns pulse width. The energy of the laser beam was measured by a Coherent energy/power meter (model no. EPM 200). The relation used to determine the LDT value is $P = E/\tau\pi r^2$ where E is the energy, τ is the pulse width, and r is the radius of the spot.[43] Laser damage involves interaction of high-power laser radiation with a crystal, and there are many processes like physical,

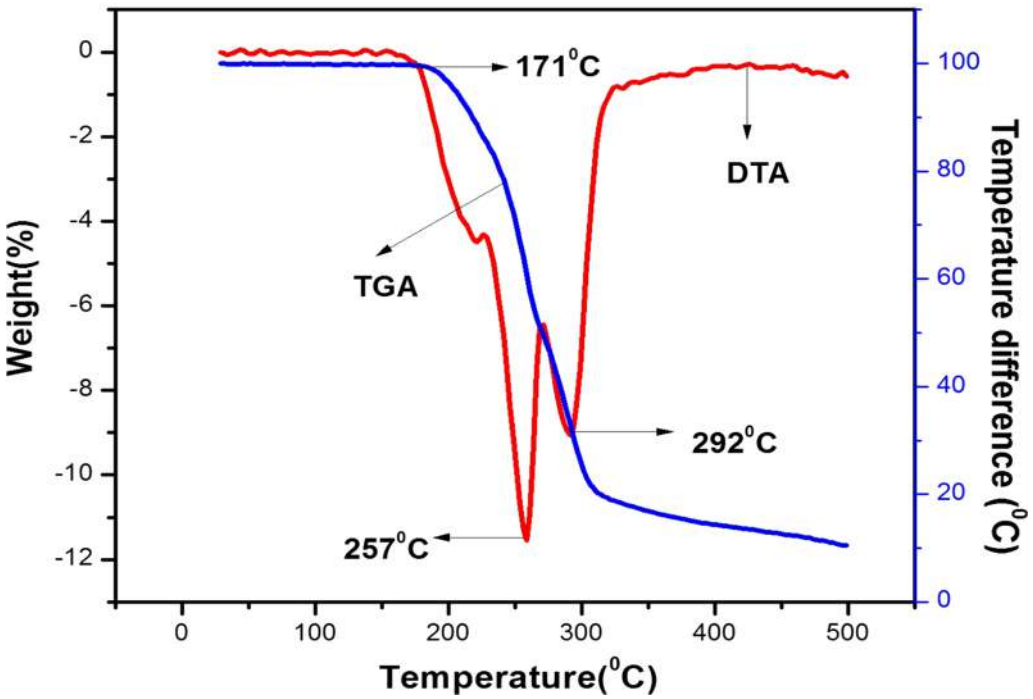


Fig. 9 TG-DTA thermogram of LAO crystal.

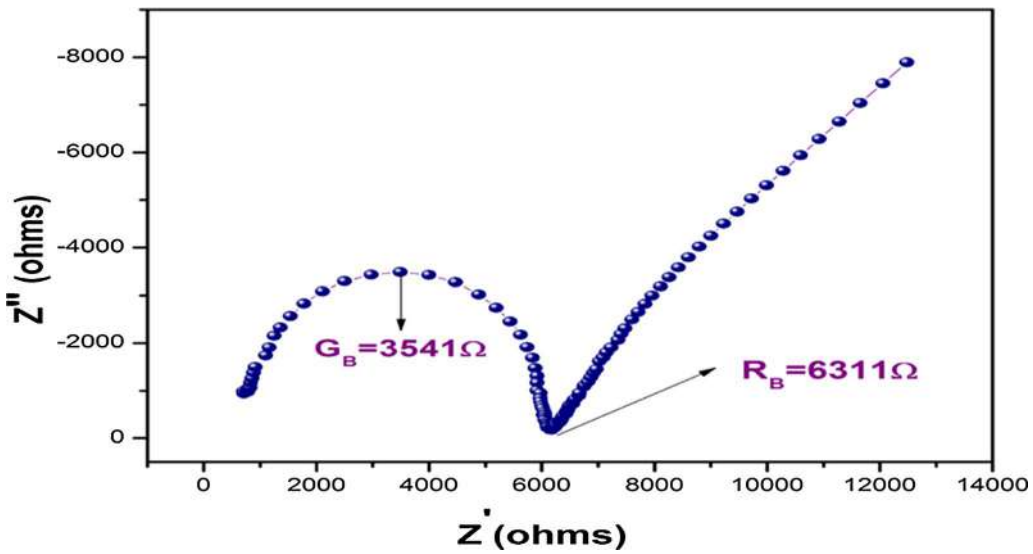


Fig. 10 Impedance spectrum of the grown LAO crystal.

chemical, optical, thermal and other processes involved. The determined value of LDT for LAO crystal is 2.492 GW/cm^2. It is observed that the LDT value of LAO crystal is greater than that of KDP (LDT value of KDP is 0.2 GW/cm^2). Since the LDT value of LAO crystal is large, this crystal could be used for NLO and laser device fabrication.

Conclusion

LAO crystals of high purity were produced using the slow evaporation solution method. The single-crystal XRD analysis indicated that the LAO crystal system is orthorhombic. FT-IR spectroscopy analysis was used to

determine the functional groups of LAO. In the UV-Vis-NIR transmittance study, the bandgap energy was found to be 4.2 eV. The fluorescence study revealed a green emission spectrum. The Vickers microhardness study was carried out to determine the hardness number (H_V), yield strength (σ_y) and stiffness constant (C_{11}), and confirmed that the LAO crystal was a soft material. Thermal stability experiments (TG/DTA) confirmed that the formed LAO crystal exhibited a reasonable degree of thermal stability up to 171°C. SHG efficiency was approximately 0.52 times that of KDP. The LDT value of the LAO crystal was found to be 2.492 GW/cm^2. Due to all of these characteristics, LAO crystals may be a viable material for nonlinear optical applications.

Acknowledgments The authors wish to express their gratitude to several research centres, including the SAIF-IIT, Madras, the ACIC-St. Joseph's College, Tiruchirappalli, the NCIF-National College, Tiruchirappalli, and Alagappa University, Karaikudi, for their contributions. The authors would like to express their gratitude to the APRC – Sacred Heart College, Tirupattur, the CIF – Pondicherry University, Pondicherry, and the IISC – Bangalore for their full-fledged assistance in carrying out the characterization measures for this research project.

Funding There is no funding involved with the current study.

Conflict of interest There is no conflict of interest in the work as declared by the authors.

References

1. Y. Mori, Y.K. Yap, T. Kamira, M. Yoshimura, and T. Sasaki, Recent development of nonlinear optical borate crystals for UV generation. *Opt. Mater.* 19, 1–5 (2002).
2. R.J. Mani, P. Selvarajan, H.A. Devadoss, and D. Shanthi, Second-order, third-order NLO and other properties of L-alanine crystals admixtured with perchloric acid (LAPA). *Optik.* 126, 213–218 (2015).
3. S. Li, K. Wu, G. Su, and Y. He, Nonlinear optical properties of some derivatives of stilbene and vinyl cyanide. *Opt. Mater.* 20, 295–299 (2002).
4. D. Shanthi, P. Selvarajan, K.K.H. Durga, and S.L.M. Ponmani, Nucleation kinetics, growth and studies of β-Alanine single crystals. *Spectro Chem. Acta Part A* 110, 1–6 (2013).
5. S. Manivannan and S. Dhanuskodi, Synthesis, crystal growth, structural and photosensitive properties of an organic NLO material. *J. Cryst. Growth* 262, 473–478 (2004).
6. J.U. Maheswari, C. Krishnan, S. Kalyanaraman, and P. Selvarajan, Characterization of potassium bromide crystals grown in the aqueous solution of picric acid. *Physica B Condens. Matter* 502, 32–38 (2016).
7. M.L. Caroline, R. Sankar, R.M. Indira, and S. Vasudevan, Growth, optical, thermal and dielectric studies of an amino acid organic nonlinear optical material: L-alanine. *Mater. Chem. Phys.* 114, 490–494 (2009).
8. A.M. Petrosyan, R.P. Sukiasyan, H.A. Karapetyan, S.S. Terzyan, and R.S. Feigelson, Growth and investigation of new nonlinear optical crystals of LAP family. *J. Cryst. Growth* 213, 103–111 (2000).
9. L. Mizoguchi, A.T. Varela, F.D. Nunes, V.S. Banat, F.F.A. Melo, J.M. Filho, and S.C. Zulia, Optical properties of L-alanine organic crystals. *Opt. Mater.* 6, 147–152 (1996).
10. P. Jayaprakash, P. Sangeetha, C.R.T. Kumari, and M.L. Caroline, Investigation on the growth, spectral, lifetime, mechanical analysis and third-order nonlinear optical studies of L-methionine admixtured D-mandelic acid single crystal: a promising material for nonlinear optical applications. *Physica B Condens. Matter* 518, 1–12 (2017).
11. J.D. Bernal, The crystal structure of the natural amino acids and related compounds. *Z. Crystallography.* 78, 363–369 (1931).
12. A.S.J.L. Rose, P. Selvarajan, and S. Perumal, Studies on growth and characterization of an NLO crystal: L-alanine hydrogen chloride (LAHC). *Mater. Chem. Phys.* 130, 950–955 (2011).
13. D. Haleshappa, B. Raghavendra, A. Jayarama, K.Q. Ching, C.K. Huey, and S.P. Parutagouda, Structural, photoluminescence, physical, optical limiting, and hirshfeld surface analysis of polymorphic chlorophenyl organic chalcone derivative for optoelectronic applications. *J. Mol. Struct.* 1232, 130053 (2021).
14. C.-I. Urit, P. Ramasamy, and P. Manyum, Comparative study on l-alaninium maleate single crystal grown by Sankaranarayanan-Ramasamy (SR) method and conventional slow evaporation solution technique. *J. Crystal Growth* 312, 2369–2375 (2010).
15. D. Balasubramanian, R. Jayavel, and P. Murgakoothan, Studies on the growth aspects of organic L-alanine maleate: a promising nonlinear optical crystal. *Nat. Sci.* 1, 216–221 (2009).
16. C.R. Raja and A.A. Joseph, Crystal growth and characterization of new nonlinear optical single crystals of L-alaninium fumarate. *Mater. Lett.* 63, 2507–2509 (2009).
17. C.J. Raj, S. Dinakaran, S. Krishnan, B.M. Boaz, R. Robert, and S.J. Das, Studies on optical, mechanical and transport properties of NLO active L-alanine formate single crystal grown by modified Sankaranarayanan-Ramasamy (SR) method. *Opt. Commun.* 281, 2285 (2008).
18. A. Wojciechowski, K. Ozga, A.H. Reshak, R. Miedzinski, I.V. Kityk, J. Berdowski, and Z. Tylczyński, Photoinduced effects in L-alanine crystals. *Mater. Lett.* 64, 1957–1959 (2010).
19. A.H. Reshak, N.M. Abbass, J. Bila, M.R. Johan, and I. Kityk, Noncentrosymmetric sulfide oxide MZnSO (M = Ca or Sr) with strongly polar structure as novel nonlinear crystals. *J. Phys. Chem. C* 123, 27172–27180 (2019).
20. A.H. Reshak, Spin-polarized second harmonic generation from the antiferromagnetic CaCoSO single crystal. *Sci. Rep.* 13, 46415 (2017).
21. A.H. Reshak and S. Auluck, The influence of oxygen vacancies on the linear and nonlinear optical properties of Pb7O(OH)3(CO3)3(BO3). *RSC Adv.* 7, 14752 (2017).
22. A.H. Reshak, Novel borate CsZn2B3O7 single crystal with large efficient second harmonic generation in deep-ultraviolet spectral range. *J. Alloy. Compd.* 722, 438–444 (2017).
23. A.H. Reshak and S. Auluck, Two haloid borate crystals with large nonlinear optical response. *Phys. Chem. Chem. Phys.* 19, 18416 (2017).
24. B.S. Naouel, O. Tarik, B. Michael, H.R. Ali, and A. Sihem, Untangling electronic, optical and bonding properties of hexagonal bismuth borate SrBi2B2O7 crystal for ultraviolet optoelectronic applications: an ab initio study. *J. Alloys Comp.* 803, 1127–1135 (2019).
25. R. Mahiaoui, T. Ouahrani, A. Chikhaoui, A. Morales-García, and A.H. Reshak, Electronic, bonding, linear, and nonlinear optical properties of Na2MGe2Q6 (M=Cd, Zn, Hg; Q=S, Se), Na2ZnSi2S6, and Na2ZnSn2S6 two metal-mixed chalcogenide compounds: insights from an ab initio study. *J. Phys. Chem. Solids* 119, 220–227 (2018).

26. A.H. Reshak, Novel ternary semiconductor CdLa2X4 (X=S or Se) single crystal with efficient second harmonic generation in the visible spectral range. *J. Alloy. Compd.* 728, 241–252 (2017).
27. A.H. Reshak, Lithium borate Li3B5O8(OH)2 with large second harmonic generation and high damage threshold in the deep-ultraviolet spectral range. *Phys. Chem. Chem. Phys.* 19, 30703–30714 (2017).
28. V. Kathiravan, G.S. Kumar, S. Pari, and P. Selvarajan, Influence of dye doping on the structural, spectral, optical, thermal, electrical, mechanical and nonlinear optical properties of L-histidine hydrofluoride dihydrate crystals. *J. Mol. Struct.* 1223, 128958 (2021).
29. S. Dhanuskodi, K. Vasantha, and P.A.A. Mary, Structural and thermal characterization of a semiorganic NLO material: L-alanine cadmium chloride. *Spectrochimica Acta Part A Mol. Biomol. Spectrosc.* 66, 637–642 (2007).
30. M. Nageshwari, P. Jayaprakash, C.R.T. Kumari, G. Vinitha, and M.L. Caroline, Growth, spectral, linear and nonlinear optical characteristics of an efficient semiorganic acentric crystal: L-valinium L-valine chloride. *Physica B Condens. Matter.* 511, 1–9 (2017).
31. D. Shanthi, P. Selvarajan, and S. Perumal, Growth, linear optical constants and photoluminescence characteristics of beta-alaninium picrate (BAP) crystals. *Optik* 127, 3192–3199 (2016).
32. M. Nageshwari, C.R.T. Kumari, P. Sangeetha, G. Vinitha, and M.L. Caroline, Third order nonlinear optical, spectral, dielectric, laser damage threshold, and photoluminescence characteristics of an efficacious semiorganic acentric crystal: L-Ornithine monohydrochloride. *Chin. J. Phys.* 56, 502–519 (2018).
33. F. Hammer, *Inorganic Chemistry* (New Delhi: Sarup Book Publishers Private Ltd., 2009).
34. M.P. Mohamed, S. Sudha, P. Jayaprakash, G. Vinitha, M. Nageshwari, P. Sangeetha, C.R.T. Kumari, and M.L. Caroline, Growth and characterization of L-histidinium fumarate fumaric acid monohydrate single crystal: a promising second and third-order nonlinear optical material. *Chin. J. Phys.* 60, 581–597 (2019).
35. S.P. Rathee and D.S. Ahlawat, Spectroscopic and thermo- electrical investigation of single crystals of L-Ornithine monohydrochloride grown by SEST. *Optik* 136, 249–258 (2017).
36. K. Meera, R. Muralidharan, A.K. Tripathi, R. Dhanasekaran, and P. Ramasamy, Growth of thiourea-doped TGS crystals and their characterisation. *J. Cryst. Growth* 260, 414–421 (2004).
37. K. Pichan, S.P. Muthu, and R. Perumalsamy, Crystal growth and characterization of third-order nonlinear optical piperazinium bis(4-hydroxybenzenesulphonate) (P4HBS) single crystal. *J. Crystal Growth* 473, 39–54 (2017).
38. Y. Le Fur, R. Masse, M.Z. Cherkaoui, and J.F. Nicoud, *Zeitschrift fur Crystallogr.* 210, 856–860 (1995).
39. M. Ram, synthesis and electrical properties of $(LiCo_{3/5}Fe_{1/5}Mn_{1/5})VO_4$ ceramics. *Solid Stated Sci.* 12, 350–354 (2010).
40. E.A. Hurtado-Aviles, M. Trejo-Valdez, J.A. Torres, C.J. Ramos-Torres, H. Martínez-Gutiérrez, and C. Torres-Torres, Photo-induced structured waves by nanostructured topological insulator Bi2Te3. *Opt. Laser Technol.* 140, 107015 (2021).
41. S. Sahoo, R.N.P. Choudhary, and B.K. Mathur, Structural, ferroelectric and Impedance spectroscopy properties of Y^{3+} modified Pb $(Fe_{0.5}\ Nb_{0.5})O_3$ ceramics. *Phys. B* 406, 1660–1664 (2011).
42. K. Ramachandran, A. Raja, V. Mohankumar, M.S. Pandian, and P. Ramasamy, Growth and characterization of 4-methyl-3-nitrobenzoic acid (4M3N) single crystal by using vertical transparent Bridgman-Stockbarger method for NLO applications. *Physica B Condens. Matter* 562, 82–93 (2019).
43. P. Vivek and P. Murugakoothan, Linear and nonlinear optical properties of a new organic NLO l-asparaginium l-tartarate (AST) single crystal. *Optik Int. J. Light Electron Opt.* 124, 3510–3513 (2013).

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

J Mater Sci: Mater Electron

Synthesis, growth, and two-photon absorption induced optical limiting action of cytosinium benzoate single crystal

P. Jaikumar[1,*], T. Balakrishnan[2,*], K. Ramamurthi[3], M. Saravanan[4], and T. C. Sabari Girisun[4]

[1] *PG & Research Department of Physics, National College (Autonomous), Affiliated to Bharathidasan University, Tiruchirappalli, Tamil Nadu 620001, India*
[2] *Crystal Growth Laboratory, Department of Physics, Thanthai Periyar Government Arts and Science College (Autonomous), Affiliated to Bharathidasan University, Tiruchirappalli, Tamil Nadu 620023, India*
[3] *School of Physics, Bharathidasan University, Tiruchirappalli, Tamil Nadu 620024, India*
[4] *Nanophotonics Laboratory, Department of Physics, Bharathidasan University, Tiruchirappalli, Tamil Nadu 620024, India*

Received: 6 April 2022
Accepted: 14 June 2022

© The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2022

ABSTRACT

Two-photon absorption induced optical limiting action was demonstrated in cytosinium benzoate (CB) under nanosecond laser (532 nm, 9 ns, and 10 Hz) excitation. Intensity dependent open aperture Z-scan experiment exposed the presence of reverse saturable absorption ascribed due to sequential two-photon absorption. Initially CB single crystals were grown at room temperature by slow evaporation solution technique. Single crystal XRD shows that CB belongs to monoclinic crystal system with $P2_1/c$ space group. Fourier Transform Infrared spectrum was recorded to identify the presence of functional groups. Thermal studies shows that the crystal is stable upto 168 °C. Vickers microhardness studies confirm that the grown crystal was belongs to soft material category. Etching study shows linear rectangular etch patterns (5 s) and well defined stacking planes (10 s) for water etchant. Optical studies demonstrate that CB crystal possess lower cut-off (287 nm) and moderate linear transmittance in visible region. The optical energy band gap of CB crystal was estimated from photoluminescence studies as 3.1 eV. CB with higher two-photon absorption coefficient (1.26×10^{-10} m/W) and lower onset limiting threshold (1.92×10^{12} W/m^2) can be a potential candidate for developing laser safety devices under nanosecond green laser excitation regime.

Address correspondence to E-mail: jaikumarphy@nct.ac.in; balacrystalgrowth@gmail.com

https://doi.org/10.1007/s10854-022-08607-w
Published online: 07 July 2022

1 Introduction

Organic nonlinear optical materials with strong optical nonlinearity and a two-photon absorption mechanism are in high demand for optical switching, optical bistability, optical limiting, optical telephony, optical computing, higher harmonic generation, and other applications. Cytosine is one of the naturally occurring nucleo bases found in deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) and it exist in several forms through combination with dicarboxylic acids such as oxalic acid, malonic acid, and succinic acid [1, 2]. Gdaniec et al. [3] reported the crystal and molecular structure of cytosine trichloroacetate. The single crystal structure of cytosinium hydrogen maleate was elucidated and reported by Benali–Cherif et al. [4]. Marsh et al. [5] reported that the compound of cytosine-5-acetic acid crystallizes in monoclinic system with space group $P2_1/c$. Sridhar and Ravikumar [6] reported the supramolecular hydrogen bonded networks in cytosinium succinate and cytosinium 4-nitrobenzoate cytosine monohydrate single crystals. Jaikumar et al. [7] reported the growth, optical, thermal, mechanical, and nonlinear optical properties of cytosinium hydrogen selenite. The nucleobase cytosine can be protonated in homo base pair associations to form protonated cytosine derivatives. In this line of search, Perumalla et al. [8] elucidated the three dimensional X-ray crystal structure of cytosinium benzoate (CB) and reported that it belongs to monoclinic system. These organic materials exhibit interesting nonlinear optical properties due to the delocalized π-electrons along the molecular backbone [9]. The strength of the NLO process depends on the polarizability of the delocalized π-electron cloud through molecular engineering of electron donor and acceptor groups on opposite sides of the molecule at the appropriate positions in the chromophore [10]. As notable research is not made on the bulk crystal growth and their NLO properties, the present work reports on the growth of CB single crystals from aqueous solution by slow evaporation technique and their structural (single crystal and powder XRD, FTIR), thermal (TG–DTA, DSC), mechanical (microhardness,), optical (UV–Vis-NIR and photoluminescence), dielectric, and etching studies. The single beam Z-scan technique using nanopulsed Nd:YAG laser beam was performed to demonstrate the nonlinear absorption and optical limiting response of the grown CB crystals.

2 Experimental

2.1 Synthesis

Cytosinium benzoate was synthesized by dissolving cytosine and benzoic acid taken in 1:1 molar ratio in double distilled water. The solution was stirred continuously for 3 h using a temperature controlled magnetic stirrer. The prepared solution was allowed to evaporate at room temperature to yield CB salt. The synthesized salt was recrystallized twice to improve the purity of the material. The reaction mechanism of synthesis CB is depicted in Scheme 1.

2.2 Crystal growth

Saturated aqua solution was prepared at room temperature using the synthesized salt. The filtered solution was taken in a beaker and the mouth of the beaker was covered with a few pricked polythene sheet to control the rate of evaporation. Then it was placed in a dust free atmosphere to allow evaporation at room temperature. Single crystals of CB with $7 \times 4 \times 2$ mm^3 size were harvested after a growth period of 20 days. The grown single crystals of CB are shown in Fig. 1.

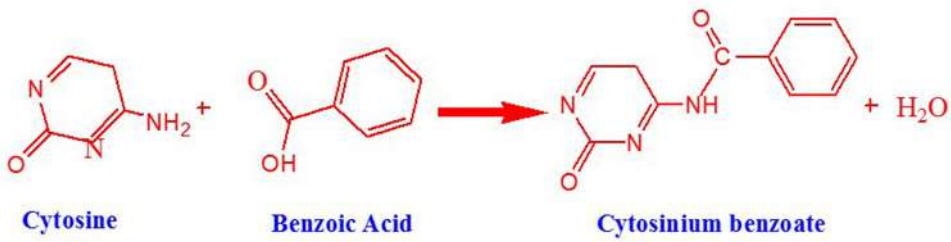
3 Results and discussion

3.1 Structural characterization

Single crystal X-ray diffraction analysis was carried out using an Enraf Nonius CAD4 X–ray diffractometer with graphite monochromated Mo K_α (λ = 0.71073 Å) radiation. The obtained unit cell parameters for CB crystal reveals that it belongs to monoclinic crystal system. The values of the unit cell parameters of the present work (Table 1) agree well with the reported values [8].

Powder X-ray diffraction is a powerful tool to analyze the phase purity by the identification of crystal planes from the observed diffraction patterns [11]. Grown CB crystals were powdered and subjected to powder X-ray diffraction analysis using a BRUKER X-ray diffractometer with the Cu K_α radiation (λ = 1.5406 Å) in the 2θ range of 10°–80° at the scanning rate of 1°/min. The recorded XRD pattern indexed with the help of XRD data analysis software (XRDA) and is shown in Fig. 2. From the X-ray

Scheme 1 The reaction mechanism involved in the synthesis of CB



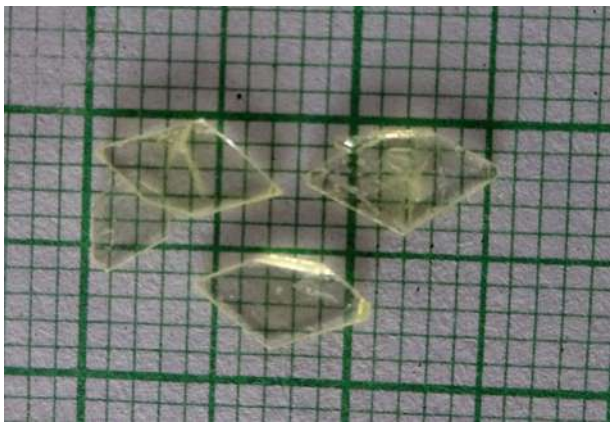


Fig. 1 As grown CB single crystal

Table 1 Unit cell parameters of cytosinium benzoate

Parameter	Present work	Reported [8]
a (Å)	13.037	13.060
b (Å)	7.434	7.450
c (Å)	22.438	22.410
α, *β*, *γ*	$\alpha = \gamma = 90°$	$\alpha = \gamma = 90°$
	$\beta = 91.84°$	$\beta = 91.91°$
Volume (Å^3)	2186	2179
Space group	–	$P2_1/c$

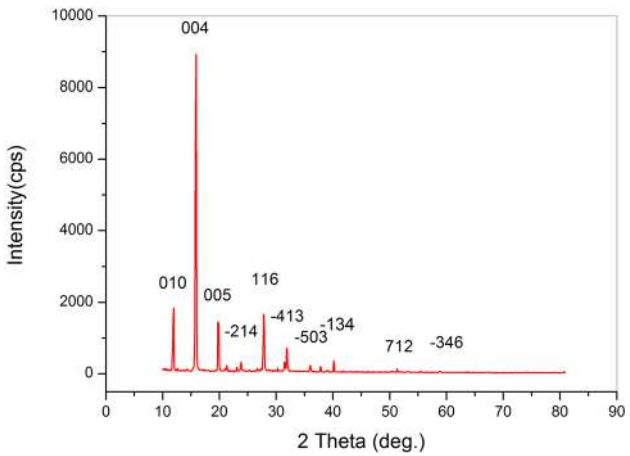


Fig. 2 Powder XRD pattern of CB crystal

diffraction patterns, it is observed that CB crystallizes themselves with high degree of crystallinity.

Fourier transform infrared (FT-IR) spectrum was recorded employing Perkin Elmer FT-IR spectrometer in the spectral range of 400–4000 cm^{-1} by the pressed KBr pellet technique and is shown in Fig. 3. The NH_2 asymmetric and symmetric stretching vibrations are observed at 3383 and 3199 cm^{-1} respectively. The vibration modes observed at 3066 cm^{-1} is assigned to C–H stretching vibration. The peak observed at 1653 cm^{-1} is assigned to NH_2 scissoring vibration. The presence of carbonyl C=O stretching vibration appears as a doublet at 1596 and 1514 cm^{-1}. The C=C stretching vibration is observed at 1449 cm^{-1}. The absorption peak appeared at 1380 cm^{-1} is due to the C=C stretching of the grown crystal [12]. The absorption peaks at 1244 and 1136 cm^{-1} indicates the presence of C–N stretching vibration. The band at 1020 cm^{-1} is corresponds to the NH_2 asymmetric rocking vibration [13]. The weak peaks around 950 and 793 cm^{-1} are due to the C=O inplane bending vibrations. The peak at 588 cm^{-1} is assigned to NH_2 wagging vibration. The twisting vibration occurred at 524 cm^{-1} [14]. Thus the FT-IR spectral studies confirm the formation of CB.

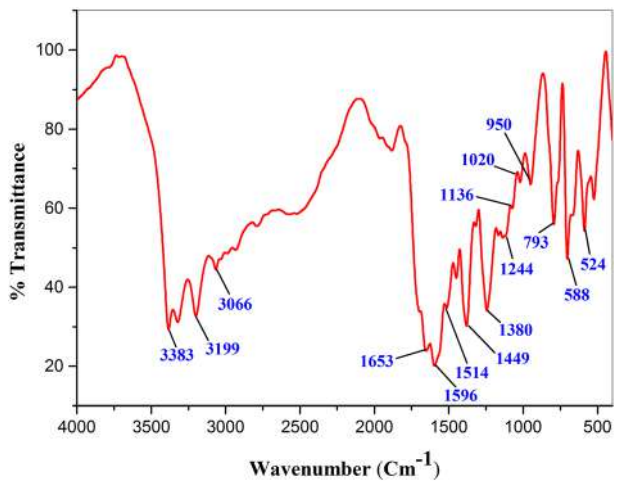


Fig. 3 FTIR spectral band assignment of CB single crystal

3.2 Thermal studies

Thermogravimetric analysis (TGA) and differential thermal analysis (DTA) were carried out employing Q600 SDT and Q20 DSC thermal analyzer in the temperature range from 30 to 1000 °C in nitrogen atmosphere at a heating rate of 10 °C/min. The TGA/DTA curves of CB are shown in Fig. 4. Powdered CB single crystal weighing 11.751 mg was used. The decomposition takes place in two steps. The first step of decomposition of the sample starts at 168 °C and ends at 318 °C. In this temperature interval a total weight loss of 52.01% is observed which corresponds to the removal of benzoate molecule. Between 318 and 1000 °C a gradual second step of decomposition arises due to loss of cytosinium molecules. In DTA trace two endotherms are observed. The first endothermic peak is observed at 168 °C. These endothermic peak corresponds to the decomposition stages. The differential scanning calorimetry (DSC) was recorded between 30 and 1000 °C in nitrogen atmosphere and the spectrum is also shown in the same Fig. 4. There is a minor endothermic peak at 168 °C and a major peak at 318 °C which correspond to the loss of CB molecule. The DSC peaks support the observation found in TGA/DTA. Thus CB is found to be thermally stable upto 168 °C which reveals that it can be used for NLO devices in the thermally stable region.

3.3 Mechanical and etching studies

The Vickers microhardness studies of CB single crystal were carried out by employing Shimadzu HMV-2000 hardness tester fitted with a diamond pyramidal indenter attached with an incident light microscope. The indentations were made for different loads with a constant indentation time of 3 s for all trials. The load P was varied between 5 and 200 g and the diagonal lengths of indentation impressions were measured. The Vickers microhardness number (H_v) was calculated using the standard relation [15]. Figure 5a presents the variation of hardness number as a function of applied load. It can be observed from Fig. 5a that the hardness number increases with increase in the applied load which confirms the reverse indentation size effect [16]. When the load exceeds 200 g multiple cracks were developed on the surface of the crystal due to the release of internal stresses locally generated by indentation. The Meyer's law relates the load and indentation diagonal length. In describing indentation size effect the Meyer's power law was employed and the Meyers index (n) was calculated from the curve drawn between log P and log d (Fig. 5b). The slope of the straight line in Fig. 5b gives $n \approx 2.8$. Onitsch [17] inferred that the value of n between 1 and 1.6 represents hard materials and n above 1.6 represents soft material. Thus the microhardness study reveals that CB belongs to soft material category [18, 19].

Yield strength of a material is the point at which the material transforms to plastic mode and is the

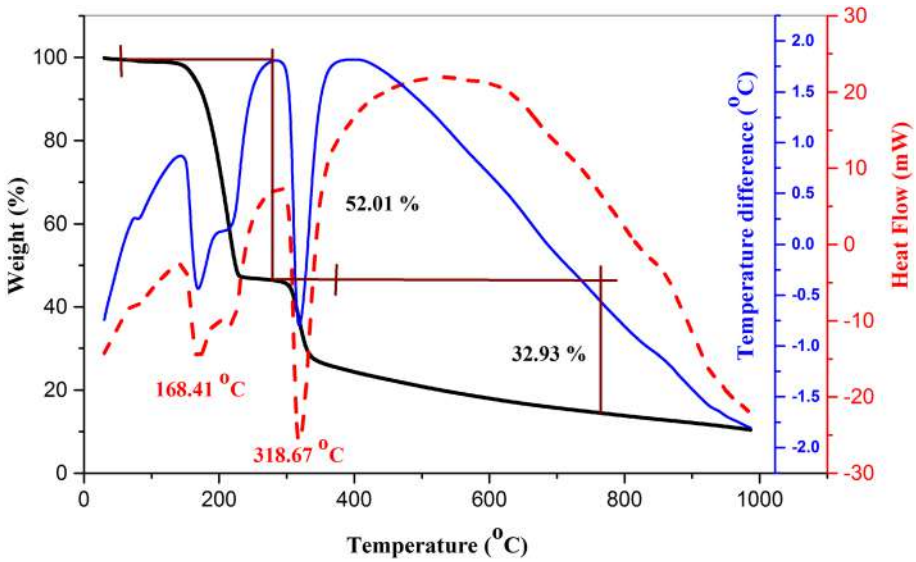


Fig. 4 TGA/DTA/DSC of CB single crystal

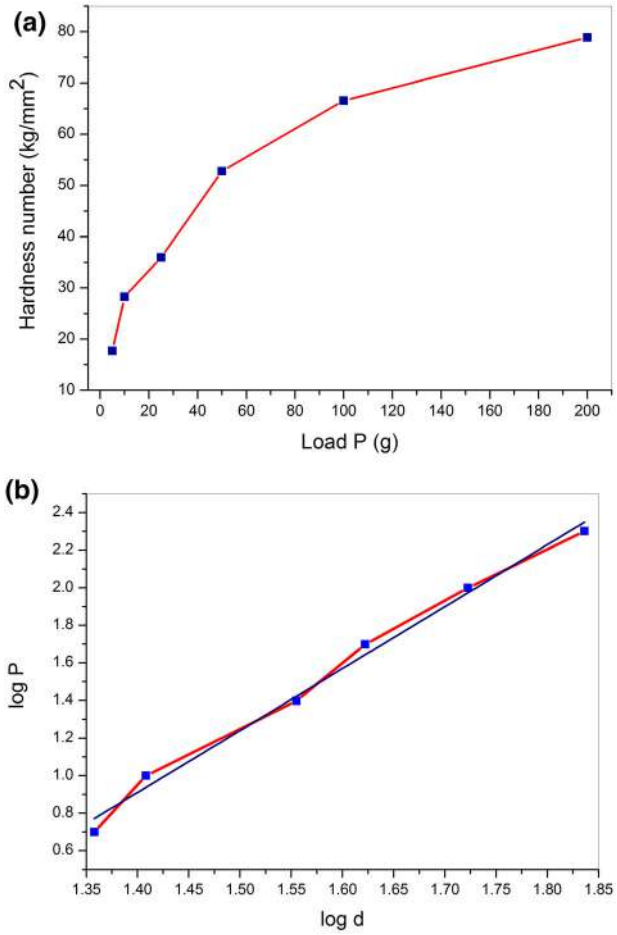


Fig. 5 **a** The variation of microhardness values vs. load for CB crystal. **b** The variation of log P vs. log d for CB crystal

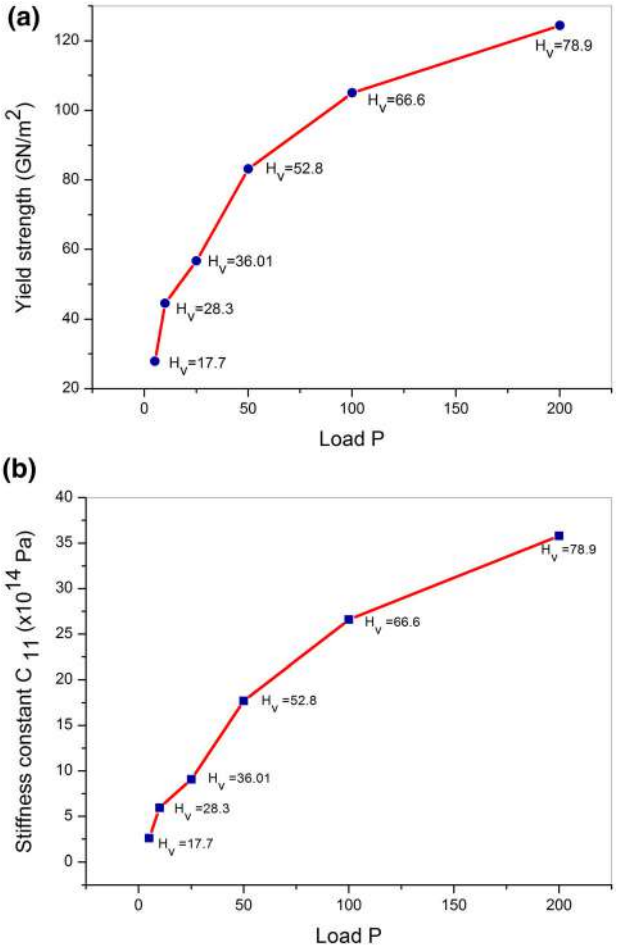


Fig. 6 **a** A plot showing yield strength (σ_y) vs. load P. **b** The variation of stiffness constant (C_{11}) vs. load P

measure of elastic limit of the material. By using hardness values of CB single crystal the yield strength σ_y can be calculated using the relation [20]

$$\sigma_y = \frac{H_v}{2.9}(3-n)\left[\frac{12.5(n-2)}{1-(n-2)}\right]^{n-2}.$$

The above equation is suitable only for the materials whose Meyer's index value lies between 2 and 3 ($2 < n < 3$). As the Meyers index of CB crystal is 2.8 the above equation is used to calculate yield strength. A plot drawn between the applied load (P) and yield strength (σ_y) is shown in Fig. 6a. Elastic stiffness constant (C_{11}) was calculated from the Wooster's empirical relation [20] which gives the basic idea about the tightness of bonding between neighboring atoms.

$$C_{11} = (H_V)^{\frac{7}{4}}.$$

A plot drawn between stiffness constant (C_{11}) and applied load (P) for the CB crystal is shown in Fig. 6b. Stiffness constant increases with increasing load. Thus Vickers test confirm the mechanical stability of the title compound, which is one of the essential criteria for developing NLO devices.

Etching study is one of the versatile method for analyzing the growth pattern, visualizations of dislocations and defects present in the crystals. The microphotographs were taken on the etched surface of the as grown crystal of CB using optical microscope with Motic camera in the reflection mode. In this work double distilled (DD) water was used as the etchant. Etching of the crystal surfaces was carried out by immersing the crystal in DD water for 5 s and 10 s at room temperature. After the respective times the samples were carefully wiped using tissue paper to remove the etchant from the surfaces. The photographs of etch patterns developed for 5 s and 10 s

are shown in Fig. 7a and b, respectively. When the crystal was etched for 5 s, micropits with mixed etch patterns are observed on the crystal surface. When the etching time is increased to 10 s well defined rectangular etch pits of various sizes are observed on the crystal surface (Fig. 7b).

3.4 Optical and dielectric studies

The optical transmittance was recorded using Perkin Elmer Lambda 35 double beam UV–Vis–NIR spectrophotometer in the wavelength range of 190–1100 nm. It can be seen from the transmission curve presented in Fig. 8 that the lower cut off wavelength of CB lies at 287 nm and crystal is transparent in the region of 420–1100 nm which makes it a potential candidate for NLO applications. The credence between absorption co-efficient (α) and photon energy ($h\upsilon$) was used to calculate the optical

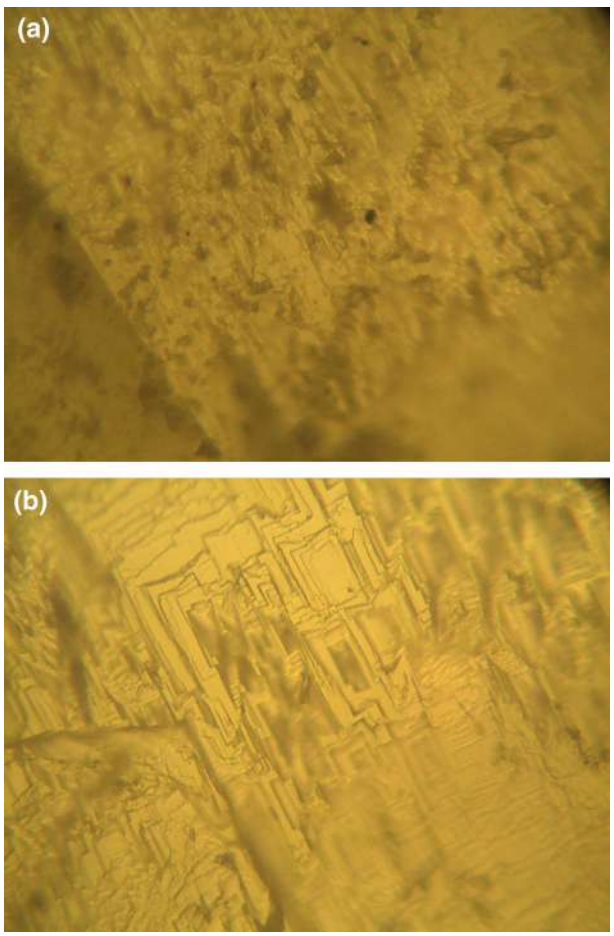


Fig. 7 **a** Etch pits observed on the surface of as grown CB crystal etched for 5 s. **b** Etch pits observed on the surface of as grown CB crystal etched for 10 s

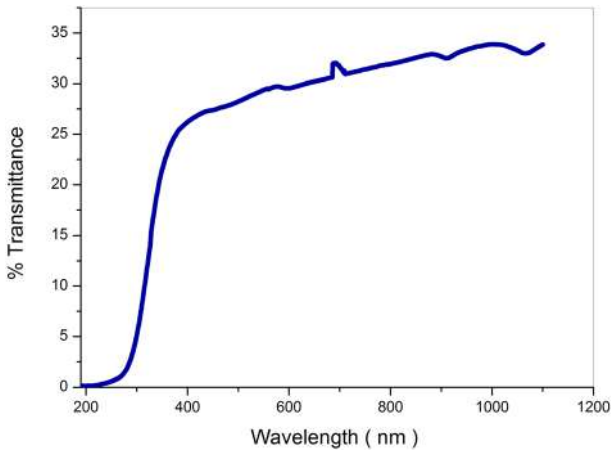


Fig. 8 UV–Vis–NIR transmittance spectrum of CB

energy band gap from Tauc's relation [21]. The graph between $h\upsilon$ and $(\alpha h\upsilon)^2$ was drawn and is shown in Fig. 9. From the graph the optical band gap (E_g) was obtained by extrapolating the linear part of the curve to X-axis. Estimated optical energy band gap of CB crystal is 3.6 eV.

Photoluminescence (PL), a non-destructive tool, can be used to characterize the defects, vacancies and other imperfections present in the grown crystal. In PL study absorption of light gives rise to bound excited state from which it returns to its ground state abiding in the analysis of color center creation mechanism [22]. The luminescence spectrum of CB crystal was recorded using Carry Eclipse fluorescence spectrometer at room temperature. The emission spectrum obtained in the wavelength range of 290–530 nm is shown in Fig. 10. A sharp peak observed with a maximum intensity at 394 nm (inset

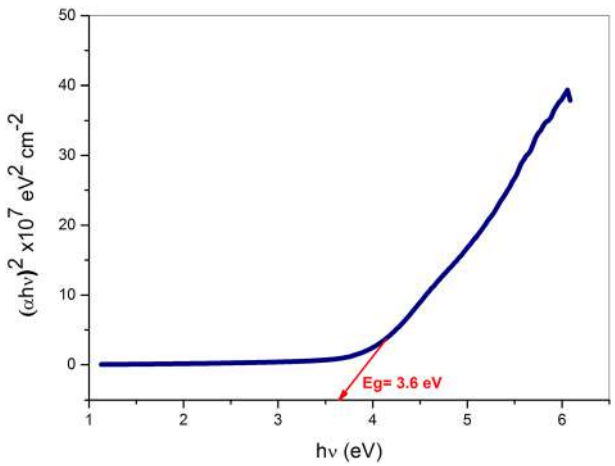


Fig. 9 Plot of $(\alpha h\upsilon)^2$ versus $h\upsilon$ for CB single crystal

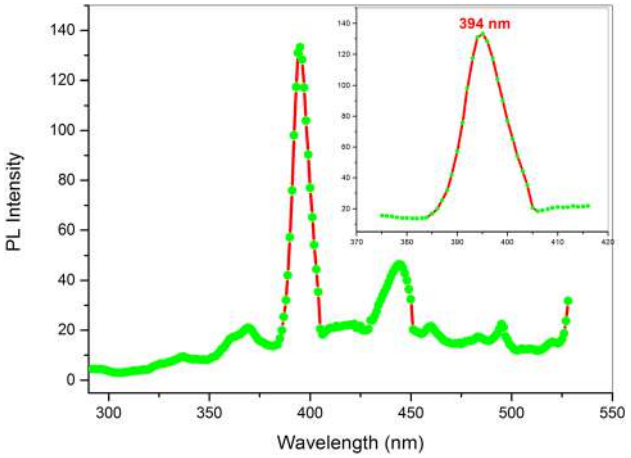


Fig. 10 Photoluminescence spectrum of CB

in Fig. 10). The result indicates that the CB crystal has blue fluorescence emission. The optical band gap was calculated using the maximum emission wavelength (394 nm) of CB crystal. The optical band gap energy of CB crystal derived from PL study is found to be 3.1 eV.

The dielectric property of the grown crystal was studied using HIOKI 3532 50 LCR HITESTER instrument at different temperatures for various frequencies. Optically transparent good quality CB single crystal with 2 mm thickness was chosen for dielectric study. The sample was given silver paste coating on the opposite faces and placed between two copper electrodes and thus a parallel plate capacitor was formed. Using the LCR meter the capacitance of the sample was measured at different temperatures in the frequency range from 50 Hz to 2 MHz. The dielectric constant (ε_r) of the crystal was calculated using measured capacitance of the material. The variation in dielectric constant as a function of frequency is shown in Fig. 11a. The graph evidently shows that the dielectric constant value decreases with increasing frequency at all temperatures. But at higher frequencies ε_r remains nearly constant. It is well known that the dielectric mechanism of the crystal is associated with space charge, orientation, electronic, and ionic polarizations, which depend on frequencies. The high value of dielectric constant of CB crystal at low frequencies is due to the polarization contribution from dipolar orientation. In the high frequency region the dielectric constant decreases slowly as the polarization loss is significant in this region [23]. The Fig. 11a reveals that the dielectric constant increases with increase in the temperature.

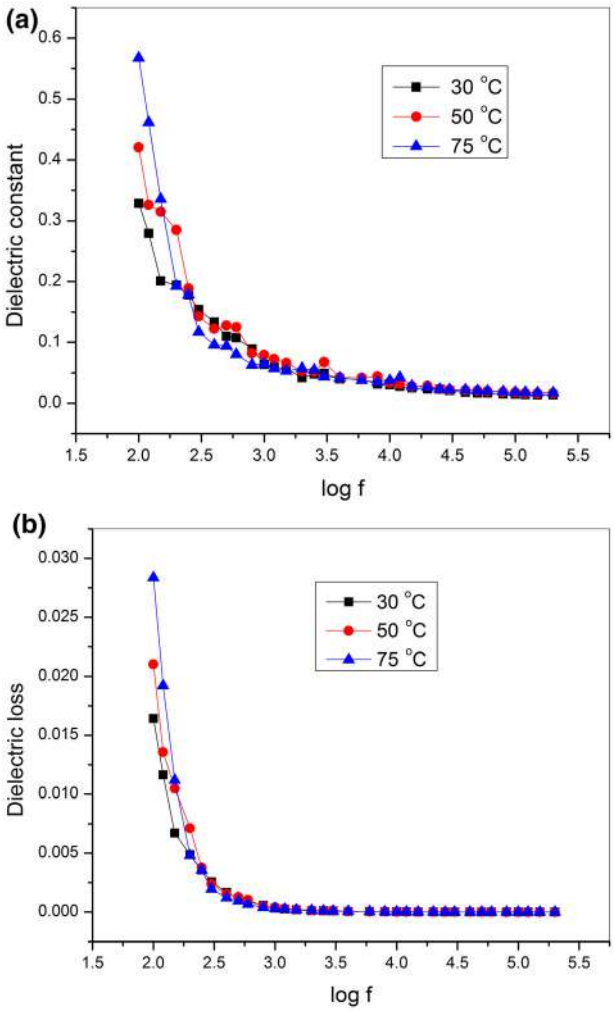


Fig. 11 **a** Variation of dielectric constant with log frequency at various temperatures. **b** Variation of dielectric loss with log frequency at various temperatures

This may be attributed to space charge polarization near the grain boundary interfaces [24]. The dielectric loss of the sample is determined using the standard relation from the dissipation factor [25]. The variation of dielectric loss observed in the CB crystal as a function of log f is presented in Fig. 11b. One can observe from the figure that the dielectric loss is high at low frequencies and further it decreases with increase in the frequencies. The behavior of low value of dielectric loss with high frequency for CB crystals suggests that the crystal possesses enhanced optical quality with lesser defects, which is the desirable property which make nonlinear optical materials for suitable applications.

3.5 Nonlinear optical studies under Nd:YAG laser excitation

Numerous techniques are developed for studying nonlinear absorption of materials. Among them Z-scan technique has gained rapid acceptance by the nonlinear optics community as it is the most widely adopted, sensitive, and simpler method for investigating the nonlinear absorption coefficient in variety of materials [26]. In the Z-scan experiment, choice of laser excitation decides the exploring domain of nonlinearity associated with a material. Here in the employed Z-scan experiment, a frequency doubled Q-Switched Nd: YAG nanopulsed laser (532 nm, 9 ns, and 10 Hz) was used as source of excitation. Further as it is aimed to explore nonlinear absorption of material, Z-scan experiment was performed in open aperture mode. Here the Gaussian beam was focused using a convex lens (focal length 10 cm, beam radius 16.9 μm, and Rayleigh length 1.69 mm) and transmittance was measured along the direction of propagation as a function of position of sample.

Recorded open aperture Z-scan pattern of CB is shown in Fig. 12. The presence of valley like pattern about its focus confirms that CB exhibits reverse saturable absorption (RSA). All RSA materials possess a higher absorption cross section for excited states compared to that for the ground state. Hence the materials with high RSA response are widely used for optical limiting applications such as telecommunication, sensing, and certain military appliances [27]. In order to identify the underlying mechanism responsible for observed nonlinear absorption, Z-scan experiment was performed at different peak intensities (0.61–2.46 $\times$ 10^{12} W/m^2).s

The Z-scan curves obtained for CB was numerically fitted to nonlinear transmission equation for a two-photon absorption process given by [20]

$$T = (1-R)^2 \frac{\exp(-\alpha L)}{\sqrt{\pi} q_0} \int_{-\infty}^{+\infty} In[\sqrt{1} + q_0 \exp(-t^2)] dt,$$

where T is the normalized transmittance of the sample and L and R are the sample length and surface reflectivity, respectively. α is the linear absorption coefficient, and $q_{0=\beta(1-R)I_{0L_{eff}}}$, I_o is the incident intensity, and β is the nonlinear absorption coefficient. As the theoretical curve fit is found matching well with experimental data, the observed reverse saturable absorption can be ascribed to be of two-photon absorption process. In general, the absorption process is termed as effective two-photon absorption as it originates from single step genuine two-photon or two step (excited state) sequential two-photon absorption [28]. To identify the underlying mechanism the intensity dependent Z-scan measurement is evaluated and at the chosen peak intensities the sample exhibits two-photon absorption (2PA). From the best numerical fit data, estimated 2PA coefficient at different on-axis peak intensities of I_0 = 0.61 $\times$ 10^{12} m/W^2, 1.23 $\times$ 10^{12} m/W^2, and 2.46 $\times$ 10^{12} m/W^2 is 0.91 $\times$ 10^{-10} m/W, 0.99 $\times$ 10^{-10} m/W, and 1.26 $\times$ 10^{-10} m/W, respectively. Early reports on materials such as C60 (fullerenes) [29], metal nanoclusters, semiconductors, and polymers [30, 31] show the 2PA type absorptive nonlinearities involving real excited states. As two-photon absorption coefficient is sensitive to peak intensities, CB exhibits sequential two-photon absorption involving excited state absorption. From UV–Visible absorption spectrum, it can be observed that CB shows absorbance state at the excitation wavelength and hence in two-photon absorption process, the electron absorbed two photons sequentially leading to excited state absorption [32].

In providing protection to eyes and optical sensors from laser damage, optical limiting based devices gain much importance. Additionally the efficient control over the optical limiting devices find applications in pulse generations and shaping. For passive optical limiting applications, reverse saturable absorption (RSA) materials are shown to be suitable [33]. The optical limiters constructed by RSA materials decrease the transmittance at high input energy and transparent for low input energies which are the essential requirement for an ideal optical limiter. A limiting graph portrayed between the input- and output-fluence of the CB sample irradiated at 532 nm using 9 ns laser pulses is shown in Fig. 12. From this figure the onset optical limiting threshold for I_0 = 0.61 $\times$ 10^{12} m/W^2, 1.23 $\times$ 10^{12} m/W^2, and 2.46 $\times$ 10^{12} m/W^2 is 3.09 $\times$ 10^{12} W/m^2, 2.92 $\times$ 10^{12} W/m^2, and 1.92 $\times$ 10^{12} W/m^2, respectively. Thus it can be concluded that the strong excited state absorption induced optical limiting action observed in CB crystal reveals that it may be a potential candidate for developing laser safety devices under nanosecond green laser excitation regime.

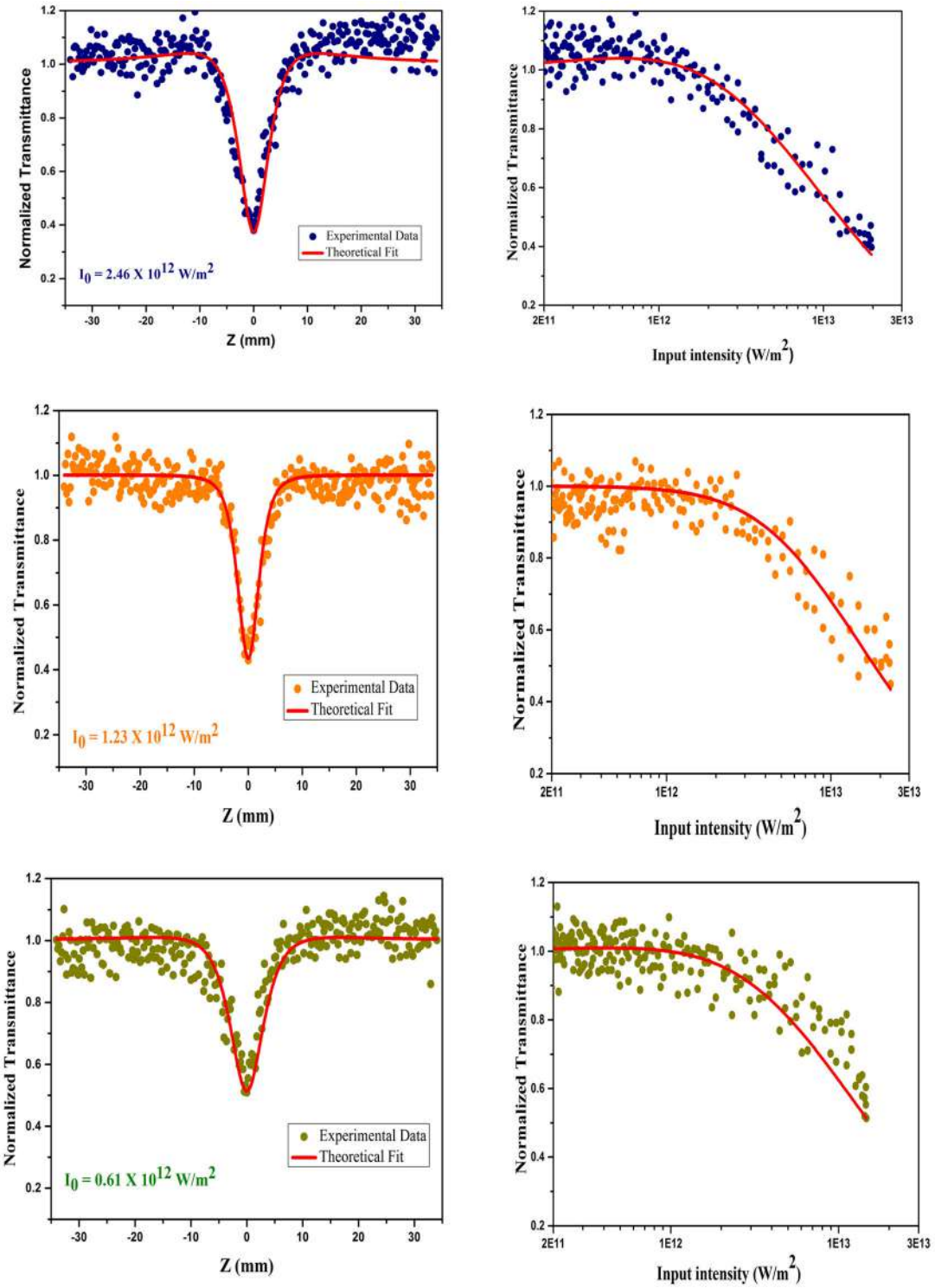


Fig. 12 Open aperture Z-Scan and optical limiting curves of CB crystal

4 Conclusion

Optical quality single crystal of CB was grown by slow evaporation technique. The single crystal XRD pattern reveals that the grown crystal belongs to monoclinic crystal system. The presence of various functional groups identified by FT-IR spectral analysis confirm the formation of the CB. The UV–Vis–NIR studies show that the CB is transparent in the visible range of 420–1100 nm. The band gap energy of CB crystal is 3.6 eV. The dielectric study shows that the dielectric constant and dielectric loss decreases with increasing frequency at different temperatures. Increase in the Vickers microhardness with increase of load exhibits reverse indentation size effect (RISE). Thermal analyses of CB reveal the two stage weight loss in the sample and CB crystal is thermally stable up to 168.5 °C. Etching studies reveal rectangular etch pits. The photoluminescence study shows that the crystal has near blue emission. Nonlinear optical studies were carried out using open aperture Z-scan (Nd: YAG 532 nm, 9 ns and 10 Hz) technique showed that optical nonlinearity is primarily of a reverse saturable absorption (RSA) nature. Intensity dependent ($I_0 = 0.61 \times 10^{12}$ m/W^2, 1.23×10^{12} m/W^2 and 2.46×10^{12} m/W^2) nonlinear absorption studies show that CB possess higher 2PA coefficient (0.91×10^{-10} m/W, is 0.99×10^{-10} m/W, and 1.26×10^{-10} m/W) and lower onset optical limiting threshold (3.09×10^{12} W/m^2, 2.92×10^{12} W/m^2, and 1.92×10^{12} W/m^2). Thus CB is identified as a potential candidate for developing optical limiting devices in the nanosecond green laser excitation regime.

Acknowledgments

The authors are thankful to Sophisticated Analytical Instrument Facility (SAIF), Indian Institute of Technology Madras, India for single crystal XRD analysis. The authors gratefully acknowledge to Central Instrumentation Facility (CIF), Pondicherry University, India for TG/DTA/DSC and FTIR analysis.

Author contributions

All authors contributed to the study conception and design. Conceptualization; Investigation; Writing—Original draft: (PJ), Project administration; Supervision, Writing—Review: (TB), Project administration: (KR), Conceptualization; Writing: (MS), Investigation: Supervision: (TCSG). All authors read and approved the final manuscript.

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that they have no known competing financial interest or personal relationships that could have appeared to influence the work reported in this paper.

References

1. J.D. Watson, F.H. Crick, Genetical implications of the structure of deoxyribonucleic acid. Nature **171**, 964–967 (1953). https://doi.org/10.1038/171964b0
2. T. Lee, P.Y. Wang, Screening, manufacturing, photoluminescence, and molecular recognition of co-crystals: cytosine with dicarboxylic acids. Cryst. Growth Des. **10**, 1419–1434 (2010). https://doi.org/10.1021/cg901412d
3. M. Gdaniec, B. Brycki, M. Szafran, Crystal and molecular structure of cytosine trichloroacetate. J. Mol. Struct. **195**, 57–64 (1989). https://doi.org/10.1016/0022-2860(89)80158-9
4. N. Benali-Cherif, W. Falek, A. Direm, Cytosinium–hydrogen maleate–cytosine (1/1/1). Acta Crystallogr. Sect. E **65**, 03058–03059 (2009). https://doi.org/10.1107/S1600536809046571
5. R.E. Marsh, R. Bierstedt, E.L. Eichhorn, The crystal structure of cytosine-5-acetic acid. Acta Cryst. **15**, 310–316 (1962). https://doi.org/10.1107/S0365110X62000791
6. B. Sridhar, K. Ravikumar, Supramolecular hydrogen-bonded networks in cytosinium succinate and cytosinium 4-nitrobenzoate cytosine monohydrate. Acta Crystallogr. Sect. C

64, 0566–0569 (2008). https://doi.org/10.1107/S010827010802979X
7. P. Jaikumar, S. Sathishkumar, T. Balakrishnan, K. Ramamurthi, Growth, structural, optical, thermal and mechanical properties of cytosinium hydrogen selenite: a novel nonlinear optical single crystal. Mater. Res. Bull. **78**, 96–102 (2016). https://doi.org/10.1016/j.materresbull.2016.02.019
8. S.R. Perumalla, E. Suresh, V.R. Pedireddi, Nucleobases in molecular recognition: molecular adducts of adenine and cytosine with COOH functional groups. Angew. Chem. Int. Ed. **44**, 7752–7757 (2005). https://doi.org/10.1002/ange.200502434
9. C.C. Evans, M.B. Beucher, R. Masse, J.F. Nicoud, Nonlinearity enhancement by solid-state proton transfer: a new strategy for the design of nonlinear optical materials. Chem. Mater. **10**, 847–854 (1998). https://doi.org/10.1021/cm970618g
10. B.F. Levine, C.G. Bethea, Second and third order hyperpolarizabilities of organic molecules. J. Chem. Phys. **63**, 2666–2682 (1975). https://doi.org/10.1063/1.431660
11. B.D. Cullity, *Elements of X-ray Diffraction* (Addison-Wesley, Boston, 1956)
12. N.B. Colthup, L.H. Daly, S.E. Wiberley, *Introduction to Infrared and Raman Spectroscopy* (Academic Press, New York, 1990)
13. K. Ataka, M. Osawa, In situ infrared study of cytosine adsorption on gold electrodes. J. Electroanal. Chem. **460**, 188–196 (1999). https://doi.org/10.1016/S0022-0728(98)00375-1
14. K. Nakamoto, *Infrared and Raman Spectra of Inorganic and Coordination Compounds* (Wiley, New York, 1978)
15. K. Sangwal, B. Surovska, Study of indentation size effect and microhardness of $SrLaAlO_4$ and $SrLaGaO_4$ single crystals. Mater. Res. Innov. **7**, 91–104 (2003). https://doi.org/10.1080/14328917.2003.11784768
16. X.J. Ren, R.M. Hooper, C. Griffiths, J.L. Henshall, Indentation-size effects in single-crystal MgO. Philos. Mag. A **82**, 2113–2120 (2002). https://doi.org/10.1080/01418610208235721
17. E.M. Onitsch, Mikroskopie **95**, 12–14 (1956)
18. S. Karan, S.P. Sen Gupta, Vickers microhardness studies on solution-grown single crystals of magnesium sulphate heptahydrate. Mater. Sci. Eng. A **398**, 198–203 (2005). https://doi.org/10.1016/j.msea.2005.03.016
19. B.W. Mott, *Micro Indentation Hardness Testing* (Butterworths, London, 1956)
20. W.A. Wooster, Physical properties and atomic arrangements in crystals. Rep. Prog. Phys. **16**, 62–82 (1953). https://doi.org/10.1088/0034-4885/16/1/302
21. J. Tauc (ed.), *Amorphous and Liquid Semiconductors* (Plenum, New York, 1974)
22. A. Philominal, S. Dhanuskodi, R. Philip, Optical limiting characteristics of dichloridobis (1-ethyl-2, 6-dimethylpyridinium-4-olate-κO) zinc (II). Curr. Appl. Phys. **12**, 401–404 (2012). https://doi.org/10.1016/j.cap.2011.07.037
23. P. Smyth, *Dielectric Behavior and Structures* (McGraw-Hill, New York, 1995)
24. C. Desai, A.H. Patel, Dielectric studies of ferroelectric rubidium hydrogen tartrate single crystals. J. Mater. Sci. Lett. **8**, 361–363 (1989). https://doi.org/10.1007/BF00725524
25. S.K. Arora, V. Patel, B. Amin, A. Kothari, Dielectric behaviour of strontium tartrate single crystals. Bull. Mater. Sci **27**, 141–147 (2004). https://doi.org/10.1007/BF02708496
26. P.B. Chapple, J. Staromlynska, J.A. Hermann, T.J. Mckay, R.G. Mcduff, Single-beam Z-scan: measurement techniques and analysis. J. Nonlinear Opt. Phys. Mater. **6**, 251–293 (1997). https://doi.org/10.1142/S0218863597000204
27. M. Saravanan, T. C. Sabari Girisun, Enhanced nonlinear optical absorption and optical limiting properties of superparamagnetic spinel zinc ferrite decorated reduced graphene oxide nanostructures. Appl. Surf. Sci. 392, 904– 911 (2017). https://doi.org/10.1016/j.apsusc.2016.09.109.
28. C. Babeela, M.A. Assiri, T.C. Sabari Girisun, 2PA and 3PA induced broadband limiting of Cr^{3+} doped BaB_2O_4 nanostructures. Opt. Mater. **95**, 109267–109276 (2019). https://doi.org/10.1016/j.optmat.2019.109267
29. R.L. Sutherland, *Handbook of Nonlinear Optics*, 2nd edn. (Marcel Dekker, New York, 2003)
30. J. Wang, W.J. Blau, Solvent effect on optical limiting properties of single-walled carbon nanotube dispersions. J. Phys. Chem. C **21**, 2298–2303 (2008). https://doi.org/10.1021/jp709926r
31. I. Cohanoschi, M. Garci, C. Toro, F.K.D. Belfield, F.E. Hernandez, Three-photon absorption of a new series of halogenated fluorene derivatives. Chem. Phys. Lett. **430**, 133–138 (2006). https://doi.org/10.1016/j.cplett.2006.08.093
32. M. Abith, T.C. Sabari Girisun, Excited state absorption induced optical limiting action of MoS_2-rGO nanocomposites. J. Mol. Liq. **341**, 117337 (2021). https://doi.org/10.1016/j.molliq.2021.117337
33. M. Saravanan, T.C. Sabari Girisun, Nonlinear optical absorption and optical limiting properties of cadmium ferrite. Mater. Chem. Phys. **160**, 413–419 (2015). https://doi.org/10.1016/j.matchemphys.2015.05.009

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

என்றும் மகாகவி பாரதி

பதிப்பாசிரியர்

முனைவர் ச. ஈஸ்வரன்

தமிழாய்வுத்துறை

தேசியக்கல்லூரி (தன்னாட்சி)

ஆற்றல்சால் தகுதி பெற்றது

தேசியத் தர மதிப்பீட்டில் 'ஏ+' (3.61) தகுதி பெற்றது

திருச்சிராப்பள்ளி - 620 001

பதிப்புரை

''பல வேடிக்கை மனிதரைப் போலே
நான் வீழ்வே னென்று நினைத் தாயோ?''

(மகாகவி பாரதியார்)

மகாகவி பாரதியாரைப் பற்றி எண்ணற்ற கட்டுரைகளும் நூல்களும் எழுதப்பட்டிருந்தபோதும் மகாகவி பாரதியாரை நம்மால் இன்னமும்கூட மிகச் சரியாக வரையறுத்துவிட இயலவில்லை.

நமது வரையறைகளுக்கு மீறியவராகவே அவர் காலந்தோறும் இருந்துவருகிறார். அவரின் படைப்புகளையும் அவரின் பணிகளையும் அவற்றால் விளைந்தவற்றையும் நம்மால் எந்த ஒரு சிமிழுக்குள்ளும் அடைத்துவிட முடியவில்லை. அதுதான் பாரதியின் ஆளுமை. எதிலும் அடைத்துவிட முடியாத பரந்துபட்ட, காலந்தோறும் வளர்ந்து வரும் ஆளுமை அவர்.

மகாகவி பாரதியாரைப் பற்றிப் பன்முக ஆய்வினை மேற்கொண்ட பாரதிப் பற்றாளர்கள் 27 பேரின் சிந்தனைகளைத் தொகுத்திருக்கிறார் பதிப்பாசிரியர் முனைவர் ச. ஈஸ்வரன் அவர்கள்.

இந்த 27 பார்வைக் கோணங்களும் நமக்குப் பாரதியைப் புதிய கண்ணோட்டத்தில் அணுக உதவுகின்றன. இமயமலைத் தொடரின் சிகரங்களை வெவ்வேறு திசைகளிலிருந்து வெவ்வேறு தொலைவுகளிலிருந்து பார்ப்பது போலத்தான் இதுவும்.

அப்படிப் பார்ப்பவர்களுக்கு இமயமலைத் தொடர் வெவ்வேறு தரிசனங்களைத் தருவதைப் போலவே, மகாகவி பாரதியாரும் தன்னுடைய ஆளுமையைப் பற்றி ஆய்வுசெய்யும் ஒவ்வொருவருக்கும் வெவ்வேறுவிதமான தரிசனத்தை அளிக்கிறார். 'எத்தனைக் கோணமோ அத்தனை பார்வை' என்று கூறும் அளவுக்கு 'மகாகவி பாரதியாரின் ஆளுமை' திகழ்கிறது.

இனிவருங்காலத்தில் மகாகவி பாரதியாரைப் பற்றி மேலும் மேலும் எண்ணற்ற கட்டுரைகள் எழுதப்படும். அதற்கு இந்தக் கட்டுரைத் தொகுப்பு முன்னோடியாக அமையும்.

'என்றும் மகாகவி பாரதி' என்ற இந்த நூலினைப் பதிப்பித்துள்ள முனைவர் ச. ஈஸ்வரன் அவர்களுக்குப் பதிப்பகத்தின் சார்பில் பாராட்டுகளைத் தெரிவித்துக் கொள்கிறோம்.

- பதிப்பாளர்

Shri K. Raghunathan B.Sc,. B.L.
Secretary

NATIONAL COLLEGE (Autonomous)
NATIONALLY RE-ACCREDITED AT 'A+' GRADE BY NAAC
COLLEGE WITH POTENTIAL FOR EXCELLENCE
TIRUCHIRAPALLI - 620 001, TAMILNADU.

வாழ்த்துரை

நம்நாட்டின் விடுதலைக்கு முன்பே விதையாகி, வேர்விட்டுக் கிளைத்து இன்று மிகப்பெரிய விருட்சமாக வளர்ந்து, நூற்றாண்டுகளைக் கடந்து வெற்றிநடை போட்டுவரும் கல்லூரி எங்கள் தேசியக்கல்லூரி.

பாரதப்பிரதமர் அவர்களால் பாராட்டப்பெற்ற எம் கல்லூரியின் மிகச் சிறந்த துறைகளுள் தமிழாய்வுத்துறையும் ஒன்று.

"வாழிய செந்தமிழ்! வாழ்க நற்றமிழர்!!
வாழிய பாரத மணித்திருநாடு !!!"

என்று நாட்டுப்பற்றையும் மொழிப்பற்றையும் தமது இருகண்களாகப் போற்றியவர் பாரதியார். மகாகவி பாரதியாரின் சிந்தனைகளை வருங்காலத் தலைமுறைக்கு எடுத்துச் செல்வதாக அமையும் இந்நிகழ்வில் எம் கல்லூரித் தமிழாய்வுத்துறைப் பேராசிரியர்கள் பாரதியார் குறித்து எழுதிய திறனாய்வுக்கட்டுரைகள் அடங்கிய நூல் வெளியீடும், இலக்கியச்செல்வர் முனைவர் இரா. மாது தலைமையில் சிறப்புப் பட்டிமன்றமும் நடைபெறவுள்ளது. இந்த நிகழ்ச்சி நன்முறையில் நடக்க என் மனம் கனிந்த வாழ்த்துகள்

"பாரதியின் புகழ் தழைக்கட்டும் !
தமிழாய்வுத்துறை சிறக்கட்டும் !!"

வாழ்த்துக்களுடன்

Dr. R.Sundararaman M.Com.,B.Ed.,Ph.D.,
Principal

NATIONAL COLLEGE (AUTONOMOUS)
NATIONALLY RE-ACCREDITED AT 'A+' GRADE BY NAAC
COLLEGE WITH POTENTIAL FOR EXCELLENCE
TIRUCHIRAPALLI - 620 001, TAMILNADU
EMAIL: PRINCIPAL@NCT.AC.IN

வாழ்த்துரை

நாட்டுப்பற்றை தம் எழுச்சிமிகு கவிதைகளின் வழியே ஊட்டிய தேசியக்கவிஞர் பாரதியார்.

"**அச்சமில்லை,**
அச்சமில்லை,
அச்சமென்பதில்லையே"

என்று தீமைகளை எதிர்த்துத் துணிவுடன் நம்மைப் போராடச் சொன்னவர் அவர்.

பாரதியார் என்றாலே நம் நினைவுக்கு வருபவை அவர் மிடுக்காகக் கட்டிய முண்டாசும், முறுக்கு மீசையும் தான். அப்படி கம்பீரத்திற்கும், துணிச்சலுக்கும் இலக்கணமாகத் திகழும் பாரதியாரின் நினைவு நூற்றாண்டைப் போற்றி, பாரம்பரியப் பெருமைகள் மிகுந்த எம் கல்லூரியின் தமிழாய்வுத் துறை சிறப்பான விழாவிற்கு ஏற்பாடு செய்துள்ளதை எண்ணி மனம் மகிழ்கிறேன்.

தமிழாய்வுத் துறையின் முன்னைப் பேராசிரியர் 'கல்விக்கடல்', 'கம்பன்மாமணி' இரா.இராதாகிருஷ்ணன் அவர்களின் மகனார் முனைவர் இரா.மாது தலைமையில் பட்டிமன்றமும் நடைபெற உள்ளது. இந்த விழா சிறக்க என் உளப்பூர்வமான வாழ்த்துகளையும் பாராட்டுதல்களையும் தெரிவித்துக் கொள்கிறேன்.

வாழ்க பாரதி!
வளர்க தமிழ்த்துறை!!

வாழ்த்துக்களுடன்

முதல்வர்

முனைவர் **ச. ஈஸ்வரன்,**
தமிழாய்வுத்துறைத் தலைவர்
தேசியக்கல்லூரி (தன்னாட்சி),
திருச்சிராப்பள்ளி- 620001.

நன்றியுரை

மகாகவி பாரதியார் நினைவு நூற்றாண்டு விழா எம் கல்லூரியில் 09.09.2021 அன்று நிகழ இருப்பது மிகவும் மகிழ்வாய் இருக்கிறது.

சின்னசாமி ஐயருக்கும் இலக்குமி அம்மாளுக்கும் அருந்தவப் புதல்வராய் எட்டயபுரத்தில் 11.12.1882 இல் அவதரித்து, தம் பதினோராம் வயதில் எட்டையபுர அரசவையில் அருந்தமிழ்க் கவிபாடி, 'பாரதி' என்ற பட்டம் பெற்று, 11.09.1921 இல் அமரரானவர் மகாகவி.

பன்மொழிப் புலமை, இயற்கை ஈடுபாடு, இரக்க உணர்வு, சாதி வெறுப்பு, மொழிப்பற்று, நாட்டுப்பற்று என்றவாறு பன்முகம் கொண்டவர் மகாகவி பாரதியார். 'இந்தியா' என்ற வார இதழைச் சொந்தப் பொறுப்பில் நடத்தியவர். ''எட்டையபுரத்தைவிட்டு மகாகவி பாரதியார் மதுரைக்கு வந்தபோது அவருக்கு உதவிய அரிய நண்பர் பண்டித ம.கோபால கிருஷ்ணய்யர் ஆவார்'' என்கிறார் கவியோகி சுத்தானந்த பாரதியார். மதுரை சேதுபதிப் பள்ளியில் மகாகவி பாரதியாருக்குத் தமிழாசிரியர் பணி கிடைக்க உதவியவர் பண்டித ம.கோபால கிருஷ்ணய்யர். (பண்டித ம.கோபால கிருஷ்ண அய்யர் அவர்கள் எம் தமிழாய்வுத்துறையின் முதல் துறைத்தலைவராகப் பணிபுரிந்தவர் (1919-1927))

''நல்ல தமிழ் எழுதக்கூடிய உணர்ச்சிமிக்க வாலிபர் எனக்கு வேண்டும். சுதேசமித்திரன் பத்திரிகையில் எனக்குத் துணை செய்ய ஒருவர் தேவை என்று சுதேசமித்திரனின் இதழ் ஆசிரியர் ஜி. சுப்ரமணிய ஐயர் கேட்க, ''இதோ தங்கமான உணர்ச்சிக்கனல்'' என்று பாரதியாரை அறிமுகப்படுத்தியவர் ம.கோபால கிருஷ்ண

அய்யர் ஆவார். இவ்வாறாக பல் சிறப்புகள் பெற்ற மகாகவி பாரதியாரின் நினைவு நூற்றாண்டு விழாவினை ஒட்டி, 'என்றும் மகாகவி பாரதி' எனும் இந்நூல் எம்துறையால் எழுதப் பெற்று வெளியிடப்படுகிறது.

இந்நூலினை எழுத அனுமதி நல்கிய எம் கல்லூரித் தலைவர் மதிப்புமிகு பத்மவிபூஷண் டாக்டர் வி.கிருஷ்ணமூர்த்தி, செயலர் மதிப்பிற்குரிய கா.ரகுநாதன், மதிப்புயர் முதல்வர் முனைவர் இரா. சுந்தரராமன் ஆகியோருக்கு முதற்கண் இதயம் நிறைந்த நன்றி மலர்களை உரித்தாக்கி மகிழ்கிறேன்.

எம் துறைப் பேராசிரிய நண்பர்கள், அச்சிட்டு, அழகுற வழங்கிய MJ பப்ளிக்கேஷன் நிறுவனத்தார், எம் கல்லூரி முதல்வர் வெளியிட நூலைப் பெற்றுக் கொண்ட திருமிகு இலக்கியச்செல்வர் முனைவர் இரா.மாது (எம் தமிழாய்வுத்துறையில் பணிபுரிந்த மதிப்புமிகு பேரா.இரா.இராதாகிருஷ்ணன் (1951-1984) அவர்களின் திருமகனார்) உள்ளிட்ட அனைவருக்கும் அன்பு நிறை நன்றிகளைப் படைத்து ஆனந்தம் அடைகிறேன்.

''சேமமுற வேண்டுமெனில் தெருவெல்லாம் தமிழ் முழக்கம் செழிக்கச் செய்வீர்'' என்று முழங்கிய மகாகவியின் புகழ் - பார் உள்ளளவும் நீர் உள்ளளவும் வாழ்க! வாழ்க!

இப்படிக்கு,

01.09.2021 **(ச.ஈஸ்வரன்)**

வளர்ந்து வரும் தமிழாய்வுத்துறை

MISSION (நோக்கம்)

- மொழித்திறனை வளர்த்தல்
- மனித மாண்பினைப் பயிற்றுவித்தல்
- ஆளுமைப் பண்பினைப் பெற வழிகாட்டல்

VISION (தொலைநோக்கு)

- தமிழ் இலக்கிய விழுமியங்களை உணர்த்தல்
- வாழ்வியல் ஒழுக்க நெறிகளை விளங்க வைத்தல்
- மொழியைப் பிழையின்றிப் பேசவும் எழுதவும் செய்தல்

தமிழாய்வுத் துறையில் பணிபுரிந்தோர்

திருமிகு. **ம.கோபாலகிருஷ்ணய்யர்** - 1919 - 1927 (முதல் துறைத்தலைவர்)

திருமிகு. **எஸ்.சீதாராமய்யர்** - 1926 - 1956

திருமிகு. **ஆர்.வாசுதேவ சர்மா** - 1926 - 1937

திருமிகு. **தி.சே.பாலசுப்பிரமணியம்** - 1937 - 1939

திருமிகு. **சிவகுருநாதப்பிள்ளை** - 1951 - 1984

திருமிகு. **இரா.இராதாகிருஷ்ணன்** - 1952 - 1954

திருமிகு. **ஆர்.பார்த்தசாரதி** - 1954 - 1955 (இந்திராபார்த்தசாரதி)

திருமிகு. **குமாரசுவாமிராஜா** - 1955 - 1956

திருமிகு. **எஸ். முருகேசன்** - 1956 - 1991

திருமிகு. **த. மணிமாறன்** - 1956 - 1958

திருமிகு. **தெ. துரைசாமி** - 1957 - 1986

திருமிகு. **சிவராமகிருஷ்ண சாஸ்திரி** - 1966 - 1967

முனைவர். **கு.திருமேனி** - 1967 - 1968

திருமிகு. **ஆர். அரங்கராசன்** - 1968 - 1975

முனைவர். **கே.ஏ. இராசாராமன்** - 1966 - 1967

திருமிகு. **ஆர். கிருஷ்ணமூர்த்தி** - 1967 - 1968

முனைவர். **சோ. சத்தியசீலன்** - 1968 - 1975

முனைவர். **நா. இராசகோபாலன்** - 1968 - 1985

திருமிகு. **வி.சுப்பிரமணியன்** - 1968 - 1972

திருமதி. **பிலோமினாமேரி** - 1968 - 1970

திருமிகு. **ச. ஆறுமுக முதலியார்** - 1968 - 1970

முனைவர். **மகா. வேங்கடராமன்** - 1969 - 1990

திருமிகு. **கு. மாதவன்** - 1964 - 1996

முனைவர். **வி.நாகராசன்** -1965 - 2000

முனைவர். **ந. சேசாத்திரி** - 1966 - 2003

முனைவர். **ம. இலக்குமிநாராயணன்** - 1969 - 2002

திருமிகு. **சுப. வேங்கடராமன்** - 1970 - 2002

முனைவர். **வே. இராமசுப்பிரமணியன்** - 1975 - 2004

முனைவர். **இரா. இரகோத்தமன்** - 1976 - 2007

முனைவர். **ச. கணேசன்** - 1985 - 2004

முனைவர். **சொ.சற்குணம்** - 1986 - 2006

முனைவர். **அ.வ. இராசகோபாலன்** - 1986 - 1992

முனைவர். **ஆ.செகந்நாதன்** - 1985 - 2004

முனைவர். **சி. சித்ரா** - 1998 - 2007

முனைவர். **கு. இராசரத்தினம்** - 1985 - 2015

முனைவர். **இரா. சபாபதி** - 1997 - 2016

முனைவர். **சாமி. திருமாவளவன்** - 1992 - 2017

துறைத்தலைவர்களாகப் பணிபுரிந்தோர்

திருமிகு. **ச. ஆறுமுக முதலியார்** - எம்.ஏ.,பி.ஓ.எல் - 1968 - 1970

திருமிகு. **தி.சே. பாலசுப்பிரமணியன்** - எம்.ஏ. - 1970 - 1974

முனைவர். **கு.திருமேனி** - எம்.ஏ., பி.ஓ.எல்., புலவர், பிஎச்.டி - 1974 - 1986

திருமிகு. **தெ. துரைசாமி** எம்.ஏ., - 1986 - 1991

திருமிகு. **கு. மாதவன்** பி.ஏ., (ஆனர்சு) - 1991 - 1996

முனைவர். **வி.நாகராசன்** எம்.ஏ., வித்துவான்,பி.ஓ.எல்., பி.எட்., பிஎச்.டி- 1996 - 2000

திருமிகு. **சுப. வேங்கடராமன்** எம்.ஏ.எம்.பில் - 2002 - 2002

முனைவர். **ந. சேசாத்திரி** எம்.ஏ., எம்.பில்., பிஎச்.டி - 2002 - 2003

முனைவர். **வே. இராமசுப்பிரமணியன்** எம்.ஏ., எம்.பில்., பிஎச்.டி - 2003 - 2004

முனைவர். **இரா. இரகோத்தமன்** எம்.ஏ., பிஎச்.டி - 2004 - 2007

முனைவர். **கு. இராசரத்தினம்** எம்.ஏ., எம்.பில்., பிஎச்.டி - 2007 - மே 2015 வரை

முனைவர். **ச. ஈஸ்வரன்** எம்.ஏ., எம்.பில்.,பிஎச்.டி- ஜீன் 2015 முதல்

தமிழாய்வுத்துறையில் பணிபுரிவோர்

1.	முனைவர் ச.ஈஸ்வரன்	இணைப்பேராசிரியர் & தமிழாய்வுத்துறைத்தலைவர்
2.	முனைவர் ந.மாணிக்கம்	இணைப்பேராசிரியர்
3.	முனைவர் சி.காந்தி	இணைப்பேராசிரியர்
4.	முனைவர் இரா.இரவிச்சந்திரன்	இணைப்பேராசிரியர்
5.	முனைவர் சா.நீலகண்டன்	உதவிப்பேராசிரியர்
6.	முனைவர் ஆ.முருகானந்தம்	உதவிப்பேராசிரியர்
7.	முனைவர் இரா.சுந்தரவேல்	உதவிப்பேராசிரியர்
8.	முனைவர் அ.கிருஷ்ணன்	உதவிப்பேராசிரியர்
9.	திரு. தி.இராமதாஸ்	உதவிப்பேராசிரியர்
10.	முனைவர் இரா.பத்மா	உதவிப்பேராசிரியர்
11.	முனைவர் க.புவனேஸ்வரி	உதவிப்பேராசிரியர்
12.	முனைவர் ஜெ.புவனேஸ்வரி	உதவிப்பேராசிரியர்
13.	முனைவர் இரா.இராஜா	உதவிப்பேராசிரியர்
14.	முனைவர் மு.சுபாஷினி	உதவிப்பேராசிரியர் (நிர்வாக நியமணம்)

பணிமுறை –1 (சுயநிதிப் பிரிவு)

15.	முனைவர் இரா.மாணிக்கவாசகன்	உதவிப்பேராசிரியர்
16.	முனைவர் கோ.இராமச்சந்திரன்	உதவிப்பேராசிரியர்
17.	முனைவர் கூ.முத்தையன்	உதவிப்பேராசிரியர்
18.	முனைவர் ச.கருத்தான்	உதவிப்பேராசிரியர்
19.	முனைவர் க.முருகேசன்	உதவிப்பேராசிரியர்
20.	முனைவர் ப. சுமதி	உதவிப்பேராசிரியர்
21.	முனைவர் இரா. பவனா	உதவிப்பேராசிரியர்
22.	முனைவர் ந.ஆனந்த்	உதவிப்பேராசிரியர்
23.	திருமதி கு.சிந்து	உதவிப்பேராசிரியர்

பணிமுறை – 2 (சுயநிதிப் பிரிவு)

24.	முனைவர் ஆ.இராஜலட்சுமி	உதவிப்பேராசிரியர்
25.	முனைவர் சு.ஷீலா	உதவிப்பேராசிரியர்
26.	முனைவர் வெ.ஜெயலெட்சுமி	உதவிப்பேராசிரியர்
27.	முனைவர் கோ.தங்கச்செல்வி	உதவிப்பேராசிரியர்
28.	முனைவர் மு.சித்திரப்பிரியா	உதவிப்பேராசிரியர்

பொருளடக்கம்

1.	இதழாளர் பாரதி	முனைவர் ந.ஆனந்த்	1
2.	பாரதியின் மொழியுணர்வுச் சிந்தனைகள்	முனைவர் கோ.இராமச்சந்திரன்	7
3.	பாரதியின் தேசப்பற்று	முனைவர் கோ.இராமச்சந்திரன்	12
4.	என்றும் மகாகவி	தி. இராமதாஸ்	17
5.	பாரதியின் பன்முகப்பார்வை	முனைவர் ஆ. இராஜலெட்சுமி	22
6.	பேராசிரியர் வே. சாரநாதன் பார்வையில் மகாகவி பாரதியார்	முனைவர் இரா. இராஜா	27
7.	பாரதியார் கவிதைகளில் காணலாகும் இறையுணர்வுச் சிந்தனைகள்	முனைவர் ச.ஈஸ்வரன்	35
8.	பாரதி வாழ்கிறான்	முனைவர் சி. காந்தி	41
9.	பாரதியார் கவிதைகள் வெளிப்படுத்தும் இனப்போராட்டத் தீர்வுகள்	முனைவர் அ.கிருஷ்ணன்	48
10.	மனதிலுறுதி வேண்டும்	முனைவர் மு.சித்ரப்பிரியா	55
11.	பாட்டுக்கொரு புலவனின் இன்பம்	கு.சிந்து,	59
12.	பாரதி போற்றும் பெண்மை	முனைவர் சு. சீலா	65
13.	பாரதியின் ஆறில் ஒரு பங்கு சிறுகதையில் சமத்துவம்	முனைவர் இரா.சுந்தரவேல்	69
14.	பாரதியின் புதுமை நோக்கு	முனைவர் மு.சுபாஷினி	78
15.	பாரதி சுட்டும் அறநெறிகள்	முனைவர் ப.சுமதி	86
16.	பாரதியின் பக்திநிலை	முனைவர் கோ. தங்கச்செல்வி	93
17.	ஞானரதத்தில் தேசத்தின் போக்கும் ஞானத்தின் நோக்கும்	முனைவர் சா.நீலகண்டன்	97
18.	மானுட உயர்வும் இன்பமும்	முனைவர் இரா.பத்மா	103

19.	பாரதியின் உளவியல் பார்வை	முனைவர் இரா. புவனா	110
20.	பாரதி – புதிய பார்வை	முனைவர் க. புவனேஸ்வரி	115
21.	பாரதியின் பன்முக ஆளுமை	முனைவர் ஜெ. புவனேஸ்வரி	121
22.	பாரதி காட்டும் சமத்துவம்	முனைவர் ந.மாணிக்கம்,	127
23.	பாரதியும் பாரதமும்	முனைவர் இராம.மாணிக்கவாசகம்	132
24.	கவித்தேரின் சாரதி	முனைவர் கூ. முத்தையன்	137
25.	பாரதியின் பல்துறைச்சிந்தனைகள்	முனைவர் ஆ. முருகானந்தம்	143
26.	பாரதியின் பெண்கள் விடுதலைக் கும்மி	முனைவர் க. முருகேசன்	153
27.	பாரதியாரின் கவிதைகளில் மரபுக் கட்டுடைப்புச் சிந்தனைகள்	முனைவர் வெ. ஜெயலெட்சுமி	160
28.	பாரதியும் புதுமையும்	ப. நவசக்திவேல்	166
29.	பாரதி போற்றிய தேசியத் தலைவர்கள்	வெ. சங்கரநாராயணன்	172
30.	பாரதியின் பன்முகப் பார்வை	பெ. ஜோதி	178
31.	சித்திர விளக்கங்களின் முத்திரை மற்றும் முன்னோடி பாரதி	திருமதி. ச.கண்ணம்மாள்	184
32.	பாரதி முரண்	செ. மணிகண்டன்	184

1. இதழாளர் பாரதி

முனைவர் ந.ஆனந்த்,
உதவிப் பேராசிரியர், தமிழ்த்துறை,
தேசியக் கல்லூரி, திருச்சிராப்பள்ளி.

முன்னுரை

சின்னஞ்சிறு வயதிலேயே கவிதை பாடிக் கவிஞராக உருவாகிய பாரதி, ஒரு விடுதலைப் போராளி. சமூகச் சிந்தனையாளர். ஆன்மீக ஞானி, பெண்ணியப் புரட்சியாளர் எல்லாவற்றையும் உள்ளடக்கிய மகாகவிஞர் இவர். சக்கரவர்த்தினி, இந்தியா, விஜயா, சூரியோதயம், கர்மயோகி, தர்மர் ஆகிய தமிழ் இதழ்களிலும் பாலபாரதா என்னும் ஆங்கில இதழிலும் பணியாற்றினார். இக்கட்டுரையில் பாரதியார் பணியாற்றிய இதழ்கள் மட்டுமே இடம் பெறுகின்றன.

இதழியல் இலக்கணம் – மேனாட்டறிஞர் கருத்து

மனிதன் அறிவு வளர்ச்சி பெற்ற காலத்திலிருந்து மற்றவர்களைப் பற்றியும், அவர்களது செயல்களைப் பற்றியும் அறிந்து கொள்ளும் ஆர்வம் உடையவனாக இருந்திருக்கின்றான். முதலில் செய்கையின் மூலமாகவும், மொழிவளர்த்த பின் பேச்சின் துணையோடும் செய்திகளை அறிந்து பரப்பியும் வந்திருக்கிறான். எழுதக் கற்றுக் கொண்டவன். அச்சுப் பொறியைக் கண்டுபிடித்தும் செய்திகளின் பரப்பளவு மிகுந்தது. போக்குவரத்து வளர்ச்சி, அறிவியல், தொழில் நுட்ப முன்னேற்றம் இவற்றின் விளைவாக மின்னணுக் கருவிகளின் துணையோடு இலக்கண வரம்புக்குள்ளடங்காத அளவிற்கு இதழியல் வளர்ச்சி பெற்றுள்ளது.

ஹரால்டு பெஞ்சமின்

ஹரால்டு பெஞ்சமின் என்ற ஒரு புகழ் பெற்ற அமெரிக்க இதழியல் பேராசிரியர், "பொது நோக்குடைய இதழியல் துறை ஓர் ஆற்றல் மிக்க கருவியாகும். அதன் மூலம்தான் இன்றைய

சமுதாயம் அதனுடைய வழிகளை வகுத்துக் கொண்டும் மாற்றிக் கொண்டும் தெளிவான வரையறுக்கப் பெற்ற வளரும் மனித நலன் என்னும் இலட்சியத்தை நோக்கி நடை பயில்கின்றது" என்று குறிப்பிடுகின்றார்.

லார்டு கிரே

"பத்திரிகை தான் நாட்டுச் சுதந்திரத்தின் மிகப் பெரிய காவலாளி," என்று கருதுகிறார்.

மேத்யூ அர்னால்டு

"இதழியல் அவசரத்தில் பிறக்கும் இலக்கியம்" என்று புகழ்கின்றார்.

இதழியல் விளக்கம்

ஜெர்னலிசம் என்ற ஆங்கிலச் சொல்லின் தமிழாக்கம் இதழியல் எனப்படும். டையர்னல் *Diurnald* என்ற இலத்தின் சொல்லில் இருந்து பிறந்தது 'ஹசர்னல்', டையர்னல் என்றால் அன்றாடம் என்று பொருள் 'ஹசர்னல்' என்பது அன்றாடம் நடந்ததை எழுதி வைக்கும் ஏடு.

எவர், என்ன, எப்போது, எங்கே, ஏன், எப்படி என்ற கேள்விகளுக்கான விடையே இதழியல் ஆகும்.

பத்திரிக்கைக்கு எழுதுதல், பத்திரிகைகளை நடத்துதலே இதழியல் என சேம்பர்ஸ் இருபதாம் நூற்றாண்டு அகராதி சொல்கிறது. செய்திகளைத் திரட்டுவதும் பரப்புவதும் இதழியல் ஆகும்.

இதழாளர் – பாரதி (சுதேசமித்திரன்)

தேசச் செய்திகளைப் பாமரர்களும் அறிந்து கொள்ள 1882 ஆம் ஆண்டு சுதேசமித்திரனை ஜி.சுப்பிரமணிய ஐயர் தொடங்கினார். 1882-இல் வார இதழாகவும், 1887-இல் வார இருமுறையாகவும், 1889-இல் நாளிதழாகவும் வளர்ச்சியுற்றது மக்களிடைய அரசியல் உணர்வு, தேசிய உணர்வு, காந்தியத்தைப் பரப்புவதையும் கண்ணுங்கருத்துமாக எடுத்துக் கொண்டார். இவர் தமிழ்ப் பத்திரிகை உலகின் தந்தை எனப் போற்றப்படுகிறார். மகாகவி பாரதியார் இதில் சிலகாலம் துணை ஆசிரியராகப் பணியாற்றினார்.

துணை ஆசிரியராகப் பணியாற்றிய பாரதிக்குத் தொலைவரியில் (தந்தியில்) வரும் செய்திகளையும், ஆங்கில

இதழ்களில் வரும் செய்திகளையும், சொற்பொழிவுகளையும் தமிழில் மொழியாக்கம் செய்வது, அச்சுப் பிழை திருத்துவது முதலியனவே பாரதியின் பெரும்பான்மைப் பணிகளாக இருந்தன.

இவ்விதழ் எட்டுப்பக்கங்களைக் கொண்டது. 9 அங்குல நீளமும் 12 அங்குல அகலமும் கொண்டதாக தொடக்க காலத்தில் இதழ் அமைந்தது. முதலிரண்டு பக்கங்களும் விளம்பரங்களைக் கொண்டுள்ளது.

இந்தியா – இதழ்

தீவிரவாதிகளின் கருத்தை ஆதரிக்கப் பாரதியார் சுதேசமித்திரன் பத்திரிகையில் இருந்து வெளியேறி 1906-இல் 'இந்தியா' என்ற இதழை ஆரம்பித்தார். இது 1910-ஆம் ஆண்டு வரை நடந்தது. இது சுதந்திர உணர்வைப் பாமரரிடையேப் பரப்புவதில் செய்த சேவை மகத்தானது. கேலிச் சித்திரங்களை வரைந்து கார்ட்டூன் மூலம் கருத்துக்களைப் பதிய வைக்கும் உத்தியைப் பாரதியார் இந்தியா பத்திரிக்கையில் கையாண்டார். 80-ஆண்டுகளுக்கு முன் வாரம் 4000 பிரதிகள் விற்றது இந்தியா. மகாகவி பாரதியார் 'இந்தியா' இதழை ஒரு புரட்சிகரமான இதழாக நடத்தியதை அவர் அறிவித்திருந்த சந்தா விவரத்திலிருந்தே அறிந்து கொள்ளலாம். ஹஇந்தியா' இதழுக்கு அவர் அறிவித்திருந்த ஆண்டு சந்தா விவரம்

எல்லா கவர்ன்மென்டாருக்கும் ரூ.50

ஜமீன்தார்கள் ராஜாக்களுக்கு ரூ.30

மாதம் ரூ.200 க்கு மேற்பட்ட வருமானம் உள்ளவர்களுக்கு ரூ.15

மற்றவர்களுக்கு ரூ.3

சக்கரவரத்தினி

பாரதி சுதேசமித்திரனில் உதவி ஆசிரியராகப் பணியாற்றும் போது 1905-ஆகஸ்டில் தொடங்கப்பட் 'சக்கரவர்த்தினி'யில் ஆசிரியராகப் பணியேற்கும் வாய்ப்பைப் பெற்றார். தொடக்கத்தில் சக்கரவர்த்தினி இதழ் சுதேசமித்திரன் அச்சகத்திலிருந்து வெளிவந்தது. பாரதி இவ்விதழில் இளமை மணம், சதி என்னும் உடன்கட்டை ஏறும் வழக்கம், வரதட்சணை, கைம்பெண்கொடுமை ஆகியவற்றை எதிர்த்தும் பெண்கல்வி, பெண்ணுரிமை ஆகியவற்றை வலியுறுத்தியும் கட்டுரைகள் வெளியிட்டார். சுதேச மித்திரன் செய்தித்தாள்களில் எழுத முடியாத நீண்ட கட்டுரை, கவிதை ஆகியவற்றை எழுதச்

சக்கரவர்த்தினி பாரதிக்குப் பயன்பட்டது. ஆசிரியர் பொறுப்பில் பாரதி முழுமையாக ஈடுபட்டுப் பட்டறிவு பெறச் சக்கரவர்த்தினி உதவியது.

பாலபாரதா ஆர்யங் இண்டியா

இது பாரதியை ஆசிரியராகக் கொண்ட ஆங்கில இதழாகும். இவ்விதழ் வார இதழா, மாத இதழா எப்போது தோன்றியது என்பது பற்றிப் பலவேறுப்பட்ட கருத்துக்கள் கூறப்பட்டன. 'பாலபாரதா' மாத இதழ் என்று கூறியுள்ளார் பாரதி 1907 நவம்பர் முதல், மாத இதழாக இவ்விதழ் வெளிவந்தது. மேலும் இதற்கு முன் வார இதழாகப் பாரதியை ஆசிரியராக கொண்டு வெளி வந்தது. ஆங்கில இதழின் ஆசிரியர் என்ற முறையில் பாரதியின் புகழ் இந்தியா முழுவதும் பரவியது.

இவ்விதழில் விவேகானந்தர், அரவிந்தர் ஆகியோரின் கட்டுரைகள் அதிகம் இடம் பெற்றன. பாரதியின் ஞானகுரு நிவேதிதா தேவியும் பாரதியோடு மடல் தொடர்பு கொண்டதோடு பெயரிடாமல் கட்டுரைகளும் எழுதினார்.

விஜயா

புதுச்சேரியில் விஜயாவைத் தொடங்கும் முன்னரே இந்தியா இதழைத் தொடங்கி விட்டார். அதன் காரணமாக விஜயா இதழுக்காக தனியொரு அச்சுக்கூடம் தேவையற்றதாக கருதப்பட்டு இந்தியா இயங்கி வந்த அச்சுக்கூடமே விஜயாவுக்குமானது. இதனால் மிக முக்கியமான செய்திகள் இந்தியாவிலும், விஜயாவிலும் ஒரே மாதிரியான தன்மையில் வெளிவந்தன. சென்னையில் விஜயா இதழ் 1909 ஜீலையில் நின்று விட்டது. அதன் பின்னர் புதுவையில் வெளியான இந்தியா இதழில் விஜயா பற்றிய விளம்பரம் வெளி வந்துள்ளது. இதனைப் போன்று விஜயா இதழுக்கான ஏஜெண்டுகள் தேவை என்பதற்கான விளம்பரம் ஒன்று இந்தியா இதழில் காண முடிகின்றது. சான்றாக 'விஜயா' என்றும் தலைப்பில் ஏஜெண்டுகள் வேண்டும். மிக உதாரமான சன்மானங்கள் கொடுக்கப்படும். தினந்தோறும் புதுச்சேரியில் பிரசுரமாகும் தமிழ்ப்பத்திரிகை, தமிழ் ஜனங்கள், அறிவு, செல்லம் வல்லமை முதலிய நன்மைகள் அனைத்தும் பெற வழிகாட்டுகிறது. ஆண்டு சந்தா ரூ10 ரூபாய்தான்.

இந்தியா 23.03.1910 இதழில் விஜயா நாளிதழையும் வார இதழாக மாற்றம் போவதாகவும் பிரிட்டிஷ் இந்திய அரசியல்

தொடர்பாக எதுவும் இனி எழுதப் போவதில்லை என்றும் அறிவித்துள்ளார் பாரதியார். எனவே 'விஜயா' இதழ் வீரியமான தன்மையில் வெளிவந்துள்ளதை அறிய முடிகிறது.

கர்ம யோகி

'கர்ம யோகின்' என்ற ஆங்கில வார இதழைக் கல்கத்தாவில் அரவிந்தர் 1909-இல் வெளியிட்டதைத் தொடர்ந்து, 1910-இல் 'கர்மயோகி' என்ற தமிழ் மாத இதழைப் பாரதி வெளியிட்டுள்ளார். இந்த இதழில் பாரதி 'ஆரியநாகரிகம்' 'நமது சொந்தநாடு' 'ஒற்றுமையே வலிமை' என்றெல்லாம் கட்டுரைகள் எழுதினார். 'பதஞ்சலி யோக சூத்திரம்' சமஸ்கிருதத்தில் இருந்ததைச் சுவாமி விவேகானந்தர் ஆங்கிலத்தில் மொழி பெயர்த்தார் மூலலத்துக்கும் விவேகானந்தருடைய மொழி பெயர்ப்புக்கும் சில இடங்களில் முரண் இருக்கிறது என்பது பாரதியாரின் எண்ணம். மூலத்திலிருந்தே அவர் யோக சூத்திரத்தைத் தமிழில் மொழி பெயர்த்து பகுதி பகுதியாகக் 'கர்மயோகி' பத்திரிக்கையில் வெளியிட்டார். 'கர்மயோகி' தொடங்கி சுமார் இரண்டு வருடங்கள் நடந்தது. இந்தப் பத்திரிகையும் நாட்டுப் பற்றைத் தூண்டும் விதத்தில் எழுதி வந்தது.

தர்மம்

கல்கத்தாவில் அரவிந்தர் நடத்திய 'தர்மா' என்ற இதழை அடியொற்றிப் புதுவையில் பாரதி 'தர்மம்' என்ற இதழை நடத்தினார். இது பெயருக்கு ஏற்ப இலவசமாக வழங்கப்பட்டது. உறுப்பினர் கட்டணம் (சந்தா) கிடையாது. நன்கொடை ஏற்கப்பட்டது. 'தர்மம்' இதழின் ஆசிரியராகப் பதிவு செய்யப்பட்டவர் ஆர்.கிருஷ்ணசாமி அய்யர் என்பவர் இது ஒரு மாத இதழ்' வ.வே.சு.ஐயரின் வீட்டு முகவரியைத் தாங்கி இந்த இதழ் வெளியானதாகத் தெரிகிறது.

சூரியயோதயம்

1908-ஆம் வருடம் 'சூரியோதயம்' எனும் தமிழ் வார இதழ் புதுச்சேரியிலிருந்து வெளிவந்தது. இந்தப் பத்திரிகை 300 பிரிதிகள் வரை விற்றதாகவும் தெரிகிறது. 1908-இல் நின்று போன இந்த இதழ் மீண்டும் பாரதியாரால் 1910-லிருந்து வெளியிடப்பட்டது. இந்த இதழில் ஆசிரியர் நீலகண்ட பிரம்மச்சாரி என்றாலும் பாரதியாருடைய கட்டுரைகள் இந்த பத்திரிகையில் வெளிவந்தன. 'சூரியோதயம்' இதழும் ஆங்கில ஆட்சியாளர்களின் தடையுத்தரவினால் நிறுத்தப்பட்டது. அதே

பெயரில் 1910-இல் பாரதி சைகோன் சின்னையா நாயுடு உதவியுடன் இந்த இதழைத் தொடங்கியிருக்கிறார்.

முடிவுரை

தமிழகத்தில் நடைபெறும் அனைத்து வகையான நிகழ்வுகளையும் உடனுக்குடன் மக்களுக்கு எடுத்துக் கூறும் ஊடகமாக செய்தித்தாள் அமைகிறது. சுவையான, அற்புதமான நிகழ்வுகள் நடைபெற்றால் அவற்றை செய்தியாக எழுதி அனுப்புவோருக்குத் தகுந்த சன்மானம் கொடுக்கும் வழக்கத்தைப் பத்திரிக்கைத் துறையில் ஏற்படுத்திய முதல் இதழாசிரியர் பாரதியார் என்பது குறிப்பிடத்தக்கது.

பார்வை நூல்கள்

1. இதழியல் ஓர் அறிமுகம் – முனைவர் ச.ஈஸ்வரன் முனைவர் இரா.சபாபதி
2. இதழியல் கலை – டாக்டர் மா.பா.குருசாமி
3. இதழாளர் பாரதி – முனைவர் பா.இறையரசன்

2. பாரதியின் மொழியுணர்வுச் சிந்தனைகள்

முனைவர் இரா.இரவிச்சந்திரன்,
இணைப்பேராசிரியர், தமிழாய்வுத்துறை,
தேசியக்கல்லூரி (தன்னாட்சி), திருச்சிராப்பள்ளி–01.

முன்னுரை

எட்டையபுரத்தில் சின்னச்சாமிக்கும் இலக்குமி அம்மையாருக்கும் மகனாகப் பிறந்தவர் சுப்பிரமணியன். சுப்பையா என்று எல்லோராலும் அழைக்கப்பெற்ற இவர் இளம் வயதிலேயே தம் கவித்திறத்தால் 'பாரதி' என்ற பட்டம் பெற்றார். தமிழ் தமிழர் நலம், நாட்டு விடுதலை, பெண் விடுதலை, தீண்டாமை ஒழிப்பு எனப் பன்முகப் பார்வையில் கவிதை பாடியவர். பத்திரிக்கை வாயிலாக விடுதலை உணர்வைத் தூண்டியவா மகாகவி சுப்பிரமணிய பாரதியார். அவரது கவிதைகளில் இடம்பெற்றுள்ள மொழியுணர்வுச் சிந்தனைகளை எடுத்துக் கூறுவதே இக்கட்டுரையின் நோக்கமாகும்.

தமிழ்மொழியைத் தவிர ஆங்கிலம், பிரஞ்ச், சமஸ்கிருதம், தெலுங்கு, இந்தி, எனப் பதினான்கு மொழிகளில் புலமை பெற்றவர் பாரதியார், ஆதலால்

> "**யாமறிந்த மொழிகளிலே தமிழ்மொழி போல்**
> **இனிதாவது எங்கும் காணோம்,**
> **பாமரராய், விலங்குகளாய், உலகனைத்தும்**
> **இகழ்ச்சி சொலப் பான்மை கெட்டு,**
> **நாமமது தமிழரெனக் கொண்டு இங்கு**
> **வாழ்ந்திடுதல் நன்றோ? சொல்லீர்!**
> **தேமதூரத் தமிழோசை உலகமெலாம்**
> **பரவும்வகை செய்தல் வேண்டும்**"

என்று உணர்ந்து பாடியதோடு மட்டுமல்லாமல் தேமதுரத் தமிழோசையை உலகமெல்லாம் பரப்பியவர். தமிழ் போன்று இனிமையான மொழி வேறொன்றும் இல்லை என்று கூறுகிறார். தமிழை மட்டுமல்ல தமிழ்நாட்டைப் பற்றியும்,

"செந்தமிழ் நாடெனும் போதினிலே - இன்பத்
தேன் வந்து பாயுது காதினிலே"

என்று பாடியுள்ளார். பாரதியின் சிந்தனை முழுக்க தமிழைப் பற்றியும் தமிழ் நாட்டைப் பற்றியும், தமிழகத்திலுள்ள வளங்கள் பற்றியுமே இருந்துள்ளது என்பதை அவரது கவிதைகள் உணர்த்துகின்றன. 'உள்ளத்து உள்ளது கவிதை' என்பதற்கேற்ப தம் உள்ளம் முழுக்க

"எண்ணிய முடிதல் வேண்டும்
நல்லவே எண்ணல் வேண்டும்,
திண்ணிய நெஞ்சம் வேண்டும்
தெளிந்தநல் லறிவு வேண்டும்"

என்ற சிந்தனையே அவரிடத்திலுள்ளது. தூய நெஞ்சமும் நல்லறிவும் பெற்றவரால் எதையும் வெற்றிகரமாக செய்து முடிக்க முடியும் என்பதை வெளிப்படுத்துகிறார். எந்த ஒரு செயலையும் திட்டமிட்டு செய்ய வேண்டும் அக்காரியம் முடியும் வரை சோர்வறியாது ஓயாமல் உழைக்கவேண்டும் என்பதை,

"நமக்கு தொழில் கவிதை நாட்டிற்கு உழைத்தல்
இமைபொழுதும் சோராதிருத்தல்"

என்ற கவிதை வழி கூறுகின்றார். 'உழைப்பே உயர்வு' என்பதற்கேற்ப உழைப்பின் மேன்மையை சுட்டிக் காட்டி பாரதியார் எங்கும் எந்த சூழ்நிலையிலும் என் தாய்தமிழை பேச மறப்பேன் என்பதை,

"பட்டினி கிடந்து
பசியால் மெலிந்து
பாழ்பட நேர்ந்திடினும்
கட்டி இழுத்து
கால் கை முறிந்து
அங்கம் பிளந்து
இழந்து துடித்திடினும்
பொங்கு தமிழை பேச
மறப்பேனோ...."

என்று பாடியுள்ளார். இவற்றிலிருந்து பாரதியார் தமிழ் மீதும் தமிழ்நாட்டின் மீதும் எவ்வளவு பற்று மிகுந்தவராய் இருந்துள்ளார் என்பதை அறியமுடிகிறது. தமிழ்மொழியைக் கற்றால் மட்டும் போதாது அதை பிறர் நாட்டவரும் வணங்கச் செய்தல் வேண்டும் என்பதை,

''பிறநாட்டு நல்லறிஞர் சாத்திரங்கள்
தமிழ்மொழியிற் பெயர்த்தல் வேண்டும்
இரவாத புகழுடைய புதுநூல்கள்
தமிழ்மொழியில் இயற்றல் வேண்டும்
மறைவாக நமக்குள்ளே பழங்கதைகள்
சொல்வதிலோர் மகிமை இல்லை,
திறமான புலமையெனில் வெளிநாட்டோர்
அதை வணங்கச் செய்தல் வேண்டும்''

என்று பாடுகிறார். பன்மொழிப் புலமை பெற்ற பாரதி பலநூல்களைக் கற்றுத் தேர்ந்தவராக மட்டுமல்லாமல் பிறரும் பலநூல்களைக் கற்கவேண்டும் என்பதை உணர்ந்து வெளிப்படுத்தி அவற்றுள் தமிழ்மொழியை பிறநாட்டவரும் வணங்கும்படிச் செய்யவேண்டும் என்கிறார். அதுமட்டுமல்லாமல் புகழ் பெற்ற புலவர்களைத் தம் கவிதை வழி குறிப்பிடுகிறார்.

''யாமறிந்த புலவரிலே கம்பனைப் போல்,
வள்ளுவர்போல், இளங்கோ வைப்போல்,
பூமிதனில் யாங்கணுமே பிறந்ததிலை,
உண்மை, வெறும் புகழ்ச்சியில்லை,
ஊமையராய்ச் செவிடர்களாய் குருடர்களாய்
வாழ்கின்றோம், ஒரு சொற் கேளீர்!
சேமமுற வேண்டுமெனில் தெருவெல்லாம்
தமிழ்முழக்கம் செழிக்கச் செய்வீர்!''

என்று பாடிய பாரதியார் கம்பர், வள்ளுவர், இளங்கோவடிகள் ஆகியோர் தம் கவிதைகளின் வாயிலாக தமிழைத் தமிழ்மொழியை எவ்வாறு செழிக்கச் செய்துள்ளனர் என்பதனை வெளிப்படுத்துகிறார். தமிழ் மொழியை எல்லா இடங்களிலும் பரப்பவேண்டும் பட்டி தொட்டிகளெல்லாம் தமிழ்மொழியின் சிறப்பை எடுத்துரைக்க வேண்டும் என்கிறார் பாரதியார். அவருடைய கனவு இன்று நனவாகி இருப்பதுதான் தமிழ்மொழிக்குச் 'செம்மொழி' என்ற அரியாசனமாகும்.

''கல்வி சிறந்த தமிழ்நாடு-புகழ்க்
கம்பன் பிறந்த தமிழ்நாடு''
''வள்ளுவன் தன்னை உலகினுக்கே தந்து
வான் புகழ் கொண்ட தமிழ்நாடு-நெஞ்சை
அள்ளும் சிலப்பதி காரமென்றோர்-மணி
யாரம் படைத்த தமிழ்நாடு''

என்று பாடிய பாரதியின் வைரவரிகள் பொய்த்துப் போகவில்லை. இன்று உலகமொழிகள் அனைத்திலும் திருக்குறள் மொழிபெயர்க்க பட்டிருக்கிறது. உலகப்பொதுமறை எனப் போற்றி புகழப்பட்டு வருகிறது. கம்பன் வீட்டுக் கட்டுத்தறியும் கவிபாடுகிறது.

"மனதி லுறுதி வேண்டும்
வாக்கினி லேயினிமை வேண்டும்
நினைவு நல்லது வேண்டும்
நெருங்கின பொருள் கைப்பட வேண்டும்
கனவு மெய்ப்பட வேண்டும்"

என்று பாடிய பாரதியின் கனவு மெய்ப்பிக்கப்பட்டிருக்கிறது என்றால் மிகையாகாது. தூய நெஞ்சமும் நல்ல மன உறுதியும் ஒருவருக்கு அவசியம் என்பதை தம் கவிதைவழி வெளிப்ப டுத்துகிறார்.

"காலை எழுந்தவுடன் படிப்பு-பின்பு
கனிவு கொடுக்கும் நல்ல பாட்டு,
மாலை முழுவதும் விளையாட்டு-என்று
வழக்கப்படுத்திக் கொள்ளு பாப்பா"

"சொல்லில் உயர்வுதமிழ்ச் சொல்லே-அதைத்
தொழுது படித்திடடி பாப்பா!"

"சாதிகள் இல்லையடி பாப்பா குலத்
தாழ்ச்சி உயர்ச்சி சொல்லல் பாவம்"

என்று பாடி குழந்தைகள் படிக்க வேண்டும், விளையாட வேண்டும் என்பதோடு தமிழ்மொழியின் உயர்வு குறித்தும் பாடுகிறார். மேலும், அனைவரும் ஒன்றே என்பதை குழந்தை மனதில் விதைக்கிறார். இந்த விதை வளர்ந்து மரமான பிறகும் மாறக்கூடாது அனைவரும் சமம் என்பதை உணர்த்துகிறார்.

தமிழ்த்தாய்க்குப் பாமாலை பாடி புகழ்மாலை சூட்டியவர் மகாகவி சுப்பிரமணிய பாரதியார். பாட்டுத் திறத்தாலே இந்த வையகத்தைப் பாலித்த பாரதி,

"வாழ்க நிரந்தரம் வாழ்க தமிழ்மொழி
வாழிய வாழியவே !"

என்றும்

"புவியனைத்தும் போற்றிட வான் புகழ்ப்படைத்துத்
தமிழ்மொழியை புகழி லேற்றும்
கவியரசர் தமிழ்நாட்டுக் கில்லையெனும்
வசையென்னாற் கழிந்த தன்றே!"

என்னும் பாடி தமிழ்மொழிக்கும் தமிழ்நாட்டிற்கும் சிறப்பினைப் பெற்றுத் தந்தவர் பாரதியார். புதுக்கவிதையின் முன்னோடியாகத் திகழ்ந்து புதுக் கவிஞர்களுக்கு வழிகாட்டியவர் பாரதியார்.

''உள்ளத்தில் உண்மையொளி யுண்டாயின்
வாக்கினிலே ஒளியுண்டாகும்
வெள்ளத்தின் பெருக்கைப்போல் கலைப்பெருக்கம்
கவிப்பெருக்கம் மேவு மாயின்
பள்ளத்தில் வீழ்ந்திருக்கும் குருடரெல்லாம்
விழிப்பெற்றுப் பதவி கொள்வார்
தெள்ளுற்ற தமிழமுதின் சுவைகண்டார்
இங்கமரர் சிறப்புக் கண்டார்''

என்று பாடி தெள்ளுற்ற தமிழின் சிறப்பினைச் சுவையினை உணர்ந்தவர். உணரச் செய்தவர் பாரதியார்.

தொகுப்புரை

''கல்தோன்றி மண்தோன்றா காலத்தே வாளொடு
முன்தோன்றிய மூத்த குடி'' நம் தமிழ்க்குடி

என்பதற்கேற்ப இரண்டாயிரம் ஆண்டுகள் பழமை வாய்ந்த மொழி நம் தமிழ்மொழி. அத்தகைய தமிழ்மொழியின் சிறப்பினைப் பாரதியார் தம் பாடல்கள் வழி வெளிப்படுத்தியுள்ளார். பாரதி என்ற பெயரில் மட்டுமல்லாமல் இளசை சுப்பிரமணியன், சாவித்திரி, சி.சு.பாரதி, வேதாந்தி, நித்தியதீரர், உத்தம தேசாபிமானி, ஷெல்லிதாசன், காளிதாசன், சக்திதாசன், ரிஷிகுமாரன், காசி, சரஸ்வதி, பிச்சுக்காளிதாசன், செல்லம்மா, கிருஷ்ணன் எனப்பல புனைப்பெயர்களில் இதழ்களின் வாயிலாகத் தமிழை வளர்த்தவர். மகாகவி, மக்கள்கவிஞர், தேசியகவி, விடுதலைக்கவி, முனன்றி புலவன், தமிழ்க்கவி, உலக கவி, தற்கால இலக்கிய விடிவெள்ளி, பைந்தமிழ்த்தேர்ப்பாகன், சிந்துக்குத் தந்தை எனப் பல சிறப்புப் பெயர்களைப் பெற்ற சுப்பிரணிய பாரதியார் "சொல்லின் உயர்வு தமிழ்ச் சொல்லே" என்பதை தம் கவித்திறன் வாயிலாக வெளியிட்டிருப்பதை இக்கட்டுரையின் வாயிலாக அறியமுடிகிறது.

பார்வை நூல்

1. பாரதியார் கவிதைகள், பாவை பப்ளிகேஷன்ஸ், 142, ஜானி ஜான்கான் ரோடு, இராயப்பேட்டை, சென்னை–600 014, ஐந்தாம் பதிப்பு ஆகஸ்ட் 2003.

3. பாரதியின் தேசப்பற்று

முனைவர் கோ.இராமச்சந்திரன்,
உதவிப்பேராசிரியர் / ஆய்வுநெறியாளர்,
தமிழ்த்துறை, தேசியக்கல்லூரி (தன்னாட்சி),
திருச்சிராப்பள்ளி–01.

முகவுரை

பாரதியார் இருண்டு கிடந்த மக்கள் வாழ்வில் தம் எழுத்துகளால் வெளிச்சம் கொடுத்து "**இனி ஒரு விதி செய்வோம்**" என்று பாடிய புதுமை கவிஞன், மக்களின் மனங்களில் நீங்கா இடம் பிடித்த கவிஞன் என்றால் அது பொய்யல்ல, பாரதி காட்டிய வழியில் நாமும் தேசப்பற்று கொண்டு வாழ வேண்டும்.

விடுதலைப் போராட்டத்திற்காக நாட்டு மக்களைத் தட்டி எழுப்புவதற்காகப் பாடப்பட்ட போதிலும் தேசியப் பாடல்களில் பல இன்னும் உணர்ச்சி ஊட்டும் கவிதைகளாக உள்ளன.

> **"பாரத தேசம் என்று பெயர் சொல்லுவார்- பிடிப்**
> **பயம் கொல்லுவார் துயர் பகை வெல்லுவார்"**[1]

என்ற பாடல் இன்றும் பாடக் கேட்டால் நாட்டுணர்ச்சியைத் தூண்டுவதாக உள்ளது இன்று பெரிதும் பேசப்படும் நாட்டு ஒருமைப்பாட்டுக்கு உரிய பல கருத்துக்களை அதில் விதைத்துள்ளார்.

சிந்து நதியின்மேல் நல்ல நிலவின் ஒளியில் கேரள நாட்டு இளம் பெண்களுடன் அழகான தெலுங்குப்பாட்டுகளைப் பாடித் தோணிகள் ஓட்டி விளையாடுவோம் என்கிறார். கங்கை நதிக்கரையில் விளையும் கோதுமைப் பண்டங்களை வாங்குவதற்காகக் காவிரிக் கரையில் விளைந்த வெற்றிலையை விற்போம் என்கிறார். சிங்கம் போன்ற மராட்டிய புலவர்களின் கவிதைகளைப் பெற்றுக் கொண்டு அவற்றிற்கு பரிசாக தமிழ்நாட்டு யானைத் தந்தங்களை அளிப்போம் என்கிறார். காசியில் பேசும் புலவர்களின் பேச்சைக் கேட்பதற்குக் காஞ்சிபுரத்தில் தகுந்த கருவிகளை அமைப்போம் என்ற

அவர்தம் கனவு உண்மையாகிவிட்டது. வங்காளத்தில் கங்கையில் ஓடிவரும் வெள்ளம் மிகுதியாக இருப்பதால் அங்குள்ள ஊர்கள் அழியாதபடி செய்து, அந்த நீரைத் தெற்கே திருப்பி வறட்சியான மத்திய நாடுகளில் பயிர் செய்ய வேண்டும் என்றும் பாடினார்.

தேசப்பற்று

ஒவ்வொரு மனிதனும் பெற்ற தாயைப் பேணிக்காப்பது போன்று தம் தாய் நாட்டையும் அன்புடன் நேசித்துக் காக்க வேண்டும் என்கிறார். பாரதத்திற்கு ஈடான பெருமை மிக்க நாடு வேறு எதுவுமில்லை என்பதை,

"பாரத நாடு பழம்பெரும் நாடே
பாடுவம் இஃதை எமக்கில்லை ஈடே"[2]

என்று உணர்த்துகின்றார்.

நாடு என்றால் அனைவராலும் நேசிப்பதற்கான நன்மைகள் நிறைந்திருக்க வேண்டும். தீராத பசியும் தொலையாத பகையும், தீராத நோயையும் தன்னிடம் சேராது துன்பமின்றி இனிதாய் இருப்பதே நாடாகும்.

"உறுபசியும் ஓவாப்பிணியும் செறுபகையும்"[3]

என்பது திருக்குறள். இக்கருத்தினை பாரதியார் தம் கவிதைகளில் பல இடங்களில் எடுத்துக் காட்டியுள்ளார். ஒவ்வொரு மனிதனுக்கும் பிறந்த நாட்டின் மீது பற்றுதல் இருக்கும் என்றாலும் அந்நிய கலாச்சாரத்தினால் மக்களுக்கு நம் முன்னோர்களின் வீரத்தை உணர்த்துகிறார் பாரதி,

"தீரத்திலே படை வீரத்திலே- நெஞ்சில்
ஈரத்திலே உபகாரத்திலே
காரத்திலே மிகு சாத்திரங் கண்டு
தருவதிலே உயர்நாடு"[4]

என்ற கவிதை உணர்த்துகின்றது. பாரதி தாம் பிறந்த மண்ணின் மீது அளவு கடந்த பற்று கொண்டவர். நம் முன்னோர்கள் மகிழ்ச்சியுடனும், ஒற்றுமையுடனும் வாழ்ந்த பாரத நாட்டை போற்றி வணங்க வேண்டும். முன்னோர்களின் பெருமையை வெளிகாட்ட வேண்டும் எனக் கருதிய பாரதி,

"எந்தையும் தாயும் மகிழ்ந்து குலாவி
இருந்ததும் இந்நாடே- அதன்
முந்தையர் ஆயிரம் ஆண்டுகள் வாழ்ந்து
முடிந்ததும் இந்நாடே"[5]

என்று பாரத நாட்டின் பெருமையை உணர்த்துகிறார். ஒரு முறை பாரதி கடையத்திற்கு வந்திருந்தார் புகைவண்டியை விட்டு கீழே இறங்கியதும் மண்ணில் படுத்துப்புரண்டார். அப்பொழுது அங்கு வேடிக்கை பார்ப்பவர்களைப் பார்த்து என் தாய் நாட்டு மண் அல்லவா? ஆகவே விழுந்து பார்த்தேன் எவ்வளவு சுகமாக இருக்கிறது. தெரியுமா? என்று கேட்டதாக வரலாறு கூறுகிறது6. இதன் மூலம் பாரதி நாட்டை எந்த அளவிற்கு நேசித்தார் என்பதை அறியமுடிகிறது.

சீர்திருத்தம்

பாரதியாரின் புரட்சி மனப்பான்மை - சீர்திருத்த மனப்பான்மை அவருடைய வாழ்வில் விளங்கியது போலவே பாடல்களிலும் தெளிவாகிறது. அரிஜனர் ஒருவரிடம் அந்தக் காலத்திலேயே புதுச்சேரியில் தம் உறவினர்போல் ஆர்வம் கொண்டு அன்பு செலுத்தினார். அவருக்குப் பூனூல் அணிவித்து அன்று முதல் அவரைப் பிராமணர் என்று பாராட்டினார். குழந்தைகளுக்காக எழுதிய பாப்பா பாட்டில் அந்த இளம் மனங்களில் சாதி வேறுபாடு வேரூன்றக் கூடாது என்ற உயர்ந்த நோக்கம் கொண்டவர் அதனை,

"சாதிகள் இல்லையடி பாப்பா- குலத்
தாழ்ச்சி உயர்ச்சி சொல்லல் பாவம்"[6]

என்று கூறுகிறார்.

சமுதாயத்தில் ஒருவரை அவரின் இனம், நிறம், மொழி, சமயம், போன்ற காரணங்களால் வேறுபாடு காட்டுதல் தவறானதாகும். மனித உரிமைகள் பாகுபாடுயின்றி அனைவருக்கும் சமமாக வழங்கப்படல் வேண்டும் என்பதே மேலோர்களின் எண்ணம். ஆயினும் சாதிப்பாகுபாடுகள் இச்சமுதாயத்தில் நிகழ்ந்த வண்ணமே உள்ளன. மனிதர்களிடையே வேறுபாடுகள் களையப்படவேண்டுமென்றால் சாதிப்பாகுபாட்டைவிட்டு சமநிலையான சமுதாயமாக விளங்க வேண்டுமென்பதை,

"ஏழையென்றும் அடிமையென்றும்
எவனுமில்லை சாதியால்
இழிவுகொண்ட மனிதரென்று
இந்தியாவில் இல்லையே"[7]

என்று கூறுகிறார் பாரதி.

சமதர்மம் (சமஉரிமை)

மனித வாழ்வில் எல்லோருக்கும் உணவு, உடை, உறையுள் தேவைப்படும். வயிற்றுக்கு உணவில்லாத போது மனித உயிர்களால் வேறு எத்தகைய உணர்வுகளையும் ஏற்க இயலாது பசி, பட்டினி ஆகியவை மனித சமுதாயத்தின் வீழ்ச்சிக்குக் காரணமாகிவிடும் அதனை உணர்ந்த கவிஞர் ஒவ்வொரு மனிதனுக்கும் உணவு வேண்டும் அவ்வுணவினை அளிக்க இயலாத சமுதாயம் இருந்தும் பயனில்லை. அவை இருப்பதைவிட இல்லாமல் இருப்பதே சிறந்தது என்பதை கவிஞர்,

"தனியொருவனுக்கு உணவில்லையெனில்
ஜெகத்தினை அழித்திடுவோம்"[8]

என்று கூறுகிறார்.

மனித உயிர்க்கு அடிப்படைத் தேவைகளுள் மற்றொன்று ஆடை, இதன் முக்கியத்துவத்தை உணர்த்தவே ஆடையில்லாத மனிதன் அரை மனிதன், ஆள்பாதி ஆடைபாதி என்ற பழமொழிகள் வழங்கி வருகின்றன. ஆடையின் பஞ்சத்தைப் போக்குவதற்காகப் பாரதியார் பட்டினால் ஆடைகளும் பஞ்சினால் உடைகளும் நெய்து வீதியெல்லாம் குவித்துப் போட வேண்டும் அவற்றை மக்கள் மனம் குளிர அணிந்து மகிழ வேண்டும் என்று தம் கவிதையில்,

"பட்டினில் ஆடையும் பஞ்சில் உடையும் பண்ணி
மலைகளென வீதி குவிப்போம்"[9]

என்கிறார் பாரதி.

மனிதனின் அடிப்படை வசதிகளுள் இருப்பிடமும் ஒன்று. இருப்பிட வசதியின்றி வாழும் மக்களைக் கண்டு மனம் குமுறுகின்றார். தம் காலகட்டத்தில் தம்முடைய மன வேதனையை நேரடியாகக் கூறமுடியாமல் மக்கள் வாழ்வதற்குக் காணி நிலமாவது வேண்டுமென பராசக்தியிடம் வேண்டுகிறார்.

"காணிநிலம் வேண்டும் பராசக்தி
காணிநிலம் வேண்டும்"[10]

என்கிறார் பாரதி.

சமுதாயத்தில் மக்கள் அனைவருக்கும் சமஉரிமை இன்றி அடிமைகளாக வாழ்கிறார்கள் என்பதை மக்களுக்கு உணர்த்தி இந்நிலையை மாற்றி புரட்சிகரமான சமுதாயம் வேண்டுமென தம் கவிதை வழியாகப் பாரதியார் வேண்டுகிறார்.

சமுதாயத்தில் செல்வங்கள் யாவும் மக்கள் அனைவருக்கும் பொதுவாக்க வேண்டும். அப்பொழுது தான் ஏற்றத்தாழ்வுகள் இன்றி அனைவரும் சமஉரிமை பெறமுடியும் என்பதை எடுத்துக்காட்டுகிறார்.

"முப்பது கோடி ஜனங்களின் சங்கமம்
முழுமைக்கும் பொதுஉடைமை"[11]

ஒப்பில்லாத சமுதாயம் உலகத்துக்கொரு புதுமை என்று பாரதி கூறுகிறார்.

தீர்வாக

சமுதாயத்தில் நிலவிய கொடுமைகளை மக்களுக்கு எடுத்துக் கூறியவர் பாரதியார். தீர்க்க இயலாத சிக்கல்களுக்குத் தீர்வுகளைக் கூறி மக்களிடையே விழிப்புணர்வை ஏற்படுத்தியவர். மக்களின் உணர்வுகளைத் தன்னுடைய கவிதைகளில் வெளிக்கொண்டு வந்தவர் பாரதி. விடுதலைப் போராட்டத்திற்குத் தன்னுடைய கவிதை வழியாகத் தட்டி எழுப்பியவர். அனைவரும் சமமான உரிமை பெற்று சமுதாயத்தில் உயரவேண்டும் என்ற கருத்தைப் பறைசாற்றியவர் பாரதியார் ஆவார்.

மேற்கோள் நூல்கள்:

1. பாரதியார் கவிதைகள், ப 127
2. பாரதியார் கவிதைகள், ப 128
3. திருக்குறள் – குறள், 734
4. பாரதியார் கவிதைகள், ப 126
5. மேலது, ப – 125
6. மேலது, ப – 242 பாடல்.15
7. மேலது, ப – 56
8. மேலது, ப – 41
9. மேலது, ப – 22
10. மேலது, ப – 112
11. மேலது, ப – 40

4. என்றும் மகாகவி

தி. இராமதாஸ்,
உதவிப்பேராசிரியர், தமிழாய்வுத்துறை,
தேசியக்கல்லூரி (தன்னாட்சி), திருச்சிராப்பள்ளி–01.

ஒரு படைப்பாளன் தான் வாழும் காலத்திற்கேற்றனவற்றைத் தமது படைப்புகளில் திறம்படக் காட்டுவானாயின் அவன் அந்தக் காலத்தால் கொண்டாடப்படக் கூடியவன் என்பதற்கு மாற்றுக் கருத்தில்லை. அவ்வாறே! ஒரு படைப்பாளன் எல்லாக் காலத்திற்கும் ஏற்றதைத் தம் படைப்புகளில் திறம்படக் காட்டியிருப்பானாயின் அவன் எல்லா காலத்திலும் கொண்டாடப்படக் கூடியவனே. மகாகவி பாரதி இதில், இரண்டாம் ரகம்.

பாரதி கவிதைகள் எல்லா காலத்திற்கும் ஏற்றவாறு நின்று மனித குலத்தைக் கரம்பிடித்து வழிநடத்திச் செல்கிறது. எனவேதான் அவன்; அவன் காலத்தில் மட்டுமல்ல நம் காலத்திலும், நம் காலத்தில் மட்டுமல்ல இன்னும் எதிர் வரும் எதிர்காலத்திலும் என எல்லா காலத்திலும் கொண்டாடப்படக் கூடிய ஒரு தீர்க்கதரிசியாகவே திகழ்ந்து வருகிறான்.

அவனை எவ்வளவு புகழ்ந்தாலும் ஏற்புடையதாகவே இருக்கும். 'பாரதி புதிய அறம்பட வந்த மறவன்.... பாரதி மக்கள் கவி. மானுடம் பாட வந்த மாக்கவி. புதுநெறி காட்டிய புலவன். தன்னைப் பின்பற்றித் தமிழ் வளர்க்க ஒரு பரம்பரையைத் தோற்றுவித்த உயர்கவி. எண்ணத்தாலும் எழுத்தாலும் இந்திய சிந்தனைக்கு வளம் சேர்த்தவர்' என்று ச.மெய்யப்பன் புகழ்வதில் எள்ளளவும் பிழையில்லை. காரணம் அவன் கவிதைகள் மனித குலத்தின் மாபெரும் சிக்கல்களை மையப்படுத்தியிருப்பதுடன் அவற்றைக் களைவதற்கு வழிகாட்டவும் செய்கிறது. அவற்றில் சிலவற்றை மட்டும் நாம் இக்கட்டுரையில் காண்போம். அவையாவன;

1. *சாதி, மதம்*
2. *பசி*
3. *கவலை*
4. *பல்லுயிர் நேயம்*

சாதி, மதம்

சாதி, மதம் என்பது இன்று நேற்றல்ல, வர்ணாசிரம தர்மங்கள் வந்த நாள் முதலாகத் தொன்றுதொட்டு வந்து மனித குலத்திற்குத் தொல்லை தரும் ஒன்றாகவே இன்று வரையில் இருந்து வருகிறது. இதனைப் பாரதி தம் கவிதைகளில் பரவலாகவே காட்டுகிறார்.

சாதி குறித்துச் சிந்தனை செய்யும் பாரதி, செய்யும் தொழில்களில் இருந்து வர்ணங்களைக் கொணர்ந்தது போன்று வணங்கும் சாமியில் இருந்து இவர்கள் சாதியைக் கொண்டு வந்திருப்பார்கள் என்று எண்ணுகிறார். எனவே இங்குச் சாமியின் பெயரால் சாதி வளர்வதைத் தடுக்க எண்ணும் அவர்; அறிவொன்றே தெய்வம் என்று தம் கவிதையில் மனித குலம் நோக்கி அறைகூவல் விடுக்கின்றார். அதனை,

"ஆயிரந் தெய்வங்க ளுண்டென்று தேடி
அலையு மறிவிலிகான் - பல்
லாயிரம் வேத மறிவொன்றே தெய்வமுண்
டாவிஅமனல் கேளீரே?
மாடனைக் காடனை வேடனைப் போற்றி
மயங்கும் மதியிலிகாள் - எத
னூடுநின் றோங்கு மறிவொன்றே தெய்வமென்
றோதி யறியீரோ?"

என்ற அவர் கவிதை வரிகளால் உணர முடிகிறது. சாதிப் பிரிவுகள் பேசுகிறவர்கள் எப்படிப்பட்டவர்கள் என்பதை,

"சாதிப் பிரிவுகள் சொல்லி-அதில்
தாழ்வொன்றும் மேலென்றும் கொள்வார்
நீதிப்பிரிவுகள் செய்வார்"

என்று அடையாளம் காட்டுகின்றார். சாதியக் கொடுமைகளால் மனம் நொந்த பாரதி தாம் வேண்டும் விடுதலையில்,

"பறைய ருக்கு மிங்கு தீயர்
புலைய ருக்கும் விடுதலை
பரவரோடு குறவ ருக்கு
மறவ ருக்கும் விடுதலை"

என்று பாடுகின்றான். இவ்வாறாய் சாதிகள் குறித்தும் அவற்றின் நிலை குறித்தும் தம் கவிதைகளில் காட்டும் பாரதி இறுதியில் சாதி என்னும் கொடுமைகளே வேண்டாம் என்பதை,

"சாதிக் கொடுமைகள் வேண்டாம் - அன்பு
தன்னிற் செழித்திடும் வையம்"

இந்த உலகம் செழிக்க சாதி வேண்டியதில்லை. அன்பே வேண்டுவது என்பதை வலியுறுத்துகிறார். இவ்வாறாகச் சாதிகள் குறித்துக் கூறும் பாரதிமதம் குறித்துக்கூற வருகையில்,

"மெள்ளப் பலதெய்வம் கூட்டி வளர்த்து
வெறுங்க தைகள்சேர்த்துப் - பல
கள்ள மதங்கள் பரப்புதற் கோர்முறை
காட்டவும் வல்லீரோ?"

என்று கூறுகின்றார். இதில் சாதியின் அடையாளம் தெய்வம் என்றதைப்போல தெய்வங்களின் கூட்டு மதம் (சாதிகளின் கூட்டும்) என்று அவர் காட்டுவதைக் காண முடிகிறது. மேலும் மதம் என்பது வெறுங்கதைகளின் பிணைப்பு என்பதுடன் அது மதம் பரப்புதற்கான ஒரு முறை என்றும் அவர் காட்டுகின்றார். இக்கவிதை அடிகளில் காணும் 'காட்டவும்' என்னும் இழிவும்மையின் வழி அவருக்கு மதங்கள் புனையும் கதைகளின் மேல் எத்தகைய வெறுப்பு தமக்கு உண்டு என்பதைக் காட்டிவிடுகிறார்.

பசி

தம் பிள்ளைகளின் பசித்துயர் கண்ட பாரதி பராசக்தியிடம் 'இந்த உலகத்தை உய்விக்க வந்த என்னை வெறும் உப்புக்கும் புளிக்கும் பாட வைத்துவிடுவாய்ப் போலிருக்கிறது. மாகாளி, பராசக்தி நான் உன்னை எச்சரிக்கிறேன். நான் நாத்திகனாய் மாறிவிடுவேன் ஜாக்கிரதை' என்று தன் கட்டுரைப் படைப்பில் காளியிடம் எச்சரித்ததும் உண்டு.

இவை பொதுவாக மனித குலத்தில் நிகழும் நிகழ்வுகளில் ஒன்று. ஆனால் அவர் பிறர் பசி குறித்து எண்ணுகையில் 'நெஞ்சு பொறுக்குதில்லையே' என்கிறார். அதனுடன்,

"கஞ்சி குடிப்பதற்கிலார்-அதன்
காரணங்கள் இவையென்னும் அறிவுமிலார்
பஞ்சமோ பஞ்சமென்றே-நிதம்
பரிதவித் தேஉயிர் துடிதுடித்து
துஞ்சி மடிகின்றாரே"

என்றும் கூறுகின்றார். இதில் 'துஞ்சி மடிகின்றாரே' எனப் பாடுகையில் பாரதி 'துஞ்சி... என்றே அவன் உச்சரித்திருப்பானோ என்று எண்ணத் தோன்றுவதுடன் கவிதையின் இறுதி அடிகளான

"இவர்
துயர்களைத் தீர்க்கவோர் வழியிலையே"

என்ற வரிகளை எழுதும் முன் பாரதி எழுதுவதை நிறுத்திவிட்டு எழுந்து நின்று கைகளைப் பிசைந்தபடி அவன் அங்குமிங்கும் அலைந்திருப்பானோ என்று எண்ணத் தோன்றுகிறது. இதனையடுத்து பசி குறித்துக் கூறும் அவன் கவிதையான,

"இனியொரு விதி செய்வோம் - அதை
எந்த நாளும் காப்போம்
தனியொ ருவனுக் குணவிலை யெனில்
ஜகத்தினை யழித்திடுவோம்"

என்னும் கவிதையில் பசியை அவன் எவ்வாறு பார்க்கிறேன் என்பதை உணர முடிகிறது.

கவலை

மனித குலத்தை மயங்குறச் செய்து மதியிழக்கச் செய்யும் உணர்வுகளுள் ஒன்று கவலை. இந்தக் கவலையால் ஆவது ஒன்றுமில்லை. ஆயினும் மனிதகுலம் பெரும்பாலும் கவலையை விரும்பிச் சுமக்கும் பொதியாகவே இன்று வரைச் சுமக்கின்றனர். இது குறித்துக் கூற வரும் பாரதி,

"சென்றதினி மீளாது மூடரே நீர்
எப்போதுஞ் சென்றதையே சிந்தை செய்து
கொன்றழிக்குங் கவலையெனும் குழியில் வீழ்ந்து
குமையாதீர்! சென்றதனைக் குறித்தல் வேண்டா"

என்று கூறுவதுடன் மட்டும் விட்டுவிடவில்லை. பின்னர்

"இன்றுபுதி தாய்ப் பிறந்தோ மென்றுநீவிர்
எண்ணமதைத் திண்ணிமுற இசைத்துக் கொண்டு
தின்றுவிளை யாடியின்புற் றிருந்து வாழ்வீர்
தீமையெல்லாம் அழிந்துபோம், திரும்பிவாரா"

என்றும் கூறுகிறார். மேலும் கவலைத் துறந்து வாழ்வதே சொர்க்கம் என்பதை உணர்த்த வரும் அவர்,

"கவலை துறந்திங்கு வாழ்வது வீடன்று
காட்டு மறைகளெல்லாம் - நீவிர்
அவலை நினைந்துமி மெல்லுதல் போலிங்
கவங்கள் புரிவீரோ?"

என்று காட்டுவதுடன் கவலையால் துயர் உறும் மனிதரின் மேல் ஒரு சலிப்புத் தன்மையும் கொள்ளுகின்றார்.

பல்லுயிர் நேயம்

பாரதி கழுதையைக் கட்டி அணைத்தான் என்பதிலும் மதம் கொண்ட யானையிடத்தும் உறவாடிச் சென்றான் என்பதிலும் தன்வீடு பட்டினி கிடக்க தன் மனைவி வீட்டில் வைத்திருந்த அரிசியை இரைத்து காக்கைக் குருவிக்குப் பசி போக்கினான் என்பதிலும் அவன் மனிதனைக் கடந்து மற்ற உயிர்களிடத்து எத்தகைய அன்பு கொண்டவனாய் இருந்திருக்கக் கூடும் என்பதை உணர முடியும். ஆனால் இவற்றையெல்லாம் காட்டிலும்,

''பேசாப் பொருளை பேசநான் துணிந்தேன்
கேட்கா வரத்தைக் கேட்கநான் துணிந்தேன்
மண்மீ துள்ள மக்கள், பறவைகள்,
விலங்குகள், பூச்சிகள், புற்பூண்டு, மரங்கள்
யாவுமென் வினையா லிடும்பைத் தீர்ந்தே
இன்புற் றன்புட னிணங்கிவாழ்ந் திடவே
செய்தல் வேண்டும். தேவ தேவா
ஞானா காசத்து நின்றுநான்
பூமண் டலத்தில் அன்பும் பொறையும்
விளங்குக, துன்பமு மிடிமையு நோவுஞ்
சாவு நீங்கிச் சார்ந்தபல் லுயிரெலாம்
இன்புற்று வாழ்க' என்பேன்! இதனை நீ
அங்ஙனே யாகுக என்பாய், ஐயனே!''

என்னும் இக்கவிதை அடிகள் பாரதியின் பல்லுயிர் நேயத்திற்கு ஒரு மிகச் சிறந்த சான்றாக இருப்பதை உணர முடிகிறது.

இவ்வாறாய்த் தன்னுயிர் அன்றி பிற உயிர்களை நேசிப்பதாலும் தன் துயர் அன்றி பிறர் துயருக்கு வருந்துவதுடன் அதைக் களைய முற்படுதாலும் எல்லா காலத்திற்கும் ஏற்புடையச் செய்திகளை அவர் கவிதைகளின் அதிகப்படியான கருப்பொருளாய் கொண்டிருப்பதாலும் '**பாரதி**' எனக்கு என்றும் மகாகவி என்றே படுகின்றார்.

■

5. பாரதியின் பன்முகப்பார்வை

முனைவர் ஆ. இராஜலெட்சுமி
உதவிப்பேராசிரியர், தமிழ்த்துறை,
தேசியக் கல்லூரி (தன்னாட்சி), திருச்சிராப்பள்ளி–620 001.

முன்னுரை

சமுதாயத்தில் இருந்து முகிழ்ப்பது இலக்கியம். சமுதாயத்தைப் பற்றியதாகத் திகழ்வது அதன் இயல்பு. அப்படி சமுதாயம் பயன்பெறவேண்டும் என்று எண்ணியவர் மகாகவி பாரதியார். பாரதி எழுத்துலகில் நுழைந்த காலகட்டத்தில் இந்தியா அடிமையுற்ற நாடாய் இருந்தது. நாட்டின் விடுதலைக் குறித்தும், நாட்டு மக்களின் முன்னேற்றம் குறித்தும் இவரது படைப்புகள் அமைந்தன. பழமையின் பண்பாட்டில் காலூன்றி நின்று கொண்டு புதுமைக்கு வித்திட்டவர் பாரதி. சாதி, சமயபேதம் பாராட்டாதவர். பேதம் பாராட்டுகின்ற நூல்களையெல்லாம் குப்பைகள் என்று புறக்கணித்தவர் வெறுத்தவர்.

"உய்வகைக் காட்டும் உயர்தமிழுக்கும்
புதுநெறி காட்டிய புலவன் பாரதி"

என்று பாட்டுத் திறத்தினால் இந்த உலகத்தைப் பாலித்திட வேண்டும் என்று கனவு கண்டவர்.

பாரதி எனும் பதம்

அறிவுத் தெய்வமாக விளங்கும் கலைமகளின் பல்வேறு பெயர்களுள் ஒன்று பாரதி.

"கலைமகள் பனுவலாட்டி, காயத்ரி ஞானமூர்த்தி
உலகமாதா பிராமி ஒள்ளிய வெள்ளை மெய்யாள்
இலகுவெண் கலசமுற்றாள் பாரதி இசைம டந்தை
மலரயன் மனைவி வாக்காள் வாணி நாமகள் பேராமே"

என்று பாரதியின் பெயர்களைச் சூடாமணி நிகண்டு பகர்கின்றது. இதனை மெய்ப்பிக்கும் வண்ணம் கம்பதேவனும்,

"பாதாம் புயத்திற் பணிவார் தமக்குப் பலகலையும்
வேதாந்த முத்தியுந் தந்தருள் பாரதி"

என்று கலைமடந்தையை 'பாரதி' என்று சிறப்பித்துக் கூறுவதைக் காணமுடிகிறது. அந்தப் 'பாரதியின்' தவப்புதல்வன் தான் இந்த பாரதி. 'பாரதி' என்ற சொல்லைப் பற்றி அறியாதார் உண்டோ? ஒவ்வொரு தமிழன் உள்ளத்திலும் ஒலிக்கும் ஒண்டமிழ்ப் புலவன் அவன். அறியாமை இருளைப் போக்கிய கவிஞர். பார் முழுவதும் அறிந்த மக்கள் கவிஞன்.

ஒழுக்கநெறி

'பிறப்பொக்கும் எல்லா உயிர்க்கும்' என்கிறார் வள்ளுவர். உயர்ந்த குடியில் பிறந்ததன் காரணமாக அவர்கள் உயர்ந்த குடியினராக வாழமுடியாது. தாழ்ந்த குடியில் பிறந்ததால் அவர்கள் தாழ்ந்த குடியினராகவே வாழமாட்டார்கள். உயர்வும் தாழ்வும் அவர்களது செயலில் ஒழுக்கத்தில் உள்ளது. உயர்ந்த குடியில் பிறந்தவர்களாக இருந்தும் அவர்கள் ஒழுக்கம் தவறினால் தாழ்ந்த குடியாகிவிடுகின்றனர். (குலத்தில்) தாழ்ந்த குடியில் பிறந்திருந்தும் நல்ல ஒழுக்கங்களைப் பின்பற்றினால் அவர்கள் உயர்ந்த குடியைச் சேர்ந்தவர்களேயாவர். உயர்வுக்கும் தாழ்வுக்கும் ஒழுக்கமே அடிப்படை காரணமாகிறது. இதனை,

"சீலம் அறிவு தரும்.. இன்று
சிறந்தவர் குலத்தினால் சிறந்தராக
மேலவர் கீழவர் என்றே வெறும்
வேடத்திற் பிறப்பினில் விதிப்பனவாம்
போலிச்சுவடியை யெல்லாம் - இன்று
பொசுக்கி விட்டால் எவர்க்கும்
நன்மை யுண்டென்பான் "

என்று பாடுகிறார்.

பொதுவுடைமைச் சமூகம்

ஆண்டான் அடிமை, ஏழை பணக்காரன் என்கிற பேதத்தை எதிர்க்கிறார். ஏழையைச் சுரண்டி பிழைக்கும் வர்க்கத்தையும் கண்டிக்கிறார். ஏழைக் கோழைகளாகவும் அடிமைகளாகவும் இருப்பது கண்டு குமுறுகிறார். உழுதுண்டு வாழ்ந்தால் எல்லாம் சிறக்கும். பிறரைச் சுரண்டி வாழவேண்டாம் என்பதனை,

"வயிற்றுக்குச் சோறுண்டு கண்டீர் - இங்கு
வாழும் மனிதர் எல்லோருக்கும்

பயிற்றி உழுதுண்டு வாழ்வீர் - பிறர்
பங்கைத் திருடுதல் வேண்டாம்''

என்று உழைப்பினைச் சுரண்டும் முதலாளிகளை எச்சரிக்கிறார்.

பெண்ணியநிலை

பண்டைக் காலத்தில் பெண்கள் மதிக்கப்பட்டனர். இடைக்காலத்தில் பெண்களை இழிகுலத்தோராக எண்ணிக் கொடுமைப்படுத்தும் வழக்கம் தோன்றியது. இந்தக் கொடுமையைக் கண்டு பாரதி நெஞ்சங் குமுறினார்.

''பெண்ணுக்கு ஞானத்தை வைத்தான் - புவி
பேணி வளர்த்திடும் ஈசன்
மண்ணுக்குள்ளே சில மூடர் - நல்ல
மாதர் அறிவைக் கெடுத்தார்''

என்று பெண்ணினத்தின் விடுதலை வேண்டியும் பாடினான்.

மூடநம்பிக்கைக்கு எதிர்ப்பு

நம்நாட்டில் எதில் குறைவிருந்தபோதிலும் மூடநம்பிக்கைகளில் என்றும் குறை இருந்ததில்லை. சூன்யம், பேய், மதத்தின் மீது கொண்ட மூடநம்பிக்கைகளைக் கண்டித்த கவிஞர் கடவுளின் பேரைச் சொல்லி செய்யும் மூடப்பழக்க வழக்கங்களைக் கண்டிப்பதோடு அவ்வாறு செய்வது தெய்வத்துக்குப் பழி என்று இடித்துரைத்தார்.

''வஞ்சனைப் பேய்கள் என்பார் - இந்த
மந்திரவாதி யென்பார் - சொன்ன
மாத்திரத்திலே மனக்கிலி பிடிப்பார்
யந்திர சூனியர்கள் இன்னும்
எத்தனை ஆயிரம் இவர் துயர்கள்!

என்று மூடநம்பிக்கைக் கொண்டோரை இகழ்ந்துரைக்கிறான். மேலும், சோதிடம் தனை இகழ்' என்றும் பாடுகிறார் அந்தக் கவியரசன்.

உழைப்பே உயர்வு

உழைப்பாளி மக்களின் உரிமை முழக்கத்தை சுதந்திரக் கொடியில் பொறிக்க வேண்டுமென்றார் பாரதி. உழைக்கும் மக்களைப் படைப்புக் கடவுளாகப் போற்றியவர். தொழிலாளி களையும், உழவர்களையும் மற்ற உழைப்பாளிகளையும் பார்த்து.

நீங்கள்தான் கண்கண்ட தெய்வம் என்கிறார் கவிச்சக்கரவர்த்தி. இதனை,

"உழவுக்கும் தொழிலுக்கும் வந்தனை செய்வோம்-
வீணில்
உண்டு களித்திருப்போரை நிந்தனை செய்வோம்"

என்று பாடி உழைக்காதவர்களை இழிவாகவும் விமர்சிக்கிறார்.

பெரிதினும் பெரிது

வெற்று வார்த்தைகளைப் பேசாமலும், வேதாந்தம் பேசாமலும் வாழ்ந்த கவி பாரதி. பராசக்தியிடம் பெரியதொரு வேண்டுதலையும் வைக்கிறார்.

"காணி நிலம் வேண்டும் - பராசக்தி
காணி நிலம் வேண்டும் - அங்கு
தூணில் அழகியதாய் நன்மாடங்கள்
துய்ய நிறத்தினதாய் - அந்தக்
காணி நிலத்திடையே-ஓர் மாளிகை
கட்டித் தரவேணும்."

என்று ஒரு பெரிய பட்டியலை நமக்குத் தயாரித்துத் தருகிறார் அந்த ஒப்பில்லாக் கவிஞன். தனக்கு எது வேண்டும் என்பதில் தெளிவு, கடவுளிடமும் சமரசம் செய்து கொள்பவரில்லை பாரதி இதை,

"எனக்கு வேண்டும் வரங்களை
யிசைப்பேன் கேளாய் கணபதி"

என்றும் பாடுகிறார்.

தேசியம்

மொழிப்பற்றுள்ளவர்கள் தேசியத்தை எதிர்த்தார்கள். தேசப்பற்றுக் கொண்டவர்கள் மொழியினை ஆதரிக்கவில்லை. ஆனால் நம் கவியோ, மொழியையும் தேசத்தையும் ஒருங்கே நேசித்தவர். இதனை அவரது இவ்வரிகள் மெய்ப்பிக்கும். நாடு குறித்தும், மொழிக்குறித்தும் பேசுகையில்,

"செந்தமிழ் நாடெனும் போதினிலே - இன்பத்
தேன்வந்து பாயுது காதினிலே"

என்றும்,

''பாருக்குள்ளே நல்ல நாடு-எங்கள்
பாரத நாடு''

என்றும் பாடியிருக்கிறார்.

பாரத நாட்டை பெற்றெடுத்த அன்னையின் வடிவமாகவும் காண்கிறார்.

''முப்பதுகோடி முகமுடையாள்
உயர் மொய்ம்புற ஒன்றுடையாள்''
''செப்புமொழி பதினெட்டுடையாள் - எனில்
சிந்தனை ஒன்றுடையாள்''

என்று பாரதநாட்டைப் புகழ்ந்துரைக்கிறார் பாரதி.

முடிவுரை

பாரதியின் பார்வையில் நாடும் நாட்டு மக்களும் பயனுறவேண்டும் என்பதனைத் தெளிவாக இந்தப்பாடல்களில் காணமுடிகிறது. பாரதி காண விரும்பிய சமூகம் அடிமைகளற்ற சமூகம், பொதுவுடைமை சமூகம் ஆகும். பெண்களின் முன்னேற்றத்திற்கு வித்திட்டவர். உழைக்கும் வர்க்கத்தைப் போற்றியும் பாடுகிறார். படிப்பவரை வீறுகொள்ளச் செய்யும்படி பாடலை இயல்பாகவும் கருத்தோட்டத்துடன் இவர் அமைத்துள்ளது மிகவும் சிறப்புடையதாகும். பாரதியின் பாடல்கள் வீரரசம் கலந்த எழுச்சியூட்டும் பாடல்களாக அமைந்துள்ளன.

பார்வை நூல்கள்

1. பாரதியார் கவிதைகள் – சி. சுப்பிரமணியபாரதி, உமா பதிப்பகம், 58, ஐயப்ப செட்டித்தெரு, மண்ணடி, சென்னை –1
2. பல்வேறு கோணங்களில் பாரதி – சீனி. விசுவநாதன், 2, மாடல் ஹவுஸ், சி.ஐ.டி. நகர், சென்னை–5
3. பாரதியின் தமிழியம்– முனைவர் சொ. ஏழுமலய், புதுச்சேரிக் கூட்டுறவுப் புத்தகச் சங்கம், புதுச்சேரி
4. பாரதியின் பரிமாணங்கள்– எம்.ஏ. பழனியப்பன், அறிவுப் பதிப்பகம், 142, ஜானி ஜான் கான் ரோடு, ராயப்பேட்டை, சென்னை–14.

■

6. பேராசிரியர் வே. சாரநாதன் பார்வையில் மகாகவி பாரதியார்

முனைவர் இரா. இராஜா,
உதவிப்பேராசிரியர், தமிழாய்வுத்துறை,
தேசியக்கல்லூரி (தன்னாட்சி),
திருச்சிராப்பள்ளி–1

புவிஅனைத்தும் போற்றிடவான் புகழ்படைத்துத்
தமிழ்மொழியைப் புகழில்ஏற்றும்
கவியரசர் தமிழ்நாட்டுக்கு இல்லைஎனும்
வசைஎன்னால் கழிந்த தன்றே? - **பாரதியார்**

நவீனத்தமிழ் முன்னோடிகளுள் தலைசிறந்த ஆளுமை சி. சுப்பிரமணிய பாரதியார் (1882-1921). பன்மொழி அறிஞர், கவிஞர், நாட்டுப்பற்றாளர், அரசியல் விமர்சகர், பெண்ணியச் சிந்தனையாளர், ஆன்மிகவியலாளர், கல்வியாளர், உரையாசிரியர், உரைநடையாசிரியர், கதையாசிரியர், இதழியலாளர், ஓவியர், மொழிபெயர்ப்பாளர், கலை இலக்கியத் திறனாய்வாளர் போன்ற பன்முகத் திறன்களைப் பெற்றவராகப் பாரதியார் விளங்கியுள்ளார். இருந்தும் அவரின் நவீன அறிவினை அன்றைய தமிழ்ச் சமூகம் முழுமையாக உள்வாங்கவில்லை. இருபதாம் நூற்றாண்டின் உலகப் பெருங்கவிஞர்கள் வரிசையில் இடம்பெறத்தக்கத் தகுதிகள் பல பாரதியாருக்கு உண்டு. ஆனாலும், கால் நூற்றாண்டுக்கும் மேலாகத் தமிழக வரலாற்றில் அவர் குறித்துப் பல சர்ச்சைகள் எழுப்பப்பட்டன. பாரதியார் வாழ்ந்த காலத்திலும், வாழ்க்கைக்குப் பின்னரும் அவரின் எழுத்துகள் பெருமளவு விமர்சிக்கப் பட்டுள்ளன. ஆங்கிலேயர் ஆட்சியில் சிலகாலம் அவை தடைசெய்யப்பட்டன. நம்மவர்கள் ஆட்சியில் சில காலம் அவை அலட்சியம் செய்யப்பட்டன. அவற்றையெல்லாம் புறங்கண்டு அவரின் ஈடில்லா எழுத்துகளே அவரை மகாகவி என்று அடையாளப்படுத்திக் கொண்டன. பாரதி குறித்த திரிபு வாதங்களை ஒழித்து அவருக்குரிய சமூக மதிப்பினை

உண்டாக்கப் பல்வேறு அறிஞர்கள் துணை நின்றுள்ளனர். இறுதியில் மாற்றுச் சிந்தையரும் பாரதியின் புலமையை ஒப்புக்கொண்டனர்.

ஒருவகையில் தமிழ்ச் சமூகத்தில் தம் அன்பர்களாலும் வம்பர்களாலும் பாரதியார் தொடர்ந்து வாசிக்கப்பட்டுக் கொண்டே இருந்துள்ளார். 1982 ஆம் ஆண்டு பாரதியார் பிறப்பு நூற்றாண்டைக் கொண்டாடியபோது அவரின் எழுத்துகளை மொத்தமாக, காலவரிசை அடிப்படையில் தொகுக்க வேண்டிய தேவையை முன்மொழிந்து சான்றோர்கள் அதனை நிறைவேற்றினர். இப்பொழுது பாரதியாரின் நினைவு நூற்றாண்டைப் போற்றிக் கொண்டாடுகின்றனர். இவ்வேளையில் அவரின் படைப்புகள் தொடர்ந்து வாசிக்கப்பட்டு (விமர்சிக்கப்பட்டு) வரும் வரலாற்றைத் தொகுத்து அறிய வேண்டியுள்ளது. அதன் ஒரு பகுதியாக, பாரதியார் குறித்த ஆங்கிலப் பேராசிரியரும் கவிஞரும் கல்வியாளருமான வே. சாரநாதனின் (1892–1948) வாசிப்பை இவ்வெழுத்துரை அடையாளப் படுத்துகிறது.

பேராசிரியர் வே. சாரநாதன்

கும்பகோணம் அருகில் அமைந்த திருச்சேறை என்னும் சிற்றூரில் வேங்கடசேஷாத்திரிநாத ஆச்சாரியார்–இராகவா இணையருக்கு 06-01-1892 இல் இளைய மகனாக சாரநாதன் பிறந்தார். தம் இரண்டாம் அகவையிலேயே தந்தையை இழந்த இவருக்கு இவரின் அண்ணன் ஸ்ரீ ஐயனாச்சாரியார் (எ) நம்பி ஆதரவாக இருந்தார். சில ஆண்டுகளிலே அவரும் மறைய, தம் இளமைக்காலத்தை மிகுந்த துயரங்களுடன் சாரநாதன் கடந்து வந்துள்ளார். இவர் ஆரம்பப் பள்ளியைக் கும்பகோணத்திலும், நடுநிலைப் பள்ளியை வீரவநல்லூரிலும், உயர்நிலைப் பள்ளியை அம்பாசமுத்திரத்திலும் (1906) முடித்துள்ளார். இவர் பயின்ற பள்ளிகளில் எல்லாம் இவரின் திறனைக் கண்டு மெச்சிய ஸ்ரீ பார்த்தசாரதி ஐயங்கார், ஸ்ரீ டி.எஸ். சதாசிவ ஐயர் போன்ற ஆசிரியர்கள் இவரைக் கைத்தூக்கி விட்டுள்ளனர். புதுமுக வகுப்பைத் திருநெல்வேலி இந்துக் கல்லூரியில் (1909) முடித்து, அங்கேயே பி.ஏ. பட்டப் படிப்பைப் பயிலத் தொடங்கித் தம் உடல்நிலையின் பொருட்டு, சென்னை மாநிலக் கல்லூரியில் பி.ஏ. பட்டத்தினை (1912) நிறைவு செய்தார். தொடர்ந்து திருச்சிராப்பள்ளி புனித வளனார் கல்லூரியில் ஆசிரியராகப் (Tutor) பணிபுரிந்து கொண்டே அங்கு எம்.ஏ. பட்ட மேற்படிப்பை முடித்தார் (1914).

முழுக்கல்வித் தகுதியுடன் திருநெல்வேலி இந்துக் கல்லூரியில் பேராசிரியராகப் பணியில் சேர்ந்தார். கே. ஏ. நீலகண்ட சாஸ்திரி உள்ளிட்ட தகுதி மிகுதி படைத்த தம் சக பேராசிரிய நண்பர்கள் ஐவருடன் சேர்ந்து அன்று புனேயில் சிறப்பாகச் செயல்பட்ட பெர்குஸ்சன் கல்லூரிக்கு இணையாக திருநெல்வேலி இந்துக் கல்லூரியை வளர்க்க முன்முயற்சி எடுத்தார். சில காரணங்களால் 1918 இல் அங்கிருந்து பணி விலகிப் பச்சையப்பன் கல்லூரியில் ஓராண்டு பேராசிரியராகப் பணி செய்தார். பின்னர் 1919 ஆம் ஆண்டு திருச்சியில் தேசியக்கல்லூரி தொடங்கப்பட்டபோது அங்கு ஆங்கிலத்துறைத் தலைவராகப் பணியமர்ந்தார். இரண்டே ஆண்டுகளில் 1921 இல் தம் 29 ஆம் அகவையில் அக்கல்லூரியின் முதல்வர் பொறுப்பை ஏற்றார். 26 ஆண்டுகள் அக்கல்லூரியின் முதல்வராக இருந்து அதன் வளர்ச்சிக்கு அரும்பாடு பட்டார். உயர்கல்வி குறித்த தம் கனவினை நனவாக்கும் களமாகத் தேசியக்கல்லூரியை உருவாக்கினார். 02-01-1947 இல் பணி நிறைவு பெற்றார்.

ஆங்கிலப் பேராசிரியரான வே. சாரநாதன் சிறந்த கவிஞராகவும் திறனாய்வாளராகவும் செயல்பட்டார். அகில இந்திய வானொலியில் ஆங்கிலத்திலும் தமிழிலும் பல உரைகள் ஆற்றியுள்ளார். காவேரி, கிராம ஊழியன் போன்ற இதழ்களில் கட்டுரைகள் எழுதியுள்ளார். இவற்றினூடே இவரின் ஆங்கிலம் மற்றும் தமிழ் இலக்கியம் குறித்த புலமை வெளிப்படுகின்றது. பணி நிறைவுக்குப் பிறகும் எழுத்துப் பணியைத் தொடர்ந்து செய்துள்ளார். 1948 மே மாதம் ஓய்வெடுப்பதற்காகப் பெங்களூ சென்ற இவர் அங்கு உடல்நலக் குறைவு ஏற்பட்டு 02-06-1948 இல் இயற்கை எய்தினார்.

எஸ். தோத்தாதிரி ஐயங்கார், எம்.எஸ். வேங்கடராமன், ஜி. வரதாச்சாரியார், பி.எஸ். ராமசுவாமி, எஸ். சீதாராமய்யர் ஆகியோரைப் பதிப்பாசிரியராகக் கொண்டு 1949 இல் வே. சாரநாதனின் நினைவு மலர் வெளியிடப்பட்டது. 2010 ஆம் ஆண்டு எஸ். ரவீந்திரன், வீ. நடராஜன் ஆகியோரைப் பதிப்பாசிரியராகக் கொண்டு சாரநாதன் பொறியியல் கல்லூரி அம்மலரை மறு வெளியீடு செய்துள்ளது.

வே. சாரநாதனின் பாரதி வாசிப்பு

வே. சாரநாதனின் நினைவு மலர் இரண்டு பகுதிகளைக் கொண்டது. அதன் முதற்பகுதியில் சாரநாதன் பற்றிய இரங்கல் குறிப்புகள் இடம்பெற்றுள்ளன. இரண்டாம் பகுதியில்

சாரநாதனின் ஆங்கிலம் மற்றும் தமிழ்ப் படைப்புகள் தொகுத்தளிக்கப்பட்டுள்ளன. அவற்றுள் மகாகவி பாரதியார் பற்றிய குறிப்புகள் விரவிக் காணப்பெறுகின்றன. அவற்றைப் பின்வருமாறு வகைப்படுத்திக் கொள்ளலாம்.

1. பாரதியைப் பற்றிய நேரடியான மதிப்புரைகள்

2. பிற கட்டுரைகளில் எடுத்தாளப்படும் பாரதி மேற்கோள்கள்

3. கட்டுரைக்குத் தலைப்பாகப் பாரதியின் கவிதை வரியைச் சூட்டுதல்

பாரதியைப் பற்றிய நேரடியான மதிப்புரைகள்

சுப்பிரமணிய பாரதி, கவி வாக்கு என்னும் இரண்டு கட்டுரைகளைப் பாரதியார் குறித்துச் சாரநாதன் எழுதியுள்ளார். அவற்றின் ஆண்டு பற்றிய குறிப்பினை அறிய முடியவில்லை. சுப்பிரமணிய பாரதி என்னும் கட்டுரை பாரதியாரைப் பற்றிய ஓர் அறிமுகமாக அமைகிறது. அதில் "பாரதியின் திருநாள் இன்று தமிழ் நாடெங்கும் கொண்டாடப்படுகிறது" என்னும் குறிப்பு இடம்பெற்றுள்ளது. அதனைக் கொண்டு பார்க்கும்போது இக்கட்டுரை பாரதி மணிமண்டபம் திறக்கப்பட்ட 11-10-1947 காலத்தை ஒட்டியதாக அமையலாம். இக்கட்டுரையில் தமிழின் நவீனப் போக்குகளின் தோற்றுவாயைச் சாரநாதன் சுட்டுகிறார். மயூரம் வேதநாயகம்பிள்ளை, மகாவித்துவான் மீனாட்சி சுந்தரம்பிள்ளை, இராமலிங்க அடிகளார் போன்றோர் 19 ஆம் நூற்றண்டில் தமிழின் முக்கிய ஆளுமைகளாகச் செயல்பட்டுள்ளதை எடுத்துக் காட்டியுள்ளார். அந்த வரிசையில் பாரதியின் வீச்சு அளப்பரியதாக உள்ளதைச் சுட்டிக்காட்டுகிறார். சாரநாதன் எடுத்தாண்டுள்ள பாரதியார் கவிதைகள் பற்பல. அவற்றின் இடங்களை அவர் சுட்டவில்லை. அவை பாரதியின் எந்தப் படைப்புகளிலிருந்து எடுக்கப்பட்டுள்ளன என்பதை இக்கட்டுரையாசிரியர் அடையாளப்படுத்தியுள்ளார். சாரநாதனின் பாரதி வாசிப்பை அடைவு செய்து பார்க்கும்போது வியப்பு மேலிடுகிறது. ஆங்கிலப் பேராசிரியராக இருந்தும் பாரதி கவிதைகளில் அளவில்லாத பற்றுடையவராக விளங்கியிருப்பதை அவர்தம் மேற்கோள்கள் காட்டுகின்றன.

1. பாரதியார் தமிழ்த்தாய் வாழ்த்து
2. மனதில் உறுதி வேண்டும்
3. வேதாந்த பாடல்கள்
4. செந்தமிழ்நாடு

5. காணி நிலம் வேண்டும்
6. கண்ணன் பாட்டு
7. பாஞ்சாலி சபதம்
8. தெய்வீகப் பாடல்கள் – ஊழிக்கூத்து
9. நந்தலாலா பாட்டு

ஆகிய பகுதிகளிலிருந்து எடுத்தாளப்பட்டுள்ளன. இதனால் சாரநாதனின் பரந்துபட்ட பாரதி வாசிப்பு வெளிப்படுகிறது.

கவி வாக்கு என்னும் கட்டுரை பாரதியாரின் ஆளுமையை மதிப்பிடும் வகையில் அமைந்துள்ளது. மகாகவி பாரதியாரை,

1. கவிஞன்
2. தேசாபிமானி
3. சித்தன்
4. உலகிற்கெல்லாம் நண்பன்

என நான்கு நிலைகளில் சாரநாதன் பகுத்துப் பார்க்கிறார். முதல் கட்டுரையில் இடம்பெறாத

1. பல்சுவைப் பாடல்கள் – முரசு
2. ஞானப் பாடல்கள் – தெளிவு
3. விடுதலை வேண்டும்
4. விநாயகர் நான்மணி மாலை
5. பாரத சமுதாயம்
6. ஆத்ம ஜயம்
7. தேசிய கீதங்கள்
8. பாரதி அறுபத்தாறு

ஆகிய பகுதிகளிலிருந்து மேற்கோள்கள் காட்டப்பட்டுள்ளன. பாரதியாரை இளைஞர்களுக்கு அறிமுகப்படுத்தும் நோக்கில் இக்கட்டுரை அமைந்துள்ளது.

பாரதியாரின் புலமைச் சிறப்பினை எடுத்துரைக்கும் அதே வேளையில் அவரின் எளிய வாழ்வு, நாட்டுப்பற்று, தெய்வ பக்தி ஆகியவற்றையும் இளைய சமூகம் உள்வாங்கிக் கொண்டு முன்னேற வேண்டும் என்பதை சாரநாதன் வலியுறுத்துகிறார். கம்பன், திருவள்ளுவர் வரிசையில் வைத்து பார்க்கப்பட வேண்டியவர் பாரதியார் என்று தம் கட்டுரையை நிறைவு செய்கிறார். ஆக தமிழ்ச்சமூகத்தில் பாரதியை நிலை நிறுத்தும்

முயற்சியாகவே சாரநாதனின் இக்கட்டுரையைப் பார்க்க வேண்டியுள்ளது. ஓர் ஆங்கிலப் பேராசிரியராக இருந்தும் ஆழங்கால் பட்ட பாரதி வாசிப்பாளராக வே. சாரநாதன் விளங்கியுள்ளதை அவரின் கருத்துகளும் மேற்கோள்களும் மெய்ப்பிக்கின்றன.

பிற கட்டுரைகளில் எடுத்தாளப்படும் பாரதி மேற்கோள்கள்

சர்வஜித் மாசித் திங்கள் காவேரி இதழில் காந்தியடிகள் குறித்து வே. சாரநாதன் ஒரு கட்டுரை எழுதியுள்ளார். அதன் தொடக்கமாக "அச்சமில்லை அச்சமில்லை" என்னும் பாரதியின் வரி மேற்கோளாக அமைந்துள்ளது.

திருவள்ளுவர் பற்றிய கட்டுரை ஒன்றில் "முத்துக்குளிப்பது தென் கிழக்கிலே" (தென் கடலிலே) என்னும் பாரதியார் வரியை மேற்கோள் காட்டியுள்ளார்.

உ.வே. சாமிநாதய்யரின் தமிழ்ப்பணி குறித்து எழுதுமிடத்தில் சாரநாதன், "முத்துக்குளிப்பது ஒரு தென் கடலிலே மொய்த்து வணிகர் பல நாட்டினர் வந்தே", "திறமான புலமையெனில் வெளிநாட்டோர் அதை வணங்கச் செய்தல் வேண்டும்" என்னும் பாரதி வரிகளை மேற்கோள்களாக எடுத்தாண்டுள்ளார்.

கட்டுரைக்குத் தலைப்பாகப் பாரதியின் கவிதை வரியைச் சூட்டுதல்

பாரதியின் புகழ் பெற்ற கவிதை வரியான "தனியொருவனுக்கு உணவில்லையெனில்" என்பதைச் சாரநாதன் தம் கட்டுரை ஒன்றுக்குத் தலைப்பாக வைத்துள்ளார். இந்திய மக்களின் சமூக வாழ்வியலை அரசியல் நிலை நின்றும் இலக்கிய வழி நின்றும் அக்கட்டுரையில் அலசுகின்றார். "உண்பது நாழி உடுப்பவை இரண்டே" என்னும் புறநானூற்று மேற்கோளுடன் தொடங்கும் இக்கட்டுரை "எட்டுத்திசையும் பறந்து திரிகுவை" என்னும் பாரதியாரின் சிட்டுக்குருவி என்னும் வேதாந்த பாடலுடன் நிறைவு பெறுகிறது.

பாரதியார் கவிதைகள் வாசிப்பு வரலாறும் சாரநாதனும்

தமிழ்ச் சமூகத்தில் பாரதியார் கவிதைகள் நூறு ஆண்டுகளுக்கு மேலாக வாசிக்கப்பட்டு வருகின்றன. அவ்வாசிப்பு வரலாற்றைப் பல நிலைகளில் பகுத்துப் பார்க்க இடம் உண்டு.

1. பாரதி வாழ்ந்த சமகாலத்திய வாசிப்பு
2. பாரதியின் இறப்புக்கும் இந்திய விடுதலைக்கும் இடைப்பட்ட கால வாசிப்பு
3. பாரதியின் படைப்புகள் நாட்டுடைமையான பின்னர் நிகழ்ந்த வாசிப்பு
4. பாரதி பிறப்பு நூற்றாண்டை ஒட்டிய வாசிப்பு
5. பாரதியார் பற்றிய நவீனக் கோட்பாட்டு வாசிப்பு
6. பள்ளி, கல்லூரிப் பாடத்திட்டங்களில் நிகழ்ந்து வரும் பாரதியார் வாசிப்பு

போன்ற பல்வேறு நிலைகளில் பகுத்து ஆராய முடியும்.

பரலி சு. நெல்லையப்பர், மு. இராகவையங்கார், வ.வே.சு. ஐயர், ஏ.வி. சுப்பிரமணிய ஐயர் போன்றோர் பாரதி வாழ்ந்த சமகாலத்திய வாசிப்பாளர்களில் குறிப்பிடத்தக்கவர்கள். தொடர்ந்து வ. ராமசாமி, பாரதிதாசன், சோமசுந்தர பாரதியார், பா. ஜீவானந்தம், சி.என். அண்ணாதுரை, பெ.கோ. சுந்தரராஜன், கு.ப.ரா., எஸ். வையாபுரிபிள்ளை போன்றோர் பாரதி வாசிப்பை அடுத்த நிலைக்கு எடுத்துச் சென்றவர்களாவர். சீனி விஸ்வநாதன், ர.அ. பத்மநாபன், இரா.ஆ. வேங்கடாசலபதி, ய. மணிகண்டன் போன்றோர் பாரதியின் படைப்புகளை முழுமையாக வெளிக்கொணர முயன்றவர்கள். இவர்களுள் சீனி விஸ்வநாதனின் பணிகள் அளப்பரியன. தொ.மு.சி. ரகுநாதன், க. கைலாசபதி, கா. சிவத்தம்பி, அ. மார்க்ஸ் போன்றோர் பாரதி குறித்த மார்க்சிய ஆய்வுகளை முன்னெடுத்தவர்கள்.

இவ்வகையில் சாரநாதன் கல்விப் புலத்தில் இருந்து கொண்டு பாரதியாரை இளைஞர்களிடம் கொண்டு சேர்ப்பதில் முனைப்புக் காட்டியுள்ளார். சர்வசித்து ஆண்டு (1947)-ஆடி மாத காவேரி இதழில் இளைஞருக்கு எழுதிய கடிதப் பகுதியில்,

> "மனிதரின் வாலிபப் பருவத்தில் இலக்கியம் பிரதானம். ஆனால், சொந்த நாட்டு இலக்கியங்களில் பரிபூர்ணமான அபிருசி ஏற்பட்ட பிறகுதான் அன்னிய நாட்டு இலக்கியங்களில் வாலிபரின் கவனம் செல்ல வேண்டும்"

எனக் குறிப்பிடுகிறார். அவர் ஓர் ஆங்கிலப் பேராசிரியராக இருந்தபோதிலும், அவருக்குத் தாய்மொழி மீது கொண்ட ஈடுபாட்டினை மேற்கண்ட பகுதி விளக்குகிறது. அதனால் சாரநாதனின் தாய்மொழியாம் தமிழில் அன்று ஒப்பற்று

விளங்கிய மகாகவி பாரதியார் மீது பெருங்காதல் ஏற்பட்டதில் வியப்பில்லை.

துணைநின்ற நூல்கள்

1. கார்த்திகேசு சிவத்தம்பி ரூ அ. மார்க்ஸ். 2008. பாரதி மறைவு முதல் மகாகவி வரை, நியூ செஞ்சுரி புக் ஹவுஸ், சென்னை.
2. குருசாமி ம.ரா.போ. (ப.ஆ.). 2001. பாரதி பாடல்கள் ஆய்வுப் பதிப்பு, தமிழ்ப்பல்கலைக்கழகம், தஞ்சாவூர்.
3. Ravindran S., (Ed.). 2010. Principal Saranathan Memorial Volume (1949), Saranathan College Of Engineering, Tiruchirappalli.

7. பாரதியார் கவிதைகளில் காணலாகும் இறையுணர்வுச் சிந்தனைகள்

முனைவர் ச.ஈஸ்வரன்,
இணைப்பேராசிரியர் தலைவர்,
தமிழாய்வுத்துறை, தேசியக்கல்லூரி (தன்னாட்சி),
திருச்சிராப்பள்ளி–01

முன்னுரை

"பாட்டினைப்போல் ஆச்சர்யம் பாரின்மிசை இல்லையடா" என்றும், "ஆசை தரும் கோடி அதிசயங்கள் கண்டதிலே ஓசை தரும் இன்பம் உவமையிலா இன்பமடா" என்றும் கவிதை இன்பத்தைப் போற்றிப் புகழும் பாரதியாரின் கவிதைகளில் காணலாகும் இறையுணர்வுச் சிந்தனைகளை இக்கட்டுரையில் தொகுத்துக் காண்போம்.

பாரதியாரின் உலகளாவிய பார்வையில் பக்தியும் ஒன்று. மகாத்மாவையும், திலகரையும் மனித தெய்வங்களாகக் கண்ட பாரதியார் பரம்பொருளாம் இறைவனைப் பல கவிதைகளில் வியந்து போற்றியும், வேண்டியும் பாடியுள்ளார். சிற்சில இடங்களில் மூடபக்தியைச் சாடவும் தவறவில்லை. பயத்தினின்று விடுதலை பெற இறையருளை இறைஞ்சுகின்றார். மனம் நன்னிலை பெறல் வேண்டி "நாசமின்றி நமை நித்தம் காப்பார் இயேசு" (ப.77) எனவும், "எல்லாரும் வந்தேத்தும் அளவில் யமபயம் கெடச் செய்பவன் அல்லா" (ப.178) எனவும் துதித்துப் பரவுகின்றார். விநாயகர், முருகன், சிவன், கண்ணன், கலைமகள், திருமகள், பராசக்தி, ராதை, காளி, முத்துமாரி உள்ளிட்ட பரம்பொருள் மீது கவிதைகளைப் புனைந்து தம் பக்தி உணர்வினைப் புலப்படுத்தியுள்ளார். அத்துடன் சூரியன், சந்திரன் முதலிய இயற்கையையும் வணங்கிப் போற்றியுள்ளார்.

பக்தியுடையார் காரியத்தில் பதறார், மிகுந்த பொறுமையுடன் வித்து முளைக்கும் தன்மைபோல், மெல்லச் செய்து பயனடைவார்" (ப.99) என்கிறார் மகாகவி. நம்பினோர் கெடுவதில்லை எனும் வாக்கின் மூலம் இறை நம்பிக்கையின்

சிறப்பினை உணர வைக்கின்றார். சித்திரம் தெளியும், செய்யும் செயல்கள் யாவும் சிறப்புற அமையும். நெஞ்சில் சஞ்சலம் நீங்கி உறுதி தோன்றிடும், கல்வி வளரும், பிணி நீங்கி, பல காரியம் கையுறும், உடல் சொன்னபடி நடக்கும் என "பக்தியினாலே இந்தப் பாரினில் எய்திடும் மேன்மைகள் கேளடீ" (ப.90) எனும் கவிதையில் உணர்த்துவதன் வாயிலாக பக்தியின் மாண்பினை உணரலாம்.

விநாயகர்

விநாயகப் பெருமானை வழிபடலால் அச்சம் தீரும், அமுதம் விளையும் என்கிறார். உலகத்து உயிர்கள் எல்லாம் வாழவேண்டும் என அனைவருமே விரும்புவர். பாரதியார் விநாயகப் பெருமானிடம் வேண்டும்போது மக்களிடம் மட்டுமின்றி விலங்குகள், புழு, பூச்சிகள், பசுமையான மரங்கள் முதலியனவெல்லாம் தம் சொல்லால் துன்பங்கள் நீங்கி ஒன்றுடன் ஒன்று பகையின்றி ஒத்து வாழவேண்டும் எனக் கேட்கிறார். "கணபதி ராயன் – அவனிரு காலைப் பிடித்திடுவோம்" (ப.163) என்கிறார்.

"நமக்குத் தொழில் கவிதை, நாட்டிற்கு உழைத்தல்
இமைப்பொழுதும் சோராது இருத்தல்-உமைக்கு இனிய
மைந்தன் கணநாதன் நம்குடியை வாழ்விப்பான்
சிந்தையே இம்மூன்றும் செய்" **(ப.99)**

என்று சுட்டுகிறார் மகாகவி.

முழுமுதற் கடவுளுக்கே தம் ஆவி, உள்ளம் போன்றனவற்றை ஈந்ததாகவும், ஆதலால் தமக்கு புகழ், செல்வம், அழகு முதலியனவற்றைத் தரும்படியும் வேண்டுகிறார். புதுச்சேரியில் உள்ள மணக்குள விநாயகரையும் போற்றித் துதிக்கின்றார்.

"அபயம் அபயம் அபயம் நான் கேட்டேன்
நோவு வேண்டேன் நூறாண்டு வேண்டினேன்" (ப.97)

என விநாயகப் பெருமானிடம் நூறாண்டுகள் வாழ வேண்டிய பாரதியார் தம் முப்பத்தொன்பதாவது வயதிலேயே (1882, டிசம்பர் 11 முதல் 1921 செப்டம்பர் 11 வரை) நம்மைவிட்டு நீங்கினாலும், காலத்தை வென்ற சாகாவரம் பெற்ற கவிதைகளை நம்மிடம் தந்து சென்றுள்ளதை எண்ணல் வேண்டும். புதியதோர் சத்தியயுகம் காண வேண்டி இவர் கண்ட கனவுகள் நம் கண்முன்னே சிறிது சிறிதாக நனவாகிக் கொண்டு வருதலையும் உணரல் நன்றாம்.

முருகனைப் போற்றல்

முருகனைத் தமிழ்க்கடவுள் என்று தமிழ் இலக்கியங்கள் சிறப்பித்துக் கூறும் முருகப் பெருமானின் வீரம் நிறைந்த திருவிழிப் பார்வையும், வெற்றி வேலும், மயிலும், நின்று எந்நேரத்திலும் தம்மைக் காத்து வருவதாக மகிழ்வுடன் உரைக்கிறார் பாரதியார். "**வாகையே சுமக்கும் வேலை, வணங்குவது எமக்கு வேலை**" *(ப.107)* எனப் பாடுகின்றார். முருகன் பாட்டு, முருகக் கடவுள் மீது கிளித்தூது, வள்ளிப்பாட்டு போன்றனவற்றைப் பாடியுள்ளார்.

பெண்ணே தெய்வம் எனல்

"எத்தனை கோடி இன்பம் வைத்தாய்! எங்கள் இறைவா!" (ப.109) என்று பாரதி பெண் தெய்வத்திற்கே அதிக முக்கியத்துவம் அளித்துள்ளார். பராசக்தி, கலைமகள், காளி, திருமகள் உள்ளிட்ட பெண் தெய்வங்களைப் போற்றிப் பல கவிதைகள் புனைந்துள்ளதை அறிய முடிகிறது. எல்லாமே சக்தியின் வடிவம் என்பது பாரதியின் அசைக்க முடியாத நம்பிக்கை,

"**காணி நிலம் வேண்டும் - பராசக்தி**
காணி நிலம் வேண்டும் - அந்தக்
காணி நிலத்திடையே-ஓர் மாளிகை
கட்டித்தர வேண்டும்
பாட்டுக் கலந்திடவே-அங்கேயொரு
பத்தினிப்பெண் வேணும் - என்றன்
பாட்டுத் திறத்தாலே - இவ்வையத்தைப்
பாலித்திட வேணும்" **(ப.112)**

என்றெல்லாம் பராசக்தியிடம் கேட்கிறார். தாய்க்கு மேல் இங்கே ஓர் தெய்வம் உண்டோ? என்ற பாரதி, நல்லதோர் வீணை செய்து அதை நலம் கெடப் புழுதியில் எறிவதுண்டோ சொல்லடி சிவசக்தி என்கிறார். மேலும் எனை அறிவுடன் படைத்துவிட்டாய், வல்லமை தாராயோ இம்மாநிலம் பயனுற வாழ்வதற்கே" என்று சிவசக்தியிடம் விண்ணப்பிக்கிறார்.

பாரதியாரது தெய்வப் பாடல்களில் சக்திப் பாடல்கள் முக்கியத்துவம் கொண்டவை. கல்கத்தாவிலே சகோதரி நிவேதிதா அவர்களைப் பார்த்தபோது சக்தி நிலையை (சொரூபம்) நேரில் கண்ட தன்மை (பரவசம்) ஏற்பட்டதால் "ஸ்வதேச கீதங்களை" நிவேதிதாவிற்குச் சமர்ப்பணம் செய்துள்ளார் பாரதியார்.

அன்னையை வேண்டல்

நல்லதையே எண்ணல் வேண்டும். இவ்விதம் எண்ண நல்லருள் புரிய வேண்டும். செய்த பாவங்கள் எல்லாம் பரிதிமுன் பனியே போல் அழிந்திட வேண்டும் என்கிறார்.

"எண்ணிய முடிதல் வேண்டும்,
நல்லவே எண்ணல் வேண்டும்
திண்ணிய நெஞ்சம் வேண்டும்
தெளிந்த நல்லறிவு வேண்டும்" **(ப.114)**

தெரிந்தோ, தெரியாமலோ, அறிந்தோ, அறியாமலோ நாம் செய்த சகல பிழைகளையும் பொறுத்துக் காத்து அருள் பாலித்திடுமாறு துதிக்கின்றார் மகாகவி பாரதியார்.

மகாசக்திக்கு விண்ணப்பித்தல்

நம்மிடம் உள்ள குறைகளையெல்லாம் மகாசக்தியிடம் எடுத்தோதி அவைகளையெல்லாம் நீக்கிவிடு, போக்கிவிடு என உரைக்கின்றார்.

"பந்தத்தை நீக்கிவிடு-
அல்லால் உயிர்ப் பாரத்தைப் போக்கிவிடு
சிந்தை தெளிவாக்கு-
அல்லால் இதைச் செத்த உடலாக்கு" **(ப.113)**

என்று விண்ணப்பிக்கிறார்.

எல்லாம் சக்திக்கே சமர்ப்பணம் செய்தல்

உடலில் உள்ள அனைத்து உறுப்புகளையும் சக்திக்கு அர்ப்பணம் செய்கின்றார். "பகையைச் சக்தி தனக்கே கருவியாக்கு-அது சாதனைகள் யாவினையும் கூடும்" *(ப.120)*

"சக்தி என்று சொல்லு - கெட்ட சஞ்சலங்கள் யாவினையும் கொல்லு" *(ப.126)* என்கிறார் பாரதியார். எண்ணும் காரியங்கள் அனைத்திலும் வெற்றிகிட்ட காளிதேவியினை வேண்டுகிறார்.

"தேடியுனைச் சரணடைந்தேன், தேசமுத்துமாரி
கேடதனை நீக்கிடுவாய்! கேட்ட வரம் தருவாய்!
நம்பினார் கெடுவதில்லை, நான்கு மறை தீர்ப்பு
அம்பிகையைச் சரண்புகுந்தால் அதிக வரம் பெறலாம்" **(ப.146)**

என முத்துமாரியன்னையின் புகழினைப் பாடிப் பரவுகின்றார்.

நீக்கமற நிறைந்திருத்தல்

கண்ணபிரானைப் பாடும்போது பார்க்கும் இடங்களில் எல்லாம் நீக்கமற நிறைந்திருப்பதாகப் புகழ்கின்றார்.

"காக்கைச் சிறகினிலே நந்தலாலா-நின்றன்
கரிய நிறம் தோன்றுதையே, நந்தலாலா
பார்க்கும் மரங்கள் எல்லாம் நந்தலாலா!"-நின்றன்
பச்சை நிறம் தோன்றுதையே நந்தலாலா!" **(ப.146)**

"கண்ணன் திருவடி எண்ணுக மனமே, திண்ணம் அழியா,
வண்ணம் தருமே" **(ப.147)**

என்னும் கவிதையின் மூலம் கண்ணபிரானின் பெருமைகளையெல்லாம் பறைசாற்றுகின்றார். திருமகளைத் துதிக்கும்போது "ஈடு நினக்கோர் தெய்வமுண்டோ? எனக்கு உனையன்றிச் சரணமுண்டோ?" (ப.153) என்று உரைக்கின்றார்.

கலைமகளைப் போற்றுதல்

கலைமகள் அறிவுக்கு அரசி, அவள் இருக்கும் இடம் வெள்ளைத் தாமரை என்றாலும், அவள் வீணையில் எழும் நாதத்திலும், பாவலர் உள்ளத்திலும், வஞ்சமில்லாத தொழில் புரிவோர் இடத்திலும் இருப்பதாகத் தெரிவிக்கிறார்.

"வெள்ளைத் தாமரைப் பூவில் இருப்பாள்
வீணை செய்யும் ஒலியில் இருப்பாள்
கொள்ளை இன்பம் குலவு கவிதை கூறுபாவலர்
உள்ளத்து இருப்பாள்" **(ப.157)**

"மனதில் உறுதி வேண்டும், வாக்கினிலே இனிமை
வேண்டும், பெண் விடுதலை வேண்டும்,
பெரிய தெய்வம் காக்க வேண்டும்"

எனக் கேட்கிறார் மகாகவி பாரதியார்.

பக்தி மாண்பினைச் சிறப்பித்தல்

இப்புவியில் உள்ளோர், "திருமாலும், சிவனும் ஒன்றே என உணரல் நன்று" என்கிறார். (ப.142). மேலும் "உயிர்களெல்லாம் தெய்வமின்றிப் பிறவொன்றில்லை" எனவும், "எழுதுகோல் தெய்வம், இந்த எழுத்தும் தெய்வம்" (ப.261) என்றும் உரைக்கிறார்.

"யாரும் பணிந்திடும் தெய்வம் - பொருள் யாவினும்
நின்றிடும் தெய்வம்

வலிமையுடையது தெய்வம் - நம்மை வாழ்ந்திடச்
செய்வது தெய்வம்'' **(ப.206)**

எனக் கூறும் பாரதியார். வெற்றி எட்டுத்திக்கும் எட்டடக் கொட்டும் முரசே" எனும் கவிதையில்,

''ஊருக்கு நல்லது சொல்வேன் - எனக்கு உண்மை
தெரிந்து சொல்வேன்
சீருக்கு எல்லாம் முதலாகும் ஒரு தெய்வம் துணை
செய்ய வேண்டும்'' **(ப.20)**

எனத் தெரிவிக்கின்றார். "ஓடி விளையாடு பாப்பா" பாட்டில்,

''உயிர்களிடத்து அன்பு வேணும் - தெய்வம்
உண்மையென்று தானறிதல் வேண்டும்'' **(ப.203)**

எனக் கூறுகிறார்.

பாஞ்சாலி சபதத்தில் வாணியை வேண்டும்போது, "தெளிவுடன் அறிந்திடவும் மொழிந்திடவும் ஒளி வளரும் தமிழ்வாணி உதவவேண்டும்" (ப.352) எனக் கேட்கின்றார். "வண்டிக்காரன் பாட்டில்"-காட்டு வழியில் கள்ளர் பயமிருந்தால் வீட்டுக்குல தெய்வம் வீரம்மை காக்கும் எனவும், கள்ளர் நெருங்கினால் கறுத்த மாரியின் பெயர் சொன்னால் காலனும் அஞ்சுமடா" (ப.192) என்றும் உரைத்திருப்பதன் வாயிலாக பாரதியாரின் பக்தி உணர்வினை நன்கு அறிய முடிகிறது.

முடிவுரை

''வகுத்தான் வகுத்த வகையல்லால் கோடி
தொகுத்தார்க்கும் துய்த்தல் அரிது'' **(குறள் - 387)**

எனும் வள்ளுவர் வாக்கிற்கிணங்க பக்தி உணர்வினை நம் உள்ளத்து வளர்த்து அறியாமையினின்றும், தீய எண்ணத்தினின்றும் விடுபட்டு வாழ முயல்வோமாக!

பாரதியின் புதிய ஆத்திசூடி வழி, கற்றது ஒழுகி, செய்வது துணிந்து செய்து, நீதிநூல் பயின்று, நாளெல்லாம் (நல்)வினை செய்து, புதியன விரும்பி (ப.201) சிறப்புடன் வாழ்வோமாக!

துணை நின்ற நூல்

1. பாரதியார் கவிதைகள், கிரி டிரேடிங் ஏஜன்ஸி வெளியீடு, 10, கபாலீஸ்வரர் சன்னதி தெரு, மயிலாப்பூர், சென்னை–600 004, முதற்பதிப்பு, மே.2000.

8. பாரதி வாழ்கிறான்

முனைவர் சி. காந்தி,
இணைப்பேராசிரியர், தமிழாய்வுத்துறை,
தேசியக்கல்லூரி (தன்னாட்சி),
திருச்சிராப்பள்ளி–01.

நூறாண்டு நினைவுகளில் பாரதி வாழ்கிறான்
நூறு நூறு ஆண்டுகளில் பாரதி வாழ்ந்திருப்பான்
வீறுகொள் சிந்தையிலும் விடுதலை வேட்கையிலும்
ஊறுஇன்றி இருந்திடுவான் இறப்பில்லை நிலைத்திடுவான்.

எட்டய புரத்திலே ஏடெடுத்து படித்திட்டான்
பட்டமாகப் பாரதியெனும் பெயரைப் பெற்றிட்டான்
கடயத்தில் வாழ்ந்தபோது கழுதையொடு புரண்டிட்டான்
விடயத்தில் ஆகப்பெரும் மேதையாய் உருவெடுத்தான்

மொட்டைத் தலையிலே முண்டாசைச் சுற்றினான்
வெட்டுவாள் மீசையை மேல்புறமாய் ஏற்றினான்
பொட்டை நெற்றியிலே நெருப்புபோல் தீற்றினான்
சட்டை யால்மெலிவு காணாதுஏ மாற்றினான்.

நெட்டை மரங்களென பயந்தோரைத் தூற்றினான்
சட்டம் செய்திடுவார் பெண்களென சாற்றினான்
தொட்டில் குழவிமேல் பேரன்பை ஊற்றினான்
நாட்டை உயர்த்திடவே நற்கடமை ஆற்றினான்

ஆங்கிலக் கல்வியினால் அறிந்ததையும் புரிந்ததையும்
பாங்குடன் பகிர்ந்திட்டுப் பாமரத்தைப் போக்கிட்டான்
தீங்கிலாக் கல்விமூலம் தன்மானம் கற்க சொன்னான்
யாங்கினும் சமத்துவம் விடுதலை பரவச்செய்தான்.

பேரறிவு சமுதாயம் பகுத்தறிவு நியாயம்
ஆறறிவு மனிதனுக்கு அடையாளம் அதுவென்றான்
ஊரறிய உலகறிய உண்மைகளை உரைத்திட்டான்
யாரறிந்தார் பாரதியை வாழ்ந்திட்ட காலத்திலே.

சாத்திரம் பொய்சொன்னால் சதிஎன்று சொல்லிவிட்டான்
கோத்திரம் குலமெல்லாம் கெடுதலென அறிவித்தான்
ஆத்திரம் கொண்டவனாய் அடக்குமுறை ஒழிகஎன்றான்
காத்திரம் குறையாத கவிஞனாய் நிலை கொண்டான்.

.....

ஆயிரம் யுகத்துக்கு அனல்மூட்டிச் சென்றான்
பாயிரம் பாடியே பொய்மைகளை வென்றான்
ஆவியினை ஆயுளினை அவனிக்கே தந்தான்
பூவினையும் வாளாக்கும் பாரதியும் அவன்தான்

காவியங்கள் கற்பனைகள் கவிமணிகள் கோர்த்தான்
பாவிகளும் வீடுபெறும் சிந்தனைகள் சேர்த்தான்
ஓய்வின்றி சோர்வின்றி உரிமைபெற உழைத்தான்
மாய்ந்தாலும் தியாகத்தால் உயிர்பெற்று நிலைத்தான்
உயிரினும் பெண்மையை இனிதுதான் என்றான்
வயிரத்து உறுதியைப் பெண்களுக்குத் தந்தான்.

மனிதரில் கீழ்மையில்லை மேன்மையில்லை என்றான்
புனிதனாய் ஆவது பேருழைப்பு வழியிலே
பள்ளத்தில் வீழ்ந்திருப்போர் படியேற்ற சொன்னான்
உள்ளத்துக் கயவர்களை எதிரியாக்கிக் கொண்டான்.
சுயசாதி கடிந்ததிலே பாரதிபோல் எவருமில்லை
சுயநலமாய் சமுதாயக் கேடுசெய்த மிகச்சிலரை
பேராசைக் காரனடா பார்ப்பான் எனப்புகன்றான்
யாரானாலும் கொடுமை செய்வான் என்றும் சொன்னான்.
வேதத்து உண்மைகளைப் பாடினான் புராணப்
போதத்துப் பொய்மைகளைப் புழுகுகளைச் சாடினான்.
சடங்குகள் சாத்திரத்தில் ஒளிந்திருக்கும் சுரண்டல்கள்
மடங்களில் புழுத்திருந்த மடமைகள் அனைத்தையும்
விளக்குமாறு கூட்டி வெளியேற்றி அடித்தான்.
விளக்கென்னும் அறிவுகொண்டு விரட்டினான் இருளென்ற
உள்ளத்து அறியாமை; ஒளிபெற்றான் வாய்ஊமை
துலக்கினான் பழங்களிம்பு தீயறிவு அழுக்குகளை
விலக்கினான் வந்துசேரும் பாசானம் பொய்க்கதைகள்
மேடுபள்ளம் சமமாக்கி முன்னேற வேண்டுமென
பாடுபட்டான் ஆங்கதனைப் பாட்டிலே பாடிவைத்தான்.

தலைகுனிதல் இல்லாமல் தலைநிமிர்ந்து நெஞ்சுயர்த்தும்
நிலைவருதல் வேண்டுமே நிலங்கிடந்தோர் பெண்களுக்கும்
அலையலையாய் கற்பனைகள் அடுக்கடுக்காய் சிந்தனைகள்
இளைத்தோர்க்கு நலிந்தோர்க்கு எல்லார்க்கும் ஆயுதங்கள்
பலருக்கும் பலகல்வி பயிற்றிட வேண்டுமென்றும்
சிலருக்கு மட்டுமே கல்வியுண்டு என்கின்ற
மோசடிகள் மாயவேண்டும்; மேன்மேலும் அயல்கல்வி
வாசிப்பும் வேண்டுமென வரகவிஞன் சொல்லிவைத்தான்.
அறிவே செல்வமென அரசகவி சொன்னான்
நெறிகள் நியாயத்தால் நிமிரும் வாழ்வென்றான்.

.....

கடலன்ன பேரன்பை எல்லார்க்கும்
கணக்கின்றி வழங்கினான் - உழைக்கும்
உடல்கொண்ட மானுடர் யாவரையும்
உண்மையிலவன் விரும்பினான் - வெல்லும்
அடல்ஏறை நாட்டின் புதல்வனுக்கு
உரமேற்றும் வழியினைக் காட்டினான் - ஒருவனின்
குடல்வயிறை வெம்பசிதான் பற்றினால்
கெடுப்போம் இவ்வுலகு என்றான்.
பழங்கதையை வகுந்தெடுத்து நலமான
புத்தறிவு கொண்டுவந்தான் - பாஞ்சாலி
பழமைமாற்றி பாரதத்தின் வீரமிகுப்
பெண்ணாகப் பிறக்க வைத்தான் - அடிமைப்
பழக்கங்கள் விலங்கினைப் பூட்டுமெனப்
பாரதத்தைக் காட்டிச்சொல்லி விட்டான் - பேதத்தால்
விழுந்துவிடும் தேசங்கள்; நம்பேதத்தால்
வெள்ளையன் அரசாண்டான் என்றான்.

வேதங்கள் காப்பியங்கள் பழங்கதைகள்
வியாக்கியானம் அனைத்தும் காட்டி
போதங்கள் காட்டியது எதுவென்றால்
வறுமைதீர்த்து சமநிலை காண்பதற்கு
நாதங்கள் பாடினான்; முரசெடுத்து
முழங்கினான்; பழங்கதையில் அவன்
பாதங்கள் ஊன்றினாலும் மண்ணிலே
பாரதிதான் தேடினான் கிருதயுகம்.

.....

பெண்கள் படித்துப் பட்டத்தைப்
பெற்றுவிட்டு அரசிகளாய்
மண்ணாள வெண்டுமென்றான் - பாரதி
மண்ணிலறம் தழைக்கு மென்றான்.
விண்ணில் பறக்கின்றார்கள் பெண்கள்
விலங்கொடித்து நடக்கின்றார்கள் - இதைக்
கண்ணில் பார்த்துவிட்டால் பாரதி
களிகொள்வான் மிகமன மகிழ்வான்.

.....

மாறாத சனாதன நிலத்தில் நின்று
மாறுபட்டு சிந்தித்து வானளவு உயர்ந்தான்
ஊறாத அடைபட்ட செய்யுள் கிணற்றிலே
ஓயாது கவியூற்றை முகர்ந்து எடுத்திட்டான்
மீறாத வரம்புகளில் கவிதைகளைப் புனைந்தான்
மீறும் சிந்தனையை அவற்றினிலே கலந்தான்
ஆறாத புண்போன்ற தீண்டாமைக் கொடுமையினை
ஆழக்குழியிலே புதைக்க வேண்டுமென்றான்.

.....

பாரதியின் சத்தியத்தை-அன்று
படித்தவன் ஏற்கவில்லை
பாமரன் படித்திட்டான் - இன்று
பாரதி உயிர்ப் பெற்றான்
எங்கள் கவிஞன் என்று-ஏழை
எளியோர் சொன்னார்கள்
தங்களின் பிறப்பு என்று-தாய்
தமிழர்கள் உணர்ந்தார்கள்.

நல்வாழ்வு வாழவில்லை-பாரதி
நாட்டு வாழ்வு மறக்கவில்லை
குலத்தமிழர் வாழ்வுகாக்க-எவரும்
பாரதிபோல் பிறக்கவில்லை
நிலமெல்லாம் கால் நடந்தான் - அவன்
நிற்குமிடம் ஒன்றில்லான்
உலகுவிட்டுப் போனபின்பு-சிலர்
ஒத்துக் கொண்டார் கவிஞனென.

.....

தமிழன்னை அமுதருந்தி தமிழுக்கு மகனானான்
தந்தைபோல் பாடுபட்டு தேசத்துக் கவியானான்
இமிழ்கடல் நிலம்பாடி உலகக்குடி மகனானான்
எல்லையிலா மனப்பரப்பால் பிரபஞ்ச மனிதனானான்.
கவிதையினால் உலளந்தான்; நிலம் கடந்தான்
சிந்தையிலே வானுயர்ந்தான்; அதற்கு மேலே
புவிசுமந்தான் பேரன்பு ஊறுகின்ற நெஞ்சினிலே
பாரதிதான் எங்கள்வழி பாரதிதான் எங்கள்விழி.

.....

கண்ணனை மனிதனாக்கி தாயாக்கிப் பலவித
வண்ணங்களில் உறவாக்கி வம்சபெருமை யினைத்தன்
எண்ணங்களின் பிழிவாக்கி ஆறாக ஓடவிட்டான்
பண்ணிசையில் நேசமதைப் பாரதி பாடிவைத்தான்.

தோழன் என்றான் தாயும் என்றான்
அழகான காதலியாய் ஆக்கிக் கொண்டான்
குழந்தை என்றான் காதலன் என்றான்
பழகிய சொந்தமென பாசம் தந்தான்.

சேவகனின் பணியாள்போல் சேவகம் செய்துவிட்டான்
காவல் செய்ய ஆணையிடும் கோமானாய் மாறிவிட்டான்
ஆவல்கொள் குழந்தையிடம் அன்னையாய் உருவெடுத்தான்
பூவுலகின் உறவனைத்தும் பெரும்நெஞ்சில்
வாரிக்கொண்டான்.

பொங்கிப் பெருக்கெடுத்த பெரும்பாச வெள்ளத்தை
பங்கமிலா உறவுஎனும் வாய்க்காலில் ஓடவிட்டான்
எங்கும் உறவுகாண ஏக்கங் கொண்டுவிட்டான்
மங்காது கண்ணன்புகழ் பாரதி இருக்கும்வரை.

தாயாகிக் கொஞ்சி தன்அன்பைப் பொழிந்திடுவான்
சேயாக அழுது தாய்மடியில் புரண்டிடுவான்
ஓயாத தொல்லை ஓடிவரும் பிள்ளையிடம்
காயாத உள்ளமுடன் கரம்சேர்த்து அணைத்திடுவான்.

முணங்கினான் கண்ணன் விளையாடும் பிள்ளையெனில்
வணங்கினான் தந்தையெனில் வயப்பட்டு கண்ணம்மாவொடு
பிணங்கினான் தாயிடம் சிணுங்கினான்; தோழனுக்கு
இணங்கினான்; கண்ணனை இன்னனம்கவி வழங்கினான்.

சேவகனுக்கு ஆணையிட்டு சேவை பெற்றிடுவான்
காவல்மன்னன் அடிபணிந்து கடமை ஆற்றிடுவான்
தேவக் கண்ணன் சீடனுக்குப் பாரதியே குருவானான்
தூயனாம் பாரதிக்குக் கண்ணனேசற் குருவாவான்.

அரசன் அடிமைகள் அத்தனை உறவுகளைச்
சரிநிகர் சமமாக்கி சமத்துவம் கண்டிடுவான்
பிரிவுஇல்லை பேதமில்லை நாட்டின் செல்வங்கள்
உரிமைதான் எல்லார்க்கும் உண்மைதான் பொய்யில்லை.

.....

திக்கு எட்டிலும் செய்தி எட்டிட
கொட்டி முழக்கிடு முழக்கிடு முரசே!
பக்தி போலிகள் அழிந்து போவென்று
பாரதி சொன்னதை உலகு அதிர்ந்திட
வெடித்து முழங்கிடு முழங்கிடு முரசே!

சாதி உள்ளது எனச்சொல்லிடும்
சின்னசாதிப் பயல்களின்
காதின் செவிப்பறை கிழிந்து விழும் வரை
கொட்டி முழங்கிடு முழங்கிடு பறையே

சுரண்டிப் பிழைப்பவன் பிறரை ஏய்ப்பவன்
செத்துப் போவான் எனப் பாரதி
உரைத்ததை உலகுக் கேட்டிட
உரத்துக் கொட்டுக கொட்டுக முரசே.

பெண்ணைத் தாழ்த்திடும் ஆண்மை இழந்திட்ட
மொன்னைப் பயல்எலாம் அழிந்து போகணும்
என்று கூறிய பாரதி செய்தியை
எழுச்சி கொண்டு அறைக பறையே!

கல்வி விற்றிடும் கயவன் இருக்கிறான்
கல்விக் கொள்ளையன் அனைத்துப் பேருக்கும்
கொள்ளி போடணும் என்று சொல்லியே
கொட்டி முழங்குக முழங்குக பறையே!

அன்பு ஒன்றுதான் உலகை நடத்திடும்
உறவு ஒன்றுதான் வாழ்வை இயக்கிடும்
என்று சொன்னவன் பாரதி என்றுதான்
உரத்துக் கொட்டுக கொட்டுக முரசே!

அன்று வெள்ளையன்! இன்று கொள்ளையன்
ஏய்ப்போன், பொய்ப்போன், அறக் கொலைஞன்
பெண்நலம் மிதிப்போன், மழலைச் சிதைப்போன்
கயவன், சுரண்டன், கல்விக் கொள்ளையன்
யாவரும் வீழ்க வீழ்க வென்று
சாவுஅடி கொட்டுக ! கொட்டுக ! பறையே!
தீம்திரிகிட! தீம்திரிகிட! தீம்திரிகிட!

9. பாரதியார் கவிதைகள் வெளிப்படுத்தும் இனப்போராட்டத் தீர்வுகள்

முனைவர் அ.கிருஷ்ணன்,
உதவிப்பேராசிரியர், தமிழாய்வுத்துறை,
தேசியக்கல்லூரி(தன்னாட்சி), திருச்சிராப்பள்ளி–01

முகவுரை

மக்கள் தங்களுக்குத் தகுந்தாற் போன்ற வேலைகளைச் செய்வதற்காக பண்டையோர் வருணபாகுபாடு செய்தனர். ஆனால் அப்பிரிவினைப் பிற்காலத்தோர் சாதி, இன வேறுபாடுகளாகப் பிரித்து நாட்டைக் கூறுபோட்டனர். இதனைக் கண்ணுற்ற பாரதி மனம் வெதும்பி வேதனைக்குள்ளானார். அந்த வேதனையின் தாக்கமே கவிதைகளாக வெளிப்பட்டு நின்றன.

மக்கள் அனைவரும் மானுடத்தின் சிறப்பினை உணர்ந்து, ஒன்றுபட்டு சகோதரர்களாக வாழவேண்டுமென்பதே பாரதியின் உயரிய நோக்கமாகும். தம் உள்ள கருத்துகளை அனைத்து மக்களும் புரிந்துக் கொண்டு ஒற்றுமை மேலோங்கினாலே நாடு முன்னேற்றமடையும் இல்லையெனில் பிளவுபட்டு நொறுங்கி அழிய நேரிடும் எனப் பாரதியார் கருதினார். இந்திய சமூகத்தில் மனிதர்கள் இயற்கை அளித்திருக்கும் வளங்களையும் தம் சிந்தனை செல்வத்தையும் கொண்டு சச்சரவுகளின்றி சுதந்திரத்துடன் ஓரினமாக வாழும் அமைதியான தேசத்தைக் காண்பதே பாரதியின் விருப்பமாகும். எனவே பாரதி தம் கவிதைகளின் வாயிலாக சாதி, மத, இனபோராட்டங்களைச் சுட்டிக்காட்டி அதற்கான தீர்வுகளையும் கூறியுள்ளதை ஆராயும் நோக்கில் இக்கட்டுரை அமைகிறது.

இனம் என்பதன் பொருள்

இனம் என்பதற்குச் சுற்றம், குலம், கூட்டம், திறள், வகுப்பு, குழு, நிறை, மந்தை, சம்பந்தம் என்பன பல பொருள்களுண்டு.

"பொது பெரும்பிரிவு மனித, தாவர, இனம், இனக்கலவரம் தொகுப்பில் அல்லது பட்டியலில் உள்ள ஒன்று அடையாளம்"[1] என்கிற க்ரியாவின் தற்காலத் தமிழ் அகராதி விளக்குகிறது.

"இனமையக் கொள்கை" எனப்படும் "மனித குலத்தின் மிகப் பெரிய பிரிவே இனமாகும். இவ்வகைக் குழுவினர் உடற்கூற்று அமைப்பாலும், உருவத் தோற்ற அமைப்பாலும் மரபுவழித் தொடரும் ஒத்த பண்புகளைக் கொண்டிருப்பர். இம்மரபுவழி பண்புகளை அடிப்படையாகக் கொண்டே மனித இனம் பலவாறாகப் பிரிகிறது. இம்மரபுவழிப் பண்புகளையே இனவகைப்பாட்டின் அலகுகளாக இனவியலார் பயன்படுத்துகின்றனர்"[2] தற்போதைய வழக்கில் வர்க்கம், மதம், சாதி, நிறம் என்ற பொருள்களிலும் இனம் என்ற சொல் கையாளப்படுகிறது. ஒரு குறிப்பிட்ட நிலப்பகுதியில் சில நோக்கங்களுக்காகச் சேர்ந்து தொழில் வழக்காறுகள் பழக்க வழக்கங்கள் போன்வற்றால் பிரிந்து வாழும் கூட்டத்தை இனம் என்று கொள்வது பொருந்தும்.

இனப் போராட்டங்களுக்கான காரணங்கள்

பரந்த நிலப்பரப்பில் பல்வேறு பிரிவினர்கள் சேர்ந்து வாழும் சூழலில் ஒவ்வொரு தனி மனிதனின் உணர்வுகளும் எண்ணங்களும் மேலோங்கும்பொழுது மாறுபாடுகள், கருத்து முரண்பாடுகள் ஏற்படுவது தவிர்க்கவியலாதது. அறிவு ஜீவிகளிடையேகூட கருத்து வேறுபாடு எழுவது இயற்கைதான். இது காலப்போக்கில் வளர்ந்து மத, சாதிய, இன உணர்வாக வெடிக்கும். இதனால் போராட்டங்கள் ஏற்படுகின்றன. தன் முனைப்பு, ஆளுமை, அதிகாரம் தாங்களே உயர்ந்தவர்கள், தங்கள் பண்பாடே சிறந்தது. தற்பெருமை அடிமைப்படுத்தும் மனப்பான்மை போன்றவை இனப் போராட்டங்களுக்கான காரணிகளாக வெளிப்படுகின்றன. இனமையக் கொள்கை உணர்ச்சியில் தாங்களே என்ற உயர்வு மனப்பான்மையே காணப்படும். இவ்வுணர்வானது வெவ்வேறு பண்பாட்டைச் சேர்ந்தவர்களுக்கிடையேயும், சில சமயங்களில் "ஒரே பண்பாட்டுச் சமூகத்தைச் சேர்ந்த வெவ்வேறு மதம் அல்லது குழுக்களுக்கிடையேயும் காணப்படும். பெரும்பன்மையோர் தம்முடைய பண்பாட்டு முறைகளே உயர்வானவை மற்றவை தாழ்வானவை என்று கருதுகிறார்கள். இம்மாதிரியான தங்கள் இனத்தின் பண்பாட்டு முறைகளே பிற பண்பாட்டு முறைகளைவிட உயர்ந்தவை என்று கருதும் மனப்பான்மையே "இனமையக் கொள்கை என்பர்"[3]

பாரதி வாழ்ந்த நாள் கொஞ்சம் எனினும் அவரது பன்மொழிப் புலமையும், அகன்ற, ஆழமான வாசிப்பும், பத்திரிக்கைத் தொழிலும், பல மாநிலங்களில் வாழ்ந்த வாழ்க்கையும் சமூகத்தையும், உலக நட்புகளையும் அறிய வாய்ப்பாயிற்று.

பாரதி கருதும் உலக ஒற்றுமை

தனது பரந்த மனப்பான்மையாலும் உயரிய சிந்தனையாலும் தம் படைப்புகளில் வெளிப்படுத்தியுள்ள கருத்து வளத்தால், உலக மகா கவிஞர்களுள் ஒருவராகப் பாரதி மதிக்கப்படுகின்றார். உலக மக்களனைவரும் மத, இன, போர் வெறிகளைவிடுத்து, ஒன்றுபட்டு வாழவழி வகைகளைக் கூறியுள்ளார்.

"உலகிடைத் தோன்றிய
மானிடரெல்லாம் சோதரர் மானுடர்
சமத்துவமுடையர் சுதந்திரம் சார்ந்தவர்"[4]

என்ற வரிகள் இதற்குச் சான்றாகும். புதிய ருஷ்யா என்ற பாடலில் 'ஸார்' சக்கரவர்த்தியின் வீழ்ச்சியால் 1917-இல் ரஷ்யாவில் ஏற்பட்ட புரட்சியை,

"ஆகாவென் றெழுந்தது பார் யுகப்பரட்சி
கொடுங்கோலன் அலறி வீழ்ந்தான்"[5]

என்று யுகப்புரட்சியாகக் கண்டு அந்நாட்டின் இன ஒற்றுமைக்குக் குரல் கொடுத்துள்ளார்.

அமெரிக்க நிற வெறி, ஆப்கானிஸ்தான் புத்தர் சிலை உடைப்பு போன்றவற்றிற்குத் தீர்வுக் காணவும், ஐ.நா. சபையின் சமாதான நடவடிக்கைகளுக்கும் பாரதியின் இன ஒற்றுமைச் சிந்தனை தீர்வாக அமையும் என்பதில் ஐயமில்லை. கல்வியிலும், பொருளாதாரத்திலும், வளர்ச்சியடைந்த அமெரிக்கா, ரஷ்யா, இத்தாலி போன்ற நாகரிக நாடுகளில் கூட மத, இனப் போராட்டங்கள் நிகழ்ந்து கொண்டிருக்கின்றன. கல்வியாளர் பாரதியின் இச்சீரிய சிந்தனைகளை உலகளவில் பரப்பி, மனிதன் மனிதனாக வாழ, மண்ணுலகம் தழைக்க வழிவகை செய்ய வேண்டும்.

உலகில் மத, இனச் சண்டைகள் நீங்கி ஒற்றுமையுடன் இன்பமாக வாழ்வதற்கு ஒரே வழி சகோதர மனப்பான்மையுடன் கூடிய யாருக்கும் தீங்கிழைக்காத, நெறியேயாகும் என்பது பாரதியின் தெளிவான கோட்பாடாகும். இதனைப்

"பாருக்குள்ளே சமத்தன்மை-தொடர்
பற்றுஞ் சகோதரத்தன்மை
யாருக்கும் தீமை செய்யாது-புவி
பொங்கும் விடுதலை செய்யும்"[6]

என்ற பாடல் புலப்படுத்துகிறது. வலியார் எளியாரை வம்புக்கழைத்துச் சண்டைகள் இட்டு மடிவதை,

"உடன் பிறந்தார்களைப் போவே-இவ்
உலகில் மனிதரெல்லோரும்
திடங்கொண்டவர் மெலிந்தோரை-இங்கு
தின்று பிழைத்திடலாமோ"[7]

என வினவுகிறார். எல்லாரும் ஒரு தாய் வயிற்றில் பிறந்த பிறப்புகள் என்ற தூய எண்ணத்துடன் வாழவேண்டும் என்று அறிவுறுத்துகிறார்.

தேச ஒற்றுமை

பாரத சமுதாயம் உலகில் ஒப்பில்லாத, புதுமையான சமுதாயம் எனத் தன் நாட்டின் பெருமைகளைப் புகழ்ந்து கூறியுள்ளார். நாட்டுப்பற்று தேசவிடுதலை, மக்களிடையே ஒற்றுமை, சமுதாய மேம்பாடு, பெண்கள் முன்னேற்றம் எனத் தன் வாழ்க்கையை அர்ப்பணித்தவர். "நாடு மேனிலை எய்தவும் நாட்டு மக்கள் நலமுற்று வாழவும் வழிகாட்டுவதற்காகவே அவர் கவிதைகளும் வசனமும் எழுதினார்"[8] என்கிறார் வல்லிக்கண்ணன். தென்னகத்தில் பிறந்து வடக்கே காசியிலும் வாசம் செய்தவர். இந்தியச் சதந்திரப் போராட்டத்தின்போது பல மாநிலங்களில் நடந்த மாநாடுகளில் பங்கெடுத்துக் கொண்டவர். இந்திய மக்கள் ஒன்றுப்பட்டுப் போராடி விடுதலையடைந்து ஒற்றுமையோடு வாழவெண்டுமென அழுத்தமாகக் குரல் கொடுத்தவர்.

"ஒன்றென்று கொட்டுமுரசே-அன்பில்
ஓங்கென்று கொட்டு முரசே
நன்றென்று கொட்டு முரசே இந்த
நாளில மாந்தருக் கெல்லாம்"[9]

என இந்திய மக்கள் அனைவரும் ஒன்றே. ஒருவருக்கொருவர் உண்மையான அன்பு செலுத்த வேண்டுமென்கிறார். இந்தியாவில் பிறந்த அனைவரும் பாரதத் தாயின் பிள்ளைகள், ஒரு தாயின் பிள்ளைகளுக்குள் மறந்தும் மதத்தால், இனத்தால், சாதியால் வேற்றுமை பாராட்டுதல் கூடாது என்கிறார்.

"கிறிஸ்துவர்களின் பார்ஸிகளாயினும், முகமதியராயினும் எங்கிருந்து வந்து எந்த இஷ்ட தெய்வத்தைக் கொண்டாடிய போதிலும் பாரத பூமியிலே பிறந்து, வளர்ந்து இதையே சரணாகக் கொண்ட மனிதர்களையெல்லாம் பாரத சாதியிலே சேர்த்துக் கணக்கிட வேண்டும். இது ஒரே ஜாதி பிரிக்க முடியாதது. அழிவில்லாதது"[10] *என்கிறார்.*

மக்கள் தங்களுக்குள் இனப்பிரிவினை பாராட்டுகளால் வரும் விளைவு அழிவாகத்தான் இருக்குமென்பதை,

"நாலு வகுப்புமிங் கொன்றே-இந்த
நான்கின் ஒன்று குறைந்தால்
வேலை தவறிச் சிதைந்தே-செத்து
வீழ்ந்திடு மானிடச் சாதி"[11]

என எச்சரிக்கிறார்.

தமிழக ஒற்றுமை

அமைதிப் பூங்காவான தமிழகத்தில் தற்பொழுது எங்கு நோக்கினும், மத, சாதிய கலவரங்களும் மோதல்களும் உள்ளன. சாதியச் சங்கங்களும், காட்சிகளும், புற்றீசல்போல் முளைக்கின்றன. சாதிய அமைப்புகளின் ஊர்வலங்கள், சண்டைகள் போன்றவற்றால் பொது மக்களின் இயல்பு வாழ்க்கைப் பாதிப்புக்குள்ளாகிறது. அரசு மற்றும் தனியார் உடைமைகள் சேதப்படுத்தப்படுகின்றன. தமிழகத்தில் நீண்ட காலமாகப் பிரிவுகள் பல இருந்த போதிலும் தற்பொழுது நிகழ்வதுபோலச் சண்டைகளும், மோதல்களும் நடந்ததாக இல்லை. பாரதி காலத்தில் இது போன்ற போராட்டங்கள் ஓரளவு இருந்துள்ளன என்பதை,

"ஜாதிச் சண்டை போச்சோ?-உங்கள்
சமயச் சண்டை போச்சோ"[12]

எனக் கேட்பதன்வழி அறிய முடிகிறது. எதிர்காலத்தில் இனங்களுக்கிடையே ஆதிக்க மனப்பான்மை, அரசியல் மற்றும் சமூக மோதல்கள் ஏற்பட்டுப் பிரிவினை வாதத்தால் ஒற்றுமைக்குக் குந்தகம் விளையும் என்பதை உணர்ந்த பாரதி, இனப் போராட்டங்களின் ஆபத்து குறித்து எச்சரிக்கை மணி அடிப்பதுபோல,

"ஜாதி வேற்றுமைகளை வளர்க்காதே ஜாதி இரண்டொழிய வேறில்லை என்ற பழந்தமிழ் வாக்கியத்தை வேதமாக் கொள்"13

என அறிவுறுத்துகிறார். சாதியை நோய்க்கு ஒப்பாகக் கொள்கிறார்.

மருந்துகள் கற்ற மருத்துவர்கள் தமிழ்ச் சாதியின் நோய்க்குத் தலையசைத் தேகினார் என்று சாதிப்பிரிவுகளின் தீய விளைவுகளுக்குத் தீர்வு காண விரைகிறார். ஆறில் ஒரு பங்கு எனும் கவிதையில் "நாம் நமக்குள்ளே ஒரு பகுதியினை நீசர்கள் என்று பாவித்தோம். தீண்டாத வகுப்பினர் என்று பாவித்தோம் தீண்டாத வகுப்பினர் என்று ஒதுக்கினோம். இப்போது நம்மெல்லோரையும் உலகத்தார் இழிந்த நீசர்களாகக் கருதுகிறார்கள்"[14]எனத் தம் காலத்திலிருந்த ஜாதியால் வேற்றுமை காணும் போக்கை இடித்துரைக்கிறார்.

குழந்தைகள் வளர்ந்து பெரியவர்களாய் வரும் காலத்திலாவது, இச்சாதிய வேறுபாடுகள் நீங்கும் என்ற நம்பிக்கையில்,

"சாதிகள் இல்லையடி பாப்பா
குலத் தாழ்ச்சி உயர்ச்சி சொல்லல் பாவம்"

எனக் குழந்தைகளின் நெஞ்சங்களில் இக்கருத்தைப் பதிய வைத்துள்ளார். சாதியப் பிரிவுகளை உடைத்து நொறுக்கி ஒற்றுமையுடன் கூடியிருக்கின்ற,

"தகரென்று கொட்டு முரசே! பொய்மைச்
சாதிக் கொடுமைகள் வேண்டாம்
அன்பு தன்னிற் செழித்திடும் வையம்
ஒன்றுண்டு மானுட சாதி-பயின்று
உண்மைகள் கண்டவர் இன்பங்கள் சேர்வார்"[15]

என்று சாதிகளை ஒழிப்பதன் வாயிலாகக் கிட்டும் பயன்பாடுகளைப் பாரதியார் குறிப்பிடுகின்றார்.

நிறைவுரை

இத்தரணியில் மக்கள் ஒன்றுபட்டு அமைதியாகவும், இன்பமாகவும், வளமாகவும் வாழத் தடையாக இருப்பவை மதங்களாலும், சாதிகளாலும் எழும் இனப் போராட்டங்களே என்பது பாரதியின் தீர்க்கமான எண்ணமாகும். உலகளவில் வர்க்கப்பிரிவு, நிறவேற்றுமை இந்தியாவில் மதவாதம், தமிழகத்தில் சாதிப்பிரிவுகள் மேலோங்கியிருப்பதைக் கண்டு வருந்தியுள்ளார். இவ்வினப் பிரிவுகளால் எழும் மோதல்களைக் களைந்து உலக மக்கள் அனைவரும் சகோதரத்துவத்துடன்

வாழ்வதற்கு பாரதியாரின் கவிதைகள் வழிகோலும் என்பதை இக்கட்டுரையின் வாயிலாக எடுத்துரைக்கப்படுகிறது.

சான்றெண் விளக்கம்

1. க்ரியாவின் தற்காலத் தமிழ் அகராதி, ப.120
2. வாழ்வியற் களஞ்சியம், தொகுதி–4, பக்–589–590
3. மேலது நூல், ப.587
4. பாரதியார் கவிதைகள், பக்.83–84
5. பாரதியார் கவிதைகள், புதிய ருஷ்யா, ப.133
6. பாரதியார் கவிதைகள், முரசு, ப.523
7. மேலது நூல், ப.523
8. பாரதிக்குப்பின் தமிழ் உரைநடை, வல்லிக்கண்ணன், ப.25
9. பாரதியார் கவிதைகள், முரசு, ப.524
10. பாரதியும் பாரத தேசமும், ம.ப.பெரியசாமித்தூரன், ப.87
11. பாரதியார் கவிதைகள், முரசு, ப.520
12. பாரதியார் கவிதைகள், ப.92
13. மகாகவி பாரதியார் நூற்றாண்டு மலர், அ.ம.கழகம், ப.168
14. மேலது நூல், ப.198
15. பாரதியார் கவிதைகள், ப.521–525

துணைநூற்பட்டியல்

1. க்ரியாவின் தற்காலத் தமிழ் அகராதி, க்ரியா பதிப்பகம், சென்னை–41, மறுபதிப்பு–2008.
2. வாழ்வியற் களஞ்சியம், தமிழ்ப் பல்கலைக்கழகம், தஞ்சாவூர், முதற்பதிப்பு–1986.
3. பாண்டி.மு, தேசியமும் தமிழும், கவிதா வெளியீடு, சென்னை– 17, முதற்பதிப்பு–2008.
4. பாரதம் போற்றும் பாரதியார் கவிதைகள், லியோ புக் பப்ளிஷர்ஸ், சென்னை–35, மறுபதிப்பு–2011.

■

10. மனதிலுறுதி வேண்டும்

முனைவர் மு.சித்ரப்பிரியா
தமிழ்த்துறை, உதவிப்பேராசிரியர்
தேசியக்கல்லூரி (தன்னாட்சி),
திருச்சிராப்பள்ளி–01.

முன்னுரை:

பொற்காலமாம் சங்க காலத்தில் பெண்களை அன்பு, பொறுமை, அடக்கம், தியாகம், வீரம் நிறைந்தவர்களாகவே காட்டினர். பெண்களுக்கும் எல்லா உரிமைகளும் வழங்கப்பட்டது. இதற்கு (உரிமையானவள்) என்ற சொல்லை உதாரணமாகக் கூறலாம். கல்வியிற் சிறந்த பல பெண்பாற் புலவர்களும் வாழ்ந்தனர். பெண்களின் வீரத்தைப் 'புறநானூறு எடுத்தியம்பும்' ஆனாலும் பரத்தையிற் பிரிவு ஆணாதிக்கத்தை எடுத்தியம்புவதாகவே உள்ளது.

நாட்டின் நதிகள் அனைத்திற்கும் பெண்ணின் பெயர்கள் சூட்டி வழிப்பட்டனர். புலவர் பெருமக்கள் பெண்மைக்கு ஏற்றம் தந்து பாராட்டி, புகழ்ந்து, போற்றிப் பாதுகாப்பதாக இருந்தாலும் பெண்கள் உரிமையின்றி அடிமை நிலையில் இருந்ததை இலக்கியங்கள் வழி அறிய முடிகிறது.

மன்னர்கள் பல பெண்களை மணந்தனர். பெண் அந்தப்புர அழகியாக, நாட்டியப் பெண்ணாக கணிகையராக அவதாரம் எடுத்து அவதியுற்றாள். குழந்தை மணம் செய்து பெண்களைப் போகப் பொருளாக மாற்றி, கைம்மை நோன்பு, சதி எனும் உடன்கட்டை ஏறுதல் போன்ற கொடுமைகள் நடந்த காலத்தில்,

> **''சேற்றி லேபுதி தாகமு ளைத்ததோர்**
> **செய்யத் தாமரைத் தேமலர் போலொளி**
> **தோற்றி நின்றனை பாரத நாட்டிலே''**

எனப் புதுமைப் பெண்ணின் புரட்சிக்கு வித்திட்டவர் பாரதியார்.

பெண்கல்வி:

பாரதி வாழ்ந்த காலம் பெண்ணுரிமை மறுக்கப்பட்ட காலம் இன்று பெண்ணுக்கு கிடைத்திருக்கும் உரிமைகள் பலரின் உழைப்பு மற்றும் தியாகம். எந்த உரிமையும் இல்லாமல், பெண்கல்வி மறுக்கப்பட்டு வீட்டுச்சிறையில் அடைக்கப்பட்ட காலத்தில் பெண்ணடிமைக்கு முக்கியக் காரணம் பெண் கல்வியின்மையே என்றுணர்ந்த பாரதி,

"நிலத்தின் தன்மை பயிர்க்குள தாகும்
நீசத்தொண்டும் மடமையும் கொண்டதாய்
தலத்தில் மாண்புயர் மக்களைப் பெற்றிடல்
சாலவே யரியாதவதோர் செய்தியாம்"

என்ற வரிகளைச் சுட்டுகிறார். பெண்கல்வி அறியாமையைப் போக்கி அக்குடும்ப வாரிசுகளையும் மேன்மையுறச் செய்யும்.

"பெண்கள் அறிவை வளர்த்தால் - வையம்
பேதமை அற்றிடும் காணீர்"

உலகம் பேதமை நீங்கி வாழ பெண் கல்வி முக்கியம் என்பதை பாரதி விளக்கியுள்ளார்.

கற்புநிலை:

'அச்சம், மடம், நாணம், பயிர்ப்பு' பெண்ணுக்கு வேண்டும். இத்தகைய பெண்களே கற்புடைவர்கள் என்று ஆணாதிக்கச் சமூகம் அடக்குமுறை ஆயுதமாகக் 'கற்பு' என்ற வார்த்தையை வழங்கியது. அவ்வொழுக்கம் பெண்ணுக்கு மட்டுமே உரிமையுடையதாகக் கூறிய நிலையில்,

"கற்பு நிலையென்று சொல்லவந்தார் - இரு
கட்சிக்கும் அஃது பொதுவில் வைப்போம்"

என்றும்,

"ஆணெல்லாம் கற்பைவிட்டுத் தவறு செய்தால்
அப்பொழுது பெண்மையுங்கற் பழிந்திடாதோ
நாணமற்ற வார்த்தையன்றோ? வீட்டைசுட்டால்
நலமான கூரையுந்தான் எரிந்திடாதோ"

என்றும் முழங்கியவர் பாரதியார்.

இல்லறம் என்னும் பெண்ணறம்:

மகாசக்தியான பெண் சிறந்தால் குடும்பமும் சிறக்கும். அவளே இல்லற வாழ்க்கையின் உயிர்நாடி அவள்

இல்லையென்றால் மனித சமுதாயமே இல்லை. தாய்மை, ஆண்டவன் பெண்மைக்கு வழங்கிய வரப்பிரசாதம். இல்லறம் என்பது பெண்ணறம் என்பதை,

"காதலொருவனைக் கைப்பிடித்தே யவன்
காரிம் யாவினும் கைகொடுத்து
மாதர றங்கள் பழமையைக் காட்டிலும்
மாட்சி பெறச் செய்து வாழ்வமடி..."

என்ற வரிகள் மூலம் வலியுறுத்தியுள்ளார். பெண்மை என்பது தன்மை, இன்பம், அன்பு, நற்புண்ணியம், அமைதி, ஆசை, காதல் என்பனவற்றைத் தரவல்லது. பெண்மை துன்பம் தீர்த்து இன்பம் சேர்க்கும் வலிமை தந்து வான்புகழ் கொள்ளும். மானம் காத்து மாட்சிமை நல்கும் என்பதைத் தன் கவிதைகளின் வழி உணர்த்தியவர் பாரதியார்.

"வலிமை சேர்ப்பது தாய்முலைப் பாலடா
மானஞ் சேர்க்கும் மனைவியின் வார்த்தைகள்"

பல்துறை வல்லுநர்கள்:

இன்று பெண்கள் அரசியல், மருத்துவம், தொழில்துறை, விஞ்ஞானம், காவல்துறை போன்ற பல்வேறு துறைகளில் சிறந்த வல்லுநர்களாக விளங்கி வருகின்றனர். இவையனைத்தும் நடைபெற வேண்டும். அப்பொழுதுதான் சமுதாயத்தில் பெண்களுக்கு விடுதலை, சமத்துவம், நாகரிகம், விழிப்புணர்வு, புகழ், மதிப்பு போன்ற அனைத்தம் கிடைக்கும் என்று விரும்பியவர் பாரதி.

"சாத்திரங்கள் பற்பல கற்பராம்
சவுரியங்கள் பலபல செய்வராம்
மூத்த பொய்மைகள் யாவும் அழிப்பராம்
மூடக்கட்டுகள் யாவுந் தகர்ப்பராம்"

சரிநிகர்:

பெண் மூடப்பழக்க வழக்கங்களுக்கும் முடக்கிவைக்கும் மரபுக் கட்டுகளுக்கும் எதிரானவள் என்றாலும் பழம் மரபான குடும்ப அமைப்பிற்கு எதிரானவள் அல்லள். புதுமைப் பெண்களின் விருப்பம் ஆணாதிக்கம் அற்ற ஆண்-பெண் சமத்துவ சமூகத்தை உருவாக்குவதேயாகும். இதையே,

"ஆணும் பெண்ணும் நிகரெனக் கொள்வதால்
அறிவில் ஓங்கி இவ்வையம் தழைக்குமாம்"

என்று பாரதியும் குறிப்பிடுகிறார்.

முடிவுரை:

பாரதி கண்ட புதுமைப்பெண் இன்று வலம்வந்து கொண்டிருக்கிறாள்.

"நிமிர்ந்த நன்னடை நேர்கொண்ட பார்வையும்
நிலத்தில் யார்க்கும் அஞ்சாத நெறிகளும்"

கொண்டு,

"பட்டங்கள் ஆள்வதும் சட்டங்கள் செய்வதும்
பாரினில் பெண்கள் நடத்த வந்தோம்"

என்றுகூறி வீரநடை போட்டு நடந்தாலும் பெண்களின் தன்நிறைவு என்பது பாலியல் வன்கொடுமைகள் நீங்கும் நாளிலேயே கிட்டும்தனையுணர்ந்தே பாரதி,

"மனதிலுறுதி வேண்டும்
வாக்கினி லேயினிமை வேண்டும்
நினைவு நல்லது வேண்டும்....
கனவு மெய்ப்பட வேண்டும்
பெண் விடுதலை வேண்டும்
பெரிய கடவுள் காக்க வேண்டும்"

என்று முழங்கியுள்ளார்.

■

11. பாட்டுக்கொரு புலவனின் இன்பம்

கு.சிந்து,
உதவிப்பேராசிரியர், தமிழ்த்துறை,
தேசியக்கல்லூரி (தன்னாட்சி), திருச்சிராப்பள்ளி– 01

முன்னுரை:

சுதந்திரப் போராட்டத்தில் கனல் தெறிக்கும் விடுதலைப் போராட்டக் கவிதைகள் வாயிலாக மக்களின் மனதில் நீங்கா இடம்பிடித்தவர் பாட்டுக்கொரு புலவன் பாரதி. தமிழ் மொழியின் எழுத்துக்களால் சமூகத்தின் தலையெழுத்தையே மாற்ற நினைத்த போராளி. தன்னுடைய தேசிய உணர்வுள்ளப் பாடல்களால் நாட்டு மக்களை ஒருங்கிணைத்த காரணத்தால் தேசியக்கவியாகவும் போற்றப்பட்டார். பாரதியின் ஒரு குறிப்பிட்ட எல்லைக்குட்படாமல் பரந்த இந்த பாரதத்தின் சிந்தனைகளுக்குத் திறவு கோலாகவும் விளங்கியதால் 'மகாகவி'யாகவும் போற்றப்படும் பெருமைக்குரியவராவார். இவரின் நோக்கம் சுயநலப்பட்டது அன்று பொதுநலம் மிகுந்தது. பிறர் வாழ்தல், பிறர் துயர் தீர்த்தல், பிறர் நலம் வேண்டுதல் என தனக்குட்படாதவற்றையே இன்பமாகக் கருதினார்.

பாரதி வாழ்ந்த கால சூழலில் இந்தியாவில் "விடுதலை வேட்கை மேலோங்கி இருந்தது. சமுதாய சீர்கேடுகள் மலிந்திருந்தது இதனை சீர் செய்ய 100 ஆண்டுகள் வாழ இறைவனிடம் விண்ணப்பிக்கிறார். ஆனால் தாம் வாழ்ந்ததோ வெறும் 39 ஆண்டுகள். மட்டுமே. குறைவான ஆயுளோடு நிறைவான புகழோடு வாழ்ந்த தன்னிகரில்லாத மனிதன். "பாரதிக்கு நிகர் பாரதி மட்டுமே" இவனை உலகம் போற்றியது அவனின் நல்ல சிந்தனைகளுக்கு மட்டுந்தான். ஆகவே இக்கட்டுரையில் பாரதி கண்ட இன்பம் தன்நலம் சேர்ந்ததா பொதுநலம் மிகுந்ததா என்பதைக் கீழ்க் கண்ட கட்டுரையின் வழிக்காண்போம்.

மொழியால் பெற்ற இன்பம்

பாரதி கவிஞன் மட்டுமல்ல ஒரு சிறந்த எழுத்தாளர். அவரை எழுத்தாளர் ஆக்கியது அவரின் மொழிப்பற்று

பலமொழி கற்ற பாரதி தமிழ் மீது தீராத தாகம் கொண்டார். தன் எண்ணத்தை வெளியிட பேனா முனை ஒன்றே அவருக்கு வழிகாட்டியாக இருந்தது. அவர் 'இந்தியா' பத்திரிக்கைக்குப் பொறுப்பாளராக இருந்தார். அப்பொழுது சென்னையிலிருந்து நாட்டு நடப்புகளை எடுத்துக்கூறி மக்களுக்கு விழிப்புணர்வு ஏற்படுத்தினார்.

அப்பொழுது அவருக்கு கடும் எதிர்ப்பு கிளம்பியது. பிரிட்டிஸார் பாரதியைக் குறிவைத்து இந்தியா பத்திரிக்கையை வெளியிடும் நபரைப் பிடிவாரண்டுடன் கைது செய்யலாம் என்றது அதனால் நண்பர்கள் கூற்றுக்கிணங்க தன் சொந்த ஊஏரான புதுச்சேரியிலிருந்து தம் பணியைத் தொடர்ந்தார். அங்கனம் 'இந்தியா' பத்திரிக்கையின் எழுத்துக்கள் பிரிட்டிஸாரை வெறுப்பேற்றியது. இவ்வாறு கடும் எதிர்பிலும் பாரதியின் மொழிப்பற்றை நாம் தெரிந்து கொள்ள முடிகிறது. இதனையே,

"யாறெிந்த மொழிகளிலே தமிழ் மொழி போல்
இனிதாவது எங்கும் காணோம்"

என்று மொழியை இனிமையாகச் சுவைத்துப் பாடிய பாடல்கள் மூலம் அறியமுடிகிறது. பல மொழிகள் கற்றாலும் தமிழ்மொழியே அவருக்கு வீரத்தை ஊட்டியது. பாரதிக்கு மட்டுமல்ல அவரின் மனைவி செல்லமாளும் "பாரதி சரித்திரம்" என்ற நூலில் தன் எண்ணத்தை வெளிப்படுத்தியிருக்கிறார். "பாரதி ஓட்டலில் சாப்பிடமாட்டாராம். கையில் நாலனா இருந்தால் வாழைப்பழம் வாங்கி எல்லோரும் பசியாறுவோம். பால்காரன் கடனாக பால்விடுவான் அந்தப்பாலைக் குடித்துவிட்டு சும்மா இருப்போம். இப்படியும் சில நாள் கழியும் அரிசி இல்லையென்றால் அவருக்கு கோபம் வரும் அப்பொழுது கொடும்பசியை போக்க "அகரம் சிகரம்" என்று சொல்லி அவரின் பசியை ஆற்றுவார். இவ்வாறு தமிழ் மீது கொண்ட பற்றினால் வறுமையில் மூழ்கித் தமிழ்மொழியால் இளைப்பாறினார் பாரதி.

சிட்டுக்குருவியால் பெற்ற இன்பம்

பாரதி புதுச்சேரியில் 'இந்தியா' பத்திரிக்கையின் பொறுப்பாசிரியராக இருந்தபோது அப்பத்திரிக்கையின் தலைமையாசிரியர் மண்டபம் சீனிவாச ஆச்சாரியார் அவரின் 10 வயது மகள் யதுகிரி ஒருமுறை பாரதி இல்லாத சமயத்தில் யதுகிரி அவரின் வீட்டிற்குச் சென்றாள் வருத்தமாக இருந்த பாரதியின் மனைவியைக் கண்டு வினவியபோது. பாரதியின்

மீது இருந்த தீராக் கோபத்தை எடுத்துக் கூறலானாள். வீட்டில் அரிசியில்லை பசிக்கும், பட்டினிக்கும் இடையே நாங்கள் போராடி கொண்டிருக்கும் போது என் கணவரின் செயலே இருக்கிறது. சமயலுக்கு இருந்த அரிசியைச் சிறிது எடுத்து வெளியே திரிந்தக் குருவிகளுக்குத் தூவி மகிழ்ந்துக் கொண்டிருந்தார்.

அதனைக் கண்டவாறு ரசித்துப் பாடி மகிழ்ந்து கொண்டிருந்தார். நூான் சென்று அவரிடம் ஏன் இவ்வாறு செய்தார்கள் என்று வினவியபோது கண்கள் திறந்திருந்தும் நாம் சிட்டுக்குருவிகளை ரசிக்கவில்லை என்றால் நாம் தான் மூடர்கள் இந்தப் பறவையிடம் இருந்து நாம் கற்றுக் கொள்ள வேண்டியவை பல இருக்கின்றது. தன்னிடம் பாரதி கூறியதை யதுகிரியிடம் செல்லம்மாள் எடுத்துரைத்தாள்.

"விட்டு விடுதலை யாகிநிற் பாயிந்தச்
சிட்டுக் குருவியைப் போல
எட்டுதிசையும் பறந்து திரிகுவை
ஏறியக் காற்றில் விரைவோடு"

என பசியிலும் பாடல் பாடி மகிழ்ந்து லயித்துப்போனார். தூரத்திலிருந்த செல்லமாளும் ஐந்தறிவுக் கொண்ட சிட்டுக் குருவியைக் கண்டு ரசித்து மகிழ்ந்தார். பறவைகளின் சுதந்திரம் குறித்தும் அதனிடமிருந்தும் மானிடர்கள் கற்றுக் கொள்ள வேண்டி, பாரதி எழுதிய வரிகள் இன்னும் காலம் கடந்து நிற்கிறது. இன்று இந்த சிட்டுக்குருவி இனம் ஆக அழிந்துவிட்டது காரணம் நம்முடைய நாகரீக வளர்ச்சியும், நகரமயமாதலுமே ஆகும். பாரதி போல நாமும் பேணி வளர்த்தோமேயானால் இந்த சிறிய இனம் அழிந்திருக்க வாய்ப்பே இருக்காது. மேலேக் கூறிய வரிகள் வறுமையின் பிடியில், பசியின் கொடுமையில் பிறந்த புரட்சிகரமான வரிகள். இதனையே கட்டுரையில் சேர்த்து அனுப்பி செல்லமாளின் ஆசையை நிறைவேற்றி வயிறு நிறைந்து மனம் நெகிழ்ந்தார்கள். பாரதியின் இன்பம் பிற உயிர்களிடமே என்பது இந்த சான்றுகளின் மூலம் அறிய முடிகிறது.

"காக்கை குருவி எங்கள் ஜாதி - நீள்
கடலும் மலையும் எங்கள் கூட்டம்"

என பறவையினங்களையும் மனித இனத்தோடு சேர்த்துப் பார்த்து இன்பம் கண்டான் பாரதி.

மானுடத்தில் பெற்ற இன்பம்

"பாரில் அதிசயம் பாரதி" என்பார் கவிஞர் சுத்தானந்த பாரதியார். பாரதி எப்படி அதிசயக் கவியோ அதுபோல அவர் ஓர்அபூர்வ மானுடராகவே தான் வாழ்ந்திருந்தார் பாரதிக்கு வரலாறு எழுந்த பின்னணி சரித்திரமாகும் பாரதி மறைந்த பின்னர் தான் பாரதிக்கு வரலாறுகள் வெளிவந்தன என்பது கவனிக்கத்தக்கது ஆகும். வாழ்ந்த காலத்தில் புறக்கணிக்கப்பட்டவன் இறந்தபிறகு பாரில் போற்றப்பட்டான். பாரதி தன்னைப் பற்றி கூறும்போது,

"சுடர்மிகு அறிவுடன் படைத்துவிட்டாய்
வல்லமை தாராயோ - இந்த
மாநிலம் பயினுற வாழ்வதற்கே"

எனப் பாடி மகிழ்ந்தார். ஒரு தனி மனிதன் மீது அதீத நம்பிக்கைக் கொண்டார்.

"தனி ஒரு மனிதனுக்கு உணவில்லை எனில்
இந்த ஜகத்தினை அழித்திடுவோம்"

என்ற கூற்றினால் பாரதியின் மானிடம் பற்றிய எண்ணத்தைப் புரிந்து கொள்ள முடிகிறது. பாரதி தன்னை அறிவுடன் படைத்துவிட்டதால், பூமியில் உள்ள மக்களை நல்ல முறையில் பயனுற வாழ்வதற்கு ஏற்றப்படி வல்லமையை தருமாறு கேட்கிறார். மனிதனுக்கு மனிதன் உதவுதல் என்பது புலப்படுகிறது. மனிதன் கொண்டு மானுடத்தை வாழ வழிக்காட்டுகிறார் பாரதி. **"பாரதியின் இன்பம் மானுடர்கள் மாநிலத்தில் பயனுற வாழ்வதற்கே"** *என்பது அவரின் இன்பமாக இருந்தது.*

வழிபாட்டில் இன்பம்

பாரதி வழிபாட்டில் மிகுந்த இன்பம் பெறுகிறார். அது தனக்கான வேண்டுதல் அன்று பிறருக்காக காளியிடம் வேண்டும் பொழுது,

"கர்ம யோகம் ஒன்றே உலகில்
காக்கு மென்னும் வேதம்"

என வேண்டுகிறார். காளியிடம் பொருள் பெற்று உலகின் துன்பங்களையும், வறுமையையும் மாய்க்கத் துடித்தார். இது இவரின் வைராக்கிய உணர்வைக் காட்டுகிறது. நல்ல சிந்தனை, நல்ல உள்ளம் உடையவர்கள் இறைவனை உறுதியாக நம்பினார்கள். உயிராக ஏற்றுக்கொண்டனர். மண்ணையும்,

மைந்தர்களையும் உயிராக மதித்த பாரதி இந்த உலகில் உயிரினங்கள் இன்பமுற்று வாழ இறைவனை வேண்டுகிறார்.

''கேட்கா வரத்தை கேட்க நான் துணிந்தேன்
மண் மீதுள்ள மக்கள் பறவைகள்
விலங்குகள் பூச்சிகள் புற்பூண்டு மரங்கள்
யாவு மென வினையால் இடும்பை தீர்ந்தே
இன்பமுற்று அன்புடன் இணங்கி வாழ்ந்திடவே''

என தன்னுடைய பாடலால் விநாயகர் நான்மணிமாலையில் வேண்டிப் பாடுகிறார். பாரதியின் இன்பம் வழிபாட்டிலும் பிறரின் உயர்வுக்காகவும், வாழ்வுக்காகவும் இருந்தது என அறிய முடிகிறது.

இறப்பினும் இன்பம் காண துடித்த பாரதி

பாரதி தன் இறப்பு பற்றியும் தன் பாடல்களை வெளிப்படுத்தி எண்ணத்தைக் காட்டுகிறார்.

''காலா! என்றன்
காலருகே வாடா! சற்றே உனை மிதிக்கிறேன்
ஆலாலம் உண்டவன் அடி சரணென்ற
மார்கண்டேயன் - தனது
ஆவி கவரப் போய் நீ பட்ட பாட்டினை
அறிந்திடுவேன்''

எனப்பாடி மகிழ்கிறார். தன் இறப்பையும் எதிர்பார்ப்பையும் அழகாக எடுத்தியம்புகிறார். பாரதி தன் பூத உடம்பு நீங்கினாலும் புகழுடம்பு நிலைத்து நிற்க விரும்பி நீண்ட புகழ் வேண்டி நின்றார். அவரின் கனவும் நனவாகி விட்டது. இன்றும் நூற்றாண்டைக் கடந்து எல்லோரும் பாரதியை நினைக்காத நாளில்லை. அவருடைய பாடலால் சிந்தனை வரியால் உலாவிக் கொண்டு தான் இருக்கிறோம்.

பாடலால் பெற்ற இன்பம்

கவிஞன் உள்ளங்கனிந்து உவந்து போதெல்லாம் பாட்டு பிறக்கும். கற்றல் சிறக்கும். ஆனால் பாரதிக்கு உலகம் வறுமையால் வாடிய போதும், தீண்டாமையால் நசுக்கப்பட்ட போதிலும், வறுமையிலும், வளமையிலும், அவருக்கு பலப் புதுப்பாடல்கள் பிறந்தது. சிறு வயதிலே பாடல் பாடும் திறம் பெற்றதாலேயே 'பாரதி' என பெயர் பெற்றார்.

“அச்சமில்லை அச்சமில்லை அச்சமென்ப தில்லையே
உச்சிமீத வானிடிந்து வீழுகின்ற போதிலும்
அச்சமில்லை அச்சமில்லை அச்சமென்பதில்லையே”

என புரட்சிகரமான பலபாடல்களால் இன்பம் பெற்று இந்த வையத்தை அடிமைத் தனத்திலிருந்து மீட்டெடுத்தப் பெருமை பாரதியேச் சேரும். விண்ணையும், மண்ணையும் பாடினான். அவன் பாடிய வாய் மனிதனையும் பாடியது வண்ணப் பறவைகளையும் பாடியது. அதனால் தான் பாட்டுக்கொரு புலவனாய் இருந்து நாட்டுக்கெல்லாம் பாடி உயர்ந்தான் பாரதி.

முடிவு

“பாட்டுக் கொரு புலவன் பாரதி”யின் இன்பம் பற்றி இக்கட்டுரையின் வழி அறிய முடிகிறது. பாரதி அறிவு, தெளிவு, இவையிரண்டும் மனிதனுக்கு தேவை என்கிறார். அறிவு இருந்தால் தெளிவு பிறக்கும். தெளிவு பிறந்தால் எல்லாவற்றையும் ஒன்றாகக் காணும் பண்பு வளரும். எனவே பாரதியின் இன்பம் பிறர் நலத்தில் தான் அதிகம். சுயநலத்தில் இல்லை.

■

12. பாரதி போற்றும் பெண்மை

முனைவர் சு. சீலா
உதவிப்பேராசிரியர், தமிழாய்வுத்துறை,
தேசியக்கல்லூரி (தன்னாட்சி), திருச்சிராப்பள்ளி.

முன்னுரை

பழைமையான சாத்திரங்களையும் வேதாந்தங்களையும் கொண்டு வளர்த்த மரபுகளை தம் கவியால் மாற்றியபெருமைக்கு உரியவர் மகாகவி பாரதியார். பெண் சுதந்திரம், பெண் கல்வி, தாய்மை உணர்வு, நிமிர்ந்த நன்னடை, செம்மை மாதர் திறம், ஆண் பெண் சமம், பாரதமாதா, தெய்வபக்தி முதலானவற்றைப் பாடினார் என்பதை கூறுவதே இக்கட்டுரையின் நோக்கம் ஆகும்.

பெண் சுதந்திரம்

பெண்ணியம் பற்றிப்பலரும் உறக்கப் பேசும் இக்கால கட்டத்திலும் பாரதி மறைந்து பல ஆண்டுகள் ஆனபிறகும் கூட பெண்கள் இன்னும் முழுமையாக விடுதலை பெற இயலாத நிலைதான் நிலாவிக் கொண்டிருக்கிறது, பெண்விடுதலையை அநியாயத்தை நியாயத்தால் எதிர்ப்போம், அதர்மத்தை தர்மத்தால் ஒழிப்போம் என்று காந்தியடிகள் சொன்ன வழியைப் பின்பற்ற வேண்டும் எங்கிறார் பாரதி.

‘ஏட்டையும் பெண்கள் தொடுவது தீமையென
றெண்ணி யிருந்தவர் மாய்ந்து விட்டார்
வீட்டுக்குள்ளே பெண்ணை பூட்டி வைப்போமென்ற
விந்தை மனிதர் தலை கவிழ்ந்தார்
.....
வெட்டி விட்டோமென்று கும்மியடி’ (பெண்விடுதலைக் கும்மி)

‘மாதர் தம்மை இழிவு செய்யும்
மடமையைக் கொளுத்துவோம் பாரதியார் கவிதைகள் (ப.54)

என்கிறார் பாரதியார். விடுதலை என்பது தானே கிடைக்காது, அதை பெண்களாகிய அவர்கள் வலிந்துதான் பெறவேண்டும் என்பதை

'அக்கா அக்கா என்றால் அக்காமிளகா என்ன
அக்கா கொடுக்க சுதந்திரம் கிளியே'
பாரதியார் கவிதைகள்

என்று பெண்களை போராட அழைக்கிறார். பெண்ணின் விடுதலையையும் உரிமையையும் போராடிப் பெற வேண்டும் என வீர முழக்கமிடுகிறார்.

மாதர் கல்வி

ஆண்கள் மட்டும் கல்வி கற்றால் போதாது, பெண்களும் கற்க வேண்டும் என்பதில் உறுதியாய் இருந்தவர் பாரதி

பட்டங்கள் ஆள்வதும் சட்டங்கள் செய்வதும்
பாரினில் பெண்கள் நடத்த வந்தோம்
எட்டு மறிவினில் ஆணுக்கிங்கே பெண்
இளைப்பில்லை காணென்று கும்மியடி'

(விடுதலைக்கு கும்மி)

என பெண்கள் விடுதலைக்கும்மி இசைத்தவர் பாரதி.

'ஞானம், நல்லறம், வீரசுதந்திரம்
நற்குடி பெண்கள் பேணும் குணங்களாம்'

என்று பாடினார்.

செம்மை மாதர்

ஒவ்வொரு பெண்ணும் தன்னம்பிக்கையுடன் கூடிய நிமிர்ந்த நடையும் எவர்க்கும் நாங்கள் அடிமையில்லை எனும் படியான நேர் கொண்ட பார்வையும், பிறர்போற்றும் படியான ஞானத்துடன் விளங்க வேண்டும் என்பதனை பாரதி

'நிமிர்ந்த நன்னடை நேர் கொண்ட பார்வையும்
நிலத்தில் யார்க்கு அஞ்சாத நெறிகளும்
நிமிர்ந்த ஞானச் செருக்கு மிருப்பதால்
செம்மை மாதர் திரும்புவதில் லையாம்' (பாரதியார் கவிதைகள்)

இவ்வாறு பெண்கள் முன்னேற்றத்தையும், நாட்டு முன்னேற்றத்தையும் ஒருங்கே வைத்துப் பார்த்தார் பாரதி.

ஆணும் பெண்ணும் சமம்

பெண்புத்தி பின்புத்தி, பெண்ணறிவு என்பது பெரும் பேதைமைத்தே' என்பது பழமொழி பெண் திருமணத்திற்கு முன்னால் தந்தையைச் சார்ந்திருக்க வேண்டும். திருமணம்

ஆனபிறகு கணவனைச் சார்ந்திருக்க வேண்டும் என்று கூறும் மனுநீதி, காலங்காலமாக நிலவி வரும் பெண்ணின் நிலை, இந்நிலை மாற வேண்டும், மாற்றப்பட்ட வேண்டும் என்று விரும்பியவர் பாரதி

ஆணும் பெண்ணும் நிகரெனக் கொள்வதால்
அறிவில் ஓங்கி இவ்வையம் தழைக்குமால்
பூணு நல்லறத் தோடிங்குப் பெண்ணுக்குப்
போற்று நிற்பது தாய்சிவ சக்தியாம்'

பாரதியார் கவிதைகள் ப.184

ஒரு நாணயத்தின் இருபக்கங்கள் இருப்பது போன்று ஒரு நாடு முன்னேற்றமடைய ஆணும் பெண்ணும் சமமாக இருத்தல் வேண்டும்.

தாய்மை உணர்வு

தாய், தங்கை, தாரம், மகள் எனப்பல அவதாரம் எடுப்பவர்கள் பெண்கள். அவற்றுள் தாய்மை போற்றுதலுக்குரியது. அப்போற்றுதலுக்குரிய பண்பை ஒவ்வொரு பெண்ணும் தாயாகும் போது உணர்வாள் ஒவ்வொரு வரும் தன்னைப் பெற்ற தெய்வமாகிய தாயைப் போற்ற வேண்டும். இதனை பாரதியும்

'அன்ன மூட்டிய தெய்வமணிக் கையின்
ஆணை காட்டில் அனலை விழுங்குவோம்'

பாரதியார் கவிதைகள்

என்கிறார்.

பெண்களின் பெருமையைப்பற்றி திருவள்ளுவர்

ஈன்ற பொழுதில் பெரிதுவக்கும் தன்மகனைச்
சான்றோன் எனக் கேட்டதாய்' **(குறள்-69)**

என்னும் குறளின் மூலம் எடுத்துக்காட்டுகிறார்.

தாயைத் தெய்வம் என நினைத்து விளங்குகிறவன் வேறு இந்தக் கோயிலுக்கும் போக வேண்டியதில்லை என்பதை

தாயிற் சிறந்தொரு கோயிலுமில்லை'

(கொன்றை வேந்தன் -37)

என்று ஔவையார் கூறுவதால் அறியலாம்.

முடிவுரை

மகாகவி பாரதியார் புதிய விழிப்பின் தூதுவன், தமிழ் மறுமலர்ச்சியின் தந்தை, விடுதலைப்பண் இசைத்த வானம்பாடி, பெண்களுக்கு ஏற்படும் கொடுமை கண்டு பலவாறு குரல் கொடுத்தார், பெண் சுதந்திரம், பெண்கல்வி, பெண்விடுதலை ஆணுக்குப் பெண் இளைப்பில்லை காண், என்று விடுதலைக்கும்மி பாடுகின்றார். உறங்கிக்கிடந்த தமிழ்க்கவிதைத் துறையில் ஒரு விழிப்புணர்வையும் வேகத்தையும் ஏற்படுத்தினார்.

பார்வை நூல்கள்

1. பாரதியார் கவிதைகள்
2. பாரதியார் கவிதைகள் (ப.54)
3. மேலது
4. பாரதியார் கவிதைகள்– விடுதலைக்கும்மி
5. மேலது
6. பாரதியார் கவிதைகள் ப.184
7. மேலது
8. திருக்குறள்– 69
9. கொன்றை வேந்தன்– 37

■

13. பாரதியின் ஆறில் ஒரு பங்கு சிறுகதையில் சமத்துவம்

முனைவர் இரா.சுந்தரவேல்,
உதவிப்பேராசிரியர், தமிழாய்வுத்துறை,
தேசியக்கல்லூரி (தன்னாட்சி), திருச்சிராப்பள்ளி–01.

முன்னுரை

மக்கள் அனைவரும் ஒரே சமுதாயமாக வாழவேண்டிய தேவையைப் பாரதியார் தம் படைப்பின்வழி காட்டுகிறார். நம் தேசம் முழுவளர்ச்சி பெற வேண்டுமாயின் வகுப்புவாதங்கள், மதவாதங்கள் பிரிவினை வாதங்கள் அனைத்தும் வேரறுக்கப்பட வேண்டும். உயர் வகுப்பினராகக் கருதப்படுவோர் ஆசாரங்களையும், பழமைவாதங்களையும் பற்றக்கோடாகக் கொண்டுள்ளனர். அப்பற்று காரணமாக குடும்பத்தில் உறவுச்சிதைவும், பிரிவும் ஏற்படுகிறது. ஒட்டுமொத்த சமுதாயமும் நலம்பெற வேண்டுமாயின் சமத்துவம் என்னும் ஒற்றுமையுணர்வை வளமையுறச் செய்தல் வேண்டும் பிரபஞ்சத்தில் பல்வேறு இனமக்கள் வாழ்ந்தாலும், பேசும் மொழிகள் வேறானாலும், உணர்வுகள் உயிர்கள் யாவிற்கும் ஒன்றுதான் "பிறப்பொக்கும் எல்லா உயிர்க்கும்" செய்யும் தொழிலையும், வறுமையையும் மையமிட்டு பாகுபாடு கூடாது. பாகுபாடு இல்லாத சமத்துவத்தை, 'ஆறில் ஒரு பங்கு' சிறுகதை வாயிலாக ஆராயலாம்.

விவேகமும் சன்னியாசமும்

ஆறில் ஒரு பங்கு சிறுகதையில் முதன்மைப் பாத்திரம் கோவிந்தராஜன், இவன் கதைத்தலைவன் தந்தை துவஷ்ராமசந்திர நாயுடு, தாயார், தங்கை ரத்தனம், தம்பி அரசாங்க ரெவின்யூ டிபார்ட்மென்ட் வேலை அவன் மனைவி இரண்டு குழந்தைகள். இதில் கோவிந்தராஜனைத் தவிர அனைவரும் கௌரவ பாத்திரங்கள் சென்னைப் பட்டினத்தில் புரசைவாக்கத்தில் ஓரடுக்கு மாடி, தஞ்சாவூரில் வாழ்ந்துவரும்

ராவ்பகதூர் சுந்தரராஜீ நாயுடு கோவிந்தராஜீவின் தாயாரின் ஒன்றுவிட்ட அண்ணன். அவரது மகள்கள் மீனாம்பாள் கதைத்தலைவி, வீணை மீட்டுவதில் கெட்டிக்காரி. தன் தந்தையார் போலீஸ் உத்தியோகத்தில் நேர்மையாகப் பணிசெய்து 'ராவ்பகதூர்' என்று பட்டம் பெற்றவர். தேச விடுதலைக்கு முன்பாகவே வேலையிலிருந்து விலகிவிட்டார். சிறுவயது முதலாக கோவிந்தராஜனுக்கும் மீனாம்பாளுக்கும் விவாகம் செய்ய முடிவு செய்திருந்தார்.

கோவிந்தராஜன் தன் பதினாறு வயது வரை சென்னை கிறிஷ்டியன் கல்லூரியில் படித்து கொண்டிருந்த காலத்தில் அவனுக்கு வேதகாலத்திலிருந்து ரிஷிகள் தொடங்கி காளிதாசன், சங்கராச்சாரியார், கபீர்தாஸர், சிவாஜி, ராமதாசர் காலம் வரை முன்னும் பின்னும் இந்திய தேசத்தார் அனைவரின் நெஞ்சிலும் இழிந்த மூடபக்திகளையும், இழி நாகரிகங்கள் என்ற பெயரில் அநாகரிகங்களையும் வளர்த்துவிட்டுள்ளனர். அந்தப் படுபாதக் கொள்கையிலிருந்து விலகி பிரம்மசமாஜத்தில் சேர்ந்தான். பள்ளிப்படிப்பு நிறுத்திவிட்டு யாரிடமும் சொல்லிக் கொள்ளாமல் கல்கத்தா புறப்பட்டு சென்று பிரம்மசாஜத்தாரின் மார்க்க போதனை கற்பிக்கும் பாடசாலையில் சேர்ந்து கொண்டு சில மரபுகள் பயற்சி மேற்கொண்டார்.

பிரம்ம சமாஜ உபதேசம் பெற்று பஞ்சாப், இந்துஸ்தான் உள்ளிட்ட பிரதேசங்களில் யாத்திரை மேற்கொண்டு சொந்த ஊர் புறப்பட்டான். சென்னைக்கு வந்தவுடன் பலரும் பலவிதமாகச் சாடினார்கள்.

"சாத்திரங்கள் றொன்றும் காணார்-பொய்ச்
சாத்திரப் பேய்கள் சொல்லும் வார்த்தைநம்பியே
கோத்திரம் ஒன்றாயிரம் இருந்தாலும் - ஒரு
கொள்கையிற் பிரிந்தவனைக் குலைந்திகழ்வார்
தோத்திரங்கள் சொல்லியவர் தாம் - தமைச்
சூது செய்யும் நீசர்களைப் பணிந்திடுவார்
ஆத்திரங் கொண்டே இவன் சைவன் - இவன்
அரிபக்தன் என்று பெருஞ்சண்டையிடுவார்"
(நெஞ்சு பொறுப்பதில்லையே பாடல் - 5)

பகைமைப் பாராட்டிய சுற்றத்தார். தந்தையார் துபாஷ் ராமச்சந்திர நாயுடு வெளிவேசங்களில் சாதாரண ஜனங்களைப் போல ஆசாரங்களில் அழுத்தம் கொண்டிருப்பார் போல் காட்டிக் கொண்டு வெளிப்படையாக பிரம்ம சமாஜத்தின் மீது

ஈடுபாடு கொண்டிருந்தது கோவிந்தராஜனுக்கு மகிழ்வூட்டின. தன்னுடைய தம்பி பழைய ஆசாரங்களில் ஈடுபட்டு வெறுப்பினை வளர்ந்தான்.

இந்நிலையில் தன் தந்தையார் இறந்து போய்விட ஈம கிரியைகள் நடத்துவதில் தம்பிக்கும் தனக்கும் மிகப்பெரிய பூசல். பழைய ஆசாரங்களைக் கைக்கொள்வதில் பல மத்தியஸ்தர்கள் வைத்தும் கூட ஒப்புக்கொள்ளாதவன் அவனுக்கு ஏற்றபடி கிரியைகள் செய்து கொண்டான். தனக்கு ஏற்றபடி ஒருகுருவினை வரவழைத்து பிரம்ம சமாஜ முறைகள் படி கிரியைகள் செய்தான். இந்த ஈமகிரியைப் பற்றி அறிந்த மீனாவின் அப்பாவிற்கு கோவிந்தராஜன் மீது அதிருப்தி உண்டானது.

புரசைவாக்கம் வீட்டுக்கு மீனா வரும் போதெல்லாம் அவளின் வீணை இசையை மறக்காதவன் அவளை தன் மனத்திற்குள் சுமப்பவன் அக்கறையுடன் அவள்காட்டும் பரிவினால் பிரம்மசாரிய சங்கல்பம் கொள்ளப் போகிறாயா எனக் கேட்க "வந்தே மாதரம்" என்ற பொதுவுணர்வுக்குள் இருப்பதை அறிந்து கொள்கிறான்.

இந்த தேசத்தில் நாட்டுப்பற்று இல்லை. பிற உயிர்களைப்பற்றிய புரிதல் இல்லை பழைய ஆசாரங்களில் ஈடுபட்டு மூடநம்பிக்கைகளைப் பரப்புகின்றனர். உலக பரிணாமங்களில் நான்கு யுகங்களாகப் பகுக்கப்பட்டதில் கலியுகமே நம்மை ஆள்கிறது பாண்டவர்கள் வாழ்ந்த பூமியில் அசாதரணமான சூழல் நிலவுகிறது. நம்மை போன்ற எளியவர்கள் இந்நாட்டில் வாழ்வது ஆகாத காரியமாகிவிட்டது. மிகப்பெரிய பலம் பொருந்தியவர்களாக உள்ள பொப்பிலிராஜா மகனாகவோ, ராஜாசர் ராமசாமி முதலியார் மகனாகவோ பிறக்கவேண்டும்.

இந்து மத வேட்கை உள்ளவர்களைப் பாரதி நரிக்கூட்டம் என்று கூறுகிறார். சாதாரண மக்களை வாழவைக்க இயலாத சம்பிராதய முறைகள். மீனாவையும் கோவிந்தராஜுவையும் வஞ்சிக்கிறது தன் தந்தை ராவ்பகதூர் சுந்தரராஜுலு நாயுடு. கோவிந்தராஜுவின் பிரமச்சாரியமும், பிரம்மசமாஜமும் பிடிக்காமல் போகவே தன் மகன் மீனாவை போலீஸ் இன்ஸ்பெக்டராக பணிபுரியும் மன்னார் என்பவருக்கு விவாகம் முடிக்க துணிந்து விட்டார்.

காளிதேவியின் கிருபையால் மீனா பச்சிலை உண்டு மயக்கமுற விவாகம் தடைபட்டது. மீனாவின் கடிதம் கண்டு

மனஅமைதியற்று சன்னியாசியாக கோவிந்தராஜ் துறவு பூண்டான். பிரம்மச்சாரியாக மாறினாலும் அவனது நினைவுகள் நீங்கவில்லை. அவளது நிலையை அறிந்து உண்மையாக இருந்தான். வடநாட்டில் தஞ்சம்மடைந்த கோவிந்தன் வந்தேமாதர தர்மத்தில் ஈடுபட்டு சிறைசென்றார். மக்களுக்கு பலத்தையும் ஒற்றுமையையும் ஏற்படுத்தி சுதந்திரகனலை உருவாக்கினான். அரசாங்க அதிகாரிகளின் அடக்குமுறையால் கொஞ்சம் கூட சளிப்படையவில்லை. மாற்றாக மக்களின் மீது அக்கறையும் ஆர்வமும் கொண்டான். அங்கிருந்து லாகூர் சென்றான் லாலாலஜபதிராயைக் கண்டு அவர் கூறியபடி கோசலநாட்டில் பஞ்சத்தைத் தீர்த்தான். அங்கே சென்று வாழும பெரியோர் அஸ்வினி குமரதத்தார் என்ற தேசபக்தரைப் பார்க்க ஆவல் கொண்டான். அஸ்வினி தத்தர் பள்ளர் பறையர்களை சமூக வரம்பிற்குள் சேர்த்து உயர்வுபடுத்த முயற்சி செய்வதாக கேள்வியுற்றன். அதனால் அவரைப் பார்க்க முற்பட்டான்.

கல்கத்தா சென்று அவரின் இருப்பிடம் அடைந்தான். அங்குள்ள வேலைக்காரனிடம் விசாரித்தான். இருவரும் உரையாடினர். அப்பொழுது சிலநாட்களுக்கு முன்பு தஞ்சாவூரிலிருந்து ஒரு பெண்மணி ஒருத்தி வந்தாள். அவளை 'மந்த்ராஜ்' என்று வடநாட்டார் அழைத்தனர் சென்னைப் பட்டணத்திலிருந்து வந்ததால் மதராஸ் பிரதேசத்து ஸ்திரீ என்று அழைப்பது வழக்கம். அந்த 'மந்த்ராஜ்' கூட ஆறிலொரு பங்கு ஜனங்களை ஆசாரங்களால் இப்படி வஞ்சித்து விட்டார்கள் என்றுரைக்க வாக்கால் அறிந்தேன் என்றார்.

கோவிந்தராஜனின் மனஓட்டத்தை ஒத்திருக்கின்ற அப்பெண்ணைப் பற்றித் தீசசந்திரபாபு ஒத்தவன். அவர் கூற எங்கே அந்த பென்மணியைப் பார்க்க ஆசைப்படுகிறான். மேலும் மதராஸ் யுவதி என கேள்வியுற்றதால் சற்று பதற்றமும் உண்டாயிற்று மீனாம்பாளாக இருக்குமோ என்ற நினைவு பாடாய்படுத்தியது.

அந்தப் பெண் கோவிந்தராஜ் மனநிலை அறிந்து சென்னை யுவதிபற்றி மேலும் தீசசந்திரபாபு விவரிக்கலானான். அவள் குமரியாம் அவளை தன் அத்தை மகனுக்கு விவாகம் செய்ய பெற்றோர் முடிவு செய்தனர் ஆனால் அந்த வாலிபன் "வந்தே மாதிரம்" கூட்டத்தில் சேர்ந்து விட அவள் தகப்பனார் ஒரு போலீஸ்காரனுக்கு மணமுடிக்க முனைந்தார் அவளை வீட்டிலிருந்து தப்பிக்காமல் இருக்க பாதுகாப்பு அரண்களை

அமைத்ததாகவும் அவளைப் பார்க்க கோவிந்தராஜன் வராமல் இருக்க தடைகளை ஏற்படுத்தியதாகவும். பிறகு காளிதேவி என்ற தெய்வ அனுகிரகத்தால் பச்சிலை உண்டு பயங்கர காய்சல் உண்டாகி விவாகம் தடைபட்டதாகவும், அவளுடைய காதலன் கோவிந்தராஜ் அவள் இறந்துவிட்டதாகக் கருதி சன்னியாசம் சென்றுவிட்டதாகவும் கூறினான்.

அப்பெண் இப்பொழுது எப்படி இருக்கிறான். அவளை உடனேபார்க்க வேண்டும் என்று துணிந்து அவளிடமே நடை செலவுக்குப் பணம் வாங்கி முகவரியும் அறிந்து புறப்பட்டான். காசியில் ஹனுமந்த கூட்டத்தில் தென்னாட்டவர் வரும் இடம் சிவமடம் என்று அழைக்கப்படும் அவ்விடம் சென்று மடத்துபிள்ளைகளில் ஒருவனை துணைக்கு அழைத்துக்கொண்டு நர்வா கூட்டத்திற்குச் சென்றான். தைப்பூர் பங்களாவிற்கு இரவு எழுமணிக்கு சென்று சேர்ந்தான். அங்கே குதிரை வண்டி ஒன்று நிற்கிறது. அதில் ஆங்கிலேய சிப்பாய் உடையணிந்த வங்காளி ஒருவன் அமர்ந்திருந்தான். வண்டிப்பக்கத்தில் ஒரு கிழவரும் வேறு சிலரும் நின்று கொண்டிருந்தார்கள்.

ஏற்கனவே அசுவினி குமார தத்தரை படமாக பலஇடங்களில் கண்டதால் அவனுக்கு அடையாளம் காணமுடிந்தது. அஸ்வினி தத்தர் அங்கிருந்து வேலையாளிடம் யாரோ சன்னியாசி வந்திருக்கிறார் அவரை தாழ்வாரத்திற்குள் வரச்சொல் எனக் கூறுகின்றார்.

தத்தரும் வண்டிக்காரரும் பேசிக்கொண்டது இவன் காதில் விழுந்தது. அஸ்வினி பாபுவின் கேள்விக்கு மருத்துவர் அந்தப் பெண் துர்ப்பல நிலையில் தான் இருக்கிறாள் இன்னும் இருபத்து நான்கு மணிநேரம் இருப்பது கடினம் அந்த கணம் தப்பினால் பாதிப்பில்லை என்றார்.

ஸ்தீச சந்திரபாபு பேசியது, தத்தர் பேசியது இவனுக்கு தன் காதலி மீனாதான் இவ்விடம் இருக்கிறான் என்பன அறிந்து கொள்ள முடிந்தது. தான் தத்தரிடம் நான் ஒரு பாவி மீனாம்பாள் தம்மிடம் கோவிந்தராஜன் என்ற பெயர் சொல்லியிருப்பாள் அல்லவா? இந்தப்பாவி நான் தான் என்று கூறுகிறார்.

தத்தர் கோவிந்தராஜனை மேல்மாடத்திற்கு அழைத்து செல்கிறார். நேற்றெல்லாம் உன்னை அடிக்கடி நினைத்துக் கொண்டிருந்தேன் என்று கூறி" கிழக்கு முகமாக திரும்பி உட்கார்ந்து கண்களை மூடிக்கொள்ளும் என்றார். அவன் இருகண்களையும் மூடிக்கொண்டநிலையில் அவன் நெற்றியில்

கையாலே தடவி ஏதோ முணுமுணுத்துக் கொண்டிருந்தார். தனக்கு உறக்கம் வருவது போலவும் மீனாம்பாளை பார்க்க முடியாமல் போய்விடுமோ என வருத்தமுற்றான்.

இந்த மாயையில் இருந்து விடுபட முயற்சித்தான். கண்களைத் திறக்க முடியவில்லை. மயக்கம் மேலும் அதிகமாக அப்படியே படுத்து உறங்கிவிட்டான். அவன் வழித்த போதுதான் தெரிந்து இரண்டு நாட்கள் உறங்கிகிடந்தது.

தன்னருகில் இருந்த சேவகனிடம் மீனா எங்கே எனகேட்டான் அவன் ஒன்றும் தெரியாது என மறுமொழி கூறினான். பொல்லாத கோபம் உடல் அயர்வினால் அவனால் ஏதும் செய்ய முடியாமல் தடுமாறுகின்றான். அஸ்வினி பாபு அவனிருந்த அறைக்குள் வந்து மீனா பூரண சௌக்கியமாகிவிட்டாள். இனி அவளுக்கு "ஓரைம்பது வருடத்திற்குச் சமுத்திரத்தில் தள்ளனாலும் அவளுக்கு எவ்வித தீங்கும் கிடையாது" என்றார்.

மறுமொழியாக, கோவிந்தராஜன் அப்படியானால் நான் போகிறேன். அவள் இறந்து போகப்போகிறாள் என்ற எண்ணத்தில்தான் என் விரதத்தை மறந்து அவளைப் பார்க்க வந்தேன். அவளுடன் இருக்க வேண்டும் என்ற எண்ணம் எனக்கில்லை. நான் போய் வருகிறேன் என்று சொன்னான்.

அஸ்வினி தத்தர் சேவகனிடம் கோவிந்தராஜனுக்கு பால் கொணர்ந்து கொடுக்கும்படி வேண்டினான். அந்தப் பாலை உட்கொண்டதும் அவனுக்கு புத்துணர்வு ஏற்பட்டது. உடல் தளர்ச்சி நீங்கி தெளிவும் சௌந்தர்யமும் பெற்றான். இப்பொழுது என்ன செய்ய போகிறாய் என்று கேட்ட தத்தருக்கு அவளை ஒருமுறை பார்த்துவிட்டு அவனிடம் விடைபெற்றுக் கொண்டு செல்கிறேன் என்றான்.

மீனா உனக்கு எழுதிய கடிதத்தில் வேண்டாத ஒருவருடன் விவாகம் நடக்கப்போகிறது என்ற தாபத்தால் படபடப்பில் மறுஜென்மம் என்றெல்லாம் எழுதிவிட்டாள். நீயும் அவசரப்பட்டு கஷாயம் தரித்துக் கொண்டுவிட்டாய். உனக்கு சந்நியாசம் குருவால் கொடுக்கப்படவில்லை.இது எல்லாம் உங்கள் இருவரின் நலம் பொருட்டாகவே ஏற்பட்டது. பரிபூரணம் பெறுவதற்கு இந்தப் பிரிவு தேவையானதுதான். இன்று மாலை லதா மண்டபத்தில் மீனாம்பாள் இருப்பாள் உன் வரவுக்காகக் காத்திருப்பாள் என்று சொல்லிப்போனார்.

மாலைப்பொழுது லதா மண்டபத்தில் அவன் சந்தியாச வேசத்தைக் கலைத்து உரிய ஆடைத்தரித்து இணைந்தோம். இரண்டு ஜீவன்களும் மாதாவின் சேவைக்காக லயப்பட்டன. மீனாம்பாள் கோவிந்தராஜன் நடந்த விவாகம் – சந்நியாசம் இந்து சமய ஆசாரக் கோட்பாடுகளினால் பழமை வாய்ந்த மூடநம்பிக்கைகளால் உருவானது என்பது சாத்தியமாகிறது.

இந்துக்களின் வகுப்புவாதம்

இந்து சமாஜம் பல்லாயிரம் ஆண்டுகளாகவே என்ற இழிந்த கொள்கைகளைக் கொண்டுள்ளது. இழிந்த அநாகரிக மூடபக்திகள் இன்று வரை மக்களைத் துண்டாடுகின்றன.

ஒரே குடும்பத்தில் பிறந்த இரண்டு சகோதர்கள் மத்தியில் பழைய ஆசாரங்கள் பாதகத்தை ஏற்படுத்துகிறது. ஆறில் ஒரு பங்கு சிறுகதையில் கோவிந்தராஜன் பிரம்மசமாஜம் வந்தே மாதர கொள்கைகளால் ஈடுபட்டதால் மரணித்த தந்தை ஈமத்திரிகை கூட முறையாக செய்ய முடியாத நிலை ஏற்படுகிறது.

திருமணங்கள் கூட தடைப்பட்டுபோகிறது. விழாக்கள்கூட பூசலில் முடிகிறது. உயர்ந்த வகுப்பினர் என்ற கூறப்படுவோர் வேதங்களையும், சடங்குகளையும் முன்னிலைப்படுத்தி மூடநம்பிக்கைகளைக் கைக்கொண்டு பாகுபடுத்துகின்றனர். பெரும்பாலும் விவாதத் தொழில்புரிவோர் செய்யும் தொழிலை மையப்படுத்தி பள்ளர், பறையர் என்று வேறுபடுத்துகின்றனர். ஆறு பங்கு மக்களில் ஒரு பங்கு வாழ்கின்ற இவர்கள் அனைவருக்குமான உணவு ஆதாரங்களை வழங்குகின்றனர். எனவே இவர்கள் சாத்திரப்படி வைசியர்கள் ஆக வேண்டும் என்று பாரதியார் கூறுகின்றார்.

வேளாண்மைத் தொழிலுக்கு பிரதானம் பசு. அப்பத மாமிசத்தை உண்பது புராண காலத்திலிருந்து வருகிறது. அவ்வாறு புலால் உண்பவர்களைத் தாழ்த்திப் பார்ப்பது சரியே என்றாலும் பஞ்சம் ,நோய் போன்ற இயற்கை இடர்களுக்கு முன்பு உயர்வு தாழ்வு பார்ப்பது மடமை. ஒரு சாரரைக் கீழ்சாதியினர் என தாழ்த்திவிட்டால் நமக்கும் அதே நிலை ஏற்படும்.

நம் தேசத்தில் பள்ளர், பறையர்களுக்கு செல்வதையெல்லாம் அன்னிய நாடுகளில் பிறர் செய்கிறார்கள். சங்கராச்சாரியாரும், ஜீயர் சுவாமிகளும், ஊருக்கு வெளியே சேரிகளில் வாசம் செய்ய வேண்டும்.

நமக்குள்ளே ஒரு பகுதியாரை நாம் நீசர்களாகக் கருதினோம் என்றால் நம்மை அன்னிய தேசத்தார் அவ்வாறே கருதுவார்கள். நம்முள் உள்ள ஒரு குறிப்பிட்ட வகுப்பினரை விலக்கினோம் என்றால் நம்மவர்களின் துன்பம் தாழாமல் முகம்மதியர்களுடன் கரம்கோத்துவிடுவார்கள். பனிரெண்டாயிரம் ஆண்டுகளுக்கு முன்பு வடநாட்டிலிருந்து வந்தோர் பஞ்சாப் நாட்டில் பிரவேசித்தபோது பள்ளர், பறையர் அவர்களுடன் சேர்ந்து கொண்டதாக இதிகாசங்கள் கூறுகின்றன.

"ஊர் இரண்டுபட்டால் கூத்தாடிக்குக் கொண்டாட்டம்" என்ற பழமொழிக்கு ஏற்ப நம்மக்களை நாம் பல பிரிவுகளாகப் பிரிக்க நினைத்தால் அது அன்னிய தேசத்தார் நம்மை வீழ்த்துவதற்கு வழியாகிவிடும்.

எங்கிருந்தோ வந்த ஆங்கிலேயர்கள் பஞ்ச காலத்தில் உதவிகள் பல செய்து நூற்றுக்கணக்கான மாதர்களையும், குழந்தைகளையும் கிருத்துவ மதத்திலே சேர்த்துக் கொண்டார்கள். வருடத்திற்கு வருடம் இந்துக்களின் மக்கள் தொகை குறைந்து கொண்டே வருகிறது.

இந்து ஜனங்கள் நம் இரத்தம், சதை, எலும்பு, உயிர் என்று எண்ணி மூட ஆசாரங்களினால் அவர்களைத் துச்சமாக்கிவிடக் கூடாது. கேட்பாரின்றி பசியாலும், நோயாலும் வயது முதிர்வாலும் வாழ்ந்து கொண்டுள்ளோரை இனப்பாகுபாடு காட்டிப் பிரிக்காமல் இணைந்து வாழ முற்படுவோம்

முடிவுரை

'ஆறில் ஒரு பங்கு' சிறுகதை ஒருமைப்பாட்டுணர்வை, சாதிப்பாகுபாடுகளை, வகுப்புவாதப் பிரிவுகளைக் களையும் வண்ணம் படைக்கப்பட்டுள்ளது. குடும்ப அமைப்பில் பெற்றோரின் மூடநம்பிக்கைகளால் பிள்ளைகளின் வாழ்வு கேள்விக்குறியாகிறது. பெண்கள் முதிர்கன்னிகளாகவும் ஆண்கள் பிரம்மசாரிகளாகவும் அவலநிலைக்குத் தள்ளப்படுகின்றனர். இந்து சமாஜம் பல வகுப்புகளாகப் பிளவுப்பட்டுக் கிடக்கின்றது. அந்தணரும், அரசரும் உயர்நிலையிலும் வணிகரும், வேளாளரும் தாழ்நிலைக்குத் தள்ளப்பட்டு விளிம்புநிலையிலும், உயர்நிலையிலும் வாழ்கின்றனர். ஏற்றத்தாழ்வு நிலை மக்களிடையே நீங்கி "வேற்றுமைக்குள் ஒற்றுமை" காண வேண்டும்.

''சாதிகள் பிரிவுகள் சொல்லி-அதில்
தாழ்வென்றும் மேலென்றும் கொள்வார்
நீதிப்பிரிவுகள் செய்வார்-அங்கு
நித்தமும் சண்டை செய்வார்'' **(பாப்பா பாட்டு-7)**

என்ற வாக்கிற்கு ஒப்ப வாழ்வதே 'சமத்துவம்' என்ற உயர்ந்த சமுதாயத்தைப் பாரதி பண்படுத்திக் காட்டுகின்றார்.

■

14. பாரதியின் புதுமை நோக்கு

முனைவர் மு.சுபாஷினி
உதவிப்பேராசிரியர் (நிர்வாக நியமனம்)
தேசியக் கல்லூரி (தன்னாட்சி), திருச்சிராப்பள்ளி–01

ஆய்வுச் சுருக்கம்

பாரத தேசம் பழமையிலிருந்து வெளியேறி சிறப்புடன் வாழ்வதற்கு உழைத்தவர் ஏராளமானோர் ஆவர். பாரதியார் உலகிலுள்ள முடைநாற்றம் வீசும் மூடநம்பிக்கை முற்றிலும் ஒழிந்து புதிய தேசமாக உலா வரக் கனவு கண்டார். புதுமைப் பார்வையுடன் தொலைநோக்குச் சிந்தனைகளையும் கொண்டவர். அறிவியல் வளர்ச்சியடையாதபோதே வளர்ச்சியடைந்தாகப் பாவித்துக் கொண்டு பாடல் இயற்றியவர். "காசிநகர் புலவர் பேசும் உரைதான் காஞ்சியிலே கேட்பதற்கோர் கருவி செய்வோம்" என்று எதிர் இந்தியா இப்படிதான் இருக்க வேண்டுமென்று வளர்ச்சியடையாத காலத்திலேயே தம் கற்பனைத் திறத்தால் அறிவியல் யுகத்தைக் கண்டவர்.

சுய நலமாக மனிதர்கள் வாழும் காலத்தில் தாம் பிறந்த மண் உயரவேண்டுமென்று சிந்தித்த மகான் பாரதி ஆவார். நாட்டை முன்னேற்றமடைய செய்வதில் முதன்மைப் பங்கு கொண்டவள் பெண் என்பதை உணர்ந்த பாரதி வீட்டறையிலிருந்து வெளியேறி புதுமைகளைப் படைக்கும் புதுமை பெண்ணாக மாறுவதற்கு பற்பல கவிதைகளை வடித்து பெண்ணினத்திற்குப் பெரும் புகழ் சேர்த்தார். இன்றைய காலத்தில் பெண்கள் அனைத்து துறையிலும் முன்னேறி ஆணுக்குப் பெண் எதிலும் இழைத்தவள் இல்லை என்பதை நிரூபித்து வருவதற்குப் பாரதி காட்டிய புதிய வழியே என்றால் அவை மிகையாகாது. மக்களுக்குப் புத்துயிர் ஊட்டி புதிய பூமியாக மாற்றிட வழிமுறைகளைக் கூறிய பாரதியின் புதுமை நோக்கினை வெளிப்படுத்தும் விதமாக இக்கட்டுரை அமைந்துள்ளது.

முகவுரை

காலத்தின் தேவையினால் கவிஞன் உருவாகிறான். அக்கவிஞன் சமுதாய மாற்றத்திற்காகப் பல படைப்புகளைப் படைக்கின்றான். ஆண்டாண்டு காலமாக அடிப்பட்டுக் கிடந்த மக்கள் எழுச்சியடைய வேண்டுமென்ற உத்வேகத்தினால் புதுமையானக் கருத்துகளை வெளிப்படுத்துகிறான். அத்தகைய வகையில் தம் படைப்புகளின் வாயிலாக மக்கள் விழிப்புணர்வு கொண்டு சமுதாயம் முன்னேற அரும்பாடுபட்டவர்களுள் முதன்மையானவர் மகாகவி பாரதியாவார். பாரதியின் புதுமையானச் சிந்தனையை வெளிக்கொணருவதே இக்கட்டுரையின் நோக்கமாகும்.

பாரதியின் காலம்

அன்னை பூமி அந்நியரிடம் அடிமைப்பட்டுக் கிடந்த வேளையில் அவதரித்தவன் பாரதி. தம் மக்கள் அடிமைப்பட்டும் மதத்தால் சாதியால் மொழியால் சண்டையிட்டுக் கொண்டும், மூடப்பழக்க வழக்கங்களில் மூழ்கியிருப்பதையும் கண்டு நெஞ்சு பதைப்பதைத்து இவற்றிலிருந்து மக்கள் விடுபட்டு நல்வாழ்வு வாழ புதுவழிகாட்டியவர். இளம் ஞாயிறுபோலத் தோன்றி புதிய பூபாளம் இசைத்தவன். மாறுபட்ட பார்வையும் கொள்கையும் உடையவர்களே மாறுபட்டுச் சிந்திக்க முடியும். மாறாதது வளராது மாறுவதே வாழும் வளரும் என்பர்.

“பழையன கழிதலும் புதியன புகுதலும்
வழுவல கால வகையினானே”[1]

என்கிறது நன்னூல்.

பாரதி மாறுபட்ட புதிய சிந்தனைகள் வழியே சமுதாயத்தை மாற்றிட முற்பட்டவன். நன் மரபுகளைப் பேணுவதோடு புதுமையையும் போற்றி பழமைக்கும் புதுமைக்கும் உறவுப் பாலம் அமைத்தவன். “எப்போதும் செய்தது போலவே செய்து கொண்டிருந்தால், எப்போதும் கிடைப்பதுதான் கிடைக்கும். அடுத்த கட்ட உயர்வு என்பதை யோசிக்கவே முடியாது”2 என்பர். இதை நன்கு உணர்ந்து, வெளிபாட்டு முறையில் மாற்றத்தையும் புதுமையையும் புகுத்தியவன். பாரதியின் புதிய சிந்தனைகளும் நற்கருத்துகளும் பலத்திறத்தனவாகும். பாரதி,

“மறைவாக நமக்குள்ளே பழங்கதைகள்
சொல்வதிலோர் மகிமை இல்லை”

என்று பழம்பெருமைகளை மீண்டும் மீண்டும் பேசுவதைச் சாடுகிறார்.

"சுவை புதிது பொருள் புதிது வளம் புதிது
சொற்புதிது சோதி மிக்க
நவக்கவிதை எந்நாளும் அழியாத மாக்கவிதை"

என்று தம் புதிய பார்வையை வெளிப்படுத்துவதன்வழி அவர் புதுமைக் கவிஞன் என்பதை அறியலாம். புதுவையில் பத்தாண்டுக் காலம் வாழ்ந்த பாரதியைக் குருவாக ஏற்று அவருடைய தாசனாக வாழ்ந்த பாவேந்தர் பாரதிதாசன் பாரதியிடம் அணுக்கமாகப் பழகியவர். பாரதி எதிலும் புதிய நோக்குடையவர் என்பதை,

"அகத்திலும் எண்ணங்கள் உலகில் இன்னல்
அறுப்பவைகள், புதியவைகள், அவற்றையெல்லாம்
திகழ்ப்பாருக்குப் பாரதியார் எடுத்துச் சொல்வார்.
தெளிவாக அழகாக உண்மையாக!
உய்வகை காட்டும் உயர்த் தமிழுக்குப்
புதுநெறி காட்டிய புலவன் பாரதி"3

என்று பாடியள்ளார். தாம் மட்டுமல்லாது தன்னைப் பின்பற்றுவோரும் புதுமை நோக்குடையவராக இருக்க வழிகாட்டியுள்ளார். ஒரு புதிய பரம்பரையை உருவாக்கியவர். தனக்குப் பாரதியாரே புதிதாகப் பார்க்கும் முறையையும் புதுவித தன்மையையும் காட்டியுள்ளார் என்பதை,

சுப்பிரமணிய பாரதியார் தோன்றியென்
பாட்டுக்குப் புதுமுறை, புது நடை காட்டினார்"

என்று கூறுகிறார் பாவேந்தர். புதியநெறி காட்டிய புலவன் என்ற பகுதியில்,

"திறம்பட வந்த மறவன் புதிய
அறம்பட வந்த அறிஞன்"4

என்றும் புகழ்ந்துள்ளார்.

புதிய நடை

நடை மனிதனை இனம் காட்டும் என்பர். எழுத்தில் பேச்சில் சிலருக்கென்று தனியான நடையுண்டு. யாப்புடன் இறுகக் கட்டமைக்கப் பெற்றிருந்த தமிழ்ச் செய்யுள் நடையைத் தளர்த்தி வசன கவிதை என்ற புது வடிவத்தையும் சந்தத்தில்

சிந்துவையும் புகுத்தியவன் பாரதி. "தற்காலக் கவிதை என்பது 1906 முதல் பாரதி தொடங்கி என்று காலக் கணக்கிடலாம்." என்கிறார் சி.கனகசபாபதி.

"உய்வகை காட்டும் உயர்தமி ழுக்குப்
புதுநெறி காட்டிய புலவன் பாரதி
பைந்தமிழ்தேர்ப் பாகன் அவனொரு
செந்தமிழ்த் தேனீ சிந்துக்குத் தந்தை
கலிக்கும் கவிதைக் குயில்"[5]

என்ற பாரதியின் தமிழ் நடையைப் போற்றுகிறார் பாரதிதாசன்.

தமிழின் உரைநடை வீரமாமுனிவரிலிருந்து தொடங்கினாலும் முறையான தமிழ் உரைநடை பாரதியிலிருந்தே ஆரம்பமாகிறது. போலச் செய்தல் அடிப்படையில் காப்பியம் பார்த்துக் காப்பியமும், அந்தாதி போலவொரு அந்தாதியும், பிள்ளைத் தமிழ்போல ஒரு பிள்ளைத்தமிழும் படைக்கும் தமிழ் மரபு வழிப்பட்ட படைப்பு நிலையிலிருந்து மாறுதலாக இறவாத புகழுடைய புதிய புதிய படைப்புகளை உருவாக்கியவர் பாரதி. புதிய ஆத்திசூடி, முரசு, லட்சிய முழக்கம், பாப்பாப் பாட்டு, கண்ணன் பாட்டு, நாட்டுப்பற்று, குயில்பாட்டு, பாரத பூமி, பாஞ்சாலி சபதம், சிறு காப்பியங்கள், தனிப்பாடல்கள் எனப் புதிது புதிதாக இலக்கியங்களைப் படைத்தவர். தமிழ்ச் சிறுகதையையும் புதிய வடிவில் வெளிப்படுத்தியவர்.

"விஞ்ஞான வெளிச்சத்தில் உலகம் விடிந்து கொண்டிருந்த
வேளையில் அவன் பெற்றெடுக்கப்பட்டவன்.
தமிழுக்குப் புது இரத்தம் தந்த கவிஞனே"[6]

என விளக்குகிறார் கவிஞர் வைரமுத்து. வடமொழி சுலோகங்களையும், வேத உபநிடதக் கருத்துகளையும், பகவத்கீதையையும், பாஞ்சாலி சபதத்தையும், எளிமையாகவும், புதுமையாகவும் தந்தவன் பாரதியாவான்.

கருத்துக்கு வள்ளுவர், கதைக்கு இளங்கோ, வளமைக்குக் கம்பன், புலமைக்குக் காலமேகம், புதுமைக்குப் பாரதி. புரட்சிக்குப் பாரதிதாசன், எளிமைக்குக் கண்ணதாசன் என்று தமிழில் சிறந்த வெளிப்பாட்டுநிலை ஒவ்வொன்றிற்கும், ஒவ்வொருவரைக் கூறுவர். புதுமைக்குப் பாரதி என்பது இவண் கருதத்தக்கது. உயர் கம்பனுடன் தமிழ்க் கவிதை போயிற்று என்று ஏங்கித் தவித்த தமிழகத்தில் நானிருக்கிறேன் என நம்பிக்கை ஊட்டியவன் பாரதி என்றும் கூறுவர்.

ஆசாரத்தை, பழமையைத் தூக்கிப்பிடித்துக் கண்போலக் காக்கும் பிராமணச் சமூகத்தில் பிறந்த பாரதி பஞ்சகச்சத்தைவிடுத்து கருப்புக் கோட்டணிந்து முறுக்கு மீசையுடனும் நீண்ட தலைமுடியுடனும், தலைப்பாகைக் கட்டித் தன் புற வடிவத்திலும் புதுமைக் கோலம் பூண்டவர்.

புதிய பாரதம் புதிய நடை

இந்திய மக்கள் சாதிமதச் சண்டைகளை விட்டொழிக்கப் புதுவழி காட்டியவர். நாட்டில் நிலவும் தரித்திரம், பாவம், பயம், சூது போன்றவை தொலைந்து மக்களிடையே சுறுசுறுப்பு, வீரம், கல்வி, செல்வம், தர்மம் போன்றவைத் தழைத்தோங்கி புதிய பாரத சமுதாயமாக மேன்மையுற்று மிளிர "நல்ல காலம் பிறக்குது" எனச் சக்தி மகாகாளியைப் புதிய கோணங்கியில் வேண்டுகிறார்.

பாரதி இந்திய மக்களுக்கு மட்டுமின்றி உலகிலுள்ள மக்கள் அனைவரின் உயர்வுக்கும் குரல் கொடுத்தவன். ரஷ்யாவில் ஜார் மன்னனின் கொடுங்கோல் ஆட்சியைக் கண்டு உள்ளம் வெதும்பியவர். இமயமலை வீழ்ந்ததுபோல் மன்னன் வீழ்ந்து அழிந்து "புதிய ருஷ்யா" உருவாக வேண்டுமென ஆசைப்பட்டுள்ளார். உலகிலுள்ள மூடத்தனங்கள் அழிந்து புதிய உலகம் உதயமாக விரும்புகிறார். "மகாகவி வெறும் கவிஞர் மட்டுமல்லர். அவர் ஒரு சமூக விஞ்ஞானி. அவர் வேதகாலத்து ரிஷிகளுக்கு ஒப்பானவர். அவர் நமது தேச மக்களைக் குறித்தும் உலக மனிதரோடு நமக்கு இருக்க வேண்டிய உறவுகள் குறித்தும் பல அரிய சிந்தனைகளை நமக்கு அளித்துச் சென்றுள்ளார்"7 என்கிறார் ஜெயகாந்தன்.

மகாகவி பாரதியார் புதுமைப் பார்வையுடன் தொலைநோக்குச் சிந்தனையும் உடையவர். கனவுகள் பல கண்டவர்.

> **"கனவு மலரட்டும்! கனவு மலரட்டும்! கனவு மலரட்டும்**
> **கனவுகள் எண்ணங்களாக வடிவம் பெறுகின்றன**
> **எண்ணங்கள் செயல்களாகப் பரிணமிக்கின்றன"[8]**

என்கிறார் குடியரசு தலைவர் அப்துல்கலாம் அவர்கள். பாரதி கனவுகள் பல கண்டவன்.

> **"மந்திரங்கள் கற்போம் வினைத் தந்திரங் கற்போம்**
> **வானை யளப்போம் கடல் மீனை யளப்போம்**
> **சந்திர மண்டலத்தியல் கண்டு தெளிவோம்**

சந்தி தெருப் பெருக்கும் சாத்திரங் கற்போம்''

(பாரதியார் கவிதைகள், பாரததேசம்,ப.127)

என்ற பாரதியின் கனவிற்கிணங்க மனிதர்கள் சந்திர மண்டலத்திற்கும், பல கிரகணங்களுக்கும் சென்று பாரதியின் கனவுகள் அனைத்தும் நனவாக்கப்பட்டுமையை அறிய முடிகிறது.

இன்றைய உலகம் கணினி தொலைபேசி, இணையம், செல்லிடப்பேசி எனும் அதிநவீன கருவிகளால் சுருங்கிவிட்டது. நினைத்த நேரத்தில் எங்கும் பேசலாம். தொடர்பு கொள்ளலாம் என்ற நிலை வந்துவிட்டது. இதைத் தீர்க்க தரிசனமாக உணர்ந்த பாரதி,

''காசி நகர்ப்புலவர் பேசு முரைதான்
காஞ்சியிற் கேட்பற் கோர் கருவி செய்வோம்''

(மேலது நூல், ப.167)

என்று பாடியுள்ளார். காலத்தின் போக்கிற்கேற்ப புதுமைகளை ஏற்பதே அறிவுடைமையாகும். மத வெறியும் தீண்டாமைக் கொடுமைகளும் மிகுந்திருந்த காலத்தில் வாழ்ந்தவர். அவற்றை ஒழிக்க மனிதர்கள் அனைவரும் சமம் என்ற கோட்பாட்டை முன் வைத்துத் தாழ்த்தப்பட்ட இனத்தைச் சேர்ந்த கனகலிங்கம் என்ற சிறுவனுக்குப் பூநூல் அணிந்து புதுமை செய்தவர். படைப்பாளர்கள் பொது நோக்கிலும் பெரியவர்களை விளித்தும் கருத்துகளை வெளிப்படுத்திக் கொண்ட மரபிலிருந்து மாறி பாப்பாக்களுக்கும், இளைஞர்களுக்கும் தம் கருத்துகளை வரித்தவர் பாரதி.

புதுமைப் பெண்கள்

உலகம் செழித்தோங்க பெண்களே முதன்மையானவர் என்பதை உணர்ந்த பாரதி பெண்கள் முன்னேறி வருவதற்கு புதுமையான வழிமுறைகளைத் தம் படைப்புகளின் வாயிலாக வெளிப்படுத்திவர். பெண்கள் இல்லாள், மனைவி, குடும்ப விளக்கு, வளமை, பொறுமை போன்ற பல சிறப்புகளைப் பெற்றவர்கள். இத்தகைய பெண்ணினம் ஆண்டாண்டு காலமாய் அடிமைப்படுத்தப்பட்டு, சிந்தனை, சுதந்திரம், அலுவலக வேலை வாய்ப்பு போன்றவை மறுக்கப்பட்டு "மனையுறை மகளிராகவே" இருப்பதை நினைந்து மனம் நொந்துள்ளார். இக்கட்டுகளிலிருந்து விடுதலையாகி ஆணுக்கு நிகராகப் புதுமைப் பெண்களாக வாழ புதிய பாதைக் காட்டியவர்.

''சிறிய தொண்டுகள் தீர்ந்தடிமைச் சுருள்
தீயி லிட்டுப் பொசுக்கிட வேண்டுமாம்
ஆணும் பெண்ணு நிகரெனக் கொள்வதால்
அறிவிலா வோங்கியிவ் வையந் தழைக்குமாம்
பூணு நல்லறத் தோடியிங்குப் பெண்ணுக்குப்
போந்து நிற்பது தாய்சிவ சக்தியாம்
புதுமைப் பெண்ணிவள் சொற்களுஞ் செய்கையாம்
பொய்மை கொண்ட கலிக்குப் புதிது''
(பாரதியார் கவிதைகள், புதுமைப்பெண், பக்.218-219)

எனப் பெண்ணினத்தைச் சிறப்பித்துள்ளார்.

மகளிரின் பழமையான பொய்யான மூடப்பழக்க வழக்களிலிருந்து விடுபட்டு புத்தொளியும் புத்துணர்வும் பெற்றிடவும் இவ்வுலகம் புதிய பூமியாக மாறிடவும் கனவு கண்டவர். இன்றைய புரட்சிகர பெண்ணிய சிந்தனைகளுக்கும், எண்ணத்திற்கும் வித்திட்டவர் பாரதி. பெண்களைப் புதுமைப் பெண்களாகக் கண்டவர்.

''புதுமைப் பெண்ணவள்
போற்றி போற்றி ஜய ஜய போற்றியாம்
புதுமைப் பெண்ணொளி வாழி பல்லாண்டிங்கே!
மாற்றி வையம் புதுமையுறச் செய்து''
(பாரதியார் கவிதைகள், புதுமைப்பெண், ப.220)

என்கிறார். வையம் புதுமைபெற பெண்கள் புதுமைப் பெண்களாக மாற வேண்டுமென்கிறார். பெண்களை பொறுமை, தாய்மை, வளமை நோக்கில் நிலமகளாக, பூமாதேவியாக வழிபடுவது தமிழர் மரபாகும். இளங்கோ அடிகள் பெண்ணை "மண்மகள்" என்கிறார். இத்தகைய சிறப்பு வாய்ந்த மகளிர் புதுமையடைவதன் வழியாக உலகம் புதுமைபெறும் எனும் புதிய கோட்பாட்டை முன் வைத்துள்ளார். "பெண்மை வாழ்கவென்று கூத்தாடுவோமடா" என்று போற்றுகின்றார்.

நிறைவுரை

உலக மக்களுக்கு அனைத்து வளமையும் கிடைத்து நன்முறையில் வாழவேண்டும் என்று எண்ணிய பாரதியின் உள்ளம் போற்றுதற்குரியது. தாம் காணும் பொருட்கள் யாவும் தமக்கே கிடைத்துவிட எண்ணுகிற உலகில் பாரதி மட்டும் புதுமையுணர்வை உள்ளத்தில் விதைத்து அவை விருட்சமாக

வளர பாடுபட்டவர் என்பதை தம் படைப்புகளின் வழியாக அவருடைய எழுத்து நடை நமக்கு அறிவுறுத்துகின்றன.

சான்றெண் விளக்கம்

1. நன்னூல் சொல்லதிகாரம் காண்டிகையுரை, நூ.462
2. ஆனந்த விகடன், நாள்–18.12.2005, ப.132
3. பாரதிதாசன் கவிதைகள், ப.160
4. மேலது நூல், ப.152
5. மேலது நூல், ப.153
6. வைரமுத்து, கவியரசன் கதை,ப.144
7. ஜெயகாந்தன், பாரதி பாடம், ப.88
8. அப்துல் கலாம், எழுச்சி தீபங்கள், ப.17

துணைநூற்பட்டியல்

1. திருஞானசம்பந்தம்.ச (உ.ஆ), நன்னூல்–சொல்லதிகாரம், கதிர் பதிப்பகம், திருவையாறு–04, முதற்பதிப்பு–2009.
2. பாண்டி.மு, தேசியமும் தமிழும், கவிதா வெளியீடு, சென்னை–17, முதற்பதிப்பு–2008.
3. பாரதம் போற்றும் பாரதியார் கவிதைகள், லியோ புக் பப்ளிஷர்ஸ், சென்னை–35, மறுபதிப்பு–2011.
4. பாரதிதாசன் கவிதைகள், சாகித்திய அகாதெமி வெளியீடு, முதற்பதிப்பு–2007.

15. பாரதி சுட்டும் அறநெறிகள்

முனைவர் ப.சுமதி
உதவிப்பேராசிரியர், தமிழ்த்துறை,
தேசியக்கல்லூரி (தன்னாட்சி), திருச்சிராப்பள்ளி–01

முன்னுரை

மகாகவி சுப்பிரமணிய பாரதியார் தமிழகத்திலும் இந்தியாவின் பிற பகுதிகளிலும் ஒரு தேசியக் கவியாக அறிமுகப்படுத்தப் பட்டுள்ளார். அடையாளப்படுத்தியுள்ளார்கள். "பாட்டுக்கொரு புலவன் பாரதி" என்பது கவிமணியின் கணிப்பு. பாரதி ஒரு தமிழனாக இருந்து உலகத்தின், பாரதத்தின், தமிழகத்தின் புரட்சிகரமான அறச் சிந்தனைகளை சமுதாயம் நல்லிணக்கம் நாட வேண்டும் என்று தம் படைப்புகளில் வலியுறுத்திக் கூறியிருக்கின்றார். அவற்றை இக்கட்டுரையில் காணலாம்.

பிறப்பு

வையத்துள் வாழ்வாங்கு வாழ வேண்டிய நெறிமுறைகளை உணர்த்திய மகாகவி சுப்பிரமணிய பாரதியார் 1882இல் பிறந்து 1921இல் இயற்கை எய்தினார். இப்பூமியில் 39 ஆண்டுகளே வாழ்ந்து அரிய சாதனைகள் படைத்தவர். பதினொரு வயதில் கற்ற புலவரும் மெச்சும்படி "பாரதி" என்ற பட்டம் 1893இல் கிடைக்கப்பெற்றது. மனித வாழ்க்கைக்குத் தேவையான குறிக்கோள்களை எல்லாம் தன்னகத்தே கொண்ட மகாகவி தம் படைப்பு இலக்கியச் செல்வங்களில் கூறியதோடு தம் வாழ்வு அனுபவங்களையும் வெளிப்படுத்துகிறார்.

வணிகம்

பாரதியின் தந்தை சின்னச்சாமி ஐயருக்கு வாணிகம் செய்து பொருட்டு தொழிலில் சரிவு ஏற்பட்டு பெரும் துயர் எய்தினார். உற்றார், உறவினர் யாவரும் அவரைவிட்டு பிரியும்போது மனம் குன்றினார். தன் தந்தையின் வாழ்வைக் கண்டுணர்ந்த பாரதி

"பணம் இல்லாதவன் பிணம்" என்ற பழமொழிக்கு ஏற்ப பொருளற்றார் நிலையை எண்ணிப் பொருமினார். வள்ளுவப் பெருமானின் "பொருள் இல்லார்க்கு இவ்வுலகமில்லை" என்ற குறள்மணி பாரதியின் நெஞ்சிடை ஆழப்பதிந்துவிட்டது. இவற்றினைத் தம் சுயசரிதையில்,

''பொருளி லார்க்கிலை யுவ்வுல கென்றநம்
புலவர் தம்மொழி பொய்ம்மொழி யன்று காண்
பொருளிலார்க் கின மில்லை துணையிலை
பொழுதெலா மிடர் வெள்ளம் வந் தெற்றுமால்''

(கனவு : செய்யுள் 43 வரிகள் 1 - 4)

என்று குறிப்பிட்டு "பொருள் இல்லார் பொருள் செய்தல் முதற் கடன்" என்றும் பணிந்தார். பொருள் செய்தலும் அறவழியில் இருத்தல் வேண்டும் என்பதை பாரதி தெளிவுப்படுத்தியுள்ளார்.

எப்படியும் பொருள் சேர்க்கலாம் என்கிற மக்கள் மனப்பான்மை கொள்ளாமல், இப்படித்தான் பொருள் சேர்க்க வேண்டும் என்று பாரதி சுட்டிக் காட்டினார் "அறமொன்றே இன்பம் தரும்" என்பதைப் பாரதி,

''அறமொன்றே தரு மெய்யின்ப மென்றநவ்
லறிஞர் தம்மையனுதினம் போற்றுவேன்
பிற விரும்பி உலகினில் யான்பட்ட
பீழை யெத்தனை கோடி நினைக்கவும்
திறனழிந்தென் மனமுடை வெய்துமால்
தேசத்துள்ள இளைஞரறிமினோ !
அறமொன் றேதரு மெய்யின்ப மாதலால்
அறனை யேதுணை யென்றுகொண் குல்திரால்''

(கனவு : செய்யுள் 44 வரிகள் 1 - 8)

என்று விளக்கி அறத்தைத் துணையாகக் கொண்டு வாழ அறிவுறுத்துகிறார்.

பாரதி சுதந்திரப் போராட்ட காலத்தில் நடைபெற்ற சுதேசீய முயற்சிகின் வெற்றிக்காக 'செய்க பொருளை' என்ற வள்ளுவனின் செந்நெறியை மேற்கோளாகக் காட்டி வாழ்ந்தார். செல்வத்தால் நன்மையும் சேமமும் ஏற்படுகிறது என்பதனை,

''செல்வம் உணவைப் போன்றது புதிதாக அதைத் தேடி உழைப்போர்க்கு அது நன்மையையும் சேஷமத்தையும் உண்டாக்கும்'' (இந்தியா : 27.3.1909 ப.3)

என்று செல்வத்தின் அருமையை விளக்கியதுடன் பொருளைத் தேடும் அறநெறியினையும் பாரதி எடுத்துக்காட்டுகிறார்.

> "பொருளைத் தேடு, உனக்கு அநீதியான இம்சை செய்வோர்களை அடக்குவதற்கு இதுபோலக் கடமையுடைய ஆயுதம் வேறு கிடையாது" (இந்தியா : 27.3.1909 ப. 3)

இந்தக் கருத்துரையைப் பாரதி அடிமை நாட்டில் சுதந்திரம் பெறுவதற்குரிய காலப் பகுதியில் கூறியுள்ளார். உலகியல் வாழ்க்கைக்குச் செல்வம் இன்றியமையாதது அவற்றை இறைநெறியில் சம்பாதிக்க வேண்டும் என்கிறார்.

மக்கட்பேறு

மானிடர்கள் பெறவேண்டிய பேறுகளில் மிகச்சிறந்தது நன்மக்கட்பேறு ஆகும். பொருளை அறவழியில் சேர்ப்பது எத்துணை முக்கியமோ அதைப்போலவே நல்ல புதல்வர்களைப் பெறுவதும் ஆகும். இந்த உண்மையை வள்ளுவர்,

> "**எழுபிறப்பும் தீயவை தீண்டா பழிபிறங்காப்**
> **பண்புடைய மக்கட் பேறின்**"

என்கிற குறளால் விளக்கியுள்ளார் இந்த அறம் கருத்தினை பாரதி 'மக்களின் மாட்சிமை" என்ற கட்டுரையில்,

> "கேவலம் புதல்வனைப் பெற்றுவிட்டதனாலேயே ஈன்றோர்க்குப் புகழ்ச்சியுண்டாவதில்லை சத்புத்திரனாயும், சன்மார்க்க வழியறிந்து நடப்பவனாயும், கண்டோர் புகழும் காருண்ய முள்ளவனாயும் மிருக்க வேண்டும்" (இந்தியா : 31.7.1909 ப.3)

என்று கூறுகையில் நல்லறிவுள்ள புத்திரர்களால் நற்பயன் பெறுவதால், நன்மக்கட் பேறு கிடைக்கிறது என்ற அறிநெறி மாண்பினை உலகிற்கு உணர்த்துகிறார்.

"மகனைச் சான்றோன் ஆக்குதல் அவன் தந்தையின் கடமை" என்று புறநானூறு பாடல் கூறுகிறது. தன் மகன் சான்றோன் எனக்கேட்ட அளவில் தாய் மகிழ்ச்சி அடைவாள் இதனை வள்ளுவர் குறளில்

> "**ஈன்ற பொழுதில் பெரிதுவக்கும் தன்மகனைச்**
> **சான்றோ னெனக் கேட்ட தாய்**"

என்று வள்ளுவர் கூறியுள்ளார். பாரதி ஒரு தாயின் துயரத்தை எடுத்துக் காட்டும் வகையில்,

"மாயும் பொழுதிற் பெரிதழுங்குந் தன் மகனைக்
கீழோன் எனக்கேட்ட தாய்"

(சக்ரவர்த்தினி 1906 ஜனவரி இதழ் – பக். 124 – 125)

என்ற குறளை பாரதி புனைந்தளித்தார். கல்விப் பயிற்சி மக்களுக்கு அருள்தன்மையை அளிக்கும். அறிவு விலாசத்தால் மக்கள் மனப்பக்குவம் பெறுவார்கள். இதனைப் பாரதி "அறிவை வளர்த்திட வேண்டும் என்றும் பயிற்றிப் பல கல்வி தந்து பாரை உயர்த்திட வேண்டும் என்றும் முரசு கொட்டி கல்வியின் சிறப்பையும், நன்மையையும் பெற அறவழியில் நின்று குரல் கொடுத்துள்ளார்.

கல்வி

ஒரு நாட்டின் பெருமைக்குச் சான்றாக விளங்குகிறவர்கள் அந்நாட்டின் பெண்மக்கள் என்பதை நன்கு உணர்ந்தார் பாரதி. எனவே பெண்ணுக்கு ஞானத்தை ஊட்டி வளர்க்க வேண்டும் என்பதை எல்லோரும் தலையாயக் கடமையாகக் கொள்ள வேண்டும் இவற்றினை,

"பெண்கல்வியறிவை வளர்த்தால் - வையம்
பேதைமை யற்றிடும்...." (முரசு: செய்யுள் 10 வரிகள் 3 – 4)

என்று பெண்கல்வியின் அவசியத்தைக் கூறுவதோடு, பெண்கள் பெற வேண்டிய அறநெறி மாண்பினையும் உணர்த்தியுள்ளார். பெண்களின் நலனையும், சிறப்பையும், அறிவு நலத்தையும் புரிவதைப் பெருநோக்கமாகக் கொண்டு பாரதியின் "சக்ரவர்த்தினி" என்ற பத்திரிக்கை வானில் ஒளி நட்சத்திரமாகத் திகழ்ந்தது. இவருடைய இலட்சியக் குறளாக,

"பெண்மை யறிவுயரப் பீடோங்கும் பெண்மைதான்
ஒண்மையுற வோங்கும் உலகு" **(சக்ரவர்த்தினி 1905 ஆகஸ்ட் இதழ்-ப.1)**

என்று பாரதி கூறும் பொழுது பெண்கள் பெறும் கல்வியறிவு அவர்களின் அறநெறி மாண்பினை அடைவதோடு, இவ்வையகத்தினில் புகழையும் ஓங்கச் செய்வர் என்கிறார்.

"தையலை உயர்வு செய்" என்று புதிய ஆத்திசூடியில் போதனை செய்த பாரதி,"பெண்மை வாழ்க" எனவும் "பெண்மை உயர்ந்தால் எல்லாம் உயரும்" என்றும் பெண்களின் அறநெறி வாழ்விற்கு குரல் கொடுத்துள்ளார். சக்கரவர்த்தினி இதழில் பாரதி கல்விச் சிறப்பை விளக்கியதோடு விக்டோரியா மகாராணியின் சரிதையிலிருந்து நம்நாட்டு மாதர்கள் அறிந்து

கொள்ள வேண்டும். பெண்ணரசியின் கல்வித் திறனைச் பாரதி எடுத்துக் காட்டியுள்ளார்.

"விலங்கொடு மக்கள் அனையார் இலங்குநூல்
கற்றாரோடு ஏனை யவர்"

என்று தெய்வப்புலவர் கூறுகிறார். மக்கள் யாவரும் கல்வித் திறமை கொண்டவர்களாக அறநெறி மாண்போடு விளங்க வேண்டும் என்பதனை,

"படிப்பு இல்லாதவர்களையும் படித்தவர்களையும் ஒப்பிட்டு நோக்கினால் மிருகங்களையும் மனிதர்களையும் ஒப்பிடுவது போலமென்கிறார். வள்ளுவர் கல்வியற்றோர் ஆணாயிருப்பினும் பெண்ணாயிருப்பினும் வண்டி இழுக்கத்தான் பயன்படுவார்கள் என்பது திருவள்ளுவருடைய கருத்து"

(சக்ரவர்த்தினி 1905 ஆகஸ்ட் இதழ் – பக். 5 – 6)

என்று மகாகவி கல்வி அறிவைப் பெறுவதுடன் அதனால் அடையும் அறநெறி மாண்பையும் கூறுகிறார்.

ஒழுக்கம்

கற்றறிந்தவர்களாக இருப்பதுடன் தம் மனம் மக்கள் அறிந்த உண்மையின்படி ஒழுக வேண்டும் சொல் வேறாகவும் செயல் வேறாகவும் உள்ளவர்களுடன் உறவு கொள்வது பாவம் என்பதனை திருவள்ளுவர்,

"கனவினும் மன்னது மன்னோ வினைவேறு
சொல்வேறு டையார் தொடர்பு"

என்று கூறியுள்ளார். இதை விளக்க வந்த பாரதி அறிவினால் எட்டிய உண்மைகளை மனிதர் ஒழுக்கத்திலே கடைபிடித்து வாழ முற்பட வேண்டும் என்று மக்களிடம் உணர்த்தினார்.

"இனிய சொற்களைச் சொல்வதினின்றும் நன்மைகள் விளைவது கண்டும் மானிடர் ஒருவருக்கொருவர் கொடுஞ் சொற்கள் வழங்குவது மடமை" என்பது உலகத்தில் சாதாரண அனுபவமுடையவர்களுக்குக் கெல்லாம் தெரியும்"

(பாரதியார் கட்டுரைகள் 1940– பக். 46 – 47)

பாரதி மனித குலத்திற்குப் பொது நெறிகளைக் சொல்லிக் கொடுஞ்சொற்களும், கோபச் செயல்களும் நீங்கியவர்களாக வாழ வேண்டும் என்பதனை புலப்படுத்தியுள்ளார்.

குடும்ப வாழ்வு

குடும்ப நாகரீகத்தில்தான் தேசநாகரீகமும் அடங்கியுள்ளது என்பதை நம்பியவர் பாரதி. தொட்டில் பழக்கம் சுடுகாடு வரை என்பது போல 'வீட்டுப் பழக்கம் தான் நாட்டில் தோன்றும்' எனவே குடும்பத்தவர் கூடித் துன்பமில்லாமல் வாழ வேண்டும். வீட்டில் எப்பொழுதும் அன்பு, அமைதி, நிலவ வேண்டும். கணவன் மனைவி கருத்தொருமித்து இல்வாழ்க்கை வாழ வேண்டும் இவற்றினை வள்ளுவர்,

> **"இல்வாழ்வான் என்பான் இயல்புடைய மூவருக்கும்**
> **நல்லாற்றின் நின்ற துணை"**

என்று கூறுகிறார். குடும்ப வாழ்வின் சிறப்பை நன்குணர்த்த வேண்டி பாரதி கட்டுரையில்,

> "வீடு துன்பமாக இருப்பதன் மூலகாரணம் கணவனுக்கும், மனைவிக்கும் மனப்பொருத்தமில்லாமை ஸ்திரீ–புருஷ விரோதத்தால் உண்டாகும் துன்பங்களோ வீட்டுத் துன்பங்களுக்குக் கெல்லாம் ஆதாரம்"
>
> (பாரதியார் கட்டுரைகள் 1940– பக். 496)

என்கிறார். தேசம் இல்லாவிட்டால் குடும்பம் கிடையாது என்ற உண்மையை புலப்படுத்துவதுடன் ஒருவனும் ஒருத்தியும் இல்வாழ்க்கையில் நீடித்து ஒன்றாக வாழ முற்படுவதேயாகும் என்ற நீதியை 'உள்ளங்கை நெல்லிக்கனி' போல எடுத்துக் கூறியுள்ளார்.

ஒற்றுமை

இந்தியநாடு சுதந்திரம் பெற வேண்டுமானால் நாமெல்லாரும் ஒருதாய் வயிற்றுப் பிள்ளைகள் என்கிற எண்ணம் கொண்டு விடாமுயற்சியுடன் பாடுபட வேண்டும். நன்றி மறவாமையும், நாவடக்கமுந்தான் சமூகத்தின் நாகரீகச் சின்னங்கள் என்பதனை இளையத் தலைமுறையினர்க்குப் பாரதி எடுத்துரைத்துள்ளார்.

> **"தீயினாற் சுட்டபுண் உள்ளாறும் ஆறாதே**
> **நாவினாற் சுட்ட வடு"**

என்கிறார் திருவள்ளுவர். குறள் வழியில் நன்றி மறவாமை என்று சொல்லி பாரதி.

> "தேசியவாதிகள் தம்முன் கருத்து மாறும்போதும் கண்டனம் செய்தல் அவசியமாகத் தோன்றும் போதும் தம் இனத்தாரை

மாறாத மன வருத்தத்துக்குட்படுத்தக் கூடிய கொடூர பாஷை சொல்வதை முற்றிலும் தவிர்த்து விடுதல் நன்று''

(பாரதி தமிழ் 1953 பக். 352)

கொடூர பாஷை பயன்படுத்துவதைத் தவிர்த்து தேசநலனுக்காக அறநெறியுடன் ஒற்றுமையுணர்வுடன் செயல்பட வேண்டும் என்பதை தம் கட்டுரையில் உணர்த்தியுள்ளார்

முடிவுரை

"அறிவை நல்ல வழியில் உழைப்பவனுடைய உடம்பு முயற்சியில்லாமல் சோம்பிப் படுத்திருக்க நியாயமில்லை" என்று கூறியவர் பாரதி. தமிழ்நாட்டு மக்களை அறைகூவி அழைத்து தேசமுன்னேற்றத்திற்கு எவனிடத்தும் அறநெறிப் பண்பு தலைத்தோங்கியிருக்க வேண்டும். அத்துடன் மனித குலம் வேற்றுமைகள் யாவும் மறைந்து வாழ வேண்டும் என எண்ணி மகத்தான அறச்சிந்தனைகளை மானுட வளர்ச்சிக்கும் தேச நலனுக்கும் பாரதியார் தம் படைப்புச் செல்வங்களில் உணர்த்தியுள்ளதை இக்கட்டுரையின் வாயிலாக அறிய முடிகிறது.

பார்வை நூல்கள்:

1. திருவள்ளுவர், திருக்குறள், கழக வெளியீடு சென்னை 1983 (மறுபதிப்பு)
2. பாரதியார் கட்டுரைகள், பாரதி புத்தக நிலையம், மதுரை 1964
3. சீனி. விசுவநாதன், பல்வேறு கோணங்களில் பாரதி, முதற்பதிப்பு 11 டிசம்பர் 1992, சென்னை 600035.

■

16. பாரதியின் பக்திநிலை

முனைவர் கோ. தங்கச்செல்வி
உதவிப்பேராசிரியர், தமிழ்த்துறை
தேசியக் கல்லூரி (தன்னாட்சி), திருச்சிராப்பள்ளி–620 001.

முன்னுரை

பாட்டுக்கொரு புலவன் பாரதி என்று எழுத்துலகம் போற்றும்படித் தன்னுடைய எழுத்துக்கள் மூலமாக இன்றும் வாழ்ந்து கொண்டிருப்பவர் பாரதி. பாட்டிலக்கியத்தினைப் பாமர மக்களும் அறிந்துகொள்ள வேண்டும் என்ற விருப்பத்தில் வசன கவிதைகளை அறிமுகப்படுத்திய கவிஞன் பாரதி. தேசியம், பக்தி, சமய சமரசம், பெண்ணியம், பெண்விடுதலை, மொழிப்பற்று, பாப்பாபாட்டு எனப் பல்வேறு நிலைகளில் தன்னுடைய உள்ள உணர்வுகளை வெளிப்படுத்தினாலும் பாரதி படைப்புகளில் பக்திஉணர்வு சிறப்பிடம் பெற்றுத் திகழ்கின்றது. ஆழ்வார்கள், நாயன்மார்கள் பாடல்களைவிட மாறுபட்ட ஒரு சிறப்பினையும் சமய சமரச நிலையினையும் பாரதியிடம் நாம் காண முடிகின்றது. பாரதியின் பக்தி நிலையினை ஆராய்வதாக இக்கட்டுரை அமைகின்றது.

பக்தி நிலை

மனிதன் அன்புடையவனாக இருத்தல்வேண்டும் என்று விரும்பியவர் பாரதி. பரிவு, பாசம், ஆசை, காதல், பக்தி அனைத்துமே அன்பினால் விளைவன. இறைவன் மீது அன்பும் தளராத நம்பிக்கையும் வைப்பதே பக்தி. இறைவனிடம் நாம் காட்டுகின்ற பக்தியானது நமது உள்ளுணர்வால் அமைதல் வேண்டும். நமது உள்ளுணர்வினால் ஏற்படுகின்ற இறைவனைப் பற்றிய ஓர் ஆழ்ந்த நம்பிக்கையே பக்தி என்று அறியப்படுகின்றது.

தெய்வபக்தி என்பது யாது? அது எத்தன்மையது? என்பதற்குப் பாரதியார் விளக்கமளிக்கும் போது *“பக்தியாவது தெய்வத்தை நம்புதல், குழந்தை தாயை நம்புதல் போலவும், பத்தினி கணவனை நம்புவது போலவும், பார்த்த பொருளைக்*

கண் நம்புவது போலவும், தன்னைத் தான் நம்புவது போலவும் தெய்வத்தை நம்பவேண்டும்." (பாரதி நூல்கள்-மூன்றாம் தொகுதி, பக் 203-204) என வலியுறுத்துகின்றார்.

"…. ஆதலால் பக்தியே தாரகம் ஒவ்வொருவனும் தெய்வ பக்தி பண்ணலாம் ….. எல்லாரும் தெய்வ பக்தியாலே மேன்மை பெறுவார்கள். எந்தத் தொழிலும் தெய்வ பக்தியால் வெற்றியடையும் …… நம்பிக்கை வெற்றி பெறும்" (பாரதி நூல்கள், நான்காம் தொகுதி. ப. 134) என்று பக்தி செலுத்துவதால் அடையும் நன்மைகள் பற்றித் தெளிவுபடுத்துகின்றார் பாரதி.

இறைவனிடம் பக்தி கொள்வதன் மூலமாக உள்ளத்தின் சுமைகளை நீக்கி உறுதிபடைத்த உள்ளத்துடன் வாழமுடிகின்றது. ஆசைகள் நீங்கும், கல்வி வளரும், அல்லல் தீரும் உடல்வலிமை ஏற்படும். பிணி போகும் தேவையான அனைத்து நலன்களும் சேரும் என்பது பாரதியின் எண்ணம்

"பக்தி யுடையோர் காரியத்தில்
பதறார் மிகுந்த பொறுமையுடன்
வித்து முளைக்குந் தன்மை போல்
மெல்லச் செய்து பயனடைவர்"

(பாரதியார் கவிதைகள்-விநாயகர் நான்மணிமாலை-27)

என்று பக்தியின் பயனைத் தெளிவுபடுத்துகிறார்.

பக்தியின் மூலம் நல்ல பல பயன்களையும் நன்மைகளையும் அடையமுடியும். ஆனால் பக்தியானது விவேகத்துடன் கூடிய பக்தியாக இருப்பது நலம் என்பதனை,

"தெய்வ பக்திக்கு நல்ல பயனுண்டு அதனால் அதனுடன் விவேகம் சேர்ந்திருக்கவேண்டும். விவேகமில்லாதவனுடைய தெய்வ பக்திக்கு உறுதி கிடையாது. தெய்வத்திடம் ஒருவன் வரங்கேட்கப் போகையிலே முதலாவது விவேகம் கேட்கவேண்டும். விவேகம் இந்த உலகத்தில் எல்லாவிதமான செல்வங்களுக்கும் ஆரம்பம் தெய்வபக்தியால் ஒருவன் எடுத்த காரியத்தில் ஐயமடையலாம்" (பாரதி நூல் முதல் தொகுதி, ப.227) என்று தமது கட்டுரை வரிகள் மூலம் வலியுறுத்துகிறார்.

"அவனின்றி ஓர் அணுவும் அசையாது" என்று தெய்வத்தின் தன்மையினைப் பலரும் எடுத்துரைப்பர். உலகினை இயக்குவதுவும் அனைவரையும் வாழவைப்பதுவும் தெய்வமே என்பது பாரதியின் எண்ணம் இதனை,

''வலிமையுடையது தெய்வம்-நம்மை
வாழ்ந்திடச் செய்வது தெய்வம்'' **(முரசு பாடல் 25 வரி 1,2)**

என்ற வரிகள் மூலமாகத் தெளிவுபடுத்துகிறார்.

பாரதியின் கடவுள் நிலை

அனைத்துப் பொருள்களிலும் நீக்கமற நிறைந்திருப்பவன் கடவுள் அனைத்துமே கடவுளின் வடிவங்களே என்பன கடவுளைப் பற்றிய சில எண்ணங்கள். இதனை நன்கு உணர்ந்த பாரதி.

"கடவுள் பக்தியுடையவன் பிற உயிர்களுக்குத் தீங்கு நினைப்பதில்லை. எல்லா உயிர்களிடமும் அன்பு காட்டு நன்மையே செய்வார் என்ற எண்ணம் உடையவராகத் திகழ்ந்தார்."

''உயிர்களெல்லாம் தெய்வமின்றிப் பிற வொன்றில்லை
ஊர்வனவும் பறப்பனவும் நேரே தெய்வம்
பயிலுமுயிர் வகை மட்டுமின்றி இங்குப்
பார்க்கின்ற பொருளெல்லாம் தெய்வம் கண்டீர்'' **(பாரதி அறுபத்தாறு)**

என்ற பாடல்வரிகள் பாரதியாரின் கடவுள் நிலையினைத் தெளிவுபடுத்துகின்றது.

மனம், சித்தம், உயிர், காடு, மலை, அருவி, ஆறு, கடல், நிலம், நீர், காற்று, தீ, வான், ஞாயிறு, திங்கள், வானத்துச்சுடர்கள் அனைத்தையும் தெய்வங்களாகக் கண்டவர் பாரதி.

பாரதியின் பிற தெய்வ வழிபாடு

பாரதியார் சக்தி வழிபாடு உடையவர் என்றும் விநாயகர், முருகன், நாமகள், பூமகள், கண்ணன், இராமன், கோவிந்தன் எனப் பல தெய்வங்களையும் அவர் வழிபடுகின்றார். அத்துடன் தாய்த்திரு நாட்டைப் பாரத மாதாவாக்கிப் போற்றியவர்.

பாரதியின் தெய்வக் காதல்

பாரதி தனது "மூன்று காதல்" என்னும் பாடலில் தனது முதல் காதல் கலைமகள் மீது தான் என்கிறார். தனது இளம் வயதில் நாமகள் அருள் இருந்ததால் மட்டுமே தனக்குக் கவிபாடும் ஆற்றல் கிட்டியது என்பது அவரது நம்பிக்கை.

''உய்வமென்ற கருத்துடையோர்கள்
உயிரினுக்குயிராகிய தெய்வம்''

(பாரதியார் பாடல்கள், 12 சரஸ்வதி தேவியின் புகழ்)

பாரதியின் மூன்று காதலில் இரண்டாவதாகச் சிறப்பிக்கப்படும் தெய்வம் திருமகள். திருமகளைப் பற்றி திருக்காதல், திருவேட்கை, திருமகள்துதி, திருமகளைச் சரண்புகுதல் போன்ற பாடல்களையும் பாடியுள்ளார்.

பாரதி தன்னுடைய மூன்று காதலில் மூன்றாவதாக அன்னை பராசக்தியிடம் தான் கொண்டிருந்த பக்தியினைத் தெளிவுபடுத்துகின்றார். நாமகள், திருமகள், பார்வதி ஆகிய முப்பெரும் தேவியரும் ஒன்றாகி விளங்குபவளே பராசக்தி என்பது பாரதியின் எண்ணம்.

பாரதியின் ஒருமைப்பாடு

"பாருக்குள்ளே தெய்வமொன்று இதில்
பற்பல சண்டைகள் வேண்டாம்" **(பாரதியார் கவிதைகள், முரசுப்பாடல் 13)**

என்ற பாடல்வரிகள் பாரதியின் ஒருமைப் பாட்டினைக் காட்டுகின்றது.

முடிவுரை

பக்தி இலக்கியக் கவிஞர்களைவிட தெளிவான ஒரு நிலையினை பாரதியிடம் காணமுடிகின்றது. எனவேதான் பாரதியாரால் பலவகையாகப் பரவவிடும் பரம்பொருள் ஒன்றே. அதனியில் ஒளியுறுமறிவாம்" என்று பாடமுடிந்தது. அனைத்திற்கும் அடிப்படை அன்பே என்பது கவிஞரின் நிலை. அனைத்து உயிர்கள், உலகப்பொருள்கள், தேசம், மொழியினையும் தெய்வமாகப் போற்றும் பாரதியார் கனவு மெய்ப்படும்போது உலக அமைதி ஏற்படும்.

■

17. ஞானரதத்தில் தேசத்தின் போக்கும் ஞானத்தின் நோக்கும்

முனைவர் சா.நீலகண்டன்
உதவிப்பேராசிரியர், தமிழாய்வுத்துறை,
தேசியக்கல்லூரி (தன்னாட்சி), திருச்சிராப்பள்ளி–01

ஆய்வுச் சுருக்கம்

மகாகவி பாரதியார் தாம் வாழ்ந்த காலத்து நாட்டு நடப்புகளை மட்டும் பதிவு செய்த படைப்பாளி அல்ல, தொலைநோக்குச் சிந்தனைகளை மட்டும் பாடிய கற்பனைக் கவிஞன் அல்ல. உருவ வழிபாட்டுள் பரவசப்பட்டு பாடிய பக்தன் மட்டுமல்ல. பரசிவ வெள்ளத்தைப் பாடிய சித்தன் மட்டுமல்ல. இல்லறச் சுவைக்குள் ஊறித்திளைத்தவன் மட்டுமல்ல. சாதி, மத, இன அடையாளங்களைத் துறந்த ஞானி மட்டுமல்ல. மானுட மேன்மைக்காக மட்டும் பாடாமல் அனைத்து உயிர்களுக்கும் கருணையாய் கசிந்தவர் கவிஞர் பாரதியார். இத்தகைய அனைத்து பரிமாணங்களும் உள்ளடக்கிய முழுமையான படைப்பு ஞானரதம் ஆகும். ஞானம் எனும் தேர், மன அமைதிக்கான வழி, அவரவர் நிலைக்கேற்ற வாழ்வியல் அறம் என்ற கோணங்களில் மகாகவி பாரதியாரின் நோக்கும் அன்றைய இந்தியாவின் போக்கும் இக்கட்டுரையில் ஆராயப்பட்டுள்ளது.

முகவுரை

மகாகவி பாரதியாரை எந்த ஒரு தனிப்பட்ட ஆளுமைக்குள் மட்டுமாக எல்லை கட்டிவிடமுடியாது. விடுதலைக்கவி, வேதாந்தக்கவி, புரட்சிக்கவி என பன்முக நோக்குள்ள படைப்பாளி பாரதியார் ஆவார். ஞான கங்கையாகப் பொங்கியெழுந்த வாரியாக வடிந்ததல்ல பாரதியின் வரிகள். ஒவ்வொரு படைப்பும் உயிர்வதையை அனுபவித்து வறுமையிலும் அடிமைக் கொட்டடியிலும், விடுதலை வேண்டி பீறிட்டு வெளிப்பட்டவை அவரின் படைப்புகள். அவரின் ஒவ்வொரு

படைப்பிலும் விடுதலை வேட்கை இழையோடியிருக்கும். கற்பனைத் தேர் ஏறிச்செல்லும் கனவுலகப் பயணமான "ஞானரதம்" என்னும் கதையில் பாரதியாரின் நாட்டு விடுதலைப் போக்கும் ஞான விடுதலை நோக்கும் பற்றி இக்கட்டுரை வெளிப்படுத்த விழைகிறது.

> **"ஞானரதம் போலொருநூல் எழுதுதற்கு**
> **நானிலத்தில் ஆளில்லை"[1]** **பாரதிதாசன் கவிதைகள், (ப.167)**

எனப் பாரதியாரைப் பிரபஞ்ச கவிஞராக அறிவித்து பெருமிதம் கொண்டார் பாரதிதாசன். காளிதாசன், சக்திதாசன் என்ற புனைப்பெயரில் எழுதிய பாரதியார் காலைக்கடன் முடித்து கிணற்றில் தண்ணீர் இறைத்து குளித்து முடித்து ஈரவேட்டியை உலர்த்தும்போதும் ஓம் சக்தி, ஓம் சக்தி என ஓதுவதை இயல்பாகக் கொண்டவர். பாரதியார், துன்பத்தில் துவளும்போதும் பராசக்தி பார்த்தக் கொள்வாள் எனப் புதுச்சேரியில் அரவிந்தனின் தொடர்புக்குப் பிறகு தன் ஞான வாழ்கைக்கு வீரியம் கூட்டினார். நடைமுறை வாழ்க்கையின் அனைத்துச் சுவடுகளையும் மறந்துவிட்டு ஒரு கனவுலகத்தில் நம்மை ஞானரதம் ஏற்றிச்செல்லும் என்ற எதிர்பார்ப்புடன் படிப்பவர்க்கு அக்கதை ஏமாற்றத்தையே தரும். உபசாந்தி உலகம், கந்தர்வ லோகம், சத்திய லோகம் என வான மண்டலத்தை வலம்வரச் சிறகு முளைத்தபோதும் நடைமுறை இந்தியாவின் அடிமைச் சிறு மதியையும் அறியாமையையும் கண்டு கவலைக் கொள்கிறார் பாரதியார்.

ஞானம் என்பதோர் தெய்வீகரதம்

பின்மாலைப் பொழுதில் திருவல்லிக்கேணி வீரராக முதலித் தெருவில் உள்ள வீட்டில் மேல்மாடியிலிருந்த பாரதியார் "நேர்த்தியான ஒரு கதிரை வண்டியில் ஏறிக்கொண்டு கடற்கரையோரமாகத் தெற்கே அடையாற்றுக்குனுப்போய், வழியெல்லாம் காளிதாசனுடைய சாகுந்தலத்தையேனும் அல்லது ஒரு உபநிஷத்தையேனும் கொண்டுபோய் படித்து இன்பமடைந்து திரும்பினால் நல்லது. ஆனால் என்னிடம் குதிரை வண்டி கிடையாது என்ற விஷயம் அப்பொழுதுதான் ஞாபகத்திற்கு வந்தது"2 *(ஞானரதம், ப.160)*

மாலை வேளையில் கடற்கரைக் காற்றின் சுகந்தமும், குளிர்ச்சியும் உடன் இரண்டு நூல்களுடன் மகாகவியை உலாச் செல்லத் துண்டின. எனினும் வறுமை அவரின் ஆசையை அகற்றிவிட்டு ஏக்கத்தைத் தந்தது. இச்சுழல் எல்லோர்க்கும

நிகழும் இயல்புதான். எனினும் பாரதியார் இவ்வறுமைச் சுழலை வாகைக் கொள்ள ஒரு வழி சொல்கிறார்.

> "சகல மனிதர்களிடத்திலும் ஈசன் ஞானம் என்பதோர் தெய்வீகரதத்தைக் கொடுத்திருக்கின்றார். அது விரும்பிய காட்சிகளையெல்லாம் பார்த்து வரக்கூடிய வல்லமை உடையது. அதைப் பயன்படுத்தி இன்பமடையாமல் எந்த நிமிஷத்திலும் உன்னைக் கீழே தள்ளித் தீங்கு செய்யக் கூடியதாகிய இழிவு மர வண்டியிலே ஏன் விருப்பம் கொள்கிறாய்"[3] (ஞானரதம், ப.161)

பொருளில்லார்க்கு பூவுலகில் இன்பம் வாய்க்காவிட்டாலும் ஞானம் என்னும் தெய்வத் தேரேறி கற்பனை உலக இன்பங்களைக் கொள்ளலாம். சற்று விழிப்புணர்வோடும், அயராத அறிவுணர்ச்சியோடும் கனவில் கரைந்துவிடாமல் பயணித்தால் வாழ்க்கைச் சிக்கலுக்கும் வரமான தீர்வைத் தரும். வெறும் கற்பனை உலகத்துப் பயணம் தற்காலிய மனமகிழ்ச்சி மட்டும் தரும். பாரதியார் பயணித்த ஞான உலா சமூகச்சிக்கல் நால்வருணப் பாகுபாடு ஆகியவற்றிற்குத் தீர்வு தருவதாக அமைகிறது.

உபசாந்திலோகமும் மனசாந்திக்கு வழியும்

வேத உபநிடதக் கருத்துகளை எளிய மக்களுக்கு புரியும்படி எழுதுவது பாரதிக்குத் தணியாத தாகம் உண்டு. மனித குலம் தோன்றிய காலம் தொட்டு இன்று வரை மன அமைதிக்கான வழிதேடி அலைகிறது. பொருள் துய்ப்பில், இன்பத்திளைப்பில், புகழ்ச்சிக் களிப்பில் எவ்வெற்றிலும் அமைதியின் முழுமைக் காணாமல் அவதியுறுகிறது. எக்காலத்திலும் ஏற்கும் இறுதி முடிவாக பாரதியார்,

> "மானுடா மனம் இறந்த பிறகுதான் உபசந்தியுண்டு. அது இருக்கும்வரையில் கவலை நீங்கி இருக்கலாம் என்ற எண்ணம் வீண். கவலைகளாகிய அசுரர்களை இடைவிடாது பெற்றுத் தள்ளிக் கொண்டே இருக்கும் தாய் மனமேயாகும்."[4]
> (ஞானரதம், ப.167)

என மனம் இறந்தபிறகுதான் அமைதியண்டாகும். கவலைத் தீரும் என்கிறார். மேலும் உபசந்திலோகம் என்பது அனைத்து ஜீவன்களுக்கும் தாய்வீடு. அங்கு செல்லும் உரிமை சகல ஜீவராசிகளுக்கும் உண்டு. யாராலும் தடுக்க இயலாது எனக் கூறுவதன் மூலம்.

கந்தர்வலோகத்திலும் அன்னை தேசத்து அவலநினைவு.

நிகழ்காலத்து நினைவுகளை விட்டொழித்து கந்தர்வலோகத்தில் கவலையற்றுப் பறந்தவரல்லர் பாரதியார். தனக்குக் கிடைக்கும் நலமெல்லாம் தன் பிள்ளைகளுக்குக் கிடைக்க ஏங்கும் தாய்மையுள்ளம்போல கந்தர்வலோகத்தில் பர்வதகுமாரி என்னும் தேவகன்னியின் கையைப்பிடித்தபோதும் பாரதியாருக்குப் பாரத தேசத்தின் அவலநிலை நினைவுக்கு வருகிறது. ஞானம் சிற்றின்பம் அனைத்தும் வெறுத்து ஒதுக்க வேண்டும்.

ஞானம் எனும் பேரின்பம் வாய்க்க சிற்றின்ப எண்ணம் சிறிதும் கூடாது என்று கட்டுரைக்கிறார் பாரதியார். காதலிளங்கன்னி பர்வதகுமாரியுடன் கைக்கோர்த்து கந்தர்வலோகம் முழுக்க களியுலா வருகிறார். அப்போதும் பாரத தேசத்தின் பரிதவிப்பைப் பின்வருமாறு உணர்த்துகிறார்.

> ‘‘எனக்கு திடீரென்று பாரத நாட்டிலே சிலைத்தொழில் இப்போது சீர்குன்றி நாசமடைந்து போயிருக்கும் விஷயம் ஞாபகத்திற்கு வந்தது அடடா! கந்தர்வ நாட்டிற்கு வந்தும் அந்த கஷ்ட தேசத்தினுடைய ஞாபகம் மறக்கவில்லையே’’ [5]
> (ஞானரதம், பக்.200–201)

காலைக் கதிரவன் உதித்து கதிர் பரப்பி ஒளி தரும் பேரழகை விடுதலை பெற்றதுபோல் பேருண்மையானது எனக் கூறுகிறார் பாரதியார். விடுதலை கிடைத்தால் உலகம் வெளிச்சம்பெறும் எனும் தவிப்பு கந்தர்வலோகத்தில் கண் விழிக்கிறது. மண்வளம், இயற்கைவளம், மனிதவளம் யாராலும் சுரண்டப்படாமலும், ஏய்க்கும் பிழையின்றி அனைவருக்கும் பொதுவான சமூகம் அமைந்தால் அதுவே கந்தர்வலோகம் என உரை முடிகிறது. கந்தர்வலோகம் என்பது கடவுளால் படைக்க முடிகிற கற்பனை உலகமல்ல.

சமதர்ம உணர்வுடைய அனைவரும் ஒன்றுகூடி வாழும் முறையில் அவ்வுலகம் வாய்க்கும் என்பது பாரதியின் கருத்தாகும்.

சனாதன தர்மமும் தர்மலோகமும்

சாதியாலும், மதத்தாலும், வருணாசிரம அடுக்குகளாலும் மனிதர்கள் பிரிந்து கிடப்பது மனித குலத்தின் பேரழிவாகும். அதுவும் பிறப்பால் இப்பேதம் நிலைநிறுத்தப்படுவது மனிதனை மிருக நிலைக்குத் தாழ்த்தப்படுவதாகும்.

ஒருவன் தன் செய்கையாலும், தர்மத்தினாலுமே
தனது சாதிக்கு வருணாசிரமத்திற்கு-உரியவனாகிறான்
தெரியுமா? பிறவியினுல்ல''[6] **(சம்பந்தம், புதுவையில் பாரதி, ப.103)**

என்று பாரதியார் அடிக்கடி பேசுவார். அதைத்தான் ஞானரதம் கதையில் வரும் கண்வரிஷியின் உபதேசம் வழியாக தெளிவாக விளக்கியிருக்கிறார்.

ஒருவன் முன்வினைப் பயனும், தர்ம சிந்தனையும், செய்கையும் உடையவனாயிருந்தால் மட்டுமே தர்ம உலகத்தில் பிரவேசிக்க முடியும் எனக் கூறுவதின் வாயிலாகப் பாரதி அடைந்த லோகங்கள் மனித மனத்தின் எண்ண அடுக்குளாகவும், ஞானத் தேடல்களின் படிநிலைகளாவும் உரை முடிகிறது. அந்தணர், வணிகர், சத்திரியன், வணிகர், வேளாளர் எனும் பகுப்பு அவரவர் செய்யும் தொழிலால் அமைகின்றன என ஞானரதம் கதையில் பதிவு செய்த படைப்பாளியாக மட்டுமின்றி புதுச்சேரியில் கனகலிங்கம் என்பவருக்கு பூணூல் அணிவித்து கருத்துக்கும், கடமைக்கும் தூரமில்லாதவர் மெய்ப்பித்திருக்க பாரதியார் தன் இல்லத்தில் கனகலிங்கத்துக்கு நடத்திய பூணூல் வைபவத்தில் கலந்து கொண்ட அனைவரும் சென்றுவிட மிஞ்சியிருந்தவர் வா.ரா. மட்டுமே ஆவார். அவரிடம் பாரதியார்,

''இதோ பார் நாடறிந்த பார்ப்பானுக்கு
பூணூல் எதற்கு? என்பார்கள். அதனால்
உனக்கும் எனக்கும் வேண்டாம் புது பார்ப்பான்
கனகலிங்கத்துக்கு பூணூல் தேவை. ஒரு தடவை
மாரியம்மன் கோயிலுக்குப் போய் பாரேன்.
அவன் செய்யும் அந்தணர் தொழிலை
அதனையும் எத்தனை ஒழுங்காய் செய்கிறான் தெரியுமா?''[7]

(சம்பந்தம், புதுவையில் பாரதி, ப.103)

இந்த எண்ணத்தின் ஈடேற்றத்தால்தான் பாரதியார் அணிந்திருந்த பூணூலையும் கழற்றிவிட்டார். புதுச்சேரி மாரியம்மன் கோயில் பூசாரியான கனகலிங்கத்தை செந்தண்மைப் பூண்டொழுகும் அந்தணனாக அடையாளம் கண்ட பாரதி பூமண்டலத்தில் சமத்துவப் பாதையில் ஞானரதம் செலுத்திய சாரதியாகிறார்.

நிறைவுரை

கந்தர்வலோகத்திற்கும், பூலோகத்திற்குமான வேறுபாடுகளைக் காணும் பாரதியார். அறிவுத்தேடலும்,

உழைப்பும் பூமண்டலத்து மனிதர்களுக்கிடையே இருப்பதால் இவ்வுலகம் கந்தர்வலோகத்தைவிட உயர்ந்தது எனக் கூறுகிறார்.

அருவியின் இயற்கையழகும், மனிதனின் கோயிலின் கலையழகும், சித்திரச் சாலையின் செயற்கையழகும், பாடிய விதத்தில் வானுலகும், பூவுலகும் இணையானது. உழைப்பால்தான் அண்டம் இயங்குகிறது. சுகந்த மாளிகையின் மெத்தையில் சாய்ந்தது. தாயின் மடியில் சாயந்தது போலிருந்தது என்பதன்வழி தாயின் மழலை தேவலோகத்துக்கு இணையானது.

"காதலும், ரிஷிதன்மையும் நன்று போலத்தான்",

சிற்றின்பத்தில் பரிபூரணம் கண்டவன் ஞானத்தின் இலக்கணம் அறிய முடியும். கந்தர்வலோகத்தில் பர்வதகுமாரியுடன் காதலை அனுபவித்த பொழுதைவிட தர்மலோகத்தில்தான் மனம் அமைதி அடந்தது என்றதின் மூலம் வாழ்க்கையின் பல கட்டங்களை உணர்ந்து கடக்க வேண்டும் என்கிறார். கந்தர்வலோகத்தில் ஞானத்தைத் தேடுவது எனப் பக்குவப்பட்ட மனத்தின் படிநிலைகள் என்பனவும், சிற்றின்பமும், பேரின்பமும், தேசவிடுதலையும், ஞான விடுதலையும், "யோகத்தின் போக்கும், யோகத்தின் நோக்கும்" இரு சக்கரங்களாக்குகிறார் பாரதியார்.

ஆய்வுக்குப் பயன்பட்ட நூல்கள்

1. சுப்பிரமணிய பாரதியார், படிக்க வேண்டிய பாரதியார் கவிதைகள் (தொகுதி–2), வெளியிடுவோர் எம்.வெற்றியரசி, ஜோசப் காலனி, சென்னை–88.
2. சம்பந்தம், புதுவையில் பாரதி, மணிவாசகர் பதிப்பகம், முதற்பதிப்பு–நவம்.1982.
3. பாரதிதாசன் கவிதைகள், ஸ்ரீ இந்து பப்ளிகேசன், சென்னை– 35, ஒன்பதாம் பதிப்பு–2011.

18. மானுட உயர்வும் இன்பமும்

முனைவர் இரா.பத்மா
உதவிப்பேராசிரியர், தமிழாய்வுத்துறை,
தேசியக்கல்லூரி (தன்னாட்சி), திருச்சிராப்பள்ளி–01

முன்னுரை

மானுடனுக்கு மகாகவி பாரதியார் சொல்வது மனிதகுலம் உயர்வதற்கான மகத்தான அறிவுரையாகும். குருவி சொல்வதாகக் கூறுகிறார். எங்களுக்குள் கீழ்மக்கள் மேன்மக்கள் என்ற பிரிவு இல்லை. நாங்கள் எல்லோரும் மன்னர்களாகவே பறந்து திரிகிறோம். எல்லா இடங்களிலும் எங்களுக்கு உணவு கிடைக்கும். எனவே உணவு பற்றிய கவலை இல்லை.

எங்களிடம் பணம் காசு இல்லை என்றாலும்கூட எங்குப் பார்த்தாலும் எமக்கான உணவுதான். இந்த ஒரு சிறிய வயிற்றை நிரப்புவதற்காக எங்களுடைய பிறவியெல்லாம் வீணாகக் கழியுமாறு கட்டுத்தளைகள் இருப்பதுபோல எண்ணிக் கொண்டு பிறருடைய ஆளுகையின் கீழ் துன்பப்படுவதில்லை. காற்றும், ஒளியும் நிறைந்த ஆகாயமே எங்கள் வீடு. எங்கள் வாழ்வில் என்றும் தாழ்வில்லை. கள்ளம் கபடமில்லை. வெறும் கர்மங்களும் சிறுமையுமில்லை.

மானுட உயர்வுக்கான வழிகள்

இழிவதற்குரிய இழிகுலங்கள் எல்லாம் உமது மனித இனத்தில் நான். எளியவர்களை வலியவர்கள் கொடுமை செய்து கொன்றழிப்பதும் இங்கில்லை என்று தொடர்ந்து கூறும் அந்த குருவி.

“தானே தளைப்பட்டு-மிகச் சஞ்சலப்படும் மனிதா
நானோர் வார்த்தை சொல்வேன் - நீ
மெய்ஞ்ஞானத்தைக் கைகொள்ளடா” (பு.பா.2)

என்று அறிவுரை பகர்வதாக அந்தப் பாடல் தொடர்கிறது.

- மெய்ஞ்ஞானத்தைக் கைக்கொள்
- விடுதலையைப்பெறு–விண்ணவர் நிலை பெறலாம்
- கீழ்மைக் குணத்தை உதறு – இன்பநிலை பெறலாம்
- அன்பினைக் கைக்கொள் இதனை உலகுக்கு எடுத்துச்சொல்
- துன்பங்கள் இனி இல்லை–துயரங்களை ஒழித்திடு
- சத்தியத்தைக் கைக்கொள் – சஞ்சலம் இல்லை
- பொய்களை, போலிவேடத்தை ஒதுக்கு–தர்மத்தைக் கைக்கொள் சங்கடம் இல்லை
- அச்சத்தை விடு–ஆண்மையைக் கைக்கொள் (பு.பா.2)

என்று பாரதி கூறுவதைப் பின்பற்றினால் இன்பமாக வாழலாம்.

பேசாப்பொருளும் கேட்காவரமும்

> **"பேசாப் பொருளை பேச நான் துணிந்தேன்**
> **கேட்கா வரத்தைக் கேட்க நான் துணிந்தேன்"** **(தோ.பா.1-32)**

என்ற வரிகளில் பாரதி கூறும் பேசாப்பொருள் எது தெரியுமா? புற்பூண்டு, மரங்கள் யாவும் என் செயலால் துன்பம் நீங்கி இன்புற்று அன்புடன் இணங்கி வாழ்ந்திடச் செய்ய வேண்டும் என்பது தான். அதற்காக ஞானமாகிய ஆகாய வெளியினில் நின்று பூமியில் அன்பும், பொறாமையும் விளங்க வேண்டும். துன்பம், சோம்பல், நோய், இறப்பு யாவும் நீங்கி பல்லுயிர்கள் எல்லாம் இன்புற்று வாழவேண்டும் என்று யாரும் கேட்கா வரத்தை நான் கேட்பேன். அங்ஙனமே ஆகுக என்று நீ அருள வேண்டும் என்று விநாயகப் பெருமானை வேண்டுகிறேன்.

"தொல்லுலக உயிர்களெல்லாம் ஒன்றே" என்று எண்ணும் பரந்த மனம் கொண்ட ஒருவனால் தான் இவ்வாறு பாட முடியும்.

மனித வாழ்வுக்கு பாரதிக் கூறும் கடமைகள்

பூமியில் தோன்றிய எல்லோருக்கும் நான்கு கடமைகள் உண்டு

- தன்னைக் கட்டுதல்
- பிறர் துயர் தீர்த்தல்
- பிறர் நலம் வேண்டுதல்
- உலகெலாம் காக்கும் ஒருவனைப் போற்றுதல்.

இதனால் அடையும் பயன்களான அறம், பொருள், இன்பம், வீடு என்னும் நான்கையும் தனக்கு நல்க வேண்டும் என்று வேண்டுகிறார். (தோ.பா.1-8)

"தன்னைத்தான் ஆளும் தன்மைநான் பெற்றிடில்
எல்லா பயன்களும் தர்மமே எய்தும்
அசையா நெஞ்சம் அருள்வாய்" **(தோ.பா.1-8)**

என்றும் தீர்மானித்து, உறுதியான நெஞ்சத்தை வேண்டுகிறார்.

"யார்க்கும் எளியனாய், யார்க்கும் வலியனாய்
யார்க்கும் அன்பனாய் யார்க்கும் இனியனாய்
வாழ்ந்திட விரும்பினேன்" **தோ.பா.1-12 (9-11)**

என்ற பாடலில் யாவர்க்கும் எளியனாக, யாவரிடத்திலும் அன்புடையவனாக, யாவருக்கும் இனியவனாக வாழ்ந்திட விரும்புவதாகக் கூறுகிறார்.

கவலை, வஞ்சனை, கரவு, புலமை, விருப்பம், ஐயம் முதலியவற்றையெல்லாம் வெறுத்து ஒதுக்க வேண்டும் என்கிறார்.

எவையெல்லாம் தமக்கு வேண்டும். எவையெல்லாம் தமக்கு வேண்டாம் என்று பட்டியலிடுகிறார்.

"நோவு வேண்டேன் நூறாண்டு வேண்டினேன்
அச்சம் வேண்டேன் அமைதி வேண்டினேன்
உடைமை வேண்டேன் உன்துணை வேண்டினேன்"

(தோ.பா.1-20 (14-16)

என்று வேண்டாத அனைத்தையும் நீக்கி, வேண்டியது அனைத்தையும் அருளுமாறு வேண்டுகிறார். அவர் வேண்டிக் கேட்பது அவருக்கு மட்டுமல்ல மானுடம் அனைத்துக்கும் சேர்த்துதான்.

"பயம் கொள்ளாதே என்று
விண்ணின் இடிமுன் விழுந்தாலும்
பான்மை தவறி நடுங்காதே
பயத்தால் ஏதும் பயனில்லை" **(தோ.பா.1-23 (2-4)**

என்று மனதுக்கு அறிவுரை பகர்கிறார்.

"ஏழை நெஞ்சமே எதற்கும் சலிப்படையாதே நேர்மையுடன் இரு
வஞ்சகம் நிறைந்த கவலைக்கு இடம் கொடுக்காதே!

(தோ.பா.1-24 (16-18)

என்று கேட்டுக் கொள்கிறார்.

நமக்குத் தொழில்,

- கவிதை இயற்றுதல்
- நாட்டிற்கு உழைத்தல்
- இமைப்பொழுதும் சோராதிருத்தல் (தோ.பா.1–25 (1–2)

என்று தன்னைத்தானே கட்டமைத்துக் கொள்கிறார். மனமே! ஐயம், துரிதம் இவற்றால் சிந்தை அழியாதே. **(தோ.பா.1-26 (3-4)** *விதை முளைக்கும் தன்மைபோல் மிகுந்த பொறுமையுடன் மெல்லச் செயலாற்றி பயனை அடைவாயாக. பக்தி உடையவர்கள் செய்யும் காரியத்தில் பதற்றத்தோடு ஈடுபடமாட்டார்கள். எல்லாமே சக்தியுடைய காரியமே என்றால் நாம் ஏன் சஞ்சலம் அடைய வேண்டும்? (தோ.பா.1-27 (1-6) என்று அறிவுரை கூறுகிறான்.*

''பொறுத்தவரே பூமியாள்வார்'' **(தோ.பா.1-28-2)**

எனவே பொங்குதல் நீங்கிப் பொறையெனக்கு அருள்வாய் **(தோ.பா.1-28-5)** *என்று வேண்டுகிறார். மனமாகிய தேசத்தை ஒரு பிழை இல்லாமல் ஆள்வதும், ஞாயிற்றின் போராளியைப் போன்ற சுடர்மிகும் அறிவோடு துன்பமின்றி வாழ்வதுமே தனது நோக்கம்* **(தோ.பா.1-28-5)** *என்று பாரதி தன்னைத்தானே செதுக்கிக் கொள்ளும் சிற்பியாகக் கண்முன் காட்சி தருகிறார்.*

எதற்கெடுத்தாலும் கவலைப்படும் மனமே, கவலையை விட்டொழிப்பாயாக. 'அச்சம்தவிர்' எனத் தொடங்கி 'வெளவுதல்' நீக்கு மூடிய உள்ள புதிய ஆத்திச்சூடியின் 110 வரிகளும் 110 அறிவுரைகளும், இளைஞர்களின் வாழ்வை உயர வைக்கும் இரும்புப்படிக் கட்டுகள்.

வீடுபேற்று நிலை

நெஞ்சமே! தவம் செய்! தவம் செய்தால் அடைய விரும்பியதை, அடையலாம். அந்த தவம் எது என்பதை 'வையத்தில் அன்பிற் சிறந்த தவமில்லை' (தோ.பா.1-37 (2-3) என்று கட்டியம் கூறுகிறார் அந்த கர்மயோகி.

பெண் விடுதலையும் பெண்மை நெறியும்

'பல்வகைப் பாடல்கள்' என்னும் தலைப்பில் சமூகம் என்னும் உட்தலைப்பில் அமைந்துள்ள புதுமைப்பெண் பெண்மை வாழ்க. பெண்கள், விடுதலைக்கும்மி, பெண் விடுதலை என்னும் இவை நான்கு பாடல்களுமே பெண்

விடுதலைப் பற்றிப் பேசக்கிடக்கின்ற புதையல்கள் என்று கூறலாம்.

மாதர்களும் சுதந்திரமும் உண்டு. அறிவு கொண்ட மனிதர்களான அவர்களை அடக்க முயல்பவர்கள் பைத்தியக்காரர்கள் ஆணும், பெண்ணும் சமம் என்று கொள்வதால் இந்த உலகம் அறிவில் மேம்பட்டு செழிக்கும். நல்லறம் பணி பெண் வடிவில் இங்கு இருப்பவள் நமது தாய் சிவசக்தியே ஆவாள். நாணமும், அச்சமும், நாய்களுக்குரிய குணங்களாகும். ஞானம், வீரசுதந்திரம், நல்லறம் இவை நற்குடிப் பெண்களின் குணங்களாகும்.

நிலத்தின் தன்மையே அந்நிலத்தில் - விளையும் பயிர்களுக்கு இருக்கும். நற்குல மாந்தர்க்கு கற்பு என்பது இயல்பான ஒன்று. கொடுமையாலும், அறிவை அழித்தும் கற்பைக் காக்க விரும்புவது தீது. வேதநெறிப்படி மானிடர் வாழ்ந்த காலத்திலேயே, புதுமைப் பெண்களின் இத்தகைய பேச்சும், செய்கையும் பொதுவான வழக்கமே.

நிமிர்ந்த நன்னடை, நேர்கொண்ட பார்வை, யார்க்கும் அஞ்சாமை ஞானச்செருக்கு இவையெல்லாம் செம்மையாக! மாதர் பண்புகள் ஆகும். பேரிருளம் அறியாமையில் ஆழ்ந்து, துன்பத்தோடு அறிவுக் கலைகள் இன்றி வாழ்வதைக் காறி உமிழ்ந்து ஒதுக்குவதே பெண்மை நெறியாகும். (ப.பா.4)

புதுமைப் பெண்ணின் இயல்பும் உழைப்பும்

பலப்பல சாத்திரங்கள் கற்று, பலப்பல சவுகரியங்கள் செய்து காலம் காலமாக இருந்துவரும் பொய்மைகளை அழித்து மூடக்கட்டுகளைத் தகர்த்து கடவுளுக்கு இனியதாக மனிதர்களின் செயல்கள் அனைத்தும் உருவாக்குவதே (ப.பா.4-9.1-6) புதுமைப் பெண்களின் இயல்பாகப் பாரதி கூறுகிறார்.

உலக வாழ்க்கையின் நுட்பங்களை அறிய பற்பல வகையான நூல்களைக் கற்றிட, நான்கு திசைகளிலும் உள்ள நாடுகள் அனைத்துக்கும் சென்று புதுமைகளை இங்கு கொணர்ந்து நமது பாரததேசம் முன்னேற இவர்கள் உழைக்க வேண்டும். (ப.பா-4-8 (1-16) என்று அறிவுறுத்துகிறார்.

பெண்ணும் பெருமையும்

'பெண்மை வாழ்க' என்னும் பாடலில்,

'துன்பம் தீர்வது பெண்மையினாலடா' (ப-பா-5-2 (3) இல்லத்தில் வாழும் மகளிருள் முக்கியமானவர் இருவர் ஒருவர்

தாய் மற்றொருவர் மனைவி. இருவரின் உயர்வைச் சொல்லும் பாரதியின் அழகிய வரிகள்,

"வலிமை சேர்ப்பது தாய் முலைப்பாலடா!
மானஞ் சேர்க்கும் மனைவியின் வார்த்தைகள்! (ப-பா-5-3(1-2)

"உயிரை காப்பது பெண்மை! உயிரினைச்
சேர்ப்பது பெண்மை!
உயிரினுக்கு உயிராய் இன்பமாகி விடுவது பெண்மை!
எனவே, உயிரினும் இந்தப் பெண்மை இனிதாம்" (பா-5.6-3)

நுண்ணிடைப் பெண் ஒருத்தி சொன்னால் நூற்றிரண்டு மலைகளையும் சாடுவோம். காதற் பெண்கள் கடைக்கண் காட்டினால் காற்றிலேறி அவ்விண்ணையும் சாடுவோம். எல்லாவற்றையும்விட,

"அன்ன மூட்டிய தெய்வ மணிக்கையின்
ஆணை காட்டில் அனலை விழுங்குவோம்" ப.பா-5-9 (1-2)

என்று பாரதி பெண்மையை உயர்வாகக் கூறுகிறார்.

"கற்பு நிலையென்று சொல்ல வந்தார்! இரு
கட்சிக்கும் அஃது பொதுவில் வைப்போம்!" ப-பா-6-5(1-2)

"மெய்யறிவும் மெய்ப்பொருளும்
நெஞ்சில்
ஒன்றுங் கவலையில் லாமலே-சிந்தை
ஊன்ற நிறுத்திக் களிப்புற்றே-தன்னை
வென்று மறந்திடும் போதினில் - அங்கு
விண்ணை யளக்கும் அறிவுதான்" க.பா-7-6(4-8)

எனப் பாரதி மெய்ப்பொருள் பற்றிக் கூறுகிறார். ஒரு சக்திக்கு ஏற்பவே நாம் இயங்குகிறமோ, தவிர தானாக இயங்கும் ஆற்றல் நமக்கில்லை என்று விளக்கம் தருகிறார்.

"தோன்றி அழிவது வாழ்க்கைத்தான் - இங்குத்
துன்பத் தொடின்பம் வெறுமையாம் - இவை
மூழ்கி நடத்தல் முறை கண்டீர்"

தோன்றி அழிவது வாழ்க்கை என்பதையும், எந்த ஒரு விஷயத்தையும் பொய்யை மெய் எனக் கொள்ளாதீர். நாம் செய்த ஊழ்வினையோடு, மற்றவர்கள் செய்கின்ற கர்ம வினைப் பயனும் நம்மை வந்து அடையும். எவ்வாறு தெரியுமா? ஆற்றில் உள்ள சிறுகுழியில் நாலாப்பக்கமிருந்தும் பலவகையான

குப்பைக் கூளங்கள் வந்து படிவதுபோல என்று உவமை கூறி அதனை விளக்குகிறார்.

"நதியிலுள்ள சிறுகுழி தன்னில்
நான்கு திக்கிலிருந்தும் பன்மாக
பதியுமாறு பிறர்செய்யுங் கர்மப்
பயனும் நம்மை அடைவ துண்டன்றோ?" **பா.ச.36-182 (5-8)**

சூத்திரனுக்கு ஒரு நீதி, தண்டச்சோறு உண்ணும் பார்ப்பனனுக்கு ஒரு நீதி என்று சாத்திரம் சொல்லுமானால் அது சாத்திரம அல்ல. சதி என்று கூறுகிறார். *(பு.பா-1-13)*

தன்னைச் சேர்ந்தவர்கள், நண்பர்கள், ஊர்க்காரர் (உற்றவர், நட்டவர், ஊரவர்) இவர்களுக்கு உண்மைகளைக் கூறி, இனியன செய்தலே நற்றவம் என்கிறார். *(பு.பா-1-15)* தன் அருகே இருப்பவர்களின் துன்பத்தினைப் பார்க்க மனம் தாளாதவனே புண்ணிய மூர்த்தி. உலக மக்கள் அனைவரையும் சரிசமமாகச் சீர்ப்படுத்தி அவர்கள் நலம்பெறும் வகையில் உழைப்பவனே யோகி. ஊர்மக்களுக்காக உழைத்திடலே யோகம். நலம் ஓங்கிட தன்னை வருத்திக் கொள்ளுதலே யாகம். போர் செய்வதற்காக நிற்கும்போதும், உள்ளக் கொந்தளிப்பு இல்லாத நிலையே மெய்ஞானம். பு-பா.*(1-15,16,17)* என்று விளக்குகிறார்.

முடிவுரை

அன்ன மூட்டிய தெய்வ மணிக்கையின்
ஆணைக் காட்டில் அனலை விழுங்குவோம்
கன்னத் தேமுத்தம் கொண்டு களிப்பினும்
வையைத் தள்ளும்பொற் கைகளைப் பாடுவோம்

நமக்கு உணவூட்டிய தெய்வமாகிய தாய் தன் ஒளி பொருந்திய கரத்தால் சுட்டிக்காட்டி உத்திரவிட்டால் நெருப்பைக் கூட உட்கொள்வோம். காதலியர் கன்னத்திலே முத்தமிட்டு இன்பமடையும் வேளையிலும், எங்களுடைய கரங்களை விலக்கி ஒதுக்கும் அவர்களுடைய அழகு மிக்கக் கரங்களால் பாடிப் புகழ்வோம்.

■

19. பாரதியின் உளவியல் பார்வை

முனைவர் இரா. புவனா
உதவிப் பேராசிரியர், தமிழ்த்துறை
தேசியக் கல்லூரி, திருச்சி 620 001.

முன்னுரை

இருபதாம் நூற்றாண்டின் ஈடு இணையற்ற இந்திய எழுத்தாளர்களுள், தமிழ்க் கவிஞர்களுள் துருவ நட்சத்திரமாக ஒளி வீசித் திகழ்பவர் சுப்ரமணிய பாரதியார். இவரைத் தமிழ் கூறும் நல்லுலகம் 'மகாகவி' என்றும் 'தேசியகவி' என்றும் 'புரட்சிகவி' என்றும் 'வரகவி' என்றும் பலவாறாகச் சிறப்பித்து அழைக்கும். கள்ளையும், தீயையும், காற்றையும், வானத்தையும் கலந்து குழைத்து படைத்த இவரது கவிதைகள் இலக்கியத் துறையில் மறுமலர்ச்சியையும், சமூக வாழ்வில் விழிப்புணர்ச்சியை ஏற்படுத்தினாலும், தனிமனித வாழ்வில் உடல் ரீதியாக அல்லது மனரீதியாக ஏற்படும் தாக்கங்களுக்கும், மன அழுத்தத்திற்கும் சரியான வழிகாட்டியாக பாரதியார் திகழ்கிறார். மன அழுத்தம் முதலான உணர்ச்சி போராட்டங்களை எதிர் கொள்ள முடியாத பலர் தவறான முடிவுகளை வாழ்வில் மேற்கொண்டு, துன்பத்தில் துவண்டு இறுதியில் இறக்கிறார்கள். அத்தகைய உளவியல் சிக்கலுக்கான தீர்வை பாரதியின் கவிதைகளை கொண்டு ஆய்வதே இக்கட்டுரையயின் நோக்கமாகும்.

குடும்பம்

ஒரு சமுதாயத்தின் அடிப்படை அலகு குடும்பம் ஆகும். குடும்பத்திலுள்ள உறுப்பினர்களை அன்பு என்னும் இழையினால் பிணைக்கப்படும் பொழுதுதான் சமுதாயம் ஒற்றுமையுடன் செயல்பட முடியும். ஆங்கிலத்தில் "உாயசவைல டிநபைளெ யவ ாழஅந" என்று கூறுவார்கள். இதை முரசு என்னும் பாடலில்

"ஒற்றைக் குடும்பம் தனிலே-பொருள்
ஓங்க வளர்ப்பவன் தந்தை
மற்றைக் கருமங்கள் செய்வதே-மனை
வாழ்ந்திடச் செய்பவர் அன்னை

ஏவல்கள் செய்பவர் மக்கள் - இவர்
யாவரும் ஓர் குலமன்றோ?
மேவிய னைவரும் ஒன்றாய் - நல்ல
வீடு நடத்துதல் கண்டோம்''

என்று குடும்பத்தினர்கள் அனைவரும் ஒருமித்த சிந்தனையுடன் அவரவர்களுடைய கருமங்களுடன் தருமங்களுடனும் செயல்பட வேண்டும் என்று குறிப்பிடுகின்றார்.

ஆனால் குடும்பத்தினர்களிடையே ஒற்றுமையில்லை என்றால் பகை தோன்றும் என்பதை

''ஐந்து தலை பாம்பென்பான் - அப்பன்
ஆறுதலை என்று மகன் சொல்லிவிட்டால்
நெஞ்சு பிரிந்திடுவார் - பின்பு
நெடுநாள் இருவரும் பகைத்திருப்பார்''

என்று குறிப்பிடுகிறார்.

பெண்கள் குடும்ப அமைதியைக் கெடுத்துப் பேராபத்தில் சிக்கிவிடக் கூடாது. கணவன் – மனைவி இருவரும் பரஸ்பரமான உறவு இருந்தால் தான் குடும்பம் நன்முறையில் இயங்கும் என்பதை பெண்கள் விடுதலைக் கும்மியில் எடுத்துரைக்கின்றார்.

''காதலொருவனைக் கைப்பிடித்தே யவன்
காரியம் யாவினும் கை கொடுத்து
மாதரறங்கள் பழமையைக் காட்டிலும்
மாட்சிபெறச் செய்து வாழ்வமடி''

இல்லறமே நல்லறம்

தனிமனித ஒழுக்கத்தில் இல்லறம் நல்லறமாக அமைந்தால் மன அழுத்தத்திற்கு வாய்ப்பில்லாமல் வீடும் நாடும் சிறந்து ஓங்கும் என்பது பாரதியின் கருத்து.

''செத்த பிறகு சிவலோகம் வைகுந்தம்
சேர்ந்திடலாமென்றே எண்ணியிருப்பார்
பித்த மனிதர் அவர் சொல்லும் சாத்திரம்
பேயுரை யாமொன்றிங்கு ஊதடா சங்கம்''

என்று சங்கு கவிதையில் ஆணையிடுகின்றார். மேலும்

''காவித் துணி வேண்டா கற்றைச் சடை வேண்டா
பாவித்தல் போதும் பரமநிலை எய்துவதற்கே''

என்று இல்லறத்தில் இருந்து கொண்டே தியானமஇ் யோகம் ஆகியவற்றின் மூலம் பரம நிலை அடையலாம் என்று வழிகாட்டுகின்றார்.

மனித வாழ்வில் உளவியல் சிக்கலுக்கு ஆணிவேராக இரண்டை குறிப்பிடுகிறார்கள். அவையாவன

1) அச்சம்
2) கவலை

இதற்கு பாரதியார் தரும் தீர்வை பற்றி ஆய்வோம்.

அச்சம்

அச்சம் என்பது ஓர் உணர்ச்சிமயமான மனநிலை. மருட்சியும், நடுக்கமும் கலந்த ஒரு மெய்ப்பாடு. தொல்காப்பியர் எட்டுவகை மெய்ப்பாடுகளுள் ஒன்றாக அச்சத்தைக் குறிப்பிடுகின்றார். (தொல். பொ. 251)

திருவள்ளுவர் அச்சமே கீழ்மக்களின் ஆசாரம் என்றும (1075) அச்சமுடையவர்க்கு அரண் இல்லை என்றும் (534) கூறியிருக்கிறார். பயந்து கொண்டு வாழ்தல் பேதைமையாகும். அறிவுள்ளவன் அச்சப்படக் கூடாது என்பது பாரதியாரின் கருத்து. இதை

"நெஞ்சில் கவலை நிதமும் பயிராக்கி
அஞ்சி உயிர் வாழ்தல் அறியாமை"

என்று மகாசக்தி வெண்பாவில் கூறுகின்றார். அதே போல அச்சத்தைப் பேயாகவே உருவகிக்கின்றார் ஒரு பாட்டில்

"பயமென்னும் பேய்தன்னை அடித்தோம்"

என்று கூறி ஜய பேரிகை கொட்டுகின்றார்.

இன்பத்தையும் துன்பத்தையும் தருவது மனம். தீதும் நன்றும் பிறர்தர வாரா என்பது கணியன் பூங்குன்றனாரின் பொன்மொழி. அச்சம் மனதில் குடியேறும் போது மனம் நரகம் ஆகிறது. அஞ்சாமை மனதில் ஆசனம் கொள்ளும் போது தான் மனம் சொர்க்கம் ஆகின்றது. இதனை பாரதி வசன கவிதையில்

"அச்சமே நரகம் அதனைச் சுட்டு
நல்லதை நம்பி நல்லதே செய்க"

என்று தெளிவாகக் கூறுகின்றார். அதனால் அச்சம் மனத்தைப் பற்றிக் கொள்ளும் போது நாடித்துடிப்பும், இரத்த அழுத்தமும்

அதிகரிக்கின்றன. இரத்த நாளங்களோ, இதயமோ வெடித்து மரணம் சம்பவிக்கின்றது.

"இடருற்று மடிந்தவர்கள் கோடி கோடி
எதற்குமினி அஞ்சாதீர் புவியிலுள்ளீர்!
....."

இந்த அச்சத்தை நீக்குவதற்கு சில மனச் சிகிச்சை முறைகளை உளவியலார் எடுத்துரைப்பார். ஆனால் பாரதி அச்சத்தினை அழிப்பதற்கு ஒரே வழி பக்தி மார்க்கமே என்று பல பாடல்களின் வழியே வலியுறுத்துகின்றார்.

"சக்தி தனையே சரணங்கொள்ளு-என்றும்
சாவினுக்கோர் அச்சமில்லை தள்ளு?"

என்று பாடுகிறார். மேலும்

"அச்சமில்லை அச்சமில்லை
அச்சமென்ப தில்லையே!
உச்சி மீது வானிடிந்து
வீழுகின்ற போதிலும்
அச்சமில்லை அச்சமில்லை
அச்சமென்ப தில்லையே"

கவலை

"கலியைப் பிளந்திடக் கையோங்கினோம் - நெஞ்சில்
கவலை யிருளனைத்தும் நீங்கினோம்"

என்ற பாடலில் கவலையற்று வாழ்வதே சொர்க்கம் என மீண்டும் வலியுறுத்துகின்றார்.

"சென்றது இனி மீளாது மூடரே நீர்
எப்போதுஞ் சென்றதையே சிந்தைசெய்து
கொன்றழிக்குங் கவலையெனும் குழியில் வேண்டா
இன்று புதிதாய்ப் பிறந்தோம் என்று நீவிர்
எண்ணமதைத் திண்ணமுற விசைத்துக் கொண்டு
தின்று விளையாடி இன்புற்று வாழ்வீர்
தீமையெலாம் அழிந்துபோம் திரும்பி வாரா"

என்று மனதை நோக்கிக் கூறுவது மக்கள் எல்லோருக்கும் அமர வாழ்வுக்கு வழிகாட்டும் வாசகங்கள் ஆகும்.

மேலும் பாரதியார் ஜீவகாருண்ய ஒழுக்கத்தையும் போற்றியவர். எல்லா உயிர்களும் பரமாத்மாவின் அம்சங்களே

என்பதை நன்கறிந்து அவற்றை மனம் மொழி மெய்களால் எவ்வகையிலும் துன்புறுத்தாமல் இயன்ற வழிகளில் எல்லாம் அவற்றிற்கு நன்மையைச் செய்து வர வேண்டும் என்பது இந்து மதத்தின் மூல தர்மம் என்று விளக்கியுள்ளார்.

"காக்கை குருவி எங்கள் ஜாதி-நீள்
கடலும் மலையும் எங்கள் கூட்டம்
நோக்கும் திசையெல்லாம் நாமின்றி வேறில்லை
நோக்க நோக்கக் களியாட்டம்"

என்று தனது தத்துவப் பார்வையை வெளிப்படுத்தியுள்ளார்.

முடிவுரை

"உங்களின் தோல்வி
எங்கே ஒளிந்துள்ளது
தெரியுமா?
பிரச்சனை வரும்
போது அல்ல..

பிரச்சனைகளை கண்டு
நீங்கள் பயந்து விலகும் போது...."

என்ற வரிகளுக்கு உதாரணமாகவே மனிதர்கள் இருக்கின்றார்கள். பிரச்சனைகள் இல்லாத உயிர்கள் பூவுலகில் இல்லை. அதனால் அப்பிரச்சனைகளை கண்டு தற்கொலை எண்ணம் கொள்ளாமல் மனதை சரியான வழியில் செலுத்தினால் வளர்ச்சி பாதையில் சென்று வெற்றி பெறலாம் என்றும் பிற உயிர்களிடம் அன்பு கொண்டு பழகும் பொழுது மனமானது கவலைகளை மறந்து மன அழுத்தத்தை தவிர்க்கலாம் என்பதை பாரதியாரின் கவிதைகளை எடுத்து கூறி இக்கட்டுரையில் விளக்கப்பட்டுள்ளது.

■

20. பாரதி – புதிய பார்வை

முனைவர் க. புவனேஸ்வரி
உதவிப்பேராசிரியர், தமிழாய்வுத்துறை,
தேசியக்கல்லூரி (தன்னாட்சி), திருச்சிராப்பள்ளி–01

முகவுரை

நம் இந்திய நாடு முழுமையுமே ஆங்கிலேயரிடம் அடிமைப்பட்டு உணர்ச்சியற்ற மரக்கட்டைகளாகக் கிடந்த மனிதகுலத்திற்குத் தன் பட்டயக் கவிதைகளால் உணர்ச்சி ஊட்டி, நன்னெறி காட்டிய, எட்டயபுரத்து நற்றமிழ் மறவன் பாரதி. தன் வயிற்றுக்குச் சோறில்லாத சூழல்களில் கூட அதைப் பற்றி எண்ணாமல், உலகத்து உயிர்கட்கெல்லாம் உணவிடல் வேண்டும் என்று பாடியவன். பதுமைகளாக இருந்த மக்களுக்குப் பொதுவுடைமையை முழங்கி பூமியைப் புரட்டிப்போட்டுப் புதுமை செய்யவும், புரட்சிகள் புரியவும் வாருங்கள், என்று இளைய பாரதத்தை அழைத்தவன். அடிமையின் மோகத்தை மாற்றி, விடுதலை உணர்வினை ஊட்டி, நாட்டு விடுதலை வேண்டித் தன்னகரில்லாத தன் பாட்டுத் திறத்தாலே, இந்த வையம் முழுவதையும் ஆட்டுவித்த முண்டாசுக் கவிஞன்.

மரபுக்கவிதை, புதுக்கவிதை, வசனகவிதை, கேலிச்சித்திரங்கள், சிறுவர் இலக்கியங்கள், தனிப்பாடல்கள், குறுங்காப்பியங்கள் உரைநடைகள், கட்டுரைகள், மொழிபெயர்ப்புகள், சுயசரிதை, கலைச்சொல் ஆக்கம் என்று பாரதி தன் தடம் பதிக்காமல் விட்டு வைத்த துறைகளே இல்லை எனலாம்.

இப்படி இலக்கிய வகைகளில் மட்டுமின்றிப் பாடுபொருட்களிலும் இவன் பாடாத பொருட்களே இல்லை என்று கூறும் அளவிற்கு வண்டிக்காரனுக்கும், வேலைக்காரிக்கும், அண்டை நாட்டிற்கும், அருமைத் தமிழுக்கும், பக்திக்கும், பகுத்தறிவிற்கும், பெண் விடுதலைக்கும், சீர்திருத்தச் சிந்தனைகளுக்கும் என்று பல்வேறு புதுமைகளைப் பாடினான்.

பிறர் நடந்த பாதையில் பயணித்து எளிமையாகச் சிகரத்தை எட்டியவனல்ல அவன். பலரும் பயணிக்கத் தயங்கும் பாதையில் துணிந்து, நடந்து, பற்பல புதிய வழிகளை முயன்று தானே உருவாக்கிப் பிறருக்கும் அதைப் பயன்பட வைத்து உச்சம் தொட்டவன் பாரதி.

இப்படிப் பாரதி புரிந்த புதுமைகளைப் பற்றியும், அவனது பெருமைகளைப் பற்றியும் ஆராய்ந்து அறிவதே இக்கட்டுரையின் நோக்கமாகும்.

புதுமைகள் புரிந்த புரட்சிப் புலவன்

இலக்கணச் செங்கோல், யாப்புச் சிம்மாசனத்தில் அமர்ந்து மகத்தான மரபுக்கவிதைகளை எழுதியவர்கள் ஏராளமானோர். அப்படி பாரதிக்கு முன்பே பலர் மரபுக்கவிதைகளை எழுதியிருந்தாலும் கூட அந்தத் தளத்திலும் தனக்கெனத் தனி முத்திரையைப் பதித்தவன் அவன். ஏனென்றால், மரபுக்கவிதைகள் என்றாலே கடினமான நடையில், பிறருக்கு எளிதில் புரியாத வண்ணம் இருக்க வேண்டும் எனும் எண்ணத்தை மாற்றி அதில் எளிமையைப் புகுத்திப் பாமரரும் அதைப் புரிந்து கொள்ளத் தந்தவன்.

> "இப்படி எளிதில் புரியும்படியான கவிதையை நான் பார்த்ததே இல்லை. அனைவர்க்கும் புரியும்படி எளிதாகக் கவிதை எழுத முடியும் என்று நான் நினைத்ததே இல்லை" (டாக்டர்.ச.க.இளங்கோ, பாரதிதாசன் பார்வையில் பாரதி, ப.15)

என்று புரட்சிக் கவிஞர் பாரதிதாசன் தான் தெய்வம் என்று கருதி வழிபடும் பாரதியாரின் கவிதைகளைப் பற்றிக் கூறியுள்ளார்.

புதுக்கவிஞர்களுக்கும் புதிய பாட்டை

மரபுக்கவிதைகளில் மக்கள் மனம் தொட்டதோடு மட்டுமின்றிப் புதுக்கவிதை உலகிற்குப் புதியபாடு பொருட்களை, புதிய வழிகளை அறிமுகம் செய்தவன்.

> **"சுவை புதிது, பொருள் புதிது, வளம் புதிது**
> **சொல் புதிது, சோதிமிக்க நவகவிதை!**
> **எந்நாளும் அழியாத மாக்கவிதை"** **(பாரதியார் கவிதைகள், ப.125)**

என்று நவீனக் கவிதைகளான ஹைக்கூ எனப்படும் வசன கவிதைகளுக்கும், குறுங்கவிதைகளுக்கும் வித்திட்டவர். அதனால்தான், பாரதிக்கு முந்தைய புலவர்கள் பலருக்கும் இல்லாத சிறப்பாக "பாரதி பரம்பரை" என்று ஒரு கவிதைப் பரம்பரையையே உருவாக்க அவனால் முடிந்தது.

மொழிப்பற்று

பொதுவாக தேசியச் சிந்தனையாளர்களிடம் மொழிப்பற்றுக் குறைவாகவும், மொழிப்பற்றாளர்களிடம் தேசிய உணர்வு சிறிது குறைந்தும் காணப்படும். ஏனென்றால் இரண்டும் இருவேறு தன்மையன.

நாட்டின்மீதும் சமுதாயத்தின் மீதும் அவனுக்கு இருந்த அக்கறையைப் போலவே மொழியின் மீதும் அதிக அக்கறை காட்டியவன் பாரதி. தமிழ்மொழி உலகமொழி என்பது அவன் கருத்து.

> **''மிகவும் விரைவில் தமிழின் ஒளி உலகம் முழுவதும்**
> **பரவாவிட்டால் என் பெயரை மாற்றி அழையுங்கள்''**
>
> **(தொ.ஆ.இராஜசேகரன்,பாரதியார் கட்டுரைகள், ப.125)**

என்று தமிழ்மொழியே உலக மொழிகளுக்கெல்லாம் தலைமை ஏற்கும் எனத் துணிந்து கூறிய கவிஞன்,

> **''வானம் அளந்த தனைத்தும் அளந்திடும்**
> **வண்மொழி வாழியவே''** **(பாரதியார் கவிதைகள், ப.47)**

என்பது பாரதியின் பாடல். இதில்,

> ''வானம் என்ற சொல் 'உலகம்' என்பதையே குறிக்கிறது, என சிலம்புச் செல்வர் ம.பொ.சி.தன் உரையில் கூறியுள்ளார்''
> (தமிழுக்குக் கதி மகாகவி பாரதி கட்டுரை 24.05.2011,பாரதி பயிலகம்)

இப்படித் தமிழின் பெருமையைத் தரணியில் உயர்த்தியவன் பாரதி.

மன்னனைப்படாமல் மக்களுக்காகப் பாடியவன்

முற்காலத்தில் புலவர்கள் தங்கள் வறுமையைப் போக்கிக் கொள்ள நாடாளும் மன்னரைப் போற்றிப் புகழ்ந்து பரிசில் பெறுவார்கள். ஆனால் பாரதியோ, தான் வறுமையில் வாடியபோதும் மன்னனைப் புகழாமல் மக்களையும், இந்த நாட்டையும் பற்றிச் சிந்தித்தான். எட்டயபுரம் மகாராஜா தன் மனத்திற்குப் பிடித்த பாடல்களைப் பாடினால் பாரதிக்கு வேண்டுவன செய்யத் தயாராக இருந்தார்.

> ''மகா புத்திசாலிகளும்கூட வயிற்றுப்பிழைப்பை உத்தேசித்து அசட்டுப் பணக்காரனுடைய அவலச் சொற்களில் கூட அழகும் அர்த்தமும் இருப்பதாக வர்ணிக்கும் இந்த தேசத்தில், பாரதியார் எட்டயபுரம் ராஜாவின் நன்மதிப்பைப் பெரிதாகக் கொள்ளவில்லை''
> (வ.ரா.,பாரதியார், ப.17)

இத்தோடு மட்டுமின்றி மன்னன் மகிழப் பாடமாட்டேன் என்றவர். புதுச்சேரியிலுள்ள வண்டிக்காரனுக்காகவும், தன் வீட்டில் வேலைக்காரியாக இருந்த அம்மாக்கண்ணு என்பவர் மீதும் "அம்மாக்கண்ணு பாடல்" என்று பாடலைப் பாடித் தன் நன்றியைத் தெரிவித்தார்.

பிறமொழிகளையும் மதித்தல்

நாம் நமது தாய்மொழி மீது பற்றுக் கொண்டவர்கள் என்பதால் பிறமொழிகளைக் கற்கவோ, மதிக்கவோ கூடாது என்று நம் நாட்டில் பலர் நினைக்கின்றார்கள். ஆனால், பாரதியோ, பற்பல மொழிகளையும் நாம் கற்க வேண்டும்.

"பிறநாட்டு நல்லறிஞர் சாத்திரங்கள்
தமிழ்மொழியில் பெயர்த்தல் வேண்டும்"

(பாரதியார் கவிதைகள், ப.47) எனக் கூறியவன்.

"ஹிந்தி பாஷையில் சில சப்தங்களைத் தொண்டைக்குக் கீழேயிருந்தே கொண்டு வர வேண்டியிருக்கும், இது தமிழர்க்கு ரொம்பக் கஷ்டம் ஆனால் பாரதியோ ரொம்ப அழகாக ஹிந்தி பேசுவார்,உச்சரிப்பார்" (வ.ரா.,பாரதியார், ப.19)

இப்படிப் பல மொழிகளையும் படிக்கும் போதுதான் தமிழ்மொழியின் வளமும், பெருமையும் புரியும் என்பதை அழகாகக் கூறியுள்ளார் அந்த வரகவி.

பெண்ணியச்சிந்தனை

கண்ணன்பாட்டு, குயில்பாட்டு, பாஞ்சாலி சபதம் போன்றவைகள் பாரதி தமிழுக்குத் தந்த பொக்கிஷங்கள். இதில் பாஞ்சாலி சபதத்தில் இதிகாச நிகழ்வை எடுத்துக்கொண்டு, அதைத் தன் காலச்சூழலுக்கேற்ப மாற்றி, பெண் விடுதலையையும், மண் விடுதலையையும் ஒருங்கே வைத்துப் பாடினான். இன்றைய பெண்ணியச் சிந்தனைகளுக்கெல்லாம் வித்திட்டு.

"பெண்மை வாழ்க வென்று கூத்திடுவோமடா"

(பாரதியார் கவிதைகள், ப.217)

என்று பெண் விடுதலைக்காக முதல் குரல் எழுப்பியவன்.

பாரதி சொல்

பாரதி என்ற சொல்லே பாரதிக்கு முன்பு வரை பெண்களின் பெயராக இருந்தது. "பாரதி எனும் பட்டம் பாரதியாருக்குக் கிடைத்த பிறகு ஏன்? அவருக்குப் பின்னால் சோமசுந்தர பாரதி

உள்ளிட்ட பலருக்கு அந்தப் பட்டம் கிடைத்திருந்தாலும் பாரதி எனும் சொல் மகாகவி சுப்ரமண்ய பாரதியையே குறித்தது". அப்போதிருந்து அது ஆண்களுக்கும் உரிய பெயரானது.

இப்படி பாரதி என்ற சொல் மட்டுமல்ல. பாரதியாரால் தமிழுக்குப் பல புதிய சொற்கள் கிடைத்தன.

> "விடுதலை என்ற சொல்லை நாட்டிற்கு உபயோகப்படுத்தி நான் முதலிலே கேட்டது பாரதியாரிடந்தான்" (வ.ரா.,பாரதியார், ப.8)

அதுவரை சுதந்திரம் என்ற சொல்லே புழக்கத்தில் இருந்து வந்துள்ளது. 'தந்தையர் நாடு', 'தொண்டச்சி' போன்றவையும் இப்படித்தான்.

பாரதி கையாண்ட சொற்கள் அனைத்துமே வலிமை பெற்றுத் திகழ்ந்தன. மொழியை ஆயுதமாகப் பயன்படுத்தியவன் பாரதி. அதனால்தான் மந்திரச்சொல் வேண்டும் என்கிறான்.

> "**போர்த்தொழில் பழகு**" **(பாரதியார் கவிதைகள், ப.201)**

> "**மோதி மிதித்துவிடு பாப்பா**" **(பாரதியார் கவிதைகள், ப.202)**

என்று குழந்தைளுக்குக் கூறும் அறிவுரைகளிலும், புதிய ஆத்திசூடியிலும் இப்படிப்பட்ட வலிமைமிகு சொற்களைக் காணமுடிகிறது.

பாரதமாதா திருப்பள்ளி எழுச்சி

பல்வேறு தெய்வ வணக்கப் பாடல்களைப் பாரதி பாடி இருந்தாலும் அந்தத் தெய்வங்களையெல்லாம் விட உடனடியாக நாம் வழிபட வேண்டிய தெய்வம் பாரதத்தாய் என்கிறான். எந்த மதத்தவராக இருந்தாலும் இப்போது நாம் செய்ய வேண்டியது.

> "**சேதமில்லாத ஹிந்துஸ்தானம் - இதைத்**
> **தெய்மென்று கும்பிடடி பாப்பா**" **(பாரதியார் கவிதைகள், ப.203)**

அதனால்தான் மன்னர்களையும், தெய்வங்களையும் துயில் எழுப்பப்பாடும் பள்ளியெழுச்சிப் பாடலைப் பாரதமாதாவைத் துயில் எழுப்பப் பாடுகின்றான்.

> "பாரத மாதாவுக்கு கோயில் அமைக்கவும், அந்தக் கோயிலில் பராசக்தியின் வடிவத்தில் தேச மாதேவியின் சிலையை நிறுத்தவும் சிவா விரும்பியது மகாகவி பாரதியைப் பின்பற்றித்தான்" (ம.பொ.சி., பாரதியார் பற்றி ம.பொ.சி., ப.39)

என்று சுப்ரமண்ய சிவா புதிய சமயம், புதிய தெய்வமாகப் பாரதமாதாவை நிறுவிட பாரதியின் உயரிய சிந்தனையே, தூண்டுகோலாக இருந்துள்ளது.

> "பாரதியார் ஏனைப் புலவர்போல் அல்லாது புது முறைகளையே சிருஷ்டித்தார். தமிழ்மொழியில் பாக்களும் பாவினங்களும், புது உணர்ச்சிகளுக்கும் அடித்தளம் அமைத்தவர்" (ந.க.மங்கள முருகேசன், பாரதி ஒரு வரலாற்று பார்வை, ப.19)

என்று எட்டயபுரத்தில் பாரதியுடன் 'பாரதி' பட்டம் பெற்ற பைந்தமிழ்ப் புலவர் சோமசுந்தர பாரதியார் கூறுகிறார்.

நிறைவுரை

இப்படி பாரதி அனைத்திலும் புதுமையையும், புரட்சியையும் செய்த காரணத்தினால்தான் அவர் 'வரகவி' என்றும், 'சீர்திருத்த முன்னோடி' என்றும் தம் முற்போக்குச் சிந்தனைகளின் 'காரணகர்த்தா' என்றும் பாவேந்தர் கூறியிருக்க வேண்டும் என்பதே உண்மை, வெறும் புகழ்ச்சியில்லை.

■

21. பாரதியின் பன்முக ஆளுமை

முனைவர் ஜெ. புவனேஸ்வரி
உதவிப்பேராசிரியர், தமிழாய்வுத்துறை,
தேசியக்கல்லூரி (தன்னாட்சி), திருச்சிராப்பள்ளி–01

சுப்ரமணிய பாரதியார் ஒரு தமிழ்க்கவிஞர். இந்திய சுதந்திரப்போராட்ட காலகட்டத்திலே கனல்தெறிக்கும் விடுதலைப்போர் கவிதைகள் வாயிலாக மக்களின் மனதில் விடுதலை உணர்வை ஊட்டியவர். இவர் ஒரு கவிஞர் மட்டுமல்லாமல் எழுத்தாளர், பத்திரிகை ஆசிரியர், சமூக சீர்திருத்தவாதி மற்றும் தன்னுடைய பாட்டுகளின் மூலமாக விடுதலை உணர்வுகளை மக்களிடம் தட்டியெழுப்பியவர் என பல்வேறு பரிமாணங்கள் கொண்டவர். கம்பனுக்குப் பின் தமிழ்மக்கள் உள்ளத்தில் நிலைத்து நின்று வரும் கவிஞர் சுப்ரமணிய பாரதியார் ஆவார்.

தொலைநோக்குச்சிந்தனை:

தொலைநோக்குப் பார்வை உடைய கவிஞரே சிறந்த கவிஞராக முடியும். பாரதி எதிர்கால இந்தியா வளமிக்கநாடாக வேண்டும் என்று அன்றே திட்டம் வகுத்தவர்.

“சிங்களத் தீவினுக்கோர் பாலம் அமைப்போம்
சேதுவை மேடுறுத்தி வீதி சமைப்போம்
வங்கத்தில் ஓடிவரும் நீரின் மிகையால்
மையத்து நாடுகளில் பயிர் செய்குவோம்”

(பாரதியின் தேசியகீதங்கள் - பாரததேசம்)

என்று பாடி நதிநீர் இணைப்புத் திட்டத்திற்கும், சேது சமுத்திரத் திட்டத்திற்கும் அன்றே பாதை வகுத்தார்.

அறிவியல் தொழில்நுட்பச் சிந்தனை:

“காசி நகர்ப்புலவர் பேசும் உரைதான்
காஞ்சியில் கேட்பதற்கோர் கருவிசெய் வோம்
ராசபுத் தானத்து வீரர் தமக்கு
நல்லியற் கன்னடத்துத் தங்கம் அளிப்போம்”

(பாரதியின் தேசியகீதங்கள் - பாரததேசம்)

காசியிலே அறிஞர்கள் பேசும் உரைகளைத் தெற்கேயுள்ள காஞ்சியிலிருந்தபடியே கேட்கும் கருவியை நாம் செய்வோம். ராஜபுத்திர வீரர்களுக்குக் கன்னட தேசத்துத் தங்கத்தைப் பரிசளிப்போம்!

''பட்டினில் ஆடையும் பஞ்சில் உடையும்
பண்ணி மலைகளென வீதி குவிப்போம்
கட்டித் திரவியங்கள் கொண்டுவரு வார்
காசினி வணிகருக்கு அவை கொடுப்போம்.

(பாரதியின் தேசியகீதங்கள் - பாரததேசம்)

பட்டாடை, பருத்தி உடை எல்லாம் செய்து மலை மலையாகக் குவித்துப் பல தேசத்திலிருந்தும் பணம் கொண்டு வருபவர்களுக்கு விற்று நம் நாட்டுப் பொருளாதாரத்தை பெருக்குவோம்!

ஆயுதம் செய்வோம் நல்ல காகிதம்செய் வோம்
ஆலைகள் வைப்போம் கல்விச் சாலைகள் வைப்போம்
ஓயுதல் செய்யோம் தலை சாயுதல் செய்யோம்
உண்மைகள் சொல்வோம் பல வண்மைகள் செய்வோம்.

(பாரதியின் தேசியகீதங்கள் - பாரததேசம்)

எழுத்து என்னும் ஆயுதங்கள் செய்வோம். காகிதங்களும் நாமே செய்வோம். இதற்காக ஆலைகளும், தொழில் வளர்வதற்கேற்ற கல்விச்சாலைகளும் நம் நாட்டிலேயே உருவாக்குவோம். அவற்றிலே ஓயாது உழைப்போம். உண்மையையே உயிராகக் கொண்டு நடப்போம். இல்லாதவர்க்கு உள்ளவர் கொடுத்து, இல்லை என்ற சொல் இல்லாமல் வாழ்வோம் என்று பாடினான். நாம் இப்போது தொழில்நுட்ப உலகில் வாழ்ந்து கொண்டிருக்கிறோம். ஆனால் வானொலி கூட இல்லாத காலத்தில் வாழ்ந்த பாரதி கண்ட இந்தக் கனவு வியப்பளிப்பதாகத்தான் இருக்கிறது.

பெண்மையைப் போற்றிய பெருங்கவி:

அலங்காரப் பதுமையாகவும், அடிமைப் பொருளாகவும், அடங்கிக் கிடந்த பெண்மைக்கு உரிமைக்குரல் கொடுத்தவர் பாரதி. அவர் காலத்திலும், அதற்கு முன்பும் வாழ்ந்த மற்ற கவிஞர்களும் பெண்களின் கண்ணீரைத் தொட்டுத்தான் கவிதை எழுதினார்கள். ஆனால் பாரதி பெண்களின் கண்ணீரைத் துடைக்கக் கவிதைப் படைத்தவர்.

''மாதருக்கும் இங்கே மா தருக்கு உண்டு'' என்று பெண்மையை உச்சிக்கு உயர்த்தி பெருமை சேர்த்தார்.

‘‘பூணு நல்லறத்தோடு இங்கு பெண்ணுருப் போந்து நிற்பது தாய் சிவசக்தியாம்’ (பாரதியார் கவிதைகள் – புதுமைப்பெண்)

என்று பாடிப் பெண்மையைத் தெய்வநிலைக்கு உயர்த்தினார் பாரதி.

ஆணும் பெண்ணும் சரிநிகர் சமானம் எனக் கூறி, பெண்விடுதலைக்காகவும் பெண்ணுரிமைக்காகவும் குரல் கொடுத்தார்.

‘‘கற்பு நிலையென்று சொல்ல வந்தார் இரு
கட்சிக்கும் அஃது பொதுவில் வைப்போம்
வற்புறுத்திப் பெண்ணைக் கட்டிக் கொடுக்கும்
வழக்கத்தைத் தள்ளி மிதித்திடுவோம்’’

(பாரதியார் கவிதைகள் - பெண்கள் விடுதலைக் கும்மி)

சமூகச் சீர்திருத்தவாதி:

தீண்டாமையைக் கண்டு கொதித்தெழுந்த பாரதி,

‘‘சாதிகள் இல்லையடி பாப்பா!-குலத்
தாழ்ச்சி உயர்ச்சி சொல்லல் பாவம்’’

(பாரதியார் கவிதைகள் - பாப்பா பாட்டு)

என்று பாடி சமூக ஒற்றுமைக்கு வழிகாட்டினான்.

நாட்டு மக்களிடையே ஒற்றுமையுணர்வை விதைக்க எண்ணிய பாரதி,

‘‘ஆயிரம் உண்டிங்கு ஜாதி-எனில்
அன்னியர் வந்து புகல்என்ன நீதி?-ஓர்
தாயின் வயிற்றில் பிறந்தோர் - தம்முள்
சண்டை செய்தாலும் சகோதரர் அன்றோ?’’

என்று பாடி ஒருமைப்பாட்டுணர்வை மக்களிடையே வளர்த்தார்.

இதழாசிரியர்:

பாரதியார் தன் வாழ்நாளின் பல வருடங்களைப் பத்திரிகையாளராகச் செலவிட்டார். பாரதி இளம் வயதில் தன்னுடைய வாழ்க்கையை ஒரு பத்திரிகையாளர் மற்றும் துணையாசிரியராக ‘சுதேசமித்திரன்’ என்ற பத்திரிகையில் 1904-ஆம் ஆண்டு தொடங்கினார். 1906-ஆம் ஆண்டு மே மாதம்

'இந்தியா' எனப்படும் ஒரு புதிய நாளிதழ் தொடங்கப்பட்டது. இது பிரஞ்சுப் புரட்சியின் மூன்று முழக்கங்களான சுதந்திரம், சமத்துவம் மற்றும் சகோதரத்துவம் போன்றவற்றைத் தனது குறிக்கோளாக அறிவித்தது. இது தமிழ்ப்பத்திரிகை துறையில் ஓர் புதிய பாதையை ஏற்படுத்தியது எனலாம். தனது புரட்சிகரமான முனைப்புகளை வெளியிடுவதற்குப் பாரதியார் அவர்கள் வார இதழை சிகப்புத் தாளில் அச்சிட்டுப் பிரசுரித்தார். அரசியல் கேலிச்சித்திரத்துடன் வெளியிடப்பட்ட தமிழ்நாட்டின் முதல் நாளேடு 'இந்தியா' ஆகும்.

இவர் மேலும் விஜயா, சூரியோதயம், கர்மயோகி, தர்மம் போன்ற தமிழ் இதழ்களிலும், பாலபாரதா, யங் இந்தியா ஆகிய ஆங்கில இதழ்களிலும் பணியாற்றினார்.

விடுதலைப் போராளி:

சீருடனும் சிறப்புடனும் வாழவேண்டிய பாரத சமுதாயம் அந்நியரின் ஆதிக்கப்பிடியில் சிக்கிச் சிதைந்து சீரழிந்து கிடப்பதைப் பாரதி கண்டு கண்ணீர் வடித்தார். நாடு இந்த நிலையில் இருப்பதை அவருடைய உடம்பிலுள்ள ஒவ்வொரு அணுவும் வெறுத்தது. தன் எழுச்சிமிக்க பாடல்களால் மக்களிடையே விடுதலைக்கனலை மூட்டினார்.

சுதந்திரப்போரின் அவசியத்தை மக்கள் உணராதவர்களாக இருந்தார்கள். காரணம், அப்போதைய மக்கள் உணர்ச்சியும் எழுச்சியும் கொள்ளக் கூடிய சூழ்நிலையில் இல்லை. பசியினாலும் பட்டினியாலும் வாடும் மக்களைப் பாரதி நேரில் கண்டார். அந்த அவலநிலையை,

"கஞ்சி குடிப்பதற்கிலார் - அதன்
காரணங்கள் இவையென்னும் அறிவுமிலார்"

(தேசியகீதங்கள் - பாரதநாடு)

என்று இரண்டே அடிகளில் பாடி விளக்கினார்.

"அச்சமும் பேடிமையும் அடிமைச் சிறுமதியும்
உச்சத்திற் கொண்டா ரடீ-கிளியே
ஊமைச் சனங்களடீ!
ஊக்கமும் உள்வலியும் உண்மையிற் பற்றுமில்லா
மாக்களுக்கோர் கணமும் - கிளியே
வாழத் தகுதி யுண்டோ? **(தேசிய இயக்கப் பாடல்கள் - நடிப்புச் சுதேசிகள்)**

என்று அன்றிருந்த எதார்த்த நிலையை மனம்நொந்து பாடினார்.

இலக்கியவாதி:

பாரதியின் முப்பெரும் படைப்புகளாகப் போற்றப்படுபவை கண்ணன் பாட்டு, குயில் பாட்டு, பாஞ்சாலி சபதம். இந்திய விடுதலைப் போராட்டத்தையே பாரதப்போராகவும், பாஞ்சாலியைப் பாரததேவியாகவும் உருவகப்படுத்தி மகாகவி படைத்த உன்னத படைப்புதான் பாஞ்சாலி சபதம். அழகிய இலக்கிய நயத்தையும், மிக அழகான கவிநயத்தையும் கொண்ட தமிழின் அழியாக் காவியமாகப் பாரதியின் பாஞ்சாலி சபதம் விளங்குகிறது. வியாசரின் பாரதத்தைத் தழுவி எழுதப்பெற்ற இக்காவியம் ஐந்து சருக்கங்களையும், 412 பாடல்களையும் கொண்டுள்ளது.

பாரதியார் தன் பிறப்பு முதல் இறப்பு வரையிலான சுயசரிதையை மரபுக்கவிதையில் படைத்துள்ளார். மேலும் அவர் சிறந்த பல கட்டுரைகளையும், சிறுகதைகளையும், எழுதியுள்ளார். உலக வரலாற்றிலே முதன்முதலாக பாரதியின் படைப்புகள்தான் அரசாங்கத்தால் வாங்கப்பட்டு பொதுவுடைமையாக்கப்பட்டன.

மொழிபெயர்ப்பாளர் பாரதி:

பாரதியின் மொழிபெயர்ப்புப் பணிகளுக்குக் காரணமாய் அமைவது அவருடைய பன்மொழிப் புலமை ஆகும். அவருடைய பன்மொழிப் புலமைக்குக் காரணம் அவர் சிறுவயதில் இருந்து புலம்பெயர்ந்து வாழ்ந்தமையே ஆகும். எட்டயபுரத்திலிருந்து காசிக்கும், பின்னர்க் கல்கத்தா, சென்னை, புதுச்சேரி எனப் பன்மொழிச் சூழலில் வாழ்ந்தமை அவருடைய பன்மொழி அறிவை மேம்படுத்தியது. அவர் தமிழ், சமஸ்கிருதம், தெலுங்கு, வங்காளம், இந்தி, ஆங்கிலம், பிரெஞ்சு ஆகிய மொழிகளை அறிந்திருந்தார். இதனை,

> **"யாமறிந்த மொழிகளிலே தமிழ்மொழிபோல்**
> **இனிதாவ தெங்கும் காணோம்"** **(பாரதியாரின் தேசியகீதங்கள் - தமிழ்)**

என்று பாரதி தன் கூற்றாகவே கூறுவதன் மூலம் அவர் பன்மொழிப் புலமை பெற்றவர் என்பதை நாம் அறிந்து கொள்கிறோம்.

பாரதியாரின் பகவத்கீதை தமிழ் மொழிபெயர்ப்பு நூல் 1922ஆம் ஆண்டு வெளியிடப்பட்டது. பாரதி செய்த மொழி பெயர்ப்புக்கு முன் பதின்மூவர் பகவத்கீதையை மொழி

பெயர்த்தனர். அவற்றுள் ஏழு மட்டுமே முழுமையான மொழிபெயர்ப்புகளாகும். இவற்றுள் பாரதியின் மொழிபெயர்ப்பே சிறந்தது என்பதற்கு,

> "வடக்கே தோன்றிய மாமுனி திலக மகரிஷி எழுதிய பகவத்கீதை மொழிபெயர்ப்புக்கு ஈடாக, தென்னாட்டுப் புலவர் திலகம் கவிசக்கரவர்த்தி பாரதியார் எழுதிய உரை விளங்குகிறதென்றால் பாரதியின் உரைக்கும், மொழிபெயர்ப்புத்திறனுக்கும் வேறென்ன சான்று வேண்டும்." (பாரதியார், பகவத்கீதை, பதிப்புரை. ப – 4).

எனக் கூறுவதிலிருந்து அறியமுடிகிறது. பாரதிக்குப் பின்னர் ஏழு பகவத்கீதை மொழிபெயர்ப்புகள் வெளிவந்துள்ளன. அவற்றில் இரண்டு செய்யுள் வடிவிலும், ஐந்து உரைநடை வடிவிலும் உள்ளன. ஆயினும் பாரதியின் மொழிபெயர்ப்பே சிறந்து விளங்குகின்றது.

முடிவுரை:

பாரதி என்ற மகாகவிஞன் தான் வாழ்ந்த காலத்தில் தமிழையும் தன் தேசத்து மக்களையும் நேசித்து மக்களிடையே பகுத்தறிவினை வளர்த்து இருண்டு கிடந்த மக்கள் வாழ்விற்குத் தம் எழுத்துக்களால் வெளிச்சம் கொடுத்து 'இனி ஒரு விதி செய்வோம்" என்று பாடிய புதுமைக்கவிஞன் மக்கள் மனங்களில் நீங்கா இடம்பிடித்த கவிஞன் என்றால் அது மிகையல்ல.

■

22. பாரதி காட்டும் சமத்துவம்

முனைவர் ந.மாணிக்கம்,
இணைப்பேராசிரியர், தமிழாய்வுத்துறை,
தேசியக்கல்லூரி (தன்னாட்சி), திருச்சிராப்பள்ளி–01

முன்னுரை

இருபதாம் நூற்றாண்டின் தலை சிறந்த சிந்தனையாளர்களில் ஒருவர் பாரதியார். உணர்ச்சி மயமாகப் பாடும் கவிஞர் என்பதோடு தான் வாழ்ந்த உலகின் பல்வேறு சிக்கல்களைப் பற்றி ஆய்ந்த அறிஞராகவும் விளங்கியவர். அவர் போக்கைக் களைந்து விடுதலை உணர்வுடன் சமத்துவ சமுதாய வேட்கையை வெளிப்படுத்தியவர் பாரதியார் என்பதை இக்கட்டுரையில் காண்போம்.

பக்தியும் தத்துவ நம்பிக்கையும் இருப்பதாலேயே பாரதியைப் பழமைக்குள்ளே யாரும் தள்ளிவிட முடியாது. டால்ஸ்டாய் பக்தி உடையவராகவும், கொள்கை அளவில் மார்க்சீயத்தைச் சாராதவராகவும் இருந்தாரென்றும், செயலளவில் மனிதாபிமானம் நிறைந்த முற்போக்காளராகவே ஏற்றுக் கொள்ளப்பட்டார். இறுக்கமான புறவிதிகளை மட்டும் வைத்து முற்போக்காளர்களைக் கண்டுபிடிப்பது இயலாது. தத்துவ அடிப்படையில் வேறுபாடுகள் இருப்பினும், சமூக வாழ்வின் இயல்பையும், போக்கையும் அறிந்து மானுட பரிவுடன் செயல்படுவோரே முற்போக்காளர் ஆவர்.

பாரதியை மதிப்பிடும்போது மார்க்சீயத்தின் விதிமுறைச் சட்டங்கள் பொருந்துகின்றனவா என்று மட்டும் ஆராய்வது போதாது. அவர் வாழ்ந்த காலத்தின் இடர்பாடுகளையும் தாண்டிக் கொண்டு, உலகத்தைத் தம் கவிதையாகிய கரங்களால் அளக்க முயன்றிருக்கிறார் என்பதே அவரிடம் நாம் வியக்க வேண்டிய முற்போக்குத் தன்மையாகும்.

"வரலாற்றுப் போக்கில் சமூக மாற்றங்கள் நிகழும்போது சிந்தனை மாற்றங்கள் ஏற்படுகின்றன".[1] அந்நியராட்சி இந்தியாவில் விளைவித்த சமூக மாற்றங்களால் தமிழ்க் கவிதை உலகம் பெற்ற சிந்தனை மாற்றத்தையே பாரதி வெளிப்படுத்துகிறார். வர்க்க முரண்பாடுகளைப் பற்றி முதல்வாதச் சிந்தனைகள் பாரதிக்கு இருந்ததில்லை. கருத்து முதல்வாதியாகவே பாரதி இருந்தார். எனினும் உலகே மாயம் என்று கருதிவிடாமல், மண்ணிலேயே விண்ணைப் படைக்க முடியும் என்ற நம்பிக்கையோடு பாரதி வாழ்ந்தார். அடக்கப்பட்டவர்களும், ஒடுக்கப்பட்டவர்களும் தம் உரிமைகளுக்காகப் போராடுவது அவசியம் என்பதை பாரதி தம் பாடல்களில் பலவிதமாக வெளிப்படுத்தியிருக்கிறார்.

உழைக்கும் வர்க்கத்தையும், உழைப்பையும் மார்க்சீயம் போற்றுகிறது. உழைக்கும் தொழிலாளரை 'பிரம தேவன் கலை' என்று பாராட்டியவர் பாரதியார்.

"அரும்பு வேர்வை யுதிர்த்துப் புவிமேல்
ஆயிரம் தொழில் செய்திடுவீரே
பெரும்புகழ் நுமக்கே இசைக்கின்றேன்
பிரமதேவன் கலை இங்கு நீரே"[2]

என்று பாடினார். அடிமைத்தனத்தால் தாழ்வுற்றுக் கிடக்கும் இந்திய மண்ணை வளமுறச் செய்வதற்கு விடுதலையும், உழைப்பும் தேவை என்றார். '**பாரததேசம்**' என்ற கவிதையில் அவர் விரிக்கும் சாதனைப் பட்டியல் சலிப்பில்லாத உழைப்பால் விளையவேண்டிய செயல்களாகும். பெரிய தொழில்புரட்சியையே தம் கவிதைகளில் அவர் படைத்திருக்கிறார். உழவுக்கும், தொழிலுக்கும் வந்தனை செய்வோம் என்று அறிவுறுத்தினார் பாரதியார். பிஜித்தீவுக் கரும்பு தோட்டத்தில் உழைத்து உழைத்து ஓடாகிப் போனவர்களுக்காக அவரது இதயம் அழுகிறது.

"கரும்புத் தோட்டத்திலே-அவர்
கால்களும் கைகளும் சோர்ந்து விழும்படி
வருந்துகின்றனரே.....".[3]

என்று உழைத்து உழைத்து வாடிப்போன பெண்களுக்கு அனுதாபக் குரலில் அஞ்சலி செலுத்துகிறார். உழைக்கும் வர்க்கத்திடம் பாரதி காட்டிய ஈடுபாடு நிகரற்றது என்பதில் ஐயமில்லை.

பொதுவுடைமைக் கொள்கையை இதயபூர்வமாக வரவேற்றவர் பாரதியார். '**பொதுவுடைமை**' என்ற சொல்லை முதன் முதலில் கவிதையில் கையாண்டவர் பாரதியார்.

"முப்பது கோடி ஜனங்களில் சங்கம்
முழுமைக்கும் பொது உடைமை
ஒப்பில்லாத சமுதாயம்
உலகத்துக்கொரு புதுமை"[4]

என்று 'பாரத சமுதாயம்' பற்றிப் பாடுகிறார். சுரண்டல் இல்லாத நிகரமை வாழ்க்கையை அவர் வரவேற்கிறார். மார்க்சீயத்தின் உயிர் நாடியான பிரபஞ்ச மாற்றக் கொள்கைக்கேற்ப பாரதியும் மாற்றங்களை விரும்புகிறார். பாட்டன் வெட்டிய கிணறு என்பதற்காக உப்புத் தண்ணீரையே குடிப்பதில் அவருக்கு இசைவில்லை. முன்பிருந்தோர் என்ற காரணத்தினாலேயே பொய்ம்மைகள் மெய்ம்மைகளாகிவிடா என்பதைப் பாரதியார் அறிந்தவர். அதனால்தான் சுரண்டல் நிறைந்த பாரத நாட்டின் நிலையை மாற்றியாக வேண்டும் என்ற விருப்பத்துடன்,

"இனியொரு விதிசெய்வோம் - அதை
எந்த நாளும் காப்போம்
தனியொருவனுக் குணவிலையெனில்
ஜகத்தினை அழித்திடுவோம்"[5]

என்று சூளுரைக்கிறார். எல்லாரும் ஓர் நிலை எய்த வேண்டும் என்ற குரல் விஞ்ஞான சோஷலிசத்தையே நினைவூட்டுகிறது.

'**வரப்போகும் யுகம்**' *எனும் தலைப்பில் எழுதிய ஆங்கில கட்டுரையில்* **சோஷலிசம்** *பற்றிப் பாரதியார் எழுதுவதை சி. ரகுநாதன் குறிப்பிடுகிறார்.*

'**சோஷலிசம்**' *என்று மேலை நாட்டினர் குறிப்பிடுவது என்னவென்று இங்கு தெளிவாகப் புரிந்து கொள்ளப்படவில்லை என்றாலும் மேலை நாட்டுக்கும் சரி, கீழைநாட்டுக்கும் சரி கௌரவமான வாழ்க்கை நடத்துவதற்கு ஒரே ஒரு மார்க்கம் தான் உள்ளது.* **"உலகைப் பொதுவுடைமையாக்கி, அதில் சக தொழிலாளர்களாகவும், கூட்டுப் பங்காளிகளாகவும் வாழ்வதே அந்த மார்க்கமாகும்."[6]**

> "மார்க்சீயத்தின் அனைத்துக் கூறுபாடுகளையும் கற்றறிய வாய்ப்பின்றிப் போனாலும், அதன் முடிவான இலக்காகிய பொதுவுடைமைக் கொள்கையைப் பற்றிக் கொண்டவர் பாரதியார்.
>
> உருசியப் புரட்சியே இந்தத் தெளிவைப் பாரதிக்கு ஊட்டியது. 1905–1907 ஆம் ஆண்டுகளின் உருசியப் புரட்சியின் குழந்தையே பாரதி என்று சி.ரகுநாதன் குறிப்பிடுகிறார்."[7]

'**சுதந்திரப் பள்ளு**' எனும் கவிதையில்கூட, சுரண்டலும் ஏற்றத்தாழ்வும் அற்ற நிகரமை சமுதாயத்தையே அவர் காட்டுகிறார். 'எல்லோரும் சமம் என்பது உறுதியாச்சு', 'எல்லோரும் ஒன்றென்னும் காலம் வந்ததே', 'பூமியில் எவருக்கும் இனி அடிமை செய்யோம்' என்ற தொடர்கள் சுதந்திரம் என்பதற்குப் பாரதியார் கண்ட பொருள் சுரண்டல் நீங்கிய உரிமை வாழ்வு என்பதே என்று புரிய வைக்கின்றன. '**விடுதலை**' என்ற கவிதையிலும் சமத்துவ வாழ்வையே பாரதியார் பெரிதும் வலியுறுத்துகிறார்.

''வாழிகல்வி செல்வம் எய்தி
மனமகிழ்ந்து கூடியே
மனிதர் யாரும் ஒருநிகர்
சமானமாக வாழ்வோம்''[8]

என்பவை பாரதியாரின் வைரவரிகள். வந்தேமாதரம் பாடும்போதும்,

''எப்பதம் வாய்த்திடு மேனும் நம்மில்
யாவருக்கும் அந்த நிலைபொது வாகும்
முப்பது கோடியும் வாழ்வோம்''[9]

என்று பாரதியார் அறிவுறுத்துகிறார்.

புரட்சிகள் தோன்றும் தருவாயில் கடவுள் கட்டளைகளை மனிதன் ஏற்கவில்லை என்பது சரித்திரமாகும். பாரதியோ புரட்சிக்கும் கடவுள் அருளையே காரணமாக்குகிறார். இத்தகைய நுண்ணிய முரண்பாடுகள் பாரதிக்கும், மார்க்சீயத்திற்கும் இடையில் நிலவுகின்றன. அதனால்தான் "பாரதி பிரஞ்ஞை பூர்வமான பொதுவுடைமைவாதி அல்ல"[10] என்று திறனாய்வாளர்கள் கூறுகின்றனர். இக்கூற்றால் பாரதிக்கு எந்தக் குறையும் நேராது. மார்க்சீயம் கொள்கை நடைமுறைப்படுத்தப் பெற்றுக் கொண்டிருந்த காலத்திலேயே அதைப் பற்றி மிகச் சரியாகவும், நுணுக்கமாகவும் புரிந்து கொள்ள முயன்றவர் பாரதியார். மார்க்சீய நெறியை முறைப்படி கற்காவிடினும் அந்நெறி செயலாவதைக் கண்டு மகிழ்ந்தவர். "ரஷ்யப் புரட்சி நடந்து முடிந்து நான்கே ஆண்டுகள் தாம் பாரதி வாழ்ந்தார் எனினும், சோஷலிசத்தின் தன்மைகளைக் கூரிய சிந்தனையோடு உணர்ந்து கொள்ள வல்லவராய் இருந்தார்."[11] போர்க் குணம் செறிந்த மனிதாபிமானத்துடன் பொதுவுடைமை வரவேற்றுப் பாடியதில், இந்தியக் கவிதை உலகுக்கே பாரதி முதன்மையானவர் ஆகிவிடுகிறார்.

பாரதி சோவியத் புரட்சியை மட்டும் வைத்துக்கொண்டு எதிர்காலத்தைப் பற்றிய கணிப்பை மிகத் துல்லியமாகச் செய்திருக்கிறார். தொழிற்சாலைகளை நிறுவ வேண்டும் என்ற பாரதியின் வேட்கை சோஷலிசத்திற்கான பாதையைச் சரியாகக் கண்டறிந்தவர் என்பதையே காட்டுகிறது.

"நாட்டு விடுதலை இயக்கத்தோடு, தொழிலாளர் இயக்கமும் வளர்வது சுரண்டலை வேரறுக்க இன்றியமையாதது"[12] *என்று இந்திய நிலையைப் பற்றி ஸ்டாலின் கூறுகிறார். பாரதியின் கவிதைகளில் விடுதலை உணர்வுடன், சமத்துவச் சமுதாய வேட்கையும் வெளிப்படுகிறது. இதனாலேயே மக்கள் கவிஞராக அவர் மதிக்கப்படுகிறார். பாரதியாரின் படைப்புகள் புதுமையின் புறப்பாடாகப் பாராட்டப்படுகின்றன.*

சான்றெண் விளக்கம்

1. நா.வானமாமலை, மார்க்சீய அழகியல், ப.16
2. பாரதியார் கவிதைகள், ப.213
3. மேலது நூல், ப.89
4. மேலது நூல், ப.40
5. மேலது நூல், ப.41
6. சி.ரகுநாதன், அக்டோபர் புரட்சி, ப.35
7. மேலது நூல், பா.5
8. பாரதியார் கவிதைகள், ப.56
9. மேலது நூல், ப.18
10. க.கைலாசபதி, சமூகவியலும் இலக்கியமும், ப.115
11. கான் தந்தின் சரோதோவ்,ஏ.எ.மூர்த்தி ரூ பாக்கியம் சுந்தரம் (மொ.பெ.ஆ) லெனினியமும் முதலாளித்துவத்திலிருந்து சோஷலிசத்திற்கான மாற்றம் பற்றிய பிரச்சனைகளும், ப.430
12. ஜே.வி.ஸ்டாலின், லெனினிசத்தின் அடிப்படை அம்சங்கள், ப. 47

23. பாரதியும் பாரதமும்

முனைவர் இராம.மாணிக்கவாசகம்,
உதவிப்பேராசிரியர் / ஆய்வு நெறியாளர்,
தமிழாய்வுத்துறை,
தேசியக்கல்லூரி (தன்னாட்சி), திருச்சிராப்பள்ளி–01.

'பரதம் என்பது ஒரு வகையான கூத்து. பாட்டு, ராகம், தாளம் இவை கலந்த ஒரு கூத்திற்குப் பரதம் என்று பெயர். இக்கலை பாரத நாட்டிற்கே உரியது. சிவபெருமானுடைய கூத்து பரதக்கூத்து ஆகும். சிவபெருமான் இக்கூத்தை விரும்பி ஆடுகிற காரணத்தால் சிவபெருமானுக்குப் பரதன் என்று பெயர் பரதனுடைய சம்பந்தமுடைய நாடாகையால் இந்நாட்டிற்குப் பாரதம் என்று பெயர் வந்தது. இந்த பாரததேசத்திற் கென்று தனியான சிறப்பும் பண்பாடும் உள்ளன. இந்நாட்டின் கலாச்சாரம் பண்பாட்டை அந்நியர்கள் சீர்குலைப்பதைக் கண்ட பாரதி மனம் வருந்திப் பலப்பல பாடல்களைப் பாடுகிறார். நாட்டிற்கென்றே தனது கவிதைப் பணியை முழுமையாக அற்பணித்தவர் பாரதியார் எனில் அது மிகையன்று. இந்த நாட்டு மக்கள் மீதும், மொழிமீதும் பண்பாட்டின் மீதும் மிகுந்த அக்கறை கொண்டவர் பாரதி.

ஞானத்தில் சிறந்த நாடு

மற்ற நாடுகளில் செல்வம், தொழில் முதலான பல்வகை வளங்கள் உண்டு. ஆனால் ஞானம் இருக்காது. ஞானத்தைப் பெற வேண்டுமென்றால் அவன் பாரத நாட்டிற்கு தான் வரவேண்டும். அதுவும் தென்னாட்டிற்கு வந்தால் தான் முழுமையான ஞானத்தைப் பெற முடியும். திருவள்ளுவர் முதலான சான்றோர்களும், மாணிக்கவாசகர் முதலான ஞானிகளும் தோன்றிய நம் பாரதநாடு. சித்தர்கள் நம்முடைய நாட்டில் தோன்றி அரிய செய்திகளையெல்லாம் வெளியிட்டுள்ளனர். இத்தகைய சிறப்பு நம் பாரத தேசத்திற்கே இருப்பதால் பாரதநாட்டின் பெருமையை விளக்க வந்த பாரதி, முதன்முதலில் 'ஞானத்திலே சிறந்த நாடு பாரதநாடு' என்று கூறியுள்ளார் மேலும் ஞானத்திற்கு அடையாளம் மோனமாகும்.

ஞானம் இருந்தால் மட்டும் போதாது மானம் இருக்க வேண்டும். இதற்கு அடுத்தபடியாகத் தானம் இருத்தல் வேண்டும்.

பிறருக்கு உணவை ஊட்டி மகிழ்கின்ற ஒரு பண்பாடு பாரத நாட்டிற்கே உண்டு. இதனைப் பட்டினத்தார், "பசித்தோர் முகம் பார்" என்று கூறினார்.

"மண்டிணி ஞாலத்து வாழுநர்க் கெல்லாம்
உண்டி கொடுத்தோர் உயிர் கொடுத்தோரே"

என்பது மணிமேகலை 'பகுத்துண்டு பல்லுயிர்ஓம்புதல்' என்பது தான் உலக இலக்கியங்கள் அனைத்திலும் கூறப்பட்ட முதல் தருமம் என்கிறார் திருவள்ளுவ நாயனார் இந்த அரிய பண்பாட்டினை நாம் இன்று வரை பின்பற்றி வருகிறோம். திருமணம், காதணிவிழா, உபநயனம் முதலான அனைத்து நிகழ்ச்சிகளிலும் 'விருந்து' மிக முக்கியமான இடம் பெருவதை இன்றும் காண்கிறோம். இந்தப் பண்பாட்டை மகாகவி உலகமக்களுக்கு எடுத்துரைக்கின்றான்.

கானம் :

கானம் என்ற சொல்லுக்கு 'இசை' என்று பெயர். சிலப்பதிகாரம் என்னும் நூலில் இசை பற்றிய அரிய குறிப்புகள் வருகின்றன. ஒரு கானகத்தில் தினை விளைந்து உள்ளது. தலைவன் தலைவி இருவரும் அப்பயிரைப் பாதுகாத்து வருகின்றனர், அங்கே தலைவன் களைப்பினால் உறங்கி விடுகின்றான். அந்த வழியே வந்த யானை தினைப் பயிரை மேய்கிறது. அந்தத் தலைவி யானையை விரட்ட முயல்கிறாள். கருவியைத் தட்டி யானையை விரட்டினால் உறங்குகிற கணவன் எழுந்து விடுவான். ஆகவே கணவன் உறக்கம் கெட்டுவிடாது இருக்க வேண்டும் என்று எண்ணிய தலைவி குறிஞ்சி என்ற பண்ணைப் பாடுகின்றாள். அப்பொழுது அந்த யானை மெய்மறந்து தினைப் பயிரை உண்பதை மறந்து பேசாமல் நிற்கிறது.

"பெருவரை மருங்கில் குறிஞ்சி பாடக்
குரலும் கொள்ளாது நிலையிலும் பெயராது
படாஅப் பைங்கண் பாடுபெற்று ஒய்யென
மறம்புகல் மழகளிறு உறங்கும்" **(அகம் - 102)**

இத்தகைய அரிய இசைக்கலையைத் தமிழ்நாட்டில் உள்ள அனைத்து மக்களும் அறிந்து வைத்திருந்தனர் என்பது குறிப்பிடத்தக்கது.

குழந்தைக்கு ஒரு பெண்மணி தாலாட்டுப் பாடுகின்றாள். அந்த இசையைக் கேட்டுத் தேவர்கள் தங்கள் வாகனத்தில் வந்து இறங்கி இசை கேட்டு நிற்கின்றனர். இக்காட்சியைத் திருஞானசம்பந்தர் தேவாரத்தில் பதிவு செய்கிறார்.

"பண்ணமர்ந்த மென் மொழியார்
பாலகரைப் பாராட்டும் ஓசைகேட்டு
விண்ணவர்கள் வியப்பெய்தி
விமானத்தோடும் மிழியும் மிழலையாமே" **(தேவாரம்)**

இவ்வாறாகத் தாலாட்டு முதலாக அனைத்து வேலைகளையும் இசையோடு கலந்து செய்து வந்திருக்கின்றனர். இந்த இசைக்கலை வேறு நாடுகளுக்கு இல்லை என்பதைப் பாரதி நன்கு சுட்டிக் காட்டுகிறார்.

"ஞானத்திலேயே பர மோனத்திலே உயர்
மானத்திலே அன்ன தானத்திலே
கானத்திலே அமுதாக நிறைந்த
கவிதையிலே உயர் நாடு"

இந்த உலகில் வீரம், புகழ், தொழில் முதலான அனைத்துத் துறைகளிலும் சிறந்து விளங்கிய நாடு பாரதநாடு. இவை போன்ற கலைகளையும், தொழில் வளங்களையும் மேம்படுத்த வேண்டும் என்பது பாரதியின் அவா.

பழம்பெரு நாடு

இமயமலை, கங்கையாறு, உபநிடதங்கள் இவையெல்லாம் பாரத நாட்டின் உடமைகளாகும். இது பழம்பெரும் நாடு. முனிவர்கள், வாழ்கின்ற நாடு.

"மன்னும் இமயமலை எங்கள் மலையே
மாநில மீதுபோற் பிறிதிலையே
இன்னறு நீர்க்கங்கை யாறெங்கள் யாறே
இங்கிதன் மாற்பிற்கு எதிரெது வேறே
பன்னரும் உபநிட நூலெங்கள் நூலே
பார்மிசை யேதொரு நூல்இது போலே
பொன்னொளிர் பாரத நாடெங்கள் நாடே
போற்றுவம் இஃதை எமக்கிலை ஈடே
மாரதவீரர் மலிந்தநன் னாடு
மாமுனிவோர் பலர் வாழ்ந்த பொன்னாடு
நாரத கான நலந்திகழ் நாடு

நல்லன யாவையும் நாடுறு நாடு
பூரண ஞானம் பொலிந்தநன் னாடு
புத்தர் பிரானருள் பொங்கிய நாடு
பாரத நாடு பழம்பெரு நாடே
பாடுவம் இஃதை எமக்கில்லை ஈடே''

செந்தமிழ் நாடு

பாரதி தமிழ் நாட்டைப் பற்றிக் கூறும்போதெல்லாம் வெறுமனே கூறாமல் செந்தமிழ்நாடு என்று கூறுகிறார். பாரத நாட்டிற்கு இதயமாக விளங்குவது தமிழ்நாடு. இந்த நாட்டைப் பற்றிக் கூறும்போதே ஒரு சக்தி பிறக்கிறது என்பது அவருடைய நாட்டுப்பற்றை வெளிப்படுத்துகிறது.

''செந்தமிழ் நாடெனும் போதினிலே - இன்பத்
தேன்வந்து பாயுது காதினிலே
தந்தையர் நாடென்ற பேச்சினிலே-ஒரு
சக்தி பிறக்குது மூச்சினிலே''

வேதம் நிறைந்த நாடு

'வேதம்' என்பது பாரத நாட்டின் சொத்தாகும். வேதத்தில் எல்லா அறங்களும் உள்ளன. வேதத்தில் இல்லாத அறங்கள் வேறெங்கும் இல்லை. சிட்சை, கல்பம், வியாகரணம், நிருத்தம், ஜோதிடம், சந்தஸ் ஆகிய ஆறு அங்கங்களும் உள்ளன. இதில் இல்லாத கல்வியே இல்லை எனலாம். வேற்று நாட்டு மக்கள் இங்கே வரும்போது இங்குள்ள வேதத்தைப் பற்றியே அதிகமான ஆய்வு மேற்கொள்கின்றனர். ஆனால் இங்குள்ளவர்கள் வேதத்தை அதிகம் கருத்தில் கொள்வதில்லை. நம் பழங்கால மக்கள் வேதத்தைப் பெரிதும் போற்றி வந்தனர். வேதத்திற்கு 'ஒழுக்க நூல்' என்றே பெயர். திருமூலர் திருமந்திரத்தில், வேதத்தை 'அறநூல்' என்று கூறுகிறார்.

''வேதத்தை விட்ட அறமில்லை வேதத்தில
ஓதத் தகும் அறம் எல்லாம் உளதர்க்க
வாதத்தை விட்டு மதிஞர் வளமுற்ற
வேதத்தை ஓதியே வீடு பெற்றார்களே (திருமந்திரம்)

பாரத தேசத்தில் தமிழ்நாட்டிலேதான் வேதம் அதிகமாக மதிக்கப்பெற்றது என்பது யாவரும் அறிந்ததே. தமிழ் பாடுவதற்காகவே அவதாரம் செய்தவர் திருஞானசம்பந்தர். அவர் பல இடங்களில் வேதத்தைப் பற்றிக் குறிப்பிடுகிறார்.

“ஓமென்று மறை பயில்வார்”

“அருநெறிய மறை வல்ல முனி”

“வேதத்தின் மந்திரத்தால்
வெண்மணலே சிவமாகப்
போதத்தால் வழிபட்டான்
புள்ளிருக்கு வேர்ரே”

“அங்கமும் வேதமும் ஓது நாவர்”

“மறை யோதும் எங்கள் பரமன்”

இவருடைய அவதாரத்தைப் பற்றிச் சொல்லும்போது சேக்கிழார் “வேதநெறி தழைத்தோங்க என்றுதான் தொடங்குகிறார். உயர்ந்த அறங்களை எடுத்துரைக்கும் வேதம் நமக்கு மிக மிக முக்கியமாகையால்,

“வேதம் நிறைந்த தமிழ்நாடு” என்று பாரதி பாடுகின்றார். பாரதியின் கவிதைகளைப் பராபட்சமின்றி உள்ளதை உள்ளவாறு கற்று, பிறருக்கு எடுத்துரைக்க வேண்டும் என்பதே இக்கட்டுரையின் முடிபாகும்

■

24. கவித்தேரின் சாரதி

முனைவர் கூ.முத்தையன்,
உதவிப்பேராசிரியர் ஆய்வு நெறியாளர்,
தமிழ்த்துறை, தேசியக்கல்லூரி (தன்னாட்சி),
திருச்சிராப்பள்ளி – 01.

முகவுரை

"இங்கிவனை யான் பெறவே என்ன
தவஞ் செய்து விட்டேன்"

பாரதி தேசிய வரலாற்றில் முக்கியப்பங்கு வகித்தவர். இலக்கிக் கலையை இயக்கக் கருவியாக்கிக் கொண்டவர். தமிழரிடையே தேசிய உணர்வை உருவாக்க முயன்றவர். சொல்லாலும், செயலாலும் மாந்தரின் சோர்வு நீக்கி ஒரு புத்தெழுச்சி கண்டவர். இளமையில் கண்ட கனவுகளை எதிர்ப்புகளுக்கும், இன்னல்களுக்கும், இயலாமைக்கும் இடையில் நனவாக்க வழி வகுத்தவர். பிள்ளைப் பருவத்திலேயே கவிமழைப் பொழிந்த அருட்கவி. இளமையிலேயே பலராலும் போற்றப்பட்டு 'பாரதி' என்னும் விருதும் பெற்றவர். தன் கவியாற்றலைத் தனியுரிமையாக்காது பலரும் பெற்றுப் பயன்பெறும் வண்ணம் பொதுவுரிமை அளித்தவர்.

இவர் இலக்கிய வாதியாக மட்டுமின்றி வாழ்வியல் ஞானியாகவும் அமைந்தவர். எவ்வுயிரையும் தன்னுயிர்போலக் கருதியவர். சமயக் காழ்ப்பின்றி எம்மதமும் சம்மதம் எனக் கொண்டவர். சக்தியையும், தேசத்தையும் வேறுபாடறக் கண்டவர். அதனால் நாட்டுக்கு இறைமை உயர்வு அளித்தவர். நாடும் இறைமையும் வாழ்வின் மையமானால் நாமும் நாடும் முழுநிலை ஈடேற்ற நிலை பெற முடியும் எனக் காட்டியவர். இத்தகைய பல சிறப்புகளுக்கும், இயல்புகளுக்கும் இலக்கணமுமான 'பாரதி' எனும் சொல் கலைமகளைக் குறித்தது. இச்சொல்லின் பல பொருள்நிலையை அகராதிகள், சரசுவதி, பைரவி, பண்டிதன், சொல், மரக்கலம் எனத் தமிழ்ப

பேரகராதி குறிக்கின்றது. பாரதி எனும் சொல், நாமகளைக் குறித்த நிலையில் நாவன்மையுடைய புலவரைச் சுட்டுகின்றது.

வாழ்வியல்

"படைப்பு நமது கண்ணுக்குத் தெரியாது;
அறிவுக்கும் தெரியாது.
சாவு நமது கண்ணுக்குத் தெரியும்;
அறிவுக்குத் தெரியாது.
வாழ்க்கை நமது கண்ணுக்குத் தெரியும்;
அறிவுக்கும் தெரியும்.
வாழ்க்கையாவது சக்தியைப் போற்றுதல்
இதன் பயன் இன்பமெய்தல்"

மனிதனின் வாழ்க்கை பிறப்பு முதல் இறப்பு வரை உள்ள இடைப்பட்ட காலத்தைக் குறிக்கிறது. அவனுள்ளே ஏற்படும் உள் மனத்திலும், புறத்திலும் ஏற்படும் மாற்றங்களையும், வளர்ச்சியையும் உட்கொள்வது அவனுள்ளே ஏற்படும் மனம், மொழி, மெய் இவையே அவன் செயற்பாடுகளாகிறது. அதனிடையே ஆக்கங்களை மட்டுமின்றி அழிவுகளையும், ஏற்றங்களையும், வீழ்ச்சிகளையும், துன்பங்களையும், இன்பங்களையும், உறுப்பாகவும், தீமைகளையும், நன்மைகளையும் உண்டாக்கி அமைகிறது. வளமும் வறுமையும் பெறுகின்றது. இதுவே அவன் காணும் கனவு நனவுமாக உருவாகின்றது. அறிவு என்பது வாழ்வில் மட்டும், மனிதன் முன்னேற்றமடைந்தால் போதாது, அகப்புற வாழ்வின் எல்லா நிலைகளிலும் அவன் முதன்மை நிலை பெறுதல் வேண்டும் என்று உணர்ந்தவர்.

தன்னை முற்றிலும் இழந்து பிறருக்காகவே வாழ்பவன் சமுதாய வாழ்வில் ஒளிவீசும் சுடரோனாகிறான். இதனை,

"சிந்தையி லறமுண்டாம் - எனிற்
சேர்ந்திடுங் கலிசெயும் மறமு முண்டாம்
மெய்த்தவர் பலருண்டாம் - வெறும்
வேடங்கள் பூண்டவர் பலருமுண்டாம்
உய்த்திரு சிவஞானம் - கனிந்
தோர்ந்திடு மேலவர் பலருண்டாம்
பொய்த்தவிந் திரசாலம் - நிகர்
பூசையும் கிரியையும் புலை நடையும்
கைத்திடு பொய்ம்மொழியும் - கொண்டு
கண்மயக் காற்பிழைப் போர் பலராம்"

மனிதர் பலவகைப்பட்டவர். எனவேதான் மனித வாழ்க்கையும் பலவகைப்படுகின்றது. பல வகையான மனிதரைக் கண்ட பாரதியும் வெவ்வேறுபட்ட வாழ்க்கைகளைச் சுட்டுகிறார். அறவாழ்வு சிறப்புடையது என்ற எண்ணத்தில் அறம் கருக்கொள்ளும்போது வாழ்வும் அறமுடையதாகின்றது. சிந்தையில் அறம் நிலை கொள்ளாதபோது செயல்கள் மறமுடையதாகித் திரிகின்றன.

இதனால் வாழ்வியல் தீமையுடையதாகின்றது. இத்தகு சுவையில்லாத சூழலிலும் வாழ்வின் சிறப்புப் பற்றிய நம்பிக்கையும், சிந்தனைகளும் எழுகின்றது. வாழ்க்கையில் எளிமையும் சிறப்புமுடையதாகக் கருதும் சூழலாக இது அமைகின்றது. பாரதி பல சூழல்களிலும் வாழ்வை இன்பங்கள் நிறைந்ததாகக் காட்டுகின்றார். இதனை,

"இவ் வுலகம் இனியது....
மனிதர் மிகவும் இனியர்
இளமை இனிது. முதுமை நன்று
உயிர் நன்று சாதல் இனிது.."

எல்லாம் இன்பமயமான ஓர் உலக வாழ்வு இங்குச் சுட்டப்படுகின்றது. இன்பம் கருக்கொண்ட சிந்தனையால் வாழ்வில் இன்பம் தெளிகின்றது. எங்கும் எதிலும் இன்பம் தெரிகின்றது எனத் தம் கவிப்புலமையைப் பல நிலைகளில் வெளிப்படுத்தியவர்.

தன்னம்பிக்கையுடன் முன்னேறும் வாழ்நிலையில் இவ்வின்ப நிறைவுகள் காணப்படல் இயல்பு. இனிமையுடையதான வாழ்க்கை வெற்றியுடையதாகவும் அமைதலை வாழ்வியல் நம்பிக்கையின் உச்சமெனலாம். கிடைக்கும் என்ற நம்பிக்கை ஒரு பாலாகக் கிடைத்தது எனும் தேற்றும் அதன் மணி முடியாகின்றது. இதனை,

"எடுத்த காரியம் யாவினும் வெற்றி
எங்கு நோக்கினும் வெற்றிமற் றாங்கே
விடுத்த வாய்மொழிக் கெங்கணும் வெற்றி
வேண்டினேனுக் கருளினன் காளி ....
எண்ணு மெண்ணங்கள் யாவினும் வெற்றி
எங்கும் வெற்றி எதனினும் வெற்றி"

இங்கும் நம்பிக்கைசார் வாழ்வும் இறைமையும் கொண்டுப் புலப்படுகின்றது.

"பிழைக்கும் வழி சொல்ல வேண்டுமென் றொலொடு
பேச்சினி லேசொல்லு வான்
உழைக்கும் வழி வினை யாளும் வழி பயன்
உண்ணும் வழி யுரைப்பான் ....
மழைக்குக் குடை பசி நேரத் துணை வென்றன்
வாழ்வினுக் கெங்கள் கண்ணன்"

என அடிப்படை வாழ்வு நிலையில் அனைத்துயிரின் வாழ்வும் ஒன்றுதான். உணவு, உறக்கம் என அமைகிறதென்று வகைப்படுத்துகிறார். வாழ்வுக்கு உடல் இன்றியமையாதது. எனினும் உடலுக்கு உணவு இன்றியமையாதது.

ஒவ்வொரு மனிதனுக்கும் உணவு வேண்டும். அவ்வுணவுகூட அளிக்க இயலாத மனித சமூகம் இருந்தும் பயனில்லை என்ற எண்ணத்தை,

"இனியொரு விதி செய்வோம் - அதை
எந்நாளும் காப்போம்
தனியொருவனுக்குக் குணவில்லை யெனில்
ஜகத்தினை அழித்திடு வோம்"

உணவுக்காகப் போராட்டம் எழும் வாழ்வு நிலை கூடாது. வளமுடைய இயற்கை உணவை வாரி வழங்கும்போது, பொது உரிமை மனநிலையுடன் அதனைப் பகுத்துண்ண வேண்டும். அதனை வலியவர் தம்முள் பங்கிட்டுப் பிறரை வறியவர் ஆக்கலாகாது என்பர்.

"மனித ருணவை மனிதர் பறிக்கும்
வழக்கம் இனியுண்டோ?
மனிதர் நோக மனிதர் பார்க்கும்
வாழ்க்கை இனியுண்டோ?"

என மொழிகிறார். மீண்டும் அதனை வலியுறுத்திப் பிறிதோரிடத்தில்,

"வயிற்றுக்குச் சோறுண்டு கண்டீர் - இங்கு
வாழும் மனிதரெல் லோருக்கும்
பயிற்றி உழுதுண்டு வாழ்வீர்-பிறர்
பங்கைத் திருடுதல் வேண்டாம்"

உணவு அளித்தல் என்பதை அரசியல் கடமையாகவும் சுட்டுகின்றார்.

“மனிதி லுறுதி வேண்டும்
வாக்கினி லேயினிமை வேண்டும்
நினைவு நல்லது வேண்டும்
நெருங்கின பொருள் கைப்பட வேண்டும்
கனவு மெய்ப்பட வேண்டும்
கைவசமாவது விரைவில் வேண்டும்
தனமும் இன்பமும் வேண்டும்
தரணியிலே பெருமை வேண்டும்”

மனதில் உறுதி கருக்கொள்ளும்போது ஆற்றல் உருவாகின்றது. நினைந்த காரியத்தைச் செய்ய இயலும் என்ற திட நம்பிக்கையே அவ்வுறுதியளிக்கிறது. ஆற்றல் செயலாகி வெளிப்படுகின்றது. முயற்சி உந்து சக்தியாகி, ஊக்கமூட்டிச் செயல் வெற்றி அளிக்கின்றது. எண்ணிய காரியம் முடிகின்றது. நினைத்தது நடக்கின்றது. நெருங்கிய பொருள் கைப்படுகின்றது. என்று முடிக்கின்றார். மேலும்,

எதிர்காலத்தைப் பற்றிய கனவுகள் நனவாக மாறுவதே சிறந்த ஆற்றலின் அடையாளம். அதுவே பெருமையுடையது கனவுகள் வெறும் கற்பனையாக நின்றுவிடலாகாது. உள் மனத்தில் காட்சிப்பட்டவை புறத்தே கைப்படல் வேண்டும். நினைவுகளெல்லாம் திட்டங்கள் எல்லாம் விரைவில் மெய்யாகின்ற நிலையிது.

“எண்ணிய முடிதல் வேண்டும்
நல்லவே எண்ணல் வேண்டும்
திண்ணிய நெஞ்சம் வேண்டும்
தெளிந்த நல்லறிவு வேண்டும்
பண்ணிய பாவமெல்லாம்
பரிதி முன் பனியே போல
நண்ணிய நின் முன் இங்கு
நசித்திடல் வேண்டும் அன்னாய்”

நன்மையை நாடித் தீமையை விலக்குவதாக வாழ்க்கை அமைகின்றது. அதற்கு உதவுவதாக இறைமை இங்குச் சுட்டப்படுகிறது.

நிறைவு

“மானுட ஜென்மம் பெறுவதற் கரிதெனும்
வாய்மை உணர்ந்தாரேல் - அவர்
ஊனுடல் தீயினும் உண்மை நிலை தவற
உடன்படு மாறுளதோ!”

மனிதராகப் பிறப்பது அரிது. கிடைத்தற்கரிய உயிர் வாழ்வைப் பெற்ற மனிதன் அதனை உயிர்வாழ்வாக நிலைத்திருக்கச் செய்ய வேண்டும். பல உயிரினங்களைவிடவும் அவனுக்கு மிகுந்த ஆற்றல் இயற்கை வழங்கி உள்ளது. அதனை நல்வழியில் செலுத்தி ஆக்கங்கள் பல புரிதல் தேவை. மனிதன் ஆறறிவுடையவன். அவனிடமே எல்லாத் தீய பண்புகளும் இருக்கின்றன. அவற்றைக் களைதல் வேண்டும். வாழ்வைச் செவ்வகைப்படுத்த வேண்டும். சோம்பலின்றி உழைக்க வேண்டும். எப்போதும் உழைத்துக் கொண்டே இருப்பவனுக்குத் தீயவைகளை எண்ண நேரம் கிடைப்பதில்லை; அடுத்தவன் வாழ்வில் குறுக்கிடப்படுவதும் இல்லை. உழைத்தவன் முன்னேறுகிறான். அவன் கடமைகளைப் புரிந்து மேலே மேலே சென்று கொண்டே இருக்கிறான். மேலே செல்பவனுக்கு வானம் 'அணியது' அண்மையிலே இருக்கிறது.

முதன்மை நூல்கள்

1. பாரதியார் கவிதைகள், பூம்புகார் பிரசுரம், சென்னை–1976.

2. வேடிக்கைக் கனவுகள், ராமன் பதிப்பகம், சென்னை–1977.

துணைமை நூல்கள்

1. கவியரசர் பாரதி, ப.அருணாசலம், பாண்டியன் அச்சகம், சிதம்பரம்– 1966.

2. பாரதியார் கவிதைகள், டி.வி.எஸ்.மணி, வானவில் பிரசுரம், சென்னை–1980.

■

25. பாரதியின் பல்துறைச்சிந்தனைகள்

முனைவர் ஆ.முருகானந்தம்,
உதவிப்பேராசிரியர், தமிழ்த்துறை,
தேசியக்கல்லூரி (தன்னாட்சி), திருச்சிராப்பள்ளி–620 001.

முகவுரை:

"புவியனைத்தும் போற்றிடவான் புகழ்படைத்துத்
தமிழ்மொழியைப் புகழில் ஏற்றும்
கவியரசர் தமிழ்நாட்டுக் கில்லையெனும்
வசையென்னாற் கழிந்த தன்றே"

என்று பெருமிதத்துடன், ஏன் களிப்புடன் கூறிக்கொண்ட பெருங்கவிஞர். புதுநெறி காட்டிய புலவன், பாரதியின் கவிதைகளில் இடம்பெற்ற நயங்களை பளிச்சிட்டுக் காட்டுவதுடன் பல்வேறு ஆளுமைக் கோலங்களை ஒரு பல்லுருவம்காட்டியில் (மயடநனழளஉழிந) காணும் காட்சிகளைப் போல் கண்டு மகிழலாம். என்றாலும்,

"பாவின் நயமெல்லாம் - யானும்
பகர வல்லேனோ
ஆவின் பாற்சுவையை-நாழி
அளந்து காட்டிடுமோ?"[1]

என்ற கவிமணியின் அளவுகோலின் வரையறையை எண்ணிப் பார்த்தேயாகவேண்டும். அப்போதுதான் அந்த அளவுகோலின் எல்லையை உண்மையிலேயே உணரமுடியும். கவிதைகளைச் சுவைத்துக் கூறுகின்றவர்களின் கருத்துக்களெல்லாம் அவர்களுடைய உணர்ச்சியையே சார்ந்திருக்கும் என்று கொண்டால் இங்குக் காணப்பெறும் கூற்றுக்கள் வாதத்திற்கு இடம்தரா. பல்லாண்டுகளாகயான் பாரதியாரின் பாடல்களைச் சுவைக்கும்போது குமிழியிட்டெழுந்த என்னுடைய இக்கருத்துக்கள் பன்முகத்தன்மையில் பகுக்கப்பட்டு இக்கருத்தரங்கில் சமர்ப்பிக்கப்படுவதில் நான் பெரும்பேறு பெற்றேனாவேன்.

பாரதீயம்:

பாரதியாரின் காலம், நாட்டில் பெரும் குறைகள் மலிந்து நிலவிய காலம். நம் நாடும் அன்னியர் ஆட்சியில் அடிமை நாடாக அடங்கிக் கிடந்தது. நாட்டுமொழிகளின் வளர்ச்சிக்கும் அவற்றின் ஆக்க வழிகட்கும் வாய்ப்பே இல்லாமல் நலிவுற்ற நிலை. மக்கட்சமுதாயத்தில் ஆணின் கையே மேலோங்கி இருந்தது. பலவகைகளிலும் பெண்ணுரிமை அடிமைப்பட்டே இருந்தது. தற்சாதிப்பற்று என்ற கொடுமை சமூகத்தை ஆட்டிவைத்தது. இத்தகையச் சூழ்நிலையில் தான் பாரதியாரின் பாடல்கள் முகிழ்ந்தன. இந்நிலையில், நாட்டுப்பற்று, விடுதலைவேட்கை, தாய்மொழிப்பற்று, சமுதாயமுன்னேற்றம், பழமையில் புதுமை, பெண்விடுதலை, அறிவுத்தெளிவு என்பன யாவும் பாரதீயங்ளாகப் பல்கிப்பெருகின.

புதுமைக்கவிஞர்:

எதிலும் 'புதுமை' என்ற ஒரு தனித்துவத்தைக் காண்டால் அரிது. 'தனிப்புதுமை' என்பது குதிரைக்கொம்பு. பழமையானவற்றைப் புதுக்கின பிறகு அவற்றைப் 'புதுமை' என்று புகழாரம் சூட்டுகின்றோம். பாரதியாரையே நாடு 'புதுமைக்கவிஞர்' என்று போற்றுகின்றது. அவரும் சுவை புதிது, பொருள் புதிது, என்றும் பெருமைப்பட்டுக்கொள்கின்றார். மக்கள் யாவரும் ஒருமனத்துடன் இப்பெருமைக்கு அவர் உரியவராகின்றார். என்று ஒப்புக்கொள்கின்றார்கள். ஆழ்ந்து நோக்கினால் பாரதியாரிடம் நாம் காணும் புதுமைகள் யாவும் பழமையின் அடிப்படையிலேயே அமைந்தவை என்பதை அறியலாம். அவற்றுள் சில,

நாட்டுப்பற்று:

தாம் பிறந்த நாடு தமிழகமாயினும் பாரதியார் கண்ட நாடு, பாரதநாடு. அவரின் தேசியப்பார்வை மிகவும் விரிந்ததே. தமிழகத்தையும் பிற பகுதிகளையும் பாரதப் பெருநாட்டின் அகப்பகுதிகளாகவே உற்றுநோக்கினார். "**யாதும் ஊரே யாவரும் கேளிர்**"**2** என்ற மரபுவழிவந்த தமிழ் மகனிடம் குறுகியநோக்கம் எங்ஙனம் இருக்கும். இந்நிலையில் தான் அவருடைய விரிந்த மனநிலையைக் காணமுடிகின்றது என்றால் மிகையாகா.

"எந்தையும் தாயும் மகிழ்ந்து குலாவி
இருந்ததும் இந்நாடே-அதன்
முந்தையர் ஆயிரம் ஆண்டுகள் வாழ்ந்து

முடிந்ததும் இந்நாடே - அவர்
சிந்தையில் ஆயிரம் எண்ணம் வளர்த்து
சிறந்ததும் இந்நாடே''[3]

என்று நாட்டுப்பற்றோடு பாடுவதையும்,

''முப்பது கோடி முகமுடையாள் உயிர்
மொய்ம்புற வொன்றுடையாள் - இவள்
செப்புமொழிபதி னெட்டுடையாள் எனிற்
சிந்தனை ஒன்றுடையாள்''-[4]

என்று படைத்துக் காட்டும்போது வேற்றுமையில் ஒற்றுமை (ரெவைல மை னைஎநசளவைல) காணும் கவிஞரின் பாரதப் பண்பைக் காணமுடியும். மேலும்

''பேரிமய வெற்புமுதல் பெண்குமரி ஈறாகும்
ஆரியநா டென்றே யறி''[5]

என்று இந்த எல்லையைக் குறிப்பிடும் நிலையில் பாரதியின் பாடல்கள் தமிழ்மொழிக்குப் புதியவை: தமிழர்களிடம் அகன்ற நோக்கை விரந்த பார்வையை உண்டாக்கக் கூடியவை எனத் துணியலாம்.

விடுதலை வேட்கை:

விடுதலையே பாரதியின் தாரக மந்திரம். நாட்டுப்பற்றிலிருந்து கிளைத்தெழுந்த ஒரு கொள்கை. **'வந்தே மாதரம் ஒன்றே தாரகம்'** *என்பது இதன் உயிராய பண்பு.*

''வீர சுதந்திரம் வேண்டிநின் றார்பின்னர்
வேறொன்று கொள்வா ரோ?-என்றும்
ஆரமு துண்ணுதற் காசைகொண் டார்கள்ளில்
அறிவைச் செலுத்துவா ரோ?''[6] *என்று கேட்கின்றார்.*

நாயன்மார்களுக்கும், ஆழ்வார்கட்கும் பக்தி அவர்தம் மூச்சாக இருந்தது போலவே, இவருக்கு விடுதலையே மூச்சாக இருந்தது. சிட்டுக்குருவியைப் போலவே எப்போதும் விடுதலையை வேண்டி நிற்பவர். என்பதை,

''விட்டு விடுதலை யாகிநிற் பாயிந்தச்
சிட்டுக் குருவியைப் போலே''[7]

என்று தம் மனத்திற்குக் கட்டளையிடுகின்றார். தெய்வத்தை நோக்கி, 'தெய்வமே, எனக்கு இரண்டு சிறகுகள் கொடுக்க மாட்டாயா? பாழ்பட்ட மனிதர் கூட்டத்தையும், அதன்

கட்டுகளையும், நோய்களையும் துன்பங்களையும், பொய்களையும் உதறி எறிந்துவிட்டு நான் இச்சைப்படி வானத்திலே பறந்து செல்ல மாட்டேனா?' என்று வினாத்தொடுக்கும் நிலையில் அவரது விடுதலை வேட்கையை அறியமுடியுமன்றோ!

இனப்பற்றும் மொழிப்பற்றும்:

பாரதியார் ஒரு பச்சைத்தமிழ் மகன். தமிழகத்தைப் பிறப்பிடமாகக் கொண்டவர். மொழியையும், இனத்தையும், நாட்டையும் இணைத்துப் பாடுவதே இவரது இணையற்றக்கொள்கை. '**பாருக்குள்ளே நல்ல நாடு பாரத நாடு**' என்று பறைசாற்றியதைப் போலவே, "தாமறிந்த மொழிகளிலே தமிழ்மொழி போல் இனிய மொழியை எங்கும் கண்டதில்லை என்று வீறு பேசுகின்றார்".[8] தமிழைப் பற்றி இவர் இயற்றிய நான்கு பாடல்களும் தமிழர்களால் பொன்னேபோல் போற்றப்பட வேண்டியவை, தமிழர்கள் வீறுகொண்டெழுதல் வேண்டும், மொழியை வளப்படுத்த வேண்டும், இலக்கியச் செல்வத்தை, கலைச்செல்வத்தைப் பெருக்குதல் வேண்டும், எனும்பொழுது

> "**சொல்லில் உயர்வுதமிழ்ச் சொல்லே : - அதைத்**
> **தொழுது படித்திடடி பாப்பா**"[9]

என்று தமிழின் உயர்வைச் சின்னஞ்சிறு குழந்தைகளின் பிஞ்சு மனங்களில் படியசெய்வதிலிருந்து அவரின் மொழிப்பற்று விளங்கும்.

> "**ஆதிசிவன் பெற்று விட்டான் - என்னை**
> **ஆரிய மைந்தன் அகத்தியன் என்றோர்**
> **வேதியன் கண்டு மகிழ்ந்தே - நிறை**
> **மேவும் இலக்கணம் செய்து கொடுத்தான்**"[10]

என்று தமிழ்த்தாயின் வாயில் வைத்தே தமிழின் தெய்வப் பிறப்பை எடுத்துரைப்பர். இலக்கணத்தின் பழமையையும் பகர்வார். தெள்ளுதமிழ்ப் புலவோர்கள் தீஞ்சுவைக் காவியங்களைச் செய்து தந்ததையும் நம் திருச்செவி சாற்றுவார் அன்றோ! இவ்வுலகில் அமரவாழ்வு பெறவேண்டுமென்றால் '**தெள்ளூற்ற தமிழமுதத்தின் சுவை காணுங்கள்**' இன்பத்தேன் வேண்டுமென்றால் '**செந்தமிழ் நாடென்று செப்புங்கள்**' என்று மொழியின் மேலும், நாட்டின் மேலும் வைத்து நல்லுரை பகர்வார். இதுவே இவரது இனப்பற்றுக்கும் மொழிப்பற்றுக்கும் தக்கதொரு சான்றாக அமையும்.

பெண்ணுரிமை:

பெண்ணுரிமை பாரதியாரின் உயிர் நிலையானது. ஆணும்,பெண்ணும் இருகண்களையொத்தவர் என்பது கவிஞரின் கருத்து.

"ஆணும் பெண்ணும் நிகரெனக் கொள்வதால்
அறிவி லோங்கிஇவ் வையம் தழைக்குமாம்"[11]

என்று இக்கருத்திற்கு அரண் அமைத்துக் காட்டுவார். மேலும்,

"வலிமை சேர்ப்பது தாய்முலைப் பாலடா
மானம் சேர்க்கும் மனைவியின் வார்த்தைகள்
கலிய ழிப்பது பெண்க ளறமடா!
கைகள் கோத்து களித்து நின் றாடுவோம்"[12]

என்ற நிலையில் இந்த அரணுக்கும் வலியூட்டுவார். காலத்திற்கேற்ப, நடைமுறைக்கேற்பப் பெண்ணினத்திற்கு வேண்டும் ஆண்மைக் கூறுகளைப் புதிய பார்வையுடன் எடுத்துக்காட்டுபவர் நம் புதுநெறி காட்டும் புலவன். இக்காலக் கவிஞர்களுள் பெண்மை வாழ்க! என்று கூத்திடும் முதற்கவிஞர் இவரே. பெண்மை வெல்க! என்று முழுமனதுடன் விழைபவரும் இவரே.

"உயிரைக் காக்கும், உயிரினைச் சேர்ந்திடும்
உயிரினுக் குயிராய் இன்ப மாகிடும்
உயிரினும் இந்தப் பெண்மை இனிதடா:
ஊது கொம்புகள்: ஆடுகளி கொண்டே"[13]

என்று பெண்ணுரிமைக்குப் பாடுபடும் தொண்டர்கட்கு உயிர்ப்பு ஊட்டுகின்றார். 'போற்றி தாய்' என்று தாளங்கள் கொட்டவும், பொற்குழல் ஊதவும் அவர்களைப் பணிக்கின்றார். ஆணினம் பெண்ணினத்திற்கு விதித்துள்ள வேலிகளைத்தகர்த்தெறிந்துவிட்டு அவ்வினம் பூட்டிய தடைகளை அவிழ்த்தெறிந்து விட்டு பெண்ணினத்தை 'விடுதலைக் கும்மி'யடிக்குமாறு கட்டளையிடுகின்றார் என்றால் அவர் பெண்ணுரிமைக்காக ஆற்றியப் பணிகள் தெளிவுப்படும்

அறிவுத்தெளிவு:

வருவதை முன்னுணர்ந்து ஆற்றலோடு நவிலும் அறிவுத்தெளிவு தனிப்பெரும் புலமைக் கூறு பாரதியாரிடத்தில், அவருடைய கவியிடத்தில் காணமுடியும்.

"மறுவில் செய்தி மூவகைக் காலமும்
நெறியின் ஆற்றிய அறிவன்"[14]

என்று தொல்காப்பியர் கணியனை உணர்த்தினதாலும் இக்கூறு கவிஞனுக்கும் உரியது என்று கொள்ளலாம். சென்ற கால வரலாற்றைச் சிந்தித்து நிகழ்காலப் போக்கை கூர்ந்து நோக்கும் கவிஞன் எதிர்காலத்தில் நிகழப்போவதை மயங்காது அறுதியிட்டுக் கூறும் நிலையில்,

"ஆடுவோமே பள்ளுப் பாடுவோமே,
ஆனந்த சுதந்திரம் அடைந்து விட்டோமேன்று"[15]

என்று நாடு விடுதலையடைவதற்குப் பல்லாண்டுகளுக்கு முன்னரே பாடிய பெருமை இக்கவிஞனுக்கு உண்டு.

"இழிவு கொண்ட மனித ரென்பது
இந்தியாவில் இல்லையே"[16]

என்றும்,

"எங்கும் சுதந்திரம் என்பதே பேச்சு-நாம்
எல்லோரும் சமமென்பது உறுதியாச்சு"[17]

என்றும் முன்னரே கூறிச்சென்ற கவிஞரின் வருவதுரைக்கும் திறன் நம்மை இறும்பூது எய்தச் செய்கின்றது என்றால் அது மிகையல்லவோ?

இயற்கை எழில்:

பாரதி இயற்கையோடு பழகும் திறன் வாய்ந்தவன். இயற்கையை அவன் தழுவும் விதமே தனித்தன்மை வாய்ந்தது. உறங்குகின்ற குழந்தையைத் தாய் அதன் உறக்கம் கெடாமல் தழுவிக்கொண்டு தன்னை மறந்திருப்பதுபோல்க் கவிஞனும் இயற்கையை அதன் இன்ப சமாதி கலையாமல் தழுவுவான். அதோடுமட்டுமல்லாமல் தன்னையும் உடனே மறந்திடுவான். இன்பப்பெருக்கில் மிதப்பான். அதிலிருந்து வெளிவந்தவுடன் பாடுவான். சில சமயம் ஆடவும் செய்வான். இந்த இன்ப நிலையிலிருந்து பாரதியார் பாடிய பாடல்கள் இயற்கை எழில்களைக் காட்டுகின்றன. சான்றாக,

"கானப் பறவை கலகலெனும் ஓசையிலும்
காட்டு மரங்களிடைக் காட்டும் இசைகளிலும்
ஆற்றுநீ ரோசை அருவி யொலியினிலும்
நீலப் பெருங்கடலெந் நேரமுமே தானிசைக்கும்

…. … … … … … … … …

… … … … … … … … …

நாட்டினிலும் காட்டினிலும் நாளெல்லாம் நன்றொலிக்கும்
பாட்டினிலும் நெஞ்சைப் பறிகொடுத்தேன்”[18]

எனும்பொழுது இயற்கையில் தோய்ந்து பாடுங்கால் நம்மையும் அதில் தோயவைத்துவிடுகின்றார்.மேலும்,

“நிலாவையும் வானத்து மீனையும் காற்றையும்
நேர்பட வைத்தாங்கே
குலாவும் அமுதக் குழம்பைக் குடித்தொரு
கோலவெறி படைத்தோம்,
உலாவும் மனச்சிறு புள்ளினை எங்கணும்
ஓட்டி மகிழ்ந்திடுவோம்”[19]

என்ற பாடலில் நிலவு, நட்சத்திரங்கள், காற்று ஆகியவற்றைப் புகழ்ந்துரைக்கின்றார்.

கடவுட் கொள்கை:

பாரதியாரின் கவிதைகளை மேலோட்டமாகப் படித்தாலே அவர் ‘சக்தி வழிபாட்டாளர்’ என்பது தெளிவுபெறலாம். பண்டையோர் வழிபடும் எல்லாக் கடவுளர்களைப்பற்றிப் பாடல்கள் காணப்பெற்றாலும், ‘**சர்வம் விஷ்ணு மயம் ஜகத்**’ என்ற பண்டைய மரபு இவரிடம் ‘**சர்வம் சக்திமயம் ஜகத்**’ என்ற புது மரபாகின்றது. சக்தியைப்பற்றி அதிகமான எண்ணிக்கையில் பாடல்கள் காணப்படுவதே இதனைத் தெளிவாக்குகின்றது. இதற்கு அரணாகவும் அமைகின்றது.

“எண்ணிற் கடங்காமல் எங்கும் பரந்தனவாய்
விண்ணிற் சுடர்கின்ற மீனையெல்லாம் - பண்ணியதோர்
சக்தியே நம்மைச் சமைத்ததுகாண்”[20]

என்ற பாடல் பகுதி இதனை நன்கு மெய்ப்பிக்கும். மேலும் ‘சக்தி’ என்ற பாடற்பகுதியில் அனைத்தும் சக்தி வடிவாம் என்ற தத்துவத்தை அலசிக்காட்டுகின்றார்.

கல்வி பற்றிய சிந்தனை:

கல்வி பற்றிய சிந்தனைகளை இவர்பாடல்களில் காணலாம். கோயில்களை எழுப்புவதில் பெரும்பங்கு கொண்டது நம் நாடு. தற்கால வாழ்விற்குக் கல்வி மிகவும் இன்றியமையாதது என்பதை நன்கு உணர்ந்த கவிஞர் எல்லா நிலைகளிலும் பள்ளிகள்,

கல்லூரிகள், பல்கலைக்கழகங்கள் இன்றியமையாதவை என்பதை,

"வீடு தோறும் கலையின் விளக்கம்
வீதிதோறும் இரண்டொரு பள்ளி
நாடு முற்றிலும் உள்ளன ஊர்கள்
நகர்க ளெங்கும் பலபல பள்ளி"[21]

என்ற கவிதையில் புலப்படுத்துகின்றார். இளைஞர்கள் கல்வி கற்று பயன்பெறவேண்டும்,தம்மையும் உயர்த்தித் தாம் பிறந்த நாட்டையும் உயர்த்த வேண்டும் என்பது கவிஞர்கண்ட கனவு. வறுமையின் காரணமாக இளைஞன் ஒருவன் கல்வி பெறுவதற்குத் தடை ஏற்படக்கூடாது என்பதையும் சிந்தித்துள்ளார் கவிஞர். செல்வர்களும் அரசும் இதற்கு வழிவகை செய்தல் வேண்டும் என்பதை வற்புறுத்துவது போல்,

"அன்ன சத்திரம் ஆயிரம் வைத்தல்
ஆலயம்பதி னாயிரம் நாட்டல்
பின்ன ருள்ள தருமங்கள் யாவும்
பெயர்வி ளங்கி யொளிர நிறுத்தல்
அன்ன யாவினும் புண்ணியம் கோடி
ஆங்கோர் ஏழைக் கெழுத்தறி வித்தல்"[22]

என்று பேசுவர். அப்பொழுதுதான் தமிழகம் '**கல்வி சிறந்த தமிழ் நாடு**' என்று பழம்பெரும் புகழை நிலைநிறுத்தும் என்பது கவிஞரின் நீள்நோக்கு என்றால் அது மிகையன்றோ!

இந்நிலையில் தான் அவர் அறிவியல் கல்விக்கு ஆக்கமும் ஊக்கமும் தருதல் வேண்டும் என்பதை,

"பயிற்றிப் பலகல்வி தந்து - இந்தப்
பாரை உயர்த்திடல் வேண்டும்"

என்று ஆற்றுப்படுத்துகின்றார். தாய்மொழி மூலம் அக்கல்வி பெறவாய்ப்பு அமைக்கப்பெறுமானால் பருத்தி புடைவையாய்க் காய்த்தது போன்ற பலனை எதிர்ப்பார்க்கலாம். விலைப்பாலை விட முலைப்பால் சிறந்ததன்றோ?

"சென்றிடு வீர்எட்டுத்திக்கும் - கலைச்
செல்வங்கள் யாவும் கொணர்ந்திங்கு சேர்ப்பீர்"

என்றும்,

"பிறநாட்டு நல்லறிஞர் சாத்திரங்கள்
தமிழ்மொழியில் பெயர்த்தல் வேண்டும்
இறவாத புகழுடைய புது நூல்கள்
தமிழ்மொழியில் இயற்றல் வேண்டும்"

என்றும் பாடும் நிலையோடு அல்லாமல் தொழிற்கல்வி பெருகவேண்டும், தொழிற்சாலைகள் பல்கி பெருகி தொழில்வளம் செழிக்கவேண்டும் என்றும், வானொலி பெட்டிகள் போன்ற கருவிகள் உற்பத்தி செய்யவேண்டும் என்ற ஆர்வத்தையும் மக்களுக்கு பறைசாற்றியப் பெருமகனார். இதனை,

"காசி நகர்ப் புலவர் பேசும் உரைதான்
காஞ்சியில் கேட்பதற்கோர் கருவி செய்வோம்"[23]

என்ற பாடல்அடிகளினால் அறியலாம்.

நிறைவுரை:

பாரதியார் தம் கவித்திறத்தினால் நாட்டையும் நாட்டு மக்களையும், நல்வழியில் பீடு நடை போட, நாட்டுப்பற்று, மொழிப்பற்று, இறைவழிபாடு, கற்றலின்மேன்மை, இயற்கையைப் போற்றும், வணங்கும் மனநிலை, பெண்கல்வி, பெண்ணடிமைத்-தனத்தை வெறுத்தல், பெண்களுக்கான உரிமைக்குப் போராடுதல், அறிவுத்தெளிவு, முன்னோர் மொழிபோற்றி அவர்களின் வழியில் நடக்கும் மாண்பு முதலிய பல்லாற்றானும் பாரதியாரின் கவிதைகள் மனத்திற்கு இன்பமளிப்பதைவிட உள்ளத்திற்கு அமைதி அளிப்பதாக அமைகின்றன. கவிஞர் பன்முகத்தன்மை கொண்டவர் என்பதை பாரதியாரின் கவிதைகள் வாயிலாகவே தெளிவு பெறலாம், மனித சமூகம் பண்பட்டு மேன்மையடைய வேண்டும் என்ற கவிஞரின் பன்முகப்பார்வையான இக்கனவினை நாம் நனவாக்குவோமாக!

வாழ்க பாரதி ! வளர்க நின் புகழ் !!

சான்றெண்விளக்கம்

1. கவிமணி, மலரும்மாலையும்–பாரதியும் பட்டிக்காட்டானும்–22
2. புறம் – 192
3. பாரதியார் கவிதைகள். நாட்டு வணக்கம் – 1
4. மேலது, எங்கள் தாய் – 3
5. மேலது, பாரததேவியின் திருத் தசாங்கம் – 2
6. மேலது, தே.கீ. சுதந்திரப் பெருமை–9
7. மேலது, வே.பா.விடுதலை–சிட்டுக்குருவி

8. மேலது, தே.கீ. தமிழ் – 1
9. மேலது, பாப்பா பாட்டு–12
10. மேலது, தே.கீ. தமிழ் – 2
11. மேலது, புதுமைப்பெண் – 4
12. மேலது, பெண்மை–3
13. மேலது, பெண்மை–6
14. தொல்காப்பியம் – பொருள் – புறத்திணை–74 இளம்
15. மேலது, தே.கீ– சுதந்திரப்பள்ளு–பல்லவி
16. மேலது, விடுதலை–2
17. மேலது, பாரத மாதா நவரத்தின மாலை–5
18. மேலது, குயில் பாட்டு, 29–44
19. மேலது, த. பா. நிலாவும் வான்மீனும் காற்றும் – 1
20. மேலது, தோ. பா. மகாசக்தி வெண்பா–4
21. தே மேலது, தோ. பா. வெள்ளைத் தாமரை–6
22. மேலது, தோ. பா. வெள்ளைத் தாமரை–9
23. மேலது, தே.கீ. பாரததேசம் – 7

26. பாரதியின் பெண்கள் விடுதலைக் கும்மி

முனைவர் க.முருகேசன்,
உதவிப்பேராசிரியர் / ஆய்வு நெறியாளர்,
தமிழ்த்துறை, தேசியக்கல்லூரி (தன்னாட்சி),
திருச்சிராப்பள்ளி–01.

முன்னுரை

கலைகள் மனித மனத்தில் எழும் உணர்ச்சிப் பெருக்கால் எழுச்சியுற்றுப் பலவிதமான புறச்செயல்களைச் செய்து அவ்வுணர்வின் போக்கையும், தன்மையினையும் பிறருக்குப் புலப்படுத்திக் காட்டும் இயல்புடையது. இத்தகைய மிகையுணர்ச்சியில் ஒரு ஒழுங்கு அமையும்போது அதில் ஒருவகையான இன்பமும் கவர்ச்சியும் ஏற்படுகிறது. அவ்வுணர்வு பலவகையான கலைகளாக உருவெடுத்துக் கண்ணுக்கும், செவிக்கும், கருத்துக்கும் பயன் நல்குகின்றன.

நாட்டுப்புறக் கலைகள் காலப்போக்கில் பல விதமான ஒழுங்குகளையும், கட்டுப்பாடுகளையும் ஒப்புக்கொண்டு சீர்மையடைந்து செம்மைக் கலைகளாக உருமாறியமைந்துள்ளன. இத்தகைய செம்மையும், சீர்மையும் பெற்று உருவானவற்றைத் திருந்திய கலைகள் (Classical Arts) என்று வகைப்படுத்துவர்.

சிறு குழந்தைகள் தங்கள் உணர்ச்சிகளைப் பேச்சால் கூற இயலாது கைகளைச் சேர்த்துக் கொட்டி ஒலியை எழுப்பி உணர்த்துவதைக் காணலாம். 'பிள்ளைத்தமிழ்' இலக்கிய வகையில் நான்காம் பருவமாக அமைந்த சப்பாணிப் பருவம் என்பதை அமைத்துப்பாடி இருப்பதைச் சிந்திக்கலாம். 'பாணி' என்பது 'கை' ஆகும். கையுடன் கைசேர்வது சப்பாணி ஆயிற்று. கை கொட்டி களிப்புடன் வாய்திறந்து பாடுவதும் இணைந்து கலைத் தன்மைக்கு ஆட்படுத்தப்படுவதின் வாயிலாகக் கும்மிப்பாடல் கலையாகத் தோன்றி வளரத் தொடங்கியது.

நாட்டுப்புற மக்கள் தாங்கள் விரும்பிய வண்ணம் பாடல்களைப் புனைந்து வாய்மொழியாகப் பாடி ஆடி மகிழ்வர்.

பல பெண்கள் இணைந்து இன்ப உணர்வைப் புலப்படுத்தும் பாங்கில் கைகொட்டி களித்துப் பல பொருள் குறித்து வரும் கதைப்பாடல்களைச் சமய சமூக விழாக்களில் சுவை ததும்பப் பாடியும், கலை சிறக்க ஆடியும் பிறரை மகிழ்விக்கும் கலையே கும்மியாகும். இதைக் கும்மியடித்தல், கொம்மி கொட்டுதல், கொப்பி கொட்டுதல் எனப் பல நிலைகளில் இதற்குப் பெயரிட்டு வழங்கினர்.

கும்மி பெயர்க் காரணம்

"கும்மி கசிந்தடி யுங்கோ" **(கவிராச குருசாமி, மாடன் கும்மி, பா.1)**

"கும்மி யடி யுங்கடி" **(இசுலாமியக் கும்மிப்பாட்டு, பா.2)**

"பதம்பாடிக் கொம்மி யடி யுங்கடி"

(கோயிற்கொம்மி என்னும் பீரக்கக்கொம்மி, பா.1)

"கொம்மி கொட்டுமின்" **(குன்றக்குடி குமரன் கும்மி சுவடி)**

'கொம்மை கொட்டல்' என்று தொடரும் நமது சிந்தனைக்குரியதாய் இருக்கிறது. அகநானூறு, குறுந்தொகை போன்ற தொகை நூல்களிலும், சிலப்பதிகாரத்திலும் 'கொம்மை' என்ற சொல்லானது 'திரட்சி' அல்லது 'பருத்த' என்னும் பொருளில் கையாளப்படுகிறது.

பதினெண் கீழ்க்கணக்கு நூலான பழமொழியில்,

"நோற்ற பெருமை யுடையாருங் கூற்றம்
புறங்கொம்மை கொட்டினார் இல்"

என்று வருமிடத்தில் 'கொம்மை' என்ற சொல் 'கைகளைக் குவித்துக் கொட்டுதல்' என்ற பொருளைத் தருகிறது.

சீவக சிந்தாமணியில்,

"குஞ்சரம் கூற்றொடு கொம்மை கொட்டுவ"

என்று கூறுமிடத்திலும்,

"கூற்றத்தைக் கொம்மை கொட்டிக்
குலத்தொடு முடியுமென்பர்" **(சீவக சிந்தாமணி, பா.2230, 1109)**

என வரும் இடத்திலும், நைடதத்திலும், 'கூற்றொடு கொம்மை கொட்டிக் குனிக்கும்' என்று புகழும் சூழ்நிலையிலும் 'கொம்மை' முன்னர் குறிப்பிட்ட பொருளிலேயே பயன்படுத்தப் பெற்றுள்ளது.

'கும்முதல்' என்பதற்குக் 'கைக்கொட்டுதல்' என்று பொருள் விளக்கம் தரப்பட்டுள்ளது. 'கும்மாளம் போடுவது' என்பது குதித்து ஆடுவது என்ற பொருளில் இன்றும் நாட்டுப்புறங்களில்

வழங்கி வருகிறது. இவற்றை அடிப்படையாகக் கொண்டு கைகொட்டி ஆடுவதைக் 'கும்மி' என்று அழைத்திருக்கலாம்.

பெரும்பாலான கும்மிகளைப் பெண்களே ஆடிப்பாடி வருகின்றனர். 'ஓயில் கும்மி' என்ற வகையே ஆண்கள் ஆடுவர். 'சண்டைக்கும்மி' என்ற ஒரு வகைக் கும்மியும் உள்ளது. கும்மிப் பாடல்களை முதலில் குழுவின் தலைவி பாடுவாள். அவளைத் தொடர்ந்து மற்றவர்கள் பாடுவார்கள். அனைவரும் சேர்ந்தும் பாடுவதுண்டு.

பல வகையான நாட்டுப்புறப் பாடல்களின் அமைப்புகளைப் பக்திப்பாடல்கள், காப்பியப் படைப்புகள் ஆகியவற்றில் புகுத்தி இருப்பது போன்று கும்மி பாடல் அமைப்பையும் சில ஆசிரியர்கள் பின்பற்றிப் பாடியுள்ளனர். இராமலிங்க அடிகள் சண்முகக்கொம்மி, நடேசர் கொம்மி என்று பாடியுள்ளார். கோபால கிருஷ்ண பாரதி சிதம்பரக் கும்மியை இயற்றியுள்ளார். சுப்ரமணிய பாரதியாரும் கும்மி மெட்டில் அருமையான பாடல்களைப் புனைந்துள்ளார். நாமக்கல் கவிஞர் இராமலிங்கம்பிள்ளை, கவிமணி தேசிக விநாயகம்பிள்ளை ஆகியோரும் கும்மிப் பாடலமைப்பில் இனிய பாடல்களைப் படைத்துள்ளனர். பாரதிதாசன் பில்கணியத் தழுவலில் கும்மிப்பாட்டைப் பாடியிருப்பதைக் காணலாம். 1981 இல் எழுதப்பட்ட 'அன்னை' என்ற கவிதை நூலில் கும்மிப்பாடலாக அம்மனைக் குறித்து ஒரு பாடலைக் குமரிச்செழியன் என்ற கவிஞர் பாடியுள்ளார்.

பாரதியென்னும் கவித் 'தீ'

விடுதலையுகக் கவிஞர் பாரதி ஒரு புதிய உலகத்தைக் காண வேண்டுமென்ற ஆவேசத்துடன் புறப்பட்ட ஆண்மையாளர். தான் காண விரும்பிய புது உலகத்தைப் புதுமை உலகத்தைக் கவிதைச் சாசனமாக வடித்துத் தந்துவிட்டுப் போயிருக்கிறான். பாரதியின் கவிதைகள் அனைத்தும் தமிழ் மக்களுக்கு மட்டுமின்றி பாரத சமுதாயத்திற்கு மட்டுமின்றி மனித சாதி அனைத்துக்குமான எழுதி வைத்துச் சென்றிருக்கும் உரிமைச் சாசனமென்றால் அக்கூற்று மிகையல்ல. பாரதி தனக்குப்பின் ஒரு பாட்டுக்குயில் பட்டாளத்தை விதைத்துவிட்டுச் சென்றிருக்கிறார். 'பாரதியின் கவிதைகள் சமூக மாற்றத்திற்கான விதைகள்' என்ற கூற்றுக்கும் அவர் பாடிய பாடுபொருள்களையே சான்றாக்கலாம். பாரதியின் பெண்மைய பாடுபொருளே இக்கட்டுரையின் பாடுபொருளாகிறது.

"அன்ன சத்திர மாயிரம் வைத்தல்
ஆலயம் பதினாயிர நாட்டல்
.....
அன்ன யாவினும் புண்ணியங்கோடி
ஆங்கோ ரேழைக் கெழுத்தறிவித்தல்" **(பாரதி பாடற் தொகுதி)**

ஓர் ஏழைக்கு எழுத்தறிவித்தலைவிட உயர்ந்த சேவை உலகத்தில் கிடைவே கிடையாது என்ற கருத்தினை அழுத்தந்திருத்தமாகக் கூறுகின்றார் பாரதி.

தேசியக்கல்வி என்ற ஒன்று இல்லாத காரணத்தால் தான் இந்தத் தேசம் முன்னர் பெரும் வீழ்ச்சிக்கு இலக்காகியது என்பது பாரதியின் பொதுக்கருத்தாகும். தேசியக்கல்வி முழுக்கத் தாய்மொழிக் கல்வியாக இருக்க வேண்டும் என்ற பாரதியின் கனவு இன்றளவும் கனவாகவே இருக்கின்ற காரணத்தினால் தான் நமது இளைஞர்களிடம் போதிய அறிவினையும், பொறுப்புணர்ச்சியையும் காண முடியவில்லை என்று ஆதங்கப்படவும் செய்கிறார்.

பக்திபாடல்கள், தேசப்பாடல்கள், தன் வரலாறும், பிற பாடல்களும், கற்பனையும், கதையும், பொதுப்பாடல்கள் எனப் பல பொருண்மைகளில் பாப்புனைந்து பாரினைப் பாலித்திடச் செய்கிறார். சுவாமி நிவேதிதா தேவியைச் சந்தித்தப்பின் பாரதி பெண் விடுதலைக்குப் பெருங்குரலெடுத்து கூவுகின்றார்.

"ஆணும் பெண்ணும் நிகரெனக் கொள்வதால்
அறிவி லோங்கியிவ் வையந் தழைக்குமாம்
.....
திமிர்ந்த ஞான செருக்கு மிருப்பதால்
செம்மை மாதர் திறம்புவ தில்லையாம்" **(பாரதியின் புதுமைப்பெண்)**

என்று பெண்மைக்கு இலக்கணம் பகர்கிறார்.

பெண்கள் விடுதலைக் கும்மி

பெண் கல்வி கற்றால் சமூகம் மாற்றத்திற்குட்பட்டு மேன்மைகள் விளையுமென்று கருதிய பாரதி 'பெண்கள் விடுதலைக்கும்மி' என்ற தலைப்பிலான கவிதையில் 'கும்மியடி தமிழ்நாடு முழுவதுங் குலுங்கிக் கைகொட்டிக் கும்மியடி' என்ற கும்மிப்பாட்டில் பெண் விடுதலைக்கான விதையை விதைக்கிறார். மேலும் பெண் கல்வியைத் தடுக்க நினைக்கும் பழமைவாதிகளைப் 'பிசாசு' என்று விளித்துச் சாடுகிறார். மேலும்,

"ஏட்டையும் பெண்கள் தொடுவது தீமையென்
றெண்ணி யிருந்தவர் மாய்ந்து விட்டார்
வீட்டுக்குள்ளே பெண்ணைப் பூட்டி வைத்த
விந்தை மனிதர் தலைகவிந்தார்" **(பெண்கள் விடுதலைக்கும்மி, பா.2)**

என விதந்தோதுகின்றார்.

மாட்டினை அடித்து ஏர் மற்றும் கவனைப் பழக்குகின்ற வழக்கத்தினை வீட்டினிலும் காட்டிட நினைந்தோர்களை வெட்டி விட்டோமென்று நிலமதிர கும்மியடி பெண்ணே கும்மியடியென்று வேண்டுகிறார். நல்ல விலை கொண்டு நாயை விற்போர் அந்த நாயிடம் யோசனை கேட்பதுண்டோ? அதே நிலையில் நம்மைக் கொல்லத் துணிவின்றி நம்மையும் அந்நிலையிலே கூட்டி வைத்தாரிடம் சாட்டையைச் சொடுக்குகிறார்.

கற்பு நிலை

"இந்த இப்பிறவியில் இருமாதரை என் சிந்தையாலும் தொடேன்" *என்று ஏகபத்தினி விரதனான இராமனை மனத்தில் வரித்துக் கொண்ட பாரதி பெண்களுக்கு மட்டுமின்றி ஆண்களுக்கும் கற்பறம் உண்டென்பதைத் தம் பாடலில் சுட்டுகிறார். இதனை,*

"கற்பு நிலையென்று சொல்லவந் தாரிரு
கஷிக்குமஃது பொதுவில் வைப்போம்"

(பெண்கள் விடுதலைக்கும்மி, பா.5)

பெண்ணின் விருப்புரிமைக்கு முக்கியத்துவம் தருவதோடு அப்படிப்பட்ட வழக்கத்தினைத் தள்ளி மிதித்திடும் மனவலி கிடைத்ததெனக் கும்மியடிக்கிறார்.

பெண் கல்வியால் சமூக மாற்றம் நிகழுமென்பதையும் பட்டங்கள் ஆள்வதும் சட்டங்கள் செய்வதையும் பெண்களின் பெருமைக்கு அணிகலன்களாக்குகிறார். இதனை,

"பட்டங்க ளாள்வதுஞ் சட்டங்கள் செய்வதும்
பாரினிற் பெண்கள் நடத்தவந்தோம்
எட்டு மறிவினி லாணுக்கிங் கேபெண்
இளைப்பில்லை காணென்று கும்மியடி"

(பெண்கள் விடுதலைக்கும்மி, பா.6)

இப்பாடலால் உந்தப்பட்ட பாவேந்தர் கல்விபெறா நிலை பெண்மைக்கு இழுக்கென்கிறார். இதனை,

"ஆமாமாம் கல்வியிலான் ஆவி யிலாதவனே"

(பாரதிதாசனின் வீரத்தாய்)

வீரத்தாய் காவியத்தில் முன்மாதிரியான பெண்ணாகச் சித்தரிக்கிறார். பத்தொன்பதாம் நூற்றாண்டின் தொடக்கத்தில் அன்னை முத்துலெட்சுமி ரெட்டி, ஜோதிராவ் புலே, சாவித்திரி பாய் புலே போன்றோர் பாரதியோடு இயைந்து பெண்கல்விக்குக் குரல் கொடுத்ததோடு மட்டுமல்லாமல் அவர்களின் முன்னேற்றத்திற்கு ஏணிப்படியாகவும் திகழ்ந்தவர்களாகிறார்கள்.

பாரதியின் கவிதையால் ஏற்பட்ட மறுமலர்ச்சி இன்று பெண்கல்வியில் தேசிய அளவில் முக்கியத்துவம் பெற்ற மாநிலமாகத் திகழ்வதற்கும் ஆண்கள் சராசரி தேர்ச்சி விகிதத்தைவிட பெண்கள் தேர்ச்சி விகிதம் அதிகரித்திருப்பதற்கும் பாரதியின் பங்கு போற்றுதற்குரியதாகிறது. இந்திய நீதித்துறையின் உயர் அங்கீகாரமான உச்சநீதிமன்ற தலைமைப்பெண் நீதிபதியாக அடுத்து ஒரு பெண்தான் அலங்கரிக்கப்போகிறார். இந்த வரலாற்றுக்குத் தொடக்கப் புள்ளி பாரதியே எனலாம். மேலும்,

வேதங்கள் படைக்கவும் நீதிபதியாக விளங்கி, நீதியை நிலைநாட்டவும், பெண்களால் இயலுமென்பதையும் பாரதியின் கனவு தற்போது நினைவாக நிலைத்திருக்கும் சீர்மையுடையதாகும். சாதம் சமைக்கச் செய்யும் பெண்மை தெய்வச்சாதி சமைத்திடவும் செய்ய முனையும் ஆற்றலை அங்கீகரிக்கச் செய்கிறார்.

"வேதம் படைக்கவும் நீதிகள் செய்யவும்
.....
சாதம் படைக்கவுஞ் செய்திடுவோம் தெய்வச்
சாதி படைக்கவுஞ் செய்திடுவோம்"

(பெண்கள் விடுதலைக்கும்மி, பா.7)

என்று கும்மியிசைக்கிறார். மேலும்,

காதலென்பது இரு உடற்கலப்பாக இல்லாமல் உள்ளக் கலப்பாகத் திகழ்வதையே காதலென்று வரையறுக்கிறார். தம் ஆடவரின் செயல்கள் அனைத்திலும் தம்மால் இயன்ற பங்களிப்பு இருக்க வேண்டுமென விரும்பும் விருப்பத்தையும் தம் பாடலில் வெளிப்படுத்துகிறார்.

"காதலொருவனைக் கைப்பிடித்தே யவன்
காரியம் யாவினும் கைகொடுத்து" **(பெண்கள் விடுதலைக்கும்மி, பா.8)**

ஆடவர் பெண்டிர் இருவரின் விருப்புரிமையோடு செய்யப்படும் செயல் வெற்றிக்கு இலக்காகும் என்று கும்மியிசைக்கும் பாரதியின் கருத்து போற்றப்பட வேண்டியதாகிறது.

பாரதியின் பெண்கள் விடுதலைக்கும்மியால் இன்று விளைந்த நற்பலன்கள் பலவாகும். பெண்கல்வியில் இந்தியளவில் இரண்டாமிடம் பெறும் நிலையில் தமிழகம் சிறப்பிடம் பெற்றுத் திகழ்கிறது. புதுக்கோட்டை கல்வி நிலையத்தில் திரைச்சீலை போடப்பட்டு படித்து மருத்துவரான முத்துலெட்சுமியாரால் தமிழக சட்டசபையில் தேவதாசி ஒழிப்பு முறைக்கு அவர் கொடுத்த உரிமைக்குரல் பாரதியின் பெண் பின்பமாகப் பார்க்கப்பட வேண்டியதாகிறது. இன்று கல்வியில் மகளிருக்கு எனத் தனிக் கல்வி நிறுவனங்களுக்கும், பொதுக்கல்வி நிலையத்திலும் பெண்களின் கல்வி இன்றளவின் பன்மடங்கு உயர்ந்து ஓங்கி நிற்பதற்குப் பாரதியின் பெண் விடுதலை விதையானது இன்று விருச்சமாகி நற்பலனை நல்குகிறது.

முடிவுரை

பகுத்தறிவு கண் கொண்டு தமிழக பெண்ணிய விடுதலைக்கு வித்திட்ட முன்னத்தி ஏருக்குச் சொந்தக்காரர் பாரதியேயாவார். மடமைகளையும், பழமைவாதிகளின் பண்பற்ற பழக்க வழக்கங்களையும் தம் பாவால் வேரறுத்த பண்பாளராகத் திகழ்கிறார். அனைத்து சாதியினரும் அர்ச்சகராகலாம் என்ற தமிழக அரசாணையால் ஓதுவார் பணியினைப் பெற்று பெண்கள் தம்முடலுள் கருவறை வைத்திருக்கும் நிலையில் இறைவன் குடி கொள்ளும் கருவறைக்குள் நின்று ஆராதிக்கும் நிலை பாரதியால் பெற்ற பெறு வெற்றியாகவே கருத முடிகிறது. இக்கட்டுரையில் கும்மி பெயர்க்காரணம், பாரதி என்னும் கவித்தீ, பெண்கள் விடுதலைக்கும்மி, கற்புநிலை என்ற உட்தலைப்புகளில் இவ்வாய்வு நிகழ்த்தப்பட்டுள்ளது.

பயன்பட்ட நூல்கள்

1. பாரதியார் கவிதைகள், மணிவாசகம் பதிப்பகம், சிதம்பரம்.
2. பாவேந்தர் கவிதைகள், பாரதி பதிப்பகம், சென்னை.
3. டாக்டர் ஏ.என்.பெருமாள், கும்மிப்பாடல்கள், உலகத் தமிழாராய்ச்சி நிறுவனம், சென்னை.
4. வசந்தா இராமநாதன், பாரதியார் கனவுகள் கற்பனைகள், அருயோதயம், சென்னை.

27. பாரதியாரின் கவிதைகளில் மரபுக் கட்டுடைப்புச்சிந்தனைகள்

முனைவர் வெ. ஜெயலெட்சுமி
உதவிப்பேராசிரியர், தமிழ்த்துறை,
தேசியக் கல்லூரி (தன்னாட்சி,)
திருச்சிராப்பள்ளி–620 001.

தமிழ் இலக்கிய உலகில் அறிவுப்பெரும் மரபைத் தோற்றுவித்தவர்களில் குறிப்பிடத்தக்கவர் பாரதியார். ஆக்கப்பூர்வமான சமூகச் சிந்தனை மரபைப் பாரதிக்குமுன், பாரதிக்குப் பின் எனப் பகுத்துப் பார்க்கும் அளவிற்கு நற்சிந்தனை மரபினை உருவாக்கியவர் பாரதியார். காலங்காலமாக மனித இனம் தொடர்ந்து வாழ்வியலில் பயன்படுத்தும் வழக்காறுகள் நிலைத்து நிற்கும்போது அது மரபாக தொடர்ந்து மக்களால் கடைபிடிக்கப்படுகிறது. இம்மரபு இருவகையாக அமைகின்றது. ஒன்று நன்மரபு மற்றொன்று தீ மரபு ஆகும். நன்மரபுகள் மனிதகுலத்தை நல்வழியில் வழிநடத்தக்கூடியவை. தீ மரபுகள் மனிதகுலத்தைப் பிரித்துப் பாகுபடுத்திச் சமூகத்தைப் பாழ்படுத்துபவை.

காலந்தோறும் இவ்விருமரபுகளும் சேர்ந்தே வளர்ந்து வந்திருக்கின்றன. இம்மரபுகளில் தீய மரபுகளைக் காலந்தோறும் தோன்றிய அறிவாளிகள் விமர்சனம் செய்ததோடு அதனை மாற்றியும் புதிய மரபுகளையும் வளர்த்தெடுத்து வந்துள்ளனர். அவ்வடிப்படையில் பல தீய மரபுகளை பாரதி தன் கவிதைகளின் வழியாகக் கட்டுடைத்துள்ளார். அப்படிக் கட்டுடைத்த மரபுகளை ஆய்ந்து விளக்குவதே இக்கட்டுரையின் நோக்கமாகும்.

கல்வி மரபு

இந்திய மரபில் கல்வி குறித்த மரபுகளும் கல்வி பயில்வோர் குறித்த மரபுகளும் காலங்காலமாக இருந்து வரும் மரபாகும். தொல்காப்பியர், கல்வி குறித்த மரபை முன்வைக்கும்போது

"ஓதலும் தூதும் உயர்ந்தோர் மேன" **(தொல்.அகத்திணையியல் நூற்-28)**

என்ற நூற்பாவின்வழி எடுத்துரைக்கிறார். இந்நூற்பாவிற்கு உரை கூறியுள்ள உரையாசிரியர்களில் பலர் ஓதல் என்பதைக் கல்வியாகக் கொண்டு, நால் வருணப் பாகுபாட்டின் அடிப்படையில் "அந்தணர்களுக்கு உரியது" என்றுரைத்துள்ளனர். கல்வியுரிமை என்பது அனைத்துச் சமூகத்தினர்க்கும் உரியதாக இருந்ததில்லை என்பதை வரலாறு நெடுகிலும் காணமுடிகிறது.

கல்வி என்பது பெரும்பாலும் குருகுலக் கல்வியாகவே இருந்தது. "குருகுலம் என்பதனை ஒரு குலத்தைச் சார்ந்தவர்கள் குறிப்பாகப் பிராமணர்கள் குருவிடம் சென்று தங்கிப் பயிலும் அமைப்பு எனலாம்" என்ற (கே.பழனிவேலு, கல்வி வரலாறு சில பார்வைகள் ப.4) கே. பழனிவேலுவின் கருத்து இங்கே சுட்டத்தக்கது. ஒரு வகையான உயர் வகுப்பினர்களுக்கே கல்வி கிடைத்துள்ளது. ஆங்கிலேய மிஷினரிகளின் வருகைக்குப் பிறகே ஆதிக்க வகுப்பினர் தவிர்த்த பலருக்கும் உரியாதாகக் கல்வி பரவியது. இதிலும் வசதிபடைத்தவர்களுக்கு உரியதாகவே கல்வி இருந்தது. இதைச் சமூகமும் ஏற்றுக்கொண்டு பின்பற்றியுள்ளது. பாரதி இத்தகைய மரபினைக் கடுமையாகச் சாடினார்.

"வயிற்றுக்குச் சோறிட வேண்டும்-இங்கு
வாழும் மனிதருக்கெல்லாம்
பயிற்றிப் பலகல்வி தந்து-இந்தப்
பாரை உயர்த்திட வேண்டும்" **(பாரதியார் கவிதைகள்-ப.439)**

கல்வி என்பது உயர் சாதியினருக்கும், பணம் படைத்தவர்களுக்கும் உரியதாக இருந்த மரபைக் கண்டு மனம் வெதும்பி அதைக் கட்டுடைக்கிறார் பாரதி.

தொன்மைக்காலம் முதற்கொண்டு பாரதியின் காலம் வரை பெண்களுக்கு கல்வி என்பது மறுக்கப்பட்டே வந்திருக்கிறது. பெண்கள் என்பவர்கள் வீட்டிற்கு மட்டுமே உரியவர்கள், அவர்களுக்குக் கல்வியும் அதனால் கிடைக்கும் அறிவும் தேவையற்றது எனும் மரபை தீய மரபினைக் கடைபிடிப்பவர்கள் கெட்டியாகப் பிடித்து வைத்திருந்தனர்.

"ஏட்டையும் பெண்கள் தொடுவது தீமையென்று
எண்ணியிருந்தவர் மாய்ந்துவிட்டார்.
வீட்டுக்குள்ளே பெண்ணைப் பூட்டிவைப்பேமென்ற
விந்தை மனிதர் தலைகவிழ்ந்தார்" **(பாரதியார் கவிதைகள் - ப. 448)**

"பட்டங்கள் ஆள்வதும் சட்டங்கள் செய்வதும்
பாரினில் பெண்கள் நடத்த வந்தோம்
எட்டும் அறிவினில் ஆணுக்கிங்கே பெண்
இளைப்பில்லை காணென்று கும்மியடி"

(பாரதியார் கவிதைகள் - ப.449)

பாரதி மேற்கண்ட கவிதையின்வழி காலங்காலமாக மறுக்கப்பட்ட பெண்கல்விமரபை உடைத்துப் பெண்கள் கல்வி பயில்வது கட்டாயம் எனும் புதிய மரபை கட்டமைக்கிறார்.

அடிமைத்தனம்

பண்டைக் காலந்தொட்டு இன்றுவரை சமூகத்தைப் பீடித்துள்ள மிகக்கொடுமையான நோய் அடிமைத்தன நோயாகும். மக்களுள் ஒரு சாரார் ஆதிக்கம் செலுத்துபவராகவும் மற்றொரு சாரார் அடிமைத்தனத்தில் மூழ்கிக்கிடப்பவராகவும் இருக்கின்றனர். ஆண்டான் அடிமை முறை தொன்மைக் காலத்திலிருந்து இன்றுவரை இருந்துகொண்டுதான் இருக்கிறது. பாரதியார் வாழ்ந்த காலத்தில் சாதிய அடிமைமுறை இருந்திருக்கிறது. ஆங்கிலேயே அடிமைமுறை இருந்திருக்கிறது. இதைச் சரியென்றும் மக்கள் அதனை ஒரு மரபாகவும் கடைபிடித்து வந்துள்ளனர். பாரதி இம்மரபைத் தன் கவிதைகளின் வழி உடைத்தெறிகின்றார்.

"பார்ப்பாரை அடிமை என்ற காலமும் போச்சே-வள்ளைப்
பரங்கியைத் துரை என்ற காலமும் போச்சேடி- பிச்சை
ஏற்பாரைப் பணிகின்ற காலமும் போச்சே-நம்மை
ஏய்ப்போருக்கு ஏவல்செய்கின்ற காலமும் போச்சே"

(பாரதியார் கவிதைகள் - ப.128)

நாடும் நாட்டுமக்களும் சுதந்திரத்திற்காகப் போராடிக் கொண்டிருந்த சூழலில் பாரதி இத்தகைய கவிதைகளைப் படைத்தளித்துள்ளார். நாடு சுதந்திரம் பெற்றபிறகு சமநிலையான சமுதாயமே இருக்கவேண்டும். பழைய பாழ்பட்ட மரபுகள் எல்லாம் உடைக்கப்படவேண்டும் என்பதில் பாரதி தின்னமாக இருந்தார்.

அடிமைத்தனத்தில் குறிப்பிடத்தக்கது பெண்ணடிமைத்தனமாகும். ஆண்களுக்குப் பெண்கள் அடங்கி ஒடுங்கி இருக்கவேண்டும். ஆணுக்கான சுதந்திரம் உலகளாவியது பெண்ணுக்கான சுதந்திரம் என்பது இல்லத்துக்குள்ளே அதிலும் ஒரு மூலைக்குள்ளேயே இருக்கவேண்டும் என்பது உலக

ஆணாதிக்கப் பொதுப்புத்தியாகும். "பெண் உரிமை வாய்ந்தவளாகவும் மதிப்பு வாய்ந்தவளாகவும் கருதப்பட்டு வந்த காலம்மாறி, உபரி உற்பத்திப் பெருக்க காலத்தில், ஆண் சேமித்து வரும் சொத்துகளுக்கு வாரிசு உருவாக்கித்தரும் பணியை பெண் ஏற்கும் நிலை உருவாக்கப்பட்டது. உற்பத்தி சக்திகள் பெருகிய காலத்தில், சமுதாய நிறுவனமாகத் தனிச்சொத்து உருவாகியது என மார்க்சியலார் கருதுகின்றனர். இதையடுத்து, அத்தனிச்சொத்தைப் பெருக்கி, சந்ததியினரிடம் ஒப்படைக்கும் சமூக நிறவனங்களாக ஒருதாரமணமும், அதன் அடிப்படையில் குடும்பமுறையும் உருவாகின. இதனால் தாய்வழிச் சமூகத்தின் எச்சசொச்சங்களும் துடைத்தெறியப்பட்டுவிட்டன. அதன்பிறகு பெண்களின் கடமைகள், ஆண்களின் ஆசைகளை நிறைவேற்றுவதும், குழந்தைகளைப் பெற்று வளர்ப்பதும் என்பது சமூக நியதியாகவிட்டது" (அரங்க மல்லிகா, தமிழ் இலக்கியமும் பெண்ணியமும், ப. 100)) என்ற அரங்கமல்லிகாவின் கருத்தும் இங்கே சுட்டத்தக்கது. பாரதிக்கு முன்பும் பாரதியார் காலத்திலும் பெண்கள் அடிமையாகவே பாவிக்கப்பட்டனர். இதனை பாரதியார் கண்டிக்கிறார்,

"பெண்ணுக்கு ஞானத்தை வைத்தான்
புவி பேணி வளர்த்திடும் ஈசன்
மண்ணுக்குள்ளே சிலமூடர்-நல்ல
மாதரறிவைக் கெடுத்தார்" **(பாரதியார் கவிதைகள். ப. 434)**

"கண்கள் இரண்டினில் ஒன்றைக்-குத்திக்
காட்சி கொடுத்திடலாமோ?
பெண்கள் அறிவை வளர்த்தால்-வையம்
பேதமை பெற்றிடுங் காணீர்" **(பாரதியார் கவிதைகள் ப. 434)**

இதுபோன்ற கவிதைகளின் வழியும் கட்டுரைகள் வழியாகவும் பெண் அடிமைத்தனமரபு துடைத்தெறியப்பட வேண்டும் என்கிறார்.

சமய ஓர்மை

தமிழ்மரபுலகில் பலசமயங்கள் உண்டு. சங்கஇலக்கிய காலத்தில் சமயக் காழ்ப்புணர்ச்சி கிடையாது. மக்கள் ஒற்றுமையுடன்தான் வாழ்ந்துள்ளனர். அதன்பிறகான காலங்களில் மக்கள் சமூகம் பெரும் சமயக்காழ்ப்புணர்ச்சிக்கு ஆட்பட்டது. காப்பியகாலத்தில் சமணம், பௌத்தம் இடையே பெரும் போட்டி நிலவியது. பக்தி இலக்கியக் காலகட்டத்தில் சமணம், பௌத்தம், சைவம், வைணவம் இடையே போட்டியும்

பூசல்களும் நிலவி வந்தன. புத்திலக்கிய காலம் வரை இம்மாதிரியான சூழலே தொடர்ந்தது எனலாம். இசுலாமியர், ஆங்கிலேயர் வருகைக்குப் பிறகு கிறித்துவம், இசுலாம், இந்து சமயங்களுக்கிடையே பூசல் நிலவி வந்தது வருகிறது. ஒவ்வொரு சமயத்தாரும் தாங்கள் சார்ந்திருந்த சமயத்தவரே, சமயக்கடவுளே உயர்ந்தவர் என்று பரப்பினர். பரப்பியதோடு மட்டுமின்றி பூசலும் இட்டுக்கொண்டனர். இதைக்கண்ட பாரதி மனம்வெதும்பி இச்சமய மரபு குறித்தும் புத்துரை வகுத்தார். இருபதாம் நூற்றாண்டில் இருந்த சமயச்சூழலை தி.சு நடராசன் பின்வருமாறு சுட்டுகிறார். "வைதீக இந்து சமயத்திற்குத் தனியானதொரு பிரச்சனை. பவுத்தத்தையும் சமணத்தையும் வீழ்த்திவிட்டு "தனிக்காட்டு ராஜாவாக"-சுயமுரண்பாடுகளுடன்-வாழ்ந்துகொண்டிருந்த சமயத்திற்கு, சர்வதேச அளவில் வலுவாக உள்ள கிறித்துவத்தையும் இசுலாத்தையும் எதிர்கொள்ளவேண்டிய நிலை ஏற்பட்டது. (தி.சு.நடராசன், தமிழின் பண்பாட்டு வெளிகள் ப.109) இப்படிப்பட்ட சண்டையிடும் சமயத்தவர்களைப் பார்த்து,

"தெய்வம் பலப்பலசொல்லி-பகைத்
தீயை வளர்ப்பவர் மூடர்
உய்வதனைத்திலும் ஒன்றாய்-எங்கும்
ஓர் பொருளாவது தெய்வம்" **(பாரதியார் கவிதைகள் ப. 434)**

என்ற கவிதையின்வழி சமயப்பூசலைச் செய்பவர்கள் மூடர் என்கிறார் பாரதியார். தெய்வம் என்பது ஓர்மைத்தன்மையுடையது என்கிறார். மேலும்,

"எல்லாரும் ஓர் குலம் எல்லாரும் ஓர் இனம்
எல்லாரும் இந்திய மக்கள்
எல்லாரும் ஓர் நிறை எல்லாரும் ஓர்விலை
எல்லாரும் இந்நாட்டு மன்னர்-நாம்
எல்லாரும் இந்நாட்டு மன்னர் ஆம்
எல்லாரும் இந்நாட்டு மன்னர் - வாழ்க" **(பாரதியார் கவிதைகள் ப. 95.)**

என்று புதிய ஆக்கமரபைப் படைக்கிறார். பாரதியார் வேறுபாடு நிரம்பிய மரபை மாற்றவேண்டும், புதிய ஓர்மை நெறியான மரபை உருவாக்க வேண்டும் என முழக்கமிட்டார்.

முடிவுரை

மனிதகுலம் காலங்காலமாக பயன்படுத்தும் வழக்காறுகள் தொடர்ந்து மக்கள்பயன்படுத்தும்போது அவை மரபுகளாக

ஆக்கம் பெறுகின்றன. இம்மமரபுகளில் மனித குலத்திற்கு ஆக்கம் செய்பவை நன்மரபுகளாகவும் தீமை செய்பவை தீய மரபுகளாகவும் கருதப்படும்.

தமிழ்ச்சமூகமும், உலக சமூகமும் காலங்காலமாக பின்பற்றி வந்த தீய மரபுகளைக் கட்டுடைத்து பாரதியார் புதிய ஆக்க நன்னெறி மரபுகளைக் கட்டமைத்தார் .

காலங்காலமாக ஆதிக்க இனத்திற்கும் ஆண்களுக்கும் உரியதாக இருந்த கல்விமரபை உடைத்து அனைவருக்கும் உரியதான மரபாக இருக்கவேண்டும் எனக் கட்டமைத்தார் பாரதியார்.

காலந்தோறும் நிலவிய அடிமைத்தனமரபு, சாதியமரபு, சமயக்காழ்ப்பு மரபு ஆகியவற்றை உடைத்து பாரதியார், புதிய அடிமைத்தனமற்ற, சாதியமற்ற, சமயக் காழ்ப்பற்ற ஓர்மை மரபு உருவாக வேண்டும் என கவிதைகளின் வழியும் வாழ்வியல் வழியும் வலியுறுத்தி வந்தார் என்பதை அறிய இயலுகிறது.

துணை நூல்கள்.

1. உஷாராணி. சு.(உ.ஆ), பாரதியார் கவிதைகள், வர்த்தமானன் பதிப்பகம், சென்னை, மூன்றாம் பதிப்பு , ஜீன் 2019.
2. அரங்க மல்லிகா, தமிழ் இலக்கியமும் பெண்ணியமும், நியுசெஞ்சுரி புக் ஹவுஸ் (பி.லிட்), சென்னை. நவம்பர்–2002.
3. இளம்பூணர் (உ.ஆ), தொல்காப்பியம், பொருளதிகாரம், கழக வெளியீடு, சென்னை.
4. நடராசன் தி.சு, தமிழின் பண்பாட்டு வெளிகள், நியு செஞ்சுரி புக் ஹவுஸ் (பி.லிட்), சென்னனை.2006.
5. பழனிவேலு.க, கல்வி வரலாறு சில பார்வைகள், பனி வெளியீட்டகம், புதுச்சேரி, மே–2001.

28. பாரதியும் புதுமையும்

ப. நவசக்திவேல்,
தேசியக் கல்லூரி (பகுதிநேரம்)
முனைவர் பட்ட ஆய்வாளர்,
தமிழாய்வுத் துறை, திருச்சிராப்பள்ளி.

முன்னுரை

பத்தொன்பதாம் நூற்றாண்டில் இந்தியாவில் வாழ்ந்தகவிஞர்களுள் மிகவும் குறிப்பிடத்தக்கவர் மகாகவி சுப்ரமணிய பாரதியார் ஆவார். தமிழ்க் கவிதையில் புதுமையையும், எளிமையையும் புகுத்தியவர். இந்திய விடுதலைக்காகப் பலகவிதைகளை எழுதியுள்ளார். அதுமட்டுமல்ல சமூகத்தில் காணப்படும் ஏற்றத் தாழ்வுகளைப் போக்க முற்போக்குச் சிந்தனைகளைத் தன் கவிதையின் பாடுப்பொருளாகப் பயன்படுத்தியவர். பாரதியின் கவிதைகள் தொடர்ந்துதமிழ்ச் சமூகத்தால் அவதானிப்பு செய்யப்படுகின்றது. இக்கட்டுரையானது பன்முக நோக்கிலே அவருடைய கவிதைகளை ஆராய்கின்றது.

பாரதியும் தமிழ்க் கவிதையும்

தமிழ் இலக்கியக் கவிதை வரலாறு பாரதிக்கு முன், பாரதிக்கு பின் எனபகுக்கும் அளவிற்கு தமிழ்க் கவிதையின் மையமாகயிருப்பவர் பாரதியார். பாரதியின் கல்வி, குடும்பச் சூழல், நாட்டின் சூழல் போன்றகாரணிகள் அவரை பெருங்கவிஞராக உருவாக்கியது. "தமிழ்நாட்டில் நிலவுடைமைச் சமூகமான நிலப்பிரபுத்துவ அமைப்பிற்கு மாற்றாக முதலாளித்துவ அமைப்பு உருவான போது சமூகத்தில் நடுத்தரவர்க்கம் தோன்றியது. அத்தகைய சூழலில் நடுத்தரமக்கள் நிலப்பிரபுத்துவத் தன்மையைப் பெற்றனர்.

இவர்களின் அடிமனஅல்லது இலட்சிய வெளிப்பாடுகள் தமிழில் புனைவியம் *(Romaticism)* அல்லது வீறுணர்ச்சிக் கவிதை

இயக்கத்தின் தோற்றுவாயாக அமைந்தது". (ஷெல்லியும் பாரதியும் – ஒருபுதியபார்வை ப.162) பாரதியார் தமிழிலக்கியத்தில் புனைவிய இயக்கத்தைத் தோற்றுவித்த கவிஞராவார். இத்தகைய இயக்கமானதுஒரு அக இயக்கமாகவும் விளங்கியது. பாரதியின் சுதந்திர மனோபாவம், விடுதலை வேட்கை, மேல்நாட்டுக் கல்வி, இலக்கியப் பயிற்சி போன்றவை அவரை அக இயக்கத்தின் கர்த்தாவாக நிலைநிறுத்தியது.

வீறுணர்ச்சிக் கவிதைகள் இயற்கை, கற்பனை, கனவு, தனிமனித சுதந்திரம் போன்ற கூறுகளுக்கு முக்கியத்துவம் அளிக்கின்றன. பாரதியின் கவிதைகளிலும் இத்தகைய தன்மை காணப்படுகின்றது.

புத்தொளிக்காலம் ஐரோப்பாவில் ஏற்பட்ட பிறகு சுதந்திரம், சமத்துவம், சகோதரத்துவம் என்ற கருத்துக்கள் உலகமெங்கும் பெரும் தாக்கத்தினை ஏற்படுத்தின. தமிழ்ச் சூழலில் மன்னர்களின் பொழுது போக்காக இருந்த கவிதையானது மாற்றமடைந்து மக்களுக்கான கவிதையாக உருப்பெற்றது. இதனை தமிழ்க் கவிதைப் பரப்பில் செயல்படுத்தியவர் பாரதியார்.

"சுவைபுதிது., பொருள் புதிது., வளம் புதிது
சொற் புதிதுசோதிமிக்க நவ கவிதை" **(பாரதியார் கவிதைகள் ப.275)**

"உள்ளத்திலே உண்மையொளி யுண்டாயின்
வாக்கினிலே ஒளியுண்டாம்" **(பாரதியார் கவிதைகள் ப.45)**

என்பது வீறுணுர்ச்சி கவிதையின் வெளிப்பாடு எனலாம். பாரதியின் கருத்தியலானது முற்போக்காகவும் சில இங்களில் பழமையை மீட்டெடுப்பதாகவும் (பாஞ்சாலிசபதம்) காணப்படுகின்றன.

பாரதியும் இயற்கை மையவாதமும் :

வீறுணர்ச்சிக் காலக்கவிஞர்கள் இயற்கையை மையப்படுத்திக் கவிதைகளை எழுதினர். இயற்கை என்பது அவர்களின் கற்பனையை அதிகரிக்கும் மூலமாகக் காணப்படுகின்றது. எனவே இவ்வகையிலான கவிதைப் படைப்புகள் இயல்பாகவே உருவாகின்றன. பாரதி இயற்கையைத் தனதுகவிதையின் ஊற்றுக் கண்ணாகவே கொண்டிருந்தார். இயற்கையை இரசிப்பது, சிலாகிப்பது, மூழ்கிப்போவது, தன்னிலை மறப்பது போன்ற தன்மைகளில் தனது இயற்கை மையவாதக் கவிதைகளைப் படைத்துள்ளார்.

இயற்கையை இரசிப்பது போன்ற கவிதை

"காலைப் பொழுதினிலே கண்விழித்து மேனிலைமேல்"
மேலைச் சுடர்வானை நோக்கிநின்றோம் விண்ணகத்தே
கீழ்த்திசையில் ஞாயிறுதன் கேடில் சுடர்விடுத்தான்
பார்த்தவெளியெல்லாம் பகலொளியாய் மின்னிற்றே"

(பாரதியார் கவிதைகள் ப.202)

"தீக்குள் விரலைவைத்தால் நந்தலாலா
நின்னைத் தீண்டும் இன்பம் தோன்றுதை யேநந்தலாலா"

(பாரதியார் கவிதைகள் ப.140)

என்ற கவிதை வரிகள் சிலாகிப்பது அதாவது இயற்கையை உய்த்து உணர்வதாகும். இக்கவிதையை வடிவியல் சிந்தனையாளர் உணர்ச்சிப் படிமம் என்று அழைக்கின்றனர்.

பாரதிகவிதைகளில் மனிதமையவாதம்

தனிமனித சுதந்திரம் என்ற சிந்தனை, விடுதலையுணர்வும் பாரதியிடம் மனித மையவாதக் கவிதைகள் தோன்றக் காரணமாக அமைந்தன. "Humahikm" என்ற சொல் தமிழில் மனிதநேயம் என வழங்கப்படுகின்றது. இதன் பொருள் மனிதன் மீது அன்பு செலுத்துவதாகும். ஆனால் மனிதமையவாதம் என்பது இவ்வுலகில் உள்ள எல்லாவற்றையும் விடமனிதன் மேலானவன் என்ற கருத்தியலாகும். பாரதியின் கவிதைகள் மனித மையவாதத்தை வலியுறுத்துகின்றன.

"இனியொரு விதி செய்வோம்
அதை எந்தநாளும் காப்போம்"

"தனிமனிதனுக்கு உணவில்லை எனில்
ஜகத்தினை அழித்திடுவோம்" **(பாரதியார் கவிதைகள் ப.40)**

என்ற கவிதைகள் மூலம் அவரின் மனித மையவாதம் வெளிப்படுகின்றது.

சமய ஏற்பும் – மறுப்பும்

பாரதியார் பராசக்தி, கண்ணன், காளி, விநாயகர் போன்ற இந்துசமயக் கடவுள்களைப் புகழ்ந்து பாடியுள்ளார். அதுமட்டுமல்லாது பிறசமயக் கடவுள்களான இயேசுகிறிஸ்து, அல்லா போன்ற கடவுள்களைப் பாடியுள்ளார். பாரதியார்

பெருந்தெய்வங்களைப் பாடியுள்ளர். ஆனால் சிறுதெய்வ வழிபாட்டில் அவருக்கு உடன்பாடில்லை. இதற்கு விதிவிலக்காக முத்துமாரியம்மனை மட்டும் பாடியுள்ளார்.

''இயேசு கிறிஸ்து''
ஈசன் வந்து சிலுவையில் மாண்டான்
எழுந்துயிர்த்தனன் நாள் ஒரு மூன்றில்...

(பாரதியார் கவிதைகள் ப.169)

அல்லாவைப் பற்றி

''பல்லாயிரம் பல்லாயிரம் கோடியண்டங்கள்
எல்லாத் திசையிலு மோரெல்லையில்லாவெளிவானிலே
நில்லாது சுழன்றோடநியமஞ் செய்தருள் நாயகன்....''

(பாரதியார் கவிதைகள் ப.170)

சிறு தெய்வங்கள்

''மாடனை காடனை வேடனைப் போற்றி
மயங்கும் மதியிலிகாள் .....'' **(பாரதியார் கவிதைகள் ப.175)**

''மெல்லப் பலதெய்வம் கூட்டிவளர்த்து
வெறுங் கதைகள் சேர்த்துப் - பல
கள்ளமதங்கள் பிறப்பதற் கோர் மறை
காட்டவும் வல்லீரே'' **(பாரதியார் கவிதைகள் ப.176)**

என பாரதியின் சமய ஏற்பு மற்றும் மறுப்பினை காணமுடிகின்றது.

பாரதியும் தமிழ்த்தேசியமும்

பாரதியார் அகண்ட பாரதத்தைத் தன் கவிதைகளில் சிறப்பித்துப் பாடியுள்ளார். எனினும் பாரதிமையப்படுத்துவது தமிழ்த் தேசியமாகும். "தமிழ்நாடு" என தமிழ் கூறு நல்லுலகை அழைப்பது உண்டு. இந்தக் கருத்தியலானது சிலப்பதிகாரம், தொல்காப்பியம் போன்றபழமையான இலக்கியங்களிலிருந்து தொன்று தொட்டுக் காணப்படுகின்றது. அதனைத் தனித்துவமாக தனது கவிதைகளில் கையாண்டவர் பாரதி, அதேபோல் மாநிலசுயாட்சி என்ற முழக்கங்கள் தற்பொழுது எழுகின்றன. அதனை முன்பே கவிதையாக எழுதியவர் பாரதியார்.

''வந்தேமாதரம் என்போம்
எங்கள் மாநிலத்தாயை வணங்குதும் என்போம்''

(பாரதியார் கவிதைகள் ப.01)

பாரதமணித் திருநாடு என்று இந்திய தேசியத்தைப் பாடும் பாரதியார் செந்தமிழ்நாடு, தமிழ்த்தாய், தமிழ், தமிழ்மொழி வாழ்த்து, தமிழ்ச்சாதி, வாழிய செந்தழிழ் என்ற தலைப்புகளில் அவர் கட்டமைப்பது தமிழ்த் தேசியமாகும். ஏனெனில் பாரதியிடம் திராவிடக் கருத்தியல் தொடர்பான சிந்தனைசில இடங்களில் மட்டுமே காணமுடிகின்றது. (இந்தியாவில் மொழிவழி மாநிலங்கள் பிரிக்கும் முன்பே செந்தமிழ்நாடு என்று ஒரு கருத்தியலை உருவாக்குகின்றார். அண்டை மாநிலமான கேரளாவை சேரநாடு என்று தான் அழைக்கின்றார்).

பாரதி கவிதைகளில் படிமம் குறியீடு

பொதுவாகதமிழ் இலக்கியத்தில் உவமை, உருவம் என்றகவிதைக் கருவிகளின் பயன்பாடு அதிகமாக உள்ளது. ஆனால் இதனை விடபடிமம், குறியீடு போன்ற கருவிகளைச் சிறப்பானதாகக் கருதமுடியும். ஏனெனில் புதிய அனுபவத்தைப் படைக்கும் கவிஞன் பயன்படுத்தும் கருவியாக படிமம், குறியீடு காணப்படுகின்றது.

படிமம் என்றசொல் தமிழ் இலக்கியத்தில் காணப்படுகின்றது. இருந்தபோதும் 'Image' என்ற ஆங்கில சொல்லின் தமிழாக்கமாக இதனை இக்காலத் திறனாய்வாளர்கள் பயன்படுத்துகின்றனர். இதன் பொருளாவது காட்சிப்படுத்தலாகும். 'காட்சிப்படுத்துதல்' என்பது கவிதையின் மிகமுக்கியமான கூறாகும். "எஸ்ரா பவுண்ட் என்ற அறிஞர் வாழ்நாளில் ஏதாவது ஒரே ஒரு புதிய படிமத்தைப் படைப்பதே படைப்பாளிகளின் வெற்றிஎனக் கூறுகின்றார்." (தமிழ் இலக்கியவரலாறு ப.276)

படிமம்

> **"தீக்குள் விரலைவைத்தால் நந்தலாலா - நின்னைத்**
> **தீண்டுமின்பம் தோன்றுதையேநந்தலாலா"**

(பாரதியார் கவிதைகள் ப.140)

என்றவரிகள் படிமமாகவும், மனிதப்படுத்தலாகவும் அமைகின்றன.

'குறியீடு' என்பது சொல்லப்பட்ட சொல்லுக்குத் தொடர்பில்லாத வேறு பொருள்களைச் சுட்டி அதிலிருந்து இன்னொரு பொருளை உருவாக்கித் தருவதாகும். ஏனெனில் குறியீடு என்பது பன்முகப் பொருளாற்றல் உடையதுஎனலாம்.

பாரதியாரின் "அக்கினிக் குஞ்சு" என்ற கவிதை குறியீட்டுக் கவிதையாகும். இதில் 'அக்னிக்குஞ்சு' என்ற சொல்லாட்சி

குறியீடாக அமைந்து புரட்சி, வேதாந்தக்கனல் போன்ற பல பொருளைத் தரவல்லது.

முடிவுரை :

பாரதியின் கவிதைகள் அவருடைய பன்முகத்திறனின் வெளிப்பாடு எனலாம். புதிதாகக் காணப்படும் கோட்பாடுகளின் அடிப்படையில் இவரின் கவிதைகள் மறுவாசிப்பு செய்யும் பொழுது இதனுடைய வீச்சு மேலும் அதிகரிக்கும் என்பதை இக்கட்டுரை வெளிப்படுத்தியுள்ளது.

துணைநூற் பட்டியல்

1. ஷெல்லியும் பாரதியும் – ஒரு புதிய பார்வை ஜான்.சாமுவேல் ஜி. – இரண்டாம் பதிப்பு 2000 ஆசியவியல் நிறுவனம் சென்னை – 600 119
2. தமிழ் இலக்கிய வரலாறு சு.ஆனந்தன் – 15ஆம் பதிப்பு 2017 – கண்மணி பதிப்பகம், திருச்சி – 620 002.
3. பாரதியார் கவிதைகள் – சுப்பிரமணிய பாரதி சி. மகாகவி – முதற்பதிப்பு 2009 சுவாமி மலை பதிப்பகம் சென்னை – 600 017.

29. பாரதி போற்றிய தேசியத் தலைவர்கள்

வெ.சங்கரநாராயணன்,
முனைவர் பட்ட ஆய்வாளர் (பகுதி நேரம்), தமிழாய்வுத்துறை,
தேசியக்கல்லூரி (தன்னாட்சி), திருச்சிராப்பள்ளி–01

முன்னுரை

இருபதாம் நூற்றாண்டின் தலை சிறந்த இந்தியக் கவிஞர்களில் மகாகவி சுப்பிரமணிய பாரதியாரும் ஒருவர். தமிழ்நாட்டில் பிறந்து தாய்நாட்டிற்கும் தமிழ்மொழிக்கும் பெருமை சேர்த்தவர். எட்டயபுரத்தில் டிசம்பர் 11, 1882-இல் பிறந்து, செப்டம்பர் 11, 1921-இல் மறைந்தார். 'பாட்டுத் திறத்தாலே இவ்வையத்தைப் பாலித்திட விரும்பியவர்'. 'பாட்டுக்கொரு புலவன் பாரதி' என்று புகழ்ந்துரைக்கப்பட்டவர். இவர் குறித்துப் புரட்சிக்கவிஞர் பாரதிதாசன் கூறும்,

''தமிழகம் தமிழுக்கு தகும் உயர்வளிக்கும்
தலைவனை எண்ணித் தவங்கிடக் கையில்
இலகு பாரதிப் புலவன் தோன்றினான்
பைந்தமிழ்த் தேர்ப்பாகன், அவனொரு
செந்தமிழ்த் தேனீ, சிந்துக்குத் தந்தை,
குவிக்கும் கவிதைக் குயில், இந்நாட்டினைக்
கவிக்கும் பகையைக் கவிழ்க்கும் கவிமுரசு
நீடுதுயில் நீக்கப் பாடி வந்தநிலா
காடு கமழும் கற்பூரச் சொற்கோ
கற்பனை ஊற்றாம் கதையின் புதையல்
திறம்பாட வந்த மறவன் புதிய
அறம்பாட வந்த அறிஞன் நாட்டிற்
படரும் சாதிப் படைக்கு மருந்து!
மண்டும் மதங்கள் அண்டா நெருப்பவன்
அயலார் எதிர்ப்பு அணையா விளக்கவன்''[1]

என்ற கவிதை வரிகள் உண்மை வெறும் புகழ்ச்சியில்லை. பாரதியின் சிந்தனை, செயல், எண்ணம், எழுத்து முதலியவை

எல்லாம் சாதி, மதம், மொழி, இனம் கடந்து, தேசிய நீரோட்டத்தில் கலந்திருந்தது. இவரது கவிதைகளுக்குக் காலம்தான் 'கருப்பொருள்' வழங்கியது. தாய்நாட்டின் பெருமைகளைத் தன் பாடல்களின் மூலம் வெளிப்படுத்திய பாரதி, தேசியத் தலைவர்கள் பலரையும் போற்றிப் பாடியுள்ளார். அதனை, இக்கட்டுரையில் காணலாம்.

தேசியத் தலைவர்கள்

பழம்பெரும் நாடான பாரததேசம் முகலாயர், ஆங்கிலேயர் முதலிய அன்னிய ஆளுகையில் அவதிப்பட நேர்ந்தபோது,

> "**ஆயிர முண்டிங்கு ஜாதி-எனில்**
> **அன்னியர் வந்து புகலென்ன நீதி**"[2]

என்று அவர்களை எதிர்த்து நாடெங்கும் பல தலைவர்கள் மக்களைத் திரட்டி குரல் கொடுத்தனர். அறவழியிலும் பிறவழியிலும் இந்திய விடுதலைக்காகத் தொடர்ந்து போராடி வெற்றி பெற்றனர். பாரதியும் விடுதலைப்போரில் தம்மை ஈடுபடுத்திக் கொண்டார். நாட்டு மக்களிடம் விடுதலை உணர்ச்சியை ஊட்டும் பாடல்கள் பல புனைந்தார். அவர் மனதைக் கவர்ந்த தலைவர்கள் பலர் குறித்தும் தம் பாடல்களில் போற்றிப் புகழ்ந்துரைத்தார். மகாத்மா காந்தி, தாதாபாய் நவுரோஜி, பாலகங்காதர திலகர், லாலா லஜபதிராய், வ.உ.சிதம்பரனார் போன்றோர் பாரதி போற்றிய தலைவர்களில் குறிப்பிடத்தக்கவர்கள் ஆவர்.

1.மகாத்மா காந்தி

மகாத்மா காந்தி குஜராத் மாநிலத்தின் 'போர்பந்தர்' என்ற ஊரில் அக்டோபர் 2, 1869-ஆம் ஆண்டு பிறந்து, இந்திய விடுதலைக்காகத் தம் வாழ்நாளை அர்ப்பணித்து,'தேசபிதா'வாக வாழ்ந்து ஜனவரி 30, 1948-இல் மறைந்தார். பல்வேறு சாதி, மத, இன வேறுபாடு கொண்ட இந்தியர்கள் அனைவரையும் ஒருங்கிணைத்துத் தலைமையேற்று நடத்திய '**சத்தியாக்கிரகம்**' என்றழைக்கப்பட்ட இவரது அறவழிப் போராட்டமே இந்திய நாட்டு விடுதலைக்கு வித்திட்டது. இந்தியர் அனைவரும் மகாத்மா காந்தியின் பின்னால் அணிவகுத்து நின்றதும், அறவழிப் போராட்டமும் அவரை உலகத் தலைவர்களில் ஒருவராக்கியது. பாரதியார், காந்தியடிகளைத் தலைவராக மட்டும் அல்லாமல் தெய்வப் பிறவியாகவே எண்ணியுள்ளார். இதனை '**மகாத்மா காந்தி பஞ்சகம்**'என்ற தலைப்பிலமைந்த,

''வாழ்க நீ! எம்மான் இந்த
வையத்து நாட்டி லெல்லாம்
தாழ்வுற்று வறுமை மிஞ்சி
விடுதலை தவறிக் கெட்டுப்
பாழ்பட்டு நின்ற தாமோர்
பாரத தேசந் தன்னை
வாழ்விக்க வந்த காந்தி
மஹாத்மா நீ வாழ்க! வாழ்க!
அடிமைவாழ் வகன்றிந் நாட்டார்
விடுதலை யார்ந்து செல்வம்
குடிமையி லுயர்வு, கல்வி
ஞானமும் கூடி யோங்கிப்
படிமிசைத் தலைமை யெய்தும்
படிக்கொரு சூழ்ச்சி செய்தாய்
முடிவிலாக் கீர்த்தி பெற்றாய்
புவிக்குளே முதன்மை யுற்றாய்!''3

என்ற பாடல்வரிகள் உணர்த்துவதைக் காணலாம். மேலும், அறவழி, ஒத்துழையாமை போன்ற காந்தியின் போராட்ட வழிமுறைகளையும் தம் பாடல்களில் குறிப்பிட்டுள்ளார்.

2. தாதாபாய் நவுரோஜி

இந்திய தேசியக் காங்கிரஸின் தலைவராக மூன்று முறை செயல்பட்டவர். மராட்டிய மாநிலத்தின் நவ்சாரியில் செப்டம்பர் 4, 1825-இல் பிறந்து, ஜீன் 30, 1917-இல் தம் 91-வது அகவையில் இயற்கை எய்தினார். ஐக்கிய அரசாங்கத்தின் நாடாளுமன்ற உறுப்பினராக (1892–1895) இருந்தவர். கல்வியாளர், அரசியல்வாதி, வணிகர் எனப் பன்முக ஆளுமை மிக்கவர். இவரது **'பிரிட்டிஷ் தன்மையுற்ற ஆட்சியும் இந்தியாவின் வறுமையும்'** *என்ற நூல் இந்தியாவின் பிரச்சினைகளைத் தீர்ப்பதற்கு அன்னிய நாடுகளின் கட்டுபாடுகள் அற்ற ஒரு* **'சுயசார்பு பொருளாதாரம்'** *உருவாக்கப்பட வேண்டும் என்ற எண்ணத்தை அனைவரிடமும் உருவாக்கியது.*

மகாத்மா காந்தி, பாலகங்காதர திலகர் போன்ற தலைவர்கள் தங்களின் வழிகாட்டியாக இவரையே குறிப்பிட்டனர். பாரதியாரும் இவர்மீது பெருமதிப்பும், மரியாதையும் கொண்டிருந்தார். **'தாதாபாய் நவுரோஜி'** *என்ற தலைப்பில் கவிதை இயற்றியுள்ளார்.*

"மாதாவாய் விட்டலற வதைச்சிறிது
மதியாதே வாணாள் போக்குந்
தீதாவார் வரினுமவர்க் கினியசொலி
நன்குணர்த்துஞ் செவ்வி யாளன்
வேதாவா யினுமவனுக் கஞ்சாமே
உண்மைநெறி விரிப்போ னெங்கள்
தாதாவாய் விளங்குறுநற் றாதாபாய்
நவுரோஜி சரணம் வாழ்க!"[4]

என்று வாழ்த்தியவர். "எமது பாரத நாட்டுப் பெண்பல்லார் வயிற்றிலுமந் நவுரோஜி போற்புதல்வர் பிறந்து வாழ்க" என்று தம் விருப்பத்தினையும் வெளிப்படுத்துகிறார்.

3. பாலகங்காதர திலகர்

பாலகங்காதர திலகர் இந்திய விடுதலைப் போராட்டத்தில் மக்களின் ஆதரவைப் பெற்ற தலைவர்களில் முதன்மையானவர். **'இந்திய தேசிய இயக்கத்தின் தந்தை'** *என அழைக்கப்பட்டவர். மராட்டிய மாநில இரத்தினகிரியில் ஜீலை 23, 1856-இல் பிறந்து, ஆகஸ்டு 1, 1920-இல் மறைந்தார். விடுதலைப் போராட்ட வீரர்கள் அடிக்கடி சிறை செல்வதைக் கண்டு, அவர்களுக்காக வாதாடி, அவர்களைக் காப்பாற்ற வேண்டும் என்ற எண்ணத்தில் சட்டம் பயின்றவர். இந்தியாவிலேயே அதிகம் விற்பனையான* **'கேசரி'** *என்னும் மராத்திமொழிப் பத்திரிகையையும்,* **'மராட்டா'** *என்னும் ஆங்கிலமொழிப் பத்திரிகையையும் நடத்தினார். "சுதந்திரம் எனது பிறப்புரிமை அதை அடைந்தே தீருவேன்" என முழங்கியவர். பாரதியார் இவர்மீது மிகுந்த பற்றினைக் கொண்டிருந்தார்.*

"வாழ்க திலகன் நாமம்! வாழ்க! வாழ்கவே!
வீழ்க கொடுங் கோன்மை! வீழ்க! வீழ்கவே!"

என்பது 'வாழ்க திலகன் நாமம்' என்ற பாடலின் பல்லவி ஆகும். மேலும், திலகர் என்ற பெயரின் பெருமையை,

"துன்ப மென்னுங் கடலைக் கடக்குந் தோணி யவன்பெயர்
சோர் வென்னும் பேயை யோட்டுஞ் சூழ்ச்சி யவன்பெயர்
அன்பெ னுந்தே னூறித் ததும்பும் புதுமலர் யவன்பெயர்
ஆண்மை யென்னும் பொருளைக் காட்டும் அறிகுறி
யவன்பெயர்"[5]

என்றெல்லாம் சரணத்தில் குறிப்பிடுகிறார்.

4. லாலா லஜபதிராய்

லாலா லஜபதிராய் பஞ்சாப் மாநிலத்தின் '**துதிகே**' என்னும் சிற்றூரில் ஜனவரி 28, 1865-இல் பிறந்து, சைமன் குழுவை எதிர்த்து நடந்த கருப்புக்கொடி ஆர்ப்பாட்டத்தில் தடியடிபட்டு காயமுற்று உடல்நலமின்றி நவம்பர் 27, 1928-இல் மரணமடைந்தார்.'பஞ்சாப் சிங்கம்' என்று அழைக்கப்பட்டவர். இவரது போர்க்குணத்தை அடக்க நினைத்த ஆங்கிலேய அரசாங்கம் 1907-இல் கைது செய்து பர்மாவில் உள்ள மண்டேலேக்கு நாடு கடத்தியது. இதனைப் பாரதியும் தம் கவிதையில்,

''விண்ணகத்தே யிரவிதனை வைத்தாலும்
அதன்கதிர்கள் விரைந்து வந்து
கண்ணகத்தே யொளிதருதல் காண்கிலமோ?
நின்னையவர் கனன்றிந் நாட்டு
மண்ணகத்தே வாழாது புறஞ்செய்தும்
யாங்களெலா மறக்கொ ணாதெம்
எண்ணகத்தே லாஜபதி யிடையின்றி
நீவளர்தற் கென்செய் வாரே?''[6]

என்று குறிப்பிடுகின்றார். மேலும்,'லாஜ்பத்ராய் பிரலாபம்' என்ற தலைப்பில்,

''சீக்கரெனு மெங்கள்விறற் சிங்கங்கள் வாழ்தருநல்
ஆக்கமுயர் குன்ற மடர்திருக்கும் பொன்னாடு''

எத்தனை ஜன்மங்கள் இருட்சிறையி லிட்டாலும்
தத்துபுனற் பாஞ்சாலந் தனில்வைத்தால் வாடுகிலேன்''7

என்றும் கண்ணிகள் பல பாடியுள்ளார்.

5. வ.உ.சிதம்பரனார்

இந்திய விடுதலைப்போரில் பங்குபெற்ற தமிழர்களில் வ.உ.சிதம்பரனார் குறிப்பிடத்தக்கவர். ஆங்கிலேயரை எதிர்த்துத் தூத்துக்குடியில் '**சுதேசி கப்பல்**' விட்டவர். அதனால் பொருளாதார இழப்பைச் சந்தித்தவர். சிறந்த வழக்கறிஞர் தமிழ்ப் பற்றாளர். செப்டம்பர் 5, 1872-இல் தூத்துக்குடி மாவட்டம் ஒட்டப்பிடாரத்தில் பிறந்து, நவம்பர் 18, 1936-இல் மறைந்தார். ஆங்கிலேய அரசாங்கத்தால் இரட்டை ஆயுள் தண்டனை விதிக்கப்பட்டுக் கோவை மத்திய சிறையில் செக்கிழுத்துத் துன்பப்பட்டார். 'செக்கிழுத்த

செம்மல்"கப்பலோட்டிய தமிழன்' என்ற அடைமொழிகளுக்குச் சொந்தக்காரர். பாரதியார் இவர்மீது அளவற்ற அன்பு கொண்டிருந்தார். ஆங்கிலேய அரசாங்கத்தால் வ.உ.சிதம்பரனார் சிறையில் அடைக்கப்பட, மகாகவி பாரதியோ,'வ.உ.சிக்கு வாழ்த்து' என்ற தலைப்பில்,

"வேளாளன் சிறை புகுந்தான் தமிழகத்தார்
மன்னனென மீண்டான் என்றே
கேளாத கதைவிரைவிற் கேட்பாய்நீ
வருந்தலைஎன் கேண்மைக் கோவே!
தாளாண்மை சிறிதுகொலோ யாம்புரிவேம்
நீஇறைக்குத் தவங்கள் ஆற்றி
வாளாண்மை நின்துணைவர் பெறுகெனவே
வாழ்த்துதிநீ வாழ்தி! வாழ்தி!"[8]

என்று வாழ்த்துப்பா பாடுகிறார்.

முடிவுரை

இந்திய விடுதலைக்காகப் போராடியதோடு மட்டுமின்றி, தமிழர்களைத் தம் பாடல்களின் வாயிலாக விடுதலை உணர்வைப் பெறவைத்தவர் பாரதியார். தம் காலத்தில் போராடிய தேசியத் தலைவர்களான மகாத்மா காந்தி, தாதாபாய் நவுரோஜி, பாலகங்காதர திலகர், லாலா லஜபதிராய், வ.உ.சிதம்பரனார் போன்றோரின் தியாகத்தையும் சிறப்பையும் வெளிப்படுத்தி உள்ளதை அவரது பாடல்களின் வழி அறிய முடிகிறது.

சான்றெண் விளக்கம்

1. பாரதிதாசன், பாரதிதாசன் கவிதைகள், பாரதி புதுநெறி காட்டிய புலவன், பா.1
2. பாரதியார், பாரதியார் கவிதைகள், பாரதநாடு, பா.1
3. பாரதியார், பாரதியார் கவிதைகள், மகாத்மா காந்தி பஞ்சகம், பா.1,2
4. பாரதியார், பாரதியார் கவிதைகள், தாதாபாய் நவுரோஜி, பா.4
5. பாரதியார், பாரதியார் கவிதைகள், வாழ்க திலகன் நாமம், பா.1
6. பாரதியார், பாரதியார் கவிதைகள், லாஜ்பத்ராய் துதி, பா.1
7. பாரதியார், பாரதியார் கவிதைகள், லாஜ்பத்ராய் பிரலாபம், கண்ணிகள்: 14, 20
8. பாரதியார், பாரதியார் கவிதைகள், வ.உ.சிக்கு வாழ்த்து, பா.1

30. பாரதியின் பன்முகப் பார்வை

பெ.ஜோதி,
முனைவர் பட்டஆய்வாளர் (முழுநேரம்)
தமிழாய்வுத்துறை,
தேசியக்கல்லூரி (தன்னாட்சி), திருச்சிராப்பள்ளி.

முன்னுரை:

தமிழன்னையின் தவப்புதல்வராய் 11.12.1882-ஆம் ஆண்டு எட்டயபுரத்தில் தோன்றியவர் பாரதியார். மொழிப்பற்றுமிக்க கவிஞராக இவர் விளங்கினார். நமக்குத் தொழில் கவிதை, நாட்டிற்குழைத்தல், இமைப்பொழுதுஞ் சோராதிருத்தல் என்று கூறியபாரதியாரின் பல்வேறு சிந்தனைகள் பற்றியும் இக்கட்டுரையில் ஆய்வுசெய்யப்படுகிறது.

பாரதியாரின் நாட்டுப்பற்று:

தாம் பிறந்தபொன் நாட்டினைப் பாரதியார் மகிழ்ச்சியோடுபாடிப் போற்றுகின்றார்.

"எந்தையும் தாயும் மகிழ்ந்துகுலாவி
இருந்ததும் இந்நாடேஅதன்
முந்தையர் ஆயிரம் ஆண்டுகள் வாழ்ந்து
முடிந்ததும் இந்நாடே" **பாரதியார் கவிதைகள் - ப.113**

என்றுகுறிப்பிட்டகவிஞர்

பூரணஞானம் பொலிந்தநன் ணாடு
புத்தர் பிரானருள் பொங்கியநாடு
பாரதநாடுபழம்பெரும் நாடே
பாடுவம் இஃதை எமக்கில்லை ஈடே". **பாரதியார் கவிதைகள் - ப.118**

என்று பாடிப் பரவசப்படுகிறார்.

பாரத நாட்டைப் பாடியது போன்றேதமிழ் நாட்டின் சிறப்பினையும் 'செந்தமிழ் நாடு' என்ற கவிதையில் தேனொழுகக் குறிப்பிடுகின்றார். செந்தமிழ் நாடெனும் சொற்றொடர், அவர்

செவிகளைக் குளிரவைக்கின்றது. தந்தையர் நாடு என்ற சொற்றொடர், அவர் உணர்ச்சியைக் கிளறி வேக மூட்டுகின்றது.

''செந்தமிழ் நாடெனும் போதினிலே - இன்பத்
தேன்வந்துபாயுதுகாதினிலேஎங்கள்
தந்தையர் நாடென்றபேச்சினிலேஒரு
சக்திபிறக்குது மூச்சினிலே!'' **பாரதியார் கவிதைகள் - ப.138**

என்கிறார்.

பாரதியின் பெருமித உணர்வு:

'தமிழ்க்கவிஞர் பரம்பரையில் பிறந்தோமே! என்ற பெருமித உணர்வும் பாரதியாருக்குப் பொங்கி வழிகின்றது. 'கம்பன் பிறந்தநாட்டிலே, வள்ளுவன் வாழ்ந்த நாட்டிலே, நெஞ்சை அள்ளும் சிலப்பதிகாரம் எழுந்த நாட்டிலே நாமும் பிறந்தோமே! என்றதருக்கும் அவர்க்குண்டு. இம்மூன்று கவிஞரிலும் சிறந்த புலவர்கள் உலகில் எங்கேனும் பிறந்ததுண்டா என்றால், 'இல்லை' என்று உறுதியாகப் பதில் கூறுகிறார் கவிஞர் பாரதி.

''யாமறிந்த புலவரி லேகம்பனைப் போல்
வள்ளுவர் போல் இளங்கோவைப் போல்
பூமிதனில் யாங்கணும் பிறந்ததில்லை
உண்மைவெறும் புகழ்ச்சியில்லை'' **பாரதியார் கவிதைகள் - ப.141**

என்ற அடிகளில் தமிழ்ப் புலவர்களை உலக அரங்கில் உயர்வாக ஏற்றிப் புகழ்ந்துள்ளார்.

தாய் மொழிபால் தணியாதகாதல் கொண்டவர் நம் தமிழ்க்கவிஞர் பாரதியார். இதனை,

''யாமறிந்த மொழிகளிலே தமிழ்மொழி போல்
இனிதாவது எங்கும் காணோம்!''. **பாரதியார் கவிதைகள் - ப.141**

என்று கூறியதிலிருந்து அறியலாம்.

பாரதியின் சமுதாயப் பெருநோக்கு:

மனித இனத்தின் மீது மாறாத பற்றுக் கொண்டவர் பாரதியார். 'ஒன்றுபரம் பொருள் நாம் அதன் மக்கள் உலகின் பக்கேணி' என்ற அடிகளில் உலகமக்கள் அனைவரையும் ஒரு கூட்டமாக ஒரு சமுதாயமாக எண்ணிப் பார்க்கும் பெருநோக்கினைக் காணலாம். அதோடு மட்டுமன்றி ஆறறிவு இல்லாத அஃறிணை உயிரினங்களையும் தம்மோடு சேர்த்துக் கவிஞர் கவிதை வெறிகொண்டு பாடுகின்றார்.

"காக்கை குருவி எங்கள் சாதி நீள்
கடலும் மலையும் எங்கள் கூட்டம்
நோக்குந் திசையெல்லாம் நாமன்றி வேறில்லை
நோக்க நோக்கக்களியாட்டம்!" **பாரதியார் கவிதைகள் - ப.92**

இதுபோன்று இந்திய நாட்டினர் அனைவரும் ஒருமைப்பாட்டு உணர்வோடு ஒன்றிய சிந்தையினராய் வாழவேண்டும் என்பதனைப் பெரிதும் வற்புறுத்துகிறார் பாரதியார் 'எங்கள் தாய்'என்ற கவிதையில்,

"முப்பதுகோடி முகமுடையாள் உயிர்
மொய்ம்புறம் ஒன்றுடையாள் - இவள்
செப்பு மொழிபதி னெட்டுடையாள் எனிற்
சிந்தனை ஒன்றுடையாள்." **பாரதியார் கவிதைகள் - ப.121**

என்று குறிப்பிட்டுள்ளார். 'வாழ்ந்தால் முப்பது கோடிமக்களும் ஒருநிறையாய் வாழவேண்டும். இன்றேல், ஒருங்கே மடிய வேண்டும்' என்று ஆவேசத்தோடு பேசுகிறார் பாரதியார்.

"எப்பதம் வாய்த்திடுமேனும் - நம்மில்
யாவர்க்கும் அந்தநிலைபொதுவாகும்
முப்பதுகோடியும் வாழ்வோம் - வீழில்
முப்பதுகோடிமுழுமையும் வீழ்வோம்!" **பாரதியார் கவிதைகள் ப.112**

வடகோடி மக்களும் தென்கோடி மக்களும் இணைந்து மகிழ்ந்து வாழ வேண்டும் என்பதனை அழகியதொரு கற்பனைக் காட்சியாய்க் கவினுறவடித்துக் காட்டுகின்றார். பொதுமை வேட்கை பொலியும் கவிஞர். 'நிலவொளிபாயும் சிந்துநதியிலே சேரநாட்டின் எழில் இளமங்கையர் அழகுப் படகினிலே அமர்ந்திருக்க இனிமையான தெலுங்கு மொழியில் இன்பப் பண்ணிசைத்துத் தோணிகளோட்டிவரலாம்.' என்று ஆசை பொங்கக் கூறுகிறார்.

"சிந்து நதியின் மிசைநிலவினிலே
சேரநன் னாட்டிளம் பெண்களுடனே
சுந்தரத்தெலுங்கினில் பாட்டிசைத்துத்
தோணிகளோட்டி விளையாடிவருவோம்" **பாரதியார் கவிதைகள் - ப.116**

வடக்கேகாஷ்மீரம் தொட்டுத் தெற்கே கன்னியாகுமரி வரை வாழ்கின்ற மாந்தர் அனைவரும் 'ஒருகுலமாய் ஒருநிறையாய் ஒருவிலையாய் எல்லோரும் இந்நாட்டு மன்னராய் வாழவேண்டும் என்பது அவர்தம் பேராவல் அவர் கனவு காணுகின்ற புதிய சமுதாயத்தில் ஒருவர் உணவை ஒருவர்

பறியாமல் மனிதர் நோகமனிதர் பாராமல் தனியொருவர் தன் உணவுக்குப் பிறரை எதிர்பாராமல் வாழவேண்டும் என்று கூறி

"முப்பது கோடி ஜனங்களின் சங்கம்
முழுமைக்கும் பொதுவுடைமை
ஒப்பில்லாத சமுதாயம்
உலகத்துக்கொரு புதுமைவாழ்க!" **பாரதியார் கவிதைகள் - ப.135**

என்று புதிய சமுதாயத்தினைப் படைத்துக் காட்டுகிறார்.

பாரதியின் விடுதலைச் சிந்தனை:

நாட்டு விடுதலைப் பாடல்களில் கவிஞர் உணர்வின் இமயமாய் விளங்குகிறார். என்றுதணியும் இந்தச் சுதந்திரதாகம்? என்ற வினாவினை எழுப்பி மக்கள் மனதில் சுதந்திரக்கனல் கொழுந்து விட்டெரியச் செய்கிறார். தொண்டுசெய்யும் அடிமை! உனக்குச் சுதந்திர நினைவோடா? என்று ஆங்கிலேயன் பாரத மக்களைப் பார்த்துக் கேள்வி கேட்பது போல எண்ணி அதற்கு மறுமொழியாக 'சொந்தநாட்டில் பிறர்க்கடிமை செய்தே துஞ்சிடோம் - இனி அஞ்சிடோம்' என்றுமார் தட்டிப் பேசுகிறார். வீரசுதந்திரம் வேண்டிநின்றார் பின்னர் வேறொன்று கொள்வாரோ?" என்று கூறி 'எத்தனை இன்னல்கள் வந்துற்றாலும் சுதந்திர தேவியைத் தொழுதிடல் மறக்கிலேன்' என்றார்.

பாரதியின் ஒற்றுமை உணர்வு:

புன்மைத் தொழிலாம் அடிமைத்தொழில் செய்தகாலம் கழிந்தொழிந்தது தொல்லை இகழ்ச்சிகள் தீர்ந்தன. புதிய பாரதத்தினைக் காணவேண்டும். அதற்குமக்கட்குத் தேவையான அடிப்படைப் பண்பு ஒற்றுமை. அந்த ஒற்றுமை என்ற ஒன்று மட்டும் அமைந்து விட்டால் பிறகு வாழ்வின் முன்னேற்றத்திற்கு வேறொன்றும் தேவையில்லை. இதனைப் பாரதியார்,

"ஒன்றுபட் டால்உண்டுவாழ்வேநம்மில்
ஒற்றுமைநீங்கில் அனைவர்க்கும் தாழ்வே
நன்றிதுதேர்ந்திடல் வேண்டும் - இந்த
ஞானம்வந் தாற்பின்நமக்கெதுவேண்டும்?"

பாரதியார் கவிதைகள் - ப.112

என்று இடித்து அறிவுரை கூறியுள்ளார்.

பெண்மையைப் போற்றுதல்:

நாட்டிற்கு அடுத்த நிலையில் மனித சமுதாயத்தின் ஒருபாதிப் பகுதியான பெண்டிர் சமுதாயம் முன்னேற வேண்டும்

என்பதில் பாரதியாருக்குப் பெருவேட்கை இருந்தது. பெண்ணை அடிமையாக நடத்தியவர்கள், '**ஏட்டையும் பெண்கள் தொடுவது தீமையென்று எண்ணியிருந்தவர்கள் மாய்ந்து விட்டார்கள்**' என்று கூறிப்புதிய பெண்மையினைப் போற்றி வரவேற்கின்றார். 'நிமிர்ந்த நன்னடை நேர்கொண்டபார்வை நிலத்தில் யார்க்கும் அஞ்சாதநெறிகள் நிமிர்ந்தஞானச் செருக்கு' முதலிய நற்பண்புகளின் உறைவிடமாகப் புதிய பெண்ணுலகத்தினைக் காணுகின்றார். பட்டங்கள் ஆள்வதும் சட்டங்கள் செய்வதும் வேதம் படைப்பதும் நீதிகள் செய்வதும் சாதம் படைப்பதும் தெய்வச்சாதி படைப்பதும் ஆன செயல்களைச் செம்மையே நடத்துதல் இனி அவர் தம் சீரிய அறப்பணிகளாகும்.

காதலன் ஒருவனைக் கடிமணம் புரிந்து கொண்டு அவனுக்கு எல்லாத் துறைகளிலும் துணைநின்று முன்னையினும் சிறக்கப் பெண்ணறத்தைப் பேணி வளர்ப்போம் என்று புதுமைப் பெண்கள் முழக்கஞ் செய்வதாகப்

'பெண்கள் - விடுதலைக் கும்மி'பாடியுள்ளார் பாரதியார்.

"காதலொருவனைக் கைப்பிடித் தேஅவன்
காரியம் யாவினும் கைகொடுத்து
மாதரறங்கள்பழமையைக் காட்டிலும்
மாட்சிபெறச் செய்துவாழ்வமடி!" **பாரதியார் கவிதைகள் - ப.57**

முடிவுரை:

பாரதியார்இ உள்ளம் அள்ளும் தெளிவான கவிதைகள் பலவற்றை இயற்றித் தமிழுலகிற்குத் தந்துள்ளார். தேசியக் கவிஞராய் இருந்து நாட்டின் விடுதலைக்கும் முன்னேற்றத்திற்கும் ஒருமைப்பாட்டிற்கும் உரியவான கவிதைகளைத் தந்துள்ளார். சமுதாயச் சீர்திருத்த கவிஞராய் விளங்கிப் பெண்ணுரிமைப் பாடல்களைப் பாடிப் புதியதொரு சமுதாயத்தினைப் படைத்துள்ளார். தாய்மொழிப்பற்றும் தமிழ்நாட்டுப்பற்றும் நிறைந்த கவிஞராய் பாரதியார் விளங்கி நமக்கெல்லாம் நல்வழி காட்டுகின்றார். பாரதியாரின் பாடல்களைப்பாங்குறப் படிப்போர் அவரைப் '**பாட்டுக் கொருபுலவன் பாரதி**' என்றும் '**புதுநெறி காட்டிய புலவன் பாரதி**' என்றும் நெஞ்சம் குளிரப் பாராட்டி மகிழ்வர் என்பது உண்மையாகும்.

ஆய்விற்குப் பயன்பட்ட நூல்கள்:

1. பாரதி கவித்திறன் – டாக்டர். மு கோவிந்தசாமி, மூன்றாம் பதிப்பு : 1968, பாரிநிலையம், சென்னை – 620 001

2. கட்டுரை வளம் – டாக்டர் சி. பாலசுப்பிரமணியன், நான்காம் பதிப்பு : 1975, நறுமலர் பதிப்பகம். சென்னை.600 029.

3. முத்துக்குவியல் – ச. சதுக்கத்துல்லா, முதற் பதிப்பு : 1992, 'தமிழ்த்தேவகம்' தமிழ் சமயப்பணி நிறுவனம், திருச்சிராப்பள்ளி. 620 023.

4. பாரதியார் கவிதைகள் – மகாகவி. சி. சுப்பிரமணியபாரதி, பதிப்பாசிரியர். இராமலட்சுமணன், பன்னிரெண்டாம் பதிப்பு : 2007, உமாபதிப்பகம், சென்னை. 600 001.

31. சித்திர விளக்கங்களின் முத்திரை மற்றும் முன்னோடி பாரதி

திருமதி. ச. கண்ணம்மாள்
முனைவர் பட்ட ஆய்வாளர் (பகுதிநேரம்)
தமிழ்த்துறை, தேசியக் கல்லூரி,
திருச்சிராப்பள்ளி – 620 001.

'ஊருக்கு நல்லது சொல்வேன், உண்மை தெரிந்து உரைப்பேன்' என்று முழங்கிய பாரதியார் பத்திரிக்கையாளர்களுக்கு ஒரு முன்னோடி. எட்டையபுரத்திலே பிறந்து எட்டுத்திக்குகளிலும் தன் சிந்தனை விதைகளைத் தூவியவர். எதை எழுத வேண்டுமென்பதில் தெளிவான பார்வை கொண்டிருந்தவர். கவிதைகள் பாடல்கள், கட்டுரைகள தலையங்கங்கள் சித்திர விளக்கங்கள கடிதங்கள் சொற்பொழிவுகள் மதிப்புரைகள் சரித்திரங்கள் சிறுகதைகள் மொழிபெயர்ப்புகள் என அனைத்து வகைப்பட்ட இலக்கிய வடிவங்கள் வாயிலாகவும் விடுதலை உணர்வைத் தூண்டியவர். சுதேசியம், ஆங்கில ஏகாதிபத்திய எதிர்ப்பு, ஒன்றுபட்ட தேசிய எழுச்சியின் தடைக்கற்கள், சாதிய ஒடுக்குமுறை, பெண் அடிமைத்தனம், புதியபாரதம் படைத்தல், முதலான பொருண்மைகளில் படைப்புகளைத் தந்தவர். இக்கட்டுரை "சித்திர விளக்கங்களின் முத்திரை மற்றும் முன்னோடிபாரதி" எனும் கருத்தை விளக்குவதாக அமைகின்றது.

கருத்துப் படங்களின் அறிமுகம்

தமிழ் மொழியில் கருத்துப்படங்களை முதன்முதலாக அறிமுகப்படுத்திய பெருமை பாரதியைச் சாரும். இவர் பணியாற்றிய 'இந்தியா' என்ற இதழில் தான் முதல் கருத்துப்படம் வெளியானது. தொடர்ந்து, தேசபக்தன் விநோதினி, காந்தி போன்ற இதழ்களிலும் கருத்துப்படங்கள் வெளிவரத் தொடங்கின.

நாளிதழ்கள் மற்றும் பருவ இதழ்களில் கருத்துப்படம் என்பது கவனத்திற்குரியதாக அமையும். கவிதை கட்டுரை

தலையங்கம் போன்ற வடிவங்களில் வெளிப்படுத்தும் கடினமான கருத்துக்களையும் புரிதல்களையும் எளிமையாக உணர்த்தும் வகையில் இயல்பாக அமையும் ஓவியம் கருத்துப்படமாகும். பொதுவாக இந்தக் கருத்துப்படம் பொதுமக்களின் எண்ணங்களைப் பிரதிபலிப்பதாக அமையும். உணர்த்துதல், நகைச்சுவை, கற்பனை ஆகிய இவையேகருத்துப் படங்களின் முக்கியக் கூறுகளாகும். கருத்துப்படங்களை*Cartoon*' என்றும், கேலிச் சித்திரங்களை '*Caricature*' என்றும் அழைப்பர். கருத்துப்படங்களின் வளர்ச்சியே கேலிச்சித்திரம் எனவும் சொல்லப்படுகிறது. உரைப்பாரின்றி புரிந்து கொள்ளும் சித்திரங்களுக்கு, மேலும் விளக்கங்களைக் கொடுத்து, குறியீடுகளைத் தெளிவுபடுத்தி உணர்த்துகின்றார் பாரதியார்.

மொழிநடை:

மொழி நடை என்பது இதழியல் துறையில் கவனிக்கத்தக்கது. பொது மக்களுக்கான வாசிப்புச் சூழலுக்குத் தகுந்தாற் போல் வாசிக்கத் தூண்டுவதாக மொழி நடை அமைதல் வேண்டும். பாரதியாரின் மொழிநடை இலக்கிய வடிவங்களைப் பொறுத்து மாறுபடுகிறது. கருத்துப்பட விளக்கங்களில் பாரதியாரின் மொழிநடை ஆங்கிலம் மற்றும் சமஸ்கிருதம் கலந்தநடையாக இருக்கின்றது.

ஆங்கில அரசாங்கத்தை, ஏகாதிபத்தியத்தை எதிர்க்கும் நோக்கில் கருத்து விளக்கங்கள் அமைவதால் ஆங்கில மொழியையும் பயன்படுத்தியிருக்கிறார். *Government, IIustation, Loyalty, Governor, Greed* போன்ற ஆங்கிலச் சொற்களையும் ஸ்வதந்திரம், ஸமத்துவம், ஸஹோதரத்துவம், ஸ்வராஜ்யம், ஸ்திரீகள், ஸத்தியம், ஐக்கியம், ஸூர்யோதயம், ரதம், வினோதம், ஜனம் முதலான சமஸ்கிருதச் சொற்களை மிகுதியாகக் கையாண்டுள்ளார்.

இன்று வழக்கிலிருக்கும் 'ஒலிபெயர்ப்புமுறை' யை முன்பே பயன்பாட்டிற்குக் கொண்டுவந்தவர் பாரதி. கன்சர்வேட்டிவ் பார்ட்டி, லிபரல் பார்ட்டி ஆகிய சொற்களைப் பயன்படுத்தியுள்ளார்.

'சூரியனைப் பார்த்து நாய்கள் குரைத்தல், எள்ளு விதைத்தால் எள்ளுவிளையும், கொட்டினால் தேள் கொட்டாவிட்டால் பிள்ளைப்பூச்சி, தன் செயல் தனக்கேகெடுதி, சுதேசிய எலிக்கு அஞ்சும் ஆந்தைகள், கேடுவரும் பின்னேமதி கெட்டுவரும் முன்னே, ஆயிரம் காக்கைகளுக்கு ஓர்கல்' என்பன

போன்ற பழமொழித் தொடர்களையும் பேச்சுவழக்குத் தொடர்களையும் தலைப்புகளாக வைத்துள்ளார்.

மஹிளரத்னங்கள்:

பெண்ணை ஒப்பில் உயர் அவதாரமாக, நல்லறத்தோடு கூடியதாய் சிவசக்தியாக, ஆண்களை முக்திக்கு ஆற்றுப்படுத்தும் வழிகாட்டியாக கண்டமையான் பெண் விடுதலைக்கும் பெண் உரிமைக்கும் அறைகூவல் விடுத்து புதுமைப் பெண்களுக்கான பாட்டையை அமைத்துக் கொடுத்தார் பாரதி.

09/10/1909 அன்று வெளிவந்த 'இந்தியா' இதழில் இடம் பெற்ற கருத்துப்படம் யாதெனில், பெண்கள் குதிரைகளின் மீது அமர்ந்து கொண்டு 'போலோ' எனப்படும் பந்து விளையாட்டை விளையாடுகிறார்கள்.

பாரததேசத்துப் பெண்களுக்கு நாகரிகம் தெரியாது படிப்பறிவு கிடையாது வித்தைகள் தெரியாது அவர்கள் அடிமைகள் என்று ஐரோப்பா, அமெரிக்கா முதலான நாடுகள் இழிவாக நினைத்துக் கொண்டிருக்க, இந்தியாவில் மொகலாயர் ஆட்சியில் ஆசியப் பெண்கள் வெளிநாட்டவர்களின் விளையாட்டை விளையாடுவது அவர்களின் கருத்தைப் பிழைப்பிப்பதாக அமைந்திருந்தது.

'ஜனானாவில் (மூலையில்) அடைத்துப் பூட்டப்பட்டிருக்கும் ஸ்திரீகளுக்கு என்ன தெரியுமென்று வீண் அரட்டை அடித்து தூஷணஞ் செய்து திரிபவர்களின் வாய்க்கு ஆப்பிட வேண்டி இச்சித்திரம் பதிப்பிக்கப்பட்டது.

நமது பாரத நாடு ஸாமான்யமான பெண்களைத் தானாபெற்றது? சம்பராஸுர வதத்தில் கைகேயிதசரதருக்குத் தேரோட்டினாள். அச்சாணி விழுந்து விட்டபோது தனது கையை அச்சாணியாகப் பிடித்தாள். நரகாஸுரவதத்தில் ஸ்ரீகிருஷ்ணர் களைத்து விட்டபோது ஸத்தியபாமாயுத்தம் செய்தாள். மகாராணி அகல்யாபாய் ஷோல்தார் நாட்டில் அரசாட்சிசெய்தாள். ராணிலட்சுமிபாய் 1857-இல் யுத்தம் செய்தாள். ஸீதா, துரொபதி, மீராபாய், ஆண்டாள், மங்கையர்க்கரசி, காரைக்கால் அம்மையார், லட்சுமிபாய், மொல்லாதேவி, மனோன்மணி, தேவிசவுதாராணி, சகுந்தலா, ஸாவித்ரி முதலான பல மஹிளரத்னங்களை மகளிர்களாய்ப் பெற்றிருக்கிறாள் ஸ்ரீ பாரத மாதா என்பதை அவர்கள் அறிவார்களாக' என்ற பாரதியாரின் விளக்கம் அவருணர்ந்த

பெருமிதத்தை நம்மிடம் கடத்திச் செல்கிறது. பெண் விடுதலைக்கு முன்னெடுத்த பாரதியார் காலத்திலே அவர் தந்துள்ள சாதனைப் பெண்களின் எண்ணிக்கை வியக்கத் தக்கதாகின்றது.

ஏகாதி பத்திய எதிர்ப்பு:

ஆங்கிலேயர்களின் ஏகாதி பத்தியத்தை எதிர்ப்பதே இந்தியர்களின் தலையாயக் கடமையாக இருக்கவேண்டும் என வலியுறுத்துகிறார் பாரதி. 30/03/1907 அன்று 'இந்தியா' இதழில் வெளியான ஒரு சித்திரம், ஒரு யானையின் மீது வெள்ளைக்கார துரை(John bull) அமர்ந்திருந்தார். யானையின் முதுகிலேஎண்ணற்ற மூட்டைகள். வெள்ளைக்காரத்துரை யானையின் மீதுள்ள சுமையைக் குறைக்கும் பொருட்டு ஒரே ஒரு சிறிய மூட்டையைத் தூக்கிப் போட எத்தனிக்கிறார்.

இவ்விடத்து, யானையை இந்தியாவிற்கும் மூட்டைகளை வரிச்சுமைக்கும் வெள்ளைக்காரத்துரையை ஆங்கிலேய ஏகாதி பத்தியத்திற்கும் தூக்கி எறிவதற்காக வைத்திருக்கும் மூட்டையை 'உப்புவரி' என்றும் உருவகப்படுத்தியுள்ளதைப் பாரதி தெளிவுபடுத்தி உரைக்கிறார். மேலும் சிறு உப்பு வரிச்சுமையை மட்டும் குறைத்ததாராளமனம் படைத்த துரை யானையின் பலம் அறியாமல் செயல்படுவது கேலிக்குரியது என்கிறார்.

'அடடா! துரையின் கருணையை என்ன சொல்வோம்! துரை இப்படி சந்தோஷமடைந்து கொண்டிருக்கிறார். யானை மனதிலே என்ன ஹடம் வைத்துக் கொண்டிருக்கிறதோ யார் அறிவார்' என தனக்கே உரியமிடுக்கேறிய நடையில் ஏகாதி பத்தியத்தை எதிர்க்கிறார். 'ஆயிரம் காக்கைகளுக்கு ஓர் கல்' போதும் எனவும் குறிப்பால் சாடுகின்றார்.

சுதேசிய, சுயராஜ்ய, சூரியோதயம்:

'சுயராஜ்யம் எனது பிறப்புரிமை' என்று முழங்கிய பாலகங்காதரதிலகரைப் போற்றி நாட்டிலே சுயராஜ்ஜியம் என்ற சூரியோதயம் தோன்றிவிட்டால் அடிமைத்தனம் நீங்கும் வழி பிறக்கும் என்பது பாரதியாரின் திண்ணம். ஆதவனை வெறுப்பாறுண்டோ?

16/03/1907 அன்று வெளி வந்த சித்திரத்தில் சூரியன் உதயமாவதைப் பார்த்து ஒருபுறம் ஹிந்துக்கள், முகமதியர், பார்சிகள், கிறிஸ்துவர் என அனைவரும் வணங்கி மகிழ்கிறார்கள். மறுபுறம் நாய்கள் நான்கைந்து நின்று கொண்டு குரைக்கின்றன. அந்த நாய்களின் தலையிலே வெள்ளைக்காரர்களின்

அடையாளமான தொப்பி அணியப்பட்டுள்ளது. அது ஆங்கிலேயப் பத்திரிகைகளைச் சுட்டி நிற்கிறது.

'இந்தியாவிலே வெளிச்சம் வருவதைக் கண்டவுடனே இந்நாய்களுக்குக் கோபமுண்டாகிறது. பலமாகக் குரைப்பதினால் எழுச்சிகொண்ட சூரியன் மறுபடியும் சமுத்திரத்துள்ளே அமுங்கிவிடுவென்பது இந்நாய்களின் நம்பிக்கை!'.

'சூரியனைக் கண்டுநாய் குலைத்தால் நாயின் வாய்க்குச் சேதமேயல்லாமல் சூரியனுக்குச் சேதமுண்டோ?' என்கிறார்.

மற்றொருவிளக்கத்தில் 'தெய்வீகத் தலைவரான ஸ்ரீமான் அரவிந்தகோஷ் அவற்றையெல்லாம் விழுங்கி ஜாதீய முயற்சிகளைக் கைக்கொண்டிருக்கும் பாரத ஸ்வராஜ்யக் கட்சியினைக் காத்துமஹா தேஜஸ்வினியாய் விளங்கிவருகிறார்' என்றும் குறிப்பிடுகின்றார்.

காங்கிரஸ் கட்சியில் நிலவியகருத்து முரண்பாடுகளை விவரிக்கும் சித்திரம் 27-04-1907 அன்று 'இந்தியா' இதழில் வெளியானது. ஸ்வராஜ்யம்எனும் இடத்தை நாடிச் செல்லும் வண்டியை இரண்டு காளைகள் இழுத்துச் செல்கின்றன. ஒரு காளைபடுத்துக் கொள்கிறது. ஒருகாளை செல்ல முயற்சிக்கிறது. இதுதான் சித்திரம்.

இதனை விளக்க முற்பட்டபாரதியார் 'திலகருடன் ஈடுகொடுக்க முடியாமல் தட்டுத் தடுமாறி இடைவெளியில் மேத்தா என்கிற காளைபடுத்துக் கொண்டு நகரமுடியாமல் கையில் பேனா கொண்டு விண்ணப்பம் எழுத ஆரம்பித்துவிட்டது. திலகரைக் குறிக்கும் காளை உற்சாகத்துடனும் இறுமாப்புடனும் தலைநிமிர்ந்தது ஸ்வராஜ்யமாகிய இடத்தைக் கண்டுமுக்காரம் போடுகிறது' எனப் பதிவுசெய்கின்றார். அதாவது காங்கிரஸ் கட்சியில் இருந்த இருவேறுபட்ட கட்சியினரை (அ) கொள்கையாளர்களைப் பற்றி சாமானியர்களுக்கும் புரியும் வண்ணம் கருத்து முரண்களை எடுத்துரைக்கும் திறன் பெற்றவர் என்பது புலப்படுகிறது.

ராஜபக்தியும் தேசபக்தியும்:

நடிக்கும் ஜனத்தவர்களையும் தேசபக்தர்களையும் கண்டிக்கிறார் பாரதி. பாரததேசத்தில் ராஜாக்களும் ஜமீன்தார்களும் மிராசுதார்களும் ஸ்வதேசிய கொள்கையை விடுத்து ஆங்கிலேயர்கள் தரும் உத்தியோகப் பதவிகள் மற்றும்

பட்டங்கள் எனும் மோகவலையில் மாட்டித் தவிக்கின்றனர் எனவும் கடிந்துரைக்கின்றார்.

07-11-1908 அன்று'இந்தியா' இதழில் வெளியான கருத்துப்படத்தில் கூண்டுச் சிறைக்குள்ளே "திலகர்" என்ற சிங்கம் அடைக்கப்பட்டது. வெளியே நான்கு மனிதர்கள், நாய்கள் போலும் வெள்ளைக்காரன் ஒருவன் வீசிக்கொண்டிருக்கும் எலும்புத் துண்டுகளைக் கடித்துக் கொண்டிருக்கின்றன.

இச்சூழல் ஒருபுறமிருக்க. 31-10-1908 அன்று 'இந்தியா' இதழில் வெளிவந்த சித்திரம் ராஜ பக்தியை விவாதிப்பதாக அமைந்துள்ளது. வாய்ப்பூட்டும் கைவிலங்குகளும் இடப்பட்ட ஜனங்களின் முன்பாக ஈட்டியைக் கையிலே வைத்துக் கொண்டு ஒருவன் 'ஸலாம்' போடச் சொல்லி மிரட்டுகிறான். இது எங்ஙனம் விசுவாசமாகும் அல்லது ராஜபக்தியாகும் என வினவுகிறார் பாரதி.

இந்த இரு சூழல்களையும் ஒப்பிடும் போதுசிலர் அடிமைகளாகிறார்கள், சிலர் அடிமைகளாக்கப்படுகின்றனர் என்ற புரிதலை உணர்த்தும் பாரதி, அடிமையாகிறவர்களைக் கண்டித்தும் அடிமைகளாக்கப்படுகிறவர்களின் மீது இரங்கியும் இனி ராஜ பக்திதேவையே இல்லை தேசபக்தியே தேவை என முழங்குகிறார்.

ஒற்றுமையே பலம்:

ராஜாக்கள் எப்போது அதர்மம் செய்யத் தலைப்படுகிறார்களோ அப்பொழுதே ஜனங்களிடம் ஸ்வதந்திர லஷ்மிக்கு அபிமானம் பிறந்துவிடுகிறது. எனவே ராஜாக்கள், ஜமீன்தார்கள், மிராசுதார்கள் என அதிகாரம் படைத்த பொறுப்பிலே இருப்பவர்கள் அதர்மத்தை கடைப்பிடித்தல் ஆகாது. மேலும் பல்வேறு இன, மத, மொழி, கலாச்சார, பண்பாடுகளைக் கொண்ட இந்தியமக்கள் ஒன்றுபட்டு வாழும் போதுதான் வெற்றியைப் பெறமுடியும் என்ற கருத்தையும் வலியுறுத்துகின்றார்.

04-12-1909 அன்று 'இந்தியா' இதழில் வெளிவந்த சித்திரம் ஆறுமுகப் பெருமானை அரவணைக்கும் சக்தி. கருத்து பேதங்கள் செயல் பேதங்களாகும். செயல் பேதங்கள் ஒற்றுமையைக் குலைக்கும் தன்மை உடையது.

"ஸ்ரீ பாரதமாதாதனது ஆறு பிள்ளைகளையும் ஒரே பிள்ளையாகும் படிதழுவி ஒன்று சேர்க்கிறாள். ஐக்கியமே பலம் பிரிவே பிளவு". வங்காளம், பம்பாய, சென்னை, மத்தியமாகாணங்கள், பஞ்சாப், ஐக்கிய வடமேற்கு மாகாணங்கள் ஆகிய ஆறும் ஒரே மனோநோக்கத்துடன் ஒற்றுமையாக இருக்கவேண்டும் என்பது பாரதியாரின் விழைவாகும்.

"ஒன்றுபட்டால் உண்டு வாழ்வு- நம்மில்
ஒற்றுமை நீங்கிடில் அனைவருக்கும் தாழ்வே"

உழைக்க முனைதல்:

மாகாணங்களையெல்லாம் ஒன்று படபார்த்த பாரதி, மாகாணங்களுக்குள்ளே நிலவும் சிற்சில குறைபாடுகளையும் சுட்டிச் செல்கின்றாா். 17-07-1909 அன்று 'இந்தியா' இதழின் முதற்பக்கத்திலே ஒரு சித்திரம் இடம் பெறுகிறது. அதில் மேலே ஒரு கப்பல் அதை எல்லோரும் இயக்குகின்றனர். கீழே ஒரு கப்பல் அதை சிலர் மட்டும் இயக்கிபலர் கை கட்டிவேடிக்கைப் பார்க்கின்றனர். இதற்கு பாரதியார் இப்படி விளக்கம் தருகின்றார்.

"வங்காளத்தில் மேற்படி (சுதேச புகைக்) கப்பல்களை நடத்தப் பெருகனவான்கள் எல்லாரும் சிறிதும் பால் மாறாது வேலை செய்கிறார்கள். தென் இந்தியாவிலோ சிலரே ஊக்கத்தோடு வேலை செய்கிறார்களேயொழிய பெரும்பாலும் தனிகர்கள் கைகட்டி வேடிக்கை பார்த்துக் கொண்டு சும்மாவிருக்கிறார்கள். இதுதான் வங்காளத்தில் அவர்கள் லாபமடைவதற்கும் நாம் நஷ்டமடைவதற்கும் காரணம்" எனக் குற்றம் சாட்டிசோம்பேறித்தனத்திற்கு ஆட்பட்டு வேடிக்கை மனிதராய் வீழ்ந்து போகாமல் உழைக்கமுனைதல் வேண்டும் என்றுஉறுதிபடஉரைக்கின்றார்.

நந்தனாரின் ஆத்ம ஸ்வதந்திரம்

நாட்டின் விடுதலையை மட்டும் பாடியவரன்று பாரதி. ஆன்ம விடுதலை குறித்தும் பாடியிருக்கிறாா். 25-15-1909 அன்று 'இந்தியா' இதழில் வெளியான கருத்துச் சித்திரம் நந்தனாரைக் குறிப்பிடுவதாக அமைந்திருந்தது.

"பறையனாய்ப் பிறந்தாலும் பரகதியடையலாம்
உறுதிமாத்திரம் வேண்டும் உனக்குண்டோ? "

'ஆத்மாவைப் பற்றிய தளைகள் நீங்கிசிவ ஸ்வதந்திரம் பெறும் உரிமை பறையனுக்கில்லை என்று இகழ்ந்தவர்கள்

வார்த்தைகளைப் பொய்யாக்கும் படிநந்தனார் யாகாக்கினி (தன்னலமறுப்பு என்றதீ)யில் வீழ்ந்து ஸ்வதந்திரம் பெறுகின்றார்'.

புராணத்திலே இடம் பெற்றநந்தனாரைக் குறியீடாகக் கொண்டுமக்கள் தன்னலம் விடுத்து பொதுநலத்தோடு செயல்படும் போதுதான் 'சுதந்திரம்' என்பதை அடையமுடியும் எனவும் குறிப்பால் உணர்த்திச் செல்கின்றார்.

முடிவுரை:

"ஆயிரம் வாளால் செய்யமுடியாததை ஒர் எழுதுகோல் செய்யும்" "வாளின் முனையைவிடவும் பேனா முனை வலிமை யானது"என்றதொடர்களுக்குஏற்றாற் போலபாரதியாரின் ஒவ்வொருவார்த்தைகளும் ஆயிரமாயிரம் அஸ்திரங்களுக்கு நிகரானவை. யாருக்கும் அடிபணியாமல் நிமிர்ந்த நன்னடையோடும் சுயமரியாதையோடும் பத்திரிக்கை தர்மத்தோடும் தனது சித்திர விளக்கங்களினால் பாமரமக்களிடையேயும் விடுதலைக்கனலை மூட்டியவர் பாரதி. சுடர் விளக்காயினும் தூண்டுகோல் தேவைப்படுவது போலே பாரதியாரின் சித்திர விளக்கங்கள் ஒவ்வொன்றும் நம் மனத்துள் மதிப்புடை உணர்வுகளை எழுப்புவனவாகவே அமைந்துள்ளன.

பாரதியை வாசிப்போம்!
பாரதத்தை நேசிப்போம்!

துணைநின்றவை:

1. இதழியல் கலைடாக்டர் மா.பா.குருசாமி– e book
2. மகாகவிபாரதியார் கவிதைகள் – நியுசெஞ்சுரி புக் ஹவுஸ்,சென்னை.
3. Mahakavibhathiyar. info – esource

32. பாரதி முரண்

செ. மணிகண்டன்
முனைவர்பட்ட ஆய்வாளர்,
தமிழாய்வுத்துறை, தேசியக்கல்லூரி (தன்னாட்சி),
திருச்சிராப்பள்ளி – 01.

பத்தொன்பதாம் நூற்றாண்டில் தோன்றி இருபதாம் நூற்றாண்டின் தொடக்கத்தில் மறைந்த ஒப்பற்ற கவிஞர் பாரதியார். இலங்கையிலிருந்து வந்த புலவர் ஒருவர் எட்டையபுரத்தின் இரு கவிச் சிறார்களுக்குப் பாரதி என்னும் பட்டத்தை 1893 ஆம் ஆண்டு வழங்கினார். ஒருவர் வ.உ.சி.யின் (1872 1936) சுதேசி நாவாய்ச் சங்கத்தில் (16-10-1906) கணக்கராகப் பணியாற்றியவரும் தமிழறிஞருமான ச. சோமசுந்தர பாரதி (1879-1959); மற்றொருவர் இந்திய விடுதலைக் கனலைத் தமது பாட்டுத் திறத்தால் பரப்பிய மகாகவி சுப்பிரமணிய பாரதியார் (1882-1921). வாழ்வின் பல முரண்பாடுகளைத் தம் எழுத்தாற்றலால் களைந்த பாரதிக்கு அவர் இறந்த பின் நிகழ்ந்த வரலாற்று முரண்களை இக்கட்டுரை துளக்குகின்றது.

பதினொன்றா? பன்னிரண்டா?

பாரதிக்கு நினைவு நூற்றாண்டைக் (2021) கொண்டாடி விட்டோம். பாரதியார் மறைந்த பிறகு அவரது நினைவு நாளை செப்டம்பர் மாதம் பதினோராம் தேதியாக (11-09-1921) நினைவு கூர்ந்து வருகின்றனர். பாரதி குறித்த அரசு விழாக்களும் இத்தேதியில் கொண்டாடப்பட்டு வருகின்றன. சிலர் செப்டம்பர் 11 என்றும் சிலர் செப்டம்பர் 12 என்றும் கூறி வருகின்றனர். பாரதி நினைவு நாளில் இவ்விவாதம் இலக்கியத் தளத்தில் ஆண்டுதோறும் தொடர்ந்து எழுப்பப்பட்டுக் கொண்டே வருகின்றது. எனவே இது எங்கு நேர்ந்த பிழை? என்பதையும் எது உண்மையான தேதி? என்பதையும் ஆராய்வது தேவையாகின்றது.

பாரதி குறித்த வரலாற்று நூல்களில் அவர் மறைந்த தேதி குறித்த பல்வேறு முரண்பாடுகளைக் காண முடிகின்றது.

பாரதியாரின் சிறுவயது தோழராகிய சோமசுந்தர பாரதியார், பாரதியார் குறித்து எழுதிய பாரதி சரித்திரம் என்னும் நூலை 1955 ஆம் ஆண்டு மதுரை கன்னித் தமிழ்ப் பதிப்பகம் வெளியிட்டுள்ளது. இந்நூல் ஆசிரியர் சோமசுந்தர பாரதியார் என்று கையெழுத்தினால் எழுதப்பட்டுள்ளது. இந்நூலில் நாவலர் சோமசுந்தரனார் எழுதிய சரித்திரச் சுருக்கம், வி. சக்கரைச் செட்டியார் எழுதிய ராஜிய வாழ்வு, பரலி சு. நெல்லையப்பர் (1889-1971) எழுதிய பாரதியார் தமிழ்ப்புலமை என்னும் மூன்று கட்டுரைகள் இடம் பெற்றுள்ளன. எனவே இந்நூல் ஒருவரால் எழுதப்படவில்லை . இஃது ஒரு கட்டுரைத் தொகுப்பு நூலாகக் கொள்ளலாம்.

இந்நூலில் பாரதியார் இறப்பு நாளை அவரது நெருங்கிய நண்பரான சோமசுந்தர பாரதியார் 1921-ஆம் வருஷம் செப்டம்பர் மாதம் 11 - சென்னை , திருவல்லிக்கேணியில் இவர் உயிர் துறந்தார் (பாரதியார் சரித்திரம், ப. 22) என்று குறிப்பிடுகின்றார். இதே கருத்தை வி. சக்கரைச் செட்டியாரும் குறிப்பிடுகின்றார். இவ்விருவரினும் ஒரு படி மேலே போய் பரலி சு. நெல்லையப்பர் சென்னை திருவல்லிக்கேணியில் துளசிங்கப் பெருமாள் கோயில் வீதியில் ஒரு வீட்டில் 1921-ஆம் ஆண்டு செப்டம்பர் மாதம் 10-ஆம் தேதி இரவு பாரதியார் உயிர் துறந்தார் (பாரதி சரித்திரம், ப. 47) என்று குறிப்பிடுகின்றார். பாரதியாரின் இறுதிச் சடங்கில் பங்கேற்றவர்களுள் வி. சக்கரைச் செட்டியாரும், பரலி சு. நெல்லையப்பரும் ஆவர். ஆனால் இருவரும் வெவ்வேறு தேதியைக் குறிப்பிடுகின்றனர்.

இன்று எட்டயபுரத்தில் ஆண்டுதோறும் 12-09-1921 அன்றுதான் பாரதியார் நினைவு தினம் கொண்டாடப்பட்டு வருகின்றது. தூத்துக்குடி மாவட்டம் கோவில்பட்டியைச் சேர்ந்த பாரதியார் நினைவு அறக்கட்டளையின் நிறுவனர் முத்து முருகன் பாரதியாரின் நினைவு தினம், அவர் இறந்ததிலிருந்து 91 ஆண்டுகள் வரை செப்டம்பர் 11-ம் தேதியில் தான் அனுசரிக்கப்பட்டு வந்தது. ஆனால் கடந்த 5 ஆண்டுகளுக்கு முன்பு பாரதி மீது பற்று கொண்ட ஒருவர் பாரதியின் நினைவு தினம் செப்டம்பர் 11-ம் தேதியா? 12-ம் தேதியா? எனத் தகவல் அறியும் உரிமைச்சட்டத்தில் கேள்வி எழுப்பினார். "1921, செம்டம்பர் 11-ம் தேதி நள்ளிரவு 1.30 மணிக்குப் பாரதி மறைந்ததால், அது 12-ம் தேதியாகி விடுகிறது. எனவே பாரதியார் நினைவுநாள் செப்டம்பர் 12-ம் தேதிதான்" எனச் சென்னை மாநகராட்சி பதில் வழங்கியதோடு பாரதியாரின் இறப்புச்

சான்றிதழ் நகலையும் வெளியிட்டது. அதன் அடிப்படையில் தூத்துக்குடி மாவட்ட செய்தி மக்கள் தொடர்புத்துறையின் பராமரிப்பின் கீழ் எட்டயபுரத்தில் உள்ள பாரதியாரின் பிறந்த வீடு மற்றும் மணிமண்டபத்தில் உள்ள கல்வெட்டுகளில் பாரதியாரின் பிறந்த வீடு மற்றும் மணிமண்டபத்தில் உள்ள கல்வெட்டுகளில் பாரதியின் மறைவு நாளை 11-ம் தேதியிலிருந்து 12-ம் தேதியாக திருத்தம் செய்யப்பட்டது (Vikatan.com, 11tr Sep.2017) என்னும் செய்தியைக் குறிப்பிடுகின்றார்.

ம.ரா.போ. குருசாமி (11 ஆம் நாள் இரவு) - 12 ஆம் நாள் யாமம் - 1-30 மணி அளவில் புகழுடம்பு எய்துதல் அகவை 39 (பாரதி பாடல்கள் ஆய்வுப் பதிப்பு, Ixox) என்னும் செய்தி 12ஆம் தேதியை உறுதி படுத்துகின்றது. இதற்கு மேலும் ஒரு அரணாக பாரதியின் மகள் சகுந்தலா பாரதி ஆயிரத்துத் தொள்ளாயிரத்து இருபத்தொன்றாம் ஆண்டு செப்டம்பர் மாதம் பதினொன்றாம் தேதி சாயங்காலம், விளக்கேற்றும் நேரம், இன்றிரவு தப்பினால் தான் பிழைப்பார். அதாவது இனிமேல் நம்பிக்கையில்லை என்று வைத்தியர் சொல்லிவிட்டார் (ர்ttp://ilakkiyapayilagam.blogspot.com) என்னும் செய்தி செப்டம்பர் 12ஆம் தேதியை உறுதிபடுத்துகின்றது. முத்து. குணசேகரன் என்பார் இரண்டு மாதங்களுக்குப் பின்னர்ப் (1921 ஜூன் மாதம் பார்த்தசாரதி கோயில் யானையால் தூக்கி வீசப்பட்டார்) பாரதி கடுமையான நோய்வாய்ப்பட்டார். இப்பொழுது சீதபேதியும் சேர்ந்து கொண்டது. 1921 செப்டம்பர் 21 ஆம் தேதி நடுநிசியில் பாரதிக்கு உடல்நிலை மிகவும் கவலைக்கிடமானது (சிந்தனைச் சிற்பி ம. சிங்காரவேலரும் தந்தை பெரியாரும், ப. 52) என்று குறிப்பிடுகின்றார். இதில் 21ஆம் தேதி எனக் குறிப்பிட்டிருப்பது எழுத்துப் பிழையாதல் வேண்டும். எனவே 12ஆம் தேதியாகக் கொண்டால் இவரது கருத்துப்படி பாரதியார் மறைந்த தினம் 1921, செப்டம்பர் 12 என்றாகிறது.

க. கைலாசபதி (1933-1982) விபுலானந்தரும் பாரதியாரும் என்னும் கட்டுரை ஒன்றில் நம்மிடம் உள்ள அன்பினால் அல்லவா யானை நசுக்கவில்லை என்று சொன்ன பாரதி 1921 செப்டம்பரில் குணமானார். ஆனால் செப்டம்பர் 12ஆம் திகதி வயிற்றுக் கடுப்பு நோயால் அதிகாலை புகழுடம்பு எய்தினார் (விபுலாநந்த அடிகளார் நூற்றாண்டு, விபுலாநந்தம், ப. 30) என்று குறிப்பிடுகின்றார். எனவே, பாரதியாரின் நெருங்கிய நண்பர் சோமசுந்தர பாரதியார், வி. சக்கரைச் செட்டி ஆகியோர் செப்டம்பர் 11 எனக் குறிப்பிடும் செய்தியும், பரலி சு.

நெல்லையப்பர் செப்டம்பர் 10 எனக் குறிப்பிடும் செய்தியும் தவறானவையாகக் கருத இடமுள்ளது. ஒருவேளை அவர்கள் தமிழ் நாள் கணக்குப்படி குறிப்பிட்டார்களா? அஃதாவது ஒருநாள் என்பது சூரிய உதித்து அடுத்த நாள் சூரியன் உதிக்கும் வரை ஒருநாள் (காலை 6 மணிமுதல் அடுத்த நாள் காலை 6 மணிவரை) எனக் கணக்கிடப்படுகிறது. அதன் அடிப்படையில் செப்டம்பர் 11 என்று குறிப்பிடுவது அடுத்தநாள் (12.9.1921) சூரிய உதயம் வரை செல்லுபடியாகும். எனவே இதன் அடிப்படையில் செப்டம்பர் 11 எனப் பதிவிட்டுவிட்டனரா எனத் தெரியவில்லை . மேலும் அவர்கள் தரும் குறிப்பில் ஆங்கில தேதியைக் குறித்திருப்பதும் தமிழ் மாதமோ தேதியோ இடம்பெறவில்லை என்பதைக் காணும்போதும் அவர்கள் தமிழ்வழித் தேதி அடிப்படையில் பாரதியார் மறைவு குறித்துக் தம் கருத்தினைக் கொண்டிருக்கவில்லை எனக் கருத இடமுள்ளது.

பாரதியார் இறந்தபோது அவர் முகத்தில் மொய்த்த ஈக்களை விட இறுதிச் சடங்கில் பங்கேற்றவர் எண்ணிக்கைக் குறைவு என்று அறியப்படும் செய்தி அவர் இறந்தபோது அந்நாளைச் சிலரே ஞபாகம் வைத்திருக்கக்கூடும். ச. சோ. பாரதியார், பரலி சு. நெல்லையப்பர் ஆகியோர் பாரதி குறித்த கட்டுரைகளைப் பின்னாளில் எழுதியிருக்கக்கூடும் எனவே பாரதியார் இறப்பு குறித்த அவர்களது நிலைப்பாடு வெவ்வேறாக அமைந்துவிட்டது. இவர்களது கூற்றையே பின் வந்தோறும் செப்டம்பர் 11ஆம் தேதியாக நினைத்து விட்டனர்.

இதேபோல் இலங்கை நாட்டுத் தமிழ் அறிஞர் விபுலானந்த அடிகள் (1892 1947) பிறந்த தினம் குறித்து இதே சர்ச்சைக்குரிய கருத்து நிலவி வருகின்றது. விபுலானந்தர் பிறந்தது 26-3-1892 என்றும், 27-3-1892 என்றும் வலைதளங்களில் கருத்து நிலவுகின்றது. விபுலானந்தரின் நெருங்கிய நண்பர்களான க. வெள்ளைவாரணரும் (1917-1988), நீ. கந்தசாமியாரும் (1898-1977) 29-3-1892 என்று குறிப்பிடுகின்றனர். இவர்களது கருத்தை மறுத்த இலங்கைப் பேராசிரியர் சி. தில்லைநாதன் பிறப்புப் பத்திரத்தில் இவரது பிறந்த தினம் 3-5-1892 (உள்ளக்கமலம், ப. 3) என்று குறிப்பிட்டுள்ளார். ஆனால் க. கைலாசபதி சாமித்தம்பிக்கும் கண்ணம்மையாருக்கும் புதல்வனாக 16-3 1892இல் பிறந்து 'மயில்வாகனன்' என்னும் பிள்ளைத் திருநாமத்துடன் வளர்ந்தார் சுவாமி விபுலானந்தர் அவர்கள் (விபுலாநந்த அடிகளார் நூற்றாண்டு, விபுலாநந்தம், ப. 16) என்று குறிப்பிடுகின்றார்.

இவ்வாறு தமிழ் வரலாற்றில் பல்வேறு அறிஞர்கள் பிறப்பு, இறப்பு குறித்த முறையான தகவல் திரட்டல் இல்லாததைக் காணும்போது யாரை நொந்து கொள்வதென்றே தெரியவில்லை. பாரதியார் நினைவு நாளை அவரது இறப்புச் சான்றிதழில் குறிப்பிட்ட தேதியைக் (12-09-1921) நினைவு நாளாகக் கொண்டாடுவதுதான் சரியானதாகத் தோன்றுகின்றது. பாரதி குறித்த அரசு விழாக்களும் இத்தேதியில் கொண்டாடுவதுதான் முறையானதாக அமையும்.

வரலாற்றில் மறைக்கப்பட்ட பாரதி நண்பர்?

பாரதியார் வாழ்வில் எண்ணற்ற நண்பர்கள் தேசிய அளவிலும் தமிழக அளவிலும் இருந்துள்ளனர். பாரதி தம் இறுதி நாட்களைச் சென்னையிலுள்ள திருவல்லிக்கேணியில் கழித்தார் என அறிகிறோம். பார்த்தசாரதி கோயில் யானையால் தாக்கப்பட்ட பாரதியார் உடல் நலக்குறைவு ஏற்பட்டு, பின்னர் பிற நோய்களும் தொற்றிக் கொண்டதன் காரணமாக உயிரிழந்தார். பாரதி இறக்கும் தருவாயில் அருடன் இருந்தவர்கள் சிலரே. இச்செய்தியை முத்து குணசேகரன் 1921 செம்டம்பர் 12ஆம் தேதி நடுநிசியில் பாரதிக்கு உடல்நிலை மிகவும் கவலைக்கிடமானது. பாரதி இப்பொழுது முழுமையும் மயக்க நிலையிலேயே இருந்தார். சிங்காரவேலர் வீட்டில் குடியிருந்த எருக்கூர் நீலகண்ட பிரம்மச்சாரி, பரலி சு. நெல்லையப்பர், பாரதியின் உறவினர் இலட்சுமண ஐயர் இன்னும் சிலரும் உடன் இருந்தனர். இரவு, ஒரு மணிக்குமேல் சிங்காரவேலர் பாரதியைப் பார்க்க மிகவும் கவலையுடன் வந்து நின்றார். இப்பொழுது பாரதியின் மயக்கம் தெளிந்தது. அருகில் நின்றிருந்த சிங்காரவேலரைப் பாரதி மிகவும் பரிவுடன் பார்த்து அவரை அருகே வரவேண்டுமென அழைத்தார். அருகில் சென்றமர்ந்த சிங்காரவேலர் மடியில் பாரதி பரிவுடன் தலை சாய்த்துக் கொண்டு குடிப்பதற்கு ஏதாவது வேண்டுமென்று சைகை செய்தார். வீட்டினர் கொடுத்த பாலைச் சிங்காரவேலர் கொடுத்தார். சிங்காரவேலர் மடியில் சாய்ந்த பாரதி மீளாத் துயிலில் ஆழ்ந்தார் (சிந்தனைச் சிற்பி ம. சிங்காரவேலரும் தந்தை பெரியாரும், பக். 52, 53) என்று குறிப்பிடுகின்றார்.

மேலும் பாரதியாரின் இறுதிச் சடங்கில் வி. ஹரிஹர சர்மா, எருக்கூர் நீலகண்ட பிரமச்சாரி, சிங்காரவேலர் நண்பர் சக்கரைச் செட்டி, சுரேந்திரநாத் ஆர்யா, சிங்காரவேலர் குடும்ப நண்பர்கள் மண்டையம் சீனிவாச்சாரி, சின்ன காஞ்சிபுரம் கிருஷ்ணசாமி சர்மா ஆகியோர் பங்கேற்றவர்களாகக்

குறிப்பிடுவதுடன் பாரதியின் ஈமச் சடங்கிற்குச் செலவிட்டவர் ம. சிங்காரவேலர் என்னும் குறிப்பையும் கொடுக்கின்றார். இப்படிப் பாரதியின் நெருங்கிய நண்பராக விளங்கியவரும் பொதுவுடமைவாதியுமான சிங்காரவேலர் (1860-1946) பாரதி குறித்த வரலாற்று நூல்களில் இடம்பெறாமை வருந்தத்தக்க ஒன்றே. அந்த மகாகவிஞன் பாரதியின் உயிர் இந்த மடியிலேதான் பிரிந்தது என்று சிங்காரவேலர் அடிக்கடித் தனது பேத்திகளிடம் (ம. ஜெய்பாய், ம. ஜ¨பாய்) புலம்பிய செய்தியையும் அவர் குறிப்பிட்டுள்ளார். இச்செய்தியைத் தவிர ம. சிங்காரவேலர் குறித்த பதிவுகள் பாரதி குறித்த வரலாற்று நூல்களில் இடம்பெறவில்லை என்பதும் நினைவுகூரத்தக்கது.

சிக்க வைத்தாரா சீனிவாசனை?

பாரதியார் 9-5-1906 அன்று தொடங்கப்பட்ட இந்தியா என்னும் இதழில் துணை ஆசிரியராகப் பணியாற்றியதோடு ஆங்கிலேயருக்கு எதிராகத் தொடர்ந்து கட்டுரைகள் எழுதி வந்துள்ளார். இவ்விதழ் குறித்து முத்து. குணசேகரன், 1906 இல் நடத்தப்பட்டு வந்த தமிழ் வார இதழ் 'இந்தியா' தொல்லைக்கு உள்ளாக்கப்பட்டது. அதன் ஆசிரியர் திருவல்லிக்கேணி மண்டையம் சீனிவாசன் கைது செய்யப்பட்டார். இந்தியாவின் பத்திரிகை உரிமையாளர் மண்டையம் திருமலாச்சாரியும், இந்தியா பத்திரிகை ஆசிரியர் பாரதியாரும் கைதாகாமல் புதுச்சேரிக்குத் தப்பிச் சென்றனர். சிந்தனைச் சிற்பி ம. சிங்காரவேலரும் தந்தை பெரியாரும், ப. 39) என்று குறிப்பிடுகின்றார். இதில் அவர், சீனிவாசன் என்பாரை ஆசிரியராகக் குறிப்பிடுவதால் பாரதியார் இவ்விதழில் துணை ஆசிரியராக இருந்திருக்க வேண்டும். 1906 ஆம் ஆண்டு தொடங்கப்பட்ட இந்தியா இதழில் 29.02.1908 முதல் 27.06.1908 வரை (ஏறக்குறைய மூன்று மாதங்கள்) வெளிவந்த 20 கட்டுரைகள் ஆங்கில அரசுக்கு எதிரானவையாகக் கருதப்பட்டு இதழின் ஆசிரியர் சீனிவாசனுக்கு ஐந்து ஆண்டு சிறை தண்டனை கிடைத்தது. பாரதியாரும் இந்தியா இதழின் உரிமையாளர் மண்டையம் திருமலாச்சாரியும் புதுச்சேரிக்குத் தப்பிச் சென்று விட்டனர் என்று கூறப்படுகின்றது.

இது பற்றி ஸ்ரீ இராமானுஜலு நாயுடு என்பார் தம்மை நம்பிய ஒருவரை ஆபத்தில் சிக்கவைத்துவிட்டுத் தாம் தூரப் போய்விட்டமை பாரதியாரின் சரித்திரத்தில் ஒரு களங்கமேயாகும் (விடுதலை வேள்வியில் தமிழகம், ப. 323). என்று குறிப்பிடுகின்றார். பாரதியாருடன் தப்பிச் சென்ற

இந்தியா இதழின் உரிமையாளர் மண்டையம் திருமலாச்சாரியை குற்றம் சாட்டாமல் வறுமையின் பிடியில் வயிற்றுப் பிழைப்பிற்காகவும் தேச நலனுக்காகவும் எழுதி வந்த பாரதியார் மேல் பழிபோடுதல் எவ்வாறு பொருந்தும்? இத்தனைக்கும் பாரதியார் துணை ஆசிரியரே.

இங்கு ஒரு செய்தியை ஒப்பிட்டுப் பார்க்க வேண்டும். தமிழக வரலாற்றில் திருநெல்வேலி புரட்சி 13-3-1908 அன்று வெடித்தது. வந்தே மாதரம் இதழைத் தொடங்கிய வங்கதேச வீரர் பிபின் சந்திரபால் கைது செய்யப்பட்டு ஆறு மாதங்களுக்குப் பிறகு விடுதலையானார். இதனை நாடு முழுவதும் சுயராஜ்ய நாளாகக் கொண்டாடினர். தூத்துக்குடி திருநெல்வேலிப் பகுதிகளில் வ.உ.சிதம்பரனாரும் அவரது நண்பர்கள் சுப்பிரமணிய சிவாவும் பத்மநாபனும் இவ்விழாவைக் கொண்டாட திருநெல்வேலி ஆட்சியரிடம் அனுமதி கோரினர். அனுமதி மறுக்கப்படவே அதனை மீறி பொதுக்கூட்டம் நடத்தியதால் மூவரும் கைது செய்யப்பட்டனர். இதனை அறிந்த மக்கள் கொந்தளித்து தொழிற்சாலை வேலை நிறுத்தம், கடையடைப்புப் போராட்டம் போன்றவற்றை நடத்தினர். காவல் நிலையம், நகராட்சி அலுவலகம் முதலியன சூறையாடப்பட்டன. இதில் நடந்த துப்பாக்கிச் சூட்டில் 4 பேர் உயிரிழந்தனர்.

இதன்காரணமாக ஆங்கில அரசு இந்திய விடுதலையாளர்கள் மீது மேலும் கோபம் கொண்டிருந்ததோடு, விடுதலை உணர்வை மக்களிடம் கொண்டு சேர்க்கும் இதழ்களின் மீது தனது அடக்குமுறையை ஏவிவிட்டிருந்தது. இப்புரட்சி நடந்த காலகட்டமும் (13-3-1908), இந்தியா இதழில் (29.02.1908 முதல் 27.06.1908 வரை) வெளிவந்த கட்டுரைகளும் ஒத்த காலத்தன. எனவே, அன்றிருந்த சூழலில் தப்பித்தால் போதும் என்ற நிலை இருந்தது. ஸ்ரீ இராமானுஜலு நாயுடு சீனிவான் கைதானதற்குப் பாரதியைக் காரணம் காட்டுவதும், சீனிவாசனை ஆபத்தில் சிக்க வைத்தவர் என வருணிப்பதும் பொருந்தாது என்பதை மனசாட்சி உள்ளவர் நினைத்துப் பார்க்க வேண்டும்.

நிறைவாக

1935 இல் கல்கி ரா. கிருஷ்ண மூர்த்தி (1899-1954) பாரதியாரை எழுத்து வாசனை இல்லாதவர் என்றும், மகாகவி அல்ல. அவரை நல்ல கவி என்று வேண்டுமானால் சொல்லாம் என்று எழுதினார். இதனைப் பாரதி பற்றாளர்களால் ஏற்றுக்கொள்ள

முடியவில்லை. கல்கிக்கு எதிராகப் பாரதி மகாகவியே என்னும் கருத்தை நிலைநாட்ட வ. ராமசாமி (1889-1951) ஜி சுப்பிரமணிய அய்யரால் (1855-1916) 1882 இல் தொடங்கப்பட்ட சுதேசிமித்திரனில் கட்டுரைகள் பல எழுதி வந்ததோடு தனது சீடர்களான பி.எஸ். ராமையா, ந. சிதம்பரசுப்பிரமணியன் போன்றோரைக் கல்கிக்கு எதிராக எழுதத் தூண்டினார். இதற்கு மணிக்கொடி இதழ் (17-09-1933) எழுத்தாளர்கள் துணை நின்றனர். கு.ப. இராஜகோபாலன் (1902-1944) பாரதி மகாகவியே என்னும் நூலை எழுதி வெளியிட்டார். இவ்வாறு பாரதி இறப்பிற்குப் பிறகு அவர் மீதான முரண்பாடுகள் எழுத்துலகம் தொடர்ந்து விவாதித்து வந்துள்ளது. எனவே மேற்கண்ட பல்வேறு சான்றுகளின் அடிப்படையில் பாரதியாரின் மறைவு தினத்தை 12-09-1921 என்று கொண்டாடுவது பொருத்தமானதாகும். பாரதியாரின் வரலாற்றில் அவரது நெருங்கிய நண்பரான ம. சிங்காரவேலருக்கு உரிய இடம் கொடுக்கப்பட வேண்டும். ஸ்ரீ இராமானுஜலு நாயுடு அன்றிருந்த சூழலை எண்ணிப் பார்க்காமல் பாரதியாரின் மேல் ஒரு வரலாற்றுப் பிழையை ஏற்படுத்தி விட்டார். இவையெல்லாம் இனியாவது திருத்தப்பட வேண்டும்.

பார்வை நூல்கள்

1. உள்ளக்கமலம், 1992. வவுனியா, இலங்கை : சுவாமி விபுலானந்தர் நூற்றாண்டு விழாச் சபை.
2. சோமசுந்தர பாரதியார் ச. சோ., 1955. மதுரை: கன்னித்தமிழ்ப் பதிப்பகம்.
3. குணசேகரன் முத்து., 2012. சிந்தனைச் சிற்பி ம. சிங்காரவேலரும் தந்தை பெரியாரும், சென்னை : உலகத்தமிழாராய்ச்சி நிறுவனம்.
4. குருசாமி ம.ரா.போ. (ப.ஆ.), 2013. பாரதி பாடல்கள் ஆய்வுப் பதிப்பு, தஞ்சாவூர்: தமிழ்ப் பல்கலைக்கழகம்.
5. wttp://ilakkiyapayilagam.blogspot.com
6. www.vikatan.com
7. wttp://ta.m.wikipedia.org

Cape Comorin Publisher

Website: www.capecomorinpublisher.com

Certificate

This is to certify that the chapter titled பூர்ணிமை சிவகாசை தொகுப்பு உணர்த்தும் விளிம்புநிலை மக்களின் authored by முனைவர் மு. சுபாஷினி, வாழ்வியல் நிலை உதவிப் பேராசிரியர், நடுவண்பொன்னை, தேசியக்கல்லூரி, திருச்சி is published in the book titled விளிம்பு நிலை மக்கள் is published on

February 2022.

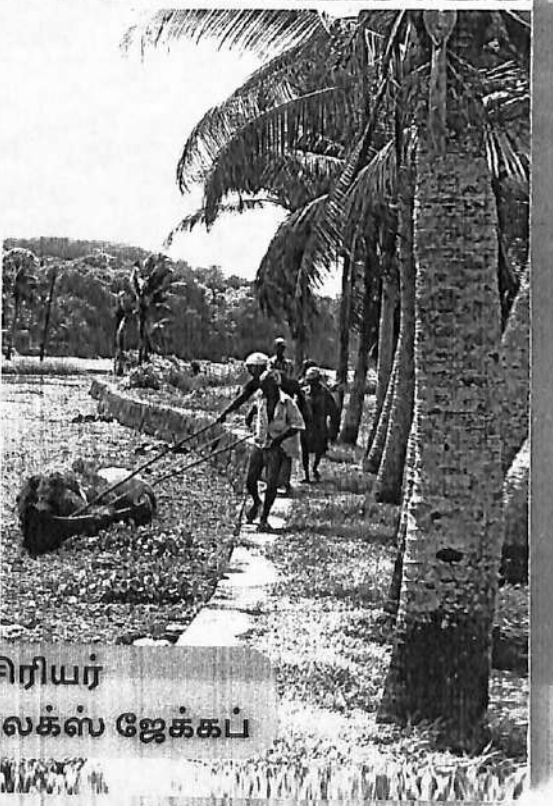
Dr. S. Alex Jacob
Editor

Dr. R. S. Regin Silvest
Managing Director

CAPE COMORIN PUBLISHER
Kanyakumari
Tamil Nadu, India
www.capecomorinpublisher.com
: 9442818648

Email: capecomorinpublisher@gmail.com Contact +91 9442818648

விளிம்பு நிலை மக்கள்



பதிப்பாசிரியர்

முனைவர். எஸ். அலக்ஸ் ஜேக்கப்

TITLE : விளிம்பு நிலை மக்கள்

ISBN : 978-93-91553-93-7

Editor : முனைவர் எஸ்.அலக்ஸ் ஜேக்கப்

Price : 350/- INR

Edition : First Edition

Moth & Year : February 2022

Total Pages : 210

Published by : Cape Comorin Publisher
Kanyakumari, Tamilnadu, India

Website : www.capecomorinpublisher.com

Printed at : NSK Digital World
Kanyakumari, Tamil Nadu, India

Copyright © 2022 by Cape Comorin Publisher, All rights Reserved.

No part of this publication may be reproduced or transmitted in any form o
by any means, electronic or mechanical, including photocopying, recording
or any other information storage and retrieved without prior permission in
writing from the publishers.

Concerned author is solely responsible for his views, opinions, polici
copyright infringement, legal action, penalty or loss of any kind regardi
their content. The publisher will not be responsible for any penalty or loss
any kind if claimed in future. Contributing author has no right to demand a
royalty amount for his content.

பதிப்பாசிரியர் உரை

ஒரு மையத்தின் ஓரத்தினை விளிம்பு எனலாம். சாதி, சமூக, பொருளாதார, தொழில் ரீதியாக முன்னேறியவர்கள் மேல்தட்டு மக்கள் எனவும் இவற்றில் கீழ்நிலையிலுள்ளவர்கள் அடித்தட்டு அல்லது விளிம்பு நிலை மக்கள் எனவும் அடையாளப்படுத்தப்படுகின்றனர். பொதுவாக சமூகத்தில் பின்தங்கிய நிலையினராக உள்ளவர்களை விளிம்புநிலையினர் எனலாம். ஒடுக்கபடட நிலையிலுள்ள பெண்களும் இதில் அடங்குவர். மேல்தட்டு சாதியில் பிறந்த பெணணாக இருந்தாலும் அவள் ஆணாதிக்கத்தினால் ஒடுக்கப்படுபவளாக இருந்தால் அவளும் விளிம்புநிலைப் பெண்ணாகவே கருதப்படுவாள்.

விளிம்புநிலை வாழ்க்கை என்பது எப்போது வேண்டுமானாலும் தடம் புரளலாம். பாதுபாப்பற்ற சமூக மதிப்பில்லாத ஒரு வாழ்வியலையே விளிம்பு நிலை சித்தரிக்கின்றது. விளிம்புநிலை என்ற சொல் இலக்கிய வட்டத்தில் இன்றைய நிலையில் அதிகம் பேசப்படுகின்ற சொல். நாம் இன்றைய வாழ்க்கையைச் சீர்படுத்த முந்தைய வரலாற்றை அறிவது அவசியம். தமிழர்தம் வரலாற்றை ரசனையோடு இலக்கியங்கள் பதிவு செய்துள்ளன. தமிழ்ச் சூழலில் தமிழரும் இம்மண்ணும் அடைந்த வெற்றிதோல்விகளை இவ்இலக்கியங்களை ஆராய்வதன் வழியாக உணர இயலும். விளிம்புநிலை மக்கள் குறித்தும் தமிழ் இலக்கியங்கள் பதிவு செய்துள்ளன. ஆகவே, காலந்தோறும் விளிம்பு நிலையில் தள்ளப்பட்ட மக்களின் வாழ்வியலையும் விளிம்புநிலையினர் குறித்த விரிவான அறிமுக்கத்தையும் இன்றைய உலகிற்கு எடுத்தியம்பும் நோக்கில் இப்புத்தகத்தைப் பதிப்பித்து வெளியிடுகிறேன்.

அன்புடன்
முனைவர். எஸ்.அலக்ஸ் ஜேக்கப்
பதிப்பாசிரியர்

உள்ளடக்கம்

01. விளிம்பு நிலையில் பீடித்தொழிலாளர்கள் (முக்கூடல், ஆலங்குளம்)
திரு. உ.தி. சங்கரநாராயணன் பி லிட்

02. தமிழ்ச்செல்வியின் கீதாரி நாவல் காட்டும் விளிம்பு நிலை மக்கள்
முனைவர் ச.பொன்ஜெயந்தி

03. தோல் நாவலில் விளிம்புநிலை மாந்தர்கள்
திருமதி. செள. சுருதி

04. 'இடக்கை' புதினத்தில் விளிம்புநிலை மாந்தர்கள்
முனைவர் க.புவனேஸ்வரி

05. பூர்ணிமை சிறுகதை தொகுப்பு உணர்த்தும் விளிம்புநிலை மக்களின் வாழ்வியல் நிலை
முனைவர் மு.சுபாஷினி

06. கி.ராஜநாராயணனின் கிடை புதினத்தில் விளிம்புநிலை மக்கள்
முனைவர் இரா.சுந்தரவேல்

07. செங்கல் சுள்ளை – விளிப்பு நிலை மக்களின் வாழ்வு ஓவியங்கள்
முனைவர். ஜா. லாலி ஏதேஷ்

08. பாக்கியம் சங்கருடைய நான்காம் சுவரில் விளிம்புநிலை மக்கள்
முனைவர் அர.மீனா

09. மூங்கில் தெப்பம் - வெளிப்படுத்தம் விளிம்பு நிலை வாழ்வியல் கூறுகள்
முனைவர். ஜா.ஞா.ஜோணி ஜெப மலர்

10. அஞ்ஞாடி நாவலில் விளிம்பு நிலை மக்கள்
ஜெ.சுஜாதா

11. எரியும் பனிகாடு நாவலின் வழி தோட்டத் தொழிலாளர்களின் வாழ்வியல்
மு.சத்தியா

12. குறத்திமுடுக்கு நாவலில் பாலியல்தொழிலாளர் வாழ்வியல்
முனைவர் க.ஜெயசீலன்

3. 'பிஞ்சுகளும் போரிடும்' மற்றும் "அண்ணன் எப்பச் சாகும்' சிறுகதைகளில் விளிம்பு நிலை மக்கள்" 89
முனைவர் செ.நாகஜோதி

4. சங்க கால குடிகளும் விளிம்பு நிலையும் 97
முனைவர். மு.விஜயசாரதா

5. தகனம் நாவலில் காணலாகும் விளிம்புநிலை மக்களின் வாழ்வியல் 102
முனைவர்.ச.சதானந்தவேல்

6. "தேடல் நாவலில் விளிம்பு நிலை மாந்தர்கள்" 109
கு.சிந்து

7. பூமணி சிறுகதைகளில் விளிம்பு நிலை மக்களின் வாழ்வியல் 112
முனைவர்.ந.சுசிலா

8. அழகிய பெரியவனின் தீட்டு குறுநாவலில் விளிம்புநிலை மக்கள் வாழ்வியல் 119
அ. சகாய ஜெசி கலா

9. இராஜம் கிருஷ்ணனின் "கரிப்பு மணிகள் நாவலில் உப்பளத் தொழிலாளர்களின் நிலை" 128
முனைவர் சு. சீலா

10. அன்றாட வாழ்க்கையில் விளிம்பு நிலை மக்களின் போராட்டங்கள் 134
திருமதி. செ. இராஜேஸ்வரி

சல்மாவின் மனாமியங்களில் விளிம்புநிலை மாந்தர்கள் 138
முனைவர் ஆ. இராஜலெட்சுமி

12. கூட்டுக்குஞ்சுகள் சுட்டும் குழந்தைத்தொழிலாளர்கள் 144
முனைவர் கோ.இராமச்சந்திரன்

13. ஜெயகாந்தன் படைப்புகளில் விளிம்புநிலை மக்கள் 152
முனைவர் கூ.முத்தையன்

மணல்வீடுகள்' புதினத்தில் பெண்ணின் அவலநிலை 159
முனைவர் கோ. தங்கச்செல்வி

கோவேறு கழுதைகள் புதினத்தில் சலவைத் தொழிலாளர் அவலநிலை 163
முனைவர் க. முருகேசன்

பூர்ணிமை சிறுகதை தொகுப்பு உணர்த்தும் விளிம்புநிலை மக்களின் வாழ்வியல் நிலை

முனைவர் மு.சுபாஷினி,
உதவிப்பேராசிரியர், தமிழாய்வுத்துறை,
தேசியக்கல்லூரி (தன்னாட்சி), திருச்சிராப்பள்ளி

ஆய்வுரை

மனித சமுதாயத்தில் பல்வேறு தொழில்கள் இருப்பினும் எல்லா தொழில்களும் ஒரு போல் ஆகிவிடாது. தொழில் செய்வதைப் பார்க்கும்போதே ஒரு அவலத்தையும், வெறுப்பையும் தரும்படியான தொழில்கள் ஏராளமானவை. குடும்பத்தை நடத்துவதற்குப் போதுமான வருமானம் இல்லாத தொழில்களும், அதனால் மனித சமூகம் படும் துன்பத்தையும் வெளிக்கொணரும் வகையில் படைப்பாளர்கள் விளிம்புநிலை மக்கள் பற்றிய கருத்துகளைக் கூற முனைந்துள்ளனர். சமுதாய மாற்றத்துக்கு விழிப்புணர்வை ஏற்படுத்த கையாளுகின்ற உத்திமுறையில் பற்பல நூல்கள் வெளிவந்துள்ளன. இத்தகைய வரிசையில் ஆசிரியர் க.வீரபாண்டியன் அவர்கள் தம் 'பூர்ணிமை' சிறுகதை தொகுப்பில் எத்தகைய விளிம்புநிலை மக்களைப் பற்றிய கருத்துகளைப் பதிவுசெய்துள்ளார் என்பதை ஆராயும் நோக்கில் இவ்வுரை அமைந்துள்ளது.

விளிம்புநிலை மக்களின் வாழ்வியலை பேசுகிறது க.வீரபாண்டியன் அவர்களின் 'பூர்ணிமை' எனும் சிறுகதை தொகுப்பு. ஆசிரியர் இந்திய ஆட்சிப்பணி அதிகாரியாக இருந்த போதிலும், தம்மை எழுத்தாளராகவே வெளியுலகிற்கு அடையாளம் காட்டுவதில் முனைப்புடையவர். வாழ்வின் கடைக்கோடி நிலையில் இருக்கும் மக்களையே விளிம்புநிலை மக்கள் என்பர். அம்மக்களின் வாழ்வியலையும், வாழ்க்கை மாற்ற முடியாத இழிவான சூழல்களையும் வெளிக்கொணரும் நோக்கில் 'பூர்ணிமை' சிறுகதை தொகுப்பில் ஒவ்வொரு கடைநிலை தொழில் செய்யும் மக்களின் வாழ்க்கை முறையைச் சமுதாய மக்கள் உணரும்படி செய்துள்ளார். 'பூர்ணிமை' 12 சிறுகதை தொகுப்பு காலத்தால் அழியாத கல்வெட்டு எழுத்துகளாகப் பதிவுபெற்றுள்ளன.

கடனால் அல்லல்படும் மக்களின் வாழ்வியல்

நூற்பாலைப் பணியாளராக வேலை பார்த்து வருபவன் இராமன். மாத வருமானம் குடும்பத்திற்குப் போதுமானதாக இல்லாமல் இருப்பதால், அரசு அதிகாரிகள், மற்றும்

ஜனித்த [illegible] வந்து [illegible] வ
னுவைத்து [illegible] வாழ்க்கை நடத்தும் [illegible]
வாழ்ந்த.

சந்தனா டீச்சருக்கு கல்யாணம் இராமன் மட்டுமல்லாது வனஜாவை போ
துப்பரிவு வேலை பார்க்கும் வசுமதியே கடன்பட்டிருக்காதோ இருந்து வந்த
இந்தக் கல்யாண காலங்களாய் தலைமுறை தலைமுறையாய் நிரந்தர பெ
துன்பங்களுக்கு அடையாளங்களாக ஆணாண் வாங்கும் சம்பளம் மற்றும்
நாடகங்களுக்கு தாக்கப் பிடிக்காததால் மீதம் உள்ள நாடகளை ஒடுவதற்கு
கல்யாணச் சடங்குகளை நடத்தினர்.

இத்தகைய சூழலில் மாத இறுதியில் குப்பனும், பெருமாளும் இரா
விட்டுவிட்டுக் கடன்வாங்க முந்திக் கொண்டு சென்றனர். இராமன் தனக்குக் க
கிடைக்காமல் போய்விடுமோ என்ற அச்சத்தில் மிதிவண்டியைத் தம்முடைய
பலத்தையும் பயன்படுத்தி அழுத்தினான் தான் மிதிவண்டியில் செல்லும்போ
கந்துவட்டிக்காரன் 'வழிவிட்டான்' வீட்டை விரைந்து அடைத்துவி வேண்டும்
எண்ணத்தில் மூச்சடக்க மிதிவண்டியை அழுத்திச் சென்றான். அப்போது
உறவினருள் ஒருவன் முன்பு வழிவிட்டானிடம் கடன்வாங்கியதையும். அவனு
உடல்நலக் குறைவால் 6 மாதங்கள் வேலைக்குச் செல்லாததாலும். வட்டித் தி
இருப்பதாலும் வழிவிட்டான் வீட்டின் முன்பு தெரிய வேண்டும் என்பதற்கா
வழிவிட்டான் மனைவி விளக்குமாற்றைக் கொண்டு அடித்துத் தீர்த்தாள். தம் மக
கிராமத்துக்குப் பண்ணை வேலைக்குச் சோத்துவிட்டு தன்னை விடுவித்துக் கொ
நினைவுக்கு வரத் திடுக்கிட்டான் இராமன்.

தாம் சிந்தனையை ஓடவிட்ட வண்ணம் நினைத்துப் பார்த்துக் கொ
வருமோதே வழிவிட்டான் வீட்டை வந்தடைந்தான். கேட்டில் நின்று சாமி. சா
என்று கத்தினான். ஆள் நடமாட்டம் எதுமில்லாததால் ஜன்னல் வழியே வீட்டை
பார்த்தான். அவ்வளவு நாட்களில் அன்று தான் வழிவிட்டான் வீட்டின் உட்புற
பார்க்கும் பாக்கியம் இராமனுக்குக் கிடைத்தது. அவ்வழியே முதாட்டி வ
அவளிடமிருந்து 'வழிவிட்டான்' விளக்குத்தாண வங்கிக்குச் சென்றிருப்பதை அ
பேங்கிற்கு மிதிவண்டியைத் திருப்பி வேகமாகச் சென்றான். பணம் வாங்கிப் போ
போனால் மனைவி சுப்பம்மாள் கத்தித் தீர்த்துவிடுவாள். அவளும் பாவம்
செய்வாள் ஒரு நாளைக்கு இரண்டு வேளை உணவு உண்ணுவதே பெரும்
இருக்கிறது எப்படிக் குடும்பம் நடத்துவாள் என்று எண்ணியதும் மிதிவண்டி
ஆரம்பித்தது.

'விளக்குத்தாண' கிளை வங்கி என்றவுடன் இராமனும் முன்னொரு
நடந்த சம்பவம் நினைவுக்கு வந்தது. அதை எப்படி மறக்க முடியும் விடியற்
மெயின் ரோட்டின் கடைகள் திறப்பதற்கு முன்பு பெருக்கிச் சுத்தம்
வேண்டுமென்று இராமன் சரசுவென்று வேளையில் கவனத்தைச் செலுத்தி
'சின்னு' கையில் தகர முறம் கொண்டு இராமன் குவிக்கும் குப்பைகளை அ
சூப்பைத் தொட்டியில் போட்டுக்கொண்டே வந்தாள். அப்போது 'சின்னு'

ட்டம் அதிகமாகிக் கொண்டே இருக்கிறது. பாலித்தின் கப் போன்ற பொருட்களின்
ப்பைகள் நாளுக்குநாள் அதிகரித்து வருகிறது. ஆனால் நம்ப சம்பளம் மட்டும்
திகரிக்கவே இலலை என்று மனக்குமுறலோடு கூறினாள்.

**"இவ்வளவு கூட்டமும் எங்க இருந்துதான் கிளம்பி வருதோ. சிரிப்பும்
கும்மாளமுமாக ஆணும், பெண்ணும் குழந்தைகள், முதியவர்கள்
கும்பல் கும்பலாகச் செல்வதைப் பார்த்து இராமனிடம் சொன்னாள்
சின்னு. நாளுக்குநாள் கூட்டம் பெருகிக்கிட்டேதான் போகுது.
எல்லாக் கடைகளிலும் கூட்டம் நெறைஞ்சு வழியுது. அட்டைப்
பெட்டிகளும் பிளாஸ்டிக் பேப்பரும் மலைக்கணக்கா குவியுது.
ஆனா, வீதியில் திரியிற நம்ம பொழப்பு மட்டும் எல்லாக்
காலத்துக்கும் தொடங்குன இடத்துலேயே அப்படியே நிக்குது
அவள் தனக்குத்தானே சொல்லி விசனப்பட்டுக் கொண்டாள்"**

(தீராக்கடன், ப.16)

னு கூறியதற்கு இராமன் பணம் படைத்தவன் அனுப்பவிக்கிறான் என்று ஒரு
த்தை மட்டும் கூறிவிட்டு, விறுவிறுவென வேலையில் இராமன் கவனத்தைச்
லுத்தினான். திடீரென அலறல் சத்தம் கேட்டது.

'மச்சக்காளை' என்ற கந்துவட்டிக்காரன் பணம் கேட்டுச் சின்னுவைப்
பக்கத்திலிருந்து உதைத்ததில், தூசு நிறைந்த தார்ச்சாலையில் கையிலிருந்த
ர முறம் ஒரு புறமும், கூடையில் நிறைந்த குப்பைகள் ஒரு புறமும் சிதறி
ந்தன. கைகளிலும், முகத்திலும் காயங்கள் ஏற்பட்டன. சட்டையின் கொக்கிக்
ன்று விழுந்ததில் சின்னு வெட்கிப்போனாள். 'மச்சக்காளை' சின்னுவைப் பார்த்து
ம் தர முடியாதவள் எதுக்குடி கைநீட்டி இளித்துக் கொண்டு வாங்கிங்க என்றான்.
தைதுடன் அசிங்கமான வார்த்தைகளை அள்ளித் தெளித்தான். உடனே சின்னுக்குத்
அவமானப்படுவது பொறுக்க முடியாமல் பதிலுக்கு.

**"ஏலேய் மச்சக்காளை. உனக்கு நல்லா இல்லை சொல்லிப்புட்டேன்.
ஆமா.... வாங்குன காசுக்கு வட்டிப் போட்டு கட்ட வேண்டிய பணத்தைக்
கட்டியாச்சு. நீ போடுற கடப்பாரை வட்டிக்கும், சம்மட்டி வட்டிக்கும்
எவளாவது ஏப்ப சாப்பயா வருவா. அவகிட்ட வச்சுக்க. தெருவைக்
கூட்டறவதானே என்ன பண்ணுவான்னு நெனச்சுட்டியா. எங்கிட்ட
வச்சுக்கிட்ட ஒன்னோட டங்குவார அத்துருவேன் பாத்துக்க"**

(தீராக்கடன், ப.17)

அவனை அடிப்பதைப் போல கையை நீட்டி எகிற ஆரம்பித்தாள். சின்னுவின்
வேதனையைவிட மச்சக்காளை மீதுள்ள கோபம் அவள் கண்களில் இரத்தம்
துத் தீக்கங்குகளாகப் பரவின. அவள் கை நீளும்போது, மச்சக்காளை முகத்தில்
முறத்தின் வழியே அவன் மூக்கைச் சுரண்டிவிடுவதைப்போல மச்சக்காளை
ந்தான்.

**"எங்க அப்பன் காலத்துல ஒன்னோட அப்பன் கொடுத்த கடனுக்கு
நான் வந்து குடுத்தும் முடியல. கணக்கு வழக்கு என்னன்னு எனக்கு**

இப்பவே தெரிஞ்சாகணும். எத்தனைத் தலைமுறையானாலும் எங்க
கடன் தீரவே தீராதா? ஐயாயிரம் ஒவா கொடுத்துட்டு மொத்த
இரத்தத்தையே உறிஞ்சி எடுத்தரலாம்னு
ஏண்டா இப்படிக் காட்டேரி மாதிரி அலையிறீங்க? இவ்வளவு காலம்
உறிஞ்சி குடிச்சும் தாகம் அடங்கலையா? எல்லாரையும் போல
என்னையும் நெனச்சியா? மகனே வகுந்துருவேன் வகுந்து"

(தீராக்கடன், ப.18)

என்றாள் சினுவின் ஆவேசத்திற்கு ஈடுகொடுக்க முடியாத மச்சக்கா
'காட்டுசிறுக்கி' என்னை கவனித்துக் கொள்கிறேன் என்று கூறி
இடத்தைவிட்டு அகன்றான். அன்று நடந்த நிகழ்வுகளை இராமன் மிதிவ
அழுத்திக் கொண்டே நினைத்துப் பார்த்துக் கொண்டு வந்தான். மச்சக்காளை
சின்னு கொடுத்த பதிலடிபோல வழிவிட்டானையும் எனறைக்காவது தாக்
வேண்டும் என்று மனத்தில் எண்ணிக் கொண்டான்.

வாழ வழிவிடாத கந்துவட்டிக்காரனுக்கு 'வழிவிட்டான்' என்று எவன்
பெயர் வைத்தானோ என்று பலமுறை தனக்குத்தானே கேள்வி எழு
கொள்வதுண்டு. இராமன் மிதிவண்டியை அதிவேகமாக அழுத்துவதில் பாதங்
வலி எடுத்தன. மூச்சிரைத்து வியர்வை சொட்ட சொட்ட இதயமே நிற்குமள
விரைந்து சென்றான்.

இராமன் பெறப்போகும் மாதச்சம்பளம் வட்டித் தொகைப் போக சொ
ஆனாலும். அந்தப் பணம் தான் சுப்பம்மாளையும். ஐந்து குழந்தைகள
பட்டினியிலிருந்து காப்பாற்றும் என்பதற்காக இராமன் பரபரப்பும். பதற்
சஞ்சலமும் அடைய காரணமாகும். பணத்தைப் பெற்றுவிட வேண்டும் என
முனைப்புடன் இருந்தான். இராமன் நெருங்க சிறிது தூரத்தில் க
சட்டைக்காரர்கள் கும்பலாகக் குழுமியிருந்தனர். அதில் தம்மைப் போன்று
செய்பவர்களும் நின்றனர். இராமனுக்குக் கலக்கம் ஏற்பட்டு அருகில்
மிதிவண்டியிலிருந்து இறங்கினான். உடல் நடுங்கியது. கை விரல்கள் ஆ
எட்டிப்பார்த்ததில் வழிவிட்டான் சாலையில் இரத்தம் வழிந்தோட இறந்து கிட
விபத்தில் அடிபட்டவன்போல தெரியவில்லை. உடல் முழுவதும் காயங்கள் இ
கண்களைத் திருப்பிக் கொண்டான் இராமன்.

"பணம் கொடுக்க வேண்டியவன் பாடையில் போய்விட்டான்" என்
சொல்லி சுப்பம்மாளையும். குழந்தைகளையும் சமாதானப்படுத்த முடியுமா?
தான் இப்போதைக்கு இராமனின் வேதனையாக இருந்தது.

குப்பன் இராமனின் காதோரத்தில் கந்துவட்டிக் கொடுமையைக் க
யாரோ ஒரு குரூப் கொலை செய்துவிட்டார்கள் என்பதைக் கூறினான். இர
கண்கள் மறுபடியும் உறுதிப்படுத்திக் கொள்ளும் விதமாய் பிணமான
நோக்கித் திரும்பியது. ஏரத்தில் கிழிந்திருந்த நீலநிற அட்டை தன்னுடைய
பாஸ்புக் தான் என்பதை உறுதிப்படுத்திக் கொண்டான்.

பாஸ்புக் வழியே இவர்களின் இரத்தத்தை உறிஞ்சிய வழிவிட்டானின்
ரத்தத்தை இராமனின் பாஸ்புக் உறிஞ்சிக் கொண்டிருப்பதைப் போல
அவனுக்குத் தெரிந்தது"

(தீராக்கடன், ப.22)

வறுமையில் வாடும் மக்களுக்கு வறுமைக்குப் பணம் கொடுத்து
தவுவதுபோல காண்பித்து, அவர்களின் வாழ்க்கையையே தன் வசப்படுத்திக்
தாண்டு, தொன்று தொட்டு தலைமுறை தலைமுறையாக அதிக வட்டி வசூல்
ய்து, அரை வயிற்றுக் கஞ்சிக்கே அள்ளாடும் விளிம்புநிலை மக்களின் சார்பில்
ல் கொடுக்கும் முகமாகவும், கந்துவட்டியை ஒழிக்க வேண்டும் என்ற சமூக
க்கறைக் கொண்டவராகவும் ஆசிரியர் 'தீராக்கடன்' சிறுகதையின் வாயிலாக
க்க முழக்கமிடுகிறார்.

ழிவுநீர் கடலுள் மூழ்கும் தொழிலாளியின் அவலம்

மனித சமுதாயத்தில் கடைநிலை தொழில்களுள் கழிவுநீர் அடைப்பைச்
தம் செய்யும் தொழில் மிகவும் கொடுமையானது. ஏனெனில் தம் உயிரைப் பணயம்
த்து செய்வதாகும். வாழ வழியின்று வறுமை கோரத்தாண்டவமாடும்போது, தம்
ம்பத்தைக் காக்க இந்தத் தொழிலைவிட்டால் வேறு கதியல்லாமல் செய்து வரும்
ழிலே இத்தொழில். அந்தக் கொடுமையான வேலை செய்பவர்களின்
ழ்க்கையில் அவலங்களை வெளி உலகிற்கு வெளிச்சமிட்டுக் காட்டவே ஆசிரியர்
வக்குழி' என்ற சிறுகதையைப் படைத்துள்ளார்.

'வேலு', 'குமார்' என்ற இரு தொழிலாளிகளை ஏ.இ.(கோபால்) மற்றும்
ண்டராக்டர் ஏதோ அடைப்பு எடுக்க வேண்டுமென்று விடிந்தவுடனே அழைத்துச்
ன்றனர். கூலி கொடுக்கும் முதலாளி கூறியபடி கழிவுநீர் கடலுக்குள் மூச்சடைத்து
னினர். நாற்றம் குடலைப் பிடுங்கி எறிவதைப் போல இருந்தது. அதோடு அதிலுள்ள
வுநீர் கசடுகளும், மலங்களும் சேர்ந்து வழவழக் கொழகொழவென்று உடல்
ழுவதும் ஊர்வது போன்ற ஒரு உணர்வு ஏற்பட்டது. மயக்கம் ... கிறுகிறுப்பு..
லசுற்றல் ஏற்பட்டது. காண்ட்ராக்டர் நாளிதழ் வாசித்துக் கொண்டே வேலையை
ங்கடா என்று கூறி கத்தினான். மறுபடியும் பற்களைக் கடித்துக் கொண்டு மூச்சை
க்கி உள்ளே நீந்தி, நாடி பைப்பில் இடித்து நிற்க கையை நீட்டிப் பார்த்தான்.
கே ஒரு திடப்பொருளும், பைப்பும் தென்பட்டது. தம் மூச்சை வெளிவிடாமல் முழு
தையும் செலுத்தி வலுவாகக் கொண்டு இழுத்தான். பைப்பும் சேர்ந்து வந்தது.
போடு சேர்ந்து வேகமாகப் பாய்ந்து வந்த கழிவுநீர் நாசிக்குள் இறங்கி
யிரலைத் தாக்கியது. மூச்சை அடக்கியிருந்தவன் மூச்சை இழுத்துவிட்டான்.
க் கடித்துக் கொண்ட பற்கள் வாய் திறந்து கொண்டது.

**"வழவழ கொழகொழவோடு சேர்ந்து கழிவுநீர் தொண்டைக் குழாயைத்
தாண்டி குடலுக்குள் சென்று தன்னை நிறைத்துக் கொண்டது. மூன்றாம்
முறை இழுத்துவிட்ட பெருமூச்சு இறுதி மூச்சானது. துடிதுடித்து நடுங்கிய
உடல் துள்ளி துள்ளி ஆடி அடங்கியது"**

(சவக்குழி, ப.72)

வேலு தூங்கி மேலே வந்தான். இது போன்று இரண்டு இவர்கள் இன
சுகதேவியோ பேரன் வேலு சிறுவனாதினாலோ, அவன் அப்பாவின் சித்தப்பா
போனது தெரிந்து வீட்டிற்குக் கொண்டு வரப்பட்டார். அவருடைய கழுவுக் கட்டுக
பலங்களும் ஆங்காங்கே ஓட்டியிருந்தன பிரேதப் பக்கத்தில் கூட செல்ல முடி
நாற்றம் வடிக்கடி நாற்றம் தெரியாமலிருக்க பனீர் தெளிக்கப்பட்டது வேலு
சிறாதவோடு சேர்ந்து பனீர் தெளித்தான். அவன் அங்கு சற்று நேரமும் உட்
முடியவில்லை. எழுந்து சென்றான் ஆனால் காலத்தின் தேவை அவனையும் அ
கழிவுநீர் சுத்தம் செய்யும் நாற்றத்தில் வேலை பார்க்க வைத்து. அந்நாற்றத்
உடல் பழகியது

வேலுவின் உடல மருத்துவமனைக்குப் பிரேத பரிசோதனைக்குக் கொ
செல்லப்பட்டது. போலீஸ்காரன் வேலுவின் உடலை நோக்கிச் சென்றான். ப
இருக்க. அதற்கு மேல் அந்நாற்றம் அவனை நகரவிடவில்லை. முகத்தில் ம
அணிந்திருந்தும் நாற்றம். வீச்சம் தாங்காமல் வெளியே ஓடி வந்தான். பிணவ
அருகில் நாவல் மரத்தடியில் ஒலமிட்டு அழுகைக் குரல் கேட்
உறவினராகத்தான் இருக்கும் என்று நினைத்து நேரே சென்றான்.

போலீஸ்காரன், மக்களைப் பார்த்து செத்துப் போனவருக்கு யார் சொ
என்று கேட்க. இவள்தான் என்று ஓர் அம்மா வேலுவின் மனைவியைக் கைகாட்
உன் பெயர் என்ன? என்றவுடன அப்பெண் 'சுனிதா' என்று அழுது கொ
கூறினாள். காது, மூக்கு, கழுத்து என்ற இடங்களில் ஒரு பொட்டளவும் பொன்
இல்லை கண்களைச் சுற்றிக் கருவளையமும், செம்படை தலையும், கழுத்து,
வலுமபு வெளியே தள்ளிக் கொண்டு இருந்தது. வெளிரிப்போன தாலிக்கயிறு
இரண்டு ஊக்குகள் மட்டுமே தொங்கிக் கொண்டிருந்தன. ஒட்டிப்போன வயிறு
வேலுவின் மனைவியை ஆசிரியர் குறிப்பிடும்போது. ஏழை என்பவன் எவ்
கேவலப் பொருளாக இவ்வுலகில் வாழ்ந்து வருகிறான் என்
வலியுறுத்தியிருப்பதை உணர முடிகிறது.

"வேலுவின் உடலை பிரேத பரிசோதனை செய்ய வந்த மரு
பிணத்தை அறுக்கும் சேகரிடம் என்ன நாற்றம் கப்படிக்குது. நிக்க முடியல
கூறி. பிணத்தை நீர்விட்டு கழுவி, பிறகு அறைக்குப் பினாயில் தெளித்துவி
போஸ்ட்மார்ட்டம் அபறம் பண்ணிக்கலாம்" (ப.73) என்று சொலலிவிட்டு போ

சேகர், மருத்துவர் கூறியதைக் கேட்டு. முந்தாநாள் ஒரு மாத
மேலாகப் புதருக்குள் கிடந்து அழுகி நாறிபோனபு பிணத்தை இந்த டாக்டர்
போஸ்டமார்டம் செயதாரு இபா மட்டும் என்னவாம் என்று தனக்குள்ளே
கொண்டான்.

குமாரின் அண்ணனிடம் 'சுனிதா' அண்ணே... என்ன விட்டு போயி
இபபடி இவர அநியாயமா? கொன்னுட்டாங்க என்று கதறினாள் அதை
காமராஜ விறுவிறுவென்று பிணவறைக்குள் நுழைந்தான். அங்கே சேகர் பி

து நீர் ஊற்றியதைப் பார்த்து என்னய்யா? என்றான். டாக்டருக்கு நாறுதாம் அதனால
ழுவ சொன்னாரு. அப்புறம்தான் போஸ்ட்மார்ட்டம் பண்ணுவாறாம் என்றான்.

"ஒங்க டாக்டரை பேளாம, மோளாம இருக்க ஒரு மாத்திரை
கண்டுபிடிச்சுத் தரச்சொல்லுங்க" (சவக்குழி,ப.74)

என்று முனங்கிக் கொண்டே வெளியில் தூரத்தில் நின்றிருந்த போலீஸ் அதிகாரியை
நாக்கிச் சென்றான். சார், வேலுவோட சேர்த்து, இந்தக் காண்டராக்டர் கீழ் இது
லாவது சாவு. அவர் மேல கேஸ் போடணும் என்றவுடன், போலீஸ்காரன்
பாஸ்ட்மாட்டம் முடியட்டும் ஆகவேண்டியதைப் பாருங்க. அப்புறம் பார்ப்போம்
ன்றான். போஸ்ட்மாட்டம் முடிந்து எல்லோரும் வெளியே வந்தனர். மருத்துவர்,
போலீஸ்காரர் மக்களை நெருங்கியபோது, அவர்களிடமிருந்த நாற்றம் வீசியது. சிலர்
கத்தைச் சுளித்தனர். ஆனால் **"அது வழக்கமான பிணநாற்றம் அல்ல. ஆம் அது**
ித மலத்தின் நாற்றம்" என்று ஆசிரியர் க.வீரபாண்டியன் அவர்கள் கழிவுநீர்
ங்கிய குழிக்குள் சென்று அடைப்பை எடுப்பதில் அடைப்பின் வழியே
ளியேறுவது கழிவுநீர் மட்டுமல்ல, அதை எடுப்பவரின் உயிரும் தான் **'கழிவுக்குழி**
வர்களுக்குச் சவக்குழி' ஆகிறது என்ற நிதர்சனத்தை ஆசிரியர் வெளிச்சமிட்டுக்
ட்டியுள்ளார்.

லியல் தொழிலாளிகளின் வாழ்வியல்

சமுதாயத்தில் மனிதர்கள் செய்யும் வேலையில் மலம் அள்ளுவதைவிட
ாடுமையான தொழில் பெண்கள் தங்களின் உடலைப் பணத்திற்கு விற்கும்
லியல் தொழிலாகும். பார்ப்பவர்களுக்கு மிகவும் அருவெறுப்பைத் தரக் கூடிய
ழில் மற்றும் அவர்களைக் கண்டாலே ஒதுங்கி போகும் அவலநிலையில்
ப்போரே பாலியல் தொழிலாளிகளாகும். பாலியல் தொழிலிலும் பற்பல மனிதர்கள்
விதமான வகையில் உள்ளனர். பாலியல் பெண்கள் ஊரிலோ, வேறு ஆடவரிடமோ
ல் காட்சியை முடித்துவிட்டு வரும் பாலியல் தொழில் செய்யும் பெண்கள், அடுத்து
வரோடு கூடுவது **'இரண்டாம் காட்சி'** என்ற கருத்தை ஆசிரியர் பதிவு
துள்ளார்.

'இரண்டாம் காட்சி' சிறுகதையில் 'லலிதா' என்ற பாலியல் தொழில்
யும் பெண் தமக்கு நிகழ்ந்த நிகழ்வுகளைச் சொல்லும் விதமாக ஆசிரியர்
ரபாண்டியன் அவர்கள் அமைத்துள்ளார்.

லலிதாவும், வீணாவும் இரவு கடைசி இரயிலில் வரும் ஆடவர்களைத்
லைப்படுத்திக் கொள்ள விழுந்து அடித்து ஓடினர். ஆனால் இரயிலில் இறங்கிய
னவரும் சென்றுவிடுகின்றனர். பேருந்து நிலையதிதல் ஏதேனும் 'பார்ட்டி'
க்குமா? என்று நோட்டம்விடும்போது புதிதாக வந்த சப் இன்ஸ்பெக்டர் ரொம்ப
மையாக நடப்பவர் என்பதை ஏட்டு அவர்களிடம் கூறி, அந்த இடத்தைவிட்டு
ட்டியடித்தார். என்னங்க சார் எங்க பொழப்புல மண்ண அள்ளிப் போடுறீங்க என்று
க் கொண்டே வந்தார்கள். லலிதா, வீணாவைத் தியேட்டர் பக்கம் போனால்
வது கிடைக்கும். அதை விட்டுவிட்டால், இன்று சாப்பாட்டுக்குச் சிங்கிதான்

1003 / 1134

அடிக்கனும் என்று விரைந்தபோது இவன் பிடி மார்க் என்ற பிடிபாகெட் காரனென்று சந்திக்கின்றனர்.

'மார்க்' திடம் தியேட்டர் உள்ளே நுழைய அனுமதி வாங்கித் தரு
கேட்கின்றனர் மேனேஜர் அண்ணாமலை முன்பொரு காலத்தில் என்னிடம் ஐ
பார்ட்டிதான். ஆனால் அவன் புதிது. புதிதாக இருக்கும் பெண்களைலிதா தியேட்டரில் அமர்ந்து படம் பார்த்தாள். காதல் காட்சியைக் கண்டவுடன்
அனுபவிப்பான பழைய ஆட்களை அவன் கண்டு கொள்ளமாட்டான். இருந்தபோதிலும்வதற்குள் ஆனந்தத்தோடு நளினத்துடன் அசைந்தான்.
மேனேஜர் முன்பு லலிதாவிடம் வந்து போனதால் அவனுடைய நம்பருக்கு
செய்தாள். ஆனால அவன் எடுக்கவில்லை 'மார்க்' போன் செய்ததில் டிக்கெட் வ
தான் உள்ளே செல்ல வேண்டும் என்று திடடவட்டமாகக் கூறிவிட்டான். 'மார்
தியேட்டர் நெரிசலில் வெளிவருபவரிடம் பிடிபாகெட் அடிக்க தயாராகினான். லலிதாவரை எந்த ஆடவர்மீதும் வராத ஈர்ப்பு அவன்மீது வந்தது. அப்போது லலிதா,
வீணாவுக்கு உள்ளே செல்ல டிகெ வாங்கிக் கொடுத்துவிட்டு, 'மார்க்' அவனு
தொழிலைப் பார்க்கச் சென்றான்.

எப்போதும் காமம் நிறைந்து தளும்பி வழிந்தோடும்
வாடிக்கையாளர்கள்தான் எனக்கு அறிமுகம். இந்தக்
கூட்டத்துக்கு நடுவில் காம இச்சையில் அவன்
அணுகவில்லை என்று உள் மனம் சொல்லியது.
ஒரு கட்டத்தில் என்னோடு பேசுவதிலும், பகிர்ந்து
கொள்வதிலும் அளவில்லாத ஆனந்தத்தை
அடைவதாகச் சொன்னான்"

(இரண்டாம் காட்சி, ப.58)

லலிதாவும், வீணாவும் தியேடரில் இடைவேளையின்போது வெளி
ஆடவர்களை உடல் அழகைக் காணபித்துத் தன்வசப்படுத்துவதில் தீவிரமானா
வீணா ஓர் ஆடவனைக் கண்டு கண் ஜாடை செய்து புன்முறுவலைவிட்
திருமணமானவனாக இருந்தால் அவன் சிரித்தவுடனே கூச்சமின்றி உடன் வந்துவி
ஆனால் இவனோ வாலிபன் என்பதால் கூச்சத்தில் நெளிந்தான். வீணா போராடி
வழியாக அவனைச் சரிகட்டினாள். அவளுக்கு ஆள் கிடைத்துவிட்டது. 'லலிதா'
ஜீன்ஸ் பேண்ட் அணிந்திருந்தவனுக்கு வலை வீசினாள். ஆனால் அவ
சொத்தெல்லாம் வித்து நடுத்தெருவுக்கு வந்த பஞ்ச பரதேசியாக ஒரு தாடிக்
லலிதாவையே பார்த்தான். இவனால் ஒரு பைசாகூட தர முடியாது என்று எண்
கொண்டு, தன் பார்வை முழுவதையும் ஜீன்ஸ் பேண்ட் அணிந்தவன் மீதே இரு
லலிதாவின் அருகே நெருக்கமாக வந்து நின்ற தாடிக்காரன் எவ்வளவு?
கேட்டான். அவன் பக்கத்தில் வந்தபோது தான் அவனை உற்றுப்பார்த்தால் 35
மதிக்கத்தக்கவன் என்று அறிந்தேன் என்றாள் லிலதா.

"நல்லவனாகத் தெரிந்தான். ஆயிரம் ரூபாய் யாரும்
கேட்டுவிடாதபடி குரலை சின்னதாக்கி மெதுவாகச் சொன்னேன்
சட்டைப்பையிலிருந்த பணத்தை எடுத்தான். அதிலிருந்து
ஆயிரம் ரூபாய் தாளை எடுத்துக் கொடுத்தான். கேட்ட
வேகத்தில் அவன் பணத்தை எடுத்துக் கொடுத்ததில்
ஆச்சர்யம். தப்புக்கணக்கு போட்டுவிட்டேன் என்று உரைத்தது"

(இரண்டாம் காட்சி, பக்

கூட்டத்திற்கும், இரைச்சலுக்கும், சலசலப்புக்கும் நடுவில் தாடிக்காரன் செ
பணம் கணவன் மனைவிக்குக் கொடுப்பதைப்போல இருந்தது. தாடிக்காரன்
கொடுத்தவுடனே தியேட்டர் உள்ளே போய் படம் பார்க்கலாம் என்று கூறி
லலிதா ஆச்சர்யம் அடைந்தாள்.

"அதுக்கெல்லாம் நேரம் கிடையாது. முடித்துக் கொண்டு அடுத்த
வாடிக்கையைப் பார்த்துப் போக வேண்டுமென அவசரப்படுத்தினேன்"

லலிதா கூறுவதன் வாயிலாக பாலியல் தொழில செய்பவர்களின் தொழில்முறையைத் தெளிவுபடுத்துகிறார் ஆசிரியர்.

தாடிக்காரன் இன்னொரு ஆயிரம் ரூபாயை எடுத்துத் திணித்தான். இரண்டாயிரம் சம்பளத்தை எண்ணி உள்ளூர மகிழ்ச்சியடைந்தாள். தாடிக்காரனோடு

தாடிக்காரன் தாம் பெற்ற அனுபவங்கள் முழுவதையும் சொல்லிக்கொண்டே வந்தான். அவனுடைய பேச்சில் லலிதா லயித்துப்போனாள்.

லலிதா வெளியில் செல்லலாம் என்று எண்ணும்போது திரையில் வெளிச்சமிட்டு தாடிக்காரன் முகத்தில் படும்போது, முகமும், தாடியும் பிரகாசமான வகளும் செல்லவிடாமல் லலிதாவைப் பிடித்து இழுத்தது.

"சரி கிளம்பலாம் என்றான் தாடிக்காரன்.
"எங்கே?" என்று கேட்டேன்.
"உனக்கு பணம் குடுத்துட்டு எங்க
கூட்டிட்டுப் போவாங்க" எனக் கேட்டபோது
அவனின் முகத்தை நெருக்கத்தில் பார்த்தேன்.
காட்சி முடிந்து எல்லோரும் எழுந்து வெளியேற
ஆரம்பித்தனர். கூட்டத்திற்குள் என் கையைப் பிடித்து
இழுத்தபடி கூட்டத்தை இடித்துக்கொண்டு முன்னேறி
நடந்தான். அவனின் இறுக்கமான பிடியில் வளையல்கள்
மணிக்கட்டை அழுத்த எனக்கு வலி எடுத்தது"

(இரண்டாம் காட்சி, ப.59)

தாடிக்காரன் லலிதாவிடம் நல்லவன்போல் போலி வேடமிட்டு இறுதியில் வனின் உண்மையான முகத்தை 'இரண்டாம் காட்சி' காட்சிப்படுத்துகிறது.

பாலியல் தொழில் செய்பவர்களைச் சமுதாயம் மிகவும் கேவலமாகவும், ாக்கைப் பொருளாகவும் பணம் வாங்குவதால் அவர்களின் மனத்தையும், லையும் எப்படியும் புண்படுத்தலாம் என்ற எண்ணத்தையும் கொண்டிருப்பதை ண்டாம் காட்சி' சிறுகதை வெளிச்சமிட்டுக் காட்டுகிறது.

'பூர்ணிமை' சிறுகதை தொகுப்பில் 12 சிறுகதைகள் அடங்கியுள்ளன. அவற்றுள் மூன்று சிறுகதைகள் மட்டுமே ஆய்வுக்கு எடுத்துக் கொள்ளப்பட்டுள்ள

நிறைவுரை

மனித சமுதாயத்தில் உயர்ந்த நிலையில் இருக்கும் மக்களைப் ப பேசுவதற்கு ஏராளமானோர் உள்ளனர். ஆனால் கடைநிலையில் கவலைக்கிட சூழல்நிலைகளில் தங்கள் குடும்பத்தை நடத்துவதற்கு ஒவ்வொரு ப படைத்தவரிடம் கையேந்தி வட்டிக்குக் கடன்வாங்கிப் பிழைப்பு நடத்துவதோடு ஏ பேசும் கேவலச் சொற்களும் வாங்கிக் கொண்டு மிகவும் குறைந்த சம்பளத் சகிக்க முடியாத வேலையைப் பார்க்கும் மக்களைப் பற்றி எடுத்துக் கூற முன் ஆசியர்களுள் ஒருவர்தான் க.வீரபாண்டியன் அவர்கள். வறுமை கோட்டிற்குக் இருக்கும் மக்களின் வாழ்க்கை முறையை இச்சிறுகதைகள் சுட்டிக்காட்டும் வித ஆசிரியர் க.வீரபாண்டியன் அவர்கள் மிகச் சிறப்பான முறையில் உணர்வு பூர்வ படைத்தளித்துள்ளார். 'கந்துவட்டிக்காரன்' கொடுமையால் பொருளாதார நிச்சய வாழ்வில் சிக்கித் தவிக்கும் தூய்மை பணியாளர்கள் பற்றித் 'தீராக்கடன்' சிறுகதை பேசுகிறது. கழிவுநீர் கால்வாயைச் சுத்தப்படுத்தும் தொழிலாளி பின்பு அவரது உடலை போஸ்ட்மார்ட்டம் செய்யவே தயங்கும் அவலநிலை 'சவக்குழி' எனும் சிறுகதை கூறுகிறது. பாலியல் தொழிலாளியின் உ குமுறல்களையும் அக்கறையாக நடந்து கொள்வது போல நாடகம் ஆடி பின்பு உண்மை முகத்தைக் காட்டும் மனிதர்களின் இழிவான குணத்தை 'இரண்டாம் க என்ற சிறுகதை வெளிச்சமிடுகிறது. பூர்ணிமை' சிறுகதை தொகுப்பு சமுதாயத் சீர்திருத்தும் ஓர் அற்புத கருவியாக அமைந்துள்ளதை இக்கட்டுரையின் வாய் எடுத்துரைக்கப்படுகின்றது.

பயன்பட்ட நூல்

பூர்ணிமை, க.வீரபாண்டியன், டிஸ்கவரி புக் பேலஸ், சென்னை-78.
முதற்பதிப்பு:ஆகஸ்ட்-2018.

RAJA PUBLICATIONS
No. 10 (Upstair), Ibrahim Nagar, Khajamalai, Tiruchirappalli - 620 023

இராஜா பப்ளிகேஷன்ஸ்
10, முதல் தளம், இப்ராஹிம் நகர், காஜாமலை, திருச்சிராப்பள்ளி - 620 023

www.rajapublications.com

நடத்தும்

முதல் பன்னாட்டுக் கருத்தரங்கம்

(இணைய வழி Online Mode)

தமிழ் இலக்கியங்கள் - 2022

இக்கால இலக்கியத் தமிழாய்வு

திருமிகு/திருமதி/முனைவர்

முனைவர் ச. ஈஸ்வரன்
இணைப்பேராசிரியர் & தலைவர்
தமிழாய்வுத்துறை, தேசியக்கல்லூரி (தன்னாட்சி),
திருச்சிராப்பள்ளி-620001.
தமிழ்நாடு, இந்தியா.

தமிழ் இலக்கியங்கள்-2022 என்னும் பொருண்மையில் நடைபெற்ற பன்னாட்டுக் கருத்தரங்கில்

பாரதியின் உரைநடைத்திறன்

என்ற தலைப்பில் ஆய்வுக்கட்டுரை வழங்கினார் எனச் சான்றளிக்கப்படுகிறது.

zoom Topic: தமிழ் இலக்கியங்கள் - 2022
(RAJA PUBLICATIONS, TRICHIRAPPALLI, TAMIL NADU, INDIA)
Time: May 30, 2022 10:00 AM Mumbai, Kolkata, New Delhi
Join Zoom Meeting
https://us05web.zoom.us/j/86056190927
pwd=bnxMRW9GTmsyL252T3p0SWhbWpLZz09Meeting
ID: 860 561 9092 Passcode: ONLINE2022

கருத்தரங்க இணை ஒருங்கிணைப்பாளர்
முனைவர் செ. முருகநாதன்
உதவிப்பேராசிரியர்.(அ.ப.பணிநிரவல்)
தமிழ்த்துறை, அரசு மகளிர் கலை & அறிவியல் கல்லூரி
பருகூர் - 635 104

கருத்தரங்க ஒருங்கிணைப்பாளர்
முனைவர் மு. சாந்திகாட்சாயினி
இணைப்பேராசிரியர், முதுகலை & தமிழாய்வுத்துறை
ஜமால் முகமது கல்லூரி(தன்னாட்சி)
திருச்சிராப்பள்ளி - 620 020

இராஜா பப்ளிகேஷன்ஸ்

10, முதல் தளம், இப்ராஹிம் நகர், காஜாமலை,
திருச்சிராப்பள்ளி – 620 023

நடத்தும்

முதல் பன்னாட்டுக் கருத்தரங்கம்

தமிழ் இலக்கியங்கள் - 2022

பிப்ரவரி 2022-ல் நடைபெறவிருக்கிறது.

ஆய்வாளர் எடுத்துக்கொள்ள வேண்டிய களங்கள்:

1. சங்க எட்டுத்தொகை இலக்கியங்கள்
2. சங்கப் பத்துப் பாட்டு இலக்கியங்கள்
3. பதினெண் கீழ்க்கணக்கு நூல்கள்
4. தொல்காப்பியம், சிலப்பதிகாரம், மணிமேகலை, முத்தொள்ளாயிரம், இறையனார் அகப்பொருளுரை
5. கம்பராமாயணம், வில்லிப்புத்தூரார் பாரதம், சீறாப்புராணம், குணங்குடி மஸ்தான் சாகிபு. தேம்பாவணி, இரட்சணிய யாத்திரிகம்,
6. புதின இலக்கியம்
7. சிறுகதை இலக்கியம்
8. கவிதை இலக்கியம்
9. நாடக இலக்கியம்
10. பிற...

கட்டுரைகள் அனுப்பக் கடைசி நாள் 31.01.2022

1007 / 1134

ச. சுல்தான்
22.06.2022

இக்கால இலக்கியத் தமிழாய்வு

online seminar on 30-05-2022 நிகழ்வு

முதன்மைப் பதிப்பாசிரியர்
முனைவர் மு. சாதிக் பாட்சா

பதிப்பாசிரியர் குழு
முனைவர் ஞா. செந்தில்
முனைவர் செ. முருகநாதன்
முனைவர் ந. ரகு

251-261

RAHMAN BOOKS
No. 4/1, T.V.S. Nagar Main Road (Rajam Street),
Race Course Road, A.M. Complex,
Kajamalai, Tiruchirappalli - 620 023.
Mob : 63858 47242

நூற்குறிப்பு

நூலின் பெயர்	:	இக்கால இலக்கியத் தமிழாய்வு
பதிப்பாசிரியர்கள்	:	முனைவர் மு. சாதிக் பாட்சா முனைவர் செ. முருகநாதன் முனைவர் ஞா. செந்தில் பேரா. ந. ரகு
பதிப்பு	:	2022
உரிமை	:	பதிப்பகத்தாருக்கு
நூலின் அளவு	:	டெம்மி (1 x 8)
பக்கங்கள்	:	(vi) + 346 = 352
அச்செழுத்து	:	10.5 புள்ளிகள்
தாள்	:	மேப்லித்தோ
விலை	:	ரூ. 250/-
வெளியீடு	:	RAHMAN BOOK SHOP No. 4/1, T.V.S. Nagar Main Road, (Rajam Street), Race Course Road, A.M. Complex, Kajamalai, Tiruchirappalli - 620 023. Mob : 63858 47242
ஒளி அச்சு	:	விஷ்ணு கிராபிக்ஸ், திருச்சி-23.
அச்சிட்டோர்	:	தி பிரிண்டிங் ஹவுஸ், திருச்சி-23.
ISBN No	:	978-93-91573-35-5

- *Name of the Book : Ikkala Ilakkiyath Thamizaivu*
- *Compiled by : Dr. M. Sadik Batcha, Dr. G. Senthil*
  Dr. S. Muruganathan, Dr. N. Raghu
- *Size : 1/8 Demmy*
- *Printing Point : 11*
- *Pages : (vi) + 346 = 352*
- *Edition : 2022*
- *Rate : Rs. 250/-*
- *Computer Printing : Vishan Graphics, Trichy-23.*
- *Copy Rights : Raja Publications, Trichy-23.*
- *Publisher : Rahman Book Shop, Trichy-23.*
- *Offset Printing : The Printing House, Trichy-23.*
- *ISBN No : 978-93-91573-35-5*

பொருளடக்கம்

1. அறிஞர்கள் மேற்கொள்களும் புதினத்தின் கதைக்களமும் 1
   தமிழ்ப் பிரியா
2. இறையன்புவின் "ஆத்தங்கரை ஓரத்தில்" 8
   சமுதாய வெளிப்பாடுகள்
   ப. வரலட்சுமி
3. தமிழ்க் குறுங்கதைகளின் வரையறைகளும் போக்குகளும் 15
   முனைவர் ச.. அ. சையத் அகமது பிரோக
4. பெருமாள் முருகன் படைப்புகளில் சமூகச் சிக்கல்கள் 20
   அ. அன்னமேரி
5. எஸ்.ரா.வின் 'துயில்' நாவலில் பசிப்பிணியும் மருத்துவமும் 28
   முனைவர் ப. முருகன்
6. எஸ். ராமகிருஷ்ணன் நாவல்களில் பெண்ணியம் 35
   அ. அருள்மேரி
7. க. வேணுகோபாலின் படைப்புகளில் பெண்கள் 41
   அ சுபைதா ஹசீனா
8. மலேசிய நாட்டு வாய்மொழித் தமிழ் இலக்கியம் 49
   கு. தமிழ்மொழி
9. மனவிலங்கு புதினத்தில் கடித உத்தி 54
   க. தேவி & முனைவர் சீ மேபெல் ஜோதி ராணி
10. கண்மணி குணசேகரனின் படைப்புகளில் மண்ணும் மக்களும் 60
    ந. ரகு
11. நா. பார்த்தசாரதியின் வஞ்சிமாநகரம் 67
    அ. ரதிரூபி & முனைவர் சா. தேவநேசம் மேபல்
12. டி. செல்வராஜ் நாவல்களில் பெண்ணியமும் சிக்கல்களும் 75
    சு. உமாதேவி
13. பரதவர் தொழிலும் தொழில்சார் வாழ்வியலும் 82
    முனைவர் ச. சுந்தர்
14. கல்மரம் நாவலில் தொழிலாளர் நிலை 89
    முனைவர் க. கார்த்திகேயன்

15. நெடுங்குருதி நாவலில் நாட்டுப்புற வழிபாடுகள் 97
முனைவர் இரா. சுப்ரமணியன்

16. வைரமுத்து நாவல்களில் கிராமியப் பண்பாடு 105
முனைவர் சீ. சிவக்குமார்

17. எட்டுத்திக்கும் மதயானை நாவலில் சமுதாயச் சித்திரிப்பு 113
முனைவர் செ. முருகநாதன்

18. கு. தமிழ்ச்செல்வி படைப்புகளில் மொழிநடை 120
முனைவர் அ. ஜோதிரவீந்திரன்

19. தமிழன்பன் கவிதைகளில் இயற்கை படிமங்கள் 129
ம. கலா

20. சிறுகதையின் தோற்றம் வாழ்க்கையின் தொடர்கதையே 135
ஏ.எஸ். தமிழரசி

21. சொல்வனம் கவிதைகளில் இயற்கை 142
கி. தென்னரசு & முனைவர் செ. பாலு

22. குறிஞ்சி சீத்தாராமனின் சிறுகதைகளில் சமூகப்பார்வை 148
முனைவர் க. ஸ்ரீதேவி

23. தோப்பில் முகமது மீரான் சிறுகதைகளில் சமுதாயம் 159
சார்ந்த பன்முகப் பார்வை
மர்பி. து

24. தேனி சீருடையானின் 'தீர்ப்பு' சிறுகதை உணர்த்தும் 165
மதுப்பழக்கத்தால் ஏற்படும் குடும்ப மற்றும் சமூகச் சீர்கேடுகள்
இரா. ஜான்சி ராணி

25. தாமரைசெம்மலர் சிறுகதைகளில் மனிதநேயம் 169
சு. பூங்கோதை

26. விந்தன் சிறுகதைகளில் பெண்ணியச் சிந்தனைகள் 175
நீ. பொற்செல்வி & முனைவர் ப. கல்பனா

27. எழுத்தாளர் சோ. தர்மன் சிறுகதைகளில் 183
சுற்றுச்சூழலியல் கருத்தாக்கம்
மு. சுரேஷ்குமார்

28. ஓர் இரவு எனும் நாடகத்தின் கட்டமைப்பு 188
முனைவர் மா. சிவபாக்கியம்

29. 'சிகரங்களில் உறைகிறது காலம்' 195
கவிதைத் தொகுப்பில் புலப்பாட்டு உத்திகள்
முனைவர் செ. முருகநாதன்

30. யுகபாரதி கவிதைகளில் படிமம் 203
முனைவர் ச. சுந்தர்

31. இரயில் பயணங்கள் போல இலக்கியத்தில் 211
சிறந்த சிறுகதைகள்
ஏ.எஸ். தமிழரசி

32. பெண்ணிய நோக்கில் 'சிகரங்களில் உறைகிறது காலம்' 221
கவிதைகள்
முனைவர் கோ. ரங்கராஜன்

33. புதுக்கவிதைகளில் பெண்ணடிமை ஒழிப்பும் 229
பெண் விடுதலைச் சிந்தனையும்
நா. லீலாவதி

34. புதுக்கவிதைகளில் மகளிர் உரிமைகள் 237
முனைவர் ப. முருகன்

35. மனித வாழ்வு மேன்மையடைய பாரதியார் கூறிய வழிகள் 244
த. விஜயலெட்சுமி & முனைவர். அ. மோகன்ராஜ்

36. பாரதியின் உரைநடைத்திறன் 251
முனைவர் ச. ஈஸ்வரன்

37. அப்துல் ரகுமான் கவிதைகளில் மாற்று மரபு 261
போர. மு. மாலதி & முனைவர். ப. கண்ணுமுத்து

38. அழகின் சிரிப்பில் உவமையும் உருவகமும் 269
முனைவர் ச. செந்தில்குமார்

39. கு. தமிழ்ச்செல்வி படைப்புகளில் 276
விளிம்புநிலை மாந்தர்கள் (மாணிக்கம் நாவலை முன்வைத்து)
முனைவர் அ. ஜோதிரவீந்திரன்

40. கவிஞர் வி. சி. அமுதன் கவிதைகளில் தமிழர் உணர்வும் 282
சமூக அக்கறையும்
முனைவர் கோ. பா. தாட்சாயிணி

41. பாரதியாரின் முற்போக்கு சிந்தனைகள் 291
முனைவர் ஜெ. குணசேகரன்

1009 / 1134

மாத்திரமே உடனே செய்யும் போது அதில் வெற்றி நிச்சயமாகக் கிடைக்கும். அதைத் தூங்கப் போட்டுவிட்டு பிறகு செய்யப் போனால் ஆரம்பத்தில்இருந்த வேகம் குறைந்துவிடும். எதிர்காலம் நம் கையில் இல்லை, நிகழ்காலமே நம் கையில் உள்ளது. காலத்தின் பயனை உணர்ந்து சோம்பலால் காலத்தை வீணாக்காமல் உட்பகைகளை வென்று வாழ்வில் வெற்றியடைவோம.

முடிவுரை:

வாழ்வின் மேன்மையான வெற்றிகளை அடைய பாரதிக் கூறிய வழிகளான அறவழியில் பொருள் சேர்த்து இன்பமாக வாழ்ந்து வீடுபேறு அடைவதை மனிதப் பிறவி கிடைத்ததன் நோக்கமாகக் கொண்டு. எங்கும், எப்போதும், எதிலும் உண்மையைப் பேசி, மனித வாழ்வில் ஏற்படும் வீண் கவலைகளையும் பயத்தையும் விட்டொழித்து, நம்மிடம் உள்ள உட்பகைகளான பயம், சந்தேகம், சோம்பல் இவைகளை நீக்கி நல்வாழ்வு வாழ்வோம், வெற்றியடைவோம்.

பாரதியின் உரைநடைத்திறன்

முனைவர் ச. ஈஸ்வரன்
இணைப்பேராசிரியர் & தலைவர்
தமிழாய்வுத்துறை, தேசியக்கல்லூரி (தன்னாட்சி),
திருச்சிராப்பள்ளி-620001.
தமிழ்நாடு, இந்தியா.

251

முன்னுரை

திருநெல்வேலியில் உள்ள எட்டயபுரத்தில் 11.12.1882 பிறந்தவர் பாரதியார் 12.09.1921 வரை வாழ்ந்தவர். தம்முடைய 39 வயது குறுகிய வாழ்க்கைக்குள் உலகப் புகழ்பெற்று 'மகாகவி' என்று அழைக்கப்பட்டவர்.

கவிதைகளோடு, வேடிக்கை நவதந்திரக் கதைகள், சின்னசங்கரன் கதை, சந்திரிகையின் கதை போன்ற நெடுங்கதைகள், ஞானரதம் எனும் தலைப்பிலான தத்துவச்சித்திரங்கள், நாடகங்கள், கட்டுரைகள், பகவத்கீதை மொழிபெயர்ப்புகள் என பல்வேறு இலக்கிய வகைமைகளிலும் தம் முத்திரையைப் பதித்தவர் பாரதியார். கவிஞராக மட்டுமின்றி இதழாசிரியராகவும் சிறந்த திறனாய்வாளராகவும் பாரதியார் விளங்கியுள்ளார். சுதேசமித்திரன் இதழில் பணியாற்றினார். சக்ரவர்த்தினி, இந்தியா ஆகிய இதழ்களைத் தொடங்கி நடத்தினார்.

பாரதியின் உரைநடைத்திறன் குறித்து இலி ஆராயலாம்.

1.1. கவிதைப் பண்பு வாய்ந்த நடை

மகாகவி பாரதியின் உரைநடையுள் கவிதைத் தன்மை வாய்ந்த நடையும் ஆங்காங்கே தென்படும். சான்று; "சிறிய தானியம் போன்ற மூக்கு; சின்னக் கண்கள்; சின்னத்தலை; வெள்ளைக் கழுத்து, அழகிய மங்கல் வெண்மை நிறமுடைய பட்டுப் போர்த்த வயிறு; கருமையும் வெண்மையும் கலந்த சாம்பல் நிறத்தாலாகிய பட்டுப் போர்த்த முதுகு; சிறிய தோகை; துளித் துளிக்கால்கள். இத்தனையும் சேர்த்து ஒரு பச்சைக் குழந்தையின் கைப்பிடியிலேயே பிடித்துவிடலாம். இவ்விதமான உடலைச் சுமந்து கொண்டு என் வீட்டில் இரண்டு உயிர்கள் வாழ்கின்றன. அவற்றில் ஒன்று ஆண் மற்றொன்று பெண்"[1] என்று எழுதியுள்ளார் மகாகவி.

பாரதியார் புதுப்புது நெறிகளையும் புதுப்புது நயங்களையும் கையாண்டு உரைநடையை எழுதினார் என்பதற்கு இப்பகுதி தக்க சான்றாகும். இங்கே சொற்களும் சொற்றொடர்களும் தனித்தனி வாக்கியங்களாக நின்று கருதிய பொருளைச் செவ்வனே புலப்படுத்துகின்றன.

1010 / 1134

ஞாபகத்திற்கு வருகிறது. ஒரு ஜப்பானியத் தாய் தனது பல குமாரர்களை போர்க்களத்தில் இறக்கக் கொடுத்துவிட்டுப் பிறகு ஒருநாள் அழுது கொண்டிருந்தாள். அவளிடம் சென்று ஒருவர் `அம்மா ஏன் அழுகிறீர்கள்? உமது பிள்ளைகள் மஹாகீர்த்திகரமான மரணத்தையல்லவா அடைந்திருக்கிறார்கள்?' என்று ஆறுதல் கூறினார். அதற்கு அத்தாய் `ஐயா நான் இறந்துபோன மக்களின் பொருட்டாக அழவில்லையே. என் தாய்நாட்டிற்கு கீர்த்திவரும் பொருட்டாக பலியிடுவதற்கு இன்னும் பிள்ளைகள் இல்லாமல் போய்விட்டதென்று வருத்தமடைகிறேன்' என மறுமொழி தந்தாளாம்"[5] என்றவாறு பாரதியார் பத்திரிகையில் எழுதிப்பதிவிட்டுள்ளார்.

இப்பகுதி பாரதியார் `இந்தியா' பத்திரிக்கையில் 1906-ஆம் ஆண்டில் `அழியாப் புகழ் கொண்ட பழங்காலத் தமிழ் மாது' என்னும் தலைப்பில் எழுதிய கட்டுரையிலிருந்து எடுக்கப்பெற்றதாகும். இவ்வகை நடைவழி பாரதியாரின் பத்திரிக்கை நடையின் தாக்கத்தை உணரமுடிகிறது.

1.5. சொல்லாட்சித்திறன்

`பொது உடமை' `மனித ஜாதி' `பின் காப்பாளர்' `தோல் பொம்மைகள்' `வாக்குச்சீட்டு' `புருஷ விதவை' `படிப்பாளி' `பொருளாளி' `கிருஸ்து சித்தர்' ஆகியவை பாரதியார் கையாண்டுள்ள புதிய சொல்லாட்சிகள். பாரதியார் சொற்களைத் தேர்ந்தெடுக்கும் போது எவ்வளவு கண்ணுங் கருத்துமாக இருக்கின்றார்; எத்தனை கோணங்களில் இருந்து அலசி ஆராய்ந்து பார்க்கிறார் என்பதற்கு ஓர் எடுத்துக்காட்டு வருமாறு.

"நடுவிலே ஒரு ரசமான வார்த்தை `மெம்பர்' என்பதற்குச் சரியான தமிழ்ச்சொல் எனக்கு அகப்படவில்லை. இது ஆச்சரியத்திலும் ஆச்சர்யம். `அவயளி' சரியான வார்த்தை இல்லை. `அங்கத்தான்' சுட்டிவராது. `சபிகன்' சரியான பதந்தான். ஆனால் பொது ஜனங்களுக்குத் தெரியாது. யாரேனும் பண்டிதர்கள் நல்ல பதங்கள் கண்டுபிடித்துக் கொடுத்தால் புண்ணியமுண்டு. அரைமணி நேரம் யோசித்துப் பார்த்தேன். `உறுப்பாளி?' எதெல்லாமோ நினைத்தேன். ஒன்றும் மனத்திற்குப், பொருந்தவில்லை. என்ன செய்வேன்: கடைசியாக `மெம்பர்' என்று எழுதிவிட்டேன். இன்னும் ஆர அமரயோசித்து சரியான பதங்கள் கண்டு பிடித்து மற்றொரு முறை சொல்லுகிறேன்"[6] என்கிறார் பாரதியார்.

புதிய சொல்லாட்சியைப் புனைந்த பாரதியார் நிறைவாக அச்சொல்லை ஒலிபெயர்த்து (மெம்பர்) எழுதியதை அறிய முடிகிறது.

"உள்ளதிலே நேர்மையும் தைரியமுமிருந்தால் கை பிறகு தானாகவே நேரான எழுத்து எழுதும். தைர்யம் இல்லாவிட்டால் வசனம் தள்ளாடும. சண்டி மாடு போல ஒரிடத்தில் வந்து படுத்துக்கொள்ளும். வாலைப் பிடித்து எவ்வளவு திருகினாலும் எழுந்திருக்காது"[7] என்கிறார் பாரதியார். எனவே நடைத்திறம் வாய்க்கப்பெற நேர்மையும் துணிவும் தெளிவும் தேவையென்ற பாரதியாரின் சிந்தனையை உணர முடிகிறது.

1.6. நகைச்சுவை உணர்வு வாய்ந்த நடை

பாரதியின் வாழ்க்கையில் இறுதிவரை இரண்டு உணர்வுகள் மேலோங்கி நின்றன. "ஒன்று கடவுளிடம் பக்தி; இன்னொன்று அபரிதமான நகைச்சுவை. பாரதியாருக்கு இரண்டும் நெருக்கடி காலங்களில் துணையாக நின்றன"[8] என்பார் வ. ரா.

பாரதியாரின் உரைநடை நூல்களில் அவரது நுட்பமான நகைச்சுவை உணர்வைப் பரக்கக் காணலாம். `ஆறில் ஒரு பங்கு' என்னும் கதையில் ஒரு கதைமாந்தரைக் குறித்து எழுதும்போது பாரதியார் பின்வருமாறு எழுதுகின்றார்: "அவருக்கு நான்கு வருடங்களுக்கொரு முறை ஆபீஸில் பத்து ரூபாயும் வீட்டில் இரண்டு குழந்தைகளும் ப்ரமோஷன்" என்று குறிப்பிடுகிறார். பாரதியார் `வேடிக்கைக் கதை'களில் கதை மாந்தர்களுக்குச் சூட்டியுள்ள பெயர்கள் நகைச்சுவை நயம் மிக்கவை. `மிளகாய்ப்பழச்சாமி' `வாழைப்பழச்சாமி' `கடற்கரையாண்டி' `நெட்டையன்' `கட்டையன்' `சொரியன்' `கரியன்' `கலியுக கடோற்கசன்' போன்ற பெயர்கள் இவ்வகையில் குறிப்பிடத்தக்கவை.

பாரதியார் நகைச்சுவை நனி சொட்டச் சொட்ட எழுதியுள்ள ஒரு பகுதி `ஞானரதத்தில் உள்ளது. மண்ணுலகில் நடுத்தரக் குடும்பத்தில் நிலவும் அன்றாடக் காட்சியை அவர் அங்கதச் சுவையோடு எழுதியுள்ளார்.

"ஏன் மனைவியைக் கூப்பிட்டேன். அவள் வந்து ஏன் என்றாள். `தலைநோவு பொறுக்க முடியவில்லை. கொஞ்சம் மிளகு அரைத்துக் கொண்டு வா' என்றேன். "ஆமாம் இரண்டு நாளைக்கு ஒரு முறை இதொரு பொய்த் தலைவலி வந்துவிடும். என்னை வேலை ஏவுவதற்காக அதெல்லாம் சரிதான். பால்காரி வந்து மத்தியானம் பணம் கேட்டு விட்டுப்போனாள். ராயர் வீட்டு அம்மாள் குடக்கூலிக்கு ஞாபகப்படுத்தச் சொன்னாள். ராயர் நேற்றே சொன்னாராம். இந்த மாதம் குழந்தைக்கு காப்பி வாங்க ரூபாய் தருவதாகச் சொல்லியிருந்தீர்கள். என்னைத் தான் ஏமாற்றுகிறது வழக்கமாகவே போய்விட்டது. இன்னும் அது இது என்று ஆயிரம் கணக்கு சொன்னாள். `தலைநோவு தீர்ந்து போய்விட்டது. நீ

1011 / 1134

தயவுசெய்து கீழே போகலாம்' என்று வணக்கத்துடன் தெரியப்படுத்திக் கொண்டேன். "ஆமாம் ஊர்க்காரர் கூடவெல்லாம் ஓயாமல் பேசித் தொண்டைத் தண்ணீரை வற்ற வைத்துக் கொண்டிருக்கலாம். நான் ஒரு வார்த்தை பேச வந்தால் உடனே கோபம் வந்துவிடும்' என்றாள். அவள் மனதில் என்னைச் சமாதானப்படுத்துவதாக எண்ணம்"[9] என்றவாறு எழுதியுள்ளார் பாரதியார். இதன்வழி உரைநடையில் அன்றாட நிகழ்வுகளை நகைச்சுவை உணர்வு கலந்து எழுதும் பாரதியின் நடை நுட்பத்தினைப் படித்து மகிழலாம்.

1.7.எளிய நடை (வாழ்க்கை நிகழ்ச்சிக் குறிப்புகள்)

சிறுசிறு வாழ்க்கைக் குறிப்புகளையும் நொடிக்கதைகளையும் செய்தித் துணுக்குகளையும் பயன்படுத்தி எழுதிச் செல்வது பாரதியாரின் நடையில் காணலாகும் பிறிதொரு சிறப்பியல்பாகும்"[10] என்பார் மோகன்.

"ஆங்கில பாஷையில் Lord Byron என்ற மகாகவி ஒரு சமயத்தில் மனிதரை `நாய்கள்' என்று கூறினார் பிறகு உண்மையில் மனிதரைக் காட்டிலும் நாய் ஜாதி மேம்பட்டதாகையால் மனிதரை நாய்களே என்று சொல்லியதிலிருந்து நாய் ஜாதிக்கு அவமரியாதை செய்வதாக ஏற்படுமாகையால் அதை மாற்றி `ஓ மனிதரே' என்று கூப்பிட்டார்"[11] என்கிறார் பாரதியார்.

அருமை, எளிமை, அழகு, தெளிவு, உணர்ச்சி, வேகம், உயிரோட்டம், புரட்சி, புதுமை ஆகிய பண்புகளைப் பாரதியாரின் கவிதை நடையில் காண்பது போலவே அவரது உரைநடையிலும் காணமுடிகிறது. எங்கும் உணர்ச்சி; எங்கும் தெளிவு; எங்கும் புதிய வேகம்; எங்கும் உயர்ந்த நோக்கம் போன்றவையே அவருடைய கட்டுரைகளிலும் மேலோங்கி நிற்கின்றன.

1.8. விளக்கநடை (கதைப் பொருண்மை)

பாரதியின் எழுத்துக்களில் விளக்கநடைக் கூறுகளும் காணப்பெறுகின்றன. "பாட்டுத் திறத்தாலே இவ்வையத்தைப் பாலித்திட" நினைத்தவர் பாரதியார். சமுதாய விழிப்புணர்ச்சியைக் கவிதைக் கருவியால் மட்டும் கொணர முடியாது என நினைத்தாரோ என எண்ண வாய்ப்புண்டு. ஏன்? அவர் அவ்வாறு நினைத்திருக்கக் கூடும்? பாரதியாரே தம் கட்டுரைப் பகுதியில் கூறுகிறார். "உண்மையான கவிதைக்குத் தமிழ்நாட்டில் தக்க மதிப்பில்லை. இங்கிலீஷ் பாஷையிலிருந்து கதைகள் மொழிபெயர்த்துப் போட்டால் பலர் வாங்கி வாசிக்கிறார்கள்"[12] என்கிறார் பாரதியார். அவரது இக்கருத்தை மனதில் கொண்டால் அவர், தம் கருத்து வெளிப்பாட்டிற்குக் கதைகளைத் துணையாக்கியமை புலனாகும்.

`நவதந்திரக் கதைகள்', பஞ்சதந்திரக்கதைகள் போல் அறிவூட்டக் கூறப்படுவன என்றாலும் பஞ்ச தந்திரக் கதைகள் மூட மைந்தர்களுக்குக் கல்வியறிவு ஊட்டக் கற்பிக்கப்பட்டவை. "தாய், மனைவி முதலிய ஸ்திரீகள் நமக்கு வெறுப்புண்டாகத் தகுந்த வார்த்தை பேசும்போது வாயை மூடிக்கொண்டு பொறுமையுடன் கேட்டுக் கேட்டுப் பழகவேண்டும்"[13] எனச் கட்டுகிறார் பாரதியார். இவ்வாறு சிந்திப்பதற்குரியனவாய் தாம் எண்ணிய சில கருத்துக்களை விளக்க பாரதியாருக்குக் கதைகள் உதவின.

`சின்ன சங்கர'னில் அக்காலப்பிரபுக்களின் பொறுப்பற்ற சிற்றின்ப வேட்கையுள்ள வாழ்வினையும் அவர்க்கு இச்சகம் பேசும் சமஸ்தான வித்வான்களின் போலி வாழ்வையும் வஞ்சப் புகழ்ச்சியுடன் நிந்தித்து, அக்கால நடப்பியல் நிலையைப் படம் பிடிப்பார். கலப்புத் திருமணம் விதவைத் திருமணம் என்ற இரண்டு இலட்சியங்களைக் காட்ட எழுந்த கதை `சந்திரிகையின் கதை'. வாழ்க்கை வரலாற்றையும் கதையையும் குழைத்துத் தந்த சித்திர்மான `சின்ன சங்கரன் கதை' இன்றும் அங்கதம் எனப்படும் நையாண்டி இலக்கியத்திற்குச் சான்றாய்த் திகழ்கிறது.

1.9. தத்துவநடை (வேதாந்தி)

பாரதியார் படைப்புகளில் தத்துவநடைக் கூறுகளும் இடம்பெற்றுள்ளன. பாரதியார் ஒரு வேதாந்தி. வேதாந்தத்தில் பல கூறுகள் இருந்தாலும் அவர் குறுகிய நோக்குடைய வேதாந்தி அல்ல. ஏனென்றால் அவருக்குக் கர்மம் பக்தி ஞானம் ஆகிய மூன்று மார்க்கங்களிலும் நம்பிக்கை உண்டு. ஆகையினால் தான் கீதையைப் பற்றிக் கீழ்க்கண்டவாறு கூறுகிறார்.

"ஈசனை சரணாகதி அடைந்து இக லோகத்தில் மோக்ஷ சாம்ராஜ்யத்தை எய்தி, நிகரற்ற ஆனந்தக் களிப்பில் மூழ்கி வாழும்படி வழிகாட்டுவதே பகவத்கீதையின் முக்கிய நோக்கம் ஆதலால் இ.்து ஞான சாஸ்திரம். இ.்து மோஷ சாஸ்திரம். இ.்து அமரத்துவ சாஸ்திரம்"[14] என்று குறிப்பிடுகிறார் பாரதியார்.

தத்துவச்சிந்தனைகளை மகாகவி பாரதியாரின் பகவத்கீதை மொழிபெயர்ப்பு நூலின் மூலமாக உணரமுடிகிறது.

1.10. மணிபிரவாளநடை(சுதந்திரம்)

வடமொழியும் தமிழும் கலந்த நடையை மணிபிரவாளநடை என்பர். பாரதியார் எழுத்தில் காணப்பெறும் மணிபிரவாளநடைக்குச் சான்று:- `சுதந்திரம்' (விடுதலை) பற்றிய பாரதியின் எண்ணத்தைப் பின்வரும் பகுதி தெரிவிக்கும். `ஸ்வதந்திரமாவது யாதெனில்,

பாரதியார் எழுதிய கதைகளும் கட்டுரைகளும் பல. அவர் மக்களுக்கு அறிவு தரும் நோக்குடன் பல வகைப்பட்ட பொருளைப் பற்றியும் எழுதியுள்ளார். தம்முடைய வாழ்க்கையில் பெற்ற அனுபவங்களையும் கற்பனையில் தோன்றிய காட்சிகளையும் பிறவற்றையும் உணர்ச்சிக் கலப்புள்ள நடையில் படைத்துள்ளார். அவர் உள்ளத்தில் குடிகொண்டிருந்த நேர்மை, துணிவு போன்ற பண்புகள் அவர் நடையிலும் பிரதிபலிக்கின்றன. எதைக்கூற எடுத்துக்கொண்ட போதும் அதைத் தன்னம்பிக்கையோடும் உறுதியோடும் கூறுவதால் அவரது வசனநடை 'நேரான தமிழ் நடை'யாகக் காணப்படுகிறது. அவரது எழுத்து நடை 'தட்டுத்தடையின்றி நேராகப் பாய்ந்து செல்லும் தன்மை'யினவாக விளங்குகின்றது. இவ்வகை நடைக்கு ஓர் எடுத்துக்காட்டு.

> "தமிழா தெய்வத்தை நம்பு! பயப்படாதே! உனக்கு நல்ல காலம் வருகின்றது. !.
>
> தமிழா பயப்படாதே! ஊர்தோறும் தமிழப் பள்ளிக்கூடங்கள் போட்டு ஐரோப்பிய சாஸ்திரங்களை எல்லாம் தமிழில் கற்றுக்கொடுக்க ஏற்பாடு செய்.
>
> ஜாதி வேற்றுமைகளை வளர்க்காதே! "ஜாதியிரண்டொழிய வேறில்லை" என்ற பழந்தமிழ் வாக்கியத்தை வேதமாகக் கொள். பெண்ணை அடிமை என்று கருதாதே. !. வேதங்களை நம்பு! அவற்றின் பொருளைத் தெரிந்து கொண்ட பின் நம்பு. . !

தமிழா உன் வேலைகள் அனைத்திலுமே பொய்க்கதைகள் மிதமிஞ்சிவிட்டன. உனது மதக்கொள்கைகள், லௌகிகக் கொள்கைகள், வைதிக நடை எல்லாவற்றிலுமே பொய்கள் புகுந்து தலைதூக்கி ஆட இடங்கொடுத்து விட்டாய். இவற்றை நீக்கிவிடு. வீட்டிலும் வெளியிலும் தனிமையிலும் கூட்டத்திலும் எதிலும் எப்பொழுதும் நேர்மையிருக்க வேண்டும் உண்மையிருக்க வேண்டும். நீயும் பிறரை வஞ்சிக்கலாகாது. பிறரும் உன்னை வஞ்சிக்கலாகாது. பிறர் பிறரை வஞ்சிப்பதையும் நீ இயன்றவரை தடுக்க வேண்டும்..."[2] என்கிறார் பாரதி. இதன்வழி மகாகவியின் ஆற்றொழுக்குப் பொருந்திய நேரான நடையினைப் படித்து இன்புறலாம்.

1.3.பேச்சு வழக்கு நடை

மகாகவி பாரதியின் நடையினுள் இடையிடையே பேச்சுவழக்குச்சொற்கள் காணப்படுகின்றன.

> என்னுடைய கட்சி. எந்த விஷயம் எழுதினாலும் சரி, ஒரு கதை அல்லது ஒரு தர்க்கம், ஒரு சாஸ்திரம், ஒரு பத்திரிகை விஷயம் எதை எழுதினாலும் வார்த்தை சொல்லுகிற மாதிரியாகவே அமைந்துவிட்டால் நல்லது"[3] என்பது பாரதியார் கருத்து. ஆதலின் பேச்சு வழக்கத்தைத் தழுவி எழுதும் இடங்களிலெல்லாம் அவர் நடையிலே ஒரு வேகமும் உயிரோட்டமும் இருத்தலைக் காணமுடிகிறது.
>
> "ஒரு விரதமெடுத்தால் அது கலையவிடக்கூடாது. இன்னொருதரம் எதற்காகச் சொல்ல வந்தேன் தெரியுமா? ஒருவன் யோகசாஸ்திரம் பழகப் போய் மௌனவிரதம் எடுத்தான். அப்பொழுது அவனுக்கு வயிற்றுக் கடுப்பு வந்தது. பல கடன்காரர்கள் வந்தார்கள். ஒரு புது சிஷ்யன் வந்தான். இராத்திரியில் இரண்டு திருடர்கள் வந்தார்கள். இரண்டு விருந்துகள் வந்தன. ஒரு கல்யாணம் வந்தது. அவசியமாகப் போய்த் தீர வேண்டியது. இன்னும் பல விஷயங்கள் வந்தன. ஒரே நாளில் அவன் மௌன விரதத்தைக் கலைத்துவிட்டான். உடனே வயிற்றுக்கடுப்பு தீர்ந்து போய்விட்டது. கடன், சிஷ்யன், திருடன், கல்யாணம் தீர்ந்துபோய்விட்டன. மறுநாள் ஒரு தொல்லையுமில்லை. பிறகு 'ஹா! இந்த ஸங்கடங்களெல்லாம் இத்தனை விரைவாகத் தொலைந்து போமென்று தெரிந்தால் நான் மௌன விரதத்தைத் கலைத்திருக்க மாட்டேனே என்று சொல்லி அந்த மனிதன் வருத்தப்பட்டான். எல்லா விரதங்களுக்கும் இதுதான் விதி"[4]

இப்பகுதியில் 'வார்த்தைச் சொல்லுகிற மாதிரியாகவே அமைந்து' நிற்கும் பண்பினைப் பார்க்கலாம். மேலும், படிப்பதற்கும் புரிந்து கொள்வதற்கும் எளிமையாக இருத்தலையும் உணரமுடிகிறது.

1.4. பத்திரிக்கை நடை

பாரதியார் இலக்கியங்களைப் படைத்தபோது கையாண்ட நடை வேறு. பத்திரிகை ஆசிரியராக இருந்து பத்திரிக்கைகளில் கையாண்ட நடை வேறு. அவர் பத்திரிகையில் எழுதியவை அப்பத்திரிகையைப் படிப்போர் பொருளறிதற்கென எழுதப் பெற்றவை; ஆதலின் அவற்றில் அவர் கையாண்ட நடை வழக்கில் இருந்த சாதாரண நடையாகும். அவர் காலத்தில் வடமொழிச்சொற்களை விரவி எழுதிச் செல்லும் போக்கு இருந்தது. அந்நடை வகைக்கு ஓர் எடுத்துக்காட்டு.

> "மேலே கூறப்பட்ட தமிழ் மாதைப் பற்றிப் பேசுமிடத்து சென்ற ருஷ்ய-ஜப்பானிய யுத்தத்தின் போது நிகழ்ந்த ஒரு செய்தி

ஒவ்வொருவனும் தனது சொந்த நலத்திற்கேனும் பிறர் நலத்திற்கேனும் அனுகூலமான காரியங்களைச் செய்வதில் (அவனுடைய செய்கைகள் மற்றவர்களுக்கு அக்கிரமான இடையூறுகள் விளையாதிருக்கும் வரை) முழு உரிமை பெற்றவனாக அவனுக்கு எவ்விதமான தடைகளேனும் இமிசைகளேனும் மற்றவர்களினால் ஏற்படக் கூடாதென்ற தர்மம்"[15] என்று இந்தியா பத்திரிகையில் 1907 இல் பாரதியார் எழுதியுள்ளார். நாமும் விடுதலை பெற்றும், பெற்ற விடுதலையால் பிறருக்குத் துன்பம் நிகழ்த்தாதவாறு இருத்தலே நல்லதாம் என்ற பார்தியாரின் கருத்தினை இதன் வழி உணர முடிகிறது.

பெண்ணியம்

பெண்ணியம் குறித்தான பாரதியின் சிந்தனையைப் பின்வரும் பகுதி உரைக்கும். "மாதர்கள் உதவியின்றி ஒரு தேசமும் எவ்விதமான அபிவிருத்தியும் அடைய முடியாது. தேசத்தின் எந்தவிதமான பெருங்காரியமும் கைகூடி வரவேண்டுமானால். அதற்கு ஸ்திரீகளின் மனோபலம் இல்லாமல் தீராது. ஈசுவரனுக்கே தேவிதான் சக்தியென்று மதவாதிகள் சொல்லும் உண்மைப் பொருளை மறந்துவிடக் கூடாது. சக்தியாகிய தேவி இல்லாத விஷயத்தில் புருஷன் ஒன்றும் செய்ய முடியாதென்பது மத விவகாரங்களில் மாத்திரமன்று எல்லா விவகாரங்களிலும் உண்மையேயாகும்."[16] என்று இந்தியா இதழில் பாரதியார் குறிப்பிட்டுள்ளார். ஆணும் பெண்ணும் இணைந்து குடும்பமாக, சரிநிகர் சமானமாக வாழ்தலையே பாரதியார் விரும்பியுள்ளதை இப்பகுதி வழி அறிய முடிகிறது.

1.11. நம்பிக்கை ஊட்டும் நடை

`தத்துவம்' என்னும் தலைப்பின் கீழ் அமைந்த அவருடைய உரைநடை நம்பிக்கையை விதைக்கும் பாங்கில் அமைந்துள்ளது.

"நம்பிக்கை உண்டானால் வெற்றி உண்டு. அந்த நம்பிக்கையின் முக்கிய லக்ஷணம் என்னவென்றால், விடாமுயற்சி, மனத்திற்குள் நிலைத்த நம்பிக்கை இருந்தால் செய்கை தடைபடுமா? முயற்சி தூங்குமா? இடுக்கண் பயமுறுத்துமா? உள்ளம் சேருமா? ராமானுஜர் சோழ நாட்டிலிருந்து போகும் காலத்தில் தம்பின்னே தம்மைப்பிடித்துச் செல்லும் பொருட்டு குதிரைப்படை வருகிறது என்று கேள்விப்பட்ட பொழுது தம்முடன் வந்த சிஷ்யர்களிடம் சொல்லிய மந்திரம் நல்ல மந்திரம். கொடுமை செய்யும் கூற்றமும் என் கோலாடி குறுகப் பெறா எனது கோல் ஆடும்போது எமனும் கிட்ட வரமாட்டான் என்று சொன்னார். அதற்குப் பெயர்தான் நம்பிக்கை" என்று உரைத்த மகாகவி பாரதியார் அதையே மேற் கோளாகவும் காட்டியுள்ளார்). "இவ்வுரைநடைப் பகுதி பாரதியே நேரில் நம் முன் அமர்ந்து பேசி

நம் முன்னே நம்பிக்கையை ஊட்டுவது போல அமைந்துள்ளது. `செய்கை தடைப்படுமா? முயற்சி தூங்குமா" எனத் தொடர்ந்து வினாக்களை எழுப்பிச் சிந்தனைத் திறத்தை வளர்த்து நம்முன்னே நம்பிக்கையை ஊட்டுகிறார் பாரதி. பாரதியின் உரை நடைத்திறத்தின் சிறப்புகுரிய பண்பு இதுவே"[16] என்கிறார் சுந்தரமூர்த்தி

1.12. விவரிப்பு நடை (சக்தியே பெண்மை அனைத்தும்)

பாரதியின் படைப்புகளில் விவரிப்பு நடையும் காணப்பெறுகிறது. மகாகவி பாரதியார் பெண்ணினத்தின் மீது கொண்ட மதிப்பினைப் பின்வரும் பகுதி வெளிப்படுத்தி நிற்கிறது. "தம்பி தமிழ்நாடு வாழ்க! என்றெழுது தமிழ்நாட்டில் நோய்கள் தீர்க! என்றெழுது தமிழ்நாட்டில் வீதிதோறும் தமிழ்ப் பள்ளிக்கூடங்கள் மலிக! என்றெழுது. இந்தப் தமிழ்ப் பள்ளிக்கூடங்களிலே நவீன கலைகளெல்லாம் பயிற்சி பெற்று வளர்க! என்றெழுது!. தமிழ்நாட்டில் ஒரே ஜாதிதான் உண்டு. அதன் பெயர் தமிழ் ஜாதி. அது ஆர்ய ஜாதி என்ற குடும்பத்திலே தலைக்குழந்தை என்றெழுது! ஆணும் பெண்ணும் உயிரின் இரண்டு கண்கள் என்றெழுது!. அவை ஒன்றிலொன்று தாழ்வில்லை என்றெழுது! பெண்ணைத் தாழ்மை செய்தோன் கண்ணைக் குத்திக் கொண்டான் என்றெழுது! `தொழில்கள் தொழில்கள் தொழில்கள் என்று கூவு' வியாபாரம் வளர்க பாத்திரங்கள் பெருகுக! முயற்சிகள் ஓங்குக! ஸங்கீதம், சிற்பம், யந்திர நூல், பூமிநூல், வானநூல், இயற்கை நூலின் ஆயிரம் கிளைகள் இவை தமிழ்நாட்டிலே மலர்ந்திடுக! என்று முழங்கு!. `சக்தி சக்தி சக்தி என்று பாடு'[18] என்று நெல்லையப்பருக்குப் பாரதியார் எழுதிய கடிதத்தில் கூறியுள்ளார். மகாகவி பாரதியாரின் உரைநடைத்திறம் இவ்வாறு விரிவாக அமையப் பெற்றுள்ளது.

தொகுப்புரை

உரைநடை பற்றிய பாரதியாரின் கொள்கைகளைப் பின்வருமாறு அறியலாம்.

1. உலகில் எந்த மொழிகளைக் காட்டிலும் தமிழ் உரைநடை, தெளிவாக இருக்கும்படி எழுத வேண்டும்.
2. கூடியவரை பேசுவது போல எழுதுவது தான் உத்தமம்.
3. எந்தப் பொருள் பற்றி எழுதினாலும் வார்த்தை சொல்லுகிற மாதிரி அமைய வேண்டும்.
4. மக்களுக்குப் பழக்கமில்லாத பொருள் பற்றி எழுதும்போது சொற்றொடர் முதலில் தத்தளித்தாலும் வாய்க்கு வழங்கும்படி எழுத வேண்டும்.
5. சொல்ல வந்ததை நேராக எழுதும் திறமை வேண்டும்.

1014 / 1134

பாரதியாரின் கட்டுரைகளில் கவிதைப் பண்பு வாய்ந்த நடை காணப்படுகிறது. எளிய உணர்ச்சியூட்டும் தருக்க ரீதியான நடை என்று அவரது நடைத்திறன் போற்றப்படுகின்றது. பாரதியார் கற்பனைச் சித்திரங்களை உருவாக்கிய போது கையாண்ட நடையிலே புதுமையும் உயிர்த்துடிப்பும் இயைந்து காணப்படுகிறது.

சான்றெண் விளக்கம்

1. பாரதியார் கட்டுரைகள், ப. 204
2. மேலது, ப. 200
3. மேலது, ப. 252
4. மேலது, ப. 367
5. மேலது, பாரதி தரிசனம் - 1 ப. 310
6. பாரதியார் கட்டுரைகள், ப. 192
7. மேலது, ப. 253
8. வ. ரா. , மகாகவி பாரதியார், ப. 142
9. பாரதியார் கதைகள், ப. 56
10. மோகன். இரா, பாரதி இயல், (மணிவாசகர் பதிப்பகம், சென்னை) ப. 148
11. பாரதியார் கட்டுரைகள், ப. 61
12. மேலது, ப. 62
13. பாரதியார், சந்திரிகை கதை, ப. 253
14. மேலது ப. 26
15. விசுவநாதன். சீனி, பாரதியின் வித்தியாசமான பார்வைகள், ப. 98, இந்தியா- 09-02- 1907, ப. 6
16. மேலது, ப. 100, (இந்தியா, 09. 02. 1907, ப. 6)
17. சுந்தரமூர்த்தி. இ, பாரதி நடையியல், (ஐந்திணைப் பதிப்பகம், சென்னை) ப. 34
18. ஜீவானந்தம். பா, தாமரை (மலர் 23- இதழ்6), பாரதி நூற்றாண்டுச் சிறப்பிதழ்-நெஞ்சையப்பருக்கு மடல், ப. 199

அப்துல் ரகுமான் கவிதைகளில் மாற்று மரபு

போர. மு. மாலதி
பகுதி நேர முனைவர் பட்ட ஆய்வாளர்,
தமிழாய்வுத்துறை,
அரசு கலைக் கல்லூரி (த), கரூர்-5.
தமிழ்நாடு, இந்தியா.

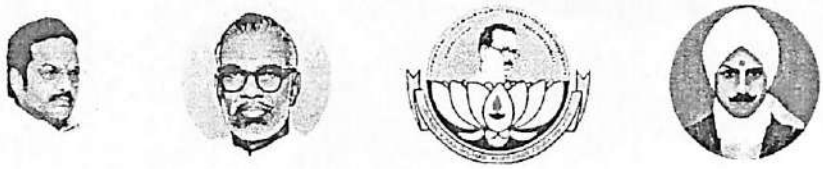
முனைவர். ப. கண்ணுமுத்து
இணைப்பேராசிரியர், தமிழாய்வுத்துறை,
அரசு கலைக் கல்லூரி (த), கரூர் - 5.
தமிழ்நாடு, இந்தியா.

முன்னுரை:

மரபுகள் எல்லாம் என்றும் மாறாததாக எல்லா காலகட்டத்திற்கும் பொருத்தமுடையதாக அமைவதில்லை. கால மாற்றத்திற்கேற்ப மக்களின் சிந்தனையும், வாழ்க்கையும் மாறும் தன்மை கொண்டவை. எனவே, ஒரு காலகட்டத்தில் பயன்பாடுடையதாக கருதப்பட்ட மரபுகள் பிறிதொரு கால கட்டத்தில் பயன்றறதாக மாறிவிடுகிற நிலை ஏற்படுகின்றது. இதனையே இன்றைய தலைமுறையினர்கள் (மரபில் மாற்றம்) மாற்று மரபு என்கின்றனர். முன்னோர்கள் பின்பற்றினார்கள் என்பதற்காகப் பயனற்ற மரபுகளைப் பின்பற்ற முடியாது. என்வே, அம்மரபில் மாற்றம் செய்யவேண்டிய அவசியம் உண்டாகின்றது. அதனால் மரபு சார்ந்த பழக்க வழக்கங்களில் மாறுதல்கள் ஏற்பட்டு அலை மாற்றுமரபு என்று அழைக்கப்படுகின்றன. 'பழையன கழிதலும் புதுவன புகுதலும்' என்ற வரிகளுக்கு ஏற்ப பழைய மரபு சார்ந்த (விஷயங்கள்) கருத்துகள் முற்றிலும் அழிந்து விடாமலும், புதிய கருத்துக்களை ஏற்றுக்கொள்ளும் விதமாகவும் உடையதையே நாம் மாற்றுமரபு என்கிறோம். (அப்துல் ரகுமான்) கவிஞன் தம் கவிதைகளில் மாற்றுமரபாக எதை எதைக் குறிக்கின்றார் என்பதை தெரிந்துகொள்ளும் வகையில் இவ்வியல் அமைகின்றது.

பெண்களின் நிலை:

சங்க காலத்தில் பெண்கள் ஆண்களோடு சரிநிகர் சாமனமாய் வாழ்ந்தனர். ஆடவரோடு பெண்களும் கல்வி கற்று சிறந்தனர். பெண்கள் வீரம் மிக்கவராய் திகழ்ந்தனர். கற்பில் சிறந்து விளங்கினர். காதலைப் போற்றினர். மன்னர்களுக்காக தூது சென்றனர். ஆணுக்குப் பெண் அடிமை என்னும் நிலை இடைக்காலத்தில் ஏற்படத்



பாரதிதாசன் பல்கலைக்கழகம்

பாரதிதாசன் உயராய்வு மையம்

திருச்சிராப்பள்ளி – 620 024.

(Accredited with A+ Grade by NAAC in the Third Cycle)

" சோதனை கடந்து சுதந்திரம் அடைந்தோம்
சாதனை புரிந்து சரித்திரம் படைப்போம் "

தினத்தந்தி நிறுவனர் **சி.பா. ஆதித்தனார் அறக்கட்டளை**

இரண்டு நாள் தேசிய கருத்தரங்கம்

சான்றிதழ்

பாரதிதாசன் உயராய்வு மையத்தின் சார்பாக **21.10.2021** மற்றும் **22.10.2021** அன்று " தினத்தந்தி இதழ் அன்றும் இன்றும் " " என்றும் பாரதியார் " என்ற தலைப்புகளில் நடைபெற்ற இரண்டுநாள் தேசிய கருத்தரங்கில் திருமிகு/ முனைவர் ச. ச.ஸ்ரீதரன், இணைப்பேராசிரியர், தமிழ்த்துறை, தேசியக் கல்லூரி, திருச்சிராப்பள்ளி. அவர்கள் பாரதியும் பகீதியும்

.....

என்ற தலைப்பில் கருத்தாளாராக கலந்து கொண்டமைக்காக / கட்டுரை வாசித்தமைக்காக/பங்கு பெற்றமையைப் பாராட்டி இச்சான்றிதழ் வழங்கப்படுகிறது.

முனைவர். க. கோபிநாத்
பதிவாளர்

முனைவர். அ. கோவிந்தராஜன்
ஒருங்கிணைப்பாளர்

ISSN 2394-2428 Vol 40-47

பாரதிதாசன் பல்கலைக்கழகம்

பாரதிதாசன் உயராய்வு மையம்

திருச்சிராப்பள்ளி – 620 024.

(Accredited with A+ Grade by NAAC in the Third Cycle)

" சோதனை கடந்து சுதந்திரம் அடைந்தோம்
சாதனை பிரிந்து சரித்திரம் படைப்போம் "

தினந்தந்தி நிறுவனர் **சி.பா. ஆதித்தனார் அறக்கட்டளை**

இரண்டு நாள் தேசிய கருத்தரங்கம்

பொருள் : **" தினத்தந்தி இதழ் அன்றும் இன்றும் "**
" என்றும் பாரதியார் "

நாள் : 21.10.2021 வியாழக்கிழமை **இடம் : பட்டமளிப்பு விழா அரங்கம்**

வரவேற்புரை

முனைவர். **அ. கோவிந்தராஜன்** அவர்கள்

இயக்குனர் பாரதிதாசன் உயராய்வு மையம்

திருச்சிராப்பள்ளி – 620 024

தலைமையுரை : **" தினத்தந்தி இதழ் அன்றும் இன்றும் "** & **" என்றும் பாரதியார் "**

நூல் வெளியிடுபவர் :

மாண்பமை துணைவேந்தர்

முனைவர் **ம. செல்வம்** அவர்கள்

திரு. M.R. **இரகுநாதன்** அவர்கள்

பாரதிதாசன் பல்கலைக்கழக ஆட்சிக்குழு உறுப்பினர் மற்றும் தாளாளர்,
மீனாட்சி இராமசாமி கல்வி நிறுவனங்கள், தத்தனூர் அஞ்சல், அரியலூர் மாவட்டம்

முனைவர் **சா. சேகர்** அவர்கள்

பாரதிதாசன் பல்கலைக்கழக ஆட்சிக்குழு உறுப்பினர் மற்றும் முதல்வர்,
மீனாட்சி இராமசாமி கலை மற்றும் அறிவியல் கல்லூரி, தத்தனூர் அஞ்சல், அரியலூர் மாவட்டம்

நாள் : 21.10.2021 **அமர்வு : 1** **பிற்பகல் : 2.00 முதல் 4.00 வரை**

அமர்வு தலைவர்

முனைவர் **அ. இலக்குமிபிரபா** அவர்கள்

பேராசிரியர், தாவரவியல் துறை தலைவர் & நாட்டு நலப்பணித்திட்ட ஒருங்கிணைப்பாளர்,
பாரதிதாசன் பல்கலைக்கழகம், திருச்சிராப்பள்ளி – 620 024.

கட்டுரையுரையாளர்கள்

முனைவர் **சி. வீரமணி** அவர்கள்

என்றென்றும் தினத்தந்தி

முனைவர் **ரெ. நல்லமுத்து** அவர்கள்

மக்கள் நலனில் தினத்தந்தி நாளிதழ் தலையங்கம்

முனைவர் **ப. விஜயலெட்சுமி** அவர்கள்

தினத்தந்தி நாளிதழில் பெண்ணிய சிந்தனைகள்

நாள் : 22.10.2021 அமர்வு : 2 முற்பகல் : 10.30 முதல் 12.00 வரை

அமர்வு தலைவர்

முனைவர் **அ. பழனிச்சாமி** அவர்கள்

பேராசிரியர் பாரதிதாசன் பல்கலைக்கழக விடுதிகளின் முதன்மை நிர்வாகி &
விவேகானந்தர் உயராய்வு மையம்,
பாரதிதாசன் பல்கலைக்கழகம், திருச்சிராப்பள்ளி – 620 024.

கட்டுரையுரையாளர்கள்

முனைவர் **ச. ஈஸ்வரன்** அவர்கள்

பாரதியும் பக்தியும்

முனைவர் **ம.ப. தமிழ்ச்செல்வி** அவர்கள்

பாரதி கண்ட பாரதம்

முனைவர் **மு. பாலகிருட்டிணன்** அவர்கள்

பாரதியாரின் கதைகளில் கருத்துக் கட்டமைப்பு

நாள் : 22.10.2021 அமர்வு : 3 பிற்பகல் : 12.00 முதல் 1.30 வரை

அமர்வு தலைவர்

முனைவர் **ஜெ. சந்திரகலா** அவர்கள்

தமிழியியல் துறை,
பாரதிதாசன் பல்கலைக்கழகம், திருச்சிராப்பள்ளி – 620 024.

கட்டுரையுரையாளர்கள்

முனைவர் **தி. நெடுஞ்செழியன்** அவர்கள்

தமிழர் வாழ்வியலோடு தினத்தந்தி

முனைவர் **இரா. இரவிச்சந்திரன்** அவர்கள்

மகாகவியின் தமிழ் முழக்கம்

முனைவர் **து. புஷ்பவள்ளி** அவர்கள்

பாரதியின் பெண்ணிய பார்வை

நாள் : 22.10.2021 நிறைவரங்கம் பிற்பகல் : 2.30 முதல் 5.00 வரை

தலைமையுரையாற்றி சான்றிதழ்கள் வழங்குபவர் :

முனைவர் **க. கோபிநாத்** அவர்கள்

பதிவாளர், பாரதிதாசன் பல்கலைக்கழகம், திருச்சிராப்பள்ளி – 620 024.

சிறப்புரை

முனைவர் **மு. செல்வம்** அவர்கள்

பாரதிதாசன் பல்கலைக்கழகம் ஆட்சிக்குழு உறுப்பினர்,
பேராசிரியர் தலைவர், வணிகவியல் மற்றும் நிதிக்கல்வியியல் துறை,
பாரதிதாசன் பல்கலைக்கழகம், திருச்சிராப்பள்ளி – 620 024.

நன்றியுரை

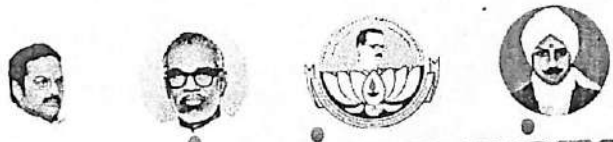
முனைவர் **அ. கோவிந்தராஜன்** அவர்கள்

இயக்குனர், பாரதிதாசன் உயராய்வு மையம்,
பாரதிதாசன் பல்கலைக்கழகம், திருச்சிராப்பள்ளி – 620 024.

இவன்,

பாரதிதாசன் உயராய்வு மையம்

பாரதிதாசன் பல்கலைக்கழகம், திருச்சிராப்பள்ளி – 620 024.



பாரதிதாசன் பல்கலைக்கழகம்

பாரதிதாசன் உயராய்வு மையம்

திருச்சிராப்பள்ளி – 620 024.

(Accredited with A+ Grade by NAAC in the Third Cycle)

" சோதனை கடந்து சுதந்திரம் அடைந்தோம்
சாதனை பிரிந்து சரித்திரம் படைப்போம் "

தினந்தந்தி நிறுவனர் **சி.பா. ஆதித்தனார் அறக்கட்டளை**

இரண்டு நாள் தேசிய கருத்தரங்கம்

பொருள் : **" தினத்தந்தி இதழ் அன்றும் இன்றும் "**
" என்றும் பாரதியார் "

நாள் : 21.10.2021 வியாழக்கிழமை — இடம் : பட்டமளிப்பு விழா அரங்கம்

வரவேற்புரை

முனைவர். **அ. கோவிந்தராஜன்** அவர்கள்

இயக்குனர் பாரதிதாசன் உயராய்வு மையம்

திருச்சிராப்பள்ளி – 620 024

தலைமையுரை : **" தினத்தந்தி இதழ் அன்றும் இன்றும் "** & **" என்றும் பாரதியார் "**

நூல் வெளியிடுபவர் :

மாண்பமை துணைவேந்தர்

முனைவர் **ம. செல்வம்** அவர்கள்

திரு. **M.R. இரகுநாதன்** அவர்கள்

பாரதிதாசன் பல்கலைக்கழக ஆட்சிக்குழு உறுப்பினர் மற்றும் தாளாளர்,

மீனாட்சி இராமசாமி கல்வி நிறுவனங்கள், தத்தனூர் அஞ்சல், அரியலூர் மாவட்டம்

முனைவர் **சா. சேகர்** அவர்கள்

பாரதிதாசன் பல்கலைக்கழக ஆட்சிக்குழு உறுப்பினர் மற்றும் முதல்வர்,

மீனாட்சி இராமசாமி கலை மற்றும் அறிவியல் கல்லூரி, தத்தனூர் அஞ்சல், அரியலூர் மாவட்டம்

நாள் : 21.10.2021 — அமர்வு : 1 — பிற்பகல் : 2.00 முதல் 4.00 வரை

அமர்வு தலைவர்

முனைவர் **அ. இலக்குமிபிரபா** அவர்கள்

பேராசிரியர், தாவரவியல் துறை தலைவர் & நாட்டு நலப்பணித்திட்ட ஒருங்கிணைப்பாளர்,

பாரதிதாசன் பல்கலைக்கழகம், திருச்சிராப்பள்ளி – 620 024.

கட்டுரையுரையாளர்கள்

முனைவர் **சி. வீரமணி** அவர்கள்

என்றென்றும் தினத்தந்தி

முனைவர் **தெ. நல்லமுத்து** அவர்கள்

மக்கள் நலனில் தினத்தந்தி நாளிதழ் தலையங்கம்

முனைவர் **ப. விஜயலெட்சுமி** அவர்கள்

தினத்தந்தி நாளிதழில் பெண்ணிய சிந்தனைகள்

22.10.2021

மகாகவி
பாரதியாரும்
தமிழர் தந்தை
சி.பா. ஆதித்தனாரும்

40-47

முனைவர் அ. கோவிந்தராஜன்

வசந்தா பதிப்பகம்
26 குறுக்குத் தெரு, ஜோசப் காலனி,
ஆதம்பாக்கம், சென்னை - 600 088.

22-10-2021

1020 / 1134

பொருளடக்கம்

வ. எண் கட்டுரை தலைப்பு & கட்டுரையாளரின் பெயர்

1. மகாகவி பாரதியார் மற்றும் தமிழர் தந்தை சி.பா. ஆதித்தனாரின் இதழியல் பணிகள்
முனைவர் அ. கோவிந்தராஜன்
உதவிப்பேராசிரியர் & இயக்குநர் (பொ)
பாரதிதாசன் உயராய்வு மையம்
பாரதிதாசன் பல்கலைக்கழகம்
திருச்சிராப்பள்ளி - 620 024 ப. 1 - 16

2. தமிழர் வாழ்வியலோடு "தினத்தந்தி" நாளிதழ்
முனைவர் தி. நெடுஞ்செழியன்
தலைவர்
சி.பா. ஆதித்தனார் தமிழியல் ஆய்வுக் கழகம்,
எண்.4/142, ஜே.எம். நகர்
அய்யனார் நகர் முதல் தெரு
குண்டூர், திருச்சிராப்பள்ளி - 620 007 ப. 17 - 26

3. பாரதியாரும் பக்தியும்
முனைவர் ச. ஈஸ்வரன்,
இணைப்பேராசிரியர் & தலைவர்,
தமிழாய்வுத்துறை,
தேசியக்கல்லூரி (தன்னாட்சி),
திருச்சிராப்பள்ளி - 620 001 ப. 27 - 33

4. பாரதி கண்ட பாரதம்
முனைவர் ம. ப. தமிழ்ச்செல்வி
இணைப்பேராசிரியர், தமிழ்த்துறை
சீதாலட்சுமி இராமசுவாமி தன்னாட்சிக் கல்லூரி
திருச்சிராப்பள்ளி - 620 002 ப. 34 - 47

வ. எண் கட்டுரை தலைப்பு & கட்டுரையாளரின் பெயர்

5. [illegible]
முனைவர் வி. வீரமணி
இணைப்பேராசிரியர், அயலதியியல் துறை
தமிழ்ப் பல்கலைக்கழகம்
தஞ்சாவூர் - 613 010. ப. 48 - 53

6. மகாகவியின் தமிழ் முழக்கம்
முனைவர் இரா. இரவிச்சந்திரன்
இணைப்பேராசிரியர்
தமிழாய்வுத்துறை
தேசியக்கல்லூரி (தன்னாட்சி)
திருச்சிராப்பள்ளி - 620 001. ப. 54 - 58

7. சி.பா. ஆதித்தனார் இதழியல் பணியின் மகத்துவம்
முனைவர் பொ. இரமேஷ்
உதவிப் பேராசிரியர், தமிழாய்வுத்துறை
பெரியார் ஈ.வெ.ரா. கல்லூரி (தன்னாட்சி)
திருச்சிராப்பள்ளி - 620 023. ப. 59 - 67

8. பாரதியின் பெண்ணியப் பார்வை
முனைவர் து. புஷ்பவள்ளி
உதவிப்பேராசிரியர், தமிழ்த்துறை
சீதாலெட்சுமி இராமசுவாமி தன்னாட்சிக் கல்லூரி
திருச்சிராப்பள்ளி - 620 002. ப. 68 - 76

9. பாரதியாரின் கதைகளில் கருத்துக் கட்டமைப்பு
முனைவர் மு. பாலகிருஷ்ணன்
உதவிப்பேராசிரியர் & துறைத்தலைவர்
அரசு கலை மற்றும் அறிவியல் கல்லூரி
குரும்பலூர், பெரம்பலூர் - 621 107. ப. 77 - 88

1021 / 1134

பாரதியாரும் பக்தியும்

முனைவர் ச. ரஸ்வரன்,
இணைப்பேராசிரியர் & தலைவர்,
தமிழாய்வுத்துறை,
தேசியக்கல்லூரி (தன்னாட்சி),
திருச்சிராப்பள்ளி - 620 001.
செல்: 94860 66384

முன்னுரை

"பாட்டினைப்போல் ஆச்சர்யம் பாரின்மிசை இல்லையடா" என்றும், "ஆசை தரும் கோடி அதிசயங்கள் கண்டதிலே ஓசை தரும் இன்பம் உவமையிலா இன்பமடா" என்றும் கவிதை இன்பத்தைப் போற்றிப் புகழும் பாரதியாரின் கவிதைகளில் காணலாகும் பக்திச் சிந்தனைகளை இக்கட்டுரையில் தொகுத்துக் காண்போம்.

பாரதியாரின் உலகளாவிய பார்வையில் பக்தியும் ஒன்று. மகாத்மாவையும், திலகரையும் மனித தெய்வங்களாகக் கண்ட பாரதியார் பரம்பொருளாம் இறைவனைப் பல கவிதைகளில் வியந்து போற்றியும், வேண்டியும் பாடியுள்ளார். சிற்சில இடங்களில் மூடபக்தியைச் சாடவும் தவறவில்லை. பயத்தினின்று விடுதலை பெற இறையருளை இறைஞ்சுகின்றார். மனம் நன்னிலை பெறல் வேண்டி "நாசமின்றி நமை நித்தம் காப்பார் இயேசு" (ப. 77) எனவும், "எல்லாரும் வந்தேத்தும் அளவில் யமபயம் கெடச் செய்பவன் அல்லா" (ப. 178) எனவும் துதித்துப் பரவுகின்றார். விநாயகர், முருகன், சிவன், கண்ணன், கலைமகள், திருமகள், பராசக்தி, ராதை, காளி, முத்துமாரி உள்ளிட்ட பரம்பொருள் மீது கவிதைகளைப் புனைந்து தம் பக்தி உணர்வினைப் புலப்படுத்தியுள்ளார். அத்துடன் சூரியன், சந்திரன் முதலிய இயற்கையையும் வணங்கிப் போற்றியுள்ளார்.

பாரதியாரைப் பொறுத்தவரை பக்தியானது மிகுந்த நெருக்கத்துடன் விரும்பி முனைக்கும் நட்புமைபோல், [illegible] செய்யும் பரவசமாகவும் (ப. 99) என்கிறார் மகாகவி. நம்பிக்கை கெடுதியினை தரும் வாக்கின் முனம் நிறை நம்பிக்கையின் சிறப்பினை உணர வைக்கின்றார். சிந்தனைத் தெளியும், செயலும் செயல்கள் மாவும் சிறப்புற அமையும். நெஞ்சில் சஞ்சலம் நீங்கி உறுதி தோன்றும், கலவி வளரும், பிணி நீங்கி, பல காரியம் கையுறும், உடல் சொன்னபடி நடக்கும் என "பக்தியினாலே இந்தப் பாரினில் எய்திடும் மேன்மைகள் கேளடி" (ப. 90) எனும் கவிதையில் உணர்த்துவதன் மூலமாக பக்தியின் மாண்பினை உணரலாம்.

விநாயகர்

விநாயகப் பெருமானை வழிபடலால் அச்சம் தீரும், அமுதம் விளையும் என்கிறார். உலகத்து உயிர்கள் எல்லாம் வாழவேண்டும் என அனைவருமே விரும்புவர். பாரதியார் விநாயகப் பெருமானிடம் வேண்டும் போது மக்களிடம் மட்டுமின்றி விலங்குகள், புழு, பூச்சிகள், முகமையான மரங்கள் முதலியனவெல்லாம் தம் சொல்லால் துன்பங்கள் நீங்கி ஒன்றுடன் ஒன்று பகையின்றி ஒத்து வாழவேண்டும் எனக் கேட்கிறார். "கணபதி ராயன் - அவனிரு காலைப் பணித்திடுவோம்" (ப. 163) என்கிறார்.

"நமக்குத் தொழில் கவிதை, நாட்டிற்கு உழைத்தல்
இமைப்பொழுதும் சோராது இருத்தல்-உமைக்கு இனிய
மைந்தன் கணநாதன் நம்குடியை வாழ்விப்பான்
சிந்தையே இம்மூன்றும் செய்" (ப. 99)

என்று சுட்டுகிறார் மகாகவி.

முழுமுதற் கடவுளுக்கே தம் ஆவி, உள்ளம் போன்றனவற்றை [illegible]தாகவும், ஆதலால் தமக்கு புகழ், செல்வம், அழகு முதலியனவற்றைத் தரும்படியும் வேண்டுகிறார். புதுச்சேரியில் உள்ள [illegible]குள விநாயகரையும் போற்றித் துதிக்கின்றார்.

"அபயம் அபயம் அபயம் நான் கேட்டேன்
நோவு வேண்டேன் நூறாண்டு வேண்டினேன்" (ப. 97)

என விநாயகப் பெருமானிடம் நூறாண்டுகள் வாழ வேண்டிய பாரதியார் தம் முப்பத்தொன்பதாவது வயதிலேயே (1882, டிசம்பர் 11 முதல் 1921 செப்டம்பர் 11 வரை) நம்மைவிட்டு நீங்கினாலும், காலத்தை வென்ற சாகாவரம் பெற்ற கவிதைகளை நம்மிடம் தந்து சென்றுள்ளதை எண்ணல் வேண்டும். புதியதோர் சத்தியயுகம் காண வேண்டி இவர் கண்ட கனவுகள் நம் கண்முன்னே விரிந்து விரிதாக நனவாகிக் கொண்டு வருதலையும் உணரல் நன்றாம்.

முருகனைப் போற்றல்

முருகனைத் தமிழ்க்கடவுள் என்று தமிழ் இலக்கியங்கள் சிறப்பித்துக் கூறும் முருகப் பெருமானின் வீரம் நிறைந்த திருவிழிப் பார்வையும், வெற்றி வேலும், மயிலும், நின்று எந்நேரத்திலும் தம்மைக் காத்து வருவதாக மகிழ்வுடன் உரைக்கிறார் பாரதியார். "வாகையே சுமக்கும் வேலை, வணங்குவது எமக்கு வேலை" (ப. 107) எனப் பாடுகின்றார். முருகன் பாட்டு, முருகக் கடவுள் மீது கிளித்தூது, வள்ளிப்பாட்டு போன்றனவற்றைப் பாடியுள்ளார்.

பெண்ணே தெய்வம் எனல்

"எத்தனை கோடி இன்பம் வைத்தாய்! எங்கள் இறைவா!" (ப. 109) என்று பாரதி பெண் தெய்வத்திற்கே அதிக முக்கியத்துவம் அளித்துள்ளார். பராசக்தி, கலைமகள், காளி, திருமகள் உள்ளிட்ட பெண் தெய்வங்களைப் போற்றிப் பல கவிதைகள் புனைந்துள்ளதை அறிய முடிகிறது. எல்லாமே சக்தியின் வடிவம் என்பது பாரதியின் அசைக்க முடியாத நம்பிக்கை,

"காணி நிலம் வேண்டும் - பராசக்தி
காணி நிலம் வேண்டும் - அந்தக்
காணி நிலத்திடையே - ஓர் மாளிகை
கட்டித்தர வேண்டும்
பாட்டுக் கலந்திடவே - அங்கேயொரு
பத்தினிப்பெண் வேணும் - என்றன்
பாட்டுத் திறத்தாலே - இவ்வையத்தைப்
பாலித்திட வேணும்" (ப. 112)

எனில்லாம் பராசக்தியிடம் கேட்கிறார். [illegible]க்கு மேல் இவர் ஓர் தெய்வம் உண்டென்று பாரதி, "நம்மைத்தாள் வீணை செய்து அதை நலம் பெற புதுநெறியில் எறிவதுன்னிடம் சொல்லடி சிவசக்தி" என்கிறார். மேலும் சென்ற துறிவுடன் படைத்துவிட்டாய், வல்லமை தாராயோ இம்மாநிலம் பயனுற வாழ்வதற்கே" என்று சிவசக்தியிடம் விண்ணப்பிக்கிறார்.

பாரதியாரது தெய்வப் பாடல்களில் சக்திப் பாடல்கள் முக்கியத்துவம் கொண்டவை. கல்கெத்தாவிலே சகோதரி நிவேதிதா அவர்களைப் பார்த்தபோது சக்தி நிலையை (சொரூபம்) நேரில் கண்ட தன்மை (பரவசம்) ஏற்பட்டதால் "ஸ்வதேச கீதங்களை" நிவேதிதாவிற்குச் சமர்ப்பணம் செய்துள்ளார் பாரதியார்.

அன்னையை வேண்டல்

நல்லதையே எண்ணல் வேண்டும். இவ்விதம் எண்ண நல்லருள் புரிய வேண்டும். செய்த பாவங்கள் எல்லாம் பரிதிமுன் பனியே போல் அழிந்திட வேண்டும் என்கிறார்.

"எண்ணிய முடிதல் வேண்டும், நல்லவே எண்ணல் வேண்டும்
திண்ணிய நெஞ்சம் வேண்டும் தெளிந்த நல்லறிவு வேணடும்"

(ப. 114)

தெரிந்தோ, தெரியாமலோ, அறிந்தோ, அறியாமலோ நாம் செய்த சகல பிழைகளையும் பொறுத்துக் காத்து அருள் பாலித்திடுமாறு துதிக்கின்றார் மகாகவி பாரதியார்.

மகாசக்திக்கு விண்ணப்பித்தல்

நம்மிடம் உள்ள குறைகளையெல்லாம் மகாசக்தியிடம் சேர்த்தோதி அவைகளையெல்லாம் நீக்கிவிடு, போக்கிவிடு என உரைக்கின்றார்.

"பந்தத்தை நீக்கிவிடு-அல்லால் உயிர்ப் பாரத்தைப் போக்கிவிடு
சிந்தை தெளிவாக்கு-அல்லால் இதைச் செத்த உடலாக்கு"

(ப.113)

என்று விண்ணப்பிக்கிறார்.

1023 / 1134

எல்லாம் சக்திக்கே சமர்ப்பணம் செய்தல் உட்பட்ட உள்ள அனைத்து உறுப்புகளையும் சக்திக்கு அர்ப்பணம் செய்கின்றார். "பலகையைச் சக்தி தனக்கே கருவியாக்கு - உறு சாதனைகள் யாவினையும் கூடும்" (ப. 120) "சக்தி என்ற கொல்லுங்கோட்ட கருச்சலங்கள் யாவினையும் கொல்லு" (ப. 126) என்கிறார் பாரதியார். என்னும் காரியங்கள் அனைத்திலும் வெற்றிகிட்ட காளிதேவியினை வேண்டுகிறார்.

"தேடியுனைச் சரணடைந்தேன், தேசமுத்துமாரி
கேடதனை நீக்கிடுவாய்! கேட்ட வரம் தருவாய்!
நம்பினார் கெடுவதில்லை, நான்கு மறை தீர்ப்பு
அம்பிகையைச் சரண்புகுந்தால் அதிக வரம் பெறலாம்"(ப.146)

என முத்துமாரியன்னையின் புகழினைப் பாடிப் பரவுகின்றார்.

நீக்கமற நிறைந்திருத்தல்

கண்ணப்பிரானைப் பாடும்போது பார்க்கும் இடங்களில் எல்லாம் நீக்கமற நிறைந்திருப்பதாகப் புகழ்கின்றார்.

"காக்கைச் சிறகினிலே நந்தலாலா - நின்றன்
கரிய நிறம் தோன்றுதையே, நந்தலாலா
பார்க்கும் மரங்கள் எல்லாம் நந்தலாலா!" - நின்றன்
பச்சை நிறம் தோன்றுதையே நந்தலாலா!" (ப. 146)

"கண்ணன் திருவடி எண்ணுக மனமே, திண்ணம் அழியா, வண்ணம் தருமே" (ப. 147)

என்னும் கவிதையின் மூலம் கண்ணபிரானின் பெருமைகளையெல்லாம் பறைசாற்றுகின்றார்.

திருமகளைத் துதிக்கும்போது "ஈடு நினக்கோர்
தெய்வமுண்டோ? எனக்கு உனையன்றிச் சரணமுண்டோ?"
(ப. 153)

என்று உரைக்கின்றார்.

கலைமகளைப் பற்றுதல்

கலைமகளின் அறிவுக்கு ஆதாரமாக, அவள் இருக்கும் இடம் வெள்ளைத் தாமரை என்றும், அவள் வீணையில் எழும் நாதத்திலும், பாவலர் உள்ளத்திலும், வஞ்சமில்லாத தொழில் புரிவோர் இதயத்திலும் இருப்பதாகத் தெளிவிக்கிறார்.

"வெள்ளைத் தாமரைப் பூவில் இருப்பாள்
வீணை செய்யும்ஒலியில் இருப்பாள்
கொள்ளை இன்பம் குலவு கவிதை கூறுபாவலர்
உள்ளத்து இருப்பாள்"

(ப. 157)

"மனதில் உறுதி வேண்டும், வாக்கினிலே இனிமை வேண்டும், பெண் விடுதலை வேண்டும், பெரிய தெய்வம் காக்க வேண்டும்" எனக் கேட்கிறார் மகாகவி பாரதியார்.

மாண்பினைச் சிறப்பித்தல்

இப்புவியில் உள்ளோர், "திருமாலும், சிவனும் ஒன்றே என உணரல் நன்று" என்கிறார். (ப. 142). மேலும் "உயிர்களெல்லாம் தெய்வமின்றிப் பிறவொன்றில்லை" எனவும், "எழுதுகோல் தெய்வம், தீந்தமிழ் எழுத்தும் தெய்வம்" (ப. 261) என்றும் உரைக்கிறார்.

"யாரும் பணிந்திடும் தெய்வம்-பொருள் யாவினும்
நின்றிடும் தெய்வம்
வலிமையுடையது தெய்வம்-நம்மை வாழ்ந்திடச்
செய்வது தெய்வம்" (ப. 206)

எனக் கூறும் பாரதியார். "வெற்றி எட்டுத்திக்கும் எட்டக் கொட்டும் முரசே" எனும் கவிதையில்,

"ஊருக்கு நல்லது சொல்வேன் - எனக்கு உண்மை
தெரிந்து சொல்வேன்
சீருக்கு எல்லாம் முதலாகும் ஒரு தெய்வம் துணை

1024 / 1134

செய்ய வேண்டும்" (ப. 20) எனத் தெரிவிக்கிறார்.

"ஒடி விளையாடு பாப்பா" பாட்டில்,

"உயிர்களிடத்து அன்பு வேறும் - தெய்வம்
உண்மையென்று நானிதல் வேண்டும்" (ப. 203)

எனக் கூறுகிறார்.

பாஞ்சாலி சபதத்தில் வாணியை வேண்டும்போது, "தெளிவுடன் அறிந்திடவும் மொழிந்திடவும் ஒளி வளரும் தமிழ்வாணி உதவவேண்டும்" (ப. 352) எனக் கேட்கின்றார்.

"வண்டிக்காரன் பாட்டில்" - காட்டு வழியில் கள்ளர் பயமிருந்தால் வீட்டுக்குல தெய்வம் வீரம்மை காக்கும் எனவும், கள்ளர் நெருங்கினால் கறுத்த மாரியின் பெயர் சொன்னால் காலனும் அஞ்சுமடா" (ப. 192) என்றும் உரைத்திருப்பதன் வாயிலாக பாரதியாரின் பக்தி உணர்வினை நன்கு அறிய முடிகிறது.

முடிவுரை

"வகுத்தான் வகுத்த வகையல்லாமல் கோடி
தொகுத்தார்க்கும் துய்த்தல் அரிது" (குறள்-387)

எனும் வள்ளுவர் வாக்கிற்கிணங்க பக்தி உணர்வினை நம் உள்ளத்து வளர்த்து அறியாமையினின்றும், தீய எண்ணத்தினின்றும் விடுபட்டு வாழ முயல்வோமாக!

பாரதியின் புதிய ஆத்திசூடி வழி, கற்றது ஒழுகி, செய்வது துணிந்து செய்து, நீதிநூல் பயின்று, நாளெல்லாம் (நல்)வினை செய்து, புதியன விரும்பி (ப. 201) சிறப்புடன் வாழ்வோமாக!

துணை நின்ற நூல்

- பாரதியார் கவிதைகள், கிரி டிரேடிங் ஏஜன்ஸி வெளியீடு, 10, கபாலீஸ்வரர் சன்னதி தெரு, மயிலாப்பூர், சென்னை - 600 004, முதற்பதிப்பு, மே. 2000.

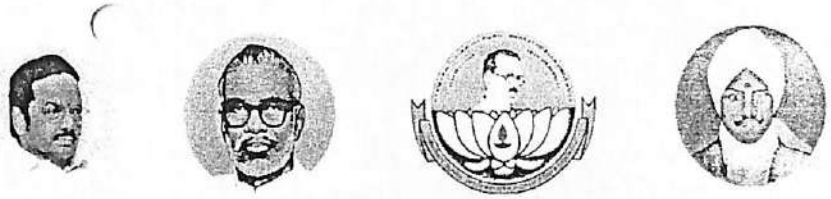
பாரதி கண்ட பாரதம்

முனைவர் ம.ப. தமிழ்ச்செல்வி
இணைப்பேராசிரியர், தமிழ்த்துறை
சீதாலட்சுமி இராமசுவாமி கல்லூரி
திருச்சிராப்பள்ளி - 620 002.
கைப்பேசி: 9944060602
மின்னஞ்சல்: mptamils@gmail. com

தமிழ் மண்ணில் ஈரம் பாய்ச்சி வீரம் விளைத்த சொல் உழவன். இப்புவி உள்ள காலம் வரை எல்லோராலும் மறக்க முடியாத மனிதன். தமிழ்மொழி பெற்றெடுத்த தவப்புதல்வன். எட்டயபுரத்தில் பிறந்த பட்டயக் கவிஞன் முறுக்குமீசைக்காரன். முண்டாசுக்கவிஞன். சுதந்திரதாசன். நவீன தமிழ்க்கவிதைகளுக்குத் தகப்பன். தமிழ், தமிழர் நலன், பெண் விடுதலை, தீண்டாமை போன்றவற்றிற்காக ஒரு நூற்றாண்டுக்கு முன்னரே தனது கவிதைக் குரலெடுத்து உரக்க கத்தியவன். தேசபக்தி என்னும் புதிய நெருப்பை மூட்டி விடுதலைப் போராட்டக் களத்தில் நின்று அதனைத் தெய்வபக்தியாக்கி செந்தமிழ்நாட்டையும் இன்தமிழையும் வீரத்தமிழனையும் வாயாரப் புகழ்ந்தவன். பாரதம் எனச் சொல்லி மனித குலத்தை வாழ்விக்கக் கவிதையைக் கையிலெடுத்த காவலன். எண்ணும் காரியங்கள் யாவற்றிலும் தேசத்திற்காக வெற்றி கிட்ட வேண்டியும் நாட்டு மக்களின் பிணியும் வறுமையும் நீங்கவும் மானிட இனத்தை ஒன்றாய்க் கூட்டி பார் முழுதும் பயன்பெறவும் கவிதை பாடியவர். உழைப்பைப் புகழ்ந்து உழைப்பால் உயர்ந்தவர். கவிதைக் குரலை பாரதநாட்டினிலும் கூட்டினிலும் நாளெல்லாம் நன்றாக ஒலிக்கச் செய்தவர்.

பாரதநாடு

உலகிலுள்ள மிக தொன்மையான வாழும் கலாச்சாரங்களில் காலத்தால் பழமையானதும் உயிர்த்துடிப்பில் புதியதுமான பாரத



பாரதிதாசன் பல்கலைக்கழகம்

பாரதிதாசன் உயராய்வு மையம்

திருச்சிராப்பள்ளி – 620 024.

(Accredited with A+ Grade by NAAC in the Third Cycle)

" சோதனை கடந்து சுதந்திரம் அடைந்தோம்
சாதனை புரிந்து சரித்திரம் படைப்போம் "

தினத்தந்தி நிறுவனர் **சி.பா. ஆதித்தனார் அறக்கட்டளை**

இரண்டு நாள் தேசிய கருத்தரங்கம்

சான்றிதழ்

பாரதிதாசன் உயராய்வு மையத்தின் சார்பாக **21.10.2021** மற்றும் **22.10.2021** அன்று **" தினத்தந்தி இதழ் அன்றும் இன்றும் "** **" என்றும் பாரதியார் "** என்ற தலைப்புகளில் நடைபெற்ற இரண்டுநாள் தேசிய கருத்தரங்கில் திருமிகு/

முனைவர் ச. ஈ.ஸ்ரீதரன், இணைப்பேராசிரியர், தமிழ்த்துறைத் தலைவர், தேசியக் கல்லூரி, திருச்சிராப்பள்ளி.

அவர்கள் பாரதியும் பகிர்தியும்

.....

என்ற தலைப்பில் கருத்தாளாராக கலந்து கொண்டமைக்காக / கட்டுரை வாசித்தமைக்காக/பங்கு பெற்றமையைப் பாராட்டி இச்சான்றிதழ் வழங்கப்படுகிறது.

முனைவர். க. கோபிநாத்
பதிவாளர்

முனைவர். அ. கோவிந்தராஜன்
ஒருங்கிணைப்பாளர்

பாரதிதாசன் உயராய்வு மையம்

பாரதிதாசன் பல்கலைக்கழகம்

திருச்சிராப்பள்ளி – 620 024.

முனைவர் **அ.கோவிந்தராஜன்**
உதவிப்பேராசிரியர் & இயக்குநர் (பொ)

தொலைபேசி : 0431 - 2407072
செல் பேசி : 99409 33534
விரிவு எண் : 616

நாள்: 22.10.2021

வருகைச் சான்றிதழ்

முனைவர் ச.ஈஸ்வரன், தலைவர் மற்றும் இணைப்பேராசிரியர், தமிழாய்வுத்துறை, தேசியக் கல்லூரி(தன்னாட்சி), திருச்சிராப்பள்ளி அவர்கள் பாரதிதாசன் பல்கலைக்கழக பாரதிதாசன் உயராய்வு மையத்தில், சி.பா.ஆதித்தனார் அறக்கட்டளை இரண்டு நாட்கள்(21.10.2021,22.10.2021) தேசியக் கருத்தரங்கில் "தினத்தந்தி இதழ் அன்றும் இன்றும் - என்றும் பாரதியார்" எனும் பொருண்மையில் 'பாரதியும் பக்தியும்' என்ற தலைப்பில் கட்டுரை வாசித்தார் எனச் சான்றளிக்கப்படுகிறது.

22/10/2021

பாரதிதாசன் உயராய்வு மையம் திருச்சிராப்பள்ளி-620 024 பாரதிதாசன் பல்கலைக்கழகம்

முனைவர் இரா. இரவிச்சந்திரன்
இணைப் பேராசிரியர்
தமிழாய்வுத்துறை
தேசியக்கல்லூரி (தன்னாட்சி)
திருச்சிராப்பள்ளி - 620 001.

மகாகவி பாரதியாரும் தமிழர் தத்தை சி.பா.ஆதித்தனாரும்

வசந்தா பதிப்பகம்

மகாகவி பாரதியாரும் தமிழர் தத்தை சி.பா.ஆதித்தனாரும்

முனைவர் அ.கோவிந்தராஜன்

1028 / 1134

திலைக்கச்செய்வதோடு மன அழுத்தத்தை போக்கும் மருந்தாகவும் இது அமைகிறது எனலாம்.

பத்திரிக்கைகள் படைக்களங்களை கட்டி எழுப்பக்கூடியவை ஆகும். எங்கயோ நடக்கக் கூடிய ஒரு சிறு சம்பவத்தையும் செய்தியாகத் தாங்கி மக்களிடம் கொண்டுச் செல்வதனால் படிப்போர் மனதில் மகிழ்ச்சியையும், சோகத்தையும், வெறுப்பையும், மன அமைதியின்மையையும் ஒருசேரக் கொண்டு சேர்க்கிறது. இதன் மூலம் படிப்போரின் மன ஓட்டத்தினை கண்டறிந்து தம் வாசகர்களை தக்கவைத்து புதுப்பித்துக் கொள்ளவும் பத்திரிக்கைகள் துணை நிற்கின்றன. அதில் தினத்தந்தி தம் வாசகர்களை தன்வயப்படுத்தி வைத்துக்கொள்வற்கு நிகழ்வுகளை வரிசைப்படுத்தி ஒழுங்கமைத்துக் கொடுப்பதில் எப்போதும் முதலிடத்தில் உள்ளது. இதனால் தினத்தந்தி உள்ளூர் மட்டுமல்ல பிற மாநிலங்களிலும் பட்டிதொட்டி எல்லாம் தம் கருத்தழாம் மிக்க கருத்தினால் வியாபித்து நிற்கின்றன.

வரலாற்று நூல்களை காலத்தின் கண்ணாடி என்பர். நாளிதழ்கள் நிகழ்கால வரலாற்றுக்களின் பக்கமாகும். எனவேதான் இதழ்களை "இன்றையச் செய்தி நாளைய வரலாறு" என்பர். அத்தகைய சிறப்பு மிக்க நாளிதழ்களில் தனித்துவமானதும் நன்பகத்தன்மையதுமான இதழாகத் திகழ்வது தினத்தந்தியாகும். இன்றைய நவீனத் தொழில் நுட்ப முறையில் பல என்னற்ற வகையில் செய்திகளை அறியும் வசதி இருந்தாலும், அச்சில் வரும் செய்தியை நாம் நம்பகத்தன்மையுடன் முழுதாக ஏற்றுக் கொள்கிறோம். அந்த வகையில் தினத்தந்தி இதழின் வெற்றிக்கு முக்கியக் காரணமாக அமைவது தினத்தந்தியின் அமைப்பு முறையும் அதன் செய்திகளில் உண்மைத்தன்மையுமே என்றுக் கூறலாம்.

அடிக்குறிப்பு விளக்கம்

1. திருக்குறள் கருத்துரை, இரா. இளங்குமரனார், தமிழ்மண் பதிப்பகம் - பக் -210-211
2. மேலது-பக்-210-211

1029 / 1134

மகாகவியின் தமிழ் முழக்கம்

முனைவர் இரா. இரவிச்சந்திரன்,
இணைப்பேராசிரியர்,
தமிழாய்வுத்துறை,
தேசியக்கல்லூரி (தன்னாட்சி),
திருச்சிராப்பள்ளி - 620 001.

முன்னுரை

தேசியக்கவி, சிந்துக்குத் தந்தை, விடுதலைக்கவி, மகாகவி, பாட்டுக்கொரு புலவன், சீட்டுக்கவி, தற்காலத் தமிழ் இலக்கியத்தின் விடிவெள்ளி, செந்தமிழ்த்தேனீ, பைந்தமிழ்த் தேர்ப்பாகன், நீடுதுயில் நீக்கப்பாடி வந்த நிலா எனப் பல சிறப்புப் பெயர்களைப் பெற்றவர் சுப்பிரமணிய பாரதியார். இவர் எட்டயபுரத்தில் சின்னசாமிக்கும், இலக்குமி அம்மையாருக்கும் மகனாக 11.12.1882 இல் பிறந்தார். இவருடைய தாத்தா ராமசாமியிடம் தமிழைக் கற்று இளம் வயதிலேயே கவிபாடும் திறனைப் பெற்றதால் "பாரதி" என்ற பட்டம் பெற்றவர். எட்டையபுர சமஸ்தானத்தில் தமிழ்ப் புலவராக இருந்த காந்திமதிநாதபிள்ளை அவர்கள் "பாரதி சின்னப் பயல்" என்ற ஈற்றடியைக் கொடுத்து வெண்பா இயற்றச் சொன்னார். உடனே,

> "ஆண்டி விளையவனென் றைய அருமையினால்
> ஈண்டின்றென் றன்னைநீ யேந்தினையில்-மாண்புற்ற
> காரதுபோ லுள்ளத்தான் காந்திமதி நாதற்குப்
> பாரதி சின்னப் பயல்" - (பாரதியார் கவிதைகள், ப. 567)

என்று பாடி அவையிலுள்ளோர் அனைவரையும் வியக்கச் செய்தவர் மகாகவி பாரதியார் ஆவார்.

தமிழ்மொழி, தமிழ்நாடு, பாரதபூமி, சுதந்திரப்பேறு

ஆகியனவற்றை மட்டுமே சிந்தித்தவர் பாரதியார். தமிழ், ஆங்கிலம், பிரெஞ்சு, சமஸ்கிருதம், தெலுங்கு, இந்தி ஆகிய பல மொழிகளைக் கற்றறிந்தாலும் தம் தாய்மொழியாகிய தமிழ்மொழியே இனியமொழியாகவும் சிறந்த மொழியாகவும் எண்ணியதோடு மட்டுமல்லாமல்,

"யாமறிந் மொழிகளிலே தமிழ்மொழிபோல்
இனிதாவது எங்கும் காணோம்"

என்று பாடியவர். உலகச் செம்மொழிகளுள் தொன்மையான மொழி நம் தமிழ்மொழி. அதைத் தமிழ்நாட்டிலுள்ள தெருக்களில் எல்லாம் ஒலிக்கச் செய்ய வேண்டும். தமிழை உலகமெல்லாம் பரவும் வகை செய்தல் வேண்டும் என்று,

"தேமதுரத் தமிழோசை உலகமெல்லாம்
பரவும் வகை செய்தல் வேண்டும்"

என்று தம் கவிதை வழி வெளிப்படுத்தியுள்ளார். தமிழ்மொழி இனியமொழி, இலக்கணச் சிறப்புமிக்க மொழி, தொன்மையான மொழி என்று கூறிய பாரதியார் இதனை வளப்படுத்த புகழ் தரக்கூடிய புதிய நூல்களைப் படைக்க வேண்டும். அதை வெளிநாட்டார் வணங்கச் செய்ய வேண்டும் என்பதை,

"பிறநாட்டு நல்லறிஞர் சாத்திரங்கள்
தமிழ்மொழியிற் பெயர்த்தல் வேண்டும்,
இறவாத புகழுடைய புதுநூல்கள்
தமிழ்மொழியில் இயற்றல் வேண்டும்,
மறைவாக நமக்குள்ளே பழங்கதைகள்
சொல்வதிலோர் மகிமை இல்லை,
திறமான புலமையெனில் வெளிநாட்டோர்
அதை வணங்கஞ் செய்தல் வேண்டும்"
(பாரதியார் கவிதைகள், ப. 432)

என்று பாடியதோடு மட்டுமல்லாமல் அதை நிருபித்துக் காட்டியவர் பாரதியார். எல்லோரும் விரும்புகின்ற எல்லா காலத்திற்கும்

பொருந்துகின்ற இலக்கியங்களைப் படைக்க வேண்டும் என்கிறார். உலகிலுள்ள பல்துறை அறிஞர்களின் சிந்தனைகளை, அவர்களது நூல்களைத் தமிழில் மொழிபெயர்க்க வேண்டும். அவற்றைத் தமிழர்கள் அறிந்து கொள்ள வேண்டும் என்று சிந்தித்தவர் பாரதியார்.

"சென்றிடுவீர் எட்டுத் திக்கும் கலைச்
செல்வங்கள் யாவும் கொணர்ந்து இங்குச் சேர்ப்பீர்"

என்று பாடியவர். உலகிலுள்ள பல நாடுகளுக்கும் சென்று அங்குள்ள கலைச்செல்வங்களை எல்லாம் சேகரித்துக் கொணர்ந்து தமிழைத் தமிழ்நாட்டை வளப்படுத்துங்கள் என்கிறார் பாரதியார். தமிழ், தமிழர்நலன், பெண்விடுதலை, தீண்டாமை ஒழிப்பு ஆகியவற்றிற்காகத் தம் கவிதை வழி குரல் கொடுத்தவர் பாரதியார்.

நம் தாய்மொழியான தமிழ்மொழி வாயிலாகவே குழந்தைகள் கல்வி கற்க வேண்டும் என்றவர். இதனையே பாப்பா பாட்டில் வெளிப்படுத்துகிறார். தாய்நாட்டின் பெருமை ஆகியனவற்றை உரைத்த பாரதியார் தமிழ்ச் சொற்களின் பெருமையைக் கூறுகின்றபோது,

"சொல்லில் உயர்வு தமிழ்ச் சொல்லே
அதைத் தொழுது படித்திடடி பாப்பா"

என்று பாப்பா பாட்டில் பாடியுள்ளார். ஆங்கிலேயர் ஆட்சிக் காலத்தில் தமிழுக்குரிய இடம் கிடைக்காததை எண்ணி வருந்தியவர் பாரதியார். தமிழைக் கற்காமல் பிற மொழிகளைக் கற்பவரையும் அவ்வாறு கற்பதையே பெருமையாகக் கருதுவோரையும்,

"வேறுவேறு பாஷைகள் கற்பாற் நீ
வீட்டு வார்த்தை கற்கிலாய் போ போ போ"

என்று சாடுகின்றார். தமிழுக்குரிய இடம் கிடைக்க வேண்டும். தமிழர்களுக்குத் தமிழ் உணர்வை ஊட்ட வேண்டும். அப்பொழுதுதான் நம்மொழி வாழும் என்று கூறியவர் பாரதியார். எங்கும் எதிலும் எல்லா இடங்களிலும் தமிழ் ஒலிக்க வேண்டும்ஞு எல்லாத் துறைகளிலும் தமிழைப் புகுத்த வேண்டும் என்று பாடியவர் மகாகவி சுப்பிரமணிய பாரதியார் ஆவார்.

மனிதன் தன் எண்ணத்தை வெளிப்படுத்த உறுதுணையாக அமைவது மொழி. மொழி உணர்வு இல்லாதவன் பகுத்தறிவு அற்ற விலங்குகளுக்குச் சமமானவன் என்கிறார் பாரதியார். வடமொழியில் காளிதாசனையும் ஆங்கிலத்தில் ஷெல்லியையும் பல மொழிகளில் பல்வேறு புலவர்களையும் அறிந்த பாரதியார்,

"யாமறிந்த புலவரிலே கம்பனைப்போல்
வள்ளுவர்போல் இளங்கோ வைப்போல்
பூமிதனில் யாங்கணுமே பிறந்ததில்லை"

என்று பாடுகிறார். பதினான்கு மொழிகளைக் கற்றறிந்த பாரதியார் பல்வேறு புலவர்களின் நூல்களைக் கற்றுத் தேர்ந்த பாரதியார் நம் தமிழ்ப் புலவர்கள்போல் வேறு எங்கும் பிறக்கவில்லை என்று உறுதியாகக் கூறுகிறார். இதனால் பாரதி தமிழ் மீதும் தமிழ்ப் புலவர்கள் மீதும் கொண்டிருந்த பற்று வெளிப்படுகிறது. தமிழ்நாட்டைப் பற்றிக் குறிப்பிடுகின்றபோது,

"செந்தமிழ் நாடெனும் போதினிலே-இன்பத்
தேன்வந்து பாயுது காதினிலே"

என்று பாடுகின்றார். ஆக தம் எண்ணம் முழுக்க தமிழ், தமிழர்நலன், தமிழ்நாடு ஆகியவையே இருந்துள்ளது என்பதை அவரது கவிதைகள் வாயிலாக அறிய முடிகிறது.

"ஊமையராய்ச் செவிடர்களாய்க் குருடர்களாய்
வாழ்கின்றோம்எனு ஒருசொற் கேளீர்!
சேமமுற வேண்டுமெனில் தெருவெல்லாம்
தமிழ் முழக்கம் செழிக்கச் செய்வீர்!"

என்று பாடியவர். தமிழர்கள் தமிழர் என்ற பெயரை மட்டும் கொண்டிருந்தால் போதாது. நாம் ஊமையராய், செவிடராய், குருடராய் வாழ்கின்றோம் இதனை மாற்ற தெருவெல்லாம் "தமிழ் முழக்கம்" செழிக்கச் செய்வீர் என்று வலியுறுத்தியவர் பாரதியார்.

தொகுப்புரை

தேமதுரத் தமிழோசை உலமெல்லாம் பரவ வேண்டும். தெருவெல்லாம் தமிழ் முழக்கம் செழிக்கச் செய்ய வேண்டும் என்ற மகாகவியின் கனவு இன்று நனவாகியுள்ளது. நம் தாய்மொழியான தமிழ்மொழி 2004 ஆம் ஆண்டு செப்டம்பர் திங்கள் 17 ஆம் நாள் செம்மொழியாக அறிவிக்கப்பட்டது. அதன் விளைவு பிறநாட்டிலுள்ள பல பல்கலைக்கழகங்களில் தமிழ் இருக்கை அமைக்கப்பட்டுள்ளன. தமிழகத்திலுள்ள பல்கலைக்கழகங்களும் மத்திய பல்கலைக்கழகங்களும் தமிழைச் செழிக்கச் செய்கின்றன. பலர் தமிழ்வழியில் கல்வி கற்று தமிழையும் தமிழ்மொழியையும் வளம் கொழிக்கச் செய்கின்றனர். நமக்குத் தொழில் கவிதை, நாட்டிற்கு உழைத்தல் இமைப்போதும் சோராதிருத்தல் என்று பாடிய பாரதியார் கவிதை, சிறுகதை, உரைநடை, பத்திரிகைப்பணி எனப் பன்முகப்பார்வையில் "தமிழ் முழக்கம்" செய்தவர். அதனால் தான் பாரதிதாசன் பாரதியாரை,

"பைந்த மிழ்த்தேர்ப் பாகன், அவனொரு
செந்தமிழ்த்தேனீ, சிந்துக்குத் தந்தை
குவிக்கும் கவிதை குயில்
.....
தமிழால் பாரதி தகுதி பெற்றதும்
தமிழ், பாரதியால் தகுதி பெற்றதும்"

என்று புகழ்ந்து பாடுவது சாலப் பொருத்தமாகும்.

பாரதிதாசன் பல்கலைக்கழகம்

பாரதிதாசன் உயராய்வு மையம்

திருச்சிராப்பள்ளி – 620 024.

(Accredited with A+ Grade by NAAC in the Third Cycle)

" சோதனை கடந்து சுதந்திரம் அடைந்தோம்
சாதனை புரிந்து சரித்திரம் படைப்போம் "

தினத்தந்தி நிறுவனர் **சி.பா. ஆதித்தனார் அறக்கட்டளை**

இரண்டு நாள் தேசிய கருத்தரங்கம்

சான்றிதழ்

பாரதிதாசன் உயராய்வு மையத்தின் சார்பாக **21.10.2021** மற்றும் **22.10.2021** அன்று **" தினத்தந்தி இதழ் அன்றும் இன்றும் "** **" என்றும் பாரதியார் "** என்ற தலைப்புகளில் நடைபெற்ற இரண்டுநாள் தேசிய கருத்தரங்கில் திருமிகு/ முனைவர் இரா. இரவிசீனிதிரன், இணைப்பேராசிரியர், தமிழ்த்துறை, தேசியக் கல்லூரி, திருச்சி. அவர்கள் மகாகவியின் தமிழ் முழக்கம் ..... என்ற தலைப்பில் கருத்தாளாராக கலந்து 'கொண்டமைக்காக / கட்டுரை வாசித்தமைக்காக/பங்கு பெற்றமையைப் பாராட்டி இச்சான்றிதழ் வழங்கப்படுகிறது.

முனைவர். க. கோபிநாத்
பதிவாளர்

முனைவர். அ. கோவிந்தராஜன்
ஒருங்கிணைப்பாளர்

பாண்டியன் கல்வி அறக்கட்டளை

தொல்லியல் தொன்மையும் அருங்காட்சியகச் சிறப்பும்

சான்றிதழ்

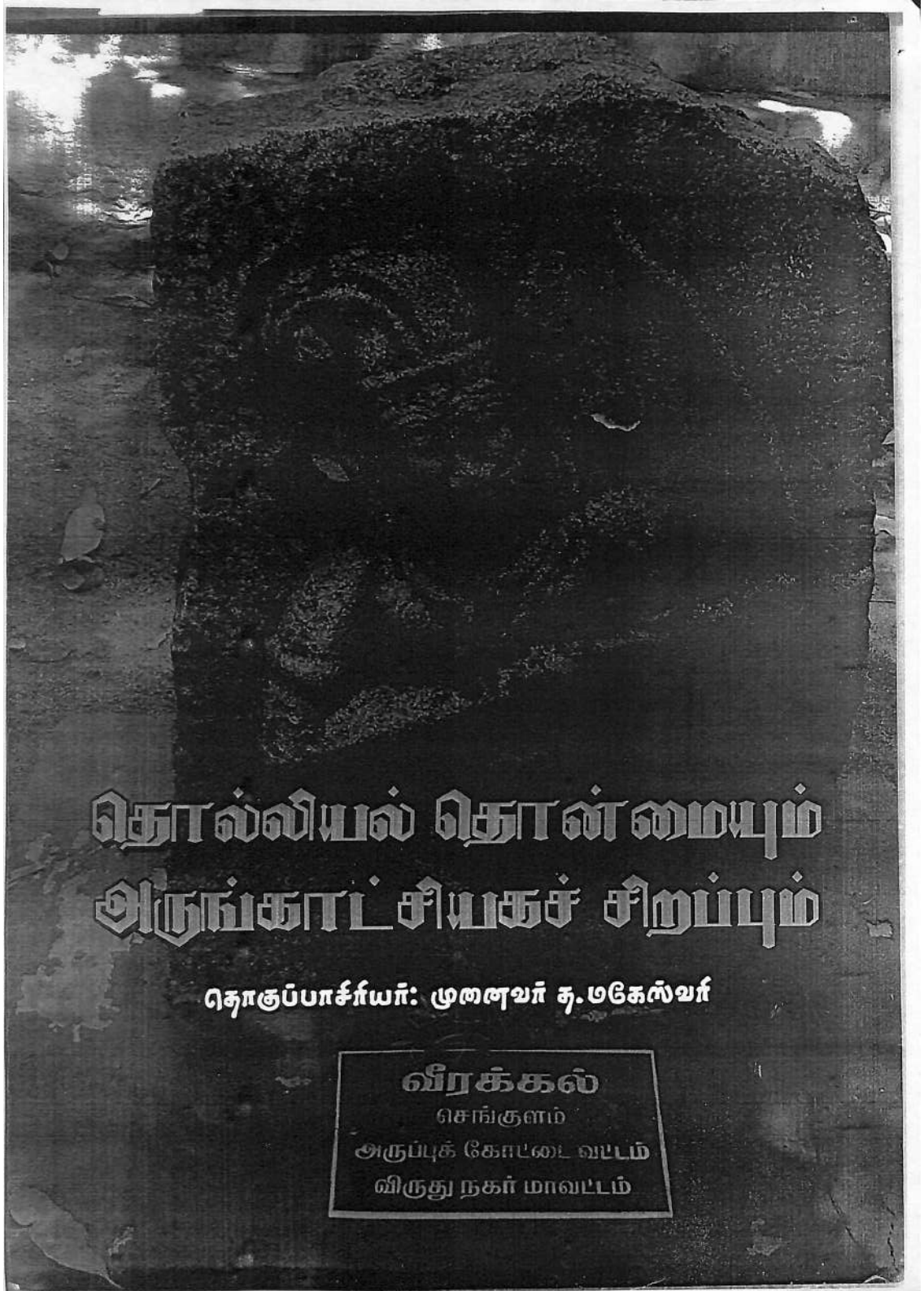
முனைவர் மு.சுபாஷினி, உதவிப்பேராசிரியர், தமிழ்த்துறை, தேசியக் கல்லூரி, திருச்சிராப்பள்ளி , தமிழ்நாடு, இந்தியா அவர்கள் **பாண்டியன் கல்வி அறக்கட்டளை** சார்பாக **தொல்லியல் தொன்மையும் அருங்காட்சியகச் சிறப்பும்** எனும் பொருண்மையில் 15.05.2021 அன்று நடந்த பன்னாட்டுக் கருத்தரங்கத்தில் **புதுக்கோட்டை அரசு அருங்காட்சியகத்தின் தகவல்கள்** எனும் தலைப்பில் புத்தகப்பகுதிக்கான ஆய்வுக்கட்டுரையை வழங்கிச் சிறப்பித்தார் என்று சான்றளிக்கப்படுகிறது.

ISBN : 978-93-91115-49-4

28.11.2021
விருதுநகர்

நிறுவனர் & பதிப்பாசிரியர்

முனைவர் த. மகேஸ்வரி



தொல்லியல் தொன்மையும் அருங்காட்சியகச் சிறப்பும்

தொகுப்பாசிரியர்: முனைவர் சு. மகேஸ்வரி

வீரக்கல்
செங்குளம்
அருப்புக் கோட்டை வட்டம்
விருது நகர் மாவட்டம்

Title Verso

TITLE OF THE BOOK	: Archaeology and Museum Studies
புத்தகத்தலைப்பு	: தொல்லியல் தொன்மையும் அருங்காட்சியகச் சிறப்பு
EDITORS	: Dr D. Maheswari
தொகுப்பாசிரியர்	: முனைவர் த.மகேஸ்வரி
Part I - FIRST EDITION	: 2021
பகுதி I - முதல் பதிப்பு	: 2021
PAGES/ பக்கங்கள்	: 250 (Part I - 1– 100)
PRICE/ விலை	: 500
ISBN NUMBER	: 978-93-91115-49-4
CONTACT NUMBER	: 8526769556

PUBLISHER : Pandian Educational Trust.
3/350, Veterinary Hospital Backside,
Virudhunagar – 626001.
Tamil Nadu

PRINTER : Maheswari Publishers.
3/350, Veterinary Hospital Rear side,
Virudhunagar – 626001.
Tamil Nadu

பதிப்புரிமை/Copyright © 2021 – முனைவர் த.மகேஸ்வரி /Dr. D.MAHESWARI

அனைத்து பதிப்புரிமையும் பெறப்பட்டுள்ளது. இப்புத்தகத்தின் எப்பகுதி பதிப்பகத்தின் அனுமதியின்றி அச்சிடப்படக்கூடாது. ஆசிரியர்கள் உடனடி பதிப்புரி பரிமாற்றத்தைப் பெறலாம்.

All rights reserved. No part of this book should be transmitted to any type of prin material without proper permission from the publisher. Authors' can get instant copyri transfer.

உள்ளடக்கம்

வ.எண்	தலைப்பு	ப.எண்
1.	பண்பாட்டுச் சின்னங்களும் தொல்லியல் தரவுகளும் முனைவர் சு. அய்யனார்	1-10
2.	சேதுபதி மன்னர்களும் இராமலிங்க விலாசமும் – ஓர் ஆய்வு முனைவர் ஜெ. கவிதா	11-21
3.	சோழர்கள் வரலாறு காட்டும் சோழபுரம் அருங்காட்சியக அற்புதம் திரு. த. குருநாதன்	22-33
4.	கல்வெட்டுகள் மற்றும் செப்பேடுகள் காட்டும் பராந்தக நெடுஞ்சடையப் பாண்டியனின் வரலாறு முனைவர் செ. சாந்தி	34-47
5.	இலங்கை அருங்காட்சியகத்தில் தொல்லியல் சிறப்பு திருமதி. சாந்தினி அருளானந்தம்	48-58
6.	புதுக்கோட்டை அரசு அருங்காட்சியகத்தின் தகவல்கள் முனைவர் மு. சுபாஷினி	59-73
7.	தரணிப்பாடும் தமிழர்தம் அடையாளங்கள் முனைவர் து. பத்மபிரியா	74-83
8.	புதுக்கோட்டை அரசு அருங்காட்சியகம் - ஒரு வரலாற்று நினைவகம் முனைவர் ஆ. இராஜலெட்சுமி	84-97

தி அங்கு
முடிகின்றது.
ட்சியகங்கள்
லையையும்
போது நாடு

வரலாறு.

ச்சு

டகள், AB

-91115-49-4

Information about Pudukkottai Government Museum

Dr M. Subashini, Assistant Professor, Department of Tamil, National College (Autonomous), Trichy -01.
ORCiD: https://orcid.org/0000-0002-9679-0662
DOI: 10.5281/zenodo.5748054

Abstract

A museum is a memorial place for the people who lived and keeps the remnants to remember them. The museum helps greatly to support the culture alive and well today. There are many ethnic groups of people in the vast world, so many events happen and happen around people. The museum is a repository of events that have taken place in the past and are taking place in each period. It is not possible to go and see the events that take place in each area in person. The museum serves as a documentary on the cultural history of the people who lived in the area and all of which are invisible to the naked eye. The museum is very useful to the extent that one can see and feel the plants, animals, birds, marine life, etc. standing nearby. In large cities, such as colleges and libraries, the museum collects and preserves rare items and preserves them in a separate room or glass case for years to come. A museum is a great tool for uncovering the traditions of the people who lived in the area, the artefacts left behind by the ancestors, the surprising events of the present and the unseen information. Each museum will have its own uniqueness. This review article aims to reveal the rare information found in the Pudukkottai Government Museum.

Keywords: Pudukkottai, Government Museum, Information.

References

[1] J. Rajamugammadu, *Pudukkottai History*, Pudukkottai Historical and Cultural Research Center, Pudukkottai, 2010.
[2] J. Rajamugammadu, *Pudukkottai District History*, Pudukkottai Historical and Cultural Research Center, Pudukkottai, 1992.

Witness of the field study

[1] Mr. C. Muthu – Museum In charge
[2] P.S. Seker – Museum In charge
[3] P. Sowndarajan – Museum In charge
[4] S. Shankar – Museum In charge
[5] S. Bharathi - Museum In charge
[6] G. Ashok Kumar - Museum In charge

ISBN: 978-93-91115-49-4

புதுக்கோட்டை அரசு அருங்காட்சியகத்தின் தகவல்கள்

முனைவர் மு.சுபாஷினி, உதவிப்பேராசிரியர், தமிழ்த்துறை, தேசியக் கல்லூரி, திருச்சிராப்பள்ளி – 01
ORCID: https://orcid.org/0000-0002-9679-0662
DOI: 10.5281/zenodo.5748054

ஆய்வுச்சுருக்கம்

அருங்காட்சியகம் என்பது மக்கள் வாழ்ந்த அடையாளங்களின் நினைவகமாகும். பண்பாடு, கலாச்சாரம் இன்றளவிலும் மாறாமல், மறையாமல் நிலைத்து நிற்பதற்கு அருங்காட்சியகம் உற்ற துணையாகிறது. பரந்து, விரிந்த இவ்வுலகில் ஏராளமான மக்களும், மக்களைச் சுற்றிப் பல அற்புதங்களும் நிகழ்ந்து கொண்டிருக்கின்றன. ஒவ்வொரு காலக்கட்டத்திலும் நடைபெற்ற, நடைபெற்றுக் கொண்டிருக்கின்ற நிகழ்ச்சிகளைப் பலரும் அறியும் ஒரு பெட்டகமாக அருங்காட்சியகம் திகழ்கின்றது. ஒவ்வொரு பகுதிகளிலும் நடக்கும் சம்பவங்களை நேரில் சென்று காண இயலாது. கண்ணில் உடனடியாகப் பார்க்க முடியாதவற்றை அந்தந்தப் பகுதிகளில் வாழ்ந்த மக்களின் பண்பாடு வரலாற்றினை ஆவனப்படுத்தி அவற்றை அளைவரும் அறிய எதுவாக அருங்காட்சயகம் விளங்குகிறது. தாவரங்கள், விலங்குகள், பறவைகள், கடல்வாழ் உயிரினங்கள் போன்றவற்றை அருகில் நின்று பார்த்து உணரும் அளவிற்கு அருங்காட்சியகம் பயனுள்ளதாக அமைகிறது. பெரிய நகரங்களிலும், கல்லூரி மற்றும் நூலகம் போன்ற இடங்களிலும், அரிதான பொருட்களைச் சேகரித்து, தனி அறையிலோ, கண்ணாடி பெட்டிகளிலோ பதப்படுத்தி வைத்து, பல ஆண்டுகள் சென்றாலும், சிதைவுறாதபடி அருங்காட்சிய நிறுவனம் பாதுகாத்து வருகின்றன. அந்தந்தப் பகுதிகளில் முன்னோர் விட்டுச்சென்ற பொருட்களையும், தற்காலத்தில் வியப்பூட்டும் நிகழ்வுகளையும், கண்டுணராத தகவல்களையும் வெளிக்கொணரும் மாபெரும் சாதனமாக அருங்காட்சியகம் அமைந்துள்ளது. ஒவ்வொரு அருங்காட்சியகத்திலும் ஒவ்வொரு தனிச்சிறப்பு இருக்கும். அத்தகைய வகையில் புதுக்கோட்டை அரசு அருங்காட்சியகத்தில் கண்டுணர்ந்த அரிய தகவல்களை வெளிப்படுத்தும் நோக்கமாக இவ்வாய்வுக் கட்டுரை அமைத்துள்ளது.

குறிப்புச்சொற்கள்: புதுக்கோட்டை, அரசு அருங்காட்சியகம், தகவல்கள்

ISBN: 978-93-91115-49-4

முகவுரை

வைத்தல் ஆகிய நோக்கங்களுக்காக அமைக்கப்பட்ட அமைப்பே அருங்காட்சியகமாகும். இது சமுதாய வளர்ச்சிக்குப் பெரிதும் துணை செய்கிறது. மக்கள் காண இயலாதவற்றைக் கண்டு அறிந்து கொள்ளும் தகவல் தொடர்பு சாதனமாக அருங்காட்சியகங்கள் இயங்குகின்றன. இந்தியாவின் முதல் அருங்காட்சியகம் 1814 இல் கொல்கத்தாவில் தொடங்கப்பட்டது. இந்தியாவின் அனைத்து வகையான பண்பாட்டையும் வெளிக்கொணரும் வகையில் இது அமைக்கப்பட்டுள்ளது. எனவே இதனை "இந்திய அருங்காட்சியகம்" எனக் குறிப்பிடுவர். அதனை தொடர்ந்து சென்னையில் 1851 இல் 16. 26 ஏக்கர் பரப்பளவில் "சென்னை அருங்காட்சியகம்" அமைந்துள்ளது. அந்தந்த வட்டார மக்கள் வாழ்ந்த வாழ்வின் பண்பாடுகளையும், கலாச்சாரத்தையும் எதிர்கால மக்கள் உணரும் வகையில் அந்தந்தப் பகுதியில் அருங்காட்சியகத்தை அரசு ஏற்படுத்தி வைத்திருக்கின்றது.

புதுக்கோட்டை அருங்காட்சியகம்

பழங்காலத்தில் வாழ்ந்த தொண்டைமான் மன்னர் ஆட்சி காலத்தில் 1910 ஆம் ஆண்டில் தொடங்கப்பட்டது. நுழைவாயிலில் தொண்டைமான் மன்னர் பரம்பரை பரம்பரையாக ஆட்சி செய்த கால வரையறையுள்ள புகைப்படம் வைக்கப்பட்டுள்ளது. இறுதி மன்னரான ராஜ கோபால தொண்மான் போர் புரிந்த போர்க்கருவிகள், ஆடைகள், போர்வாள் போன்றன காட்சிப்படுத்தப்பட்டுள்ளன.

பண்டைக் காலத்தில் வாழ்ந்த மக்கள் பயன்படுத்திய பொருட்கள் அகழாய்வுகள் மூலமாக தோண்டியெடுக்கப்பட்டுள்ளன. அப்பொருட்கள் அக்கால மக்களின் வாழ்க்கை முறை பற்றி அறிந்து கொள்வதற்காகப் பாதுக்கப்படுகின்றன. கலாச்சார தொடர்ச்சியைப் பேணுவதில் பாரம்பரியமாக மதிப்புகளின் முக்கியத்துவத்தை வலியுறுத்துவதை இந்த அருங்காட்சியகம் நோக்கமாகக் கொண்டுள்ளது.

அருங்காட்சியகம் ஒரு சமூக கலாச்சார நிறுவனமாகும். இதில் கலை, அறிவியல், தொழில்நுட்பம் மற்றும் மனித செயல்பாட்டின் பிற பகுதிகளின் நினைவுச் சின்னங்கள் சேகரிக்கப்பட்டு, ஆய்வு செய்யப்பட்டு சேமிக்கப்படுகின்றன. அரிதானப் பொருட்களின் தனிப்பட்ட சேகரிப்பிலிருந்து எடுக்கிறது.

தாவரங்கள், பறவைகள், விலங்குகள், கனுக்காலிகள், பாம்புகள், கைக்கருவிகள், ஆபரணங்கள், திருமாலின் தசாவதாரம், பனை மரத்தால் செய்த

ISBN: 978-93-91115-49-4

ஏற்றசால், டைனோசர், புதைபடிவ மரம் போன்ற பல அரிய பொருட்களைப் புதுக்கோட்டை அருங்காட்சியகம் வெளிப்படுத்துகிறது.

பறவைகள்

இறக்கைகள் கொண்ட இருகாலியைப் பறவை என்பர். இவை முதுகெலும்புடைய முட்டையிட்டு இனப்பெருக்கம் செய்வனவாகும். பறப்பதற்கு ஏதுவாகக் காற்றைகளையும், இலேசான "பொள்" எலும்புகளையும் கொண்டவை. இத்தகைய பறவையினங்களின் இயல்புகளைக் கண்முன் கண்டுணரும் வகையில் புதுக்கோட்டை அருங்காட்சியகத்தில் வைக்கப்பட்டுள்ளன.

இவ்வருங்காட்சியகத்தில் எண்ணற்ற பறவையினங்களைப் பதப்படுத்திப் பார்வைக்கு வைத்துள்ளதால் அறிந்திட இயலாத அரிய வகை பறவை இனங்களாகக் கண்டு கழிக்கவும், அதனுடைய பெயர்களைத் தெரிந்து கொள்ளவும் புதுக்கோட்டை அருங்காட்சியகம் வாய்ப்பாக அமைகிறது.

மாமிசம் உண்ணும் பறவைகள், இருவாய் குருவி, கொக்கு மற்றும் நாரை இனம், காட்டுக்கோழி, ஆந்தைகள், பூஞ்சிட்டு மைனா இனம், மயில், வெள்ளை மயில், வெளவால், ஊர்க்குருவி, தினைக்குருவி, சிட்டுக்குருவி, சிறகி, நீர்க்காக்கை, கிளி போன்றனவும், பறவையின் முட்டைகளுள் நெருப்புக்கோழி முட்டை, ஈமு கோழி முட்டை, காக்கை முட்டை, வாத்து முட்டை, மயில் முட்டை, பினந்தின்னி கழுகு முட்டை, கௌதாரி முட்டை போன்ற பறவைகளின் முட்டைகளும் பார்வைக்கு வைக்கப்பட்டுள்ளன.

மாமிசம் உண்ணும் பறவைகள்

மாமிசம் உண்ணும் பறவைகள் பகலில் இரை தேடுபவை. மாமிசங்களை உண்பதற்கேற்ப உடமைப்புகளைக் கொண்டுள்ளன. அலகுகள் வலுவாகவும், வளைந்தும், மேல்தாடை, கீழ்த்தாடையைவிட நீளமாகவும் காண்படும். வலுவான

பாதங்களையும், நகங்களையும் கொண்டிருக்கும். இவை சிறந்த பார்வை திறன் கொண்டவை. இத்தகைய அதிசய பறவைகளைப் பார்வைக்கு வைத்துள்ளனர்.

இருவாய் குருவி

இருவாய் குருவியின் அமைப்பு காண்போரை வியக்க வைக்கிறது. மிகப் பெரிய அலகு. அதன்மீது தொப்பி போன்ற அமைப்பு என்ற புதுமையான தோற்றத்தை இப்பறவைப் பெற்றுள்ளதால் வேறு பறவைகளிலிருந்து நன்கு வேறுபட்டு தோற்றமளிக்கிறது. இவை காடுகளிலுள்ள உயர்ந்த மரங்களில் வாழ்கின்றன. இப்பறவை மரப்பொந்துகளில் கூட்டை அமைக்கிறது. பெண் பறவை முட்டையிடுவதற்கு முன், மரப்பொந்தினுள் சென்ற பிறகு, ஆண் பறவை ஈர மண்ணில் சிறு இடைவெளிவிட்டு மரப்பொந்தை அடைகிறது. முட்டைகள் பொறிக்கப்பட்டு, குஞ்சுகள் தாமே பறந்து செல்லும்வரை பெண் பறவைக்கும், குஞ்சுகளுக்கும் ஆண் பறவை உணவூட்டுகிறது.

கொக்கு மற்றும் நாரை இனம்

கொக்கு, நாரை, கரண்டி மூக்கன் ஆகியவை இவ்வினத்துள் அடங்கும். இவை மிதமானது முதல் மிகப் பெரிய அளவு வரை காணப்படும் பறவைகளாக உள்ளன. இவற்றின் கழுத்து, கால்கள் மற்றம் அலகுகள் நீளமானதாக இருக்கும். இதன் பாதங்களில் சிறிதளவு சவ்வு காணப்படும். வேகமாகப் பறக்க ஏதுவாக இவற்றின் இறக்கைகள் நன்கு வளர்ந்திருக்கும். இவை சதுப்பு நிலப் பகுதிகளில் வாழும். இப்பகுதிகளில் காணப்படும் மரங்களில் கூட்டம் கூட்டமாகக் காணப்படும்.

காட்டுக்கோழி

காட்டுக்கோழிகள் இந்தியாவின் தென் மற்றும் மேற்குப் பகுதிகளில் காணப்படுகின்றன. இவைகள் அதிக கூட்ச சுபாவம் கொண்டவைகளாக இருப்பதால் சிறு சலசலப்பிற்கும் ஓடி ஒளிந்துக் கொள்ளும். தனியாகவும், கூட்டமாகவும் காணப்படும். இவை உணவைத் தேடி அதிகாலையிலும், பின் மாலையிலும் வெளியில் வரும். வெயில் சூட்டிலிருந்து தப்புவதற்கு அடர்ந்த புதருக்குள் வசிக்கும்.

ஆந்தைகள்

ஆந்தைகள் பறவைகளுக்கென்று தனிப்பட்ட பண்புகள் உள்ளன. இவைகள் இரவில் இரைதேடும் மாமிசப் பட்சிகளாகும். கொக்கிப் போன்ற அலகுகளையும், வலுவான கால்களையும், வளைந்த நகங்களையும் கொண்டுள்ளன. கண்கள்

பெரிதாகவும், முன்னோக்கிப் பார்க்கக் கூடியதாகவும் அமைந்துள்ளன. விசித்திரமான புறச்செவிகள் காணப்படுகின்றன. உணவையும், பயிர்களையும் பாழ்படுத்தும் விலங்குகளை அழிப்பதன் மூலம் இவைகள் மனிதனுக்கும் விவசாயத்திற்கும் பெரிதும் துணைபுரிகின்றன.

பூஞ்சிட்டு மைனா இனம்

பூஞ்சிட்டு மைனா இனம், காகம், குருவி, மைனா, பனங்காடை, தூக்கனாங்குருவி முதலிய பெரும்பாலான வகைகள் உள்ளடக்கியதாகும். இவற்றில் பெரும்பாலானவை அளவில் சிறியதாகவும், பளிச்சிடும் வண்ணம் கொண்டவைகளாகவும் இருக்கும். இவை பெரும்பாலும் மரத்தில் வாழ்பவை.

முட்டைகள்

அருங்காட்சியகத்தில் பல வகையான பறவைகளின் முட்டைகளைப் பாடம் செய்து வரிசைப்படுத்தப்பட்டுள்ளன. அவற்றுள் நெருப்புக்கோழி முட்டை மிகப் பெரிய அளவில் இருப்பதை இவ்வருங்காட்சியகத்தின் வழியாக அறிந்து கொள்ள முடிகிறது. ஈமு கோழி முட்டை கரிய சாம்பல் நிறத்தோடு இருப்பதை அறிவதற்கான வாய்ப்பை இந்த அருங்காட்சியகம் ஏற்படுத்தியுள்ளது. மேலும் வாத்து முட்டை, காக்கா முட்டை, செம்போத்து முட்டை, மயில் முட்டை, பிணந்தின்னி கழுகு முட்டை, கௌதாரி முட்டை போன்ற எண்ணற்ற முட்டைகளைக் காட்சிக்கு அரசு நிறுவனம் அமைத்துள்ளது.

கணுக்காலிகள்

உலகில் காணப்படும் விலங்குகளில் கணுக்காலிகள் தொகுதியிலுள்ள விலங்குகளே அதிக எண்ணிக்கையில் உள்ளன. இதில் நண்டுகள், இறால்கள், பூரான்கள் அடங்கும். இவைகள் 20,000 அடி உயரத்திலுள்ள மலைகளிலும், 18,000 அடி ஆழமுள்ள கடலிலும் காணப்படுகின்றன. இதன் உடலமைப்பு இரு பக்க சமச்சீரமைப்பும், கண்டங்களுடனும் ஜோடியான கால்களையும் கொண்டிருக்கும்.

புதுக்கோட்டை அரசு அருங்காட்சியகத்தில் நண்டு, நத்தை, எட்டுக்கால் பூச்சிகள், இறால், பூரான், மரவட்டை, சிலந்தி போன்ற வகைகளைப் பதப்படுத்திப் பார்வைக்குக் கண்ணாடி பாட்டிலில் வைக்கப்பட்டுள்ளன. அவற்றை நோக்கும்போது நேரே பார்க்கும் உணர்வை ஏற்படுத்துகிறது. மேலும் மெல்லுடலிகள், சங்கு வகைகள், வண்டுகள், வண்ணத்துப்பூச்சிகள் போன்றவையும் காட்சிப்படுத்தப்பட்டுள்ளன.

ISBN: 978-93-91115-49-4

மெல்லுடலிகள்

மெல்லுடலிகள் என்பது முதுகெலும்பற்ற விலங்குகளின் இரண்டாவது மிகப் பெரிய தொகுதியாகும். இவை மெலிதான ஓடுகளைக் கொண்டிருக்கும். அவை நத்தை, சிப்பி, ஆக்டோபஸ் போன்றன அடங்கும். மெல்லுடலிகள் இவ்வாறு தான் இருக்கும் என்பதை இவ்வருங்காட்சிகம் எடுத்து மொழிகிறது.

வண்ணத்துப்பூச்சிகள்

பட்டாம் பூச்சி அல்லது வண்ணத்துப்பூச்சி அல்லது வண்ணாத்திப்பூச்சி என்பது பல நிறங்களில் கண்ணைக் கவர்வனவாகும். பல வண்ணங்களில் இருப்பதால் வண்ணத்துப்பூச்சி என்பது பெயர் பெறுகின்றன. இது முட்டையிலிருந்து குடம்பி நிலையில் புதுவாக அல்லது மயிர்க் கெட்டியாக உருமாறி, பின்னர் கூட்டுப்புழு எனப்படும் உறங்குநிலைக்குச் சென்று பின்னர் அழகான பட்டாம் பூச்சியாய் உருமாற்றம் அடைகிறது. இத்தகைய அரிய பூச்சிகள் அனைத்தையும் அருங்காட்சிகம் அமைத்து வைத்து அதிசயிக்க வைக்கிறது.

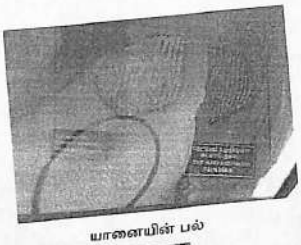
விலங்குகள்

இயற்கையும், விலங்குகளும் ஒன்றோடு ஒன்று பின்னிப் பிணைந்த சூழலே மனிதர்களின் வாழ்வியல் சூழலைச் சிறப்பாக வழி நடத்துகிறது. உலகில் உள்ள காடுகளையும், கடல்களையும், நீர்நிலைகளையும் வாழ்வாதாரமாகக் கொண்டு பல்லாயிரக்கணக்கான உயிரினங்கள் விலங்குகள் வாழ்ந்து வருகின்றன.

மரநாய்

முள்ளெலி

கருங்குரங்கு



யானையின் பல்

மரநாய்

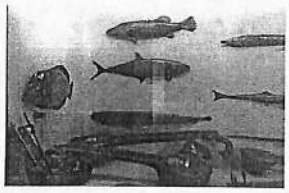
பண்டை மக்கள் செல்வங்களைப் பெருக்குவதற்கு விலங்குகளைப் பழக்கித் தங்களின் தேவைகளை நிறைவு செய்தனர். போர் செய்தல், வேட்டையாடுதல், உணவிற்கு என மக்களின் வாழ்வோடு ஒன்றிய விலங்குகளின் தன்மைகளைப் பட்டியலிட்டு காட்டும் வண்ணம் அருங்காட்சியகம் விலங்குகளைப் பதப்படுத்தி, அதன் உதிரி பாகங்களையும், சில விலங்குகளின் முழுமையான உருவத்தையும் கண்முன்னே காண்பன போன்ற காட்சியாக அமைத்துள்ளது. அருங்காட்சியகத்தில் கண்ட விலங்குகளாவன யானையின் மண்டை ஓடு, பாதம், கிழ்த்தாடை, தொம்பு போன்றனவும், புதுக்கோட்டை மாவட்டத்தில் பிறந்த இரட்டை எருமைக்கன்று மற்றும் இரண்டு உடலும் ஒரு தலையும் ஒட்டியவாறு உள்ள ஆட்டுக்குட்டியும் என்ற வகையில் அதிசய பிறவிகளைப் பதப்படுத்தி மக்கள் பார்வைக்கு வைக்கப்பட்டுள்ளது. இதன் வழி விலங்குகள் இவ்வாறெல்லாம் பிறந்துள்ளன என்பதை மக்கள் அறிய முடிகிறது. எலி, முள்ளெலி, முழுமையான வடிவில் உள்ள கீரிப்பிள்ளை, சிறுத்தையின் மண்டை ஓடு, காட்டுப்பன்றியின் கிழத்தாடை, நாயின் மண்டை ஓடு, பூனையின் மண்டை ஓடு

ISBN: 978-93-91115-49-1

மரநாயின் மண்டை ஓடு, பதப்படுத்திய முழு உருவத்திலுள்ள கருங்குரங்கு, மான் நின்றபடியான எலும்புக்கூடு போன்றனவும், மரநாயின் இயற்கை அமைப்பில் மரநாய் மரம், செடி, கொடி போன்ற காடுகளுக்கிடையே இருப்பதைப் போன்ற உண்மைத் தோற்றத்தைப் படம் பிடித்துக் காட்டுகின்றன.

குறுத்தெலும்பு மீன்கள்

திருக்கை மீன்



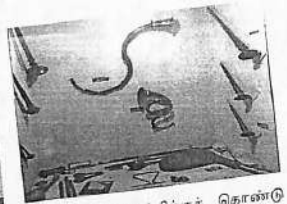
சுறா மீன்

விரால் மீன்

சுறா, திருக்கை மற்றம் தட்டை மீன்கள் குறுத்தெலும்பு மீன்களாகும். இவற்றின் எலும்புகள் மற்ற மீன்களில் காணப்படும் எலும்புகளைப் போலில்லாமல் குறுத்தெலும்பாகக் காணப்படும். செதில்கள் பறவை போலும், செவுள் திறப்புகள் மூடப்படாமலும், மேல் தடுப்பு கீழ்த்தடுப்பைவிட நீளமாகவும், வால் வரை வளர்ந்தும் காணப்படும். இப்பண்புகள் எலும்பு மீன்களில் காணப்படுவதில்லை. சுறா மற்றும் திருக்கை மீன்கள் கடல்வாழ் உயிரினங்களாக இருந்தாலும் சிலவகை மீன்கள் நதிகளிலும் காணப்படுகின்றன. சுறா மீன்கள் நன்றாக நீந்தக் கூடியவை. மாமிச பட்சியாகும். இத்தகைய குறுத்தெலும்பு மீன்களையும், அதன் எலும்புகளையும் அருங்காட்சியகம் வழியாக அறிய முடிகிறது.

ISBN: 978-93-91115-49-1

இசைக்கருவிகள்



இசை மக்களின் மனத்தை மகிழ்ச்சி வெள்ளத்திற்குக் கொண்டு செல்லும் என்பதை அறிந்த பண்டையோர் தங்களின் அறிவு நுட்பத்தைப் பயன்படுத்தி பலவகையான கருவிகளைப் பயன்படுத்தினர். பழங்கால மனிதன் முதன்முதலாகக் கண்டறிந்த இசைக்கருவி "புல்லாங்குழல்" ஆகும். பண்டையோர் பயன்படுத்திய இசைக்கருவிகள் ஏராளமாக வைக்கப்பட்டுள்ளன. ஜால்ரா, ஆயர் புல்லாங்குழல், நெடுங்குழல், டல்சிமர், மகுடி, துந்திரு, மாக்கோணம், தடி, துருத்தி, சங்கு, நாதஸ்வரம் போன்றவும், பழங்கால பயன்பாட்டில் இருந்து, தற்போது இல்லாத மனித முன்கை எலும்பால் செய்த குழல் போன்றவை அறிந்திடாத இசைக்கருவிகளும் மனத்தை உருக வைக்கும் எண்ணற்ற முறையில் பயன்படுத்திய இசைக்கருவிகளையும் எடுத்துக் காட்டும் வகையில் அருங்காட்சியகம் அடையாளமாக இருப்பதை அறிய முடிகிறது.

கைவினைப்பொருட்கள்

கைவினை என்பது சிறிய கையோடு உழைப்பின் உதவியுடன் தொழில்த்துறை பொருட்களின் உற்பத்தியை அடிப்படையாகக் கொண்ட ஒரு உற்பத்தி நடவடிக்கையாகும். கைவினைக் கலைகள் வழியாக ஏழை மற்றும் நடுத்தர மக்கள் தங்களின் அன்றாட தேவைகளை நிறைவு செய்வதற்காக அருகில் கிடைக்கும் மூலப்பொருட்களைக் கொண்டு பொருட்களைத் தயாரிக்கும் ஒரு வகை கலையாகும். இக்கலைப் பண்டைய காலத்தில் பெரிதும் போற்றப்பட்டது. அக்கால

ISBN: 978-93-91115-49-4

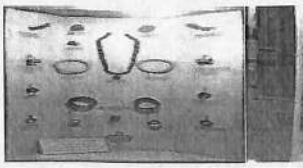
கலையறிவையும், நுட்பமான முறையில் தொழில் நுணுக்கத்தில் மக்கள் வாழ்ந்த உண்மையையும் வெளிப்படுத்துவதைப் புதுக்கோட்டை அருங்காட்சியகம் வரலாற்றப் பெட்டகமாக விளங்குகிறது.

மண்பாண்டங்கள், பனையோலை பெட்டிகள், மரத்தில் செய்த தேர், பொம்மைகள், சிற்பங்கள், பட்டு நெசவு, நெட்டி வேலை, உலோக வேலை, மாக்கல் வேலை, பனை மரத்தால் செய்த ஏற்றச்சால் போன்ற எண்ணற்ற கைவினைப் பொருட்களைத் தயாரிப்பதில் கைத்தேர்ந்தவர்களாக இருந்தமைக்கு மிகச் சிறந்த சான்றாக இவ்வருங்காட்சியகம் அமைத்துள்ளது.

பனை மரத்தால் செய்த ஏற்றச்சால்

பழங்கால மக்கள் இயற்கை வளம் செழிக்க வாழ்ந்தனர். அவர்கள் இயற்கையோடு இயைந்து வாழ்ந்த வாழ்விற்கு அடையாளமாக மரங்களினால் செய்த கருவிகள் பயன்பாட்டில் இருந்து வந்துள்ளன என்பதற்குச் சான்றாகப் பனை மரத்தால் செய்த நீர் இறைக்கும் சால் புதுக்கோட்டை அருங்காட்சியகத்தில் வைத்து பண்டைய மக்களின் அடையாளத்தைப் பறைசாற்றியுள்ளனர். அக்கருவி தமிழர் வாழ்ந்த அடையாளச் சின்னமாக நிலைத்த புகழுடன் விளங்குகிறது என்பதை இதன் வழி உணர முடிகிறது.

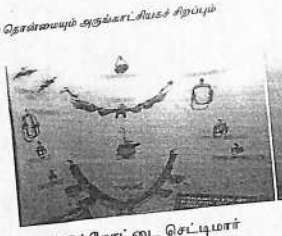
ஆபரணங்கள்



செட்டிமார் அணிகலன்கள்

நரிக்குற மகளிர் அணிகலன்கள்

ISBN: 978-93-91115-49-4



நாட்டுக்கோட்டை செட்டிமார்

முகம்மதியர் அணிகலன்கள்

அணிகலன்கள்

மக்களின் அழகை மேம்படுத்துவதில் அணிகலன்கள் சிறந்த இடத்தை பெறுகின்றன. எனவே மக்கள் தங்களை அழகுபடுத்துவதைப் பழங்காலம் முதல் இக்காலம் வரை பெரிதும் விரும்புகின்றனர். புதுக்கோட்டையை ஆண்ட தொண்டைமான் மன்னர்கள் அணிந்த அணிகலன்களும், மக்கள் அணிந்த அணிகலன்களான நாட்டுக்கோட்டை செட்டிமார் அணிகலன்கள், நரிக்குற மகளிர் அணிந்த அணிகலன்கள், இஸ்லாமிய பெண்கள் அணிந்த அணிகலன்கள் என்ற வகையில் மக்கள் பயன்படுத்திய அணிகலன்களும் காட்சிக்கு வைத்துள்ளதை அறிவதன் வாயிலாக மக்களின் ஆபரணங்களைக் காண முடிகிறது.

தசாவதாரம்

திருமால் அதர்மத்தை அழித்து தர்மத்தை நிலைநாட்டுவதற்குப் பத்து அவதாரங்கள் எடுத்து உலகின் மக்களைக் காத்தார் என்று புராணங்கள் கூறுகின்றன. அதை மெய்ப்பிக்கும் பொருட்டு புதுக்கோட்டை அருங்காட்சியகத்தில் திருமாலின் பத்து அவதாரங்களின் உருவச்சிலைகளைக் காண முடிகிறது.

ISBN: 978-93-91115-49-4

1.மச்சாவதாரம் 2.கூர்ம அவதாரம் 3.வராக அவராதம் 4.நரசிம்ம அவதாரம் 5.வாமன அவதாரம் 6.பரசுராம அவதாரம் 7.ராம அவதாரம் 8.பலராம அவதாரம் 9.கிருஷ்ண அவதாரம் 10.கல்கி அவதாரம் என்று திருமால் உலக உயிர்களைத் துன்பங்களிலிருந்து காப்பாற்றி நல்வாழ்வு வாழ பத்து அவதாரங்களை இப்புவியில் நிகழ்த்தினார். இவற்றை நினைவுபடுத்தும் விதமாகவும், வைணவ சமயத்தைப் புதுக்கோட்டையை ஆண்ட தொண்டைமான் மன்னர்கள் பெரிதும் போற்றிக் காத்ததற்கான அடையாளமாகவும் திருமால் எடுத்த தசாவதாரத்தின் உருவச்சிலைகள் உள்ளன. இதன்வழி புராணங்கள் கூறும் செய்திகளை இவ்வருங்காட்சியகம் சான்றுரைக்கிறது.

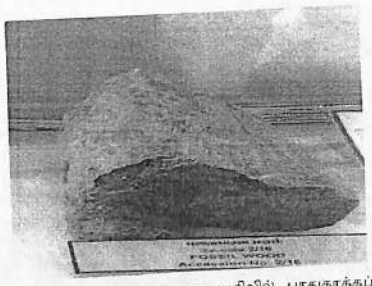
முதுமக்கள் தாழி

முதுமக்கள் தாழி என்பது பண்டைய தமிழகத்தில் இறந்தவர்களின் உடல்களை அவற்றில் இட்டு, மண்ணில் புதைக்கப் பயன்படுத்தப்பட்ட புதைகலன்கள் அல்லது ஈமத்தாழிகள் என்பர்.

முதுமக்கள் தாழி (மண்ணால் செய்த மிகப் பெரிய அளவிலான மண்பாண்டம்) பல இடங்களில் அகழாய்வு செய்து கண்டெடுக்கப்பட்டு வருகின்றன. தமிழர்களின் வாழ்வில் முதுமக்கள் தாழி பயன்படுத்தியமைக்கான அடையாளமாகப் புதுக்கோட்டை அருங்காட்சியகத்தில் பெரிய அளவிலான முதுமக்கள் தாழி பொதுமக்கள் பார்வைக்காக வைக்கப்பட்டுள்ளன. இதிலிருந்து இந்தத் தாழிக்குள் முதியவர்களை அடக்கம் செய்த செய்திகளைக் கண்டுணர புதுக்கோட்டை அருங்காட்சியகம் தமிழர் நடையாளமாக நிற்பதை உணர முடிகிறது.

ISBN: 978-93-91115-49-4

புதைபடிவ மரம்



புதைபடிவ மரம் என்பது புதைபடிவ பதிவில் பாதுகாக்கப்படும் மரமாகும். தாவரத்தின் பகுதியாக இருக்கும் மரங்கள் காலம் செல்லச் செல்ல சிறந்த முறையில் பாதுகாக்கப்படுகிறது. புதைபடிவ மரம் கல் பாறையாக மாறலாம். தாவர மரத்தண்டுகள் செறிவுற்ற கரைந்த கனிமங்களுடன் காணபடும். ஈரமான படிவுகளில் புதைக்கப்படும்போது புதைபடிவ மரங்கள் உருவாகின்றன. பிராண வாயு பற்றாக்குறை மரங்கள் சிதைவதைக் குறைக்கிறது. இது தாதுக்கள் மூலம் தாவர செல் சுவர்களை அகற்றிவிட்டு வெற்றிடங்களை நிரப்பச் செய்கிறது. இதில் கல்லாகிய மரம் என்பது "புரைகளினமாக்குதல்" முறையில்லாத வறண்ட குளிர்ந்த அல்லது வெப்பமான சூழலில் உருவாகின்றன. இதிலுள்ள அசல் செல்கள் அல்லது திசுக்கள் மாறாமல் பாதுகாக்கப்படுகின்றன. மரங்களின் வயதை மரக்காலவரிசை முறைப்படி அறிந்துக் கொள்ளலாம். புதைபடிவ மரங்கள் பற்றிய ஆய்விற்கு "பாலியோலைலாலஜி" என்று பெயர். இங்குக் காட்சிக்கு வைக்கப்பட்டுள்ள புதைபடிவ மரமானது புதுக்கோட்டை நகரம் "நரிமேடு" என்ற இடத்தில் புறப்பரப்பில் கிடைத்தது.

நிறைவுரை

புதுக்கோட்டை அருங்காட்சியகம் பண்டையோர் வாழ்ந்த அடையாளங்களையும், கிடைத்தற்கரிய தகவல்களையும், அப்பகுதியில் நிகழ்ந்த பல வகையான அதிசயங்களையும், புதுக்கோட்டையை ஆண்ட மன்னர்களின் சிறப்புகளையும், அகழாய்வு வழியாகக் கிடைத்த பொருட்களையும், முன்னோர் வாழ்ந்த பண்பாடு, கலாச்சாரம், வாழ்வின் மரபுகளையும் எடுத்துரைக்கும் விதமாக இவ்வருங்காட்சியகம் விளங்குகிறது.

ISBN: 978-93-91115-49-4

துணைநூற்பட்டியல்

[1] ஜெ. ராஜாமுகம்மது புதுக்கோட்டை வரலாறு புதுக்கோட்டை வரலாற்றுப் பண்பாட்டு ஆய்வு மையம் புதுக்கோட்டை 2010.

[2] ஜெ. ராஜாமுகம்மது புதுக்கோட்டை மாவட்ட வரலாறு புதுக்கோட்டை வரலாற்றுப் பண்பாட்டு ஆய்வு மையம் புதுக்கோட்டை 1992.

களஆய்விற்குத் துணைநின்றவர்கள்

[1] திரு சி. முத்து – காட்சிக்கூட காவலர்

[2] P.S. சேகர் - காட்சிக்கூட காவலர்

[3] பா. சௌந்தர்ராஜன் - காட்சிக்கூட காவலர்

[4] S. சங்கர் - காட்சிக்கூட காவலர்

[5] S. பாரதி - காட்சிக்கூட காவலர்

[6] G. அசோக்குமார் - காட்சிக்கூட காவலர்.

ISBN: 978-93-91115-49-4

தொகுதி-9 எண் 4
Vol. 9 No. 4

புரட்டாசி-மார்கழி 2052
October - December 2021

ISSN : 2321-984X

Chief Editor
Dr. M. Sadik Batcha

Advisory Editor
Dr. N. Chandra Segaran

Editorial Board
Dr. MAM. Rameez
Dr. Jeyaraman
Dr.A. Ekambaram
Dr. G. Stephen
Dr. S. Chitra
Dr. S.Senthamizh Pavai
Dr. Aranga. Pari
Dr. A. Shunmughom Pillai
Dr. P. Jeyakrishnan
Dr. S. Easwaran
Dr. Kumara Selva
Dr. A. Palanisamy
Dr. Ganesan Ambedkar
Dr. Kumar
Dr. S. Kalpana
Dr. T. Vishnukumaran
Dr M. N. Rajesh
Dr. M. Ramakrishna
Dr. Govindaraj
Dr. Uma Devi
Dr. Senthil Prakash
Dr. M. Arunachalam
Dr. S. Vignesh Ananth
Dr. Pon. Kathiresan
Dr. S. Bharathi Prakash

நவீனத் தமிழாய்வு

(பன்னாட்டுப் பன்முகத் தமிழ் காலாண்டு ஆய்விதழ்)

Journal of

Modern Thamizh Research

(A Quarterly International Multilateral Thamizh Journal)

Arts and Humanities (...), Language Literature and Literary Theory, Tamil UGC ... Listed ... Journal

நவீனத் தமிழாய்வு

Published by

RAJA PUBLICATIONS

No. 10 (Upstair), Ibrahim Nagar, Khajamalai,
Tiruchirappalli - 620 023, Tamil Nadu, India.
Mobile : 9600535241
Website : www.rajapublications.com

30 பகுதி-8
Part -8

Modern Thamizh Research

A Quarterly International Multilateral Thamizh Journal

Published by

Raja Publications, Trichy-23.

INFORMATION FOR AUTHORS

Journal of **Modern Thamizh Research** (JMTR) is published Four (Every March, June, September & December) times a year. It primarily publishes significant original papers and survey articles on important advances of Modern Thamizh research in any of the following major areas:

- Classicalism (செவ்வியல்)
- Modernism (நவீனம்)
- Folklorism (நாட்டுப்புறவியல்)
- Archeologism (தொல்லியல்)
- Spritualism (பக்தியியல்)
- Traditioanlism (மரபியல்)
- Mediaism (ஊடகவியல்)
- Journlism (இதழியல்)
- Translationism (மொழிபெயர்ப்பியல்)
- Comparativism (ஒப்பியல்)
- Educationalism (கல்வியல்)
- Histroism (வரலாற்றியல்)

Submission of the Manuscript

1. Manuscripts written in Thamizh or English may be submitted in two copies to the Chief Editor **Dr. M. Sadik Batcha, Associate Professor of Tamil, PG and Research Department of Thamizh, Jamal Mohamed College (Autonomous), Tiruchirappalli - 620 020, Mobile : 9600535241, Email : ms_batcha@yahoo.co.in**

2. Manuscripts should be prepared according to the following format.
   The first page of the manuscript should contain
   (i) the title
   (ii) the name(s) and institutional affiliation (s) of the author(s).
   (iii) an abstract of not more than 100 words; and
   (iv) a footnote giving the name and present address of the author to whom proofs and reprint order form should be addressed.
   (v) References at the end of the manuscript should be arranged in alphabetical order of the first letter of the surname and styled and punctuated according to the following format.
   [1] author(s) name(s), "Book name", Page, Edition number, Published Year, Book Publisher address.

Publication of Accepted Papers

The editorial Policy of JMTR is to give a clear reply about the acceptance of all submitted papers, within a period of one month.

Subscription Rate of JMTR

Libraries, Institutions and other establishments can subscribe for JMTR through publisher : **Raja Publications, 10, (Upstair), Ibrahim Nagar, Khajamalai, Tiruchirappalli - 620 023, Thamizh Nadu, India. Mobile : 9600535241 website : rajapublications.com** and in case of any difficulty they should write to the Chief Editor at address given elsewhere.

Subscription Rates	Years	Printed
(Year) India INR ₹	1 Years Only	2000
Other Countries INR ₹	1 Years Only	3000
(Life) India ₹	10 Years Only	10,000
Other Countries INR ₹	10 Years Only	20,000

நவீனத் தமிழாய்வு
(பன்னாட்டுக் காலாண்டு ஆய்விதழ்)

Journal of
Modern Thamizh Research
(A Quarterly International Journal)

தொகுதி-9 எண் 4
Vol. 9 No. 4

புரட்டாசி-மார்கழி 2052
October - December 2021

ISSN : 2321 - 984X

Chief Editor
Dr. M. Sadik Batcha

Advisory Editor
Dr. N. Chandra Segaran

Editorial Board
Dr. MAM. Rameez
Dr. Jeyaraman
Dr.A. Ekambaram
Dr. G. Stephen
Dr. S. Chitra
Dr. S.Senthamizh Pavai
Dr. Aranga. Pari
Dr. A. Shunmughom Pillai
Dr. P. Jeyakrishnan
Dr. S. Easwaran
Dr. Kumara Selva
Dr. A. Palanisamy
Dr. Ganesan Ambedkar
Dr. Kumar
Dr. S. Kalpana
Dr. T. Vishnukumaran
Dr M. N. Rajesh
Dr. M. Ramakrishnan
Dr. Govindaraj
Dr. Uma Devi
Dr. Senthil Prakash
Dr. M. Arunachalam
Dr. S. Vignesh Ananth
Dr. Pon. Kathiresan
Dr. S. Bharathi Prakash

30 பகுதி-8 Part -8

சான்றிதழ்

Certificate

This is to certify that Dr. / Mr. / Ms.

முனைவர் இரா. பத்மா
உதவிப்பேராசிரியர். தமிழாய்வுத்துறை. தேசியக்கல்லூரி (தன்னாட்சி).
(பாரதிதாசன் பல்கலைக்கழகத்துடன் இணைவு பெற்றது).
திருச்சிராப்பள்ளி-01. தமிழ்நாடு. இந்தியா.

has Published a paper titled

கலித்தொகையில் பெண்களின் பழக்க வழக்கங்களும் அவர்களின் நிலையும்

Sl.No. 392 402
Pages : 2707-2711

Published by

RAJA PUBLICATIONS
No. 10 (Upstair), Ibrahim Nagar, Khajamalai,
Tiruchirappalli - 620 023. Tamil Nadu, India.
Mobile : 9600535241
Website : www.rajapublications.com

Chief Editor
Dr. M. Sadik Batcha
Associate Professor
PG and Research Department of Thamizh
Jamal Mohamed College (Autonomous)
Tiruchirappalli - 620 020, Tamil Nadu, India
Mobile : 94434 17242, Email : ms_batcha@yahoo.co.in

கலித்தொகையில் பெண்களின் பழக்க வழக்கங்களும் அவர்களின் நிலையும்

முனைவர் இரா. பத்மா
உதவிப்பேராசிரியர், தமிழாய்வுத்துறை, தேசியக்கல்லூரி (தன்னாட்சி),
(பாரதிதாசன் பல்கலைக்கழகத்துடன் இணைவு பெற்றது),
திருச்சிராப்பள்ளி-01, தமிழ்நாடு, இந்தியா.

ஆய்வுச் சுருக்கம்

"சங்க காலம் ஒரு பொற்காலம்". பண்பாட்டுச் சிறந்த காலம். பெண்களைப் போற்றியக் காலம். என்றாலும் பெண்கள் ஆண்களைச் சார்ந்தும், ஆண்களுக்குக் கீழே, சமமாகவில்லை. சங்ககாலச் சமுதாய நிலை ஆண்வழிச் சமுதாயமாக இருந்துள்ளது. கலித்தொகையில் இடம்பெறும் பெண்களின் நிலையை இக்கட்டுரையில் காணலாம்.

கலைச் சொற்கள்

ஒழுகை – வண்டி; சிலைவல்லான் - மன்மதன்; முரற்சி – திரிந்த தயிர்; தெருமரல் - மனக்கலக்கம், கழற்சி; தேச – செயற்கையழகு; வண்ணம் - இயற்கையழகு; வான்துகள் - வெண்மையான கற்பொடி

முன்னுரை

சங்ககாலம் ஒரு பொற்காலம். பண்பாட்டில் சிறந்தும், பெண்களைப் போற்றியும், பெண்பாற் புலவர்களும் சிறந்து விளங்கிய காலமாக இருந்தாலும், பெண்கள் ஆண்களுக்குக் கீழே வாழ்ந்தனர். ஆண்களைச் சார்ந்து வாழும் நிலையே. ஆணுக்குப் பெண் சமமாக மதிக்கப்படவில்லை. மக்கட்பேற்றிலே மகன் பெறுதலே முதன்மையாகக்க கருதப்பட்டாலும். மறுமை உலகுக்கும் மகனே கடமை செய்யத் தகுதியுடையவன் என்பதும் ஆண்வழிச் சமுதாயமாகவே இருந்தது? என்பதைக் கலித்தொகையில் இடம்பெறும் பெண்களின் பண்புகளையும், பழக்க வழக்கங்களையும் இக்கட்டுரையில் காண்போம்.

கலித்தொகை – ஓர் அறிமுகம்

எட்டுத்தொகை நூல்களில் பா வகையால் பெயர்பெற்ற நூல்கள் கலித்தொகையும், பரிபாடலும் ஆகும்.

"இருந்தமிழே உன்னால் இருந்தேன் இமையோர்
விருந்தமிழ்தம் என்றாலும் வேண்டேன்?"
(தமிழ்விடு தூது

என்ற தமிழ் விடுதூது தமிழ் மொழியின் பெருமைகளைப் பறைசாற்றுகிறது. அத்தகைய பெருமையுடைய தமிழ்மொழியின் அகமரபுகளை எடுத்தியம்புவதற்காகப் பயன்படுத்தப்பட்ட ஒருவகை யாப்புதான் கலிப்பாவாகும்.

பழக்க வழக்கங்கள்

பழக்கம் என்ற சொல் 'பழகுதல்' அல்லது 'பயிற்சி பெறுதல்' என்கிற பொருளில் வழங்கப்பெறுகிறது. இது ஒரு வகையான கல்வியாகும். ஒன்றைக் கற்றுக்கொள்வதே பழக்கம் என்று கூறலாம். பழக்கத்தினுடைய தொடர்ந்த நிலையே 'வழக்கம்' என்று குறிப்பிடுகிறோம். தனிமனித பழக்க வழக்கங்கள் மாறக்கூடிய தன்மையைப் பெற்றிருக்கின்றன. ஆனால் சமுதாயப் பழக்க வழக்கங்களும், நம்பிக்கைகளும் அடிக்கடி மாறக்கூடியனவாக இல்லை. சமுதாயம் தான் இருக்கும் நிலைக்கு ஏற்பச் சில பழக்க வழக்கங்களை உருவாக்கிக் கொள்கிறது. தனிமனிதன் அதைப் பின்பற்றி வாழத் தலைப்படுகிறான். பழக்கம் என்பது தனிமனிதனிடம் இயல்பாக வாய்ந்தமைந்த நடத்தையைக் குறிக்கும் என்று எஸ்.கே.பாஸ்கல் கிஸ்பர்ட் கூறுகிறார்.

சங்ககால மகளிர் மேற்கொண்ட பழக்க வழக்கங்களால் அவர்களின் வாழ்க்கைக் கண்ணோட்டங்களையும், உயர்ந்த நோக்கங்களையும் அறியலாம்.

உடை

சங்ககாலப் பெண்கள் பலவகையான ஆடைகளை அணிந்திருந்தனர். நாகரீக வாழ்வின் இன்றியமையாக் கூறாசக ஆடை அணிதல் அமைந்தது. தமிழகத்தில் பழங்காலத்திலேயே மூதாட்டியாரும், கைம்பெண்டிரும் நூல் நூற்றல் தொழிலை மேற்கொண்டிருந்தனர். அதனால் அவர்களைப் 'பருத்திப் பெண்டிர்' என்று புறநானூறு கூறுகிறது.

'பருத்திப் பெண்டிர் பனுவலன்ன' (புறநானூறு)

'ஆடையில்லா மனிதன் அரை மனிதன்' (பழமொழி)

'ஆள்பாதி ஆடைபாதி' (பழமொழி)

போன்ற பழமொழிகள் ஆடையின் இன்றியமையாமையினை உணர்த்துகின்றன. சங்க இலக்கியம் தழையாடையை மிகுதியாகச் சிறப்பித்துள்ளது. சமுதாய பொருளாதார வளர்ச்சிக்கேற்ப ஆடை அணிவதிலும் மாறுபாடுகள் தோன்றி வருகின்றன.

அணிகலன்கள்

பண்டைய தமிழகத்தில் விரும்பிய அளவு அணிகலன் செய்வதற்கேற்ற பொன், வெள்ளி, முத்து, மணி முதலியனவும், அவற்றைச் செய்வதற்கேற்ற கருவிகளும், கைதேர்ந்து பொற்கொல்லரும், அணிபவரும் இருந்தனர்.

'பலவுறு கண்ணுள் சிலகோல் அவிர்தொடி'

என்னும் பகுதி அக்காலத்து அணிகல வினைத்திறத்தைக் காட்டும். சங்க மகளிர் இயற்கை அணிகலன்களையும், செயற்கை அணிகலன்களையும் அணிந்திருந்தனர். தழை, மலர் போன்றவற்றை அணிகலன்களாக அணிந்தனர். சூரர மகளிர் காதுகளில் அசோகின் தளிர்களைச் செருகிக் கொண்டதை,

"வண்காது நிறைந்த பிண்டி யொண்டளிர்
நுண்பூன் ஆகந்திளைப்ப"
(திருமுருகாற்றுப்படை)

என்று திருமுருகாற்றுப்படை கூறுவதன் வாயிலாக அறிய முடிகிறது.

தொய்யில் எழுதும் பழக்கம்

சங்ககாலப் பெண்கள் தங்கள் மார்பின் மீதும், தோள்மீதும் சந்தனம் போன்ற வண்ணக் குழம்பினால் இலை, கொடி, மலர் போன்ற ஓவியங்களும், கரும்பும் எழுதிக் கொள்வது உண்டு எனத் தெரிகிறது. இவற்றைத் தன் காதலிமார்க்குக் காதலர்கள் எழுதினர் என்று குறிக்கப்பட்டுள்ளது. இதனை,

"தீநெய்தல் நெறிக்கவும் வல்லவன்
நெடுமென்தோன்"

"பெய் அம்பு ஈர்க்கவும் இளமுலைமேல்
தொய்யில் எழுதவும் வல்லன், தன்கையில்
சிலை வல்லான், போலும் செறிவினான்"
(கலித்.நெய்தற் கலி:16)

என்றும் பாடல் கூறுகிறது. இவற்றைத் தம் காதலிமார்க்குக் காதலர்கள் எழுதினர் என்று குறிக்கப்பட்டுள்ளது. ஆனால் இது போன்ற மார்பும், பகுதியில் இல்லாத நெற்றி, கை, புறங்கை, விரல்களில் பச்சைக் குத்திக்கொள்ளும் பழக்கம் இன்றும் காணப்படுகிறது. இது உலகம் முழுவதும் உள்ளடக்கம் என்று வாழ்வியல் களஞ்சியம் குறிப்பிடுகிறது.

குரவையாடல்

குரவையாடல் என்பது காதலையோ, போர் வெற்றியையோ பலர் வட்டமாக நின்று கைக்கோர்த்து கொண்டு ஆடுவது குரவைக் கூத்தாகும். இவர்களுக்கு இன்பப் பொழுது போக்காக குரவையாடும் பழக்கம் இருந்து வந்துள்ளது. ஏறு தழுவி வெற்றிபெற்றவனுக்கும் அவ்வாற்றால் அவனுக்குப் பரிசிலாக ஆய்மகட்கும் முல்லை நிலத்தார் வெற்றி விழாவாகக் கொண்டாடி குரவையாடியதை,

"தண்ணுமைப் பாணி தளரா தெழுஉக
பண்ணமை யின்சீர்க குரவையுள் தெண் கண்ணி"
(கலித்.முல்லைக்கலி:102)

என்று கலித்தொகை பாடல் கூறுவது கொண்டு சங்காலப் பெண்கள் குரவையாடி மகிழ்ந்தனர் என்பதை அறிய முடிகிறது.

கண்ணாடி பார்த்தல்

பெண்கள் ஆடையணிந்து வாசைன முதலியன பூசிக்கொண்டு கோலம் புனைகின்ற காலத்து தங்கள் இயற்கை அழகையும், செயற்கை அழகையும் நோக்குவதற்குக் கண்ணாடியை உபயோகித்தனர். தன்னைப் பார்த்து தன் அணிகளைத் திருத்தி அமைத்த செய்தியை,

"வாசநற நெய் ஆடி, வான்துகள்
மாசு அறக்கண்ணாடி வயக்கி, வண்ணமும்,
தேசும், ஒளியும் திகழ நோக்கி,
வாச மணத்தவர் வாய்க் கொள்வோரும்"
(பரிபாடல், பா-12)

என்று பரிபாடல் கூறுகிறது.

ஆடை வெளுத்தல்

ஆடை வெளுத்துக் கொடுக்கும் தொழிலில் பெண்கள் தான் இடம் பெற்றிருந்தனர். இதில் ஆடவரும் பங்கேற்றனரா? என்பதை அறிய இயலவில்லை. ஊரவரின் ஆடையை வெளுப்பவள் பெண்கள் என்ற செய்தி,

"நாடி நின் தூது ஆடி துறை செல்லாள், ஊரவர்
ஆடை கொண்டு ஒலிக்கும்"
(கலித்.மருதக்கலி,பா.6)

என்று கலித்தொகை கூறுகிறது.

இல்லறத்தில் பெண்கள்

ஒத்த தலைவனும் தலைவியும் பலரறிய மணந்து கொண்டு வாழ்வதையே இல்லற ஒழுக்கம் என்று சான்றோர் குறிப்பிட்டனர். அவர்கள் சமுதாயத்தில் கணவன் மனைவியாக ஒரு குடும்பமாக வாழும் வாழ்க்கைக்கு 'அன்பே' அடிப்படையாய் அமையும் என்பதை,

"அன்பும் அறனும் உடைத்தாயின் இல்வாழ்க்கை
பண்பும் பயனும் அது" (குறள்-45)

என்று திருவள்ளுவர் கூறுகின்றார். அன்பு ஊற்றெடுக்குமிடத்தில் துன்பமோ, வறுமையோ வாழ்வைச் சுடுவதில்லை. எந்நாளுமே இன்பமே உண்டகும். வழியிடை ஏற்படும் துன்பத்தை எண்ணாமல் தலைவனுடைய துன்பத்திற்குத் துணையாகத் தன்னையும் அழைத்துச் செல்லும் இன்பத்தையே தலைவி விரும்புவதாக,

"துன்பம் துணையாக நாடின், அதுவல்லது
இன்பமும் உண்டோ எமக்கு"

என்று கலித்தொகை பெண்களின் உறுதியைக் கூறுகிறது.

தலைவியின் துன்பநிலைகள்

கலித் தொகையில் அகப் பொருளில் தலைவனும் தலைவியும் சிறப்பிடம் பெற்று திகழ்பவர்கள். தலைவி தன் எண்ணங்களைத் தோழி வாயிலாகவே தெரிவிக்கின்றாள். அவருடைய பிரிவுத் துன்பத்தை உணர்த்துவனவாக அமைந்துள்ளன. பிரிந்து சென்ற தலைவன் இளவேனில் பருவம் வந்ததும் தலைவன் வரவில்லை. அப்பருவத்தில் மலர்கள் பூத்துக் குலுங்கின. மயில்கள் ஒருசேர ஆடுவன போல நின்ற காட்சியைக் கண்டு தலைவி மிகவும் துன்புறுவதை இப்பாடல் மூலம் அறியலாம்.

"நொந்து நகுவன போல் நந்தின, கொம்பு
நைந்து உள்ளி
உகுவது போலும் என்நெஞ்சு; எள்ளி
தொகுபு உடன் ஆடுவ போலும் மயில்
தலைவியின் காதல் வாழ்வு"
(கலித்.பாலைக்கலி,பா.32)

தலைவியின் காதல் வாழ்வில் தலைவி, தலைவன் திருமணம் செய்து கொள்ளாமலே இருவரும் பழகி வந்தனர். இவ்வொழுக்கம்

காரணமாக நிறங்கெட்டு மெலிந்த அவள் உடல் பலருக்கும் அவளது காதலைக் கூறுவதாய் நின்றது. பசலை நோயால் காணப்பட்டாள் என்பதன் மூலம் அவளது காதல் வாழ்வு தெளிவாகின்றது.

"பல்நாளும் படர்அட பசலையால்
உணப்பட்டான்"

என்ற கலித்தொகை பாடல் மூலம் அறிய முடிகிறது.

கனவில் தோழியின் பங்கு

களவொழுக்கத்தில் முக்கிய பங்கு வகிப்பவள் தோழி. இயற்கைப் புணர்ச்சி, இடந்தலைப்பாடு, பாங்கற் கூட்டம் என்ற மூன்று நிலைகளில் தலைவியைக் கூடிய தலைவன், நான்காவது நிலையாகத் தோழியின் உதவியை நாடுகின்றான். இதனை பாங்கியற் கூட்டம் என்பர்.

தோழியின்றி களவொழுக்கம் நீளாது என்றும், ஐந்திணை இலக்கியம் படைப்போருக்குத் தோழி என்னும் பாத்திரம் இன்றியமையாதது என்றும், தோழியிற் புணர்ச்சிக்குரிய துறையே புலவனின் நெஞ்சை கவர்ந்தன என்றும் கூறுவர் வ.சுப.மாணிக்கம்.

கற்பில் தோழியின் பங்கு

தலைவன் இளவேனில் காலத்து வருவேன் எனக் காலங்குறித்து பிரிந்தவன். அக்காலம் வந்ததும் தலைவி நமக்கு அருள் செய்ய வேண்டிய காலத்து அருள் செய்யவில்லை என்றாள். அது கேட்ட தோழி ஆற்றுவதற்காக,

"தெருமரல் வாழி, தோழி நம் காதலர்
பொருமுரண் யானையர் போர் மலைத்து எழுந்தவர்
செருமேம்பட்ட வென்றியர்
வரும் என வந்தன்று அவர் வாய்மொழித் தூதே" (கலித்.பாலைக்கலி,பா.25)

தலைவன் நாடுகளை வென்ற வெற்றியை உடையவர். ஆதலின் அவருடைய மெய்மொழியாகிய தூதுவிடுவர் என்று சொல்லி வந்ததைக் காண். ஆதலால் தலைவன் விரைவில் வருவர் என்று கூறித் தேற்றுகின்றாள் என்பதை கலித்தொகைப் பாடல் மூலம் அறியலாம்.

பரத்தையர் வாழ்க்கை

பரத்தையர் தம்மை நாடி இன்பம் துய்ப்போர் தரும் பொருளால் வாழ்க்கை நடத்தினர். அதனால் பொருட்பெண்டிர் எனவும் கூறப்பட்டனர். மற்ற பெண்களை நோக்கும்போது சமூகத்தில் மதிப்புக் குறைவே எனினும், சமுதாயத்தில் செல்வாக்குப் பெற்றவர்களாக இருந்துள்ளனர் என்பதை மதுரைக்காஞ்சியில் குறிப்பிடப்பட்டு உள்ளது. பிற நாட்டிலிருந்து கவர்ந்து வரும் பெண்கள் பரத்தையாக்கப்படுவதுண்டு. இதற்குப் போர்நெறி வழிவகுத்தது. போர் வெற்றியின் பொழுது பொருள்களோடு பெண்களும் அடிமைப்படுத்திக் கொண்டு வரப்பட்டதைப் பதிற்றுப்பத்தின் பதிகத்தின் பாடல்,

"வாலிழை கழித்த நறும்பல் பெண்டிர்
பல்லிருங் கூந்தற் முரற்சியால்
குஞ்சல் வொழுகை பூட்டி"
(பதிற்றுப்பத்து, ஐந்தாம்பத்து, பா.15)

என்று குறிப்பிடுகிறது.

முடிவுரை

கலித்தொகையில் பெண்களின் நிலையும், பழக்கவழக்கங்களும் என்ற தலைப்பில் கலித்தொகை ஓர் அறிமுகம், பழக்க வழக்கங்கள், அவர்களின் வசதிக்கேற்ப ஆடை உடுத்தினர். மேலும் தலையணி, கையணி, காதணி, விரலணி போன்ற பலவகையான அணிகலன்களும் அணியும் பழக்கமும், மன்னனை வாழ்த்திக் குரவையாடி மகிழ்ந்தனர். தொய்யில் எழுதும் பழக்கமும், கண்ணாடி பார்த்தலும், ஆடை வெளுப்பதும் ஆண்களைவிட பெண்களே சந்ததியினரைக் காத்தளிக்கும் பொறுப்பும் பெண்களுக்கே அதிக பங்கிருந்ததையும் அறிய முடிகிறது. தலைவியின் இல்வாழ்க்கை, தலைவியின் காதலுக்கு தோழியின் பங்கு, கற்பில் தோழியின் பங்கு, பரத்தையர் போன்ற

"தண்ணுமைப் பாணி தளரா தெழுஉக
பண்ணமை யின்சீர்க குரவையுள் தெண் கண்ணி"
(கலித்.முல்லைக்கலி:102)

என்று கலித்தொகை பாடல் கூறுவது கொண்டு சங்காலப் பெண்கள் குரவையாடி மகிழ்ந்தனர் என்பதை அறிய முடிகிறது.

கண்ணாடி பார்த்தல்

பெண்கள் ஆடையணிந்து வாசைன முதலியன பூசிக்கொண்டு கோலம் புனைகின்ற காலத்து தங்கள் இயற்கை அழகையும், செயற்கை அழகையும் நோக்குவதற்குக் கண்ணாடியை உபயோகித்தனர். தன்னைப் பார்த்து தன் அணிகளைத் திருத்தி அமைத்த செய்தியை,

"வாசநற நெய் ஆடி, வான்துகள்
மாசு அறக்கண்ணாடி வயக்கி, வண்ணமும்,
தேசும், ஒளியும் திகழ நோக்கி,
வாச மணத்தவர் வாய்க் கொள்வோரும்"
(பரிபாடல், பா-12)

என்று பரிபாடல் கூறுகிறது.

ஆடை வெளுத்தல்

ஆடை வெளுத்துக் கொடுக்கும் தொழிலில் பெண்கள் தான் இடம் பெற்றிருந்தனர். இதில் ஆடவரும் பங்கேற்றனரா? என்பதை அறிய இயலவில்லை. ஊரவரின் ஆடையை வெளுப்பவள் பெண்கள் என்ற செய்தி,

"நாடி நின் தூது ஆடி துறை செல்வாள், ஊரவர்
ஆடை கொண்டு ஒலிக்கும்"
(கலித்.மருதக்கலி,பா.6)

என்று கலித்தொகை கூறுகிறது.

இல்லறத்தில் பெண்கள்

ஒத்த தலைவனும் தலைவியும் பலரறிய மணந்து கொண்டு வாழ்வதையே இல்லற ஒழுக்கம் என்று சான்றோர் குறிப்பிட்டனர். அவர்கள் சமுதாயத்தில் கணவன் மனைவியாக ஒரு குடும்பமாக வாழும் வாழ்க்கைக்கு 'அன்பே' அடிப்படையாய் அமையும் என்பதை,

"அன்பும் அறனும் உடைத்தாயின் இல்வாழ்க்கை
பண்பும் பயனும் அது" (குறள்-45)

என்று திருவள்ளுவர் கூறுகின்றார். அன்பு ஊற்றெடுக்குமிடத்தில் துன்பமோ, வறுமையோ வாழ்வைச் சுடுவதில்லை. எந்நாளுமே இன்பமே உண்டகும். வழியிடை ஏற்படும் துன்பத்தை எண்ணாமல் தலைவனுடைய துன்பத்திற்குத் துணையாகத் தன்னையும் அழைத்துச் செல்லும் இன்பத்தையே தலைவி விரும்புவதாக,

"துன்பம் துணையாக நாடின், அதுவல்லது
இன்பமும் உண்டோ எமக்கு"

என்று கலித்தொகை பெண்களின் உறுதியைக் கூறுகிறது.

தலைவியின் துன்பநிலைகள்

கலித் தொகையில் அகப் பொருளில் தலைவனும் தலைவியும் சிறப்பிடம் பெற்று திகழ்பவர்கள். தலைவி தன் எண்ணங்களைத் தோழி வாயிலாகவே தெரிவிக்கின்றாள். அவருடைய பிரிவுத் துன்பத்தை உணர்த்துவனவாக அமைந்துள்ளன. பிரிந்து சென்ற தலைவன் இளவேனில் பருவம் வந்ததும் தலைவன் வரவில்லை. அப்பருவத்தில் மலர்கள் பூத்துக் குலுங்கின. மயில்கள் ஒருசேர ஆடுவன போல நின்ற காட்சியைக் கண்டு தலைவி மிகவும் துன்புறுவதை இப்பாடல் மூலம் அறியலாம்.

"நொந்து நகுவன போல் நந்தின, கொம்பு
நைந்து உள்ளி
உகுவது போலும் என்நெஞ்சு; எள்ளி
தொகுபு உடன் ஆடுவ போலும் மயில்
தலைவியின் காதல் வாழ்வு"
(கலித்.பாலைக்கலி,பா.32)

தலைவியின் காதல் வாழ்வில் தலைவி, தலைவன் திருமணம் செய்து கொள்ளாமலே இருவரும் பழகி வந்தனர். இவ்வொழுக்கம்

நிலைகளில் வாழ்ந்து, அந்தந்த நிலைகளில் இல்லறத்தில் கணவனிடமும், விருந்தினரிடமும் மிகுந்த அன்புடையவராகத் திகழ்கிறார்கள் என்பதை இக்கட்டுரையின் வாயிலாக அறியலாகினோம்.

சான்றெண் விளக்கம்

1. ச.வே.சுப்பிரமணியன், தமிழ்விடுதூது, ப.51
2. திருக்குறள், குறள்.45
3. குறிஞ்சிக்கலி, பா.12
4. வ.சுப.மாணிக்கம், தமிழ்க்காதல்,. ப.58
5. பதிற்றுப்பத்து, பா.5

பார்வை நூல்கள்

1. கலைகளஞ்சியம், தொகுதி ஏழு, தொகுதி நான்கு
2. புறநானூறு
3. கலித்தொகை
4. திருக்குறள்

துணைநூற்பட்டியல்

1. வ.சுப.மாணிக்கம், தமிழ்க்காதல், பாரி நிலையம், சென்னை.
2. விசுவநாதன், கலித்தொகை, நியூ செஞ்சுரி புக் ஹவுஸ், சென்னை.
3. ச.வே.சுப்பிரமணியன், தமிழ்விடு தூது

Chief Editor

Dr. M. SADIK BATCHA
Associate Professor
Department of Thamizh
Jamal Mohamed College (Autonomous)
Tiruchirappalli - 20,Tamil Nadu, India.

Advisory Editor

Dr. N. CHANDRA SEGARAN
Assistant Professor of Thamizh
Centre of Indian Languages,School of Language,
Literature &Culture Studies
Jawaharlal Nehru Univeristy
New Delhi - 610 067, India

Editorial Board

Dr. MAM. Rameez
Head
Department of Languages
Snr. Lecturer, Grade-I in Thamizh
South Eastern University of Srilanka
Oluvil, Srilanka # 32360.

Dr. S. Chitra
Professor & Head
Dept. of Thamizh
Bharathiar University
Coimbatore - 641 046
Tamil Nadu, India.

Dr. P. Jeyakrishnan
Director (MSCDCS)
Department of Thamizh
University of Kerala
Karyavattom,
Thiruvananthapuram - 695 581
Kerala, India.

Dr. Ganesan Ambedkar
Associate Professor
Department of Dravidian &
Computational Linguistics,
Dravidian University,
Kuppam 517 426
Andhra Pradesh, India.

Dr M N Rajesh
Assistant Professor,
Department of History,
School of Social Sciences,
University of Hyderabad,
Dr C R Rao Road, Gachibowli,
Hyderabad -500046 Telangana state

Dr. Senthil Prakash
Assistant Professor
Department of Thamizh
Basha Bhawan
Visva Bharathi, Santhinikethan
West Bengal-731 235, India.

Dr. S. Bharathi Prakash
Assistant Professor
Tamil
PSG College of Arts & Science
Avinashi Road, Civil Aerodrome Post,
Coimbatore-641 014.

Dr. Jeyaraman
Professor and Head
Department of Thamizh
Thiruvalluvar University,
Vellore - 632 115
Tamil Nadu, India.

Dr. S.Senthamizh Pavai
Professor& Director
Centre for Tamil Culture
Alagappa University
Karaikudi – 630 003
Tamil Nadu, India.

Dr. S. Easwaran
Associate Professor & Head
Department of Thamizh
National College (A)
Tiruchirappalli - 620 001
Tamil Nadu, India.

Dr. Kumar
Assistant Professor of Thamizh
School of Social Sciences & Humanities
Central University of Tamilnadu
Tiruvarur-610 005, Tamil Nadu, India.

Dr. M. Ramakrishnan
Assistant Professor,
Centre for Tribal Folklore,
Language & Literature,
Central University of Jharkhand,
CTI Campus, Brambe, Ranchi – 835 205
Jharkhand, India

Dr.M. Arunachalam
Associate Professor
P.G. & Research Dept. of Thamizh
E.V.R.College (A)
Tiruchirappalli - 620 023,
Tamil Nadu, India.

Dr.A. Ekambaram
Professor
Department of Tamil Literature
Madras University
Chennai - 600 005
Tamil Nadu,India.

Dr. ARANGA. PARI
Professor
Department of Thamizh
Annamali University,
Chidambram-608 002
Tamil Nadu, India.

Dr. Kumara Selva
Associate Professor & Head
Department of Thamizh
Nesamani Memorial Christian College
Marthandam - 629 165
Kanyakumari, Tamil Nadu, India.

Dr. S. Kalpana
Assistant Professor
Department of Tamil Studies
Annamalai University
Annamalai Nagar - 608 002
Tamil Nadu, India

Dr. Govindaraj
Assistant Professor
Center for Bharathidasan Studies
Bharathidasan University
Tiruchirappalli - 620 024
Tamil Nadu, India.

Dr. S. Vignesh Ananth
Assistant Professor in Thamizh
Department of Indian Languages,
Faculty of Arts,
Banaras Hindu Univeristy
Varanasi - 221 005,Uttar Pradesh

Dr. G. Stephen
Professor & Head
Department of Thamizh Studies
Manonmaniam Sundaranar University
Tirunelveli-627 012
Tamil Nadu, India.

Dr. A. Shunmughom Pillai
Associate Professor
Department of Folklore
Tamil University
Thanjavur – 613 010
Tamil Nadu, India

Dr. A. Palanisamy
Professor
Dept. of Physical Education and Yoga
Bharathidasan University
Tiruchirappalli – 620 024
Tamil Nadu, India

Dr. T. Vishnukumaran
Assistant Professor
Department of Thamizh
Dravidian University, Kuppam,
Andra Pradesh - 517 426, India.

Dr. Uma Devi
Assistant Professor
Department of Modern Indian
Languages and Literary Studies
University of Delhi
Delhi - 110 007, India.

Dr. Pon. Kathiresan
Assistant Professor in Thamizh
Ganesar College of Arts & Science
Melaisivapuri-622 403
Pudukkottai (Dt.)
Tamil Nadu, India.

தொகுதி-9 எண்-4
Vol. 9 No. 4
புரட்டாசி-மார்கழி 2052
October - December 2021
ISSN : 2321 - 984X

353. பொ. அறிவழகன்	செங்கோட்டை ஓலை ஆவணத்தில் காணப்படும் வரி நிர்வாக முறை	2439-2442
354. கா. கணநாதன்	முக்கூடற்பள்ளு வழி மராட்டியர் வேளாண்மை	2443-2449
355. நா. பிரபு & சி. சந்திர காந்தி	கமழ்ச்சி சிறுகதைத் தொகுப்பில் உத்திகள்	2450-2464
356. ஏ. இராஜசேகர்	பத்துப்பாட்டில் உணவும் உற்பத்தியும்	2465-2480
357. மு. அப்துல் சமது & மே. நாகூர் கனி	இஸ்லாமியப் பார்வையில் - அஞ்சுவண்ணம் தெரு	2481-2487
358. VARSHA, C.V & WILSON V. A	ANALYSIS OF ATHLETIC BURNOUT AMONG NATIONAL LEVEL TRACK AND FIELD MALE ATHLETES IN KERALA	2488-2494
359. B. MATHIVANAN	SOCIAL STATUS IMPROVEMENT BY PERIYAR AND AMBEDKAR RELATED WITH GREAT REVOLUTIONARY MODERN THOUGHT IN TAMILNADU (INDIA)	2495-2499
360. கோ.ப. நல்லசிவம்	பொருளதிகாரத்தில் மெய்ப்பொருட் கோட்பாடு	2500-2505
361. இரா. ரமாதேவி & கு. சந்திரசேகரன்	இனியவை நாற்பது உணர்த்தும் இல்லறவியல்	2506-2510
362. கி. வீரமணி & கு. சந்திரன்	பெண்ணிய எழுத்தாளர் ஒளவை நிர்மலா	2511-2516
363. நி. அமிருதீன் & க. சாகுல் ஹமீது	நற்றிணையும் நெல்லும்	2517-2521
364. RAJIYA SULTAN	GOOD PRACTICES OR WOMEN EMPOWERMENT IN HIMACHAL PRADESH	2522-2527
365. ஜெ. மணிகண்டன் & சி. கண்மணி	நாலடியாரில் மகளிர் மீக்கூறு	2528-25 3
366. ANOOP SEBASTIAN	AEROBIC TRAINING AS THE BACKBONE OF THE WELL- BEING OF INDIVIDUALS	2534-
367. ஆ. வனிதா	இரட்டைக்காப்பியங்களில் இயற்கை இகந்த நிகழ்ச்சிகள்	2541-254.
368. மு. கிருஷ்ணமூர்த்தி	தமிழ் இதழியல் வரலாற்றில் செந்தமிழ் இதழின் பங்களிப்புகள்	6-2551
369. இரா. தங்கலதா	பேராசியரியர் அ.சீனிவாச ராகவனின் மொழிபெயர்ப்பு உத்திகள்	-2557
370. G. PREMA & K. GOVINDARAJ	THE CONTRIBUTION OF WOMEN ASSOICAITON ON SOCIAL AV 'ENING DURING 20th CENTURY IN TAMIL	25 2562
371. சிவ ஆதிரை & தீ.சி.கே. இராஜசேகர்	கம்பனில் ஆட்சிமுறை	2563
372. மு.முகமது அலி ஜின்னா	கவிக்கோவின் படைப்புகளி டவுள் துவம்	2572-2576
373. து. கிருஷ்ணன் & சி.ஜெயபால்	நற்றிணை காட்டும் உணவு கள்	2577-2584
374. இரா. சங்கர் & தி. முத்து	மோகமுள் புதினத்தில் தி. ஜா ராமனின் இசை டி	2585-2592
375. தி. திவ்யா & மாதவி	திருவெ ப்நல்லூர் தல ாறு	2593-2597
376. சு. அ ாமி	இல கண ல களில் கைக்கிளை	2598-2605
377. கா. நாதன்	திரு விழுமங்களில் தன்ன சார்புகள்	2606-2612
378. ஜெயட வள்ளி ராஜ்	இல கியத்தில் ந ம்பிக்கைகளும பழக்கவழக்கங்களும்	2613-2617
379. ஏ. இர சேகர்	பத்து ாட்டின் மைப்பியல்	2618-2626
380. ஏ.பாஸ்க மேஷ்	மேலா ான்னுசாமி சிறுகதைகளில் விளிம்புநிலை மாந்தர்கள்	2627-2633
381. க. ஜலேந், & ஜ.	மதுரைக்கூ க் காட்டும் இல்லறம்	2634- 2643
382. க. இரமேச	றை உத்திகள் நோக்கில் செவ்வியல் இலக்கியங்கள்	2644-2648
383. செ. கயல்க.	வள்ளுவம் உணர்த்தும் தனிமனித அறங்கள்	2649-2653
384. கோ. கலைம கா. கணநாச	'வீரயுகநாயகன் வேள்பாரி'யில் இடம்பெறும் கொற்றவைக்கூத்தின் தன்மைகள்	2654-2659
385. பெ. காளியானந்தம் & கா. கணநாதன்	திருவள்ளுவர், கபீர்தாஸ் பாடல்களில் அறக்கோட்பாடுகள்	2660-2664
386. கு. நித்தியா & நா. குமாரி	சூர்யகாந்தன் சிறுகதைகளில் விளிம்பு நிலையில் பெண்கள்	2665-2671
387. கூ. லதா	திருஞானசம்பந்தர் பாடல்களில் (மனம்) உளவியல் பார்வை	2672-2677
388. பா. சங்கீதா	அறம் காக்கும் புறம் என நிறுவும் இயன்மொழித்துறை	2678-2682
389. செ. நாகேஸ்வரி	கண்மணி குணசேகரன் சிறுகதைகளில் பெண்களின் மனக்குமுறல்	2683-2689
390. த. விஜயலெட்சுமி & அ. மோகன்ராஜ்	பாரதிக் கண்ட பாரத சமுதாயம்	2690-2696
391. தி. கோவிந்தராஜீ & ச. குமரேசமூர்த்தி	பொதுத்தளங்களில் பெண் கவிஞர்கள்	2697-2706
392. இரா. பத்மா	கலித்தொகையில் பெண்களின் பழக்க வழக்கங்களும் அவர்களின் நிலையும்	2707-2711
393. ச. சுந்தர்	மருதத்திணையில் பரத்தையர் பிரிவும் தலைவியின் ஊடலும்	2712-2716
394. இரா. பிரவீணா	தமிழ், மலையாள உயிர்ப்புணர்ச்சியில் யகர வகர உடம்படுமெய்கள்	2717-2723
395. கோ. ரங்கராஜன்	பூமணியின் அஞ்ஞாடி நாவலில் கிராமிய வாழ்வியல்	2724-2728
396. கா. ரெங்கராஜ்	மானாவாரி மனிதர்கள் புதினத்தில் குறியீடும் காட்சியும்	2729-2734
397. அ. அலுடயானா	வள்ளுவர் கூறும் அரசனின் நிலைகள்	2735-2742
398. வ. கணபதிராமன்	எட்டுத்திக்கும் மதயானை புதினத்தில் விளிம்புநிலையினர் வாழ்வியல்	2743-2748
399. செ. முருகநாதன்	'மானாவாரி மனிதர்கள்' புதினத்தில் நடப்பியல் உத்திகள்	2749-2753
400. செ. யோகம்	பதினெண்கீழ்க்கணக்கில் நீர் மேலாண்மை	2754-2757
401. S. ரோஜாமணி	பெண்ணிய முன்னேற்றத்திற்கான வழிவகைகள்	2758-2766
402. ஜெ. காமாட்சி	கி.ராஜநாரயணன் கரிசல் காடு - ஒரு பார்வை	2767-2770

Published by : Raja Publications, No. 10, Upstair, Ibrahim Nagar, Khajamalai, Tiruchirappalli - 620 023, Tamil Nadu, India. Mobile : 9600535241
Printed : The Printing House, Tiruchirappalli - 620 023, Tamil Nadu, India. Ph : +91 - 431-2420121

மருதம் கலை இலக்கிய ஆய்வு மையம், கும்பகோணம்.

[மருதம் கல்வி அறக்கட்டளை, பதிவு எண்.95/2019, UDYAM-TN-22-0004191]

சுவாமி தயானந்தா கலை மற்றும் அறிவியல் கல்லூரி, மஞ்சக்குடி.

[Affiliated To Bharathidasan University, Thiruchirapalli. UGC Recognized U/S 2(f) & 12 (b)

தமிழ்நாடு - இந்தியா

பன்னாட்டுக் கருத்தரங்கம்

ISBN : 978-81-950822-4-7

நாள் : 17.10.2021

இடம் : கும்பகோணம்

சான்றிதழ்

திரு/செல்வி/திருமதி சி.சாயாதேவி, உதவிப்பேராசிரியர், தமிழ்த்துறை, தேசியக் கல்லூரி, அவர்கள் "துறைதோறும் தமிழ்" என்னும் பொருண்மையில் கும்பகோணம் மருதம் கலை இலக்கிய ஆய்வு மையம் நடத்திய பன்னாட்டுக் கருத்தரங்கில் ஆய்வாளராக / அமர்வுத்தலைவராக / பார்வையாளராகப் பங்கேற்று சிலப்பதிகாரம் காட்டும் சிவபெருமானின் திருப்புகழ் என்னும் தலைப்பில் ஆய்வுக்கட்டுரை வழங்கிச் சிறப்பித்தார் எனச் சான்றளிக்கப்படுகிறது.

தலைவர்	செயலாளர்	முதல்வர்	ஒருங்கிணைப்பாளர்

துறைதோறும் தமிழ்

பதிப்பாசிரியர்கள்
முனைவர் மா.கோ.பெரியசாமி
முனைவர் செ.கனேசமூர்த்தி
முனைவர் சு.அ.சம்பத்குமார்

வெளியீடு
மருதம் கலை இலக்கிய ஆய்வு மையம்
கும்பகோணம் - 612001.
சுவாமி தயானந்தா கலை மற்றும் அறிவியல் கல்லூரி.
மஞ்சக்குடி, திருவாரூர் - 612610.

தமிழ்நாடு - இந்தியா.
அக்டோபர் - 2021

29. இலக்கிய நயத்தோடு கணிதத்தைப் போதிப்பதால் தமிழ்மொழி வளர்ச்சி அடையும் 204
- ஆசிரியர் சதீஸ்குமார் (எ) குவாங் லியோங் யீ

30. துறைதோறும் தமிழ் பயில்வோர்க்கான பணி வாய்ப்புகள்
- முனைவர் ம.சரவணகுமார், 210

31. இலக்கியங்களில் நெய்தல் நிலப் பண்புகள் 214
- திரு. மு. சரவணன்,

32. போகர் ஏழாயிரத்தில் உலோகங்கள் 221
(Metals in Bhogar 7000 Book)
- முனைவர் சித்ரா

34. அழகியபெரியவன் கதைகளில் மகளிர் நிலை 231
- திருமதி த.சுபத்ரா

35. அகநானூற்றில் பண்பாட்டுக் கூறுகள் 237
- முனைவர் ச. சுபாஷ்

36. சமயக்குரவர்கள் உணர்த்தும் சிவபெருமானின் சிறப்புகள்
- முனைவர் மு.சுபாஷினி 246

37. ஐம்பெருங் காப்பிங்களில் இசைச் சிறப்பு 252
- திருமதி. சுஜாதாரிரஞ்சன்

38. கவிஞர் மீராவின் கவிதைகளில் மானுடச் சிந்தனைகள்
- வெரமேஷ் 258

39. சங்க இலக்கியங்களில் முல்லைத் திணையின் வாழ்வியல் கூறுகள் 266
- செ. இராஜலட்சுமி

40. சூர்யகாந்தனின் மானாவாரி மனிதர்கள் கதையில் பழக்கவழக்கங்கள் 271
- ஆ.இம்மானுவேல்

41. கீதாரி - புதினத்தில் தமிழ்ப் பண்பாட்டுப் பதிவுகள் 277
- செ.பேபி ஸ்டெல்லா

42. அகநானூற்றினால் அறியலாகும் அரசர்கள் 283
- முனைவர் செ.மாரிமுத்து

43. சுந்தரவல்லி சொல்லாத கதை நாவலின் வாழ்வியல் மற்றும் பண்பாட்டுக் கூறுகள். 292
- க.செந்தில்குமார்

சமயக்குரவர்கள் உணர்த்தும் சிவபெருமானின்

சிறப்புகள்

முனைவர் மு.சுபாஷினி
உதவிப் பேராசிரியர் (நிர்வாக நியமனம்)
தமிழாய்வுத்துறை, தேசியக்கல்லூரி,
திருச்சி 1
Cell No: 9042453877
E-Mail kofi.kaavya@gmail.com

முகவுரை

மக்களின் மனத்தைப் பக்குவப்படுத்துவதற்கு தோன்றியதே பக்தி இலக்கியமாகும். ஊரெல்லாம் ஒன்றுகூடி கருத்துகளைப் பரிமாறிக் கொள்வதற்கு கோயில் என்ற அமைப்பை உருவாக்கினர். அக்கோயிலில் மக்கள் மகிழ்ச்சியோடு இருந்தனர். சமுதாயத்தில் நற்சிந்தனைகளும், நல்லெண்ணங்களும் வித்தாக விதைத்தால்தான் சிறந்த விருட்சம் உண்டாகும். இக்கருத்தை அடியொற்றியே இறையடியார்கள் இறைவனைப் பற்றியும், இறைவனை அடையும் வழிமுறைகள் பற்றியும் மக்களிடையே கொண்டு செல்வதற்கு தேவாரப் பாடல்களைக் கோயில்களில் இசைத்தனர். அவர்களுள் சமயக்குரவர்கள் எனப்படும் நால்வர்களின் பாடல்கள் சிவபெருமானின் சிறப்புகளை எவ்விதத்தில் குறிப்பிட்டுள்ளன என்பதை ஆராயும் நோக்கில் இக்கட்டுரை அமைந்துள்ளது.

சிவம்

சிவம் என்பதற்கு பலர் பல்வேறு பெயர்களைக் குறிப்பிடுகின்றனர். தென்னகத்தில் உள்ளோர் இறைவனைச் சிவம் என்றே வழங்குகின்றனர். சிவம் என்னும் சொல் வடமொழி மற்றும் தென்மொழியில் வழங்கப்படுகிறது. தென்மொழி வல்லார் சிவம் என்பதற்கு செம்மையென்று பொருள் கூறுவர். செம்மை என்னும் சொல் பல பொருள்களை உடையது. ஆனால் இந்த எடுத்துக் கொண்ட பொருளுக்கேற்ப செம்மையென்பதற்குச் செந்நிறம் என்ற ஒரு பொருளை மட்டும் எடுத்துக் கொள்ளப்படுகிறது. அச்செம்மையனே சேயென்றும் சிவமென்றும் நம் முன்னோர்கள் போற்றினார்கள்.

பொன்னாற் மேனியனே
புலித்தோலை அரைக்கசைத்து
மின்னார் செஞ்சடைமேல்
மிளிர் கொன்றை அணிந்தவனே [1]

(சுந்தரர் தேவாரம், ஏழாம் திருமுறை, பா.239)

என்று சுந்தரரும்,

செம்பொனாற் செய்தழகு பெய்தார் போலுஞ்
செஞ்சடையெம் பெருமானே தெய்ய நாறும் [2]

(அப்பர் தேவாரம், ஆறாம் திருமுறை,பா.41)

என்று அப்பரும் கூறுவது குறிப்பிடத்தக்கது.

செய்ய மேனியனே செய்வகை அறியேன் என்றும், **செஞ்சுடர் வெள்ளமே** என்றும், **ஆடகச் சீர்மணிக் குன்றே** என்றும், **செக்கர்போலுந் திருமேனி திகழ் நோக்கிச் சிலிர்சிலித்து** என்றும் **வாதவூரடிகள் இறைவன் தீவண்ணத்தைச் சிறப்பித்துக் கூறுகிறார்.** [3] (திரு.வி.க., சைவசமயம், ப.116) சிவனெனு நாமம் தனக்கேயுடைய செம்மேனி எம்மான் என்று அப்பர் சுவாமிகளும் விளக்கியிருத்தல் இங்கே நோக்கத்தக்கது.

இறைவனது இயற்கை வடிவஞ் செக்கச் செவேலெனத் திகழ்தல் நோக்கி மாணிக்கவாசகனார், **தென்னாடுடைய சிவனே போற்றி** [4] (மாணிக்கவாசகர், போற்றித்திருவகவல், பா.அடி.64) என்று கூறுவதோடு அச்சிவனே மற்ற நாடுகளின் இயற்கைக்கேற்ப வெவ்வேறு பெயரான் குறிக்கப்படும் இறைவன் என்பதை அறிவுறுத்தப் பொதுவாக **எந்நாட்டவர்க்கும் இறைவா போற்றி** [5] (மாணிக்கவாசகர், போற்றித்திருவகவல், பா.வரி.65) என்றருளிச் செய்தார். இவற்றிலிருந்து இறைவன் என்னும் தமிழ்ச் சொல்லுக்கு எங்கும் நிறைந்துள்ள ஒருவன் என்பது பொருளாகும்.

சிவனை உணரும் வழி

இறைவன் ஆதியும் அந்தமும் இல்லாத அருட்பெருஞ்ஜோதி என்று ஆன்றோர் கூறுவர். சிவன் மாற்ற மனங்கடந்தவன், போக்கு வரவுமிலாப் பண்பியல்பு ஒரு நாமம் ஒருருவ மில்லாதவன் என்ற கூற்றை உரைத்த மேலோர்கள் சிவனை உணராது கூறமுடியாது; என்பதால் உணர்ந்த நிலையைக் கூறுகின்றனர்.

இறைவனை உரையுணர்வு இறந்து நின்ற உணர்வதோர் உணர்வே என வருஉம் இன்னுரையை உற்று, உற்று ஊன்றி நோக்கினால் உணர்வதோர் உணர்வே என்று முழுமுதற்பொருளை நம் பெருமான் மிகிக்கிறார். எந்நிலையில் நின்று அச்செம்பொருளை உணர்வது பற்றி மனம் உலகிற்கு உணர்த்தியுள்ளார் என்பதை அடிகள் அறிவுறுத்துகிறார். நினைப்பற நினைக்கும் நிலையில் இறைவ னல்லாமல் பிறிதுமற்றின்மை எனும் காட்சி பெறுதல் கூடும். இந்நிலை நின்றே மாணிக்கவாசகனார் இறைவனுடைய உண்மையை எடுத்துரைக்கிறார்

உருவ வழிபாடு

உருவ வழிபாடு மிகவும் இன்றியமையாதது என்பதை உணர்ந்த மெய்யடியார்கள் உருவ வழிபாட்டை உலகிற்கு வலியுறுத்திச் சென்றார்கள். மனம் பற்றி நிற்பதற்கு ஒன்று வேண்டும். அவ்வொன்றே உருவ வழிபாடு. எவ்வுருவாயினும் அவ்வுருவத்தை இறைவன் திருப்படம் என்று மனத்தால் கொண்டால் போதும் [6] (திரு.வி.க.சைவசமயம்,ப.132). இயற்கையை உடலாகக் கொண்ட இறைவனைக் குறிக்கும் எப்படத்தையும் நல்லறிவு கொண்டு ஆராய்ந்து பார்த்தால் அறிவு விளக்கம் அவரவர் நிலையைப் பொறுத்து நிற்கிறது. உருவமென்பது கல், மண் வடிவங்கள் மட்டுமன்று; எண்ணம், ஒலி, எழுத்து, சொல் எல்லாம் உருவத்தின்பாற் பட்டனவே. எண்ணத்தால் இறைவனை வழிபடுவதுவும் உருவ வழிபாடாகும். நெஞ்சம் நிலைத்து நிற்றற்கு ஒரு கொழுகொம்பு வேண்டற்பாலதே. மனம் ஒன்றில் ஒன்றி ஒன்றி உள்ளொளி பெருகும் என்று இறைவனை ஆழ்ந்துணர்ந்தவர்கள் உரைப்பதை அறியமுடிகிறது.

சிவபெருமானின் திருவுருவம்

இறையின் வடிவம் செம்மை நெருப்பு ஆகும். செம்மையின் விளிம்பில் நீலமிருத்தலைக் காணமுடிகிறது. செம்மைக்கும் நீலத்திற்கும் உள்ள தொடர்பு நிறத்தியல் வல்லார்க்கு நன்கு புரியும். செம்மையில் நீலமும் நீலத்தில் செம்மையும் மிளிர்தல் இயல்பு. எல்லா நிறங்களும் இவ்விரண்டில் ஒடுங்குதலும் உண்டென்பதையும் உணர்த்துகிறது.

செம்மை வெம்மையைக் குறிப்பது; நீலம் தண்மையைக் குறிப்பது. வெம்மை (Heat), தண்மை (Cold) என்னும் இரண்டாலும் உலகம் ஆக்கப்பட்டு இயங்கி வருகிறது. இவ்வெம்மை ஆணென்னும், தண்மை பெண்ணென்றும் உள்ளத்தில் பதியுமாறு உருவகிக்கப்பட்டன [7]. (திரு.வி.க, சைவசமயம், ப.134) அனைத்திற்கும் அடிப்படையாய் விளங்கும் இறைவன்பால் வெம்மைக் கூறும் உண்டு. தண்மைக் கூறும் உண்டு. இக்கூறுகளின் பரிணாமமாக உலகம் இருத்தலைக் கண்கூடாகக் கண்டு உணர்வதை இதன் வழியாக அறியமுடிகிறது.

இயற்கை இறைவனுடல்

இறைவன் இயற்கையை உடலாக் கொண்ட உயிர் என்பதை நந்தமிழ்ப் பெரியோர்கள் செவ்வான உள்ளங்கை நெல்லிக் கனியாக விளங்கச் செய்திருக்கிறார். அந்த திருவாக்குகளில் இரண்டொன்று வருமாறு உலகமே வடிவமாக யோனிகள் உறுப்பதாக என்பது அருணந்தி சிவாசாரிய

இருநிலனாய்த் தீயாகி நீருமாகி
இயமான னாயெறியுங் காற்று மாகி
அருநிலைய திங்களாய் ஞாயிறாகி
ஆகாச மாயட்ட மூர்த்தி யாகிப்
பெருநலமுங் குற்றமும் பெண்ணும் ஆணும்
பிறருருவந் தம்முருவந் தாமே யாகி
நெருநலையாய் அன்றாகி நாளை யாகி
நிமிர்புன் சடையடிகள் நின்ற வாறே [8]

(அப்பர் தேவாரம், ஆறாம் திருமுறை, பா.919)

என்று அப்பர் தேவாரத்தில் சிவபெருமான் பூமியாகவும், நெருப்பாகவும், நீராகவும், இயமானன், காற்று எனவும், சந்திரன், சூரியனாகவும், ஆகாசமாவும் விளங்கும் மூர்த்தியானவர். நன்மை மற்றும் தீமையைத் தம் ஆற்றலால் விளைவிப்பவர். ஆண், பெண் மற்றும் அலி எனவும், இயல்பான ஒளி உருவமும், நேற்று, இன்று, நாளை என்னும் மூன்று காலமும் ஆகி நிலைத்து நிற்பவர் இயற்கையில் தோன்றும் அனைத்துமாக இருப்பவர் இறைவன் என்பதை இப்பாடல் வரிகளின் வாயிலாக அறியமுடிகிறது.

திருஞானசம்பந்தர் தம் பாடல்களில் பலவிடங்களில் இயற்கையையும், இறைவனையும் அழகுறப் பாடியுள்ளார்.

எழுந்துலகை நலிந்துலலும் அவுணர்கள்தம்
புரமூன்றும் எழிற்கணாடி
உழுந்துருளும் அளவையின் ஒள்ளெரிகொளவெஞ்
சிலை வளைத்தோன் உறையும் கோயில்
கொழுந்தரள நகைக்காட்ட கோகநத
முகம் காட்டக் குதித்து நீர்மேல்
விழுந்தகயல் விழிகாட்ட விற்பவளம்
வாய்காட்டு மிழலையாமே [9]

(சம்பந்தர் தேவாரம், முதல் திருமுறை, பா.1428)

உலகத்து மக்களுக்கு துன்பத்தை அளித்த அசுரர்களின் முப்புரத்தை ஒடிபொழுதில் அழித்த ஈசன் முத்து போன்ற புன்னகையும், தாமரை போன்ற முகமும், மீன் போன்ற கண்ணும் கொண்ட மகளிர் வாழும் திருவிழிமிழலையில் அழகுற வீற்றிருக்கின்றான் என்று சம்பந்தர் இயற்கையோடு கலந்திருக்கும் இறைவனை வருணிக்கின்றார். இறைவனின் உடலாக இயற்கை இலங்குதலை மாணிக்கவாசகரும் ஓதியிருக்கிறார் என்பதை

நிலம்நீர் நெருப்புஇ ரிர நீளவிகம்பு நிலாப்பகலோன்
புலனாய மைந்தனோடு எண்ணெகையாய்ப் புணர்ந்து நின்றான
உலகே ழெனத்திசை பத்தெனத்தான் ஒருவனுமே
பலவாகி நின்றவர் தோணோக்கம் ஆடாமோ [10]
(மாணிக்கவாசர், திருவாசகம், பா.319)

என்ற பாடல் அடிகள் வாயிலாக உணர முடிகிறது.

இயற்கையின் பெண் ஆண் கூறுகள்

இயற்கையின் வடிவாக உள்ள இறைவன் தன்மை வெம்மையெனும் பெண் ஆண்மயமாக இருத்தலை உணர்ந்த ஞானசம்பந்தப் பெருமான் அந்நுட்பத்தை, **தோடுடைய செவியன்** என்று பாட்டுப் படமாக வரைந்து காட்டுகிறார். தோடுடைய செவியன் என்பது இயற்கை இறையின் பெண் ஆண் தன்மையை விளக்கும் எழுத்தோவியமாகும். இவ்வோவியத்தைப் பெருக்கிப் பார்த்தால் அது தன்மை வெம்மையாகப் பொலியும் வாக்குமனங் கடந்த இறைவனது இயற்கை வடிவத்தை உணர்த்துதலைக் காணமுடிகிறது. இவ்வியற்கைப் பெருவடிவை மாணிக்கவாசகர் தன்னடைய முதிர்ந்த நுட்பத்தினால் கனிந்த மனத்தில் எழுந்த ஒவியமொன்றை தருகிறார்.

தோலும் துகிலும் குழையும் சுருள்தோடும்
பால்வெள்ளை நீறும் பசுஞ்சாந்தும் பைங்கிளியும்
சூலமும் தொக்க வளையும் உடைத்தொன்மைக்
கோலமே நோக்கிக் குளிர்ந்துஊதாய் கோத்தும்பி [11]
(மாணிக்கவாசர், திருவாசகம், பா.232)

இயற்கையின் தன்மை வெம்மை என்று சொல்லால் பிரித்துக் கூறலாமேயன்றிப் பொருளால் பிரித்துக்கூற இயலாது. வெம்மையில் தன்மையும், தன்மையில் வெம்மையும் பிரிவின்றிக் கிடக்கின்றன. அவ்விரண்டும் ஒன்றன் கூறுபாடுகள். அப்பிரிவின்மையை உணர்த்தவே **தோடு** என்று பெண்மையையும், **செவியன்** என்று ஆண்மையையும் ஆளுடைய பிள்ளையார் பொறித்திருத்தல் வியப்பினை ஆழ்த்துகின்றது.

உருவ வருணனை

இறைவனின் அழகை எண்ணி எண்ணி இரசித்தவர்கள் இறையடியார்கள். அவர்கள் ஒவ்வொரு அங்கத்தையும் அழகுபட தம் பாடல்களில் பதிவுசெய்துள்ளனர். பெருங்கருணையான் மக்கள் நெஞ்சில் புகுந்த முறையில் ஆண்டவன் அழகு வடிவைப் பாட்டோவியமாகப் பலப்பல வடித்துள்ளனர். இறைவனின் அழகு புத்தியைக் கவர்கின்றன; உள்ளம் கவர்கின்றன; புலன்களைக் கவர்கின்றன என்ற நோக்கில் அப்பர் தில்லை பெருமானின், உருவத்தை அழகுபட வருணிக்கும் காட்சி

குனித்த புருவமும் கொவ்வைச் செவ்
வாயிற் குமிண் சிரிப்பும்
பனித்த சடையும் பவளம் போல்
மேனியில் பால்வெண் ணீறும்
இனித்தம் உடைய எடுத்தபொற்
பாதம் காணப்பெற்றால்
மனித்தப் பிறவியும் வேண்டுவ
தேஇந்த மாநிலத்தே [12]
(அப்பர் தேவாரம், நான்காம் திருமுறை, பா.783)

என்கிறார் அப்பர் பெருமான். இறைவனின் வளைந்த புருவமும், கோவைப் பழம் போன்ற சிவந்த வாயும், இனிய புன்னகையும், குளிர்ச்சியான சடையும், பவளம் போன்ற சிவந்த மேனியும், திருமேனியில் பூசபெற்ற பால் போன்ற வெண்மையான திருநீறும், உயிர்களுக்கு நன்மை சேர்ப்பதற்காகத் தூக்கப்பட்ட திருவடியும் ஆகிய இவற்றைக் கொண்டு புரியும் திருநடனக் காட்சியைக் காண்பதால் இவ்வுலகத்தில் மனித பிறவி எடுத்ததன் பலன்களை அடைந்த மகிழ்வினை அடைவர் என்று அப்பர் பெருமான் தம் பாடல்களின் வாயிலாக எடுத்துரைக்கிறார்.

நிறைவுரை

இறைவனின் பக்தியில் தங்களையும், வாழ்க்கையையும் அர்ப்பணித்தவர்கள் இறையடியார்கள் ஆவர். அவர்கள் இறைவனை முழுமையாக உணரபெற்றவர்கள். அவற்றுள் சமயக்குரவர் நால்வரும் சிவனையும், சிவனின் அருளையும் பெற்று, அவற்றை உலகுக்கு உணர்த்தினர்கள். சிவம் என்னும் சொல் சிவப்பு என்ற பொருளில் உள்ளதையும், மனித வாழ்க்கையில் ஆணும் பெண்ணும் ஒருவரோடு ஒருவர் ஒன்றிணைந்தவர் என்பதை உணர்த்துவதற்கே இறைவன் சிவசக்தி என்ற ஓர் உருவமாகக் காட்சியளிக்கிறார் என்பதையும், இறைவன் இயற்கையாக உள்ள அனைத்து பொருள்களிலும் நிறைந்துள்ளான் என்பதை இக்கட்டுரையின் வாயிலாக எடுத்துரைக்கப்படுகிறது.

பார்வை நூல்கள்

1. நா.வி.கலியாணசுந்தரனார், சைவசமயம், மணிவாசகர் பதிப்பகம், சென்னை.
2. சுவாமிசுகம் (உ.ஆ), பன்னிரு திருமுறைகள் மூலமும் உரையும், சேகர், வித்தமானன் பதிப்பகம், சென்னை.
3. வ.த.இராமசுப்பிரமணியம் (உ.ஆ), பன்னிரு திருமுறைகள் மூலமும் உரையும், [illegible] சுவாமிகள், நான்கு, ஆறு திருமுறை, வித்தமானன் பதிப்பகம், சென்னை.
4. வி.நாராயண், வேலுப்பிள்ளை (உ.ஆ), பன்னிரு திருமுறைகள் மூலமும் [illegible] சுந்தரமூர்த்தி சுவாமிகள், ஏழாம் திருமுறை, வித்தமானன் பதிப்பகம், சென்னை.
5. வ.த.இராமசுப்பிரமணியம் (உ.ஆ), பன்னிரு திருமுறைகள் மூலமும் உரையும், [illegible] சுவாமிகள், முதல் திருமுறை, வித்தமானன் பதிப்பகம், சென்னை.

செம்மொழித் தமிழின் மொழி வளமும் இலக்கிய ஆளுமையும்

(ஆய்வுக் கட்டுரைகள்)

தொகுப்பாசிரியர் :

உடையார்கோயில் குணா

பதிப்பாசிரியர்கள் :

முனைவர் **ச.திருநாவுக்கரசு**

முனைவர் **சீ.செஞ்சுலட்சுமி**

முனைவர் **கோ.பத்மவிலாசினி**

முனைவர் **ச.லட்சுமி**

முனைவர் **அ.ஜெலிந்தாராணி**

முனைவர் **இரா.பத்மா**

செம்மொழி நாள் பன்னாட்டு மாநாடு - 2021 - சென்னை

தமிழ்த்தாய் அறக்கட்டளை வெளியீடு - 39

செம்மொழித் தமிழின் மொழி வளமும் இலக்கிய ஆளுமையும்

செம்மொழி நாள் பன்னாட்டு மாநாடு - 2021 - சென்னை

ISBN 978-81-909877-5-2

உள்ளே...

1. சங்க இலக்கியப் பனுவல்களில் செவ்வியல் கூறுகள்
முனைவர் இரா.பத்மா 1

2. பத்துப்பாட்டில் செவ்வியல் பண்புகள்
பா.மோகன் 7

3. குறுந்தொகை காட்டும் குறிஞ்சித்திணைச் சமூகம்
முனைவர் கி.காயத்ரி 16

4. தொல்காப்பிய உவம உருபுகளும் அதன் வளர்ச்சிப் படிநிலைகளும்
முனைவர் சு.லட்சுமி 29

5. நாலடி நானூற்றில் கல்வி
திருமதி. பொ.விஜயராணி 39

6. செம்மொழித் தமிழ் இலக்கியங்களிலிருந்து தனித்துவமான சிந்தனைகள்
முனைவர் நடா இராஜ்குமார் 46

7. கண்ணதாசன் திரையிசைப்பாடல்களில் தொடை இலக்கணம்
ராஜகுமாரி. ஈ 54

8. பாரி என்னும் பேராளுமை
முனைவர் சீ.செஞ்சுலட்சுமி 65

9. செம்மொழிகளுள் தமிழின் தனிச்சிறப்புகள்
கி.மோனிஷா 74

10. உயர்தனிச் செம்மொழியின் தகுதிப்பாடுகள்
ர.சந்தியா 81

11. இலக்கணம் வழுவா செம்மொழி
முனைவர். இரா.சொர்ணபமிலா 88

12. குமரி மாவட்ட நெய்தல் நில ஆளுமை குறும்பனை சி.பெர்லின்
முனைவர் நே.நிஷாநேவிஸ் 95

13. முத்தமிழறிஞர் கலைஞரின் மொழிப்பற்றும் இலக்கியப் பணியும்
ச.தேவி ரமா 103

14. செவ்வியல் பண்புகளை அடையாளம் காட்டும் நீதிஇலக்கியங்கள்
பா. விஜயலெட்சுமி 111

15. தமிழ் செம்மொழியான வரலாறு
முனைவர் அ.கணேசன் 118

சங்க இலக்கியப் பனுவல்களில் செவ்வியல் கூறுகள்

முனைவர் **இரா.பத்மா**
உதவிப் பேராசிரியர்
தமிழாய்வுத்துறை
தேசியக்கல்லூரி,
திருச்சிராப்பள்ளி-620001.

முன்னுரை :

தமிழ்மொழி ஒரு செம்மொழி என்று கூறிப்பிட்டப்போது இது. உலகறிய, நாடறிய, உள்ளூர் மக்கள் அறிய, உயர்ந்த அதிகாரிகளும் அறிய தமிழ்மொழி இன்று செம்மொழி என வலம் வருகிறது. செம்மொழியாக வாழ்ந்து வந்தபோதிலும், இப்போது தான் நடுவண் அரசினால் "சொம்மொழி" என்று அறிவிக்கப்பட்டுள்ளது. செம்மொழிக்குரிய தகுதிகளையும் பண்புகளையும் பெற்றுவிட்டது. செவ்வியில் தன்மை என்பது தொண்மை, ஒத்திசைவு, தெளிவு தன்னடக்கம், கண்ணியம், இலட்சியம், பொதுமை, பகுத்தறிவு ஒழுங்கு, கண்ணோட்டம் போன்ற உறுப்பினங்களைத் தொகுப்பாகக் கருதப்படுகிறது. சங்க இலக்கியங்களில் செவ்வியல் பார்வையை பற்றி இக்கட்டுரையில் காணலாம்.

செவ்வியல் காலம்

இந்நிலையில் இது தோன்றிய காலத்தையும் சங்க இலக்கியங்கள் தோன்றிய காலத்தையும் இணைத்துத் தமிழ்

இலக்கழிய வரலாற்றின் செவ்வியல் காலம் எனக் கூறலாம். இக்காலம் பகுதியில் தோன்றிய திருக்குறள் சிலப்பதிகாரம் ஆகிய நூல்கள் தோன்றிய காலப்பகுதியையும் இணைத்து இக்காலப் பகுதியை தமிழ் இலக்கிய வரலாற்றின் செவ்வியல் காலம் எனக்கு கூறலாம்.

காலப்பகுப்பு இந்நிலையில் தமிழ் இலக்கிய வரலாற்றுக் காலப்பகுதிகளைப்

1. பழங்காலம்
2. செவ்வியல் காலம்
3. சமய இலக்கிய காலம் (இடைக்காலம்)
4. தற்காலம்

எனப்பிரித்து கூறலாம். இவற்றை ஆராய்ந்த போது செவ்வியல் பண்புகள் ஆசிரியரின் சங்க இலக்கியங்கள் செவ்வியல் இலக்கியங்களே என்ற நூல் செய்திகளின் அடிப்படையில் கூறப்படுபவை.

சிறந்த குறிக்கோள் :

செவ்வியல் இலக்கியங்கள் சிறந்த குறிக்கோள்களுடன் கூடிய நிலையில் செம்மருந்து நிற்கும். பொதுவாக எந்தவொரு கலையாக்கமும் சிறந்த குறிக்கோளுடன் விளங்கவேண்டும் என்பது அரிஸ்டாட்டில் போன்றவர்களின் எண்ணம்.

செவ்வியல் மொழி இலக்கியங்கள் :

உலக அரங்கில் இன்று செம்மொழியாகக் கருதப்படும் மொழிகள் கிரீக், லத்தீன், ஹீப்ரு, சமஸ்கிருதம் அகியமொழிகளே. இந்நிலைகளுடனும், இம்மொழி இலக்கியங்களுடனும், சங்க இலக்கியங்களை ஒப்ப வைத்து ஆராய்வது உண்மையில் உலகரங்கில் (அல்லது) செவ்வியல் மொழி. அதில் தமிழ் இலக்கியத்தின் பெற்றுள்ள செவ்வியல் பண்புகளைப் பற்றியும் இக்கட்டுரையில் காண்போம்.

செவ்வியல் பார்வை :

செவ்வியல் நிலையில், சங்க இலக்கியம் செவ்வியல் இலக்கியமா? செவ்விய இலக்கியத்தின் பண்புகள் என்னென்ன இலக்கணங்கள் என்னென்ன? என்பன போன்ற வினாக்களை எழுப்பி அப்பணர்புகள் சங்க இலக்கியத்தில் காணக்கிடக்கின்றன. அவை எவ்வெவ்வாறு உள்ளன. அவை எவ்வாறு உள்ளன? அப்படி இருந்தும் ஏன் இதுவரையிலும் அந்த வகையில் சங்க இலக்கியத்தில் காணப்படுகிறது.

சங்க இலக்கியங்கள் செவ்வியல் இலக்கியங்களே:

செந்தமிழ், செம்மொழி, செவ்வியல் மொழி இவற்றுள் இரண்டும், பழமை சார்ந்த வழக்குகள், செவ்வியல் மொழி என்பது செம்மை நேர்த்தியான மொழி என்பன. இலக்கியத்தின் பல்வேறு பளர்புகள் நன்கு ஆராயப்படுகின்றன. டாக்டர் ச.அகத்தியலிங்கம் தாம் எழுதி சங்க இலக்கியங்கள் தொடர்பான ஒரு நூலை சங்க இலக்கிய செவ்வியல் இலக்கியங்களே" என்ற தலைப்பில் எழுதியிருக்கிறார். டாக்டர் தமிழண்ணால் எழுதிய நூல் ஒன்றிற்கு தமிழ் செவ்வியல் மொழி என்று பெயரிட்டிருந்தார். நாம் ஒரு புதிய கருத்துருவை பயன்படுத்தும்பொழுது தமிழ் வேர்ச் சொல்லிலிருந்து புதிய சொல்லை உருவாக்குதல், புதிய சொல் வளத்தை உயர்த்துவதாகும். நாமும் செவ்வியல் மொழி" என்று அழைக்கப்படுகிறோம்.

சங்க இலக்கியத்தில் தொன்மை

"இரப்பான் வெகுளாமை வேண்டும்"

தமக்குப் பரிசில் தரதாமதித்த அதியமான் நெடுமான் அஞ்சிடம் ஔவையார் வெகுண்டு எமது இசைக்கருவிகளைத் தோளில் சுமந்தோம். இசைக் கலங்கள் கொள்ளும் பைகளைக் கட்டிக் கொண்டோம். மரத்தை வெட்டி தொழில் செய்யும் தச்சன் மக்கள், மழுவோடு காட்டுக்குள் சென்றால் அவர்கள் வாழ்வில் குறை எதுவும் நேராது அதேபோல எமக்கு

எத்திசைச் செலினும், அத்திசைசோறே (புறம் 206)

என்று கூறிவிட்டுப் புறப்படுகிறார்.

அதியமான் தனக்கு உரிய நேரத்தில் பரிசு கொடுக்காமல் தன்னை மதிக்காது கொடுத்த பரிசிலை ஏற்காது

"யாங்கறிந்தனனோ, தாங்கு அருங்காவலன்
காணாது ஈந்த இப்பொருட்டு யான் ஓர்
வாணிகப் பரிசிலன் அல்லேன், பேணி,
தினை அனைத்து ஆயினும் இனிது (புறம் 208)

தான் ஒரு வணிகப்பரிசிலன் அல்லன் என்ற கம்பீரமாகப் பிரகடனப்படுத்துகிறார்.

பாடுபொருள்கள். சிறந்த ஒரு குறிக்கோள் வாழ்க்கையைச் செம்மையாக உழைப்பதே அகப்பாடல்கள்.

மனித வாழ்வை மனித வாழ்வாக்கும் இலக்கியம்

இந்நிலையில் மனித வாழ்வை மனிதவாழ்வாக ஆக்கும் மகத்தான இலக்கியம் அது. சொல்லும் பொருளிலும் சொல்லும் முறையிலும் தலைசிறந்து விளங்கும் இலக்கியம் சங்க இலக்கியம் என்றால் அது மிகையாகாது.

நிலைபேறு

நிலைபேறு என்பதும் செவ்வியல் இலக்கியத்திற்கு வேண்டிய ஒன்று. சிறந்த இலக்கியமாக ஒரு காலத்தில் விளக்கிக் காலப்போக்கில் வாழ்விழந்து அழிந்துவிடும். எந்த இலக்கியமும் செவ்வியல் இலக்கியமாகக் கருதப்படக்கூடாது. "வாழையடி வாழையாக வாழும் இலக்கியமே" செவ்வியல் இலக்கியமாகக் கருதப்படவேண்டும்.

முடிவுரை :

"சகாவரம் பெற்ற இலக்கியம் சங்க இலக்கியம் "

சங்க இலக்கியம் தமிழ் மக்களால் சாகாவரம் பெற்ற இலக்கியம் என்று கூறப்படுவது (அகத்திய இலக்கியம் 2002) சங்க இலக்கியம் அன்று முதல் இன்று வரை 2300 ஆண்டுகளுக்கும் மேலாகத் தமிழ் மக்களின் இதயத்தில் குடிகொண்டு வருவது. எட்டுத் தொகையுமட், பத்துப்பாட்டும், இன்று தமிழ் அறிஞர்களிடையே சிரோடும், செல்வாக்கோடும் வாழும் இலக்கியங்கள் தமிழ் இலக்கியம் என்றாலே அது சங்க இலக்கியமே என்று எண்ணும் அளவுக்கு தமிழ் அறிஞர்களிடையே அது வாழுகின்றது என்று சொன்னால் மிகையாகாது.

துணை நூல்கள் :

1. அகத்திய லிங்கம் ச., 1968, உலக மொழிகள் பகுதி1, பாரிநிலையம்
2. அகத்திய லிங்கம், 1995, திராவிடமொழிகள் பகுதி1, சென்னை
3. அகத்திய லிங்கம், 1995, ஒருமுகப்போக்கும் கவிதை உருவ ஆக்கமும், உ.த.ஆ. நிறுவனம்
4. அகத்திய லிங்கம், 199,7 சங்க இலக்கியங்கள் செவ்வியல் இலக்கியங்களே, ம.வா.பதிப்பகம், சென்னை.

பத்துப்பாட்டில் செவ்வியல் பண்புகள்

ஆய்வாளர் **பா.மோகன்**

முழுநேர முனைவர் பட்ட ஆய்வாளர்,
தமிழ்த்துறை, நிர்மலா மகளிர் கல்லூரி (தன்னாட்சி),
கோயமுத்தூர் - 641 018

ஆய்வுநோக்கம்

ஓர் இனம் இவ்வுலகில் பல்லாண்டு நிலைத்து நிற்பதற்கும் அதன் நாகரிக வளர்ச்சிக்கும் துணை செய்பவைகளில் மொழி இன்றியமையாத கூறாகும். உலகில் பல மொழிகள் காணப்பட்டாலும் அவைகளில் சில மொழிகளே சிறப்புக்குரியதாக விளங்குகின்றன. அதற்குக் காரணம் அம்மொழிகளில் உள்ள செவ்வியல் இலக்கியங்களே ஆகும். செவ்வியல் இலக்கியங்களுக்கு என்று சில அடிப்படை பண்புகளை அறிஞர்கள் வகுத்துள்ளனர். அவ்வகையில் பத்துப்பாட்டில் காணப்படும் செவ்வியல் பண்புகளை வெளிப்படுத்துவதே இவ்வாய்வின் நோக்கமாகும்.

ஆய்வு நெறிமுறை

இவ்வாய்வில் விளக்கமுறை ஆய்வு, தொகுப்புமுறை ஆய்வு என்ற இரு ஆய்வு நெறிமுறைகள் கையாளப்பட்டுள்ளது.

ஆய்வு எல்லை

சங்க இலக்கிய பத்துப்பாட்டில் உள்ள ஆற்றுப்படை அல்லாத நூல்களான மதுரைக்காஞ்சி, முல்லைபாட்டு,

இலக்கழிய வரலாற்றின் செவ்வியல் காலம் எனக் கூறலாம். இக்காலம் பகுதியில் தோன்றிய திருக்குறள் சிலப்பதிகாரம் ஆகிய நூல்கள் தோன்றிய காலப்பகுதியையும் இணைத்து இக்காலப் பகுதியை தமிழ் இலக்கிய வரலாற்றின் செவ்வியல் காலம் எனக்கு கூறலாம்.

காலப்பகுப்பு இந்நிலையில் தமிழ் இலக்கிய வரலாற்றுக் காலப்பகுதிகளைப்

1. பழங்காலம்
2. செவ்வியல் காலம்
3. சமய இலக்கிய காலம் (இடைக்காலம்)
4. தற்காலம்

எனப்பிரித்து கூறலாம். இவற்றை ஆராய்ந்த போது செவ்வியல் பண்புகள் ஆசிரியரின் சங்க இலக்கியங்கள் செவ்வியல் இலக்கியங்களே என்ற நூல் செய்திகளின் அடிப்படையில் கூறப்படுபவை.

சிறந்த குறிக்கோள் :

செவ்வியல் இலக்கியங்கள் சிறந்த குறிக்கோள்களுடன் கூடிய நிலையில் செம்மருந்து நிற்கும். பொதுவாக எந்தவொரு கலையாக்கமும் சிறந்த குறிக்கோளுடன் விளங்கவேண்டும் என்பது அரிஸ்டாட்டில் போன்றவர்களின் எண்ணம்.

செவ்வியல் மொழி இலக்கியங்கள் :

உலக அரங்கில் இன்று செம்மொழியாகக் கருதப்படும் மொழிகள் கிரீக், லத்தீன், ஹீப்ரு, சமஸ்கிருதம் அகியமொழிகளே. இந்நிலைகளுடனும், இம்மொழி இலக்கியங்களுடனும், சங்க இலக்கியங்களை ஒப்ப வைத்து ஆராய்வது உண்மையில் உலகரங்கில் (அல்லது) செவ்வியல் மொழி. அதில் தமிழ் இலக்கியத்தின் பெற்றுள்ள செவ்வியல் பண்புகளைப் பற்றியும் இக்கட்டுரையில் காண்போம்.

செவ்வியல் பார்வை :

செவ்வியல் நிலையில், சங்க இலக்கியம் செவ்வியல் இலக்கியமா? செவ்விய இலக்கியத்தின் பண்புகள் என்னென்ன இலக்கணங்கள் என்னென்ன? என்பன போன்ற வினாக்களை எழுப்பி அப்பணர்புகள் சங்க இலக்கியத்தில் காணக்கிடக்கின்றன. அவை எவ்வெவ்வாறு உள்ளன. அவை எவ்வாறு உள்ளன? அப்படி இருந்தும் ஏன் இதுவரையிலும் அந்த வகையில் சங்க இலக்கியத்தில் காணப்படுகிறது.

சங்க இலக்கியங்கள் செவ்வியல் இலக்கியங்களே:

செந்தமிழ், செம்மொழி, செவ்வியல் மொழி இவற்றுள் இரண்டும், பழமை சார்ந்த வழக்குகள், செவ்வியல் மொழி என்பது செம்மை நேர்த்தியான மொழி என்பன. இலக்கியத்தின் பல்வேறு பளர்புகள் நன்கு ஆராயப்படுகின்றன. டாக்டர் ச.அகத்தியலிங்கம் தாம் எழுதி சங்க இலக்கியங்கள் தொடர்பான ஒரு நூலை சங்க இலக்கிய செவ்வியல் இலக்கியங்களே" என்ற தலைப்பில் எழுதியிருக்கிறார். டாக்டர் தமிழண்ணால் எழுதிய நூல் ஒன்றிற்கு தமிழ் செவ்வியல் மொழி என்று பெயரிட்டிருந்தார். நாம் ஒரு புதிய கருத்துருவை பயன்படுத்தும்பொழுது தமிழ் வேர்ச் சொல்லிலிருந்து புதிய சொல்லை உருவாக்குதல், புதிய சொல் வளத்தை உயர்த்துவதாகும். நாமும் செவ்வியல் மொழி" என்று அழைக்கப்படுகிறோம்.

சங்க இலக்கியத்தில் தொன்மை

"இரப்பான் வெகுளாமை வேண்டும்"

தமக்குப் பரிசில் தரதாமதித்த அதியமான் நெடுமான் அஞ்சிடம் ஔவையார் வெகுண்டு எமது இசைக்கருவிகளைத் தோளில் சுமந்தோம். இசைக் கலங்கள் கொள்ளும் பைகளைக் கட்டிக் கொண்டோம். மரத்தை வெட்டி தொழில் செய்யும் தச்சன் மக்கள், மழுவோடு காட்டுக்குள் சென்றால் அவர்கள் வாழ்வில் குறை எதுவும் நேராது அதேபோல எமக்கு

எத்திசைச் செலினும், அத்திசைசோறே (புறம் 206)

என்று கூறிவிட்டுப் புறப்படுகிறார்.

அதியமான் தனக்கு உரிய நேரத்தில் பரிசு கொடுக்காமல் தன்னை மதிக்காது கொடுத்த பரிசிலை ஏற்காது

"யாங்கறிந்தனனோ, தாங்கு அருங்காவலன்
காணாது ஈந்த இப்பொருட்டு யான் ஓர்
வாணிகப் பரிசிலன் அல்லேன், பேணி,
தினை அனைத்து ஆயினும் இனிது (புறம் 208)

தான் ஒரு வணிகப்பரிசிலன் அல்லன் என்ற கம்பீரமாகப் பிரகடனப்படுத்துகிறார்.

1068/1134

ISBN :978-81-927107-8-5

- மகாகவி பாரதியார் ஆய்வுக்கோவை
- தமிழ்ப் பல்கலைக்கழக ஓலைச்சுவடித்துறையும் கோவைக் கௌமார மடாலயத் தவத்திரு கஜபூசைச் சுந்தரசுவாமிகள் தமிழாய்வு மையமும் இணைந்து நடத்திய அனைத்துலக மகாகவி பாரதியார் நூற்றாண்டு நினைவு மாநாட்டு வெளியீடு
- திருவள்ளுவராண்டு 2053, ஆனித் திங்கள் 11,12 (25-26.06.2022)
- முதற் பதிப்பு : சூன் 2022
- பக்கங்கள்: 352
- பதிப்பாசிரியர்

  முனைவர் மோ.கோ. கோவைமணி
  தலைவர், ஓலைச்சுவடித்துறை
  தமிழ்ப் பல்கலைக்கழகம், தஞ்சாவூர்.

  சிரவையாதீனப் புலவர் கு. சிவலிங்கம்
  தவத்திரு கஜபூசைச் சுந்தரசுவாமிகள் தமிழாய்வு மையம்
  கௌமார மடாலயம், கோவை.

விலை : ரூ.300/=

நூல் கிடைக்குமிடம்:

சிரவையாதீன முதல்வர்,
கௌமார மடாலயம், சின்னவேடம்பட்டி,
கோயமுத்தூர் - 641 006.

பதிப்பகம் : பாமொழி பதிப்பகம், தஞ்சாவூர்.

அச்சாக்கம் : சௌமி அச்சகம், கோவை.

(கட்டுரைக் கருத்துகட்குக் கட்டுரையாளரே பொறுப்பு)

முனைவர் வி. திருவள்ளுவன்
துணைவேந்தர்

தமிழ்ப் பல்கலைக்கழகம்
தஞ்சாவூர் - 613 010

SE/RRC

அணிந்துரை

செந்தமிழ் நாடெனும் போதினிலே இன்பத்
தேன்வந்து பாயுது காதினிலே எங்கள்
தந்தையர் நாடென்ற பேச்சினிலே ஒரு
சக்தி பிறக்குது முச்சினிலே

என்று உணர்ச்சி மிக்க கவிதைக்குச் சொந்தக்காரர் மகாகவி சுப்பிரமணிய பாரதியார். பாரதியார் என்று சொன்னாலே அவனுடைய நேர்கொண்ட பார்வையும் முறுக்கு மீசையும் முண்டாசும் கோலும்தான் நம் கண்முன் தோற்றமளிக்கின்றன.

நிமிர்ந்த நன்னடை நேர்கொண்ட பார்வையும்
நிலத்தில் யார்க்கும் அஞ்சாத நெறிகளும்

என்று தன்னுடைய கவிதையிலேயே அதற்குச் சான்றுகாட்டி நிற்கின்றார். இதற்கெல்லாம் காரணம் அவன் வாழ்ந்த காலத்தில் இருந்த சமூக நிலைப்பாடுதான். தேவையற்ற சமூக நிலைப்பாட்டை மாற்றுவதற்குப் பாரதி தான் எடுத்துக்கொண்ட ஆயுதம் கவிதை. கவிதையால் காவியம் படைக்கலாம், கவிதையால் காலத்தால் நிலைக்கலாம் என்று காட்டி,

''யாமறிந்த மொழிகளிலே தமிழ்மொழி போல்
இனிதாவது எங்கும் காணோம்!

என்று தமிழின் மேன்மையைப் பரைசாற்றியவர். ஒரு காலத்தில் பெண்கள் படிப்பதற்குத் தடைவிதித்தனர். அதைவிட கொடுமை, புத்தகங்களைத் தொடுவதே பாவம் என்று நினைத்தனர். இவற்றையெல்லாம் தகர்த்தெறிந்து பெண்களை முன்னேற்றப் பாதையில் அழைத்து வந்தவர் பாரதி. சட்டமன்றம், பாராளுமன்றம் போன்ற இடங்களுக்குச் சென்று சட்டங்கள் செய்வதும் நாட்டு நிர்வாகத்தில் சமப்பங்கு வகுத்திடவும் ஆணுக்கு நிகராகப் பெண்கள் செயல்பட முடியும் என்று கனவு கண்டவர். பாரதியின் பாடல்களில் புரட்சியும், விழிப்புணர்வும் பெரும்பாலான பாடல்களில் அமைந்திருக்கின்றன. அதுவும் குறிப்பாகப் பெண்களின் சுதந்திரம், உரிமைகள், கல்வி, அவர்களது வாழ்வின் முன்னேற்றம், அச்சமின்மை விழிப்புணர்வு போன்றவை குறித்த பாரதியின் சிந்தனை புரட்சிகரமானது.

பாரதியார் புதுமைப் பெண்கள் உருவாவதற்குத் துணைநின்றது அரசியலோ. பொருளாதாரமோ, மரபோ, மதமோ அல்ல அவர்களை உருவாக்கியது கல்வி என்று பெண் கல்வியின் முக்கியத்துவத்தைப் பாரதியார் தம் பாடல்களில் பல இடங்களில் சுட்டிச் செல்கின்றார்.

'சாத்திரங்கள் பலப்பல கற்பாராம் சவுகரியங்கள் பலப்பல செய்வாராம் மூத்த பொய்மைகள் யாவும் அழிப்பாராம் மூடக் கட்டுகள் யாவுந் தகர்ப்பாராம் கடவுளர்க் கினிதாகச் சமைப்பாராம் ஏத்தி ஆண்மக்கள் போற்றிட வாழ்வாராம் இளைய நங்கையின் எண்ணங்கள் கேட்டிரோ' என்று புதுமைப் பெண்களின் சாதனைகள் ஆண்கள் அனைவரும்

விரும்பிப் போற்றும் வகையில் சிறந்திருக்கும் என்றவர் பாரதியார். ஆண்களே கல்வி கற்க விரும்பாத காலத்தில் பெண் கல்வி பற்றிப் பாரதியார் பேசியது பெரும் புரட்சியாகக் கருதப்படுகிறது.

பாரதியார் நமது நாட்டு விடுதலைக்காவும், நாட்டு மக்களின் அறியாமையைப் போக்கவும், பெண் விடுதலைக்காகவும் தமிழ் மொழியை நிலைநாட்டவும் தமது கவிதைகளில் பயன்படுத்தப்பட்ட சொல்லாட்சி மிகவும் அருமையான ஒன்று.

பல்முகப் புலவன் பாரதியின் ஆளுமைத் திறத்தால் தமிழுக்கும் நமிமருக்குமான ஏற்றம் சிந்தனைகள் ஓங்கி வளர்ந்ததோடு அவருக்கென்று ஒரு கவிஞர்கள் கூட்டமே உருவானதுதான் பெரும் சிறப்பாகும்.

பாரதி தனது காலத்தில் இருந்த சமூக நிலைமையைப் புரிந்துக்கொண்டு, அதற்கு மாற்றாக நேர் எதிர்த் திசையில் தனது பயணத்தைத் தொடங்கியவர்.

பாரதி காலச் சமூகம் என்பது மனிதர்களிடையே வேற்றுமை நிறைந்ததாக முரண்பாடுகள் நிறைந்ததாக அரசியல், சமூக, பொருளாதார விடுதலை அற்றதாக இருந்ததால் அவற்றைக் களைய பெரும் முயற்சி எடுத்துக்கொண்டவர். தாம் பிறருக்கு அரசியல் அடிமைகளாகவும், பொருளாதார அடிமைகளாகவும், சமூக அடிமைகளாகவும் இருப்பதைக் கூட உணர முடியாத மக்களாய் இருந்த காலம்தான் பாரதியின் வீச்சுக்கு மூல காரணம்.

இத்தகு பாரதியாரின் நூற்றாண்டு நினைவைப் போற்றும் வகையில் அவரது படைப்புகள், எண்ணங்கள், ஆற்றல்கள் குறித்து அனைத்துலக பாரதியார் நினைவு மாநாட்டின் இயக்குநராக இருந்து நடத்தும் தமிழ்ப் பல்கலைக்கழக ஒலைச்சுவடித்துறைத் தலைவர் முனைவர் மோ.கோ. கோவைமணி அவர்கள் இம்மாநாடு நடைபெறுவதற்கு அடித்தளமிட்டு செயல்வீரராக இருக்கும் அவர்களுக்கு என்னுடைய வாழ்த்துக்கள்.

கடந்த இருபத்தைந்து ஆண்டுகளில் கோவை சிரவையாதீனம், கௌமார மடாலய தவத்திரு கஜபூசைச் சுந்தரசுவாமிகள் தமிழாய்வு மையத்துடன் தமிழ்ப் பல்கலைக்கழக ஒலைச்சுவடித்துறை நெருங்கிய தொடர்பு உண்டு. இதுவரை நான்கு மாநாடுகள் மற்றும் கருத்தரங்குகளை நடத்தி வண்ணச்சரபம் தண்டபாணி சுவாமிகள் ஆய்வு மாலை (இரண்டு தொகுதிகள்), முருக இலக்கிய ஆய்வு மாலை (இரண்டு தொகுதிகள்). திருக்குறள் ஆய்வுமாலை, முருக இலக்கிய ஆய்வுக்கோவை (இரண்டு தொகுதிகள்) என வெளியிட்டுள்ளன. இப்போது மகாகவி பாரதியார் ஆய்வுக்கோவை என்னும் இது ஐந்தாவது மாநாடு மற்றும் கருத்தரங்கு வெளியீடாக அமைந்திருப்பது பாராட்டுக்குரிய ஒன்று.

தஞ்சாவூர்

06.06.2022

வி. திருவள்ளுவன்

தவ. குமரகுருபர சுவாமிகள்
சிரவை ஆதீனகர்த்தர்

கௌமார மடாலயம்
கோவை - 641 006

ஆசியுரை

"பைந்தமிழ்த் தேர்ப்பாகன் அவனொரு
செந்தமிழ்த் தேனீ சிந்துக்குத் தந்தை
குவிக்கும் கவிதைக் குயில்இந் நாட்டினைக்
கவிழ்க்கும் பகையைக் கவிழ்க்கும் கவிமுரசு
நீடுதுயில் நீக்கப் பாடி வந்தநிலா"

எனப் பாரதியின் சிறப்பைப் பாரதிதாசன் குறிப்பிடுவார். அத்தகுச் சிறப்பு வாய்ந்த பாரதி முருகனிடம் வேண்டும் பொழுது

"வருவாய் மயில் மீதினிலே
தருவாய் நலமும் தகவும் புகழும்
தவமும் திறமும் தனமும் கனமும்"

என வேலன் பாட்டில் பாடியுள்ளார். முருகப் பெருமானே! மக்களுக்கு உடல்நலம், நற்குணம், புகழ், வல்லமை, செல்வம் ஆகியவற்றை வழங்கியருள்வாயாக! என வேண்டுகிறார்.

"தமிழால், பாரதி தகுதி பெற்றதும்
தமிழ், பாரதியால் தகுதி பெற்றதும்"

என்பதற்கேற்ப தமிழின் மேன்மைக்கு வித்திட்டவர் பாரதியார்.

குருவருளால் சிரவையாதீனமும், தஞ்சை தமிழ்ப் பல்கலைகழகத்தின் ஒலைச்சுவடித்துறையும் இணைந்து இதுவரை நான்கு கருத்தரங்குகளை நடத்தியுள்ளோம்.

1998 சூன் திங்களில் வண்ணச்சரபம் தண்டபாணி சுவாமிகள் ஆய்வுக் கருத்தரங்கம் நடைபெற்றது. அதில் 171 ஆய்வாளர்கள் தங்கள் ஆய்வுக் கட்டுரைகளை வழங்கினார்கள். அவை இரு தொகுதிகளாக வெளியிடப்பட்டன.

2007 திசம்பர் திங்களில் முருகன் இலக்கிய ஆய்வுக் கருத்தரங்கம் நடைபெற்றது. 170 ஆய்வுக் கட்டுரைகள் இரு தொகுதிகளாக வெளியிடப்பட்டன.

2013இல் திருக்குறள் ஆய்வுக் கருத்தரங்கம் நடைபெற்றது. அதில் 144 ஆய்வுக் கட்டுரைகள் ஒரு தொகுதியாக வெளியிடப்பட்டன.

2018இல் நான்காவது அனைத்துலக முருக இலக்கியக் கருத்தரங்கம் நடைபெற்றது. 242 ஆய்வுக் கட்டுரைகள் இரு தொகுதிகளாக வெளியிடப்பட்டன.

தற்போது 25-26 மே 2022இல் அனைத்துலக மகாகவி பாரதியின் நூற்றாண்டு நினைவு மாநாடு மற்றும் கருத்தரங்கம் நடைபெறவுள்ளது. 72 ஆய்வுக் கட்டுரைகளை ஒரே தொகுதியாக இம்மாநாட்டில் வெளியிடப்படவுள்ளன.

தம் கவிதைகள் வழி மக்களுக்கு ஊட்டியதோடு மட்டுமல்லாமல் கவிதைகளைச் சுவையுடன் எதுகை, மோனை, இயைபு என அழகியல் தன்மையுடன் படைத்தவர். விளக்க நடை, மேற்கோள் நடை, வினா நடை, எளிய தொடர் நடை, ஆங்கிலச் சொற்களைக் கையாளுதல், வடமொழிச் சொற்கலப்பு போன்ற பல்வேறு மொழிநடையினைப் பாரதியார் பின்பற்றுவதில் வல்லவர்.

ஆங்கிலேயர்கள் ஒரு நூற்றாண்டுக்கும் மேல் இந்தியப் பெருநாட்டில் நடத்திய கொள்ளை, போர்கள், படுகொலைகள் மற்றும் நெசவுத் தொழில், வர்த்தகம், விவசாயம், நீர்ப்பாசன முறைகள், கால்நடைகள், மனித வளம், கல்வி முதலியவற்றில் ஏற்படுத்திய சேதம் ஆகியவை கொஞ்ச நஞ்சமல்ல. அதன் விளைவாக ஏற்பட்ட பஞ்சம், வறுமை, பசி, பட்டினி, நோய், கல்லாமை, அதனால் ஏற்பட்ட பட்டினிச் சாவுகள், மனித சேதங்கள், கால்நடைச் சேதங்கள் கணக்கிலடங்காது. இந்தச் சூழ்நிலையில் விழிப்புணர்வு ஏற்படுத்துவதற்கு எழுந்த கவிஞனே பாரதி. இந்த உணர்வின் அடிப்படையிலேயே பாரத தேசத்தை அடிமைப்படுத்தி வைத்திருந்த ஆங்கிலேயர் ஆட்சியை எதிர்த்து முழங்கியவர்.

பாரதி தனது காலத்தில் இருந்த சமூக நிலைமையைப் புரிந்துக்கொண்டு, அதற்கு மாற்றாக நேர் எதிர்த் திசையில் தனது பயணத்தைத் தொடங்கியவர். பாரதி காலச் சமூகம், மனிதர்களிடையே வேற்றுமை நிறைந்ததாக முரண்பாடுகள் நிறைந்ததாக அரசியல், சமூக, பொருளாதார விடுதலை அற்றதாக இருந்ததால் அவற்றைக் களைய பெரும் முயற்சி எடுத்துக்கொண்டவர். மகாகவி பாரதியாரால் தமிழுக்கு அறிமுகப்படுத்திய ஐக்கூக் கவிதை வடிவம் இன்று புற்றீசல்போல் பல்கிப் பெருகித் தமிழின் இலக்கிய வளத்தைப் பெருக்க வித்திட்டவர்.

பாரதியார் காலத்தில் பெண்களுக்குக் கல்வி மறுக்கப்பட்டது. பல உரிமைகள் மறுக்கப்பட்டன. பல கொடுமைகள் இழைக்கப்பட்டன. அவற்றைக் கண்ட பாரதிக்கு நெஞ்சம் துடித்தது. நாட்டு விடுதலையைத் தன் பாட்டு வரிகளால் சுட்டிக் காட்டிய பாரதி அதனோடு பெண் விடுதலைக்கும் குரல் கொடுத்தவர். என்நாடு, என் மக்கள் எனக் குறுகிய நோக்கு உடையவராக இல்லாமல் நம்நாடு நம்மக்கள் எனக் கருதி, கடலைப்போல விரிந்த உள்ளம் படைத்தவராக விளங்கிய பாரதிக்கு இவ்வாண்டு நினைவு நூறாண்டு.

பாரதியாரின் நூற்றாண்டு நினைவைப் போற்றும் விதமாக தஞ்சாவூர், தமிழ்ப் பல்கலைக்கழக ஒலைச்சுவடித்துறையும் கோயமுத்தூர் தவத்திரு கஜபூசைச் சுந்தர சுவாமிகள் தமிழாய்வு மையமும் இணைந்து கோவை கௌமார மடாலயத்தில் 25-26.06.2022ஆம் நாள்களில் அனைத்துலக மகாகவி பாரதியார் நினைவு நூற்றாண்டு மாநாடு மற்றும் கருத்தரங்கு நடைபெறுகிறது. இவ்வாய்வுக் கோவையில் எழுபத்திரண்டு கட்டுரைகள் இடம்பெற்றுள்ளன.

இம்மாநாடு சீரும் சிறப்புமாக நடைபெறுவதற்கு அனைத்து உதவிகளையும் நல்கிய தமிழ்ப் பல்கலைக்கழகத் துணைவேந்தர் பேராசிரியர் முனைவர் வி. திருவள்ளுவன் அவர்களுக்கும், இம்மாநாடு கோவை கௌமார மடாலயத்தில் நடப்பதற்கு ஆசீர்வதித்த குருமகா சன்னிதானம் தவகுமரகுருபர சுவாமிகள் அவர்களுக்கும் நன்முறையில் பதிப்பித்த தஞ்சை பாமொழி பதிப்பகத்திற்கும், செம்மையாய் அச்சிட்டு வழங்கிய கோவை சௌமி அச்சகத்திற்கும், இம்மாநாட்டுக்குக் கட்டுரைகள் வழங்கிய பேராளர்கள் அனைவருக்கும் பலப்பல நன்றிகள்.

கோவை
06.06.2022

பதிப்பாசிரியர்கள்

பொருளடக்கம்

	அணிந்துரை	003
	ஆசியுரை	005
	பதிப்புரை	007
1	மகாகவி பாரதியும் மகான் வண்ணச்சரபம் தண்டபாணி சுவாமிகளும் - ஒப்பீடு தவத்திரு முனைவர் குமரகுருபர சுவாமிகள்	013
2.	பாரதி பாடல்களில் சமய உணர்வு சு. அய்யப்பன்	019
3.	பாரதியாரின் குயில் பாட்டில் உவமைகள் முனைவர் நா. அருண்குமார்	022
4.	பாரதியின் கவிதைகளில் சமயப் பொதுமைகள் அ. அன்புநிதி	027
5.	OTHER LANGUAGE INFLUENCE IN BHARATHI'S SONGS Mrs.M.ANNALAKSHMI	030
6.	இந்திய மெய்யியல் கோட்பாடும் பாரதியும் முனைவர் த. ஆதித்தன்	037
7.	பாஷ்யகாரரும் பாரதியும் முனைவர் தி.ந. ஆராவமுதன்	040
8.	பாரதி பார்வையில் ஸ்வர்ணகுமாரி மு. இசக்கியம்மாள்	042
9.	பாரதியார் கவிதைகளில் அழகியல் முனைவர் இரா. இரவிச்சந்திரன்	046
10.	பாரதியார் "சுயசரிதை" ஒரு கண்ணோட்டம் முனைவர் ப. இராசமாணிக்கம்	050
11.	பார் போற்றும் பாரதி முனைவர் இரா. இராஜவினோதா	055
12.	பாரதியும் தத்துவமும் முனைவர் ச. ஈஸ்வரன்	058
13.	பாரதியாரின் படைப்புகளில் சாதி மறுப்பு முனைவர் பெ.இளையாபிள்ளை	062
14.	பாரதி கண்ட பாரத தேசம் முனைவர் ஜெ. உமா	067
15.	பாரதி பாடல்களில் புதிய உவமைகள் கா. உமாபதி	070
16.	பாரதியார் கட்டுரைகளில் தனிமனித வாழ்வியல் முனைவர் த. கண்ணன்	076
17.	புவிமிசை என்றுமிருப்பேன் முனைவர் பி. கரிகாலன்	081

பாரதியும் தத்துவமும்

முனைவர் ச. ஈஸ்வரன்
இணைப்பேராசிரியர் ரு தமிழ்த்துறைத் தலைவர்
தேசியக் கல்லூரி (தன்னாட்சி), திருச்சி

உலகியல் தத்துவமும் வாழ்வியல் தத்துவமும் தமிழ் இலக்கியங்களில் யாண்டும் விரவிக்கிடக்கின்றன. மகாகவி பாரதியாரின் கட்டுரைகளில் தத்துவங்களின் நுட்பமும், அவற்றால் மனிதன் மேம்படலும் குறித்த கருத்துக்குவியல்கள் காணக்கிடைக்கின்றன. இக்கட்டுரையில் 'மகாகவியின் கட்டுரைகளில் காணலாகும் தத்துவச் சிந்தனைகளை'த் தொகுத்தறியலாம்.

நல்லெண்ணம்

வேதாந்தம், சித்தாந்தம், போதாந்தம், நாதாந்தம், யோகாந்தம், கலாந்தம் எனும் (ஷடாந்தங்களே) ஆறும் இறைவனை விளக்குவனவாயும், இறைவனின் வடிவாயும் உள்ளன. அமைதியான தியானம் ஒவ்வொருவரையும் நன்னெறி காட்டி மனதினைப் பக்குவப்படுத்தும். மனதினுள் வேண்டாத தீய எண்ணங்களை இட்டு நிரப்பாது நற்சிந்தனைகளைக் கொண்டு நின்றால் ஏற்றம் பெறலாம். இதனைப் பின்வருமாறு பாரதியார் தெளிவித்துள்ளார். 'அ.'.தெப்படி என்றால் நமது உள்ளத்திலே தீரத்(தைரியம்) தன்மை, அமைதி, பலம், தேஜஸ், சக்தி, அருள், பக்தி, சிரத்தை போன்ற இந்த எண்ணங்களையே நிரப்ப வேண்டும். இவற்றையெல்லாம் நான் எனது உடைமையாக்கிக் கொள்வேன். இவற்றுக்கு எதிர்மறையான சிந்தனைகள் எனது அறிவினுள்ளே நுழைய இடங்கொடுக்க மாட்டேன் என்று ஒவ்வொருவரும் உறுதி செய்துகொள்ள வேண்டும்'(பாரதியார் கட்டுரைகள், ப.6) என்பதன் வழி மனதில் நல்ல எண்ணத்தை ஏற்றி வாழ்ந்தால் இன்பமே எந்நாளும் என்பதைத் தெளியலாம்.

மூட பக்தி

"மனம் இறை நம்பிக்கையுடன் இருப்பின் நேர் பாதையில் செல்லும் மூடபக்தி கூடாது என்பது பாரதியாரின் எண்ணம். அதனைப் பின்வருமாறு தெளிவுபடுத்தியுள்ளார். பக்தி உடையார் காரியத்தில் பதறார் மிகுந்த பொறுமையுடன் வித்து முளைக்கும் தன்மைபோல், மெல்லச்செய்து பயனடைவார்" (பாரதியார் கவிதைகள், ப.99) என்கிறார் பாரதியார்.

"இந்த மூட பக்திகளிலே மிகவும் தொன்மையான அம்சம் யாதெனில், எல்லாச் செய்கைகளுக்கும் நாள், நட்சத்திரம், வர்ணம் முதலியன பார்த்தல்" (பாரதியார் கட்டுரைகள், ப.8) என்று உரைப்பதன் மூலம் மூட பக்தி எண்ணத்தினால் சிற்சில சமயங்களில் காரியம் செய்யாது ஒத்திப் போடுகிறோம் என்கிறார் பாரதியார். இத்தகைய மூட பக்திகளெல்லாம் ஒழிக்கப்பட வேண்டும் என விரும்புகிறார் பாரதியார்.

சக்தியின் அருள்

சிவனுக்கு ஒரு ராத்திரி அது சிவராத்திரி. அவன் தேவி சக்திக்கு நவராத்திரி (ஒன்பது). இந்த நவராத்திரி நாட்களில் சக்தியை நம்பிக்கையுடன் வழிபட்டால் உயர்வு பெறலாம் என்பது பாரதியின் கருத்து.

"ஒன்பது நாளும் தியான்ம், கல்வி, தவம் இவற்றிலே செலவிடத் தவறியில்லாதோர் கடைசி ஒன்பதாம் நாள் மாத்திரமே விரதம் காக்க வேண்டும், இந்த வார்த்தையின் பொருள் மிகவும் தெளிந்தது. சக்தியால் உலகம் வாழ்கிறது நாம் வாழ்வை விரும்புகிறோம் ஆதலால் நாம் சக்தியை வேண்டுகிறோம்" (பாரதியார் கட்டுரைகள், ப.23) என்று 'நவராத்திரி' எனும் தலைப்பிலான கட்டுரையில் புலப்படுத்தியுள்ளார்.

"தமிழா, தெய்வத்தை நம்பு. பயப்படாதே. உனக்கு நல்ல காலம் வருகின்றது" (பாரதியார் கட்டுரைகள், ப.133) என்று, 'தமிழுக்கு' எனும் தலைப்பின் கீழ் எடுத்துரைத்துள்ளார் பாரதியார்.

நம்பிக்கை

'நம்பிக்கை வை நலம் பெறுவாய்' என்பது ஆன்றோர் வாக்கு. "நம்பினார் கெடுவதில்லை நான்குமறை தீர்ப்பு" (பாரதியார் கவிதைகள், ப.151) என்பது பாரதியின் வாக்கு. நம்பிக்கையுடன் இருப்பின் நினைத்த காரியம் கைகூடும். தன்னம்பிக்கைதான் ஒவ்வொருவருக்கும் ஏற்றம் பெற வழிகாட்டும். இதனைப் பின்வருமாறு எடுத்துரைத்துள்ளார் பாரதியார்.

"நம்பிக்கை உண்டானால் வெற்றி உண்டு. அந்த நம்பிக்கையின் முக்கிய லக்ஷணம் என்னவென்றால் விடாமுயற்சி. மனத்திற்குள் நிலைத்த நம்பிக்கை இருந்தால் செய்கை தடைப்படுமா? முயற்சி தூங்குமா? இடுக்கண் பயமுறுத்துமா? உள்ளம் சோருமா? ராமானுஜர் சோழ நாட்டிலிருந்து போகும் காலத்தில் தம் பின்னே தம்மைப் பிடித்துச் சொல்லும் பொருட்டு, குதிரைப்படை வருகிறது என்று கேள்விப்பட்டபொழுது, தம்முடன் வந்த சிஷ்யர்களிடம் சொல்லிய நல்ல மந்திரம் : 'கொடுமை செய்யும் கூற்றமும் என் தோலாடி குறுகப்பெறா - எனது கோல் ஆடும் பொழுது எமனும் கிட்ட வரமாட்டான்' என்று சொன்னார். அதற்குப் பெயர்தான் நம்பிக்கை" (பாரதியார் கட்டுரைகள், ப.31) என்று, 'காமதேனு' எனும் தலைப்பிலான கட்டுரையினுள் சத்ரபதி சிவாஜி, திருநாவுக்கரசர் (அப்பர்) போன்றோரின் வாழ்வியலைச் சான்றுகள் தந்து நம்பிக்கையின் சிறப்பினை உரைத்துள்ளார்.

மானிட விரதம் (அன்பும் பண்பும்)

ஒவ்வொருவர் வாழ்விலும் பற்பல ஏற்றம், இறக்கம், பயம், துன்பம், கொடுமை, சிக்கல் என்பவை ஏற்படுவதுண்டு. இவைகளிலிருந்து விடுபட்டு வாழ்வதற்குத் தேவையானவற்றைப் பாரதியார் பின்வருமாறு எடுத்துரைத்துள்ளார்.

"அடா, மனிதர்களே, எத்தனை சாத்திரங்களுக்கும், கட்டுப்பாடுகளுக்கும், நம்மனிதர் உட்பட்டிருக்க வில்லையா? ஒரு புதுக்கட்டுப்பாடு செய்து கொள்வோமே. அந்தக் கட்டுப்பாடு யாதெனில்: ஒருவருக்கொருவர் மனத்தாலும் தீங்கு நினைப்பதில்லை. ஒருவருக்கொருவர் பயப்படல் இல்லை. மானிடரே இந்த விரதம் எடுத்துக் கொள்ளுங்கள். இது பிழைக்கும் வழி'. (பாரதியார் கட்டுரைகள், ப.59) என்று, 'ஜன வகுப்பு' எனும் தலைப்பிலான கட்டுரையினுள் அன்பு தான் அனைவருக்கும் வேண்டும் என்பதைத் தெளிவுபடுத்தியுள்ளார் பாரதியார். ஏசு கிறிஸ்துவின் வார்த்தை, இஸ்லாம் மார்க்கத்தின் மகிமை போன்ற தலைப்புகளிலான கட்டுரைகளில் அன்பின் மாண்பினைப் போற்றியுள்ளார் மகாகவி. 'தெய்வமே துணை' என்று இருப்போர் ஓயாமல் தொழில் செய்து கொண்டிருப்பார்கள். தெய்வ பக்தி உண்மையானால் பரோபகாரம் அங்கே இருக்கும். பரோபகாரம் இல்லாத இடத்தில், தெய்வ பக்தி வேஷத்தைத் தவிர வேறொன்றுமில்லை"

(பாரதியார் கட்டுரைகள், ப.84) என்றுரைத்துள்ளார் பாரதியார். அன்பு இருப்பின் பிறருக்கு உதவி செய்யும் நல் எண்ணம் மனதினுள் உதிக்கும். இறைவன் தேடி வந்து அருள்புரிவான் என்பன போன்ற தத்துவச் சிந்தனைகளைப் பாரதியாரின் கட்டுரைகள் புலப்படுத்துகின்றன. "பாப புண்ணியங்களுக்கு இணங்க மானிடரின் கர்மத்தினுடைய பலனாக அடுத்த ஜென்மத்தில் உயர்ந்த பிறப்பேனும் தாழ்ந்த பிறப்பேனும் கிடைக்கும் என்பது நமது தேசத்துப் பொது நம்பிக்கை. பாவம் செய்யும் ஒருவனை, 'நீ அடுத்த ஜென்மம் மிருகமாய் பிறப்பாய்' என்றால் அவனுடைய மனம் பதைக்கிறது" (பாரதியார் கட்டுரைகள், ப.113) என்று 'புனர்ஜென்மம்' என்ற கட்டுரையுள் குறிப்பிட்டுள்ளார் பாரதியார்.

இல்லற வாழ்வு சிறப்புற்றுத் திகழ ஆணும் பெண்ணும் ஒற்றுமையுடனும் அன்புடனும் இருத்தல் வேண்டும். இதனை, "ஸ்திரீகள் புருஷரிடம் அன்புடன் இருக்க வேண்டினால் புருஷர் ஸ்திரீகளிடம் அசையாத பக்தி செலுத்த வேண்டும். பக்தியே பக்தியை விளைவிக்கும்" (பாரதியார் கட்டுரைகள், ப.245) என்கிறார் பாரதியார். 'பெண் விடுதலை' (பெண் விடுதலைக்கு - தமிழ்ப்பெண்கள் செய்யத்தக்கது யாது? 'தமிழ்நாட்டு மாதருக்கு' என்பன) குறித்தும் பல கட்டுரைகளை எழுதியுள்ளார் பாரதியார்.

புராணமும் வாழ்வியலும்

இராமாயணம், மகாபாரதம் போன்ற இதிகாசக் காப்பியங்களைப் படித்தும், கேட்டும் அதன் வழி நடத்தல் சிறப்பானதாம். புராணங்கள் உணர்த்தும் வாழ்வியல் விழுமியங்களான அறம் வெல்லும் பாவம் தோற்கும் தருமமே வெல்லும் பிறன்மனை நயவாமை, சகோதர பாசம், பெரியோர் சொல் கேட்டல், சரணாகதி, அனைவரையும் ஒன்றாகப் பாவித்தல் என்பன போன்றவற்றை ஒவ்வொருவரும் அறிதலும் அவைகளின் வழி ஒழுகுதலும் நன்மையே பயக்கும். இதனையே பாரதியார்,

"இதிகாச புராணங்களில் உள்ள, வீரர் ஞானிகள் முதலியோரின் குணங்களை நாம் பின்பற்றி நடக்க முயல வேண்டும், உண்மை, நேர்மை, வீர்யம், பக்தி முதலிய வேதரிஷிகளின் குணங்களையும் ஸ்வதேச பக்தி, ஸ்வஜனாபிமானம், சர்வ ஜீவதயை முதலிய புராதன வீரர்களின் குணங்களையும் பிள்ளைகளுக்கு நன்றாக உணர்த்த வேண்டும். சிபிச் சக்கரவர்த்தி புறாவைக் காப்பாற்றும் பொருட்டாக, தன் சதையை அறுத்துக் கொடுத்த கதை முதலியவற்றின் உண்மைப் பொருளை விளக்கிக் காட்டி மாணாக்கர்களுக்கு ஜீவகாருண்ணியமே எல்லா தருமங்களிலும் மேலானது என்பதை விளக்க வேண்டும். ஏழைகளுக்கு உதவி புரிதல், கீழ் ஜாதியாரை உயர்த்தி விடுதல் முதலியனவே ஜன சமூகக் கடமைகளில் மேம்பட்டன என்பதைக் கற்பிக்க வேண்டும்" (பாரதியார் கட்டுரைகள், ப.358) என்ற பாரதியாரின் வாக்கின்படி, நல்லன படித்தல், படிக்க வைத்தல், அதன் வழி நடத்தல், பிறருக்கு உதவிகள் பல புரிதல், உயர்த்தி விடுதல் என்பன போன்ற வாழ்வியல் அறங்களை மனதினுள் நிலை நிறுத்துதல் சாலச் சிறந்ததாம்.

குயில் பாட்டும் தத்துவமும்

பாரதியாரின் கட்டுரைகளில் தத்துவச் சிந்தனைகள் பொதிந்து கிடப்பதைப் போல, 'குயில்பாட்டு' வேதாந்தமாகப் புனையப்பெற்றுள்ளது. தத்துவம் என்பது வாழ்வோடு பிண்ணி சரியாகச் சிந்திப்பதற்கு மட்டுமன்று முறையாக வாழவும் வழிகாட்டுகிறது. (பதி என்னும் பரமாத்மாவைப் பசு என்னும் ஜீவாத்மா அடைய பாசம் என்னும் புற உலக மாயைகள் தடைக்கற்களாக உள்ளன. 'குயில் பாட்டில்' சேர இளவலும், கவிஞரும்

[illegible]கவும், சின்ன குயிலியும், குயிலும் பசுவாக்கும், பாச சக்திகளாக மாடன், நெட்டைக் [illegible]ன் வல்லாம் அமைகின்றன. இறைவனை அடையும்போது உலக மாயை தடையாக [illegible]னாலும், ஆணவம், கன்மம், மாயை என்ற மூன்றையும் ஆன்மா கடக்க என்ன [illegible]கிறது என்பதையும் மறைபொருளாகச் சுட்டியுள்ளார் பாரதியார். உண்மையான [illegible]புக்கு வந்தத் தடை வந்தாலும் அதைக் கடக்கும் சக்தி அதற்கு உண்டு என்று [illegible]யிலுவர்-குயிலி-சேரன் பாத்திரம் மூலம் உணரலாம். மறுபிறப்புக் குறித்தும் பாரதியார் [illegible]பாட்டில் புனைந்துள்ளார்.

முடிவுரை

நிலையில்லாத இந்த வாழ்வில் நிலைத்த புகழ் அடைய தத்துவச் சிந்தனைகள் [illegible]காட்டுகின்றன. பாரதியாரின் கட்டுரைகளிலும் குயில்பாட்டிலும் தத்துவக் கருத்துக்கள் [illegible]ள்ளன. இத்தத்துவ போதனைகளை மனதில் நிலைநிறுத்தி வாழ்ந்தால் ஆனந்தமே [illegible]ளும் கிட்டும் என்பது உறுதி.

துணைநின்ற நூல்கள்

1. பாரதியார் கவிதைகள், அருணா பப்ளிகேஷன்ஸ், சென்னை-49, முன்றாம் பதிப்பு, மே-2011.
2. மகாகவி பாரதியார் கட்டுரைகள், வானதி பதிப்பகம், தி.நகர், சென்னை-17, முதற்பதிப்பு, டிசம்பர் 1981.

பாரதியார் கவிதைகளில் அழகியல்

முனைவர் இரா. இரவிச்சந்திரன்,
இணைப்பேராசிரியர், தமிழ்த்துறை,
தேசியக்கல்லூரி (தன்னாட்சி), திருச்சி – 620 001

பத்தொன்பது, இருபதாம் நூற்றாண்டுகளில் வாழ்ந்த விடுதலைக் கவிஞர்களில் குறிப்பிடத்தக்கவர் மகாகவி சுப்ரமணிய பாரதியார் ஆவார். இவர் எட்டயபுரத்தில் சின்னச்சாமிக்கும், இலக்குமி அம்மையாருக்கும் மகனாகப் பிறந்தார். இவருடைய தாத்தா ராமசாமியிடம் தமிழைக் கற்று இளம் வயதிலேயே கவிபாடும் திறம் பெற்றார். பாரதி என்ற பட்டத்தையும் பெற்றார். முப்பத்தொன்பது ஆண்டுகளே வாழ்ந்த இவர் புதுக்கவிதையின் முன்னோடியாகவும் மரபுக்கவிதைப் பாடுவதில் வல்லவராகவும் திகழ்ந்துள்ளார் என்பதற்கு அவருடைய கவிதைகளே சான்றுகளாகின்றன.

எட்டயபுரத்தில் தமிழ்ப் புலவராக இருந்த காந்திமதி நாதப்பிள்ளை பாரதியைச் சோதிக்க "பாரதி சின்னப் பயல்" என்னும் ஈற்றடியைக் கொடுத்து வெண்பா இயற்றச் சொன்னார். ஒரு சில நிமிடங்களே சிந்தித்த பாரதி,

"ஆண்டி விளையவ னென்றைய அருமையினால்
ஈண்டின்றென் றன்னைநீ யேந்தினையால் - மாண்புற்ற
காரதுபோ லுள்ளத்தான் காந்திமதி நாதற்குப்
பாரதி சின்னப் பயல்"

என்று நேரிசை வெண்பா பாடி அங்கிருந்த அனைவரையும் வியக்கச் செய்தார். இந்த யாப்பிற்குள் காந்திமதி நாதனின் சிறுமை எண்ணம், அதை அவர் பக்கமே பாரதி திருப்பிவிட்ட திறம், நிகழ்வுச் சூழல், பாரதி இளம்பருவம் ஆகியன நுட்பமாக உணர்த்தப்படுகின்றன. வெண்பா கட்டுமானத்தின் புற அழகியலோடு உள்ளடக்கம் சார்ந்த உணர்த்தும் அழகியல் சிறப்புடையதாக இருக்கிறது. வெண்பா மட்டுமல்ல கவிதை அழகியலான எதுகை, மோனை, இயைபு எனத் தம் கவிதைகளில் இயற்றிப் பாட்டுத் திறத்தாலே இந்த வையகத்தை பாலித்தார் பாரதி. அழகியல் என்பது அழகின் தன்மையை ஆராய்வதும் கலைப்படைப்புகளில் அழகை இனம் கண்டு இரசிப்பதும் சுவையுடன் படைப்புகளைப் படைப்பதும் பற்றிய இயலாகும். (தமிழ் விக்கிபீடியா). மேற்கண்ட அழகியல் தன்மை அனைத்தும் பாரதியார் பாடல்களில் மிகுந்து காணப்படுகின்றன.

பாரதியார் விடுதலை வேட்கையைத் தம் கவிதைகள் வழி மக்களுக்கு ஊட்டியதோடு மட்டுமல்லாமல் கவிதைகளைச் சுவையுடன் எதுகை, மோனை, இயைபு என அழகியல் தன்மையுடன் படைத்துள்ளார். இந்தத் தொடை நயங்கள் செவிகளுக்கு இன்பம் அளிப்பதோடு அந்நயங்கள் சார்ந்த சொற்களின் பொருண்மை இதயத்தில் ஊடுருவி உணர்வில் கலந்து அறிவைத் தூண்டி செயல்பட வைக்கும் வல்லமையைக் கொண்டிருக்கின்றன. ஓரடிக்கும், மற்றோர் அடிக்கும் உள்ள பொருத்தத்தை விளக்குவது முதற்தொடை அல்லது தலைமைத் தொடையாகும். இவ்வாறன்றி ஓர் அடியின்கண்

அமைந்த சீர்களில் காணும் பொருத்தத்தைத் தொடை விகற்பம் என்று கூறுவர். இவ்விருவகைத் தொடைகளும் செய்யுளில் சிறப்பிக்கும் ஓசை நயத்திற்கும் அடிப்படையாய் விளங்குகின்றன. இக்கருத்துப் பற்றியே,

"தொடையற்ற பாட்டு நடையற்றுப் போகும்" (யாப்பருங்கலக்காரிகை, ப.562)

என்னும் பழமொழி அமைந்துள்ளது. ஓசை நயத்துடனும், பொருட் சுவையுடனும் கவிதைகளைப் படைப்பதில் பாரதிக்கு நிகர் பாரதியே என்பதை அவர்தம் கவிதைகள் வழி அறியலாம்.

அடிதோறும் முதல் எழுத்து ஒன்றிவரத் தொடுப்பது மோனை. இரண்டாம் எழுத்து ஒன்றிவரத் தொடுப்பது எதுகை. இறுதி எழுத்து அல்லது சொல் ஒன்றிவரத் தொடுப்பது இயையு ஆகும். இதனையே யாப்பருங்கலக்காரிகை,

"வழுவா வெழுத்தொன்றின் மாதே யெதுகை
எழுவா யெழுத்தொன்றின் மோனை யிறுதி யியையிரண்டாம்"
(யாப்பருங்கலக்காரிகை, நூற்.16)

என்ற நூற்பா விளக்குகிறது. பாரதியார் முதல் எழுத்து ஒன்றி வருமாறு பல கவிதைகள் படைத்துள்ளார். சான்றாக,

"காக்கைச் சிறகினிலே நந்தலாலா! நின்றன்
கரியநிறம் தோன்றுதையே நந்தலாலா!
பார்க்கும் மரங்களெல்லாம் நந்தலாலா! நின்றன்
பச்சை நிறம் தோன்றுதையே நந்தலாலா!
கேட்கும் மொலியிலெல்லாம் நந்தலாலா! – நின்றன்
கீத மிசைக்குதடா நந்தலாலா!
தீக்குள் விரலை வைத்தால் நந்தலாலா! நின்னைத்
தீண்டும் இன்பம் தோன்றுதடா நந்தலாலா!
(பாரதியார் கவிதைகள், பக்.214-215)

என்று பாடுகிறார். மேற்கண்ட கவிதையில் முதல் எழுத்து ஒன்றி வந்திருப்பதைக் காணமுடிகிறது. மேலும்,

"ஆடுவோமே – பள்ளுப் பாவோமே;
ஆனந்த சுதந்திரம் அடைந்துவிட்டோமென்று.
உழவுக்கும் தொழிலுக்கும் வந்தனை செய்வோம் - வீணில்
உண்டுகளித் திருப்போரை நிந்தனை செய்வோம்.
விழலுக்கு நீர் பாய்ச்சி மாயமாட்டோம் - வெறும்
வீணருக்கு உழைத்துடலம் ஓயமாட்டோம்".
(பாரதியார் கவிதைகள், பக்.48-49)

என்று விடுதலை இயக்கப் பாடலைப் பாடியுள்ளார். சுதந்திரம் அடைவதற்கு முன்பே சுதந்திரம் அடைந்துவிட்டோம் என்று பாடிய கவிஞர் மகாகவி சுப்பிரமணிய பாரதியார் ஆவார். நம் உழைப்பு வெறும் புல்லுக்குப் பாய்ச்சிய நீர் போல் இருத்தல் கூடாது என்பதைச் சுட்டிக்காட்டியுள்ளார். இவ்வாறு முதல் எழுத்து ஒன்றிவரப் பாடியது மட்டுமல்லாமல் இரண்டாம் எழுத்து ஒன்றி வருவது போலவும் கவிதை பாடியுள்ளார்.

"உலகத்தில் உண்மைபொளி யுண்டாயின்
வாக்கினிலே ஒளியுண் டாகும்
வெள்ளத்தின் பெருக்கைப்போல் கலைப்பெருக்கும்
கவிப்பெருக்கும் மேவு மாயின்
பள்ளத்தில் வீற்றிருக்கும் குருடரெல்லாம்
விழிப்பெற்றுப் பதவி கொள்வார்
தெள்ளுற்ற தமிழமுதின் சுவைகண்டார்
இங்கமரர் சிறப்புக் கண்டார்" (பாரதியார் கவிதைகள், ப.432)

மேற்கண்ட அறுசீர் விருத்தப்பாவில் அடிதோறும் இரண்டாம் எழுத்து(ள்) ஒன்றி வந்திருப்பதைக் காணலாம். இதனையே இலக்கணத்தில் 'எதுகை' என்று குறிப்பிடுவர். பாரதியார் கவிதைகளில் பெரும்பான்மையான கவிதைகள் எதுகையே வந்துள்ளதைக் காணமுடிகிறது. கவிதையின் பொருண்மை மாறாமலும், யாப்பிலக்கண அமைப்பிலும் கவிதை வடித்திருப்பது பாரதியின் கவித்துவத்தையும், இலக்கண நுண்மையையும் காட்டுகிறது.

சேரநன் னாட்டிளம் பெண்க ளுடனே
சுந்தரத் தெலுங்கினிற் பாட்டி சைத்துத்
தோணிக ளோட்டிவிளை யாடி வருவோம்
கங்கை நதிப்புறத்துக் கோதுமைப் பண்டம்
காவிரி வெற்றிலைக்கு மாறு கொள்ளுவோம்
சிங்க மாராட்டியர்தம் கவிதை கொண்டு
சேரத்துத் தந்தங்கள் பரிச ளிப்போம்" (பாரதியார் கவிதைகள், ப.54)

என்னும் பாடலடிகளில் இரண்டாம் எழுத்து ஒன்றி வந்திருப்பதைக் காணமுடிகிறது. எத்தகைய பாடல்கள் இயற்றினும் ஓசையும் நயமும் மாறாமல் யாப்பின் அமைப்பை ஒட்டியே பாடியுள்ளார் பாரதியார். விடுதலை வேட்கையைத் தம் பாடல்கள் வழி வெளிப்படுத்தியுள்ளார்.

செந்தமிழ் நாடெனும் போதினிலே - இன்பத்
தேன் வந்து பாயுது காதினிலே – எங்கள்
தந்தையர் நாடென்ற பேச்சினிலே ஒரு
சக்தி பிறக்குது மூச்சினிலே (பாரதியார் கவிதைகள், ப.430)

என்ற பாடலில் எதுகையும் இயைபும் இணைந்து வந்துள்ளதைக் காணலாம். ஒரே பாடலில் இரு இலக்கண அமைப்புகள் கொண்டு பாடும் திறம் பெற்றவர் பாரதியார் என்பதை மேற்கண்ட பாடல் சான்றாகிறது. இயைபு என்பது இறுதியில் ஒன்றி வருவதாகும். மேற்கண்ட பாடலில் இறுதி எழுத்து ஒன்றி வந்திருக்கிறது. இதே போல,

"எண்ணி முடிதல் வேண்டும்
நல்லவே எண்ணல் வேண்டும்
திண்ணிய நெஞ்சம் வேண்டும்
தெளிந்த நல்லறிவு வேண்டும்"

என்ற பாடலில் "வேண்டும்" என்ற சொல் இயைபாக வந்திருப்பதைக் காணலாம். அரசியல், தெய்வம், தமிழ்மொழி, காதல், சமூகம் எனப் பல்பொருண்மையில் பாடியுள்ள பாரதியார் கவிதைகளில் அழகியல் கூறுகள் பொருண்மை சார்ந்து இயல்பாக அமைந்து

நிற்பதைப் பார்க்கிறோம். கவி உள்ளமும், மொழித்திறனும், உயிரிய கொள்கைகளும் கொண்ட பாரதியின் கைகளில் பொருளும், உணர்வும், உணர்ச்சியும், தேர்ந்த சொற்களும், அழகியல் கூறுகளும் கூடி முயங்கிப் பேரூற்றாகப் பொங்குகின்றன. பாரதியின் கவிதைகளின் அழகுக் கூறுகள் கவிதைகளின் உதிரிக் கூறுகளாக இல்லாமல் வாசிப்போர் மனங்களிலும், அறிவிலும், பொருண்மைகளைக் கடத்தும் கருவிகளாகவும் ஏற்க வைக்கும் நயக் கூறுகளாகவும் அமைந்துள்ளதைச் சிறப்பாகக் குறிப்பிட வேண்டும்.

'இலக்கை' இயம்புவது இலக்கியம் என்பர். சங்கப் புலவர்களும், கவிஞர்களும் தம் இலக்கைக் கவிதை, புனைகதை, உரைநடை என ஏதோ ஒரு வடிவத்தில் சமூகத்திற்கு வெளிப்படுத்தியுள்ளனர். சங்கப் பாடல்கள் அனைத்தும் ஆசிரியப்பா, கலிப்பா என்ற இலக்கண வடிவத்தைப் பெற்று வந்துள்ளன. பின்னர் வந்த இலக்கியங்கள் குறள்வெண்பா, வெண்பா போன்ற வடிவத்தில் அமைந்துள்ளன. அவ்வகையில் பாரதியாரும் தம் கவிதைகளில் எதுகை, மோனை, இயைபு போன்ற இலக்கணத்தை அழகியல் நுண்மையோடு படைத்துள்ளார் என்பதை அறியமுடிகிறது. "எமக்குத் தொழில் கவிதை நாட்டிற்கு உழைத்தல் இமைபொழுதும் சோராதிருத்தல்" என்பதைச் சொன்னதோடு மட்டுமல்லாமல் நிருபித்துக் காட்டியவர் மகாகவி பாரதியார் ஆவர்.

பார்வை நூல்கள்

1. பாரதியார் கவிதைகள், பாவை பப்ளிகேஷன்ஸ், சென்னை – 14.
2. யாப்பருங்கலக்காரிகை, முனைவர் ச. திருஞானசம்பந்தம்(உ.ஆ), கதிர் பதிப்பகம், திருவையாறு.

பாரதியார் "சுயசரிதை" ஒரு கண்ணோட்டம்

முனைவர் ப. இராசமாணிக்கம்
உதவிப் பேராசிரியர், தமிழ்த்துறை
மன்னர் சரபோஜி அரசு கல்லூரி (தன்னாட்சி)
தஞ்சாவூர் - 613 005.

அருளாளர்கள், ஞானிகள், கவிஞர்கள், சான்றோர்கள் இன்னபிற பெரியோர்கள் உலகில் தோன்றுவதும், மறைவதும் காலச் சக்கரத்தின் நியதி. அவர்கள் அருளிச் செய்த விட்டுச் சென்ற எச்சங்களும், தடயங்களும் காலத்தால் அழியாத பிரம்ம சூத்திரங்களாகப் புவியில் ஆட்சி செய்து வருகின்றன என்பது மறுக்க முடியாத உண்மை. அவைகள் சுத்தமான, மாசுபடாத, கலங்காத, பிழைபடாத மெய்ஞானமாக உலகில் பவனி வருகின்றன. அப்படிப்பட்ட ஞானத்திற்குச் சொந்தமான உடலும் உயிரும், உள்ளமும் அறிவும் தம்முள் ஒருங்கிணைந்து ஒரு மகா சக்தியாக, தமிழ் நாட்டின் விடிவெள்ளியாகத் தோன்றிய மகாகவி சுப்பிரமணிய பாரதியாரின் நவ கவிதைகளின் உள்ளே ஊடுருவிச் சென்று அவரின் கவி உள்ளக் கருத்தை ஆராய முற்படுவதே இக்கட்டுரையின் நோக்கமாகும். பாரதி ஒரு கவிஞன் மட்டுமல்ல, ஒரு யோகி என்பதைப் பல பாடல்கள் மூலம் நிருபித்துள்ளார். மனதை அடக்கி யாள்பவனே ஞானி; மனம் செல்லக்கூடிய வழியில் செல்லாமல் மனதைக் கட்டுப்படுத்தி மனதிற்குத் தலைவன் ஆகும் முறையை,

பேயாய் உழலும் சிறுமனமே
பேணாய் என்சொல் இன்றுமுதல்
நீயாய் ஒன்றும் நாடாதே
நினதுதலைவன் யானே காண் (வே.பா.22, மணப்பெண்)

என்று அருமையாகப் பாடுகின்றார். அலையும் மனதை அமைதிப்படுத்தும் வழியைக் கூறுகின்றார்.

பாரதியின் கவிதைகள் என்ன காற்றில் மறையக் கூடிய வர்ண ஜாலங்களா? இல்லை நீரில் தோன்றி சட்டென உடையக் கூடிய நீர்க் குமிழிகள் போன்றனவா? அல்லது தாள இலய ஓசைகள் கொண்ட கவித்துவம் மட்டும் நிறைந்த கவிதைகளா? ஒருக்காலும் இல்லை. பிரபஞ்சத்தின் சூனியத்தை வென்று மானுடம் படைக்க வந்த வாழ்வியல் கவிதைகளாகும்.

பாரதியின் சுயசரிதையில் 'கனவு' எனும் தலைப்பில் காணலாகும் 49 பாடல்களை மட்டும் எடுத்துக் கொண்டு இவ்ஆய்வு பயணிக்கிறது. 1. பாரதியின் பிள்ளைக் காதல், 2. பாரதி கூறும் கைக்கிளை இலக்கணம், 3. பாரதி கண்ட காதல், 4. பாரதியின் ஆங்கிலமொழி வெறுப்பு, 5. பால்ய விவாகம் (அ) குழந்தை திருமண எதிர்ப்பு ஆகிய உட்தலைப்புகளின் கீழ் பாரதியின் மனோநிலை ஆராயப்பட்டுள்ளது.

கவிஞர்கள் என்றாலே கனவுகளுக்குப் பஞ்சமில்லை. கவிதையின் அடிநாதமே கற்பனை கலந்த கனவுதானே. வாழும் மாந்தர்க்குக் கனவில்லை எனில் அவன் நடமாடும் செத்த பிணம் தானே? கனவுகள் இல்லாத கவிஞர்களும் இல்லை. ஞானிகளும் இல்லை. அப்படிப்பட்ட மோன மயக்கம் கலந்த கனவு பாரதிக்கு உண்டு என்பதைச் சுயசரிதையில் பதிவு செய்கிறார்.

பாரதியின் பிள்ளைக் காதல்

பாரதி சமுகத்தில் தீர்க்க வேண்டிய, களைய வேண்டிய, உருவாக்க வேண்டிய வேறு பிரச்சினைகளை, தீர்வுகளை அவரின் பல பாடல்கள் வெளிப்படுத்துகின்றன. இது அவருக்கிருந்த கடமையுணர்ச்சியையும், பொறுப்புணர்ச்சியையும் வெளிப்படுத்துவதாகவே அமைகின்றன. இருப்பினும் காதல் பற்றிய அபிப்பிராயம் பாரதிக்குத் தனிப்பட்ட முறையில் உண்டு என்பதைப் பல பாடல்கள் பறைசாற்றுகின்றன. ஆணுக்கும் பெண்ணுக்கும் காதல் வருவது இயற்கை. குறிப்பாக குமரப் பருவத்தில் காதலுக்கு ஆட்படாதவர்களே இல்லை. உணர்ச்சிக் கொந்தளிப்புகள் மன ஒருமைப்பாட்டையே நிலைகுலையச் செய்துவிடும். தடுமாற்றத்தை ஏற்படுத்தும். அசாத்திய துணிவையும் கொடுக்கும். ஒவ்வொருவரின் வாழ்விலும் வசந்தத்தையும், சூறைக்காற்றையும் வீசச் செய்வது காதல். இவ்வாறான காதலுக்கு பாரதியும் விதிவிலக்கல்ல என்பதை அவரே தமது சுயசரிதையில் பதிவு செய்கிறார்.

காதல் என்பது ஒருதலைக் காதலாக இருந்தால் அது கடலில் பிறந்த ஆலகால விஷத்தைப் போல துன்பம் தரக்கூடியது என்றும், அக்காதல், காதல் கொண்டோர் இருவரிடமும் இருக்குமானால் சாகாவரம் தரக்கூடிய அமிர்தத்தைவிட உயர்வானது என்றும் கூறுகின்றார். இதனை,

காதலென்பதும் ஓர்வயின் நிற்குமேல்
கடலில் வந்த கடுவினை யொக்குமால்
ஏத மின்றி யிருபுடைத் தாமெனில்
இன்னமிர்தும் இனைசொல் லாகுமோ?
ஒதொணாத பெருந்தவம் கூடினோர்
உம்பர் வாழ்வினை யெள்ளிடும் வாழ்வினோர்
மாதரார்மிசை தாமுறுங் காதலை
மற்ற வர்தரப் பெற்றிடும் மாந்தரே (சுயசரிதை, பா.15)

என்று காதலின் இலக்கணத்தைப் பதிவு செய்கிறார்.

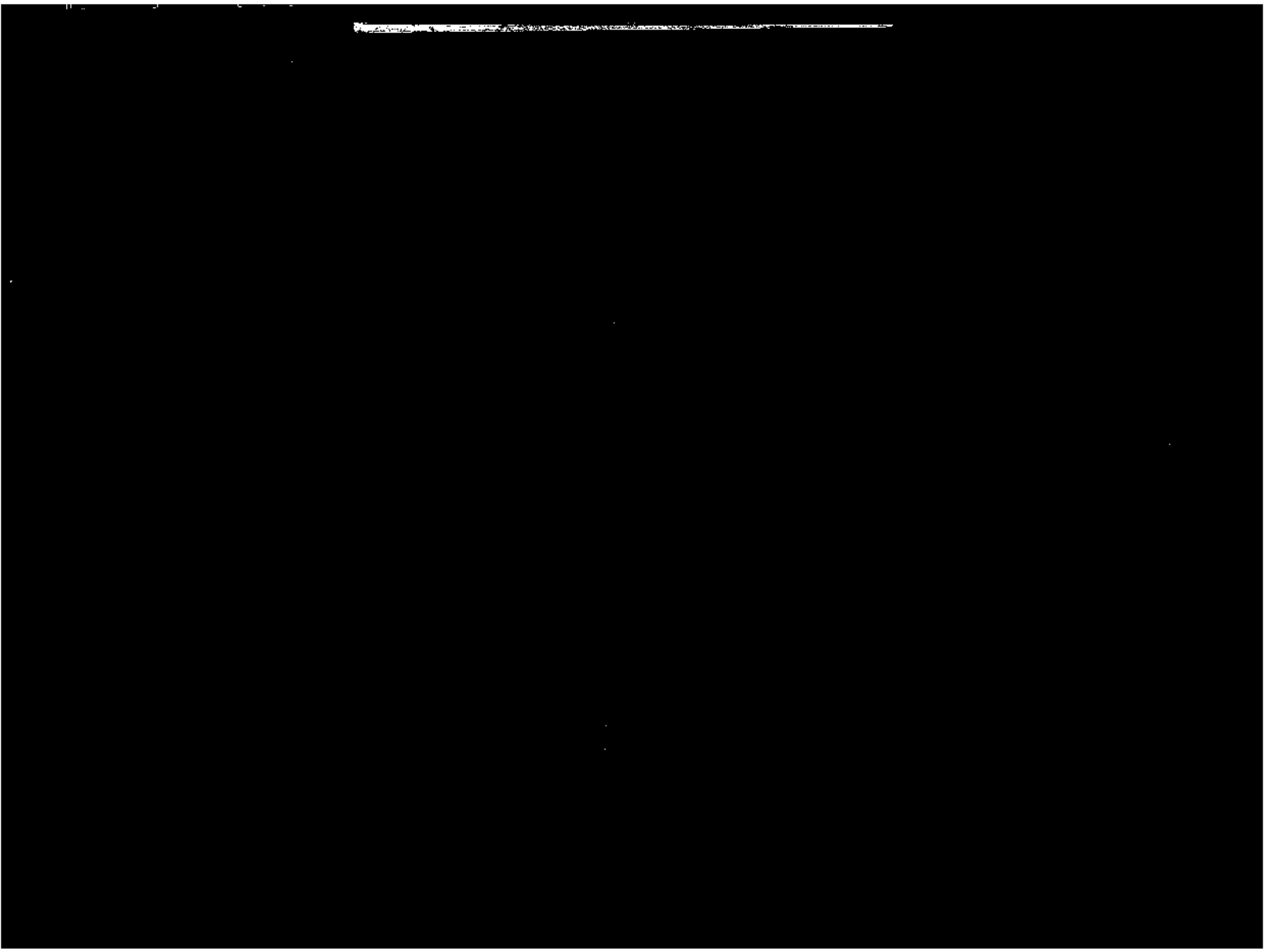
பாரதி கூறும் கைக்கிளை இலக்கணம்

தொல்காப்பியர் காதலின் கைக்கிளைக்கான இலக்கணம் வகுத்தார். ஆனால் பாரதியோ ஒருதலைக் காதலின் உணர்ச்சி உந்துதலையும், காதல் வயப்பட்ட தனிமனிதன் அவதிப்படும் மனக்கொந்தளிப்பையும் உணர்ச்சி பிரவாகமாக எடுத்தோதுகின்றார். உளவியல் மருத்துவர் போன்று மிக அனாயசமாக மாதரின் காதல் கிடைக்கப் பெறாத மனத்துன்பத்தைக்,

கைக்கி ளைப்பெயர் கொண்ட பெருந்துயர்க்
காத ல.:து கருதவந் தீயதால் (பா.16:7-8)

என்று நினைத்துப் பார்க்கவே கொடுமை நிறைந்தது என்று கூறுகின்றார். மேலும் இதனை, மேகங்களாய் மறைக்கப்பட்ட சந்திரன், உறையச் செய்யும் கொடிய பனியின் அடியில் சிக்குற்ற மென்மையான மலர், கசப்பான வேம்பின் சுவை கலந்திட்ட பால், பார்வைத்திறன் இல்லாத அழகிய நீண்ட கண்கள், பொய்மைக்கு இடம் கொடுத்து ஒதுங்கித் துன்பப்படும் மெய்மையைப் போன்றதாக அமையும் பல்வேறு உவமைகளைப் பொருத்திக் காட்டி கைக்கிளையின் கொடுமையைக் கூறுகின்றார். ஆகையால் கொடிய கைக்கிளை என்னும் காதலை என்னால் பாட இயலாது என்றும் திட்டவட்டமாகக் கூறுகின்றார்.

1075 / 1134



விளிம்பு நிலை மக்கள்

பதிப்பாசிரியர்

உள்ளடக்கம்

01. விளிம்பு நிலையில் பீடித்தொழிலாளர்கள் (முக்கூடல், ஆலங்குளம்)
திரு. உ.தி. சங்கரநாராயணன் பி லிட்

02. தமிழ்ச்செல்வியின் கீதாரி நாவல் காட்டும் விளிம்பு நிலை மக்கள்
முனைவர் ச.பொன்ஜெயந்தி

03. தோல் நாவலில் விளிம்புநிலை மாந்தர்கள் 1
திருமதி. செள. சுருதி

04. 'இடக்கை' புதினத்தில் விளிம்புநிலை மாந்தர்கள் 2
முனைவர் க.புவனேஸ்வரி

05. பூர்ணிமை சிறுகதை தொகுப்பு உணர்த்தும் விளிம்புநிலை 2
மக்களின் வாழ்வியல் நிலை
முனைவர் மு.சுபாஷினி

06. கி.ராஜநாராயணனின் கிடை புதினத்தில் விளிம்புநிலை மக்கள் 3
முனைவர் இரா.சுந்தரவேல்

07. செங்கல் சுள்ளை – விளிப்பு நிலை மக்களின் வாழ்வு 4
ஓவியங்கள்
முனைவர். ஜா. லாலி ஏதேஷ்

08. பாக்கியம் சங்கருடைய நான்காம் சுவரில் விளிம்புநிலை மக்கள் 4
முனைவர் அர.மீனா

09. மூங்கில் தெப்பம் - வெளிப்படுத்தம் விளிம்பு நிலை வாழ்வியல் 5
கூறுகள்
முனைவர். ஜா.ஞா.ஜோணி ஜெப மலர்

10. அஞ்ஞாடி நாவலில் விளிம்பு நிலை மக்கள் 6
ஜெ.சுஜாதா

11. எரியும் பனிகாடு நாவலின் வழி தோட்டத்
தொழிலாளர்களின் வாழ்வியல்
மு.சத்தியா

12. குறத்திமுடுக்கு நாவலில் பாலியல்தொழிலாளர் வாழ்வியல்
முனைவர் க.ஜெயசீலன்

ுகின்றனர் ஜாதியக் கொடுமைகள் என்பது நாடு முழுவதும் பர
ன்றன இதற்கு கடுமையான சட்டங்களை அரசாங்கம் இயற்றினாலும் அத
த பயமுமின்றி உள்ளது தீண்டாமையைப் பின்பற்றுவது தண்டனைக்குரி
ும், அதை பழக்கவழக்கங்கள் கடைபிடித்து வருகின்றனர்.

தோல் நாவல் மூலம் தலித்தை மக்களுக்கு ஏற்படும் அவலங்
ட்டுள்ள. சாதிய அடக்கு முறையால் இன்னல்களுக்கு ஆளாகும் த
ன் நிகழ்வுகளை தோல் நாவலில் டி.செல்வராஜ் எடுத்துப் பேசுகிறார்.அ
, தலித்தியப் பிரச்சினைகள் போன்று மையமாக வைத்து இக்கட்டுரை
செய்யப்பட்டுள்ளது.

ுறிப்பு

செல்வராஜ்.டி., தோல் நாவல், ப.168.
மலது ப. 169
மலது ப. 60
மலது ப. 76
மலது ப. 65
மலது ப. 66
மலது ப. 71
மலது ப. 79
மலது ப. 78
மலது ப. 79
மலது ப. 108
செல்வராஜ்.டி., தோல் நாவல், ப..
மலது ப. 108
ுய்வுக் கோவை 2009 பாகம் 4 ப. 2191.
ரங்க மல்லிகா, தமிழ் இலக்கியமும் பெண்ணியமும், ப.37.
செல்வராஜ்.டி., தோல் நாவல், ப.14.
செல்வராஜ்.டி., தோல் நாவல், ப.17.

'இடக்கை' புதினத்தில் விளிம்புநிலை மாந்தர்கள்

முனைவர் க.புவனேஸ்வரி,
உதவிப்பேராசிரியர், தமிழாய்வுத்துறை,
தேசியக்கல்லூரி (தன்னாட்சி), திருச்சிராப்பள்ளி

முகவுரை

தற்காலத்தில் இலக்கிய உலகில் அதிகமாகப் பேசப்படுகின்ற சொற்களில் விளிம்புநிலை என்பதும் ஒன்றாகும். சாதி சமய பொருளாதார தொழில் நிலைகளில் கீழ்த்தட்டில் உள்ளவர்களை விளிம்புநிலை மாந்தர் என்கிறோம். சமூகத்தில் நிலவும் அவலங்களைத் தங்கள் எழுத்துக்களில் வடிப்பதன் மூலம் அந்த அவலங்களுக்கு எதிரான போராட்டங்களில் பங்கேற்க முடியும். இப்படித் தன் எழுத்துக்கள் வாயிலாக விளிம்புநிலை மக்கள் பற்றி முதன் முதலில் தன் படைப்புகளில் எடுத்துக்காட்டியவர் எழுத்தாளர் பிரபஞ்சன் ஆவார். அவரைத் தொடர்ந்து பல்வேறு எழுத்தாளர்கள் தங்கள் படைப்புகளில் விளிம்புநிலை மாந்தர்கள் குறித்துப் பதிவு செய்தனர். இந்த விளிம்புநிலை மக்களில் 'மூன்றாம் பாலினத்தவர்கள்' எனப்படும் திருநங்கைகளின் நிலையையும் அவர்கள் வாழ்வின் போராட்டங்கள், ஏக்கங்களையும் எடுத்துக்காட்டுவதே இக்கட்டுரையின் நோக்கமாகும்.

எஸ்.இராமகிருஷ்ணனின் படைப்பு

இன்றைய எழுத்தாளர்களில் எஸ்.இராமகிருஷ்ணனுக்கும் அவரது எழுத்துக்களுக்கும் சிறப்பிடம் உண்டு. வாழ்வியல் எதார்த்தங்களை அழகாகத் தன் படைப்புகளில் வடிப்பதில் வல்லவர் இவர். 'இடக்கை' என்ற இவரது வரலாற்றுப் புதினத்தில் இடம்பெறும் 'அஞ்யா' எனும் திருநங்கை வாயிலாக மூன்றாம் பாலினத்தவர்கள் படும் துன்பங்களையும் திருநங்கைகள் மட்டுமன்றிப் பெண்களின் அவலநிலையையும் அழகாக நம் மனதில் பதியும் வண்ணம் இவர் இந்த 'இடக்கை' எனும் புதினத்தில் படம் பிடித்துக் காட்டியுள்ளார்.

மூன்றாம் பாலினத்தவர் எனப்படும் திருநங்கைகளின் வாழ்க்கை மிகவும் கொடுமையானதாக, வலிகளும், ஏக்கங்களும் நிறைந்ததாகவே இருக்கிறது. சிறுவயதிலிருந்தே இந்தச் சமுதாயம் அவர்களை, தப்பிப் பிறந்த ஜீவன்களாகவே பார்த்து வருகிறது.

"சந்திப்பிழை போன்ற
சந்ததிப்பிழை நாங்கள்
காலத்தின் பேரேட்டை
கடவுள் திருத்தட்டும்"
(நா.காமராசன், காகிதப் பூக்கள், ப.25)

என்று திருநங்கைகள் பற்றி நா.காமராசன் எழுதிய இந்தக் கவிதை திருநங்கைகளி நிலையை நமக்கு நன்கு உணர்த்துகிறது.

'அஜ்யா'வின் பாத்திரப்படைப்பு

'இடக்கை' என்ற இந்தப் புதினத்தில் 'அஜ்யா பேகம்' என்ற இந்த கதாபாத்திரத்தை 'அரவாணி' என்று அறிமுகப்படுத்துகின்றார் ஆசிரி எஸ்.இராமகிருஷ்ணன். ஔரங்கசீப் மாமன்னராக ஆட்சி செய்யும் வேளையில் அவ அந்தப்புரப் பணிப் பெண்களில் தலைமை சான்றவளாக 'அஜ்யா பே திகழ்கிறாள். அரவாணியாக அவள் அடைந்த துன்பங்களையும், ஆட்சிகள் மாற மு காட்சிகளும் மாறும் என்பதையும் இவள் வழியே காட்சிப்படுத்துகிற எஸ்.இராமகிருஷ்ணன்.

அஜ்யாவின் பிறப்பு

ஆக்ராவில் ஒரு நெசவாளியின் வீட்டில் ஆண் குழந்தையாகப் பிறந்தபோ 'அஜ்யா'வின் பெயர் 'இம்ரான்' எனப்பட்டது. இம்ரானுக்கு ஆரம்பம் முதலே பெண் மீது அன்பும் மரியாதையும் இருந்தது. ஒரு நாள் அவன் தன் தங்கையின் உடை எடுத்து அணிந்து கொண்டபோது அவனுக்கு அதிக ஆனந்தத்தைத் தந்தது.

"எதுக்குடா பெண்பிள்ளையாக ஆசைப்படுறே? நான் படுற கஷ்டம் போதாதா
(இடக்கை, ப.5

என்று பெண்களின் அவலநிலையை எண்ணி அவன் தாய் அழுதாள்.

இம்ரான் அஜ்யாவாக மாற்றம் பெறல்

அந்த நிகழ்விற்குப் பிறகு அவன் பெண்களின் உடையை உடுத்துவ வழக்கமாக்கிக் கொண்டான். அப்படிப் பெண் உடை அணிந்து அருகில் உள் பாழடைந்த மண்டபத்தில் விளையாடிக் கொண்டிருந்தபோது தர்ஷனுடன் அவனுக்கு பழக்கம் ஏற்பட்டது. தர்ஷன் 'இம்ரானைப்' பெண் என்றே கருதினான். அவளி அழகை ஆராதனை செய்தான்.

**"தர்ஷன் தன்னைப் பெண் என்று நம்புவதும் ஆராதனை
செய்வதும் அஜ்யாவிற்கு மிகவும் பிடித்திருந்தது" (இடக்கை, ப.57)**

நாளடைவில் இருவரின் நட்பும் தொடர்ந்தது. மகிழ்வான இந்தச் சூழ் நீடிக்கவில்லை. தர்ஷனைத் தேடி வந்த சில பையன்கள், அஜ்யா பற்றி உண்மையை அறிந்தனர்.

"எதுக்குப் பொம்பளப்பிள்ளை மாதிரி நடிக்கிறே" (இடக்கை, ப.60)

என்று அந்தப் பையன்கள் மாறி மாறி அவளை அடித்து மிதித்தனர். வலி பொறுக்க முடியாமல் அஜ்யா அழுதாள். அதையும் தாண்டி அவள் மனத்தை பாதித்தது தர்ஷனின் நடவடிக்கைதான்.

"பேடிப்பயலே இனி இங்கே வராதே" (இடக்கை, ப.65)

என்று தர்ஷன் கூறிய வார்த்தைகள் தன்னைப் போன்ற அரவாணிகளின் நிலை என்ன என்பதை அஜ்யாவுக்கு உணர்த்தியது.

சமுதாயத்தில் இப்படித்தான் மூன்றாம் பாலினத்தவர்கள் கேலியும் கிண்டலும் செய்யப்படுவதோடு அடி உதைக்கும் ஆளாகின்றனர். அதனினும் அவலம் தங்கள் மீது அன்பும் அக்கறையும் செலுத்த யாரும் இல்லையே என்ற ஏக்கமே அவர்களை நிலைகுலையச் செய்துவிடுகிறது. தன்னைப் பெற்றவர்களாலேயே வெறுத்து ஒதுக்கப்படும் அஜ்யா, தன்மீது அன்பு செலுத்திய தர்ஷனின் மனமாற்றத்தால் மனமுடைந்து போவதை நாவலாசிரியர் எஸ்.இராமகிருஷ்ணன் சிறப்பாகக் காட்சிப்படுத்தியுள்ளார்.

ஔரங்கசீப்பின் சகோதரி

ஔரங்கசீப்பின் நான்காவது மனைவி 'உதய்பூரிமகல்' இவரது தோழியாக அஜ்யா ஆனபின்பே நல்ல வாழ்க்கையும், சுதந்திரமும் கிடைக்கிறது. ஆனால் அதுவே பிற பணிப்பெண்கள் அஜ்யாவை வெறுக்கக் காரணமாகின்றது. கொடுங்கோலனாக அனைவராலும் அறியப்படும் ஔரங்கசீப், அஜ்யாவின் உண்மை அன்பில் நெகிழ்ந்து போகிறார். ஒரு கட்டத்தில் அவளைத் தன் உடன் பிறந்த சகோதரியாக ஏற்கிறார்.

**"இந்த உலகம் பெண்களின் அன்பால்தான் உயிர்ப்புடன் இருக்கின்றது.
அதை உணராத ஆண்களில் நானும் ஒருவன்" (இடக்கை, ப.105)**

என்று கூறும் ஔரங்கசீப்

**"என்னை 'சகோதரன்' என்றே அழைக்க வேண்டும்
அதைக் கேட்டபின்பே என் உயிர் நீங்கும்" (ப.120)**

என்கிறார். அதன் பிறகு அஜ்யா தன் பணிவிடைகளால் அவரின் உடலும், உயிரும் காக்கின்றாள்.

நம்பிக்கை நல்கியவள்

ஔரங்கசீப் தன் இறுதி நாட்களில் தான் அதுவரை பத்திரமாகப் பாதுகாத்து வைத்திருந்த சில பொருட்களை அஜ்யாவிடம் அளிக்கின்றார்.

"என் பணப்பை அஜ்யாவிடம் உள்ளது. அதில் நான் தொப்பிகள் தைத்துக் கிடைத்த 4 ரூபாயும் 2 அணாவும் உள்ளன. இதில்தான் இறந்த என் உடலை மூட சவத்துணிவாங்கப்படல் வேண்டும்"
((இடக்கை, ப.48)

என்று ஔரங்கசீப் தன் இறுதி விருப்பத்தை அஜ்யாவிடம் கூற அந்த நம்பிக்கையைக் காக்கும் போரில் அஜ்யா தன் உயிரையே பணயம் வைக்கிறாள். ஔரங்கசீப்பின் மகனால் அதிக சித்ரவதைளுக்கு ஆளானபோதும் அரசரின் விருப்பத்தை நிறைவேற்றிய பின்பே அவள் உயிர் பிரிகிறது.

இப்படி ஒரு கொடுமையான மன்னனின் மனத்தை மாற்றக்கூடிய வல்லமை அந்த அரவாணியின் அன்பிற்கு இருந்தது. ஆனால் அதிகாரம் மிக்கவர்களின் ஆட்சி வெறி அந்த 'அஜ்யா' எனும் அரவாணியைக் கொன்றுவிட்டது என நூலாசிரியர் மூன்றாம் பாலினத்தவரான விளிம்புநிலை மக்கள் பற்றி விளக்குகிறார்.

தண்டனைகள்

அரண்மனைப் பணிகளில் குறிப்பாக அந்தப்புரங்களில் அரவாணிகளே பணிப்பெண்களாக இருப்பார்கள். ஆனால் அவர்கள் பல துன்பங்களுக்கு ஆளாக்கப்படுவார்கள் என்பதை.

"பேரரசனின் படுக்கை அறைக்குள் சேவகம் செய்யும்
அரவாணிகளின் நாக்கு துண்டிக்கப்பட்டிருக்கும்" ((இடக்கை, ப.96)

என்று அஜ்யா தன் நெருங்கிய தோழியிடம் கூறுவதன் வாயிலாகவும்,

"நோயுற்ற நாட்களில் கூட அவர்களுக்கு ஓய்வு கிடையாது"
((இடக்கை, ப.96)

என்ற கூற்றைப் பார்க்கும்போது, உடல்நலமில்லாத நிலையில் கூட அவர்கள் பணி செய்ய வேண்டி இருந்தது என்பதையும், அதற்கும் ஒருபடி மேலாக, இந்த அரவாணிகள் தங்கள் மனவுணர்வுகளை, சோகத்தை, பணிக்கொடுமைகளைக்கூட பாரிடமும் கூற முடியாது மனநோய் பிடித்தவர்களாக மாறித் தற்கொலை செய்து கொள்ளும் நிலைக்குத் தள்ளப்பட்டார்கள் என்பதையும் பல இடங்களில் ஆசிரியர் பதிவு செய்துள்ளார்.

"சில அரவாணிகள் தனக்குத் தானே அழுது கொண்டும்,
பேசிக்கொண்டும் இருந்தார்கள்" (இடக்கை, ப.102)

என்கிறார். இப்படி உடலாலும், மனதாலும் அரவாணிகள் துன்புறுத்தப்பட்ட நிகழ்வுகளைத் தம் படைப்புகளின் வாயிலாகப் பதிவு செய்துள்ளார் ஆசிரியர்.

மூன்றாம் பாலினத்தவர்கள்

அரவாணிகள் எனப்படும் மூன்றாம் பாலினத்தவர்கள் பற்றித் தமிழில் எழுந்த முதல் நாவல், எழுத்தாளர் சு.சமுத்திரம் எழுதிய 'வாடாமல்லி'. 'நூர் முகம்மது' எனும் ஆண் தன் ஆணுடம்பில் இருக்கும் பெண் தன்மையைச் சமுதாயம் ஏற்க மறுத்தபோது, தமிழகத்திலிருந்து மும்பை சென்று பால் மாற்று அறுவை சிகிச்சை செய்து கொண்டு 'நூரி' என்ற பெண்ணாக மாற்றம் பெறுகிறாள். அவள் ஆண் வாழ்வில் சந்திக்கும் அடுக்கடுக்கான அவலங்களைத் "திருநங்கையர் உலகம்" எனும் புத்தகம் மிகச் சிறப்பாகச் சொல்லியிருக்கும். அதுபோலவே 'சுயம்பு' எனும் ஆண் தன் கல்லூரிப் பருவத்தில் பெண் தன்மையை உணரும்போது பெண்களாலேயே அவமானப்படுத்தப்படுகிறான். பின்னர் பல்வேறு இன்னல்களுக்கு ஆளாகி, திருநங்கையர்கள் படும் பல்வேறு விதமான சிக்கல்களையும் எதிர்கொள்வதை சு.சமுத்திரம் தன் நாவலான வாடாமல்லியில் சிறப்பான முறையில் பதிவு செய்துள்ளார்.

'பிரியாபாபு' எனும் எழுத்தாளர் தனது 'மூன்றாம் பாலின் முகம்' எனும் புத்தகத்தில் அரவானின் கதையையும், அரவாணிகள் நிலையையும் எடுத்துச்

சொல்கிறார். அதில் சில பத்திரிகைகள் திருநங்கைகளைத் தாசிகள்போல் நினைப்பதையும், சில காவல்துறை அதிகாரிகள் அவர்களை அதற்காகவே பயன்படுத்த நினைப்பதையும் துல்லியமாகக் காட்சிப்படுத்தி இருப்பார்.

இப்படித் தமிழ் இலக்கிய உலகில் அரவாணிகள் பற்றிய படைப்புகள், மூன்றாம் பாலினத்தவர்களே தங்கள் அவலங்களை எடுத்துச் சொல்லும் படைப்புகள் இன்றைய சூழலில் அதிகரித்து வருகிறது. அப்படிப்பட்ட நிலையில் எழுத்தாளர் எஸ்.இராமகிருஷ்ணன் எழுதிய **'இடக்கை'** எனும் வரலாற்றுப் புதினத்தில் இடம்பெற்றுள்ள **'அஜ்யா'** எனும் அரவாணியின் நிலையையும், அவளது போராட்ட மனப்பான்மையையும், தியாக உணர்வையும் மிகவும் சிறப்பாகவும், சரியான புரிதலோடும் ஆசிரியர் பதிவுசெய்துள்ளார். இவரது மற்றொரு படைப்பான 'தேசாந்திரி' புத்தகத்தில் கூவாகத் திருவிழா பற்றியும், அரவான் கதை குறித்தும் இவர் கூறிச் சென்றிருப்பார்.

ஆக மொத்தத்தில் அதிகம் பேசப்படாமல் இருந்த இந்த விளிம்புநிலை மாந்தர்கள் பற்றிப் பல்வேறு படைப்புகள் வரத் தொடங்கி இருப்பது, மூன்றாம் பாலினத்வர்களின் நிலையை இந்தச் சமுதாயத்திற்கு எடுத்துச் சொல்லி, நல்ல மாற்றங்கள் அவர்களின் வாழ்வில் ஏற்படக் காரணியாக அமையலாம் என்று நாம் எண்ணமிடலாம்.

பெண்ணின் நிலை

இந்த மூன்றாம் பாலினத்தவர்களில் ஆணாக இருந்து பெண்ணாக மாறியவர்களைத் 'திருநங்கைகள்' என்றும், பெண்ணாய்ப் பிறந்து ஆணாக மாறுகிறவர்களைத் 'திருநம்பிகள்' என்றும் அழைப்பார்கள். இந்தச் சமுதாயத்திலும், இலக்கியப் படைப்புகளிலும் கூட 'திருநங்கைகள்' பற்றியே அதிக அளவில் பேசப்பட்டுள்ளதே தவிர, 'திருநம்பிகள்' பற்றிப் பேசப்படுவதில்லை. ஏனெனில் அதிலும் பால் பாகுபாட்டில் திருநம்பிகளின் நிலை திருநங்கைகளைவிடவும், மிகமிக மோசமாக உள்ளது.

"ஆணுக்கு ஒரு நீதி
பெண்ணுக்கு ஒரு நீதியா மாலிக்"
(இடக்கை, ப.137)

என்று 'அஜ்யா' ஔரங்கசீப்பிடம் பெண்களின் நிலை குறித்துக் கேட்கும் கேள்வி திருநம்பிகளுக்கும் பொருந்தும். இன்று பெண்கள் பல துறைகளில் முன்னேறி இருந்தாலும், பல சாதனைகள் புரிந்திருந்தாலும், சமுதாயத்திலும், குடும்பத்திலும் அவர்கள் நிலை பின்தங்கியே உள்ளது. ஆணின் செயல்பாடுகளை ஏற்கும் சமுதாயம், பெண் செய்யும் ஒவ்வொரு செயலையும், கேள்விகளோடும், சந்தேகத்தோடும் எதிர்நோக்குகிறது. அதனால் தான் விளிம்புநிலை மாந்தர்களில் பெண்களும் இடம்பெற்றுள்ளார்கள் என்பதை நினைக்கும்போது, இந்தச் சமுதாயம் அடைய வேண்டிய மாற்றங்கள் இன்னும் நிறைய இருக்கிறது என்பதை உணர முடிகின்றது. பால்பாகுபாட்டை மறந்து அனைத்து மனிதர்களும் மதிப்புடன் வாழத் தொடங்கும் நாளில் தான் இந்தச் சமுதாயம் உண்மையான ஏற்றத்தைப்பெறும்.

முடிவுரை

'இடக்கை' எனும் புதினத்தில் நாவலாசிரியர் எஸ்.இராமகிருஷ்ணன் 'அஜ்ய எனும் அரவாணியின் வாயிலாக, மூன்றாம் பாலினத்தவர்களை இந்தச் சமூக புறந்தள்ளுவதையும், அவர்களைக் கேவலப்படுத்துவதையும் பெற்றவர்கள் அவர்களை வெறுத்து ஒதுக்குவதையும், அரவாணிகளுக்கு மனதளவிலு உடலளவிலும் உண்டாகும் மாறுபாடுகளைப் புரிந்து கொள்ள மறுப்பதையு அவர்களுக்குக் கொடுமையான தண்டனைகள் வழங்கப்படுவதையும் தம் நாவலி சித்திரித்துக் காட்டியுள்ளதன் வாயிலாக விளிம்புநிலை மாந்தர்களின் வாழ்வியல் அவலங்களை நாம் உணர்ந்து கொள்ள வழிவகை செய்துள்ளார் என்பதே உண்மை

துணைநின்ற நூல்கள்

1. எஸ்.இராமகிருஷ்ணன், 'இடக்கை' (புதினம்), தேசாந்திரி பதிப்பகம், சென்னை
2. நா.காமராசன், காகிதப்பூக்கள் (கவிதை), முல்லை புத்தக நிலையம், சென்னை

பூர்ணிமை சிறுகதை தொகுப்பு உணர்த்தும் விளிம்புநிலை மக்களின் வாழ்வியல் நிலை

முனைவர் மு.சுபாஷினி,
உதவிப்பேராசிரியர், தமிழாய்வுத்துறை,
தேசியக்கல்லூரி (தன்னாட்சி), திருச்சிராப்பள்ளி

முகவுரை

மனித சமுதாயத்தில் பல்வேறு தொழில்கள் இருப்பினும் எல்லா தொழில்களும் ஒன்று போல் ஆகிவிடாது. தொழில் செய்வதைப் பார்க்கும்போதே ஒரு அவலத்தையும், அருவெறுப்பையும் தரும்படியான தொழில்கள் ஏராளமானவை. குடும்பத்தை நடத்துவதற்குப் போதுமான வருமானம் இல்லாத தொழில்களும், அதனால் மனித சமுதாயம் படும் துன்பத்தையும் வெளிக்கொணரும் வகையில் படைப்பாளர்கள் விளிம்புநிலை மக்கள் பற்றிய கருத்துகளைக் கூற முனைந்துள்ளனர். சமுதாய மக்களுக்கு விழிப்புணர்வை ஏற்படுத்த கையாளுகின்ற உத்திமுறையில் பற்பல படைப்புகள் வெளிவந்துள்ளன. இத்தகைய வரிசையில் ஆசிரியர் க.வீரபாண்டியன் அவர்கள் தம் 'பூர்ணிமை' சிறுகதை தொகுப்பில் எத்தகைய விளிம்புநிலை மக்களைப் பற்றிய கருத்துகளைப் பதிவுசெய்துள்ளார் என்பதை ஆராயும் நோக்கில் இகட்டுரை அமைந்துள்ளது.

விளிம்புநிலை மக்களின் வாழ்வியலை பேசுகிறது க.வீரபாண்டியன் அவர்களின் 'பூர்ணிமை' எனும் சிறுகதை தொகுப்பு. ஆசிரியர் இந்திய ஆட்சிப்பணி அதிகாரியாக இருந்த போதிலும், தம்மை எழுத்தாளராகவே வெளியுலகிற்கு அடையாளம் காட்டுவதில் முனைப்புடையவர். வாழ்வின் கடைக்கோடி நிலையில் இருக்கும் மக்களையே விளிம்புநிலை மக்கள் என்பர். அம்மக்களின் வாழ்வியலையும், வாழ்க்கை நடத்த முடியாத இழிவான சூழல்களையும் வெளிக்கொணரும் நோக்கில் 'பூர்ணிமை' எனும் சிறுகதை தொகுப்பில் ஒவ்வொரு கடைநிலை தொழில் செய்யும் மக்களின் வாழ்க்கை முறையைச் சமுதாய மக்கள் உணரும்படி செய்துள்ளார். 'பூர்ணிமை' எனும் 12 சிறுகதை தொகுப்பு காலத்தால் அழியாத கல்வெட்டு எழுத்துகளாகப் பதிவாகியுள்ளன.

கந்துக்கடனால் அல்லல்படும் மக்களின் வாழ்வியல்

துப்புரவுப் பணியாளராக வேலை பார்த்து வருபவன் இராமன். மாத வருமானம் குடும்பத்திற்குப் போதுமானதாக இல்லாமல் இருப்பதால், அரசு அதிகாரிகள், மற்றும்

Eco. Env. & Cons. 28 (1) : 2022; pp. (582-587)
Copyright@ EM International
ISSN 0971–765X

DOI No.: http://doi.org/10.53550/EEC.2022.v28i01.087

Stress response of biomolecules (carbohydrate, protein and lipid profiles) in fresh water Major carp *Catla catla* exposed to Triphenyl phosphate

D. Gayathri[1] and L. Shakila[2]

PG & Research Department of Zoology, Ethiraj College For Women, Egmore, Chennai 600 008, T.N., India

(Received 27 October, 2021; Accepted 24 December, 2021)

ABSTRACT

Plasticizers are the xenobiotics which have attracted the attention in recent days due to their extensive use in commercial applications and growing concern of potential threat they pose to human health and ecosystem. Triphenyl phosphate (TPP), is an organophosphate flame retardant widely used as a plasticizer in hydraulic fluids, lacquers and varnishes, which is highly toxic to non – target organisms. Fish has been used as indicator species for aquatic pollution. *Catla catla* an Indian major carp is an edible fish and is highly sensitive to slight stress. Recently, fish biomarkers are widely used to determine the impact caused to aquatic ecosystem by release of these chemicals. *Catla catla* were exposed to sublethal concentrations (0.25mg/l , 0.5 mg/l and 1 mg/l) of TPP to determine the alterations in the biomolecules (protein, carbohydrate and lipid profiles) in tissues (gill, muscle and intestine). Significant alterations in the protein, carbohydrate and lipid profiles in tissues were observed which are indications of severe cellular damage in tissues of respective organs of TPP treated fish. The results of the present investigation suggest that gill, muscle and intestine exposed to Sub lethal concentrations (0.25mg/l , 0.5 mg/l and 1 mg/l) of TPP were adversely affected with increasing concentration of toxicant which are highlighted by significant alterations in protein, carbohydrate and lipid profiles. Thus, the study can be effectively used as a potential biomarker for monitoring of organophosphorus plasticizer in aquatic environment. Further, TPP should be used with caution to protect natural waters and aquatic organisms.

Key words : *TPP, Catla catla, Plasticizer, Toxic effect.*

Introduction

Anthropogenic activities concerning with xenobiotics such as plasticizers, pharmaceuticals, personal care products, pesticide residues and gasoline additives can result in the release of toxic organic compounds with carcinogenic, teratogenic and endocrine disrupting properties into the environment. Plasticizers are xenobiotics that are of particular concern because of the extent of their use in commercial applications and growing recognition of the potential threats they pose to human health and ecosystems (Gomez – Hens and Aguilar - Caballos, 2003; Rahman and Brazel, 2004). Plasticizers are used to impart properties such as flexibility and workability to plastics for effective processing and tailoring of plastic formulations (Sears *et al.,* 1982). Their importance as industrial chemicals is demonstrated by their rate of use, which is estimated to be millions of tonnes annually. Unfortunately, plasti-

([1]Research Scholar, [2]Assistant Prof.)
Corresponding author's email: gayathridharmaraj01@gmail.com

cizers are not chemically bound to the polymers and can migrate from plastic products during normal use and following their disposal (Fromme *et al.*, 2002). Thus, many studies have shown their wide distribution in the environment and their occurrence in surface waters that receive treated effluents (Fromme *et al.*, 2002; Fauser *et al.*, 2003; Gavala *et al.*, 2003; Martineen *et al.*, 2003). One of the most common organophosphate compound is, TPP which is a phosphate ester, having much utility both as a flame retardant in polyurethane foam and as a plasticizer in hydraulic fluids, lacquers and varnishes (CPSC, 2005; Van der Veen and de Boer, 2012). Due to the fact that TPP is not bonded to manufactured products, it is relatively prone to be released into the environment through leaching, abrasion and volatilization (Wei *et al.*, 2015).

Hence, in the present investigation, an attempt has been made to observe changes in biochemical parameters like protein, carbohydrate and lipid profiles of vital organs like gill, muscle and intestine of Indian major carp *Catla catla*, exposed to sub – lethal concentrations (0.25 mg/l, 0.5 mg/l, 1 mg/l) of TPP technical grade, for an exposure period of 15 days.

Materials and Methods

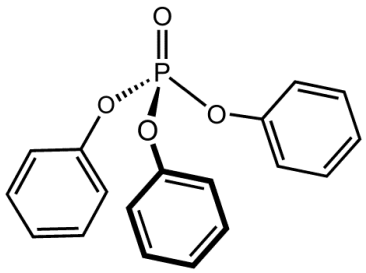
Animal selection and acclimatization

Catla catla, is the experimental animal model used in this study which belongs to the family of Cyprinidae of order cypriniformes. They are economically important South Asian fresh water fish, native to rivers and lakes. It is one of the most important aqua - cultured species. It is also grown in polyculture ponds with rohu and mrigal carp. It is a surface and mid water feeder. The experimental fish fingerlings of size (2 – 4 g), were procured from Tamil Nadu Fish Seed Farm, Poondy, Thiruvallur and shifted in plastic bags containing fresh water filled with oxygen to the research laboratory. The fingerlings of fish were immersed in 0.1% $KMnO_4$, for 2 – 3 minutes in order to sterilize before acclimatization.

The process of acclimatization was carried out for a period of one week in glass aquaria of size 30cm x 60 cm x 45 cm filled with water before the start of the experiment in the laboratory. Ten fish fingerlings were kept in each glass aquaria. The fish fingerlings were fed with commercial artificial feed at 2 - 3 % of wet body weight during the acclimatization period. Proper aeration was supplied continuously to all the glass aquaria with electric air pump. The water in the glass aquaria was replaced with fresh water for every two days.

Physio – Chemical Properties of TPP

The present study involves TPP, technical grade as chemical, whose toxic effect in tissues of vital organs of Cattla carp has been evaluated.

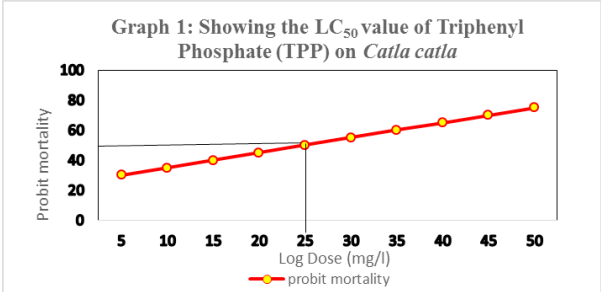


Structure of TPP

TPP is the chemical compound with the formula $OP(OC_6H_5)_3$. This colourless solid is the ester (tri ester) of phosphoric acid and phenol. It is used as a plasticizer and a fire retardant in a wide variety of settings and products. It has been used as a flame retardant for a variety of materials, including electronic equipment, PVC, hydraulic fluids, glues, in nail polishes, and casting resins. TPP is also used as a plasticizer in lacquers, varnishes, and hydraulic fluids.

Determination of LC_{50}

Determination of LC_{50} was done according to Behreus and Karbeur (1953). In the present study 72hrs LC_{50} bio – assay method was followed in which the ten fishes per group was placed at various concentrations of TPP in each group. The mortality rate was observed and recorded at time intervals of 24 hrs, 48 hrs, 72 hrs. The concentration of TPP which gave 50% mortality at 72 hrs was taken as the LC_{50} value. The percentage mortality was converted



into probit values and plotted against the log dose values (graph 1).

Experimental Design For Sub – Lethal Study

The sub – lethal study was carried out by placing five groups which contain ten fishes per group and the group comprising of group – I – Control, group – II – Acetone treated, group – III – Sublethal treated with 0.25 mg/l of TPP, group – IV – sublethal treated with 0.5 mg/l of TPP and group – V – treated with 1 mg/l of TPP in 20 litres of water. The water in the glass aquaria was changed for every two days and freshly prepared toxicant was added to maintain the concentration of TPP at constant level. The experiment was carried out for a duration of 15 days.

Biochemical analysis

The biochemical parameters such as quantitative analysis of proteins, carbohydrates and lipids were estimated by standard procedures in three tissues – gill, muscle and intestine of the healthy fish (Control) and of those from the fish exposed to sub-lethal concentrations (0.25mg/l, 0.5 mg/l and 1 mg/l) of TPP for a period of 15 days before sacrifice for the biochemical analysis. The proteins, carbohydrates and lipids were estimated by methods of Lowry *et al.,* (1951), Roe (1955) and Folch *et al.,* (1957) respectively.

Results

Protein

The protein content in the tissue extracts of gill, muscle and intestine of the group I control were found to be 0.106 mg/g, 0.151 mg/g and 0.120 mg/g, respectively. In group II treated with acetone the protein concentration of tissue extracts of gill, muscle and intestine was found to be 0.119 mg/g, 0.109 mg/g and 0.115 mg/g, respectively. In group III treated with TPP (0.25 mg/l) the protein content in the tissue extracts of gill, muscle and intestine was noted as 0.096 mg/g, 0.098 mg/g and 0.095 mg/g respectively. In group IV treated with TPP (0.5mg/l) the protein content in the tissue extracts of gill, muscle and intestine was observed as 0.093 mg/g, 0.090 mg/g and 0.098 mg/g, respectively. In group V treated with TPP (1 mg/l) the protein content in the tissue extracts of gill, muscle and intestine was found to be 0.084 mg/g, 0.089 mg/g and 0.086 mg/g, respectively. (Graph 2). The mean and SD values of group I, II, III, IV and V were found to be significantly different from control at 5% probability level (Table 1).

Carbohydrate

The carbohydrate content in the tissue extracts of gill, muscle and intestine of the group I control were found to be 0.07 mg/g, 0.05 mg/g and 0.06 mg/g respectively. In group II treated with acetone the carbohydrate concentration of tissue extracts of gill, muscle and intestine was found to be 0.06 mg/g, 0.04 mg/g and 0.05 mg/g respectively. In group III treated with TPP (0.25 mg/l) the carbohydrate content in the tissue extracts of gill, muscle and intestine was noted as 0.05 mg/g, 0.03 mg/g and 0.04 mg/g respectively. In group IV treated with TPP (0.5mg/l) the carbohydrate content in the tissue extracts of gill, muscle and intestine was observed as 0.04 mg/gm, 0.02 mg/g and 0.03 mg/g respectively. In group V treated with TPP (1 mg/l) the carbohydrate con-

Table 1. Effect of sub lethal dosages of Triphenyl Phosphate (TPP) (0.25, 0.5, 1 mg/g B.wt.) on protein (Gill, Muscle and Intestine) of *Cattla cattla* (15 days)

Treatment	Protein (mg/g of wet tissue)		
	Gill	Muscle	Intestine
Group I - Control	0.119 ± 0.012	0.151 ± 0.010	0.12 ± 0.008
Group II - Treated Acetone	0.106 ± 0.011	0.109 ± 0.005	0.115 ± 0.007
Group III - Treated Triphenyl phosphate (0.25 mg/gm b.wt)	0.096 ± 0.003	0.098 ± 0.003	0.095 ± 0..006
Group IV - Treated Triphenyl phosphate (0.5 mg/gm b.wt)	0.093 ± 0.005	0.09 ± 0.008	0.094 ± 0.01
Group V - Treated Triphenyl phosphate (1 mg/gm b.wt)	0.084 ± 0.007	0.089 ± 0.009	0.086 ± 0.01

Values are expressed as mean ± standard deviation.
Significantly different from Control at 5% probability level.

tent in the tissue extracts of gill, muscle and intestine was found to be 0.03 mg/g, 0.01 mg/g and 0.02 mg/g, respectively. (Graph 3). The mean and S.D values of group I, II, III, IV and V were found to be significantly different from control at 5% probability level (Table 2).

Lipid

The lipid content in the tissue extracts of gill, muscle and intestine of the group I control were found to be 0.37 mg/g, 0.323 mg/g and 0.365 mg/g respectively. In group II treated with acetone the lipid concentration of tissue extracts of gill, muscle and intestine was found to be 0.212 mg/g, 0.176 mg/g and 0.237 mg/g, respectively. In group III treated with TPP (0.25 mg/l) the lipid content in the tissue extracts of gill, muscle and intestine was noted as 0.183 mg/g, 0.143 mg/g and 0.172 mg/g respectively. In

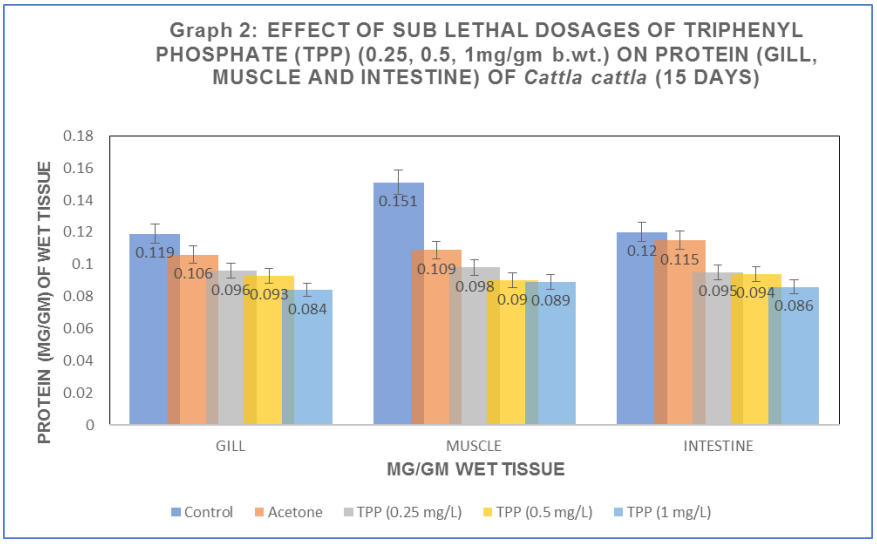


Table 2. Effect of Sub Lethal Dosages of Triphenyl Phosphate (Tpp) (0.25, 0.5, 1mg/g B.wt.) on Carbohydrate (Gill, Muscle and Intestine) of *Cattla cattla* (15 days)

Treatment	Carbohydrate (mg/g of wet tissue)		
	Gill	Muscle	Intestine
Group 1- Control	0.07 ± 0.008	0.05 ± 0.01	0.06 ± 0.01
Group 2- Treated Acetone	0.06 ± 0.003	0.04 ± 0.009	0.05 ± 0.01
Group 3- Treated Triphenyl phosphate (0.25 mg/g b.wt)	0.05 ± 0.006	0.03 ± 0.005	0.04 ± 0..005
Group 4- Treated Triphenyl phosphate (0.5 mg/g b.wt)	0.04 ± 0.009	0.02 ± 0.004	0.03 ± 0.005
Group 5- Treated Triphenyl phosphate (1 mg/g b.wt)	0.03 ± 0.003	0.01 ± 0.005	0.02 ± 0.005

Values are expressed as mean ± standard deviation.
Significantly different from Control at 5% probability level.

Table 3. Effect of sub lethal dosages of triphenyl phosphate (TPP) (0.25, 0.5, 1mg/g b.wt.) on lipid (gill, muscle and intestine) of *cattla cattla* (15 days)

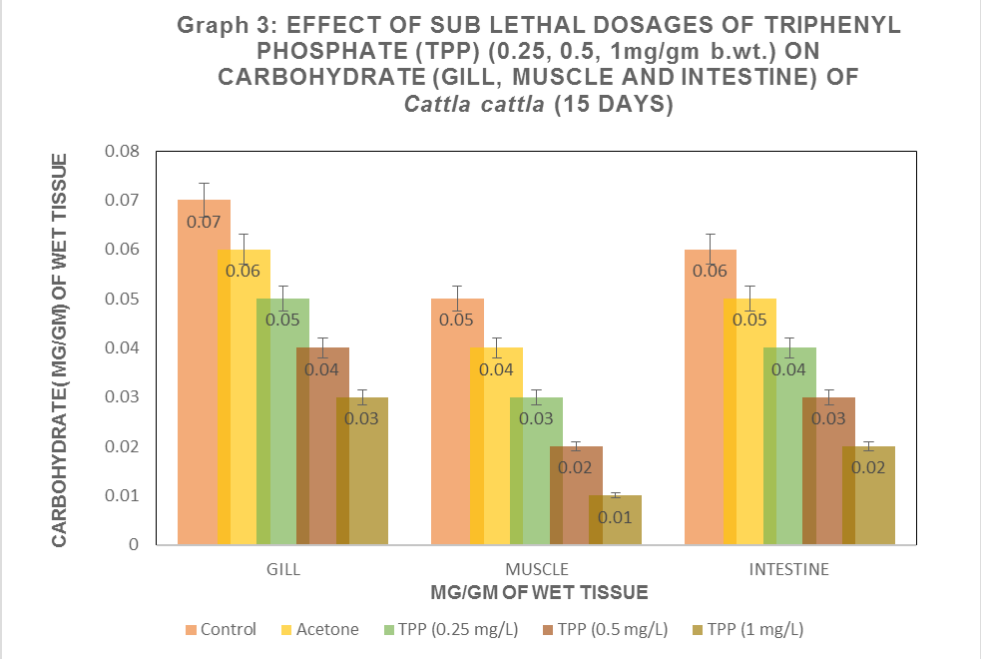
Treatment	Lipid (mg/gm of wet tissue)		
	Gill	Muscle	Intestine
Group 1- Control	0.37 ± 0.007	0.323 ± 0.05	0.365 ± 0.03
Group 2- Treated Acetone	0.212 ± 0.02	0.176 ± 0.06	0.237 ± 0.008
Group 3- Treated Triphenyl phosphate (0.25 mg/g b.wt)	0.183 ± 0.009	0.143 ± 0.007	0.172 ± 0.008
Group 4- Treated Triphenyl phosphate (0.5 mg/g b.wt)	0.143 ± 0.007	0.101 ± 0.004	0.155 ± 0.006
Group 5- Treated Triphenyl phosphate (1 mg/g b.wt)	0.091 ± 0.01	0.076 ± 0.005	0.078 ± 0.04

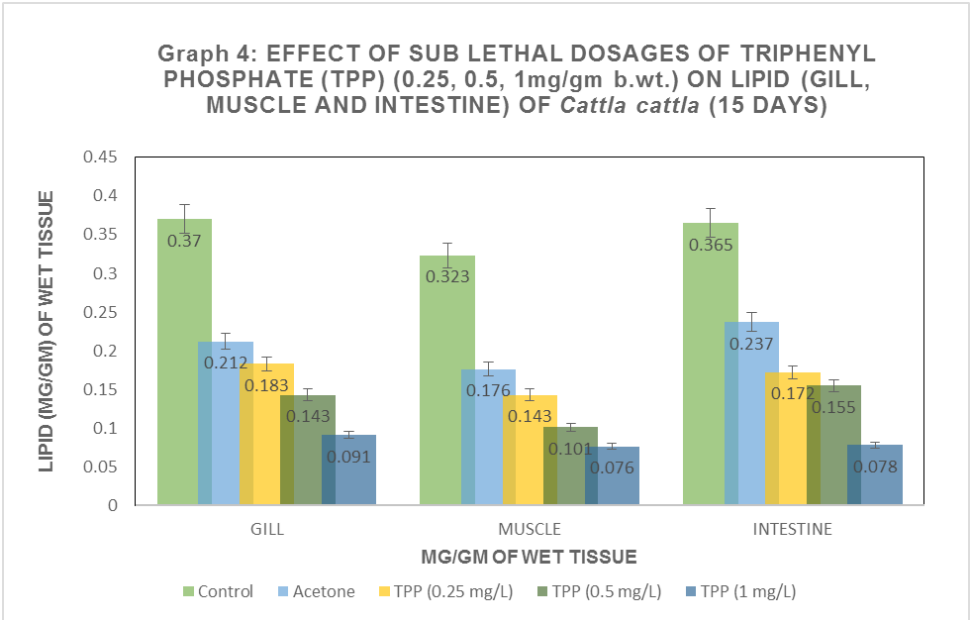
Values are expressed as mean ± standard deviation.
Significantly different from Control at 5% probability level.

group IV treated with TPP (0.5mg/l) the lipid content in the tissue extracts of gill, muscle and intestine was observed as 0.143 mg/g, 0.101 mg/g and 0.155 mg/g respectively. In group V treated with TPP (1 mg/l) the lipid content in the tissue extracts of gill, muscle and intestine was found to be 0.091 mg/g, 0.076 mg/g and 0.078 mg/g respectively (Graph 4). The mean and SD values of group I, II, III, IV and V were found to be significantly different at 5% level (Table 3).

Discussion

During the present investigation TPP caused significant decline in the protein, carbohydrate and lipid levels in tissues of gill, muscle and intestine of *Catla catla* at different sub lethal dosages (0.25 mg/l, 0.5 mg/l, 1 mg/l). The toxicant TPP act as a kind of strees and the organism respond by developing necessary potential occurring in body to give first indi-





cation of stress. During stress an organism needs sufficient energy which is supplied from reserve food materials such as proteins, carbohydrates , lipids, etc., Decrease in protein, carbohydrate and lipid content was observed throughout the exposure period. The sub lethal exposure results shows that there is a progressive decline in the protein, carbohydrate and lipid profiles of *Catla catla*, with respect to increase in concentration of TPP. The toxicity of TPP showed a direct correlation with concentration and time of exposure. Similar observation has been observed by Singh and Bhati (1996), Agarwal, (1992); Vutukuru, (2005), Sastry and Sunita, (1983) and Gill and Pant, (1981).

Conclusion

The present investigation provides a tool for assessing the toxic nature of TPP, which produced pronounced changes in biochemical parameters (proteins, carbohydrates and lipids) in *Catla catla*, with respect to increase in concentration and exposure time period. This change may probably affect the enzyme mediated bio-defense mechanism of these fishes. Hence, it may be concluded that the use of TPP must be checked especially near the water bodies. Further research should focus on the effect of TPP toxicity on *Catla catla,* at cellular and sub cellular levels.

References

Agarwal, S.K. 1992. Sub lethal effect of mercury chloride on some biochemical parameters of the blood in an air breathing fish *Channa punctatus* (Block.). *Journal of Environmental Biology.* 13(2) : 127-133.

Behreus, A. S. and Karbeur, L. 1953. Determination of LC50. *Arch Exp. Path. Pharmacol.* 28 : 177-183.

Fauser, P., Vikelsoe, J., Sorensen, P.B. and Carlsen, L. 2003. Phthalates, nonyl phenols and LASinan alternately operated waste water treatment plant –Fate modelling based on measured concentrations in waste water and sludge. *Water Research.* 37 : 1288-1295.

Folch, J., Lees, S. M. and Sloane – Stanley, G. H. 1957. A simple method for isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 226 : 497 – 508.

Fromme, H., Küchler, T., Otto, T., Pilz, K., Müller, J. and Wenzel, A. 2002. Occurrence of phthalates and bisphenol A and F in the environment. *Water Research.* 36 : 1419-1438.

Gavala, H.N., Alastriste - Mondragon, F. Iranpour, R. and Ahring, B.K. 2003. Biodegradation of phthalate esters during the mesophilic anaerobic digestion of sludge. *Chemosphere.* 52 : 673-682.

Gomez - Hens, A. and Aguilar-Caballos, M.P 2003. Social and economic interest in the control of phthalic acid esters. *Trends in Analytical Chemistry.* 22(11) : 847-857.

Gill, T.S. and Pant, J.C. 1981. Effect of sub-lethal concentration of mercury in a teleost, *Puntius conchonius*. Biochemical and haematological responses. *Journal of Experimental Zoology, India.* 19 : 571-573.

Lowry, O.H., Rosenbrough, N.J., Farr, R.L. and Randall, R.J. 1951. Protein measurement with the Folin Phenol reagent. *J. Biol. Chem.* (193) : 265-275.

Marttinen, S.K., Kettunen, R.H. Sormunen, K.M. and Rintala, J.A. 2003. Removal of bis (2-ethyhexyl) phthalate at a sewage treatment plant. *Water Research.* 37 : 1385-1393.

Rahman, M. and Brazel, C.S. 2004. The plasticizer market : An assessment of traditional plasticizers and research trends to meet new challenges. Prog. *Polymer Science.* 29 : 1223-1248.

Roe, J. R. 1955. The determination of sugar in blood and spinal fluid with anthrone reagent. *J. Biol. Chem.* 20: 335-343.

Sastry, K.V. and Sunita, K. 1983. Enzymological and biochemical changes produced by chronic exposure in teleost fish, *Channa punctatus*. *Toxicology Letters.* 16 : 9-15.

Sears, J.K. and Darby, J.R. 1982. *The Technology of Plasticizers,* John Wiley and Sons, New York.

Singh, S. and Bhati, D.P.S. 1996. Evaluation of liver protein due to stress under 2,4-D. Intoxication in *Channa punctatus* (Bioch). *Bulletin of Environmental Contamination Toxicology.* 53 : 149-152.

United States Consumer Product Safety Commission (CPSC), 2005. Nomination of FR Chemicals for NTP Testing.

Van der Veen I, de Boer, J. 2012. Phosphorus flame retardants: properties, production, environmental occurrence, toxicity and analysis. *Chemosphere.* 88(10) : 1119 – 1153.

Vutukuru, S.S. 2005. Acute effects of Hexavalent chromium on survival, oxygen consumption, hematological parameters and some biochemical profiles of the Indian major carp, *Labeo rohita. International Journal of Environmental Research and Public Health.* 2(3) : 456-462.

Wei, G.L., Li, D.Q., Zhuo, M.N., Liao, Y.S., Xie, Z.Y., Guo, T.L., Li, J.J., Zhang, S.Y. and Liang, Z.Q. 2015. Organophosphorus flame retardants and plasticizers: sources, occurrence, toxicity and human exposure. *Environ. Pollut.* 196 : 29–46.

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.13 / Issue 73 / August / 2022 *International Bimonthly (Print)* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Comparative Analysis of Biochemical Composition of Commercial Freshwater and Marine Fishes from Tiruchirappalli, Tamil Nadu

M.Sivasankari[1] M. Ragavi [1], D. Gayathri[2]* and V. Gokula[3]

[1]II M.Sc., PG and Research Department of Zoology, National College, Trichy, Tamil Nadu, India
[2]Assistant Professor, PG and Research Department of Zoology, National College, Trichy, Tamil Nadu, India.
[3]Associate Professor and Head, PG and Research Department of Zoology, National College, Trichy, Tamil Nadu, India.

Received: 07 May 2022 Revised: 03 June 2022 Accepted: 05 July 2022

***Address for Correspondence**
D. Gayathri
Assistant Professor,
PG and Research Department of Zoology,
National College, Trichy,
Tamil Nadu, India.
Email: gayathridzoo@nct.ac.in

This is an Open Access Journal / article distributed under the terms of the **Creative Commons Attribution License (**CC BY-NC-ND 3.0) which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. All rights reserved.

ABSTRACT

Many parameters impact the biochemical compositions of fishes, including biological state in the life cycle, food patterns, and environmental elements, as well as sex, size, maturation stages, and season, all of which have an impact on the compositions and quality of aquacultured or wild fish. The Present study is carried out to find the biochemical composition of five species of Freshwater fishes namely, *Etroplus suratensis*, *Cirrhinus reba*, *Oreochromismos sambicus, Cyprinus carpio, Channa striata* and 5 species of Marine fishes namely, *Lethrinus nebulous*, *Lutjanus compechanus, Caranx melampygus, Sardinella longiceps,* and *Elops saurus* from Tiruchirappalli, Tamil Nadu. This study aims to estimate the amount of Protein, Glucose, Cholesterol and Urea present in tissues of vital organs like gill, muscle, kidney and liver in this ten species of Freshwater and Marine fishes. Protein content was found to be lowest in muscles of *Sardinella longiceps* (0.148 ± 0.02) gm/ dl and highest in muscles of *Channa striata* (78.973 ± 0.01) gm/dl. Glucose content in liver was estimated highest in *Sardinella longiceps* (208.8 ± 0.02) mg/dl and lowest in kidneys of *Caranxme lampygus* (0.473 ± 0.03) mg/dl. Urea content was predicted to be highest in the muscles of *Sardinella longiceps* (249.51 ± 0.01) mg/dl and lowest in gills of *Elopes saurus* (1.28 ± 0.02) mg/dl. Cholesterol is highest in gills of *Etroplus suratensis* (381.062 ± 0.03) mg/dl and lowest in liver of *Caranxme lampygus* (28.77 ± 0.01) mg/dl. The results revealed that highest amount of protein content present in muscles of *Channa striata* which is a freshwater fish, higher levels of Glucose and Urea were present in liver and muscles of *Sardinella longiceps*, which is a marine fish and highest cholesterol content was estimated in gills of *Etroplus suratensis*, which is a freshwater fish.

Keywords: Biochemical Composition, Protein, Glucose, Urea and Cholesterol.

INTRODUCTION

Fish is a key source of food for humans, supplying a considerable portion of the animal protein diet as well as great dietary supplies of highly unsaturated fatty acids and polyunsaturated fatty acids, particularly the omega-3 fatty acids, eicosapentaenoic acid, and docosahexaenoicacid [1]. Fish intake is becoming more popular as a result of its high polyunsaturated fatty acid content. Furthermore, fish intake has been linked to health benefits, as the long-chain chain polyunsaturated fatty acid has gained attention due to the control of chronic coronary artery disease[2], enhancement of retina and brain development[3], reduced risk of developing breast cancer, rheumatoid arthritis, multiple sclerosis, asthma, psoriasis, inflammatory bowel disease [4], and control of prostaglandin synthesis [5]. A clear idea on the biochemical makeup of fish is highly useful in a variety of situations. Today, there is a growing awareness of the need of eating nutritious foods, and fish is gaining popularity as a result of its unique nutritional benefits. In this context, nutritionists and dieticians must have a thorough grasp of the biochemical elements of fish. Animal meals contain fish and fisheries products. A thorough survey on the biochemical makeup is also an indispensible factor in this situation for the formulation of fishery products and goods.

Another critical area where correct biochemical composition information is required is in the processing and preservation of fish and fishery products. Fish is a perishable item, and quality decline is caused by changes in numerous elements such as proteins, cholesterol, etc. Information on the biochemical ingredients will surely assist a processing technologist in determining the best processing and storage conditions to ensure optimal quality preservation. Hence, the present investigation is done to study the biochemical components of fishes such as proteins, glucose, urea and cholesterol in vital tissues such as gills, muscles, liver and kidney in ten species of fishes (5 Freshwater species and 5 Marine species) from Tiruchirappalli, Tamil Nadu.

MATERIAL METHODS

FISH COLLECTION AND PREPARATION

In December 2021, a total of 200 individual fish, 20 of each species, were caught from four different fish markets in Tiruchirappalli namely, Kallanai, Cauvery Paalam, Gandhi Market and Vengur Market. These species were chosen because of their diverse habitat, year-round availability, and economic significance (www.fishbase.org), According to local fishermen knowledge, as people living around the shoreline have consumed the fish species on a regular basis throughout the years. Ten different species of fishes belonging to freshwater and marine habitat were sampled (Fig 1 & Fig 2). Table 1 contains information about the fish species. The fishes were wrapped in aluminum foil, stored in an icebox, and brought to the Department of Zoology laboratory, National College, Tiruchirappalli, where they were dissected or frozen (–20 C).

BIOCHEMICAL ANALYSIS

The Biochemical Analysis such as quantitative analysis of proteins, glucose, cholesterol and urea were estimated by standard procedures in four tissues of vital organs namely gill, muscle, liver and kidney of the experimental fishes given in Table 1.Glucose estimation was done by Enzymatic (GOD/POD), Photometric, Trinder Reaction, Fixed Time and End Point, Urea was estimated according to Photometric test enzymatic according to modified Berthelot, End point, Cholesterol was estimated according to "CHOD-PAP": enzymatic photometric test, Trinder, End Point and Protein was estimated according to Photometric test corresponding to Biuret method, end point. All the analysis were carried out in ROBONIK semi auto analyser (VER – RBK 2.2.010(ATP1200219RPK)).

QUALITY ASSURANCE AND QUALITY CONTROL

Throughout the experiment, medical gloves, glassware, and metal instruments which were utilised to treat the samples, and the dissecting tools were cleaned three times with distilled water before use.

STATISTICAL ANALYSIS

For statistical analysis and charting, Microsoft Office Excel 2010 and SPSS 16 were used. To investigate interspecies and geographic differences, a one-way ANOVA test was performed at significance level of 0.05.

RESULTS

The Freshwater and Marine fish species used in the present investigation are given below in Table 1. The Glucose content in the tissue extract of liver in *Sardinella longiceps* was estimated to be highest (208.8 ± 0.04) mg/dl and lowest in the tissue exracts of Kidney in *Caranxme lampygus* which was estimated to be (0.473 ± 0.04) mg/dl. The Glucose content measured in the ten species of Freshwater and Marine Fishes were indicated in table 2 and Graph 1 respectively. The Protein Content in the tissue extracts of muscles in *Sardinellalongiceps* was found to be the least (0.148 ± 0.04) gm/dl and reported to be the highest in the tissue extracts of muscles in *Channastriata* (78.973 ± 0.04) gm/dl. The Protein content analysed in the ten species of Freshwater and Marine Fishes were indicated in table 3 and Graph 2 respectively.

The Urea Content was reported to be high in the tissue extracts of muscles in *Sardinella longiceps* (249.512 ± 0.05) mg/dl and lowest in tissue extract of gills in *Elopssaurus* (1.28 ± 0.05) mg/dl. The Urea content analysed in the ten species of Freshwater and Marine Fishes were indicated in table 4 and Graph 3 respectively. The Cholesterol levels were estimated to be highest in tissue extracts of gills in *Etroplus suratensis* (381.062 ± 0.02) mg/dl and lowest in tissue extracts of liver in *Caranxme lampygus* (28.775 ± 0.03) mg/dl. The Cholesterol content estimated in the ten species of Freshwater and Marine Fishes were indicated in table 5 and Graph 4 respectively.

DISCUSSION

The ten Species of fish harvested in local aquatic bodies by fishermen and widely consumed by local rural people in Tiruchirappallimay provide substantial sources of affordable nourishment, particularly for rural people. The nutritional value of various fishes fluctuates, based on their biological contents such as protein, fat, vitamins, and Mineral content. Fish is said to be a great source of omega-3 fatty acids, contains high-quality protein, especially important amino acids, Methionine and lysine. Aside from the excellent nutritional value, In addition to their monetary worth, fish proteins offer beneficial functional qualities, including water-holding capacity, gelling, emulsification, and textural qualities for goods such as fish mince and surimi. Quality criterions like water retention capacity and gelling characteristics is critical to establish the textural properties of the items.

Proteins are necessary for the body's growth and development, as well as its repair and replacement of worn-out tissues. The protein composition of fish varies according to species, nutrient intake, and muscle type. Protein plays a crucial function in biological processes such as metabolic activation, diffusion, and structural support, as well as growth and differentiation regulation.

Protein depletion suggests fast use of energy stores to fulfil the energy demands of the environment, and protein content degradation may be attributed to increased proteolytic activity [6] or to changes in membrane porosity. After different pollutant treatments, there was a decrease in content in *Heteropneustes* fossils and *Anabas testudineus*[7].Fish proteins have a significant health benefits because they include all amino acids in the proper proportions, particularly lysine, as well as sulphur-containing amino acids like methionine and cysteine, which are lacking in plant proteins. In the present investigation, the fresh water fish *Channa striata* had the highest concentrations of proteins in its muscular tissues based on the type of protein distribution in tissues of vital organs like gill, muscle,

liver and kidney among the 10 fishes scrutinized in the current study. The cholesterol content of fish varies according on species and season. Fish, on the other hand, has less fat than red meat. Lipoproteins are the most common and cost-effective type of energy storage in fish, and they may be stored in a variety of organ [8]. The main component of fat is cholesterol, which typically varies from 0.2 to 255. As the fat level increases, so does the water content, and vice versa. It is worth noting that the decrease of cholesterol from the liver, which leads to a rise of cholesterol in the gonads through circulation, is dependent on the spawning phase [9]. In the present investigation, the freshwater fish, *Etroplussuratensis* had the highest concentrations of Cholesterol in its gill tissues based on the type of cholesterol distribution in tissues of vital organs like gill, muscle, liver and kidney among the 10 fishes evaluated in the study.

Monosaccharides are protoplasmic fundamental chemicals that play a role in energy storage and release. They are chemically described as aldehyde or ketone derivatives of higher polyhydric alcohols or substances that give these derivatives upon hydrolysis. The major sugars in animal cells are glucose, fructose, mannose, sucrose, galactose, maltose, lactose, and glycogen. Shobana Manoharan *et al.,* 2017 investigated *Lepidocephalichthys thermalis*, an IUCN least care category consumable freshwater fish with indigenous taste and aroma identified native to Tamil Nadu [10].According to Sumi *et al.,* 2016, seafood is a good source of metabolically important proteins, Glucose, vitamins, trace minerals, and polyunsaturated fatty acids [11].In the present research study, the Marine fish, *Sardinella longiceps* had the highest concentrations of Glucose in its liver tissues based on the type of Glucose distribution in tissues of vital organs like gill, muscle, liver and kidney among the 10 fishes analysed in the study.

The rise in urea level in fishes provides solid evidence for the activation of the second process of ammonia detoxification, ureogenesis by fishes. The current study made on 10 species of both Freshwater and Marine fishes from Tiruchirrappalli, reported an highest concentration of Urea levels in the muscles tissues of Marine fish, *Sardinella longiceps*. The significance of the present study lies in fact that alterations in the level of biomolecules such as Glucose, Protein, Cholesterol and nitrogenous waste material like Urea could be used in risk assessment of pollutant exposure in both Freshwater and Marine ecosystem of Tiruchirappalli, Tamil Nadu.

CONCLUSION

The Present investigation provides a tool for assessing the quality of both Freshwater and Marine ecosystem in Triuchirappalli, Tamil Nadu, which in turn may result in pronounced changes in the biochemical parameters like Protein, Glucose, Cholesterol and nitrogenous waste products such as Urea, with respect to increase in concentration of environmental pollutants and their exposure time periods. Hence, it may be concluded that this changes may probably affect the enzyme mediated bio-defense mechanism of these fish population in the future.

DECLARATION OF COMPETING INTEREST

The authors state that they have no known competing financial interests or personal ties that may seem to have influenced the work described in this study.

ACKNOWLEDGEMENTS

The authors of this paper collectively thank the management National College (Autonomous), Tiruchirappalli , Tamil Nadu, India.

REFERENCES

1. Huynh MD, Kitts DD, Hu C, Trites AW. 2007. Comparison of fatty acid profiles of spawning and non-spawning Pacific herring, Clupeaharenguspallasi. Comparative Biochemistry and Physiology. B.; 146:504-511.

2. Ward OP, Singh A. 2005. Omega-3/6 fatty acids: alternative sources of production. Process Biochemistry;40:3627-3652.
3. Crawford MA. 1993. The role of essential fatty acids in neural development: implications for perinatal nutrition.American Journal of Clinical Nutrition; 57:703S710S.4.
4. Simopoulos AP. 2002. Omega-3 fatty acids in inflammation and autoimmune diseases. Journal of American College Nutrition; 21:495-505.
5. Gibson RA. 1983. Australian fish – an excellent source of both arachidonic acid and 3 polyunsaturated fatty acids. Lipids; 18:743-752.
6. Gita P, Yeragi SG. 1998. Alterations in metabolic constituents in the marine edible Crab, Scylla serrata exposed to ethion. J. Aqua. Biol.; 13(1-2):94-96.
7. GeetaBhaskar. 1997.Effects of alloxan monohydrateadministration on the biochemical Constituents of the fish *Anabas testudineus* (Bloch.) Part I, J. Eco toxicol. Env. Mon.; 7:27-32.
8. Guijarrow AL. Lopez-Patino MA, Pinillos ML Sorna E, De Pedro N. Alonso- Gomez AL *et al.,*2003. Seasonal changes in haematology and metabolic resources in the tench. J. Fish Biol.; 62;803-815.
9. Joshi BD. 1998. Sex relation annual variations in the cholesterol contents of some tissue of a teleost, Wallagoattu, U.P. J. Zool.; 8(2):145-150.
10. ShobanaManoharan, RaghavanKuppu, Ramesh Uthandakalaipandian. 2017. Bioprospecting the Anti-microbial Properties of *Lepidocephalusthermalis* (V.), Journal of Threatened Taxa; 270-277.
11. Sumi ES, Vijayan DK, Jayarani R, Navaneethan R, Anandan R, Mathew S. 2016. Biochemical composition of indian common small pelagic Fishes indicates richness in nutrients capable of Ameliorating Malnutrition and Age –Associated disorders; Journal of chemical biology and therapeutics. 1:2.

Table 1: Depicting Fish Species used in Present Investigation

Species Name	Sample	Habitat
Etroplus suratensis	20	Freshwater
Cyprinus carpio	20	Freshwater
Oreochromis mossambicus	20	Freshwater
Channa striata	20	Freshwater
Cirrhinus reba	20	Freshwater
Elops saurus	20	Marine
Lutianus compechanus	20	Marine
Caranx melampygus	20	Marine
Sardinella longiceps	20	Marine
Lethrinus nebulous	20	Marine

Table 2: Representing Glucose Concentration of Ten species of Marine and Freshwater Fishes

S.No.	Fish Species Name	Glucose Concentration in mg/dl			
		Gill	Liver	Muscle	Kidney
1.	*Sardinella longiceps*	105.739 ± 0.02	208.8 ± 0.04	185.11 ± 0.05	204.327 ± 0.06
2.	*Lutjanus campechanus*	23.2 ± 0.04	33.286 ± 0.05	22.498 ± 0.03	48.082 ± 0.02
3.	*Caranx melampygus*	2.89 ± 0.06	8.294 ± 0.07	6.09 ± 0.05	0.473 ± 0.04
4.	*Lethrinusnebulosus*	59.314 ± 0.02	20.445 ± 0.03	42.612 ± 0.07	35.51 ± 0.06
5.	*Elopssaurus*	23.755 ± 0.04	41.845 ± 0.05	46.759 ± 0.06	14.988 ± 0.02
6.	*Etroplussuratensis*	20.571 ± 0.02	26.906 ± 0.04	7.706 ± 0.03	11.837 ± 0.05
7.	*Cirrhinusreba*	20.506 ± 0.01	6.596 ± 0.03	18.335 ± 0.02	13.388 ± 0.06
8.	*Oreochromismossambicus*	29.731 ± 0.03	21.388 ± 0.02	15.282 ± 0.04	18.498 ± 0.06

9.	*Cyprinuscarpio*	30.645 ± 0.02	45.853 ± 0.04	50.269 ± 0.03	20.024 ± 0.01
10.	*Channastriata*	40.453 ± 0.05	76.69 ± 0.04	35.665 ± 0.03	50.616 ± 0.01

Table 3: Representing Protein Concentration of Ten Species of Marine and Freshwater Fishes

S.No.	Fish Species Name	Protein Concentration in gm/dl			
		Gill	Liver	Muscle	Kidney
1.	*Sardinella longiceps*	0.424 ± 0.02	0.625 ± 0.03	0.148 ± 0.04	0.199 ± 0.05
2.	*Lutjanus campechanus*	0.836 ± 0.06	1.2 ± 0.07	0.309 ± 0.03	1.556 ± 0.02
3.	*Caranx melampygus*	0.972 ± 0.03	0.677 ± 0.02	1.041 ± 0.01	0.877 ± 0.04
4.	*Lethrinus nebulosus*	8.639 ± 0.06	4.373 ± 0.04	0.922 ± 0.03	1.196 ± 0.02
5.	*Elopss aurus*	0.176 ± 0.05	0.484 ± 0.04	0.279 ± 0.02	0.097 ± 0.03
6.	*Etroplus suratensis*	0.745 ± 0.01	6.584 ± 0.02	0.042 ± 0.04	0.409 ± 0.03
7.	*Cirrhinus reba*	0.36 ± 0.03	0.029 ± 0.04	0.091 ± 0.05	0.714 ± 0.02
8.	*Oreochromis mossambicus*	1.149 ± 0.04	1.903 ± 0.05	0.23 ± 0.03	0.922 ± 0.02
9.	*Cyprinus carpio*	0.439 ± 0.03	2.877 ± 0.02	0.395 ± 0.01	1.924 ± 0.05
10.	*Channas triata*	50.744 ± 0.02	40.452 ± 0.03	78.973 ± 0.04	30.638 ± 0.05

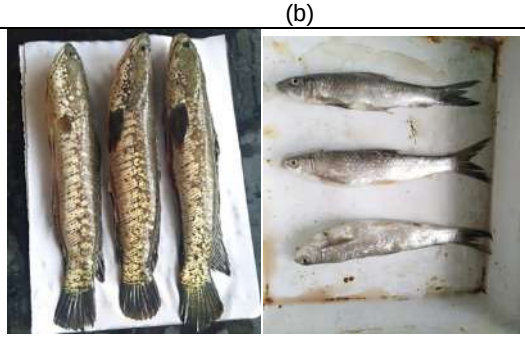
Table 4: Representing Urea Concentration in Ten Species of Marine and Freshwater Fishes

S.No.	Fish Species Name	Urea Concentration in mg/dl			
		Gill	Liver	Muscle	Kidney
1.	*Sardinella longiceps*	179.397 ± 0.04	163.096 ± 0.03	249.512 ± 0.05	201.509 ± 0.06
2.	*Lutjanus campechanus*	6.695 ± 0.07	8.41 ± 0.06	3.213 ± 0.03	10.793 ± 0.04
3.	*Caranx melampygus*	10.024 ± 0.05	71.391 ± 0.06	21.138 ± 0.04	31.172 ± 0.02
4.	*Lethrinus nebulosus*	113.053 ± 0.06	238.678 ± 0.02	185.659 ± 0.01	126.355 ± 0.03
5.	*Elopss aurus*	1.28 ± 0.05	10.43 ± 0.04	3.953 ± 0.03	2.696 ± 0.06
6.	*Etroplus suratensis*	69.431 ± 0.03	71.833 ± 0.02	11.989 ± 0.01	10.658 ± 0.04
7.	*Cirrhinus reba*	19.839 ± 0.02	6.546 ± 0.01	11.198 ± 0.03	6.085 ± 0.06
8.	*Oreochromis mossambicus*	39.865 ± 0.03	40.638 ± 0.04	30.511 ± 0.02	24.412 ± 0.05
9.	*Cyprinus carpio*	7.51 ± 0.05	17.692 ± 0.04	13.757 ± 0.03	21.166 ± 0.02
10.	*Channas triata*	16.071 ± 0.03	14.769 ± 0.02	18.254 ± 0.01	10.22 ± 0.04

Table 5: Representing Cholesterol Concentration of Ten species of Marine and Freshwater Fishes

S.No.	Fish Species Name	Cholesterol Concentration in mg/dl			
		Gill	Liver	Muscle	Kidney
1.	*Sardinella longiceps*	140.266 ± 0.03	137.879 ± 0.02	130.813 ± 0.01	146.051 ± 0.06
2.	*Lutjanus campechanus*	43.187 ± 0.08	99.538 ± 0.05	71.594 ± 0.03	47.113 ± 0.02
3.	*Caranx melampygus*	40.619 ± 0.04	28.775 ± 0.03	99.769 ± 0.02	70.39 ± 0.05
4.	*Lethrinus nebulosus*	180.84 ± 0.06	145.725 ± 0.07	116.567 ± 0.08	122.402 ± 0.02
5.	*Elopss aurus*	142.263 ± 0.01	169.977 ± 0.04	101.617 ± 0.05	139.03 ± 0.03
6.	*Etroplus suratensis*	381.062 ± 0.02	215.038 ± 0.03	215.243 ± 0.04	236.951 ± 0.05
7.	*Cirrhinus reba*	264.203 ± 0.03	294.688 ± 0.04	186.143 ± 0.02	323.788 ± 0.01
8.	*Oreochromis mossambicus*	196.767 ± 0.04	215.243 ± 0.02	142.956 ± 0.01	138.106 ± 0.03
9.	*Cyprinus carpio*	174.365 ± 0.03	291.917 ± 0.02	139.954 ± 0.01	276.674 ± 0.04
10.	*Channas triata*	174.207 ± 0.02	183.869 ± 0.01	161.397 ± 0.03	130.107 ± 0.04

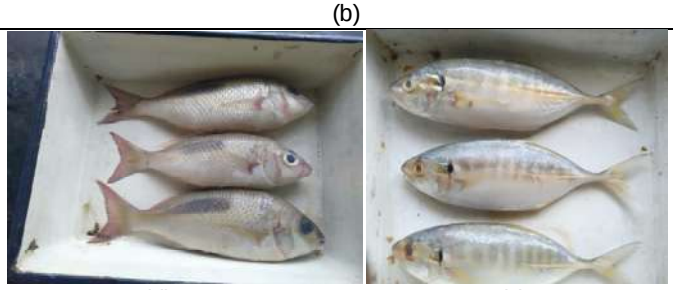
(a) (b) (c)



(d) (e)

Fig. 1: Freshwater fish species collected from different sampling sites –
a) *Etroplus suratensis* b)*Cyprinus carpio* c) *Oreochromismos sambicus* d)*Channa striata* e) *Cirrhinus reba*

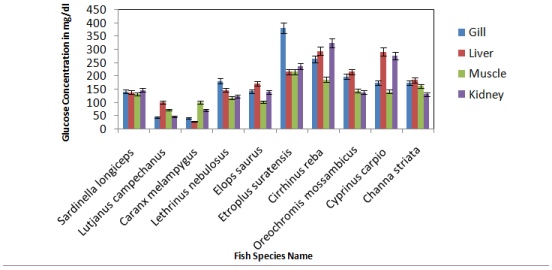
(a) (b) (c)



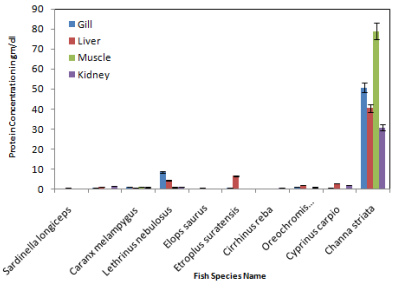
(d) (e)

Fig. 2: Marine fish species collected from different sampling sites –
a)*Elops saurus* b)*Lutianus compechanus* c)*Caranxmelam pygus* d) *Sardinella longiceps* e)*Lethrinus nebulous*

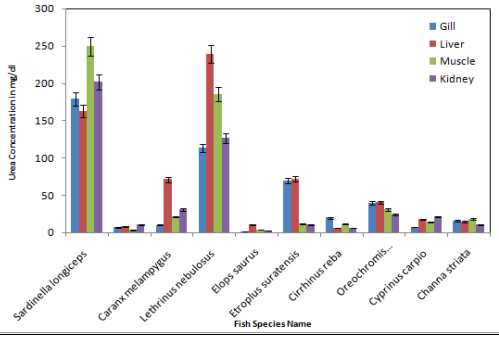
Graph 1: Representing Glucose Concentration in Ten Species of Freshwater and Marine Fishes



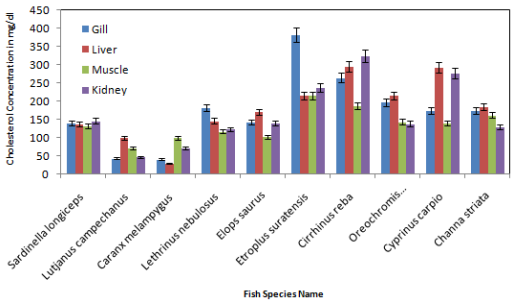
Graph 2: Representing Protein Concentration in Ten Species of Freshwater and Marine Fishes



Graph 3: Representing Urea Concentration in Ten Species of Freshwater and Marine Fishes



Graph 4: Representing Cholesterol Concentration in Ten Speices of Freshwater and Marine Fishes



ISBN: 978-93-91768-31-7

RECENT TRENDS OF INNOVATIONS IN CHEMICAL AND BIOLOGICAL SCIENCES VOLUME I

EDITOR

DR. BASSA SATYANNARAYANA

MR. MUKUL BARWANT

Bhumi Publishing

FIRST EDITION: 2022

RECENT TRENDS OF INNOVATIONS IN CHEMICAL AND BIOLOGICAL SCIENCES

VOLUME - I

(ISBN: 978-93-91768-31-7)

Edited by

Dr. BASSA SATYANNARAYANA

Assistant Professor

Department of Chemistry,

Govt M.G.M P. G. College, Itarsi, MP-461111

E-mail: satyanarayana.bassa@gmail.com

Mr. MUKUL BARWANT

Assistant Professor

Department of Botany,

Sanjivani Arts Commerce and Science College,

Kopargaon, Ahmednagar, Maharashtra, India

E-mail: mukulbarwant97@gmail.com

Bhumi Publishing

2022

CONTENT

Sr. No.	Book Chapter and Author(s)	Page No.
1.	**ETHNOMEDICINAL PLANTS USED FOR RESPIRATORY DISORDERS IN REASI DISTRICT, JAMMU AND KASHMIR (INDIA)** Tarun Thakur and Bharty Kumar	1 – 6
2.	**INORGANIC METAL OXIDE-POLYMER NANOCOMPOSITES FOR NEAR INFRA-RED (NIR) REFLECTIVE COATING APPLICATIONS** Giable George	7 – 13
3.	**BIOLOGICAL ACTIVITY OF TRADITIONAL INDIAN MEDICINAL PLANTS** D. Gayathri	14 – 48
4.	**ROLE OF SACRED GROVES IN BIODIVERSITY CONSERVATION WITH SPECIAL REFERENCE TO UTTARAKHAND** Satya Raj Singh	49 – 55
5.	**QUALITATIVE EVALUATION OF VARIOUS EXTRACTS OF MEDICINALLY RICH PLANT- *COMMIPHORA MUKUL* RESIN** Shweta Hingwasiya and Shailbala Baghel	56 – 62
6.	**TOXIC EFFECTS OF ENVIRONMENTAL CHEMICAL MIXTURES** Anita Singh	63 – 72
7.	**PHARMACOLOGICAL POTENTIAL OF SELECTED PLANTS OF EUPHORBIACEAE FAMILY** Reena Gami	73 – 78
8.	**PHYTOCHEMISTRY AND APPLICATIONS OF ACTIVE INGREDIENTS OF MEDICINAL PLANTS** Yogesh Chandra Nainwal and Tarun Mohan	79 – 85
9.	**INTRODUCTION TO MEDICINAL CHEMISTRY - AN OVERVIEW** Sarabjot Kaur Makkad	86 – 91

Chapter 3

BIOLOGICAL ACTIVITY OF TRADITIONAL INDIAN MEDICINAL PLANTS

D. GAYATHRI

Department of Zoology, National College, Trichy, Tamil Nadu, India

Corresponing Author E- Mail: gayathridzoo@nct.ac.in

ABSTRACT

Herbal medicines and phytomedicine have been employed in healthcare systems, since ancient times. Various nations, ethnic groups, and cultures from throughout the world have traditionally employed medicinal herbs. Plants are a potential source of therapeutic compounds with great pharmacological value and significant application in the advancement of healthcare. In both developing and developed countries, the use of herbal medicines to treat many human ailments has enormous potential. Numerous indigenous medicinal plant varieties offer protection against a variety of serious ailments. Secondary metabolites from plants are also becoming quite important in the pharmaceutical and nutraceutical sectors. Products made from traditional system of medicinal plants are reasonably priced, have few adverse effects, and pose little danger. In reality, plants deploy secondary metabolites as defense mechanisms. It strengthens human immunity while also providing defense against bacteria, viruses, parasites, and other serious disorders. It recognizes dangerous compounds entering human body from external environment and destroys them. The plants that are historically utilized for illness prevention and treatment are the subject of the current research. In India, there is a large supply of these medicinal plants with a variety of claimed pharmacological effects. This chapter focuses on those indigenous medicinal plants that can provide defense against a range of human illnesses. A sizable portion of the world's population may find this chapter useful in gaining insight about naturally occurring preventative and therapeutic substances in the traditional medicinal plants of India.

KEYWORDS: Medicinal plants, Phytochemicals, Herbal medicine, Pharmacological properties.

INTRODUCTION

Plant-derived therapeutic items are the most popular, accepted, and acknowledged type of medicine in today's human society worldwide. The basic sources of herbal, pharmacological, and nutraceutical formulations are phytocompounds, which are created by plants in a variety of ways from primary metabolites. These plant's therapeutic and nutritional needs are met by a variety of phytomolecules that have specific biological effects on the human organ system. Different plant components are used by people to create phytomedicine, which serves as a

remedy for contemporary cultures, civilizations and society. Different traditional medicinal plants would be the finest alternative source to acquire high-quality and essential herbal formulations for the treatment and prevention of many ailments [1, 3]. Indian medicinal plants have long been considered a viable source for the treatment of several ailments [4]. Ethno botanicals, natural materials, and their derivatives have demonstrated potential efficacy in the treatment of variety of disorders [5]. Consuming fruits, vegetables, ethno medicinal plants, or their products, in a very efficient manner, generates the defenses against many diseases, since medicinal plants are an incredible source of various phytochemicals, including flavonoids, tannins, terpenoids, polyphenols, steroids, alkaloids, glycosides, chlorophyll, carotenoids, proteins, minerals, and vitamins, which have potent antioxidant properties as well as other biological activities [6, 7]. Around the world, more than 80,000 different species of plants are utilized as medicines, and the majority of them are passed down orally from generation to generation. It implies that the foundation of traditional or folklore remedies is the use of medicinal herbs. Traditional medicinal herbs are currently receiving major attention from the mainstream medical community or healthcare delivery system. Many people in underdeveloped or developing nations continue to use traditional medicines as their main source of treatment for a variety of maladies. These indigenous medicinal herbs are widely utilized as a strong source of alternative medicines to treat a variety of medical conditions. The mainstays of the primary healthcare system for humans are the medicinal plants, due to their accessibility, acceptance, compatibility and cost [8, 9]. In order to create safe and effective herbal formulations, medicinal and aromatic plants are important and significant sources of primary and secondary metabolites that are utilized as templates for lead optimization procedures [10].

Numerous medical professionals and researchers frequently suggest using numerous medicinal plant species or their derivatives to gain protection from a variety of health issues due to their curing potential, ease of availability and cost-effectiveness. The plants examined in this chapter have a long history of use as medicines and are abundant in a variety of bioactive compounds. These herbs are extremely effective against a number of life threatening illnesses, including bacterial and viral infections, diabetes, renal and lung cancer [11, 12]. Hundred species of Indian traditional medicinal plants (herbs, shrubs and trees) are being compiled through this chapter and are shown in the accompanying table. These are incredibly powerful for both preventing and curing a variety of illnesses. By using contemporary pharmacological technology and procedures, these medicinal plants can be exploited to create herbal medicines and efficient nutraceuticals.

Table 1: Some Important Indian Traditional Medicinal Plants and Their Healing Properties against Various Human Diseases

Sr. No.	Botanical Name	Family	Common Name	Phytochemical compounds	Ethnomedicinal Uses	Pharmacological Activities
1	*Ocimum sanctum*	Lamiaceae	Holy basil	Phenolics, flavonoids, phenyl propanoids, terpenoids, fatty acid, ursolic acid, apigenin, luteolin, steroids, eugenol, and carvacrol.	It is used for nausea, cold and flu, fever, indigestion, stress, anxiety, earache, bronchitis, malaria, diabetes, asthma, heart disease, snakebite antidote, hepatitis, tuberculosis, genitourinary disorders, migraine, acne, Anti-aging purposes.	Antimicrobial Antioxidant Anti-tussive Anti-inflammatory Anti-diabetic Anti-pyretic Anti-arthritic [13 14]
2	*Azadirachta indica*	Meliaceae	Neem	Phenolic compounds, alkaloids, glycosides, terpenoids, flavonoids, azadirachtin, nimbin, azharon, nimbidol, azadirone, melianol, steroids, and tannins.	This plant parts are used for the treatment of rheumatism, asthma, fever, worm infestations, bacterial infection, tuberculosis, diarrhea, jaundice, dysentery, to promote healing, measles, smallpox, inflamed gums, urinary Diseases.	Antimicrobial Anti-cancer Anti-fertility Anti-inflammatory Anti-malarial Antioxidant Neuro-protective Insect repellent [15]
3	*Mentha piperita*	Lamiaceae	Mint	Flavonoids, carbohydrates, alkaloids, phenols, coumarin, saponin, steroids, essential oils (menthol, menthone, pulegone, Menthofuran) azulenes, cholene, carotenes and tannins.	It is used in indigestion, diarrhea, hyperacidity, anemia, morning sickness, bad breath and gum problem, Irritable bowel syndrome, tuberculosis, bronchitis, eczema, acne, anxiety, Crohn's disease, colitis, gallbladder, liver Complaints.	Antibacterial Antioxidant Anti-inflammatory Anti-pyretic Anti-cancer Anti-parasitic Anti-diarrheal Anti-tussive [16]

4	*Zingiber officinale*	Zingiberaceae	Ginger	Phenolic compounds, flavonoids, steroids, triterpenoids, glycosides, tannins, saponins, proteins, carbohydrates, amino acids and proteins,volatile oil.	This plant is used to treat stomach upset, nausea, vomiting, nose bleeds, rheumatism, coughs, chest congestion, cholera, cold, diarrhea, dropsy, stomachache, and baldness,snakebite and toothache.	Analgesic Anthelmintic Anti-arthritic Anti-cancer Anti-diabetic Anti-hypertensive Antimicrobial Antioxidant [15]
5	*Allium sativum*	Amaryllidaceae	Garlic	Alkaloids, glycosides, saponins, flavonoids, steroids, proteins, carbohydrates, oils, reducing sugars and acidiccompounds.	It is used as natural immunity booster, reduces high blood pressure, diabetes, cardiovascular diseases, and reduces stress, cure nerve problems, skin darkening, and blisteringpurposes.	Antioxidant Anti-cancer Anti-inflammatory Antimicrobial Anti-diabetic Atherosclerosis Anti-hypertensive [13]
6	*Curcuma longa*	Zingiberaceae	Turmeric	Carbohydrates, protein, curcumin, tannins, alkaloids, saponins, flavonoids, terpenoids and cardiac glycosides, coumarins, and steroids.	It is used to cure burns, cuts, improves digestion, dissolves gallstone, relieves arthritis, prevents cancer, helps in Alzheimer, and inhibits bacteria, virus, fungi, and parasites, cures acne, pimples, allergy, andpores.	Antioxidant Antimicrobial Antiseptic Anti-inflammatory Anti-carcinogenic Anti-allergic Anti-diabetic [13]
7	*Allium cepa*	Amaryllidaceae	Onion	Flavonoids, carbohydrates, glycosides, proteins, alkaloids, saponins, acid compounds, reducing sugars, oils and tannins.	It is mainly used for common cold, normalizes blood pressure, prevent diarrhea, inhibits microbial infection, increases appetite, treats diabetes, inhibits cancer cell growth, stimulates respiratory tract,helpful in swollen feet, hair growth	Anti-diabetic, Anti-cancer Antimicrobial Antioxidant Anti-platelet Anti-inflammatory Anti-thrombotic Anti-hypertensive [13]

8	*Spinacia oleracea*	Amaranthaceae	Spinach	Flavonoids, carotenoids, β-carotene, apocyanin, ascorbic acid, proteins, amino acids, steroids, tannins, carbohydrates, anthroquinones, coumarin, and saponins.	It is used in digestion, liver and kidney diseases, diuretic, laxative, inflammation of the lungs, bowels, sore throat, ring worm scabies, constipation, anemia, diabetes, fever, brain and heart disease, urinary infection, gallstones.	Anti-diabetic, Antioxidant Anti-inflammatory Anti-carcinogenic Antimicrobial Protection against Gamma Radiation Hepato-protective [17]
9	*Carica papaya*	Caricaceae	Papaya	Flavonoids, papain, tannins, saponins, alkaloids, phenolic compounds and phytosterols, alkaloids, saponins, and glycosides.	It prevents dengue fever, cancer cell growth, facilitates digestion, menstrual pain, anti- malarial, and helps in IBS, used in skin problems, arthritis, dressing wounds, and cure dyspepsia.	Wound healing Nephro-protective Anti-inflammatory Anthelmintic Antimicrobial Anti-diabetic Analgesic [13]
10	*Actinidia deliciosa*	Actinidiaceae	Kiwi	Flavonoids, polyphenols, anthocyanin, carotenoids, alkaloids, tannin, proteins, triterpenoids, carbohydrate, and minerals.	Kiwi is used for good digestive health, hair growth, and strong bone, in eye disease, pregnancy period, stress, high blood pressure, cancer, to reduce stone in kidney, lower blood Sugar level, and better sleep.	Anti-diabetic Hepato-protective Dermatological Activity Antioxidant Anti-inflammatory Cardio-protective ACE Inhibitor [18]
11	*Camellia sinensis*	Theaceae	Green tea	Flavonoids, tannins, caffeine, polyphenols, boheic acid, theophylline, theobromine, anthocyanins, and gallic acid.	Green tea helps in weight loss and metabolism, lowers blood sugar level, used in heart disease, esophageal cancer, high cholesterol, Alzheimer's and Parkinson's disease, helps in depression, good sleep, tooth decay, to reduce wrinkles and signs of aging.	Antioxidant Anti-carcinogenic Anti-arteriosclerotic Antimicrobial Anti-cancer Anti-diabetic Weight control Anti-hypertensive Cardio-protective [19,20]

12	*Prunus dulcis*	Rosaceae	Almonds	Flavonoids, carotenoid, phenolics, tannins, lignans, anthocyanins, phytosterols, polyphenols, and fatty acids.	Almond has memory improving activities, used for insomnia, headache, respiratory troubles, colic pain and peptic ulcer disease, constipation, enhances glow and fairness of skin, cures hair fall, itching, used in kidney stone, ladder, and strengthening of tooth.	Antioxidant Anti-aging Hepato-protective Memory Improving Anxiolytic Activity Hypoglycemic Anti-inflammatory Immuno-stimulant [21]
13	*Cuminum cyminum*	Apiaceae	Cumin	Alkaloid, coumarin, anthraquinone, flavonoid, glycoside, protein, resin, saponin, tannin and steroid.	It is used against jaundice, lung diseases, nosebleeds, menstrual pains, remedy for colic or diarrhea, digestive problems, stimulates saliva production, excretion of bile, increase production of breast milk, used in menstrual cramp, inhibit formation of tumors, lowers blood sugar, to treat insomnia, fever.	Antimicrobial Anti-inflammatory Antioxidant Anti-diabetic Anti-platelet Anti-cancer Hypotensive Bronchodilatory Anti-amyloidogenic Anti-osteoporotic [13]
14	*Marsilea quadrifolia*	Marsileaceae	Marsilea	Polyphenols, tannins, saponins, flavonoids, steroids, terpenoids, alkaloids, carbohydrates, and proteins.	It is used to treat cold, respiratory troubles, hypertension, insomnia, anemia, in migraine, epilepsy, diarrhea, bronchitis, hepatitis, to treat snake bite, skin diseases, and inflammation, enhances memory, lowers blood sugar.	Anti-tussive Analgesic Anti-diabetic Anti-pyretic Antimicrobial Anti-tumor Anti-inflammatory Hepato-protective Antioxidant [22]

15	*Nigella sativa*	Ranunculaceae	Black cumin	Alkaloids, carboxylic acid, coumarins, phenol, resin, saponin, and steroid, protein, fat, and carbohydrates.	It has been used for the treatment of respiratory problems, digestive tract, and kidney, liver function, cardiovascular system, asthma, inflammatory diseases, externally its oil is used as antiseptic, anesthetic, to stop vomiting, in dropsy, and in treatments of worm and skin eruptions.	Antimicrobial Anti-inflammatory Anti-hyperlipidemic Anti-cancer Anti-diabetic Anti-hypertensive Wound healingactivity Gastro-protective Antioxidant Analgesic [13]
16	*Lactuca sativa*	Asteraceae	Lettuce	Phenols, flavonoids, triterpenoid, saponins, anthocyanin, β-carotene, ascorbic acid, riboflavin, niacin, carotine, iodine, andfluorine.	The milky leaves of the plant contain 'lactucarium' is used in medicine for its anodyne, digestive, diuretic, hypnotic, narcotic, sedative, to treat insomnia, anxiety, neuroses, dry cough, pain, drowsiness, preventsmicrobial attacks.	Hypoglycaemicactivity Anxiolytic effect Sedative Effect Anti-inflammatory Antioxidant Anti-aging Antimicrobial [23]
17	*Citrus limon*	Rutaceae	Lemon	Alkaloids, flavonoids, limonoids, steroids, terpenoids, carotenoids, tannic acids and phenolic compound.	Lemon is active against bacteria, reduces inflammation, against fever, prevents scurvy, ulcer, urinary diseases, an antidote against poison, prevents bad breath, body odor, lowers blood pressure, vomiting, liver disorder, enhances immunity, for glowing skin.	Antioxidant Anti-scorbutic Anti-migraine Diuretic Anti-inflammatory Antimicrobial Anti-carcinogenic Anti-pyretic Hypertension Antidote Anti-ulcer [24]

18	*Piper nigrum*	Piperaceae	Black pepper	Alkaloids, flavonoid, cardiac glycosides, piperine, piperatine, piperidine, protein, carbohydrats, essential oils, and tannins.	It prevents asthma, arthritis, bronchitis, used in infection that causes diarrhea, headache, stuffy nose, sinus infection, weight loss, menstrual pain, cancer, nerve pain, itchy skin, and people inhale pepper oil to prevent falls, to help quit smoking.	Antioxidant Antimicrobial Anti-inflammatory Anti-thyroid Anti-tumor Anti-asthmatics Anti-diarrheal Anti-depressants [13,15]
19	*Mangifera indica*	Anacardiaceae	Mango	Alkaloids, flavonoids, saponins, tannins, phenols, ascorbic acid, carotenoids, glycosides and phytosterols.	The leaves are used in the burns and scalds; chewed to strengthen the gums, used for skin ailments, asthma and cough, bark is used for the treatment of fever cholera, rheumatism, to treat ulcerated tongue.	Analgesic Antimicrobial Anti-convulsant Anti-inflammatory Anti-cancer Gastro-protective Hepato-protective [13,15]
20	*Malus domestica*	Rosaceae	Apple	Phenolics, flavonoids and carotenoid, saponin, glycosides and steroid, carbohydrates, and proteins.	Used in kidney stones, constipation, blood formation, diarrhea, high blood pressure, cardiac problems, useful in sore eyes, inflammation, Alzheimer's, cancer, diabetes, gallstone, obesity loss.	Antioxidant Antimicrobial Anti-inflammatory Anti-diabetic Anti-depressant Anti-asthmatic Anti-cancer [13]
21	*Musa paradisiaca*	Musaceae	Banana	Alkaloids, flavonoids, tannins, phenolic compounds, carotenoids, anthocyanins, and cardiacglycosides.	It cures depression, treats emotional sickness, increase blood and cures anemia, reduces risk of blood pressure, menstrual cramp, helps to reduce morning sickness, constipation, ulcers, mosquito bites.	Anti-diabetic Anti-hypertensive Antioxidant Anti-diarrheal Antimicrobial Anti-cancer Anti-ulcer [13]

22	*Coriandrum sativum*	Apiaceae	Coriander	Flavanoids, alkaloids, tannins, saponin, terpenoids, sterol, carbohydrate, protein, and fat.	It treats indigestion, worm infections, rheumatism, convulsion, insomnia, anxiety, pain in the joints, used as a diuretic plant, in skin disease, effective in constipation, irritable bowel syndrome, diarrhea, measles, toothaches, nausea, hernia.	Antioxidant Diuretic Anti-diabetic Sedative Antimicrobial Anti-convulsant Anthelmintic Anti-mutagenic [13]
23	*Crinum asiaticum*	Amaryllidaceae	Spider Lily	Alkaloids, flavonoids, tannins, phenols, cardiac glycosides, triterpenes, steroids, saponins, hamayne, palmilycorine, lycoriside, ambelline, crinine, powelline, and ungeremine.	It is used to treat injury and inflamed joints, carbuncles and cancer, to treat wounds by poisoned arrows, bites and stings, swellings, fever, inflammation, piles, to stop bleeding and to treat gonorrhea, induces vomiting, toothache, ulcers.	Analgesic Antimicrobial Anti-inflammatory Antiviral Anti-cancer Anti-tumor Anti-mitotic membrane stabilizing [13, 15]
24	*Cicer arietinum*	Fabaceae	Chick Peas	Alkaloids, carbohydrates, proteins, amino acids, fixed oils, phytosterols, phenolic compounds and tannins, flavonoids, glycosides, saponins, and amino acids.	Used for insufficient milk or sperm, kidney stones, urine problems, menstruation, treats bronchitis, sunstroke, snake bites, diabetes, hypertension, itchy skin, tumor, increases bone health, controls blood pressure, prevent cancer, develops memory and thinking power.	Antioxidant Anti-hypertensive Anti-cancer Anti-inflammatory Antimicrobial Anti-diabetic Anti-pyretic Anti-cancer Estrogenic [13]

25	*Ipomoea aquatica*	Amaranthaceae	Water spinach	Flavonoids, amino acids, alkaloids, lipids, steroids, saponins, phenols, reducing sugars, tannins, β-carotene, glycosides, and minerals.	It helps to reduce cholesterol, treats jaundice and liver damages, anemia, useful for indigestion, constipation, diabetes, prevents heart attack, lowers blood pressure, boosts immunity and treats ulcer, menstruation pain, fever, rejuvenates skin.	Antioxidant Anti-inflammatory Antimicrobial Anti-diabetic Anti-diarrheal Antiseptic Anti-cancer [13]
26	*Citrus aurantiifolia*	Rutaceae	Lime	Alkaloid, glycoside, saponin, tannin, flavonoids, steroids, terpenoids, limonene, pinene, sabinene, protein, ascorbic acid and polyphenols.	It is used in headaches, cold, fever, insomnia, indigestion, to treat diarrhea, chest pain, snake bite, cancer, itching, dandruff, skin diseases, to glow skin, boiled root with alcohol is drunk to abort foetus, It prevents from microbial attack, allergy.	Antimicrobial Anti-cancer Antioxidant Immuno-modulatory Anti-obesity, Anti-fertility Anthelmintic Anti-pyretic Hepato-protective Cardio-protective [24]
27	*Citrus sinensis*	Rutaceae	Orange	Phenolics, flavonoids, carbohydrates, alkaloids, tannins, fixed oils and lipids, sugars, proteins, terpenoids, steroids, and amino acids, ascorbic acid and carotenoids.	The fruit is appetizer and blood purifier, used to treats bilious diarrhea, acne, skin disease, itching, obesity, enlarged spleen, constipation, coughs, dyspepsia, prevents digestive tract ailments, nerve disorder, blood pressure, it relieves headache and cures rheumatism.	Antimicrobial Anti-parasitic Anti-proliferative Insecticidal Relaxant, Sedative Anxiolytic Protective of UV Anti-osteoporotic Anti-obesity Hypo- cholesterolemic Antioxidant [24]

28	*Aegle marmelos*	Rutaceae	Stone apple	Phenols, flavonoids, alkaloids, cardiac glycosides, saponins, terpenoids, steroids, carbohydrate, tannins, and proteins.	Fruits and leaves are used for wound healing, curing digestive disorders, ulcers, headache, hypertension, diabetes, promotes digestion, fight against cholera, diarrhea, to cure constipation, scurvy, skin disorders, liver, heart, kidney problems.	Antimicrobial Anti-cancer Anti-inflammatory Laxative, Hepato-protective Insecticidal Anti-diabetic Anti-diarrheal Anti-ulcer Antioxidant [25]
29	*Petroselinum crispum*	Apiaceae	Parsley	Flavonoids, volatile oils, proteins, minerals, carotenes, carotenoids, ascorbic acid, coumarins, tocopherol, myristicin, essentials oils and phenolic compounds, Vitamin-A, B and C.	Used to treat asthma, bladder infections, bruises, cough, cracked or chapped skin, digestive problems, swelling, insect bites, Prevents tumor, liver disorder, kidney stones, high blood pressure, diabetes, obesity, anemia, osteoarthritis.	Antioxidant Anti-diabetic Anti-platelet Antimicrobial Hypotensive Anti-pyretic Gastro-protective Cyto-protective Laxative Analgesic [26]
30	*Cymbopogon citratus*	Gramineae	Lemon Grass	Alkaloids, glucosides, phenols, saponins, flavonoids, tannins, terpenoids, resins, citral, citronellal, cymbogonol, α-terpineol, citronellic acid, α-camphorene, geranial, isoscoparin.	Used to treat digestive problems, cramping pains, coughs, fever, to reduce swelling, improve blood circulation, bladder problems and leprosy, oil is used as an insect repellent, detergent, carminative for cholera.	Analgesic Anthelmintic Antimicrobial Anti-neoplastic Anti-malarial Antioxidant Hepato-protective Hypoglycaemic Radio-protective [13,15]

31	*Aloe barbadensis*	Asphodelaceae	Aloe vera	Flavonoids, alkaloids, aloin, carbohydrate, tannin, steroid, triterpenoids, glycosides, saponins, salicylic acids, amino acids, and beta- carotene.	Treatment of pimples, acne and mouth ulcers, laxative, wash for piles, burns, edema, pain, swellings and wounds, Juice used to increase menstrual flow and for eye disease, used in case of any type of skin problem.	Angiogenic Anti-inflammatory Anti-cancer Antimicrobial Antioxidant Dermatitis Gastric mucosal Wound healing [15]
32	*Capsicum annuum*	Solanaceae	Chili	Flavonoids, carotenoids, phenolics, capsaicin, solasdine, scopoletin, capsaicinoids, and ascorbic acid.	It is used to cure toothache, also used to increase blood circulation and stimulate gastric activities, used to treat arthritis, neuralgia, lumbago and chilblains.	Antimicrobial Anti-cancer, Antioxidant Anti-protozoal Immuno-modulatory Pesticidal [15]
33	*Ananas comosus*	Bromeliaceae	Pineapple	Flavonoids, saponins, tannins, alkaloids, terpenoids, anthraquinones, carbohydrate, steroids, phenols and glycoside.	Pineapples are rich in nutrients, low in calories, fights with oxidative stress, to treat indigestion, reduce cancer, boosts immunity, suppress inflammation, to recover surgery, kill parasites, gout, bronchitis, ulcer, cystic fibrosis.	Anti-inflammatory Hypolipidemic Laxative, Diuretic Antimicrobial Anti-obesity, Anti-diarrheal Antioxidant [13]
34	*Centella asiatica*	Apiaceae	Pennywort	Flavonoids, tannins, alkaloids, carbohydrate asiaticoside, asiatic acid, brahmic acid, brahmoside, asiaticin, centellicin, terpenoids, sterols, triterpenoids and saponins.	Used to cure diarrhea, diuretic, gravel, leprosy treatment, stones, wound healing the plant is used in cooling drinks when boiled, treatment of nervous system, skin, and blood diseases, treatment for cancer, circulatory stimulant, headache, urinary discharges, peptic ulcer and sore throat.	Antimicrobial Anti-depressant Antioxidant Anti-thrombotic Anxiolytic Gastro-protective Immuno-modulatory Nerve-regenerative Wound healing [13, 15]

35	*Agaricus bisporus*	Agaricaceae	Mushrooms	Alkaloids, flavonoids, phenols, tannins, saponins, terpenoids, cardiac glycosides, proteins, carbohydrates, fibers, and ascorbic acid.	It is effective in diabetes, cancer, hardening of arteries, to cure digestive problems, ulcer, hepatitis B, colitis, high cholesterol, osteoporosis, asthma.	Anti-cancer Anti-diabetic Antimicrobial Antioxidant Anti-ulcer Anti-hepatitis Hepato-protective [27]
36	*Trigonella foenum-graecum*	Fabaceae	Fenugreek	Flavonoids, alkaloids, terpenoids, steroids, saponins, anthocyanins, tannins, volatile oils, phenol, carbohydrates, fat, amino acids and quinones.	It is used to lower blood sugar, reduce menstrual cramps, sexual problems, used as antacid, treats dysentery, stomach disturbances, respiratory infections, fever, and hormonal disorders, induce labor pain, and inducelactation, hair growth.	Carminative Gastric stimulant Anti-diabetic Hypolipidemic Anti-ulcer Antimicrobial Antioxidant Anti-inflammatory Antacid [28]
37	*Chrysanthemum morifolium*	Asteraceae	Chrysanthemum	Alkaloids, phenols, flavonoids, caffeoylquinic acids, terpenoids, aardiac glycosides, steroids, tannins and saponins.	It is used to cure chest pain, diabetes, and fever, cold, swelling, cancer, to treat headache, inflamed eyes, and pain in the throat, boil syndrome, vertigo, tonic, urinary stones, and swelling feet.	Antimicrobial Antioxidant Anti-cancer Anti-inflammatory Anti-tussive Anti-ulcer Anti-diabetic Gastro-protective [29]

38	*Phyllanthus emblica*	Phyllanthaceae	Indian Gooseberry	Falvonoids, saponins, tannins, cardiac glycosides, alkaloids, anthraquinones, phlobotanins steroids, andcarbohydrates.	This plant is used for the treatment of jaundice, inflammation, diabetes, cough, asthma, bronchitis, dyspepsia, anemia, cures colic, peptic ulcer, skin diseases, cardiac disorders, dizziness, snake bite, vomiting, and gonorrhea.	Antimicrobial Laxative Anti-diabetic Anti-diarrheal Analgesic Anti-pyretic Hepato-protective Cardio-protective Anti-cancer[13]
39	*Boerhaavia diffusa*	Nyctaginaceae	Punarnava	Phenols, flavonoids, terpenoids, reducing sugar, ligans, organic acids, boeravinones, flavone, isoflavone, flavonol, xanthone, lignin, purine nucleoside, and sterols.	It is beneficial in treating obesity, dropsy, liver diseases, bacterial, fungal infections, intestinal worms,and bronchial tubes, treats asthma, ulcers, skin diseases, swelling, anemia, nervous weakness, paralysis, constipation, cough, mild laxative, and improves appetite, cure jaundice.	Laxative Cardio tonic Stomachic Anthelmintic Anti-inflammatory Antimicrobial Anti-metastatic Immuno-modulatory Anti-proliferative Anti-estrogenic Gastro-protective [30]
40	*Coccinia grandis*	Cucurbitaceae	Ivy Gourd	Alkaloids, glycosides, saponin, amyrine, lupeol, cucubbitacin, cephalandrol, cephalandrine, flavonoids, saponin and polyphenols.	It is useful treatment for diabetes, cancer, eye problems, skin eruptions, it is laxative, treats headache, rheumatism, high blood pressure, endocrine system disorder, stimulate weight loss, malaria, helps in constipation, hypertension, skin wounds, infections.	Anti-pyretic Anti-ulcer Antioxidant Hepato-protective Anti-cancer Anti-malarial Anti-diabetic Analgesic Anti-dyslipidemic [15]

41	*Nyctanthes arbor-tristis*	Oleaceae	Night-flowering Jasmine	Flavonoids, terpenoids, saponins, steroids, carbohydrates, cardiac glycosides, alkaloids, proteins and tannins.	It is used in various ailments like fever, enlargement of spleen, malaria, blood dysentery, gastritis, anemia, also used to treat infection of scalp, piles, skin disease, and stem bark cures rheumatic joint pain, eye disease, internal injury, diarrhea, other infections.	Anti-allergy Anti-inflammatory Anti-filarial Antimicrobial Antioxidant Anti-cancer Anti-diabetic Anti-malarial Anti-leishmanial [31]
42	*Euphorbia hirta*	Euphorbiaceae	Asthma Weed	Alkaloid, flavonoids, saponins, terpenoids, tannin, polyphenols, sugar, amino acids, euphorbon, and euphosterol.	It is used to cure asthma, calculus, cough, eruptions, headache, hypertension, measles, nausea, stomachache, warts and wounds, athlete's foot, dysentery, enteritis, fever, gas, itch, skin conditions.	Analgesic Antimicrobial Anti-diarrheal Anti-inflammatory Anti-platelet Anxiolytic Gastro-protective Molluscicidal [32]
43	*Moringa olifera*	Moringaceae	Drumstick	Alkaloids, flavonoids, steroids, tannins, saponins, glycosides, carotenoids, ascorbates, tocopherols, beta-sitosterol, moringine, and kaempferol.	It is used to treat ear infection, eye infection, toothache, cold, HIV, male impotency, blood sugar, it helps to treat high blood pressure, fever, typhoid, malaria, indigestion, diarrhea, boost immunity, cure skin diseases, treats snake bite, improves lactation in mother.	Oxidative DNA damage Cardio-protective Anti-proliferation Hepato-protective Hypolipidaemic Hypercholesterolemic Antioxidant Anti-inflammatory Antinociceptive Antimicrobial [33]

44	*Hygrophila spinosa*	Acanthaceae	Swamp Weeds	Alkaloids, flavonoids, tannins, saponins, steroids, terpenoids, phytosterol, minerals, polyphenols, proanthocyanins, mucilages, ascorbic acid, amino acids, and glycosides.	It is used to treat biliousness, blood disorders, gonorrhea, diarrhea and fever, urinary affections, rheumatism, hepatic obstruction, urinary calculi, inflammations, eye diseases, cough, joint pains, anemia, abdominal disorders.	Anti-inflammatory Anti-tumor Anti-pyretic Hepato-protective Diuretic Antioxidant Antimicrobial Analgesic [34]
45	*Bacopa monnieri*	Plantaginaceae	Waterhyssop	Flavonoid, tannin, phlobatannin, saponin, steroid, cardiac glycoside, polyphenol, carbohydrateand alkaloids.	It contains antioxidants, protects against cell damage, prevents Alzheimer's, Parkinson's disorders, reduces inflammation, cure diabetes, heart & kidney disease, boosts brain function, prevent anxiety, stress, lower blood pressure, Inhibit tumor cells, epilepsy.	Anti-depressant Anti-anxiety Anti-cancer Anti-inflammatory Antioxidant Anti-ulcer Analgesic Hypertensive Anti-diarrheal [13]
46	*Justicia adhatoda*	Acanthaceae	Malabar Nut	Alkaloids, anthraquinoes, flavonoids, carbohydrate, proteins, saponins, phytosterols, triterpenoids,and polyphenols.	Different parts of the plants have been used in cough, cold, asthma, bronchitis, tuberculosis, allergy, wound healing, it prevents intestinal parasites, dysentery, helps in skin disease, scabies etc. chest congestion, breathing problems, spasms.	Anti-tussive Hepato-protective Antimicrobial Anti-ulcer Anti-inflammatory Abortifacient Cardio-protective Thrombolytic [35]

47	*Bryonia alba*	Cucurbitaceae	Bryony	Alkaloids, saponins, steroids, triterpenoids, cucurbitane, triterpene, glycosides, carbohydratesand proteins.	It gives relief to constipation, upset stomach, fluid retention, arthritis, inflammation, headache, migraine, prevents cancer, lung disorder, vomiting, wounds, ulcer, edema, rheumatism, epilepsy, snakebite, gout pain.	Analgesic Anti-inflammatory Anti-pyretic Antimicrobial Anti-viral Anti-tumor Antidote Anti-arthritic [36]
48	*Cinnamomum zeylanicum*	Lauraceae	Cinnamon	Alkaloids, flavonoids, saponins, terpenoids, cinnzeylanin, cinnzeylanol cinnamic acid, phenolic acids, tannins, and essential oils.	It is useful in upset stomach, gastrointestinal problems, diarrhea, morning sickness, cold, vomiting, urinary tract infections, vaginal infections, diabetes, and nose bleeds, colic, prevents head lice, pain-killer, mouth freshener, and cures skin problems.	Antimicrobial Anti-inflammatory Anti-cancer Antioxidant Anti-diarrheal Anti-diabetic Cardio-protective Gastro-protective [37]
49	*Vitis vinifera*	Vitaceae	Grapes	Phenolic acids, flavonols, flavon-3-ols, myricetin, peonidin, flavonoids, quercetin, tannin, anthocyanin, cyanidin, ellagic acid, and proanthocyanidins.	Leaves are used in diarrhea, unripe fruit astringent, used in throat affections, laxative, stomachic, consumption, uterine tumors, and hardness of the liver, fresh fruits, eaten or processed into wine, juice.	Antioxidant Hepato-protective Anti-carcinogenic Anti-bacterial Anti-viral Anti-diabetic Cardio-protective [38]
50	*Thymus vulgaris*	Lamiaceae	Thyme	Flavonoids, phenols, terpenoids, protein, fat, essential oils, saponins, steroids and cardiac glycosides.	Thyme is effective in anxiety, arthritis, bad breath, bronchitis, colds, sore throat, colic, diarrhea, dermatitis, gingivitis, earache, hair loss, menstrual cramps, oral thrush, reduce inflammation, swelling oftonsils.	Anti-inflammatory Anti-anxiolytic Antimicrobial Anti-tussive, Antioxidant Anti-spasmodic Insecticidal activity[39]

51	*Laurus nobilis*	Lauraceae	Bay Leaves	Alkaloids, flavonoids, tannins, limonien, cineol, linalool, terpinol, essential oil, phenols, coumarins, steroids, triterpenes and saponins.	It has been used to treat indigestion, bronchitis, influenza, upper respiratory tract disorder, arthritic, used to improve appetite, prevent cancer, urinary ailments, rheumatic pain, renal disease, migraine, skin irritation, stimulate hairgrowth.	Anti-cancer Antimicrobial Antioxidant Anti-tussive Cardio-protective Gastro-protective Nematicidal Insecticidal [13]
52	*Elettaria cardamomum*	Zingiberaceae	Cardamom	Flavonoids, carbohydrates, proteins, minerals, lipids, essential oils, terpenoids and carotenoids.	It is used to treat indigestion, vomiting, congestion of lungs, to prevent stomach pain, gripping, colic, heart burn, stomach disorder, stones, arthritis, sore muscle, scanty urination, cystitis, fight against skin disorders.	Antioxidant Anti-inflammatory Antimicrobial Anti-cancer Insecticidal Gastro-protective Anti-ulcer Anti-asthmatic [13]
53	*Diospyros virginiana*	Ebenaceae	Persimmon	Alkaloids, proanthocyanidins, flavonoids, tannins, β- carotene, zeaxanthin, phenolics, carotenoids, and dietaryfiber.	It is used to treat bloody stools, cancer, dysentery, diarrhea, for making spirits and beer; fruits are used in treatment of diarrhea, cancers, in the treatment of thrust and sore throats.	Antipyretic effect Anti-inflammatory Anti-diarrheal Gastro-protective Anti-cancer Antimicrobial [40]
54	*Withania somnifera*	Solanaceae	Ashwagandha	Flavonoids, phenolics, alkaloids, steroidal lactones, tannins, terpenoids, carbohydrates, and saponins.	It is useful in curing the bronchial asthma, chronic fever, dysentery, arthritis, emetic syndrome, insect bites, gastric, cardiovascular, hepatic disorders, nervous exhaustion, insomnia, loose teeth, diabetes, male infertility, fibromyalgia, Parkinson disease.	Anti-stress Anti-arthritic Neuro-protective Analgesic, Anti-tumor Anti-ulcer, Antioxidant Antimicrobial Abortifacient [41]

55	*Tinospora cordifolia*	Menisper maceae	Gilory	Alkaloids, flavonoids, glycosides, steroids, phenolics, aliphatic compounds, polysaccharides, protein, terpenoid, lignans, and steroids.	It is used to boost immunity, to treats heart related issues, infertility, swine flu, dengue, constipation, reduces stress, anxiety, tonsils, boosts memory, rheumatoid arthritis, improves the vision, reduces sign of aging, dark spots, pimples, fin lines.	Neuro-protective Anti-ulcer, Anti-diarrheal Analgesic, Aphrodisiac Immuno-modulatory Anti-inflammatory Gastro-protective [42]
56	*Artemisia vulgaris*	Asteraceae	Artemisia	Flavonoids, coumarins, sesquiterpene, lactones, volatile oils, insulin, alkaloids, volatile oils (camphor, camphene, caryophyllene) and essential oils.	It is used as an immunity booster, itching, burns, colic, diarrhea, constipation, irregular menstrual periods, to treat headache, fatigue, sleeping disorders, depression, asthma, improves digestion, metabolism.	Antimicrobial Anthelmintic Anti-inflammatory Anti-hypertensive Hepato-protective Antiseptic Anti-spasmodic [13]
57	*Vitex negundo*	Lamiaceae	Lagundi	Flavonoids, carbohydrates, proteins, amino acids, saponins anthraquinones, tannins, triterpenes, lignin and polyphenolics.	It is used to treat oozing from ear, obesity, diabetes, muscular pain, skin disease, cold, diarrhea, swelling of gums, arthritis, joint pain, colon cancer, effective in chronic bronchitis, scrofulous sores, used in burns, and angina.	Anti-inflammatory Antispasmodic Antimicrobial Anti-pyretic Anthelminthic Rejuvenative Tranquilizer Anti-arthritic [13]
58	*Glycyrrhiza glabra*	Fabaceae	Liquorice	Flavonoids, saponin, isoflavonoids, stilbenoids, coumarins, glycosides, carbohydrates, starches, phenolics, alkaloids, proteins, pectin, lipids, tannins, and steroids.	It improves the symptoms of eczema, sore throats, bad breath, dental plaque, indigestion, ulcer, hepatitis, kidney problems, liver injury, obesity, Parkinson disease, stomach ulcer, and enlarged ovary with cysts, infections, cancer, tuberculosis, chronic fatigue.	Anti-tumor Antimicrobial Anti-inflammatory Anti-diabetic Anti-ulcer, Anti-cancer Antioxidant Hepato-protective Anti-allergic [13]

59	*Hibiscus rosa-sinensis*	Malvaceae	China Rose	Flavanoides, tannins, anthraquinones, quinines, phenols, alkaloids, terpenoids, saponins, cardiac glycosides, mucilages, anthocyanin, myristic acid, and campesterol.	The plant is used to treat headaches respiratory and digestive tracts, skin problems, mumps, to relieve from fever, astringent for excessive menstruation, used to treat gonorrhea, diarrhea.	Anti-anxiety, Anti-cancer Anti-fertility Antioxidant Hepato-protective Hypolipidaemic Anti-diarrheal Wound healing [15]
60	*Murraya koenigii*	Rutaceae	Curry Plant	Alkaloids, flavonoids, phenols, saponins, tannins, reducing sugars, terpenoids carbohydrates, and steroids.	It treats diabetes, anemia, eye related problems, improves vision, fights against infection and germs, burns excess fat, It is useful in digestion, constipation, diarrhea, dysentery, piles, nausea, bloating, It prevents cancer, toothache, improves memory, skin, hair, respiratory problems.	Antioxidant Anti-diabetic Anti-inflammatory Anti-tumor Neuro-protective Anti-cancer, Antimicrobial Anti-diarrheal Blood purifying Gastro-protective Anti-depressant [13]
61	*Punica granatum*	Punicaceae	Pomegranate	Phenolics, flavonoids gallotannins, lipid, ellagic acid, gallic acid, punicalins, punicalagins, tannins Punicafolin, punicalagin, friedelin, and betulic acids.	It is used for diarrhea, dysentery, vomiting, eye pain, gastrointestinal disturbances, used to relieve itch, used to treat diarrhea, colitis, dysentery, leucorrhoea, paralysis, to treat piles, yellowish discharge from the vagina, nose bleed.	Anthelmintic Antimicrobial, Anti-cancer Hypoglycemic Anti-diarrheal Anti-fertility, Antioxidant Anti-convulsant Gastro-protective Hepato-protective [15]

62	*Averrhoa carambol*	Oxalidaceae	Carambola	Alkaloids, phenols, flavonoids, protein, tannins, steroids, carbohydrates and glycosides.	It is used for skin disorder, fevers, high blood pressure, diabetes, coughs, rheumatism, asthma, colic, used to treat chicken pox, ringworm infection, to relieve gastritis, angina, malaria, intestinal worms, diarrhea, and hemorrhoids.	Antioxidant Anti-inflammatory Anti-tumor Antimicrobial Anti-diabetic Anti-diarrheal Hypertensive Hypoglycemic Anti-ulcer [43]
63	*Syzygium aromaticum*	Myrtaceae	Clove	Alkaloids, glycoside, steroids, carbohydrates, terpenoids, saponins, tannins and phenolic compound.	It reduces plaque on the teeth, excessive sweating of the palms, pain, blood sugar, bacterial infections, it helps in hangovers, diarrhea, gas, dyspepsia, nausea, vomiting, swelling, cholera, earache, mosquito repellent, bone stronger.	Antimicrobial Antioxidant Anti-carcinogenic Analgesic Anti-inflammatory Anti-thrombotic Anesthetic Anti-pyretic Anti-diabetic [13]
64	*Zea mays*	Poaceae	Corn	Polyphenols, flavonoids, anthocyanins, glycosides, carotenoids, maizenic acid, maysin, rutin, and polysaccharides.	It is used to cure diabetes, cystitis, gout, prevents nose bleeding, menorrhagia, ulcer, swellings, rheumatic pain, cancer, warts, bladder infections, kidney stones, heart failure, high blood pressure, high cholesterol.	Antioxidant Antimicrobial Anti-obesity Anti-diabetic Anti-proliferative Hepato-protective Renal protective Anti-inflammatory Anti-cancer [15]

65	*Nelumbo nucifera*	Nelumbo naceae	Sacred lotus	Alkaloids, flavonoids, phenols, tannins, steroids carbohydrate, protein, fatsand glycosides.	The leaves are used to treat sunstroke, dysentery, fever, dizziness and vomiting of blood. It is also used as an antidote for mushroom poisoning and for small pox;it is used to treat cholera, worm infestation, vomiting, and exhaustion.	Anti-anxiety Antimicrobial Anti-diarrheal Anti-inflammatory Hepato-protective Antioxidant Anti-proliferative Anti-pyretic [15]
66	*Ricinus communis*	Euphorbia ceae	Castor Bean	Flavonoids, glycosides, alkaloids, steroids, terpenoids, ricinoleic acid, ricinine, ferulic acid, syringic acid, cinnamic acids, and stigmasterol.	It is used for anal prolapsed, arthritis, constipation, facial palsy, strabismus, to treat deafness, abscesses, headache, skin problems, bleeding, constipation, boils, piles warts, dandruff, and hair loss.	Anti-fertility Antioxidant Antipsychotic Anti-inflammatory Hepato-protective Haemaglutination Insecticidal [15]
67	*Bryophyllum pinnatum*	Crassulac eae	Goethe plant	Alkaloids, saponins, flavonoids, carbohydrate, sterols, fatty acids, monoarylphenolics and tannins.	The paste of the leaves are externally used for the treatment of boils, insect bites, burns, to treat dysentery, bleeding piles, fresh wounds, blood mixed diarrhea, to reduce swelling,asthma, cold, ulcer, earache, kidney stones.	Antioxidant Anti-pyretic Anti-allergic Antimicrobial Sedative, Analgesic Anti-diarrheal Anti-ulcer [44]
68	*Oxalis corniculata*	Oxalidaceae	Creeping Woodsorr el	Flavonoids, alkaloids, tannins, phenols, carbohydrates, reducing sugar, proteins, sterols, cardiac glycosides and ascorbic acid.	It is used for liver and digestive problems, headache, cures digestive problems, to stop bleeding from cuts, used as good appetizer, piles, dyspepsia, swelling, helps in insomnia, an antidote for snake bite, mercury, arsenic poisoning.	Anti-diarrheal Anti-diabetic Antimicrobial Anti-inflammatory Anti-ulcer, Anti-epileptic Wound healing Anti-scorbutic [45]

69	*Trianthema portulacastrum*	Aizoaceae	Horse purslane	Phenols, flavonoids, alkaloids, cardiac glycosides, tannins, steroids, saponins, triterpenes, lipids, carbohydrates, and proteins.	This plant has analgesic, laxative, stomachic, used in cardiac diseases, bronchitis, inflammations, piles, cancer, obstruction of the liver asthma, amenorrhea, dropsy, edema, rheumatism, anemia, ulcer, night blindness.	Antioxidant Anti-inflammatory Anti-carcinogenic Hepato-protective Anti-hyperglycemic Antinociceptive Antimicrobial [46]
70	*Scoparia dulcis*	Scrophulariaceae	Sweet Broom	Flavonoids, alkaloids, tannins, triterpenes, hexacosonal, β-sitosterol, ketone-dulcitone, amellin, carbohydrates, protein, saponins, and steroids.	It is used to treat cough, diarrhea, bronchitis, jaundice, menstrual disorder, and malaria, skin infections, used to cure headache, liver disorders, burns, anemia, urinary tract disorders, ulcer, snake bite, migraine, hepatitis, and hypertension.	Antimicrobial Anti-inflammatory Anti-malarial Anti-diabetic Anti-diarrheal Antioxidant, Anti-cancer Anti-hypertensive [47]
71	*Psidium guajava*	Myrtaceae	Guava	Flavonoids, alkaloids, anthocyanins, carotenoids, essential oils, fatty acids, lectins, phenols, saponins, tannins, triterpenes and Vitamin C.	It cures diarrhea, reduce fever, gastroenteritis, diabetes, wounds, prevents cancer, skin aging, high blood pressure, colitis, vomiting, obesity, used to treat nail, and enhances hair growth, cure heart disease, and high cholesterol.	Anti-diabetic Anti-diarrheal Antimicrobial Antioxidant, Hepato-protective, Anti-cancer Anti-inflammatory Cardio-protective [13]
72	*Artocarpus heterophyllus*	Moraceae	Jackfruit	Phenolic compounds, flavonoids, stilbenoids, arylbenzofurons, carotenoids, volatile acid, sterols, vitamins, protein and tannins.	The decoction from its seed, root and bark is used to treat digestion, diarrhea, and dysentery. Root extract used as a remedy of skin disease, asthma, fever, used to cure wounds. It heals abscesses, ear problems, ulcer, gall stones, diabetes, reduce pain, builds muscle.	Anti-inflammatory Antioxidant Hepato-protective Wound healing Anti-carcinogenic Anti-ulcer, Anti-diabetic Anti-diarrheal Antimicrobial [48]

73	*Catharanthus roseus*	Apocynaceae	Periwinkle	Alkaloids, flavonoids, terpenoids, saponins, steroids, carbohydrates, anthaquinone, glycosides, carbohydrates and protein.	It is used to treat leukemia, Hodgkin's lymphoma, and increases blood flow in brain, cures hypertension, dizziness, cranial traumas, menstrual irregularities, chronic constipation, dyspepsia, malaria, dengue, skin diseases, wasp stings, toothache, and diabetes.	Anti-carcinogenic Anti-diabetic Antioxidant Antimicrobial Anthelmintic Anti-sterility Anti-diarrheal Hypertensive Wound healing [15]
74	*Tagetes erecta*	Asteraceae	Marigold	Flavonoids, cadinol, carotenoids, isorhamnetin, saponins, triterpenes, quercetin, kaempferol, quercetagetin, phenolics and syringic acid.	It detoxifies the digestive system, heals viral inflammations, treats urinary, liver, gallbladder ailments, cure sore throats, toothaches, measles, mumps, heals cuts, scrapes, treats sunburn, diaper rash, insomnia.	Antiseptic Wound healing Analgesic Antispasmodic Anti-inflammatory Antimicrobial Stomachic Anti-ulcer [13]
75	*Heliotropium indicum*	Boraginaceae	Indian Heliotrope	Flavonoids, steroids, tannins, alkaloids, saponins, glycosides, terpenoids, phenols, and carbohydrates, and phytosterols.	Heliotrope is used to heal skin infections, stomach problems, poisonous animal bites, nervous disorders, Prevents dandruff, treats eye diseases, cure wounds, inflammation, tumor, malaria, vein disease, kidney stones, gum infection, ring worm infection.	Anti-inflammatory Antioxidant Anti-cataract Anti-tumor Anti-ulcer Anti-fertility Diuretic Antimicrobial Analgesic [49]

76	*Tectona grandis*	Verbenaceae	Teak	Alkaloids, triterpenoids, steroids, lignans, fatty esters, phenolic compounds, tannin, carbohydrate, flavonoids, and physterols.	It is useful in bronchitis, dysentery, hyperacidity, diabetes, leprosy, nerve and skin disease, to treat headache, ringworm, promote hair growth.	Anti-inflammatory Antimicrobial Antioxidant Analgesic Diuretic Laxative [10]
77	*Leucas aspera*	Lamiaceae	Thumba	Alkaloids, phytosterols, flavonoids, saponins, phenols, glycosides, oleanolic acid, ursolic acid, triterpenoids and beta-sitosterol.	Used orally as stimulant, anthelmintic, laxative, and diaphoretic, to treat inflammation, dyspepsia, and jaundice, diarrhea, used orally for gastroenteritis, cholera, malaria, syphilis, leprosy, and dysentery.	Anti-inflammatory Antimicrobial Antioxidant Hepato-protective Anti-diabetic Anti-diarrheal [50]
78	*Lantana camara*	Verbenaceae	Indian Lantana	Flavonoids, phenols, saponins, alkaloids, tannin, anthocyanins, flavones, isoflavones, coumarins, lignans, catechins, carotenoids, carbohydrates, proteins, glycosides, and steroids.	Used to treat cuts, rheumatisms, catarrhal infection, tetanus, abdominal viscera, sores, measles, malaria, chicken pox, asthma, ulcer, swelling, bilious fever, used in the treatment of skin, itches.	Anti-cancer Anti-inflammatory Anti-diabetic Anthelmintic Antimicrobial Hepato-protective Antioxidant, Larvicidal [15]
79	*Oldenlandia corymbosa*	Rubiaceae	Diamond Flower	Alkaloids, sterols, terpenes, flavonoids, saponin, glycosides, cyanogenics, tannins, resins, lactones, quinines, phenolic acids, anthocyanidins, and volatile oils.	To treat skin sores, ulcers, sore throat, bronchitis, gynecologic infections, pelvic inflammatory diseases, fever, jaundice, diarrhea, heat eruption, hepatitis, pneumonia, urinary infection, colic, constipation.	Anti-diabetic Anti-malarial Anti-inflammatory Abortifacient Antimicrobial Antioxidant Hepato-protective [51]

80	*Centipeda minima*	Asteraceae	Spreading Sneezeweed	Alkaloids, flavonoids, tannins, phenolic compounds, glycosides, fats, terpenes and carbohydrates.	It is used to prevent pain in joints, used against hepatitis, diabetes mellitus, malaria, opium poisoning, and bronchitis, in night blindness, ring worm, and fever.	Anti-angiogenic Anti-proliferative Anti-arthritic, Anti-diabetic Antioxidant Anti-inflammatory [13]
81	*Canna indica*	Cannaceae	Canna	Alkaloids, steroids, carbohydrates, proteins, flavonoids, terpenoids, cardiac glycosides, β– carotene, essential oil, lignin, diterpenes, steroids and saponins.	Used as diuretic, diaphoretic, demulcent and stimulant, flowers are used to cure eye disease, to treat dropsy, tonsillitis, malaria, diarrhea and dysentery, fever, diabetics, cancer, bruises.	Antimicrobial Anti-inflammatory Analgesic Immuno-modulatory Anti-diarrheal Anti -pyretic Anti-cancer [52]
82	*Acalypha indica*	Euphorbiaceae	Acalypha	Alkaloids, catachols, flavonoids, phenolics, tannin, saponins, triterpenoids, steroids, glycosides, anthraquinone, proteins, and amino acids.	Used in curing diabetes, leprosy, jaundice and heart disease, Used to treat asthma, ulcers, bronchitis, headache, cough dysmenorrheal, and skin disease, Also used for constipation, tummy ache, gastrointestinal irritant.	Anti-inflammatory Antimicrobial Anti-cancer Anti-diabetes Anti-obesity Anti-venom Hepato-protective [53]
83	*Sida cordifolia*	Malvaceae	Country Mallow	Flavonoids, lignin, glycosides, alkaloids, saponins, phytosterols, fixed oils, reducing sugar, and fatty acids.	Used as astringent, diuretic, tonic, cystitis, hematuria, fever, leucorrhoea, and disease of nervous system, paralysis, to treat piles, throat disease, phthisis, and sciatica.	Analgesic Anti-inflammatory Antimicrobial Hepato-protective Anthelmintic Anti-diabetic [13]

84	*Sesbania sesban*	Fabaceae	Sesban	Alkaloids, anthocyanins, polyphenols, flavonoids, phytosterol, triterpenoids, Vitamins, tannins, saponins, and glycosides.	It is used to treat diabetics, inflammation, pains, diarrhea, scorpion sting, emmenagogue astringent, skin disease, leucoderma, menorrhagia, and for spleen enlargement.	Anti-inflammatory Antioxidant Anthelminthic Anti-diabetic Antimicrobial Dermatitis Anti-pyretic Antimicrobial [54]
85	*Daucus carota*	Apiaceae	Carrot	Phenolics, carotenoids, alkaloids, flavonoids, terpenoids, polyacetylenes, ascorbic acid, uccinic acid, α- ketoglutaric acid, lactic acid, and glycolic acid.	It is used as a diuretic and treats many urinary problem kidney stone, It also used in digestive disorders such as indigestion, gas formation, also used to treat skin disease, and cancer.	Anti-inflammatory Anti-oxidant Anti-cancer Antimicrobial Anti-flu Anti-anxiety Antispasmodic [13]
86	*Cocos nucifera*	Arecaceae	Coconut	Phenols, tannins, leucoanthocyanidins, flavonoids, triterpenes, steroids, alkaloids, lpids, phytates and saponins.	Used for the treatment of diarrhea, arthritis, uterine diseases, bronchitis, liver complaints, diuretic, laxative, to treat diseased skin, teeth.	Analgesic Anti-inflammatory Antimicrobial Laxative Diuretic Hepato-protective Anti-diabetic [15]
87	*Andrographi spaniculata*	Acanthaceae	Green Chiretta	Alkaloids, steroids, flavonoids, tannins, triterpenoids, quinones, protein, and sugars.	It is used to treat diabetes, high blood pressure, ulcer, leprosy, bronchitis, colic, influenza, malaria, blood purifier, cold, constipation, fever, liver disorders, loss of appetite, urinary tract and lung infections, low sperm count.	Anti-inflammatory Anti-hyperglycemic Hepato-protective Antimicrobial Anti-cancer, Anti-viral Gastro-protective Cardio-protective [8]

88	*Phoenix dactylifera*	Arecaceae	Date Palm	Carotenoids, flavonoids, polyphenols, isoflavons, lignans, alkaloids, saponins, terpenoids, tannins, cardiac glycosides, and sterols.	Used for the treatment of memory disturbances, paralysis, loss of consciousness, inflammation, nervous disorders, diuretic, sore throat, colds, bronchial asthma, to relieve fever, cystitis, gonorrhea, liver and abdominal troubles.	Anti-ulcer, Anti-cancer Anti-diarrheal Hepato-protective Antioxidant, Anti-inflammatory, Antimicrobial Anti-hyperlipidemic [55]
89	*Morinda citrifolia*	Rubiaceae	Indian Mulberry	Alkaloids, octanoic acid, terpenoids, anthraquinones, sitosterol, carotene, Vitamin-A, flavones glycosides, amino acids, caproic acid, ursolic acid, rutin and proxeronine.	It is used in diabetes, anxiety, and high blood pressure, also used to treat in colds, flu, asthma, bronchitis, piles, depression, mental disorder, improved high frequency hearing, cure heart disease, leprosy, kidney problems and urinary discharges.	Antimicrobial, Antitumor Hypertensive activity, Mental health and improved high frequency hearing, Anti-convulsant Anti-fertility [15]
90	*Saraca asoca*	Fabaceae	Ashoka	Glycoside, flavonoids, tannins, saponins, carbohydrates, phenols, steroids, lignins, catechol, leucopetargonidin and leucocyanidin.	Used in treating hemorrhagic, uterine pain, tumors, worm infestations, bacterial infections, skin problems, leucorrhoea, internal, menstrual cycle and uterine disorders, diabetes, diseases of the blood, enlargement of abdomen.	Antimicrobial Anti-implantation Anti-tumor Anti-progestational Anti-inflammatory Antioxidant, Anti-cancer [13]
91	*Ficus benghalensis*	Moraceae	Banyan	Steroids, flavonoids, tannins, phenolics, leucocyanidin, glycosides, proteins, carbohydrates, fatty acid, amino acid, terpenoids, Vitamin C, and anthraquinones.	It is used to treat diabetes, seminal weakness, leucorrhoea, nervous disorders, burning sensation, ulcers, vomiting, vaginal complains, fever, inflammations, leprosy, syphilis, dysentery, inflammation of liver, applied for reducing pimples.	Analgesic, Anti-pyretic Anti-ulcerogenic Anti-inflammatory Antimicrobial Anti-diabetic Anti-convulsant Anti-diarrheal Hepato-protective Larvicidal [56]

92	*Tridax procumbens*	Asteraceae	Coatbuttons	Flavonoids, alkaloids, hydroxycinnamates, tannins, phytosterols, lignans, carotenoids saponins, phenols and anthocyanins.	Used to treat diabetes, insect repellent, diarrhea, hemorrhages, hair loss, wounds, inflammation, cough, stomachache, epilepsy, gastrointestinaldisorders.	Anti-diabetic Antimicrobial Antiviral, Anti-oxidant Hypo-lipidaemic Anti-hypertensive [57]
93	*Solanum nigrum*	Solanaceae	Black Night Shade	Alkaloids, flavonoids, steroids, tannins, phlobatannins, phenols, solanidine, tigogenin, tomatidenol, uttronins and solanigroside.	Used in wounds, cancerous growths, piles, abdominal pain, dysentery, inflammation, asthma, ulcer, neuralgia, heavy female discharge, diarrhea, diuretic, dysentery, febrifuge.	Anti-inflammatory Anti-diabetic Antimicrobial Antinociceptive Anti-cancer Hepato-protective [15]
94	*Terminalia arjuna*	Combretaceae	Arjuna	Flavonoids, tannins, phenols, phytosterols, saponins, alkaloids, lactones, terpenoids, Carotenoids, stilbenes, lignans, tannins and glycosides.	Used for the treatment of heart diseases, hypertension, to decrease blood pressure, hemorrhages, ulcers, tumors, earaches, dysentery, sexual diseases, urinary tract infection	Antimicrobial Antioxidant Anti-carcinogenic Anti-mutagenic Gastro-protective Anti-hypertensive Anti-atherogenic [58]
95	*Mimosa pudica*	Mimosaceae	Sensitive Plant	Alkaloids, flavonoids, cardiac glycosides, polyphenols, saponins, tannins, terpenoids, quinines, and coumarin.	Used to treat blood and bile, cure piles, jaundice, leprosy, ulcer and smallpox, dysentery, inflammations, asthma, insomnia, premenstrual syndrome.	Anti-diabetic Antioxidant Anti-hepatotoxin Anti-inflammatory Wound healing Anti-fertility [59]
96	*Momordica charantia*	Cucurbitaceae	Bitter Gourd	Flavanoids, saponins, terpenoids, coumarins, emodins, alkaloids, proteins, glycosides, anthraquinones, anthocyanins, andsteroids.	Used to treat diabetes, abortifacient, gout, jaundice, abdominal pain, leprosy, laxative, piles, pneumonia, psoriasis, purgative, rheumatism, melanoma, cancer and lymphoma.	Anti-diabetic Antimicrobial Antioxidant, Anti-tumor Anti-inflammatory Hypo-lipidaemic [60]

97	*Pterocarpus marsupium*	Fabaceae	Malabar Kino	Alkaloids, glycosides, flavonoids, flavonols, poly, phenols pterostilbene, tannins, lignans, stilbenes, sterols, and terpenoids.	Used in fractures, constipation, hemorrhages, arthritis, sores, boils, stomach pain, cholera, leprosy, toothache, tumors, dysentery, fever.	Anti-diabetic Antimicrobial Antioxidant Anti-cancer Anti-diarrheal [61]
98	*Gymnema sylvestre*	Apocynaceae	Gurmar	Alkaloids, flavanoids, glycosides, tannins, triterpene, saponins gymnemic acids, phenols, anthraquinones, and phtoseterois.	It is used in the treatment of jaundice, constipation, asthma, bronchitis, conjunctivitis, renal, calculi, laxative, jaundice, piles, colic pain, eye troubles, cardiac, and respiratory diseases.	Anti-diabetic Anti-arthritic Antimicrobial Hepato-protective Anti-cancer Anti-hyperlipidemic[62]
99	*Enicostemma littorale*	Gentianaceae	Indian Gentian	Flavonoids, steroids, quinones, cardiac glycosides, saponins, quinine, tannins, phenols, and alkaloids.	Used for treating diabetes, stomachic, tonic, reduce fever, tonic for appetite loss, improves kidney function, blood pressure, and malaria.	Anti-diabetic Antimicrobial Antioxidant Anti-inflammatory Anti-tumor [63]
100	*Clerodendrumphlomidis*	Lamiaceae	Arna	Flavonoids, glycosides, terpenoids, saponins, tannins, lignin, phenols, alkaloids, glycosides, phenylethanoid, steroid, anthraquinones, and cyanogenic acid.	Used to treat inflammation, diabetes, nervous disorder, rheumatism, urinary disorders, asthma, febrifuge, coughs, venereal infections, skin diseases, elephantiasis, tropical burns, and malaria.	Anti-diabetic Antimicrobial Antioxidant Anti-inflammatory Anti-cancer Anti-diarrheal [64]

SIGNIFICANCE OF MEDICINAL PLANTS IN PHARMACOLOGY AND PHYTOCHEMISTRY

The current chapter provides research and explanations in a concise manner on a variety of ethno medicinal plants from India that have a variety of pharmacological or therapeutic qualities that can aid to lower the risk of life-threatening disorders in humans. Numerous types of phytochemicals can operate as potent defences against various illnesses or as potent immune boosters. Studying these traditional medicinal plants, aids in developing our understanding of biological processes that will benefit human welfare and healthcare in the future. The careful treatment of these species of medicinal and aromatic plants is required for these innovative objectives. It is also necessary to cultivate and preserve them methodically. The direction of the chapter reveals the pharmacology's future developments as they emerged from observational and experimental therapies for complementary and alternative medicines. Since these plants are a rich source of dietary fibre, minerals, and vitamins, as well as significant primary and secondary metabolites, they are also employed as functional food supplements to support better health. On the basis of pharmacology, phytotherapy and phytochemistry, the plant species shown enormous promise for future usage in the pharmaceutical and nutraceutical industries [1, 5, 65,67].

CONCLUSION

The current chapter focuses on certain significant Indian traditional medicinal plants and their extensive potential for phytochemicals, pharmacology, therapeutics, and nutritional qualities. These medicinal plants have been utilised as a significant medicinal, pharmacological, therapeutic, pharmaceutical, and nutritional source for many disorders, which can be inferred through this chapter. Comprehensive research studies would provide a detailed understanding of the historically used Indian medicinal herbs, demonstrating their superior value for curing various illnesses or combating other serious, life-threatening conditions.

REFERENCES

[1] Satapathy KB, Mishra PK, Jena GSJP.(2020). Micro-botany of plants used in rituals in Jajpur district of Odisha. International Journal of Botany Studies; 5(4):1-8.

[2] Al-Snafi AE. (2017). Pharmacology and Therapeutic Potential of Euphorbia hirta (Syn: Euphorbia pilulifera) - A Review. IOSR Journal of Pharmacy; 7(3):07-20.

[3] World Health Organization. (1991). “Guidelines for Assessment of the Herbal Medicines”. Programme on Traditional Medicine. WHO, Geneva, 56-91.

[4] Ghosh P, Das P, Mukherjee R, Banik S, Karmakar S, Chatterjee S. (2018). Extraction and quantification of pigments from Indian traditional medicinal plants: A comparative study between tree, shrub, and herb. International Journal of Pharmaceutical Sciences and Research; 9(7):3052-3059.

[5] Kumar R, Sharma V, Kumar S. (2020). Ethnobotanical study of herbal medicinal plants of Billawar region, Jammu and Kashmir, India. International Journal of Botany Studies; 5(2):19-23.

[6] Ghosh P, Dutta A, Biswas M, Biswas S, Hazra L, Nag SK, Sil S. (2019). Phytomorphological, chemical and pharmacological discussions about Commelina benghalensis Linn. (Commelinaceae): A Review. The Pharma Innovation Journal; 8(6):12-18.

[7] Ghosh P, Das C, Biswas S. (2020). Phytochemical Composition Analysis and Evaluation of *In-vitro* Medicinal Properties and Cytotoxicity of Five Wild Weeds: A Comparative Study. F1000Research, 9:493.

[8] Md. Hossain S, Urbi Z, Sule A, Rahman KMH. (2014). Review Article Andrographis paniculata (Burm. f.) Wall.ex Nees: A Review of Ethnobotany, Phytochemistry, and Pharmacology. The Scientific World Journal, 28.

[9] Ghosh P, Biswas S, Dutta A, Biswas M, Das S, Das C. (2019). Evaluation of phytochemical constituents and antioxidant property of leaf acetone extracts of five herbaceous medicinal weeds. Journal of Pharmaceutical Sciences and Research; 11(8):2806-2813.

[10] Ramesh B Nidavani, Mahalakshmi AM. (2014). Pharmacology of Tectona grandis Linn. Short Review. International Journal of Pharmacog and Phytochemical Research; 6(1):86-90.

[11] Sarkar S, Mondal M, Ghosh P. (2020). Quantification of total protein content from some traditionally used edible plant leaves: a comparative study. Journal of Medicinal Plant Studies; 8(4):166-170.

[12] Ghosh P, Chatterjee S, Das P. (2019). Natural habitat, phytochemistry and pharmacological properties of a medicinal weed-Cleome Rutidosperma DC. (Cleomaceae): A comprehensive review. International Journal of Pharmaceutical Sciences and Research; 10(4):1605-1612.

[13] Khare CP. (2006). Indian Medicinal Plants: An Illustrated Dictionary. Berlin/Heidelberg: Springer Verlag.

[14] Banik S, Mukherjee R, Ghosh P. (2018). Estimation of plant pigments concentration from Tulsi (Ocimum sanctum Linn.): A six months study. Journal of Pharmacognosy and Phytochemistry; 7(4):2681- 2684.

[15] Ling KH, Kian CT, Hoon TC. (2009). A Guide to Medicinal Plants: An Illustrated, Scientific and Medicinal Approach. World Scientific Publishing Co. Pvt. Ltd.

[16] Paul R, Datta KA. (2011). An updated overview on Peppermint (Mentha Piperita L.). International Research Journal of Pharmacy; 2(8):1-10.

[17] Mehta D, Belemkar S. (2014). Pharmacological Activity of Spinacia oleracea Linn. A Complete Overview. Asian Journal of Pharmaceutical Research and Development, 2 (1).

[18] Tyagi S, Nanher A.H, Sahay S. (2015). Kiwifruit: Health benefits and medicinal importance. Hind agricultural and training institute; 10(2):98-100.

[19] Shrivastava RR, Pateriya P. (2018). Green tea- A short review. International Journal of Indigenous Herbs and Drugs ; 3(2):12-21.

[20] Das C, Kothari S, Muhuri A, Dutta A, Ghosh P, Chatterjee S. (2019). Clove Based Herbal Tea: development, phytochemical analysis and evaluation of antimicrobial property.Journal of Pharmaceutical Sciences and Research; 11(9):3122-3129.

[21] Abdullah Khalid M, Hussain MK. (2017). Badam (Prunus amygdalus Bail.): A fruit with medicinal properties. International Journal of Herbal Medicine; 5(5):114-117.
[22] Soni P, Singh L. (2012). Marsilea quadrifola Linn. - A valuable culinary and remedial fern in Jaduguda, Jharkhand, India. Int J of Life Science and Pharma Research; 2(3):99-104.
[23] Ali W, Hamiduddin, Ahmad A, Tukh- E- Kahu. (2016). (Lactuca Sativa Linn.): pharmacological and phytochemical profile and uses in unani medicine. Journal of Pharmaceutical and Scientific Innovation; 5(1):1-4.
[24] Sidana J, Saini V, Dahiya S. (2013). A Review on Citrus- "The Boon of Nature". International Journal of Pharmaceutical Sciences Review and Research; 18(2):20-27.
[25] Upadhyay RK. (2015). Bel plant: A source of pharmaceuticals and ethno medicines. International Journal of Green Pharmacy; 9 (4): 204-222.
[26] Chauhan ES, Aishwarya J. (2018). Nutraceuticals potential of Petroselinum Crispum: A Review. Journal of Complementary Medicine and Alternative Healthcare: 7 (2).
[27] Atila F, Owaid MN. (2017). The nutritional and medical benefits of Agaricus bisporus: a review. Journal of Microbiology, Biotechnology and Food Sciences; 7 (3): 281-286.
[28] Wani SA, Kumar P. (2018). Fenugreek: A review on its nutraceutical properties and utilization in various food products. J of the Saudi Society of Agricultural Sciences; 17(2):97-106.
[29] Shahrajabian MH, Sun W, Zandi P. (2019). A review of Chrysanthemum, the eastern queen in traditional Chinese medicine with healing power in modern pharmaceutical sciences. Applied Ecology and Environmental Research; 17(6):13355-13369.
[30] Essiett UA, Okoko AI. (2013). Comparative nutritional and phytochemical screening of the leaves and stems of Acalypha fimbricata Schum. & Thorn. And Euphorbia hirta Linn. Bulletin of Environment, Pharmacology and Life Sciences; 2(4):38-44.
[31] Srivastava R, Trivedi D, Shukla G. (2018). Nyctanthes arbor- tristis: A Wonder Indian herbal drug needs healthcare attention; 5(3):4527-4530.
[32] Ghosh P, Ghosh C, Das S, Das C, Mandal S, Chatterjee S. (2019). Botanical description, phytochemical constituents and pharmacological properties of Euphorbia hirta Linn. A Review. International Journal of Health Sciences and Research; 9(3):273-286.
[33] Saini RK, Sivanesan I. (2016). Phytochemicals of Moringa oleifera: a review of their nutritional, therapeutic and industrial significance. Springer; 6:203.
[34] Kshirsagar AD, Ingale KG, Vyawahare NS, Thorve VS. (2010). Hygrophila spinosa: A comprehensive review. Pharmacognosy Reviews; 4(8):167-171.
[35] Singh KJ, Huidrom D. (2013). Ethnobotanical uses of medicinal plant, Justicia adhatoda L. by Meitei community of Manipur, India. Journal of Coastal Life Medicine; 1(4):322-325.
[36] Kujawska M, Svanberg I. (2019). From medicinal plant to noxious weed: Bryonia alba L. (Cucurbitaceae) in northern and eastern Europe. Journal of ethnobiology and Ethnomedicie; 15(22):1-12.
[37] Rao PV, Gan SH. (2014). Cinnamon: A Multifaceted Medicinal Plant. Evidence-Based Complementary and Alternative Medicine, 12.
[38] Kanagarla, Kuppast IJ. (2013). A review on benefits and uses of Vitis vinifera (Grape). Research and Reviews in Biosciences; 7(5):175-180.

[39] Reddy PV, Reddy RV, Varsha PV, Satyam S. (2014). Review on Thymus vulgaris Traditional Uses and Pharmacological Properties. Medicinal and Aromatic Plants, 3:3.

[40] Briand CH. (2005). The common persimmon (Diospyos virginiana L.): The history of a underutilized fruit tree (16th- 19th centuries). Hunt Institute for Botanical Documentation; 12(1):71-88.

[41] Verma SK, Kumar A. (2011). Therapeutic uses of Withania Somnifera (Ashwagandha) with a note on Withanolide and its Pharmacological actions. Asian Journal of Pharmaceutical and Clinical Research; 4(1):1-4.

[42] Bharathi C, Reddy AH, Nageswari G, Sri Lakshmi B, Soumya M, Vanisri DS. (2018). A Review on Medicinal Properties of Tinospora cordifolia. International Journal of Scientific Research and Review; 7(12):585- 598.

[43] Saghir SA, Sadikun A, Khaw AW. (2013). Star fruit (Averrhoa carambola L): From traditional uses to pharmacological activities. Boletin Latinoamericano del Caribe de Plantas Medicinales y Aromaticas; 12(3):209- 219.

[44] Nagaratna A, Hegde PL. (2015). A comprehensive review on Parnabeeja [Bryophyllum pinnatum (lam.) Oken]. Journal of Medicinal Plants Studies; 3(5):166- 171.

[45] Sarkar T, Ghosh P, Poddar S, Choudhury S, Sarkar A, Chatterjee S. (2020). Oxalis corniculata Linn. (Oxalidaceae): A brief review. J of Pharmacognosy and Phytochemistry; 9(4):651-655.

[46] Poddar S, Ghosh P, Sarkar T, Choudhury S, Sarkar A, Chatterjee S. (2020). Phytochemical, Ethnobotanical and Phytopharmacological Discussions about Trianthema portulacastrum Linn.: A Brief Review. Journal of Pharmaceutical Sciences and Research;12(7):899-903.

[47] Sarkar A, Ghosh P, Poddar S, Sarkar T, Choudhury S, Chatterjee S. (2020). Phytochemical, Botanical and Ethnopharmacological Study of Scoparia dulcis Linn. (Scrophulariaceae): A Mini Review. The Pharma Innovation Journal; 9(7):30-35.

[48] Baliga MS, Shivashankara AR, Haniadka R, Dsouza J, Bhat HP. (2011). Phytochemistry, nutritional and pharmacological properties of Artocarpus heterophyllus Lam (Jackfruit): A review. Food Research International; 44(9):3123.

[49] Ghosh P, Das P, Das C, Mahapatra S, Chatterjee S. (2018). Morphological characteristics and phyto- pharmacological detailing of Hatishur (Heliotropium indicum Linn.):A concise review. Journal of Pharmacognosy and Phytochemistry; 7(5):1900- 1907.

[50] Prajapati MS, Patel JB, Modi K, Shah MB. (2010). Leucas aspera: A review, Pharmacognosy Reviews; 4(7): 85-87.

[51] Das S, Mondal N, Mondal S, Ghosh P. (2019). Botanical features, phytochemical and pharmacological overviews of Oldenlandia corymbosa Linn. A brief review. The Pharma Innovation Journal; 8(2):464-468.

[52] Al-Snafi AE. (2015). Bioactive Components and Pharmacological Effects of Canna indica - An Overview. International Journal of Pharmacology and Toxicology; 5(2):71-75.

[53] Nag A, Anoop M, Sharma K, Verma K. (2018). Acalypha Indica L. an Important Medicinal Plant with Antimicrobial agents: a Review. International Journal of Research and Analytical Reviews, 5(4).

[54] Goswami S, Mishra KN, Singh RP, Prashant Singh, Singh P. (2016). Sesbania sesban, A Plant with Diverse Therapeutic Benefits: An Overview. Journal of Pharmaceutical Research & Education; 1(1):111- 121.

[55] Vyawahare N, Pujari R, Khsirsagar A, Ingawale D, Patil M, Kagathara V. (2009). Phoenix dactylifera: An update of its indigenous uses phytochemistry and pharmacology. International Journal of Pharmacology, 7(1).

[56] Patel R, Gautam P. (2014). Medicinal Potency of Ficus bengalensis: A Review. International Journal of Medicinal Chemistry and Analysis; 4(1):53-58.

[57] Ghosh P, Biswas S, Biswas M, Dutta A, Sil S, Chatterjee S. (2019). Morphological, Ethnobiological and Phytopharmacological Attributes of Tridax procumbens Linn. (Asteraceae): A Review. International J of Scientific Research in Biological Sciences; 6(2):182-191.

[58] Alam A, Anchan K, Iwuala E. (2019). Contemporary medicinal uses of ethnomedicinally important plant: Arjuna [Terminalia arjuna (Roxb.) Wight and Arn.]. Annals of Phytomedicine: An International Journal; 8(1):63-69.

[59] Joseph B, George J, Mohan J. (2013). Pharmacology and traditional uses of Mimosa pudica. International Journal of Pharmaceutical Sciences and Drug Research; 5(2):41-44.

[60] Bortolotti M, Mercatelli D, PolitoL. (2019). Momordica charantia, a Nutraceutical Approach for Inflammatory Related Diseases. Frontiers in Pharmacology.

[61] Devgun M, Nanda A, Ansari SH. (2009). Pterocarpus marsupium Roxb. - A comprehensive review. Pharmacognosy Reviews; 3(6):359-363.

[62] Tiwari P, Mishra BN, Sangwan NS. (2014). Phytochemical and pharmacological properties of Gymnema sylvestre: An Important Medicinal Plant. BioMed Research International, 8.

[63] Saranya R, Thirumalai T, Hemalatha M, Balaji R, David E. (2013). Pharmacognosy of Enicostemma littorale: A review. Asian Pacific Journal of Tropical Biomedicine; 3(1):79-84.

[64] Wang JH, Luan F, He XD, Wang Y, Li MX. (2018). Traditional uses and pharmacological properties of Clerodendrum phytochemicals. Journal of Traditional and Complementary Medicine; 8(1):24-38.

[65] Dutta A, Biswas S, Biswas M, Ghosh P, Ghosh C, Das S. (2019). Phytochemical screening, anti-oxidant and anti- microbial activity of leaf, stem and flower of Rangoon creeper: A comparative study. Journal of Medicinal Plants Studies; 7(2):123-130.

[66] Das C, Dutta A, Muhuri A, Kothari S, Ghosh P. (2020). Biochemical analysis and evaluation of antimicrobial properties of Theaflavin and Flavonoids-rich Extract (TFE) and its nanoconjugates: A comparative study. International Journal of Pharmaceutical Sciences and Research; 11(8):3690-3701.

[67] Singh R. (2015). Medicinal plants: A review. Journal of Plant Sciences; 3(1-1):50-55.