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**RECENT TRENDS OF  
INNOVATIONS IN CHEMICAL  
AND BIOLOGICAL SCIENCES  
VOLUME IV**

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**EDITORS**

**DR. BASSA SATYANNARAYANA**

**MR. MUKUL MACHHINDRA BARAWNT**



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## **PREFACE**

Chemical sciences and Biological science play an important role in the evolutionary concept of the living world. This book *Recent Trends Innovation Chemical and Biological Science: An Approach towards Qualitative and Quantitative Studies and Applications* is a considerable effort taken by different authors in the discipline to provide new methodologies of research, its applications, and practical inducements of chemical sciences and Biological Science. The various themes in the book such as application of biological organisms, ethnomedicinal used in different human disorder, biological activity of Indian medicinal plants, Ethnobotanical study, Ecofriendly energy, Transplastomic plants, Role of Sacred Groves in Biodiversity Conservation, Medicinal property rich plants comphora and Different traditional parts in India its application. It covers topic from environment science like effect of toxic chemical on environment. Also covered point from pharmacognosy like as the pharmacological property of Euphorbiaceae. It cover topic like phytochemistry biochemistry and active ingredients Indian medicinal plants. From chemical science subject like organic and inorganic and as well as applied chemistry included such as the Inorganic Metal Oxide-Polymer Nanocomposites For Near Infra-Red, Qsar: A Useful Tool Of Computational Chemistry For Designing New Drug And Predicting Their Biological Activities It also cover there under medicinal and computational chemistry This book acts as an intermediary manual between Chemical sciences with other disciplines paving a way for ideas to new research in the respective arena. The experiments described in the boom chapters are such as should be performed by everyone beginning the study of chemistry, and would also serve as an excellent introduction to a course of qualitative and quantitative analysis. All scientists, academicians, researchers, and students working in the fields of chemistry, biology, physics, materials science, and engineering, among other fields, will find this book quite valuable.

This book with valuable book chapters from eminent scientists, academicians, and researchers will surely be a part of almost information for the coming new research taken by the researchers in the field of chemical sciences and other disciplines in the future.

**DR. BASSA SATYANNARAYANA**

**MR. MUKUL MACHHINDRA BARAWNT**

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Chapter

1

TRANSPORT AND THERMODYNAMIC PROPERTIES OF  
ASSOCIATED FLUID MIXTURE AT VARIOUS TEMPERATURES

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**ABSTRACT**

Density, viscosity, surface tension and refractive index are the basic data used in various physico-chemical analyses of multicomponent fluid mixtures. Various predictive models were used for the computation of these thermodynamic and transport properties. In this work Prigogine-Flory-Patterson (PFP) model based on non-associated process was used to evaluate viscosity, excess viscosity, excess volume and surface tension and Lorentz-Lorentz relation was used to evaluate the refractive index of binary mixture of 2-propanol and 2-phenyl ethanol over the entire composition ranges from temperature 298.15-318.15K and atmospheric pressure. The computed results were compared with literature values. Excess viscosity and Excess volume were also computed to observe the extent of molecular interactions between the binary components. Lorentz-Lorentz relation deals a fair agreement with measured values while result computed from PFP model are very compatible to measured values but still modifications are required.

**KEYWORDS:** viscosity, surface tension, refractive index, Binary mixture, Excess function.

**INTRODUCTION**

In chemical industry the knowledge of transport and thermodynamic properties of liquid mixtures have a considerable importance in many technological areas such as process simulations and liquid-liquid extraction and molecular dynamics (Wang *et al.*, 2005 and Mchaweh *et al.*, 2004)). Density, viscosity, surface tension and refractive index are intensive thermodynamic properties. Which do not depend upon the quantity of the substances? These transport and thermodynamic properties consist of a wide application in pharmaceutical, agro-chemicals and petroleum industry. Experimental as well as theoretical study of viscosity and Excess viscosity (Awasthi *et al.*, 2022) of non-polar liquid mixture help us to understand the transport behaviour of protic and aprotic solvents. Excess volume and surface tension (Awasthi *et al.*, 2019) were evaluated at different temperatures for different alcohols. Refractive index is an intensive thermodynamic property, which does not depend on the amount of the substance. It is basically used for the identification and confirmation of the purity of substance. The prediction of refractive index of binary and multicomponent liquid mixtures (Pandey *et al.*, 1999) is essential for designing various physico-chemical calculations in terms of heat and material transfer. Refractive index depends upon frequency of the light used. Normally Yellow

light of sodium lamp is used for the measurement of pure liquid and liquid mixtures. Refractive index also depends on temperature. Its value changes with change in temperature. Theoretical interpretation of refractive index at different temperatures has its own importance (Mehra *et al.*, 2003). Various theoretical relations such as Argo, Eyring, Heller, Newton, Weiner and Lorentz-Lorentz relations are used for the theoretical calculation of refractive index. Lorentz-Lorentz relation (Lorentz *et al.*, 1980) found to be most suitable for the theoretical evaluation of refractive indices of binary liquid mixtures. A critical review of literatures reveals that various researchers have performed experimental and theoretical analysis of polar-nonpolar liquid mixtures (Shukla *et al.*, 2011). In the continuation of previously published work (Awasthi *et al.*, 2017). This paper is concerned with the theoretical evaluation transport and thermodynamic properties of aforementioned polar binary liquid mixture at different temperatures. Transport and surface properties were evaluated from most popular statical liquid state model (Flory *et al.*, 1965) and refractive index was evaluated from Lorentz-Lorentz relation from 298.15-318.15K over the entire composition range and compared with the literature values (Ching Ta *et al.*, 2007). Excess volume and excess viscosity were also computed from Flory model and play a significant role in understanding of molecular interactions between the binary components. To carry out such calculations a formula sheet for both the aforesaid models. The purpose of this work was to analyses and predicts the extent of molecular interactions by the application of non-associated liquid state model to polar binary liquid mixture.

## THEORETICAL MODELING

### PRIGOGINE-FLORY-PATTERSON MODEL

#### DETERMINATION OF VISCOSITY

Macedo *et al.*, 1965 and Litovitz *et al.*, 1965 proposed a hypothesis by considering activation energy and free volume theory for the probability of viscous flow and extended to the solution.

$$\begin{aligned} &\text{Gibbs free energy of mixing } \Delta G_m^R \\ \Delta G^{\#} &= x_1 \Delta G_1^{\#} + x_2 \Delta G_2^{\#} - \Delta G_M^R \end{aligned} \quad (1)$$

For pure components and the solution, the viscosities  $\eta_i$  are defined as

$$\eta_i = A \exp[\Delta G_i^{\#} / RT + (v_i - 1)^{-1}] \quad (2)$$

Where  $\tilde{v}_i$  is reduced volume, taking logarithms

$$\ln \eta_i = \ln A + \frac{\Delta G_i^{\#}}{RT} + (\tilde{v}_i - 1)^{-1} \quad (3)$$

Applying equation (3) to solution and pure component one obtained

$$\Delta \ln \eta = \ln \eta_{sol} - (x_1 \ln \eta_1 + x_2 \ln \eta_2) \quad (4)$$

If equation (1) and (2) are applied to equation (4) the activation energy is eliminated and  $\Delta \ln \eta$  is related to the free energy of mixing

$$\Delta \ln \eta = -\Delta G_M^R / RT + \frac{1}{\tilde{v}-1} - \frac{x_1}{\tilde{v}_1-1} - \frac{x_2}{\tilde{v}_2-1} \quad (5)$$

If reduced volume ( $\tilde{v}$ ) of two components are not the same, then  $\Delta \ln \eta \neq 0$  even if  $\Delta G_m^R = 0$ .

$$\Delta G_M^R = x_1 P_1^* v_1^* \left[ \left( \frac{1}{\tilde{v}_1} - \frac{1}{\tilde{v}} \right) + 3\tilde{T}_1 \ln \left( \frac{\tilde{v}_1^{-1/3} - 1}{\tilde{v}^{-1/3} - 1} \right) \right] + x_2 P_2^* v_2^* \left[ \left( \frac{1}{\tilde{v}_2} - \frac{1}{\tilde{v}} \right) + 3\tilde{T}_2 \ln \left( \frac{\tilde{v}_2^{-1/3} - 1}{\tilde{v}^{-1/3} - 1} \right) \right] + \frac{x_1 v_1^* \theta_2 X_{12}}{\tilde{v}_1} \quad (6)$$

Put the value of Gibbs free energy of mixing in equation (5), we get the viscosity

$$\ln \eta = x_1 \ln \eta_1 + x_2 \ln \eta_2 - [x_1 P_1^* v_1^* \left\{ \frac{1}{\tilde{v}_1} - \frac{1}{\tilde{v}} \right\} + 3\tilde{T}_1 \ln \left\{ \frac{\tilde{v}_1^{-1/3} - 1}{\tilde{v}^{-1/3} - 1} \right\} + x_2 P_2^* v_2^* \left\{ \frac{1}{\tilde{v}_2} - \frac{1}{\tilde{v}} \right\} + 3\tilde{T}_2 \ln \left\{ \frac{\tilde{v}_2^{-1/3} - 1}{\tilde{v}^{-1/3} - 1} \right\} + x_1 v_1^* \theta_2 X_{12} / \tilde{V}_1] / RT + 1/(\tilde{v}-1) - x_1/(\tilde{v}_1-1) - x_2/(\tilde{v}_2-1) \quad (7)$$

Where  $P^*$ ,  $v^*$ ,  $\tilde{v}$ ,  $\tilde{T}$ ,  $\theta$  and  $X_{12}$  are the characteristic pressure, characteristic volume, reduced volume, reduced temperature, site fraction and interaction parameters respectively

#### DETERMINATION OF SURFACE TENSION

Surface tension of the binary liquid mixture is expressed by Flory's statistical theory as:

$$\sigma = \sigma^* \tilde{\sigma}(\tilde{v}) \quad (8)$$

Where  $\sigma$ ,  $\sigma^*$  and  $\tilde{\sigma}$  are the surface tension, characteristic surface tension and reduced surface tension respectively.

According to Patterson and Rastogi (Patterson *et al.*, 1970) characteristic surface tension:

$$\sigma^* = k^{1/3} P^{*2/3} T^{*1/3} \quad (9)$$

Where, k is the Boltzmann constant and  $P^*$  and  $T^*$  are the characteristic pressure and temperature.

Prigogine and Saraga (Prigogine *et al.*, 1952) formulated an equation of reduced surface tension which is presented as:

$$\tilde{\sigma}(\tilde{v}) = \left[ M \tilde{v}^{-5/3} - \frac{\tilde{v}^{1/3}}{\tilde{v}^2} \ln \frac{\tilde{v}^{1/3} - 0.5}{\tilde{v}^{1/3} - 1.0} \right] \quad (10)$$

Here  $\tilde{v}$  is reduced volume and M is the fraction of nearest neighbors. It's most suitable value lies in ranges from 0.25 to 0.31.

Reduced volume can be calculated by the help of following expression

$$\tilde{v} = \frac{V}{x_1 v_1^* + x_2 v_2^*} \quad (11)$$

Where V is molar volume of binary liquid mixture.  $x_1$  and  $x_2$  are mole fractions of respective liquids and  $v_1^*$   $v_2^*$  are characteristic volume of individual liquids.

$$V = \frac{M_1 x_1 + M_2 x_2}{\rho_{mix}} \quad (12)$$

Where  $\rho_{mix}$  is the density of binary liquid mixture, characteristic. Temperature of binary liquid mixture can be calculated from the following expression.

$$T^* = \frac{P^*}{\frac{\Psi_1 P_1^*}{T_1} + \frac{\Psi_2 P_2^*}{T_2}} \quad (13)$$

Where  $P^*$  is the characteristic pressure of binary liquid mixtures can be expressed as

$$P^* = [\Psi_1 P_1^* + \Psi_2 P_2^* - (\Psi_1 \theta_2 X_{12})] \quad (14)$$

Where  $\psi_1$  and  $\psi_2$  is segment fraction,  $\theta_2$  is the site fraction of component 2 and  $X_{12}$  is the interaction parameters.

$$\psi_2 = \frac{X_2}{X_2 + X_1 (V_1^* / V_2^*)} \quad (15)$$

$$\psi_1 = 1 - \psi_2$$

Site fraction is given by the following expression

$$\theta_2 = \frac{\psi_2}{\psi_2 + \psi_1 (V_2^* / V_1^*)^{1/3}} \quad (16)$$

$$\theta_1 = 1 - \theta_2 \quad (17)$$

The interaction parameter  $X_{12}$  is obtained by adopting familiar Berthelot relationship  $\eta_{ij} = (\eta_{ii} \eta_{jj})^{1/2}$  can be expressed as

$$X_{12} = P_1^* [1 - (P_2^* / P_1^*)^{1/2} (v_2^* / v_1^*)^{1/6}]^2 \quad (18)$$

## DETERMINATION OF REFRACTIVE INDEX

### LORENTZ-LORENTZ RELATION

Lorentz-Lorentz (L-L) relation depends upon the densities of pure liquid and density of binary mixture can be given as:

$$\left[ \frac{n_m^2 - 1}{n_m^2 + 2} \right] \frac{1}{\rho_m} = \left[ \frac{n_1^2 - 1}{n_1^2 + 2} \right] \frac{W_1}{\rho_1} + \left[ \frac{n_2^2 - 1}{n_2^2 + 2} \right] \frac{W_2}{\rho_2} \quad (19)$$

This relation can be used to another form, where volume fraction ( $\phi_i$ ) of individual components is taken into consideration.

$$\left[ \frac{n_m^2 - 1}{n_m^2 + 2} \right] = \left[ \frac{n_1^2 - 1}{n_1^2 + 2} \right] \phi_1 + \left[ \frac{n_2^2 - 1}{n_2^2 + 2} \right] \phi_2 \quad (20)$$

Where ( $n_1, n_2$ ) and  $n_m$  are the refractive indices of pure liquids and liquid mixture respectively.  $W_1, W_2$  are the weight fractions and  $\rho_1, \rho_2$  are densities of pure liquids in the mixture. The weight fraction of  $i$ th component in the mixture may be defined as:

$$w_i = \frac{\phi_i \rho_i}{\rho_{ij}} = \frac{M_i}{M_{ij}} x_i \quad (21)$$

## RESULTS AND DISCUSSION

Table 1 represent the experimental and theoretical results of viscosity and surface tension evaluated for binary liquid mixture of 2-propenol and 2-phenyl ethanol by statistical liquid state model of Flory and refractive index by Lorentz-Lorentz relation over the entire composition range and 1atmospheric pressure from 298.15-318.15K. Excess volume and Excess viscosity evaluated from 298.15-318.15K over the entire composition range presented in table 2. A careful observation of table 1 reveals that the density of binary liquid mixture decreases with increase in mole fraction of 2-propenol in the binary system with increase in temperature which indicate that molecular association becomes weak

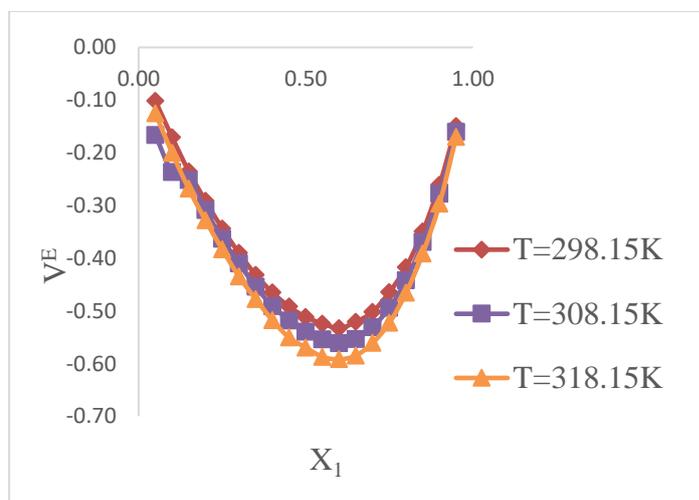


Fig. 1: Variation of Excess volume with temperature

At higher temperatures. The theoretical values of viscosity and surface tension evaluated from Flory model based on non-association process over the entire composition range are very compatible with experimental results. Theoretical results evaluated from Lorentz- Lorentz relation over the entire

**Table 1: Experimental and theoretical values from 298.15-318.15K**

$X_1$	$\rho^{\text{MIX}}$	$V^{\text{M}}$	$\eta^{\text{EXP}}$	$\eta^{\text{Flory}}$	$\sigma^{\text{Exp}}$	$\sigma^{\text{Flory}}$	$n^{\text{Exp}}$	$n^{\text{L-L}}$
<b>T=298.15K</b>								
0.05	1.01	117.95	10.67	10.37	38.10	34.46	1.53	1.53
0.10	1.00	115.71	9.93	9.45	36.90	33.70	1.52	1.52
0.15	0.99	113.48	9.26	8.61	35.80	32.94	1.52	1.52
0.20	0.99	111.26	8.62	7.86	34.70	32.18	1.51	1.51
0.25	0.98	109.05	8.00	7.19	33.63	31.43	1.51	1.51
0.30	0.97	106.84	7.41	6.58	32.61	30.68	1.50	1.50
0.35	0.96	104.63	6.86	6.02	31.60	29.93	1.49	1.49
0.40	0.95	102.43	6.35	5.52	30.60	29.18	1.49	1.49
0.45	0.94	100.24	5.86	5.07	29.60	28.43	1.48	1.48
0.50	0.93	98.06	5.41	4.65	28.62	27.68	1.47	1.47
0.55	0.92	95.88	4.98	4.28	27.65	26.93	1.47	1.46
0.60	0.91	93.71	4.57	3.94	26.70	26.18	1.46	1.46
0.65	0.89	91.56	4.17	3.63	25.80	25.43	1.45	1.45
0.70	0.88	89.41	3.79	3.34	24.98	24.68	1.44	1.44
0.75	0.87	87.29	3.44	3.08	24.20	23.92	1.43	1.43
0.80	0.85	85.17	3.10	2.85	23.45	23.17	1.42	1.42
0.85	0.84	83.07	2.81	2.63	22.75	22.41	1.41	1.41
0.90	0.82	81.00	2.54	2.43	22.10	21.64	1.40	1.40
0.95	0.80	78.95	2.28	2.25	21.50	20.88	1.39	1.39
<b>T=308.15K</b>								
0.05	1.00	118.78	7.05	6.88	37.70	34.78	1.52	1.52
0.10	0.99	116.55	6.59	6.30	36.58	33.98	1.52	1.52
0.15	0.99	114.36	6.15	5.78	35.47	33.18	1.51	1.51
0.20	0.98	112.14	5.73	5.31	34.38	32.39	1.51	1.51
0.25	0.97	109.92	5.34	4.88	33.28	31.60	1.50	1.50
0.30	0.96	107.71	4.97	4.49	32.23	30.81	1.50	1.50
0.35	0.95	105.50	4.62	4.14	31.19	30.03	1.49	1.49
0.40	0.94	103.29	4.29	3.82	30.17	29.24	1.48	1.48
0.45	0.93	101.10	3.96	3.52	29.16	28.46	1.48	1.47
0.50	0.92	98.91	3.67	3.26	28.16	27.68	1.47	1.47
0.55	0.91	96.73	3.39	3.01	27.17	26.90	1.46	1.46

0.60	0.90	94.56	3.12	2.79	26.20	26.12	1.45	1.45
0.65	0.89	92.40	2.88	2.58	25.30	25.34	1.45	1.44
0.70	0.87	90.26	2.63	2.40	24.48	24.56	1.44	1.43
0.75	0.86	88.13	2.41	2.22	23.68	23.78	1.43	1.43
0.80	0.84	86.01	2.20	2.07	22.90	23.00	1.42	1.42
0.85	0.83	83.92	2.01	1.92	22.20	22.21	1.41	1.41
0.90	0.81	81.85	1.86	1.79	21.50	21.43	1.40	1.39
0.95	0.79	79.80	1.69	1.66	20.80	20.64	1.38	1.38

**T=318.15K**

0.05	0.99	119.75	4.95	4.81	37.20	35.01	1.52	1.52
0.10	0.99	117.51	4.65	4.42	36.13	34.18	1.51	1.51
0.15	0.98	115.27	4.37	4.07	35.05	33.35	1.51	1.51
0.20	0.97	113.05	4.10	3.76	33.97	32.53	1.50	1.50
0.25	0.96	110.82	3.85	3.47	32.88	31.71	1.50	1.50
0.30	0.95	108.61	3.60	3.21	31.80	30.90	1.49	1.49
0.35	0.94	106.40	3.36	2.97	30.75	30.09	1.49	1.48
0.40	0.93	104.19	3.13	2.75	29.70	29.28	1.48	1.48
0.45	0.92	101.99	2.91	2.55	28.68	28.47	1.47	1.47
0.50	0.91	99.81	2.71	2.37	27.68	27.67	1.47	1.46
0.55	0.90	97.62	2.52	2.20	26.68	26.87	1.46	1.46
0.60	0.89	95.45	2.34	2.05	25.70	26.06	1.45	1.45
0.65	0.88	93.29	2.16	1.91	24.83	25.26	1.44	1.44
0.70	0.86	91.15	1.99	1.78	24.00	24.46	1.43	1.43
0.75	0.85	89.02	1.82	1.66	23.20	23.66	1.42	1.42
0.80	0.83	86.91	1.67	1.55	22.45	22.86	1.41	1.41
0.85	0.82	84.82	1.53	1.44	21.70	22.06	1.40	1.40
0.90	0.80	82.75	1.42	1.35	20.97	21.25	1.39	1.39
0.95	0.78	80.71	1.30	1.26	20.18	20.45	1.38	1.38

Composition range deals a faire agreement with experimental findings at various temperatures. The molar volume of such binary liquid mixture increases with increase in temperature which indicates the breaking of intermolecular association between the binary components. Excess volume as shown.

In figure 1 indicate that as the temperature increases Excess volume decreases. Whereas the Excess viscosity as shown in figure 2 increases with increases in temperature. Trends for all the Excess viscosity curves are similar which indicate that association between the polar binary liquids becomes poor at higher temperature.

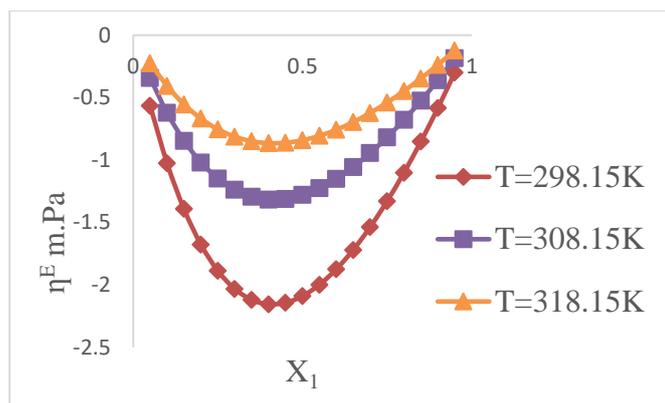


Fig. 2: Variation of Excess viscosity with temperature

Table 2: Excess volume and Excess viscosity from 298.15-318.15K

$X_1$	$V^E$			$\eta^E$		
	298.15K	308.15K	318.15K	298.15K	308.15K	318.15K
0.05	-0.10	-0.17	-0.12	-0.56	-0.34	-0.22
0.10	-0.17	-0.24	-0.20	-1.02	-0.62	-0.41
0.15	-0.24	-0.25	-0.27	-1.39	-0.84	-0.55
0.20	-0.29	-0.31	-0.33	-1.68	-1.02	-0.67
0.25	-0.34	-0.36	-0.38	-1.89	-1.15	-0.75
0.30	-0.39	-0.41	-0.43	-2.03	-1.24	-0.81
0.35	-0.43	-0.45	-0.48	-2.12	-1.29	-0.85
0.40	-0.47	-0.49	-0.52	-2.15	-1.32	-0.86
0.45	-0.49	-0.52	-0.55	-2.14	-1.31	-0.86
0.50	-0.51	-0.54	-0.57	-2.09	-1.28	-0.84
0.55	-0.52	-0.55	-0.59	-2.00	-1.22	-0.81
0.60	-0.53	-0.56	-0.59	-1.87	-1.15	-0.76
0.65	-0.52	-0.55	-0.58	-1.72	-1.06	-0.70
0.70	-0.50	-0.53	-0.56	-1.54	-0.94	-0.62
0.75	-0.46	-0.49	-0.52	-1.33	-0.82	-0.54
0.80	-0.42	-0.44	-0.47	-1.10	-0.68	-0.45
0.85	-0.35	-0.37	-0.39	-0.85	-0.52	-0.35
0.90	-0.26	-0.28	-0.30	-0.58	-0.36	-0.24
0.95	-0.15	-0.16	-0.17	-0.30	-0.18	-0.12

## CONCLUSION

It can be concluded that the computed results of various thermodynamic and transport properties are very consistent to the experimental findings. Excess volume and Excess viscosity under varying condition of temperature and mole fraction provide fruitful information regarding the intermolecular association at different temperatures. Lorentz- Lorentz relation over the entire composition range deals a faire agreement with experimental findings at various temperatures.

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**ABSTRACT:**

In the present study, Lichen species were collected and identified based on morphological features to examine its growth type, thallus color, vegetative and sexual reproductive parts. Spot tests, TLC and micro-crystallography were the chemical analysis which was carried out on lichen fragment and their extract to identify it in species level. Phytochemical screening was done using standard procedure. Antioxidant activities were performed using the free radical scavenging activity of 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) and ABTS assay at 5 different concentrations against control. The studied species was identified as *Parmotrema melanothrix*. Usnic acid and Atranorin were the compounds identified using TLC and micro-crystallography. *P. melanothrix* revealed the presence of alkaloids, flavonoids and tannins. The antioxidant activity increased with the increasing amount of extracts (from 5µg to 100µg). The result obtained in the present study indicates that *P. melanothrix* can act as potential source of natural antioxidant.

**KEYWORDS:** Lichen, phytochemical screening, TLC, micro-crystallography, DPPH, ABTS, antioxidant.

**INTRODUCTION:**

Lichens are symbiotic organisms consisting of a fungus partner and a photosynthetic organism, either an alga or Cyanobacteria (Grube and Berg, 2010; Bates *et al.*, 2011). More than 20,000 known species of lichens have been identified and inhabit diverse ecosystems ranging from Arctic tundra to desert climates (Oboh and Ademosun, 2006). They are ubiquitous on barks, stems, leaves and in soil but often grow in habitats that are less favourable for higher plants (Vrablikova *et al.*, 2006).

Lichens are valuable plant resources and are used as medicines, food, fodder, dyes perfume, spice and for miscellaneous purposes. More than one thousand primary and secondary metabolites with identified structures are currently known in lichens (Molnár and Farkas, 2010). Lichens and lichen products have been used in traditional medicine worldwide, including the Indian system of medicine. These lichen species are said to effectively cure dyspepsia, bleeding piles, bronchitis, scabies, stomach disorders and many disorders of blood and heart (Saklani and Upreti, 1992; Lal and Upreti, 1995; Negi *et al.*, 1996).

The use of lichens in medicine is based on the fact that they contain unique and varied biologically active substances, mainly with antimicrobial actions. These substances are used in

lichen chemotaxonomy (i.e., their classification in terms of chemical features) and they are of interest as natural antibiotics.

Lichen metabolites exert a wide variety of biological actions including antibiotic, antimycotic, antiviral, anti-inflammatory, analgesic, antipyretic, antiproliferative and cytotoxic effects (Molnar and Farkas, 2010; Huneck., 1999; Manojlovic et al., 2002; Manojlovic et al., 2010; Manojlovic, Vasiljevic, and Markovic., 2010; Shukla et al., 2010). Lichens have been found to contain a variety of secondary lichen substances with strong antioxidant activity. Therefore, it is of great interest to carry out the identification process and screening of the selected lichen species in order to validate their use and to reveal the active principle present in it. Systematic screening of them may result in the discovery of novel active compounds.

#### **MATERIALS AND METHODS:**

##### **COLLECTION OF SAMPLE**

Lichen species were collected during December 2018 from Ooty, Tamil Nadu, coordinates at 11.4064°N to 11.436160°N and 76.6032° E to 76.696408° E with average altitude of 2,240 meters. Collected foliose form of lichen was soaked in water and washed, then it was dried and certain quantity of sample was powdered by using pestle and mortar for the extraction process. Remaining parts were preserved in acid-free packets for further use.

##### **PREPARATION OF LICHEN EXTRACT**

6 gms of sample was subjected to Soxhlet extraction using methanol as solvent. The extract was filtered and then concentrated under reduced pressure in a rotary evaporator. The dried extract was dissolved in DMSO (5 % dimethyl sulphoxide) and stored for further studies.

##### **IDENTIFICATION OF SAMPLE**

Collected lichen sample was identified on the base of their morphology and chemical characteristics.

##### **IDENTIFICATION BASED ON MORPHOLOGY**

Morphological study on lichen includes studying its growth type, presence and absence of rhizines or cilia in its vegetative part and the presence and absence of Apothecia and Perithecia in its reproductive part and the thallus color ([http:// www.Ces.iisc.ernet.in](http://www.Ces.iisc.ernet.in) 2014; Sudarshan P. B et al., 1997).

##### **Identification based on chemical test**

##### **SPOT TEST**

The colour test is also known as spot test. Presence of certain substances in its thalloid body reacts with the applied chemicals which results in change in colour on thallus surface. The colour change is denoted by a positive (+) symbol and no change in colour is denoted by a negative (-) symbol. The chemicals routinely used are as follows:

##### **'C' test**

A drop of freshly prepared  $\text{Ca}(\text{OCl})_2$  or  $\text{NaOCl}$  solution was applied on lichen fragment. Aromatic compounds with two free-OH- Meta groups on the thallus react to this solution which results in appearance of yellow colour.

#### **'K' test**

10-25% aqueous solution of potassium hydroxide was used. Quinonoid lichen pigments react to this solution and produce dark red colour (Ahmadjian, Hale, 1974).

#### **'P' test**

1-5% ethanolic solution of p-phenyl-enediamine was used, which reacts with aromatic aldehydes and gives yellow to red colour on tested fragment.

#### **'KC' test**

K solution applies on the lichen fragment immediately followed by C solution. Some depsides and depsidones if present on the thallus can produce red colour after applying this chemical (Ahmadjian, Hale, 1974).

#### **TLC-Visualization**

Methanolic lichen extract was spotted on heat-activated silica coated TLC Aluminium sheets (Silica gel 60 F254, Merck, Germany) and were run by TEF solvent (Toluene: Ethyl acetate: Formic acid; 65.5 ml: 41.5 ml: 4 ml) (Verma, 2011). Developed bands were visualized under UV chamber. The developed bands were also visualized by different chemical reagents in order to have accurate identification by their present phytochemicals and secondary compounds. Firstly, developed plates were sprayed with 10% Sulphuric acid solution, which is the most classic and useful method to identify lichen substances.

Thereafter, to analyze phenolic nature of lichens as secondary compounds, plates were sprayed with 1% ferric chloride solution in 50% methanol. Moreover, a solution of Vanillin-sulfuric acid was used to identify steroidal lichen substances. Another reagent used in this regard was p-Anisaldehyde solution with 97% sulfuric acid to reveal phenol, terpenes, sugars and steroidal nature of available compounds. Iodine granules were used to make iodine-vaporized plates, which cause the phenolic compounds to turn brown in color.

Finally, all the gathered information was matched with reference keys in various literatures (Awasthi (1988), (1991), (2007)) had mentioned different result of spot test in lichen species of India.

#### **Micro-Crystallography**

A small piece of sample was placed on slide and lichen substance was extracted by drop wise adding crystallizing solution: GAW; Glycerol: Ethanol: Water (1:1:1) (Ahmadjian and Hale, 1974). After light heating of slide, that was observed under microscope and captured the image. Then the capture image was compared with reference literature (Culberson, 1969 and 1970; Huneck and Yoshimura; 1996 and Hale, 1974).

#### **Phytochemical screening of lichen**

The methanolic lichen extract obtained was qualitatively tested for the presence of various phytochemical constituents (Harborne, 1973; Yadav and Agarwala, 2011; Yadav and Agarwala, 2013).

#### **Test for Tannins**

10 ml of bromine water was added to the 0.5 gm of methanolic extract. Decoloration of bromine water showed the presence of tannins.

### **Test for Alkaloids**

5ml of sample was dissolved in diluted hydrochloric acid and filtered.

#### **Mayer's test**

The filtered sample was treated with Mayer's reagent (potassium mercury iodide). Formation of the yellow coloured precipitate indicates the presence of alkaloids.

### **Test for glycosides**

#### **Liebermann's test**

2 ml of acetic acid and 2 ml of chloroform was mixed with 2 ml of methanolic extract. The mixture was then cooled and then adds 2 ml of conc.H<sub>2</sub>SO<sub>4</sub>. Appearance of violet colour indicates the presence of glycosides.

### **Test for Flavonoids**

#### **Alkaline reagent test**

2 ml of 2.0 % NaOH mixture was mixed with 2 ml of methanolic extract. Concentrated yellow colour was produced, which becomes colourless when 2 drops of diluted HCl added to the mixture.

### **Test for Terpenoids**

2.0 ml of chloroform was added with 5 ml of methanolic extract and the solution was concentrated by evaporating it in the water bath and then 3 ml of Conc. H<sub>2</sub>SO<sub>4</sub> was added. Change in brown colour indicates the presence of terpenoids.

## **DETERMINATION OF ANTIOXIDANT ACTIVITY**

### **DPPH ACTIVITY**

#### **REAGENT PREPARATION**

0.01mM DPPH solution was prepared by dissolving 4 mg of DPPH in 100 ml of ethanol.

#### **WORKING PROCEDURE**

Different volumes (5-100 µg) of sample extracts were made up to 40 µl with DMSO and 2.96 ml DPPH (0.1 mM) solution was added. The reaction mixture was incubated in dark condition at room temperature for 20 mins. After 20 mins, the absorbance of the mixture was read at 570 nm. 3ml of DPPH was taken as a control.

$$\% \text{ RSA} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Whereas, RSA is the Radical Scavenging Activity; Abs control is the absorbance of DPPH radical + ethanol; Abs sample is absorbance of DPPH radical + sample extract. (Slinkard and Singleton, 1977).

### **ABTS Decolorization assay**

The working solution of ABTS radical was made by reacting ABTS (9.5 ml, 7 mM) with potassium persulfate (245 µl, 100 mM) and raising the volume to 10 ml with distilled water. The solution was kept in dark at room temperature for 18 hrs. and then diluted with potassium phosphate buffer (0.1 M, pH 7.4) to an absorbance of 0.70 (+0.02) at 734 nm.

Samples were prepared in methanol with dilutions 5-100 µl per ml. A sample (10 µl) was placed in a test tube mixed thoroughly with 2.99 ml ABTS radical working solution. Absorbance of the resulting clear mixture was recorded at 734 nm.

The percentage antioxidant activity of the sample was determined by using the following formula

$$\text{Percentage of antioxidant activity} = [(Ac-As) / Ac] \times 100$$

Where Ac and as are the absorbance of the control and sample respectively. The control was prepared by adding 10 µl of methanol in place of the sample.

**RESULTS AND DISCUSSION:**

**IDENTIFICATION BASED ON MORPHOLOGY**

The collected lichen sample was subjected to morphological identification. The morphology of the lichen is depicted in the table 1.

**Table – 1: Morphology of Parmotrema Melanothrix**

S. No	Morphology	Observed
1	Thallus colour	Grey
2	Type	Foliose (Corticolous)
3	Cilia	Present
4	Isidia	Absent
5	Soredia	Absent
6	Rhizines	Present
7	Apothecia	Present

The similar morphological identification was reported earlier by Hengameh and Raj Kumar (2017). The morphology of lichen Cladonia subradiata was corticolous, squamules type with horizontal primary thallus and simple greenish white podetia (stalklike outgrowth of the thallus).

**Identification based on chemical test**

**Spot test**

The medulla and cortex region of the lichen was slightly scrubbed with the help of needle and the chemicals were applied on that to note colour change. The colour change helps in the identification of the sample by referring the identification keys. The result is depicted in the table 2 and figure 1.

**Table – 2: Spot Test on Lichen Species**

Lichen species		C		K		P		KC	
Parmotrema melanothrix									
Cortex	Medulla	-	-	+	-	-	-	+	-



**Figure 1: Spot test on Parmotrema melanothrix**

The similar work was done in *Heterodermia leucomelos* (L.) by Hengameh and Rajkumar (2017). The Lichen was identified by spot test and the result was K+, C-, KC- and P-. Lichen was identified with the help of identification keys.

#### **TLC (Thin layer chromatography)**

The compounds which were present in the sample was separated and identified. The identified compounds are present in the table 3 and figure 2.

**Table 3: Lichen Compounds Identified Using TLC**

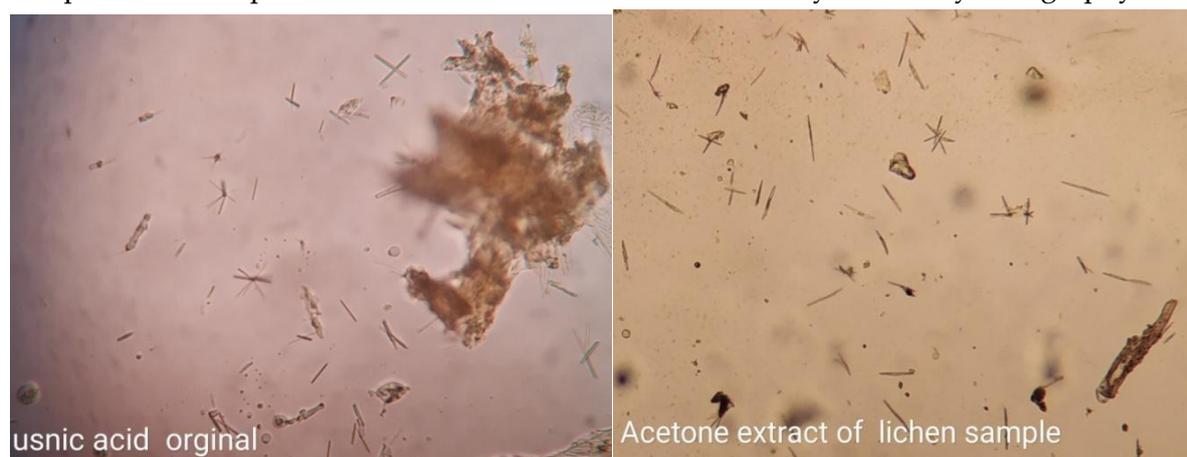
S. No	Lichen	Compounds
1.	<i>Parmotrema melanothrix</i>	Usnic acid and Atranorin



**Figure 2: Lichen compounds separated in TLC**

### **Micro- crystallography**

The known usnic acid was compared with the separated compounds of selected sample and the sample shows the presence of usnic acid and it is confirmed by micro- crystallography.



**Figure 3: Micro-crystallography of Lichen sample**

The similar work was carried out by Hengameh and Rajkumar (2017). The micro-crystallography showed presence of Fumarprotocetraric acid in *Cladonia subradiata* (vain.), Zeorin, atranorin, norstictic acid in *Heterodermia leucomelos* (L.) and psoromic acid in *Herpothallon tobler*.

### **Phytochemical screening**

The preliminary phytochemical screening of *Parmotrema melanothrix* was done to detect the presence of active chemical constituents like alkaloids, flavonoids, terpenoids, glycosides and tannins. The result is showed in the table 4.

**Table 4: Phytochemical Screening of Methanolic Extract**

S.No	Parameter	Colour appeared	Result
1.	Flavonoids	Pink colour	Present
2.	Alkaloids	Cream colour	Present
3.	Terpenoids	No colour	Absent
4.	Glycosides	No colour	Absent
5.	Tannins	Greenish black precipitate	Present

The similar work was done in different extracts of *Cyperus compressus* L. Showed the presence of alkaloids, tannins and saponins which were the major phytochemicals.

#### **Determination of antioxidant activity**

Antioxidant activity may be due the presence of terpenoids, tannins and flavonoids. Based on the present results of phytochemical screening, DPPH antiradical activity is possible to affirm that *Parmotrema melanothrix* can be used as a source of natural antioxidants. Antioxidant activity was done by two assays namely DPPH and ABTS on 5 different concentrations against control. The result is depicted in the table 5.

**Table – 5: Antioxidant Activity of *Parmotrema Melanothrix***

S.No	Name of the Test	5µg	10 µg	20 µg	50 µg	100 µg	IC <sub>50</sub>
1.	DPPH	3.2%	8.5%	21%	46.2%	88%	50.87%
2.	ABTS	2.0%	6.7%	15.8%	33.6%	71%	71.02%

The similar work was done by Dzomba et al. (2012) on Phytochemicals, antioxidant and antibacterial properties of a lichen species *Cladonia digitata*. It showed the reduced number of DPPH free radicals and the absorbance decreased with increasing concentration of extract (10 µg/ml to 200 µg/ml).

#### **CONCLUSION**

The work comprises the Identification, Phytochemical analysis and Antioxidant Properties of lichen in the species of *Parmotrema melanothrix*. Identification of lichen was done by two ways on the bases of morphology and chemicals. The External appearance of *Parmotrema melanothrix* was Grey in colour and foliose type. Cilia, Rhizines, and Apothecia were Present and Isidia, Soredia were absent. Spot test result showed the presence and absence of colour formation (C-, K+, P-, KC) in *Parmotrema melanothrix*. Thin Layer Chromatography and micro-crystallography confirms the presence of usnic acid and Atranorin. Phytochemical screening confirmed the presence of alkaloids, flavonoids and tannins. DPPH assay and ABTS assay was prepared in five different concentrations (5µg, 10 µg, 20 µg, 50 µg, 100 µg) and the result was better in 100 µg of DPPH (88%) and ABTS (71%).

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**Chapter**

**3**

**PHYTOHARMONAL IN NEED OF CALLUS STIMULATION  
STUDIES ON GOTU KOLA (CENTELLA ASIATICA L.), AN  
IMPORATANT HERBAL CURATIVE**

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**ABSTRACT**

*Centella asiatica* L. is a traditional phytotherapeutic herb called saraswathaku in Telugu or Gotu kola with wide-ranging medicinal value, commonly used in Southeast Asian countries. This plant is familiar with its neuroprotective activity, which has been used to improve memory and is considered a brain tonic. The present work is aimed to optimize the in-vitro culture conditions of *Centella asiatica* leaf explants for callus induction and standardize the sterilization procedures for the callus initiation. Mild concentrations of Bavistine (50 mg/lit) and mercuric chloride (0.1%) are the effective sterilizing agents that control the fungal and bacterial contamination of the cultures. The phytohormones like Kinetin with 2, 4- D and BAP with 2, 4- D were used for callus initiation from leaf explants. Among all the hormonal combinations used in the culture media, the combination of BAP and 2, 4-D was suitable for callus induction. BAP was more appropriate than Kinitine for callus initiation and growth. Green color callus was induced and proliferated after 25 days of inoculation of leaf explants on MS culture medium with BAP (1mg/lit) + 2,4-D (0.5 mg/lit).

**KEYWORDS:** *Centella asiatica* L., Leaf explants, Callus, Bavistine, Phyto-hormones.

**INTRODUCTION**

*Centella asiatica* L, a perennial herbaceous creeper belonging to the Apiaceae family, is native to India, Srilanka, Malaysia, and other parts of Asia. It is commonly known as Gotu kola in Chinese, Brahmi in Hindi, and Manduukaparani in Ayurveda (Gohil *et al.*, 2010). Since ancient times, it has been utilized as an essential folk medicinal herb by natives of Asia, Southern and middle Africa, the southeastern United States, and Australia (Bandara *et al.*, 2011). It is commonly used as an ingredient in salads and summer drinks, "Thandayee," prepared in India. The stems are slender, green to reddish-green, interconnecting one plant to another for self-nutrition (Phototropism). It has long-stalked, green, reniform leaves with rounded apices which have smooth texture with palmately netted veins. The leaves are around 2-3 cm in size. *Centella* grows in India up to 600 –1800 meters above sea level (Tiwari *et al.*, 2000; Patra *et al.*, 1998) on

moist, clayey soils, forming a dense green mat. Stems and leaves of these plants are the most popular parts to be used as traditional drugs. Centella is an important medicinal plant used to treat asthma, bronchitis, elephantiasis, catarrhal gastritis, kidney troubles, leucorrhoea, skin disease, and non-specific urethritis (Nurlaily *et al.*, 2011). Antibacterial, anti-feedent, anti-filarial, anti-stress, anti-tuberculosis activities, and wound healing properties were also reported (Nasution *et al.*, 2018). The plant contains secondary phytochemicals like sterols, lipids, saponins, triterpenic acids, nitrogen-containing constituents, and flavonoids (Chew *et al.*, 2011). The callus tissue is produced experimentally from a small excised portion called the explant of a healthy living plant. The explants are cultured sterile in-vitro under controlled conditions on a nutrient medium containing specific phytohormones for their sufficient growth. During culture conditions, the excised plant tissue loses its structural integrity and changes entirely to a rapidly multiplying, the unorganized mass of cells called the callus tissue. The present study is aimed to standardize the sterilization protocol, induce callus initiation in leaf explants and predict the suitable medium for initiation of callus.

## **MATERIALS AND METHODS**

### **CULTURE MEDIUM PREPARATION**

The culture medium is composed of inorganic salts, sugar, iron, vitamins, amino acids, growth substance (hormones), and a carbohydrate supply. To prepare 1 liter of MS medium, 30gms cane sugar and 8 grams of Agar-Agar are taken, and other required nutrients are in suitable concentrations. The pH of the medium is adjusted to 5.6-5.8. The culture medium is dispensed into culture tubes and plugs the tubes with non-absorbent cotton. Medium is finally sterilized by autoclaving.

### **STERILIZATION PROTOCOL**

The diseased free young leaves of the *Centella asiatica* L plants were collected from growing in pots in the medicinal garden of UCSS, Saifabad, Osmania University campus, Hyderabad. Selected plant materials were first rinsed under tap water for 20 minutes, followed by a gentle swirl in double-distilled water. Explants were then immersed in an aqueous solution of tween-20, a liquid detergent. The detergent acts as a wetting agent and allows the entire surface of the explants to be exposed to an antimicrobial agent. After this, they were soaked in an aqueous solution containing 0.2 % Bavistine for 4 minutes with constant shaking. Then the explants were taken to laminar airflow. The explants were washed twice with sterile double distilled water for 10-12 minutes, shaking in the laminar flow. After this treatment, the explants were sterilized with 0.1% mercuric chloride aqueous solution for 2-3 minutes. Then the explants were removed from the sterilizing solution and rinsed thoroughly three times with autoclaved water. Further, these explants were then cut at the distal ends and were finally inoculated in MS media with different hormonal concentrations.

### **HORMONAL CONCENTRATIONS FOR CALLUS INDUCTION**

The present study mainly focused on various types of hormones, and combinations were tried to initiate the callus from the leaf explants (Tiwari *et al.*, 2000). The medium employed was MS Basal with different concentrations and combinations of phytohormones such as Kinetin, BAP & 2, 4-D. various combinations of 2, 4-D and Kinetin were used to initiate the callus (Table 1). Leaf

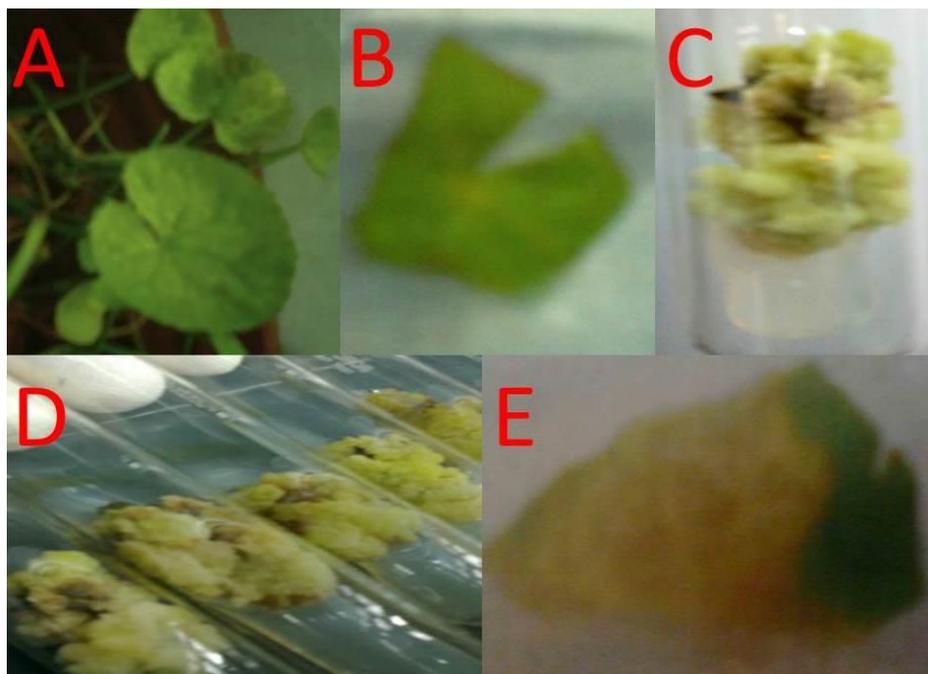
segments of appropriate size are inoculated directly into the culture medium. After inoculation, the culture tubes were properly capped with sufficient labeling. The culture tubes are then incubated at 25± 20°C and 12 hrs of light.

**Table 1: Culture medium types**

Culture Medium	Auxin	Cytokinin
MS1	2,4D-1mg/l	Kinetin1mg/l
MS2	2,4D-1mg/l	Kinetin2mg/l
MS3	2,4D-1mg/l	Kinetin3mg/l
MS4	2,4D-2mg/l	Kinetin1mg/l
MS5	2,4D-2mg/l	Kinetin2mg/l
MS6	2,4D-2mg/l	Kinetin3mg/l
MS7	2,4D-3mg/l	Kinetin1mg/l
MS8	2,4D-3mg/l	Kinetin2mg/l
MS9	2,4D-3mg/l	Kinetin2mg/l
MS10	2,4D-1mg/l	BAP 2mg/l

**Table 2: Response of the explants in culture media**

Culture Medium combinations	Change in Explant (15 days)	Callus initiation	Color of the callus
MS1	Explant color changed from pale yellow to white	-	-
MS2	Explant color changed from pale yellow to white	-	-
MS3	Explant color changed from pale yellow to white	-	-
MS4	Explant color changed from pale yellow to white	-	-
MS5	Explant color changed from pale yellow to white	-	-
MS6	Explant color changed from pale yellow to white	-	-
MS7	Explant color changed from pale yellow to white	-	-
MS8	Explant colored changed from pale yellow to white	-	-
MS9	Explant colored changed from pale yellow to white	-	-
MS10	Explant turned greenish white in color with slight bulging	Callus observed after 25 days	Greenish white callus



**Figure 1: A-Plant materials, B-Leaf explant inoculation, C-Callus Induction, D-Bulk callus in white color**

## RESULTS AD DISCUSSION

Based on the earlier reports of sterilization procedures for *Centella asiatica* L., the inclusion of fungicide like Bavistine was found to be more effective. To avoid the growth of microorganisms contamination during culturing of the explants. In the present study, the usage of Bavistine and mercuric chloride successively as sterilizing agents effectively controlled contaminations of the cultures, and therefore we conclude that the two sterilizing agents can be recommended for the sterilization of *Centella asiatica* leaf explants. Patra *et al.*, 1998 have described sterilization treatment for *Centella*, which includes a wash with 2% (v/v) teepol and surface sterilization in 0.1% (w/v) mercuric chloride for 20 minutes and finally rinsing with sterile distilled water. Banerjee *et al.* (1999) has also reported a different sterilization treatment.

Callus tissue is an unorganized, proliferative mass of cells produced from isolated plant cells, tissue or organs grown aseptically on artificial nutrient medium in glass vials under controlled experimental conditions. Both auxin and cytokinin are required for indefinite growth and cell division in callus culture. The absolute amounts of plant hormones (auxins&cytokinins) are different for different explants from the same plant and depend on factors like the size of explants, the genotype of the explants, and the physiological state of the donor plant. It also explants the composition of culture medium and environment under which cultures are grown i.e., light, temperature, and humidity. Phytohormones play a vital role in the initiation and growth of the callus. The callus initiation can be observed when there is compatibility between the exogenous supply of the hormones in the medium and endogenous levels of the hormones present in the explants.

The media types from 1 to 9 contain the hormonal combination of 2, 4-D, and Kinetin as Auxins and cytokinin sources. While MS medium 10 includes BAP instead of Kinetin as cytokinin source. Table 2 shows the growth pattern of the explants in the culture conditions of the leaf

explants. It was observed that the leaf explants are grown in the nutrient medium containing 2,4D and Kinetin after 15 days, changed their color from green to pale yellow or white (Fig1-D), and did not respond further to initiate the callus.

The MS10 nutrient medium with BAP as a Cytokinin source and 2,4D showed a positive response of callus initiation after 25 days of culture. Callus induction could not be observed in the nutrient medium having the hormonal combination of 2, 4-D and Kinetin. The result shows that BAP as a cytokinin source is most suitable for callus initiation and growth rather than the Kinetin.

Green globular callus induction was also reported in *Centella asiatica* on MS medium supplemented with NAA and Kinetin (Shalini 2004). Shashikala *et al.*, 2009 described that the frequency of callus induction was more on MS media supplement with Naphthalene acetic acid (NAA) (0.5 mg<sup>-1</sup>)+kinetin (2.0 mg<sup>-1</sup>) and NAA (0.5 mg<sup>-1</sup>)+Benzyl Amino Purine (BAP) (2.0 mg<sup>-1</sup>). Stem and leaf explants of greenhouse-grown plants were used to regenerate from callus cultures of *Centella asiatica* (Patra *et al.*, 1998). Banerjee *et al.*, 1999, used 5-6-month-old glass house-grown plants of *Centella* for in vitro multiplication from leaf explants. Tiwari *et al.*, 2000 have reported micro propagation of *Centella* using nodal segments.

## CONCLUSION

There has been increasing demand for herbal medicines daily due to fewer side effects than synthetic drugs, highlighting the need for medicinal plants' conservation and propagation and their in-vitro conservation of germplasm. *Centella asiatica* L is an important medicinal plant used in several Ayurvedic preparations. Due to overexploitation, *C. asiatica* leads to gradual depletion. Tissue culture techniques can play a crucial role in the rapid multiplications of the elite clones and germplasm conservation of *Centella asiatica* L.

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## CONFLICT OF INTREST

All the authors reviewed the article, contributed equally ad there is no conflict of interest.

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### ABSTRACT

Biomolecules predominantly contains carbon atom in their structures. Hence, it is considered as element of life. It is involved in formation of carbon based-compounds and in the generation of structural complexity. The arrangement of simple to complex bimolecular states provides interesting insights of structural plan of a cell of living biological organism. The bio molecules are built according to the structural hierarchy ranging from the precursor metabolic molecules, simple organic building blocks, complex bio molecules, supra molecular complexes, supra molecular assemblies, sub cellular organelles and cellular organization of life. The cell is considered as basic unit of life which is capable of showing all the characteristics of a living state.

**KEYWORDS:** Biomolecules, Structural hierarchy, Cellular Organization.

### INTRODUCTION

Chemicals or molecules present in the biological organisms are known as Bio molecules. These bio molecules are mostly made up carbon based compounds. The biomolecules present in living organisms are organized around carbon. So, it is the most common versatile and the most predominant element of life. The prevalence of carbon is due to its unparalleled versatility in formation of stable covalent bonds through electron pair sharing. The carbon has the ability to form the covalent bond and it is tetrahedral nature (Fig.1). It contributes to the formation of compounds by combining with other atoms such as nitrogen, oxygen, hydrogen, phosphorous, sulphur etc. As a result of chemical bonding between atoms and the force of attraction between two (or) more atoms they are held together in a molecule.

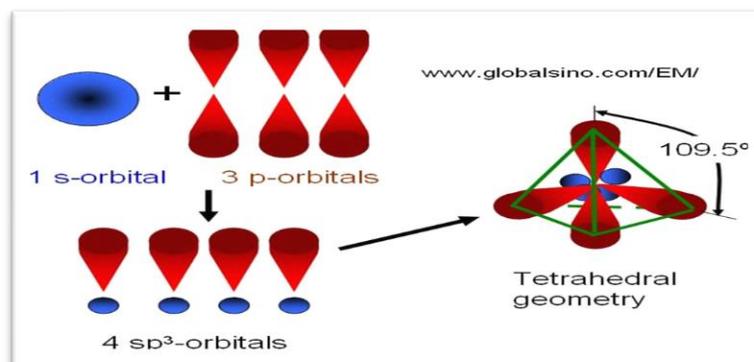


Figure 1: Tetrahedral nature of carbon

## COMPOSITION OF BIOMOLECULES

The biomolecules are built according to the structural hierarchy ranging from the precursor metabolic molecules, simple organic building blocks, complex biomolecules, supra molecular complexes, supra molecular assemblies and cellular organization of life

### PRECURSOR METABOLIC MOLECULES OF BIOMOLECULES

Bio molecules are built from the smaller precursor molecules of organic and inorganic origin, they are of low molecular weight substances, which maintain the identity of that compound is called molecule. The organic molecules include the molecules such as methane, hydrocarbons, reduced compounds of carbon dioxide, protonated species of carbon such as methanol, acetonitrile (Geppert, Wolf & Vigren *et al.*, 2007) etc. The inorganic compounds or substances includes the in-organic molecules such as water, oxygen (Fig.2), inorganic oxides (Lucia gigli *et al.*, 2020), carbon dioxide, in-organic nitrogen compounds such as nitrates, ammonium, di-nitrogen, phosphorous, Chlorine, iodine etc. The inorganic biological elements or polycationic biological macromolecules or metal ions include sodium, potassium, calcium, magnesium, iron etc.

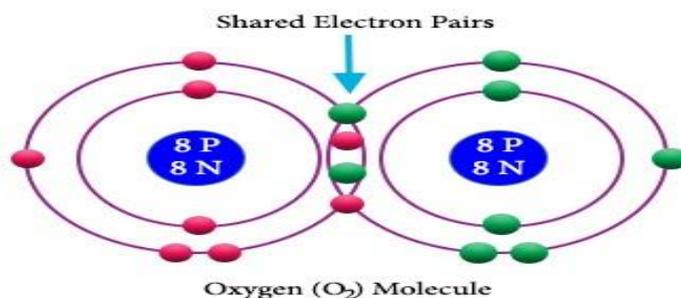


Figure 2: Structure of oxygen molecule

### SIMPLE ORGANIC BUILDING BLOCKS OF BIOMOLECULES

The bio molecules mostly contain hydrocarbon backbones and exhibits structural diversity. Their 3-dimensional properties due to the arrangements of atoms around the hydrocarbon backbone and their stability corresponds to the bond distances and bond angles in order to adopt conformations, which are essentially strain-free. In carbon-based biomolecules and elements are recombined in different ways to form different biologically important molecules. Mostly, Covalent bond holds together the molecules and the structure of the molecule is unique and has specific aspect of identity. Biomolecules are made up of collection of set of molecules joined together which are the structural units and their atoms combine, by sharing of electrons. They act as a simple organic building block which includes amino acids, sugars, nucleotides, fatty acids and glycerol.

### AMINO ACIDS

The organic molecules which contain amino group at one end and carboxylic group at other end. Based on the substituent groups the amino acids can be classified into acidic amino acids, basic amino acids, neutral amino acids, there are about 20 standard amino acids predominantly present in many biological organisms.

## **CARBOHYDRATES**

The carbohydrates are the organic substances containing poly hydroxyl aldehydes or ketones or the compounds that can be hydrolyzed to them. They are present as structural and functional elements of living organisms which include plants, animals and microorganisms. Carbohydrates are widely used as source of food for example rice, Jowar Bajra potatoes legumes and other vegetables etc. Carbohydrates acts as a structural elements for example wood is used for furniture making, cotton is used for textile industry, Paper Products etc. Carbohydrates acts as structural components are present biological organisms. For example chitin is a carbohydrate present as an exoskeleton in case of insects and crustaceans.

## **NUCLEOTIDES**

The nucleotides are organic molecules which contain nitrogenous base, pentose sugar and phosphate group associated with basic proteins. Based on the sugar component and nitrogenous base composition there are two types of nucleic acids 1. Deoxy ribose nucleic acid and 2. Ribose nucleic acids.

Nucleotides are involved in the replication, replication and expression of hereditary information. They participate in cellular signaling processes.

## **LIPIDS**

Lipids are diverse group of organic compounds, which are insoluble in water due to its predominant hydrocarbon chains in their structures. They are present in structural membranes and function as protective barrier in different organisms such as bacteria, plants, insects and vertebrates serving as a part of outer coating between the body of the organism and its environment.

## **FATTY ACIDS**

They act as the basic form of lipids which act as constituent molecules. Fatty acids are carboxylic acids with a long hydro-carbon chain(R).-contains even number of carbon atoms ranging from 4 to 36 carbons long. Based on bonds two types -saturated and unsaturated fatty acids

## **GLYCEROL**

Glycerol acts as a constituent molecule in lipids, which a three-carbon alcohol.

Complex biomolecules:

Through the covalent linkage these simple building block organic molecules can be further assembled into complex bio-molecules (Table 1) such as proteins, polysaccharides, poly nucleotides and lipids.

## **PROTEINS**

Amino acids are linked with one another and assembled to form larger molecules called as proteins. Proteins differ from each other because of its number of amino acids and sequence of amino acids. Based on the degree of complexity the proteins are divided into four levels of organization

**Primary structure:** It refers to the linear sequence of amino acids, which are joined together by peptide bonds and disulphide linkage location.

**Secondary structure:** The conformation of polypeptide chain is in the form of twisting or folding or coiling. Based on hydrogen bonding there are of two types of secondary structures in protein such as alpha helix and beta pleated sheet.

**Tertiary structure:** The 3-D arrangement of protein structure is referred to as tertiary structure. It results in the formation of compact structures such as globular, spherical or ellipsoid shapes.

**Quaternary structure:** the proteins are composed of two or more than two polypeptide chains are referred to as subunits. The subunits may be similar in homogenous quaternary structure eg: isoenzymes of lactate dehydrogenase. The subunits may be dissimilar in heterogeneous quaternary structure eg: hemoglobin. Based on the number of sub-units they may referred to as dimers, tetramer and polymer

### **POLY-SACCHARIDES**

Carbohydrates are composed of sugar units and their polymers. Based on sugar units, carbohydrates classified into three types

### **MONOSACCHARIDES**

Basic units of carbohydrates, which cannot be hydrolyzed to still smaller units. It includes molecules such as glucose, fructose, galactose etc.

### **OLIGOSACHHARIDES**

Carbohydrate chain is formed by the linear sequence of monosaccharide units, which are joined together by glycosidic bond. On hydrolysis give two to nine units of monosaccharides. It includes maltose, sucrose, lactose etc.

### **POLYSACCHARIDES**

Upon hydrolysis gives large no. of monosaccharide units ranging from hundreds to thousands. It includes starches, fibers, glycogen etc.

**Polynucleotides:** Polynucleotide chain is formed by the linear sequence of nucleotide residues joined together by phosphodiester bond is called primary structure. Based on the presence of types of nitrogenous bases- there are two types of polynucleotide chains

### **PRIMARY STRUCTURE OF DNA**

The linear sequence of chain of polynucleotides compounds present in DNA contains four types of Deoxy ribo nucleotide monomeric units. Each deoxyribonucleotide is made up of pentose sugar, phosphate and nitrogen bases. The nitrogen bases are of two types 1.purines which include adenine and guanine. 2. The pyrimidines which include cytosine and thymine.

### **PRIMARY STRUCTURE OF RNA**

The linear sequence of chain of polynucleotides compounds presents in RNA contain four types of ribonucleotide monomeric units. Each ribonucleotide is made up of pentose sugar, phosphate and nitrogen bases. The nitrogen bases are of two types 1.purines which include adenine and guanine. 2. The pyrimidines which include cytosine and uracil.

### **SECONDARY STRUCTURE OF DNA**

The two polynucleotide strands of DNA are twisted around each other and held together by hydrogen bonds between the paired nitrogenous bases and by van der Waals force of attraction between the stacked bases. The continuity of life is based on heritable information in the form of DNA.

## SECONDARY STRUCTURE OF RNA

The polynucleotide strands of RNA are folded to give rise secondary structure of RNA. RNA plays an essential role in the transfer of genetic information during protein synthesis. All types of RNA are transcribed from template DNA.

## LIPIDS

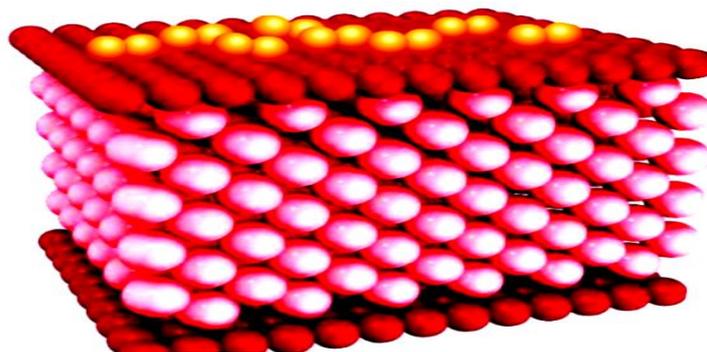
Based on structure and composition, lipids are classified into 3 different types

- **Simple lipids:** The tri-ester of three fatty acids with the alcohol glycerol
- **Compound lipids:** The esters of fatty acids with glycerol having additional group like Phosphate, nitrogen bases, protein, etc. They are of 3 types :-phospholipids, glycol-lipids, lipoproteins
- **Derived lipids:** They are obtained on the hydrolysis of simple and complex lipids. Examples include sterols, terpenes ,eicosanoids, prostaglandins etc

**Table 1: Complex biomolecules**

Large complex biomolecules	Simple organic building block biomolecule	Function
Protein	Amino acid	Basic structure and function of cell
DNA	Deoxyribonucleotide	Hereditary genetically information
RNA	Ribonucleotide	Protein synthesis
Polysaccharide	Monosaccharide	Storage form of energy
Lipids	Fatty acids & glycerol	Storage form of energy to meet long term energy demands

Supramolecular complexes: Intricate interactions among the complex bio-molecules lead to the next level of structural organization called as supramolecular complexes. Their resemblance is like complexes of protein, membrane lipids of cells and organelles (Fig: 3). their attractive forces between supramolecular complexes are dictated by non-covalent forces obtained from electron fluctuations in molecules. The hydrophobic interactions direct the folding of macromolecules such as proteins, lipids and nucleic acids into compact structures. Example: The proteins are linear sequences of covalently linked amino acids to form protein chain. The protein chain can turn, fold and coil in the three dimensions of the space to establish a specific next higher architecture of protein molecule. Hydrophobic portions of biopolymers tend to be buried inside the structure of the molecules minimizing its contact with the water surrounding. The non-covalent forces maintain the structural integrity under certain conditions of temperature, p H, relative acidity and ionic strength. They also interact with other inorganic and organic precursor molecules to involve themselves into metabolic processes, which act as intermediates in cellular metabolism, energy transformation and in the biosynthesis of various sets of building blocks.



**Figure 3: Structure of membrane lipid**

### SUPRA MOLECULAR ASSEMBLIES

The supra molecular complexes of one or more classes of macromolecules polymerize together to form specific very large supra molecular assemblies, they have the subcellular functions of the cell. The supra molecular assembling structures in turn determines the structure of cell organelles in a living cell. The supra molecular assemblies include enzyme complexes, ribosomes, chromosomes, cytoskeletal elements. For example the ribosomes contain 4 different RNA molecules and 70 different unique proteins. This is driven by the accumulation of action of weak non-covalent forces providing structural complementarity. These weak forces profoundly influence the nature of biological structure as they are built. These weak forces create interactions that are constantly forming and breaking at physiological temperature where as in its cumulative number impart stability to their structures because of weak forces generated by their collective action.

**Table 2: Non-covalent forces of biomolecules**

Non-covalent forces	Strength(KJ/mole)	Distance(nm)	Description
Vanderwaals interaction	0.4-4.0	0.3-0.6	Size and area of contact between two molecules
Hydrogen bonds	12-30	0.3	Polarity of atoms between two molecules containing H-Bond donor and H-Bond acceptor
Ionic interactions	20	0.25	Relative polarity between two molecules containing positive and negative charged species
Hydrophobic interactions	Less than 40	-	Molecular orientation and disorder in the presence of water

## **SUB-CELLULAR ORGANELLES**

Large supra molecular assemblies generally have definite membrane boundaries called as an organelle. In addition to the plasma membrane surrounding the cell, eukaryotic cells contain a variety of membrane-bound sub-cellular organelles (Steven R. Goodman., 2021). Sub cellular cell organelles and other organized structures are essential for proper specific function of a cell. They participate in metabolic reactions since many enzymes are incorporated directly into the membrane of sub cellular organelles.

## **CELLULAR ORGANIZATION**

Sub-cellular organelles organize themselves into cells, tissues, organs and finally into whole organism. Each cell acts as a characteristic basic living structural and functional unit of life. One cell is able to show all attributes of a living state such as growth, metabolism, stimulus response, replication etc. Based on the organization of their cellular structures all living cells are categorized into two types 1. Prokaryotic cells and 2. Eukaryotic cells.

## **CONCLUSION**

Thus, the arrangement of simple to complex bimolecular states provides interesting insights of structural plan of a living cell and its characteristic attributes associated in a living state.

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### ABSTRACT

The earth's environment is changing globally at all scales as per the developmental greed of human beings. The degradation of environment is all due to human activities. The climate is showing warming trends, the biodiversity is being continuously exploited at an unprecedented rate; the fisheries are at its decline in most of the world's water bodies. The soil quality of the fields is reducing due to the repeated use of chemical fertilizers. The water bodies are being polluted by industries and human beings in all over the world. The industrialization is posing a serious threat to our environment because of the higher emissions of carbon content in the atmosphere. The time has come to think and work collectively to stop all unwanted environmental changes at global level. The world's population and the global economy are growing. The increased population demands more survival amenities. For that, we will have to understand the interconnections among the environmental and developmental issues for developing and implementing the cost effective and socially acceptable policies at local, regional and global level.

**KEYWORDS:** Warming trends, Biodiversity, fisheries, Industrialization, Carbon content.

### INTRODUCTION

The major environmental issues such as extraction of natural resources, climate change, loss of biodiversity, and collapse of ecosystem, depletion of ozone at stratosphere, air pollution, water pollution, soil pollution, global warming and acid rain are of great concern from survival point of view of living creatures. Most of the countries have understood that the time has come to achieve environmentally and socially sustainable economic growth, coupled with food, water, energy, and human health at local, regional and global scale. Most of the environmental challenges are primarily demographic, economic, sociopolitical, technological, cultural and religious. Sustainable development fundamentally a question of people's need to influence their future, claim their rights and voice their concerns. Effective governance and respect for human rights are key prerequisites for empowering people to make sustainable choices keeping in their mind the environmental challenges. A progressive shift towards sustainable development is possible only when we could save our environment. The current environmental trends show that by 2025 over half of world's population will live in places that are subject to severe water crisis and by 2040 demand is projected to exceed the supply. The water quality in many parts of

world is decreasing and about fifty to sixty percent wetlands have been lost. The human induced climate change is supposed to decrease water quality and availability in many arid and semi-arid regions and also to increase the threats posed by floods and droughts in most part of the world. The emission of Green House Gases is one of the greatest threats to our environment. World emissions are currently around 50 billion tons of carbon dioxide equivalents per annum and are growing rapidly. Since the terrestrial and oceanic ecosystems are unable to absorb world's all annual emissions, the concentration of GHGs emissions in the atmosphere have increased today to over 400 ppm of CO<sub>2</sub> equivalent and increasing at the rate of around 2.5 ppm per year. Biodiversity has specific social, economic, cultural, spiritual and scientific values. The protection of biodiversity is quite essential for protecting the environment. The rapid loss of biodiversity in last few decades is jeopardizing the provisions of ecosystem services. Effective education and training programs are to be conducted by each and every country to educate people in regard of saving the environment and taking global steps to make earth green.

## METHODOLOGY



**Figure 1: Environmental Challenges**

Environmental challenges are increasing day by day and in some of the areas it is often difficult to record the environmental variation more precisely. When we talk about air pollution or radioactive pollution it gets little bit more difficult to study the variation pattern. The present study of environmental challenges is based on the data released from various national and international laboratories by Govt. of India and Govt. of foreign countries (IPCC's report 2014). The strategies are proposed to curbe the issues based on the availability of sources (Montreal and Kyoto: A tale of two protocols). The impacts of the data are correlated to reach to the increasing alarm to the nature and human beings (world water resource: A new appraisal and assessment for 21<sup>st</sup> century).

## RESULTS AND DISCUSSION

### THE GLOBAL WARMING

There are various factors which cause global warming. Industrialization is one of the major factors which contributes towards climate change. To fulfill our requirements we setup industries and increase the production but at the same time we don't see the level of pollution created by the industries. The gases thrown out by the industries and automobiles particularly the GHGs don't allow the heat radiations of earth to escape into the space thereby making the earth warmer. Emissions from industries and automobiles not only increase the temperature of

the atmosphere but also create health hazards, more specifically the respiratory problems. Our respiratory system begins to fall ill gradually due to such emissions. Vast industrialization leads to the significant increase in the release of carbon content into the atmosphere. The most advanced countries have higher rates of release of carbon content into the atmosphere. The harmful gases thrown out by the industries chemically react with our protective umbrella, the ozone and make it weaker and weaker.

The depletion of ozone allows the harmful ultra-violet rays to enter the atmosphere and make the earth warmer globally. The flights of supersonic aircrafts involve high temperature combustion of fuel. The nuclear tests during their conduction generate high temperature. All these activities generate high temperature and eventually, make the earth warmer. The temperature of the earth depends upon the rate at which energy is received from the sun and distributed to the atmosphere by various energy distribution ways like convection, conduction and evaporation etc. This is the reason why temperature of our atmosphere is not constant but it changes with the season, time of day, cloud cover and wind velocity. As per the NOAA reports, the year 2015 has been recorded as the hottest year (National climate assessment 2014). The global increase in earth's temperature is forcing our Cryosphere to melt with higher pace. The melting of glaciers assists the sea level to rise and invites floods to wash out the coastal areas.

The phenomenon of global warming is assisting the phenomenon of climate change. The scientists are speculating a number of indirect measures of climate change like study of ice cores, tree rings, glacier lengths, pollen remains and ocean sediments by the study of changes in earth's orbit around the sun (IPCC 2013 climate change). The records show that climate change varies over a wide range of time scales. The causes responsible to predict climate change are change in solar energy, volcanic eruptions and natural changes in the concentrations of Green House Gases (IPCC 2013 climate change). The climate change as a result of increased temperature allows higher rates of water evaporation which acts as a fuel to bring natural calamities like drought, storms and hurricanes (IPCC's 2014 report on climate change)

As per IPCC the net damages due to climate change are significant and are likely to increase by time (IPCC 2007 summary of policy makers). The emission of Green House Gases particularly the Hydro fluorocarbons (HFCs) is yet to be reduced substantially. The climate change negotiations between developed and developing countries have been made to ensure global cooperation by Montreal Protocol, Kyoto Protocol and Paris Agreement. In Montreal Protocol (1<sup>st</sup> Jan. 1989) eight revisions have been made so far and it is ratified by 197 countries. The Kyoto Protocol (11<sup>th</sup> Dec. 1997) also working in the same direction to cut down the emission of Green House Gases. The Paris Agreement (12 Dec. 2015) has been ratified by 63 countries. A few years back on world ozone day, India announced new collaborations on research and development to make eco-friendly refrigerants. To save the ozone layer, India has made great stride in diversifying its energy needs by moving towards renewable sources of energy like Solar and Wind. An international Solar Alliance has been launched by Govt. of India at Paris. As a result of international agreements, the ozone hole in Antarctica is recovering gradually (Arbetarbladet 12-9-2014, P-10). The climate projections show that the ozone layer will return to 1980 level between 2050 and 2070 (UNEP September 10, 2014). Among the various agreements made by

countries there are some factors for the ozone depletion where global regulations based on the Kyoto Protocol has failed to do so (Cass R. sunstein 38 ELR 10566 8/2008). As per the recent revision in Montreal Protocol the developed countries have agreed upon the curbing of 10% emission of GHGs.

### LOSS OF BIODIVERSITY AND ECOSYSTEM FUNCTIONING

Biodiversity is the degree of variation of life on earth and comprises microorganisms, plants, animals and ecosystems such as coral reef, forests, deserts etc. The conservation of biodiversity (Nature 360, 216; 1992) is very essential to conserve the ecosystem and ultimately the environment where we live in. Biodiversity provides a variety of ecosystem services that living creatures need for e.g. food, fresh water, wood, fiber and fuel. We are at the risk of loss of most of our biodiversity and the benefits given by it. In biodiversity, each species, no matter how big or small, has specific role to play in the ecosystem. The factors which affect biodiversity include temperature, altitude, precipitation and habitat. The oceanic biodiversity is less than that of terrestrial biodiversity.

Biodiversity maintains the balance of ecosystem, combats pollution, stabilizes climate, protects water resources and helps in soil formation. The biodiversity makes provisions to provide medicines for humans and animals. Besides it, biodiversity gives us various social benefits like recreation, tourism, cultural values and promotes educational research activities. Biodiversity strengthens our economic and industrial growth by providing fiber, oil, dyes and rubber. Biodiversity enhances recreational activities like bird watching, fishing, trekking etc. It inspires musicians and artists.

**Table 1: Number of described species on earth**

Number of described species on earth are under as:	
Species	Number
Bacteria	4000
Protoctists (algae, protozoa)	80,000
Vertebrates (animals)	52,000
Invertebrates (animals)	1,272,000
Fungi	72,000
Plants	270,000
Total described species (Including unknown species)	14,000,000
Source: UNEP / Global Environmental Outlook (Ref. 3)	

There are various reasons of loss of biodiversity such as habitat destruction, climate change, and invasion of exotic species, pollution, human overpopulation and deforestation. The global warming contributes a lot in the loss of biodiversity. If present rate of global warming continues, the coral reefs which are biodiversity hotspots will disappear in next twenty to forty years. Most of the species may become extinct by 2020. The loss of biodiversity adversely affects

the functioning of ecosystem of that area. Therefore, biodiversity matters a lot for proper functioning of an ecosystem. Some processes of an ecosystem may increase with biodiversity while some may decrease. Loss of biodiversity seriously threatens the services which well-functioned ecosystems provide to human beings. The loss of biodiversity can be reduced by protecting the areas where human activity is limited. No doubt, much planning is needed for creating protected areas. For that, we will have to consider all parameters of the ecosystem of that area. Many countries are not allowing the entry of foreign species which can adversely affect the ecosystems. Besides it, educating people is also a good tool to save biodiversity. We must resist all those factors which assist global warming, deforestation and climate change.

#### **ENVIRONMENTAL CHALLENGES OF HYDROSPHERE**

The hydrosphere includes the water bodies like lakes, rivers, ponds, ground water etc. Various kinds of pollutants are thrown into these water bodies through various sources. About 75 % of water pollution is caused by domestic sewage, food processing, waste water and sullage from houses. The sewage contains about 99.9% water and 0.1% solids. The solids comprise grit, metals, salts, proteins, fats and carbohydrates. The sullage from house hold work includes soaps, detergents, fats and grease. Accumulation of domestic waste in water bodies (A new appraisal and assessment for 21<sup>st</sup> century report UNESCO, 1998) retards the growth of aquatic organisms. The agricultural waste also pollutes the hydrosphere. The agricultural waste mainly includes pesticides, fertilizers, farm wastes, plants and animal's debris. Besides it, various agrochemicals used for instant plant growths, growth hormones and nutrient solutions also pollute the water bodies. The agricultural run- off is considerably rich in NPK nutrients. The run-off from nuclear power plants is also causing a serious threat to our aquatic organisms. Emissions from radioactive material hamper the growth of aquatic organisms. The major radioactive pollution in water bodies is caused by uranium, radium and plutonium. Waste from thermal power plants not only contaminates the water bodies but also elevates the temperature of water bodies and thus, the life of various aquatic organisms is adversely affected. Effluents from industries severely affect the life of aquatic organisms. The industrial waste mainly includes salts, chemicals, suspended solids, thermal constituents and cyanide etc. All these pollutants adversely affect our life. The water must be given suitable treatment before entering into any water body. We must promote globally, the rain water harvesting and water recycling processes. Chemical recovery plants must be installed to separate out chemicals from water bodies by making use of upgraded cleaner technologies.

#### **ENVIRONMENTAL CHALLENGES OF LITHOSPHERE**

The lithosphere includes the soil portion. The lithosphere acts as reservoir for water bodies, having various minerals, a producer of vegetative crops and a home of wild life. Soil portion is also polluted from various sources. With the advances made in agro technology, huge quantities of pesticides, chemical fertilizers, herbicides, weedicides and soil conditioning agents are used to increase the yield. These constituents pollute the lithosphere when used in excessive quantity. Deposition of excess nutrients causes eutrophication. Various kinds of pesticides used to control pests contaminate the natural environment of soil portion. Some chlorinated pesticides like DDT, BHC, Aldrin, Chlordane, Lindane etc. severely pollute the lithosphere.

Radioactive pollutants obtained from explosions of nuclear devices also pollute the soil. The isotopes of Potassium (K-40) and carbon (C-14) are frequently seen in soil, rocks, water and air. Hydrogen weapons on explosion produce neutron-proton reactions by which N-15 produces C-14. This C-14 participates in carbon metabolism of plants and enters into the bodies of animals and humans. Industrial effluents from chemical factories, oil refineries, textiles, distilleries and paper mills also pollute the soil. Besides this, urban waste which mainly includes dried sludge of sewage, municipal garbage and metallic pollutants such as As, Hg, Ni, Si and Co on addition to the soil produces adverse effects on crop productivity. The pathogenic biological agents such as bacteria obtained from sewage produce chronic diseases like Cholera, Typhoid and Bacillary dysentery.

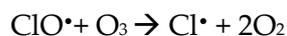
The agrochemicals should be used in limited quantity. The use of insecticides and pesticides should also be limited. The nuclear reactions must be either banned or carried out only in nuclear reactors. The industrial effluents must be treated well before disposing off into the land area. All these protective steps must be followed right from local level to global scale.

### **ENVIRONMENTAL CHALLENGES OF ATMOSPHERE**

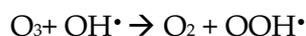
The atmosphere is basically the protective blanket of gases surrounding the earth. It absorbs most of the fatal electromagnetic radiations like UV rays IR rays and X-rays. The atmosphere plays a key role in maintaining the heat balance on earth by absorbing IR radiations received from sun and re-emitted by earth. In atmosphere, gases such as O<sub>2</sub>, N<sub>2</sub> and CO<sub>2</sub> play an important role in sustaining life on earth (Delay R. 1940 strength and structure of earth). The oxygen supports life on earth. Nitrogen is an essential macro nutrient for plants and CO<sub>2</sub> is essential for photosynthesis. Any major disturbance in the atmospheric composition may pollute it. The atmospheric pollution may take place either naturally or by anthropogenic method. The natural sources include volcanic eruptions, forest fires, smoke, deflation of sand and dust. Dispersion of bacteria, fungi, yeast and moulds in air can also pollute atmosphere naturally.

In anthropogenic sources which are man- made sources, the atmosphere may pollute by excessive deforestation, vehicular emissions, burning of fossil fuels and rapid industrialization. The primary pollutants of atmosphere are inorganic gases (such as SO<sub>2</sub>, NO<sub>x</sub>, H<sub>2</sub>S, CO, NH<sub>3</sub>, HF, and CO<sub>2</sub> etc.), particulate matter (like ash, smoke, dust, mist etc.), olefinic and radioactive compounds. The secondary pollutants are derived from primary pollutants due to chemical reactions which include SO<sub>3</sub>, NO<sub>2</sub>, O<sub>3</sub> and peroxy acetyl nitrate (PAN) etc. All these gaseous pollutants generate respiratory infections. The past centuries natural factors caused atmospheric CO<sub>2</sub> concentrations to vary within a range of about 180 to 300 ppm. (Source: NRC 2010). Besides it, the chloro-fluoro carbons used as coolant produce free chlorine which destroys the ozone layer present in stratosphere. The ozone layer acts as protective umbrella not allowing the harmful UV radiations to reach on earth. Freon-1 (CFCl<sub>3</sub>) and Freon-12 (CF<sub>2</sub>Cl<sub>2</sub>) used as propellants in aerosol spray cans, refrigerants, firefighting reagents and solvents for cleaning components of electronic device. The fluorocarbons are stable compounds and are chemically inert and thus, float in atmosphere and enter the stratosphere region where they absorb solar ultraviolet radiations and break down liberating the free atomic chlorine. The free atomic

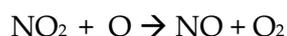
chlorine reacts with ozone and destroys it. The hole created as a result of depletion of ozone layer is called ozone hole.



It is observed that ozone depletion occurs rapidly at the poles because of the photochemical dissociation of fluorocarbons and other chlorine containing reagents in stratosphere. The Cl atoms react with ozone and form ClO• radicals which further destroy ozone layer. The NO<sub>2</sub> has the ability to eat up the ClO• radicals but due to lower temperature NO<sub>2</sub> solidifies and is not available for eating up the ClO• radicals. Besides it, the ice available at poles acts as catalyst for the photochemical decomposition of ozone. It has been reported that a very small quantity of fluorocarbons can increase earth's temperature several degree and thus, may change the climate of that area. The hydroxyl radicals generated due to photochemical reaction of organic pollutants with oxygen in the stratosphere also deplete the ozone layer.



The atmospheric nitrogen at higher temperature reacts with atmospheric oxygen and forms oxides of nitrogen. These oxides of nitrogen react with the ozone layer and destroy it.



Higher the concentration of oxides of nitrogen in the atmosphere greater is the destruction of ozone. The flights of plane generate high temperature in atmosphere due to combustion of fuel and the nuclear tests conducted by various countries also generate high temperature in the atmosphere thus, favouring the formation of oxides of nitrogen. Carbon dioxide also plays an important role in bringing up the environmental challenges by warming up the atmosphere. Carbon dioxide is mainly released into the atmosphere by combustion of fossil fuels, decomposition of organic matter and carbonates etc. Our earth receives a large amount of radiation from sun and 66% of the total received radiation is reflected back to the space. The carbon dioxide allows the short wavelength radiations which are mainly the UV and visible radiations to pass through the earth but it blocks the passage of long wavelength IR radiations which favour the heating of the environment. Thus, CO<sub>2</sub> acts as heat barrier from earth to atmosphere. It has been calculated that by doubling the CO<sub>2</sub> concentration, the temperature of earth increases by about 1.9°C. Methane gas released from coal and oil industries heats up the environment more potentially rather CO<sub>2</sub> but the concentration of CO<sub>2</sub> in atmosphere is 200 times more than methane.

The CO<sub>2</sub> level in atmosphere is 380 ppm while methane level is 1.75 ppm. Nitrous oxide released as a result of agricultural activities, industrial activities, combustion of fossil fuel and solid waste also contribute towards environmental challenges by increasing the temperature of earth globally. The oxides of nitrogen and sulphur react with water and produce acid. The precipitation of this acid from atmosphere is called acid rain which also adversely affects the living organisms on the earth.

## CONCLUSION

In order to make the earth globally fit for survival, we must think about the human activities carried out so far and their consequences in terms of sustainability of life on this planet earth. We don't have any right to deteriorate the things which nature has given us just for the lust of development. If we carry on present activities in haste of development and urbanization at the cost of environment, what would have our future generations to see? Indeed nothing. The increased rate of melting of glaciers due to global warming is taking us towards the situation where rivers will not have water to flow and thus, a food scarcity will develop. Use of CFCs as refrigerants is assisting ozone depletion thereby allowing high energy radiations to come on to the earth and thus, genetic complications will develop in various species and many species will become extinct. Loss of biodiversity will either change the functioning of ecosystems or the ecosystems will collapse. Whatever developmental activities are being carried out to cater the needs of food, fabric, energy and housing of increasing population must be done by considering nature too. We will have to chart out or develop such technologies and processes which are eco-friendly and safe to our future generations. Recently "The United Nation Climate Change Conference-November 2017" (COP 23) held on 6-17 November 2017 in Bonn, Germany was presided over by Government of Fiji. In this conference around 200 countries, except America agreed to promote the Paris agreement which took place in 2015. All these countries have agreed upon to bring down the emission of Green House Gases and to work collectively towards the resolutions taken in Paris agreement-2015. Besides this, the participating countries in COP-23 also agreed upon to finish the use of fossil fuel by the end of this century.

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### ABSTRACT

Antimicrobial resistance (AMR) is a global health threat, and antimicrobial usage and AMR in animal production is one of its contributing sources. Poultry is one of the most widespread types of meat consumed and cheapest source of protein used worldwide. The chicks of poultry are habitually grown under intensive conditions where there is necessarily of using enormous amounts of antibiotics to prevent the spread of diseases as well as for growth promotion of chicks. The above situation leads to a development of antimicrobial resistant poultry pathogens. This may also result in increased treatment cost of a farmer, thus leading to economic losses and also a source of resistant bacteria/genes that may transfer to human health when it is consumed. It may also increase the risks of AMR exposure to poultry, other domestic animals, wildlife, and human populations.

**KEYWORDS:** Antimicrobial resistance (AMR), Poultry, zoonotic bacteria.

### INTRODUCTION

Antimicrobial resistance (AMR) causes an estimated 7, 00,000 deaths annually worldwide, and every country is potentially affected by this concept. If not properly addressed, the number could grow upto 10 million per year by 2050 (Swartz, 2002). According to Allen (2014), antimicrobial resistance (AMR) is found to be a growing threat for human and animal health in future perspective. Care should be taken to treat the bacterial infections arising in the poultry stations and thus enabling the further risk of morbidity and mortality caused by resistant bacteria in poultry. While the production of agriculture crops has been rising at a rate of 1.5-2% a year, the production of eggs and chickens bred and raised specifically for meat – otherwise known as broilers – has been rising at 8-10% (FAO., 2014 & 2019). According to United States of America agency, Agricultural and Processed Food Products Export Development Authority (APEDA), India is the third-largest egg producer after China and the US, and the fourth-largest chicken producer after China, Brazil and the US.

In a developing country like India poultry is one of the highest per capita earning livestock. As a cheaper means of protein source to humans this industry will expand and countries have to shift from the subsistence to intensive farming which requires routine antimicrobials and clinical surveillances. This poultry industry growth brings benefits for both the Indian economy and nutritional security. However, it has brought with it a major public and environmental

health challenge: the development of Antimicrobial Resistance (AMR) (US FDA, 2018). Antimicrobial resistance is a major challenge for people, animals and the environment, felt most acutely in the developing world. It demands both global and local action (Bengtsson-Palme *et al.*, 2018).

#### **WHAT IS ANTIMICROBIAL RESISTANCE?**

Antimicrobial resistance (AMR) is a state which happens when germs like bacteria and a fungus develops the ability to defeat the drugs designed to kill them (Linton *et al.*, 1977). This occurs when disease causing microbes become able to counter the toxic effect that antimicrobials which are produced by antimicrobial drugs (medicine) had on them (Laxminarayan *et al.*, 2013).

#### **ANTIMICROBIAL RESISTANCE IN POULTRY**

In poultry production, the use of antimicrobial drugs is widespread. They are used to treat disease and also sometimes to prevent disease. They are also used as growth promoters to increase production and so are often added to food (Manson *et al.*, 2018). AMR when it arises in chickens it can lead to untreatable infections in animals affected, as well as in people who work with the animals and others in the chicken production and distribution network. This causes serious issues to the society and environment (Alders and Pym, 2009).

#### **MECHANISMS FOR ANTIMICROBIAL RESISTANCE**

In an antimicrobial resistance mechanism development, there are two fundamental biological pathways that assist the evolution and dissemination of resistance mechanisms includes Vertical Gene Transfer (VGT) and Horizontal Gene Transfer (HGT). In the second pathway (HGT), the genetic mechanisms facilitating the resistance can be exchanged between bacterial species and the organism which is also often described as horizontal gene transfer (HGT). HGT usually manifests through the following three mechanisms:

- (1) Transformation of the exogenous DNA from environment across the cell membrane of an organism.
- (2) Transduction of a gene transfer from one bacterium all the way through a viral medium.
- (3) By means of Conjugation, where gene transfer from a donor to a recipient cell by direct cell-to-cell contact mediated methods.

The phenomenon of acquired bacterial resistance is usually occurs by four general mechanisms namely by inactivation, target alteration, decreased permeability, and increased efflux in a bacterium. At first, spontaneous mutation of a bacterial gene from the selection pressure of antibiotics affects the target sites of membrane receptors changes typically. Secondly, this target alteration uses a stratagem to make the antibiotic ineffective to a bacterium through enzymatic degradation, commonly occurring for the antibiotics namely aminoglycosides, chloramphenicol, and beta-lactams (Jayaratne *et al.*, 1990; Kehrenberg and Schwarz, 2001). In the third step, the above effects can decrease the permeability of selectively filtering the antibiotics from entering the cell membrane in the Gram-negative bacteria. At last in the fourth mechanism, efflux pumps from the bacterium pumps and release toxic substances and many of these pumps can transport an extensive variety of compounds to sustain against the antibiotics. Further the ABR mechanism is facilitated by the circular DNA plasmids structures which contains scaffolds of Antibiotic Resistance Genomes (ARG) and Mobile Genetic Elements (MGEs) namely transposons, integrons, and insertion sequences.

## **BACKGROUND OF ANTIMICROBIAL USE IN POULTRY FARMS**

The use of antimicrobial in poultry farms was mainly a tool of growth promoting factor. A study conducted in Muvattupuzha on the antimicrobial resistance of pathogens in the poultry production environment and developing the same among humans revealed that the bacterial infections among people residing in the area were caused by similar drug-resistant *Escherichia coli* (*E. coli*). The irrational use of antibiotics in chicken, for meat production and preventing infections, has made an impact in the community by making them resistant to certain common antibiotics, including ampicillin, amoxicillin, amikacin and ofloxacin. In poultry, *E. coli* bacteria can cause colibacillosis disease, among others, that account for high morbidity and mortality. There is increasing evidence that improper use of antimicrobials in poultry is a significant factor. Antibiotics with a lower dose of the active composition of compounds can lead to resistance in organisms.

## **METHODS OF POULTRY WASTE MANAGEMENT AND ENVIRONMENTAL RESISTANCE**

Usually, a poultry farm generates a larger volume of excretion that comprise of solid waste rich in ammonia and wastewater. The Primary structure of poultry waste includes the litter with a mixture of bedding substrate, excreta, feed, feathers, shells and wastewater usually comes from disinfecting and washing hatchery and slaughter house environment (Van Boeckel *et al.*, 2017). In the livestock feedlots in Northern China there is evident that the occurrence of sulfonamide, tetracycline, penicillin, streptomycin, plasmid-mediated quinolone and macrolide resistance genes in microorganisms. Waste products of poultry farms make up the majority as manure. In many countries the above manure administration can supply a nutrient-rich source of fertilizer or a livestock feed supplement because of the presence of rich source of nitrogen (3.3% NO<sub>3</sub>), phosphorus (3.4% P<sub>2</sub>O<sub>5</sub>), and potassium (1.7% K<sub>2</sub>O). The above composition could be the best for crop fertilizer and recognized as one the best organic fertilizer collected from terrestrial food animals (Gupta *et al.*, 1997). In spite of the phenomenal applications of poultry byproducts, it is widely accepted that the poultry which were fed up with antibiotics can then transfer AMR bacteria and ARGs into the soil and plants (Elwinger *et al.*, 2016). The poultry farm solid and wastewater can also contaminate runoff and drain into the critical reservoirs. Thus spreading the resistance to ground water, surface water, soil, and fertilizer (Zhu and Johnson, 2013). These findings suggested that reduction in Anti-Microbial Use (AMU) alone cannot effectively eliminate AMR bacteria from the environment, as it requires comprehensive evaluation of environmental reservoirs in parallel way where the poultry waste removal is necessary for mitigating AMR emergence.

## **MISPERCEPTIONS ABOUT ANTIMICROBIAL RESISTANCE**

In Low to Middle Income Countries like ours, the small-scale farmers have misperceptions about the AMU and AMR emergence in an ecosystem. This awareness gap is further exacerbating that the fact of antimicrobials are typically purchased over the counter in small animal agriculture stores (Schar *et al.*, 2018). In Khartoum state of Sudan, North Africa, about 50% of small-scale farmers doesn't have the knowledge of common zoonotic diseases and ABR generation. Only the 30% of them were able to define AMR in poultry (Eltayb and Barakat, 2012). Correspondingly, a group of Peruvian veterinarians reported that inappropriate use of

AMU is widespread among the farmers of Peru. It was also associated by many barriers including the availability of antibiotics, competition with other veterinarians, economic constraints of farmers, and limited knowledge of animal diseases among farmers. It was noted that, studies have recognized that the farmers of Peru as ill-informed about the functionality of antibiotics specific to bacterial infections and application of different antibiotic classes they are using for their poultry farms (Sadiq, 2018). Improved access to quality veterinary services in Peru is necessary to alleviate misconceptions surrounding antibiotics in animal husbandry.

#### **ANTIMICROBIAL RESISTANCE IN INDIAN POULTRY FARMS**

Antibiotics are commonly provided to chickens in India in small doses to promote growth, keep disease in control and also to produce large amount of meat to meet the demand. These antibiotics are almost acting as replacement for nutrition and sanitation says the report of 12 of 18 farms, or 67% of them reported the use of antimicrobials as growth boosters in their poultry farms. Tetracycline's and fluroquinolones, antibiotics commonly used to treat cholera, malaria, respiratory and urinary tract infections in humans were most commonly used as antimicrobials. This increase the rate of antimicrobial resistance and the increase of zoonotic pathogens in the country.

#### **CONSTRAINTS ASSOCIATED WITH POULTRY PRODUCTION**

In spite of the positive outcomes of modern poultry development, there are many constraints associated with poultry production that need to be acknowledged. The constraints include predators, quality of nutrition, breeding through hybridization (genotypes), management and training, infrastructure and capital, farmer organization, governmental policies, and most relevant to public health which are the associated with biosafety and biosecurity risks (Conan *et al.*, 2012). The convergence of humans and animal species coming from several locations provides a unique opportunity for the spread of antibiotic resistance bacteria from one species to another and also will propagate in the ecosystem. Availability of poultry meat in wet markets usually comes from intensive operations with a wide Anti-Microbial Use (AMU) by the farmers. As a result, cases of 1-day old chickens harboring multidrug-resistant bacteria have been reported in Low to Middle Income Countries (LMICs). As a result of intensive small-scale poultry culture leads to rising of larger flock volumes, which can lead to potentially higher economic yields. These cascade effects highlight the necessity to integrate an opt mechanism to prevent the development of ABR in poultry.

#### **CONCLUSION**

Antimicrobial resistance is fast growing issue which must rectified in earlier stages it. Poultry is one of essential food items in the modern world and it has direct contact with human race. Increase in AMR can bring adverse effect to human race also, so a full stop must place to this environment threat. The two major ways suggested by the veterinarian around world is

- To afford early detection and diagnostics of AMR in different samples
- To establish effective biosecurity and bio contamination measures by following these methods we can control the spread of fast-growing AMR and save the humans from zoonotic diseases.

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## ABSTRACT

An abundance of bioactive compounds found in plants are utilised either directly or indirectly to treat a variety of human diseases. Tribal societies all over the world have employed plants and plant parts as an ethno-medicine to treat a range of diseases since the dawn of time. In line with WHO Surprisingly, the main healthcare needs of almost 80% of people in poor nations are now largely or entirely met by herbal medications. A wide range of ailments are treated with various plant parts, such as the root, stem, leaf, fruit, and seeds, including diabetes, hepatitis, analgesia, antipyretics, and as a chemo protective agent for cancer, among others. The therapeutic potential of several historically important medicinal herbs, such as *Curcuma longa*, *Catharanthus roseus*, *Ziziphus nummularia*, *Embllica officinalis*, *Allium sativum* L, *Psidium guajava* and *Aloe vera* will be discussed in the following chapter.

**KEYWORDS:** *Curcuma longa*, *Catharanthus roseus*, *Ziziphus nummularia*, *Embllica officinalis*, *Psidium guajava*.

## INTRODUCTION

Plant-derived medicinal products having a long history of use in ethnomedicine can be a rich source of substances for the treatment of various ailments and infectious diseases around the world for many years due to their minimum side effects and positive effects on human health. It is estimated that there are 250,000 to 500,000 species of plants on Earth. A relatively small percentage (1 to 10%) of these is used as foods by both humans and other animal species. It is possible that even more are used for medicinal purposes. According to the World Health Organization (WHO), a variety of drugs are obtained from different medicinal plants and about 80% of the world's developing population depends on traditional medicines for their primary health care needs. Kala *et al.*, 2006 documented that 80% of the world population has faith in traditional medicines, particularly plant drugs for their primary healthcare. Medicinal plants or their extracts have been used by humans since time immemorial for different ailments and have provided valuable drugs such as analgesics (morphine), antitussives (codeine), antihypertensive (reserpine), cardiotoxic (digoxin), antineoplastic (vinblastine and Taxol) and antimalarial (quinine and artemisinin). Phytomedicine are a major component of traditional system of

healing in developing countries, which have been an integral part of their history and culture (Arif *et al.*, 2009). The biosynthesis and breakdown of proteins, fats, nucleic acids and carbohydrates, which are essential to all living organisms, is known as primary metabolism with the compounds involved in the pathways known as "primary metabolites". Secondary metabolites are considered products of primary metabolism and are generally not involved in metabolic activity viz. alkaloids, phenolics, essential oils and terpenes, sterols, flavonoids, lignins, tannins, etc. (Ramawat *et al.*, 2009). The classical examples of drug discovery like morphine, quinine, digoxin, etc which replaced the extracts of their respective plants were mostly responsible for harbouring the idea that a single active ingredient must have been responsible for the bioactivity (Bhutani and Gohil, 2010). Mainstream medicine is increasingly receptive to the use of antimicrobial and other drugs derived from plants, as traditional antibiotics (products of microorganisms or their synthesized derivatives) become ineffective and as new, particularly viral, diseases remain intractable to this type of drug. Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives.

#### **CURCUMA LONGA**

The Zingiberaceae (ginger) family of plants includes the perennial *Curcuma longa*, which is indigenous to Southeast Asia. The most well-known and extensively researched Chemopreventive agent is curcumin (diferuloylmethane). This turmeric powder's yellow-orange colour comes from polyphenols that build up in *Curcuma longa*'s rhizome. The anti-inflammatory, antioxidant, anti-proliferative, anti-angiogenic, and antineoplastic properties of curcumin make it useful. By reducing oxidative stress and reducing inflammation, curcumin can protect the skin and chemo prevents photo carcinogenesis (Aggarwal *et al.*, 2003; Heng, 2010). In multiple animal models of different tumour types, such as oral cancer, mammary carcinoma, and intestinal tumours, curcumin has been shown to have an inhibitory effect on carcinogenesis. Curcumin also prevents platelets from producing thromboxane (TX) both in vitro and ex vivo.

#### **CATHARANTHUS ROSEUS**

Madagascar, an island in the Indian Ocean, is the original home of *Catharanthus roseus*. An evergreen sub herb or herbaceous plant, *Catharanthus roseus* can reach a height of 1 m. The *Catharanthus roseus* plant's principal chemically active components are alkaloids. Ajmalicine, vinceine, vineamine, raubasin, reserpine, catharanthine, and other alkaloids are primarily found in the aerial sections of plants, whereas actineo plastidemic, Vinblastine, Vincristine, Vindesine, and Vindeline Tabersonine are found in the roots and basal stem. The *C. roseus* flower contains the anthocyanin pigment known as roseindin. The stem and leaf of *Catharanthus roseus* contain the alkaloids Vinblastine and Vincristine, which have the ability to prevent the growth of various human malignancies. Vinblastine is suggested for Hodgkins disease and choriocarcinoma and is used experimentally to treat neoplasms.

#### **ZIZIPHUS NUMMULARIA WIGHT**

*Ziziphus nummularia*, used for medicinal purposes is a thorny small bush with pale-purplish stems found in India, Pakistan, Afghanistan, Egypt, Iran, Iraq, and Israel. Betulin and betulinic

acid are present within the bark and stem of *Z. nummularia* and have been shown to have antitumor activity. Betulinic acid glycosides produce differential cytotoxicity, such that cancer cell lines are more sensitive than normal cells. Betulinic acid has been suggested to induce apoptosis inhibition of angiogenesis and modulation of pro-growth transcriptional activators and effectively kill cancer cells that are resistant to other chemotherapeutic agents.

#### **EMBLICA OFFICINALIS**

The Euphorbiaceae family, which includes amla, also goes by the names *Phyllanthus emblica* Linn is native to South Asia, China, Malaysia, Pakistan, Uzbekistan, Sri Lanka, and South Asia. Because of the distinctive tannins and flavanoids in *Embllica officinalis*, which have strong antioxidant effects, it is highly prized. More than 18 substances that have been found in amla fruit have been shown to have anti-proliferative effects on uterine and stomach cancer cells. Amla is said to be useful at slowing down the ageing process because it is a strong source of vitamin C. As a diuretic, laxative, liver tonic, refrigerant, stomachic, restorative, anti-pyretic, and hair tonic as well as to cure fever and the common cold as well as to avoid dyspepsia and ulcers, amla fruit is used extensively in Indian medicine.

#### **ALLIUM SATIVUM L.**

*Allium sativum* L., a member of the Alliaceae family, is a valuable spice and a well-liked treatment for a number of illnesses and physiological conditions. Additionally, garlic increases fibrinolytic activity and inhibits platelet aggregation, minimizing clots on injured endothelium. Organosulfur chemicals found in garlic have been suggested to be responsible for the chemo preventive activity. S-allylcysteine sulfoxide (allin), a precursor of several allyl sulphide components of garlic oil, has been demonstrated to have a hypoglycemic impact in diabetic patients that is comparable to glibenclamide. It has been demonstrated that crushed garlic preparations have a broad spectrum of antibacterial action against both Gram-positive and Gram-negative bacteria. *Allium sativum*, or garlic, extracts have been demonstrated to have fungi static and fungicidal activity at high dilutions. The anthelmintic action of *Allium sativum* oil eliminates all injurious parasites in the intestine, effective in the exposure of dysentery and also acts as vermifuge.

#### **PSIDIUM GUAJAVA**

*Psidium guajava*, Guava a member of the Myrtaceae family, is widely cultivated for its fruit. Vitamin C and A concentrations are higher in guava. Guavas are also a fantastic source of pectin, a vital dietary fibre. In many regions of the world, guava is often used to treat a wide range of illnesses, including Diarrhoea, fever reduction, dysentery, gastroenteritis, hypertension, diabetes, caries, pain relief, and wounds. Due to its anti-cough properties, guava leaf extract doses might lessen the amount of coughing. Guava extracts in an organic solvent have an impact on sperm production and can be used to treat male infertility.

#### **ALOE VERA**

The Asphodelaceae family includes the perennial, drought-resistant succulent plant known as aloe vera. The word aloe, which means a bitter, shining substance, comes from the Arabic "alloeh" or the Hebrew "halal." It has a significant historical role in traditional indigenous medical systems including ayurveda, siddha, unani, and homoeopathy. Along the southern

Indian coast, *Aloe vera* grows wild. The polysaccharides found in the gel of the leaves of *Aloe vera* have been credited with many of its health advantages. Gums are healed, and gum disease, mucositis, lip fissure, and oral herpes lesions are eliminated. It also acts as an antiseptic to kill bacteria, viruses, and fungus, stimulates cell growth, detoxifies, and normalizes the body's metabolism. *Aloe vera* juice is a fantastic remedy and enhances liver function.

## CONCLUSION

"Tomorrow's medication, yesterday's customs." If marketing is not an issue, the successful growing of this plant is economically advantageous. Exploration of ethno pharmacology and conventional medicine has led to the entry of enormous medications onto the global market. The indiscriminate use of plant medicines can result in short-term negative effects as well as severe long-term toxicity. The limitations of conventional medicine are distinct. The patient's recovery and treatment require time. In order to open up new design possibilities, the aforementioned chapter explores the potential use of medicinal plants and their bioactive ingredients in both prevention and treatment.

"Tomorrow's medication, yesterday's customs." If marketing is not an issue, the successful growing of this plant is economically advantageous. Exploration of ethnopharmacology and conventional medicine has led to the entry of enormous medications onto the global market. The indiscriminate use of plant medicines can result in short-term negative effects as well as severe long-term toxicity. The limitations of conventional medicine are distinct. The patient's recovery and treatment require time. In order to open up new design possibilities, the aforementioned chapter explores the potential use of medicinal plants and their bioactive ingredients in both prevention and treatment. There are many traditional systems of medicine in the world, each with different associated philosophies and cultural origins.

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### ABSTRACT

In this work, the antifungal and antioxidant activities of zinc oxide nanoparticles (ZnO NPs) in *Barringtonia racemosa* were examined. Agar well diffusion techniques were employed to test the antifungal activity of the four pathogenic fungus *Aspergillus niger*, *Aspergillus flavus*, *Trichoderma* sp., and *Fusarium* sp. The technique developed by Brand-Williams et al. was used to assess the DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) radical scavenging activity. The results demonstrated that ZnO nanoparticles had greater activity when compared with all other fungal strains. The plant extract has a strong antifungal effect against *Aspergillus niger*. Antioxidant results showed that ZnO NP had an IC<sub>50</sub> value of 0.473mg/ml whereas ascorbic acid had an IC<sub>50</sub> value of 0.352mg/ml. As a result, ZnO NP exhibits antioxidant properties that only slightly differ from those of the standard ascorbic acid.

**KEYWORDS:** Antifungal, Zinc oxide, *Barringtonia racemosa*, antioxidant, DPPH.

### INTRODUCTION

Phytochemical, antibacterial and antifungal compounds are abundant in medicinal plants. Many potent and strong medications come from plants, which are utilised medicinally in various countries (Srivasta *et al.*, 1996). Many different medicinal plant components, each with a unique set of therapeutic characteristics, are employed to extract as raw medicines. Although some of these unprocessed substances are gathered by regional communities and traditional healers in modest amounts for use locally (Rekha *et al.*, 2013). Due to its therapeutic properties, *Barringtonia racemosa*, commonly known as Putat, fish poison tree, or powder puff tree, is a specific very valued plant species. Globally, it is discovered to be extensively spread, with populations ranging from eastern Africa and Madagascar to Micronesian and Polynesian Islands in addition to at mangrove river sites in Sarawak. This type was discovered in the Meranak river bank near Kota-Samarahan, Sarawak. The plant does have a strong history of being used in a variety of ethno - medicinal ways by several communities throughout the globe (Osman *et al.*, 2015). Large leaves, beautiful white blooms, and fruit that spread in long racemes make the mangrove tree distinctive. It stands between 4 and 8 metres tall, but may infrequently exceed 15 metres, and has a tall, unbranched stem that ends in a circular crown. Featuring white

spots, protruding dots, and lines, the bark ranges in colour from greyish brown to pink. Leaf scars can be observed on the branches. The category of this species is "underutilised crops" (Isaac *et al.*, 2018). Plant materials generally combine these secondary metabolites to produce their positive therapeutic effects. Antioxidant (Wong *et al.*, 2009), antibacterial (Nair *et al.*, 2005), antifungal (Khan *et al.*, 1987), antidiabetic (Singh *et al.*, 2008, Gupta *et al.*, 2008, Kumar *et al.*, 2008) and anti-inflammatory (Kumar *et al.*, 2008) properties are known to be found in phytochemicals. The present study deals with the antifungal and antioxidant activity of zinc oxide in *B. racemose*.

## MATERIALS AND METHODS

### ANTIFUNGAL ACTIVITY

In this investigation, the antifungal activity of zinc oxide nanoparticles (ZnO NPs) against four pathogenic fungi—*Aspergillus niger*, *Aspergillus flavus*, *Trichoderma* and *Fusarium* and their mechanism of action were examined. *Aspergillus niger*, *Aspergillus flavus*, *Trichoderma*, and *Fusarium* have been the four pathogenic fungal species that were retrieved from the stock culture of the laboratory at Xcellogen biotech India Pvt.Ltd., Thiruvananthapuram.

Agar well diffusion techniques were used to assess the antifungal properties of zinc oxide nanoparticles discharged in different forms. The antifungal activity of plants or microbial isolates is often assessed using the agar well diffusion technique. To evaluate its potency against fungi, 100 µl of zinc oxide nanoparticles were synthesized. Additionally, a culture medium with a chosen fungus was employed as a control. In order to create cultural plates for antifungal activity, sterile Potato Dextrose Agar (PDA) in 25ml autoclaved concentrations was poured onto sterile petri plates. On the surface of the media plates, a consistent 6mm well is made using a typical well borer. Wells were made on the agar plates' surface after solidification. 100 µl of various zinc oxide nanoparticle releases was put into the well, and 6 mm-sized, carefully chosen fungi were placed on the wells opposite side. The entire experiment was carried out, and the plates were incubated for 72 to 92 hours at 25 to 30°C.

### ANTIOXIDANT ACTIVITY

#### DPPH RADICAL SCAVENGING ACTIVITY

The technique was employed to assess the DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) radical scavenging activity. This test is based on the concept that when DPPH accepts an atom of hydrogen (H) out of an antioxidant molecule, it is reduced to DPPH<sub>2</sub>, changing from purple to yellow and losing absorbance at 515 nm in the process. Freshly made 0.1mM DPPH (4 mg/100 ml methanol) methanolic solution was prepared. An aliquot of plant extract (1 ml) was added to 3 ml of DPPH solution at various doses (25–300 g/ml). After 30 minutes of dark storage for reaction mixtures, a reduction in absorbance at 515 nm was observed. Activities was calculated to use a calibration curves comprised of ascorbic acid varying concentrations from 25 to 300 g/ml and expressed in mg of ascorbic acid / g of extract ( $R^2= 0.990$ ).

## RESULTS

### ANTIFUNGAL ACTIVITY

*Trichoderma* is less susceptible to the ZnO nanoparticles' antifungal effects. That is 50mm growth in the test group and 58mm growth in the control group. ZnO nanoparticles exhibit high

activity compared to all other fungi species. The plant extract does have a strong antifungal effect against *Aspergillus niger*. In the control, complete growth was seen, but only 16mm of growth was shown in the test. ZnO nanoparticles are thus an effective fungicide against *Aspergillus niger*. (Fig.1 to 4).

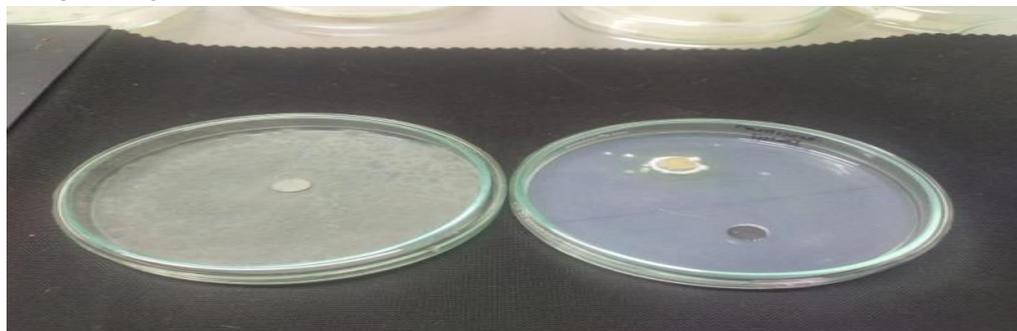


Fig.1 a) Control *Aspergillus niger* b) Anti-fungal activity of ZnO over *Aspergillus niger*

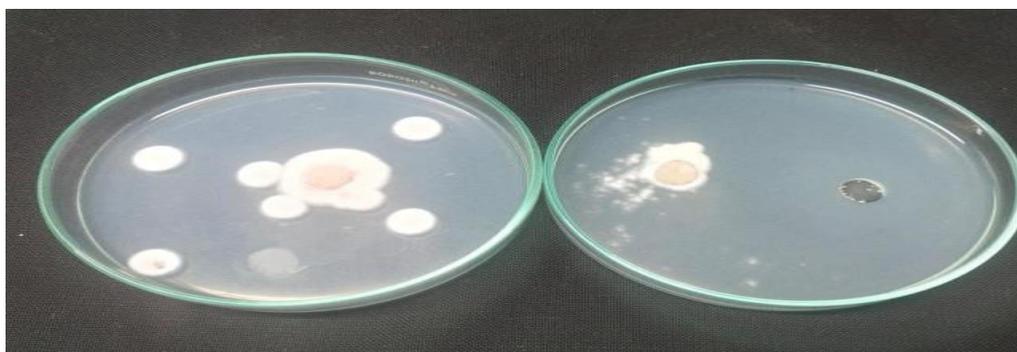


Fig.2: Control (*Fusarium*) b) Anti-fungal activity of ZnO over *Fusarium*

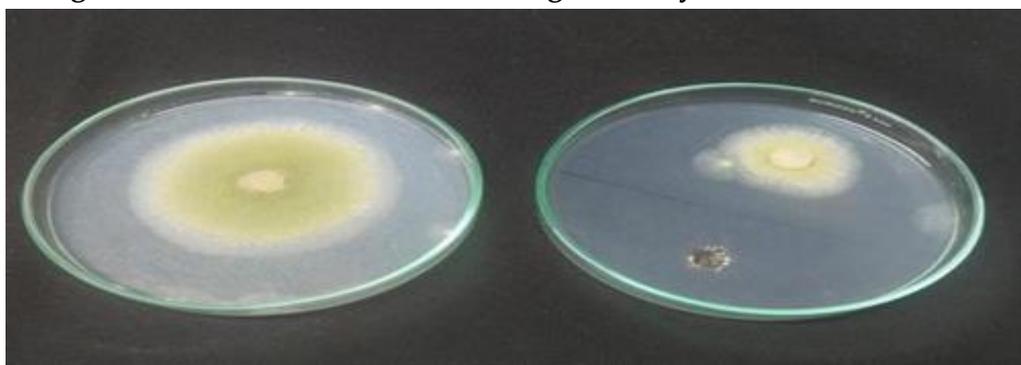


Fig.3: Control (*Aspergillus*) b) Anti-fungal activity of ZnO over *Aspergillus*

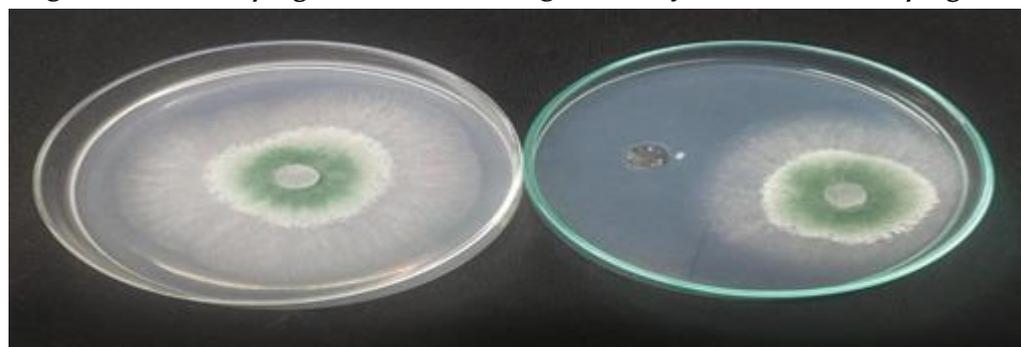


Fig.4: a) Control (*Trichoderma*) b) Anti-fungal activity of ZnO over *Trichoderma*

## ANTIOXIDANT ACTIVITY

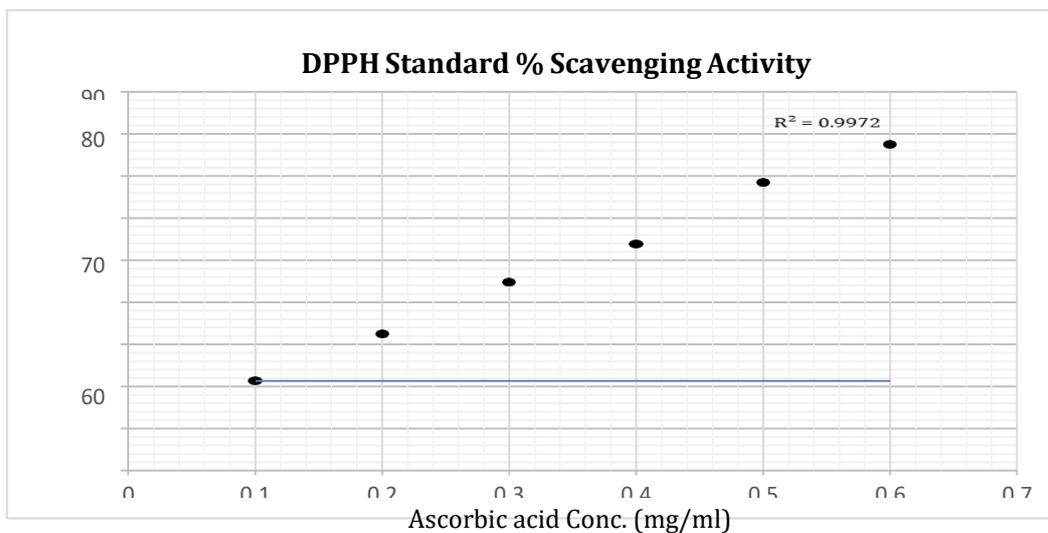
DPPH- radical scavenging activity

DPPH-radical scavenging activity of Ascorbic acid

The standard curve for the DPPH scavenging activity using ascorbic acid is shown below. (Graph1 and 2).

**Table.1: DPPH- radical scavenging activity**

Sr. No	Ascorbic Acid (vol. in ml)	Conc. (mg/ml)	Methanol (vol. in ml)	DPPH (vol. inml)	Total (vol. in ml)	OD @520nm	% Scavenging Activity
Blank	0	0	5	0	5	0	-
Control	0	0	2.5	2.5	5	0.89	-
S <sub>1</sub>	0.1	0.1	2.4	2.5	5	0.70	21.3
S <sub>2</sub>	0.2	0.2	2.3	2.5	5	0.60	32.5
S <sub>3</sub>	0.3	0.3	2.2	2.5	5	0.49	44.9
S <sub>4</sub>	0.4	0.4	2.1	2.5	5	0.41	53.9
S <sub>5</sub>	0.5	0.5	2.0	2.5	5	0.28	68.5
S <sub>6</sub>	0.6	0.6	1.9	2.5	5	0.20	77.5



**Graph 1: DPPH Assay- Ascorbic acid**

$$y=mx+c$$

$$\text{From graph, } y = 113.71x + 9.9667$$

$$\text{IC}_{50} \text{ for ascorbic acid, } 50 = mx + c$$

$$x = (50 - c)/m$$

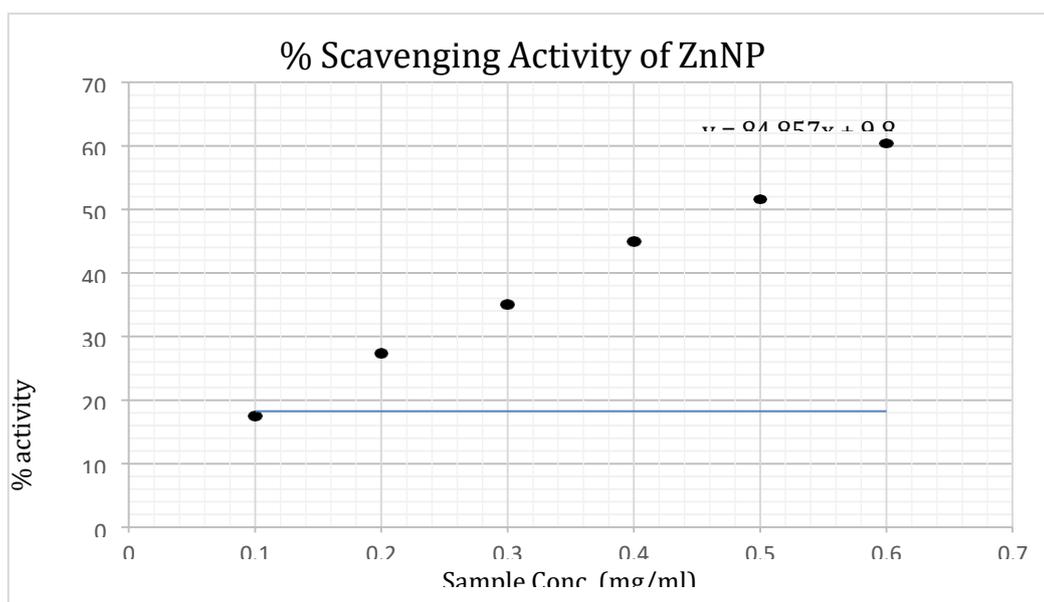
$$x = (50 - 9.9667)/ 113.71$$

$$x = 0.352 \text{ mg/ml}$$

**DPPH SCAVENGING ACTIVITY OF ZnNp**

**Table 2: DPPH scavenging activity of ZnNp sample has been presented below**

Sr. No	Sample Extract (vol. in ml)	Conc. (mg/ml)	Methanol (vol. in ml)	DPPH (vol. in ml)	Total (vol. in ml)	OD @520nm	% Scavenging Activity
Blank	0	0	5	0	5	0	-
Control	0	0	2.5	2.5	5	0.91	-
S <sub>1</sub>	0.1	0.1	2.4	2.5	5	0.75	17.5
S <sub>2</sub>	0.2	0.2	2.3	2.5	5	0.66	27.4
S <sub>3</sub>	0.3	0.3	2.2	2.5	5	0.59	35.1
S <sub>4</sub>	0.4	0.4	2.1	2.5	5	0.50	45
S <sub>5</sub>	0.5	0.5	2.0	2.5	5	0.44	51.6
S <sub>6</sub>	0.6	0.6	1.9	2.5	5	0.36	60.4



**Graph 2: DPPH Assay ZnNp**

ZnNP from graph,  $y = 84.857x + 9.8$

$$IC_{50} \text{ for ZnPS } 50 = mx + c \quad x = (50 - c)/m$$

$$x = (50 - 9.8) / 84.857$$

$$x = 0.473 \text{ MG/ML}$$

Ascorbic acid is known to have an IC<sub>50</sub> value of 0.352 mg/ml, whereas ZnO NP was observed to have an IC<sub>50</sub> value of 0.473 mg/ml. ZnO NP, thus, has antioxidant properties that only slightly coincide with those of the standard antioxidant ascorbic acid (Table 1, 2 and Fig 5).

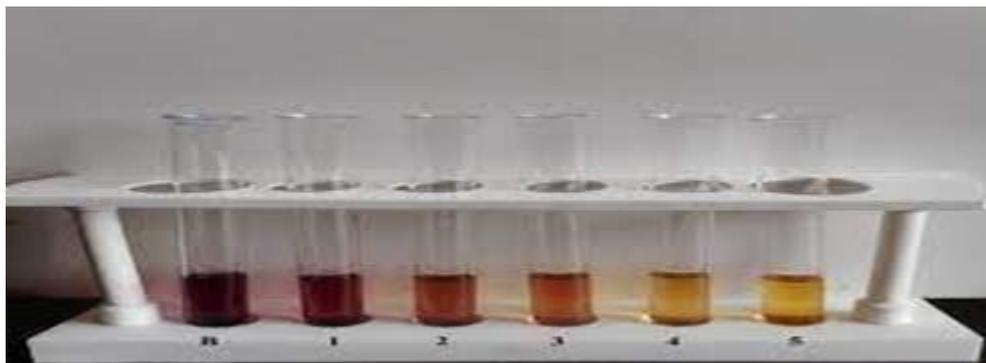


Fig. 5: DPPH Assay

## DISCUSSION

Medicinally plant extracts have already shown inhibitory actions in vitro against phytopathogenic fungus (Shalini *et al.*, 2009). According to Hussin *et al.* (2009) of the seven fungi studied, methanolic extracts from the leaf, stick, and bark of *B. racemosa* showed the most significant findings in terms of their antifungal activity. The methanolic leaf extract showed the highest level of inhibitory action (53.4%) against *Fusarium sp.*, followed by *G. lucidum* (34.57%), *Aspergillus* (32.27%), and *T. koningii* (20.99%). The particular results of the leaf extract in boiling water against *Fusarium sp.* (51.72%) as well as the bark extract in ethanol against *Rhizopus sp.* (37.50%) are equally noteworthy. Neither of the leaf, stick, nor bark boiling water extracts had any inhibitory effects on *G. tropicum* and *T. koningii*. When tried to compare to other fungi examined, *Fusarium sp.* was shown to be more susceptible to all of the extracts. The earlier investigation, which found that *Barringtonia asiatica* (leaves, fruits, seeds, stems, and root barks), the same species as *B. racemosa*, had a very strong degree of wide spectrum antifungal activity, supported the current findings of the antifungal activity (Khan *et al.*, 2002). The development of *Microsporium canis*, *Trichophyton rubrum*, and *Epidermophyton floccosum* were also seen in the methanolic extract of the *B. asiatica* flower (Locher *et al.*, 1995). However, in our investigation, ZnO nanoparticles had high activity compared to all other fungal species. The plant extract has a strong antifungal effect against *Aspergillus niger*.

Lycopene was shown to be the active ingredient in *B. racemosa*, according to the many findings. An important antioxidant called lycopene may be found in a variety of fresh fruits and vegetables. This substance is well recognized for helping to safeguard animals from the harm caused by free radicals and singlet oxygen reactive species. Lycopene has been found to have good anti-inflammatory and antioxidant activities in a mouse research (Zhao *et al.*, 2003). According to Mandana Behbahani *et al.*, (2007), the IC<sub>50</sub> values of extracts obtained from ethanol, chloroform, hexane, and -tocopherol were 32, 54, 63, and 125 g/ml, significantly. Plant extracts evaluated for their IC<sub>50</sub> values have less activity than -atocopherol. The results indicated that DPPH activity was much greater ( $p < 0.05$ ) in non-polar extract (chloroform and hexane extracts) than it does in polar extract (ethanol extract). Chloroform extract has considerably greater ( $p < 0.05$ ) DPPH activity than hexane extract among the two types of non-polar extracts. However, in our research revealed that the IC<sub>50</sub> value for ascorbic acid was 0.352

mg/ml whereas the IC<sub>50</sub> value for ZnO NP was 0.473 mg/ml. As a result, ZnO NP exhibits antioxidant properties that only slightly differ from those of the standard antioxidant ascorbic acid.

## CONCLUSION

The zinc oxide nanoparticles in *B. racemosa* have been discovered as having antifungal and antioxidant properties from the aforementioned investigation. It demonstrated a very strong DPPH radical scavenging action. These findings point to the potential of *B. racemosa* as an addition in the pharmaceutical sector, as well as in Nano drugs and preparation of cream for antifungal treatment

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## ABSTRACT

The antibacterial efficacy of ZnO nanoparticles in *Barringtonia racemosa* towards gram-positive bacteria and gram-negative bacteria was tested using *Bacillus velezensis*, *Klebsiella ozaenae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* as test microorganisms. The impact of particle size and concentration on the antibacterial ability of ZnO nanoparticles was examined using bacteriological tests such as the disc method and well diffusion agar techniques, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Standard procedures were followed in these assays, which were carried out in nutrient broth as well as nutrient agar. Additionally, the impact of various ZnO nanoparticle concentrations upon that development of microorganism's *B. velezensis*, *K. ozaenae*, *S. aureus*, and *P. aeruginosa* were studied with respect to time. The antibacterial study demonstrated that *P. aeruginosa* and *K. ozaenae* had the highest zones of inhibition (6mm). Five different doses of ZnO nanoparticles, including 0.02 g/mL, 0.04 g/mL, 0.06 g/mL, 0.08 g/mL and 0.1 g/mL were used to establish the minimal inhibitory concentration. Accordingly, the MIC value was 0.6 mg/ml. This was discovered that lowering size of the particles and raising powder concentration both boosted antibacterial activity of ZnO nanoparticles in *B. racemosa*. Antibacterial property was time-dependent but also developed progressively. There was no discernible antibacterial action in ZnO bulk powder.

**KEYWORDS:** *Barringtonia racemosa*, antibacterial, MBC, MIC, Zinc oxide nanoparticles.

## INTRODUCTION

*Barringtonia racemosa* is a small tree that typically reaches a height of 4 to 8 metres, although it may grow as high as 15 metres. Its crown is rounded, brownish bark, smooth and surface roots are spreading. In clusters towards the ends of branches, the alternate leaves are glabrous, 2–15 mm long petiole. Large, oblong, deep green leaf lamina with acute apex and serrate crenulate edges. Pharmaceutical qualities include high concentrations of phenolics and carotenoids were documented in *B. racemosa* aerial plant (lycopene,  $\beta$ -carotene). *B. racemosa* may be found throughout the coasts of Eastern Africa from South Africa via Madagascar as well as other Indian Ocean islands (Hussin *et al.*, 2009).

Active metal oxide like zinc oxide can be produced quickly, are good for the environment, and have a wide range of uses. Because of its many qualities, that have recently been discovered to

greatly rely on its morphology, it is appropriate for industrial, technological, and medicinal applications (Zheng *et al.*, 2009). A vital component for humans, animals, and microorganisms, Zinc (Zn) is among the most significant elements. DNA replication, oxidative stress, DNA repair, and cell cycle progression are all vital biological activities that Zn plays a critical part in regulating (Bisht *et al.*, 2016). All of the enzymatic groups, including oxidoreductases, isomerases transferases, lyases, ligases, hydrolyses, and, contain only this one element (Auld *et al.*, 2001). Due to their distinctive characteristics, ZnO nanoparticles (ZnO NPS) had already subsequently attracted a lot of interest. Compared to their typical Zn sources, ZnO NPs showed a higher absorption rate, decreased toxicity, and increased bioavailability and biocompatibility (Mohd *et al.*, 2019, Hosseini *et al.*, 2015). They demonstrated a broad range of biological and medicinal value, such as antibacterial (Shahid *et al.*, 2019, Khan *et al.*, 2018) antioxidant, antiprotozoal and anticancer properties. Additionally, recent research revealed the potential benefits of ZnO nanoparticles that might be used in the cattle and poultry industries (Abd Elkodous *et al.*, 2020, Zhao *et al.*, 2014).

The two major types of antibacterial agents are organic and inorganic. In comparison to inorganic antibacterial agents, organic antibacterial compounds are shown to be less stable at high temperatures and pressures (Khoobakht *et al.*, 2018). Thus, when used in the creation of micro- and nanotechnology systems for medicinal purposes, zinc oxide has shown to be a potent antibacterial agent: (Singh *et al.*, 2012) ZnO nanoparticles seemed to have more antibacterial activity than micro particles did (Khah *et al.*, 2015). Because of this, the antibacterial activity of nanoparticles is improved by increasing their surface area and concentration (Noreen *et al.*, 2018). There is currently a lack of knowledge on the mechanisms behind ZnO particles' antimicrobial properties. According to many studies, the major cause of the antibacterial activity is the production of hydrogen peroxide. However, it was also suggested that the electrostatic interactions that cause particles to adhere to the bacteria's surface might play a role (Brayner *et al.*, 2006). In this work, the antibacterial activity of ZnO nanoparticles in *B. racemosa* were investigated using bacterial culture tests such as disc and well diffusion agar methods.

## **MATERIALS AND METHODS**

### **PREPARATION OF PLANT EXTRACT**

Fresh *B.racemosa* leaves and fruits (Plates 1 and 2) were procured from the Poovar regions of Thiruvananthapuram District, Kerala. They were then thoroughly cleaned with sterile distilled water for removing any debris, drying in hot air oven at about 35–40°C for a couple of days to get rid of any moisture, and crushed into a powder. The dried plant material was successively extracted with distilled water and swirled continuously for 24 hours using a magnetic stirrer (Plate.3). For the purpose of removing plant debris and contaminants, the extracts were screened using Whatman No. 1 filter paper. The extracts had been kept in a jar for future research.

## **SYNTHESIS OF ZNO NANOPARTICLES GREEN SYNTHESIS OF ZINC OXIDE NANOPARTICLES USING BARRINGTONIA RACEMOSA LEAF EXTRACT**

The co-precipitation technique was reported by Singh et al. 2011 was used to create zinc oxide nanoparticles. Starting materials included zinc nitrate and sodium hydroxide. In 25 mL of deionized water, zinc nitrate was prepared and stirred for one hour appropriately (Plate.4). After the mixture had completely dissolved, 1 mL of leaf extract was added to the zinc nitrate solution that had been made. To achieve pH 12, 25 mL of 0.02 M NaOH were dropped into the mixture. For two hours, the mixture was stirred using a magnetic device. In order to eliminate the contaminants, the resulting white precipitate have been filtered then subsequently rinsed with distilled water and then ethanol. The purified precipitate was dried for two hours at 700°C in a Muffle furnace to produce a solid white powder at the end (Plate.5). During drying, Zn (OH) completely transformed into ZnO NPs (Plate.6).

### **APPLICATION STUDY**

#### **ANTIBACTERIAL ACTIVITY**

##### **TEST MICROORGANISMS**

*B. velezensis*, *K. ozaenae*, *S. aureus* and *P. aeruginosa* four bacterial isolates strains, were utilized to assess the antibacterial effectiveness of various plant extracts and nanoparticles. By adding a loop's worth of mother culture to test tubes containing 5 mL of nutrient broth and 24-hour incubation period at 37 °C.

##### **AGAR WELL DIFFUSION ASSAY**

By using the agar well diffusion technique, the effects of *B. racemosa* leaves and fruits was evaluated. Aseptically poured Mueller-Hinton agar medium was then let to set up in the petri plates. Using a sterile cotton swab, the tea bacterial strain was transferred to each of the separate plates. Plates were let to remain for ten minutes to allow for culture absorption. Sterile forceps were used to aseptically remove the cut agar sections after creating the wells with the use of sterile micropipette tips. The wells on each plate were filled with around 100 µl newly synthesized nanoparticles and they were left to permeate at room temperature for two hours. Negative control consisted of 50 µL of DMSO, while positive control consisted of 25 µL of typical antibiotic concentrations such as gentamicin (GEN), oxyacillin (OX), penicillin (P), and tetracycline (TE). After 24 hours of incubation, the plates were kept in the incubator at about 37°C to detect the zone of inhibition (mm). After which, choose the ones that show the zone of inhibition and found the ones that show the better zone on the concluding plate.

##### **MINIMUM INHIBITORY CONCENTRATION (MIC)**

In lowest concentrations at which any discernible growth of bacteria on the culture plates may be inhibited is known as the minimum inhibitory concentration. The reading on the culture plates following incubation was used to make this determination. Diagnostic labs primarily employ MIC to verify resistance. However, the majority of them are research instruments for figuring out novel antibiotics' in vitro activity and using the information from that study to decide out MIC break points. The Agar well diffusion method was used to determine the minimal inhibitory concentration. Mueller-Hinton agar medium was aseptically added to the

petri plates and allowed to set. We used sterile cotton to make the test bacterial strain lawns on each of the separate plates. Plates were then let to stand for 10 minutes to enable the culture to absorb. Clean micropipette tips were used to make the wells and sterile forceps were used to aseptically remove the cut agar sections. The wells were filled with 100  $\mu$ L of nanomaterials at various concentrations (0.02 g/ml, 0.04 g/ml, 0.06 g/ml and 0.08 g/ml), which were then left to diffuse at optimum temperature for two hours. The cultures were kept at 37°C for 24 hours of incubation. The nanoparticle quantity with the lowest zone of inhibition, known as the MIC, was measured.

### MINIMUM BACTERICIDAL CONCENTRATION (MBC)

The least amount of antimicrobial agent necessary to kill the bacteria after subculturing on antibiotic-free medium is known as the minimum bactericidal concentration. The Dilution Methodology (DM) is used to determine MBC. It entails incubating the indicator bacterium in different drug concentrations for 18 to 24 hours before testing for bacterial viability by subculturing on agar medium made without the antibiotic).

For the purpose of determining MBC, a dilution protocol was used. The nanoparticles were diluted in test tubes with sterile nutritive broth (5 mL) at different concentrations (0.06, 0.07, 0.08, 0.09, 0.1, and 0.5 $\mu$ g/mL). All tubes received the 20  $\mu$ L each of *B.velezensis* and *K.ozanae*, which were added and incubated the tubes at 37°C for 24 hours. On newly made nutrient-agar plates, 10 $\mu$ L of the MIC tube containing was sub cultured and incubated for 24 hours at 37°C. The lowest concentration at which no bacteria colony could develop was designated as MBC.

## RESULTS

### PREPARATION OF PLANT EXTRACT



**Plate:1 Fresh leaves  
of *Barringtonia  
racemosa***

**Plate:2 Fruits of  
*Barringtonia  
racemosa***

**Plate:3 Powdered  
plant extract stirring  
by magnetic stirrer**

Fresh leaves of *B. racemosa* were collected, and a powdered plant sample was successively extracted using three solvents—hexane, butanol, and methanol. To get rid of plant debris and contaminants, the extracts were filtered using Whatman No. 1 filter paper. To facilitate additional biological activities, the different plants extracts were fully dried and kept at 4°C.

**SYNTHESIS OF ZNO NPS BARRINGTONIA RACEMOSA LEAF EXTRACT**



**ANTIBACTERIAL ACTIVITY**

The antibacterial property of a plant extract was tested using the agar well diffusion method against two gram positive bacteria, *B. velezensis* and *S. aureus* and two gram negative bacteria, *p. aeruginosa* and *K. ozaenae*. The zone of inhibition (mm) discovered during a 24-hour incubation period. The appropriate antibiotic disc for the relevant bacterial species serves as the positive control and DMSO act as the negative control.

**Table.1: Antibacterial activity of *Barringtonia racemosa* Leaf Extract**

Organism	Zone of inhibition (in mm)			
Wells	Control (C)	Sample (S)	-Ve Control (N)	+Ve Control (P)
<i>Klebsiella Ozaenae</i>	-	6	-	8
<i>Pseudomonas aeruginosa</i>	-	6	-	10
<i>Staphylococcus aureus</i>	-	2	-	5
<i>Bacillus velezensis</i>	-	4	-	6
Organism	Zone of inhibition (in mm)			
Wells	Control (C)	Sample (S)	-Ve Control (N)	+Ve Control (P)
<i>Klebsiella Ozaenae</i>	-	6	-	8
<i>Pseudomonas aeruginosa</i>	-	6	-	10
<i>Staphylococcus Aureus</i>	-	2	-	5
<i>Bacillus velezensis</i>	-	4	-	6

Samples Including ZnO Nanoparticle And Solvents Had The Largest Zone Of Inhibition (Measured In Mm) Towards *B. velezensis*, *S. aureus*, *P. Aeruginosa*, And *K. ozaenae*. Regarding Consideration to *K. ozaenae* And *P. Aeruginosa*, A ZnO Sample Diluted In Distilled Water Demonstrated A Maximal Zone Of Inhibition (6 Mm) (Plate.9). No Bacillus Strains Was Ever Affected By DMSO As A Negative Control For Antibacterial Activity (Table.1). When Compared To ZnO Nanoparticles, Antibiotic Discs Indicated That The Zone Of Inhibition Was One Step Forward.

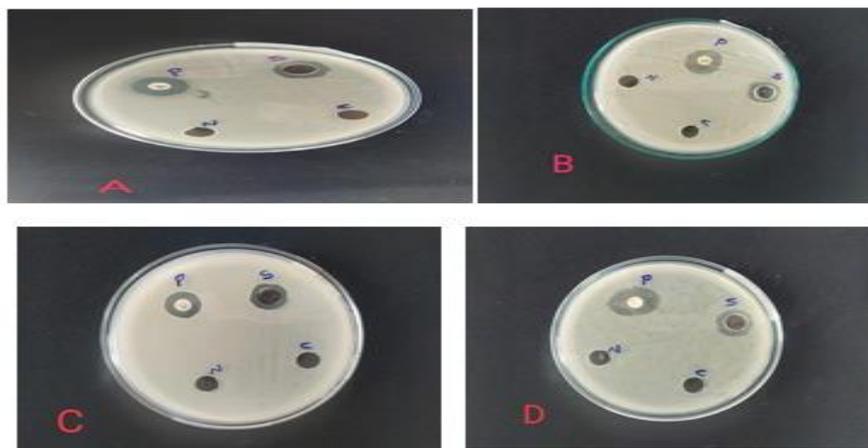


Plate 7, 8, 9: A, B, C, D shows Antimicrobial activity of ZnO nanoparticles against *B. velezensis*, *K. ozaenae*, *S. aureus*, *P. aeruginosa*

#### MINIMUM INHIBITORY CONCENTRATION (MIC)

Agar well diffusion was employed to calculate minimal inhibitory concentration. ZnO nanoparticles were put to wells in a volume of 100  $\mu$ L at various concentrations like 0.02, 0.04, 0.06, 0.08, and 1.0 g/ml, and then left in an incubator for 24 hours at 37  $^{\circ}$ C. The MIC measured on four strains of bacteria: *P. aeruginosa*, *B. velezensis*, *K. ozaenae* and *S. aureus*. It displayed a MIC of 0.04 $\mu$ g /ml including both bacterial species (Plate.10 and 11 and Table.2).

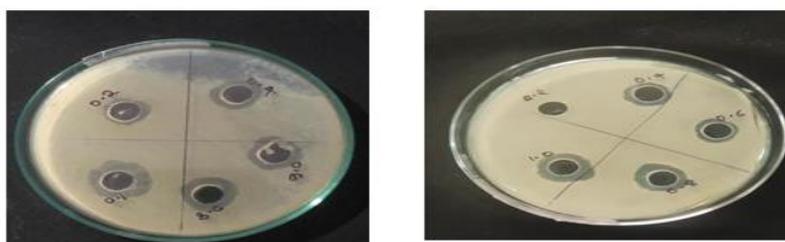


Plate.10 MIC of plant extract against a) *B. velezensis* b) *K. ozaenae*



Plate.11 MIC of plant extract against c) *S. aureus*, d) *P. aeruginosa*

**Table 2: Minimum Inhibitory Concentration (MIC) of *Barringtonia racemosa* leaf extract**

Concentration ( $\mu\text{g/ml}$ )	Zone of inhibition (in mm)			
	<i>Klebsiella pneumoniae</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Bacillus velezensis</i>
0.02	-	-	-	-
0.04	4	5	4	4
0.06	7	8	8	7
0.08	10	11	9	10
1.0	12	12	11	12

**MINIMUM BACTERICIDAL CONCENTRATION (MBC)**

The ZnO nanoparticles subsequently diluted in sterile nutritional broth (5 ml) to different concentrations (0.04 to 1.0  $\mu\text{g/ml}$ ). All tubes received the 20  $\mu\text{l}$  of *S. aureus*, which was then set for incubation at 37 °C for about 24 hours. Followed by incubation, 10  $\mu\text{l}$  of MIC solution were subcultured on newly made agar plates and incubated at 37°C for 24 hours. The minimum bacterial concentration or MBC is the level below which bacteria cannot thrive. The MBC result showed that plant extract greatly slowed bacterial growth. *S.aureus* had the lowest MBC concentration, which was 0.6  $\mu\text{g/ml}$  (Plate.12).

**Plate 12: MBC Results 0.6 $\mu\text{g/ml}$  of *Staphylococcus aureus*****DISCUSSION**

According to the research, antibacterial activity of two new clerodane diterpenoid nasimal un A and B were produced by an ethanol extract of *B. racemosa* roots (Khan *et al.*, 2001). As per studies conducted in 2006 by Brayner and others reported the antibacterial property of ZnO NPs towards *E. coli*, the inhibition of bacterial growth increased. The findings demonstrated that doses of 1.3 mM or below had no discernible impact on *E. coli* growth. ZnO NPs exhibited 100% suppress the growth of the bacteria at the dose ranges between of 3.0 to 10 mM.

According to a separate study by Jones and colleagues, ZnO NPs showed an inhibitory effect on a number of bacteria, including *S. aureus*, *S. epidermidis*, *S. pyogenes*, and *B. subtilis* (Jones *et*

al., 2008). Using qualitative and quantitative testing, Tayel *et al.*, 2011 study showed that the gram-positive bacteria were much more vulnerable to ZnO NPs and it was more efficient against gram-positive *S. aureus* than gram-negative *S. aureus* and *S. typhimurium* cell counts was decreased slowly to zero after least inhibitory concentration of ZnO NP exposure within 8 to 4 hours respectively. Examining treated cells under scanning electron microscopy revealed that they had totally disintegrated or ruptured. The findings of this research also indicated that *Pseudomonas* spp. was the most resistant strain to ZnO NPs, whereas *Bacillus cereus* was the most susceptible.

Research done by Mirhosseini and Firouzabadi (2013), ZnO NPs were shown to have antibacterial properties in food samples. Infected milk samples with *S. aureus* and *E. coli* were treated with ZnO NPs at two doses, 5 and 10 mM. As a result, after 8 hours of incubation, *S. aureus* treated with ZnO NPs in milk had a growth rate that was 2 log CFU/ml lower than only its control, whereas *E. coli* had a growth rate that was significantly less than 1 log lower. ZnO NPs had a more potent antibacterial impact on *S. aureus* than *E. coli* in milk. The results suggest that ZnO NPs have antibacterial effect in food samples.

The antibacterial activities of ZnO NPs and the inhibitory mechanisms against bacteria as well as fungus have been the subject of more investigations in recent years. Although detailed findings on the processes by which ZnO NPs work to suppress bacteria are lacking, it is well acknowledged that the inhibitory effect increases with concentration.

#### CONCLUSION:

*B. velezensis*, *S. aureus*, *P. aeruginosa* and *K. ozaenae* were used as test microorganisms to determine the antibacterial activity. Agar well diffusion test was used to conduct the experiment. Standard zone of inhibition have been determined for susceptible and resistant values using this approach, which is extensively documented. These results show that ZnO nanoparticles and the leaf extract of *Barringtonia racemosa* exhibit a broad spectrum of antibacterial properties against different potential harmful bacteria that might be used in nano drug formulations.

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Chapter  
10

THE SYNTHESIS OF ZINC SULFIDE FOR USE IN SOLAR CELLS  
BY SOL-GEL NANOMATERIALS

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ABSTRACT

There has been a meteoric rise in research into ZnS and its modification into ZnS-based composites for use in solar cell-based materials. ZnS has been successfully synthesized via traditional synthetic approaches during the past three decades, including one-pot synthesis, sol gel formation, hydrothermal preparation, and solid-state reaction. Until recently, ZnS has been further developed as ZnS-based composite materials to increase the quality of use. The advantages and disadvantages of ZnS will be examined from the perspective of solar-based materials.

**KEYWORDS:** ZnS, Composite Material, Solar Cell based materials, Solgel, Nanomaterials.

INTRODUCTION

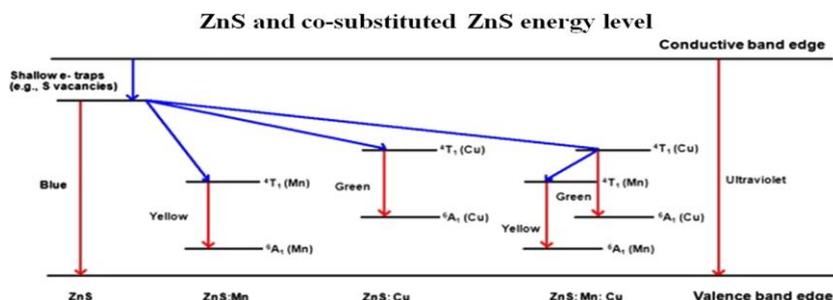
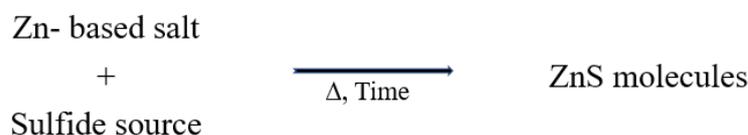


Figure 1: Exhibits the luminescent properties of ZnS and Mn and Cu-substitution of ZnS

ZnS is a well investigated material. Transition metal oxide is synthesized. One-pot, sol-gel, hydrothermal, and solid-solid interactions yield transition metal oxides. ZnS has several applications. Spheres, thick and thin films, rods, and tubes are possible. ZnS's form flexibility benefits LEDs, solar cells, active sensors, and wastewater treatment. ZnS may be formed as 3D particles, 2-D thin films, 1-D wire, rod, tube, ribbon, belt, and sheet structures, and 0-D quantum dots [1-3]. ZnS bridged bulk particles to nanoscale structures or decreased. ZnS is used in health and safety, electronics and optoelectronics, and catalytic activity in olefin-based polymeric polymers. Academic and industry researchers have studied ZnS's manufacturing path. ZnS is used in research because of its high energy conversion efficiency, although it can't

do some tasks that need little displacement [5, 7]. ZnS lacks rigidity; hence it shouldn't be used in mobile phones, flexible solar cells, or moveable sensors. It's built for a ceramic's gallery. Polyaniline, polythiophene, PSU, and PEDOT include ZnS. Conductive polymer was combined with ZnS for portability. Organic solar cells generate electricity for portable gadgets. Initial explanations of photovoltaic were theoretical [1-7].



**Figure 2: Chemical reaction of ZnS molecules**

### PREPARATION OF ZNS FROM SOLUTION BASED CHEMICAL SYNTHESIS

ZnS is a semiconductor used in electrical and optical devices with a large band gap. With band-gap energy of 3.6–3.9 eV, it may be used in UV LEDs, flat panel displays, thin film electroluminescent material, and solar panels. ZnS may be made in bulk or nanoscale spheres, rods, tubes, and wires. Chemical synthesis may readily modify ZnS, MnS, and Cu [8]. Adding guest ions makes ZnS more helpful in many applications. This implies light's properties may be used across the visible light spectrum. Blue light causes a bad response. Positive transition ions were added to Zn atoms to make ZnS active at longer wavelengths. Yellow and green are from Mn and Cu substitutions. Sn, Ni, Cd, and Fe were used to partially replace Zn [9]. Due of this, ZnS is useful in many settings. Sensors that change colour can detect deterioration in food, medical and pharmaceutical equipment, and wastewater treatment plants. Color modification is possible using electronics and optoelectronics [10]. Luminous materials benefit from colour shifts. In Fig. 1, ZnS and Mn and Cu-substituted ZnS emit light. ZnS has been explored for its structure and controlled qualities, and there are several ways to produce it with predictable size and shape [11-12].

### SOL-GEL FORMATION

One method for synthesizing ZnS involved the creation of a sol gel. In materials science, liquid gels were utilized to create solid products from very small molecules [13-17]. The process includes transforming monomers into a colloidal solution in order to create a network polymer made up of tiny, independent particles. Reagents used to create sol-gel chemistry are alkoxides of metals. The two phases of sol-gel production are sol and gel. A longer period of gel formation time was used to provide a lower-power output. In order for a sol gel to develop on ZnS, several parameters including reaction time, solvent, pH, ageing time, chemical reagents, and calcination temperature were necessary [18, 19].

The process of sol gel production has found widespread usage recently. The size and shape of ZnS depend on the molecular weight of the stabilizer. Polymers of  $5 \times 10^2 - 1 \times 10^5$  Dalton molecular weights are used as stabilizers, and some examples include PVP, PEG, and PAA. Constructing ZnS is feasible at both the bulk and quantum dot levels [20]. The use of ZnS will be crucial in many scientific disciplines. The production of ZnS sol-gel is seen in Figure 4.

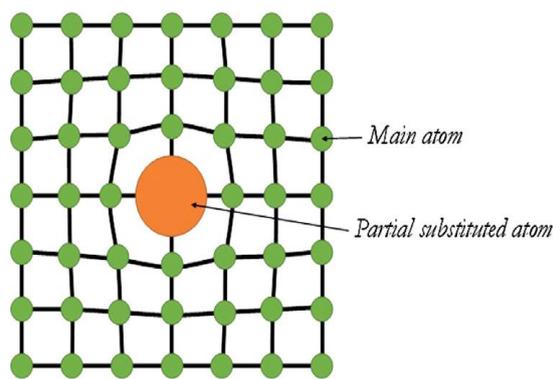


Figure 3: Partial substitution

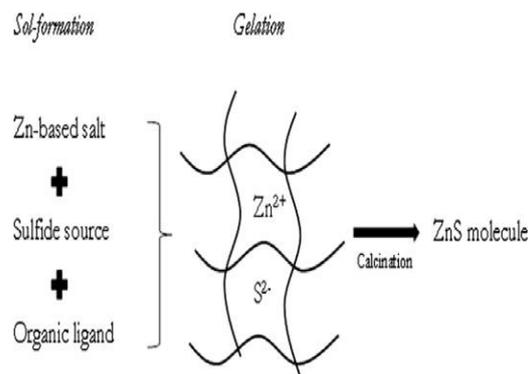


Figure 4: Sol-gel formation technique for ZnS

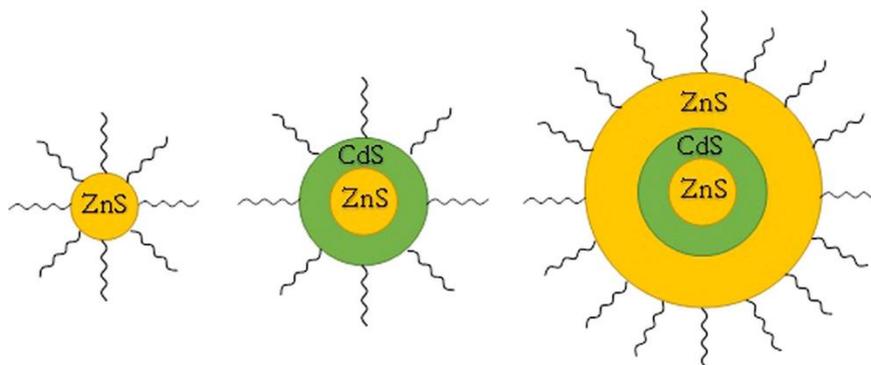


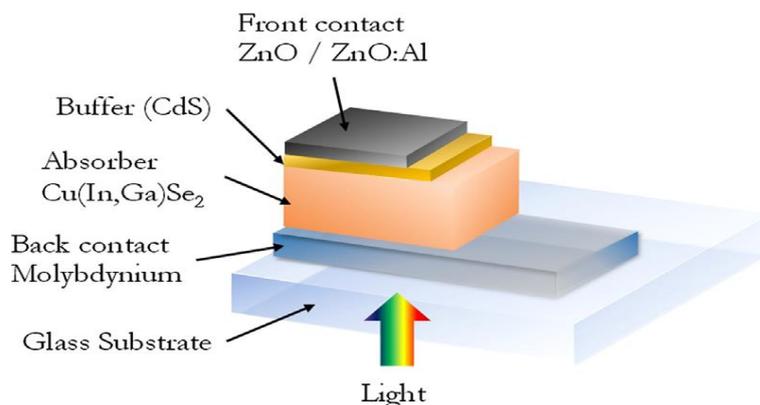
Figure 5: Modification of ZnS as core-shell composite material

### MODIFICATION OF ZNS AS COMPOSITE BASED MATERIAL

ZnS-based polymers might broaden its usage. ZnS adds optical energy. Subshells generate light. CdS improves ZnS. Widespread usage of ZnS requires mechanical advancements. ZnS adapted in-situ polymerization. In situ ZnS polymerization using polymer as matrix. The polymer matrix was mixed. Nanocomposites. ZnS composites have further uses. Ceramic ZnS. Circumstances prompted a change polymerization improved ZnS's usefulness and polymer matrixes. [21]

Synthesized ZnS-based composites had the same luminescence as pure ZnS. Energy level changes when a transition metal ion replaces Zn. Mixing two components generated ZnS. Wet chemistry made ZnS powder. Composite made from ZnS powder and polymer. ZnS composites need homogeneous powder. Mechanical, thermal, and luminous efficiency must be tested. Multiple materials affect its mechanical properties. Ceramics and polymers were in both. Reduce materialism. Fragmented clay/polymer matrix. ZnS has industrial applications. It's better for applications that demand flexibility but less relocation space.

Wet chemical synthesis is used to make ZnS. OLED emissive layer was as-synthesized ZnS. Our team created a cellulose-based OLED composite. Flexible screens can't employ synthesized ZnS powder. Substituting another metal ion for  $Zn^{2+}$  changed ZnS' luminescence. Restricted ZnS usage. Polymer matrix ZnS blocked this. Matrix was PVP. Solution included ethanol-dissolved ZnS. 310-nm-stimulated ZnS-PVP composites peak at 428 nm. Home inkjet printers printed the OLED emissive layer. Creating ZnS and PVP was difficult. ZnS increased use.



**Figure 6: CIGS thin film solar cells layer to layer device structure**

### POTENTIAL OF ZNS MATERIAL IN NOVEL GENERATION OF SOLAR CELL

Today's energy demand is rising, and industry relies on irreversible sources. Long-standing global energy concerns have led to the hunt for clean, renewable energy sources. One renewable energy viewpoint converts solar energy into electricity. Therefore, a solar cell is a potential technology. High-efficiency solar cells need expensive fabrication methods and high production costs, making solar energy more expensive than fossil fuels. Low-cost solar cells lack commercial power conversion efficiency. To improve the reliability of low-cost solar cells, researchers must find cheap, clean materials.

Nanostructured solar cell materials have multiple components. Light harvesting and light-to-electricity conversion employ active material. The passive substance helps photo-charge carrier separation and transport or retards recombination. The electrode collects charge carriers and guides them via the external circuit and load. With this notion, solar cell architectures have been built and investigated for maximum efficiency.

ZnS nanostructure is employed in dye-sensitized solar cells, quantum dot-sensitized solar cells, CIGS thin film solar cells, and organic-inorganic hybrid solar cells. ZnS nanostructures have gained interest because to their resemblance to classic semiconductors including  $\text{TiO}_2$ , CdS, and ZnO. ZnS nanostructure applications vary across innovative solar cells. In these portions, we'll discuss solar cells employing ZnS nanostructures as difference functions.

### SENSITIZED SOLAR CELLS (SSCS)

QDSSCs and DSSCs might replace solar cells. DSSCs and QDSCs absorb light differently. Optimizing charge injection, recombination, and transport enhances DSSC efficiency (PCE). Alignment of the metal oxide conduction band with LUMO and HOMO energy levels and dye sensitizer adsorption enable effective electron injection and reduce recombination. ZnO nanowires have lower DSSC effectiveness than  $\text{TiO}_2$ . Inefficient ZnO DSSCs Any oxidized dye or redox electrolyte can react with ZnO. Dye- $\text{Zn}^+$  binds, and CdS and ZnS nanostructures boost cell efficiency.

DSSC photo anodes are dye-sensitized nanocrystalline CdS or ZnS solar cells. Smaller dye-CdS particles than dye-ZnS. Inefficient ZnS nanostructure DSSC photoanode. ZnS nanobuns wrapped in RGO might be DSSC counter electrodes. CdSe nanocrystals are capped with ZnS.

Solar cells with ZnS/ZnO. Poorly functioning dye-adsorption cells. ZnO/ZnS nanowire-based DSCs performed well. ZnS nanowires decreased anode/electrolyte electron recombination. Standard dyes can't adsorb on ZnO/ZnS core/shell, reducing cell efficiency. QDSCs have a band gap, extinction coefficient, size, and structure. CdSe nanostructures enhance photocurrent by 1%. ZnS reduces QDs-sensitized TiO<sub>2</sub> photocurrent recombination. Multilayered or composite QDs-sensitizers enhance short circuit current to DSSC requirements. ZnS's valence band maximum and ZnO's conduction band minimum allow charge separation. ZnO nanostructures improve multilayer and core/shell DSSCs.

### THIN-FILM CIGS SOLAR CELLS

Thin-film CuIn<sub>x</sub>Ga<sub>(1-x)</sub>Se<sub>2</sub> cells are efficient and shown in figure 1. Metal, semiconductor, and alloy make up CIGS solar cells. CdS connects CIGS solar cells' absorber and front contact layers. The deposition procedure isn't compatible with CIGS cells having a CdS buffer layer, and Cadmium isn't eco-friendly [1, 7-10].

Due to their electrical and physical characteristics, Zn-based compounds may replace CdS in buffer layers. CBD, MBE, MOCVD may deposit ZnS buffer layer. CBD-ZnS buffer layers reduce band offset and bulk recombination. ZnS-In<sub>2</sub>S<sub>2</sub> buffer layers improve thin-film solar cell open-circuit voltage and fill factor. Zn<sup>2+</sup> ions increased solar cell performance when added to the buffer layer. ZnS deposition experiments improved CIGS thin-film solar cell efficiency [8, 18].

ZnS is used in solar cells. ZnS passivation lowers semiconductor-electrolyte corrosion and chemical instability. With an oxidized dye or redox electrolyte, ZnS reduces recombination. ZnS, not CdS, is the CIGS buffer layer. A thin buffer layer limits diffusion when depositing, making cells more robust. ZnS nanostructures are employed in hybrid ZnS nanoparticles/Si Nano tip solar cells and P3HT/ZnS/ZnO/reduced graphene oxide shell-core Nano rod arrays. ZnS solar cells have several applications. Solar cells include double-junction, triple-junction, CIGS, and hybrid. ZnS and its influence on cutting-edge solar cells, specifically its nanostructure's implications on solar cell operations, chemistry, and morphology, are still little understood [6, 11, 17].

### CONCLUSION

Numerous researchers have spent a lot of time and energy studying ZnS materials up to this point. It has been thoroughly researched that there are various synthetic approaches to many alterations in physical and chemical characteristics. In this study, a solid-state reaction-based composite based on ZnS was effectively synthesized via one pot synthesis, sol gel formation, and the hydrothermal approach. As a result, a plethora of strategies for solar cells based on ZnS were introduced. ZnS is a good material for solar cells, but to get high efficiency, it required extensive synthetic procedures and modifications, such as ZnS-based composites.

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## ABSTRACT

*Mimosa pudica*, called a sensitive plant or Thottal sinungi in Tamil, belongs to the Fabaceae family. It is well known traditional plant with several valuable secondary metabolites such as tannins, steroids, flavonoids, triterpenes, and glycosylflavones. A wide range of pharmacological effects attributed to different parts of *Mimosa pudica* such as antihyperglycemic, anti-inflammatory, antivenom, anticonvulsant immunomodulatory, antifertility, antihepatotoxic, diuretic, and possess wound healing activity. All the parts of *Mimosa pudica* have been reported regarding their medicinal properties. This review aims to summarize the pharmacological potential and therapeutic insights of *Mimosa pudica*.

**KEYWORDS:** *Mimosa pudica*, traditional plant, Pharmacological activity.

## INTRODUCTION

*Mimosa pudica* Linn. is an annual or perennial climbing plant. This is considered native to south and Central America and is also grown in India (Sanaye *et al.*, 2015). It is a common plant in moist waste, ground, lawn, and open plantations and is now widely distributed in all tropical areas. It is called as sensitive plant in English, ajalikalika in Sanskrit, lajawanti in hindi, lajjabate in bangali, hadergitte in kannada, thotach chenunggi or tottalvadi in tamil and attapatti in telugu. All parts of this plant have been reported in the treatment of numerous diseases (Lubna Azmi *et al.*, 2011). Previous research shows the medicinal properties of leaves, roots, shoots, seeds, and whole plants. The roots and leaves of *M. pudica* are almost always the best pharmacological agents as antidiabetic, antihepatotoxic, anticancer, anti-malarial and anti-bacterial, anti-ulcer, diuretic, anti-inflammatory, and anticonvulsant agents indicate an action (Rajendiran *et al.*, 2017).

*M. pudica* has been used in Ayurveda, Unani and homeopathic medicine. In Siddha herbal formulation, Thottalvadi Chooranam, a powdered form of *M. pudica* has demonstrated its antioxidants and antidiabetic activity in experimental animals (Viswanathan *et al.*, 2014). The root of *M. pudica* is bitter, pungent, cooling, noxious, and alexipharmic, In Ayurveda, it is used for the treatment of leprosy, dysentery, diseases of the vagina and uterus, inflammation, burns,

asthma, vitiligo, as well as fatigue and blood disorders. The root is used to gargle with water to relieve toothache and is also useful for treating diarrhea and urinary tract infections. (Joseph baby *et al.*, 2013). The plant is about 0.5m in height and about 0.3m wide and its stems are erect, slender, spiny, and well-branched. The leaves are bipinnate and contain one or two pinnae pairs. Nearly ten – twenty-six leaflets are present per pinna. The plant is a very sensitive one and both the pinnae and leaflet will fold when the leaves are being touched hence it derives this name (Ahmad *et al.*, 2012).

#### TAXONOMY OF *MIMOSA PUDICA*

Kingdom	:	Plantae
Order	:	Fabales
Family	:	Fabaceae/ Mimosaseae
Genus	:	Mimosa
Species	:	<i>M. pudica</i>



Figure 1: Photograph showing the plant *Mimosa pudica*

#### PHYTOCHEMISTRY

Medicinal plants are tremendous in the treatment of numerous diseases due to their active constituents. All parts of medicinal plants are effective in treating ailments and aid in the discovery of new classes of medicines. In India, 45,000 plant species have been identified as medicinal plants. The plants contribute a potential source for hypoglycemic drugs because of their phytoconstituents (Rahimi *et al.*, 2005). *M. pudica* contains a variety of secondary plant matter including alkaloids, glycosides, carbohydrates, steroids, flavonoids, phenols, quinines, fat, resin, triterpene, and c-glycoflavines (Deepa *et al.*, 2015). The ethanolic extract of *M. pudica* root contains tannin and protein (Ranjan *et al.*, 2013). In another study, the root was also reported to contain calcium oxalate crystals, ash, and mimosine (Oudhia.P, 2006). Mimosine is a toxic alkaloid that is reported in the leaf extract. The seed of a mimosa contains mucilage consisting of d-xylose and d-glucuronic acid (Joseph Baby *et al.*, 2013)

Total phenol content was estimated as gallic acid equivalents in petroleum ether, chloroform, ethanol, and aqueous extracts of mimosa. Total phenol content increased in different extracts in a concentration-dependent manner, with the highest phenol content estimated for the chloroform extract at a concentration of 60 µg, followed by the aqueous and petroleum ether extracts, while the ethanol extract was the lowest (Jagetia and Lalmangaihi, 2018) *M. pudica* has

been reported to contain beta-sitosterol, linoleic acid, oleic acid, and free amino acids. These active phytochemicals present in *M. pudica* have a variety of medicinal properties. The known medicinal properties of *M. pudica* are antitoxic, antidiabetic, antiviral, hepatoprotective, and wound healing (Kokane, 2009). It is also used for the treatment of headaches, insomnia, diarrhea, dysentery, fever, hemorrhoids, and fistulas (Ngo Bum *et al.*, 2004).

## **PHARMACOLOGICAL ACTIVITIES OF MIMOSA PUDICA**

### **ANTIDIABETIC ACTIVITY**

Rita Maneju Sunday *et al.*, (2020) demonstrated the antioxidants and antidiabetic activity of *M. pudica* seed ethanolic extracts in Wistar rats. The result showed that *M. pudica* seed extract has moderate amounts of antioxidants and scavenges free radicals 2, 2-diphenyl-1-picrylhydrazyl and nitric oxide radicals. The seed extract also reduced the fasting blood sugar, lipid peroxidation, and elevated serum insulin,  $\alpha$ -amylase, and liver antioxidants in diabetic rats treated for 21 days. In another study, the leaf extract of Mimosa has been reported as having antidiabetic and antihyperlipidemic activity. Different concentrations of mimosa were used for the study (125, 250, and 500 mg/kg body weight). The result showed a significant reduction in the levels of glucose, TG, LDL, and VLDL in animals treated with methanol extract of *M. pudica* (Subramani Parasuraman *et al.*, 2019).

### **ANTICANCER ACTIVITY**

Jagetia *et al.*, (2020) investigated the anticancer potential of *M. pudica* extracted in chloroform, ethanol, and water in cultured Dalton's ascites lymphoma cells. The cultured Dalton's ascites lymphoma cells were treated with *Mimosa pudica* (10, 25, 50, 75, 100, 150, 200, and 250  $\mu$ g/ml of chloroform, ethanol, and aqueous extract). The result of the study showed that the Chloroform, ethanol, and aqueous extracts of mimosa killed Dalton's ascites lymphoma cells in a dose-dependent manner, with the aqueous extract being the most effective compared to the ethanol and chloroform extracts.

In the earlier study, the dichloromethane (DCM) extract of *M. pudica* flowers and its isolated compound 11 $\beta$  hydroxy-3 methoxy 1,2 dehydro crinane has been investigated for its anticancer and immunostimulatory activity. Anticancer activity was performed in the Ehrlich ascites carcinoma (EAC) cell line of Swiss albino mice. In the result, Significant reductions in tumor volume ( $3.46 \pm 0.135$  mL,  $2.25 \pm 0.153$  mL, and  $1.84 \pm 0.012$ ), were reported in extracts at doses of 500 and 1000 mg/kg/day and 2.5 mg/kg/day of isolated compound. The extract and 11 $\beta$  hydroxy-3 methoxy 1, 2 dehydro crinane also increased the lifespan (59.32%, 76.39%, and 82.43%) and significantly decreased the tumor weight compared to controls. Immunostimulating activity has also been shown to be effective in the above dosage regimen (Dasgupta and Dash, 2017).

### **ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY**

Vikram *et al.*, (2012) reported that the administration of *M. pudica* leaf extract 250 and 500mg showed analgesic and anti-inflammatory activity in a dose-dependent manner. Analgesic activity was evaluated using the tail-flick method and hot plate method and acetic acid-induced writhing method. The results showed that the pethidine-administered group in the hot plate test and diclofenac-treated group in the tail flick test and the group treated with *M. pudica*

ethanol extract 250mg/kg and 500 mg/kg dose-dependently increased latency time. In the Acetic acid-induced writhing test, the oral administration of the ethanolic leaf extract significantly reduced the writhing responses induced by acetic acid. The anti-inflammatory effect of *M. pudica* was evaluated by Carrageenin-induced paw edema. Ethanol extract showed maximal inhibition of 72.31% at a dose of 500 mg/kg.

#### ANTI-CONVULSANT ACTIVITY

The leaf decoction of *M. pudica* has been reported the anticonvulsant activity at a dose of 1000-4000 mg/kg against pentylenetetrazol and strychnine-induced seizures in mice (Ngo Bum *et al.*, 2004).

#### ANTIOXIDANT ACTIVITY

The antioxidant activity was evaluated using the methanolic leaf extract of *M. pudica*. Antioxidant activity was assessed using total phenolic content, total antioxidant capacity, total flavonoid content, and DPPH (1, 1-diphenyl-2-picrylhydrazyl) assays. The crude methanol extract exhibited moderate antioxidant activity; with a total flavonoid content of 63.8 mg QE/g and total phenol content of 42.9 mg GAE/g. Total antioxidant capacity was expressed in ascorbic acid equivalents (AAE) and was 5.038 mg/g AAE in leaves. DPPH scavenging activity was measured by comparison with ascorbic acid. The IC<sub>50</sub> values for both leaf and ascorbic acid were 126.71 and 20.13 µg/mL, respectively (Kamanashis Das *et al.*, 2014).

**Table 1: Pharmacological actions of *Mimosa pudica***

Pharmacological Activity	Extract	Parts	Dose	Efficacy	Reference
Antidiabetic	Ethanol and aqueous extract	Whole plant	400 mg/kg of b.wt	The subsequent reduction of cellular glutathione and lipid peroxidation status and improved insulin production. Significantly control hyperglycemic stress.	Subashini <i>et al.</i> , 2022
	Ethanol	Leaves	100,200, 300 400 mg/kg of b.wt	Improved blood insulin level and a significant decrease in glucose level. The antidiabetic effect was reported at a dose of 300mg.	Deepa Rajendiran <i>et al.</i> , 2019
Antioxidant activity	Petroleum ether, chloroform, ethyl acetate, and methanol extracts	leaf, stem, and root	12.5, 25.0, 50.0, 100.0 and 200.0 µg/ml of the extracts	The IC <sub>50</sub> values of DPPH radical scavenging activity could be arranged as ethyl acetate > methanol > chloroform > petroleum ether for leaf and root, and as ethyl acetate > chloroform > methanol > petroleum ether for stem	Ujjwal Kumar Mondol and Islam, 2022

Antioxidant and Antimicrobial	Ethyl acetate	Leaves	12.5, 25, 50, 100, and 200 mg	The ethyl acetate extract of <i>M. pudica</i> has antibacterial activity against Gram-positive bacteria and Gram-negative bacteria. The presence of phenolic and flavonoid compounds in <i>M. pudica</i> has a significant impact on bacterial growth inhibition and has a notable potential to scavenge free radicals (DPPH). The maximum activity was found at 200mg of leaf extract.	Ashok Kumar Mandal <i>et al.</i> , 2022
Wound Healing Activity	Methanol and aqueous extract	Root	Extract used in simple ointment base 0.5% (w/w), 1% (w/w) and 2% (w/w)	The methanolic extract has been reported good wound-healing activity probably due to phenols constituents.	Dnyaneshwar <i>et al.</i> , 2009
Anti-angiogenic properties	Aqueous extract	Root	10%, 20% and 30%	The results showed that concentrations of 10%, 20%, and 30% gave significant anti-angiogenic results.	Masagca, Jimmy <i>et al.</i> , 2020
Hepato-protective	Ethanol	Leaves	200 and 400 mg/kg of b.wt	Treatment with <i>M. pudica</i> extract at 200 and 400 mg/kg b.wt. Showed a significant decrease in creatinine, urea, bilirubin, ALT, AST, and ALP level in a dose-dependent manner.	Sule <i>et al.</i> , 2017
Antivenom activity (Mucuna pruriens Seed and <i>Mimosa pudica</i> root)	Aqueous extract	Root	50 and 400 mg/ml	<i>Mimosa pudica</i> extract reduced hemolysis by 49.4%. <i>M. pruriens</i> at a concentration of 50 mg/ml only inhibited phospholipase A2 activity by 7.7% but higher concentrations up to 400 mg/ml had no antivenom	Ameh <i>et al.</i> , 2020

				against <i>N. nigricollis</i> ; at 200mg/ml. <i>M. pudica</i> extract inhibited PLA2 activity by up to 23%.	
Anticonvulsant activity	Ethanol	Leaves	50,100 and 200 mg/kg of body weight.	The alcohol extract of <i>M. pudica</i> reported significant dose-dependent protection ( $P < 0.00$ ) against the tonic-prolonged phase at all doses tested (50, 100, and 200 mg/kg), with the maximum effect found at higher doses (200 mg/kg).	Alasyam <i>et al.</i> , 2014

### CONCLUSION

Pharmacological studies performed on *M. pudica* show the great potential of this plant including antioxidant, antidiabetic, antibacterial, antifungal, anti-inflammatory, and hepatoprotective properties, which have been attributed to different parts of *M. pudica*. Traditionally it has been used for the treatment of diseases such as urogenital disorders, hemorrhoids, dysentery, and sinus and also applied to wounds. The use of natural remedies in the treatment and prevention of disease is not only safe and readily available with lesser side effects. Therefore, the development of traditional herbal medicines from natural sources must be emphasized.

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### ABSTRACT

The importance of traditional system of medicine has now been recognized all over the World. The traditional medicinal practices have become the integral part of social culture, particularly in the countries of third World. Ethno-botanical information plays an important role in identifying the active principles of various medicinal plants their efficacy and scientific evaluation. Early man on this Earth would have hunted in the jungle for plants and animals to meet with his hunger, and would have searched for some materials like skin, bark, foliage etc., to protect him from unfavorable weather. Most of the traditional practices help in sustainable use of bio-resources. Traditional practices also help in lesser damage and better preservation of individual species in their habitats, and biodiversity in general. Traditional knowledge about the land races or wild relatives of crop plants and other economic species, which are more suited to local conditions and microclimates, is useful in agricultural practices. There is also prospect of socioeconomic uplift of the indigenous communities through promotion of cottage industries based on their traditional tools, gadgets, arts, crafts and other minor forest medicines.

**KEYWORDS:** Ethanobotany, Interdisciplinary, Traditional Medicines, Bio-resources.

### INTRODUCTION

The term ethnobotany was first coined by John William Harshberger in 1895. The term ethnobotany has often been considered synonymous with traditional medicine or with economic value of the plants, in other words, ethnobotany can also be defined as the study of natural and traditional interrelationships between plants and animals including man. Initial studies on ethnobotany (in the World) in the last 75 years have been primarily devoted to the preparation of inventories of plants of certain regions and specific ethnic groups. Many scientists, naturalists and thinkers from outside the community of Ethnobotanists started emphasizing the importance of ethnobotanical inquiries and explorations. The first book on ethnobotany was written by Faulks in 1958, entitled "An introduction to Ethnobotany". Subsequently Jain (1981) published a book entitled "Glimpses of Indian Ethnobotany" with various ethnobotanical related articles of different phyto-geographical areas and tribes of India. India has been considered as on of the twelve mega-biodiversity countries of the World having rich biodiversity with wide variety of plants, animals and ecosystems including medicinal plants. India has about 45000 plant species of which plant derived medicines are from 7,500 species. These provide the Indian tribals with potentially effective herbal treatment for various

diseases. Indigenous communities still depend on uncultivated leafy vegetables, tubers, fruits and wild legumes to meet their nutritional balance in the diet. Ethnobotany is the study of the relationship between man and the plants and indicates that it is a multi-disciplinary science that requires an interdisciplinary approach and may be pursued through several avenues. The word “ethno” means a group of people sharing common origin, culture, language, customs, beliefs, traditions, etc., and Botany is the study of plants. Thus, ethnobotany is a hybrid term, with anthropological approach to plant science. For ethnobotanical study, there should be a close working relationship between botanists, ecologists, anthropologists, chemists, soil scientists and pharmacologists. While botanists are required to identify the plants, the others are required for ecology and environment, gather the data of the plants used by the ethnic groups, for phytochemical analysis note the details of soil and for testing the efficacy of native drugs. Ethnobotanists explore how plants are used for such things as food, shelter, medicine, clothing, hunting, and religious ceremonies

### INTERDISCIPLINARY NATURE OF ETHNOBOTANY

The very name Ethnobotany indicates that it is an interdisciplinary science. The word “ethno” means a group of people sharing common origin, culture, language, customs, beliefs, traditions, etc., and ‘botany’ is the study of plants. Thus, Ethnobotany is a hybrid term, with anthropological approach to plant science. For Ethnobotanical study, there should be close working relationship between botanists, ecologists, anthropologists, chemists, soil scientists, pharmacologists. While botanists are required to identify the plants, the others are required for ecology and environment, for gathering the data of the plants used by the ethnic groups, for phytochemical analysis, for noting down the details of soil and for testing the efficacy of native drugs.

Ethnobotany is within the bounds of botany & it may transgress the boundaries of botany as well.

The sub-disciplines of ethnobotany are based on different plant groups. Thus Ethno phycology, Ethnomycology, Ethnolichenology, Ethnobryology, Ethnopteridology, etc., respectively (all with the word ethno- as a prefix), indicate the study of algae, fungi, lichens, bryophytes, pteridophytes, etc., by the ethnic groups. Similarly sub-disciplines of Ethnobotany are based on different aspects of plants by tribals), ethnoecology (study of inter-relationships between tribals and living and non-living environment).

The Para botanical, Trans disciplinary or inter-disciplinary nature of ethnobotany can be divided into the following (usually with ethno as a prefix): -

- **“Ethnomedicine** is the study of plants used in medicine by tribals or ethnic groups. It is further divided into the following:
- **Ethnopharmacology:** It deals with the study of indigenous drugs and experimental investigation of potency, efficacy, etc.
- **Ethnotoxicology:** It deals with the study of plants used as fish poison, arrow poison, etc.
- **Ethnopediatrics:** It deals with the study of medicinal properties of plants used by the ethnic groups for curing the diseases of the eye.

- **Ethnogynecology:** It deals with the study of medicinal properties of plants used by the ethnic groups, for sterility, contraception, abortion, diseases of women.
- **Ethnonarcotics:** It deals with the study of plants used by ethnic groups as narcotics, hallucinogens, etc.

The ethnobotanical studies may help in the choice of species to be consumed, time of collection of the plant products, judicious exploitation of plant resources by the tribals. Further, the germ plasm of traditional races may be made.

Organized collection of plant products and on the spot conversion to transportable products (of basketry, toy making, etc.) for social and economic uplift of tribals is possible by the ethnobotanical studies.

The planned objectives and critical approaches reveal fascinating findings. For a sincere researcher in ethnobotany, it is a delight and his findings may benefit the tribals in due course.

- **Ethno agriculture:** It deals with the study of agricultural practices, dealing with origin, domestication, cultivation, etc., adopted by the ethnic group.
- **Archaeoethnobotany:** It deals with the study of pollen, wood, etc., from archaeological excavations, carving, sculpture, etc., relating to plants to get an insight into the culture and civilization of ethnic groups.

#### RELEVANCE OF ETHNOBOTANY

Since Ethnobotany has socio-economic impacts, its relevance in modern times is established particularly with the problems of conservation of ecosystem, population control, rural health, nutrition, drug usage and abuse and cottage industries – all turned to the economic uplift, there is a shift in ethnobotanical studies from descriptive to analytical type. The Ethno botanically unexplored regions, regarding plant resources are now out with large amount of information.

The aspect of conservation or balanced use of resources by the tribals, which has been observed, understood and appreciated by the Ethnobotanists, is receiving attention. The conservation of natural resources, particularly the plant wealth, by the tribals is due to avoidances, beliefs and taboos. They treat the plants as Gods or Goddesses e.e., as “Sylvan Deities”, due to mythological association. Faith and tradition are the two key things, which make the primitive people to ‘conserve the Nature’, that we too have to adopt. The report on tribal medicine should be subjected to critical scientific evaluation. New drugs may be obtained to fight the diseases. Phytochemical screening and pharmacological studies have to be intensified, in the regard so that they may pave the way for new drugs.

The tribals have small families. They employ some plants for contraception. A further enquiry, in this line, may bring the population in general, under control. The tribals use curare botanically known as *Chondrodendron tomentosum* as hunting poison. Later on it has been found by the scientific community that it contains tubocurarine, which is now being used as a painkiller and muscle relaxant. A further investigation, on other plants, may lead to the discovery of new sources in this direction.

The tribals use some plants as narcotics and hallucinogens. A further enquiry may lead to the discovery of new drugs for anesthesia. In the mountain tracks of Central India, Western Ghats, and the Himalayas and almost in very state, there are several kinds of tribals, each with a

distinct culture, religion, local beliefs, taboos and dialect. The northern belt in particular is inhabited by many tribals. It is the region of origin of the Vedas and a variety of sacred and religious documents. The ethnobotanical continuum, may link up the distant past with modern folklore.

#### **PLANTS VS TRIBAL LIFE**

The life of the tribals is intimately connected with the plants in their day-to-day activities. They are dependent on the plants for their food, clothes, shelter, medicine, beverages, binding material, oils, resins, etc.

#### **FOOD AND FOOD-CYCLE**

**A) FOOD:** Tribals are dependent on tubers, conns, bulbs of several wild plants, which are dug out and eaten. They also collect the leaves, flowers, fruits and seeds of a number of plants, as a part of food, besides basically being non-vegetarians. More than 80 plant species have been recorded as a source of underground parts. They include *Dioscorea hispida*, *Cyperus rotundus*, *Costus speciosus*, *Curcuma angustifolia*, *Nymphaea pubescens*, *Balanites aegyptica*, etc. Further, *Asparagus adscendens* roots are pickled and *Amorphophallus companulatus*, *Colacasia esculenta* and *Dioscorea esculenta* and *D. Alata* are cultivated by the tribals for their underground edible parts. About 250 plants, occurring in wild state are used by the tribals, from which the leaves and shoots are collected. They are usually cooked and consumed. They include *Achroynchia pedunculata*, *Allium sphaerocephalum*, *Murraya koenigii*, *Caralluma adscendens*, *Clausenia pentaphylla* and *Tamarindus indica*. The unripe fruits numbering about 60, occurring widely are used as vegetables and some of them are pickled. They include *Artocarpus heterophyllum*, *Atlantia monophylla*, *Cucumis melo var. agrostis*, *Dillenia indica* and wild banana (*Emsete superbum*). About 35 plants are recorded as useful foods, in which inflorescence, flowers and floral buds are consumed. They are *Carvota urens*, *Madhuca longifolia*, *Periploca aphylla*, *Bambusa bambos*, and *Ardisia griffithii*.

The seeds/grains of about 50 plants are eaten either roasted or boiled. They include *Artocarpus heterophyllum*, *Bauhinia vahlii*, *Buchnanania lanzan*, *Euryale ferox*, *Caryota mitis*, etc. Seeds of *Sorghum nepalens* and *Panicum turgidum* are taken during scarcity. The fruits of about 300 plants like *Aglaia edulis*, *Coriara nepalensis*, *Olax nana*, *Emblica officinalis*, *Feronia limonia*, *Rubus milleus*, *Hovenia dulius*, etc.

**B) FOOD CYCLE:** The dependence of tribals on forest resources for food in different seasons, and different activities in this regard can be divided into (1) Food production and (2) Food collection.

(1) Food production by hilly tribals is mainly by 'Podu cultivation', which is marked by periodic shifting to different places as the soil at a place becomes totally unproductive in 2-3 years. Podu cultivation is advantageous as the inputs (as bullocks, agricultural implements, fertilizers, etc) are nil, except the labour. Podu cultivation activity starts in December (or some other month depending on the monsoon period at a later stage) with the selection of the site. The large trees are cut, piled up in different lots and allowed to dry up during the summer. They are eventually burnt so that the ash spreads in the cleared site. When the rainy season starts, the tribals practice mixed cropping system (*Sorghum vulgare* + *Panicum miliare* + *Pennisetum*

americanum). The seeds are broadcast. The harvested crop is put on a high platform, erected on 4 wooden poles. A part of the harvested crop is consumed and the remaining is stored for future broadcast in the following year. Besides Podu cultivation, the tribals grow tuber crops like sweet potato, yam, tapioca, turmeric, ginger and others (tobacco, challis, maize, vegetables) and maintain plantations too. The tribals perform rituals like Bhoomi pooja in the beginning, propitiate Ganga Devi in the middle and Rajula Panduga in the end, of their agricultural activities.

(2) Food collection has 4 phases depending on the season and the material collected. They are (a) Tuber collection phase, when the tribals collect the tuberous underground portions of the plants like *Amorphophallus*, *Asparagus*, *Dioscorea*, *Ceropegia*, *Nelumbo*, etc., during October to March. (b) Leaves, flowers and fruit collection phase, when fruits of cucumber, Gourd, Pumpkin, leaves and shoots of *Abrus*, *Celosia*, *Portulaca*, *Chlorophytum*, *Ipomea aquatica*, and buds & flowers of *Bauhinia*, *Cassia*, *Dillenia*, etc., are collected from January to June. (c) Jeelugu Toddy phase which overlaps the above two phases, wherein the tribals collect the toddy from *Caryota urens*, 3-4 liters a day. In the collected toddy, a semi-burnt wood or bark of *Cassia fistula* is put to enhance the taste and digestive properties. While sipping the toddy, they consume the seeds of *Pisum sativum*, *Gnetum ula*, *Xylia xlyocarpa*. Thus, the Jeelugu toddy phase is a year-long activity except perhaps in heavy rainy period. (d) The Hunting phase also overlaps the first 2 phases of food collection, like the previous one. Hunting, catching and consuming the animals killed by the predators are routine and year-round. Hunting is indirectly connected with the plants as bows and arrows are made up of plants.

### INTOXICANTS AND BEVERAGES

From *Caryota urens*, the tribals get the arrack or toddy by tapping the inflorescence. It is collected in an earthenware pot about 3-4 liters a day. Wood or bark of *Cassia fistula* is half-bunt and dipped into the arrack pot, to enhance the taste. The petals of *Mahua* are collected and the country liquor is brewed at home. In the tea-like preparation, *Cymbopogon citratus* and *Basella alba* are used. The rice beer is prepared with the addition of flowers of *Madhuca longifolia*, rhizome of *Imperata cylindrica* and fruit of *Syzygium cumini*. However, Miris (a tribe) of Assam, in preparation of rice beer use the sundried and powdered parts of *Ageratum conyzoides*, *Attocarpus heterophylla*, *Centella asiatica*, *Clematis codina*, *Clerodendrum viscosum*, *Lycopodium*, *Sachhamm*, and ferment with rice cakes. In Arunachal Pradesh, the tribals use *Zea mays*, *Eleusine coracana* and *Chenopodium album* in the preparation of local drinks. Cool drinks are prepared with *Lepidogathis barbadens* and *Ocimum basilicum*.

### ROPES AND BINDING MATERIAL

These are obtained from the fibers of most of the malvaceous taxa such as *Abelmoschus moschatus*, *Abutilon indicum*, *Bambax cieba*, *Gossypium barbadens*, *Kydia calycina*, *Sida acuta*, *Urena lobata*, *Thespesia lampas*. The fibers from *Corchorus capsularis*, *Sterculia urens*, *Xylia xylocarpa*, *Helictres isora*, etc., are also used for the same purpose. The fibers of *Acacia senegal* and *Bauhinia vahlii* are used to drag the elephants. The mesh of cots is also a plant-product. *Phanera vahlii* is used in this connection.

## RESINS AND OILS

The resins are tapped from the tree trunks of *Gardenia resinifera*, *Careya arborea*, *Acacia concina*, *Sterculia urens* and *Tamarindus indica*. The oil, which is used as a luminant and cooking medium is obtained from the seeds of *Derris indica*, *Schleichera oleosa*, *Principia utilis*, *Aleurites moluccana*, *Azadirachta indica*, *Calophyllum inophyllum*, *Carcellia brachiata* and *Garcena moella*.

## POISONS AND BITES

Bows and arrows are the proud possessions of the tribals. Archery is one of their favourite games. They depend on plant material for bows, arrows and arrow poison. While a bow is a long bent culm of *Bombax arundinacea*, it is tied with fiber from *Phanera vahlii* from end to end (bow string), which offers some elasticity. The arrows are made from young stems of bamboo, at the end of which a triangular iron piece is tied and stem juice of *Antialis toxicana* is smeared. It serves as an arrow poison. For fish poison, the tribals of Garo hills of Assam throw the decoction of crushed and soaked bark of *Acacia pinnata* and *Engelhardtia picata*, roots of *Melletia pachycarpa*, and fruits of *Symplocos chinensis* and *Xeromorphis spinosa* -into the ponds. The fish get stupefied and float on water, thus facilitating easy catch. The plant material from *Alangium salvifolium*, *Barringtonia acutangula*, *Caeseria elliptica*, *Cliestanthus collinus*, *Diospyros melanoxylon* and *Securinega virosa* also serve as fish poison. Such roots of *Colocasia montana* and *Alocasia montana* are used by the tribals as tiger poison.

## METHODS OF STUDY OF ETHNOBOTANY

Ethnobotany is a synthetic or trans-disciplinary science. It involves inter alia the study of botany (taxonomy, phytogeography, ecology), anthropology, sociology, medico botany, pharmacology, phytochemistry and even psychology. Therefore, any approach to Ethnobotany should consider all these aspects. There are various methods of study of ethnobotany (1) Field work (2) scrutiny of Herbarium specimens (3) scrutiny of ancient literature and unpublished information (4) ancient sculpture in temples and sacred places (5) Archeological findings, besides analysis of tribal folklore and study of fossilized plant material.

## FIELD WORK

The fieldwork in ethnobotany is different from that of a taxonomist, because there is record of relationship of plants and habitat with the local population of tribals, in addition. In the ethnobotanical fieldwork, firstly, the tribe and their regional jurisdiction have to be noted. Secondly, familiarity has to be gained about tribals, their culture, dialect & religion and the vegetation pattern. Thirdly, the correct time and season for fieldwork have to be identified and finally proper knowledgeable informants (popularly known as Kaviraj or medical men) have to be located. These 4 steps constitute protocol. The degree of authenticity of information varies from person to person in the same area. Experienced men and women only have to be contacted. There are two ways of getting the information (1) the informants are taken to the field for collection and the uses of plants are recorded as told. (2) Alternatively, voucher specimens could be collected and shown to the informants to get informed. Plant-wise or disease-wise interview may be had. The informants are very conservative, secretive or shy. Ethnobotanical fieldwork should also involve the collection of information not only of medicinal value of plants but also of connection of plants with religion and rituals of tribals.

Several other uses of plants (such as those used in hut-making fiber-yielding, beverage, animal nutrition, fuel, arrow, poison, musical instruments, etc) in Short, the entire relationship of plants with the lives of the tribals have to be recorded, besides personal observations (such as folklore songs in which plants figure) and they may be analyzed. Plant names, have to be recorded exactly as told or spelt and verification should be made by the uses, if they are similarly spelt out. Voucher specimens with entire ethnobotanical information should be maintained. Long association, mixing, gaining confidence, and respecting the rituals of tribals are very important in the fieldwork. Schultes spent over 12 years and Rodims about 25 years, before they published the monumental ethnobotanical works of Amazon and Ovambos tribals respectively. In a general survey, it has been observed that women informants (known as Kaviraj, etc) outnumber the men. Prior to child bearing and rearing, they might have limited knowledge about the nature of plants. But later, when they face the problems with their babies, they integrate their knowledge with their mother's, mothers-in-law and relatives. The secretive nature of informants has been found to be due to 2 reasons a) They consider it as a matter of status or livelihood b) They do not want to reveal to the field worker, so that it will be known to the other tribals, in general. Some of the tribals are even inimical or hostile to the field worker. Besides the field notebook (or similar one), the entire ethnobotanical information that is recorded should be entered on the label of the herbarium sheet on which the voucher specimen is conserved,

#### **SCRUTINY OF HERBARIUM SPECIMENS**

Herbarium is a dried collection of plants, arranged according to some accepted sequence of classification. The plants are collected from far and wide, they are pressed, dried and preserved with details of uses (medicinal and otherwise), local names, botanical names, hierarchy in the system (i.e., family name), place of collection, altitude, nature of the plant (herb, shrub, tree, climber, etc), date and field number. The herbarium sheets are housed in a herbarium. There are 2.5 million specimens in Central National Herbarium (Howrah), 6.5 million in Royal Botanic Garden (Kew) and 6.0 million in Komarov Botanical Institute (Leningrad) collected from all over the world. The herbarium sheets serve as a data bank as they give the first information about the plant. Two types of ethnobotanical studies could be made by the herbarium. a) Record the uses of plants as told by the tribal people of one locality b) Record the uses of plants by different tribals of different localities. One can consolidate all the plant species for a particular disease at national or global level. Altschul (1970) scrutinized several thousands of herbarium specimens at Harvard University. He recorded the uses of 5178 less known plants. However, not much work has been done, in this line, in India. Jain & Dam (1979) from the herbarium of North-east circle of Botanical survey of India (popularly known by the name ASSAM) made a scrutiny of local names and uses of different tribals and localities regarding Solanum. They found that Solanum crassipetalum (whole plant) is used by Lepachis (a tribal group) of Sikkim as food (for human beings) and also for curing the foot and mouth disease in cattle. Similarly, the leaves of Acacia farnesiana are used for curing Catarrh, while the juice of stem bark is used for stomach pain by Lepachis. inhabiting Meghalaya and adjoining states like Assam, Arunachal Pradesh. etc.

### SCRUTINY OF ANCIENT LITERATURE

The scrutiny and survey of old and unearthed literature, unpublished literature, diaries, travelogues by adventurers. etc. give useful data of plants used by the people, their life, culture, etc., if one overcomes the constraint of correct identification and equivalence of vernacular names. In India there is a vast scope of this type of ethnobotanical study, as there is vast heritage of Vedic literature of 2000-1000 BC, epics like Ramayana, Mahabharata, a number of literary works (Dramas, poetry, etc) by Kalidasa and a host of persons. Mention of numerous plants & their role in the life of the people (as uses in cosmetics, beverages, medicine, and garments) give a glimpse of their floristic composition and vegetation type. Sarmah (1969) listed 248 botanical drugs from Atharvana Veda and Rig-Veda. Singh & Chunekar (1972) published a glossary of medicinal plants from Charaka samhita, Sushruta samhita and Ashtanga Hridayam. Hartwell (1976) of Cancer Chemotherapy National Service has done the compilation of all anti-tumor plants cited in old texts, folk medicine all over the world. Krishna Murthy (2000) gave an incisive account of plant wealth in Sanskrit literature covering the Vedas, epics, samhitas from medicinal and non-medicinal points of view. The practical problem that lies, in this aspect of ethnobotanical study, is --- finding the botanical names equivalent to local names used in ancient literature, which vary from place to place and time to time. For example, such names as Soma, Brahmi, Jatamani, Bala, Kalpavriksha, etc., have got different botanical equivalents. 'Soma' plant has at-least 20, names including *Sarcostemma* (Asclapiadaceae), *Amanita* (polyporus 'fungus), *Ephedra* (gymnosperm), etc., according to hymns of Rig-Veda. In other words, fixing the correct identity to plants mentioned in ancient literature exists because of lack of Voucher specimens. The confusion of identity can be dispelled if Ethnobotanists, pharmacognosists, botanists, anthropologists, and linguists collectively come forward so that mankind will be benefited by the ancient literature.

### ANCIENT SCULPTURE IN TEMPLES AND SACRED PLACES

India has temples of every religion and each religion flourished at one time or the other. The sculptures on temples are a potential source of information about plants. The writings on temple stones, if analyzed, give valuable information. Shakti Gupta contributed a lot on this type of ethnobotanical study. The interpretation of not only sculpture but also writings and paintings requires the assistance of experienced and trained archaeologists. The following examples of structural motifs (=designs) give important information; remain as legendary plants and are respected, worshipped or treated as sacred.

a) The tree motif in Gandhara sculpture in Lumbini garden, near Kapilavasthu, where Siddhartha (Gautama Buddha) was born, indicating a large sized flower with 5 free petals resembling that of *Shorea robusta*, has also been interpreted as that of *Bombax ceiba*, *Mangifera indica* and *Butea monosperma*. To commemorate Siddhartha's birth, young maidens sing, dance, gather flowers of Sall (*Shorea*) and throw them at each other (that is how the term *Salabhangika* has come into existence). Therefore, Sal tee is worshipped and *Salabhangika* are carved on the railing pillars of Sanchi. Further, Mahamaya (Siddhartha's mother) is depicted, sitting cross-legged behind Ashoka tree (*Saraca indica*) and therefore the tree is also worshipped.

b) The Jain goddess Ambika is depicted under a tree with a child in her arms as a motif in Jain sculpture. The tree is interpreted as *Mangifera indica* and respected as a fertility symbol. In Hindu temples, Parvati is depicted as standing under the same tree. Therefore mango tree is worshipped.

c) The tree motif resembling Banyan (*Ficus bengalensis*) is worshipped because it is associated with goddess Vatapatreshwari (=Kali) wearing a garland of skulls in sculptures of Kushan's period. It is also worshipped because Satyavan regained life after death due to the steadfastness of Sati Savitri, as shown in some temple sculptures.

d) Such motifs as lotus flowers in Sun temple (Konark), Tala (*Borassus flabillifer*) tree in Vishnu's temples. Seven Tala trees in a row in Rama's temples, Kadamba trees (*Anthocephalus cadamba*) with Krishna stealing saris of Gopikas in Krishna's temples --are all treated as sacred plants and worshipped.

e) Coconut (*COCOS nucifera*) fruit in a water pot along with a few branches of mango tree symbolizing Punakumbha is found as a common motif in many temples of Tamilnadu and on the top of Khajuraho temple (in Orissa) and is a revered one for Hindus. *Cocos nucifera* is further treated as a creation of sage Vishwamitra..

f) Sugarcane (*Saccharum officinale*) motif, as a bow of Kamadeva seen in many of the temples of South India, and Mango, Albizzia, Jasminum, Nymphaea, Saraca (as floral arrows of Kamadeva) - are all respected.

### **ARCHAEOLOGICAL FINDINGS**

The archaeological remains include hard coated seeds, charcoal, timber, which get buried along with manmade structures. Excavations of these at sites reveal certain information on new sources of medicine, food and fiber. In some cases, the archaeological excavations reveal wild races of food grains, cultivated in those times. The archaeologists and botanists should jointly take up such. Investigations. The archaeological findings may indicate the migration of human culture, origin, dispersal and domestication of agricultural crops and their types. Vishnu Mittre (1981) worked extensively in this line.

### **MAGICO-RELIGIOUS RITUALS, SOCIAL CUSTOMS & SACRED GROVES**

There is an intimate relationship between plants and human beings. There are several references of use of plants by man in the Indian epics. Plants were used by our ancestors, folklore and tribal societies. Many of the rituals are plant-based.

### **MAGICO-RELIGIOUS RITUALS**

Plants used by tribals that affect the mind were beyond their comprehension, therefore they attributed the properties to spiritual forces residing in the plants and legends were woven around them in course of time. The transportation of human mind to spiritual world was thought to be pure magic by the people. Thus, the magico-religious beliefs can be defined as a system of faith and worship of supernatural beings and attributed everything good or evil in the universe as acts of these beings of forces. Appeasement of supernatural forces was considered essential for betterment of individual or society, and was regarded as the only way out to avoid the divine retribution for their past misdeeds. Rituals are based on beliefs & faith. Tribal people of Jaunsar Bawa, who are superstitious and orthodox, object to any change or

innovation on the ground, because Gods will be displeased. God can be appeased by sacrificing a sheep or goat. This is done once in a year before the commencement of felling Cedrus Deodara trees and at the beginning and end of agricultural operations for the smooth and successful completion of the work-as a regular ritual. When, a man crosses one valley to the other, he may throw a stick or cone of Pinus on the top of the way to prevent spirits of the valley from crossing along with him to the next. People tie a piece of cloth to the tree in the sacred spots situated in deep deodar forests. A person suffering from hysteria is believed to be possessed by an evil spirit and in an attempt to cure him, with the help of uttering some mantras a magico-religious specialist holds tuberous roots of *Stephania hernandifolia* over the patient's head and rotates them around his body. Tribal people when afflicted with small pox, burn down hundreds of deodar trees as a sacrifice. On the other hand, there is a belief among several tribal communities that when one commits damage to trees, he gets death penalty by the spirits. Natural vegetation is thus conserved over generations, out of faith in good and bad powers of plants--as sacred groves. Paharia tribals of Bihar offer *Madhuca latifolia* (Mahua) liquor along with maize beer in the ear bowls to "Gossiyans" and other spirits on the occasion of festivals. Later, the liquor bowls are exchanged among the participants. In Arunachal Pradesh, some orchids are regarded as sacred by the local tribals. *Vanda coerulea* (blue Vanda), popularly known as 'Rangpu' by vanchoo tribals in Tirap dt. is the most sacred orchid and is associated with their worships and festivals. In Gargo hills of Assam, many plants are believed to have mystic and supernatural powers. For example, *Thysanolaena maxima* wards off evil dreams; *Spatholobus parvifolius* is dreaded as an evil spirited plant; *Averrhoa carambola*'s leaves are kept in houses to prevent measles and *Erythrina variegata* is a must for burying the dead bodies. During religious ceremonies, the sacrificial articles include *Lagenaria siceraria*, grains of *Oryza sativa*, and leaves of *Musa pardisiaca*, by most of the tribals. The trifoliate leaves of *Aegle marmelos*, resembling the trident of Lord Shiva, and symbolizing the creation, preservation and destruction by the folklore, however are used by tribals of Central India for snakebite and they do not regard it as a sacred one. Tribals consider the hanging roots of *Ficus bengalensis* as hair of the departed Guru and treat it as a sacred palm. They perform rice festivals under different names. Branches of *Mangifera indica* are used by tribals to avert diseases in villages. Smearing its inflorescence and inhalation makes one immune to snakebite for a year. Tribals sweep rice fields with the twigs of *Vitex* or *Cleistanthus* for a better crop.

### **SOCIAL CUSTOMS**

Tribal people in general distinguish themselves by some peculiarity in their dress (which includes headgears, tubans), ornaments, body markings, tattooing or emblems, which vary from tribe to tribe. Indo-Aryan languages, with distinct dialects are spoken by a vast majority of tribals. Those speaking Dravidian and Tibeto-Burman language are also not few in terms of communities. All tribal communities (except three) are non-vegetarians. They eat pork, beef of cow or ox. The vegetarian tribals are Toda, Rabari and Bharwad communities. Animal fat is the cooking medium in the North-east. They consume home-made alcoholic drinks but of late started taking marketed ones. Tribals generally remain outside the caste-system. Only a few of them, who are aware of caste-system claim as Kshatriya, Shudras and Brahmins. Pardhan tribe

claims to be Vaishya. The large tribal communities are divided into moieties and phrarties, mostly in central and North-east India. All are endogamous. Incidence of intercommunity hypergamy (10%) and hypogamy (6%) is reported. Then are cases where clan exogamy is broken. The practice of consanguinity of both types of marriages (With father's sister's daughter or mother's brother's daughter and elder sister's daughter) is followed. Though monogamy is predominant, polygamy (polygyny) is also found. Polyandry is also not uncommon. Bride price (dowry) in cash or kind is prevalent. Divorce for either party is permitted and remarriages are allowed. Children can stay with father or mother after divorce. Their attitude towards family planning is not favorable. The families of tribals are nuclear. The conflicts arise on account of property. The male equigeniture is preponderant. The principle of male primogeniture is not recognized universally. Women have no right to property but only right to maintenance. There are about 56 tribal communities among about a total of 500, where equal rights for sons and daughters are observed. Tribal women have larger participation in workforce. They have a role in almost all economic activities, rituals and social functions but not in social affairs. They contribute to the family income, but very few have control over the expenditure. Tribals are mainly land holding community, but landless is also seen and the latter's economy is in the hands of market forces and middlemen. There is a shift from traditional occupation like trapping of birds, pastoral activities, shifting cultivation, hunting, crafts and skin and hide work to new ones like horticulture, terrace cultivation, animal husbandry, piggery, sericulture, bee-keeping, employment in govt. and private service, masonry, small scale industry, etc. The ecological degradation has severely affected them. There has been arising in the political leadership at village panchayat, regional and national levels. Tribals follow one religion and mostly Hinduism or Buddhism. There is a remarkable spread of Christianity in North-east and Northern Indian and also in Andaman and Nicobar island tribals. Tribals are not only peasants but also craftsmen. Carving and body-tattooing remain major tribal art and ritual. Wall painting, and drawing, has emerged recently. Basketry, weaving, cloth and leather embroidery, pottery is also found. They have fondness for dance and music, which constitute a vibrant part of tribal social life. Tribals depend on springs, streams, rivulets, etc., for drinking water, and firewood for cooking. Gobar gas and solar energy have not made any impact. Their attitude towards saving is not favorable and dependence on moneylenders and shopkeepers continue to be substantial. The tribals have reacted to education favorably. Literacy level is on an increase. Education has created a new stratum of entrepreneurs, businessmen, teachers, administrators, engineers, and doctors and also in defense services.

### **SACRED GROVES**

Sacred groves are the pockets of almost climax vegetation, preserved on religious grounds. They are located in the remote tribal areas. The forests which are regarded as sacred groves indicate the true vegetation that once existed before the dawn of modern civilization. Their existence is mostly due to certain taboos and strong beliefs supplemented by mystic folklores. Sacred groves are locally known as Devavana, Devarakadu, etc. They constitute only a representation of forest in near-virgin condition. They are ancient natural sanctuaries, where all

forms of living creatures are afforded protection through the grace of some deity, who is extremely primitive. The deities are called Mother Goddesses in the form of nshaped stones, smeared with red lead, lying under a tall tree or in the open and in the latter case, any attempt to give shelter is treated as a taboo. The Goddesses are known by such names as Kalkai, Vanadevata, Shirikai, U basa, U Ryngkew, Sylvan Deity, Tree Spirit, Waghai, etc., who are very fearsome. Removal of a small twig or breaking of even dead wood from sacred grove, it is believed by tribals, may result in serious illness or death. Such strict taboos have led to their preservation in virgin form over generations. Sacred groves are situated at a distance from human settlement. The composition of vegetation corresponds to climax (vegetation) formation for that region and supports a belief that they are immune to human interference. Sacred groves may range from a group of trees to a forest of trees, spaced over as much as 20 hectares. Even the smallest grove often harbors some old and magnificent specimens of trees and climbers. Larger sacred groves are a veritable treasure trove to naturalists, supporting many species of plants, which are rare in the area and have become rarer due to deforestation. The borders of sacred groves are distinct. In some of the sacred groves of Mawphlong (Meghalaya), *Castanopsis kurzee* protects as a hedge halting the intrusion. In Maharashtra, there are over 400 sacred groves in several districts of Thana, Jalgoan, Pune, Satara, Kolhapur, etc., covering 3570 hectares because of cultural and traditional beliefs in forest deities. Sacred groves serve as a last refuge for arboreal birds and mammals. They are natural treasure houses of plants and can satisfy the aesthetic, scientific and recreational needs of mankind today. They are the hotspots of biodiversity too. Recently a survey of sacred groves in A.P. has been taken up and various universities in the state have been involved. The inventorying of sacred groves has been funded by WWF (World Wildlife Fund).

## CONCLUSION

Biological diversity that is seen today is the result of millions of years of evolutionary process. Diversity is measured in terms of genetic diversity (diversity within the species), species diversity (diversity at species level), and ecosystem diversity. Conservation of Biological diversity is essential in order to sustain the life of human beings as well as other forms of life. Human race has been dependent on plants both for their material needs and emotional needs since its evolution. The tribal communities understand all these as life sustaining resources. Therefore they not only utilize them but also conserve them. Erosion of either of this diversity would greatly affect the human kind. Hence, both the biological and cultural diversity should be considered as a unit for a meaningful conservation. Tribal communities follow well balanced and judicious conservation methods for medicinal plants by applying the strict restrictions to be followed by everybody in the community in the form of taboos or totems or worship or rituals etc., for the posterity. This has been confirmed by the tribal healers during the interaction with the author. They observed that younger generation is influenced by attractions of urban society, further they also observed that middle aged people in their communities are suffering from arthritis and diabetic and hypertension which was not seen in the older generation particularly people of 70-80 years age. Recently a study published by National nutrition monitoring bureau (NNMB) and National Institute of Nutrition of neutrino (NIN) and Indian Council of Medical

Research (ICMR) in the month of June, 2012 in Hyderabad has released a report on "Diet nutritional study of Tribals of India". The report observed that many Adivasi (Tribal) in the country suffer from hypertension, overweight, obesity problems both in tribal men and women in the states of Gujarat, Odisha, Kerala, West Bengal, Karnataka, Maharashtra, Madhya Pradesh, Tamil Nādu and Andhra Pradesh

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Chapter

13

WATER- IT HAS NO SUBSTITUTE

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**ABSTRACT**

The unique characteristics of water include; it can rage, it can gush, it can caress, it can overcome and it can disappear. In fact, water has no substitute. Water defines and shaping the global climate as large masses of water trap and transport the sun's energy across the planet over sea and sky. If we compare the climate change with shark, then water is their sharp tooth. Some incredible facts about water concluded that the fresh/potable water resources are very less in quantity and it causes scarcity of water. Moreover, available water is undergoing to pollution and future needs are increasing day by day. In this scenario, fear is a powerful change agent that can inspire us to think about water scarcity, pollution of water and future needs of water. Therefore, the main objective of this chapter is to bring awareness on water- how precious it is. Once water is seen valuable, innovations and processes will come fast to conserve it.

**KEYWORDS:** Water, Water Scarcity, Water Pollution, Water Security Technologies.

**INTRODUCTION**

The unique characteristics of water include; it can rage, it can gush, it can caress, it can overcome and it can disappear. In fact, water has no substitute. Unique chemistry of water enables the plants to move up against gravity. Moreover, water defines and shaping the global climate as large masses of water trap and transport the sun's energy across the planet over sea and sky. If we compare the climate change with shark, then water is their sharp tooth (Copeland, 2019). Water covers 75 % of the earth and composes approximately 78 % of the human body. It reflects on every food grain that we are eating for survival. Thus water is omnipresent. In contrast to the provided information, we need to accept the incredible facts about water. Which includes: 1. about 98% of all water on Earth is in the seas. Fresh water makes up fewer than 3% of all water on earth, and nearly 65% of this drinkable water is tied up in glaciers. Rivers, streams, lakes, and dams that hold freshwater contain 1% of potable water while groundwater accounts for 0.3 % (CCAO-ARWEC). 2. By 2025, 1.8 billion people will be living in countries or regions with absolute water scarcity, and two-thirds of the world's population could be living under water stressed conditions (UNDESA).3.Worldwide, every year 4 million people are dying due to water borne diseases(WHO, 2021). 4. In India, less than one thirtieth of the world's water is available to survive (AQUASTAT, 2021) and is suffering from worst water crisis now ever in the history. At present, 600 million Indians under extreme water stress and two lakh people dying every year due to inadequate access to safe water (WRI

Aqueduct; WHO Global Health Observatory). 5. As per the report of National Commission for Integrated Water Resource Development of MoWR, the water requirement by 2050 in high use scenario is likely to be a milder 1,180 BCM, whereas the present-day availability is 695 BCM. In fact, if third world war is inevitable, that would be due to water.

From the above facts, it would be concluded that the fresh/potable water resources are very less in quantity and it causes scarcity of water. Moreover, available water is undergoing to pollution and future needs are increasing day by day. In this scenario, fear is a powerful change agent that can inspire us to think about water scarcity, pollution of water and future needs of water. Therefore, the main objective of this chapter is to bring awareness on water- how precious it is. Once water is seen valuable, innovations and processes will come fast to conserve it.

### **WATER- HOW IT BECOMES SCARCE?**

India's water is in a great crisis. Now let us make our attempt to know the reasons for ongoing and future crisis. Upon independence, our leaders thought about the erstwhile famines that India was faced. So their immediate objective then is to make the India as food secure country. So they built dams, projects to encourage the farmers leaving the local water harvesting and that created a dangerous fault line. Pretty Soon, bore well revolution was came with the belief that India's water is endless (Ramesh, 2021). This entrenched emotional belief made us fail to manage our ground water. Perhaps this is one of the reason for scarcity of water that we are experiencing and major fault line in India.

80% of India's water consumed by agriculture in the year 2010, while the domestic and industrial sectors accounted for 6% each and these two sectors gained their water shape over the past decade (WRIS-CWC, 2013). Moreover, the requirement of water for domestic and industrial use will continue to grow and such requirement creates much scarcity of water, leads to crises.

The World Health Organization (WHO) estimates that a person needs more than 70 liters of water a day to live (Reed, Shaw & Chatterton, 2011), while Bureau of Indian Standards (BIS) recommends 135 litres per person per day as a lower limit (CBRWS, 1993). Many Indians get far less water than this, while in summer or drought, situation is most vulnerable and which brings the water-tanker market.

Meanwhile lack of meaningful, formal water price leads to unbridled demand, decimating India's water and leaving its populations to the tender mercies of the water-tanker market. For instance, Chennai, India's sixth -largest city in Tamilnadu state, the price some paid for water during the Chennai water crisis was substantially higher than what Singapore charges (Ramesh, 2021). Besides this, Chennai had a bitter flood experience in 2015 South India floods. This kind of extreme rain events- extreme scarcity has been in the history of Chennai. So we need to realize from Chennai case to understand what is going on across the country. In fact, in nutshell, for the rich water is peripheral. For middle class, water becomes important during summer or drought. For poor, life revolves around water.

According to World Bank Report, across the world, India is the largest ground water user with an estimated usage of approximately 230 cubic kilometers per year and it is more than quarter of the global total. Moreover, 60% of irrigated agriculture and 85% drinking water supplies

dependent on ground water resources. Ground water has become a vital source for water in rural areas of India. Reliance of people on ground water has been increasing and it leads to the construction of million private wells, eventually exploitation of ground water resources increases. This scenario has been continuing for the last five decades. There were number of factors encouraged the expansion of ground water use which includes; 1. Poor delivery service from public water supply systems to farmers, rural and urban households. 2. Affordable pump technologies to operate own tube wells. 3. Flexibility and timeliness of ground water supply...etc. The endless reliance on ground water aquifers for both drinking and irrigation purposes is now approaching its limit and to unsustainable levels of exploitation. The nationwide 2004 assessment concludes that 29 percent of groundwater blocks to be in the semi-critical, critical, or overexploited categories, with the situation deteriorating rapidly.

The Indian Rivers Inter-link project aims to manage water resources in India by linking Indian Rivers by a network of reservoirs and canals to enhance irrigation and ground water recharge, reduce persistent floods in some parts and water shortages in other parts of India. The average rainfall in India is about 4000 billion cubic metres and most of it occurs through South-West Monsoon (June-September) in a non-uniform manner across the nation. India also sees excess monsoons, floods and droughts. Despite abundant rains during July–September, some regions in other seasons see shortages of drinking water. The geographical variance in availability of natural water versus demand for irrigation, drinking and industrial water creates demand-supply gap and that has been worsening with India's rising population (IWMI, 2012). Rivers interlinking project experts claim the answers to India's water problem is to conserve the monsoon water, construct the reservoirs and deliver this water to areas in which water has scarce. But, Surprisingly even after 4 decades of start the interlinking project, we could not receive its fruits. Moreover, instead of that, we have been facing the famines, droughts and water scarcity. For instance, the experienced Chennai floods in the past, water scarcity that we are facing across the country are the live examples for water mismanagement.

Due to time lag in the execution of Rivers inter-link project, every year colossal amounts of water drains into the sea leaving the droughts, floods and plunge in the ground water levels etc., are standing as practical problems. In addition to the above executive failures, water pollution makes the scarcity of potable water in some places, where we get water from ground water resources. The waste water or polluted water percolates into the soil or evaporates. The waste accumulated in urban areas causing unhygienic conditions and releasing pollutants that leach into the surface and ground water. Major cities of India produce 38,354 million litres per day (MLD) of sewage, but the urban sewage treatment capacity is only 11,786 MLD (Kaur et al.2012). A large number of Indian rivers are severely polluted as a result of discharge of domestic sewage, agriculture sewage and aquaculture sewage. Therefore, the gap between sewage generated per day and treatment capacity of sewage per day is also organizational failure leads to scarcity of water.

## **HOW CAN WE DECREASE THE POLLUTION AND INCREASE THE GROUND WATER LEVELS?**

Nowadays Governments, Research Institutes Educational institutions etc., are with the slogan “Sustainable Development”. What is this Sustainable Development? Sustainable Development is the Development without being loss of natural resources and ecosystem failure. Governments, Institutions and many more have been working on this slogan for the last two decades, but the most important natural resource and it has no substitute i.e., water; is goes on decreasing and were failed to manage it properly. Sustainable development is interlinked to water, food and energy security. Wastewater is a key element in the water-food-energy nexus and recovery of resources can link water, nutrient and energy cycles.

Effective treatment of wastewater is indispensable for public health and sanitation, water reclamation, preventing environmental pollution and protecting water resources. Moreover, the treated wastewater is a potential resource and its reuse will offset supply and demand in water-stressed areas. Technologies which include Activated Sludge (AS) process, Granular Sludge (GS) process have already come for wastewater treatment. The bio beads or granules consisting of certain bacteria developed by scientists of Bhabha Atomic Research Centre(BARC) facilitates at Kalpakkam of Tamilnadu state which has the Madras Atomic Power Station can remove contaminants in wastewater. The patented technology will bring down the size of the treatment plant by 70%, cost by 40% while the treated water can be used for gardening and scientists call the technology as Hybrid Granular Sequencing Batch Reactor (Nancharaiah *et al.*, 2019). Moreover, this Atomic Power Station trying to commissioned the sewage treatment plant to supply water to 3200 households in the Kalpakkam Township. These types of initiations would decrease the water scarcity, there by decreases water pollution and rejuvenated the ground water levels and stops ground water pollution too. Moreover, the established sewage treatment plants should work with the intension that reduce gap between sewage generated per day and treatment capacity of sewage per day. These will definitely decrease the scarcity of water.

## **CONCLUSION: HOW CAN WE MAKE INDIA’S WATER RESILIENT?**

There is a misconception in India with Governments, Political parties, Burocratic systems and people that interlinking the Rivers will give the perennial solution to water scarcity leaving the other technologies. In fact, its executive failures and procrastination in meeting its objectives for the last four decades thrown us to number of droughts, floods and water scarcity problems across India. All this happens due to enmeshed politics with water. According to Ramesh, M., authored book “Watershed”; there are three technologies to India’s water security which includes; 1. Forests- a natural technology 2. Tanks - a traditional technology 3.Sewage Treatment- a modern technology. Of these technologies, natural and traditional technologies were forgotten by modern water engineering experts. In fact, Indian ground waters and agriculture were well managed by the kingdoms through Tank technology before the colonial rule. After Colonial rule, our new nation leaders wanted India to be food secure at any cost and it changed the water facets. After all, Rivers interlinking projects were came in to the lime light by constructing dams across the rivers. These projects are highly welcoming to cater the agriculture needs and to increase the ground water levels around their pass by. But what about

the areas in which Rivers are not being flown and how can we increase the water levels in those areas? So there is an urgent need to go for tank technology to increase the ground water levels and to harvest the rain water. Coming to the modern technology i.e., sewage treatment is highly essential in cities to overcome the water scarcity and it should execute in proportion to sewage generated per day. i.e., without gap between sewage generated per day and treatment capacity of sewage per day. Otherwise, we are living in the economic world, in this economic world what is priced becomes prized while the unpriced becomes invisible and abused. Therefore there would be a possibility of water invisible unless we priced it. If it is necessary to price the water, with a meaningful price would we can eat so much rice/wheat that we are consuming? You just think and imagine.

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**ABSTRACT**

Medicinal plant and their bioactive compounds have greater prospective in the development of drugs and consider safe for human after prolonged treatments. The medicinal plant *Clitoria ternatea* deserves high potential due to its potent bioactive secondary metabolites. *Clitoria* is an attractive climbing perennial with prominent blue or white flowers. The present study aim to investigate the phytochemical constituents and antibacterial activities of leaf extract of *C. ternatea* by cold extraction method. Primary phytochemical analyzes of carbohydrates, starches, chlorophylls, alkaloids, total phenolics, total flavonoids, tannins and steroids were performed according to standard methods. This study also showed that *Clitoria ternatea* leaf extract susceptibility of *S. aureus* natural skin flora bacteria. Qualitatively and quantitatively, the methanol extract of the leaf contains a sufficient amount of phenol, carbohydrates, tannins, flavonoids and terpenoids. Agar well diffusion assay and minimum inhibitory concentration (MIC) were used to investigate the zone of inhibition measurements based on the methanol extract of *C. ternatea* leaves for bacterial treatment. Therefore, *C. ternatea* can be used to discover bioactive natural products that can be the basis for the development of new natural herbal medicines.

**KEYWORDS:** *Clitoria ternatea*, cold Extract, Phytochemical analysis, Antibacterial activity.

**INTRODUCTION**

Infectious diseases are the number one cause of death, accounting for about half of all deaths in tropical countries. The clinical effectiveness of many existing antibiotics is threatened by the emergence of multidrug-resistant pathogens (Gossell-Williams *et al.*, 2006). *Clitoria ternatea* L. belongs to the legume family known as butterfly pea. Perennial twining plant of tropical tropics. Commonly known as 'Aparajita', 'Butterfly Pea' and 'Shankasupi'. Plants seem to be very adaptable to different ranges of temperature and humidity. They are tolerant of cold and dry conditions (Manjula *et al.*, 2013). *Clitoria ternatea* is a vital, herbaceous, persistent perennial legume. The stems are finely tangled, sparsely hairy, the base is semi-erect, 0.5-3 m long. The leaves are pin-shaped with 5 or 7 leaflets. The length of the petiole is 1.5-3 cm. Spikelets

persistent, narrowly triangular, 1-6 mm long, stipitate, prominent, 3-nerved. The length of the shafts is 1-7 cm. String nails 2 mm long. Leaflets are oval. *Clitoria ternatea* L. Var. (Blue var), clitoris, is an attractive climbing perennial with prominent blue or white flowers. It is traditionally used to treat various diseases (Mukherjee *et al.*, 2008; Arya *et al.*, 2018; Mohan *et al.*, 2022).

The development of herbal medicines has made it possible to treat the disease on a more effective scale. On the other hand, the use of antibiotics can have unwanted side effects such as kidney failure, stroke and liver damage. The study of plants as sources of treatment for incurable diseases has caused a great demand for medicinal plants. It has been reported that plants are one of the most popular sources for the discovery of antimicrobial agents. Secondary metabolites in plants are mainly responsible for antimicrobial activity. The main plant components involved are terpenoids, phenols, lectins, alkaloids, polyphenols and polypeptides (Knöppel *et al.*, 2017). The use of medicinal plants has a special potential as natural antibacterial agents. Drug use has adverse effects on the human body, leading to multidrug-resistant bacteria, which is a global public health problem (Moussa *et al.*, 2012). Due to its beneficial value as an antibacterial agent along with bioactive compounds, *C. ternatea* is also seen as a potential candidate for public health support (Figure 1). According to traditional medicine such as ayurvedic medicine using *C. ternatea*, it is widely used as a memory enhancer, antidepressant, anti-stress, sedative, anti-anxiety and sedative (Mohan *et al.*, 2022). This traditional system has long been considered as an alternative to drugs, as it is derived from non-toxic compounds. In other words, medicinal plants are used in the preparation of herbal medicines and are very powerful elements in the Indian medical system using plants as a source of medicine (Jain *et al.*, 2003). Therefore, these plant extracts play a very interesting role in helping the results of any experiment. This extract has been described as an effective natural treatment for many diseases, because its extracts have antiseptic properties (Tadesse *et al.*, 2017).



**Figure 1: Plant of *C. ternatea***

## **MATERIALS AND METHODS**

### **SELECTION AND PROCUREMENT OF *CLITORIA TERNATEA***

The plants named *Clitoria ternatea* was selected after reviewing the medicinal properties of this plant and how they can enhance the use of herbal medicine in near future. All leaves were cleaned with a brush, washed with water, dried in the sun and finally turned into a fine powder which was stored for further analysis as shown in figure 2.



Figure 2: The powder form of *Clitoria ternatea* leaves

### PREPARATION OF EXTRACT

The collected healthy and fresh leaves of *C. ternatea* were washed in tap water for ten minutes and rinsed with sterile distilled water and completely air dried. The dried leaves were grinded into fine powder. For methanol extract, 50g of powder sample was dissolved in 250 ml of 80% methanol solution in conical flask. The flasks were kept in normal room temperature for 10 days after properly sealing with parafilm. Then this extract was filtered separately by using Whatman No. 1 filter paper. Filtrate was concentrated to dryness in a rotary evaporator (Büchi Labortechnik, Germany) under reduced pressure and controlled temperature (40–50 °C) to give final extracts, which was stored at 4 °C in an airtight container until further use.



Figure 3 : The filtrate of plant extract of *C. ternatea*

### PHYTOCHEMICAL QUALITATIVE SCREENING

The sample were screened for phytochemical screening which helps to confirm the presence of the secondary metabolites in the prepared extract by the standard book (Practical Pharmacognosy by C. K. Kokate, 2004).

### QUANTITATIVE PHYTOCHEMICAL SCREENING

#### DETERMINATION OF PHENOLIC CONTENTS

The total phenolic content was determined using the Folin–Ciocalteu method of Lee *et al.*, (2015) with slight modification. Briefly, 1 ml of the extract solution (500-100 µg/ml) was mixed with 2.5 ml of 10% (w/v) Folin-Ciocaltio reagent. After 5 min, 2.0 mL of Na<sub>2</sub>CO<sub>3</sub> (75%) was subsequently added to the mixture and incubated at 50 °C for 10 min with intermittent stirring. The samples

were then cooled and the absorbance was measured using a UV spectrophotometer (Shimazu, UV-1800) against a substance without extract at a wavelength of 765 nm. The resulting data were expressed as mg/g equivalent of gallic acid in terms of mg/g dry extract (mg GAE/g).

#### **DETERMINATION OF TOTAL FLAVONOIDS CONTENT**

This analysis was performed according to the method described by Abu Bakr et al. (2009) with slight modification. Approximately 0.5 mL of freshly prepared extract was mixed with 2.25 mL of distilled water in a test tube wrapped in aluminum foil. Then 0.15 ml of 5% w/v sodium nitrate solution was added to the mixture. The solution was allowed to stand for 6 min before adding 0.3 mL of 10% w/v aluminum chloride. After 5 min, 1.0 mL of 1 M sodium hydroxide was added and the mixture was immediately vortexed. Absorbance was read at 510 nm using a UV-Visible spectrophotometer. The calibration curve was prepared using standard quercetin solution and the results were expressed in terms of milligrams of quercetin per gram of dry weight.

#### **TEST ORGANISM**

The bacteria *S. aureus* was isolated by bacterial flora of the skin by swap method and further identification of bacteria done by standard biochemical and selective media test. Bacterial strain was maintained on Nutrient Agar medium (NAM) and subcultured every month on the same media. Paraffin stocks were prepared by overlaying agar slants with it.

#### **PREPARATION OF BACTERIAL INOCULUM**

A loop full of grown culture from the slants was transferred into 5-6 ml of fresh muller hinton broth for bacteria. The broth was standardized by using sterile normal saline (0.85% NaCl) to obtain a population of  $1.5 \times 10^8$  cfu/ml by 0.5 McFarland standard methods for bacterial culture.

#### **SCREENING OF ANTIBACTERIAL ACTIVITY**

The agar diffusion method is widely used to evaluate the antibacterial activity of plant extracts (Valgas *et al.*, 2007). Similar to the method used in disk diffusion, the surface of the agar plate is inoculated by spreading a volume of microbial inoculum on the surface of the agar. Then, a hole with a diameter of 6-8 mm is drilled with a sterile cork or tip, and a volume (20-100 microliters) of the antibacterial agent or extract with the desired concentration is injected into the well. The agar plate is then incubated under conditions suitable for the test organism. The antimicrobial agent diffuses into the agar medium and prevents the growth of the tested microbial strains. The experiment was performed in triplicate and the results (mm of zone of inhibition) were expressed as their mean values.

#### **DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC)**

The minimum inhibitory concentration (MIC) value of extract of *C. ternatea* was determined by agar dilution method for bacterial culture (Akerlele *et al.*, 2008). The experiment was performed in Mueller Hinton Agar (MHA) for bacterial strain. The methanolic extract of *C. ternatea* was incorporated into specific nutrient medium at various concentrations aseptically, mixed gently in sterile Petri-dishes and then allowed to set. The surface of the agar plates were allowed to dry properly before inoculating with prepared inoculum of standardized bacterial culture. The plates were then incubated at 37°C up to 24 h for bacteria after about 30 min of inoculation. The lowest concentration preventing visible growth in each determination was taken as the minimum

inhibitory concentration. The growth of microorganisms was indicated through visual examination. All the experiments were performed in triplicate. Streptomycin sulphate served as a control for bacteria.

## RESULTS AND DISCUSSION

Fresh leaves of *Clitoria ternatea* was collected from the authorized supplier choudhary Chandrabhan Dawai Vale, Jabalpur (M.P.). The sample specimen was identified based on the taxonomical characteristics and registered by 'Herbarium' Institute of science and laboratory education, IPS Academy Indore. The dried plant samples were powdered by mechanical grinder and sieved to give particle size 40- 100 mm (Figure 2). The leaves of *Clitoria ternatea* plants were extracted with 80 % methanol solvent by cold extraction method for 10 days by the earlier reported method (Majekodunmi, 2015; Zhang *et al.*, 2018). The plant extract was filtered and then concentrated using rotary evaporator at 40 °C and transferred to glass vials, kept at 4° C before use. Investigations on the qualitative phytochemical screening of methanolic leaf extract of *Clitoria ternatea* are summarized in Table 1. Results from the current study indicated that methanolic leaf extracts of *C. ternatea* contain various types of pharmacologically active compounds. The present study coincide with the earlier finding of Salhan *et al.*, (2011) and Manalisha and Chandra, (2011) who confirmed the phytochemical analysis of methanol and ethanolic extract of *Clitoria ternatea* roots and reported the presence of tannins and resins and certain other constituents.

The result of total phenol and flavonoid content of methanolic leaf of *Clitoria ternatea* were summarized in Table 2. Earlier studied of Jaafar *et al.*, (2020), suggested that total phenolic contents were varied according to plant parts and also depend on extraction solvent. The results were also similar with the study of Aryal *et al.*, (2019) showed that the highest values of total polyphenols, flavonoids, anthocyanins and tannins were found with methanol solvent.

The amount of total phenolic content was determined with help of Folin-Ciocalteu reagent method. Gallic acid was used as a standard compound and the total phenolic content of extracts of *C. ternatea* were expressed as mg/g gallic acid equivalent (GAE) which was calculated by using the standard curve equation:  $y = 0.098x + 0.021$ ,  $R^2 = 0.995$  at 750 nm. The total phenolic content in the methanolic extract of *C. ternatea* was found  $85.59 \pm 0.46$  mg GAE/g dry weight of extract respectively. The amount of total flavonoid content was determined by using aluminum chloride reagent and quercetin used as a standard compound. The total flavonoid content of extracts of *C. ternatea* leaves was calculated by using the standard curve equation:  $y = 0.027x + 0.024$ ,  $R^2 = 0.993$  at 510 nm. The total flavonoid content was  $17.00 \pm 0.68$  (mg QE/g dry extract wt) in the methanolic extracts *C. ternatea* respectively. The present study was coincided with Rabeta and Nabil, (2013) who reported that the total phenolics compound found in methanol extracted sample was between 61.7 to 64.8 mg GAE per gram samples for *C. ternatea*. However, the methanol-extracted sample contained high TPC in the leaf part of *C. ternatea*. This situation may be caused by increasing the solubility of plant phenols by an organic solvent (methanol), which facilitates their dissolution by penetrating the plant cell structure (Moure *et al.*, 2001).

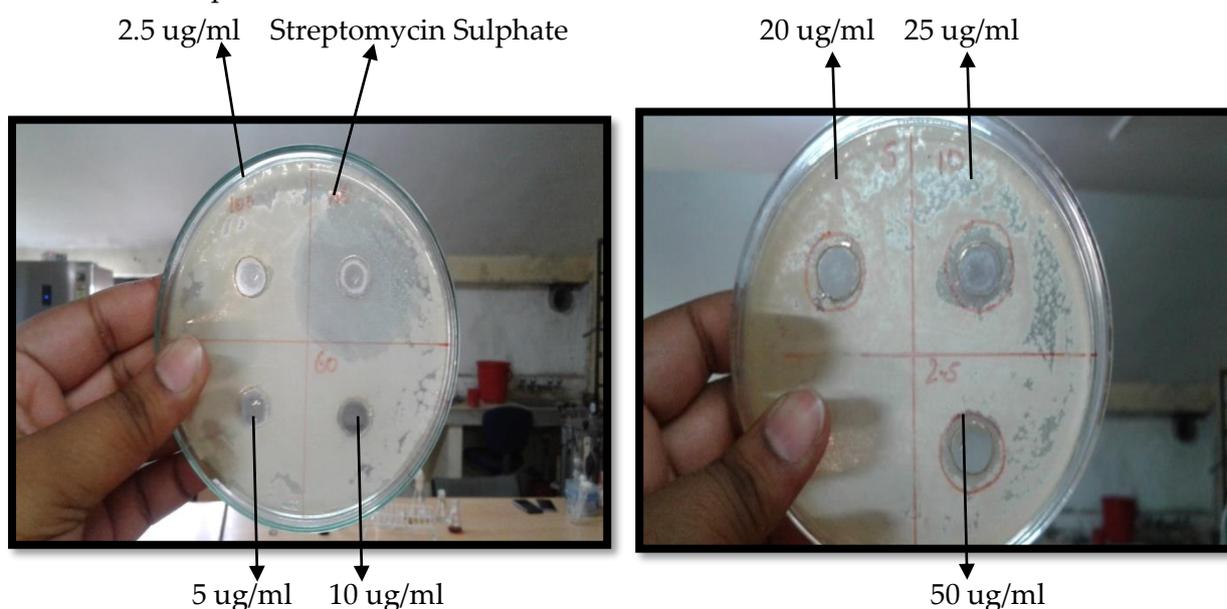
Antimicrobial activity of the extract of selected plant part was carried out by agar well diffusion method. Consequentially, the results showed that selected plant have great potential as

antibacterial compounds against *S. aureus*. The diameter of inhibition zone of *C. ternatea* extract was found to be 5 mm, 6 mm and 8 mm by using different concentrations ranging from 20 µg/ml to 50 µg/ml against *S. aureus* (Figure 4). The present results were also in the full agreement with the study of Anand *et al.*, (2011), who reported that methanolic extract of *C. ternatea* affected the activity of *Bacillus cereus* to a greater extent followed by *Klebsiella pneumoniae*, *Proteus vulgaris* and *Salmonella typhi*. The MIC was performed to determine the minimum concentration of cold methanolic leaf extract of *C. ternatea*, which inhibited the growth of bacterial strain (*S. aureus*) with MIC values ranging from 100 to 2000 µg/ml (Table 3). Agar dilution method was used to determine the MIC values according to Akerele *et al.*, (2008) with slight modification. Minimum inhibitory concentration values of methanolic extracts of *Clitoria ternatea* for *S. aureus* was found to be 1800 µg/ml and 2000 µg/ml as shown in Table 3. At this MIC concentration, no visual growth was reported up to 8 days in a petridish. Similar to the present finding, the study of Kamilla *et al.*, (2009) also demonstrated that the MIC, MBC and MFC values of *C. ternatea* extracts ranged from 0.3 mg/ml to 100.00 mg/ml. The leaf and root extracts were found to be most effective against all of the tested organisms.

**Table 1: Qualitative Estimation of Phytochemical Constituents of *Clitoria ternatea***

Sr. No.	Active components	<i>Clitoria ternatea</i> (leaves)
1	Alkaloids	-
2	Tannins	+
3	Flavonoids	+
4	Phenols	+
5	Anthroquinones	-

Note: + denotes presence and - denotes absence.



**Figure 4: Zone of inhibition of *C. ternatea* by Agar well diffusion method Against *S. aureus***

**Table 2: Quantitative Estimation of Total Phenolic and Flavonoids Content of Crude Extract of *Clitoria ternatea***

<i>Clitoria ternatea</i>	Total phenolic content (mg/gm*)	Total flavonoids content (mg/gm*)
Methanolic leaf Extract	85.59 ± 0.46	17.00 ± 0.68

\*Values are means of three independent determinations ± Standard Error Mean (SEM)

**Table 3: Minimum Inhibitory Concentration (MIC) results of Standard Drugs Streptomycin sulphate (St.S) and *C. ternatea* leaf extract (Ct LE).**

Conc. (µg/ml)	Visible growth of Microorganism	
	<i>S. aureus</i>	
	<i>C. ternatea</i> (Ct LE)	St.S
100	+4	+3
200	+4	+2
400	+3	+2
600	+3	+1
800	+2	+1
1000	+2	No growth
1200	+1	0
1400	+1	0
1600	+1	0
1800	No growth	0
2000	0	0
Control without standard drug	100 % growth	100 % growth

\*Growth was scored in the following manner: +4 very good growth; +3 good growths comparable to that of the Extract free control; +2, light growths approximately that of the control + 1, very light growths approximately that of the control; 0, No visible growths.

## CONCLUSION

From this study, it can be concluded that *Clitoria ternatea* leaf extract has antibacterial activity against normal skin flora. It is expected that the use of natural products as therapeutic agents is unlikely to induce resistance in microorganisms. This may explain the reasons for the use of the plant in the treatment of infections in traditional medicine. In this study, the assessment of higher phenolic and flavonoid contents could be a significant source of natural antioxidants. The plant could be a real and cheaper substitute for conventional drugs because the plant is easy to obtain and the extract can be easily made by a simple process of maceration or infusion. It is imperative that research continues to isolate and purify the active ingredients and their

mechanism of action which is essential in good health for understanding their ability to control diseases that have a significant impact on quality of life.

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### ABSTRACT

The management of different waste requires different kinds of procedures to handle as the different toxic compounds that might be present in one may not be present in the other. Nature recycles all types of waste materials and so then nature is capable of recycling which results in pollution. Waste management practice can differ for developed and developing nations, for urban and rural areas, and for residential and industrial producers. Management of hazardous waste residential and institutional waste in metropolitan areas is usually the responsibility of local government authorities, while management for non-hazardous commercial and industrial waste is usually the responsibility of the generator subject to local, national or international controls.

**KEYWORDS:** Environment, waste, waste types, recycle, treatment.

### INTRODUCTION

Most human activities generate waste (Brunner and Rechberger, 2014). Despite that, the production of wastes remains a major source of concern as it has always been since pre historic period (Chandler et al, 1997). In recent times, the rate and quantity of waste generation have been on the increase. As the volume of waste increases, so also does the variety of the waste increases (Vergara and Techobanoglous, 2012). Unlike the pre historic period where wastes were merely a source of nuisance that needed to be disposed of. Proper management was not a major issue as the population was small and a vast amount of land was available to the population at that time. In those days, the environment easily absorbed the volume of waste produced without any form of degradation.

Scientific waste management in urban areas, waste including human excreta and waste from polluting industries are disposed through sewers. This pollutes the environment, underground water and exposes people to infection. Open decomposition of solid waste and sewer water through existing river systems takes very long period in natural treatment while causing many health hazards. In many countries, initiatives to develop the skills through recycled waste material for producing vegetables by the poor have brought about excellent results. In some Latin American countries, vegetable production in urban areas through waste recycling have not only been able to reduce the direct and indirect costs associated with waste disposal but it has also simultaneously been able to solve the problems of urban sanitation while becoming an income generating activity as well.

### TYPES OF WASTE MANAGEMENT

Waste arises in many different forms and its characterization can be expressed in several forms. Some common characteristics used in the classification of waste includes the physical states,

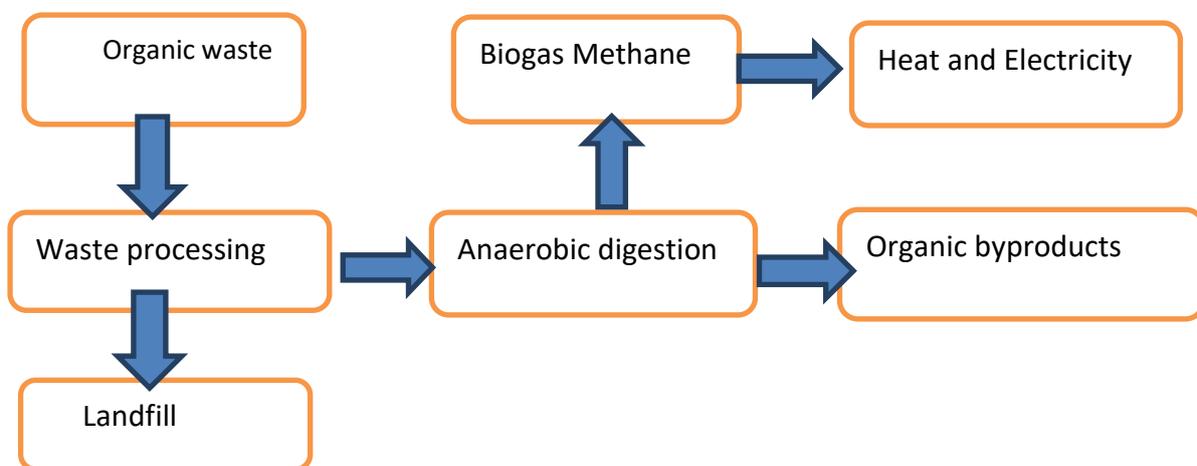
physical properties, reusable potentials, biodegradable potentials, sources of production and the degree of environmental impact (Demirbas, 2011; Dixon & Jones, 2005; White *et al.*, 1995). White *et al.* (1995) stated that waste can be classified broadly into three main types according to their physical states

1. Solid waste
2. Liquid waste
3. Gaseous waste

### MANAGEMENT OF SOLID WASTE

A Solid waste management SWM system includes the generation of waste, storage, collection, transportation, processing and final disposal. Managing solid waste generally involves planning, financing, construction and operation of facilities for the collection, transportation, recycling and final disposition of the waste. Solid waste management SWM is a basic public necessity and this service is provided by respective urban local bodies. The waste management term usually relates to materials produced by human activity, and the process is generally undertaken to reduce their effect on health, the environment. Waste management is a distinct practice from resource recovery which focuses on delaying the rate of consumption of natural resources. All waste materials whether they are solid, liquid, gaseous or radioactive fall within the remit of waste management.

Waste composition dictates the waste management strategy to be employed in particular location. Organics are putrescible, and are food for pests and insects and hence need to be collected and disposal off daily basis. The amount of recyclable like paper and plastics dictates how often they need to be collected. Organics need controlled biological treatment to be of any value, however due to the general absence of such facilities, organics do not represent any direct value to informal collections. The municipal solid waste industry has four components; recycling, composting, landfilling and waste-energy via incineration. The primary steps are generation, collection, sorting and separation, transfer, and disposal.



**Figure 1: Solid waste management**

### COLLECTION OF WASTE

The functional element of collection includes not only the gathering of solid waste and recyclable materials, but also the transport of these materials, after collection, to the location where the collection vehicle is emptied. This location may be a materials processing facility, a transfer station or landfill disposal site. Waste transfer stations are facilities where municipal solid waste is unloaded from collection vehicles and briefly held while it is reloaded onto larger long-distance transport vehicles for shipment to landfills or other treatment or disposal facilities.

## DISPOSAL OF WASTE

### A) Dumping

Dumps refer to uncovered areas that are used to dump solid wastes of all kinds. The waste is untreated, uncovered and not segregated. It is the breeding ground for flies, rats and other insects that spread diseases. The rain water run-off from these dumps contaminates the nearby land and water, thereby spreading diseases. In some countries, Open dumps are being phased out.

### B) Sanitary land fill

An alternate to landfill which will solve the problem leaching to some extent is a sanitary landfill which is more hygienic and build in a methodical manner. These are lined materials that are impermeable such as plastics and clay, and are also built over impermeable soil. Constructing sanitary landfill is very costly and they are having their own problems. Some authorities claim that often the plastic liner develops cracks as reacts with various chemical solvents present in the waste.

### C) Incineration

The process of burning waste in large furnaces is known as incineration. In these plants the recyclable material is segregated and the rest of the materials are burnt. At the end of the process all that is left behind is ash. During the process, some of the ash floats out with the hot air. This is called fly ash. Both the fly ash and ash that is left in the furnace after burning have high concentration of dangerous toxins such as dioxins and heavy metals, leaches the area and cause severe contamination.

### D) Pyrolysis

Pyrolysis is related to a form of thermal treatment where waste materials are heated to high temperature with limited oxygen availability. Pyrolysis of solid waste converts the material into solid, liquid and gas products. The liquid oil and gas can be burnt to produce energy or refined into other products.

### E) Composting

Composting is a biological process in which micro-organisms, mainly fungi and bacteria, convert degradable organic waste into humus like substance. This finished product, which looks like soil, is high in carbon and nitrogen and is an excellent medium for growing plants. The composting can significantly reduce the amount of garbage.

### F) Biogas technology

Waste materials that are organic in nature, such as plant material, food scraps, and paper products, are increasingly being recycled. These material, food scraps, and paper products, are increasingly being recycled. These materials are put through a digestion system to the biological process to decompose the organic matter and kill pathogens. The resulting stabilizer organic materials is then recycled as mulch or compost for agricultural or landscaping purposes.

## MANAGEMENT OF LIQUID WASTE

Clean and plentiful water provides the foundation for prosperous communities. We rely on clean water survive, yet right now we are heading towards a water crisis. Changing climate patterns are threatening lakes and rivers, and key sources that we tab for drinking water are being overdrawn or tainted with pollution. Safe drinking water is essential to humans and other life forms even though it provides no calories or organic nutrients. Access to safe drinking water and over 2.5 billion lack access to adequate sanitation. Water plays an important role in the world economy as it functions as a solvent for a wide variety of chemical substances and facilitates industrial cooling and transportation. Approximately 70% of the fresh water used by humans goes to agriculture.

This natural resource is becoming scarcer in certain places, and its availability is a major social and economic concern. Currently, about a billion people around the world routinely drink unhealthy water. Poor World Health Organization estimates that safe water could prevent 1.4 million child deaths from diarrhea each year.

There are two general treatment objectives with respect to wastewater

A) Reducing or minimizing the public health and hazards of waste water. These are general treatment measures aimed at preventing pathogens and other potentially harmful components from findings their way back to the consumer.

B) Eliminating, reducing or minimizing the deteriorative impact of wastewater on the receiving water quality and its environment.

A sharp distinction must be made between the term “wastewater disposal” and waste treatment”. All wastewater has to be disposed of. Some wastewater is subjected to various types of treatment before disposal, but some wastewater receives no treatment before disposal.

### **WASTEWATER DISPOSAL**

There are three methods by which final disposal of wastewater can be accomplished. The general problem areas that are of concern in final disposal are pathogenic microorganism, heavy metals and presence of biologically resistant organic compounds, such as pesticides or insecticides which can find their way into water supplies. More recently there has been interest in the use of land for both surface and subsurface disposal after wastewater treatment.

#### **SURFACE DISPOSAL**

Generally this is disposal by irrigation. This involves spreading the wastewater over the surface of the ground, generally by irrigation ditches. There is some evaporation but most of the wastewater soaks into the ground and supplies moisture with small amounts of fertilizing ingredients for plant life. This method is largely restricted to small volumes of wastewater from a relatively small population where land area is available and where nuisance problems will not be created.

#### **SUBSURFACE DISPOSAL**

By this method wastewater is introduced into the ground below its surface through pits or tile fields. It is commonly used for disposal of settled wastewater from residences or institutions where there is only a limited volume of wastewater.

### **SEWAGE TREATMENT**

#### **A) DILUTION**

Disposal by dilution is the simple method of discharging wastewater into surface water such as river, lake, ocean, estuaries or wetland. This results in the pollution of receiving water. The degree of pollution depends on the dilution, volume and quality of the water with which it is mixed

#### **B) PHYSICAL TREATMENTS**

Physical methods of wastewater treatment include sedimentation, floatation and adsorption, as well as barriers such as bar racks, screens, deep bed filters, and membranes. Physical method includes processes where no gross chemical or biological changes are carried out and strictly physical phenomena re used to improve or treat the wastewater.

#### **C) BIOLOGICAL TREATMENTS**

Biological treatment is an important and integral part of any wastewater treatment plant that treats wastewater from either municipality or industry having soluble organic impurities or a mix of the two types of wastewater sources. The obvious economic advantage, both in terms of capital investment and operating costs, of biological treatment over other treatment processes

like chemical oxidation; thermal oxidation etc. has cemented its place in any integrated wastewater treatment plant.

#### **D) CHEMICAL TREATMENTS**

Chemicals are used during wastewater treatment array of processes, which include chemical reactions, are called chemical unit processes, and are used alongside biological and physical cleaning processes to achieve various water standards. These several distinct chemical unit processes, including chemical coagulation, chemical precipitation, chemical oxidation and advanced oxidation, ion exchange, and chemical neutralization and stabilization which can be applied to wastewater during cleaning.

#### **MANAGEMENT OF GASEOUS WASTES**

The gaseous wastes are generated in to environment mainly due to anthropogenic activities. The gaseous wastes include carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>), chlorofluorocarbon (CFC), oxides of nitrogen (NO<sub>x</sub>), carbon monoxide (CO), oxides of sulphur (SO<sub>x</sub>) etc. These gaseous wastes can cause serious environmental hazards. Therefore, it is highly essential to take appropriate steps for the proper management and control of gaseous wastes in the environment.

Some important control measures are described below

- (i) The gaseous pollutant like SO<sub>2</sub>, H<sub>2</sub>S, HCl, Cl<sub>2</sub>, NH<sub>3</sub>, etc. Can be removed by absorption in (using appropriate liquid) wet scrubbers.
- (ii) The use of smokeless, solar cookers and biogas can reduce the production of smoke.
- (iii) The industries should use precipitators, scrubbers and filters to check production of particular matter.
- (iv) The emission of hydrocarbons from vehicles can be checked by the use of unleaded petrol.
- (v) There should be large scale of plantation which will reduce CO<sub>2</sub> level and increase O<sub>2</sub> level of atmosphere.
- (vi) There should be large chimneys in industries.
- (vii) The automobile emission can be controlled by:
  - (a) Control of exhaust emission,
  - (b) Control of evaporation emission,
  - (c) Control of crank case emission,
  - (d) Using engine alternative to gasoline engine,
  - (e) Use of CNG instead of diesel.
- (viii) Air cleaning devices like gravity settlers, cyclone separators, wet collectors, electrostatic precipitators etc. should be used for the cleaning of air before their discharge into atmosphere.
- (ix) Public awareness should be created regarding hazards of air pollutant accumulation in environment.
- (x) Adequate legislation (Air act) should compel people to control air pollution. Severe punishment should be specified for the defaulters.

#### **CONCLUSION**

Waste management involves a process whereby wastes are collected, transported and disposed of in the best possible way of limiting or eliminating the harmful effect of wastes. This aspect of environmental management is as important as other public amenities or infrastructures without which the life of contemporary man would be extremely difficult. This is because studies have shown a direct link between air, water and land pollution and diseases such as lung cancer, heart disease, cholera and hepatitis. In addition, climate change and eutrophication are a direct result of water and air pollution. Little wonder why there is a huge disparity in the life expectancy of people in developed and developing countries.

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## ABSTRACT

As the world is at present confronting tremendous issues concerning the atmosphere, energy, and the environment, catalysis innovations have all the earmarks of being getting critical to energy, synthesis process, and environmental areas. In the recent years, transformation of the research on catalytic activities and advanced catalyst was seen with the advancement of nanotechnology. Undoubtedly, the utilization of nanomaterials in catalysis and, all the more especially, inorganic nanoparticles has pulled in many research attempts over the globe to create imaginative and greener conventions. These nanoparticles can be used as the catalyst or as mediator and can encourage the reactant procedure in new medium such as, water. Besides, attributable to their little size and expanded surface area, nano-catalysts have obviously risen as offering an interesting candidate at the interface among homogeneous and heterogeneous catalysis, taking into consideration an expanded response rate. Furthermore, nanoparticles give extra reactant functionalities because of their interesting inherent properties (e.g., nanomagnetism, photocatalytic activity). Along these lines, in this pursuit for eco- friendly and more affordable catalyst, nano-catalysis is turning into a significant field in science, which is applied broadly in the academics and industrial areas. This brief review principally centered on portraying the major comprehension of nano-catalysis, how remarkable catalytic property and other explicit properties of nanomaterials rely upon its size and structure at the nano level.

**KEYWORDS:** Nanomaterials, Catalysis, Nano catalysis, Nano scale.

## INTRODUCTION

Catalysis assumes a focal job in chemical reaction and lies at the core of incalculable synthetic conventions, from academics at research centres level to the chemical industries level [1, 2]. By utilizing catalytic reagents, one can lessen the temperature of a reaction, diminish reagent-based waste and upgrade the selectivity of a response that conceivably maintains a strategic distance from the undesirable side responses prompting a green innovation [3]. Structuring and creating perfect catalyst is one of the significant ideas of green science. As per the standards of green science, reactant reagents (as particular as could be expected under the circumstances) are better than stoichiometric reagents [4]. Stoichiometric reagents are utilized in abundance and work just a single time while catalytic reagents utilized in modest quantities and can carry out single reaction for multiple times. To work more like nature is the base of the considerable number of standards of green science [5]. Nature unmistakably gave clues to us to carry out

environmentally friendly reactions by utilizing microorganisms and enzymes. Without catalyst, assortment of items for example medications, fine synthetics, polymers, fibers, fuels, paints, oils, and a heap of other worth added items necessary to humans, would not be possible. Catalysis contributes the mechanism by which chemical reactions happen which enables economical formation of pure materials. In this way, by utilizing catalyst fabricating conventions can be made more monetary, green and sustained. Aside from heavy metal ion catalysts which are for the most part not recoverable from framework, soft catalyst materials such as zeolites, phase transformed catalysts are emerged for industrial level applications.

Among three notable catalysis classifications for example homogeneous, heterogeneous and enzymes-based catalysis, enzymes-based catalysis is the most effective and greenest catalysis found in nature. Both the homogeneous and heterogeneous catalysis has its own benefits and negative marks because of which there is critical need of another synergist framework, which should be dynamic like homogeneous catalysis, and should likewise be effectively recoverable like heterogeneous catalysts. Nano-catalyst has joined points of interest of both the homogeneous and heterogeneous catalytic frameworks [6, 7]. Nano catalytic framework permits the quick, specific chemical conversion with higher yield combined with the simplicity of catalyst separation and recuperation. Recuperation of catalyst from the framework is most significant qualities of any catalyst before being adequate for green synthesis processes at industrial level [8, 9]. On account of nano scale, the contact among reactants and catalyst increments significantly (this is near to homogeneous catalysis). Insolubility in the reactive solvents makes the catalyst heterogeneous and thus can be isolated out effectively from the reaction mixture (this is near to heterogeneous catalysis).

### NANOPARTICLES SYNTHESIS

Nanoparticles (NPs) are particles estimated between 1-100 nm. Synthesis of stable nanoparticles estimated between 1-100 nm is the primary assignment of the Nanoscience. Nanoparticles might be integrated by different methods classes in two significant heads like (I) top-down method and (ii) bottom-up method (Fig 1).

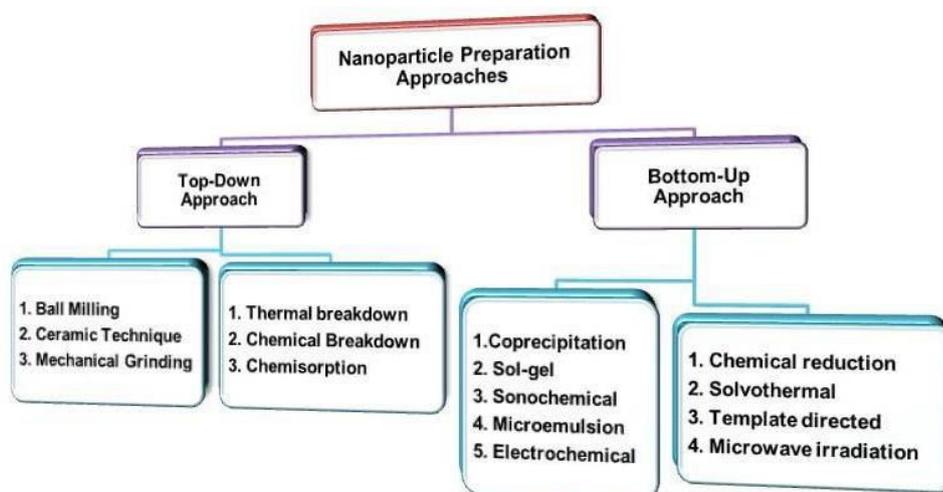
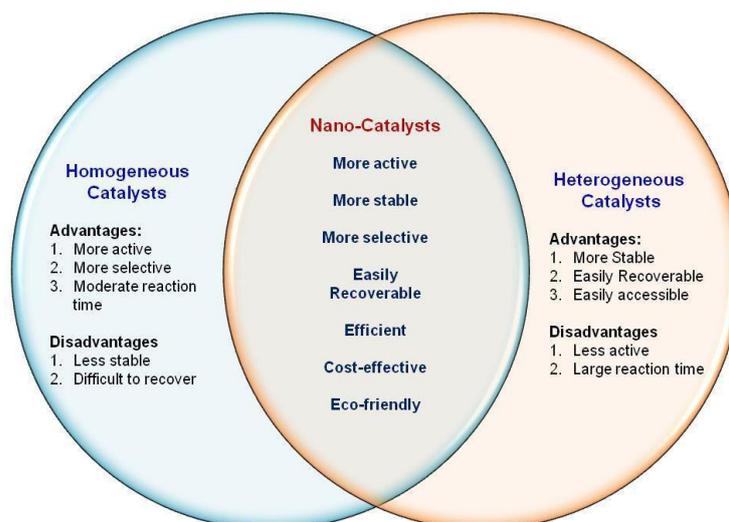


Figure 1: Summary of nanoparticle preparation methods

In literature, various methods were reported for the synthesis of nanoscale materials such as sol-gel [10, 11], sol-gel auto-combustion [12-15], hydrothermal [16-18], micro emulsion [19, 20], chemical coprecipitation [21-23], spray pyrolysis [24, 25] etc. As limitation emerges in its definition about its size, the science of nanoparticles essentially relies upon two things for example (I) union of NPs all around controlled size/shape and (ii) sub-atomic way to deal with discover more specific utilizations of NPs particularly in nano catalysis. Preparation of NPs in all around controlled size is principally made by utilizing diverse stabilizing operators for example ligands, surfactants, polymers, and so forth. Selectivity and reactivity of the NPs has a basic significance as it can impact the course of a reaction which essentially relies upon surface area of NPs. Along these lines, selectivity and reactivity of NPs relies upon essentially two concerns for example (I) control of surface structure and morphology and (ii) control of surface compositions [26, 27]. Recyclability of Nano catalyst carries on like the bottleneck for modern utilization of NPs. Magnetic NPs has risen as a vigorous, exceptionally effective and quick detachment material with numerous points of interest contrasted and product and catalyst isolation by methods in comparison with other physical techniques, for example, fluid extraction, chromatography, refining, filtration or centrifugation [28]. The Nano catalyst immobilized on overly paramagnetic nanomaterials can be effectively isolated from the items because of a solid cooperation between the magnetic nanoparticles and an outer applied magnetic field, and it very well may be effectively again dispersed without the magnetic field because of its nonappearance.

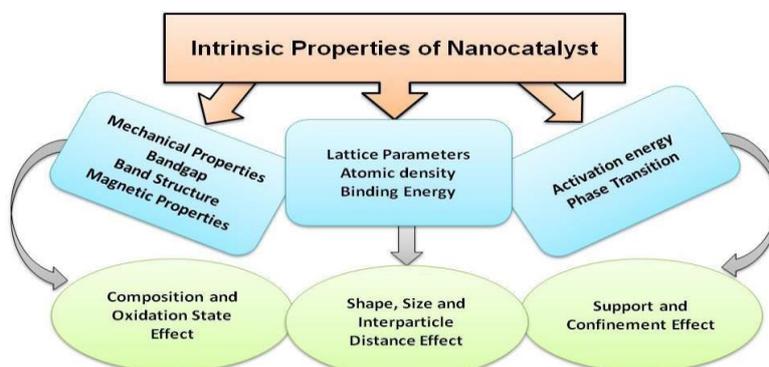
### **NANOCATALYSIS**

Catalysis is one of the pioneer utilizations of nanoparticles. Different components and materials like aluminium, iron, titanium dioxide, and silica all have been utilized as catalyst in nanoscale form in the past decades [29, 30]. In any case, suitable clarification of its gigantic synergist conduct appearing by NPs despite everything has not been completely comprehended. Enormous surface area of nanoparticles has a straight forward beneficial outcome on reaction rate and may likewise be a sensible clarification of its reactant movement. Structure and shape-dependent properties of any materials at its nanoscale size can likewise impact the reactant movement of a material. The calibrating of nano catalyst, as far as synthesis, shape and size has achieved more noteworthy selectivity. In this way the inquest here is the means by which the physical properties of nanoparticles influence their reactant properties, and how manufacture boundaries can thus influence those physical properties [31, 32]. By better comprehension of these, a researcher can design and develop nano catalyst which is exceptionally dynamic, profoundly particular, and exceptionally tough. Every one of these points of interest will empower modern synthetic responses to turn out to be more asset proficient, consume less energy, and produce less waste which helps to counter the ecological effect brought about by our dependence on synthesis process. Nanoparticles are perceived as the most significant modern catalyst and have more extensive application extending from chemical manufacturing to energy transformation and storage applications. Fig. 2 exhibits to fundamental distinction in between homogeneous, heterogeneous and nano catalysts.



**Figure 2: Difference between homogeneous, heterogeneous and nano-catalysts**

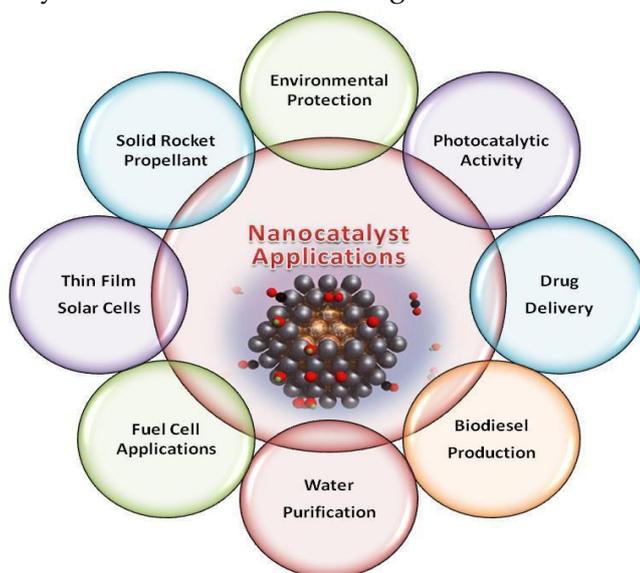
Idea driving the nano catalysis might be comprehended by considering the effect of the characteristic properties of nanomaterials on catalytic reaction as shown in Fig. 3. Characteristic properties of nanomaterials that vitally affect their synergist action might be sorted as (I) amounts that are straightforwardly identified with bond length, for example, the lattice parameter, density, and binding energy; (ii) amounts that rely upon the cohesive energy; (iii) properties that differ with the density of binding energy and (iv) properties from the joint impact of the density of binding energy and atomic cohesive energy.



**Figure 3: Intrinsic properties of Nano catalyst**

Performance of materials or a bunch of atoms shift from that of a confined particle predominantly because of the inclusion of interatomic interface. Alteration of the relative number of the under- composed surface atoms gives an extra opportunity that permits one to tune the properties of a nano- catalyst concerning that of its bulk counterpart. Consequently, contribution from the under-composed atoms and the contribution of interatomic interface can be the beginning stage of consideration to overcome any issues between an isolated atoms and a bulk material in chemical and physical exhibitions. The effect of atomic coordination decrease is huge and it brings together the exhibition of a surface, a nano-catalyst, and an amorphous state reliably as far as bond unwinding and its outcomes on bond vitality. The irregular conduct of a surface and a nano-catalyst has been reliably comprehended and efficiently detailed as elements of atomic coordination decrease and its subordinates (size reliance) on the atomic catching

potential, crystal binding intensities, and electron– phonon coupling. By absolutely controlling the size, shape, spatial dissemination, surface compositions and electronic structure, and thermal and synthetic dependability of the individual nano segments, it very well may be broadly utilized in catalysis with fresher properties and activity. Nanoscale catalysts have been the subject of significant academic and commercial exploration consideration as of late because of the various potential advantages that can accumulate through their utilization. The applications of Nano catalyst are summarized into Fig. 4.



**Figure 4: Applications of Nano catalyst**

## CONCLUSION

Nano catalysis assumes a focal job in both the academia and industry research and developments. Modern effect of nano catalysis is plainly reflected by the expanding number of nano catalysis related research articles, products, technologies and patents. Size and shape-controlled planning of metal nanoparticles are promising for greener heterogeneous synergist responses. Based on better comprehension of size and shape impacts of the nanoparticles and their communications with supportive materials or balancing out operator, today it is extremely encouraging that researchers can comprehend to address environmental, industrial and societal issues. Along these lines, this brief review may give concise information about nano catalysis and furthermore rouse innovative work in this field.

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## ABSTRACT

Mitochondrial gene editing is a promising therapeutic technology in the field of mitochondrial disease. The robustness of this technology will allow for it to be used as a therapeutic technique for a wide variety of diseases caused by mutations to the mitochondrial genome. Many “classic” mitochondrial diseases have been described that arise from single homoplasmic mutations in mitochondrial DNA (mtDNA). These diseases typically affect non-mitotic tissues (brain, retina, muscle), present with variable phenotypes, can appear sporadically, and are untreatable. Evolving evidence implicates mtDNA abnormalities in diseases such as Alzheimer's, Parkinson's, and type II diabetes, but specific causal mutations for these conditions remain to be defined. Understanding the mtDNA genotype–phenotype relationships and developing specific treatment for mtDNA-based diseases is hampered by inability to manipulate the mitochondrial genome. For now mitochondrial gene therapy appears to be only theoretical and speculative. Any possibility for gene replacement is dependent on the development of an efficient mitochondrial transfection vector. In this chapter we describe the current state of the development of mitochondria-specific DNA delivery systems. Further, we outline some unique hurdles that need to be overcome if the development of such delivery systems is to progress.

**KEYWORDS:** Mitochondrial DNA (mtDNA), Mitochondrial Replacement Therapy (MRT), In-vitro Fertilization (IVF), Genetic disorder, Heterologous and autologous mitochondria

## INTRODUCTION

### MITOCHONDRIA: A UBIQUITOUS CELL ORGANELLE

There are typically about 1000 copies of mitochondrial DNA per cell, and the percentage of these that are damaged, or mutated, will determine whether a person will suffer from mitochondrial disease or not. Usually, more than 60% of the mitochondrial DNA molecules in a cell need to be mutated for the disease to emerge, and the more mutated mitochondrial DNA a person has, the more severe their disease will be. Conversely, if the percentage of mutated DNA can be reduced, the disease could potentially be treated. <sup>(1)</sup>

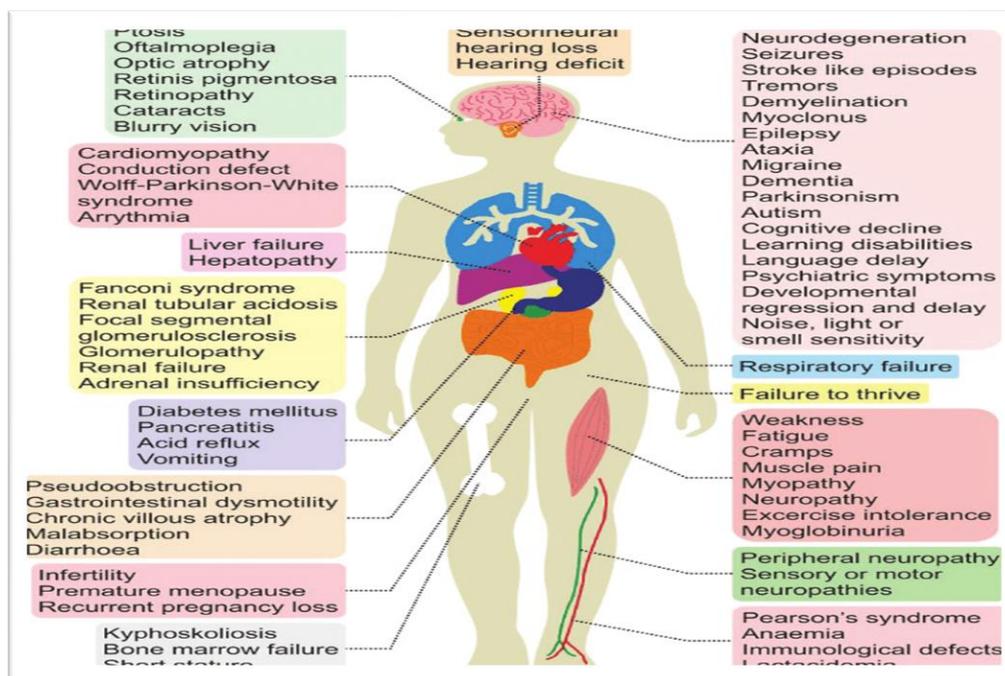
The human mitochondrial DNA (mtDNA) is a circular genome of 16,569 bp that encodes 13 proteins and the RNA components of the machinery for their translation (two ribosomal RNAs and 22 transfer RNAs). Because all 13 proteins are subunits of the enzymes that perform oxidative phosphorylation, loss-of-function mutations in any part of the mtDNA deprive the

cell of most of its ATP-synthesis capacity. Since 1988, many mtDNA mutations have been discovered that cause neural and/or muscular dysfunction. <sup>(1)</sup>

The mitochondria typical of mammalian cells respire O<sub>2</sub> during the process of pyruvate breakdown and ATP synthesis, generating water and carbon dioxide as end products. The Krebs cycle and the electron transport chain in the inner mitochondrial membrane enable the cell to generate about 36 moles (mol) of ATP per mole of glucose, with the help of O<sub>2</sub>- respiring mitochondria. Such typical mitochondria also occur in plants and various groups of unicellular eukaryotes (protists) that, like mammals, are dependent on oxygen and specialized to life in toxic environments. <sup>(2)</sup>

In contrast, the mitochondria of many invertebrates (worms like *Fasciola hepatica* and mollusks like *Mytilus edulis* being well-studied cases) do not use O<sub>2</sub> as the terminal acceptor during prolonged phases of the life cycle. These mitochondria allow the anaerobically growing cell to glean about 5 mol of ATP per mole of glucose, as opposed to about 36 with O<sub>2</sub>. The typical excreted end products are carbon dioxide, acetate, propionate, and succinate, which are generated mostly through the rearrangement of Krebs cycle reactions and the help of the mitochondrial electron transport chain. These organelles are commonly called anaerobic mitochondria. <sup>(2)</sup>

## MITOCHONDRIAL GENETICS



**Figure 1: Clinical symptoms of mitochondrial diseases.** <sup>(3)</sup>

(Mitochondrial disorders can manifest with a variety of clinical symptoms. They vary from very mild ones to very severe, mostly neurological difficulties.)

The genetics of mtDNA has a number of unique features that distinguish it from Mendelian genetics. 1<sup>st</sup> it is believed to be inherited strictly through maternal lineage, 2<sup>nd</sup> the mitochondrial genome is polyploid with multiple copies of mtDNA with each mitochondrion and several thousand per cell. In general all of the mitochondrial genomes within an individual are

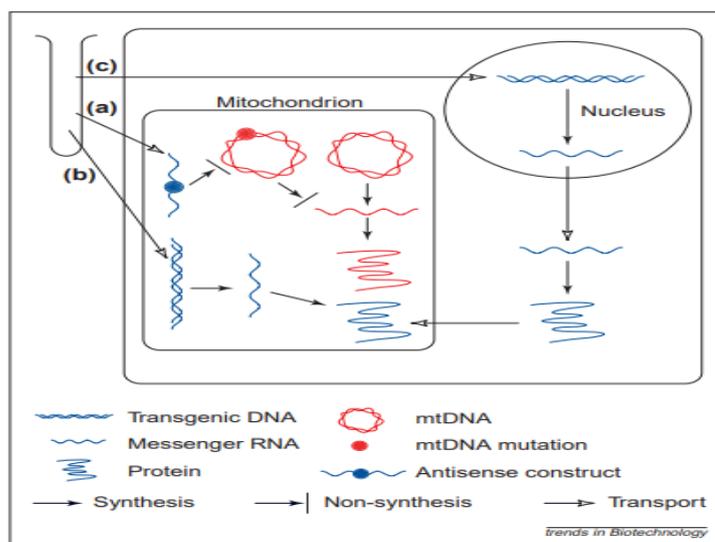
identical, a situation termed homoplasmy. However mutation occurring in one copy of mtDNA can lead to dual population of wild type and mutated DNA coexisting within the same cell a situation termed heteroplasmy. At mitosis, both the DNA (i.e. wild type and mutated DNA) are randomly segregated to each daughter cell, thereby affecting both disease expression and inheritance, further contributing wide range of clinical presentations seen in mtDNA disorders.

### GENERAL STRATEGIES FOR MITOCHONDRIAL GENE THERAPY

Gene therapy of mtDNA mutations also faces a specific difficulty in addition to those that apply to gene therapy of nuclear DNA mutations. Either the engineered DNA must be delivered to a cellular location that traditional vectors do not target (the mitochondrion) or its products must be transported to its natural location in the mitochondrion despite being synthesized in a different compartment (the nucleus in the case of RNAs, the cytosol in the case of proteins).

Three strategies have been advocated for mitochondrial gene therapy, which address this problem in different ways;

- (1) To inhibit the ability of the mutant mtDNA to replicate (Fig.1a);
- (2) To express replacement proteins from transgenic DNA targeted to the mitochondria (Fig.1b);
- and
- (3) To express modified replacement proteins from transgenic DNA targeted to the nucleus (Fig. 1c)



**Figure 2: MITOCHONDRIAL GENE THERAPY** <sup>(4)</sup>

### MITOCHONDRIAL REPLACEMENT THERAPY

Pre-implantation genetic diagnosis (PGD) in assisted human reproduction (AHR) cases is considered as an option for the prevention of mitochondrial disorders. MRT or Mitochondrial Gene Therapy (MGT) is a medical technique where defective mitochondria carried by a woman are replaced with the healthy mitochondria of a donor. MRT has been associated with a number of terms, some of which conveyed positive implications like “Mitochondrial gene therapy”, “Mitochondrial donation”, “Life-saving Treatment”, “Narratives of Hope” while some others made negative impacts like “Three parent baby”, “Three-person baby”, “Three persons DNA”, “Slippery Slope”, “Designer babies”. <sup>(4, 5)</sup>

The truth is that it is the nuclear DNA around which the whole concept of child's genetic identity and personality revolves since, nuclear DNA is the one to make a profound impact on the latter, not the mtDNA. This technique is associated with the category of techniques in which the embryo possessing the nuclear DNA of the parents is subjected to in vitro fertilization (IVF) procedure to have mtDNA of the donor female. MRT include different techniques like spindles transfer, pronuclear transfer or polar body transfer. <sup>(5)</sup>

### DIFFERENT TECHNIQUES

MRT include different techniques like spindles transfer, pronuclear transfer or polar body transfer.

#### ○ PRO NUCLEAR TRANSFER TECHNIQUE

Two zygotes are raised in vitro. One belongs to the biological parents with pronuclei and defective mitochondria and the second one having pronuclei and healthy mitochondria. The pronuclei of biological parents are taken out and transplanted into the donor's zygote (with rejected pronuclei) with healthy mitochondria by using electric pulses. The reformed zygote is transferred to the mother's womb. <sup>(6)</sup>

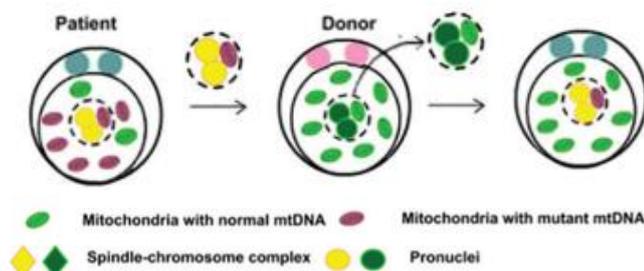


Figure 3: PRO Nuclear Transfer Technique

#### ○ MATERNAL SPINDLE TRANSFER (MST) TECHNIQUE

Maternal spindle transfer technique has been adopted and successfully executed by Dr. John Zhang and his team. The technique executed before fertilization is a form of selective reproduction similar to prenatal diagnosis and pre implantation genetic diagnosis. The maternal spindle complex at the metaphase stage is extracted from the defective egg of the mother, which is then transplanted into the perivitelline space of the enucleated donor's egg with healthy mitochondria. The reformed embryo is transplanted into the mother's womb. This approach is preferable because maternal spindle contains little cytoplasm which eventually reduces the chances of mtDNA carryover and mutations. <sup>(6)</sup>

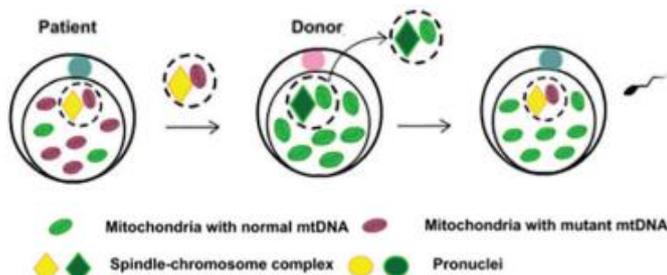
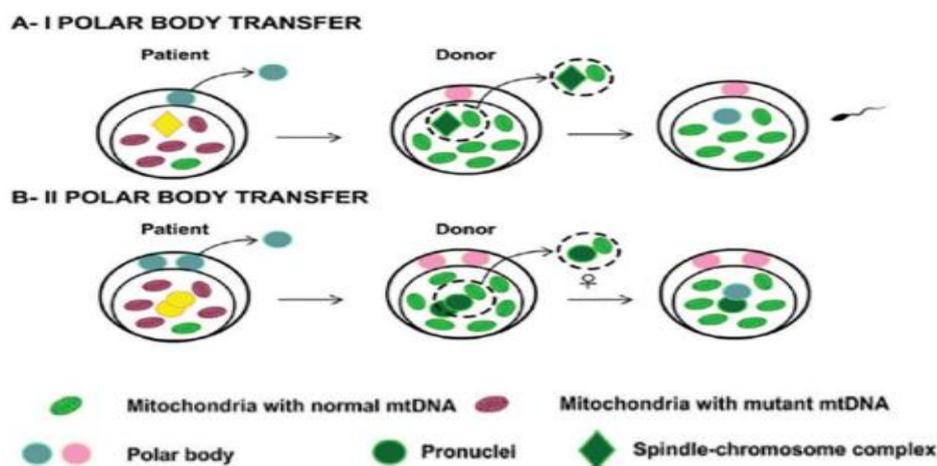


Figure 4: MST Technique

○ **POLAR BODY GENOME TRANSFER (PBT)**

It is considered as the most significant approach because of the presence of the scarce mitochondria with little cytoplasm which minimizes the possibilities of mtDNA carryover. All the nuclear content enclosed within polar bodies increases its potency to confer the re-established oocytes and zygotes by the above two methods. The idea of the usage of the polar bodies was first put forth by Wakayama and Yanagimachi but adopted by Wang and his colleagues to perform the technique in mice where, the transfer of first and the second polar body led to the normal progression of the progenies. (7, 13)



**Figure 5: Polar Body Genome Transfer (PBT)**

**PROS AND CONS OF THE THERAPY**

It would be unfair to females with mitochondrial disease to limit their conceptive range. Apart from biological relations, there is another relation that exists between mother and child, an emotional connection. There are minimal chances of risks correlated with the mixing of prospective mother’s mtDNA and donor mtDNA (8). The proofs about discrepancies are not reported yet. It is feasible to match the haplotypes of the two (9). There are little possibilities of mtDNA alterations and is unlikely to be troublesome. Egg donation can lead to ovarian hyper stimulation syndrome and its genetic kinship cannot be achieved as the whole gamete (nuclear and mtDNA is shared by a third person (10). Prenatal diagnosis is unsuccessful in heteroplasmic populations. Pre-implantation Genetic Diagnosis is appropriate for women with low levels of deficient mtDNA. The drawbacks of these alternatives illustrate the importance of MRT for couples with mitochondrial diseases (11, 12).

**CONCLUSION**

Mitochondria arose once in evolution, and their origin entailed an endosymbiosis accompanied by gene transfers from the endosymbiont to the host. Mitochondrial diseases are currently incurable, although a new IVF technique of mitochondrial transfer gives families affected by mitochondrial disease the chance of having healthy children – removing affected mitochondria from an egg or embryo and replacing them with healthy ones from a donor. Mitochondrial replacement therapy is a promising approach to prevent transmission of mitochondrial

diseases, however, as the vast majority of mitochondrial diseases have no family history, this approach might not actually reduce the proportion of mitochondrial disease in the population. MRTs were initially developed for the treatment of infertility in older women and then improved for the prevention of mitochondrial diseases. Despite reports with positive results, there are still questions about the amount of mtDNA load during the procedure. Therefore more studies must be performed in humans to prove their effectiveness in preventing the transmission of mitochondrial disorders.

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## ABSTRACT

“Cancer”, this name is enough for a patient to get frightened. This is a very deadly disease which is spread globally. The detection of this disease in early stages is a very difficult task. However, the detection in later stages is comparatively easy. After detection of cancer, the treatment of cancer is very expensive as well as full of side effects. Even after full medication, there is no guarantee of the life of the patient. There are many therapies available for the treatment of cancer like chemotherapy, radiation therapy and many more. But there are many side effects of such therapy which make the patients. Nanotech drugs can be proved as an alternative way to treat cancer as the therapy involving these drugs would minimize the side effects as well as increases the survival probability of the patients. But there are many limitations of using such drugs. More clinical trials and characterization of drugs are needed to make such use effective.

**KEYWORDS:** Cancer, Nano materials, Nano tech drugs, clinical trials, tumours.

## INTRODUCTION

Cancer is the second leading cause of death globally, accounting for an estimated 9.6 million deaths, or one in six deaths, in 2018. Lung, prostate, colorectal, stomach and liver cancer are the most common types of cancer in men, while breast, colorectal, lung, cervical and thyroid cancer are the most common among women. Cancer is a disease in which some of the body cells grow without any control. The cancerous cells spread through blood vessels and affect various parts of the body. Generally, the normal cells multiply and do their work. In due course of time the cells grow older and die. This cycle goes on but sometimes the cells grow abnormally and result in the formation of tumours. The tumours may be Nonmalignant (non-cancerous) and malignant (cancerous).

Non Malignant tumours do not spread in the body but the malignant tumours do. Cancer cells replicate rapidly and develop drug resistance quickly. Generally, chemotherapy and radiotherapy are used to cure cancer but such Therapies affect the healthy tissues of the body which results in the development of various side effects.

Thus, a new generation drug called nanotech drug can be used to fight cancer. Nano materials may offer a way to treat cancer without damaging the healthy tissues. This results in minimizing the risk of side effects on the body.

Statistics of Cancer in India-The number of cancer cases in India is estimated to be 13.9 lakh this year and may increase to 15.7 lakh by 2025, with its prevalence being marginally higher among women, according to a report.

According to the report released by the ICMR and the Bengaluru-based National Centre for Disease Informatics and Research, in India, the total number of cancer cases in men is estimated to be 6,79,421 in 2020 and may reach 7,63,575 in 2025.

Among women, the total number of incidence cases is estimated to be 7,12,758 in 2020 and likely to reach 8,06,218 in 2025. Breast cancer (2,38,908) is expected to be the most common site of cancer in 2025, followed by cancer in lung (1,11,328) and mouth (90,060).

According a combined report (2020) of ICMR and NICPR, the cancer cases in India can be summarized as -

- Estimated number of people with cancer: around 2.7 million (2020)
- Every year, new cancer patients registered: 13.9 lakhs
- Cancer-related deaths: 8.5 lakhs
- Risk of developing cancer before the age of 75 years for Indians
- Overall (both sexes included): 1 in 9
- Male: 1 in 68
- Female: 1 in 29
- Total deaths due to cancer in 2020
- Total: 8,51,678
- Men: 4,38,297
- Women: 4,13,381
- Risk of dying from cancer before the age of 75 years among Indians
- Rural males/ females: 1 in 22
- Urban males: 1 in 20
- Urban females: 1 in 24

Cancers of oral cavity, stomach and lungs account for over 25% of cancer deaths in males and cancer of uterine cervix, breast and oral cavity account for 25% cancers in females.

The top five cancers in men and women account for 47.2% of all cancers; these cancers can be prevented, screened for and/or detected early and treated at an early stage. This could significantly reduce the death rate from these cancers.

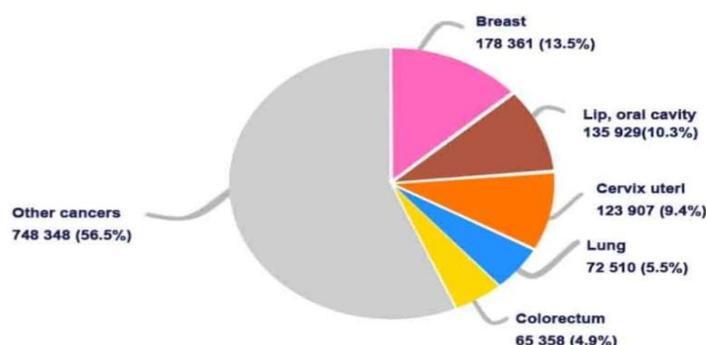


Figure 1: No.of New Cases in 2020

## **NUMBER OF PATIENTS RECEIVING CHEMOTHERAPY AND RADIATION THERAPY**

Treatment modalities comprise of radiation therapy, surgery, chemotherapy, immunotherapy and hormonal therapy. Radiation therapy remains an important component of cancer treatment with approximately 50% of all cancer patients receiving radiation therapy during their course of illness; it contributes towards 40% of curative treatment for cancer.

By 2040, the number of people living with cancer who require chemotherapy will increase significantly worldwide. The burden will be most pronounced in low- or middle-income countries, including India, home to the third largest number of people living with cancer in the world.

This is according to researchers who have conducted a study published today in *The Lancet*. The study surveyed the number of cancer cases which required chemotherapy in 2018, and to what extent such cases are likely to increase by 2040. It estimates that the number of cancer cases necessitating first-line chemotherapy treatment will increase by 53 percent, from 9.8 million in 2018 to fifteen million in 2040. 67 percent of such patients will reside in low- or middle-income countries.

## **SIDE EFFECTS OF CANCER TREATMENTS**

Cancer cells tend to grow fast, and chemo drugs kill fast-growing cells. But because these drugs travel throughout the body, they can affect normal, healthy cells that are fast-growing, too. Damage to healthy cells causes side effects. Side effects are not always as bad as you might expect, but it's normal to worry about this part of cancer treatment.

The normal cells most likely to be damaged by chemo are:

- Blood-forming cells in the bone marrow
- Hair follicles
- Cells in the mouth, digestive tract, and reproductive system
- Some chemo drugs can damage cells in the heart, kidneys, bladder, lungs, and nervous system. Some of the more common side effects caused by chemotherapy:
  - Fatigue
  - Hair loss
  - Easy bruising and bleeding
  - Infection
  - Anaemia (low red blood cell counts)
  - Nausea and vomiting
  - Appetite changes
  - Constipation
  - Diarrhoea
  - Mouth, tongue, and throat problems such as sores and pain with swallowing
  - Peripheral neuropathy or other nerve problems, such as numbness, tingling, and pain
  - Skin and nail changes such as dry skin and color change
  - Urine and bladder changes and kidney problems
  - Weight changes
  - Chemo brain, which can affect concentration and focus

- Mood changes
- Changes in libido and sexual function
- Fertility problems

Nanotech drug as an alternative cancer treatment - Since, the side effects of the drugs used in treatment of cancer as well as the radiations which are used to kill the cancer cells are very harmful, the nanotech drugs can be used as the alternative treatment for cancer. The use of these drugs results in minimizing the side effects of cancer treatment on the body. This can be considered as an achievement in the field of Medical Science.

#### **PRINCIPLE OF NANO DRUG**

During initial stages, the tumours lack their own blood vessels. They obtained Oxygen and glucose from surrounding tissues. Cells at the periphery get more nutrients from nearby cells or tissues. Thus, the peripheral region becomes rich in nourishment but the core starts starving. Due to such starvation, the core makes protein signaling to the cells. Such proteins stimulate the growth of blood vessels in the starving core of cancerous cells.

The process of development of blood vessels in the tumours is called angiogenesis. Angiogenic blood vessels supply tumours with nutrients. Because of their rapid growth, they are irregular and leaky, having more gaps in between them. Nanomaterials now play an important role as they can be incorporated in the gaps of these cells. Generally the nanoparticles of 10 to 300 nm diameter are right in size to fit onto the gaps of blood vessels. These particles get impregnated with the chemotherapeutic drugs. Thus, cancer cells could be killed without affecting the healthy cells.

Many nano medicines are under process for clinical development. Such Nano medicines could become very good technological tools which could help to destroy the tumours. Once the particles enter the tumour clues, the near IR laser could be used to destroy the cells thermally; the surroundings could also be easily destroyed. Auroshells is currently being tested in a Phase - I clinical trial for head and neck cancer.

#### **CHALLENGES**

There are many limitations of using Nano medicines for chemotherapy. The control of physicochemical properties is the major obstacle in using nano medicine. The nano medicines must be characterized in many ways before clinical trials. The characterization includes measurement of shape and size, surface chemistry and state aggregation but at the same time the characterization of nanoparticles is very difficult. It is very important to characterize the nanoparticles by using multiple methods such as electron microscopy and light scattering for size to gain detailed understanding. In order to help the cancer treatments and for cancer trials, the National cancer Institute makes use of Nanotechnology characterization Laboratory which helps the nano tech cancer treatments. The main task of NCL is to perform physicochemical characterization to test the safety of Nano material and also test the toxicity. Such tests are conducted on the animals.

#### **CONCLUSION**

The treatment of cancer is expensive and results in many side effects. But due to lack of alternative medications, the patients are compelled to go with the available medication. Thus

there is a need to search for such medication which may be cost effective and results in fewer side effects. Nano tech drugs could provide a way to get such advances in the field of Medical Sciences. Before making the entry of such drugs in the market more clinical trials are required. There must be proper characterization of such drugs for these more clinical labs must be set up to come up with proper results. There is a future of nano tech drug as an alternate of cancer treatment but for this more technological advancement is needed.

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## ABSTRACT

Plants are important for life requirements for every living being, like food, fibre, shelter and other medicinal uses. Awareness of medicinal plants utility increased as the consequence of many years of fights against illnesses. Because of which humans learned to find natural remedies in leaves, barks, seeds, fruit bodies, roots, flowers and other parts of the plants. People who live in rural areas widely use plants as medicine due to their nutritive status, healing capacity and easy availability. The basic nutrients that are present in plants are carbon, hydrogen, and oxygen. Medicinal plant contains various bio active components like minerals and phytochemicals. The phytonutrients which are present in plants are the reason for their physiological activity and their ability to cure human diseases. Lately, excessive attention in the phytochemical drugs and traditional remedies are specified worldwide and there has been an improvement in the scientific research in this field.

**KEYWORDS:** medicinal plants, nutritive status, pharmacological activity, traditional remedies.

## INTRODUCTION

In ancient periods, to cure the illness, people searched for drugs in nature, so the specific medicinal plants were discovered as the plants are the most vital sources of medicines. So far 2, 50,000 plant species reported in the world, more than 80,000 species have medicinal properties and are utilized as medicine. Medicinal plants are most important source for traditional medicine and herbal products. Most of the people depend on the natural plant medicine for their pharmacological values. Due to their medicinal property, they are used in pharmacological industries.

In India, around 3000 plant species are identified to possess medicinal character and are being utilized in our traditional systems of medicines, namely Ayurveda, Yunani, Siddha, Homeopathy etc. The plant-based drugs are derived from the whole plant or from different plant parts like leaves, stem, bark, root, flower, seed, etc., as they have numerous nutritive values. The dropping efficiency of synthetic drugs and the growing complications on their usage gives the natural drugs a significant focus. The usage of medicinal plants for the control of human illnesses is most prominent, as the plants are normally readily obtainable and no farming cost is needed.

Medicinal plants contain metabolites and nutrients that are possible sources of drugs. The nutritive status of medicinal plants is vital as they act as a significant constituent for human

energy. Plants can synthesis nutrients from carbon dioxide, water and solar energy. Plants absorb other inorganic salts through roots from soil. Nutrients found in medicinal plants are classified into two types (i) inorganic nutrients (ii) organic nutrients.

(i) **Inorganic nutrients** - Inorganic nutrients are important growth of higher plants. They include elements of potassium, chlorine, sulphur, nitrogen, manganese, copper, phosphorus, zinc, iron, calcium etc.

(ii) **Organic nutrients** – Organic nutrients are the building blocks of plant cells. They are carbohydrates, protein, proteins, fats, and vitamins.

These nutrients have most significant role in human body, so they are used in various metabolic and physiological activities. Natural plant-based nutrients are used in nutritional supplements, medications and health care products. As the medicinal plants have confirmed to contain essential nutrients, they can prevent various diseases such as heart disease, cancer, diabetes and ulcers etc. some of the important and easily available medicinal plants, their nutritive status and their therapeutic uses are discussed below.

#### NEEM (*AZADIRACHTA INDICA*)

*Azadirachta indica*, normally known as neem or Indian lilac, belongs to the family Meliaceae. It is native to the Indian subcontinent and most of the countries in Africa. It is naturally grown in hot and semi-tropical counties. Neem trees also grow on islands in southern Iran. It is the greatest beneficial traditional medicinal plant in India. Every part of the neem tree has medicinal property and is therefore commercially utilizable. Through the last five decades, apart from the chemistry of the neem compounds, significant progress has been achieved concerning the biological activity and medicinal applications of neem. Its fruits and seeds are the source of neem oil. All parts of neem leaves, flowers, fruits, seeds, roots, barks are used for the treatment of inflammation, fever, skin diseases, and dental disorders. The leaf is also used for birth control and to cause abortions.

**Table 1: Nutritive Values of Neem (*Azadirachta indica*)**

Nutritive value	percentage
Nitrogen	2-3%
Phosphorus	1%
Potassium	1.4%
Tannic acid	1.0-1.5%
Sulphur	1.07 – 1.36%
Protein	7.1%
Carbohydrate	22.9%
Vitamin A	19.6%
Vitamin B1	3.7%
Vitamin B2	3.52%
Vitamin C	3.15%



**Figure 1: Neem (*Azadirachta indica*)**

## HEALTH BENEFITS

- This may promote oral health by antiseptic, anti-inflammatory, Antioxidant, and immune-boosting properties.
- It also relieves pain and treats gingivitis, tooth decay and periodontitis.
- It contains chemicals which help to reduce sugar levels, prevent pregnancy, heal ulcers in digestive tract, kill bacteria, and prevent plaque.

### **ALOE VERA (ALOE BARBADENSIS MILLER)**

The botanical name of *Aloe vera* is *Aloe barbadensis miller*. It fit in to Asphodelaceae (Liliaceae) family. It is a shrubby or arboreous, perennial, xerophytic, succulent, pea green color plant. It grows primarily in the dry regions of Africa, Asia, Europe and America. Aloe is a cactus-like plant that cultivates in hot, dry climates. The plant is the basis of two products, gel and latex, which are gotten from its fleshy leaves. *Aloe vera* products contain several elements with potential biological and toxicological activities. Absorption of *Aloe vera* is associated with diarrhea, electrolyte imbalance, kidney dysfunction, and conventional drug interactions, episodes of contact dermatitis, erythema, and phototoxicity have been reported from topical applications. In aloe vera, polyphenols, along with some other compounds support to inhibit the growth of certain bacteria that causes infections in humans. It is known for antibacterial, antiviral, and antiseptic characters. It is helpful to heal wounds and treat skin problems.

**Table 2: Nutritive Values of *Aloe vera* (*Aloe barbadensis miller*)**

Nutritive value	Percentage
Fiber	18.5%
Protein	4.8%
Fat	2.2%
Total ash	14.4%
Carbohydrate	48.0%
Energy	231 kcal
Iron	64.8 mg/100 g
Ascorbic acid	27.0 mg/100 g



**Figure 2: *Aloe vera* (*Aloe barbadensis miller*)**

## HEALTH BENEFITS

- Used for Burns and to Heal Wounds.
- Cures Intestinal Problems.
- Reduces Arthritic Swelling.
- Heals Psoriasis Lesions.
- Used for Gum Infections.
- Heals Eye irritations.
- Used against Strains and Lung congestion.

**HOLY BASIL (*OCIMUM TENUIFLORUM*)**

*Ocimum tenuiflorum*, frequently known as holy basil, tulsi or tulasi, is an aromatic perennial plant in the family Lamiaceae. It is inborn to the Indian subcontinent and extensively cultivated plant through the Southeast Asian tropics. Tulsi is cultivated for religious and traditional medicine purposes, and also for its essential oil. It is widely used as an herbal tea, commonly used in Ayurveda, and has a place in the Vaishnava tradition of Hinduism, in which devotees perform worship including holy basil plants or leaves. Many in vitro, animal and human studies attest to tulsi having multiple therapeutic activities together with adaptogenic, antimicrobial, anti-inflammatory, cardio protective, and immunomodulatory effects.

**Table 3: Nutritive Values of Holy Basil (*Ocimum tenuiflorum*)**

Nutritive value	Percentage
Potassium	8%
Iron	17%
Magnesium	16%
Calcium	17%
Dietary fibre	6%
Protein	6%
Vitamin C	30%
Vitamin B6	10%



**Figure 3: Holy Basil (*Ocimum tenuiflorum*)**

**HEALTH BENEFITS**

- Tulsi has also been shown to counter metabolic stress through normalization of blood glucose, blood pressure and lipid levels,
- It relieves psychological stress through positive effects on memory, cognitive function through its anxiolytic and anti-depressant properties.
- Protection against infection and treating wounds, improves digestion system, Aids in losing weight, dissolving kidney stones, helps fight diabetes, dental and oral health, skin and hair benefits.
- It promotes blood circulation.
- Used for the treatment of asthma.
- Eases joint pain and lowers cholesterol.
- It decreases fertility.

**STONE BREAKER (*PHYLLANTHUS NIRURI*)**

*Phyllanthus niruri* (keezhanelli) is a widespread tropical plant usually found in coastal parts, known by the common name's gale of the wind, stonebreaker or seed-under-leaf. It is in the genus *Phyllanthus* of the family *Phyllanthaceae*. Extracts of this herb have been confirmed to have therapeutic effects in many clinical studies. Some of the most fascinating therapeutic properties include anti-hepatotoxic, anti-lithic, anti-hypertensive, anti-HIV and anti-hepatitis B. Therefore, studies relating to chemical properties and structural properties of the bioactive

phytochemicals found in *P. niruri* are very beneficial for additional research on this plant as many phytochemicals have shown preclinical therapeutic abilities for an extensive range of human diseases, including HIV/AIDS and hepatitis B.

**Table 4: Nutritive Values of Stone Breaker (*Phyllanthus niruri*)**

Nutritive value	Percentage
Phenolic	11.85mgGAE/100g
Oxalate	22.69mg/g
Phytate	5.40g/100g
Alkaloid	3.67g/100g
Tannin	0.001g/100g
Saponin	0.20g/100g
Flavonoid	1.40g/100g
Fibre	16.90/100g
Protein	14.7g/100g
Calcium	29.11g/100g
Phosphorous	69.78g/100g



**Figure 4: Stone Breaker (*Phyllanthus niruri*)**

#### HEALTH BENEFITS

- Used for jaundice, gonorrhoea and frequent menstruation.
- Used for diabetes and used as a poultice for skin ulcers, sores, swelling, and itchiness.
- Leaves extract improves kidney functions, ameliorates kidney oxidative stress, inflammation, fibrosis and apoptosis.

#### LAKSHMI TARU (*SIMAROUBA GLAUCA*)

*Simarouba glauca* normally known in India as Lakshmi Taru. *Simarouba glauca* is a flowering tree which belongs to Simaroubaceae that is inborn to Florida South America, and the Caribbean. Common names include paradise-tree, dysentery-bark, and bitter wood. The tree is well suited for warm, humid, tropical regions. The extracts from parts of the tree have been claimed to possess potent anticancer properties.

**Table 5: Nutritive Values of Lakshmi Taru (*Simarouba glauca*)**

Nutrients present	Percentage
Protein	47.7g/100g
Saponins	3.7g/100g
Alkaloids	1.01g/100g
Copper	1.43g/100g
Zinc	4.13g/100g
Iron	11.1g/100g
Phosphorus	31.1g/100g
Magnesium	3.45g/100g



**Figure 5: Lakshmi Taru (*Simarouba glauca*)**

## HEALTH BENEFITS

- The leaves and stem of Lakshmi Taru plant are widely used to treat cancer. Consuming the parts of this plant is known to boost the immunity.
- The leaves bring down the effects of chemotherapy in cancer patients.
- It has anti-tumorous, anti-bacterial properties.
- It improves mitochondrial metabolism and hence improves energy levels.
- The leaves and barks are used as digestive aid.

## CASTOR PLANT (*RICINUS COMMUNIS*)

*Ricinus communis* commonly known amanakku is a flowering plant which belongs to Euphorbiaceae. The plant's native range is Afghanistan, the Indian subcontinents Bangladesh, India, Pakistan, Nepal and Sri Lanka, Laos, Myanmar and Vietnam. These castor seeds are the vital source of castor oil. India has highest production of castor plant. The castor oil plant is used as feedstock and medicine from ancient times.

**Table 6: Nutritive Values of Castor Plant (*Ricinus communis*)**

Nutrients present	Percentage
Protein	21-48 g/100g
Soluble carbohydrate	9.1-20.5g/100g
Fat	2.5-24.5g/100g
Carbohydrates	61.04%
Fiber	6.62%
Alkaloids	7.40mg/100g
Saponins	6.69mg/100g
Calcium	1.06-5.67g/100g
Phosphorous	0.3-0.73g/100g
Magnesium	7.16mg/100g



**Figure 6: Castor Plant (*Ricinus communis*)**

## HEALTH BENEFITS

- The castor leaves have important usage to start labor during pregnancy.
- They act as a stimulant for flow of breast milk.
- They are used for constipation.
- Castor oil pastes are used to cure burns and inflammation of skin.
- Castor plant has anti-inflammatory, anti-oxidant and anti-microbial properties.
- The roots of castor plants are used for the treatment of fever, arthritis, jaundice and liver related problems.
- It stimulates digestion.

## PUNCTURE VINE (*TRIBULUS TERRESTRIS*)

*Tribulus terrestris* commonly known as puncture vine. It is called as 'sirunerinjimul' in Tamil. *Tribulus terrestris* is an herb belonging to the Zygophyllaceae family, which is home-grown in the Southern Europe, Southern Asia, Australia, and Africa. It is adapted to thrive in dry

locations in which few other plants can survive. It is native to warm temperate and tropical regions. The leaves and shoots are eaten in East Asia.

**Table 7: Nutritive Values of Puncture Vine (*Tribulus terrestris*)**

Nutrients present	Percentage
Protein	12.1g/100g
Fibre	27.5g/100g
Carbohydrate	68.6g/100g
Calcium	4.21g/100g
Phosphorous	0.245g/100g
Saponin	0.06g/100mg
Glycosides	11.7g/100mg
Flavonoids	3.63g/100mg



**Figure 7: Puncture Vine (*Tribulus terrestris*)**

#### HEALTH BENEFITS

- It is used in traditional medicine for chest pain, heart problems, dizziness, and skin and eye disorders.
- Used to expel kidney stones as diuretic and tonic.
- It enhances the immune system.
- Helps to build muscles and increase stamina and endurance.
- It has anti-fungal, anti-bacterial, and anti-inflammatory actions.
- It is useful as a general tonic and revitalizer for the liver, kidneys and urinary tract.

#### MALABAR NUT (*JUSTICIA ADHATODA*)

*Justicia adhatoda* generally known as Malabar nut, adulsa, adhatoda and is native to Asia. The plant's native range is Afghanistan, the Indian sub-continent (Bangladesh India, Pakistan, Nepal and Sri Lanka), Laos, Myanmar and Vietnam. It belongs to Acanthaceae family. The leaves, roots, flowers, and bark of this plant have been used in the treatments of cough, colds, asthma, to liquefy sputum, as a bronchodilator, bronchial catarrh, bronchitis, and tuberculosis. A number of parts of the plant are commonly used in the forms of decoctions or powders.

**Table 8: Nutritive Values of Malabar nut (*Justicia adhatoda*)**

Nutrient present	percentage
Fibre	4.9g/100g
Carbohydrate	16.1mg/g
Proteins	7.8mg/100g
Phenols	32.1mg/g
Flavonoids	37.9mg/g
Fat	5.6g/100g
Potassium	2.3g/100g
Sodium	0.26g/100g
Amino acid	14.3g/100g



**Figure 8: Malabar nut (*Justicia adhatoda*)**

### HEALTH BENEFITS

- Adathoda herbs hold a vital position in treating asthmatic conditions- Cough, cold and asthma.
- Proteins present in adathoda plays a key role in body development, hormone formation, maintaining fluid balance, enzyme formation, and development of immunity.

### CURRY LEAVES (*MURRAYA KOENIGII*)

Curry leaves (*Murraya koenigii*) are the most common herb used in Asia with vast nutritive and pharmacological profits belonging to the family Rutaceae. Their phytochemical constituents play an important role in disease management through the managing of many metabolic pathways. *M. koenigii* is a captivating house plant grown in Asia and native to Sri Lanka, Bangladesh, and India, which has been in use centuries. The dark green fresh leaflets of *M. koenigii* are broadly used in cooking primarily for their fragrance and useful medicinal properties.

**Table 9: Nutritive Values of Curry Leaves**  
(*Murraya koenigii*)

Nutritive value	percentage
Protein	6.1%
Carbohydrate	18.7%
Vitamin A	0.50%
Fibre	6.4%
Mineral matter	4.2%
Calcium	830 mg/100 g
Phosphorus	57 mg/100 g
Iron	7.0 mg/100 g
Nicotinic acid	2.3 mg/100 g



**Figure 9: Curry Leaves (*Murraya koenigii*)**

### HEALTH BENEFITS

- Various parts of *M. koenigii* are used to treat diabetes, chronic fever, dysentery, and diarrhea.
- The bark and the roots are used as a stimulant by the physicians.
- They are also used externally to cure eruptions and the bites of poisonous animals.
- Curry leaves are also used in calcium deficiency.
- It has Vitamin A, Vitamin B, Vitamin C, Vitamin B2 and calcium in plenty.
- Leafy vegetable shows various notable pharmacological activities such as activity on heart, Anti diabetic and cholesterol reducing property, antimicrobial activity, antiulcer activity, antioxidative property, cytotoxic activity, antidiarrhea activity, phagocytic activity.

### MINT (*MENTHA*)

Mint (*mentha*) is a popular natural herb which is used as remedy in many different health sicknesses. They belong to the family Lamiaceae. Mint plant originates from Europe, Asia and Australia. Nowadays they are spread worldwide. Mints have the utility as natural flavouring agent for foods, and their essential oil is used as natural medicine and perfumes.

**Table 10: Nutritive Values of Mint (*Mentha*)**

Nutritive value	percentage
Carbohydrate	15 g
Dietary fiber	8 g
Potassium	569 mg
Fat	0.9 g
Protein	3.8 g
Vitamin C	52%
Vitamin A	12%
Iron	28%
Magnesium	20%
Calcium	24%



**Figure 10: Mint (*Mentha*)**

### HEALTH BENEFITS

- The anti-oxidants and phytonutrients present in mint leaves have potential anti-bacterial properties.
- The iron, potassium and manganese present in mint leaves increases hemoglobin levels and encourages brain function.
- As the mint leaves has minimum amount of fat content, it is used for weight loss diet.
- Mints increase the digestion of foods; it has antiseptic property.
- Mint leaves are useful remedy for stuffed nose and cough.

### CONCLUSION

Medicinal plants consist of many important nutrients such as protein, fiber, calcium, phosphorous, flavonoids etc. These are very essential for human body. These nutrients help to improve human health, and it is also widely used to treat many diseases like high blood pressure, fertility, cancer, malaria etc. Most of the medicinal plants possess anticancer, antifungal, antiviral, antimalarial properties. Naturally occurring medicinal plants have no side effect. Certain medicinal herbs have disinfectant property, which destroys disease causing germs. They also inhibit the growth of pathogenic microbes that cause communicable diseases. All medicinal herbs and plants are playing a vital role in treating diseases without any side effects.

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# RECENT TRENDS OF INNOVATIONS IN CHEMICAL AND BIOLOGICAL SCIENCES

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