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RESEARCH ARTICLE

Magneto-hydrodynamic Thin-Film Nano Fluid Flow for a Non-Uniform Heat Source by using HAM

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ABSTRACT

In the current paper, based on the method of Homotopy analysis, a series solution for an unsteady BVP is obtained. The Homotopy investigation accelerates towards a guarantee of the convergence of series solution of any strong nonlinearity problem. Because of the vast range of industrial applications, in the current paper with the flow property, we have examined the effects of the parameters like magnetic field, space-dependent (A^*) and temperature-dependent (B^*) over a stretching sheet and presented the same graphically. In future, it will observe that the numerical simulations

Keywords: Stretching surface, Non-uniform heat source/sink, Magneto hydrodynamics, Nanofluid, Homotopy analysis method.

INTRODUCTION

More recently, the study of Nanofluid flow problems has received large interest among the researchers due to its extensive sort of applications in diverse field technological disciplines such as in heat exchanges, fluidization of the devices, foodstuff striating, drawing of an elastic sheet, coating of solid surfaces and in polymers and metal extraction processes. Wang [1, 2] elaborated about the hydro-dynamical essence for a flux's thin liquid film over an unstable stretching sheet analytically. The problem was subsequently expanded by Anderson et al. [3, 4]. In view of wide range of industrial applications, further investigation of the liquid film is considered to be necessary. For this reason, various investigators[5, 6] have made tremendous efforts for constructing geometries from time to time. Further, the classical problem proposed by Wang [1, 2] and of the very same Anderson et.al [3,4] some interesting





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phenomena are considered by several authors [7-22, 46-47]. The investigations of heat transfer within Nanofluid have paid much attention among researchers for a wide range is industrial applications. There are many methods through which base fluid properties will improve. Thus, Nanofluid is more constructive in various processes of pharmaceutical, hybrid-powered engines, fuel cells, and microelectronic chip cooling. Choi *et al.* [23,24] investigated the nanoparticles volume < 1%, the thermal conductivity properties increases approximately up to two times. Furthermore, the authors [25-31] have presented the heat transport phenomenon of thin-film Nanofluid. In recent years, researchers [32-39] have shown keen interest in the convergence of thin-film flow problems by homotopy analysis method (HAM) ([40-42].The outcomes obtained by HAM are more competent than the existing results for the BVPs. In the current investigations, for initial guesses, fast convergence, consistency, reliability, and accuracy in the result, the homotopy analysis technique is implemented and discussed with the aid graphs. The contributions of the arising physical variables such as Nusselt number, skin friction, magnetic field as well as non-uniform sink/heat source are discussed. The result reveals that there is an increase in thickness of thermal boundary layers in heat generation and absorption presence which is very essential in the heat controlling processes.

Formulation of the mathematical problem

The continuity, momentum, and energy equations, for unsteady 2-D flow, are

$$\frac{\partial u}{\partial x} + \frac{\partial v}{\partial y} = 0 \tag{1}$$

$$\frac{\partial u}{\partial t} + u \frac{\partial u}{\partial x} + v \frac{\partial u}{\partial y} = \frac{\mu_{nf}}{\rho_{nf}} \frac{\partial^2 u}{\partial y^2} - \sigma \frac{B^2 u}{\rho}$$
 (2)

$$\frac{\partial T}{\partial t} + u \frac{\partial T}{\partial x} + v \frac{\partial T}{\partial y} = \alpha_{nf} \frac{\partial^2 T}{\partial y^2} + \frac{q^{"}}{\rho C_{n}}$$
(3)

where

$$q'' = \frac{ku_w(x)}{m} [A^*(T_S - T_0)f' + (T - T_0)B^*]$$
 (4)

 A^* and B^* greater than zero corresponds to internal heat generation whereas A^* and B^* less than zero corresponds to internal heat absorption. The velocity components which are parallel and perpendicular to the horizontal axis are respectively represented by u and v. Here we have to make a note that, there is no liquid movement for $t \le 0$. For solving equations (1) – (3), the proper conditions are

$$u = 0, v = 0, T = T_{w}$$
 for $t \le 0$

 $(5) \rightarrow Initial conditions$

For t > 0 and $x \ge 0$, boundary conditions are as below:

$$u = U, v = 0, T = T_w \text{ at } y = 0$$
 (6)

$$\frac{\partial u}{\partial y} = \frac{\partial T}{\partial y} = 0, \quad \frac{\partial h}{\partial t} = v \quad at \quad y = h \tag{7}$$

Here we have to make a note that at the adiabatic free surface, the heat flux and viscous shear stress disappear. Let us introduce the following appropriate similarity transformations





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$$\psi(x, y, t) = \left(\frac{\upsilon_f b}{1 - \alpha t}\right)^{\frac{1}{2}} x f(\eta)$$
 (8)

$$T(x, y, t) = T_0 - T_{ref} \left[\frac{bx^2}{2\nu_f} \right] (1 - \alpha t)^{-\frac{1}{2}} \theta(\eta)$$
 (9)

$$\eta = \left\lceil \frac{b}{\upsilon_f \left(1 - \alpha t \right)} \right\rceil^{\frac{1}{2}} y \tag{10}$$

Here, $\psi(x,y,t)$ is a stream function having u and v as velocity components, which are defined as

$$u = \frac{\partial \psi}{\partial y} = \left(\frac{bx}{1 - \alpha t}\right) f'(\eta) \tag{11}$$

$$v = -\frac{\partial \psi}{\partial x} = -\left(\frac{v_f b}{1 - \alpha t}\right)^{\frac{1}{2}} f(\eta) \tag{12}$$

Here the derivative is w.r.t. η , at the γ -dimensionless film thickness is obtained by using equation (10),

$$\gamma = h(1 - \alpha t)^{-\frac{1}{2}} \left(\frac{b}{v_f}\right)^{\frac{1}{2}},\tag{13}$$

which is given by

$$\frac{dh}{dt} = -(1 - \alpha t)^{-\frac{1}{2}} \left(\frac{v_f}{h}\right)^{\frac{1}{2}} \frac{\alpha \gamma}{2} \tag{14}$$

With the help of similarity transformation (see eq. (8) - (10)), the following differential equations which are ordinary in nature are derived from the governing equations (2)–(3):

$$f''' + \phi_1 \gamma \left(f f'' - \frac{S}{2} \eta f'' - (f')^2 - (S + M) f' \right) = 0$$
 (15)

$$\theta'' + \phi_2 \left(\frac{k_f}{k_{nf}} \right) \gamma \Pr \left\{ \left(f \theta' - \frac{S}{2} \eta \theta' \right) + \left(A^* f' + B^* \theta \right) \right\} = 0$$
 (16)

Subject to

$$f(0) = 0, \quad f'(0) = \theta(0) = 1$$
 (17)

$$f''(\beta) = \theta'(\beta) = 0 \tag{18}$$

$$f(\beta) = \frac{S\beta}{2} \tag{19}$$

Here $Pr = k_f / (\mu C_p)_{nf}$ stand for the Prandtl number and $S = \alpha/b$ stand for the unsteadiness parameter of the base liquids, γ , is the film thickness and ϕ_1 , and ϕ_2 , are volume fractions and defined as below





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$$\phi_{1} = (1 - \phi)^{5/2} \left[(1 - \phi) + \phi \left(\frac{\rho_{s}}{\rho_{f}} \right) \right],$$

$$\phi_{2} = (1 - \phi) \phi \left\{ \frac{(\rho C_{p})_{s}}{(\rho C_{p})_{f}} \right\}$$

The Nussult number Nu_x characterized heat transfer rate and skin friction coefficients C_f characterized for the surface drag. Below expressions are given for $\,q_w$ – surface heat flux and τ_w – shear stress

$$\tau_{w} = -\mu_{nf} \left[\frac{\partial u}{\partial y} \right]_{y=0}$$

$$q_{w} = -k_{nf} \left[\frac{\partial T}{\partial y} \right]_{y=0}$$

$$C_{f} = \frac{\tau_{w}}{\frac{1}{2} \rho U^{2}} = \frac{2}{\left(1 - \phi\right)^{5/2}} \operatorname{Re}^{-1/2} \left\{ -f''(0) \right\}$$
(20)

$$Nu_x = \frac{xq_w}{k_f(T_w - T_0)} = \left(\frac{k_f}{k_{nf}}\right) \operatorname{Re}^{-1/2} \left\{-\theta'(0)\right\}$$
 (21)

HAM Solution

To find the homotopy analysis solution of Eqns. (15) to (16) with the appropriate BCs (Ref. (17)-(19)), we pick initial guess approximations as

$$f_0(\eta) = 1 - e^{-\eta} \tag{22}$$

$$\theta_0(\eta) = e^{-\eta} \tag{23}$$

and the auxiliary linear operators as

$$\mathcal{L}_{f} = \frac{\partial^{3}}{\partial \eta^{3}} - \frac{\partial}{\partial \eta}$$

$$\mathcal{L}_{\theta} = \frac{\partial^{2}}{\partial \eta^{2}} - 1$$
(24)

$$\mathcal{L}_{\theta} = \frac{\partial^2}{\partial n^2} - 1 \tag{25}$$

The above linear operators satisfy

$$\mathcal{L}_{f}[C_{1} + e^{\eta}C_{2} + e^{-\eta}C_{3}] = 0 \tag{26}$$

$$\mathcal{L}_{\theta}[e^{\eta}C_4 + e^{-\eta}C_5] = 0 \tag{27}$$

Here C_i is an arbitrary constant (for i = 1, 2, ..., 5).

We build the zeroth order deformation equations by choosing q as an embedding parameter is as below.

$$(1 - q)\mathcal{L}_{f}[\widehat{f}(\eta, q) - f_{0}(\eta)] = q \, \hbar_{f} \mathcal{N}_{f}[\widehat{f}(\eta, q)]$$

$$(1 - q)\mathcal{L}_{\theta}[\widehat{\theta}(\eta, q) - \theta_{0}(\eta)] = q \, \hbar_{\theta} \mathcal{N}_{\theta}[\widehat{\theta}(\eta, q)]$$

$$(28)$$

$$(29)$$

$$(1-q)\mathcal{L}_{\theta}[\widehat{\theta}(\eta,q) - \theta_{0}(\eta)] = q \, \hbar_{\theta} \mathcal{N}_{\theta}[\widehat{\theta}(\eta,q)] \tag{29}$$

With boundary conditions

$$\widehat{f}(0,q) = 0, \widehat{f}'(0,q) = 1, \widehat{f}'(\infty,q) = 0$$
 (30)

$$\widehat{\theta}(0,q) = 1, \widehat{\theta}'(\infty,q) = 0 \tag{31}$$

In above equations, the partial derivatives w.r.t. η is denoted with prime, the non-zero auxiliary parameters are represented by h_f and h_θ . Further, the differential operators N_f and N_θ , which are nonlinear, are defined as





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$$\mathcal{N}_{f}\left[\widehat{f}\left(\eta,q\right)\right] = \frac{\partial^{3}\widehat{f}\left(\eta,q\right)}{\partial\eta^{3}} + \phi_{1}\gamma\left[\widehat{f}\left(\eta,q\right)\frac{\partial^{2}\widehat{f}\left(\eta,q\right)}{\partial\eta^{2}} - \frac{S\eta}{2}\frac{\partial^{2}\widehat{f}\left(\eta,q\right)}{\partial\eta^{2}} - \left(\frac{\partial\widehat{f}\left(\eta,q\right)}{\partial\eta}\right)^{2} - \left(S+M\right)\frac{\partial\widehat{f}\left(\eta,q\right)}{\partial\eta}\right] \quad (32)$$

$$\mathcal{N}_{\theta}\left[\widehat{f}\left(\eta,q\right)\right] = \frac{\partial^{2}\widehat{\theta}\left(\eta,q\right)}{\partial\eta^{2}} + \phi_{2}\frac{k_{f}}{k_{nf}}\gamma\operatorname{Pr}\left[\left\{\widehat{f}\left(\eta,q\right)\frac{\partial\widehat{\theta}\left(\eta,q\right)}{\partial\eta} - \frac{S\eta}{2}\frac{\partial\widehat{\theta}\left(\eta,q\right)}{\partial\eta}\right\} + \left\{A^{*}\frac{\partial\widehat{f}\left(\eta,q\right)}{\partial\eta} + B^{*}\theta\right\}\right] \quad (33)$$

Clearly, we have for q = 0 and q = 1

$$\hat{f}(\eta,0) = f_0(\eta), \qquad \hat{f}'(\eta,1) = f(\eta)$$
 (34)

$$\widehat{f}(\eta,0) = f_0(\eta), \qquad \widehat{f}'(\eta,1) = f(\eta)
\widehat{\theta}(\eta,0) = \theta_0(\eta), \qquad \widehat{\theta}'(\eta,1) = \theta(\eta)$$
(34)

By using Taylor's series, and by equations (34) and (35), we have

$$\widehat{f}(\eta,q) = f_0(\eta) + \sum_{m=1}^{\infty} f_m(\eta) q^m$$
(36)

$$\widehat{\theta}(\eta, q) = \theta_0(\eta) + \sum_{m=1}^{\infty} \theta_m(\eta) q^m$$
(37)

$$f_m(\eta) = \frac{1}{m!} \frac{\partial^m \widehat{f}(\eta, q)}{\partial \eta^m} \bigg|_{q=0}$$
(38)

$$\theta_m(\eta) = \frac{1}{m!} \frac{\partial^m \widehat{\theta} (\eta, q)}{\partial \eta^m} \bigg|_{q=0}^{q=0} . \tag{39}$$

We assume that both h_f and h_{θ} are appropriately preferred in such a way that the equations (36) and (37) respectively converges at q = 1. Then, due to equations (34) and (35), we get

$$f(\eta) = f_0(\eta) + \sum_{m=1}^{\infty} f_m(\eta)$$
 (40)

$$\theta(\eta) = \theta_0(\eta) + \sum_{m=1}^{\infty} \theta_m(\eta) \tag{41}$$

For obtaining the m-thorder deformation equation the derivative of deformation equations (28) and (29) are taken which are in zeroth order m-times w.r.t q and then divide by factorial of m, to end with setting the value for q = 0, we

$$\mathcal{L}_f[f_m(\eta) - \chi_m f_{m-1}(\eta)] = \hbar_f R_{m,f}(\eta) \tag{42}$$

$$\mathcal{L}_{\theta}[\theta_{m}(\eta) - \chi_{m}\theta_{m-1}(\eta)] = \hbar_{\theta}R_{m,\theta}(\eta) \tag{43}$$

With the chosen BCs are

$$f_m(0) = f_m'(0) = f_m'(\infty) = 0$$
 (44)

$$\theta_m(0) = \theta_m(\infty) = 0 \tag{45}$$

Where
$$\chi_m = \begin{cases} 0, m \le 1 \\ 1, m > 1 \end{cases}$$
 (46)

$$R_{m,f}(\eta) = f_{m-1}^{"'} - \phi_1 \gamma \frac{S\eta}{2} f_{m-1}^{"} - \phi_1 \gamma (S+M) f_{m-1}^{'} + \phi_1 \gamma \sum_{k=0}^{m-1} \left[f_{m-1-k} f_k^{"} - f_{m-1-k}^{'} f_k^{'} \right]$$
(47)

$$R_{m,\theta}(\eta) = \theta_{m-1}^{"} - \phi_2 \frac{k_f}{k_{nf}} \gamma \Pr \frac{S\eta}{2} \theta_{m-1}^{'} + \phi_2 \frac{k_f}{k_{nf}} \gamma \Pr \left(A^* f_{m-1}^{'} + B^* \theta_{m-1} \right) + \phi_2 \frac{k_f}{k_{nf}} \gamma \Pr \sum_{k=0}^{m-1} f_{m-1-k} \theta_k^{'}$$
(48)

Here, to solve the eqs. (42) - (43) one after the other (for m = 1, 2, 3, ...), a software tool is used

CONCLUSION

This paper deals the mathematical formulation and analytical approach for nonlinear differential equations. In future, we will find out the parameter estimation and numerical simulations.





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Formulation, Characterization and Antibacterial Activity of Topical Hydrogel Containing the Composition of Fenugreek Extract and Ciprofloxacin Hydrochoride

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ABSTRACT

To formulate and evaluate antibacterial activity of topical hydrogel containing the composition of fenugreek extract and ciprofloxacin hydrochoride. The excipients were selected based on drug-excipent compatability studies. The topical hydrogel was evaluate for various parameters like pH, Viscosity, spreadability, homogeneity, In vitro drug release and antibacterial activity. The prepared gel showed between 4.7 - 6.2. which lies in the normal pH range. The viscosity of gel was found between 462±24.12-632±46.90cP. In this method the spreadability ranges from14.6±1.34-16.3±0.19q.cm/sec. The results shown that the drug content was almost greater than 85%. All developed formulation of hydrogel showed good homogeneity with an absence of lumps. The antibacterial activity of topical hydrogel containing fenugreek extract was measured against E.Coli and gel formulation F5 & F6 having the highest concentration of fenugreek extract, showed significant antibacterial activity against E.Coli. Among this F5 showed marked highest zone of inhibition (24.4mm) where as for F6 its 21.9mm . The Invitro drug release studies showed non-fickian diffusion. On the basis of the evaluation studies, we can conclude that from all the developed formulations, F5 showed comparatively good spreadability, drug content, drug release and antimicrobial effect. As he concentration of fenugreek extract increased, antibacterial activity was synergistically increased against E.Coli. Therefore it was concluded that this formulae could be very promising topical alternative compared to marketed formulations against E.Coli as fenugreek is non-toxic, compatible and economical.

Keywords: fenugreek, spreadability, antibacterial, zone of inhibition, synergistically,





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INTRODUCTION

Antibiotic resistance has required the quest for novel antibacterial drugs due to the development of bacterial resistance to currently existing antibiotics [1]. Antibiotic resistance is caused by a number of variables, including the nature of bacteria's relationship with antibiotics, the use of antibacterial agents, host features, and environmental factors. This circumstance has compelled scientists to look for new antimicrobial compounds from a variety of sources as innovative antimicrobial chemotherapeutic agents, but synthetic medications are expensive to create and have adverse side effects when compared to plant-derived drugs [2]. These antimicrobial compounds are of natural origin, and their environmental effects are expected to be minimal, allowing them to be employed as biological control agents[3]. Plant-based pharmaceuticals are gaining popularity, owing to the general idea that "green medicine" is safer and more dependable than expensive synthetic drugs, many of which have adverse side effects[4]. Fenugreek (Trigonella Foenum-gracium) is an annual plant in the Leguminosae family that is commonly cultivated in Mediterranean and Asian nations. The dried seeds have long been utilised in India, China, Egypt, and portions of Europe for their health benefits, including galactogogue, antibacterial, anti-inflammatory, insulinotropic, and rejuvenating properties [5]. Flavonoids, alkaloids, steroids, terpenoids, steroids, saponins, anthocyanin, and tannins are among the phytoconstituents found in fenugreek. Due to its beneficial chemical contents, fenugreek has been proven to have antidiabetic, anticancer, hypocholesterolemic, anti-inflammatory, antioxidant, and chemopreventive effects [7]. Fenugreek seeds and sprouts were discovered to be effective against gramme negative bacteria like E.coli and gramme positive bacteria like Staphylococcus aureus [8]. The widespread idea that the introduction of antibiotics would put an end to the spread of infectious diseases was shattered when antimicrobial drug resistance emerged. The incidence and frequency of bacteria resistant to commonly used and well regarded effective first-line medicines is on the rise. There is a great opportunity to discover new, natural plant-based antibacterial compounds for use in disease treatment or as food or cosmetic preservatives [9].

MATERIALS AND METHODS

Fenugreek seeds were procured from the local market. Metformin HCL was obtained by Enaltec Chemistry Applied, Pithampur, and Mumbai, India. Talc, Magnesium stearate, Citric acid, Sodium bicarbonate, Ethylcellulose were obtained from Loba Chem., India. Different instruments *viz*; Monsanto Hardness Tester, Roche Friabilator, Vernier Caliper, Disintegration apparatus, dissolution apparatus (Veego, Mumbai), UV spectrophotometer (Shimadzu, Japan).

Extraction of fenugreek seed

A 100 gm of crushed fenugreek seed was soaked in 500 ml of distilled water and boiled at 60°C for 4 hours using magnetic stirrer to maintain heat and stirring continuously to thick mass was obtained. It was kept aside overnight at room temperature. The mucilage was filter by using muslin cloth. Then mucilage was washed with 300 ml of absolute alcohol. The precipitated mucilage was filtered using vacuum filtration. The separated mucilage was rewashed with 200 ml of acetone. This treatment to remove pigments and to deactivate enzyme. After filtration, filtrate was dried in hot air oven at 60°C for 6 hours. The dried mucilage was to make the fine powder using mortar and pestle and it is passed through the sieve no 120.

Identification test

The extracted fenugreek seeds was test for identification. To determine the presences of mucilage (Ruthenium red test), carbohydrates (Molisch's test), starch (Iodine test), Alkaloids (Dragendroff's test), protein and amino acids (Ninhydrin test), glycosides (Keller Killiani test).

Physiochemical characterization of extract

The extracted powder was evaluated for solubility, swelling index, and pH





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Fourier Transforms Infrared (FTIR) spectroscopy of extract

Fenugreek seed extract was analyzed using Shimadzu, Japan. Samples were prepared by KBr pellet method, one part of the sample and three part of the KBr pellet were taken in mortar and triturated. The small amount of sample was taken in pellet maker and compressed at pressure 10Kg/cm² using hydraulic press. Compressed pellet was scanned at transmittance range of 4000cm-1 – 400-1.

Swelling index

1g of extract was taken 25ml of ground glass stoppered cylinder graduated over a height of 120 to 130mm in 0.5 divisions to this 25ml of water was added and this was shaken vigorously every 10min once for 1 hour and then allowed to stand for 24hr.

Fourier transforms infrared (FTIR) spectroscopy of Ciprofloxacin Hydrochloride.

FTIR of ciprofloxacin hydrochloride was analyzed using Fourier transform infrared (FTIR) spectrophotometer (Shimadzu, Japan). Samples were prepared by KBr pellet method, one part of the sample and three part of the KBr pellet were taken in mortar and triturated. The small amount of sample was taken in pellet maker and compressed at pressure 10Kg/cm² using hydraulic press. Compressed pellet was scanned at transmittance range of 4000cm⁻¹ – 400⁻¹.

Formulation of Gel

All the ingredients were collected according to the formula given in the table 1. Required amount of gelling agents Carbopol 934P, Feenugreek extract were added in water with constant stirring at 500rpm for about 2 hours. Drug was added to the above mixture. Glycerine, TEA was added to it. All the samples were allowed to equilibrate for 24 hours at room temperature prior to performing evaluation test.

Evaluation of topical hydrogel containing fenugreek extract pH

1 % of aqueous solution of gel formulation was prepared and stored for 2 hours and pH was determined using a digital pH meter.

Vicosity

Brookfiled digital viscometer was used measure the viscosity of developed gel formulations. The spindle No.6 dipped in gel sample rotated at 10 rpm 20 ± 1 °C for 15 mins. The viscosity centipoise (cp) was measured.

Spreadability

Parallel Plate Method

It is determined by the following method. The instrument consist of a Wooden block and pulley at one end. First of all a glass was fixed to the wooden block using an adhesive. About 2 gm gel was placed on the glass. Another slide of same dimension (with a hook) was placed over the fixed slide and gel got sandwiched. Almost 1Kg weight was placed over the slide to remove air entrappment. Then a weight of 30g is attached to the hook of the slide with a string a allows some time to move the top slide through the fixed slide. Notice the time (in seconds) taken up by the top slide to cover a distance of 4.6Cm. A shorter interval indicates better spreadability calculate the spreadability by using following equation.

Spreadability = M(L/T)

where as,

M- Weight in pan

L- Length moved by the glass slide

T- Time taken to separate the glass slide





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Homogenity

All developed gel formulation were allow to set in a suitable container and tested for homogenity by visual inspection and gel appearance was reported.

Drug Content:

The gel having a weight of 500mg was weighed and solubilised in 50ml of PBS pH5.5 and shake well to extract the drug and filter. The drug content was analysed spectrophotometrically at 275nm.

FTIR of Topical Hydrogel containing fenugreek extract

FTIR of Topical Hydrogel containing fenugreek extract was analyzed using Fourier transform infrared (FTIR) spectrophotometer (Shimadzu, Japan). Samples were prepared by KBr pellet method, one part of the sample and three part of the KBr pellet were taken in mortar and triturated. The small amount of sample was taken in pellet maker and compressed at pressure 10Kg/cm² using hydraulic press. Compressed pellet was scanned at transmittance range of 4000cm¹- 400·

In-vitro drug release studies

The study was done using franz diffusion cell with cellophane membrane as barrier and 100ml of PBS pH 5.5 was used in the receptor compartment, then 1 gm of gel was spread uniformly on the membrane. the drug concentration on the receptor fluid was determined spectrophotometrically against appropriate blank.

In-vitro antibacterial study

An agar well diffusion method was used for determination of antibacterial activity of topical hydrogel containing fenugreek extract.

1) Preparation of Inoculum

Fresh bacterial culture of E.coli was suspended in sterile water for 24 hours to obtain uniform suspension of microorganism

2) Preparation of Muller Hintor Agar Media

Beef extract0.3g, peptone 5.0g, muller hinton agar 15.0g were accurately weighed and transferred into a conical flask. Then add required quantity of distilled water and stirred the mixture of agar media for 2 mins set the boiling condition. Then sterilised in a autoclave at 121°C for 15mins.

3) Determination of Zone of Inhibition

An agar well diffusion method was used for determination of antibacterial activity of topical hydrogel containing fenugreek extract. In this method, transferred the 15 -20ml of a previously liquified medium into sterile test tubes and cool all the test tube at 42-45°C. One loopfull of culture was transferred in a each agar medium containing test tube and mix. Then all inoculated liquid agar media was poured into a separate sterile petri plate and allow to solidify the agar media. After solidification of the medium, required quantity of gel(1%,2% and 3%) was applied into the cavities of the agar plates and agar plates was incubated at 37°C±1°C for 24 Hours.

RESULTS AND DISCUSSION

DRUG COMPATIBILITY STUDIES

FTIR Spectroscopy of Fenugreek Extract

The FTIR spectra of isolated fenugreek extract are shown in Figure 1. It showed characteristic peaks of -OH between 3510.8 and 3156.3 cm $^{-1}$, $-CH_3$ at 2934 cm $^{-1}$, -CH stretching between 2922 and 2856 cm $^{-1}$, ether linkage at 1450-1400 cm $^{-1}$, and -CO stretching at 1018 cm $^{-1}$. These findings confirmed that the isolated seed extract was fenugreek starch extract.





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FTIR spectrum of Ciproflofloxacin

In the FTIR spectrum of cipro, one prominent peak was found between 3500 and 3450cm-1and was assigned to and OH stretching vibration another band at 3000 to 2950cm-1 represented alkene and aromatic CH stretching. The band at 1750-1700 cm-1 indicated carbonyl C=O stretching. The bands at 1300 to 1250 cm-1 suggesting bending vibration of O-H group indicate the presence of carboxylic group. In addition strong absorption in peak between 1050 and 1000cm-1 was assigned to CF group.

FTIR spectrum of carbopol

For C934, FTIR spectrum showed a peak in the range 3000-2950cm-1 representing OH stretching vibration. The prominent peak between 1750 and 1700cm-1was assigned to C=O stretching. Ethereal cross linking s indicated by prominent peak at 1160cm-1. The band between 850 and 800cm-1represent C=CH, Bending vibration of aromatic enes.

Evaluation of Topical Hydrogel Containing Fenugreek Extract

Topical hydrogel containing fenugreek extract was developed using carbapol 934P, as gelling agent, Fenugreek extract. Glycerine was used as humectants and emollient, triethanolamine. Ciprofloxacin hydrochloride was used as antibacterial agent. FTIR spectrum of ciprofloxacin hydrochloride and its gel formulation was obtained and shown in Fig4(a,b,c,d). Results of pH, Viscosity, Spreadability, drug content and homogeneity were shown in table 3. The pH value lies in the normal range which compatible with the normal pH range of skin. The pH of all formulated hydrogel was found to be between 4.7 – 6.2.which lies in the normal pH range. Rheological studies were performed on the prepared gel. The viscosity of gel was found between 462±24.12-632±46.90cP. This rheological behavior is useful for the better spreading and the application of gel formulation. The spreadability was determined by using parallel plate method. In this method the spreadability ranges from14.6±1.34-16.3±0.19g.cm/sec. The percentage was drug content was measured for all the topical hydrogel formulation. The results shown that the drug content was almost greater than 85%. All developed formulation of hydrogel showed good homogeneity with an absence of lumps.

In-vitro drug release studies

In-vitro drug release studies was using cellophane membrane by franz diffusion cell

Drug release kinetics

The results obtained from the in-vitro drug release study were fitted into various mathematical models as follows

- a) cumulative percentage drug release Vs time(Zero order rate kinetics)
- b) Log cumulative percentage drug remaining Vs Time(First order rate kinetics)
- c) cumulative percentage drug remaining Vs Square root of time (Higuchi classical diffusion model)
- d) Log cumulative percentage drug release Vs Log time (Korsemyer peppas equation)

Plots of Zero order, First order, Higuchi and Korsemeyer peppas were depicted below. The regression coefficient (R²) values were shown in figures given below: The *in-vitro* antibacterial study was performed by measuring and comparing the diameter of the zone of inhibition (mm) for the various formulations of topical gel (F1,F2,F3,F4,F5,F6). The antibacterial activity of topical hydrogel containing fenugreek extract was measured against *E.Coli* and gel formulation F5 & F6 having the highest concentration of fenugreek extract, showed significant antibacterial activity against *E.Coli*. Among this F5 showed marked highest zone of inhibition (24.4mm) where as for F6 its 21.9mm. There is not much significant difference in antibacterial activity of both the formulations. This suggest the antimicrobial potential of fenugreek extract against *E.Coli*.





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CONCLUSION

The antibacterial activity of topical hydrogel containing fenugreek extract was studied. On the basis of the evaluation studies, we can conclude that from all the developed formulations, F5 showed comparatively good spreadability, drug content, drug release and antimicrobial effect. As the concentration of fenugreek extract increased, antibacterial activity was synergistically increased against *E.Coli*. Therefore it was concluded that this formula could be very promising topical alternative to marketed formulations against *E.Coli* as fenugreek is non-toxic, compatible and economical. However further preclinical and clinical studies are required to prove its application in human.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Table:1 Composition of Hydrogel

S.NO	INGREDIENTS	F1	F2	F3	F4	F5	F6
1	Ciprofloxacin hydrochloride	5mg	5mg	5mg	5mg	5mg	5mg
2	Feenugreek Extract	2.5	4.5	6.5	8.5	10.5	12.5
3	Carbopol 934P	250mg	250mg	250mg	250mg	250mg	250mg
3	Glycerine	2ml	2ml	2ml	2ml	2ml	2ml
4	Triethnaolamine	0.4ml	0.4ml	0.4ml	0.4ml	0.4ml	0.4ml
5	Distilled Water	q.s	q.s	q.s	q.s	q.s	q.s
6	Colouring agent	5mg	5mg	5mg	5mg	5mg	5mg

Table 2: Physicochemical characterization of Fenugreek seed extract

PARAMETERS	RESULT		
State	Amorphous powder		
Odor	No characteristic odor		
Taste	Tasteless		
Color	Off white- cream-yellow color		
Identification			
Test for starch	The appearance of blue-black colour		
Test for carbohydrate (Mollish's test)	The appearance of violet ring at the junction of two liquids		
Test for tannins (Ferric chloride test)	-		
Test for alkaloids	-		
 Test for glycosides 	-		
 Test for mucilages 	+		
 Test for steroids and sterois 	-		
Test for proteins and aminoacids	-		
pH (1%w/v)	5.6		
Swelling index	35		
Solubility	Quickly dissolves in warm water, forms viscous colloidal solution, insoluble in ether, acetone, chloroform, ethanol		

Table 3: Evaluation of Topical hydrogel containing fenugreek extract for pH, Viscosity, Spreadability, Homogenity and Drug content.

Batch No	pН	Viscosity (cP)	Spreadability (g.cm/sec)	Homogenity	Drug content
F1	5.7±0.17	462±24.12	14.6±1.34	Homogenous	90.36±053
F2	6.2±0.31	517±32.06	14.9±0.30	Homogenous	87.34±0.25
F3	4.9±0.10	542±47.52	16.3±0.19	Homogenous	92.40±0.17
F4	5.3±1.12	560±89.03	15.5±0.01	Homogenous	89.03±0.01
F5	4.7±0.12	598±56.03	16.0±0.08	Homogenous	97.67±0.23
F6	5.1±0.39	632±46.90	15.2±0.05	Homogenous	91.32±0.45





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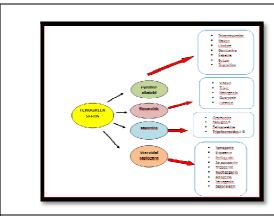
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Table 4:Evaluation of Topical hydrogel containing fenugreek extract for *In-vitro* drug release studies.

S.No	Time	CUMULATIVE % DRUG RELEASE						
5.110	(Minutes)	F1	F2	F3	F4	F5	F6	
1	0	0	0	0	0	0	0	
2	15	4.23	6.30	5.0	6.25	9.66	3.9	
3	30	10.76	9.41	12.48	11.01	12.13	12.01	
4	45	21.31	16.34	24.93	19.03	18.03	20.82	
5	60	28.22	22.69	34.62	26.79	24.56	26.32	
6	75	32.34	25.09	38.98	32.98	33.54	29.88	
7	90	37.68	29.30	47.58	38.56	47.04	38.34	
8	105	40.56	33.39	52.68	43.85	51.62	43.56	
9	120	46.95	41.82	61.45	55.78	63.86	55.65	
10	150	51.76	53.60	74.05	60.63	76.42	69.32	
11	180	58.72	64.92	79.43	69.72	80.02	76.89	
12	240	63.54	70.08	84.67	76.02	94.38	82.46	

Table5: Evaluation of topical hydrogel for antibacterial activity

Formulation	Zone of Inhibition
	Negative strain - E.coli
F1	07.3mm
F2	10.6mm
F3	12.6mm
F4	17.3mm
F5	24.4mm
F6	21.9mm



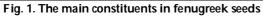




Fig. 2: Extract of Feenugreek

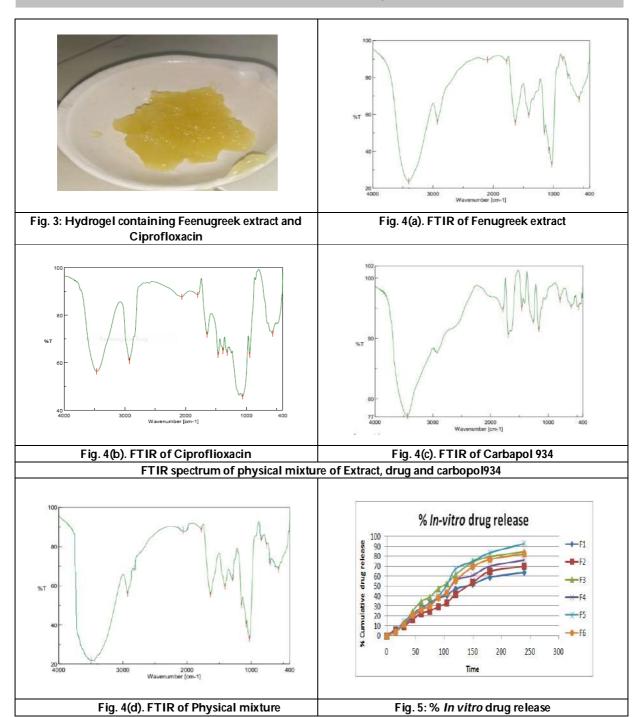




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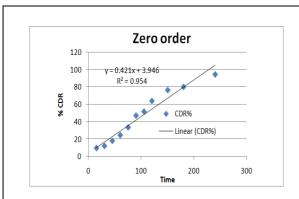




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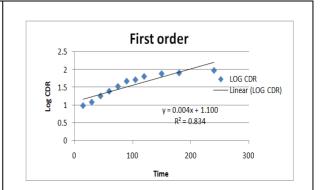


Fig. 6: Zero order

Higuchi release

120
100
80
80
60
40
20
0
5
10
15
20
Sq.root of time

Fig. 7: First order

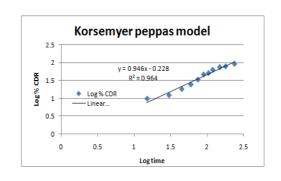


Fig. 8: Higuchi release



Fig. 9: Korsemyer peppas model



Fig. 10: A) F1

Fig. 11: B) F2

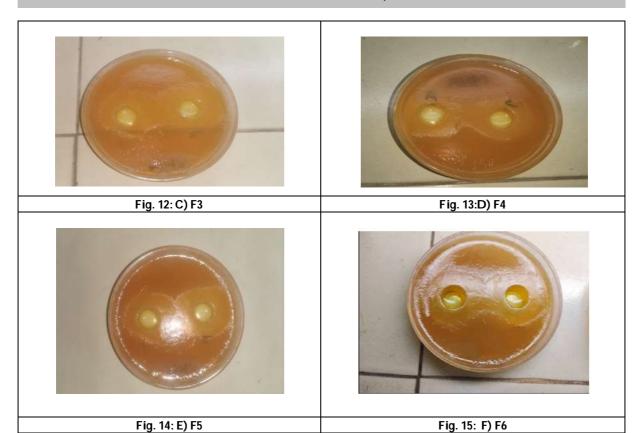




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REVIEW ARTICLE

Physiotherapy Awareness in Women's Health amongst Health Worker and in General Population: A Literature Review

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ABSTRACT

A subspecialty of physical therapy known as obstetrics and gynaecology physiotherapy promotes women's health during the period of childbearing. However, the knowledge, attitude, and practise of gynaecologists and obstetricians affect how often people need physiotherapy services. The study is aim to access the literature of knowledge, attitude and practice of physiotherapy amongst obstetricians, gynecologists and in general population. We searched google scholar and Pub Med for take a evidence article which proves that awareness, practice and attitude towards physiotherapy treatmentsin health care providers as well as general population. For that total 101 articles were searched which includes physiotherapy treatments are used to improve overall women health on pubmed and google scholar. After reading all abstract and methodology duplicates are removed. Remaining 98 reviews were screening whole methodology and result. And by screening 54 articles were exclude remaining 44 articles were re-read and excluded with reason which has not positive findings and not relevant methodology. A total of 40 studies were finally included in the review. We include article related to effect of physiotherapy in obstetrics & gynaecological conditions. According to the researches proves that physiotherapy beneficial for obstetrics& gynaecological conditions but awareness of physiotherapy treatment among health workers and general people are less. Physiotherapy during various phases of women life plays an important role in overall health of women's life. In this study we concluded from the previous studies that knowledge, attitude and practice of physiotherapy among health workers and general population is low and need to encourage.

Keywords: Physiotherapy, Gynaecologists, Obstetrician, Physiotherapist





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INTRODUCTION

Women facing so many health-related problems throughout their lifetime. Some of them are pregnancy-related low back pain, pelvic girdle pain, post-partum incontinence [1]. During Pregnancy women undergoes many physiological and psychological changes in the body; and all of these changes are normal and because of growing fetus mainly changes are noticed in abdominal and thoracic regions [2]. However, considerable numbers of women do not recover from pain after delivery. That could be the serious problem for herself, her family and the society because that could be reflected in the inability of her to perform daily activities or to earn a living and by healthrelated reduced quality of life[3]. Information from the several studies shows result that pelvic organ prolapse; urinary incontinence and fecal incontinence are very common conditions affecting women's overall health. Approximately 83,000 women were surveyed overall; mean prevalence of pelvic organ prolapse was 19.7% (range 3.4%-56.4%), urinary incontinence was 28.7% (range 5.2-70.8%) and fecal incontinence was about 7% (range 5.3-41%) [4]. The prevalence rates vary depending upon criteria used for diagnosing or clarifying the pain. Several studies have shown that approximately 50% of women have some lumbo-pelvic pain during pregnancy. Very often the pain will be disappeared after 1 to 3 months after delivery [3]. However, prevalence of urinary incontinence estimates even higher, varying between 20% and 67% during pregnancy, and 0.3% and 44% after child birth, respectively [5]. Pregnancy and vaginal delivery are considered to be one of the main risk factors in the development of urinary incontinence because pregnancy and childbirth may cause damage to the fascias, ligaments, pelvic floor muscles, and nerves supporting and controlling the bladder neck and urethra [5]. In prevention and treatment of urinary incontinence, Pelvic floor muscle training after childbirth has been demonstrated to be effective [5]. The role of physiotherapists in obstetrics and gynecology is so important. The role of the physiotherapist in obstetrics and gynecology involves pregnancy, labor, and the puerperium and the preoperative and postoperative periods [1]. Several Studies claimed that general practitioner in India are aware about physiotherapy applications in musculoskeletal condition, paediatric condition, neurological condition, cardio respiratory condition and sports conditions, but there is lack of awareness of recent advances in physiotherapy practice and its application in the area of women's health [6].

Aim and Objectives

- **Aim** The study is aim to access the literature of knowledge, attitude and practice of physiotherapy amongst health worker and in general population.
- objectives
- o To access the literature of knowledge, attitude and practice of physiotherapy amongst health worker.
- o To access the literature of knowledge, attitude and practice of physiotherapy amongst general population.

METHODOLOGY

PRISMA. Preferred reporting items for systemic review and meta-analysis. We searched google scholar and Pub Med for take an evidence article which proves that awareness, practice and attitude towards physiotherapy treatments in health care providers as well as general population are low. For that total 101 articles were searched which includes physiotherapy treatments are used to improve overall women health on pubmed and google scholar. After reading all abstract and methodology duplicates are removed. Remaining 98 reviews were screening whole methodology and result. And by screening 54 articles were exclude remaining 44 articles were re-read and excluded with reason which has not positive findings and not relevant methodology. A total of 40 studies were finally included in the review. Firstly all 101 articles were found which are related to awareness, practice and attitude towards physiotherapy treatments in health care providers as well as general population. Then which are not Then which are not relate with each other and conclusion is not positive that all are excluded from review and after the entire factor which have to included review that total number of articles are 40. There is a need to raise the level of knowledge among the obstetricians and gynaecologists working in both private and public institutions about the importance of





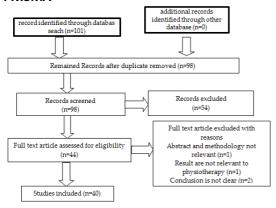
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physical therapy in managing gynaecological and obstetric patients [2]. Pelvic floor muscle strength improved significantly after intensive pelvic floor muscle training [3].

PRISMA



DISCUSSION

Utilization of Physiotherapy services depends upon knowledge, attitude and practice of gynaecologist and obstetricians [1]. Our findings indicate that consultants tend to be more aware and have better knowledge than senior registrars about the role of physiotherapy in obstetrics and gynecology due to exposure resulting from years of experience [1]. The majority (79.1%) of participants knew of the role of physiotherapists in obstetrics, with postnatal care having the highest score (98.5%), followed by antenatal care (82.1%) and parturition (56.7%). We found that 66 (98.5%) of participants knew the role of physiotherapy in uterine prolapse, 47 (70.1%) in hysterectomy, 22 (32.8%) in pelvic inflammatory disease, and five (7.5%) in cervical incompetence, indicating that participants were knowledgeable about the role of physiotherapy in obstetrics and gynecology [1]. This is in agreement with two other reports. The majority of participants strongly agreed that physiotherapy would not cause harm to patients, and 92.5% strongly agreed that physiotherapy services cannot be replaced with drugs and instructions, and that physiotherapists are competent to manage obstetric and gynecologic conditions [1]. This study shows that obstetricians had not been involving physiotherapists in the management of conditions such as pelvic inflammatory disease [1]. Four studies with strong to moderate quality were appropriate for inclusion in the review [7]. Previously, specific stabilization exercises have been found effective in the postpartum period. The findings of this review ads to the knowledge that specific stabilization exercises are also useful in the antenatal period. One study found that physical therapist-led group exercise was more beneficial than individual home-based exercise, but this may suggest an attention effect or reflect the duration of the intervention, because the study with the greatest clinical effect was for one week of individual exercise [7]. Articles were identified through titles and abstracts and if the articles were found appropriate, the full text was reviewed. the reference lists of the systemic reviews and full text articles were hand searched for potentially appropriate studies based on in-text references and titles [7]. This review has followed a systemic process and used a comprehensive quality appraisal tool, but the parameters of comprehensive quality tool, but the parameters of the search may be limiting factor of the conclusions of this review [7].

Physiotherapy Awareness amongst Health Worker and in General Population

Maqsood U, et al. in 2017 a comparative cross-sectional study conducted in private and government hospitals of Lahore in their study, from October 2016 to March 2017 the study participants were obstetricians and gynecologists working in private and government hospitals of Lahore [2]. One thirty-eight participants, including 71 consultants, 67 senior registrars participated in the study. 69 participants were from private hospitals while remaining 69 were from government hospitals. Data was collected using a pre-tested 28-items self-administrated





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questionnaire which was taken from previous research work [2]. they reported that The Obstetricians and gynecologist working in private and government hospitals are equally aware about the role of physical therapy in managing gynecological and obstetric patients and there is a need to increase their awareness level [2]. Pratik Govekar et al. (2020) in their study Awareness about Role of Physiotherapy Management During Labour among Obstetricians and Gynacologists they took total 144 obstetricians and gynaecologist in this study, Each of them was given a self-made questionnaire The present study showed that the awareness among obstetricians in less regarding role of physiotherapy in managing labour due to lack of knowledge and also due to unavailability of a qualified physiotherapist in that particular area. On the basis of result they concluded that there is a need for better interaction and communication between physiotherapist and obstetricians and gynaecologists, which candone through journals, seminars, UG/PG curriculum, social media, workshops [8]. A Kale et al. (2019) in their study Awareness about exclusive role of postnatal physical therapy A preliminary survey conducted on obstetricians at Aurangabad, Maharashtra, they conducted a survey on 105 obstetricians from Aurangabad using a questionnaire that included 8 questions. In their result they found out AII the 6 subjects who were referring patients to Physiotherapists for Postnatal exercises (Q.7) from the questionnaire who offers postnatal exercises to your clients Garbhasanskara center 17(16.919%), / Physiotherapist 6(5.71%) / Not answered 82(78.09%) were found to be serving as faculty members of the Medical College and had regular interaction with their teaching Physiotherapy department. From above information they concluded that their hypothesis on lack of awareness about the importance of individual need based (INB) protocol of PFM exercises and need of regular follow up of diastasis recti among Obstetricians was accepted. The survey also showed agreement on the poor trend of referrals for postnatal physiotherapy in India. They also recommended that It is high time that Physiotherapists in India make adequate efforts to spread awareness about the exclusive scope of postnatal physiotherapy. It is also strongly recommended that Physiotherapist should be included in each team involved in postnatal care including National programs on maternal health [9].

Ghania nazar (2021) in their study Awareness about the role of physical therapy in post-partum females among gynaecologists A cross-sectional study was conducted Data were collected from 75 gynecologists of Lahore and Sahiwal using non-probability convenient sampling. Using SPSS version 21, descriptive statistics like frequency and percentage were calculated It was shown that postpartum physical therapy services were known to 57 (76%) of gynecologists. Among them, 11 (14.7%) gynecologists do refer patients, 14 (18.7%) do not refer at all, 28 (37.3%) refer when required, and 22 (29.3%) refer clients very rarely to the physiotherapy department. It was concluded that the majority of the gynecologists were aware of postpartum physical therapy for women. But there is a lack of referral systems in our settings because they do not have any proper gynecological physiotherapy settings affiliated with them [10]. Only a few gynecologists refer patients for Physiotherapy services [10]. M Sarfraz et al. (2013) in their study Role of Physical Therapy in antenatal care as perceived by the clients-a cross sectional survey on pregnant females attending antenatal OPD they did cross sectional study and females in their nine months of pregnancy aged between 18 to 40 years were included in the study. They found out 375 patients among which 81.9% were housewives, 15.5% were working women while 2.7% were students. 83.7% of the women were aware of physical therapy out of which 95.2% women agreed that physiotherapy has positive role in antenatal care yet only 42% actually attended physiotherapy sessions and 36% were currently following exercise progamme from that they concluded that The perception and knowledge of women regarding physical therapy (exercise) during pregnancy is reasonable but a small number of subjects exercise during pregnancy [11].

Attitude towards Physiotherapy amongst Health Worker and in General Population

Nse a odunaiya (2013) et al. in their study Attitude and practices of obstetricians and gynecologists towards involvement of physiotherapists in management of obstetric and gynecologic conditions did a descriptive survey of obstetricians and gynecologists from seven hospitals in south-western Nigeria. Sixty-seven participants, including 41 senior registrars and 26 consultants, participated in the study. More consultants (73.1%) than senior registrars (46.3%) had a good knowledge of the role of physiotherapists in obstetric and gynecological practice. All participants had general knowledge of the role of physiotherapists in obstetrics and gynecology, but a limited knowledge of specific conditions amenable to treatment by physiotherapists. More senior registrars had a better attitude (73.2%) than consultants (53.8%) towards involvement of physiotherapists in their practice, and 94.0% of the obstetricians





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and gynecologists utilized the services of physiotherapists, with referral of patients (56.7%) mainly to physiotherapists working in general/state hospitals [1]. They concluded that there is a need for better interaction and communication between physiotherapists and obstetricians and gynecologists, which could be achieved through clinical meetings, seminars, and workshops [1]. Patricia W Bauer et al. (2010) studied on Exercise and pregnancy knowledge among healthcare providers To examine healthcare provider's knowledge, beliefs, and practices regarding exercise during pregnancy using a cross-sectional 31-question pen and paper survey in their study they took Ninety-three practicing healthcare providers, M.D. (n=45) and D.O. (n=14) physicians and certified nurse midwives (C.N.M., n = 34), from hospitals and birth centers around Michigan city [7]. Descriptive characteristic data, provider knowledge, beliefs, and practices regarding exercise during pregnancy, common exercise restrictions given to pregnant patients, and provider awareness of current American College of Obstetricians and Gynecologists (ACOG) exercise and pregnancy guidelines were collected [7]. From their study they find out that Overall, 99% of respondents believed that exercise during pregnancy is beneficial, 64% of all respondents believed that maternal exercise heart rate should not exceed 140 beats per minute (bpm), and 60% of M.D.s and 86% of D.O.s were not familiar with the 1994 ACOG guidelines for exercise and pregnancy (p < 0.05) [7]. And they concluded that although the providers' beliefs about exercise during pregnancy were positive, not all were aware of or followed current ACOG recommendations. Different strategies for dissemination of current research may be warranted [7].

Ruhi Sheth *et al.* (2019) in their study of Knowledge, attitude, and perception about antenatal physiotherapy among pregnant women in Ahmedabad they did A descriptive cross-sectional study in a semi government hospital. Descriptive analysis of data obtained was done from that they find out Knowledge regarding antenatal exercises was fairly low. A large number of subject population (91%, n= 91) was aware of the role of physiotherapy in general health and well-being. A sufficient number of subjects (59%, n= 59) were informed regarding the uses of physiotherapy in backache. However, only 2% of the population was familiar with the role of pelvic floor muscle strengthening in urinary incontinence [12]. The attitude of the patients toward antenatal physiotherapy was favorable (72%, n= 72), whereas the attitude toward postnatal physiotherapy was comparatively less favorable (39%, n= 39). The perception of physiotherapy was restricted to exercises (88%, n= 88), massage (63%, n= 63), and hot water fomentation (18%, n= 18) from that they concluded that Knowledge and perception regarding antenatal physiotherapy were fairly low, but the attitude of the patients was favourable [12].

Practice of Physiotherapy amongst Health Worker and in General Population

Hina Munawar *et al.* (2013)in their study Awareness of Obstetricians/ Gynecologists Regarding the Role of Physiotherapy Services in Managing Obstetric Patients they did A cross-sectional study among gynecologists and obstetricians selected from tertiary care hospitals of Karachi through non-probability convenient sampling, total sample size of the study was 300. SPSS version 17 was used to analyse data. Chi square test was used to find association for qualitative variables and p value and they concluded that Out of total, 194(64.7%) had an awareness about pre-natal physiotherapy exercises sessions but only 56(18.7%) refer their patients for the antenatal classes (p-value 0.001). From total 204(68.0%) answered yes regarding the role of postnatal exercise sessions but out of them only 60(20.0%) refer for the post-natal physiotherapy sessions regularly and 74(24.7%) occasionally (p-value=0.218).s, there was lack of referral for physiotherapy. Situation was even better in private hospitals than government hospitals with proper referral system[13]. Ali, Zeinab A.(2019) in their study Knowledge, attitude, and practice of pregnant women toward antenatal physiotherapy in Al-Qurayyat A cross-sectional study they did A descriptive cross-sectional study in over 3 months in 2019 of pregnant women attending antenatal clinics at Al-Qurayyat General Hospital, Al-Qurayyat city, KSAA self-administered survey was distributed to 134 pregnant women in any trimester. We questioned participants about their attitudes toward and knowledge of prenatal physiotherapy.

The participants' attitudes regarding physiotherapy during prenatal treatment were generally good, with 58% of them believing that antenatal exercises lower pregnancy-related problems and promote a safe birth. The patients' attitudes toward antenatal physiotherapy were mostly negative. Pregnant women's perceptions of physiotherapy were likewise unfavourable. Grom that they concluded that Knowledge regarding antenatal physiotherapy was favorable, but the attitude and perception of the patients were fairly low [14]. Loveness A Nkhata *et al.* (2015) in their





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study of Exercise practice among women attending antenatal care at the University Teaching Hospital in Lusaka, Zambia their aim of the study is to obtain information on exercise practice among women attending antenatal care at the University Teaching Hospital in Lusaka, Zambia and establish whether, educational level, number of pregnancies and cultural background had an influence on exercise practice during pregnancy they collected data using a selfadministered questionnaire in a cross sectional, exploratory study and summarized data using descriptive statistics. However, cultural background had a significant association to the women's exercise practice (p-value 0.025). From their study they concluded that pregnant women practice general physical activities of daily living such as walking and household chores during pregnancy. They do not know the specific antenatal exercises. Consequently, they are not able to practice the ideal exercise during pregnancy. This highlights the need for Physiotherapy personnel to be actively involved during antenatal to educate pregnant women on the ideal exercise activities [15]. Bayat et al. (2021) in their study Evaluation of Awareness, Adherence, and Barriers of Pelvic Floor Muscle Training in Pregnant Women: A Crosssectional Study they conducted a study on 200 pregnant women attending prenatal care clinic in their third trimester of pregnancy they interviewed with open and closed questions derived from recent literature review on PFMT. They found out that Fifty-four (27%) of studied women were familiar with PFMT, 175 (87.5%) of females thought that the UI is normal during pregnancy, and 25 females (32.05%) had experienced Urinary Incontinence episodes and had consulted with their obstetrician. Twenty-one (10.5%) of patients did the PFMT exercises before their pregnancy, 14 (66.6%) of them continued their PFMT exercises during their pregnancy, and 7 (33.4%) stopped it. Health care professionals should be more involved in-patient education process [16].

CONCLUSION

Physiotherapy during various phases of women life plays an important role in overall health of women's life. In this study we concluded from the previous studies that knowledge, attitude and practice of physiotherapy among health workers and general population is low and need to encourage.

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RESEARCH ARTICLE

Integrated FCM and CFH Approaches with Picture Fuzzy Set Representations to Study the Impacts of Cyber Pedagogy

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ABSTRACT

The recurrent pandemic waves have made the teaching community practice cyber pedagogy with the online engagements of teaching and learning and it has set the new benchmarks for performance evaluation of the learners. The pros and cons of cyber pedagogy have to be studied deeply to suggest measures to handle the hurdles of this new paradigm of teaching and learning processes and this paper intends the same. The integrated approaches of Fuzzy Cognitive Maps (FCM) with Concentric Fuzzy Hypergraph (CFH)is used to make optimal decisions on the factors of the study with picture fuzzy set representations. This paper aims to determine the core facilitating factors in exercising cyber pedagogy and the interrelation between the core factors. Among the factors chosen for study based on the primary data from different stakeholders using a questionnaire, five core factors and their interrelationship were found. The outcomes of this study will certainly benefit the academic fraternity.

Keywords: FCM, Concentric Fuzzy Hypergraph, Picture Fuzzy set

INTRODUCTION

FCM decision techniques play a vital role in determining the causal effects between the factors. FCM was developed by Kosko [1] and it is an extension of Cognitive Maps in a fuzzy environment. The theory of fuzzy set developed by Zadeh [2] resolve the hurdles caused by imprecision and uncertainty in making decisions. FCM, a directed graph with nodes and edges signifying the factors of the problem and their relationships respectively. The edge weight





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denotes the degree of the relationship between the factors and it assumes the values from [-1,1]. FCMs are categorized as simple FCM and weighted FCM based on the edge weights. The edge set of the former assumes the values from {-1,0,1} and the latter assumes the weight from [-1,1]. FCM is extensively used in different arenas to make optimal decisions [3-4]. FCMs are extended to Intuitionistic FCM (IFCM) with membership and nonmembership representations [5]. Neutrosophic Cognitive Maps (NCM) [12] are the extensions of IFCM, comprising of representations in terms of neutrosophic sets consisting of truth, indeterminacy and falsity expressions. Both IFCM and NCM are applied in different arenas of decision-making. FCMs are also integrated with fuzzy hypergraphic representations developed by Kaufmann [7]. Nivetha et al built FCM models blended with fuzzy hypergraphs[8-9]and concentric hypergraph [11]. These integrated models are two-staged, in the first stage, the number of factors associated with the decision-making problem is reduced and the core factors are considered for studying the interrelationship between the factors in the second stage. These integrated models are applied to make optimal decisions on various aspects by Nivetha et al., [8-11]. In a certain decision-making environment, the factors and their inter-association cannot be represented by intuitionistic sets or neutrosophic sets always, the representations may involve neutrality, to handle such cases, picture fuzzy sets shall be used. Picture fuzzy set (PFS) developed by Cuong and Kreinovich [6] consists of membership, non-membership and neutrality values whose sum must be less than or equal to 1. PFS representations are also applied to make optimal decisions. It is also found from the literature that the notion of PFS is not integrated with FCM and henceforth in this research paper Picture FCM is introduced to bridge this gap. This paper proposes a picture fuzzy concentric hypergraph integrated FCM model and the proposed model is validated by considering the factors contributing to the impacts of cyber pedagogy. The remaining of the paper is structured as follows: Section 2 presents FCM and the methodology; section 3 applies the proposed approach to the decision-making problem; section 4 discusses the results and concludes the paper.

FCM

A FCM is a directed graph depicting the cause and effects of the decision-making problems. Let us consider four factors [F1,F2,F3,F4] causing social distress at workspace for women especially. The four factors are taken as nodes of the FCM. The edges and the edge weights represent the interrelationship between the factors and their intensity. The graphical representation shall be represented as a connection matrix C of order 4×4. With the diagonal elements as zero. Let us consider an instantaneous vector V of the form (F1, 0,0,0) which signifies the ON position of the first factor and off positions of the other factors. The vector V is passed on to C and the resulting vector is threshold and updated by assigning 1 to the values greater than 1 and 0, otherwise. By repeating the above procedure, the fixed point or the limit cycle is obtained if two subsequent updated vectors are alike. FCM is cyclic in nature and it has directed cycles and this makes the system dynamical.

Proposed Methodology

The methodology proposed is similar to the earlier steps followed by Nivetha et al [8-11] and the representation using picture fuzzy sets makes a difference. The sequence of steps followed in making decisions is given Fig.2.1

Decision Making on the impacts of Cyber Pedagogy

This section presents the following impact factors of cyber pedagogy in both positive and negative perspective and the most core impact factors are determined.

The factors considered for the study are

- 1. Blended system of learning
- 2. Self-Paced learning
- 3. Access to Different Open Educational Resources
- 4. Development of Digital tools to be creative
- 5. Exposure to different portals of Learning
- 6. Transition towards Online system of Evaluation
- 7. Weakening of Social skills
- 8. Discrimination between have and have nots
- 9. Opportunities to Elite class





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- 10. Health ramifications
- 11. Physical and Mental Distress
- 12. Lack of focus on Psychomotor Domain
- 13. Switching between synchronous and asynchronous modes of teaching is stress to teachers
- 14. Chances of deviations and obsessions towards social media and gaming
- 15. Institutional Linkages with IT industries
- 16. Commercialization of Education
- 17. Upcoming of new learning software
- 18. Upcoming of new learning software

Stage - I

In this stage the 18 factors are reduced to five factors using concentric hypergraph approach with PFS representations. The concentric Hypergraphic representation of the factors is presented in Fig 3.1[For further a detailed description of concentric fuzzy hypergraphs, the readers shall refer [10-11]]. The expert's opinion on the significance of the factors are represented using picture fuzzy sets and the final weight corresponding to each factor is the intersection of the three picture fuzzy sets given by three experts and Table 1 consists of the respective score values.

Stage-II

In this stage the causal effects between the significant factors are obtained using FCM approach. The factors F1, F7,F12,F14,F15 are very significant. The FCM approach is applied to the following five core factors.

- C1Blended system of learning
- C2 Weakening of Social skills
- C3 Lack of focus on Psychomotor Domain
- C4 Chances of deviations and obsessions towards social media and gaming
- C5 Institutional Linkages with IT industries

Computation of Fixed Point

The fixed points obtained are presented in Table 2





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Table 2. presents the fixed points obtained on keeping each factor in ON position. The fixed point (11111) obtained states the influence of the first factor over all the other factors. The limit point (01110) states that the factors F2,F3 and F4 are interrelated and has mutual influence over one another. The limit point (10001) states that the factor F5 has only an influence on F1.

CONCLUSION

This paper proposes the Concentric Hypergraphic FCM approach with picture fuzzy sets representations to investigate the impacts of cyber pedagogy. The representations of expert's opinions using picture fuzzy sets are more realistic. The optimal decisions obtained using this proposed approach will certainly assist in framing suitable remedial measures and alternative strategies for promoting better system of education in an online environment.

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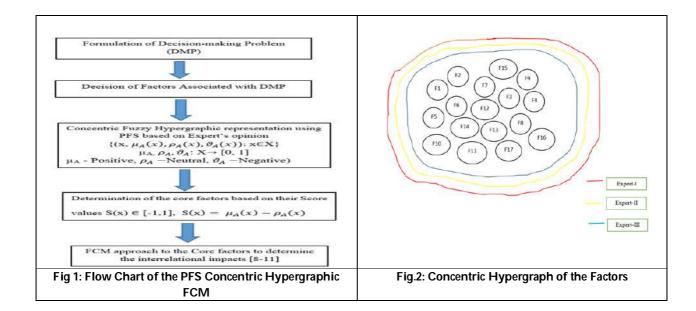
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Table 1: Score values of the Factors

Factors	Expert-I	Expert-II	Expert-III	Final Weight	Score Values
F1	(0.6,0.2,0.1)	(0.8, 0.1, 0.1)	(0.7,0.2,0.1)	(0.6,0.1,0.1)	0.5
F2	(0.7, 0.1, 0.1)	(0.4, 0.3, 0.1)	(0.4, 0.2, 0.3)	(0.4,0.1,0.3)	0.3
F3	(0.3, 0.4, 0.1)	(0.4, 0.3, 0.1)	(0.5, 0.3, 0.1)	(0.3,0.3,0.1)	0
F4	(0.4, 0.3, 0.1)	(0.5, 0.3, 0.1)	(0.3, 0.4, 0.1)	(0.3,0.3,0.1)	0
F5	(0.6,0.2,0.1)	(0.4, 0.2, 0.3)	(0.7,0.2,0.1)	(0.4,0.2,0.3)	0.2
F6	(0.4, 0.2, 0.3)	(0.4,0.2,0.2)	(0.5, 0.3, 0.1)	(0.4,0.2,0.3)	0.2
F7	(0.8,0.1,0.1)	(0.7, 0.1, 0.1)	(0.6,0.2,0.1)	(0.6,0.1,0.1)	0.5
F8	(0.7,0.2,0.1)	(0.5,0.2,0.1)	(0.6,0.1,0.1)	(0.5,0.1,0.1)	0.4
F9	(0.5,0.1,0.1)	(0.4, 0.3, 0.1)	(0.4, 0.2, 0.3)	(0.4,0.1,0.3)	0.3
F10	(0.7, 0.1, 0.1)	(0.3, 0.4, 0.1)	(0.8, 0.1, 0.1)	(0.3,0.1,0.1)	0.2
F11	(0.5, 0.3, 0.1)	(0.6,0.2,0.1)	(0.3, 0.4, 0.1)	(0.3,0.2,0.1)	0.1
F12	(0.6,0.1,0.1)	(0.7,0.2,0.1)	(0.6,0.2,0.1)	(0.6,0.1,0.1)	0.5
F13	(0.4,0.1,0.1)	(0.5, 0.3, 0.1)	(0.4, 0.3, 0.1)	(0.4,0.3,0.1)	0.1
F14	(0.7,0.2,0.1)	(0.6,0.2,0.1)	(0.8,0.1,0.1)	(0.6,0.1,0.1)	0.5
F15	(0.7,0.1,0.1)	(0.6,0.1,0.1)	(0.6,0.2,.01)	(0.6,0.1,0.1)	0.5
F16	(0.4, 0.3, 0.1)	(0.7, 0.1, 0.1)	(0.7,0.2,0.1)	(0.4,0.1,0.1)	0.3
F17	(0.7,0.2,0.1)	(0.6,0.1,0.1)	(0.5,0.1,0.1)	(0.5,0.1,0.1)	0.4

Table 2: Fixed Points

Table 2: Fixed Politis	
On position of the Factors	Fixed Point
(10000)	(11111)
(01000)	(01110)
(00100)	(01110)
(00010)	(01110)
(00001)	(10001)







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	C1	C2	C3	C4	C5
C1	0	1	1	1	1
C2	0	0	1	1	0
C3	0	1	0	1	0
C4	0	1	1	0	0
C5	1	0	0	0	0

Fig 3: The connection matrix





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ISSN: 0976 – 0997 **RESEARCH ARTICLE**

Synthesis and Characterization of Novel HDAC Inhibitors using Various Analytical Techniques

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ABSTRACT

HDAC and histone acetyltransferase (HAT) enzymes are known to have an important role in regulating gene transcription through remodeling of chromatin, which comprises histone protein-DNA complexes that tightly package to form chromosomes. Inhibitors of the enzyme histone deacetylase (HDAC) are potent epigenetic regulators with significant therapeutic potential and pleiotropic effects on cellular and systemic levels. HDAC inhibitors are clinically employed for a wide range of illnesses, ranging from hematopoietic malignancies to psychiatric disorders. The aim of the study was to synthesize, purify and characterize novel cinnamyl sulfonamide hydroxamate derivatives. In the present study, cinnamic acid moiety was modified to obtain cinnamyl sulfonamide hydroxamate (CSH) derivatives, the design evolve the concept of bioisosterism. Two derivatives, 4-Chloro aniline and 4-Fluoro aniline CSH were synthesized, purified and structure was confirmed by various spectroscopic analytical techniques like UV, NMR, IR and mass spectroscopy respectively.

Keywords: HDAC enzyme, HDAC inhibitor, Cinnamyl sulfonamide hydroxamate, 4-Chloro aniline, 4-Fluoro aniline.





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INTRODUCTION

HDAC Enzymes

Histone deacetylases (HDACs) are a super family of enzymes originally named because they remove an acetyl group from ε -N acetyl lysine amino acid. HDACs deacetylate a wide range of non histone proteins and are more appropriately termed lysine-specific protein deacetylases. Histone deacetylase (HDACs) are involved in chromatin remodeling and modification of non histone transcription regulatory protein, thus regulating the expression of genes crucial for complex biological events. Dysregulation of HDACs and aberrant chromatin acetylation and deacetylation may a role in the pathogenesis of various diseases, including cancer and inflammatory diseases [1].

HDAC Inhibitors

The family of natural and synthetic compounds known as histone deacetylase (HDAC) inhibitors has a wide range of target specificities and biological activity. Based on their structures, HDAC inhibitors are widely divided into four classes: hydroxamic acids, cyclic peptides, benzamides, and short-chain fatty acids [2]. The modulation of immunological responses by HDAC inhibitors has been demonstrated to have a wide range of effects on cellular processes. HDAC inhibitors are now being researched for their potential to enhance recovery from spinal cord injury, a frequent result of accident injury [3]. Histone deacetylase (HDAC) inhibitors activate and repress subsets of genes that are likely to be regulated by an altered acetylation status in histones [3]. Alteration in balance of histone acetylation and deacetylation may have an impact on a variety of cellular processes including cell growth, differentiation, cell death, cell-cell and cell-matrix interactions and inflammatory responses [4].

MATERIALS AND METHODS

Synthesis

Synthesize and characterize novel cinnamyl sulfonamide hydroxamate (CSH) derivatives.

General Synthetic Scheme

General synthetic scheme for (E)-N-Hydroxy-3-(4-(N-(2-(thiophen-2yl) ethyl) sulfamoyl) phenyl) acrylamide Reagents and conditions:

Step I (a) chlorosulfonic acid (HSO₃CI), 34%.; stirred at 35 °C for 4 h;

Step II (b) 4-fluoro aniline and p-chloro aniline (c) double distilled water, (d) aq. Na₂CO₃; stirred at 35 °C for 4 h; **Step III** (e) Ethyl chloroformate, (f) CH₂Cl₂, (g) anhydrous condition, *cat*. N-methylmorpholine; stirred for 5 h, (h) NH₂OH, (j) CH₃OH, (j) aq. NaOH; stirred 2 h [5].

Synthesis of (E)-3-(4-(chlorosulfamoyl) phenyl) acrylic acid

Cinnamic acid **1** (1 g, 0.006 mM) and chlorosulfonic acid (0.69 g, 0.0054M) were stirred at 35° C for 4 h. Pre-coated TLC plates were used to monitor the progress of the reaction. The viscous reaction mixture was then poured into a beaker containg ice cubes. The resulting yellow colored precipitate was filtered, washed successively with 20 mL of distilled water and dried in *vacuo* (anhydrous CaCl₂) [6].

Synthesis of (E)-3-(4-(N-(phenyl chloro) and (phenyl fluoro) sulfamoyl) phenyl)acrylic acid

4-fluoro aniline and 4-chloro aniline were added to a suspension of (E)-3-(4-(chlorosulfamoyl) phenyl) acrylic acid 2 (2.46 g, 10 mM) in 50 mL distilled water, and pH 8 was maintained using aqueous NaHCO₃. At 35° C, the reaction mixture was stirred for 4 hours. The mixture was then adjusted to have a pH of 2 by adding 12 M HCl drop by drop. The product 3 was recovered as a white precipitate after being repeatedly washed with 20 mL of water, dried, and then recrystallized using ethyl acetate [5].

Synthesis of 3-(4-(N-(phenylchloro) and (phenylfluoro) sulfamoyl) phenyl) acrylamide. Compound 3 (1.4g, 46.5mM) in 30 mL of dichloromethane (Cl₂CH₂), ethyl chloroformate (5.0g, 56mM) and N-methylmorpholine (3.5g,





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13mM) were added under anhydrous condition and the reaction mixture was then agitated at 35°C for 5 h. The complete conversion of acrylic acid to acid chloride was monitored on a pre-coated TLC plate. Further, the conversion of acid chloride to the corresponding hydroxamate derivative was completed by the addition of freshly prepared neutral solution of hydroxylamine (0.5 g, 15 mM) in 30 mL of tetrahydrofuran (THF) and the reaction mixture was then stirred at 35 °C for 2 h and further partitioned with ethyl acetate and 2M HCl. The ethyl acetate layer was repeatedly rinsed with 20 mL of distilled water before being evaporated. The residue recovered was purified by column chromatography to obtain the final product [5].

RESULTS AND DISCUSSION

Two derivatives of cinnamyl sulfonamide hydroxamate (CSH) were synthesized, characterized and structure was confirmed by various spectral analysis.

Synthesis

The derivatives 4a and 4b were synthesized as per the procedure outlined in the methodology. The physicochemical properties of the compounds were given below.

Characterization of Synthesized CSH Derivatives

(E)-N-hydroxy-3-(4-(N-(phenyl chloro)sulfamoyl) phenyl) acrylamide (4a)

Yield =2.53g (78.57%); MP 246 °C; Rf value 0.59 (chloroform:methanol; 9:1) λ max=274.50nm (ethanol)IR (KBr,cm-1); 3262 (OH), 2923 (N-H 2°), 1671 (C=O carboxylic), 1380, 1185 (O=S=O),690(C-S)cm-1,respectively. 1HNMR(400MHz, DMSO(D6)) δ 10.89(s,1H), 7.98(d,J=8.92Hz,2H), 7.59(d,J=2.56Hz,2H), 7.31(d,J=8.6Hz,3H), 7.12(d,J=4.04Hz,2H), 6.7 (d,J=3.72Hz 1H),4.1369(s,1H),4.0199(s,1H)ppm; 13C NMR (400MHz, DMSO(D6)) δ 167.58,142.34,140.38,139.07, 136.99, 130.50,129.67, 128.82, 127.73, 123.07, 122.28ppm; MS (ESI): m/z= 353.25

(E)-N-hydroxy-3-(4-(N-(phenyl fluoro)sulfamoyl)phenyl)acrylamide (4b)

Yield =2.05g(66.55)%; MP 246 °C; Rf value 0.63 (chloroform:methanol; 9:1) λ max=223(ethanol)IR (KBr-1); 3260(OH), 2676(N-H 2°), 1672.55 (C=O carboxylic), 1382.88, 1186.71 (O=S=O), 672.93 (C-S) cm-1, respectively. 1H NMR (400MHz, DMSO(D6)) δ 7.981(d,J=4.8Hz,3H),7.702(d,J=15.84Hz,2Hz,2H),7.2647(d,J=8.82Hz,3H), 6.751 (d,J=15.72Hz,2H), 4.04(s,2H), 2.37(s,1H)ppm; 13C NMR (400MHz, DMSO(D6)) δ 168.1, 152.13,142.36, 139.99,132.76,130.49,129.67, 123.26 ppm; MS (ESI): m/z= 337.19

Two derivatives of cinnamyl sulfonamide hydroxamate (CSH) 4a and 4b were synthesized with a percentage yield of 78.57% (2.53g) and 66.55% (2.05g) respectively. The progress of the reaction was monitored using pre-coated TLC plates. Chloroform: methanol solvent system was used in the ratio 9:1. The synthesized compounds were purified by column chromatography. The isolated compounds were characterized by UV, infrared (IR), Mass and nuclear magnetic resonance (NMR) spectroscopy. The IR spectrum of 4a showed the presence of hydroxyl group at 3262cm-1. The peak of secondary N-H was seen at 2923cm-1 and carboxyl group, C=O at 1671cm-1. The peaks at 1380, 1185 and 1155 indicate the presence of S=O in the compound. The peak at 690.24 showed the presence of C-S group.

The IR spectrum of 4b showed O-H group at a peak of 3260.26. The peak at 2676 indicate the presence of secondary N-H group. The peak at which C=O was present at 1672.55. The peaks at 1382.88, 1186.71 indicate the presence of S=O. The peak at 672.93 showed the presence of C-S group. In 1H NMR of 4a and 4b,10 aromatic proton displayed a complex multiplet in the range of δ 6-8. In 4a, a peak at 10ppm indicate the presence of singlet N-H group. In both compounds, peak at 4.03, 4.05, 4.01, 4.13 also showed the presence of singlet. The 13C NMR spectrum of 4a and 4b showed the presence of C=O at a peak value of 167.58 and 168.1respectively. The peaks in the range 127-142 indicate the presence of C-aromatic group. The peaks at 122.21, 123.26 showed the presence of C=C group. The mass spectrum of 4a and 4b showed molecular ion peak as base peak at m/z 352 and 336 respectively.





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CONCLUSION

Two derivatives of CSH 4a [(E)-N-hydroxy-3-(4-(N-(phenyl chloro) sulfamoyl) phenyl) acrylamide)] and 4b [(E)-N-hdroxy-3-(4-(N-(phenyl fluoro) sulfamoyl) phenyl) acrylamide)] were synthesized & characterized and the structure was confirmed by spectral analysis. The pharmacological activity of these compounds can be further assessed using various screening methods.

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Table: 1:Physico chemical properties of 4a and 4b

DATA	4a	4b	
Molecular formula	C ₁₅ H ₁₄ O ₄ N ₂ Cl	C ₁₅ H ₁₄ O ₄ N ₂ F	
Molecular weight	352	336	
Melting point(°C)	246	220	
Percentage yield(%)	73.2	65.3	
Color	Yellowish white	Yellowish white	
Solubility in water	Insoluble	Insoluble	
Solubility in organic solvent(methanol, ethanol, DMSO)	Soluble	Soluble	



sulfamoyl)phenyl)acrylamide

4b-R=ClC₆H₄

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sulfamoyl)phenyl)acrylamide

3b-R=ClC₆H₄

OH a OH OH OH OH A OH A A A, b $A = FC_6\Pi_4$

General Synthetic Scheme





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RESEARCH ARTICLE

Assessing Effect of Economic Factors on Gold Prices in India using Multiple Linear Regression - A Case Study

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ABSTRACT

The present paper makes an attempt to investigate the effect of Foreign Reserves, Crude Oil Prices, Consumer Price Index and Deposit Growth on Gold Price using Multiple Linear Regression. For the purpose of the study monthly time series data of the above-listed variables were analyzed that spanned a ten-year period from January 2011 to December 2020 taken from a publicly available data at Investing.com. The influence of the factors was investigated using IBM SPSS software version 23 considering Gold as the dependent variable. The findings reveal that all of the economic factors have a considerable impact on gold, with Foreign Reserves and Crude Oil Prices having a positive impact on gold variability, with Foreign Reserves contributing the most. Consumer Price Index and Deposit Growth, on the other hand, have a negative impact on gold variability, with the Consumer Price Index contributing the most.

Keywords: Foreign Reserves, Crude Oil Prices, Consumer Price Index, Deposit Growth, Multiple Linear Regression

INTRODUCTION

Gold has traditionally been regarded as one of the most valuable metals in human history, as well as one of the most liquid and universally accepted mediums of exchange. Since its discovery, gold has been regarded as one of the most popular investment options. Due to the features it gives, like as liquidity, security, and portfolio, this gleaming yellow metal has always captivated all kinds of investors, regardless of their gender, geographical location, or other





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factors. Gold is now a commodity that can be traded and projected (Greely & Currie, 2009). It is the world's oldest hedging investment vehicle. Gold has historically been seen as a commodity that protects against the depreciation of money's purchasing power, hence gold investments are frequently undertaken to mitigate the effects of inflation and currency depreciation. Due to this ability, gold has the quality of maintaining value. Central banks utilize gold as a reserve component, and they all have a large amount of gold in their vaults. Gold is a common component of foreign reserve holdings, particularly in oil-producing nations. When investors want to place their money in safe assets, demand for gold rises. Gold prices have been fluctuating in the Indian commodities market for a long time. As a result of these price swings, determining the implications of the changes and the relationship between gold and other economic issues has become an important field of research.

Gold and Crude oil Prices

The inflationary link between gold and crude oil is well-known. Through commodity indexes, gold and oil have a link with one another. The price of crude oil is very important in determining the price of gold. Crude oil is a vital commodity that has an impact on every country's economy. The oil-producing and exporting economies will benefit from the higher crude price, while those that import oil for domestic consumption will suffer. The amount of oil produced and consumed is used as a gauge of economic activity. Crude oil and gold prices are thought to be positively correlated, and historical evidence shows that gold and crude oil prices follow the same pattern.

Gold and Foreign Reserves

It is impossible to exaggerate the significance of foreign exchange reserves to a nation's economic health. Lack of sufficient reserves can cause a nation's economy to stagnate and leave it unable to pay for essential imports like crude oil or service its external debt (Kuppusamy *et al.*, 2019). A foreign exchange reserve has never had a single definition across the board. The majority of central banks are different from one another in terms of item coverage, asset ownership, and liquidity. Global central banks have embraced the idea of the International Monetary Fund (IMF). A country's monetary authority can use foreign reserves to fund balance of payments obligations, manage currency exchange rates in currency exchange markets, and perform other relevant tasks, according to the IMF. In India, the Reserve Bank of India (RBI) Act, 1934 empowers the RBI to act as the custodian of foreign exchange reserves and to manage reserves in accordance with established goals. The RBI accumulates foreign exchange reserves through purchases from Authorized Dealers and income on foreign exchange assets deployed. Foreign exchange reserves are also bolstered by government aid receipts.

Gold and Consumer Price Index

There is a common belief that gold can act as an inflation hedge. The monthly changes in the Consumer Price Index are used to calculate inflation, which is defined as a general rise in the price level (as opposed to an increase in the money supply). Gold would need to exhibit a negative correlation with inflation in addition to being uncorrelated in order to function as a traditional hedge against it (Fei and Adibe 2010). During the global financial crisis of 2007-2009, a negative link is discovered. During a recession, demand for both consumption and stock investments falls, resulting in a prolonged reduction in the consumer price index, which leads to a drop in the intrinsic value of asset prices, suggesting that the economy is in deflation. The price of gold, on the other hand, is rising as investors seek a secure alternative in gold.

Gold and Deposit Growth

Due to their role as financial intermediaries in bridging the gap between the society's surplus and deficit, banks serve as the main support for the expansion of the economy (Saunders and Cornett, 2011). One of the crucial criteria by which the bank contributes to economic development is deposit mobilization. Deposits play a crucial role in all aspects of banking, particularly in developing nations (Ünvan and Yakubu, 2020). Even if there are several investment opportunities with generally superior net returns (including tax incentives), the Indian banking sector is concerned since the deposit is still chosen above other options.





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Literature Survey

Several researches used various econometric techniques to look at the co-movement, co-integration, and lead-lag relationship between crude oil and gold. From April 1960 to November 1985, In order to demonstrate the strong correlation between the prices of oil and gold, Cashin et al. (1999) examined the correlations between seven different commodities. Pindyck and Rotemberg also developed the regular co-movement of oil and gold prices (1990). Fattouh looked into the asymmetries in the spread adjustment processes for commodities like oil and other metals (2010). Most studies employed inflation linkage to explain the relationship between the price of gold and the price of oil. Zang et al. investigated the co-integration connection and causality of the relationship between gold and crude oil prices (2010). A substantial positive association between the price of gold and the price of crude oil was discovered by the study to exist during the sample period. The long-term relationships between gold, crude oil, and futures contracts were examined by Narayan et al (2010). The following findings were reached: (2) Gold prices can be approximated using oil prices or the other way around. (1) Investors utilise gold as a hedge against inflation. Jana examined the connection between the price of crude oil and the price of gold (2011). She also researched fundamentals and pricing trends. She used a number of econometric techniques, such as the Vector Error Correction Model, Johansen co-integration evaluation, and Granger causality, to get to the conclusion that the two variables were causally associated. The relationships between seven commodities are examined by Cashin et al. (1999) between April 1960 and November 1985. The empirical findings of this inquiry show a strong relationship between gold and

It is demonstrated that the levels of gold and oil prices move together consistently, as Pindyck and Rotemberg theorised (1990). The asymmetry in the spread adjustment process for oil and metal commodities is studied by Ewing et al. (2006) and Fattouh (2010). Le and Chang (2011) showed that there is a long-term correlation between the prices of gold and oil, indicating that gold can be used as a hedge against inflation and that gold prices can be predicted using the price of oil. In 2010 (Narayan et al.) Investors use gold as a hedge against inflation because of market inefficiencies, and they can estimate gold market prices using information from the oil market and vice versa. Baber et al. (2013) found a high (about 900 percent) increase in gold prices in India between 2002 and 2012. They not only identified the growth, but also attempted to pinpoint the causes, including international trade, the political and financial climate, market circumstances, and its introduction into the commodity market, consumer purchasing behavior, and inflation. In addition, an empirical analysis was carried out using secondary data from the Reserve Bank of India's various databases (RBI). They discovered a positive association between inflation, gold prices, and dollar prices at first glance. The Hotelling Squared t-test was also applied, and the relationship between the three variables was found to be statistically significant.

METHODOLOGY

The primary tool for establishing a connection between a pollutant's kind, quantity, and activity of release into the atmosphere is multilinear regression analysis. Additionally, it makes it easier to determine each pollutant's contribution to the air quality index as well as to estimate emissions from various sources of air pollution. The strength of the association between each independent variable and one dependent variable is evaluated using the multilinear regression analysis technique. The multilinear regression model for a dependent variable y with observed values y_1, y_2, \ldots, y_n (where n is the sample size) and q independent variables x_1, x_2, \ldots, x_q with observed values x_1, x_2, \ldots, x_q for $i = 1, 2, \ldots, n$ is

$$y_i = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \dots + \beta_q x_{qi} + \varepsilon_i$$

The term ε_i is the residual or error for individual i and represents the deviation of the observed value of the dependent variable for this individual from that expected by the model. These error terms are assumed to have a normal distribution with variance σ^2 . The regression coefficients $\beta_0, \beta_1, \ldots, \beta_q$ are generally estimated by least squares Draper and Smith (1998). The correlation between the observed values of the dependent variable and the





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values predicted by the model, also known as the multiple correlation coefficient R, is a metric for assessing how well the model fits the data.

$$\hat{y}_i = \hat{\beta}_0 + \hat{\beta}_1 x_{i1} + \hat{\beta}_2 x_{i2} + \dots + \hat{\beta}_a x_{ia}$$

R2's value indicates how much of the dependent variable's variability was accounted for by the independent variables. The evaluation of individual regression coefficients can be done using $\hat{\beta}_j/SE(\hat{\beta}_i)$ (Pantula and Dickey,1998)

RESULTS AND DISCUSSIONS

Correlation of economic factors with gold is shown in Table-1. As the correlation coefficient > |.3| of economic factors with dependent variable Gold hence they correlate quite strongly with Gold. Multi-collinearity between economic factors is ruled out as their correlation is <.7. From the R-square value of Table-2,economic factors contribute to 93.9% of the variability of Gold. The economic factors significantly predict Gold. F (4, 93)=358.409, p<.001indicates that four factors under study have a significant impact on Gold. Moreover, R2=.939 depicts that the model explains a93.9% variance in satisfaction. The standardised regression coefficients address this problem and permit at least an attempt at comparison by expressing the coefficients in terms of a single, common set of statistically suitable units. According to Table 3, every economic factor's standardised Beta values contribute significantly to the fluctuation of gold. The Un-standardized explains how each component and Gold are related to one another. They inform us of how much each factor influences the result when the impact of all other factors are maintained constant. Gold = 71.327+(.750)Foreign Reserves + (.017) Crude oil prices- (13.162) Consumer Price Index-(4.994) Deposit Growth. From Un-standardized Coefficients in the above equation Gold is positively correlated with Foreign Reserves and Crude oil prices however, it is negatively correlated with Consumer Price Index and Deposit Growth. Squared Part Correlations indicate that Foreign Reserves contribute 29.8% variance to the total variability which is quite large when compared with 5.2% of Crude oil prices, 9.6% of Consumer Price Index and 1.9% of Deposit Growth.

CONCLUSIONS

Multilinear Regression is successful in demarcating the contributions of various economic factors to Gold. It was observed that the maximum variability in prices of Gold is significantly contributed by Foreign Reserves followed by Consumer Price Index and Crude oil prices. However, Consumer Price Index has negative effect on Gold Prices.

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Table.1: Correlation of gold with economic factors

	Correlations							
		Gold price	Foreign Reserves	Crude oil prices	Consumer Price Index	Deposit Growth		
	Gold price							
	Foreign Reserves	1.000	.851	360	666	753		
Pearson	Crude oil prices		1.000	485	384	566		
Correlation	Consumer Price Index			1.000	.537	.365		
	Deposit Growth				1.000	.598		
						1.000		

Table.2 Model Summery

	Model	df	F	Sig.	R	R Square	Adjusted R Square
1	Regression	(4, 93)	358.409	.000	.969	.939	.936

Table.3 Regression Coefficients

Economic factors	Standardized Coefficients (Beta)	t-value	t-value Sig Part Correlation		Square of Part Correlations
Foreign Reserves	.715	21.350	.000	.546	.298
Crude oil prices	.291	8.883	.000	.227	.052
Consumer Price Index	431	-12.125	.000	310	.096
Deposit Growth	197	-5.449	.000	139	.019





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RESEARCH ARTICLE

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Pharmacological Evaluation of Lapatinib for Alzheimer's Disease by In silico, In vitro and In vivo Methods

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ABSTRACT

Alzheimer's is progressive neurodegenerative disease which mainly affects older individuals. It is associated with the deposition of beta- amyloidal protein and hyperphosphorylation of tau protein in brain. The present study is done to evaluate the neuroprotective activity of Lapatinib (Tyrosine kinase inhibitor), for in vivo and in vitro study on Alzheimer's disease induced rats. In silico molecular docking studies were carried out by using Auto dock software. In vitro anti-inflammatory and anti-oxidant studies were carried out. Elevated plus maze, Morris water maze, Actophotometer and Novel object recognition test were conducted to evaluate learning and memory parameters. Various biochemical parameters such as AchE, Catalase, SOD and TBARS assay were also assessed. Histopathology examination were carried out. In silico molecular docking shows greater binding of Acetyl cholinesterase receptors (1HOY) with ligands. The Lapatinib shows potential therapeutic activity in rats through inhibiting COX, LOX and Cellular nitrite, augmenting antioxidant enzymes and inhibiting AchE and TBARS. Lapatinib shows memory improvement effects on behavioural studies such as Elevated plus maze, Morris water maze, Actophotometer and Novel object recognition test. The histopathological study confirmed the recovery. The Lapatinib (20 mg/kg) has shown effectiveness against oxidative stress and neuronal inflammations. The In silico, In vivo and In vitro studies supported that Lapatinib has Neuroprotective activity.

Keywords: Lapatinib, Neuroprotective activity, elevated plus maze, Morris water maze, Actophotometer, Novel object recognition, COX, LOX, Histopathological assessment.





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INTRODUCTION

Alzheimer's disease is an amnestic disorder with subsequent progression to other cognitive domains such as visuospatial abilities, language, and memory function. Degeneration and dysfunction of large-scale cortical systems parallel the decline of cognitive functions observed in dementia [1]. The majority of patients develop clinical symptoms at age older than 65 years and are called late-onset Alzheimer's disease, symptoms occur at age below 65 years are called earlier onset of disease. Amyloid precusor protein and presenillin proteins are responsible for early onset disease. The reduction of Apo lipoprotein E are responsible for the late onset of Alzheimer's disease[2]. The Epidermal growth factor receptor tyrosine protein kinase inhibitors (EGFR TKI's) are the targeted drugs for the cancer. TKI's has the capacity to protect the neurons against oxidative stress, suppress the neuroinflammation and the ability to promote memory, learning and cognition. EGFR TKI's particularly inhibit the protein tyrosine kinase EGFR [3].

MATERIALS AND METHODS

Drugs and Chemicals

Aluminium chloride (Aiswarya Laboratory suppliers, India), Donepezil (DNZ), Lapatinib (LTB) (Mayoora Pharmaceuticals, India), Saraswatharishtam (STM) (AryaAyurvedic medical shop, India). All other reagents used were of analytical grade.

In silico studies

In silico computer simulation studies and docking studies are proved to be the best tool used to investigate the complementarity and level of interaction at the molecular level between the compounds of natural or synthetic origin. Molecular docking was performed using AutoDock vinaPyRx software.

In vitro Anti-inflammatory activity

In vitro Cyclooxygenase [4], Lipoxygenase [5] and Cellular nitrite level[6] were carried out.

In vitro Anti-oxidant activity

In vitro estimation of reducing power [7], determination of Antioxidant activity by Phosphomolybdenum method [8] were determined.

Animals

Albino rats, 9-11 weeks old with a weight range of 250-300 g were housed in a temperature-controlled room under light/dark cycle. These were given access to pellet food and water ad libitum throughout the experiment and maintained under controlled temperature ($22\pm3^{\circ}$ C), humidity ($55\pm5\%$). The behavioral experiments were carried out. This study protocol and the number of animals required for the experiment was approved by Institutional Animal Ethics Committee (ECPS/IAEC-2-2019-5/28) and the national guidelines on the care and use of laboratory animals were followed. All experimental animals were sacrificed and their brain were harvested.

Experimental methods

The Alzheimer's disease were induced by administration of Aluminium chloride. Animals were divided into 5 groups (n=5). Group I (Control) was given daily oral doses of 10ml/kg normal saline. The remaining animals were administered with 100mg/kg AlCl₃ daily for 21 days to induce Alzheimer's disease, and were further divided into 3 groups. This 3-5 groups were the treatment groups. Group 3 receiving an oral dose of Donepezil (3mg/kg); Group 4, an oral dose of Saraswatharishtam (200mg/kg) and group 5, an oral dose of Lapatinib (20mg/kg). All groups were treated with their respective treatments for 21 days (from day 21-42). At the end of the experiment the animals were sacrificed and the brain were harvested for the biochemical and histopathological investigations [9,10] and [11].





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BEHAVIORAL ANALYSIS

Elevated Plus maze (EPM)

Elevated plus maze is a widely used behavioural study for rodents to assess the neuroprotective effects of various pharmacological agents. The apparatus consists of 2 open arms ($50 \times 10 \times 40$) and 2 closed arms ($50 \times 10 \times 40$). The 2 open arms are opposite to each other with a central square of 10 cm. Here the transfer latency time is determined. That is the time required by the animal to transfer from open arm to any one of the closed arm [12].

Morris water maze (MWM)

In the present study each group of experimental animals were subjected for acquisition training with the invisible platform. There are two basic trials, one utilizing a hidden platform. On the first experimental day rats were trained to swim in the maze (in the absence of platform) in 60 seconds. In five subsequent days the special memory of the experimental animals were assessed on the basis of the escape latency period of animals in the morris water maze [13].

Actophotometer

An Actophotometer has a square arena in which the animal moves. The locomotor activity can be measured using an actophotometer which operates with help of photoelectric cells which are connected in circuit with a counter. The total number of light beams assessing the movement of the animal [14].

Novel object recognition test (NORT)

Training (T1)

Place two identical objects in opposite quadrants of the area. 24 hour after habituation the animals were presented with 2 identical Objects and allow free exploration for a minimum of 5 min.

Testing (T2)

Place one object used during T1 (i.e., the familiar object) and one novel object in opposite quadrants of the area. Use the same locations as used during T1 for each rat. The time spent by the rat in both familiar and novel object were determined. Allow free exploration for 10 minute [15].

STATISTICAL ANALYSIS

The data were expressed as SEM and were analysed by using Graph pad software version 8.0. ANOVA is also carried out followed by Student-Newman-Keuls tests.

RESULT AND DISCUSSIONS

In Silico Molecular Docking Studies

The protein used for the docking studies are downloaded from PDB. The main PDB includes the 1HOY- acetyl cholinesterase. Figure 1 shows the docked image of Lapatinib with Acetyl cholinesterase receptor.

In vitro Studies of Lapatinibon Anti-Inflammatory assays

COX is the first enzyme in the pathway for producing Prostaglandin and thromboxane from Arachidonic acid. Inhibition of LOX can block the production of proinflammatory mediators. Percentage inhibition of COX, LOX and Cellular nitrite activity is estimated in RAW 264.7 Cell lines and compared with standard Diclofenac (DFC) that treated in same conditions. Decreased cellular nitrite level is an indication of the capacity to inhibit nitric oxide synthase, thus inhibiting the production of nitric oxide. The results were shown in Table 1.





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In vitro Studies of Lapatinib (Ltb) on Antioxidant assays (Table.2) Behavioral Analysis

Effect of LTB on behavior of animals on EPM.

The animal required less time to transfer to any one of the closed arm, improves the memory. The results of the study shows that LTB significantly reduce the transfer latency time in EPM. The result of the test were summarized in figure 2.

Effect of LTB on behavior of animals on MWM.

In MWM the time required by the animal to escape into the hidden platform were determined. The results of the study shows that LTB significantly reduce the Escape latency time in MWM. The result of the test were summarized in figure 3.

Effect of LTB on behavior of animals on actophotometer.

The locomotor activity were determined by using actophotometer. The results of the study shows that LTB significantly increases the locomotor activity in Actophotometer. The result of the test were summarized in figure 4.

Effect of LTB on behavior of animals on NORT.

If the animal recognizes the familiar object then it will spend most of its time at the novel object. The results of the study shows that the LTB significantly increases the time spent in novel object as compared to the familiar object. The result of the test were summarized in figure 5.

Biochemical Estimation

Acetyl cholinesterase inhibitors prevent the formation of AchE and used for the treatment of Alzheimer's disease. The results of the study shows that LTB significantly reduce the AchE level in blood. The antioxidant enzyme catalase protect the brain against Alzheimer's disease. The results of the study shows that LTB significantly increases the catalase level in brain. SOD is an antioxidant enzyme which reduces the oxidative stress. The results of the study shows that LTB significantly increases the SOD level in brain. In TBARSassay Malonedialdehyde levels were determined which is the major biomarker of Oxidative stress. The results of the study shows that LTB significantly reduces the TBARS level in brain. The results are summarized in Table 3.

Histopathological Examination

The Histopathological evaluations were carried out and the results are summarized in figure 6. In histopathological studies, it was visible that AICl₃ induced group shows neurodegeneration which leads to cell death in the rat brain (Figure 6). The degenerated cells were seen in the form of lesions, these lesions were seen as the main markers of cell death or apoptosis. A -Show the control group which does not shows any change in the brain section therefore there is no neuronal degeneration or cell death. B-Shows the negative control group which clearly shows the degeneration of neurons and massive death of neuron causes encephalomalacia, C- shows the standard DNZ (3mg/kg) treated group which reveals the regeneration of the neuron cells and number of lesions were also reduced. D- Represents the photographic section of brain in which animals treated with STM (200mg/kg), reveals a small change in neuronal cells compare to the negative control. E- Represents the LTB (20mg/kg) treated groups, here the lesions and degenerations were reduced due to the decrease in various cellular mediators, increasing antioxidant activity and this group shows the normal his to structure that reveals the activity of the drug in the brain.

DISCUSSIONS

In this study, the virtual screening and molecular docking of the compounds against acetyl cholinesterase (1HOY) with Auto Dock scoring functions for neuroprotective activity were determined. The LTB undergo docking studies with Acetyl cholinestrase receptor. The least binding affinity (more negative value) of the compound is considered as





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the best drug in pharmaceutics. The best docking score was shown by Lapatinb (Test drug) -7.8 with hydrogen bond interaction 5. The Standard Donepezil shows docking score -6.9 and Hydrogen bond interaction 4. This reveals the good binding of the ligands with protein (acetylcholinesterase-1HOY) and shows the better anti-alzheimer's activity of LTB. Inhibition of COX and LOX can block the production of proinflammatory mediators. The agents that inhibit these pathways are useful as potential anti-inflammatory agents. The study indicates that LTB at concentration 100mcg/ml produced significant (P<0.001) increase in anti-inflammatory activity as compared to standard Diclofenac at different concentration level, $25\mu g/ml$, $50\mu g/ml$, and $100\mu g/ml$. LOX also leads to synaptic dysfunction and cognitive impairement. LOX acts on GSK 3β , CDK5 leads to cognitive decline. The percentage inhibition of inflammation at different concentration were increased. The LTB were found to be a good inhibitor of COX and LOX therefore must possess anti-inflammatory actions. The reduction of both these enzyme were found to be in a dose dependent manner. Nitric oxide regulates the proinflammatory mediators and leads to cell death. Decreased cellular nitrite level is an indication of the capacity to inhibit nitric oxide synthase, thus inhibiting the production of nitric oxide. Decreased cellular nitrite level is an indication of the capacity to inhibit nitric oxide synthase, thus inhibiting the production of nitric oxide.

The reducing power method is based on the principle of increase in the absorbance of the reaction mixtures. The absorbance increases, the antioxidant activity also increases. Higher absorbance indicates higher reducing power. The study reveals that LTB produced significant antioxidant activity (P<0.01). The absorbance of LTB were increased with increase in concentration. The total anti-oxidant activity was also studied by Phosphomolybdenum method. In this method the absorbance increases with increase in concentration. If absorbance increases, anti-oxidant property is also increases. The assay is based on the reduction of Molybdenum VI to Molybdenum V therefore the LTB possess significant (P<0.001) increase in anti-oxidant activity. In the all five *in vitro* methods, there was an increased anti-inflammatory and anti-oxidant activity in dose dependent manner. The findings of the study suggests that LTB have the ability to prevent inflammation and oxidative stress. For the behavioral study of the animals different apparatus was used such as EPM, MWM, Actophotometer and object recognition apparatus. AlCl₃ (100 mg/kg) induced rats showed higher transfer latency (TL) values as compared to the control group. This finding suggests that Alcl₃ adversely affect the memory potentiation. LTB was able to restore the memory of the rats as the results showed.

The Morris water maze was used for determination of escape latency time shown by the animals. Rat in vehicle treated group, administered with distilled water showed a downward trend in their escape latency time (ELT). AICI₃ treated group showed significant increase in ELT when compared to the control group. The latency period of DNZ and STM was significantly reduced on day 35 and 42 (P<0.01), (P<0.001). Groups treated with LTB 20mg/kg showed the lesser time to locate the platform and significantly (P<0.05), (P<0.001) reduced the ELT period. These findings suggest that the Lapatinib increased the special memory and cognitive performance of the experimental animals. Spontaneous locomotor activity was tested using Actophotometer in rats of all groups AICI₃ treated group showed decrease in Locomotor activity. The groups were treated with DNZ and STM shows a significant (P<0.01), (P<0.01) increase in Locomotor activity on day 35 and 42. The LTB treated groups also showed a small significant (P<0.05), (P<0.01) increase in activity on both day 35 and 42. These finding suggests that LTB through a series of chemical events is able to counteract and restore the locomotor activity.

NORT is used for the determination of learning and memory. If the animal recognizes the familiar object then it will spend most of its time at the novel object. As per the study the disease induced group of animals spent more time in Familiar object than the novel objects. The groups were treated with Standard DNZ and STM significantly (P<0.001) increase the time spent in novel object. The groups which were treated with LTB and compared with the induced group, the significance were (P<0.01). These findings reveals that the drug Lapatinib improves the memory. The determination of Acetyl cholinesterase gives the measure of Acetylcholine level in the brain. The AICI3 induced group showed a significant increase in the level of AchE. The level of AchE was found to be decreased significantly (P<0.001) in the dose of 20mg/kg as compared to the induced animals. Catalase enzyme was considered to be the main regulator of hydrogen peroxidase metabolism. The antioxidant enzyme catalase protect the brain against AD. From the result obtained, the treatment with LTB increases the level of catalase significantly (P<0.01). The activity of





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SOD reduce the oxidative stress related AD. The level of antioxidant enzyme was found to be decreased in induced group of animals. The present study reveals that the DNZ increase the level of SOD significantly (P<0.001). The STM and LTB groups also attenuate SOD significantly (P<0.01). The level of TBARS was found to be increased in the induced group as compared to the control group. The treatment with the DNZ shows reduced TBARS as compared to the induced group. STM at 200mg/kg reduced TBARS level significantly (P<0.01). LTB group shows small reduction in TBARS significantly (P<0.05) when compared to the group treated AICI30nly. These finding suggest that the LTB significantly reduced the oxidative stress by increasing the level of antioxidant enzyme such as CAT and SOD and reducing the level of AchE and TBARS. Figure 7 shows the Proposed Mechanism of action of Lapatinib.

CONCLUSION

Lapatinib were shown to modulate signaling through tyrosine kinase, Cyclin dependent kinase and Mitogen activated protein kinase pathways. These signaling cascades are also critical for the control of inflammatory processes in the brain, including the activation of microglia in response to cytokines and the induction of nitric oxide production. The study revealed the potential of Lapatinib as a neuroprotective drug. However further studies have to be undertaken to establish the exact mechanism of the above mentioned actions by isolating and elucidating the structure of the active principles. Lapatinib is not only suggested as a novel strategies for the reduction of deleterious effects of neuroinflammation in the brain, but also as having a direct influence on memory acquisition, consolidation and storage through the induction of protein synthesis in neurons.

CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this investigation.

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Table 1: Effect of LTB on Cellular nitrite. The values are expressed as mean ±S.E.M, n=3, the two way ANOVA was carried out by Student Newman-keuls test, Where a,b,c Significance at concentration 25, d,e,f Significance at concentration 50 and g,h,I Significance at concentration 100mcg/ml with P<0.001, P<0.01 and P<0.05 respectively.

Anti-	Concentration (mcg/ml)							
inflammatory	2	5	5	0	100			
activity	DFC	LTB	DFC LTB		DFC	LTB		
COX	22.24±0.523	14.71±0.321c	34.63±0.407	29.24±0.493e	55.85±0.987	52.25±0.490g		
LOX	26.57±0.299	22.15±0.442a	41.36±0.601	37.96±0.571d	58.24±0.574	54.61±0.312 ^g		
Cellular nitrite	10.95±0.111	7.77±0.295b	7.56±0.381	5.70±0.236d	4.09±0.332	2.91±0.111g		

Table 2: Effect of LTB on Anti-oxidant activity. The values are expressed as mean ± S.E.M, n=3, the two way ANOVA was carried out by Student Newman-keuls test, Where a,b,c Significance at concentration 25, d,e,f Significance at concentration 50 and g,h,I Significance at concentration 100mcg/ml with P<0.001, P<0.01 and P<0.05 respectively.

		Absorbance (nm)					
Antioxidant Assays	25		50		100		
Antioxidant Assays	AA	LTB	AA	LTB	AA	LTB	
Reducing power	0.39±0.033	0.12±0.065ns	0.74±0.075	0.45±0.023e	0.90±0.060	0.68±0.81h	
Phosphomolybdenum method	0.37±0.042	0.17±0.034°	0.60±0.056	0.42±0.029e	0.90±0.085	0.7±0.87g	

Table 3: Effect of LTB on Biochemical parameters. The values are expressed as mean± S.E.M, n=6. The statistical analysis were carried out using one way ANOVA, Where a.b.c significance between control group and x.y.z significance between induced group with P<0.001, P<0.01 and P<0.05.

Group	Control	induced	DNZ	STM	LTB
AchE	891±6.5	1200.5±8.2a	925.9±6.7 ^{c,x}	968.2±8.5 ^{c,x}	987.6±9.8 ^{c,x}
Catalase	3911.7±9.3	1851.7±8.5a	3305.7±7.3c,x	3019.7±9.9c,x	2809.5±1.3b,y
SOD	18.81±0.3	7.84±1.6a	16.27±0.3c,x	12.77±0.4b,y	11.78±1.5 ^{b,y}
TBARS	15.96±1.1	33.78±0.2a	17±0.2 ^{c,x}	22.5±0.2b,y	27.15±0.9a,x



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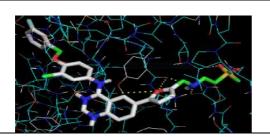


Figure.1: Docking image of Lapatinib with Acetylcholine receptor (1HOY)

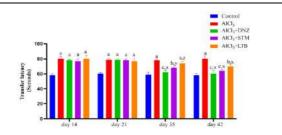


Figure 2: Effect of LTB on behavior of animals on EPM. The values are expressed as mean ± S.E.M, n=6, two way ANOVA were carried out by Student-Newman-Keuls test, Where a,b,c significance between control and x,y,z significance between induced group with P<0.001, P<0.01 and P<0.05

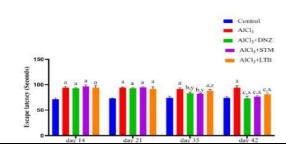


Figure 3: Effect of LTB on behavior of animals on MWM. The values are expressed as mean ± S.E.M, n=6, the statistical analysis were carried out using two way ANOVA followed by Student-Newman-Keuls test, Where a,b,c significance between control and x,y,z significance between induced group with P<0.001, P<0.01 and P<0.05.

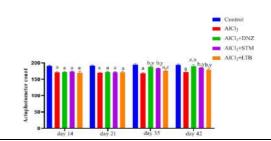


Figure 4: Effect of LTB on behavior of animals on Actophotometer. The values are expressed as mean ± S.E.M, n=6, the statistical analysis were carried out using two way ANOVA followed by Student-Newman-Keuls test, Where a,b,c significance between control and x,y,z significance between induced group with P<0.001, P<0.01 and P<0.05.

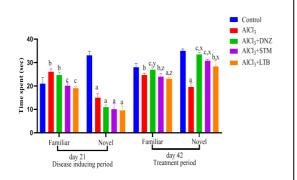


Figure 5: Effect of LTB on behavior of animals on NORT. The values are expressed as mean ± S.E.M, n=6, the statistical analysis were carried out using two way ANOVA followed by Student-Newman-Keuls test, Where a,b,c significance between control and x,y,z significance between induced group with P<0.001, P<0.01 and P<0.05.

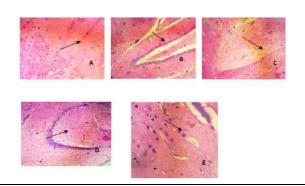


Figure 6: The histopathological examination reveals A - Control, B-AICI3 induced, C-AICI3+DNZ, D- AICI3 + STM and E- AICI3 + LTB.



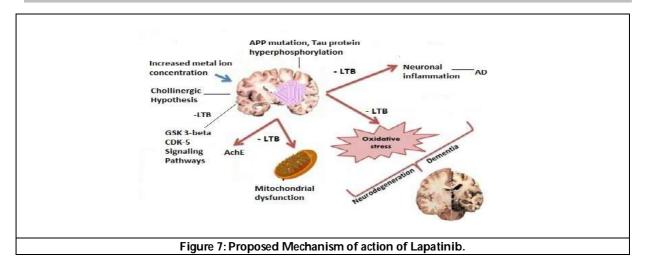


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ISSN: 0976 – 0997 **RESEARCH ARTICLE**

The Role of Socio-Demographics on Doctor Motivation and Turnover in Tamil Nadu CHCs- Smart PLS Analysis

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ABSTRACT

Tamil Nadu is the one of the states having a greater number of doctors than WHO ratio 1: 1000, yet specialists and GDMOS shortage in community health centers is a continuous pattern of rural health statistics from the year 2015 despite many policies. The study efforts to analyze the ERG (Existential, Relatedness and Growth) needs of doctors in relation with the turnover intention and to investigate the moderating effect of socio-demographic variables on the relationship between ERG dimensions and turnover intention. Structured questionnaire representing Existential, Relatedness and Growth needs was designed based on the review of the literature. Hypotheses have been set for the investigation to test the relationship and investigate the moderating effects of age, gender, years spent in a rural area, marital status, years of service and future location choice. A negative association was discovered between ERG needs and turnover intention. Absence of 'Growth needs' was shown to be the most crucial predictor of rural CHC doctors' turnover intention. The relationship between ERG motivation and turnover intention is influenced by doctors' marital status, childhood years spent in rural areas, and future location choices. Identifying these aspects will enable policymakers, academics, and public health practitioners to devise rural retention strategies for doctors.

Keywords: Rural CHC doctors, ERG dimensions, Turnover intention, Moderation, demographic variables.





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INTRODUCTION

Tamil Nadu is one of the largest states in India, having 97.35 per cent of the rural land with a rural population of 37229590 as of the 2011 census [1] .The objective of Health Sector 2023 is for Tamil Nadu to become India's number one state in terms of societal health indicators by delivering universal access to health services[2]. Rural Health Statistics, 2020 shows that there are now 179 GDMO vacancies in rural CHCs [3]. Moreover, CHCs have a massive shortage of specialists as of 2015. Currently, there is a shortage of 1312 specialists. Despite the need for 1540, just 228 have been filled [4]. Keeping rural doctors is a constant battle, so that NHP 2015 underlined the need for more rural medical students to return to their communities and enhance access to health care for the poor [5]. Also, the government took some compulsory measures for retention like mandatory rural postings and mandatory rotational postings [6]. However, the applicability of the standards is a big challenge. Tamil Nadu is one of six states with more physicians than the WHO's 1: 1000 ratio. It has four physicians per 1000 people, but abundancy in urban areas, producing rural shortages. It reflects doctors' disdain towards rural Tamil Nadu. Following the alarming facts, the study sought to find out the motivational demands, reasons for turnover intention and the moderating impacts of socio-demographic characteristics.

MATERIALS AND METHODS

Objectives of the study

- To find out the relationship between ERG needs and turnover intention as perceived by rural CHC doctors in Tamil Nādu.
- To investigate the moderating effect of socio-demographic variables on the relationship between ERG dimensions and turnover intention.

This study employs a cross-sectional analytical approach and stratified sampling technique under the probability method. Ethical approval was taken from IRB, IIHMR University.

Questionnaire design

Structured questionnaire representing Existential, Relatedness and Growth needs was designed based on the review of the literature. The items relevant to ERG needs taken from 7 validated scales. In addition, three qualitative studies' themes were itemized to form the questionnaire. The validated turnover intention scale – TIS-6 by Roodt used in this study to measure the turnover intention of rural doctors [7]. On the basis the objectives, hypotheses have been set for the investigation to test the relationship and investigate the moderating effects of age, gender, years spent in a rural area, marital status, years of service and future location choice.

RESULTS

Objective no.1

Find out the relationship between ERG dimensions and turnover intention

The reliability of questionnaires was verified to determine the Dimensions of ERG and turnover intention, tested by the Cronbach α test through SPSS software. Overall reliability of the questionnaire, i.e.,0.933 for 43 items, is highly reliable [8]. Next the dimensions of ERG were extracted through the Exploratory Factor Analysis technique.

The results of Exploratory Factor Analysis (EFA)

KMO = 0.942 > 0.5 indicated adequate sampling size to access factor structure. The data acquired for the dimensions of ERG were eligible for factor analysis since Barlett's test of sphericity was significant at 0.000 <0.05[9]. Through EFA, four components were extracted with a variance of 63.909%, and all the items were forced to form a single factor[10]. The new extracted factors were Factor 1 (Existential needs: EN) comprising of 8 items, Factor 2(Societal relatedness needs: RN_S) entailing of 5 items, Factor 3 (Family relatedness needs: RN_F) involving of 5 items and Factor 4 (Growth needs: GN) residing of 6 items describing the variances of 19.078, 15.819%, 14.517% and 14.495%





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respectively. Further to check the validity and causal relationships, the dimensions of ERG need and Turnover intention were further subjected to CFA and Structural Equation Modelling (SEM) analysis [11]. It comprises of two parts; one is measurement model and next is structural model.

Measurement Model

Before constituting the structural model, the path of the framework to be utilized for constructing the model was checked through the measurement model. The confirmatory factor analysis technique was used to determine the constructs' reliability and validity as derived after exploratory factor analysis.[12](Fig.2, Table.1) The square root of average variance extracted values and discriminant validity values were verified with the Fornell and Larcker criteria and the Heterotrait and Monotrait ratio (HT-MT ratio) [13][14]. HTMT ratio indicated the correlation values between the latent constructs to show the extent of the uniqueness of the measure. (Table 2).

Structural model

Hair et al. advocated, SEM to analyze the cause-and-effect relationship between the dependent and independent variables [15]. Dimensions of ERG needs as obtained after the Confirmatory Factor Analysis was used further in envisaging the proposed conceptual relationship of ERG dimension with turnover intention. After removing the insignificant dimension, i.e. "Family relatedness needs", the final structural model comes up with three significant dimensions and were tested with turnover intention and checked for model fit. The key standards for the internal structural model evaluation are the determination coefficient R^2 and the path coefficient (β -value) and the T-statistic value, the impact size f² and the model's predictive significance Q². Path coefficient and total effect were used to test the hypothesis. It is the most reliable source for examining multi-correlational variables [16]. Furthermore, the t-value must be larger than 1.69, and the p-value must be less than 0.05 [17]. R² levels of 0.04 to 0.16 are moderately poor in social sciences, according to Cohen and Ritchey, whereas R² values of 0.25 to 0.49 are moderately high [18][19]. The R² score in this study is 28.6 per cent, indicating that the model generated has a moderately strong effect. Hair recommends the f-values 0.35 (strong effect), 0.15 (moderate effect), and 0.02 (weak effect) [15]. GN and RNS considerably influence TOI, whereas EN has a minor impact.(Table.3) Q2 values greater than 0, 0.25, and 0.50, respectively, indicate the PLS-path model's small, medium, and significant predictive significance [16]. The Q² value is 0.271. (Table.3) Hence the model has medium predictive power. Furthermore, the SRMR was lower than the initially recommended criterion of 0.080, suggesting that the model fit was acceptable [20]. The normed fit index developed by Bentler is one of the fit metrics suggested in the SEM literature; the closer the NFI to 1, the better the fit [21]. NFI values greater than 0.9 generally indicate an acceptable fit [22]. In this study, The SRMR, i.e., 0.000 and NFI = 1, indicated the acceptable fit of the model. Table 4 shows that with every unit increase in growth needs, societal relatedness needs and existential needs, Turnover intention increased by -.852,740, and -.249 units and growth need were found to have maximum impact on the turnover intention of rural CHC doctors. (Table.4) On the other hand, growth and existential requirements were found to have an inverse connection with doctors' turnover intention. Hence, it can be deduced that there is a significant relationship between ERG dimensions with elements turnover intention. So, the alternative hypothesis H1: There is a significant relationship between Dimensions of ERG (Existential, relatedness and growth) and Turnover intention is accepted.

Objective no. 2

$\label{lem:moderating effect of Socio-Demographic Variables on the Dependent Variables. \\$

A moderator variable can be visualized as a third variable that changes the relationship between the independent and dependent variables [23]. Thus, a moderator specifies the conditions under which a given effect occurs and the conditions under which the direction (nature) or strength of an effect vary [24]. Once the relationship found between ERG dimensions and turnover intention, the final model was analyzed with moderators i.e., age, gender, marital status, years spent in rural areas, years of service, and future location choice for their tapping effects in relationship of ERG needs and turnover intention. The results of moderation show that the p value for age, gender and years of service are 0.514, 0148 and 0.209; since the values are more than 0.05, hence there is no significant moderating impact of variables age, gender, and years of service of rural doctors in influencing the dimensions of ERG concerning turnover Intention. However, for the other demographic variables i.e., Marital status, Years spent in the rural area,





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and future location choice, the p values are 0.032, 0.035 and 0.001. Since the value is less than 0.05, these variables have a significant moderating effect. Further to identify which group is having a significant effect, simple slope analysis results have been checked with Smart PLS. The focal predictors represent the negative relationship between ERG Dimensions and turnover intention for higher for married doctors and lower for unmarried doctors. The gradient slope values assess the same. Likewise, a negative relationship is strengthened for doctors who spent fewer years in rural areas and lowered for the doctors who spent more years. In addition, the negative relationship intensified for doctors having the rural choice of location in future and decreased for the doctors having the urban choice of location.

Testing of study hypotheses

Inferences were found from the results, and the study proved the following hypotheses.

- 1. There is a relationship between Dimensions of ERG and Turnover intention.
- 2. The interactive relationship between ERG dimensions and turnover intention is not affected by age.
- 3. The interactive relationship between ERG dimensions and turnover intention not affected by gender.
- 4. The interactive relationship between ERG dimensions and turnover intention affected by marital status.
- 5. The interactive relationship between ERG dimensions and turnover intention affected by years spent in rural area.
- 6. The interactive relationship between ERG dimensions and turnover intention not affected by 'years of service'.
- 7. The interactive relationship between ERG dimensions and turnover intention affected by 'future location choice'.

DISCUSSION

The study indicated a negative association between ERG motivation and doctor turnover intention, with 'Growth needs' having the most significant influence. Age, gender, and years of service did not alter the aspects of ERG demands in influencing rural CHC doctors' turnover intentions. However, marital status, years spent in a rural area, and future location choices alter interaction connections. Based on the study's findings, the Tamilnadu public health department must expand human resources, especially physicians, to assist the poor, thereby strengthening the health system's infrastructure and workforce quality. This study has various implications for health care providers and policymakers in Tamil Nadu. To meet the growth needs of doctors, accurate and rapid scholarship awarding systems without time-bound forced rural deployments are required. NHM should design more detailed training modules and refocus medical education on rural health to increase training quality. Achieving procedural justice in setting promotion requirements would improve doctor motivation. From the start of their course, rural students must be encouraged, not only admired but mentally prepared to serve their community. This rural pipeline approach should be reinforced based on the study's conclusions. Doctors' marital status is an essential element in rural retention. Due to their jobs or lack of recreation amenities, most married doctors and wives do not want to move to rural locations. On the other hand, they do not wish to stay in rural areas if they work elsewhere, especially in cities. Getting the exact location for doctor couples is difficult because the counselling process determines priorities. So, for married doctors, the government should consider creating rural posts with adequate counselling and without delays. The study found that basic rural amenities influenced their future placement choices; however, many existential demands are regarded adversely by rural doctors and specialists, such as COVID safety, social instability, isolation, communication, transportation, and water facilities. Upgrading basic facilities in rural regions is a cooperative endeavor of public health, public welfare, and rural administrative agencies.

CONCLUSION

From the perspective of the practical contribution of this study, it is anticipated that the conclusions will provide management implications to boost the motivation of Rural CHC doctors. Moreover, this study can be applied to the rural public health care setting in significant states of India where the problem is rural doctors' retention. Nevertheless, the sampling frame identified should be the same as this study to obtain reliable results.





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Table 1: Convergent Validity

Table 1.	Convergent Validity			Composite	Average
	Constructs and associated items	Loading	Cronbach's Alpha	reliability	variance extracted
	Existential needs		0.911	0.93	0.653
EN1	Housing services	0.76			•
RN2	Appreciation from patients	0.805]		
RN3	Pleasant working climate	0.824			
EN2	Working conditions with light, heat, and ventilation	0.821			
EN3	Safe and attractive working environment	0.849			
EN4	Social benefits	0.802	1		
EN5	Satisfactory physical surroundings	0.796	1		
GN3	Initial training for my learning	0.633			
	Growth needs		0.86	0.895	0.588
GN2	Sense of value what I do	0.728			
GN4	Personal growth in my work	0.804			
GN5	Opportunities for advancements in my career	0.758			
GN6	Equal opportunities for promotion	0.788]		
GN8	Promotion opportunities	0.736			
RN6	Status in the community as health care professional	0.784			
	Family relatedness needs	5	0.833	0.883	0.602
GN9	Opportunity to expand the scope of practice	0.709			
RN11	Spousal fulfillments	0.844			
RN12	Finding spouse job	0.75			
EN12	Undisrupted family life	0.762			
EN13	School for children	0.806			
	Societal relatedness need	s	0.884	0.915	0.684
RN9	Interpersonal relationship	0.803			
GN11	Chance to paying back for public concern	0.796			
GN12	Chance to work for the poorest segments	0.844			
GN7	Chance to work for other people	0.829			
RN10	Social contact at work	0.86			

Table 2: Discriminant Validity

Table 2. Discilli	able 2. Discriminant variaty								
	EN	GN	RN_F	RN_S					
		Fornell-Larcker crite	erion						
EN	0.808								
GN	0.716	0.767							
RN_F	0.522	0.597	0.776						
RN_S	0.714	0.706	0.448	0.827					
	Heterotrait – Monotrait ratio								





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EN	1.000			
GN	0.803	1.000		
RN_F	0.594	0.7	1.000	
RN_S	0.789	0.807	0.514	1.000

Table 3: Model Fit Indices For Final Model

	Beta coefficients	T Statistics	P Values	f²	Q²	R²	NFI	SRMR
GN -> TOI	-0.852	7.875	0.000	0.186				
RNS -> TOI	0.740	5.979	0.000	0.155	0.271	28.6	1	0.000
EN-→TOI	-0.249	2.611	0.009	0.019				

Table 4: Beta Coefficients For The Final Model.

		Estimate	
Turnover	<	GN	852
Turnover	<	RN_S	.740
Turnover	<	EN	249

Table 5: Moderation Effects

Moderation	Beta coefficients	Standard Deviation	T Statistics	P Values	Significance
Age*ERG -> TOI	0.039	0.06	0.653	0.514	Insignificant
Gender*ERG -> TOI	0.092	0.063	1.448	0.148	Insignificant
Marital Status*ERG -> TOI	-0.127	0.059	2.149	0.032	Significant
ERG*years rural -> TOI	0.105	0.05	2.111	0.035	Significant
ERG*Years of service -> TOI	-0.078	0.059	1.257	0.209	Insignificant
ERG*Location choice -> TOI	-0.166	0.049	3.379	0.001	Significant

Table 6: Slope Analysis Results For Significant Moderators

Table 0. Slope Allarysis Results 1 of Significant Moderators							
Marital status		Years spent in rural area		Future location choice			
Gradient of slope for Single doctors	-0.359	Gradient of slope for Less years spent in rural area -0.396		Gradient of slope for Urban choice in future	-0.305		
t-value	-8.027	t-value -8.855		t-value	-6.820		
p-value	0.000	p-value	0.000	p-value	0.000		
Gradient of slope for Married doctors	-0.486	Gradient of slope for More years	-0.291	Gradient of slope for Rural choice in future	-0.471		
t-value	-4.437	t-value	t-value -2.656		-4.300		
p-value 0.000		p-value	0.009	p-value	0.000		





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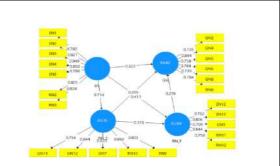


Fig. 1: Sampling method and respondents of study

Fig. 2: Measurement model

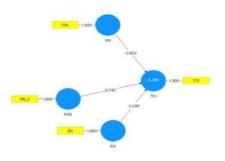


Fig. 3: Final structural model

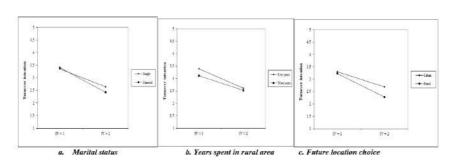


Fig. 4: Graphical comparison of moderating variables - Slope analysis





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ISSN: 0976 – 0997 **RESEARCH ARTICLE**

Perfectionism and Locus of Control: A Comparative Study of Hypertensive and Non Hypertensive People

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ABSTRACT

Recent years have seen a considerable increase in the prevalence of hypertension due to the rapid pace of modern life, decreased opportunities for physical activity, and elevated levels of mental stress. It is a pathophysiological state characterized by consistently high blood pressure (BP). Approximately 7.1 million people die each year from this illness, which affects an estimated 1 billion people around the world. In India, 23.1% of males and 22.6% of females aged 25 had high BP (WHO, 2010), and 57% of all mortalities and 24% including all CHD deaths in India can be attributed to it. By 2020, the number of deaths in India attributable to cardiovascular disease is expected to rise by 111%, according to projections. The purpose of this paper is to compare the levels of perfectionism and locus of control in people who suffer from hypertension against those who do not. Between January 2020 and November 2021, scientists from the N.K.P. Salve Institute of Medical Sciences & Research Institute and LMH, Digdoh Hills, Hingana, Nagpur, conducted their studies. This study used a sample of 60 people from the Nagpur, India, region, 30 of whom had hypertension and 30 of whom did not. N=30 in each study group; (15 males and 15 females). Only those between the ages of 40 to 50 years were considered. The samples were selected using a completely random manner. SPSS was used for the statistical analysis of the data. This study found that patients with hypertension had slightly higher internal locus of control scores than participants without hypertension. However, this distinction is not backed by any statistical evidence. According to the findings of this study, those who did not suffer from hypertension scored higher than those who did on a test of perfectionism. Although, it should be noted that the gap is not particularly





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large. The levels of internal locus of control and perfectionism in people with hypertension are not significantly different from those in people without hypertension.

Keywords: Hypertension, locus of control, Perfectionism, Blood pressure

INTRODUCTION

Blood pressure is the colloquial term for hypertension. Generally accepted medical recommendations agree that a blood pressure reading of 140 over 90 mm Hg constitutes hypertension [1]. This means that your blood pressure is above 140/90 mmHg during the systolic phase (when the heart contracts as well as pumps blood throughout the body) or indeed the diastolic phase (when the heart rests and refills by blood). Those with normal blood pressure may experience a temporary increase in readings due to acute stress, strenuous activity, or other circumstances; therefore, a diagnosis of hypertension requires multiple readings demonstrating high blood pressure over time [2,3]. In many contexts, a temporary rise in blood pressure is a typical physiological response. Hypertension can be caused by a number of reasons, including general risk factors and physical inactivity as well as by specific disorders and drugs (listed below) that helps blood pressure to shoot up [4]. In addition to it, factors such as genetics, poor stress management, attitude, etc., can increase one's vulnerability to a disease. There's a need to shed some more light on notions like perfectionism and locus of control because of the study comparing people with and without hypertension. An individual's tendency for perfectionism is a characteristic of his/her character [5]. Individuals who exhibit the personality trait of perfectionism strive for excellence in every endeavour they undertake. Perfectionist has an ideal/imaginary image in his mind of everything and he continually desires to match with it whether it is physical, emotional, social, artistic, intellectual or spiritual component of life [6,7,8].

He becomes agitated if conditions are subpar. This picture of perfection is usually nebulous, difficult to pin down, and difficult to understand. Relationships with one's parents, classmates, subordinates, and others are crucial to the formation and maintenance of perfectionist tendencies, which eventually become ingrained in the individual's character [9,10]. To be a perfectionist is to engage in a pattern of negative thinking and destructive actions with the intention of achieving impossible standards. While others may find such a style of life to be unnecessary stress, self-destructive behaviour, and exhaustion, the individual may feel it as very necessary. In today's culture, a desire for perfection is incorrectly considered as desirable and often even required for attaining one's goals. Recent research, however, has demonstrated that striving for perfection is counterproductive [11]. Trying to attain perfection can leave you feel unfulfilled and less successful than those with more reasonable goals. "Locus of control" is another significant personality attribute in individual wellness. In 1966, Julian Rotter established this framework. A person's locus of control is their sense of agency in the face of external forces [12].

Psychologists use the term "internal locus of control" to describe the belief that an individual can influence their environment. One has an external locus of control if they believe they have no say in their circumstances and that other people or events are to be blamed for their misfortune [13]. According to psychologist Philip Zimbardo's definition from 1985's Psychology and Life, "The locus of control refers to a person's outlook on whether or not they have any influence on the results of their actions (internal management orientation) or whether or not they are completely at the mercy of external forces (external control orientation). Psychologist Julian Rotter proposed a theory in 1954 that allow rewards and punishments to dictate our behaviour and shape our understanding of the motivations behind our acts. The assumptions we hold regarding the origins of our actions in turn shape our conduct and outlook. Rotter's 1966 scale for gauging internal vs. external locus of control was the first of its kind. For each item on the scale, respondents must select one of two predetermined options using a forced-choice method. Although the scale has seen extensive use, it has also been heavily criticized by some who argue that locus of control exists on a scale.





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To a 100% degree, no one has either an internal or an external locus of control. In reality, most people fall somewhere in the middle, on the spectrum that connects these two poles.

MATERIALS AND METHODS

Between January 2020 and December 2022, studies were carried out at the LMH in Digdoh Hills, Hingana, Nagpur, and the N.K.P. Salve Institute of Medical Sciences and Research Center. Patients who came to the Outpatient Department (OPD) were surveyed and their information was analyzed simultaneously. The Institutional Ethical Committee gave its blessing to the research, so it could be conducted. Every participants were requested to sign and give his or her consent for the research study.

Standards for Including

Sixty people from the Nagpur area participated in the study (30 were with hypertension and 30 were without hypertension). N=30 in each study group; (15 males and 15 females). Participants were between age group of 40 to 50 years.

Exclusion Criteria

Patients with data from other diseases or conditions, including cancer, autoimmune disorders, pregnancy, or breastfeeding were not included in this analysis.

Study Procedure

The researchers contacted all the members of sample group to measure the degree of perfectionism and locus of control (Self-Assessment Perfectionism Screening Test and Rotter's Locus of Control Scale). A personal data sheet was used to record the aforementioned generic data. Before beginning any kind of testing, they sat down with the subjects to figure out the testing plan that works best for them. Patients with hypertension were evaluated based on their medical history, recorded blood pressure readings, and current medications.

Statistical Analysis

The data was analysed statistically with SPSS 20.0 (Statistical Package for the Social Sciences). t-test was used to examine the correlation and its significance.

RESULTS

The purpose of the research was to find out if there was a any linkage between hypertension and perfectionism and locus of control in persons aged 40–50 years. There were total 60 patients, 30 of whom were females and 30 of whom were males. Table 1 includes patient demographics and clinical data. The patients' average age was 44.56 years, and their average BMI was 25.26.

DISCUSSION

This study found that patients with hypertension had slightly higher internal locus of control scores than participants without hypertension. P = 0.6803 when testing hypotheses with two tails. This change is not statistically significant using the standard methods. Even though internal locus of control is a contributor to hypertension, it appears that there are many other factors that play a much larger role. The individual's genetic makeup, parenting style, stress at work, personal habits, and personality type all have a role in the development of hypertension. Value of P for a two-tailed test is 0.1275. This difference is typically not deemed statistically significant. In this study, those without hypertension scored somewhat higher on a measure of perfectionism than those with hypertension. The researchers also aimed to comprehend the external locus of control differences between the two groups. Those who do not





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experience hypertension are less likely to have an internal than those who do. Nonetheless, there is no statistical evidence to support the claimed difference.

The findings of the study suggest

- Hypertensive and non-hypertensive persons do not significantly differ from one another in their levels of internal locus of control.
- No statistically significant difference exists in the levels of perfectionism between those with hypertension and those without the condition.
- Equal levels of perfectionism can be seen in both sexes.
- There is no variation in internal locus of control between the sexes.

Limitations

No single research study can stand on its own. That's just the first baby step toward mastering all that information. There are caveats to the present study as well. The study aimed to compare perfectionism and locus of control in individuals with and without hypertension.

- The study's sample size was too small to draw firm conclusions.
- The sample was drawn from only one hospital situated in Nagpur city.
- It was limited to participants of a certain age range only.
- Stress levels, links to other disorders like diabetes, and other potential contributors were disregarded.
- There is no consideration of socioeconomic background.
- Hypertension can also be affected by a person's cultural background, which includes their religious and political beliefs too.

CONCLUSION

The ultimate goal of study is to comprehend all aspects of life in order to improve the quality of human existence. This research will undoubtedly shed light on the linkages between perfectionism and locus of control in hypertensive patients. The study demonstrates that there is no gender difference between the two groups over the level of locus of control and perfectionism. Because of the overemphasis on sex differences, this discovery may be considered significant. The leveling of two genders has occurred in recent times on account of education, employment, and participation in social, political, and cultural life. More research into the complex web of social, psychological, and educational determinants of health problems in the general population is desirable to arrive at conclusive findings in this regards.

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Table.1:Patients' demographic & clinical data(n=60)

Clinical Profile	Mean (S.D.)
Age (years)	44.56(10.22)
Weight (kg)	69.31 (12.32)
Height (cm)	161.42 (5.95)
BMI (kg/m²)	25.26(5.89)
Demographic profile	frequency (%)
Family status	
Single	5 (8.33)
Married	36 (60)
Divorced	9 (15)
Widow	10 (16.67)
Education level	
Preliminary	8 (13.33)
High school	15 (25)
University	37 (61.67)
Profession	
Unemployed	14 (23.33)
Employed	46 (76.67)
Hypertension	
Yes	30 (50)
No	30 (50)
Sex	
Male	30 (50)
Female	30 (50)

Table.2:Comparison of locus of control and Perfectionism in Hypertensive and non-Hypertensive Group

					J .
	Group	Mean	SD	n	t-score
Locus	Hypertensive	7.53	1.76	13	t=0.41 (not
of Control	Non-Hypertensive	7.29	1.4	17	significant)
Dorfoctionicm	Hypertensive	8.06	2.31	30	t=1.54 (not
Perfectionism	Non-Hypertensive	8.93	2.04	30	significant)





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RESEARCH ARTICLE

The Effect of Mobile Apps for Health Promotion in Rural Regions of Andhra Pradesh on Health Care: A Case Study

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ABSTRACT

A well-established IT industry followed by cutting-edge technology is throwing new challenges in the industry and giving scope for complete overhaul in the country's map. In view of rapid advancements in mobile technology and its applications, at the disposal of public health care transformation of technology has been felt at global level. Using mobile technology and its applications in the developing nations like India on health care, it gives a new impetus for generating employment with the local resources available. In most of the rural areas, the use of mobile phone is felt necessary to cover the health care of the people. As such use of mobile phone coordinating with the health care services in rural areas is rapidly increasing. The mobile and wireless health care delivery systems are affiliated with m-Health applications. These m-Health applications have been helping the patients to have direct link with medical practitioners from their homes and the later are able to consult them through video calls even. In the wake of rapidly evolving health care technology, the current research focuses on the use of mobile health app to promote health among rural population and to control chronic and acute illness being suffered by them. With the growth of mobile technology, mobile health is shot into prominence to teach the patients suffering from chronic and acute illnesses.

Keywords: mHealth, Rural, mobile health app, Health promotion, Chronic illness, Acute illness





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INTRODUCTION

It has been estimated that the medical health business is served by about 44,000 health care Apps. The Ministry of Health and Family Welfare has initiated proper steps to expand the areas of public health care units using ICT, provide 104 Medical Health Services (MHS) covering rehabilitation, physiological, family planning program to address rural population specifically in Andhra Pradesh. These services also provide sufficient amount of information with respect to the availability of Medical hospitals, pharmacies, diagnostic services, rehabilitation centers, and other health care institutions. Public health applications have several advantages. The prescriptions have been digitalized, making rapid judgments on a patient with proper diagnosis with ease. Internet connection hitherto has made the task still easier with the instant accessibility right at every nook and corner of the country. Instead of travelling to far of places physically, internet applications assist to trace out the doctor himself for prescription of effective drugs to the patient. Internet of Things (IOT) is a term commonly focused on medical health care apps in the recent past. With the proper implementation of health care it has become still easier to attain more patients through conducting health care camps and offering fee concessions for instant treatments to the deserving patients. However, it is found that the rural areas are still less accessible to high-speed internet, smart phones, licensing, documenting, reimbursement, sustainability, malpractices, trust of technology and health care and on other aspects [1]. So the authors have tried to discover out the impact of mobile health among rural people, and if at all they genuinely serve the goal or just take up digitalization for the sake of it [2, 3].

Review of Literature

According to [4], outpatient health information may be acquired from health centers as well as health subentries via mobile apps. As per [5], collecting patient health data through mobile phone is unavoidable in rural settings. In their study, [6] they have found that mobile phones are rarely utilized to solve health issues in rural India. According to [7], substantial usage of mobile telephony for mobile health (mHealth) brings closer the health gap between rural and distant living individuals. As per [8], diabetes is more common in rural areas than in metropolitan areas. A high risk group was enrolled in the sixth week mHealth Diabetes Self-Management Education Program. Mobile technology and its applications are propelling global health care, according to [9]. It is difficult for doctors to give medical treatment to rural populations, according to [10]. [11] Author believes that the global spread of smart phones has created new prospects for medical education and health care institutions to aid the poor. They have found that both governments and health care organizations have needed to use mobile phone-based solutions to deal with the Covid 19 pandemic. [12] They have also suggested integrating mHealth with public health systems. According to [13], the wide applicability of m health services is a key factor in raising public awareness. [14] He has opined in his study, smart phone-based mental health apps have helped patients self-manage their mental health conditions. [15] He has noted aversions after brief usage and hence suggested the on-going use of mobile applications. [16] They believe that health-related mobile applications can also spread disinformation and damage users.

RESEARCH METHODOLOGY

Followed by a brief review of the relevant literature, the authors set out to determine the impact of mobile applications in health promotion programs.

As a result, the following objectives have been established for the study:

- 1. To investigate the impact of various socio-demographic parameters on the use of mobile health applications, the management of acute and chronic disease, and the promotion of health and welfare of the affected.
- 2. To examine the link between health promotion and mobile health app usage, as well as the interaction of mobile apps in managing and acute illness and chronic diseases.

The research strategy for this study is cross sectional and analytical in nature, since the authors have collected data from the participants during a single one-on-one session. The raw data has been acquired from ten villages in Prakasam district of Andhra Pradesh. A total of 161 samples have been utilized in this investigation, which is conducted using a random sampling approach. The validated study instrument that is utilized for data collection





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consisted of 29 different items. [5] He has used a 5-point Likert scale to assess their findings. The Hypotheses have been set to test the gender differences, impact of age, education, marital status and occupation, regular use of mobiles etc. In addition, the study has tested the relationship between the health promotion and mobile health usage in management of acute and chronic illness. The analysis has been carried out using the SPSS software.

Data Analysis

The results indicate that the Cronbach's alpha value for the 29 items taken from the scale in this study is 0.887, (Table I) the closer the reliability co- efficient gets to 1.0 will be better, thus the internal consistency reliability of the measures used can be considered better. The T test findings (Table II) demonstrate substantial variations between male and female usage of mobile health applications, management of acute and chronic diseases, but with no gender-based differences in health promotion. The results show that females have less difficulty using health applications over the males. Female have agreed that introducing applications is sufficient and in order, but stated that it takes too long, but agreed to suggest further. For reminders in respect of diabetic and blood pressure medication, men respondents favoured Whats App and SMS over female respondents. Female prefers reminders in native language, while men prefer English. In cases of chronic diseases, females choose human communication with health workers or physicians over mobile applications, but in case of acute illness, men prefer mobile communication with doctors or health professionals. It is interpreted from the above Table III that there is no significant difference between usage of health apps, management of chronic illness, acute illness and health promotion with regards to age. Since p is more than 0.005, there is no significant difference in the mean score of factors regarding perception in the usage of health apps, health promotions, and management of chronic and acute illness of different age groups. It is interpreted from the above Table IV that there is significant difference between the usage of health apps, management of acute illness with regards to education (p<0.005), and there is no significant difference in the usage of health promotion and management of chronic illness with regards to education(p>0.005).

It is interpreted from the above Table V that there is no significant difference between the usage of health apps, management of chronic illness, acute illness and health promotion with regards to marital status. Since p is more than 0.005 there is no significant difference in the mean score of factors regarding perception of usage of health apps, health promotions, management of chronic and acute illness with regards to marital status. It is interpreted from the Table VI above that there is significant difference between the usage of health apps, management of chronic illness, management of acute illness and health promotion with regards to occupation. Since p is less than 0.005 there is significant difference in the mean score of factors regarding the perception of usage of health apps, health promotions, management of chronic and acute illness of different occupations. Thus, the results of ANOVA test shows that there is a significant effect of occupational status on usage of health apps, health promotion, and management of chronic and acute illness. It is interpreted from the above Table VII that there is significant difference in management of acute illness with regards to regular use of mobile phone (p<0.005), and there is no significant difference exist between usage of health promotion and management of chronic illness in the case of regular use of mobile phone (p>0.005).

Thus, it can be interpreted that the rural people frequently use mobile phones for management of acute illness rather than for chronic illness and for their health promotion. A regression analysis was done to identify the relationship between the two variables (Table VIII A). The dependent variable is health promotion and the independent variables are mobile health app usage, chronic illness and acute illness. The changes in dependent variables is due to changes in independent variables are shown in R square. The adjusted R square is 0.607which shows that 61% variation in health promotion is due to changes in the stated critical factors and the remaining 39% are those factors which are not represented in the model. Usage of health apps, management of chronic illness and management of acute illness has a significant effect on health promotion of rural people (Table VIII A). In Table VIII B, F value is 82.456 and the p value is 0.000 <0.005 which shows that there is significant difference between the variables. The usage of mobile apps, management of chronic illness and management of acute illness has p-values 0.015, 0.006, 0.000 <0.05 are considered significant (Table VIII c). Hence it can be deduced that there is a significant effect of usage of mobile apps, management of chronic illness and management of acute illness with health promotion for elements. With a unit





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increase in usage of health apps, management of chronic illness and management of acute illness, the health promotion increases by 0.154, 0.208, and 0.552 respectively (Table VIII c). It can be interpreted that the factors such as usage of health apps, management of chronic illness and management of acute illness are significant predictors which influence the health promotion. Management of acute illness is the strongest predictor of health promotion behavior of rural people on the ground of mobile health.

DISCUSSION AND CONCLUSION

The ideal interpretation of this research demonstrates that there exists a link between utilization of health app, treatment of chronic and acute sickness using health apps and health promotion (Table IX). It implies that health promotion considerations by rural people are significantly encouraged by the introduction of mobile apps. As the findings of t-tests have unveiled that female have experienced some challenges in spending time in applications and they prefer native language for apps. Also, it is stressed by female respondents that the management of chronic sickness is not only achievable by health apps, but also, that they require dialogue with physicians and other health professionals. The ANOVA findings have suggested that educational status of rural respondents have influenced their health promotion behavior and management of acute sickness with respect to utilization of mobile applications. And also professional status is playing a crucial part in health promotion, utilization of health applications, and management of acute and chronic sickness [17]. The study has shown that rural people have utilized their mobile applications in a routine manner for the management of acute sickness rather than for chronic diseases or health promotion.

Following an examination of the findings, the authors have made several sound recommendations. It is strongly advised that mobile applications can be developed in the user's local language in order to reach the rural population who would be able to get healthcare through the apps. When it comes to using mobile applications, it is important to follow easy procedures to avoid boredom in the minds of users, which increases the likelihood of their abandoning or using the app incorrectly. People also like quick reminders to go through, which means communicating over What's App or SMS that will be more convenient than calling or sending e-mails. Designing health applications for the treatment of acute diseases is highly recommended; however, because of the management of chronic illness may not be achievable through mobile apps; it is probably that individuals may be misled if they do not check with their physicians beforehand. The findings of the study also have revealed that when education levels rise, the likelihood of health promotion and acute disease management too rises. As a result, boosting the degree of health literacy among rural populations is highly recommended [18]. It is possible to perceive this through conducting health awareness activities at the village level. Health policymakers and the government will benefit from the findings of the study since they will provide valuable insight into the consideration of digital health initiatives [19-22]. It is better to create health apps at a national level to achieve the greatest possible degree of utility and the best outcomes. The study may be extended to many remote places in order to determine the difficulties as well as compatibility in the usage of mobile applications.

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Table 1: Reliability Statistics

Cronbach's Alpha	N of Items		
0.087	29		





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Table.2: T test for Gender Differences

	GENDER	MEAN	N	DF	t- value	p- value
UHA 3	male	3.81	72	160	0.273	0.026
	female	3.79	90	160	0.273	Significant
UHA4	male	2.47	72	160	-0.613	0.047
	female	2.56	90	160	-0.013	Significant
UHA5	male	3.50	72	160	-0.325	0.000
	female	3.86	90	160	-0.323	Significant
UHA6	male	2.38	72	160	-0.3175	0.000
	female	2.83	90	160	-0.3173	Significant
UHA 10	male	3.50	72	160	-0.29	0.027
	female	3.98	90	160	-0.29	Significant
C18	male	3.82	72	160	0.560	0.016
	female	3.76	90	160	0.360	Significant
C20	male	3.82	72	160	2.387	0.000
	female	3.54	90	160	2.307	Significant
C22	male	3.78	72	160	0.425	0.005
	female	3.91	90	160	0.423	Significant
C23	male	3.13	72	160	3.446	0.000
	female	2.57	90	160	3.440	Significant
C26	male	3.81	72	160	-2.086	0.05
	female	3.97	90	160	-2.000	Significant
A29	male	4.18	72	160	-0.526	0.025
	female	4.03	90	160	-0.520	Significant

Table.3: ANOVA Test to test the impact of Age

		Sum of Squares	df	Mean Square	F	Sig.
	ВG	73.163	4	18.291	1.544	0.192
UHA	WG	1823.792	154	11.843		
ОПА	Т	1896.956	158			
	ВG	81.370	4	20.343	1.151	0.335
CI	WG	2774.605	157	17.673		
CI	T	2855.975	161			
	ВG	2.855	4	0.714	0.109	0.979
AI	WG	1029.645	157	6.558		
Ai	T	1032.500	161			
	ВG	1.228	4	0.307	0.124	0.973
HP	WG	387.217	157	2.466		
ПР	Т	388.444	161			





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Table. 4: ANOVA Test to test the impact of Education Status

		Sum of Squares	df	Mean Square	F	Sig.
	Between Groups	16.637	4	4.159	1.756	0.140
HP	Within Groups	371.807	157	2.368		
ПР	Total	388.444	161			
	Between Groups	225.811	4	56.453	5.202	0.001
UHA	Within Groups	1671.145	154	10.852		
ОПА	Total	1896.956	158			
	Between Groups	95.070	4	23.767	1.352	0.253
CI	Within Groups	2760.906	157	17.585		
CI	Total	2855.975	161			
	Between Groups	95.437	4	23.859	3.998	0.004
ΑI	Within Groups	937.063	157	5.969		
	Total	1032.500	161			

^aHP- Health promotion, ^bUHA- usage of health app, ^cCI-Chronic illness, ^dAI-Acute illness

Table.5: ANOVA to test the impact of Marital Status

		Sum of Squares	df	Mean Square	F	Sig.
	ВG	0.218	1	0.218	0.090	0.765
HP	WG	388.226	160	2.426		
	Т	388.444	161			
	ΒG	3.852	1	3.852	0.319	0.573
UHA	WG	1893.104	157	12.058		
	Т	1896.956	158			
	ВG	3.755	1	3.755	0.211	0.647
CI	WG	2852.221	160	17.826		
	Т	2855.975	161			
	ΒG	5.966	1	5.966	0.930	0.336
ΑI	WG	1026.534	160	6.416		
	Т	1032.500	161			

Table.6: ANOVA to test the impact of Occupational Status

		Sum of Squares	df	Mean Square	F	Sig.
	ВG	46.463	3	15.488	7.156	0.000
HP	WG	341.981	158	2.164		
	Т	388.444	161			
	ВG	190.852	3	63.617	5.780	0.001
UHA	WG	1706.104	155	11.007		
	Т	1896.956	158			
	ВG	287.573	3	95.858	5.897	0.001
CI	WG	2568.402	158	16.256		
	T	2855.975	161			
	ВG	158.226	3	52.742	9.532	0.000
ΑI	WG	874.274	158	5.533		
	Т	1032.500	161			





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Table.7: ANOVA to test the impact of Regular use of mobile phones

		Sum of Squares	df	Mean Square	F	Sig.
	ВG	2.029	1	2.029	0.840	0.361
HP	WG	386.415	160	2.415		
	Т	388.444	161			
	ВG	44.270	1	44.270	3.752	0.055
UHA	WG	1852.686	157	11.801		
	Т	1896.956	158			
	ВG	49.208	1	49.208	2.805	0.096
CI	WG	2806.767	160	17.542		
	Т	2855.975	161			
	ВG	100.085	1	100.085	17.174	0.000
ΑI	WG	932.415	160	5.828		
	Т	1032.500	161			

Table.8A: Regression Analysis to test the relationship between Mobile Health usage and Health Promotion

Mode	I R	R Square	Adjusted R Square	Standard error of Estimate				
1	0.784a	0.615	0.607	0.98241				
	a. Predictors: (Constant), AI, UHA, CI							

Table.8B: ANOVA

Sum of Squares	df	Mean Square	F	Sig.
238.744	3	79.581	82.456	0.000
149.596	155	0.965		
388.340	158			

Table.8C: COEFFICIENTS

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		В	Std.	Beta		
			Error			
	(Constant)	311	.857		363	.717
	UHA	.070	.028	0.154	2.461	.015
1	CI	.077	.027	0.208	2.807	.006
	Al	.338	.039	0.552	8.698	.000

Table.9 : Testing of Hypothesis

Hypotheses	Test results	
H1: There are differences between male and female in health app usage and management of illness and health promotion	Alternate hypothesis substantiated	
Ho2: There is no effect of age group in usage of mobile health apps, management of acute and chronic illness and health promotion	Accepted null hypothesis	
H3: There is an effect of education status in usage of mobile health apps, management of acute and chronic illness and health promotion	Alternate hypothesis substantiated	





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Ho4: There is no effect of marital status in usage of mobile health apps, management of acute and chronic illness and health promotion.	Accepted null hypothesis
H5: There is an effect of occupational status in usage of mobile health apps, management of acute and chronic illness and health promotion.	Alternate hypothesis substantiated
H6: There is an effect of regular use of mobile phones in usage of mobile health apps, management of acute and chronic illness and health promotion.	Alternate hypothesis substantiated
H7: There is a relationship between health promotion and mobile health app usage, management of chronic illness and management of acute illness.	Alternate hypothesis substantiated





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RESEARCH ARTICLE

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Conservative Management of Infected Follicular Cyst- A Case Report

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ABSTRACT

Follicular cysts represent one of the very disturbing lesions affecting in the jaws. Their silent nature before they cause expansion of the jaws or before getting infected secondarily to cause pain is one of the most dangerous aspects. These lesions must be diagnosed and treated promptly at an earlier stage to prevent unwanted complications. This case report presents a child of nine years old with large follicular cyst affecting mandibular posterior region. The lesion was diagnosed as infected follicular cyst after clinical and radiographic examinations which in turn was confirmed by aspiration. Looking at the size of the lesion, potential complications that might happen with enucleation and most importantly the age of the child to heal the lesion fast, marsupialization of the cyst was considered. After marsupialization the lesion was followed for two years to observe the changes. The cyst showed excellent healing and the displaced tooth erupted uneventfully.

Keywords: odontogenic cyst, follicular cyst, management of cyst





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INTRODUCTION

A cyst may be defined as a pathological cavity containing fluid, semi fluid or gaseous contents and is not created by accumulation of pus. Many of the true cysts are lined by epithelium [1]. The maxillofacial region seems to be most commonly affected by odontogenic cyst compared to any other body parts because of presence of epithelial cell rests after odontogenesis [2]. Dentigerous cyst is a type of Odontogenic cyst in which the follicular epithelial lining surrounds the crown of unerupted tooth and attaches at the neck of a tooth in a collar like fashion. Accumulation of fluid between the reduced enamel epithelium and the enamel of unerupted tooth causes expansion of follicle causing formation of cyst and results in impaction of tooth [3]. Mandibular third molars, maxillary canines, and mandibular premolars are the most common sites affected. The expansion of dentigerous cyst results from increase in cystic fluid osmolality as a result of moving of inflammatory cells and desquamated epithelial cells into the cyst lumen. Also, in some instances, the periapical inflammation extension into intra-follicular space from an overlying primary tooth cultivates into the dentigerous cyst. It remains asymptomatic until there is any acute inflammation and hence is diagnosed on routine radiographic examination [4].Dentigerous cyst usually grows slowly and has a capacity of cortical plate expansion of the involved jaw [5]. Also known as follicular cyst, it is one of the most major type of odontogenic cysts in terms of its relative frequency and also, it's enlarging potential. The follicular cyst can be defined as a distented follicle around the anatomical crown of an unerupted tooth within the bone [6].

The exact pathogenesis of follicular cyst is not known, but current hypothesis states two mechanism that possibly leads to formation of follicular cyst. The first states that the increase in size of outer layer of reduced enamel epithelium leads to formation of cleft within the epithelium which leads to accumulation of fluids except pus within the gap created by formation of cleft [7]. A second hypothesis states that passive haemodynamic pooling of fluid between the follicular lining and the crown due to arrested eruption [8]. However both hypothesis may not be independently self-explanatory but together they may relate to a variable degree for formation and development of Follicular Cyst. If the size of the cyst is larger than 2cm in diameter then the swelling of gingiva, tooth mobility, tooth displacement may be present. Radiographs usually reveal unilocular radiolucent lesion having well defined sclerotic margins usually involving the unerupted crown of tooth and root resorption may also be associated with it. Complications may involve pathologic jaw fracture, early loss of permanent teeth, bone deformities and even development of squamous cell carcinoma in few cases [9]. If cyst remains untreated, it may cause tissue destruction and deformities. Since enucleation has the drawback of being destructive in many aspects and health of jaw and developing tooth is the priority marsupialization can be the mostly choice treatment for large dentigerous cysts.

Case Report

An eight and half year-old girl reported to Pediatric Dentistry Department with chief complaint of swelling in right lower cheek region of face for three months. The swelling caused disfigurement of face and was associated with continuously dull aching pain. Extra oral evaluation showed that the swelling was hard on palpation and was mildly tender extending from left corner of lip to angle of mandible approximately 2.5cm X 2.5 cm. Intraoral examination revealed deep carious lesion with 84 and 85. The teeth were grade I mobile and non-tender on percussion. A diffuse swelling was noticed in the right buccal vestibule. The parents and guardians of the child was said to observed the swelling, which was increased in size from three months. Orthopantomogram revealed a diffuse unilocular radiolucency below the roots of 84 and 85 extending from lateral root surface of left lower canine to the roots of left lower first permanent molar measuring 3cm X 2.5cm in dimension. The radiolucency was well demarcated with clear sclerotic border. It was observed that the second premolar was inclined distally and was placed in horizontal direction radio graphically. First premolar was also inclined messially radio graphically (Image 1). Considering the clinical picture and radiographic findings it was decided to perform aspiration to check if any fluid is present in the radiolucent part to help in diagnosing it as a cyst. On aspiration 4 ml of straw coloured fluid mixed with pus and blood was obtained (Image 2A). The aspirated fluid was sent for microbiological testing. The test revealed presence of multiple pus cells and mixed microorganisms indicating the diagnosis as an infected follicular cyst (Image 2B). Observing the extent of lesion and its proximity to lower border of mandible it was decided to perform





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marsupialization of the cyst. The risks involved and the possible outcomes were explained in detail to the parents and their written consent was taken for performing the procedure. Under Inferior alveolar nerve block and long buccal nerve block, a 2cmX2cm incision was taken on the ridge mucosa in the region previously occupied by 85. The cystic cavity was entered by careful blunt dissection. All the cavity was emptied from the infected fluid using gauze pieces along with intermittent irrigation with Povidone Iodine (Wokadine® 10%) diluted with normal saline. Following emptying, a sterile gauze soaked in the solution of povidone lodine(10%) and saline was packed in the cyst cavity and the child was given a course of antibiotics and anti-inflammatory drugs(Image 3). The child was recalled after every twenty-four hours for changing the dressing for span of seven days till the signs of infection and inflammation subsided. Orthopantomogram and occlusal radiograph were advised at the end of every month to evaluate the lesion size, cortical bone expansion and progress of healing. It was clinically and radiographically observed that, at every subsequent visit the symptoms of pain diminished. Radiographically at the end of one month there were hazy radiopaque areas showing evidence of new bone formation (Image 4). The second premolar showed evidence of reorientation towards normal path of eruption. At three months follow up the lesion clearly showed evidence of beautiful healing with increased radiopaque areas indicating active bone formation inside the area of cyst (Image 5). The impacted premolar showed near normal tendency of eruption. At every subsequent visit the cyst cavity was thoroughly irrigated with povidone lodine and saline and the dressing was changes gently. At the end of two years it was noticed that there was no intra/extraoral swelling. The second premolar erupted normally in its position and was surrounded by healthy gingiva. There was clear picture of healthy bone on radiograph (Image 6). All the lesion dramatically healed with healthy bone and soft tissues. There was no evidence of pain or any other clinical problems. However, the child was advised clinical follow up every six months for next few years to tackle any problems if notice

DISCUSSION

Managing any types of lesions in children should be considered as a major task with maximum efforts to minimize any type of permanent disability. It is very common observation that in developing countries the dental diseases are badly neglected and it's especially true for primary dentition. Although infrequent, sometimes this negligence causes very large-scale damage. Follicular cysts commonly affect the premolar areas in lower arch and canine region in maxilla. Often these cysts go unnoticed due to painless destructions. The child notices when either swelling becomes obvious or when the cyst gets secondarily infected leading to acute inflammation. Moreover, the vital structures might get compressed due to the size of cyst leading to loss of sensation in the innervated part. The affected tooth usually gets pushed within the bone leading in some cases to lying in lower border of mandible or even at infraorbital border in Maxilla [1,3,8]. Treatment of these cysts include either enucleation to completely scrape the lesion from the affected site or Marsupialization where the cyst is attempted to collapse in its aggression and reduce in size[2,3]. In some cases, depending on the situation, a combination of marsupialization followed by enucleation can also be preferred. Enucleation may result in damage to peripheral vital structures like neurovascular bundles causing paresthesia and anesthesia of the innervated areas. Teeth associated with the lesion may also have to be extracted leading to permanent loss of a good tooth. In larger cysts enucleation may even cause iatrogenic perforation of mandible [2,5]. The drawbacks of enucleations can be overcome by marsupialization of the cyst particularly for large cysts where it is risky to attempt enucleation [5].

The present case was indeed a case of negligence where due to lack of pain the child as well as the parents did not seek the advice of Pediatric Dentist. The lesion had expanded to an extent that it would have perforated even the lower border of mandible after few days. The child and parents reported when the cyst was infected causing pain. Considering the age of the child and massive extent of the cyst it was decided to start with marsupialization and observe the improvements. Surprisingly radio graphically the improvements were evident immediately at the end of first month. Clinical symptoms also were drastically reduced and the child was more confident. Since it was an infected cyst it was decided to irrigate the cyst repeatedly with dilution of povidone lodine and saline. Usage of povidone lodine proved greatly beneficial in alleviating the infection and thus reducing inflammation. Every





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subsequent appointment the lesion showed evidences of healing with calcification. The direction of premolar also changed from horizontal to near normal path of eruption. At the end of two years it was observed that the cyst had completely vanished. There was good bone formation seen in orthopantomogram, affected premolar was almost in occlusion with well demarcated lamina dura around the roots. The present case is an example that many times simple procedures without using advanced resources can also give extraordinary results and comfort to children. It was possible to get such results only because of cooperation and regularity of parents to get their child in follow-up appointments. The report shows marsupialization must be the first choice in large cysts compared to enucleation.

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Fig. 1: Preoperative orthopantomogram showing diffuse unilocular radiolucency extending from lateral root surface of left lower canine to the roots of left lower first permanent molar



Fig. 2 : Shows straw coloured fluid inspiration from cystic cavity





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Fig. 3 : Shows histopathological report of the cystic fluid

Fig. 4: Preoperative and intraoperative images



Fig. 5: Postoperative orthopantomogram of patient at 1 month follow up showing hazy radiopaque areas evident of active bone formation



Fig. 6: Postoperative orthopantomogram of patient after 3 months showing increased radiopaque areas indicating active bone formation inside the area of cyst. The second premolar showed evidence of reorientation towards normal path of eruption.



Fig. 7: Postoperative orthopantomogram after 2 years showing normally erupted second premolar in its position with active formation of bone and completely healed lesion





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RESEARCH ARTICLE

Characterization Tri-Metallic Green **Synthesis** and of Alloy **Nanoparticles**

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ABSTRACT

Crystallized Nickel (Ni), Copper (Cu) and Zinc (Zn) tri-metallic nanometal alloy were prepared and stabilized the use of plant extracts of Caparis zylanica leaves in an aqueous system. Aqueous solutions of, Ni⁻², Cu⁻², Zn⁻² ions are in 1:1:1 ratio of Ni-Cu-Zn alloy changed into treated with a filtered solution of Caparis zylanica leaves extract for the formation of Ni-Cu-Zn tri-metallic alloy nano particles (Ni-Cu-ZnNP). Analysis of the feasibility of the green synthesized bio-functional nanometal alloy from plant leaves extract is specifically noteworthy. The colloidal suspensions obtained had been in stable circumstance for three weeks. The composition, morphology, size and structure of the nanoparticles have been determined by way of UV-Visible spectroscopy (UV-Vis), Fourier transform infrared spectroscopy (FTIR) and Scanning electron microscopy (SEM).

Keywords: Caparis zylanica plant extracts, green synthesis, tri-metallic alloy nanoparticles, UV-Vis, FTIR, SEM.

INTRODUCTION

Nanotechnology plays a totally crucial role in modern studies. It is the maximum capable generation that may be carried out nearly all fields inclusive of pharmaceutical, electronics, fitness care, meals and feed, biomedical technology, drug and gene shipping, chemical enterprise, electricity technological, cosmetics, environmental fitness, mechanics and space industries [1-4]. It has also been applied for the remedies of infection [5, 6], most cancers, hypersensitivity [7, 8], diabetes and irritation. Green chemistry is an implementation, improvement, design of chemical ways which includes sol gel method, chemical reaction, strong kingdom response and co-precipitation.





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Compare many products and strategies to reduce the use of hazardous to environment. To synthesize nanoparticles there are to the ones techniques green synthesis approach is one of the satisfactory technique for the manufacturing of nanoparticles in recent years. This inexperienced synthesis approach have numerous benefits over other techniques particularly fee effectiveness, simplicity, use of less temperature, the utilization of less toxic substances, moreover it is like minded for scientific programs. The nanoparticles synthesize from plant extract have been found to be blanketed through the medicinal properties of plant extract which can be used in drug, centered drug delivery and beauty programs. In this present research, *Capparis zeylanica Linn* very own to the family of *capparidaceae* typically known as Indian caper, is a mountaineering scandant shrub located throughout India and been used as a 'Rasayana' drug in the traditional medication. This flower is of fatty acids, alkaloids and flavonoids. *Capparis zeylanica Linn* was said to maintain antioxidant, antimicrobial, anti inflammatory and immunostimulant pastime. The modern investigation focused on the aqueous leaves extract of *Capparis zeylanica* used to synthesize NiCuZnNPs at various experimental conditions and thereby enhancing the significance of plant supply and involving green chemistry for the synthesis of different nanoparticles as destiny studies.

MATERIALS AND METHODS

MATERIALS

Nickel sulphate, Copper sulphate and Zinc acetate (E. Merck) used as a precursor salt and *Capparis zeylanica* plant leaves had been accrued from Thirumayam, Pudukkottai, Tamil Nadu, India. Deionized water turned into used in the course of the look at. Fresh and wholesome leaves of *Capparis zeylanica* leaves rinsed very well 2-3 instances with deionized water to eliminate dirt and undesirable particles. Leaves were dried in room temperature for 15days. The 250 ml Erlenmeyer flask containing 10g of small pieces of leaves with 100mL deionized water became heated at 60°C temperature for 20 min. Obtained leaf broth changed into filtered twice through Whatman No.1 paper and stored at fridge for in addition experiments.

METHODS

Green Synthesis of Nickel Copper Zinc Nanoparticles (Ni-Cu-ZnNPs)

In an artificial technique, Ni-Cu-ZnNPs have been received through an inexperienced reduction route. The flask containing an aqueous solution of salt NiSO₄.2H₂O, CuSO₄.2H₂O and ZnA(CO)₂.2H₂O solution turned into heated to 80°C in water bath. The ensuing darkish brown coloration answer changed into centrifuged for 15min at 6000rpm.Different spectrophotometric strategies like UV-Vis, FT-IR and SEM evaluation had been used for investigating the morphology, crystalline nature, practical group and stability of synthesized Ni-Cu-ZnNPs.

Characterization

Ultra violet spectrophotometric evaluation (Perkin Elmer-UV-WIN) was executed to research the diverse wave lengths among 300-700nm. Fourier transform infrared spectroscopy (Perkin Elmer-IR) turned into done to analyze the functional institution of the biosynthesized Ni-Cu-Zn nanoparticles within the range of 500cm⁻¹ to 4000 cm⁻¹. To examine the morphological examine, particle distribution of the biosynthesized Ni-Cu-Zn nanoparticles became decided by way of area emission scanning electron microscopy (EVO-18).

RESULT AND DISCUUSION

Phytochemical Screening

Qualitative phytochemical [9] of the following tests were performed on extracts to detect various phytochemicals present in them.

UV-Visible absorption spectrum analysis

UV-Vis absorption spectrum serves as an effective technique for studying the formation of Ni-Cu-Zn nanoparticles. Fig.2a indicates the UV-Vis absorption spectra of extract indicating strong absorption top at





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353 nm, which matches the previous work [10]. The UV-visible spectra of dispersion were recorded at distinct time intervals from the initiation of response in fig.2b. The absorption peak most at 260 nm, which can be expectantly ascribed to of Ni-Cu-ZnNPs formed [11-13].

FTIR Analysis

FTIR analysis of leaf extract and green synthesized Ni-Cu-Zn nanoparticles revealed the various chemical groups at ranges of 500–3500 cm⁻¹ shown in Fig.3a and 3b. The sharp peak of synthesized Ni-Cu-Zn nanoparticles of 3333.92cm⁻¹ indicated the presence of N-H group bond of amides [14]. The band at 2119.75cm⁻¹ was assigned of C≡ C stretching [15]. A peak around 1642 cm⁻¹ was attributed C=C stretching of alkynes. The peak appeared at 598 cm⁻¹ is due to the presence of the Ni-Cu-Zn moiety at this range. Similarly, *Capparisam zeylanica* leaf extract showed various peaks such as 3333.92, 2124.76, 1637.35, and 598 cm⁻¹.The peak observed at 3333.92cm⁻¹ is attributed to the hydroxyl group (-OH) [16]. The peak at 2124.76 cm⁻¹ indicated aliphatic C≡C stretching vibrations. The peak at 1637.35 indicated that C=O stretching, ketone and sulfur compounds respectively and 598cm⁻¹ indicated that C-CI stretching of alkyne groups.

SEM analysis

Shape and morphology of the synthesized nanoparticles had been recognized by means of scanning electron microscope analysis. The nanoparticles were tested under numerous magnifications of ×15,000,×30,000,×45,000 and ×45,000. SEM pictures of the synthesized NiCuZnNPs are proven in fig.4. It shows pretty round-formed nanoparticles. Accumulation of greater reducing moieties sure at the surface of the particles should have contributed to the formation of elongated huge round nanoparticles [17].

CONCLUSION

In the field of nanotechnology, development of dependable and eco pleasant strategies for the synthesis of metal nanoparticles is pinnacle need. The present study fulfills the goal of 'Green' synthesis of Ni-Cu-Zn nanoparticles with the resource of a simple approach. The Ni-Cu-Zn alloy nanoparticles were effectively synthesized with the beneficial resource of using novel *Capparis zeylanica* plant leaves as first time, which offers charge powerful, clean and proficient way for synthesis of Ni-Cu-ZnNPs. The traits of the obtained Ni-Cu-Zn alloy nanoparticles have been studied using UV-Vis, FTIR, SEM evaluation strategies.

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Table. 1. Plant Classification

Caparis zylanica leaf	Leaf Powder	Scientific Classification	
		Kingdom	Plantae
	The same of the sa	Phylum	Tracheophyta
		Class	Magnoliopsida
		Order	Capparales
		Family	Capparaceae(caperfamily)
		Genus	Capparis
		Species	Capparis zeylanica.L
		Common name	Ceylon coper, Indian caper
		Tamil name	Adondai, Karrottai

Table. 2 Phytochemical Screening

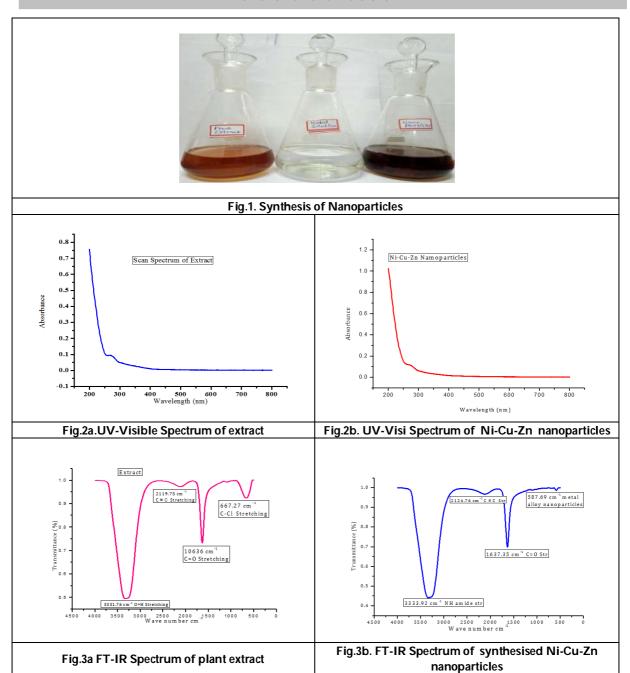
Test	Specific Test	Reagent Composition	Observed Color	Inference
Detection of	Ferric chloride	Solution when treated with a drop of Ferric	Blackish red color	Presence of
Flavonoids	Test	chloride		Flavonoids
Detection of	Mayer's Test	Extract mixed with ammonia and then with	Creamy white	Presence of
Alkaloids		chloroform solution. Dil HCl was added.	precipitate	Alkaloids
		Acid layer with a few drops of Mayer's		
		reagent.		
Detection of	-	5ml of extract, FeCl₃ was added.	Deep blue (or)	Presence of
Tannins			greenish black color	Tannins
Detection of	Keller killani's	Dissolved the extract in water with glacial	Brown ring	Presence of
Glycosides	Test	acetic acid, FeCl ₃ ,Con.H ₂ SO ₄		Glycosides
Detection of	Salkowski's of	2ml of extract, 2ml of chloroform was added,	Reddish brown	Presence of
Steroids	Steroids	followed by 3ml of H ₂ SO ₄ .	color	Steroids
Detection of	-	Extract was dissolved in 5ml of distilled	Dark green color	Presence of
PhenoIs		water. Few drops of neutral 5% FeCl ₃		Phenolic
		solution were added.		Compound.



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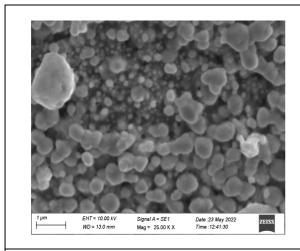




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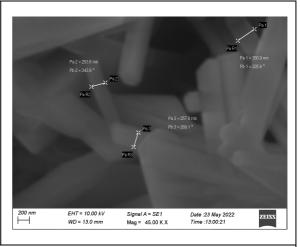


Fig. 4. SEM images of synthesized Ni-Cu-Zn nanoparticles





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RESEARCH ARTICLE

Certificate-based Protected Communication System for Cluster-based Structural Design

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ABSTRACT

Clustering Problems in MANETs consist of selecting the maximum suitable nodes of MANETs topology as cluster heads, thereby confirming that everyday nodes are related closer to cluster head, in order that community lifetime is prolonged. This approach deliberates the attacker degree of every malicious node. In order to supply the authentication and confidentiality within side the community, a threshold signature shape has been employed. Cluster-primarily based totally certificates revocation scheme has been used primarily based totally at the attacker degree of every malicious node. This recruits and gets rid of the certificate of the observed malicious neighbourhood nodes. The expected Certificate-Based Secure Communication Scheme (CBSCS) is related to the Cluster-primarily based totally Certificate Revocation with Vindication Capability (CCRVC) approach. A certificates-primarily based totally stable verbal exchange scheme has been proposed for cluster-primarily based totally structure in MANET.

Keywords: MANET, CBSCS, CCRVC, Cluster head and Cluster Member





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INTRODUCTION

The certificates primarily based totally secured verbal exchange scheme for cluster primarily based totally architecture. The clustering strategies within side the MANETs and a number of the algorithms used for such clustering with suitable protection measures. Clustering Problems in MANETs consist of selecting the maximum suitable nodes of MANETs topology as cluster heads, thereby confirming that ordinary nodes are related in the direction of cluster head, in order that community lifetime is prolonged. The clustering of cell nodes in MANETs is separated to multiple essential clusters, and is shipped geographically contiguous into the identical cluster primarily based totally on a few guide lines which might be depending on the algorithms with multiple manners for nodes which might be encompassed inside clusters. A cluster head normally capabilities as a nearby coordinator for his or her clusters, conducting intra-cluster transmission, statistics forwarding, etc. A cluster gateway is a non-cluster head node with inter-cluster links, thereby allowing them to touch neighboring clusters and ahead statistics amongst clusters.

Problem Identification

Initially, the nodes with the very best balance index are decided on as Cluster Heads (CH) and each different node develops into Cluster Members (CM). For differentiating the misbehaving nodes from well-behaved nodes, SVM has been hired. For setting apart misbehaving nodes, Fuzzy guidelines are hired that prevent intrusion. In the following paintings in securing the communique among the cluster member and cluster heads, a secure communique shape for cluster-primarily based totally structure in MANETs has been provided.

Certificate-Based Secure Communication Scheme

A certificates-primarily based totally steady communique scheme has been proposed for cluster-primarily based totally structure in MANET. In this technique, a cluster-primarily based totally certificates revocation shape is implemented that procures and gets rid of the certificate of the observed malicious community nodes. This technique deliberates the attacker stage of every malicious node. In order to supply the authentication and confidentiality within side the network, a threshold signature shape has been employed.

Certificate Authority

For loading the certificates in every cell node, a certification authority CA is placed within side the created cluster. The main reason of CA is to replace warning (Lw) and black lists(Lb). Lwaids in detecting the accused node and Lb aids in detecting the pertinent accusing node. CA appraises every listing on the idea of acquired manage packets. Moreover, CA pronounces the Lw and Lb to the whole community to cancel the certificates of nodes indexed in Lb and segregates them from the community.

Nodes Classification

The nodes come under subsequent groups on the basis of their behavior in the network:

Legitimate Nodes:

It aids in the accomplishment of secure communication with other nodes.

It includes:

Noticing attacks from malicious nodes, reproving the nodes definitely, Cancelling the certificates and Assuring network security.

Malicious Node:

It stops classifying misbehaviour and incorrectly reproaches a legitimate node revoking its certificate.

Attacker Node:

It launches attacks on its neighbours interrupting secure communications in the network.

The above nodes are additionally categorized on the basis of their consistency as follows:





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Normal Nodes:

If a node links the network and does not promote any attacks, it is referred to as normal node, and it comprises higher reliability. It has supreme aptitude to indict other nodes and announce itself as cluster head or cluster member.

Certificate Revocation

Cluster-primarily based totally certificates revocation scheme has been used primarily based totally at the attacker degree of every malicious node. This recruits and removes the certificate of the observed malicious neighbourhood nodes. The manner of revoking certificates from malicious attacker incorporates the subsequent manner: Accusing, Verification and Notification. Let AN_i be the attacker node, Pa be the accusation packet and RM be the revocation message.

The stages involved in the certificate revocation procedure are as follows:

Every neighbour node Ni detects the presence of attacks from ANi. If Ni does not find ANi in Lb Then

$$Ni \xrightarrow{Pa} CA End if$$

Ni sends Pa to CA. The format of Pa is shown below

When CA receives first Pa, it confirms the certification validation of accused node.

If certificate is legal, Then

Accused node is deliberated as malicious attacker The corresponding node is included in Lw.

$$CA \xrightarrow{RM}$$
 whole network

End if

CA broadcasts the RM that contains Lw and Lb to the entire network. The setup of RM is exposed in table below.

Threshold Signature Scheme

For providing authentication and confidentiality for secure communication, threshold signature scheme has been employed.

Let CH be the cluster head.

Let CMi (i=1,...,t1) be the cluster member in the cluster. Let Zk (k = 1,...,t2) be the group of signer.

t1 and t2 be the identity of i th and k th member. Let Q be the verifier.

Let p be the message

Let ${}^{\tau}\mathcal{E}$ be the arbitrary tokens where $1^{\leq \varepsilon \leq \tau_2}$

Let Ge be the signature.

The steps convoluted in this method are as follows:

One of Zk transmits the threshold generation request (Th_Req) to CH with signers list (Z1, Z2,...Zt2).

CH selects $^{\tau_{\mathcal{E}}}$ and transmits it to pertinent Zk in secured manner.

Each Zk generates Ge (Ge = h0(p).ge) and guesses respective token { $^{\tau}_{\varepsilon}$ }. Ge}. Each Ni transmits the message tuple to O and CHi:

 $Ni \rightarrow CHj:(p,Ke,KQ, {}^{\tau}_{\varepsilon}.Ge,HMACe(p,KQ, {}^{\tau}_{\varepsilon}.Ge))$ where Ke = public / private key pair.

CHj searches pertinent pseudonym public keys and confirms HMAC validity. Then, it confirms the signature validity. $^{\tau_{\mathcal{E}}}$. Ge using relevant tokens and CHj improves the signature Ge from $f^{CH^{j}}(x)$ produces additional (t1-t2) pointsonh0(p).





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Then, CHj carries out secret reconstruction algorithm using t2 received signatures and (t1-t2) points as follows:

h0(p).
$$f^{CH_{I}}(x) = \sum_{i \in \mathcal{N}} \eta_{i}(x) V_{i} \pmod{w}$$

$$= (1 \leq i \leq t_{2}),$$

$$\{ h_{0}(p).G_{i}, (t_{2} + 1 \leq i \leq t_{1})$$

$$V_{i} = (3.1)$$

where
$$\eta_j(x) = \prod_{j \in \mathcal{N}} \int_{\mathcal{N}} ((id_j - x)/(id_j - id_j)) = \text{Lagrange Co-efficient N} = \{1, ..., t1\} \int_{\mathcal{N}} f^{CH^j}(x)$$

If the reconstructed polynomial has h0(p). at x = 0, then CH j receives the signature as valid

If the complete messages and signatures are effective, then CH j produces verifying polynomial and additional points and transmits it to Q.

CH
$$\rightarrow$$
Q: ({ $^{\tau_e}$.Ge1,... $^{\tau_{\varepsilon}}$.Ge2},R1,...Rt2,HMACQ($\lambda_p^{CHj}(0)$, τ_e · $G_{e1,...}$ τ_e .G2et}, R1,...Rt2))

Q accomplishes the secret reconstruction as follows:

$$\lambda_{i=1}^{CH_j}(x) = \sum_{i \in N} \eta_i(x) U_{i,i}(\text{mod } w)$$

$$\begin{bmatrix} R_{i,i} & (1 \le i \le t_2), \\ h(n), G, & (i = \varpi) \end{bmatrix}$$

$$Ui =$$

$$\text{Where } \eta_i(x) = \prod_{j \in N \setminus i} ((id_i - x) / (id_j - id_i)) =$$

$$\text{Lagrange Co-efficient } N = \{1, ..., t1, \overline{\omega}\}$$

If HMAC is valid Then

Q considers the signature as valid.

End if

Simulation Results

We used a network simulator (NS2) to simulate the proposed architecture. For simulation purposes, 50 mobile nodes move over a 1000m x 1000m area with a simulation time of 50 seconds. Each node has the same transmission range of 250 meters. The simulated traffic is a constant bit rate (CBR). Table 4.1 briefly summarizes the simulation settings and parameters. The values of each are given in the table.

Performance Metrics

The anticipated Certificate-Based Secure Communication Scheme (CBSCS) is associated with the Cluster-based Certificate Revocation with Vindication Capability (CCRVC) method. The performance is assessed as per the following metrics:

Packet Delivery Ratio:

It is the ratio between the quantity of packets received and the quantity of packets sent.





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Packet Drop:

It mentions the average quantity of packets dropped during transmission

Delay:

It is the quantity of time taken by the nodes to transmit the information packets.

Overhead:

It is the average quantity of control packets bartered during the transmission

RESULTS

Figure 4.2 represents the Attackers as opposed to Delay. It indicates the put off of CBSCS and CCRVC strategies for multiple amount of attacker scenario. Until there being 2 attackers, the put off for each the schemes is nearly equal. When the range of attackers is 3, the put off for CBSCS and CCRVC schemes is discovered to be 12 and 15 respectively. Similarly, whilst the range of attackers is 5, the put off for CBSCS and CCRVC schemes is eleven and 15 respectively. It is absolutely discovered that the put off of the proposed CBSCS approach is 10% much less than that of CCRVC approach. Figure 4.3 three represents the Attackers as opposed to Delivery Ratio. It suggests the transport ratio of Certificate-Based Secure Communication Scheme (CBSCS) and Cluster-primarily based totally Certificate Revocation with Vindication Capability(CCRVC) strategies for numerous amount of attacker scenario. Initially, whilst the variety of attacker is 1, the transport ratio of CBSCS and CCRVC schemes is located to be 0.32 and 0.29 respectively. Similarly, whilst the variety of attackers is 5, the transport ratio of CBSCS and CCRVC schemes is found to be 0.35 and 0.20 respectively. It is honestly perceived that the transport ratio of the proposed CBSCS approach is 22% better than that of CCRVC approach. Figure 4.5 represents the Attackers versus Overhead. It displays the overhead of CBSCS and CCRVC methods for dissimilar quantity of attacker scenario. Initially, when the number of attacker is 1, the overhead of CBSCS and CCRVC approaches is 27000 and 33000 respectively. Similarly, when the number of attacker is 5, the overhead of CBSCS and CCRVC approaches is 30000 and 44000 respectively. It is clearly seen that the overhead of our proposed CBSCS approach is 22% less than that of CCRVC approach.

CONCLUSION

A certificates-primarily based totally steady conversation scheme has been proposed for cluster-primarily based totally structure in MANET. In this strategy, a cluster-primarily based totally certificates revocation association has been implemented that recruits and gets rid of the certificate of the detected malicious neighborhood nodes. This technique deliberates the attacker stage of every malicious node. In order to supply the authentication and confidentiality within side the community, a threshold signature shape is used. From simulation results, it's far actually visible that the proposed device improves the community protection and decreases the overhead.

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Table.1: Format of Accusation Packet

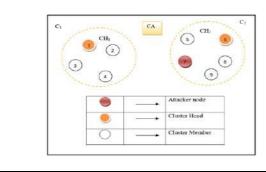
Pac	ket Type	Source ID	Accuser ID	Accused ID	Destination ID	Data Information	
-----	----------	-----------	------------	------------	----------------	------------------	--

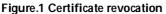
Table.2: Format of Revocation Message

Packet Type	Source ID	Data Information	Node count in Lb	Node count in Lw
Nodes in Lb are revo	oked from the networ	k successfully.		

Table.3: Simulation parameter setup

Simulation Parameters	Values			
No. of Nodes	20,40,60,80 and 100			
Area Size	1000 m X 1000 m			
MAC	IEEE 802.11			
Transmission Range	250m			
Simulation Time	50 sec			
Traffic Source	CBR			
Packet Size	512 bytes			
Routing Protocol	AODV			
Attackers	1,2,3,4 and 5			
Rate	100KB			





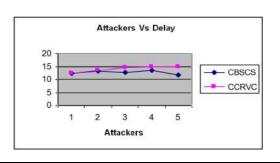


Figure.2 Attackers versus Delay

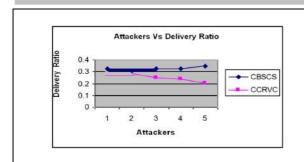




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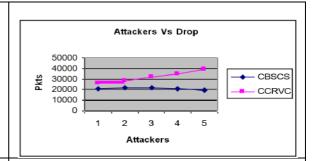


Figure.3 Attackers versus Delivery Ratio

Attackers Vs Overhead 50000 40000 Overhead - CBSCS 30000 20000 CCRVC 10000 5 3 Attackers

Figure.4 Attackers versus Drop

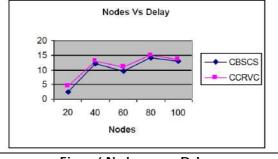


Figure.5 Attackers versus Overhead

Figure.6 Nodes versus Delay





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RESEARCH ARTICLE

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Dynamical Aspect of Mathematical Models on Epidemiology

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ABSTRACT

The SI and SEI mathematical models and their dynamical aspect has been discussed for an epidemic disease. This mathematical model emphasizes the deterministic modelling applied to the population dynamics of an epidemic disease. Here, we are using the ordinary differential equations for infectious diseases. Model have discussed at various levels of diseases as susceptible- exposed- infective with the concept of basic reproductive rate, equilibrium points and investigated their stability by using Routh's Stability criterion.

Keywords: Epidemic Disease, Mathematical Modelling, Epidemiology.

INTRODUCTION

In the history of epidemic diseases "Plague of Athens" (430-428 BC); "Bubonic Plague" (Black Death) caused by the bacterium Yersinia pestis, devastated population in Europe from the 14th-16th centuries, killing between one-half to one-third of the entire population(Langer 1970) and according to WHO reports 1000-3000 cases of Bubonic Plague every year[8]. In the early 1500s, smallpox was identified into Caribbean by the Spanish army's lad from Cortez and spread to Mexico, Peru and Brazil country. The number of deaths has been noted during the period of smallpox. While dynamical modelling on epidemic diseases can be measured back to 1760 when Bernoulli used mathematical models for smallpox. The dynamic models for infectious disease are mostly based on their compartment structure. The compartment structures for well - recognized SIR (Susceptible - Infective - Recovered) are firstly given by Kermack and Mc Kendrick in 1927 and are developed by many other bio-mathematicians in 1932 [5], [9]. Modified SIR model is providing for analysis of influenza infection with bacterial disease (Pneumonia). This model describes the proportion of the population to quarantine after being infected with influenza, and how better treatments of





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bacterial infections all would play into reducing the total number of deaths [1]. The dynamics of the compartmental modelling for an epidemic disease is an important technique of studying the behaviour of an epidemic diseases. Which is based on the specific behaviour of the population growth, to describe spreading rule of an infectious disease and the related factors and so on. For constructing mathematical models reflecting the dynamic properties of infection disease numerically, to analyse the dynamical behaviour and to do some simulations. The research result is useful to measure the growth of an epidemic disease, also, to determine the key factors of the spread of infectious disease and to seek the optimal strategies of medication the spread of an epidemic disease "Chikungunya" [2].A various types of mathematical models have been used in mathematical epidemiology. By modifying the basic SIR model, we have obtained proposed infectious diseases compartmental ODE – models for further development in the future epidemiology. Chikungunya fever is an arbovirus disease caused by chikungunya virus (CHIV), an alphavirus of Togaviridae family. It spread through mosquito bite with the following symptoms like high fever and arthralgia in 2 to 6 days. The disease is self-limiting and usually resolve within 2 weeks. This current report has been published in Frontiers in Microbiology by TRC (2021), India.

Basic forms of Compartmental Models for various type of disease

Definition of parameters of the model:

S = Susceptible Class;

I = Infective Class;

E = Exposed Class.

Model SI

In this model, when the infective cannot be recovered from infection. The governing equations are as follows

$$dS/dt = -\beta SI$$
 (i)
$$\frac{dI}{dt} = \beta SI - \alpha I$$
 (ii)

Where S(t) is the number of suspectable and I(t), the number of infectious at time t. Also, the system considered under the following initial conditions

$$S(t_0) = S_0 > 0$$
 and $I(t_0) = I_0 > 0$.

Model SEI

This model is providing the current scenario of an epidemic disease with the different terms like exposing, transmission and infection status. The governing equations are giving with the help of differential equations for the SEI model as follow:

$$\frac{dS}{dt} = \Delta - \beta SE$$
 (iii)
$$\frac{dE}{dt} = \beta SE - \alpha I$$
 (iv)
$$\frac{dI}{dt} = \alpha I$$
 (v)

Where S(t) is the number of suspectable, E(t) is the number of exposed cases and I(t), the number of infectious at time t. Also, the system considered under the following initial conditions

$$S(t_0) = S_0 > 0$$
, $E(t_0) = E_0 > 0$ and $I(t_0) = I_0 > 0$.





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Mathematical Solution of the above mathematical model

Model 1: General Solution of simultaneous differential equation of model SI is given by

$$\begin{split} & S(t) = \frac{N(N+I_0)}{N+e^{(N+I_0)\beta t}} \\ & \text{and } I(t) = \frac{(N+I_0)e^{(N+I_0)\beta t}}{N+e^{(N+I_0)\beta t}} \; ; \end{split} \tag{Vi}$$

Where S + I = N.

Here, we can obtain the numbers of susceptibility and infected person.

Model 2: General Solution of simultaneous differential equation of model SEI is given by Jacobian method

$$J = \begin{pmatrix} -\beta E & -\beta S & 0 \\ \beta E & \beta S E & -\alpha \\ 0 & 0 & \alpha \end{pmatrix} \text{ and their eigen values are }$$

$$\lambda(\lambda - \alpha) \left[\lambda - \beta(S + E)\right] = 0 \tag{Vii}$$

With the help of Routh's stability method result is obtained and appears the condition of re-susceptible of the mathematical dynamic models for the infectious diseases.

Case I: The Basic Reproduction Number

The basic reproduction number (R_0) shows the differences of contact rate and duration of infection. Mathematically we can define as

 $R_0 = Contact\ rate - Duration\ of\ infection.$

According to the criteria of the basic reproduction number (R_0) , we have the following results

S. No.	Type of Disease	Value of R_0	Conclusion of R_0
1	Endemic	= 1	Transmission occurs but the number of cases
I I	Endernic	= 1	remains constant
2	Epidemic	< 1	Transmission occurs and the number of infected
	Epidemic	< 1	cases increases
2	Pandemic > 1	. 1	When epidemic occurs at several continents also
3		> 1	known as Global Epidemic

Case II: Routh's Stability Criteria

Suppose the given polynomial is $P(\lambda) = \lambda^n + a_1 \lambda^{n-1} + ... + a_{n-1} \lambda + a_{n}$ (viii)

Where the coefficients a_i , i = 1, 2, ..., n, are all real constants. Using these coefficients, we form Hurwitz matrices H_i ; j = 1, 2, ..., n for the given system of equations as:

$$H_1 = (a_1), H_2 = \begin{pmatrix} a_1 & 1 \\ a_3 & a_2 \end{pmatrix},$$

$$H_3 = \begin{pmatrix} a_1 & 1 & 0 \\ a_3 & a_2 & a_1 \\ a_5 & a_4 & a_3 \end{pmatrix} \quad \text{ and } \quad$$





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$$H_n = \begin{pmatrix} a_1 & 1 & 0 & & 0 \\ a_3 & a_2 & a_1 & \cdots & 0 \\ a_5 & a_4 & a_3 & & 0 \\ & \vdots & & \ddots & \vdots \\ 0 & 0 & 0 & \cdots & a_n \end{pmatrix}$$

For all i > n, $a_i = 0$.

All roots of the polynomial $P(\lambda)$ are negative or have negative real part iff the determinants of all Hurwitz matrices are positive as: $\det(H_i) > 0$, j = 1, 2, ..., n.

For the polynomials of degree n = 2, 3, 4 and 5, the Routh-Hurwitz criterion is summarized as:

For n = 2: $a_1 > 0$ and $a_2 > 0$.

For n = 3: $a_1 > 0$, $a_3 > 0$ and $a_1a_2 > a_3$.

For n = 4: $a_1 > 0$, $a_3 > 0$, $a_4 > 0$ and $a_1 a_2 a_3 > a_3^2 + a_1^2 a_4$.

For n = 5: $a_i > 0$; $i = 1,2,3,4,5, a_1a_2a_3 > a_3^2 + a_1^2a_4$

and $(a_1a_4 - a_5)(a_1a_2a_3 - a_3^2 - a_1^2a_4) > [a_5(a_1a_2 - a_4)^2 + a_1a_5^2]$ and so on.

The Routh Hurwitz matrix of a stable polynomial satisfying, if f(0) > 0 is totally nonnegative.

Case III: Model "SI - Model"

The Jacobian of SI model is given by

$$J = \begin{pmatrix} -\beta I & -\beta S \\ \beta S I & \beta S - \alpha \end{pmatrix}$$

The characteristic equation of the Jacobian is given by

$$|J - \lambda I| = \begin{vmatrix} -\beta I - \lambda & -\beta S \\ \beta S I & \beta S - \alpha - \lambda \end{vmatrix}$$

So, the eigenvalues are given by

$$\lambda^2 + \lambda(\beta S - \alpha - \beta I) - \alpha \beta I = 0$$

(ix)

Using Routh's Stability Method, the coefficients a_i ; i = 1,2,...,n, are all real constants. Using these coefficients, we form Hurwitz matrices H_i : j = 1,2,...,n for the given system of equations as:

$$H_1 = (a_1) = (\beta S - \alpha - \beta I)$$

All roots of the polynomial $P(\lambda)$ are negative or have negative real part iff the determinants of all Hurwitz matrices are positive as: $\det(H_j) > 0$, j = 1, 2, ..., n. For the polynomial of degree n = 2, the Routh-Hurwitz criterion is summarized as:

$$(\beta S - \alpha - \beta I) = 2015.3752 > 0 \text{ and } \alpha \beta I = 37.40801896 > 0.$$
 (x)

Case IV: Model"SEI - Model"

The Jacobian of SEI model is given by

$$J = \begin{pmatrix} -\beta \mathsf{E} & -\beta \mathsf{S} & 0\\ \beta \mathsf{E} & \beta \mathsf{SE} & -\alpha\\ 0 & 0 & \alpha \end{pmatrix}$$

The characteristic equation of the Jacobian is given by

$$|J - \lambda I| = \begin{vmatrix} -\beta E - \lambda & -\beta S & 0\\ \beta E & \beta SE - \lambda & -\alpha\\ 0 & 0 & \alpha - \lambda \end{vmatrix}$$





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So, the eigenvalues are given by

$$\lambda^{3} + \lambda^{2} (\beta E - \alpha - \beta S) + \beta \alpha \lambda (S - E) = 0$$
 (xi)

Using Routh's Stability Method, the coefficients a_i ; i = 1,2,...,n, are all real constants. Using these coefficients, we form Hurwitz matrices H_i ; j = 1,2,...,n for the given system of equations as:

$$H_2 = \begin{pmatrix} a_1 & 1 \\ a_3 & a_2 \end{pmatrix} = \begin{pmatrix} \left(\beta E - \alpha - \beta S\right) & 1 \\ 0 & \beta \alpha (S - E) \end{pmatrix}$$

All roots of the polynomial $P(\lambda)$ are negative or have negative real part iff the determinants of all Hurwitz matrices are positive as: $\det(H_j) > 0$, j = 1, 2, ..., n. For the polynomials of degree n = 3 the Routh-Hurwitz criterion is summarized as:

$$(\beta E - \alpha - \beta S) = -1163.6556 < 0, a_3 = 0 > 0$$
and $\beta \alpha (S - E)(\beta E - \alpha - \beta S) = -325457.033 < 0.$ (xii)

Numerical Analysis of the Mathematical Models

India: In 2022 and as of 30 April, 18 856 cases, including 1 261 confirmed cases and no deaths have been reported. This is an increase of 18 519 cases since 25 April 2022.

Graphical Analysis

The Basic Reproduction Number (R_0)

The Basic Reproduction Number (R_0) is used to explain the current situation of an epidemic diseases. In an improved model SEI, if the basic reproduction number (R_0) is less than one that is $R_0 < 1$, then the disease-free equilibrium point will be stable, otherwise it will be unstable for $R_0 > 1$. The basic reproduction number (R_0) is defined for chikungunya as

$$R_0 = \frac{birth\ rate}{death\ rate} = \frac{17.1}{7.3} = 2.3425 < 3.4\ (95\%\ CL\ 2.4 - 4.2)$$
 (xii)

According to the observation of Haider N. (2020), the basic reproduction number ($R_0 = 2.32561 < 3.4$) shows that the current situation of India is stable.

DISCUSSION

A wide range of mathematical models have been used in biological mathematical modelling on epidemiology. By modifying the basic SI model, we have obtained useful result insight on the effect of viral infection during endemic. Both model SI as well as SEI have been analysed graphically. According to SI Model provides the stable situation while second SEI model provides unstable situation. This modified mathematical modelling may helpful for taking best strategy for upcoming risk, preventing and controlling of the spread of infectious diseases. Also, discussed graphically the outcomes of the research paper.

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Table 1: Definition of parameters

Parameters	Meaning	Dimension
β	Transmission rate	Per year
α	Exposed rate	Per year
δ	Infection rate	Per year
t	Time	Per year

Table 2: Values of Parameters for Chikungunya Disease



Table 3: Numerical Table for Variables

Variables	Numerical Values of cases in year 2022	Reference
S_0	17595	https://timesofindia.indiatimes.com/city/mum
E_0	8167	bai/mumbai-5-yr-high-in-chikungunya-cases-
7	12/1	in-state-but-count-under-
10	1261	reported/articleshow/87318886.cms

Table 4: Numerical Table for Parameters

Parameters	Meaning	Values
β	Transmission rate	0.1234
α	Exposed rate	0.2404
Δ	Infection rate	0.0755
T	Time	1 – 7 days

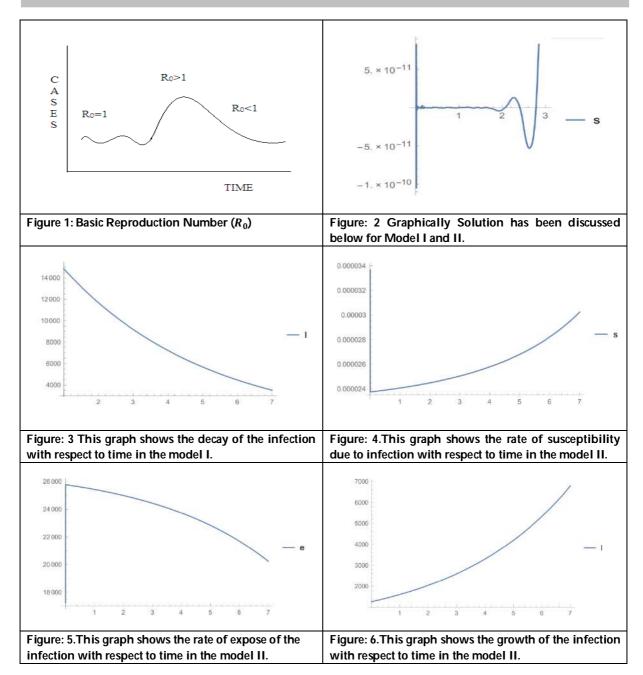




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RESEARCH ARTICLE

Active and Reactive Power Control of Hybrid Power-Flow Controller (HPFC) with Fuzzy Logic Controllers

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ABSTRACT

Many of the PFCD's are mostly used in the power-systems in order to monitor the real as well as reactive power-flow variations. Here a innovative power-flow controlling device so called Hybrid-PFC is projected in this paper, this device also belongs to the FACTS family. This device is emerged from the Unified-PFC, there is a small differentiation between both these devices that is the common dc-link. In case of HPFC there is no existence of the dc link which connects both the converters. Here in this controller the real-power swapping is done via transmission-line at the 3rd harmonic-frequency. Simulation of the controller is carried out in the Matlab / Simulink software.

Keywords: power electronics devices, Unified Power Flow Controller, Hybrid power flow controller,

INTRODUCTION

In most of the power-systems the utilization of electricity varies frequently so due to this reason it is very much essential to organize the power-flow. Here a device called Power Flow Controlling (PFC) used to adjust the parameters such as bus voltage, transmission angle and impedance of the line. The combined characteristics of FACTS and Power-Electronics PFCDs are one of the most appropriate devices which are utilized in managing power-flow[1]-[2]. One of the series compensated FACTS controller known as UPFC which can be treated as one of the commanding PFCD which can be used to correct all the above mentioned system parameters[3]. Enlarging the Unified-PFC into the innovative controller known as Hybrid-PFC is done by excluding the common DC link and series-converter distribution as given away in Fig. 1.The new concept Hybrid FACTS is employed in the series





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converter design. Here many number of low power rating converters are adopted as a replacement for a large 3-phase rated converter which results in cost reduction and increases reliability greatly [4]. These are considered as the major advantages of HPFC compared to UPFC. Figure 2 shows the configuration of Hybrid-PFC consisting shunt along with series-converters as in case of UPFC. In HPFC each converter have their individual DC-capacitor that contributes necessary DC-voltage.

HPFC Principle

Ac terminals of shunt-converter are connected with series converter via transmission line through which active power is interchanged. Power-theory of the non-sinusoidal components is adopted in this method. In Fourier analysis the non sinusoidal current and voltage is articulated as summation of sinusoidal-functions in various frequencies by means of dissimilar amplitudes. This results in the true-power as shown in equation (1).

$$P = \sum_{i=1}^{\infty} V_i I_i \cos \phi_i \tag{1}$$

Where I_i , V_i be the current as well as voltage at the i^{th} harmonic-frequency respectively, where as ϕ_i be the phase-angle among the current as well as voltage. HPFC mainly comprises of a many number of low power rating series-converters and also a shunt-converter is also utilized. Here shunt-device is operated as the Static-compensator where as the series converter acts as a Hybrid-FACTS controller[5]. Using the PWM(pulse width modulation) technique inject the current into the shunt converter to organize reactive-power and also the voltage is injected into the series converter through a dc link to monitor real-power variations inorder to inject voltage into the series converter. There are many advantages regarding D-FACTS controller, the price of the apparatus gets minimized and the reliability of the system is improved[6]. By using a single bulky controller with high rating, there is an availability of using number of low rated controllers so this the cost the equipment may get reduced. Each and every Hybrid-FACTS component is self employed and they are monitored tenuously by the PLC's(power-line communications) or wireless communication. Since the unit does not need support of phase to ground isolation, it is capable of adopted at all voltage levels. During single module failure, the Hybrid-FACTS redundancy offers an unremitting process, thus giving higher reliability compared to other FACTS devices[7].

HPFC Control

Fig. 3 shows the three types of controllers that control the multiple converters; namely central control, shunt-control as well as series-control. The constraints that are used in shunt in addition to series-control are sustained by themselves they are also called as local controllers. By the side of the system's level the central control regulates the HPFC functions. Each controller's function is discussed [8].

Central Control

Mainly this control is adopted to produce the reference-signals for the other controllers. These are generated next to the fundamental-frequency. Reactive current signal and voltage reference signal is provided for the shunt converter along with the series-converters respectively. At power system level, the central-control is used to minimize the systems oscillations, and also monitors the power-flows and also balances the components which are asymmetrical.

Series Control

This control in adopted in HPFC inorder to inject the voltages into the line when required. Here the voltage is inserted into the at the 3^{rd} harmonic-frequency. Here Vector control principle used to maintain the voltage across capacitor.

Shunt Control

Inorder to transfer the real-power, 3rd harmonic-current is inserted into the three phases of the transmission line with the help of the transformer which is connected to the AC terminals of the shunt-device. This is the main objectie of this control technique. And also it maintains the constant voltage-levels across the dc-capacitor [9].





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Comparison of PI and Fuzzy Controller

Here fuzzy as well as PI controllers are utilized. The responses obtained by both the controllers are studied under this section.

PI Controller

When PI-controller is utilized, the dc-link voltage is observed at regular intervals and is correlated with a reference value. Here an error signal can be generated which is handled in a PI controller as shown in figure below [10]-[11].

Comparing PI and Fuzzy Logic Controller (FLC)

The following are some the advantages of fuzzy- logic controllers when compared with the proportional integral(PI) controllers,

- perfect mathematical model is not mandatory
- Fuzzy controllers can handle the inputs which are unknown.
- Fuzzy controllers have enough capability to work with non-linearities .

Fuzzy-inference systems are applied in numerous applications namely computer-vision techniques, control-data categorization etc. Mainly the FIS are classified into two types they are as follows

- Mamdani-techique and
- Sugeno-techique

Among both the methods, Mamdani' technique is adopted frequently when compared to sugeno method and also this method gives more accurate results when compared with PI controller. The sampling time of this method is very low so due to this reason we are not adapting this method for real time applications. Block diagram of fuzzy-logic controller is given away in Fig.4. The control-action that is carried out by fuzzy-logic controller is determined by evaluating the linguistic rules. The different components of a fuzzy control system are fuzzification, rule base, defuzzification, and inference mechanisms. Under fuzzification procedure the crisp-values are transformed to fuzzy-values. Where as in de-fuzzification procedure fuzzy-values are changed to crisp-values . rule base consists of different rules which are applied to solve a problem. Inference mechanism mainly consists of two techniques such as mamdani and sugeno techniques by considering these two techniques we will solve a problem [12]-[13].

Simulation Results

Simulation is carried out in MAATLAB/SIMULINK software. Here mainly we are analyzing two situations they are steady-state in addition to step-response conditions. In first case using series control we are inserting a voltage-vector with d-axis and q-axis components , namely V se, d, ref=0.30V and V se, d, ref=-0.10V. The responses of HPFC under the steady-state is shown in figures d (a),d (b),d (c) where only the waveforms of one phase are shown for clarity. When a fault occurs on a power system a third harmonic current component is produced in order to eliminate the third frequency component by using a shunt converter we are introducing another third frequency component in the negative direction now both of the harmonics currents gets cancelled out then the fault can be easily rectified. Fig d (a) represents the third harmonic current component waveform after nullifying the harmonic current component using a shunt converter the resultant waveform is shown in fig.d (c). If fig.7.(a) the step-change of series converter reference voltage is shown which consists of the real as well as the reactive-power variations. And dc voltage is alleviated previously and behind the step-change. fig.7(b) and 7(c) calculates the power from the voltages as well as currents which are measured.

The data is measured in 3 phases but only one phase data is progressed in computer system using MATLAB. A LPF (low-pass filter) with 50-Hz cut-off frequency filters the measured data containing harmonic distortion in order to examine the current along with voltage at fundamental-frequency. This filter causes a 1.5 cycle delay of calculated voltage and current to the real values thus cause of delay of measured real-power in addition to reactive-power. Fig.7.(d) shows the series-converter injecting real-power along with reactive-power at fundamental-frequency. The receiving end side waveforms are represented in fig.7(e). Above fig.8(a), 8(b), 8(c), 8(d), 8(e) demonstrate the step





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response of the HPFC using fuzzy logic controller. Here the delay of measured active and reactive power is still reduced compared to PI controller.

CONCLUSION

In this paper a new PFCD from FACTS Family called HPFC was presented. Here we are using Fuzzy Logic Controllers instead of using Conventional Proportional Integral(PI) Controllers, these Fuzzy Controllers gives better response and we get accurate results compared with conventional PI Controllers. Fuzzy Controllers manages the real-Power, Reactive-Power, Bus Voltages along with Currents. The D-FACTS concept is operated in the HPFC resulting in cost reduction and high reliability compared to UPFC. Simulation is carried out in Matlab/Simulink Software.

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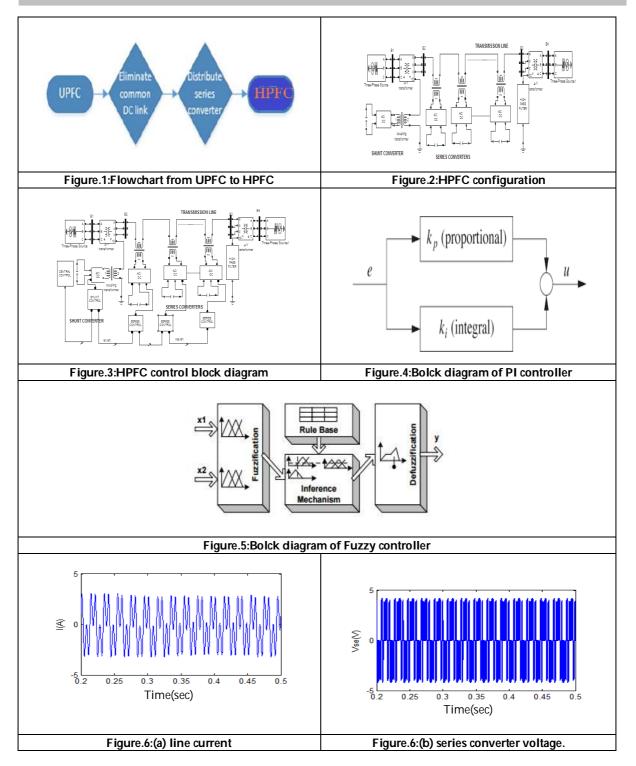




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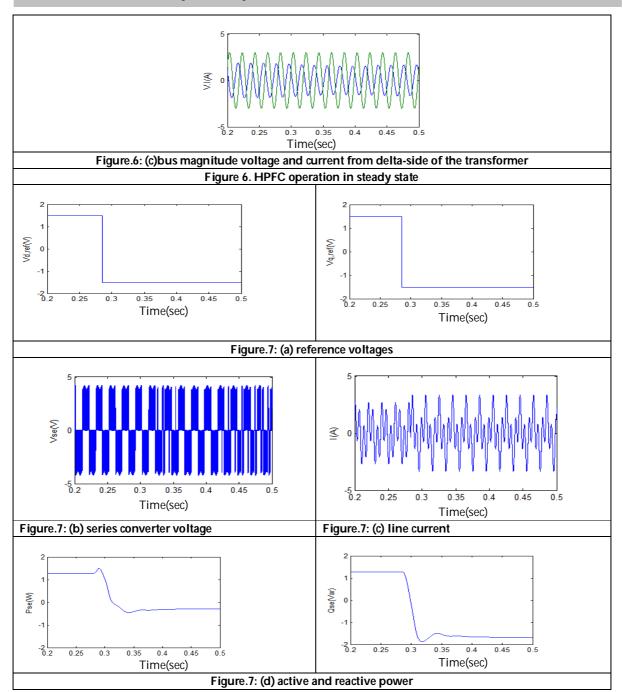




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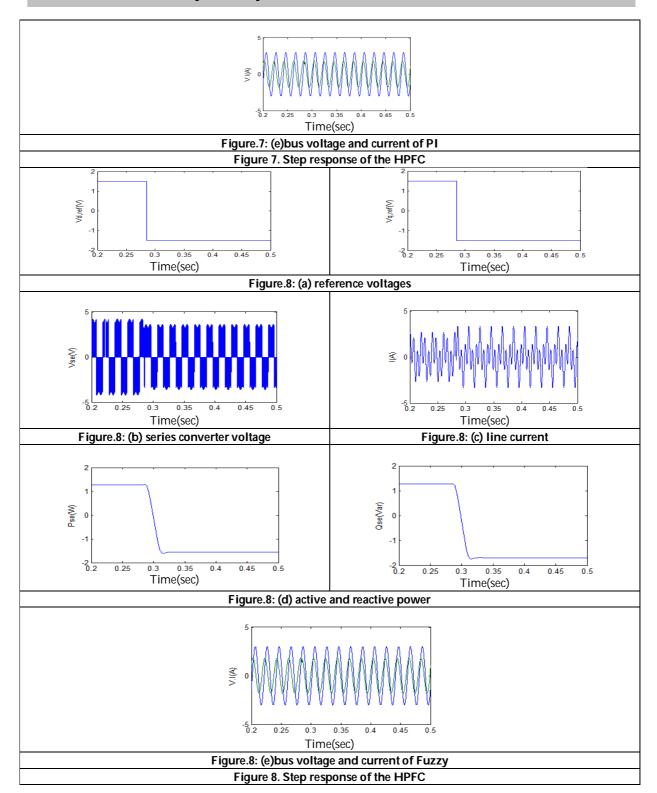




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ISSN: 0976 – 0997 RESEARCH ARTICLE

Bioavailability Enhancement of Albendazole Chewable Tablet with Improved Dissolution using Design of Experiment (DoE)

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ABSTRACT

The current research aims to design and evaluate an albendazole chewable tablet with enhanced bioavailability by a dissolution approach and optimization using a central composite design. Preformulation studies were performed for API and optimized blend. The albendazole chewable tablet was prepared by wet granulation method. Optimization of formulation by Central Composite Design and model validation using Design of Expert software (DoE). Post compression parameters such as hardness, thickness, friability, weight variation, assay, disintegration and dissolution were evaluated. The significant model was constructed using the 3² Central Composite Design with 10 experimental runs. The responses include dissolution and disintegration, and the factors used were sodium starch glycolate, croscarmellose sodium, and sodium lauryl sulphate. ANOVA was used to validate the model, and it was determined to be significant. The rapid disintegration time (130 secs) and the highest percentage of drug release (89.2%) was demonstrated by F-5 Formulation. The Korsmeyer and Peppas model of kinetics (R² = 0.9761) accurately depicts the drug release pattern in albendazole chewable tablet. In comparing the invitro dissolution profiles of the Zentel and optimised albendazole chewable tablet (F-5), a similarity factor





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(f2) of 74 and a difference factor (f1) of 4 were obtained. After performing stability studies, the optimized formulation (F-5) was found to be stable.

Keywords: Albendazole, Chewable tablet, Dissolution enhancement, Central Composite Design and Statistical Methods

INTRODUCTION

Albendazole is a benzimidazole drug that is commonly used as anti-parasitic therapy. It is an anti-helminthic and anti-filarial drug, and is listed on the WHO's list of essential medications [1]. Its chemical name is methyl [5-(propylthio)-1H-benzimidazole-2-yl] carbamate. Due to its extremely low water solubility, it exhibits poor and unpredictable absorption in the GI tract and is classified as a type II BCS class drug. Chewable tablets are an oral dosage form that should be administered by the patient after being chewed, as opposed to being swallowed whole. They need to be designed so that they are palatable and convenient to chew and swallow. The FDA recently suggested updated standards for chewable tablets that would cover all potential influences on drug bioavailability and bioequivalence, as well as essential quality characteristics like hardness, disintegration, and dissolution [2]. Drug-release characteristics are critical to the active ingredient's bio-performance, with some pharmacopoeias requiring no drug release testing at all. With the exception of the international pharmacopoeia, no pharmacopoeia contains the monograph for chewable albendazole tablets. It has been demonstrated that tablet dissolution performance is significantly correlated with the clinical efficacy of albendazole tablets. Because of the poor solubility of albendazole in aqueous media and evidence of excipient effect on dissolution, quality criteria for albendazole tablets are recommended to include disintegration and dissolution testing. Disintegration and dissolution tests have been included to the international pharmacopeia's monograph for albendazole chewable tablets [3]. It has been reported that many dissolution enhancement methods and novel formulations can increase albendazole's rate of dissolution [4,5,6]. Disintegration and dissolution parameters are optimised using central composite design (CCD) to ensure that the prepared formulation achieves its intended purpose [7]. To be a bioequivalent product, the improved formulation must meet the similarity factor (f1). The Process involved in formulation and optimization of Albendazole chewable tablet by Central Composite Design is illustrated in the figure 1. The dissolution of marketed albendazole tablets produced poor results. This demonstrates the need for the development of a new albendazole tablet, maybe a generic one with improved dissolution [8]. The designed albendazole chewable tablet was prepared using a cost-efficient, industrially scalable technique that will meet quality standards.

MATERIALS AND METHODS

Calibration Curve for Albendazole

The stock solution was prepared by weighing 25 mg of albendazole, transferring it to a 50 ml standard flask, and mixing it with acidified methanol. Further dilutions were made from the stock solution to obtain concentrations ranging from 8 μ g/mL to 12 μ g/mL and made up to the volume using 0.1 M sodium hydroxide respectively. The absorbance of the solutions was measured at 308 nm using a UV-Visible spectrophotometer using 0.1 M Sodium hydroxide as a blank[9]. The calibration curve was constructed with concentration (μ g/mL) on the X-axis and absorbance on the Y-axis.

Preformulation Studies Organoleptic Properties

Appearance

The albendazole sample was placed on butter paper and examined under a bright light.





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Taste and odour

A very little amount of albendazole was used to assess the taste with the tongue and smelled to determine the odour.

Drug - Excipient Compatatbility Study

In the study of drug-excipient compatibility, two methods were used, such as physical compatibility and chemical compatibility[10].

Physical compatibility study

The drugs were combined with the excipients and stored at 30° at 65% RH and 40° at 75% RH, respectively. Any colour change in the physical admixture was observed visually on the first day and after 1 month of storage.

Chemical compatibility study

To examine drug-excipient interactions, the pure drug, drug-excipient mixture was subjected to FT-IR investigations. Using potassium bromide, the IR spectra of the test samples were generated using the Pressed Pellet Technique.

Evaluation of Precompression Parameters Micromeritic Properties [11]

Angle of Repose

To minimize the impact of falling powder on the tip of the cone, the funnel height should be maintained at approximately 2–4 cm from the top of the powder pile as it is formed. The heap's height and radius were measured, and the angle of repose was determined using the formula below:

$$\tan \theta = \frac{\text{Height of the heap formed (h)}}{\text{Radius of the heap (r)}}$$

 $\theta = \tan^{-1} h/r$

Bulk Density

Weigh the amount of powder that occupies 60% of the volume of the dry 100 ml cylinder. Calculated the bulk density in g/mL, using the following formula:

Bulk Density =
$$\frac{\text{Mass of the powder (g)}}{\text{Bulk volume (ml)}}$$

Tapped Density

In order to measure tapped volume, the powder blend was tapped 100, 500, and 1250 times. If the difference between V100 and V500 is less than or equal to 2 mL, then V100 is the tapped volume. If the difference between V500 and V1250 is more than 2 mL, repeat in increments of 1250 taps until the difference between succeeding measurements is less than or equal to 2 mL. The following formula was used to determine the tapped density

Tapped Density =
$$\frac{\text{Mass of the powder (g)}}{\text{Tapped volume (ml)}}$$

Carr's Compressibility Index (%)

The compressibility index reflects these differences in particle interactions. Using the below formula, Carr's compressibility index was determined:

$$CI = \frac{Tapped Density - Bulk Density}{Tapped Density} X 100$$





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Hausner's Ratio

The Hausner's ratio correlates to the flow ability of a powder or granular substance. The following formula was used to determine Hausner's ratio from the bulk and tapped density

$$Hausner Ratio = \frac{Tapped Density}{Bulk Density}$$

Formulation Development

The following ingredients were sieved through #30 mesh: Albendazole (API), lactose, maize starch, sodium starch glycolate, and croscarmellose sodium. Using a polybag, mix for 10 minutes. In hot water, Povidone K-30 was dissolved. Separately, sodium lauryl sulphate and Sunset yellow supra were dissolved in water and added to the prepared binder solution. Binder solution was gradually added to the dry mix of API + excipient and uniformly mixed. The mixing procedure was repeated until a cohesive mass of granules was formed. The initial drying was done in a tray dryer at 50°C and passed through #12 mesh. The final drying was done at 50°C and passed through #30 mesh. Microcrystalline cellulose 102, sucralose, capsil-orange, and carbosil were combined with the granules after sieving through #40 mesh. Before being mixed into the granules, magnesium stearate was sieved through #80. The granules produced are used for compression of the albendazole chewable tablet.

Design of Expert

Central composite design

Design Expert V.11 (Stat-Ease Inc., Minneapolis, MN) was used to build the models, using a three-factor and two-level Central Composite design (CCD) which was detailed in table 1[12]. The CCD requires 10 experimental runs to determine the experimental error and the design's precision. With the effect of these independent variables, the dependent variable such as the percentage of dissolution must be increased and the disintegration time must be decreased. Lack of fit, ANOVA, and multiple correlation coefficient (R²) tests were used to validate the models.

Post Compression Parameters

The compressed albendazole chewable tablet was evaluated for the following parameters [13].

Hardness

Three tablets are chosen at random from the formulation and positioned between the tester's arms in a horizontal position. The tablet is broken down by applying breaking force. The hardness value noted in kg/cm².

Thickness

A tablet is placed vertically between two arms of the digital apparatus (Vernier Calliper) and the thickness and diameter are measured by observing the values on the screen.

Weigth Variation

Twenty tablets were randomly selected from the formulation and weighed on an analytical balance (King Lab Analytical Balance). Weight variation was calculated according to Indian Pharmacopeia specifications.

Friability

The Friability of tablets was evaluated by randomly selecting 6 tablets and placing them in a plastic Rosch Friabilator chamber. The friabilator drum was rotated at 25 rpm for 100 counts. The following formula calculated the percentage loss in the weight of tablets.

Friability =
$$\frac{\text{Initial weight-Final weight}}{\text{Initial weight}} \times 100$$





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Disintegration

In each of the 6 tubes of the disintegration test device (Electrolab ED-2L), a tablet was placed and the basket rack was placed in a 1L beaker of water at 37°C + 2°C. The time taken for the particles to pass through the 10 nm mesh screen was recorded.

Assay

20 tablets should be weighed and powdered. A portion of the powder containing 0.1 g of albendazole should be weighed, 150 ml of 0.1 M methanolic hydrochloric acid added, and the mixture should be shaken for 15 minutes before being diluted to 250 ml. 5.0 ml of the filtrate is collected, filtered, and then diluted to 250 ml using 0.1 M sodium hydroxide. The maximum absorbance of the resulting solution should be measured at 308 nm. Determine the $C_{12}H_{15}N_{3}O_{2}S$ content using 742 as the specific absorbance at 308 nm.

Dissolution Study of Albendazole Chewable Tablet

Six tablets were randomly selected and placed in each of the six amber-coloured vessels of dissolution apparatus type - I (Paddle) contained 900 mL of 0.1 N hydrochloric acid. Temperature was maintained at $37 \pm 0.5^{\circ}$ C. A sample of 5 ml was collected at an interval of 5, 10, 15, 20 and 30 mins and replaced with the buffer medium respectively. The collected samples were diluted with 0.1 M NaOH by making up 100 mL of volume from 2 ml of sample solution. A UV spectrophotometer was then used to measure the absorbance of the samples at 308 nm. Tolerances: NLT 80% (Q) of the labelled amount is dissolved [14].

In-vitro Release Kinetic Study

Using mathematical functions, the in-vitro release kinetics data of the best formulation F-5 were fitted to the Korsmeyer- Peppas, zero order, first order, and Higuchi model to identify potential release kinetics order and mechanism. Kinetic modelling is used to describe the formulation's dissolution profile [15].

Dissolution Profile Comparison

A method from the Indian Pharmacopeia was used to determine the dissolution profiles of a brand and test tablet containing albendazole 400 mg. Zentel was selected as Reference tablet manufactured by GSK. The reference tablet was compared with the optimized albendazole tablet in terms of dissolution.

Dissolution conditions

Apparatus 1: Paddle Type

Medium: 0.1N Hydrochloric acid, 900 mL

RPM: 75 Time: 30 min

Temperature: 37 ± 5°C

Sampling interval: 5, 10, 15, 20 and 30 mins.

Sample withdrawn: 5 mL

Statistical Methods

A difference factor (f1) and similarity factor (f2) were calculated between the test tablet and the reference tablet. f_1 is the average % difference between the amount of test brand dissolved over all time points compared to the reference brand. When the test and reference profiles are identical, the f_1 value is 0; as dissimilarity increases, the f_1 value increases. Between 0 and 100 is the value of f_2 . If the test and reference profiles are identical, the value is 100. As the dissimilarity increases, the value approaches zero [16].

Stability Study

The albendazole chewable tablets (F-5) were stored under real-time stability conditions (30°C/65% RH) and accelerated conditions (40°C/75% RH) according to the Association of Southeast Asian Nations Guidelines for Drug Product Stability in Climate Zone IVb [17]. The appearance, dissolution, and assay of tablets were all assessed.





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RESULTS AND DISCUSSION

Calibration Curve for Albendazole

The standard calibration curve was plotted as per table 2 and the linearity obtained was shown in the figure 2.

Preformulation Studies

The organoleptic property and solubility test were carried out for albendazole drug and the results were described in the table 3 and 4 respectively.

Organoleptic Properties

Solubility test:

Drug - Excipient Compatatbility Study

Physical compatibility study

The drug and excipients did not exhibit any typical differences, according to the present findings on drug excipient compatibility. The excipients chosen for the formulation were therefore determined to be compatible with albendazole and suitable for formulation development.

Chemical compatibility study

Fourier transform - infrared spectroscopy study

The drug is compatible with each excipient used in the formulation F-5, according to FT-IR spectral studies. The spectral data obtained for pure API and the F-5 blend were shown in table 5 and 6 respectively. The FT-IR spectrum of the physical mixture revealed all of the distinctive peaks of the pure API for albendazole and the F-5 blend as shown in figure 3 and 4, confirming that there was no drug-excipient interaction.

Evaluation of precompression parameters

Micromeritic properties

The formulation blend (F1 – F10) was evaluated for following parameters such as Bulk density, Tapped density, Hausner's ratio, Compressibility index and Angle of repose. The results are given below in table7. The optimised formulation blend F-5 was found to have a good flow property with an Angle of repose of 32° 24', Bulk Density of 0.559 g/cm3, Tapped Density of 0.637 g/cm3, Hausner's ratio of 1.13, and Compressibility Index of 12.22%.

Design of expert

Central composite design

The three factor two level (3²) central composite design was employed for the development of Albendazole chewable tablet[19]. The optimized formulation trials (10 RUNS) using Central Composite Design was shown in table 8.

Dissolution (%)

The DOE graph for prediction of dissolution were displayed in the figure 5, 6 and 7.

Model Validation

ANOVA for Linear model and Fit Statistics for dissolution were mentioned in table 9 and 10 respectively

Disintegration

The DOE graph for prediction of disintegration were displayed in the figure 8, 9 and 10.

Model Validation

Anova for linear model and fit statistics for disintegration were mentioned in table 11 and 12 respectively. Two disintegrants, sodium starch glycolate and croscarmellose sodium, which function as process variables X1 and X2, respectively, were used to predict the Dissolution and Disintegration time. The actual factor, sodium lauryl sulphate,





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was maintained at a level of 4.5 mg. The percentage of dissolution varies from 82 to 89.2 % and the disintegration time ranges between 130 to 270 secs. The F-5 experimental design (#5 run) with the independent variable composition ratios of sodium starch glycolate - 66.2132; croscarmellose sodium - 15; and sodium lauryl sulphate - 4.5 was determined to have the highest dissolution rate of 89.2% and the lowest disintegration time of 130 secs. Model validation has been performed by Lack of fit, ANOVA, and multiple correlation coefficient (R^2) test. As a result, the model was found to be significant (p < 0.05).

Post Compression Parameters

The prepared tablets of all formulations (F1 – F10) were evaluated for post compression parameters. These results are presented in the table13. All of the formulations had the uniform thickness. The prepared tablets had a good mechanical strength with sufficient hardness to chew. The assay value ranges between 99.17 to 102.56% Albendazole chewable tablet optimised formulation (F-5) assay results in 101.85%.

In-vitro Dissolution Study

Comparative in-vitro drug release studies of albendazole chewable tablet (F1-F10) were performed, and the findings are listed in the table 14 and depicted in Figure 11. The formulation F5 exhibits an approximate 89.2 % maximum drug release rate. In comparison to previous formulations, the two disintegrants and surfactant used had acceptable highest concentrations. These findings suggest that the disintegration time impacts the drug release percentage. The tablet's ability for rapid disintegration and availability as tiny particles for enhanced solubilization produces the highest rate of dissolution [20]. Based on its flow property, disintegration, and dissolution parameters, F5 formulation was selected as the optimized albendazole chewable tablet formulation.

In-Vitro Release Kinetic Study

Fitting to zero-order, first-order, Higuchi and Korsmeyer and Peppas models to analyze the in-vitro release kinetics of albendazole chewable tablet were shown in the figure 12. Based on the coefficient of determination (R^2), the Korsmeyer and Peppas model has the maximum regression value (R^2 = 0.9761) compared to other kinetic models. As a result, the drug release events in albendazole chewable tablet formulations are represented by the Korsmeyer and Peppas model of kinetics.

Dissolution Profile Comparison

The difference factor (f1) and similarity factor (f2) were calculated by dissolution profile comparison of zentel and optimised F-5 formulation which were detailed in table 15 and results were displayed in the figure 13. In comparing the dissolution profiles of the Zentel and optimised albendazole chewable tablets, a similarity factor (f2) of 74 and a difference factor (f1) of 4 were obtained, both of which meet the acceptance criteria for statistical methods [21].

Stability studies

The Real time and Accelerated stability studies were carried out, as shown in the table 16 and 17 respectively.

Real time stability data

Accelerated stability data

Real time stability data accelerated stability data

According to stability studies (Real-time and Accelerated), the Formulation (F-5) did not significantly change in terms of physical appearance, disintegration test, dissolution, and assay between the first and third month of storage which are illustrated in the figure 14.

CONCLUSION

The wet granulation method and the Central Composite Design were used to design and optimise the Albendazole chewable tablet. The model validation was found to be significant. The dissolution rate and disintegration time for





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the optimized formulation F-5 were found to be 89.2 % and 130 seconds, respectively. The percentage of dissolution increases when the disintegration time decreases due to an increase in the concentration of the two disintegrants positively impacting the solubility by surfactant. This indicates that the solubility of the albendazole has improved, increasing the dissolution rate. A similarity factor (f2) of 74 and a difference factor (f1) of 4 demonstrate bioequivalent product performance.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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Table 1: Execution of experimental design of albendazole chewable tablet

Factors: Formulation Variables	Levels (mg /	Tablet)	
1 actors. Formulation Variables	-1	+1	
Sodium starch glycolate	30	60	
Croscarmellose sodium	10	20	
Sodium lauryl sulphate	3	6	
Response	Acceptable	Ranges	
Dissolution at 30 mins (%)	> 80 %		
Disintegration time	NMT 15 MINS		

Table 2: Standard calibration curve for albendazole

S.No	Concentration (µg/mL)	Absorbance
1.	0	0
2. 8		0.551
3.	3. 9 0.672	
4.	10	0.731
5.	11	0.801
6.	12	0.889

Table3: Organoleptic properties of Albendazole drug

Test	Specification	Observation
Colour	White powder	White powder
Odour	Odourless	Odourless
Taste	Tasteless / Taste unknown	Tasteless





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Table4: Solubility of Albendazole in Different Solvents

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Raw Material	Solubility (µG/ML)				
	Poorly soluble in water				
Albendazole	Slightly soluble in methanol and acetonitrile				
	Very slightly soluble in ethanol				

Table5: FT-IR Spectral data for pure Albendazole drug[18]

S.NO	NO Wave Number (Cm ⁻¹) Functional G						
1.	3332.75	NH					
2.	1712.66	C=O					
3.	1623	C=C-NH					
4.	2947.01	CH ₃ -CH ₂ -CH ₂					
5.	1100.49	NHCO-O-CH₃					
6.	995.20	C=S					

Table 6: FT-IR Spectral data for Albendazole blend (F-5) [18]

S.No	Wave Number (cm-1)	Functional Group
1.	3332.75	NH
2.	1697.23	C=O
3.	1627.8	C=C-NH
4.	1010.63	CH ₃ -CH ₂ -CH ₂
5.	2954.73	NHCO-O-CH ₃
6.	948.91	C=S

Table7: Evaluation of the precompression parameters

Formulation Code	Bulk Density (g/cm³)	Tapped Density (g/cm³)	Hausner's Ratio	Compressibility Index (%)	Angle of Repose (⊖)	Flow Property
API	0.489	0.669	1.36	26.90	47° 15′	POOR
F1	0.541	0.677	1.25	20	39° 23′	FAIR
F2	0.599	0.692	1.15	13.45	34° 35′	GOOD
F3	0.489	0.652	1.34	25.45	42° 40′	PASSABLE
F4	0.502	0.612	1.21	21.90	38° 15′	FAIR
F5	0.559	0.637	1.13	12.22	32° 24′	GOOD
F6	0.512	0.603	1.17	15.09	32° 33′	GOOD
F7	0.510	0.585	1.14	12.72	33° 04′	GOOD
F8	0.520	0.597	1.14	12.96	31° 48′	GOOD
F9	0.466	0.596	1.22	18.51	36° 21′	FAIR
F10	0.464	0.556	1.20	16.66	36° 42′	FAIR

Table 8: Optimized formulation trials (10 RUNS) using Central Composite Design

S.NO	Factor 1 A: SODIUM STARCH GLYCOLATE (mg)	FACTOR 2 B: CROSCARMELLOSE SODIUM (mg)	FACTOR 3 C: SODIUM LAURYL SULPHATE (mg)	RESPONSE 1 DISSOLUTION (%)	RESPONSE 2 DISINTEGRATION (Sec)
1	23.7868	15	4.5	84	210
2	30	20	6	86.8	180





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3	60	10	6	84.1	240
4	45	7.92893	4.5	82.1	260
5	66.2132	15	4.5	89.2	130
6	45	22.0711	4.5	88.6	135
7	45	15	2.37868	86	170
8	60	20	3	87.3	150
9	45	15	6.62132	85.7	210
10	30	10	3	82	270

Table 9: Response 1 - DISSOLUTION

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Source	Sum of Squares	Mean Square	Difference factor	Mean Square	F-value	p-value	
Model	49.51	16.50	3	16.50	14.20	0.0039	Significant
A-Sodium Starch Glycolate	12.39	12.39	1	12.39	10.66	0.0171	
B- Croscarmellose Sodium	36.95	36.95	1	36.95	31.80	0.0013	
C-Sodium Lauryl Sulphate	0.1728	0.1728	1	0.1728	0.1487	0.7131	
Residual	6.97	1.16	6	1.16			
Cor Total	56.48		9				

Table 10: Fit Statistics for dissolution

Std. Dev.	1.08	R ²	0.8766
Mean	85.58	Adjusted R ²	0.8149
C.V. %	1.26	Predicted R ²	0.6435
		Adequate Precision	9.9553

Table 11: Response 2 - Disintegration

Source	Sum of Squares	Mean Square	Difference factor	F- value	p-value	
Model	20058.26	6686.09	3	13.53	0.0044	significant
A-Sodium Starch Glycolate	3747.06	3747.06	1	7.58	0.0331	
B-croscarmallose sodium	15911.20	15911.20	1	32.21	0.0013	
C-Sodium Lauryl Sulphate	400.00	400.00	1	0.8097	0.4029	
Residual	2964.24	494.04	6			
Cor Total	23022.50		9			

Table 12: Fit Statistics - Disintegration

Std. Dev.	22.23	R²	0.8712
Mean	195.50	Adjusted R ²	0.8069
C.V. %	11.37	Predicted R ²	0.6285
		Adequate Precision	9.7015





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Table13: Post compression parameters for all formulations (F1 - F10)

Formulation Code	Thickness (mm)	Hardness (kg/cm²)	Weight variation (mg)	Friability (%)	Disintegration Time (Sec)	Assay (%)
F1	6.63	8.1	999.98	0.13	210	99.21
F2	6.68	7.8	1000.02	0.16	180	100.02
F3	6.58	8.3	999.87	0.21	240	99.32
F4	6.56	8.4	1000.01	0.17	260	99.86
F5	6.78	7.5	999.95	0.12	130	101.85
F6	6.75	7.7	999.89	0.14	135	101.30
F7	6.68	7.8	999.93	0.35	170	99.17
F8	6.70	7.7	999.98	0.15	150	102.56
F9	6.62	8.1	999.98	0.19	210	99.56
F10	6.50	8.6	1000	0.28	270	100.30

Table14: Comparative In-vitro drug release studies of albendazole chewable tablet (F1 -F10)

								<u> </u>		
Time		Percentage Drug Release (%)								
	Formulation Code									
(Mins)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
5	52.98	52.15	51.56	54.32	56.94	55.15	54.45	53.15	52.83	51.15
10	64.16	64.45	63.45	62.78	68.45	65.96	64.81	63.16	64.12	60.15
15	73.56	73.18	72.19	69.19	79.78	76.96	72.17	71.09	74.87	68.92
20	77.34	78.67	77.91	75.41	84.73	83.08	78.09	78.12	79.21	74.87
30	84	86.8	84.1	82.1	89.2	88.6	86	87.3	85.7	82

Table 15: Dissolution profile comparison of Zentel and optimized F-5 formulation

C NIa	Times (NAires)	% Drug I	Released
S.No	Time (Mins)	Reference – Zentel	Test - Albendazole
1.	5	61.78	56.94
2.	10	65.79	68.45
3.	15	76.02	79.78
4.	20	82.14	84.73
5.	30	90.69	89.2
	No. of Tim	ne Points	5
Similarity Factor (f2) [value greater than 50; (50 – 100)]			74
Difference Factor (f1) [value up to 15 (0-15)]			4

Table 16: Real Time stability data of optimized Albendazole chewable tablet at 30 \pm 2° C at 65 % \pm 5% RH

S.NO	STORAGE CONDITIONS: 30 ± 2° Cat 65 % ± 5% RH						
3.140	TESTS	INITIAL PERIOD	1 ST MONTH	3 RD MONTH			
1.	Description	Complies	Complies	Complies			
2.	Average weight (mg) (Limit: 1000.0 mg ± 5.0 % Between 950.0 and 1050.0 mg)	999.95	1000.01	1000.02			
3.	Disintegration test (sec) (Not more than 15 mins)	2 mins 10 sec	2 mins 12 sec	2 mins 16 sec			
4.	Dissolution Albendazole chewable tablet	89.2	89.36	90.12			





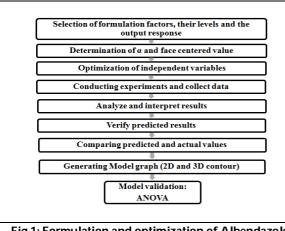
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	equivalent to Albendazole Not less than 80% of label claim is dissolved in 30 mins)			
5.	Assay Each chewable tablet contains: Albendazole 400 mg Between 370.00 and 430.00 mg (92.5% to 107.5% of Label claim)	101.85	101.40	100.02

Table17: Accelerated stability data of optimized Albendazole chewable tablet at 40 \pm 2° Cat 75 % \pm 5% RH

S.No	Sto	orage Conditions: 40 ±2%	Cat 75 %±5% RH	
3.IVO	Tests	Initial period	1st month	3 rd month
1.	Description	Complies	Complies	Complies
2.	Average weight (mg) (Limit: 1000.0 mg ± 5.0 % Between 950.0 and 1050.0 mg)	999.95	999.93	999.90
3.	Disintegration test (sec) (Not more than 15 mins)	2 mins 10 sec	2 mins 17 sec	2 mins 26 sec
4.	Dissolution Albendazole chewable tablet equivalent to Albendazole Not less than 80% of label claim is dissolved in 30 mins)	89.2	89.49	89.52
5.	Assay Each chewable tablet contains: Albendazole 400 mg Between 370.00 and 430.00 mg (92.5% to 107.5% of Label claim)	101.85	101.56	101.09



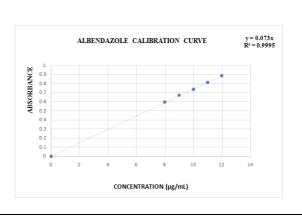


Fig 1: Formulation and optimization of Albendazole chewable tablet by Central Composite Design

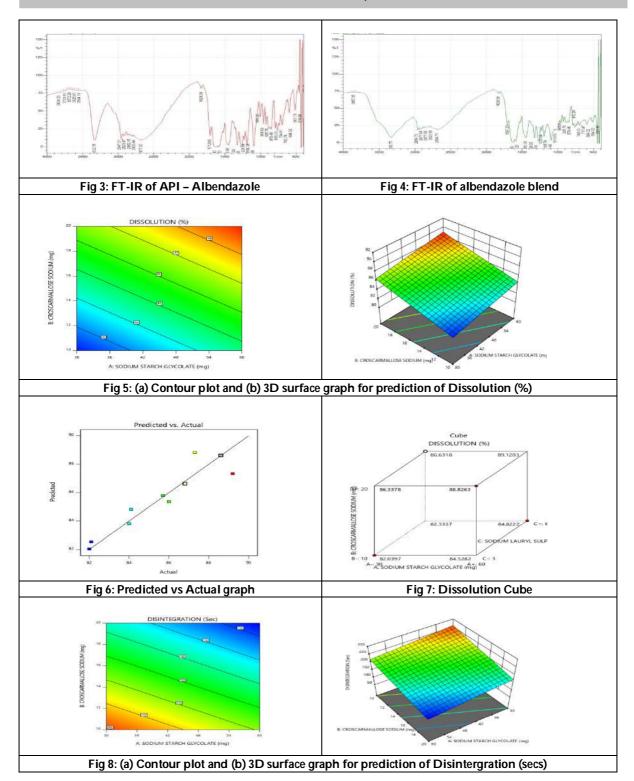
Fig 2: Standard calibration curve for Albendazole





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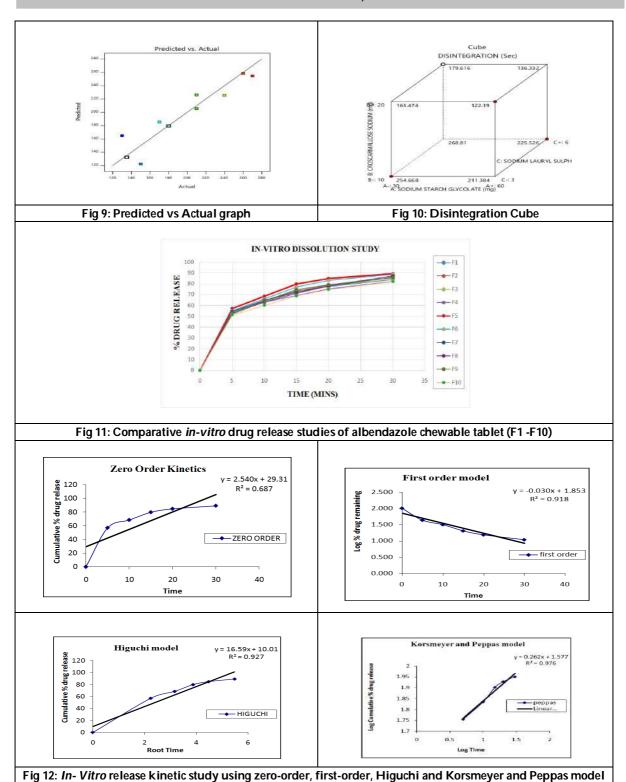






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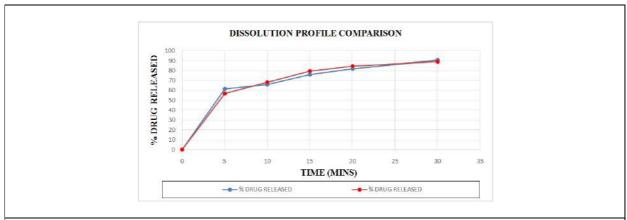
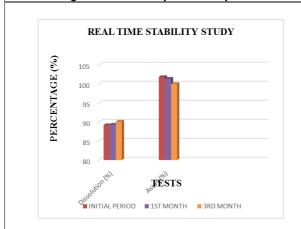


Fig 13: Dissolution profile comparison of Brand and the optimized albendazole chewable tablet



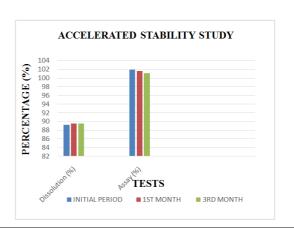


Fig 14: Real Time and Accelerated stability data of optimized Albendazole chewable tablet at 30 $\pm 2^{\circ}$ Cat 65% $\pm 5\%$ RH and 40 $\pm 2^{\circ}$ Cat 75 % $\pm 5\%$ RH respectively.





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RESEARCH ARTICLE

Antioxidant Activity of Pterospermum canescens Roxb., Leaf Extract

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ABSTRACT

The present work was designed to evaluate the antioxidant activity of Pterospermum canescens Roxb,(Sterculiaceae) of the leaf extracts (petroleum ether, chloroform and methanol) using different enzymatic assay methods like super oxide dismutase, catalase, glutathione reductase, lipid peroxidase, nitrates and nitrites. The leaf extracts (petroleum ether, chloroform, methanol) are altering the levels of super oxide dismutase, catalase, nitrite level, glutathione reductase and lipid peroxidase significantly as compared with the control group. The obtained results reveal that Pterospermum canescens Roxb leaf extracts have exhibited significant antioxidant potential (P < 0.01, P < 0.001, P < 0.01) and can be used to treat oxidative stress as well as protect the tissues.

Keywords: Pterospermum canescens Roxb, antioxidant, superoxide dismutase, catalase, nitrite, glutathione reductase, peroxidase.

INTRODUCTION

Antioxidants are secondary metabolites, found in plants (fruits and vegetables). An antioxidant is defined as a substance which inhibits or prevents oxidation of a substrate. Carotenoids, flavonoids, cinnamic acid, benzoic acid, folic acid, ascorbic acid, tocopherols and tocotrienols are the group of compounds present in plants prevents oxidation of the liable substrate [1]. Common antioxidants which are found in plants are vitamin A,C, E, and certain compounds called carotenoids (like lutein and β -carotene) [2]. These plant-based dietary antioxidants are thought to have an imperative role in the upholding of human health because our endogenous antioxidants provide insufficient





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protection against the constant and inevitable challenge of reactive oxygen species (ROS; oxidants) [3]. Oxidative stress is a causative factor for several human diseases by that result from disproportion between the formation and neutralization of pro-oxidants. Oxidative stress is initiated by free radicals, such as superoxide anions, hydrogen peroxide, hydroxyl, nitric oxide and nitrite, play a vital role in destructing various cellular macromolecules (DNA, proteins). Free radicals are formed by exogenous sources i.e. exposure of γ -radiation [4-6] and endogenous sources i.e. consumption of O₂ by mitochondria during normal aerobic respiration; destroying of bacteria and virus infected cells by phagogytic cells; degradation of fatty acids and other molecules by per oxisomes; oxidants formed during degradation of P450 of natural toxins [7,8]. Though, human cells have a wide range of protecting mechanisms to prevent the formation of free radicals and oxidative damage, which comprises of both enzymatic and non-enzymatic antioxidants like superoxide dismutase, catalase, glutathione reductase, ascorbic acid and tocopherol [9]. There is presently a worldwide expansion in the use of herbal based preparations and the active ingredient isolated from medicinal plants in health care. Approximately 60% of worldwide population still relies on medicinal plants for their primary health care [10]. With this background, this study was conducted with an objective of evaluation of antioxidant activity of Pterospermum canescens, Roxb., by enzymatic method. The genus Pterospermum Schreb., (Sterculiaceae) represent of about 40 species throughout the world, of which 12 species were reported in India [11] and 8 species have been reported in Tamil Nadu [12]. An ethnomedicinal plant *Pterospermum canescens* Roxb., (Syn. Pterospermum suberifolium Lam.) in the vicinity known as Sempulavu was distributed in all districts of TamilNadu. Ethnomedicinally, the leaves are used for headache, treatment of fractured bones, small pox [13-15] and reported for antimicrobial [16,17], antipyretic and anti-inflammatory [18, 19] properties. The plant has been reported for the presence of β -amyrin, betulin, kaempferol, lupeol, quercetin, scopoletin and β -sitosterol and α -sitosterol, 3,7,11,15tetramethyl-2-hexa decane-1-ol, ricinoleic acid, vitamin-E, phytol, α -tocopherol, diethyl phthalate, squalene, benzhydrazide-3-mthoxy-N₂-(4-henylcyclo hexylideno, benzoic acid, 4- heptyl-4-cyanophenyl ester and nhexadecanoic acid [20, 21]. After the inquiry of literatures, it was established that so far no other work has been conceded on this plant. Hence, the present study aims to develop an antioxidant lead of therapeutic interest from this selected ethno medicinal plant.

MATERIALS AND METHODS

Collection of plant material

The leaves of *Pterospermum canescens* Roxb., were collected from the Kalapet vicinity of Pondicherry and the collected plant material was botanically identified and confirmed by the Plant Taxonomist Dr.A.C.Tangavelou and the herbarium specimen (KPJ 42) was prepared and deposited at the department for future reference.

Preparation of extracts

The collected leaves were cut down into small pieces, dried under shade and by using a mixer blender coarsely powdered. Then, the powdered leaf was subjected to successive solvent extraction with organic solvents of increasing polarity (petroleum ether, chloroform and methanol) by continuous hot percolation method using soxhlet apparatus [22, 23]. The extracts were collected and distilled off on a water bath at atmospheric pressure until last trace of the solvent was removed *in vaccuo*. The resulted extracts were subjected to evaluate the antioxidant activity.

Materials required

Male Wistar albino rats of either sex (150-200 g) were used for this study. They were maintained in 12h light, 12h dark condition, at a controlled temperature ($25^{\circ} \pm 3^{\circ}$ C), humidity ($60 \pm 5^{\circ}$) and housed in the Department of Pharmacology and all experiments were performed in accordance with the Institutional Animal Ethics Committee, C. L. Baid Metha College of Pharmacy, Chennai- 600097 (IAEC/ 34/ 22/ CLBMCP/ 2011, dated on 7/2/2011). Wistar albino rats were divided into eight groups of six animals each and they were fasted overnight, during the experiment free access of water *ad libitum*. The dose of the extracts was selected on the basis of folkloric use of the plant. Group I served as control (0.9% Normal saline with 3% Tween, 2 ml/kg), Group II, III; Group IV, V and Group VI, VII are administered with methanol, petroleum ether and chloroform of *Pterospermum canescens*, Roxb., (leaf, stem, stem bark) respectively at doses of 100 mg/kg and 200 mg/kg body weight and Group VIII served as standard





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(Indomethacin, 100 mg/kg). All animals were administered with respective grouping via oral route for 42 days. At the end of treatment period, blood samples were collected from the tail vein of rats and at the same time of the day centrifuged at 10,000 rpm for 15 minutes, and stored at -20°C, for performing the biochemical assays [24].

Estimation of superoxide dismutase (SOD) Superoxide dismutase [25]

The SOD activity in supernatant was measured by the standard method. The supernatant (500 μ l) was added to 0.800 ml of carbonate buffer (100mM, pH 10.2) and 100 μ l of epinephrine (3mM). The change in absorbance of each sample was then recorded at 480 nm in spectrophotometer for 2 minutes at an interval of 15 sec. Parallel blank and standard were run for determination SOD activity. One unit of SOD is defined as the amount of enzyme required to produce 50% inhibition of epinephrine auto oxidation. The reaction mixtures are diluted 1/10 just before taking the readings in spectrophotometer.

% inhibition = $\Delta A480$ nm/min Uninhibited - $\Delta A480$ nm/ min Inhibited $\propto 100$ $\Delta A480$ nm / min Uninhibited - $\Delta A480$ nm / min Blank

Units/ mI enzyme = % inhibition x V_t (50%) x V_s

Units/ mg protein = Units/ ml enzyme

Mg of protein/ ml enzyme

Estimation of Catalase [26, 27]

For evaluating the catalase activity, 0.1 ml of supernatant was added to cuvette containing 1.9 ml of 50 mM phosphate buffer (pH 7.0). Reaction was started by an addition of 1.0 ml of freshly prepared 30 mM H_2O_2 . The rate of decomposition of H_2O_2 was measured spectrophotometrically from changes in absorbance at 240 nm. Activity of catalase was expressed as units/mg protein (μ mol of H_2O_2 /minute/mg protein). A unit is defined as the velocity constant per second. The reaction occurs immediately after the addition of H_2O_2 . Solutions are mixed well and the first absorbance (A_1) is read after 15 seconds (t_1) and the second absorbance (A_2) after 30 seconds (t_2). The absorbance is read at wave length 240 nm.

$$K = V_t \quad x \quad 2.3 \quad x \quad Log \quad A_1 \quad x \quad 100$$

$$V_s \quad \Delta_t \quad A_2$$

Where, K = rate constant of the reaction; $\Delta t = (t_2 - t_1) = 15 \text{ seconds}$; $A^1 = \text{absorbance after 15 seconds}$; $A_2 = \text{absorbance after 30 seconds}$; $V_t = \text{total volume (3 ml)}$; $V_s = \text{volume of the sample (0.1ml)}$.

Nitrates and Nitrites [28]

Nitrate levels in the tissues were measured by using Griess reaction. For nitrate estimation, $100~\mu l$ Griess reagent (1% sulphanilamide, 0.1% naphthyl ethylene diamine dichloride and 3% phosphoric acid), were mixed with leaf extracts (100 μl each), the vanadium (III) chloride (100 μl) to each well was added rapidly followed by an addition of Griess reagent and incubated at 37°C for 30 minutes. Absorbance was measured at 540nm by using microplate reader. For nitrite estimation, $100~\mu l$ of distilled water was added in place of vanadium chloride. Rest of procedure remained same as for nitrate estimation. Nitrate/nitrite standard curves were prepared by taking different concentration of nitrite ranging from 20 to $100~\mu l$ /mol. Level of nitrite are expressed as nmolg-1 tissue. The absorbance of the chromophores formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthyl ethylene diamine dichloride was recorded at 540 nm and referred to the absorbance of Ascorbic acid as a positive control treated in the same way with Griess reagent.

Nitric oxide scavenged (%) = A control
$$\frac{A \text{ test}}{A \text{ control}}$$

Where, Acontrol = absorbance of control reaction Atest = absorbance of test (extracts)





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Glutathione reductase [29, 30]

The reaction mixture containing 1 ml phosphate buffer, 0.5 ml EDTA, 0.5 ml GSSG and 0.2 ml of NADPH was made up to 3 ml with distilled water. After an addition of 0.1 ml of serum, the change in optical density at 340 nm was monitored for 2 minutes at 30 seconds interval. One unit of the enzyme activity was expressed as "n" moles of NADPH oxidized/ min/ mg protein.

Enzymatic activity (M/ min/ mI) =
$$\frac{A_{340} \times V_t}{F_s \times d_s \times V_s}$$

Where, ε = 6.22 X 10 6 M-1cm-1; d = 1 cm; V_t = Total volume (3s.1); V_s = Sample volume (0.1 ml).

Lipid peroxidase (LPO) [31, 32]

To 0.2 ml of test sample, 0.2 ml of SDS, 1.5 ml of acetic acid and 1.5 ml of TBA were added. The mixture was made up to 4 ml with water and then heated on a water bath at 95 °C for 60 minutes. After cooling, 1 ml of water and 5 ml of n-butanol / pyridine mixture were added and shaken vigorously. After centrifugation at 4000 rpm for 10 minutes, the organic layer was taken and its absorbance was read at 532 nm. The level of lipid peroxides was expressed as nmoles of MDA released/ g wet tissue.

The concentration of MDA (M) = Absorbance at $532nm \times D$

Lxε

Where, L = light path (1 cm); ϵ = extinction coefficient 1.56 x 105 M-1.Cm-1; Total volume (10 ml) D; dilution factor Volume of the sample (0.2 ml).

Statistical analysis

All biochemical results were expressed as mean \pm SEM by Dunnett's method. Statistical significance was considered at *** P < 0.001, ** P < 0.01, * P < 0.05.

RESULTS AND DISCUSSION

Super oxide dismutase - Control group of animals were exhibited a decrease of SOD level in the serum when compared with the standard group of animals. Chloroform (100 mg, 200 mg/kg) and methanol (100 mg/kg) leaf extracts were decreased the SOD level in the serum when compared with the control group of animals (Fig. 1; Table 1). Catalase- Control group of animals were exhibited decrease in catalase enzyme level in the serum when compared with the standard group of animals. Petroleum ether (100 mg/kg) and chloroform (100 mg, 200 mg/kg) leaf extracts were exhibited significant (P < 0.01, P < 0.001, P < 0.01), decrease of catalase level in the serum when compared with the control group of animals. Petroleum ether (200 mg/kg) leaf extract did not exhibit significant increase in catalase level in the serum and methanol (100 mg, 200 mg/kg) leaf extract did not show significant decrease of catalase level in the serum when compared with the control group of animals (Fig. 2; Table 1). Nitrite level - Standard group of animals were exhibited significant (P < 0.001), decrease of nitrite level in the serum when compared with the control group of animals. Chloroform (100 mg, 200 mg/kg) and methanol (100 mg, 200 mg/kg) extracts were exhibited significant (P < 0.001), decrease of nitrite level in the serum when compared with the control group of animals. Petroleum ether (100 mg, 200 mg/kg) extract did not show significant, decrease of nitrite level in the serum when compared with the control group of animals (Fig. 3; Table 1). Glutathione reductase - Control group of animals were exhibited decrease of glutathione reductase level in the serum when compared with the standard group of animals. Petroleum ether (100 mg/kg), chloroform (100 mg, 200 mg/kg), and methanol (100 mg/kg) leaf extracts were exhibited significant (P < 0.001, P < 0.001, P < 0.01 and P < 0.001), decrease of glutathione reductase level in the serum when compared with the control group of animals. Petroleum ether (200 mg/kg) and methanol (200 mg/kg) leaf extracts did not show significant decrease of glutathione reductase level in the serum when compared with the control group of animals (Fig. 4; Table 1).





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Lipid peroxidase - Standard group of animals were exhibited significant (P < 0.001), decrease of lipid peroxidase level in the serum when compared with the control group of animals. Petroleum ether, chloroform and methanol leaf extracts (100 mg, 200 mg/kg) were exhibited significant (P < 0.001), decrease of lipid peroxidase level in the serum when compared with the control group of animals (Fig. 5; Table 1).

DISCUSSION

Medicinal plants are an imperative source of antioxidants [33]. Natural occurring antioxidants boost the antioxidant power of the plasma and shrink the risk of certain diseases such as cancer, heart diseases and stroke [34]. The secondary metabolites like phenolic compounds and flavonoids from plants have been reported to be effective free radical scavengers. They are found in all parts of plants such as leaves, fruits, seeds, roots and bark [35]. The formation of different types of free radicals like hydroxyl radical (OH), super oxide radical (O), nitric oxide radical (NO) and lipid peroxyl radical (LOO) occurs continuously in the cells as an outcome of both enzymatic and nonenzymatic reactions. The human body has several mechanisms to counteract the damage generated by free radicals and other reactive species. These act on different oxidants as well as in different cellular compartments. One important line of defense is a enzymes, including glutathione peroxidase, super oxide dismutase, and catalases which decreases the concentrations of most harmful oxidants in the tissues. Cu/Zn SOD is most commonly used by eukaryotes and is important antioxidant defense in nearly all cells exposed to oxygen [36]. Here CAT activity was increased and then restored to normal levels on administration of plant extracts of P. canescens. Peroxidase is an enzyme that catalyzes the reduction of hydroperoxides, including hydrogen peroxides, and functions to protect the cell from peroxidative damage [37]. Thus, in the present research work, enzymatic oxidants such as glutathione, SOD, catalase, and peroxidase were improved in drug treated group as compared to control. This might be due to some phenolic compounds present in the extracts are involved in the removal of the free oxy radicals. The secondary metabolites like phenolic compounds and flavonoids from plants have been reported to be potent free radical scavengers. They are found in all parts of plants [35], for protecting the body against reactive oxygen species (ROS). Phenolic compounds like quercetin, kaempferol, morin, myricetin and rutin, by acting as antioxidants, exhibited valuable effects such as inflammation, allergy, viral infections, as well as in cancer [38]. Flavonoids are prominent antioxidants against free radicals and are described as free-radical scavengers [39]. This activity is ascribed due to their hydrogen-donating ability. Indeed, the phenolic groups of flavonoids serve as a source of readily available "H" atoms such that the subsequent radicals produced can be delocalized over the flavonoid structure [40]. Free radical scavenging capacity is primarily attributed to high reactivity of hydroxyl substituent's that participate in the reaction [41]. Flavonoids inhibit lipid peroxidation by acting as scavengers of superoxide anion and hydroxyl radicals. They terminate the chain radical reaction by donating hydrogen atom to a peroxy radical and forming flavonoids radical which further reacts with free radicals thus terminating propagating chain [42, 43]. Naturally, the organism has developed a defense against toxic substances such as peroxynitrite and nitrous acid. An important mechanism is catalyzed by the enzyme superoxide dismutase (SOD) which converts two superoxide anions to H₂O₂ and O₂ [44].

CONCLUSION

In conclusion, the present investigation exhibits significant antioxidant potential of *Pterospermum canscens* Roxb., leaf extract and justified the traditional and folkloric claim of the plant. Further research works are desirable to explore the therapeutic potential of the plant and to establish the responsible active constituents and possible mechanism of action.





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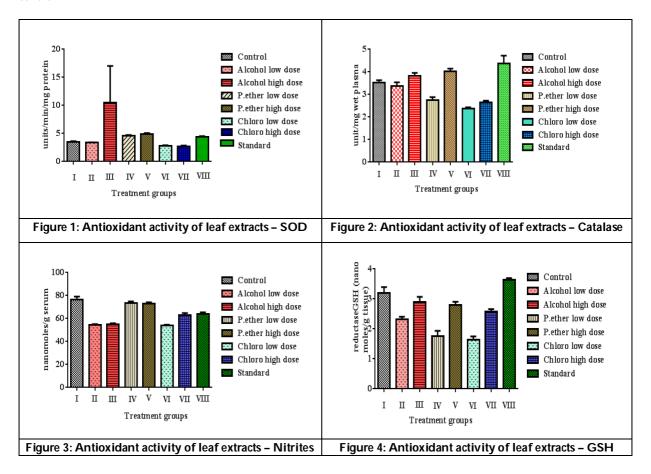
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Table 1: Antioxidant activity of leaf extracts

Treatment	SOD (Units/min/mg protein)	Catalase (unit/mg wet tissue)	Nitrate/nitrite (nano moles/g tissue)	reductase GSH (nano moles/g tissue)	MDA (nano moles/mg protein))
Control	3.55 ± 0.08	3.53 ± 0.09	76.67 ± 2.45	3.20 ± 0.19	103.20 ± 0.79***
PETL	3.36 ± 0.06	3.36 ± 0.17	54.33 ± 0.88***	2.31 ± 0.08***	82.00 ± 0.44***
PETH	10.53 ± 6.49	3.81 ± 0.14	55.17 ± 0.70***	2.90 ± 0.17	84.67 ± 1.14***
CHL	4.63 ± 0.09	2.75 ± 0.13**	73.50 ± 1.40	1.75 ± 0.18***	73.33 ± 0.88***
CHH	4.91 ± 0.16	4.03 ± 0.10	73.00 ± 1.15	2.80 ± 0.10	76.00 ± 1.41***
MEL	2.75 ± 0.16	2.38 ± 0.04***	54.00 ± 0.68***	1.65 ± 0.10***	91.00 ± 1.06***
MEH	2.71 ± 0.14	2.65 ± 0.07**	62.83 ± 1.92***	2.58 ± 0.07*	91.17 ± 1.42***
STD	4.43 ± 0.10	4.38 ± 0.34**	64.00 ± 1.46***	3.63 ± 0.06	91.00 ± 1.15***

Values shown are mean ± SEM (n= 6). *** P < 0.001, ** P < 0.01, * P < 0.05, experimental groups were compared with control







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Jaiganesh and Arunachalam Control Alcohol low dose Alcohol high dose P.ether low dose Chloro low dose Chloro low dose Chloro high dose Standard Figure 5: Antioxidant activity of leaf extracts – Lipid peroxidase





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ISSN: 0976 – 0997 **RESEARCH ARTICLE**

Sustainable Management of Forest Resource in Uttarakhand

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ABSTRACT

Forests are a critical component of a land-based ecosystem. The contributions of forests have always been important to the natural environment, and for the socio-economic well-being of anynation. The significance of forests is considerably higher in developing countries. In India, forests perform akeyrolein the environmental stability, cultivation, ecosystem for a wide range of flora, fauna and natural protection against soil erosion. However, forests are vulnerable to fire, excess grazing, pests and other insidious species and are also the prime objects for the agricultural and urban extension. The forest capital in India is exceptionally assorted due to the distinction in its geography. With the coercion of biotic stress on the forests, many forests have been either depleted or degraded. Forest conservation has become a subject of importance in the recent past for not only its role in meeting the material requirements but because of their ecological and environmental roles. To ensure sustainable development of our forests, it is important to manage forestry resources efficiently. Some important parameters required for measuring sustainable management of forest resources include forest cover, annual increment, growing stock, species composition, biomass, biodiversity, non-timber forest products, and regeneration status. With the wide range of Flora and Fauna, the state of Uttarakhand is significantly rich in Forest Resource, and it provides livelihood not only to many people but is a source of revenue for the State Government. The paper analyses the impact of forest resources on the GSDP of the Uttarakhand state and its importance on the livelihood of common people.

Keywords: Forest Economy, Sustainable Management, Uttarakhand





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INTRODUCTION

The value of forests cannot be miscalculated; forests promise our existence, from the air we inhale to the fuel we use. Apart from offering locales for animals and livings for humans, forests also provide watershed protection, avert soil erosion, and alleviate climate alteration. Forests secure us with shelter, incomes, water, food, and fuel. Beyond, the urban narrative, forests provide habitats to diverse animal species, as 80 per cent of the world's terrestrial biodiversity resides in forests, and they also offer a livelihood for different human settlement. In addition, 300 million people live in forests, including 60 million indigenous people. During 1990 and 2015, the world lost approximately 129 million hectares of forests, an area of a size equivalent to South Africa. The sad irony is that when the forests are taken away, it is not only the trees that fall but the entire ecosystem gets demolished, with a dire consequence for everyone. The earliest records of forest management date back to 700BCin Assyria, where game reserves were set aside by decree for royal hunts (Dixon and Sherman, 1991) [1]. According to Schaeffer forest management is the administration of the forests to acquire a regular yield (Schaeffer et. al., 1930) [2]. Kramer demarcated forest management as the middle and long term planning of the forest enterprise (Kramer, 1976) [3]. Stankey defined forest management as sustainability is fundamentally a socio-political construct rather than a scientific concept capable of precise, unequivocal measurements (Stanky, 1995) [4]. According to Schlaepfer and Elliott, forest management relates to find the equilibrium between production and the productive capacity of the ecosystems locally, regionally, and globally, in the short and longterms (Schlaepfer and Elliott, 2000) [5].

RESEARCH METHODOLOGY

The paper aims to analyse forest management in Uttarakhand State and its impact on the economy of the State. This research throws light on the importance of sustainable forest management in the scope of conservation of forest resource for a better tomorrow. The paper discusses the present forest resource in Uttarakhand State through critically examining the land use pattern, forest cover, forest area, flora and fauna, growing stock, carbon stock and the impact of forest resource on the gross domestic product of the state. The research approach is analytical and descriptive. The paper is based on secondary data which has been taken from annual reports published by Uttarakhand Forest Development Corporation (UAFDC), Indian State of Forest Report (ISFR)-2019 by Forest Survey of India (FSI), Department of Economics and Statistics of Uttarakhand, articles and journals [6][7][8].

Forest Resource in Uttarakhand

The Uttarakhand State is spread across an area of 53,483 square km and shares domestic borders with Himachal Pradesh in the north & Uttar Pradesh in the south, international borders with Nepal and China. As the State lies in the Western- Central Himalayan range, the typical climate and vegetation vary exceptionally with altitudes ranging from glaciers at the highest elevations to subtropical forests at the lower elevations. Major rivers such as Ganga, Yamuna, Ramganga & Sharda originates in the State. The State has 13 districts. As per the 2011 census, Uttarakhand has a population of 10.09 million. The rural-urban population is distributed as 69.77 per cent and 30.23 per cent respectively; a larger proportion of the rural population implies fewer and less evolved urban areas in the State.

Land-use pattern

The land is utilized in different ways such as cultivable land, land for settlements, forest land, pasture & grazing land, and other purposes. Figure 1 shows the land-use pattern of Uttarakhand during 2015-2019. The land-use pattern is influenced by many exogenous and endogenous parameters such as relief features, climate, soil, and density of population, as well as technological and socio-economic factors.

Forest Cover

The Uttarakhand State is prosperous in terms of forest resources the forests in Uttarakhand belong to nine Forest Type Groups, which are further divided into 43 Forest Types (Champion and Seth, 1968) [9]. Figure 2 shows the forest cover map of Uttarakhand State. According to the physiographical division, the State can be classified into





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three zones namely, the *Himalayas*, the *Shiwalik* and the *Terai* region. Figure 3 shows the forest cover in Uttarakhand. As per the Forest Survey of India, the Recorded Forest Area (RFA) of the Uttarakhand State is 38,000 km square out of which 26,547 km square is Reserved Forest, 9,885 km square is Protected Forest and 1,568 km square is Un-classed Forests (FSI, 2020) [10].

Growing Stock

Growing stock describes all available wood resources and it acts as a standard for quantifying carbon stock. Figure 4 shows the growing stock in the State. The data from FSI shows there is a decline in the growing stock in the recorded forest area of Uttarakhand. Between 2015 and 2019, the growing stock declined 7.86 per cent which is to be concerned.

Forest Area

The Forest area of Uttarakhand includes Reserved Forests (RF) and Protected Forests (PF). Figure 5 shows the recorded forest area of Uttarakhand. As of 2015, the State's recorded forest area consists of 70 per cent RF, 26 per cent PF, and 4 per cent un-classed forest.

Carbon Stock

Forests in Uttarakhand provides an efficient carbon bed for nearby industrial states. Figure 6 shows the total carbon stock of forests in Uttarakhand State. Total carbon stock of forests in the Uttarakhand state increased from 284.664 MT to 370.912 MT during 2017-2019 This growth of 30 per cent in carbon stock shows holistic plantation efforts by the Uttarakhand Forest Department and promotion of horticulture in the State.

Biodiversity

The Uttarakhand resides in the Western-Central Himalayan Biogeographic zone, the region is rich in forest resource which includes diversity in forests that ranges from tropical deciduous to alpine shrubbery. "The rich floral diversity of the state comprises 5096 species of Angiosperms and Gymnosperms" (ENVIS, 2013). Important floral species include Sal (Shore robusta), Jhingan (Lannea coromandelica), Shisham (Dalbergia disso), Haldu (Haldina cordifolia), Jamun (Syzygium cumini), Amaltas (Cassia fistula), Khair (Acacia catechu). Banj/ Oak (Quercus leucotrichophora), Burans (Rhododendron arboretum), Kaphal (Myrica esculenta), Pure stands of Chir Pine (Pinus roxburghii) and Deodar, Cedar (Cadres deodar), (Korthalsella), Himalayan white pine (Pinus wallichiana), Pindrow (Abies pindrow) and Willow (Salix sp.) Out of the total species of vascular plants, 1748 spp. species are medicinal (Samant et al., 1998) [11]. Capitalising on favourable climatic conditions and Government promotional schemes, the State experienced a record ten-fold growth in the farming of aromatic plants from 2003 to 2007. (ISFR, 2019) [10]. The entomopathogenic fungus YartsaGunbu (Ophiocordyceps sinensis) are of immense medicinal value. Buras (Rhododendron arboretum) flower juice is also significantly effective in heart-related medical treatment. The forests' of Uttarakhand State are home to a wide range of faunal species. As of 2013, 4,907 faunal species have been reported which include 959 vertebrates and 3,948 invertebrates. It also homes several pervasive and vulnerable faunal species. The sub-alpine zones are a natural sanctuary for Langur (Semnopithecus), Leopard (Panthera pardus), Kakkar (Muntia cusmuntjac), Himalayan Black Bear (Ursus thibetanus laniger), and Goral (Naemorhedus goral) The elevated regions are home to Snow Leopard (Panthera uncia), Musk Deer (Moschus moschiferus), Thar (Hemitragus jemlahicus), and Blue Sheep (Pseudois nayaur). The State also enjoys a remarkable variety of birds including Grey Quail (Ophrysia superciliosa), Peacock (Pavo cristatus), Chakor (Alectoris chukar), Kala Titar Pheasant (Francolinus francolinus), Monal Pheasant (Lophophorus impejanus), Cheer Pheasant (Catreus wallichii), and Whistling Thrush (Myophonus horsfieldii) (ENVIS, 2013) [8].

Impact of Forest Resource in Economic Development of Uttarakhand

Forests play a critical role in green growth and are also an important source of both formal and informal jobs, particularly in remote areas where there are few economic alternatives. Figure 7 shows the details of revenue generated by the forests of Uttarakhand, during 2011-12 Uttarakhand Forest Development Corporation (UAFDC) reported, ₹19078.40 Lac Royalty and tax payment to Central and State Govt., this figure grew to ₹23105.30 Lac in 2016-17 which shows a total growth of 21.12 percent [12].





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Forest-Based Industries

India is one of the leading countries involved in wood-based industries. These consist of paper and pulp, veneer and plywood, match, pencil, dendro-biomass, and saw wood industries. Figure 8 shows the sales of forest products in Uttarakhand State.

Timber

People in the country mostly use timber and other processed wood in their domestic and industrial wood requirement. Commercial felling is restricted in the State in areas that are more than 1000 meters above mean sea level (MSL).

Plantation

The plantation includes the planting of trees that are managed for commercial timber production or environmental purposes. Plantation operations are managed on both forest area and freehold land and the establishments are regulated and assisted by the Forest Department of the State.

Resin Production

Resin tapping is one of the most important revenue-generating activities of the Forest Department of Uttarakhand as the State has a substantial area under *Chirr* pine forest which exudes resin. Figure 9 shows the Pine Resin production in the State. Resin extraction not only contributes revenue to the State but also provides livelihood to a large number of people in the hills [13].

Sustainable Management of Forest Resource in Uttarakhand

The management of forest resources falls under the responsibilities of the Central and State Governments. The Indian Forest Act, 1927 provides a rigid legal framework for the protection and management of the country's forest resources but the formulation and the implementation of programs are performed by the respective State Government, hence the rules and regulations enforced by each state differ based on the unique flora and fauna that exists in those states. Some of the important Acts and Policies that are framed by the Government of India for Sustainable Management of Forest Resources followed by the Uttarakhand Forest includes, "Indian Forest Act, 1927, Forest Conservation Act, 1980, Environment Protection Act, 1986, Wildlife Protection Act, 1972, The Biological Diversity Act 2002, Schedules Tribes and Other Traditional Forest Dwellers (Recognition and Forest Right) Act, 2006 and latest The Compensatory Afforestation Fund Act, 2016" (Uttarakhand Forest, 2020) [10].

Economic development requires a balanced trade-off between human and environment since 71 percent of the State's Land is recognised as forest area of which 46 per cent is Total Forest and Tree Cover, along with this 7.54 percent of the land is not available for cultivation and only 11.68 per cent land of the State is under net sown area, which means a scarce availability of Culturable land in the State with the majority of the population livingin rural areas, thus primary sector cannot be thrust in boosting the economic growth of the state, whereas if we talk about secondary sector, Uttarakhand Government's ambitious industrial project, SIDCUL created job opportunity in the state, but was very expensive for the state govt. in terms of forest land diverted to industries. (Farooquee & Maikhuri, 2007) [14], reported that in Uttarakhand the forced conversion of forest land into the industrial area resulted in a high level of deforestation of native species such as Amaltas (*Cassia fistula*), Sisham (*Dalbergia disso*), Sal (*Shore robusta*), Jhingan (*Lanneacoroman delica*), Haldu (*Haldina cordifolia*), Jamun (*Syzygium cumini*), and Khair (*Acacia catechu*) which was the diet of local Fauna such as Monkey, Deers, and Elephants and replacement of these species with the plantation of commercial trees such as Poplar (*Populus tremuloides*), Eucalyptus (*Eucalyptus globules*) for paper and pulp industries harmed the forest's biodiversity, as a result, the leftover wild species enters nearby areas and causes human-animal conflicts.





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CONCLUSION

Forest plays an important part not only in providing livelihood to a significant portion of the population but directly and indirectly maintains the life of the Earth. Apart from timber, the forest provides clean air, freshwater, a wide range of flora which includes ethno medicinal plants, and fauna. Table 1 shows the proportion of forest resource revenue generation to the GSDP of Uttarakhand State. The GSDP of Uttarakhand is contributed 10.81 percent by the primary sector, 48.28 percent by the manufacturing sector, and 40.91 percent by the tertiary sector. The per cent contribution of the forest produce in the GSDP of the State clearly states, the underutilization of forest resource capacity in the State. Uttarakhand is the only north Indian state to have more than 60 percent of the area under forest cover that acts as atmosphere stabilizer for high carbon-emitting neighbouring States such as Delhi NCR, Uttar Pradesh and Haryana; this gives a comparative advantage to the State Government and local communities to develop forest-friendly industries such as:

- Ethno-medicinal culture,
- Jatropha (bio-fuel) culture,
- Resin Production,
- Mineral water industries
- Eco-Tourism industry

Recommendations

- Balance with plantation of commercial species in such a manner that plantation of may yield revenue but native species may also survive.
- Emphasis should be taken by the institutions, not on the plantation of trees on a certain occasion, but the maintenance of planted trees.
- Incentivise the Sustainable Development of Forest Resource in Uttarakhand State through the **grant of special** status to Uttarakhand State as State's 71 percent land is classified under Forest Area, thus, less than 29 per cent land is available for utilisation for cultivation, urbanisation, developing Socio-economic Overheads Capital (SOCs) and industrialisation.

Declaration of Conflicting Interests

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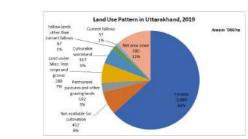
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Table.1: GSDP of Uttarakhand State and Forest Resource (2011-2016)

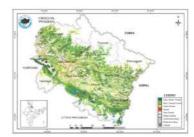
Year	GSDP at Constant Price (₹ Lakh)	Royalty Paid to State / Central Govt (₹Lakh)	Sales of Forest Products/ Services (₹ Lakh)	Pine Resin / Other Production (₹Lakh)	Total (₹ Lakh)	The proportion of Forest Resource revenue to GSDP (%)
(I)	(II)	(III)	(IV)	(V)	VI=(III+IV+V)	(VII)
2011-12	11532800	19076.4	37428.058	2421	58925.46	0.51
2012-13	13161300	18027	39918.71	2200.5	60146.21	0.49
2013-14	14907400	23955.9	59395.18	2229	85580.08	0.64
2014-15	16143900	24515.6	49940.12	2163	76618.72	0.54
2015-16	17716300	33491.2	63635.33	2343	77469.53	0.65

Source: (i) Economic & Statistical Organisation, Uttarakhand; (ii) Central Statistical Organisation, New Delhi; (iii) UADFC, 2019



Source: ISFR-2019, Forest Survey of India

Figure.1: Land-use Pattern of Uttarakhand (2015-2019).



Source: ISFR 2019, Forest Survey of India

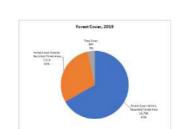
Figure.2: Forest Cover Map of Uttarakhand



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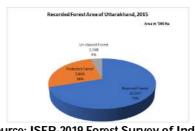


Source: Forest Survey of India

Growing Stock of Uttarakhand Forest (2015-2019)

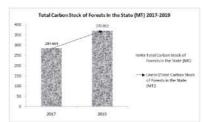
Source: ISFR-2015, 2017, 2019, Forest Survey of India

Figure.3: Forest Cover in Uttarakhand



Source: ISFR-2019 Forest Survey of India

Figure.4: Growing Stock in Uttarakhand



Source: ISFR-2017, 2019, Forest Survey of India

Figure 5: Recorded Forest Area of Uttarakhand

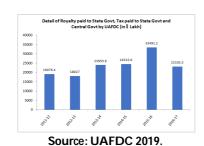
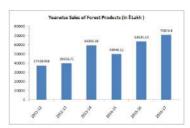


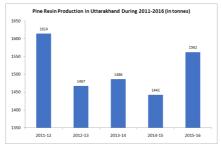
Figure. 7: Detail of Royalty paid to State Govt, Tax Paid to State Govt and Central Govt by UAFDC

Figure 6: Carbon Stock of Forests in Uttarakhand

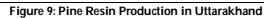


Source: UAFDC, 2019.

Figure 8: Year-wise Detail of Sales (in Lakh)



Source: ICAR-Indian Institute of Natural Resins and Gums, Ranchi (Jharkhand), 2016







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RESEARCH ARTICLE

Data Utility Evaluation in K-Anonymization through Classification **Accuracy for Privacy Preserving**

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ABSTRACT

In recent years, data privacy has received significant scholarly attention. Sensitive data fields are frequently included in datasets, and their exposure may threaten the interests of anyone connected to the data. In order to address this problem, privacy techniques can be employed to prevent the identity of a person by protecting sensitive information by anonymizing the dataset's sensitive data. The dataset can then be utilized without restriction by other parties for analysis. In this study, we examined the kanonymity privacy strategy for various k values on a variety of n number of columns of the dataset. The information loss associated with k-anonymity is then calculated. To test for a stability amongst data anonymity and data value, some machine-learning algorithms, including Naive Bayes, J48, Neural Network, and SVM, classify the anonymized files. Since the ideal k and c are determined by the classification accuracy, the k-anonymity technique can be used to anonymize the ideal number of columns in the dataset.

Keywords: Data Utility, Information loss, Privacy Protection, K-Anonymity.





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INTRODUCTION

Today's increasing internet usage [1] is a factor in the growth of big data in social and business media. Big data security and privacy are crucial because the people whose data it is could be in risk if their personal information were to leak. Data security and privacy have attracted more attention in recent years. There are two types of data: public data, which is accessible to everyone, and secret data, which is only visible to a small number of people. The interests of those with links to the dataset may be put at risk if sensitive data fields are exposed since many datasets contain both sensitive and insensitive information. With the development of technology, hackers have occasionally created new techniques to access private data on bank, government, and voter identification cards, among other places. Attackers gather information by taking advantage of the system's weakness when it is at its most vulnerable, such as at the stages of data collecting, storage, use, or processing [2]. The attackers have a fair chance of identifying the target after combining all the information. Therefore, one of the most important challenges at the moment is the protection of big data privacy [3,4]. Data privacy refers to the characteristic of keeping private sensitive data in a dataset. A privacy technique's goal is to prevent the identity of a person by de-identifying the dataset's persons in order to protect sensitive information, while allowing interested third parties to utilize the dataset without restriction for research. The inability to identify specific individuals from a dataset is a quality of anonymity that is realized via a privacy strategy. In order to maintain the anonymity of the data's subjects, personally identifiable information is removed from datasets [5,6].

Age, gender, and other characteristics of a person, such as their zip code, don't seem to be particularly sensitive in terms of privacy, but if they are linked to other characteristics, a person's identity or sensitive information may be exposed. Data anonymization is done to shield users from invasions of their privacy. To do this, a variety of privacy strategies, including k-anonymity[7,8], differential privacy[9], and sample-uniqueness [10], are already available. The need to maintain privacy is very important if one wants to earn people's confidence. Since anonymization and randomization require the removal or reorganization of personally identifying data, they cause some data loss while preserving privacy. The repository may keep data from insurance customers, people with medical records, and those who shop online, as well as information like name, national ID number, email address, zip code, and date of birth. Machine learning algorithms look for certain patterns and facts in large, complex datasets to comprehend the structure of the data within. Data mining methods [11,12] like Naive Bayes, J48, Neural Networks, and SVM can uncover important connections that improve business operations, health care, and many other fields of expertise. Large datasets that are electronically recorded are mined for patterns of strategic information that are concealed, and detecting these patterns produces important insights that are beneficial to many businesses [13,14]. Data mining plays a significant role in the recognition of the collection of large amounts of data and the ease with which it can be stored across computer systems. Machine learning employs statistical techniques to identify information that is displayed and understood by people.

The goal of this research is to retain the privacy technique known as k- anonymity to safeguard users' data in the dataset against privacy violations by attackers both inside and outside of the system. The performance of the data's usefulness in terms of classification accuracy is then assessed using machine learning techniques, both before and after privacy techniques have been applied to the dataset. The remaining part of the article is structured as follows. The construction process for the suggested algorithm is laid out in Section II. Section III presents the findings and discussion, while Section IV presents the conclusion.

METHODOLOGY

Algorithm provides a method for balancing information loss with data utility. The following are the inputs for the algorithm: dataset D containing n rows and m columns, p sensitive columns, and an accuracy tolerance of 0<€«1 for classification.



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ALGORITHM

Optimal k value for k-anonymity on the optimal n number of columns of a data set D

Input

Dataset D with the r number of rows, and the m number of columns. The s number of sensitive columns, with an Accuracy tolerance, $0 < \epsilon < 1$.

Output:

kandm number of columns.

Step 1: Calculate the classification accuracy a of dataset D applying a machine learning algorithm;

Step 2: Initialize the values: k = 1; and m = s;

For*j*= *n*-1to*p*with adecrementof1do

Fori=m/2to2with adecrementof1do

Apply k-anonymity on D with k = i and save the result in R,

Compute the classification accuracy of R;

Calculate the loss of accuracy: $\tilde{0}=^{x-y}$

If (ð<∈) then

k=i; and n=j;

return kandn;

End(If)

End(For)

End(For)

return k and n;

The delicate columns must be safeguarded. An algorithm for machine learning, such as Naive Bayes, J48, Neural Network, and SVM, calculates the classification accuracy. Algorithm 1's line numbers 5 to 11 are followed by the following:

The programme determines the dataset D's k-anonymity and sets the result to be E. The next step is to calculate E's classification accuracy. The accuracy loss is then calculated. The values of I and j are given back if the accuracy loss is less than the tolerance limit. Using an outer for loop over j from m-1 to p with a decrease of 1, and an inner for loop over I from n/2 to 2, the preceding approach is repeated. As a result, the brute-search technique makes use of k and c in exactly the right amounts. To avoid producing meaningless results that satisfy the requirement in line number 8, the loops are organised from largest to smallest values. The starting values of k and c are returned, where k = 1 denotes that no anonymity is used, if no values of k and n for the technique are obtained by fulfilling the conditions in line number. This could indicate that the tolerance is too low or that the data are too sensitive to be classified.

RESULTS

An Attribute-Relation File Format (.arff) dataset of cardiac patients that was converted to Comma-Separated Values (.csv) for this study was used for testing reasons. The website (https://github.com/renatopp/arff- datasets) was used to retrieve this dataset. The dataset had 542 rows and 1213 columns (gender, age, type of chest pain, blood pressure, cholesterol, fasting blood sugar, ECG break, maximum heart rate, angina, peak, colored vessels, class). The dataset contains both sensitive and non-sensitive data. But there is also the choice of other test datasets. The dataset was subjected to several tests using k-anonymity, which was applied to various columns of the dataset with varying values of k. The dataset's classification accuracy was then calculated using several machine learning algorithms. Tables 1,2 and Figures. 1-6 provide the test findings. After applying privacy protection to the data with k-anonymity for various values of k, the table gives the numerical results of classification accuracy obtained using





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Naive Bayes, J48, Neural Network, and SVM. The graphs show how each algorithm's classification accuracy compares for a range of k-anonymity values. The outcomes present significant data. The machine learning techniques do not significantly alter the categorization with a change in k.The classification algorithms are implemented with different number of columns to check the accuracy of data utility. The experiment is continued with Naive Bayes, J48, Neural Network, and SVM classification algorithms with column size as 10, 25 and 100. The results are as shown in the figures below:

When k-anonymity is used on three and five columns, we see that the accuracy stays relatively the same. However, when the Naive Bayes approach is applied for classification, there is a notable loss in accuracy for k-anonymity on columns eight and ten. It should be noted that the accuracy of the categorization is not always impacted by the information loss caused by k-anonymity. Perhaps the other dataset attributes offer a better connection to the result. It should be observed that as the k-value increases, more information from the dataset is lost. When additional columns are subject to k- anonymity, this loss is amplified. For instance, age information is reduced when k-anonymity is used. Ages are grouped with lower and upper boundaries when k is less than 1. This enables age-based analysis of the dataset. Higher k-values, on the other hand, result in fewer age groups, which makes age-based analysis difficult. The age data will therefore be worthless. Therefore, k-anonymity and data usefulness must be balanced. The next step is to utilize the method to determine the best values for k and c to use with the k-anonymity technique to anonymize the right number of columns c in the dataset. The ideal value was discovered to be k = 5, c = 8, and a = 0.04. According to Table III and Fig. 2, where is equal to 0.039, the accuracy with these values is 79.05%.

CONCLUSION

In this research, we propose a method for calculating the ideal value of k and the ideal number of columns c of a dataset using the k-anonymity algorithm. In order to stop system operators (internal attackers) or other third parties from acquiring sensitive patient data, the method was utilized to anonymize the sensitive data in various columns of a test dataset of cardiac issues. Three machine learning algorithms—Naive Bayes, J48, neural networks, and SVM—were used to classify the data both before and after the privacy technique was applied to the dataset in order to assess the utility of the results. In order to stop system operators (internal attackers) or other third parties from acquiring sensitive patient data, the method was utilized to anonymize the sensitive data in various columns of a test dataset of cardiac issues. Four machine learning algorithms—Naive Bayes, J48, neural networks, and SVM—were used to classify the data both before and after the privacy technique was applied to the dataset in order to assess the utility of the results. Naturally, the results become less valuable the more anonymized data there are in the dataset, as shown by the machine learning algorithm experiment. The Nave Bayes approach produces the highest accurate categorization results, according to observations. In essence, we need to strike a compromise between the utility of the data and its anonymization. When using the k-anonymity technique to anonymize a dataset, the recommended algorithm can be used to determine the ideal value of k and the ideal number of columns.

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Table.1:Classification Accuracy Before Applying K-Anonymization Algorithm for different values of K.

k	Naïve Bayes	J48	Neural Network	SVM
1	80.56	79.91	79.11	77.72
2	80.24	79.22	78.34	77.6
3	80.17	79.13	78.69	76.55
4	79.95	79.17	78.97	77.88
5	79.33	79.46	78.71	77.47
6	79.43	79.37	77.56	77.33
10	79.48	78.77	77.36	77.57
15	79.43	78.88	77.16	77.73
25	78.73	78.32	77.58	77.81
50	78.92	78.32	77.43	76.92
90	78.71	78.56	77.83	76.77
100	78.52	78.51	77.71	76.85
200	78.43	78.45	77.54	76.13

Table.2:Classification Accuracy After Applying K-Anonymization Algorithm for different values of K

K	Naïve Bayes	J48	Neural Network	SVM
1	79.56	79.81	79.02	77.43
2	80.03	79.47	78.21	77.24
3	80.03	78.18	78.42	76.36
4	79.72	79.34	76.63	77.42
5	79.12	79.02	76.55	77.21
6	79.26	78.63	77.35	77.08
10	79.31	79.12	77.21	77.42
15	78.1	78.32	76.89	77.51





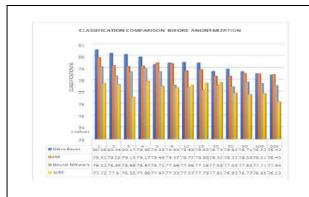
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25	78.55	79.11	76.82	77.43
50	78.75	78.12	76.76	76.81
90	78.52	78.35	77.65	76.31
100	78.21	78.01	77.53	76.71
200	78.05	77.56	76.32	75.72



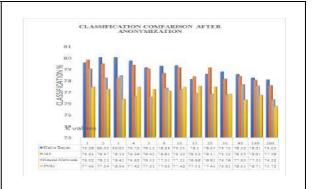
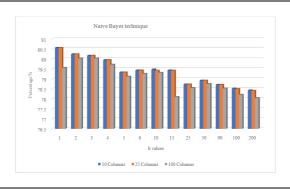


Figure 1. Classification Accuracy Before Applying K-Anonymization Algorithm for different values of K

Figure 2. Classification Accuracy After Applying K-Anonymization Algorithm For Different Values Of K



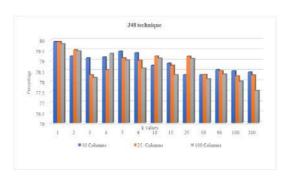
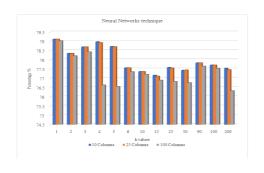


Figure 3: Naïve Bayes technique for different values of k and columns

Figure 4: J48 technique for different values of k and columns



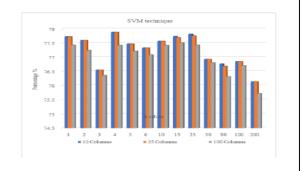


Figure 5: Neural Networks technique for different values of k and columns

Figure 6: SVM technique for different values of k and columns





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RESEARCH ARTICLE

Comparative Aspects of Image Data Analysis using Drones and Smart **Phones: A Pilot Study**

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ABSTRACT

Since drones and smart phones are so widely available to the general public and are, as we all know, growing in popularity, there has also been an increase in criminal offences such as spying. Drones are adaptable aircraft that can fly autonomously and perform a wide range of tasks. Since drones have the potential to commit crimes, it is essential to look into such activities. Drones and smart phones share many characteristics with computers, such as a Universal Serial Bus port, Central Processing Unit, memory, and camera. Drones have the special and unique ability to fly swiftly. Drones are used in the investigation in a number of ways, including crime scene administration, mapping, reconstruction, photography, videotaping, and evidence gathering. This study involves data extraction method using smart phone and recorded drone flight data, allowing us to compare the parameters in two sets of data and spot differences between them rapidly.

Keywords: Drone, smart phones, Extraction Method, Mavic Air 2S, DJI Phantom 4 pro+, Forensics.

INTRODUCTION

Most definitions define a drone as an unmanned aircraft or ship that is controlled remotely or by computers on board. A subset of digital forensics called drone forensics deals with recovering digital data or evidence from a drone under forensically sound circumstances [1]. The drone is an unmanned aircraft used for as a weapon or a toy. An overview, close-up, and mid-view range of photographs are taken of the evidence and the crime sites. A highresolution camera is necessary when working with an experienced professional. Rarely, it is challenging to capture





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evidence that is out of the photographer's line of sight, like in lakes, ponds, seas, mountainous slopes, etc. With its high-resolution camera, a drone can, however, readily take pictures of evidence that is larger than the minute evidence without disturbing the evidence. In rural settings and wooded areas, it is possible to fly it over bodies of water at a great height [2]. To capture the entire crime scene in a continuous flow Another type of documentation carried out by the staff is videotaping. A drone can be used to videotape the crime scene, just like it can be used for photography. It has the ability to record video. big, inaccessible crime scenes precisely, effectively, and quickly. The documentation is followed by a thorough look for supporting materials. Even if the search pattern methods are used, some of the evidence can be missed. A drone can look for and find the evidence, which is also required for collecting. We can digitally map the entire crime scene with the aid of LIDAR technology (Light Illumination Detection and Ranging). Highly coherent laser light from the drones equipped with this technique strikes the surface of the evidence at the crime scene. The drone takes in this light and determines how far away the crime scene is from the evidence. We can map traces of evidence like glass, bloodstains, hair, and fiber thanks to LIDAR technology. like saliva [3]. Drones are utilized in many different industries, including topography, surveillance, inspections, heritage, agriculture, fisheries, emergencies, communications, transport, media, and defense and security [4]. Drones were first primarily utilized for military purposes like surveillance, reconnaissance, and weapon delivery [5]. The main problem in metropolitan areas is the crimes in a complex environment and street crimes, which security and police cannot access quickly. To develop pleasant metropolitan cities with a unique strategy. Drone technology has a significant impact on how we live our daily lives, simplifying and minimizing issues. This technology is being developed for crime observation, surveillance, detection, and control [6,7,8].

Obstacles in drone forensics

Obstacles can be observed are Deterioration of the storage media and dispersed components, A lack of data from GPS, Ascertaining ownership, A lack of suitable digital forensics equipment, Attaching the drone's USB cable, Incompatible file systems, Issues with standardization, Permission to access, Remote tampering, Cloud computing etc...

Drone categories

Drones can be categorized in four sections Drones with multiple motors, Drones with a single rotor, Drones with fixed wings, Hybrid VTOL with Fixed Wings [10]. Drone-related issues are frequently categorized in a variety of ways, including legally, morally, and ethically. Many nations forbid the open use of drones, although other modern nations are now permitting them to be used for social good. Additionally, a trustworthy drone market is developing in Singapore, yet ethically, there are some drone issues [11]. UAVs are gradually used in a variety of civil applications, such as agriculture, remote sensing, aerial delivery, and photography. Many e-commerce businesses, like Amazon, are aiming to use UAVs for their delivery services. UAVs have recently been used in the field of wireless communication to provide network coverage and strengthen network connections in remote places. UAVs are frequently used to track public movements and social gatherings during pandemics like COVID-19 in order to reduce the risk of disease transmission and to report gatherings and lockdown violations in order to reduce the likelihood that law enforcement and health officials will contract the disease [12,13]. The 21st century is typically seen as the era of "Technology," which has made daily human activities easier. One of the 192 important new technologies, "drone," is expected to have enormous implications in the majority of fields, including urban planning, defense, healthcare, and disaster management, forecasting the weather, managing garbage, mining, telecommunications, etc. [14,15,16]. To find, gather, keep, examine, and record potential UAV occurrences, the drone forensics (DRF) domain may be important. Due to the range and multidimensionality of UAVs, the domain is heterogeneous, complicated, and confusing. There are many different DRF models and frameworks that are suggested to solve scenarios, but there isn't a unified and structured model to make it easier to manage, share, and reuse DRF tasks and activities [17,18,19,20].





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Experimental Design

Scenario Creation

At two distinct sites, flights were carried out with two different drones, at various times, and on various days. Two of the many photos that were collected were picked for the experiment. Additionally, capturing pictures from two various mobiles, at various locations, at various hours, and on various days.

Recorded Media

Image files in JPG format.

Environment setting

The environment's setting was made. The experiment was carried out on many days and in various locations. On December 6, 2021, at 10:04 AM, a DJI Phantom 4 pro+ drone was used to capture this photograph in PATHRI, 69F3+9H7, Majalgaon-Parbhani Rd, Somthana, Maharashtra 431131, India. The DJI Mavic Air 2S Drone captured the DJI 0503.JPG photograph on April 24, 2022, at 1:24 PM in Majalgaon, 55GJ+668, Maharashtra, India. The photo 20220604 144123.jpg was shot on June 5, 2022, at 5:34 PM, using a Samsung Galaxy A53 5G (shown in Fig no. [11] and image was taken by Mobile shown in Fig no. [5]) at the Government Institute of Forensic Science in Aurangabad, Maharashtra, India. The photo 20220605173407.jpg was captured on an Oppo F19 pro (Shown in Fig no. [12] and image was taken by Mobile Shown in Fig no. [7]) at 2:41 PM on June 4th, 2022 in Aurangabad, Maharashtra, India (W859+P76).

Hardware

Mobile phones

The Oppo F19 Pro and Samsung Galaxy A53 were employed in this experiment. (Table No. [2] shows the Device Information of Mobile).

Drone

DJI Phantom 4 Pro+ and DJI Mavic Air 2S Drone were employed in this experiment. (Table no. [1] shows the Device Information of Drone)

SD card

The drone has a 32GB SD card that serves as storage. The flight's films and photographs are kept on a 32GB external micro-SD card.

Software

Windows 10 laptop (Operating System).

Hashcal: This piece of software is essentially used to maintain the configuration, validity, and integrity of documents, files, and images.

MD5 and SHA 1

In this experiment, we are able to compute the MD5 and SHA 1 values of photos with the goal of maintaining the integrity of the images. with the aid of HASHCAL, an open-source program. (Fig no. [2,4,6,8] shows MD5 & SHA1 value of images). MD5 may function as a hash function. Message Digest 5 (MD5) is an algorithm that creates hashes with a 128-bit length. It is employed in forensic image processing for integrity verification. Cryptographic hash algorithms like SHA 1 are a basic procedure in digital forensics that are used to preserve digital evidence and guarantee its integrity. Each image used in this experiment will have its MD5 and SHA1 values provided after it.

Experimental Procedure

For this study, we directly extract data from a drone's micro-SD card. and after that, an analysis was done. We shall attempt to compare the data that is contained in both drones and mobile devices. the assistance of the HASHCAL





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open-source tool. A hash function could be MD5. A 128-bit hash is produced by the MD5 method, which stands for (Message Digest 5). Forensic image processing employs it to check the integrity of images. The preservation of digital evidence and guaranteeing its integrity involve the use of cryptographic hash algorithms, such as SHA 1. This approach is typical in the field of digital forensics. We will provide the MD5 and SHA1 values for each image used in this experiment after the image has been used.

Experimentation Method

For this experiment, a micro-SD card was immediately extracted by a drone. analysis was then carried out. In an effort to evaluate both, we will examine the data that is available on mobile and drones compare the data from the two sources (drone and smart phone) and see if any commonalities can be detected. For comparison, we'll use recorded cell phone image data and drone flight data, including photographs.

Data Extraction Methodology

We can extract the information utilizing a variety of techniques like:

Extraction using a chip-off extraction technique.

NAND flash, a type of memory technology that doesn't require power to preserve data, may be used in the chip-off process. The chip-off technique works around passcode-protected devices and enables the recovery of damaged equipment. The removal process is mostly destructive, and it's a little challenging to reattach the NAND flash to the PCB and get the device to work.

Steps

- 1) Physically removed the NAND memory chip from the drone using tools that either desoldered it or used a vacuum and burst of hot air to remove it.
- 2) The connectors on the underside of the chip must first be repaired because the removal operation frequently destroys them.

The chip is then put into a specialized hardware device so that it may be read (see step 3). The gadgets typically require programming for a specific NAND flash chip and have a selection of the more common chips.

Direct extraction from the circuit board technique.

board might be a drone board that regulates the motors' RPM in accordance with the input. Took the drone apart, physically removed the card, and used its inbuilt camera and the circuit board to extract data.

Extraction with the Cellebrite UFED forensic instrument.

The UFED is connected to the drone via more than 70 connections and displays the specific information needed for extraction. The extraction was completed by simply connecting a USB drive, SD card, and PC to the UFED device, copying the information, and pasting it where it was needed.

Direct data extraction from the drone's micro-SD card.

We are using the DJI Phantom 4 Pro+ (shown fig no. [9] and image was taken by Drone shown in Fig no. [1]) and Mavic Air 2s drone(shown in fig no. [10] and image was taken by Drone shown in Fig no. [3]) for this experiment. While the DJI phantom 4 pro+ lacks internal memory has no internal memory (There may be a requirement to load a micro-SD card into the drone to store the data). The drone's internal 32 GB micro-SD card is taken out, and it is then connected to a laptop or CPU. By simply right-clicking on the various drone data files and various image types present on an SD card, the copy option is visible. By selecting that, and pasting the image on the desired drive, the extraction was completed. External SD storage will appear on a laptop or desktop by simply clicking on them. There are four options from which we can choose the strategy of direct information extraction by drone's micro-SD. (Table no.[3] shows comparison of Drone and Mobile). Out of the numerous data extraction techniques listed above, the researcher used convenient Direct data extraction from drones like the DJI Phantom 4 pro+ and DJI Mavic air 2S





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micro-SD card. Researchers have used two smart phones, the Samsung Galaxy A53 5G and the Oppo F19 Pro, to capture images, and their internal memory metadata has been recovered.

Devices used in Experiment

Observations and Results

Comparative analysis and monitoring will be done for the given parameter

Around 30 plus parameters are studied here

Date Taken

The original shot's most trustworthy source is its date of capture.

Moment Spent

This indicates the best time to capture a photo.

Program Name

This information is provided in the drone's property.

Dimensions

The image's dimensions are its height and length.

Width

The image's width in pixels the measurement of anything from one edge or side to the other.

Height

One piece of information used to calculate an image's size is its height.

Horizontal Resolution

The amount of items, columns, or dots on a printed page, screen, or fixed area, such as an inch, from left to right.

Vertical Resolution

This refers to how many vertical elements can be recorded by a camera and displayed on a monitor.

Bit Depth

The amount of bits used to represent each pixel in an image is known as the bit depth.

Resolution unit

The image's X or Y resolution is measured using the resolution unit.

Color representation

This is a way for displaying the red, green, and blue colours that will be utilized on a computer display.

Compressed bits/pixels

The amount of bits required to define a single pixel determines the bit depth.

Camera manufacturer

The camera manufacturer provides a summary of the camera's designer or manufacturer.

Camera model

The camera model provides information on the drone camera's model number





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F-stop

A camera is used to measure aperture measurements.

Exposure time

This crucial camera setting, which is associated with camera sensitivity, is governed by shutter speed.

ISO speed

The measurements of the camera's sensor's sensitivity to light made according to an international standard.

Exposure bias

The amount of f-stops that are either above or below the camera's metering.

Focal length

Is the measurement of how far the image sensor is from the lens while the subject is in focus.

Max aperture

The widest that a lens may be opened is determined by its maximum aperture.

Metering mode

Depending on the amount of light entering the ISO and the camera, metering mode is the method by which a camera detects the exposure and determines the proper shutter speed and aperture should be.

Subject distance

The distance between the camera and the focus of the image.

Flash mode

Flashes can be used in slow sync, fill flash, and rear-curtain sync modes, all of which brighten portrait subjects.

Contrast

The degree of contrast between an image's constituent parts is known as contrast.

Light source

This might be either a natural or artificial source of light.

Exposure programme

This option regulates either the exposure time or the aperture.

Saturation

This term refers to the color's purity and intensity, or how light or dark it is.

Sharpness

This refers to the degree of clarity and level of detail that a photograph achieves.

White balance

used to make colours appear whiter by adjusting them to fit the colour of the light source.

Latitude

It establishes how much over- or underexposure can be tolerated while still producing passable photographs.





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Longitude

Is the measurement of a place on the planet's east or west axis.

Altitude

It makes it possible to cover wide areas.

Item type

It is used to upload JPEG, JPG, and PNG files and is a place where an image resource can be a picture of an item, an image from a form field, or a static image.

Attributes

Formats, aperture, and focal length are the controls that determine how a basic camera lens behaves as well as its depictive content and structure. According to society's needs, when digital evidence is gathered and investigations are conducted, it appears that more data is obtained via drones than from mobile devices, increasing their admissibility and authenticity in courts. As the number of parameters rises, so does the accuracy and quality of the image or video.

Forensic Tools- Numerous tools are available online, whereas just a few of them are:

Drones- Rosoka Add-on, MD-SERIES Powered By Hancom, Digital Evidence Investigator PRO, Mobile Device Investigator, and XRY Drone are the tools used in drone forensics for data recovery purose. Mobile- UFED physical analyzer, Dr.Fone for Android / iOS. Cardrecovery, PhotoRec, Recover My Files. Recuva ,Remo Recover, Undelete 360. Recoverit Data Recovery are the tools used in Mobile forensics for data recovery purose.

CONCLUSION

In this paper, the researcher compares images from Smartphone's and drones. While the drone provides a number of parameters, we noticed that just a few of those parameters are displayed on the mobile device. Because the drone camera displays a bigger number of features and enables us to view the image from the fly view, which is not possible with the smart phone camera. Researchers are attempting to show that the drone camera is much superior to the latter. In this research, smart phones and drones are used to extract data. Then, a comparison is carried out by taking important parameters, followed by applying MD5 and SHA1 hash functions, which guarantees two key uses: the detection of duplicate files and the assurance that files are undamaged and forensically sound following data recovery, finally, a tool review is accomplished.

List of Abbreviations Used

- Unmanned aerial vehicles (UAVs)
- Remote pilot aircraft systems are frequent names for drones (RPAS)
- Global Positioning System (GPS)
- Universal Serial Bus (USB)
- Drone Forensics (DRF)
- Joint Photographic Group (JPG)

Statements and Declarations

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The authors declare that no financial interests/personal relationships which may be considered as potential competing interests





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Competing interests

"The authors declares that they have no competing interests" .We are not receiving or having financial competing and non-financial competing interests.

Disclaimer

Researcher only conducts a pilot study on various e-devices. In this research, researcher never encourages / do not promote / do not advertise any specific e-devices. While conducting this research, researcher follows privacy and ethical guidelines. Also all figures and Tables are originated from the authors only.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article

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Table.1: Device Information of Drone

Sr no.	Parameters	Drone 1	Drone 2
1	Make	DJI	DJI
2	Model No.	DA2SUE1	GL300F
3	Device Name	Mavic air 2s	Phantom 4 pro +
4	Price	1,35,000	1,85,000
5	Weight(grams)	198g	1388g
6	Camera resolution	5.4k/30fps	4k/60fps
7	range	10km (6.2 miles)	7km (4.3 miles)
8	Device Serial number	3YTB8B00303TU	0AXDDCT0A20717
9	Battery Serial number	57ZPK3HEA208VZ	082ADBG0310AJ9
10	Total flight time	31 minutes	30 minutes
11	Maximum speed	42.5mph	45mph
12	Maximum altitude	16,404 feet	19,685 feet
13	Battery Model number	BWX232-3750-11.04	PH4-5870mAh-05.2V
14	Maximum data storage capacity (internal storage)	8GB	Does not have internal storage
15	Maximum charging	35.8 W	160 W
16	Maximum wind speed resistance	10.5 m/s	10 m/s

Table 2: Device Information of Mobile

abic.2. Device information of Mobile				
Sr no.	Parameters	Mobile 1	Mobile 2	
1	Model Name	Galaxy A53 5G	OPPO F19 Pro	
2	Brand	SAMSUNG	OPPO	
3	Model Number	SM-A536E/DS	CPH2285	
4	IMEI Number	584963/85/348596/9*	781436259471659*	
5	Serial Number	EDUQ53UL8Q*	T5OE7NLGA6587D3E*	

^{*} Actual information was modified for the sake of privacy and security purpose.

Table.3: Comparison of Drone and Mobile

Sr no.	Parameters	Drone 1(Mavic air 2S)	Drone 2(DJI Phantom Pro+)	Mobile 1(Galaxy A53 5G)	Mobile 2(OPPO F19 Pro)
1	Date Taken	24-04-2022	06-12-2021	04-06-2022	05-06-2022
2	Time Taken	13:21	10:04	2:41 pm	5:34 pm





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3	Program Name	10.10.39.23	v01.07.1641	-	-
4	Dimensions	5472 X 3078	5472 X 3078	4624 x 3468	4000 x 1800
5	Width	5472 pixels	5472 pixels	4624 pixels	4000 pixels
6	Height	3078 pixels	3078 pixels	3468 pixels	1800 pixels
7	Horizontal resolution	72 dpi	72 dpi	-	-
8	Vertical resolution	72 dpi	72 dpi	-	-
9	Bit Depth	24	24	-	-
10	Resolution unit	2	2	-	-
11	Color representation	8RGB	8RGB	-	-
12	Compressed bits/pixel	-	3.480083140491 47138	-	-
13	Camera maker	DJI	DJI	-	-
14	Camera Model	FC3411	FC6310	-	-
15	F – stop	f/2.8	f/2.8	-	F1.7
16	Exposure time	1/1600 sec	1/1600	1/3077 s	1/1266 s
17	ISO Speed	ISO-100	ISO-200	ISO-50	ISO-103
18	Exposure bias	-0.3 step	0 step	-	-
19	Focal Length	8 mm	9 mm	5.23 mm	4.71 mm
20	Max aperture	2.97	2.97	-	-
21	Metering mode	Average	Center- Weighted Average	-	-
22	Subject distance	-	0 mm	-	-
23	Flash mode	No flash	No flash function	No flash	No flash
24	Contrast	Normal	Normal	-	-
25	Light Source	Daylight	Fluorescent	-	=
26	Exposure program	Normal	Manual	-	=
27	Saturation	Normal	Normal	-	-
28	Sharpness	Normal	Normal	-	-
29	White balance	Auto	Auto	Auto	Auto
30	Latitude	19:10:36.448999999 9932479	19:13:31.356400 0000042498	-	-
31	Longitude	76:10:55.556999999 9715591	76:21:14.476900 0000086834	-	-
32	Altitude	561.6	380.16	-	-
33	Item type	JPG File	JPG File	JPG File	JPG File
34	Attributes	AP	AP	-	-
35	Computer	LAPTOP- CROBFE07	LAPTOP- CROBFE07	-	-
36	Size	7.13 MB	7.26 MB	6.42 MB	1.92 MB
37	IMEI Number	-	-	584963/85/3485 96/9*	781436259471659*



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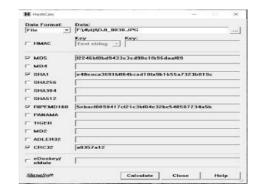


Fig. 1:The image was taken by DJI Phantom 4 pro+ Drone [DJI_0038.JPG]

Fig. 2:MD5 & SHA1 value of DJI_0038.JPG image



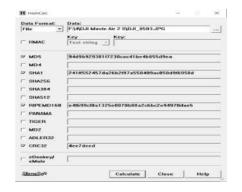


Fig.3: The image was taken by DJI Mavic air 2S Drone [DJI_0503.JPG]

Fig.4: MD5 & SHA1 value of DJI_0503.JPG image





Fig. 5: The image was taken by Samsung Galaxy A53 5G [20220604_144123.jpg]

Fig. 6: MD5 & SHA1 value of 20220604_144123.jpg image





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Fig.7: The image was taken by Oppo F19 pro



Fig.8: MD5 & SHA1 value of 20220605173407.jpg image



Fig.9: DJI Phantom 4 pro+

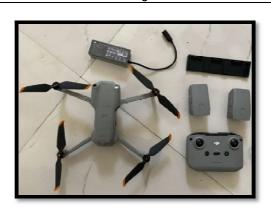


Fig.10: DJI Mavic air 2S



Fig.11: Samsung Galaxy A53 5G



Fig.12: Oppo F19 pro





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RESEARCH ARTICLE

Laboratory Investigations on Durability Assessment of Concrete **Incorporating Copper Slag as Fine Aggregates**

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ABSTRACT

The research study aims at incorporating copper slag in fine aggregate for its utilization in concrete constructions. As the production of slag during the manufacture of copper is high, this leads to major economic and environmental problem. This yearly production leads to the increment in landfills as it produces leachate which includes metals such as Copper, lead, mercury and sulphur dioxide. In this study two sets of concrete cubes were casted with 0% and 40% copper slag incorporation in fine aggregates. Initially mix design was done for Mix grade of concrete 20,25,30,35 and 40. The water cement ratio for all grades of concrete varied between 0.40 to 0.44 and the amount of polyheed is limited to 0.75% to have proper workability of 75-100mm by conducting various trails. Durability properties such as acid attack test, sulphate attack test and water absorption test are conducted. With reference to the X-ray diffraction patterns, the incorporation of slag of copper in place of river sand in concrete increased the peak intensity without affecting the composition of minerals for all the grades of concrete. This work suggests 40% copper slag is an optimum content as a partial replacement to sand for the comparable durability behavior.

Keywords: Concrete, Copper slag, Fine aggregates, Durability properties





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INTRODUCTION

Concrete is widely used in building, bridge, and highway construction and the production of precast concrete elements such as curbs, slabs, pipes, drain channels, etc. One of the commonly used construction material world wide is concrete, this is consumed around 25 x 109 MT / year. [2,3]. In the production of concrete , the major component is found to be the aggregates which occupies about 70% of the volume of the concrete. Annually about 8-12 million tonnes of aggregates is found to be consumed globally (1). There is enormous order for the natural aggregates especially fine aggregate which fills the majority portion of concrete mixture, due to the rapid growth in the metropolitan cities in developed and developing countries. Severe environmental problems like bed erosion, degradation and loss of vegetation are caused due to excavating the natural aggregates from the river bed (2, 4). Since there is tremendous growth in industries the available resources cannot fulfill its demand. Due to instant growth in the industrialization quantities of waste materials or by-products like blast furnace slag, fly ash, silica fume, aggregates from Demolition sites, solid waste, plastic Domestic waste and rubber commercial waste are produced in large quantities.(5,6). These wastes generated during the production of industrial products present severe issues and challenges worldwide in their disposals (7). The proper utilization of waste materials (slags) produced by the industries must be effectively used in order to protect the environment. This effective use of waste materials reduces the use of energy and raw natural materials (8,9). Therefore, there is an urgent need to find and utilize alternative material for aggregates by utilizing the waste materials and by-products with little or no property modification which leads to a sustainable and greener environment along with the technical advantages.(12).

One solution would be the use of copper slag aggregates as a substitute for natural aggregate in concrete mixture (10). For the production of 1 ton of copper around 2-2.2 tonnes of copper slag is generated as a waste slag and almost 109 tonnes per annum of copper slag is generated and this is disposed in landfills(11). Earlier studies reveals that the incorporation of copper slag in the river sand by varying the proportions of the slag finds possible implementation in concrete for marine application. In this study the strength of compression, strength of flexure was found to be increased similarly reduction in chloride ion penetration and sorptivity was found to be decreased. A very wide range of marine applications was found in the concrete structures based on the results of durability studies such as rapid chloride penetration test and sorptivity test (13-15). Bleeding and segregation is observed in concrete containing more than 40% copper slag as fine aggregate replacement (16). Many works revealed that the strengths of compression and tension of concrete samples made by incorporation of copper slag in fine and coarse aggregates shows better increment than that of concrete incorporated with 0% copper slag (17).

MATERIALS AND METHODS

Locally available materials i.e., cement, river sand and coarse aggregates are procured. The properties of each and every materials for the concrete mixture were characterized and is tabulated below. For this study the copper slag is made available from Sterlite Industries limited, Tuticorin, Tamil Nadu. Various study on copper slag like physical IS2386:1963 (Part-3) (22), chemical and mineralogical properties are tabulated in Table 1 & 2. Sieve analysis for both copper slag and river sand is conducted as per IS 383:1970 [9-10], [19] and is shown in the figure 1. The specific gravity of copper slag is found to be 3.54 and fineness modulus is found to be 3.95. The specific gravity of river sand is found to be 2.57 and fineness modulus is found to be 3.08. In the present study the mix proportioning of five grades of concrete i.e., M20, M25, M30, M35 and M40 are done in accordance with IS 10262.2009. Polyheed is the super plasticizer used in this study. 0.75% is the dosage of the super plasticizer fixed for all the grades of concrete to get the slump of 75-100mm. The water cement ratio is fixed for all the grades based on several trial and error method among 0.4, 0.42 and 0.44. Slump test is done on fresh concrete for all the grades of concrete to find the ease with which on can work with concrete.





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Water absorption test

The water absorption of concrete is determined by casting three cubes for each grade of concrete i.e., from M 20 to M 40 for both normal and copper slag concrete. After demoulding, initial weight of cubes are taken and then immersed in clean water for period of sixty days. After 60 days, the cubes are removed and kept for in oven. The final weight of cubes is noted down

Acid attack test

In the resistance of concrete to acid attack one cube each of various grades i.e., M20, M25, M30, M35 & M40 are casted. After demoulding, initial weight of cubes are taken and then immersed in solution for 60 days. The solution is prepared by mixing 5% Sulphuric acidv (H₂SO₄) by weight of clean water. After 60 days, the cubes are taken out and keptg for dry for 4 to 5 hours. The final weight of cubes is noted down. The cubes are tested in compression testing machine.

Sulphate attack test

The sulphate attack on concrete is where the sulphate ions attack the ingredients of cement paste. The sulphates in the form of sodium, potassium, calcium and magnesium, which are present in water or soil causes sulphate attack. The sulphates react with calcium hydroxide present in concrete and form calcium sulphate (gypsum). Then the calcium sulphate reacts with tricalcium aluminates (C₃A) to form ettringite, which occupies more volume leading to disruption of concrete. For this test, one cube each of various grades viz, 20, 25,30, 35, 40 (0% CS and 40% CS in concrete) are casted. After demoulding, initial weight of cubes are taken and then immersed in solution for 60 days. The solution is prepared by mixing 5% Sodium Sulphate (Na₂SO₄) by weight of clean water. The cubes are under the process of wetting and drying. After 60 days, the cubes are taken out and kept for dry for 4 to 5 hours. The final weight of cubes is noted down. The cubes are tested in compression testing machine. Before testing the cubes, the non-destructive tests are done i.e. UPV and Rebound Hammer tests.

X-Ray diffraction

This test gives the qualitative mineral analysis of the specimens.XRD test is widely used to identify the crystalline structure of materials. The finer powdered sample of all the grades of concrete incorporated with copper slag in fine aggregates and normal concrete is used in XRD analysis. The graph is drawn between 2Θ and Counts, thus the qualitative and quantitative measures can be done by using this analysis. This test is performed in SSCU Department, IISc Bangalore.

Scanning electron microscopy (SEM)

This instrument gives the morphological structure of the specimens for various magnification. The powdered sample of concrete specimens with and without copper slag are used for SEM analysis. Also the only coppers slag sample is also tested. The image of concrete sample with and without copper slag for different grades are taken for 100 and 250 magnifications to identify the texture as well as the shape of particles. This test is performed in SSCU Department, IISc Bangalore.

RESULTS AND DISCUSSIONS

Water absorption test

From table 6, the water absorption of concrete is determined by casting three cubes for each grade of concrete i.e., from M 20 to M 40 for both normal and copper slag concrete. After demoulding, initial weight of cubes are taken and then immersed in clean water for a period of sixty days. After 60 days, the concrete cubes are removed out and kept for dry in oven. The final weight of cubes is noted down. From the results, it can be concluded that the percentage of water absorption of copper slag concrete specimen is lower than that of the nominal concrete. Hence the copper slag concrete is less susceptible to chemical attack. The main derivative is that the incorporation of copper slag in sand reduces the pores in concrete [24].





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Acid attack test

From figure 4, the strength of compression for M20 grade of normal concrete and 40% incorporated concrete cubes immersed in acid solution is found to be 12.44N/mm² and 16.88N/mm² and the strength of compression of the same concrete cubes immersed in clean water is found to be 27.47 N/mm² and 39.44N/mm². The strength of compression for M25 grade of normal concrete and 40% incorporated concrete cubes immersed in acid solution is found to be 17.77N/mm² and 21.33N/mm² and the strength of compression of the same cubes immersed in clean water is found to be 30.82N/mm² and 42.84N/mm². The strength of compression for M30 grade of normal concrete and 40% incorporated concrete cubes immersed in acid solution is found to be 20.96N/mm² and 22.89N/mm² and the strength of compression for the same cubes immersed in clean water is found to be 37.92 N/mm² and 46.72N/mm². The strength of compression for M35 grade of normal concrete and 40% incorporated concrete cubes immersed in acid solution is found to be 24.52 N/mm² and 29.33N/mm² and the strength of compression for the same cubes immersed in clean water is found to be 40.20 N/mm² and 50.65N/mm². The strength of compression for M40 grade of normal concrete and 40% incorporated concrete cubes immersed in acid solution is found to be 29.63N/mm² and 32.44N/mm² and the compressive strength of the same cubes immersed in clean water is found to be 44.22N/mm² and 52.18N/mm². From figure 5, there is a clear indication that for the cubes immersed in the sulphuric acid solution for 60 days, the reduction in the strength of compression for the concrete incorporated with 40% is on greater side in comparison with the concrete incorporated with 40% copper slag in river sand. Overall there is a drastic reduction in the strength of compression for normal concrete and concrete incorporated with 40% copper slag in river sand which is immersed in the acid solution for 60 days in comparison with the concrete cubes (Both 40% CS and 0% CS) immersed in normal water for 56 days. The % of weight loss in normal concrete is lower than the concrete incorporated with slag of copper. The percentage reduction in the strength of concrete is on the lesser side % for 0% copper slag concrete cubes in comparison with 40% copper slag incorporated in river sand concrete. As the acid moved inside the concrete cubes, it hindered the hydration reaction which resulted in less compressive strength. [25].

Sulphate attack test

From Figure 5, the strength of compression for M20 grade of normal concrete and 40% incorporated concrete cubes immersed in sodium sulphate solution is found to be 11.26N/mm² and 17.33N/mm² and the strength of compression of the same cubes immersed in clean water is found to be 27.47 N/mm² and 39.44N/mm². The strength of compression for M25 grade of normal concrete and 40% incorporated concrete cubes immersed in sodium sulphate solution is found to be 14.48N/mm² and 20.88 N/mm² and the compressive strength of the same cubes immersed in clean water is found to be 30.82N/mm² and 42.84N/mm². The strength of compression for M30 grade of normal concrete and 40% incorporated concrete cubes immersed in sodium sulphate solution is found to be 18.23N/mm² and 20.9N/mm² and the strength of compression of the same cubes immersed in clean water is found to be 37.92 N/mm² and 46.72N/mm². The strength of compression for M35 grade of normal concrete and 40% incorporated concrete cubes immersed in sodium sulphate solution is found to be 21.3 N/mm² and 28.44N/mm² and the strength of compression of the same cubes immersed in clean water is found to be 40.20 N/mm² and 50.65N/mm². The strength of compression for M40 grade of normal concrete and 40% incorporated concrete cubes immersed in sodium sulphate solution is found to be 26.97N/mm² and 32.44N/mm² and the strength of compression of the same cubes immersed in clean water is found to be 44.22N/mm² and 52.18N/mm². The outcome of the selected slag incorporated in river sand concrete immersed in sulphate solution under the process of alternate wetting and drying presented in table 15 and 16 The addition of copper slag for the replacement of sand shows higher resistance against sulphate attack than normal concrete cubes with 0% CS in concrete.

XRD

From Figure 6 it is noticed that the X-ray diffraction which is known as the mineralogical analysis was performed using MATCH software is performed for slag of concrete and all the grades of concrete incorporated with 40 percent copper slag . the results of this qualitative analysis revealed that the slag of concrete consists of 21% silicon dioxide, 20% of Calcium oxide, 23% of Fe₂O₃, 14% Magnesium oxide, 13.7% of As₂Cd₃, which is considered as a minacious material. It is noticed that the M20 grade of concrete with 40 percent incorporation in copper slag consists of 34.5% silicon dioxide, 2.7% Magnesium oxide, 49.7% Cu₂Fe₂, 9% CaoH and 4.8% of Al. It is noticed that the M25 grade of





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concrete with 40 percent incorporation in copper slag consists 29.9% silicon dioxide, 8% Magnesium oxide, 10% Cu_2Fe_2 , 12% CaoH and 38.5% of Al. It is observed that M30 grade of concrete with 40 percent incorporation in copper slag consists 35.6% Silicon dioxide, 10% Magnesium oxide, 14.8% Cu_2Fe_2 , 11% CaoH and 18% of Al. It is observed that the M35 grade of concrete with 40 percent incorporation in copper slag consists 30.4% Silicon dioxide, 2.1% Magnesium oxide, 49.8% Cu_2Fe_2 , 11.4% CaOH and 3.3% of Al. It is noticed that the M40 grade of concrete with 40 percent incorporation in copper slag consists 39.50% Silicon dioxide, 19.4% Magnesium Palladium, 18.5% Cu_2Fe_2 , 4.6% CaoH and 19% of Al.

SEM

It is noticed from figure 7, that the particle shape is uneven for the sample of copper slag. And also its glassy texture provides good resistance to water absorption this important property enhances workability of concrete in comparison with the incorporation of 0% copper slag in concrete. From the figure of SEM shown it is noticed that the texture of copper slag incorporated in river sand concrete is more dense for all the grades of concrete cubes. Hence the strength of concrete cubes is enhanced due to this important property. From the figure of SEM it is also observed that the calcium silicate hydrate gel is surrounded around the concrete mixture. Copper slag and river sand cannot be differentiated from the concrete mixture which indicates that the copper slag blends with the river sand. The mixture of the slag used in this study and the fine aggregates in concrete cannot be isolated from the pictures of scanning electron microscope for all the sets of concrete; this indicates that the used slag in this study blends finely with river sand.

CONCLUSIONS

- 1. The water absorption test indicates that incorporation of copper slag reduces the water absorption in concrete. Thus, as water absorption is lesser in concrete it is more resistance to chemical attacks.
- 2. The results of acid attack shows that the addition of copper slag in concrete has similar resistance to acid attack as that of nominal concrete.
- 3. The results of sulphate attack shows that the addition of copper slag in concrete has better resistance to sulphate attack than the concrete with no copper slag.
- 4. From the Scanning electron microscope there is clear indication that the shape of particle is uneven. And also its glassy touch provides good resistance to water absorption this important property enhances workability of concrete in comparison with the incorporation of 0% copper slag in concrete.
- 5. Copper slag and river sand cannot be differentiated from the concrete mixture which indicates that the copper slag blends with the river sand. The mixture of the slag used in this study and the fine aggregates in concrete cannot be isolated from the pictures of scanning electron microscope for all the sets of concrete, this indicates that the used slag in this study blends finely with river sand.

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Table 1: Physical Properties of Copper slag

rable:1: 1 Hysicar 1 repetities of copper stag		
Physical Properties	Values	
Specific Gravity	3.59	
Water Absorptions [%]	0.34	
Fineness Modulus	5.89	

Table.2: Chemical Properties of Copper Slagx

Chemical Properties	Percentage [%]
Al as Aluminum trioxide	3.63
Si as Silicon dioxide	28.56
Cu as Copper oxide	0.79
Fe as ferrous oxide	58.67





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Table 3: Physical Properties of riversandx

Parameter	Value
Specific Gravity	2.51
Water Absorption [%]	0.59
Fineness Modulus	4.01

Table 4: Physical Properties of Coarse Aggregatesx

	33 3
Parameter	Value
Specific gravity	2.97
Water Absorption [%]	0.23
Fineness Modulus	3.92
Crushing Value [%]	19.23
Abrasion Value [%]	22.68
Impact Value [%]	15.98

Table 5: Slump Test results

Grade of Concrete	Percentage of copper slag Replacement [%] in river sand	Slumpir[mm]
M20	0	76
	40	88
M25	0	72
	40	82
M30	0	70
	40	80
M35	0	70
	40	78
M40	0	68
	40	73

Table.6: Water Absorption Test Results

Grade of Concrete	Saturated Weight of cubes (g)	Weight of Oven dried cubes (g)	Result of water absorption at 60 days (%)
M20+0%CS	8236.5	8040.5	2.38
M20+40%CS	8432.6	8251.3	2.15
M25+0%CS	8347.3	8169.5	2.13
M25+40%CS	8493.2	8292.3	1.74
M30+0%CS	8380.5	8200.8	2.14
M30+40%CS	8499.5	8356.8	1.67
M35+0%CS	8477.5	8336.7	1.66
M35+40%CS	8568.3	8471.5	1.13
M40+0%CS	8365.2	8238.1	1.52
M40+40%CS	8482.3	8400.2	0.97

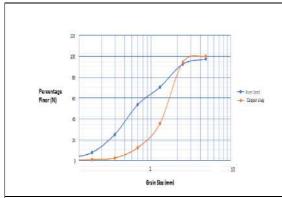




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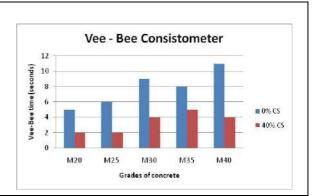
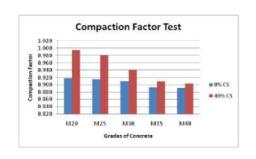


Figure.1: Sieve analysis curve of Copper Slag and River Sand

Figure.2 : Vee – Bee Consistometer



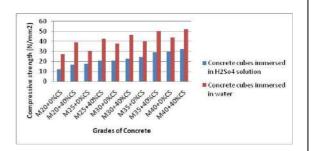


Figure.3: Compaction Factor Test

Figure.4: Graphical Representation of Acid Attack test.

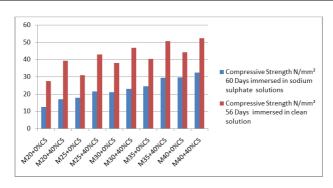


Figure.5: Graphical Representation of Sulphate Attack test.





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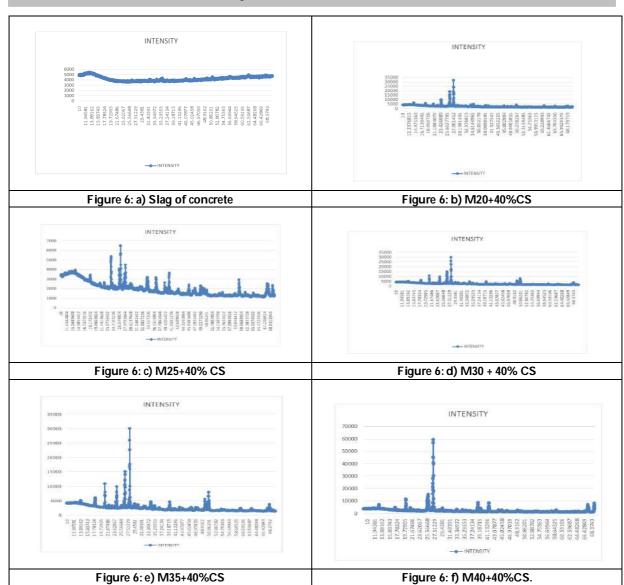




Figure 6: XRD images

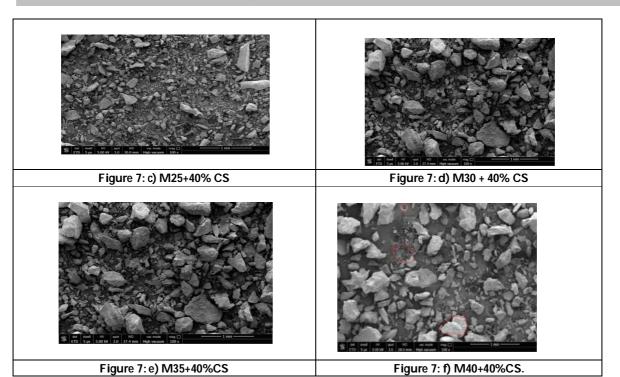




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RESEARCH ARTICLE

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Exploring the Species Diversity and Phytosociological Studies on Karankadu Mangrove Forest, Ramanathapuram District, Tamil Nadu

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ABSTRACT

The Karankadu Mangrove Forest in Ramanathapuram, Tamil Nadu, was the focus of this research, emphasizing the variety of true mangroves and their associated plants. The Karankadu mangrove forest's ethnobotanical investigation is utilized to alleviate a variety of infections, both pathogenic and nonpathogenic. The meteorological data aid in understanding and predicting organism behaviour or distribution. Phytosociology aims to achieve a sufficient empirical vegetation model using combinations of plant species that characterize discrete vegetation units. Field surveys are made to explore the mangrove diversity in Karankadu. The distribution pattern of mangroves in Karankadu Mangrove Forest was studied by quadrant analysis and standard phytosociological methods. In this attempt, climatic data were procured from the Ambient Air quality monitoring station Ramanathapuram, Tamil Nadu State Pollution Control Board. In the study area, plant diversity exhibited about nine halophytic species including five true mangroves Avicennia marina, Ceriops tagal, Rhizophora apiculata, Rhizophora mucronata and Bruguiera cylindrica and the ethnobotanical uses of the halophytic species are identified. The procured climate variables include an average annual temperature, annual rainfall, annual wind speed, annual relative humidity (RH), and Barometric Pressure (mbar) for one year (2021 - 2022) for assumption on rainfall inducing floristic richness. This study provides a pathway for biodiversity conservation and ecosystem restoration in mangrove areas. Rainfall data shows that rain induces the richness of the mangrove species. Creating proper awareness among the public is the most crucial aspect of the conservation of mangroves in Karankadu.





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Abbreviations

CT - Ceriops tagal

BC - Bruguiera cylindrica

RA – Rizhophora apiculate

RM - Rhizophora mucronata

AM - Avicennia marina

Keywords: Karankadu, Species diversity, Phytosociological analysis, Diversity indices and Rainfall Data.

INTRODUCTION

In tropical and subtropical latitudes, mangrove forests are the only forest occurring at the rendezvous point of land and sea, rendering them one of the largest abundant ecosystems on earth. Indian mangroves cover about 4827 Km2, 57% along the east coast, 23% along the west coast and the remaining 20% in the Andaman and Nicobar Islands. Thirty-nine mangrove species are known from India [1]. As the abode of rich biodiversity, their role in the sustainability of seafood species and shoreline stability, economic standing and the survival of selected communities and the context of the predicted scenarios of global warming and sea level rise, conservation of mangrove vegetation is vital [2]. The positive relations between temperature and species richness (Biodiversity) were widely documented. The impact of biodiversity on ecosystem productivity and relationships between Phosphorus and the richness of species in mangrove forests were previously documented [3]. A systematic review and meta-analysis on the interactions between climate and effects of habitat loss on biodiversity and the impact of nutrient enrichment on the loss of biodiversity and consequent declines in ecosystem productivity are only a few examples from the vast mass of documentation about the ecological impacts on Biodiversity traits in aquatic habitats [4]. The strong correlations between latitude and elevation and biodiversity reduction on a global scale have been well-documented. The most critical matter is how climate change will influence biodiversity in the future. Data on rainfall and results from phytosociological investigations were anticipated for this study's evaluation. The Shannon and Wiener Alpha Index of Biodiversity's (BDI) periodic fluctuations' correlations were examined.

The amount of green in mangrove forests, which is connected with tree height, is related to the health of the mangroves. The surrounding water stress is also correlated with the greenness of mangroves. Mangrove grows better in moist soil than in dry dirt. Therefore, it is crucial to anticipate rainfall data since this soil moisture may occur through rain, river flooding, and tidal inundation [5]. The dry and undeveloped Karankadu mangrove forest is located in Tamil Nadu's Ramanathapuram district and is a part of the Gulf of Mannar Marine National Park. The Karankadu Mangrove swamp ranges from 9°36' N latitude and 78°83'E longitude in the Palk Bay region on the southeast coast of India. The marine habitat is also enriched with exceptional marine life forms[6]. The conservation of mangroves is highly essential. Priority should be given to biodiversity conservation and ecosystem restoration in mangrove areas. The preservation of mangroves in Karankadu depends heavily on raising public awareness of the issue. An ecotourism programme sponsored by the Tamil Nadu government in the region promotes preserving the mangrove ecology. The Karankadu Community-Based Eco-tourism was created due to the entertainment activities the Tamil Nadu Forest Department conducted with rural residents [7]. Mangrove plants here are highly medicinal, and this mangrove ecosystem is one of the best places for biodiversity hotspots. Karankadu Mangroves resembles a complex coastal ecosystem and has been declared a protected area primarily because of its affluent but fragile mangrove vegetation [8]. However, currently, it is under significant threat mainly due to anthropogenic interventions such as illegal felling and destruction of mangroves and pollution. The unavailability of scientific data on species composition and vegetation structure of mangroves in the Karankadu Mangrove Forest has made it more difficult to implement conservation strategies to protect this ecosystem. Therefore, this study was mainly focused on bridging that gap.





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MATERIALS AND METHODS

The Study area

Karankadu is located in Ramanathapuram district, Tamil Nadu, which belongs to the Gulf of Mannar region of Tamil Nadu. Field surveys were made to explore the mangrove diversity and medicinal properties of the Karankadu estuary in the Ramanathapuram district. Geographically Karankadu lies between latitude 9°38'58"N and longitude 78°57'38"E.The seasonal variations affect the physiological status of the Karankadu mangroves, particularly during the North East monsoon. Climatic data (2021 - 2022) were procured from the Ambient Air quality monitoring station Ramanathapuram, Tamil Nadu, India, to assess extinction risk under the impacts of climate change. Several studies have used these to assess plant species based on their distribution [9].

Field survey and Identification of specimens

The field visit was conducted from 2021 to 2022. Plants are collected and identified on spot identification and the plants are confirmed through a Botanical Survey of India, Southern Circle, Coimbatore and with Flora of the presidency of Madras [10] and an Excursion Flora of Central Tamil Nadu, India.

Phytosociological Studies

The study was based on species-area estimation and quadrant analysis [11]. The quadrant size was fixed by the species-area curve method, and seven quadrates were selected randomly. The plant species and their individuals occurring in each quadrant were recorded. Using tailoring tape, the basal area of several mangrove species was measured at 1.37 meters above the lowest prop root. Measure the approximate height of the tree as well. According to the observations, quantitative traits, including frequency, density, abundance, relative frequency, relative density, relative dominance, and Importance Value Index, were determined (IVI). To determine the relative contribution of each species to the overall stand composition, the critical value index of each species was determined as the sum of relative density, relative frequency, and relative dominance[12]. The vegetation data were analyzed to calculate the diversity indices and species richness, Shannon– Weiner diversity (H'), Simpson index and equitability were measured[13]. Species richness was measured[14]. Frequency, density and abundance were calculated using the following formulae:

Frequency (F)	=	Number of quadrants in which the species present Total number of quadrates studied	
Abundance (Ab)	=	Number of individuals of the species in all quadrants Number of quadrants of occurrence of the species	_
Density (D)	=	Number of individuals of the species in all quadrants The total number of quadrants studied.	

In addition to this relative frequency, relative density and relative dominance were calculated using the following formulae:

		Number of individuals of a species	
Relative density	=	Total number of individuals	x100
		Total basal area of a species	
Relative dominance	=	Basal area of all species	x100
		Frequency of a species	
Relative frequency	=	<u> </u>	X100
		Sum frequency of all species	

Sum frequency of all species

Importance value Index (IVI) = Relative Density + Relative Dominance + Relative Frequency.





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Diversity indices

A biodiversity Calculator for Simpson and Shannon Indexes was used. The BDI used in this study that was selected from Young's manipulation is Alpha Biodiversity [α], known as the "Shannon Index" (short version) or "Shannon-Wiener Biodiversity Index" (full version) (BDI) [15-16].

RESULTS

Floristic Composition of True Mangroves and Mangrove associates at Karankadu Mangrove Forest

The present study was conducted in Karankadu Mangrove Forest, Ramanathapuram District, Tamil Nadu. In this attempt, the preliminary survey of the true mangrove and mangrove associate plants was documented. This study also provides a pathway for properly utilizing and conserving this beautiful gift of nature. The occurrence of five species of true mangroves representing four genera and two families was recorded during the present study (Table 1). The floristic study revealed the presence of five true mangrove species, namely *Avicennia marina* (AM), *Ceriops tagal* (CT), *Bruguiera cylindrica* (BC), *Rhizophora apiculata* (RA) and *Rhizophora mucronata* (RM) belonging to the families Acanthaceae and Rhizophoraceae. The species of *Avicennia marina* was found to be dominant and denser at Karankadu. Mangrove associates: four species of mangrove associates belonging to 4 genera and four families were recorded along the inundated and adjacent regions in the study area (Table 1). Species of *Sesuvium portulacastrum* L, *Azimatetracantha* Lam, *Suaeda maritima* (L.) Dumort and *Salicornia brachiata* Miqwere recorded abundantly at Karankadu.

Medicinal Uses of True Mangroves and Mangrove associates at Karankadu Mangrove Forest.

Ethno botany deals with studying the natural and traditional interrelationships between man and plants. An ethnobotanical study was conducted among the traditional healers and the plants used were collected and identified. Without proper medicinal facilities, the local populace is dependent on these traditional healers for immediate treatment. A floristic survey was undertaken and nine ethnomedicinal plant species belonging to 6 families were recorded from the karankadu mangrove forest. the presence of five true mangrove species, namely *Avicennia marina* (AM), *Ceriops tagal* (CT), *Bruguiera cylindrica* (BC), *Rhizophora apiculata* (RA) and *Rhizophora mucronata* (RM) and the four mangrove associates species Species of *Sesuvium portulacastrum* L, *Azimatetracantha* Lam, *Suaeda maritima* (L.) Dumort and *Salicornia brachiata* Miqwere recorded abundantly at Karankadu. A list of plant species, the plant part/s used and their mode of application reported to be efficacious for different ailments is provided in table 2. The traditional treatment used through mangrove plants has a popular method practiced in the community's social life. The authors suggest that further investigations be undertaken on the pharmacological properties and level of toxicity of potion made using mangrove plant species. This will lead to stating scientific information on the safety of consuming traditional medicines.

Meteorological Data of the Study Area

Seasonal growth patterns are related to the climate variables, including the average annual temperature, rainfall, wind speed, annual relative humidity (RH), and Barometric Pressure (mbar). The growth of mangrove trees in karankadu is affected by seasonal changes that are minimal or high as continuous throughout the year. The monthwise data collection of rainfall was noticed in table 3 and the overall average values of rainfall data were mentioned graphically in figure 4. Hence, the data results in the overall growth of mangroves were sometimes related positively to quarterly rainfall depths; seasonal diameter growth patterns were not distinctive. A reduced chance of moisture-related stress in high-rainfall, wetland environments may serve to buffer the growth of Karankadu mangroves from climatic extremes.

Phytosociological parameters of mangroves in Karankadu Mangrove Forest

Karankadu mangrove forest resembles a complex coastal ecosystem and has been declared a protected area primarily because of its affluent but fragile mangrove vegetation. However, currently, it is under significant threat mainly due to anthropogenic interventions such as illegal felling and destruction of mangroves and pollution. The





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unavailability of scientific data on species composition and vegetation structure of mangroves in Karankadu has made it more difficult to implement conservation strategies to protect this ecosystem. Therefore, this study was mainly focused on bridging that gap. Seven mangrove representative sites were selected, and the quadrat method was employed to acquire data on the species composition and regeneration potential of mangroves in each site. The floristic study revealed the presence of five true mangrove species, namely *Avicennia marina*(AM), *Ceriops tagal*(CT), *Bruguiera cylindrica*(BC), *Rhizophora apiculata*(RA) and *Rhizophora mucronata*(RM). AM was recorded as the most abundant species (64.41%) and followed by CT (19.7%), RM (9.44%), RA (7.1%) and BC (2.36). Abundances of AM, CT, RM, RA and BC were estimated as 11.14, 8.3, 6, 4.5, and 3, respectively. Stand densities of mangroves were recorded as CT-3.57, BC-0.43, RA-1.28, RM1.71, and AM-11.14, respectively. Further analysis of Frequency Density, Abundance, Relative frequency, density dominance, Importance value index (IVI), and Whit ford's Index (WI) was tabulated in Table 4.

Diversity indices of True Mangroves in Karankadu

The distribution exhibited an exponential distribution through species richness, suggesting that Karankadu is an uneven-aged forest with continuous natural regeneration. Therefore, the protection and restoration of such species must receive the highest priority in mangrove management and conservation efforts. A similarity check between species diversity was done based on the various diversity index formulas for true mangroves in Karankadu. Routledge beta-R Index showed a tremendous value of 1.67 and the lowest value was observed in the Simpsons index (D). The value 0.695 showed by the equability of the species reveals the equal distribution of species in every quadrant. Consideration of the calculated results was as follows (fig-5a d & 5b): the higher the BDI value is, the more the community is diverted, i.e. the richness of a specimen is higher and/or species are closest to balanced densities status. The index is the highest and entirely richness-dependant if all specimen densities are similar. In other words, as high as the BDI value is, the community richness is higher and specimens are balanced. This is the most typical way to study and measure biodiversity. These indices are calculated with all data provided to the calculator as a single sample. These diversity indices are used to compare the sample regions for "similarity" and other correlations of biodiversity between different quadrants.

DISCUSSION

In the research region, mangroves have been successfully planted, and a wide variety of mangroves are present. Arunprasath and Gomathinayagam's[17] study, in contrast, examined the natural mangrove vegetation and its variety in significant mangrove regions in South India. Twenty-five species, comprising 12 mangroves and 13 related mangrove plants, were identified throughout the research. The Pichavaram mangrove forest was discovered to include *Avicennia* and *Rhizophora* as the main species. Six prominent companion species were discovered, including *Sesuvium portulacastrum, Ipomoea pes-caprae*, and *Suaedamaritima*. According to Kumaravel, & Ranganathan [18], *Xylocarpus mekongenisis* is an endangered species. The variation in the mangrove floristics of Kerala in earlier studies could be attributed to the uncertainty in the classification schemes of mangroves. As per the recent review on mangrove floristics, in India based on the classification of Polidoro[19], a total of 19 species belonging to 12 genera and eight families have been recognized as true mangroves species in Kerala[20], A floristic survey revealed three *Acanthus* species in the Andaman and Nicobar Islands mangroves, India.

Acanthus ilicifolius and A. ebracteatus are shrubs, whereas A. volubilis is a climbing shrub. Only A. ilicifolius was found in the Nicobar Islands, but all three Acanthus species were found in the Andaman Islands. The IUCN Red Data List rates all three Acanthus species as the least concern[21]. On the other hand, we assessed 14 plants in the Karankadu mangrove forest, which are regarded as the least concerning (see table 1-3). Sixty-two plant species, 33 families, and 47 genera were counted. Malvaceae (eight), Acanthaceae (six), Fabaceae (four), Rhizophoraceae (four), Lamiaceae (four), and Caesalpiniaceae are the dominant families in the research (3). Mangrove habitats offer a unique and pricey range of materials, services, and goods, yet they have long been a resource that is underappreciated. These plants require immediate consideration for carrying out detailed chemical and pharmacological evaluations. Such investigations





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can lead to the finding of bioactive compounds to facilitate determination and assist in considering the efficiency of herbal remedies [22]. The most common pharmacological activities reported were antioxidant, antimicrobial, anticancer, anti-inflammatory and anti-diabetic properties. Mangroves are traditionally reported to treat ulcers, skin diseases, eye complaints and snake bites and are used to treat other common issues like fever, cough, headache, stomach ache, etc [23-24].

Ratheesh [25] implemented the Shannon-Wiener Biodiversity Index for Zooplankton Diversity (BDI) in Lake Kinneret (1970-2001). It was computed based on monthly mean densities concerning Epilimnetic temperatures Matching was found between low BDI and the high summer temperature and between higher BDI and the low winter temperature. The similar correspondence did not indicate annual means of BDI values. Surya & Hari [26] tested the potential role of the mangrove ecosystem as sinks for anthropogenic contaminants in tropical and subtropical areas has been widely recognized. India has a total area of 4461 sq. km of mangroves. It is 0.14% of the country's total geographical area. The higher population density on the Kerala coast has resulted from tremendous pressure on the natural ecosystem, partially on the mangroves. A study was conducted on the diversity and structural analysis of the mangroves in ten districts of Kerala state. A total of 15 true mangrove species belonging to 9 genera and seven families were enumerated. The true mangrove species are confined to the salty-marshy environment along backwaters. The study results consider the ten districts of Kerala; the diversity is relatively high in Kollam and Kasargod compared to other districts. The continuous anthropogenic activities threaten the survival of mangroves; this was predicted from the work of Sreeja & Khaleel [27]. The study by Gophen [28], resulted from the dominance of Sonneratia caseolaris L, distributed in all quadrates studied, followed by Rhizophora apiculata. The mangrove species Avicennia officinalis recorded the lowest density. Maximum relative basal area was represented by Sonneratia caseolaris followed by Rhizophora apiculata. Hence these species registered the highest Importance Value Index.

CONCLUSIONS AND FUTURE PERSPECTIVES

In conclusion, from the present study, mangroves have massive potential for many medicinal products and drug discovery to prevent and treat many diseases. There is a dire need for careful investigations substantiated with objective scientific and clinical evidence to ensure these plants' safe and efficient use and validate their pharmacological properties and toxicity. The Margalef index was found to be the best index for species diversity measurement in the mangrove community rather than Shannon, Simpson and Menhinick indexes. The index showed a low correlation with sample variance and normality markers as the identity of non-parametric statistics. Thus, we recommend the Margalef index for measuring the species diversity of the mangrove community. It can be used to monitor and evaluate the biodiversity assessment for the mangrove community. As a future prospect, we consider that physiochemical properties and biochemical analysis on seasonal variations can predict the impact of rainfall on plant growth which may reveal that Karankadu mangroves may or may not become the victims of climate change-inducing habitat loss.

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Table 1: Floristic Composition of True Mangroves and Mangrove associates at Karankadu Mangrove Forest

S. No	Name of the Species	Family	Habit	Common Name	Local Name
1	Ceriops tagal (Perk.) C.B. Rob.	Rhizophoraceae	Tree	Yellow Mangrove	Panrikuththi
2	Bruguiera cylindrica (L.) Blume	Rhizophoraceae	Tree	Small Leaved Orange	Kaa Kandal
				Mangrove	
3	Rhizophora apiculata Blume.	Rhizophoraceae	Tree	Tall-Stilt Mangrove	Cirugandal
4	Rhizophora mucronata Lamk.	Rhizophoraceae	Tree	Long Fruited Stilted Mangrove	Pey-Kandal
5	Avicennia marina (Forssk.)	Avicenniaceae	Small	Grey Mangrove	Kanna
	Vierh		Tree		
6	Sesuvium portulacastrumL.	Aizoaceae	Herb	Sea Purslane	Vankaravacci
7	AzimatetracanthaLam.	Salvadoraceae	Shrub	Needle Bush	Sugam Cheddi
8	Suaeda maritima (L.) Dumort	Amaranthaceae	Herb	Sea-Blite	NilaVumarai
9	Salicornia brachiataMiq.	Chenopodiaceae	Herb	Salicornia	Kolikalavuri

Table 2. Medicinal Uses of True Mangroves and Mangrove associates

S. No	Name of the Species	Family	Flowering& Fruiting	Medicinal Uses of True Mangroves	
1.	Ceriops tagal (Perk.) C.B. Rob.	Rhizophoraceae	February - July	Decoction of the bark given during childbirth and used as hemostatic.	
2.	Bruguiera cylindrica (L.) Blume.	Rhizophoraceae	December-October	Used for, low blood pressure, bleeding, hemorrhage and ulcers.	
3.	Rhizophora apiculata Blume.	Rhizophoraceae	Throughout the year	They contain antimicrobial, anticancer, antidiabetic, antidiarrhea, antiemetic, and hemostatic properties.	
4.	Rhizophora mucronata Lamk.	Rhizophoraceae	April-October	Used as an astringent and to treat diarrhea, diabetes and dysentery	
5.	Avicennia marina (Forssk.) Vierh	Acanthaceae	March-July	Used to treat snake bites, skin complaints and has antimicrobial activity.	
6.	Sesuvium portulacastrum L.	Aizoaceae	Almost for the whole year. August to September	The plant is used as a hemostatic, antiscorbutic and the best antidote for stings of venomous fish	





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7.	Azima tetracantha Lam.	Salvadoraceae	August to September	Used in the treatment of rheumatism, dropsy, stomach disorders, asthma and coughs caused by phthisis.
8.	Suaeda maritima (L.) Dumort.	Amaranthaceae	September to February	Used as a remedy for liver, heart, lipid disorders and showed antioxidant, anti- inflammatory, and antimicrobial activities.
9.	Salicornia brachiata Miq.	Chenopodiaceae	August-March	The juice of the fresh plant is an excellent diuretic.

Table 3. Meteorological Data of the Study Area

PREMONSOON							
Wind Speed Temperature Relative Barometric							
Month	(m/s)	(°C)	Humidity (%)	Pressure (mbar)	Rain (mm)		
July	1.26	30.55	60.93	1004.3	13		
August	1.23	30.31	60.46	1005.4	19.5		
September	1.19	29.94	59.72	1005.18	33.75		
MONSOON							
October	1.15	28.36	56.58	1006.38	67.75		
November	1.1	27.26	54.38	1006.84	22.5		
December	0.86	27.05	53.96	1010.59	156.5		
POST MONSOON							
January 1 27.14 54.15 1010.28 515							
February	1.02	27.22	54.3	1009.31	67		
March	1.15	29.05	57.95	1007.48	0		
SUMMER							
April	1.09	30.14	60.11	1006.74	901		
May	1.57	31.04	61.91	1003.6	0		
June	1.3	30.83	61.49	1005.35	17.75		

Table 4. Phytosociological parameters of mangroves in Karankadu Mangrove Forest

Name of the Species	Frequency (%)	Density (m²)	Abundance (%)	Relative frequency (%)	Relative density (%)	Relative dominance (%)	Importance value index (IVI)	Whit ford's Index (WI)
Ceriops tagal	42.86	3.57	8.3	20	19.68	27.84	67.52	0.19
Bruguiera cylindrica	14.3	0.43	3	6.6	2.36	9.24	18.2	0.21
Rhizophora apiculata	28.57	1.28	4.5	13.33	7.08	15.25	35.66	0.16
Rhizophora mucronata	28.57	1.71	6	13.33	9.44	16.14	38.91	0.21
Avicennia marina	100	11.14	11.14	46.66	61.41	38.96	147.03	0.11



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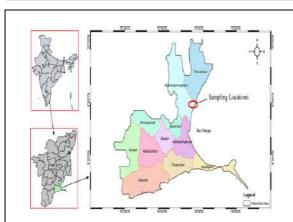
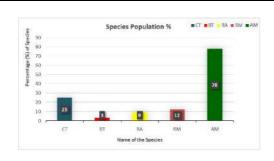




Fig. 1. Map showing the location and the sites selected in the Karankadu Mangrove Forest.



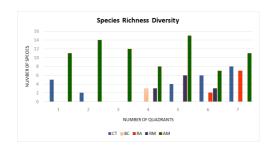


Figure 2. Diversity indices of True Mangroves in Karankadu

Figure 3. Diversity indices of True Mangroves in Karankadu

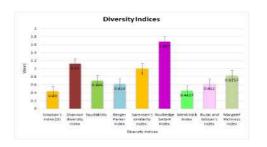


Figure 4. Average annual Rainfall on Karankadu **Mangrove Forest**

Figure 5a. Diversity indices of True Mangroves in Karankadu

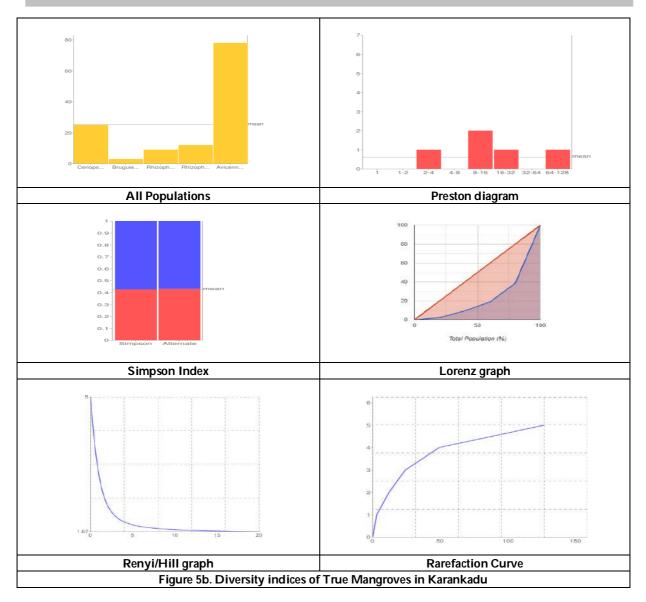




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RESEARCH ARTICLE

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Identification and Isolation of Bioactive Constituents of Hemigraphis colorata Blume for Antidiabetic Activity

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ABSTRACT

The present study was aimed to identify and isolate the bioactive constituents from Hemigraphis colorata Blume for antidiabetic activity. Although there are synthetic drugs available in the market as antidiabetic agents, majority of them having short term or long term side effects. So it is worthwhile to explore a novel herbal compound having almost no side effects. The dried leaves were extracted with hydro alcohol (70% ethanol + 30% water) and pharmacognostical and phytochemical screening were carried out according to standard procedure. The crude hydro- alcoholic extract was subjected to column chromatography for isolation by gradient elution technique. The spectral analysis of isolated compound was carried out by using IR, 1HNMR, 13C NMR, MASS spectrums. The isolated compound was screened for antidiabetic activity against diabetes induced in albino rats by a single IP dose Alloxan monohydrate. Glibenclamide (5 mg/kg) was used as standard drug. The study was carried out on a 14 day protocol and the blood glucose levels were measured on Day 0, Day 7 and Day 14 of the treatment. Dose was selected on the basis of acute oral toxicity study as per OECD guidelines. Biochemical parameters such as SGPT and SGOT were measured according to standard protocols. It was observed that the isolated compound was obtained in the ratio of chloroform: ethanol (75:25) and it has showed the presence of phytoconstituents such as carbohydrate and glycoside, and the glycosides have an aldose reducing sugar part. Acute oral toxicity study found that the isolated compound is safe up to the dose of 2000 mg/kg body weight, and the result also showed that it have better antidiabetic activity with high dose





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(400mg/kg) and moderate activity at low dose (200mg/kg) when compared with standard Glibenclamide. The present study has justified that the isolated compound exhibited significant antidiabetic activity against alloxan induced diabetic rats. As it is a newer isolated compound, it may consider as a new lead moiety for future studies.

Keywords: Hemigraphis colorata Blume, Antidiabetic activity, Glibenclamide, Alloxan

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycaemia (fasting blood glucose level > 126 mg/100 ml (6.1mmol/L)) with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycaemia of diabetes is associated with long term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels that result in significant morbidity and mortality. In India diabetic patients are increasing day by day and according to world diabetic foundation it has the world's largest diabetes population, followed by China with 43.2 million [1]. The plant Hemigraphis colorata Blume (Family: Acanthaceae) is a versatile tropical low creeping perennial herb that reaches a height of 15 to 30 cm[2], which is the native of tropical Malaysia[3]. It is a prostrate growing plant with spreading, rooting stems. Its stainy leaves are slender and lance shaped with toothed, scalloped or lobed margins. They are grayish green stained with red purple above and darker purple beneath [4]. The tiny white flowers grow intermittently throughout the year. Literally, Hemigraphis means 'half writing' because the filament of the outer stamen bear brushes. The plant is known by several name such as Aluminium plant, Cemetery plant, Metal leaf, Red flame Ivy, Waffle plant, Java Ivy etc. In Kerala, the plant is popular in the name 'murikootti' or 'murianpacha' because of its incredible potency to heal wounds. This plant possesses various medicinal properties, only a few are reported like, the whole plant or leaves are used to treat fresh wound, cuts, ulcers, inflammation and in folk medicines[5], it is used internally to cure anaemia, gallstone, diuretic, hemorrhoids, diabetic mellitus[6].

MATERIALS AND METHODS

Collection of plant material

Hemigraphis colorata is widely distributed in western ghat and almost throughout the plain areas in India. Fresh plant was collected in the month of December and authenticated (specimen No.88451) by Dr. M. Pradeep, Assistant Professor and Head, Department of Botany, University of Calicut, Kerala India. The specimen voucher was deposited in the Department of Botany, University of Calicut, itself.

Preparation of solvent extraction

The fresh leaves of *Hemigraphis colorata* Blume was cleaned and dried in the laboratory at room temperature and powdered to get a coarse powder in a mixer grinder. A sample of hydro-alcoholic leaf extract was prepared by continuous hot percolation method for 72 hour by using a Soxhlet apparatus [7]. About 25 grams of plant material packed uniformly into a thimble and extracted with 350 ml of hydro-alcohol (70% ethanol + 30% water). The extraction process was continued till the solvent in siphon tube of the apparatus become colourless. The extracts were concentrated to a dry mass by vacuum distillation. The chlorophyll of the leaf portions are removed by adding diethyl ether by using separating funnel. Again the crude extract was filtered and concentrated under vacuum and controlled temperature. After complete drying, extracted material was weighed and the extractive value in percentage was calculated with reference to the air dried sample[8]. The hydro- alcoholic leaf extracts were subjected to qualitative chemical tests for the detection of various plant constituents like carbohydrates, glycosides, flavonoids, phenolic compounds, tannins, steroids, saponins, coumarins, phytosterols, alkaloids, carbohydrates and triterpenoids [9-11].





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Isolation of the compound by column chromatography

A column of suitable size (1mx1.5inch) was chosen and packed with silica gel 100-200 mesh by adding slurry of the adsorbent in hexane. The hydro-alcoholic leaf extract of *Hemigraphis colorata* Blume was dissolved in hydro-alcohol, and mixed with silica gel 100-200 mesh and fed in to the column through a funnel. Hexane was added to the column and kept aside without disturbance for overnight for the settlement of the extract. Maximum precautions were taken to remove the air bubbles. The column was eluted with different organic solvents in the order of increasing polarity (hexane, petroleum ether, chloroform and ethanol). Fractions showing similar R_f value and identification test, were pooled together and solvents evaporated to get the residues.

Thin layer chromatography

Thin layer chromatography is an analytical method that is widely used for the separation, isolation, identification, and quantification of components in a mixture. The selection of a solvent for application of the sample can be a critical factor in achieving reproducible chromatography with distortion free zones. In general, the application solvent should be a good solvent for the sample and should be as volatile as possible and more non polar[12]. Out of various trials made, the mobile phase, and chloroform: ethyl acetate (5:5) was show better result for extract sample and n-butanol: toluene (5:5) show better result for isolated compound. Silica gel was chosen as stationary phase, since it is an efficient adsorbent for the TLC separation of most of the plant extracts and plant drug extracts[13].

Experimental animals

Albino mice of Swiss strain and Albino rats of Wistar strain were used for pharmacological and toxicological studies. These animals were the purchased stock maintained in the animal house of Devaki Amma Memorial College of Pharmacy, Chelembra, Malappuram district of Kerala, India. Female mice selected were nulliparaus and non pregnant. Female mice weighing 25 to 30 g and rats of either sex weighing 125 to 150 g were used for the study. Each animal, at the commencement of its dosing, was between 8 and 12 weeks old and their weight variation was within ± 20% of the mean weight of any previously dosed animals. The temperature in the experimental animal room was 22°C (± 3°C) and the relative humidity was between 50-60%. These animals were fed with pellet diet manufactured by Amrut laboratory, Animal Feed Company, Sangli, Maharashtra and drinking water ad libitum. They were kept in 12 hr/12 hr light/dark cycle and maintained for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions. The animal experimental protocol has been approved by our Institutional Animal Ethics Committee vide reference no: 1527/PO/a/11/CPCSEA.

Acute oral toxicity study of isolated compound

This was performed to ascertain the safe dose by the acute oral toxic class method by the Organization of Economic Cooperation and Development (OECD) 423 guidelines. *Swiss albino* mice weighing 25-40g were selected and fed with standard feed and drinking water and monitored on a regular basis. The selected animals were grouped as three in one group. The animals were fastened overnight and the test sample of the isolated compound was given orally at a starting dose of 5mg/kg body weight. Animals were observed for a period of 2 hours and occasionally for 4 hours to detect any toxic signs and mortality. Since no mortality was observed, same dose was repeated with another group of animals. The procedure was repeated for doses of 50, 300 and 2000 mg/kg in separate group of animals. From the maximum dose of 2000 mg/kg, 1/10th and 1/5th of the values were taken as treatment dose for further studies. The experiment was repeated for seven more days and also for fourteen days, no change was observed from the experiment [14].

Antidiabetic study

Healthy Wistar strain albino rats were selected and randomly divided into five groups with six animals in each group and assigned as below,

Group A: Vehicle control (normal saline).

Group B: Diabetic control (Alloxan 120mg/kg).

Group C: Diabetic + Glibenclamide (5mg/kg)

Group D: Diabetic + isolated compound of Hemigraphis colorata (low dose)





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Group E: Diabetic + isolated compound of Hemigraphis colorata (high dose)

The above treatment was carried out in each group of animals for 14 days. Blood samples were withdrawn on the 0th day, means the day on which the dosing was started, 7th day and 14th day, through the tail vein of the rats and were analyzed for the blood glucose level, using Glucometer. Blood glucose level was read from digital display of Glucometer with its customized test strips. A drop of blood obtained through tail vein was placed on inserted glucostrips on Glucometer. The method is widely used in clinical practice and appears to be sensitive and accurate. The biochemical parameters were estimated as per the standard procedure prescribed by the manufacture's instruction manual provided in the kit using auto analyser [15-19].

Statistical analysis

Results of biochemical estimation were reported as mean \pm S.E.M. The total variation present in a data was analyzed by one way analysis of variance (ANOVA). P value less than 0.05 was considered as statistically significant.

RESULTS

Table 1: percentage yield obtained from hemigraphis colorata. Table 2: preliminary phytochemical studies on hemigraphis colorata. Table 3: solvents used in column chromatography of hemigraphis colorata. Table 4: tlc study on extract of hemigraphis colorata. Table 5: tlc study on isolated compound of hemigraphis colorata. Table 6: physical examination of isolated compound. Table 7: effect of isolated compound of hemigraphis colorata blume on alloxan-induced diabetic rats. Table 8: sgpt and sgot levels in diabetic rats

DISCUSSION

The plant material was dried, powdered and subjected to continuous hot percolation by using Soxhlet extractor for the preparation of hydro-alcoholic leaf extract. The solvent was evaporated under reduced pressure. The percentage yield of extracts was found to be 14.4%w/w. The phytochemical studies of hydro-alcoholic extract of Hemigraphis colorata Blume showed the presence of phytoconstituents such as carbohydrates, glycosides, amino acids, phytosterols, flavonoids, phenolic alkaloids and terpenoids. Out of the various mobile phase combinations tried, TLC that was developed using chloroform: ethyl acetate (5:5) has shown spots for the extract. The crude hydroalcohol extract (2.0q) was subjected to analytical column chromatography containing silica gel G (100-200 mesh) as stationary phase. The elution of column by gradient elution technique with different mobile phase in the order of increasing polarity led to the isolation of phytoconstituents. After chromatographic separation and TLC studies the fractions showing similar spots were pooled together. Then the solvents were evaporated off. One major fraction was obtained; it was a yellow crystalline compound from chloroform: ethanol (75:25) fraction. The isolated compound was named as HGC, having percentage yield of 3.1%w/w. TLC was developed using toluene: n-butanol (5:5) for isolated compound of Hemigraphis colorata Blume and its R_f value was found to be 0.45. It was identified as a green fluorescent in UV chamber. The phytochemical studies of isolated compound of Hemigraphis colorata Blume has showed the presence of phytoconstituents such as carbohydrate and glycoside. The isolated compound gives positive result for Legal's test, Molisch's test, Fehling's test and negative result for Seliwanhoff's test. So, it reveals that the isolated compound was a glycoside and having an aldose reducing sugar part.

The purified compound was elucidated by means of IR, 1H NMR, ^{13}C NMR and LC-MS spectral studies. There is an intense peak at a region 3440 c.m- 1 , which indicates the presence of a hydroxyl group in the isolated compound. The peak in the region of 1638 c.m- 1 indicate the presence of C-C stretching in ring and 1400 c.m- 1 represent C=O group. From these IR data it is clear that it contains a functional group OH and aromatic ring. The 1H NMR spectra was used to find the number of proton present in a chemical compound. The sample is dissolved in CDCl₃ and value was measured in 5 ppm. 1H NMR spectral analysis of isolated compound showed peak at 6.798-6.8 indicates the $^2H_{(d)}$ of phenyl, peak at 7.111-7.127 indicates the $^3H_{(1)}$ of phenyl, 5.005 indicates the $^4H_{(m)}$ of OH, 5.009-5.865 represent the $^4H_{(m)}$ of phenyl. From these data it was understood that the isolated compound contain more than one phenyl ring. In 13 C





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NMR spectral analysis of the isolated compound shows peaks at 14.094, 18.409, 22.679, 28.956 are responsible for carbon of (CH₃- primary). The peaks in the region of 29.153-29.690 indicate the carbon of (CH₂- secondary). The peak shown at 31.922 indicates the presence of carbon in (CH- tertiary). The carbon present near to oxygen represent in the peaks are 58.472 (CH₂-O) and 114.053 (CH-O). The peaks in the region of 139.279 indicates the presence of carbon of (C=C aromats). ¹³C NMR spectra revealed that there are primary, secondary, tertiary carbons in the isolated compound and it contains one or two carbons which are attached to oxygen atom also. Mass spectra of isolated compound showed the molecular ion peak at 415 and base peak was showed at 66. Exact molecular mass of the compound is 415. From the spectral analysis of IR, ¹HNMR, ¹³C NMR, MASS spectra of the isolated compounds, we can assume that the isolated compound is a newer compound, having molecular mass 415. With more than one phenyl ring and hydroxyl group also present as a functional group.

To assess the short term toxicity of the isolated compound acute oral toxicity study was carried out as per the guidelines no: 423 given by the Organization of Economic Co-operations and Development (OECD), Paris and it revealed that the isolated compound was safe up to the dose level of 2000 mg/kg body weight of animals as no mortality was observed among the animals used. The *in-vivo* antidiabetic activity was performed for the isolated compound against Alloxan-induced Albino Rats. Alloxan was induced at a dose of 120mg/kg body weight intraperitoneal injection. The study was carried out on a 14 day protocol and the blood glucose levels were measured on Day 0, Day 7 and Day 14 of the treatment. Biochemical parameters pertaining to these activities such as SGPT and SGOT were measured according to standard protocols. Glibenclamide was taken as the standard and the results were quite comparable with it. Dose selection was made on the basis of acute oral toxicity study as per OECD and CPCSEA guidelines. The result showed that the isolated compound have better antidiabetic activity with high dose (400mg/kg) and moderate activity at low dose (200mg/kg) when compared with standard Glibenclamide. The present study demonstrated the *in-vivo* antidiabetic activity of isolated compound against Alloxan-induced Albino Rats. However, further research works are required to elucidate their possible mechanism of action.

The isolated bioactive constituent of *Hemigraphis colorata* Blume possesses significant antidiabetic activity on Alloxan-induced Albino Rats. The isolated compound was screened for the presence of hypoglycaemic and antidiabetic activity. In this study diabetes was induced by a single IP dose Alloxan monohydrate. Glibenclamide (5 mg/kg body weight) was taken as the standard and the results were quite comparable with it. Dose selection was made on the basis of acute oral toxicity study as per OECD and CPCSEA guidelines. The result showed that the isolated compound have better antidiabetic activity with high dose (400mg/kg) and moderate activity at low dose (200mg/kg) when compared with standard Glibenclamide. From these results and discussion it was found that in future this newly isolated compound can be considered as a lead molecule in antidiabetic drug discovery process.

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Table.1: Percentage Yield Obtained From Hemigraphis Colorata.

SI. No.	Name	Percentage yield
1.	Hydro-alcohol extract	14.4%w/w
2.	Isolated compound	3.1%w/w

Table 2: Preliminary Phytochemical Studies on Hemigraphis Colorata.

SI. No.	Phytoconstituents	Hydro alcohol extract	Isolated compound
1.	Carbohydrate	+	+
2.	Alkaloids	+	-
3.	Glycosides	+	+
4.	Flavonoids	+	-
5.	Saponins	+	ı
6.	Phenolic	+	ı
7.	Tannins	+	ı
8.	Steroids	_	_
9.	Triterpenoids	_	_





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Table 3: Solvents used In Column Chromatography of Hemigraphis Colorata

Fraction No.	Mobile phase	Phyto chemical test	TLC report	Nature of the residue
1-17	Hexane: Petroleum ether (100:0)	•	-	=
18-32	Hexane: Petroleum ether (75: 25)	-	-	=
33-47	Hexane: Petroleum ether (50: 50)	-	-	=
48-62	Hexane: Petroleum ether (25:75)	-	-	-
63-77	Petroleum ether: CHCl3 (100: 0)	•	-	=
78-92	Petroleum ether: CHCl3 (75: 25)	=	-	=
93-112	Petroleum ether: CHCl3 (50: 50)	-	-	-
113-128	Petroleum ether: CHCl₃ (25:75)	-	-	-
129-143	CHCl ₃ : Ethanol (100:0)	•	-	=
144-161	CHCl ₃ : Ethanol (75: 25)	+	+	Yellow crystal
162-177	CHCl ₃ : Ethanol (50: 50)	=	-	=
178-192	CHCl3: Ethanol (25:75)	-	-	=
193-207	Ethanol: Water (100: 0)	-	-	-

Table.4: TLC Study on Extract of Hemigraphis Colorata

Mobile Phase	Detecting Agents	R _f value
Chloroform: Ethyl acetate (5:5)	lodine chamber	0.68
Chloroform: Methanol (4:1)	lodine chamber	0.48

Table 5: TLC Study on Isolated Compound of Hemigraphis Colorata

SI. No.	Mobile phase	Fraction	R _f value
			0.45
1.	Toluene: n-butanol (5:5)	CHCl3: Ethanol (75:25)	0.44
			0.43
			0.28
2.	Ethyl acetate: methanol (1.3:0.5)	CHCl3: Ethanol (75:25)	0.33
			0.27

Table 6: Physical Examination of Isolated Compound

rable 6.1 mysical Examination of isolated Compound			
Colour	Yellow		
Chemical test	Legal's test, Molisch's test, Fehling's test		
Solubility	Chloroform, Ethanol		
Mobile phase	Toluene: n-butanol (5:5)		
Stationery phase	Silica gel G		
Detection method	UV chamber		
R _f Value	0.45		
Melting point	58°C		
Yield	60 mg		





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Table 7: Effect of Isolated Compound of Hemigraphis Colorata Blume on Alloxan-Induced Diabetic Rats

Group	Drug Treatment	0 th day	7 th day	14 th day
Α	Normal saline	83.38 ± 0.1206	83.38 ± 0.1206	83.55 ± 0.12
В	Alloxan (120mg/kg) (diabetic control)	282.17 ± 0.5545	291.29 ± 0.3402	302.31 ± 0.45
С	Alloxan (120mg/kg) + Glibenclamide (5mg/kg)	287.97 ± 0.3596	59.15 ± 0.4191	114.39 ± 0.37
D	Alloxan (120mg/kg) +Isolated compound of Hemigraphis colorata (200mg/kg)	263.75 ± 0.4513	171.25 ± 0.5634	119.01 ± 0.30
E	Alloxan (120mg/kg) +Isolated compound of Hemigraphis colorata (400mg/kg)	287.95 ± 0.2771	130.8 ± 0.4107	109.72 ± 0.28

Values are mean ± SEM of 6 animals each. It shows that the reduction in fasting blood glucose level by the isolated compound is significant (p<0.001) when compared with diabetic control.

Table 8: SGPT and SGOT Levels in Diabetic Rats

Groups	Drug Treatment	SGOT U/ml	SGPT U/ml
Α	Normal saline	66.47±1.28	74.32±1.45
В	Alloxan (120mg/kg) (diabetic control)	152.41±1.95	160.18±1.65
С	Alloxan (120mg/kg) +Glibenclamide (5mg/kg)	122.7±2.65	118.81±2.85
D	Alloxan (120mg/kg) + Isolated compound of Hemigraphis colorata (200mg/kg)	111.51±2.85	10819 ±2.85
E	Alloxan (120mg/kg) + Isolated compound of Hemigraphis colorata (400mg/kg)	99.18±1.65	97.15±2.25

Values are mean \pm SEM of 6 animals each.





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RESEARCH ARTICLE

Agroforestry as a Sustainable Response to Climate Change and Food Security: Evidences from India

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ABSTRACT

Agroforestry refers to techniques and tools that use woody perennials (shrubs, trees, bamboos, palms etc.) on the same land-management components as agricultural yields in certain kind of longitudinal composition or historical sequence. There are interactions between many components in agroforestry systems on both an environmental and financial level. By incorporating trees into farms and agricultural landscapes, agroforestry is defined as a vibrant environmental system for handling natural resources that differentiates and nurtures farming production for better economic, social, and ecological advantages for land clients at all scales. Smallholder growers and other rural citizensgain most from agroforestry as it improves their access to income, food, and health. Systems for agroforestry are multipurpose systems that offer wide choice of socio economic and ecological advantages. This research is an attempt to understand the agroforestry initiatives which been taken by farmers in tropical country India. India is primarily an agrarian economy and agroforestry techniques are not yet widespread in India. This research helps to understand how these initiatives can bring about a change and how agriculture can be done in a developing agrarian economy.

Keywords: Agroforestry, Riparian buffer, Windbreakers, Silvopasture





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INTRODUCTION

Agriculture has huge impact on the environment. It is linked to around one-third of the world's land usage and is a major factor in land-use shifts everywhere from the biodiverse tropics to the Arctic (Searchinger *et al.*, 2019). About fifteen percent of all worldwide emissions of greenhouse gases are linked to the production of food (Bodirsky *et al.*, 2015). Increase in both population and per capita demand point to a rise in the world's need for food. Several groups are looking towards more environmentally friendly methods of food production as a direct result of these numerous challenges (Foley *et, al.*, 2011). The fundamental and most important aim of agricultural expansion and improvement is to increase food production to meet the needs of all consumers and to increase the income of farm households. Farmers today use dangerously high levels of agrochemicals, pesticides, synthetic fertilizers, and weedicides in their fields, endangering not only human health but also soil fertility and the environment, to produce more food but of worse quality (Kumari *et, al.*,2019). The rising price of agrochemicals and fertilizers and their detrimental effects on the environment highlight the importance of transitioning to more sustainable agricultural practices and environmentally friendly farming methods.

Within the constraints of a given ecosystem and land use, the state of soil's health is defined as its ability to encourage plant and animal output, conserve, or enhance water and air quality, and foster the wellbeing of living organisms. Life in the soil is extremely sensitive to changes in land use and weather. They have a strong positive relationship with soil and ecosystem processes such as water storage, decomposition and nutrient cycling, toxicant detoxification, noxious and pathogenic organism suppression. The relationships between land management decisions and the long-term success of crops and animal populations are best demonstrated by the interdependence of soil organisms. In current years, there has been a resurgence of attention in assessing the state of our soil resources due to the growing realization of soil's crucial role in the biosphere, which includes not only the production of food and fiber but also the preservation of environmental quality on a local, national, and international scale (Glanz, 1995). Both agricultural and natural plant populations rely on healthy soil. Thus, for most land-based life, the thin level of soil protecting the earth's side signifies the distinction between persistence and annihilation (Doran et al., 1998). Today's farms rely largely on contemporary agricultural innovations such as pesticides, synthetic fertilizer, continuous irrigation, genetically modified seed, and chemical weedicides. While its usage in agriculture increases yields, careless application can reduce environmental quality and soil productivity, raising concerns about sustainability (Dar & Bhat, 2020). Soil organic matter (SOM) has decreased because of the widespread use of synthetic fertilizers, which has also led to higher acidity and more pollution in agricultural soils (Dinesh et, al., 2010). This is becoming a really serious concern. According to Project Drawdown (2020), "regenerative agriculture improves and sustains soil health by renovating carbon content, that in turn expands productivity—exactly the reverse of conservative agriculture" and that this could lead to a reduction or sequestration of 14.5-22 gigatons of CO2 by 2050. More than 100 percent of annual carbon dioxide emissions may be absorbed by adopting accessible and inexpensive organic management strategies, commonly known as renewing organic farming (Rodale Institute, 2014). Regenerative agriculture has just received a lot of interest from producers, merchants, academics, consumers, politicians and even the media. Multiple institutions, including government, business, and NGOs, are looking at regenerative agriculture(Kastner, 2016). Research is being conducted by the government to see if regenerative agriculture can support national and international climate change plans.

To meet the demands of feeding their populations, all countries must now increase their food output. Constant use of fertilizers reduces soil quality, and overusing herbicides and insecticides lead to biomagnification on humans, which in turn increases the risk of numerous diseases. The process of restoring deteriorated soil also takes a long period. As a result, the whole agronomic community is actively looking for viable alternatives to conventional farming that might potentially improve environmental and social outcomes (Rhodes, 2017). Regenerative agriculture, often known as sustainable agriculture or precision farming, aims to maximize food production while minimizing wasteful use of agricultural resources. Through regenerative agriculture, both soil and plants are revitalized. Some researchers are among the many who hold the view that regenerative agriculture may have a positive effect on both





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the short and long-term future of the global food supply through lowering emissions of greenhouse gases. A better water supply, more abundant plant life, and more productive soil are the goals of regenerative farming. According to Rhodes (2017), the fundamental goal of regenerative agriculture is to restore severely deteriorated soil to productive use. This kind of farming takes a systematic approach, encouraging farmers to work with nature rather than against it. This biological orthodoxy is based on ecological principles. Regenerative agriculture is crucial because it is our greatest metric for a fast decrease in atmospheric carbon dioxide. It is widely believed that agricultural output is one of the most significant causes of environmental deterioration worldwide. Agricultural practices are to be blamed for a lot of problems, including the release of greenhouse gases, the consumption of a lot of freshwaters, the destruction of forests, extinction of many species, and the spread of pests and diseases. Also, it will be a worldwide issue in the future decades to provide enough food for the world's growing population. It is suggested that under a climate change scenario, the key element that would expand the gap between food supply and demand is the shortage of appropriate arable land. Persistently bad management has damaged the soil in many places of the world, decreasing the acreage usable for agriculture, which in turn increases the food production gap. Keeping productive capacity stable as climates shift and environmental deterioration slows is becoming an increasingly important goal in this scenario. The term "agroforestry," which refers to the intentional integration of trees and other woody plants with agricultural and grazing systems, has been advocated as an approach to farming that can effectively addresses today's pressing environmental concerns. Fostering the multifunctionality of agricultural lands and limiting the different tradeoffs connected with food production, the ecological and economic advantages arising from the integration of the various elements that are part of an agroforestry system can help. Carbon sequestration, soil erosion, impacts on biodiversity, greenhouse gas emissions, nutrient leaching, crop protection from harsh weather, and long-term agricultural production stability are all improved by agroforestry. Benefits to society and culture are also amplified by agroforestry systems. However, the broad adoption of agroforestry systems is hampered by various obstacles, including a negative perception of trees in agricultural areas, a lack of clear definition and legislative backing, and an inability to manage complicated systems.

The recovering results of agroforestry have been established and nurtured all over time, and it is now considered as a comprehensive food production system that addresses social, ecological, and economic concerns (Leakey,1996). While the name "agroforestry" didn't appear until the 1970s, the practices it describes have been used for centuries in both colder climates and warmer ones (Nair, 1993). Varied, multifunctional agroforestry was historically created as a basis for food creation across the world via careful examination of organic forests, particularly how woods recover after disruptions such as fires or serious storms, and prosecution and error over many groups. The potential and possible of agroforestry as a restorative method is supported by indigenous knowledge (Smith, 2010). Agroforestry systems are increasingly seen as a cutting-edge solution to the problems plaguing modern agriculture (Miller & Nair,2006), such as the escalation of climatic extremes, the deterioration of soil and water quality, and the loss of biological variety (Foley et. al., 2005). A contemporary study directed by the USDA Forest Service and involving more than fifty researchers from throughout the United States shows that agroforestry systems increase crop yields, improve soil and water quality, provide animal habitation, and allow for revenue diversification (Dagar& Tewari, 2016). Soil improvement, improved water condition, increased biodiversity, protected ecosystems, and carbon sequestration are only some of the benefits of agroforestry that have been confirmed in recent review publications. This concordance indicates that the results of well-designed, well-implemented, and well-managed agroforestry systems are regenerative (Schulz, 2011).

AGROFORESTRY PRACTICES

Riparian buffer

Riparian buffers are described by Gold and Garrett (2009) as strips of planted permanent vegetation consisting of shrubs, trees, herbs, and grasslands and maintained simultaneously next to streams and water areas. By preventing bank erosion, sediment and nutrient runoff, alleviating banks, bettering water quality, and boosting biodiversity, these scattered zones protect water areas from the possible harmful effects of nearby agriculture or pasture (Stutter et. al., 2012). Even, while farmers and ranchers often plant riparian systems for the conservation advantages they





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bring, these systems may also provide perennial crops that can be harvested year after year. This multifunctional agroforestry method could achieve regenerative aims because to its conservation advantages, crop production potential, and usage of riparian regions that are not considered for production. The grasses, flowers, shrubs, and trees that make up riparian buffers have evolved through time to endure the effects of seasonal floods and strong currents, making this kind of ecosystem unique. There is a rich strip of riparian buffer on either side of a stream in the Kodagu (Coorg) district of Karnataka that is a headwaters tributary of the Cauvery River. This forest is one of the rarest and most valuable in the Western Ghats, and riparian forests are rare and valuable anywhere they can be found (Sunil, et. al., 2016).

Alley Farming

One or several lines of trees may be planted with farmed harvests in the "alleys" between the tree rows; this method, known as alley cropping or intercropping, is closely connected to silvoarable agroforestry (Gold& Garrett, 2009). Alley cropping systems have been shown to boost biodiversity (Tsonkova et, al., 2012), decrease carbon emissions, improve fertilizer usage efficiency, and decrease water runoff and soil erosion (MacFarland, 2017). It is possible to maximize potential gains by angling alley cropping systems. Soil erosion may be mitigated, for instance, by planting tree rows along to the land's natural contours. Growing a variety of crops, including both annual and perennial varieties, helps to provide steady income both now and in the future. Changing from monocultures and row crop farms to enduring agriculture systems may be facilitated using alley cropping. When two or more plant species or varieties that are mutually beneficial are cultivated together, the resulting yields may surpass those of monoculture or plantation stands (Van der Werf et, al., 2007). Reduced reliance on synthetic fertilizers is one of the many benefits of alley farming, which has been shown to boost crop yields. Additionally, it aids in enhancing the soil's physical condition. Shredded leaves may be used as a cover on top of the soil to enhance its infiltration, decrease its runoff, and increase its water usage efficiency by lowering the soil's temperature and evaporation rates. Tree rows prevent soil and water from eroding away from sloping ground by acting as a physical barrier (Paningbatan et. al. 1989). It's possible that the interspaces' shade during the fallow period and the mulch during the cropping phase might both help suppress weed development (Ssekabembe 1985). When a tree legume with several uses is utilized as a hedgerow, it not only serves these purposes but also offers feed, fuel, or stakes. Alley cropping of rice-wheat sequence is done with trees like babool trees (Vachellia nilotica) in the Modipuram regions with salt impacted alluvial soil.

Wind breakers

The purpose of a windbreak, also known as a shelterbelt, is to create a distinct microclimate by blocking the wind and reducing its speed and influence on a specified region downstream (Goodrich,2017). It is possible to establish windbreaks in areas that are already used for farming or grazing. Field windbreaks provide several benefits for agricultural land, including lessening soil erosion, boosting crop growth and output, shielding plants from wind harm, and lowering water loss via evaporation. Windbreaks may protect livestock from the sun, the cold, and the unpleasant smells (Alemu,2016) and sights that can be found on pastures. Windbreaks may prevent snow from blowing and drifting by decreasing wind velocity. Depending on the species chosen and the layout of the windbreak, they may serve other purposes in addition to reducing wind speed and reducing the risk of fire (USDA,2016). Benefiting the economy, the ecology, and the community at large, windbreaks are linear plantings of trees and plants. A windbreak's main function is to attenuate wind speed, which in turn improves conditions for soils, crops, cattle, wildlife, and humans. Protecting land, crops, cattle, etc. from the wind is a great idea. To prevent damage to crops, plants, and vegetables from high winds, windbreaker nets are used. In addition, the high concentration of salt in ocean air is detrimental to plant life. The most popular types of windbreaks used in farms are coconut (*Cocos nucifera*) and betelnut (*Areca catechu*) palms.

Silvopasture

The term silvopasturerefers to a method of agroforestry that integrates tree planting with livestock husbandry and the production of feed. There are two ways to set up a silvopasture system: (1) by planting trees on pasture, or (2) by diminishing and managing current forestland to sow fodder yields and graze cattle. In either case, trees and





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grassland are actively employed to graze cattle as part of a single integrated system. By switching from pasture to silvopasture, a farmer may diversify their income and ensure a steady stream of income from tree products such as nuts, fruit, and lumber for the medium to long term. Trees in silvopasture systems may shield cattle from the sun and reduce wind speed, which can help to keep them somewhat warmer in the winter. Among the five types of agroforestry techniques studied, silvopasture had the biggest growth potential, indicating that implementing these methods on India's current grazing lands might have significant regenerative effects. To restore and maximize output on deteriorating land, silvopasture methods were conceived and implemented. The usage of such methods was shown to increase land production by at least 2.5 times compared to the status quo of conventional approaches. Similarly, comparable systems supplied more than 10 times the biomass compared to degraded rangelands in a cycle of 10–12 years ((IGFRI, 2022). Reduced soil damage of less than a ten percent relative to clean land has been shown as an environmental benefit of these systems. Horticultural and pastoral combinations based on Guava (Psidium guajava), Bael (Aegle marmelos), and Aonla (Phyllanthus emblica) have been refined for use in rain fed areas. These mixtures were more lucrative than the sole cultivation of fruit trees in analogous settings because they ensured a steady supply of forage for animals while also ensure the safety of human nutrition via the harvest of high-quality fruits. Three shrubs Safed Babool (Leucaena leucocephala), Katurai (Sesbania aegyptiaca), and Drumstick tree (Moringa oleifera), three grass species Anjan grass (Cenchrus ciliaris), Guria grass (Chrysopogon fulvus), and Kansi (Panicum maximum), and two legumes Aparajita (Clitoria ternatea) and Naikurna (Stylosanthes seabrana) make up the silvopasture model in Bundelkhand area of Uttar Pradesh. During the winter months (January-June), 21% of the system's total annual feed supply comes from tree leaf, 15% from shrubs (February-April), and 64% from grasses (July -December) beyond cut and carry process and (March -April) by foraging method. Producing roughly 8-10 t/ha dry substance yearly and maintaining 3-4 ACU/ha/year, this approach is self-adequate for ensuring excellent feed resource during the year (IGFRI,2022). Eco-restoration efficiency was shown to be almost ten times higher than fallow land for degraded land in the tropical environment of the Bundelkhand area, where Morus alba and Acacia nilotica trees and Panicum maximum grass were cultivated using this approach.

Parklands

Parklands, additionally recognized as dispersed plants in croplands, are a frequent form of agroforestry structure that develops because of harvest production on a part of land that has become a perpetual activity (Raj & Lal, 2014). Smallholder agronomists in the emerging or undersized countries who are anguish from starvation, scarcity, and famine might greatly benefit from a system of mixing tree types into farmlands because of the productive, caring, socio-economic, and spiritual functions these trees play (Raj & Lal, 2014). To meet human needs, trees are incorporated into rural livelihood systems (Wiersum, 2004). Trees in agricultural systems require directly disposable products, as well as create revenue and improve the regional environmental condition, for example, by improving soil and their impact on micro-environment (Arnold, 1997). Trees can be broken down into distinct types: (i)plants on non-arable or unplanted land- naturally restored trees on land without harvests or grass; (ii)dispersed trees in the arable land- naturally renewed trees on cropland; (iii) trees mounting in home parks: primarily planted trees in and about the home,(iv) Boundary plants: trees that have been strategically placed to serve as barriers between different areas, whether that's across fields or inside them for aesthetic or practical reasons, (v) plant trees in amongst agricultural plants (intercropping) and (vi) plant trees in isolation (mono-cropping) on arable land (Bongers, 2010). Parkland trees, according to Dawson et al. (2014), contribute to livelihood strategies through various mechanisms, including diversification of production, forage feed for livestock (Atangana et al., 2014) that allows producers to incorporate animals in their farming method, thereby making extra income divergence and food for times when harvest yield is low also with farm implements, fuel wood, building materials, fruit, and treatment (Guyassa & Raj, 2013).

Importance of Agroforestry

Food insecurity, less unsustainable production methods, and the vulnerability of the people are all represented, but they still need to be addressed. Increased agricultural diversification and less food wastages are two potential solutions to these food-related problems. A staggering 828 million people are hungry, and there are an estimated 3.1 billion people who do not have access to a decent food (FAO, 2022). All of this points to the critical need of moving





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quickly to curb food waste and loss. It is estimated that 14% of the world's food supply is lost between the time of harvest and the point when it is sold in stores, excluding loss caused by spoilage. 8–10% of global GHGs come from lost or wasted food, which contributes to climatic instability and severe weather like droughts and floods(FAO, 2022). Crop yields, nutrient content, supply chain stability, and food security are all adversely affected by these shifts. Therefore, agroforestry systems provide biodiversity that may ensure indigenous peoples' food security. This agricultural method has the potential to improve food security by increasing farm revenue and enhancing product diversity. A further benefit is less economic risk because of diversified product lines (Hernández et. al., 2017). While food security ensures that people have enough to eat, food sovereignty helps define people's food and agricultural practices and ensures that they have the right to food via environmentally responsible production (Falkowski, et. al., 2019). Farmers prefer agroforestry systems because of the short-term advantages, such as increased food security and more stable income, rather than the long-term benefits, such reduced economic risks. With this in mind, public policies that aim to encourage the adoption of agroforestry systems should think about ways to work with plant and animal species that provide a quicker financial reward (Gosling et. al., 2020).

The provision of food for families was highlighted as the primary advantage of agroforestry systems, followed by the provision of fiber, fodder, timber, fuel, medicines, and other goods of commercial value. By adjusting the environment, we were able to pinpoint every species. Indigenous agroforestry systems are customary techniques that are crucial to the survival of this population and offer prosperity to the families that practice them. Coelho (2017) examined a silvi-pastoral system, one that allows human and animal contact. Martinelli *et. al.* (2019) modelled an agroforestry arrangement from two different points of view, landless farmers and the other in which the farmer owns some land. This allowed them to determine whether agroforestry systems would be economically viable under both situations. Net present value, equivalent uniform annual value, internal rate of return, payback period, productivity index, modified internal rate of return, cost-benefit ratio, and the capital asset pricing model were all utilized to evaluate potential investments. The research concluded that the two hypotheses' modelled agroforestry system is a feasible option that aids in the restoration of degraded regions by increasing the variety of native trees that provide environmental benefits. When the farmer already owns the land, the economic performance improves since the value of the property isn't included into the investment and external financing isn't required. Livelihoods of agroforestry farmers are more multifaceted and diverse than those of traditional farmers. Furthermore, it is proved that agroforestry farmers have a larger income than traditional farmers.

Women have a crucial role in agroforestry systems, which enhance the local microclimate and provide food and money for their families. In addition, they foster the economic and social advancement of rural women (Bose, 2017). Producing food and resources for crafts, medicines, and revenue production via agroecological agroforestry systems is a millennia-old indigenous tradition (Nair et. al., 2017). Food security for the indigenous population was ensured for a long time thanks to a farming method based on polyculture and agroforestry. Most often, agroforestry systems grow food because of a combination of human need for it and the nutritional and economic worth of the many species that make up the system (Torres et, al., 2018). Therefore, indigenous communities could devise plans to promote the adoption of more sustainable productions, such as agroforestry; the arrangement's make-up may encourage these people to consume more nutritious indigenous foods, primarily lowering the malnutrition of women and children, in addition to providing ecosystem services and conserving biodiversity. Products like handicrafts may be a by-product of agroforestry techniques. Cotta (2017) claims that in villages, half of the revenue comes from handicrafts, and those handcrafted medications are also extensively utilized. Indigenous peoples' expertise on how to treat illness with plants is invaluable; many medicinal plant species may be grown in agroforests, making them more accessible. Indigenous farmers' huge store of empirical knowledge might help boost family income and ensure they always have enough to eat. Indigenous knowledge is beneficial to biodiversity, and Vallejo et al. (2014) reaffirm this point. However, agroforestry management is affected by things like land ownership, agricultural intensification, and previous land usage. Ethnicity is another essential factor that impedes on efforts to increase plant variety and use agroforestry techniques. As a result, when introducing agroforestry systems into indigenous communities, care must be taken to respect the unique farming practices of each culture (Torres et. al.,





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2018). Indigenous peoples' traditional knowledge, as expressed via agrobiodiversity, has the potential to inform strategies for restoring habitats when biodiversity is declining (Vallejo et. al., 2014)

Need of the Hour: Agroforestry Policy For Regenerative Farming

Agroforestry is becoming more important in the field of environmental services. It is well-known that agroforestry can lessen the impacts of climate change by, in the short-term, moderating microclimates and conserving natural resources, and, in the long-term, by sequestering carbon. Food, nutrition, energy, employment, and environmental security are all issues that might be helped by agroforestry systems. A forward-thinking National Agroforestry Policy, as well as suitable research interferences, acceptable investment, appropriate extension approaches, inducements to agroforestry physicians, an allowing legal and governing setting, advertising of agroforestry crop, post-harvest treating, growth of new products, and other measures, are required to address these issues. Farmers and rural residents may use agroforestry as a powerful tool to strengthen their defenses against climate change and other natural disasters. Economic potential based on agroforestry trees may also contribute to rural development and greening.

The National Forest Policy (1988), the National Agriculture Policy (2000), the Planning Commission Task Force on Greening India (2001), the National Bamboo Mission (2002), the National Policy on Farmers (2007), and the Green India Mission (2010) all highlight the importance of agroforestry in achieving these goals. A variety of issues, however, have prevented agroforestry from achieving its full potential as a technique for developing available resources. Restraining legal requirements for harvesting and conveying trees implanted on woodlands and the usage of non-timber yield, adjoining non-existence of addition mechanisms, a lack of formal support mechanisms, a lack of quality establishing materials, insufficient study on agroforestry models appropriate across the country's numerous ecological regions, insufficient marketing structure and price discovery instruments, and a lack of post-harvest dispensation are just a few of the factors.

CONCLUSION

Instead of seeing farming as fixed one kind of cropping system, small farmers should think of their business as a portfolio of crops and trees to be grown. Convergent programming including trees, crops, water, animals, and other means of subsistence is essential for development in this direction. It seems that national agroforestry projects are lacking this integrated vision. The absence of a specific extension infrastructure means that the research findings on agroforestry that are accessible in the community and personal domains do not frequently achieve the farmers. Unfortunately, there is a severe shortage of multi-level institutional frameworks to support agroforestry. Due to a deficiency of understanding of the practical and financial data on various agroforestry representations, as well as the techno-economic limits needed by economic establishments to assess finance requirements and feasibility of the projects, institutional finance in agroforestry has not reached its full potential. Similarly, there is a lack of effort put into creating and promoting insurance options for agroforestry businesses. This dismal state of things may be attributed to a lack of education, absence of goods that are acceptable to farmers, a high cost of reward, and a murky system for claim settlements. Except for a handful of states that have either advanced exclusive marketing structure for agroforestry produce or have merged with the regulated farming commodity marketing schemes, India lacks the marketing infrastructure, including price discovery mechanisms, necessary to sell agroforestry produce. Therefore, the market is mostly in favor of the buyer, and most of the profit is going to the intermediaries.

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RESEARCH ARTICLE

Enhancing Employability Skills in Indian Classrooms: Towards Atamnirbhar Bharat in Light of National Education Policy 2020

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ABSTRACT

It is evident that the progress and prosperity of the nation depends upon the development of its human resources, which ultimately is dependent on the well productive education of today's youth. Education, being the social institution or a kind of tool enables one to know about himself, and provide him/her with knowledge and skills thus bringing the holistic personality of the individual. Holistic education aims to involve the learner's mind, body, and spirit in all aspects of learning and advances the mind and abilities of an individual towards progress, social and national development by meeting the demands of the current scenario. Thus, in order to impart the real word skills that will enable the students to lead a successful life in rapidly changing society and to produce creative and innovative ideas, vocational education is very much important. It will inculcate the crucial capabilities and skills that prove to be very useful in tomorrow's world. The present paper highlights the significance of vocational education as the need of an hour and the emphasis laid down by the Samagra Shiksha Abhiyan as well as our recent new educational policy NEP 2020 towards the integration of vocational education with the mainstream education, thereby striving in the direction of self-reliance. Different problems related to the vocational education in India and the suggestions to cater them has also been mentioned. While perceiving the role of skill-based education in assisting students, society, and as a whole for the nation, there is a great need that all the stakeholders should carefully analyse and seriously consider the strategies for skill education and training in order to yield and improve the quality of human capital of the country thereby enhancing the employability skills among students.

Keywords: Holistic education, Vocationalisation, Samagra Shiksha Abhiyan, NEP 2020.





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INTRODUCTION

In the present scenario where the unemployment is at peak and also after struggling through the Covid like situation, everybody realised the need of a skill driven society to sustain one's own livelihood as well as to make our nation Atam-nirbhar (self-reliant). Somehow, the main aim of imparting vocational education or the skill-based education is to create an Atam-nirbhar youth, thereby contributing towards Atam-nirbhar Bharat. Vocational education and skill development tends to be at the top of agenda of the Indian government because of the need to cope up with the high economic growing rates and the shortage of the laboured skills. It should also be kept in mind that, following COVID, the skill-based sector is going to see an increase in demand for jobs in sanitation, health, technology, and other areas. Students can participate in the effort of breaking the cycle of unemployment by entering plethora of different workforce vocations (Chaturvedi, 2021). Furthermore, in order to tap into the immense potential of a youthful and dynamic population, the Indian government shows a great attention in skill growth through formal vocational education and training (Agrawal, 2014).lts right to say that in order to survive in this era of technological and industrial advancement, one needs to be skilled and competent in all aspects or it can be said that the holistic development of the child is of utmost importance along with bringing out the best of their existing talent, so that they can contribute on their part towards the progress and welfare of their nation. Education related to the skill development enables people to take the ownership of learning in their own hands and hence emerge as successful leaders in their own respective fields. Vocational training, without any doubt, improves your chances of securing a job and getting promoted faster. It has become a lifesaver for many people in the era of unemployment.

Education for Holistic Development

Holistic vision of education here refers to the overall development i.e., the development of each and every potential of the individual whether it is social, emotional, physical, intellectual, spiritual, artistic or creative. Holistic educationis a method of educating pupils to confront whatever problems they may experience in their lives and academic careers. It attempts to modify learning of human kindness, personal grandeur, and the joy of living both in challenges and accomplishments. On contrary sides, considering the past times, children had received enough support from his or her family, religion, or previous tribes making them to be dependent on others. In addition to this, competition at school, afterschool activities, and societal pressure on them to look a certain way, as well as violence, all put pressure on students. All these instances not allowed them to perform on their own because they were obligated to follow the instructions of their parents or teachers. This is changed via holistic vision of education where children need to develop not only academically, but also socially, having an ability to thrive in today's world availing their own all-round capacities. They should be able to overcome the odds and meet future problems, as well as contribute to the world in which they live. Therefore, Holistic education is a method of teaching that may suit the needs of all types of students and mould future citizens who are concerned about society and the environment. It is infused with interconnectedness and connectivity (John, 2017, p-347).

Holistic education is a kind of drive in education that seeks to encompass the learner's mind, body, and spirit in all facets of learning. Its philosophy is built over the conception that individual discovers uniqueness, meaning, and reason in life by maintaining links to their community, the local home-grown environment, and benevolent principles such as kindness, consideration and peace. In this regard, an integrated scheme called 'Samagra Shiksha Abhiyan' was launched with an aim of holistic development of the child from a pre-school level to a secondary school level. Generally, vocational education can be viewed as a means of achieving a wide range of objectives in holistic approach, whereas the objective is solely economic, on the part of government to impart VET i.e., the need of Industry-driven and skill-based society to raise economy. The conception of "locally relevant education" appears essential to holistic approach to vocational training (UNESCO, 2005).

Samagra Shiksha Abhiyan: A Holistic Vision of Education

With the aim to bring change in the school education sector, MHRD on 24th May,2018 launched the Samagra Shiksha Abhiyan by merging the three schemes i.e., Sarva Shiksha Abhiyan, Rashtriya Madhyamik Shiksha Abhiyan (RMSA)





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and Teacher Education. Universal access, equity and quality, along with the advancement of vocationalisation of education, and firming up of teacher education institutions are the key objectives of the Scheme. Shri Prakash Javadekar, the union minister for Human Resource Development spearheaded the Samagra Shiksha initiative, which aims to create an integrated system for school education that assists states at all levels, from pre-school to senior secondary. He further expressed his belief that by conducting itself in a manner that treats 'school' holistically as a continuation from pre-school to primary, upper primary, secondary, and senior secondary levels, this scheme will herald a paradigm shift in the fundamental design of education. The scheme generally assists the states in implementing the Right to Education Act 2009 and to promote inclusive and equitable education thereby ensuring the attainment of SDG 4 (Sustainable Development Goal). While aligning with the Sustainable Development Goal 4.4 which talks of substantial increase in the number of adults and youths possessing relevant technical and vocational skills that will enable them to get employment, decent jobs and to emerge as entrepreneurs, NEP 2020 aims to ensure the exposure of at least 50% of the learners to vocational education in collaboration with school and higher education system by 2025.

Vocationalisation of education

Simply, Vocationalisation of education refers to initiatives taken up the schools to integrate in their curriculum those practical disciplines or courses that are likely to stimulate some interest among pupils' basic abilities, knowledge, and attitude that will encourage them to consider a career in the field as skilled workers or take up other manual labour occupations. Skill development is the acquisition or development of a skill through training or experience. Vocational education plays a significant role in preparing pupils to work, either independently (self-employed) or perhaps to get job openings in the world of work. As a result, the macro perspective of vocational education growth is focused toward the fulfillment of workforce market need. As part of the national education system, vocational high schools have undergone a paradigm shift, shifting to demand-driven from supply-based, from academic to work focused, and from learning-based to dual-based programmes (Yoto, 2016). Vocational education and training prepare a person for work in a particular field. It minimises resource and financial waste. When the attention is solely on a single region, a significant amount of money is saved. Vocational training can also be learned by school dropouts and adults. Due to a flawed educational system, the majority of people got stuck in the incorrect employment. They can analyse their own interests and pursue employment in that field if they are provided vocational instruction from the start.

Vocational Education in relation to Samagra Shiksha Abhiyan

The Government of India, through Samagra Shiksha Abhiyan is attempting to provide our students with all-round skills – academic, extracurricular, and vocational – in order to establish a strong basis for their future development into better men, who will then serve as a catalyst for India's progress. Present students, being the major asset and the human capital of our nation, are supposed to play a profound impact on the economy and sustenance of our nation, therefore, their empowerment should be the prime concern.

Integration of vocational education with general education has been recommended under Samagra Shiksha Abhiyan.

- 1. The scheme talks of the extension to the exposure of students of upper primary level to the vocational skills.
- 2. The teaching of vocational courses that are created on the work roles and are accepted by National skill qualification committee from class 9th to 12thhas also been advocated under this scheme.
- 3. National hours, age and educational qualifications would be regarded as the basis for the selection of vocational courses by the students. Vocational education, at secondary-level should be strengthened and made an integral part of the curriculum.
- 4. In addition to this, vocational modules to be referred as an additional subject for the class 9th and 10th and for class 11th and 12th, the vocational courses are to be taught as compulsory subject as per recommended under Samagra Shiksha scheme.
- 5. Provision of employability skills has also been mandated as a part of vocational courses to teach them entrepreneurial, communication, self-management and ICT skills.





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6. The scheme also talks of encouraging the foundational skills through 'Padhe Bharat Badhe Bharat'. Special emphasis has been also given to vocational and soft-skill development i.e. 'Kaushal Vikas'.

NEP 2020 and Vocational Education

Our recent National Education Policy 2020 (NEP) also talks of instilling values, skills and dispositions in order to develop a true global mind-set and supporting human rights and sustainable development. Also, the reimagining of vocation education by implementing the global standards in all vocational education institutes at all levels, and creation of labour dignity through general structural transformation, innovation, and partnership has also been suggested. Recognition, identification and fostering of each individual's unique capability should be done so as to bring the holistic development of each student by removing silos among different areas of learning, bringing integration of vocational and mainstream education, providing them flexible environment, stimulating critical and creative thinking and hence promoting respect for diversity and local context. The policy advocates for the development of a skill-based mainstream education system with locally developed content and curriculum that is accessible to all through the use of various learning modes With the vision to make the youth Atamnirbhar, NEP 2020 gives additional impetus to the integration of vocational education and training with mainstream education at school level too. Facilitating collaboration with industries, conducting short-period certification courses and establishment of incubation centres will help a lot in meeting the goals of integrating the vocation education along with mainstream education. In addition to this, the creation of NCIVE i.e., National committee for the integration of vocational education, having Vocational education experts and representatives from ministries has been recommended in NEP2020.

Integrating Indigenous Vocational Knowledge into Classrooms

The recent National Education Policy NEP 2020 of India contains a very important policy tenet that calls for the integration of Indigenous Traditional Knowledge into the curricula of both higher education and schooling. One of the guiding ideas of NEP 2020 is that it will inspire pride in India and its rich, diversified, ancient and modern culture, knowledge systems, and traditions within both the education system as a whole and the individual institutions operating within it. This vision of NEP 2020 emphasises the vital need of preserving Indigenous traditional knowledge (ITK). ITK is an essential component of a community's culture and history. It has developed over many years of consistent experimenting with daily living and the resources that are close to the community. It is the distinct, age-old local knowledge that has grown up around and within the particular circumstances of men and women who are native to a given region. Societies have developed over the years by picking up knowledge from experience and passing it on to the next generation. ITK contains the traditional technological, social, economic, and philosophical knowledge of a particular society that is based on spiritual abilities, customs, and modes of being in nature. The urgent need to strike a balance between indigenous traditions, knowledge, values, economic necessities, social pressures, and demands of national and global development was also stressed by Tusiime (2018).

According to this viewpoint, formal VE systems ought to include cultural heritage and values as the foundation for the VE curriculum, which are sustained through indigenous languages as a means of transmitting indigenous knowledge. It is important to make efforts to incorporate this knowledge into formal schooling in order to better one's understanding of the community in which they live. India has the capacity to recognise and preserve the knowledge facets crucial to its continuation and existence as a unique nation in the world. According to the policy's recommendation, States/UTs will individually establish courses that will foster local arts and crafts among schoolchildren. Training in these arts and crafts would be provided by the local craftspeople. The country's population must be able to utilise its experience and knowledge to the fullest extent for the geographical riches to be utilised to their fullest potential. Despite having abundant natural resources, a place may continue to be pitifully underdeveloped.

Other Initiatives taken by the Government of India for the promotion of vocational education:

- ✓ Pradhan Mantri Kaushal Vikas Yojana
- ✓ Udaan





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- ✓ India International Skills Centres (IISCs)
- ✓ Polytechnics
- ✓ Skill Strengthening for Industrial Value Enhancement (STRIVE)
- ✓ Advanced Vocational Training Scheme
- ✓ Craftsmen Training Scheme(CTS)
- ✓ Pradhan Mantri Kaushal Kendras(PMKK)
- ✓ Apprenticeship Training under the Apprentices Act, 1961
- ✓ Pradhan Mantri YUVA Yojana

Initiatives taken by Jammu and Kashmir Govt. for Vocational Development and entrepreneurship

- ✓ Skill Hub initiative under PMKVY
- ✓ Accelerated Transformation of Aspirations and Livelihoods (ATAL) Yojana for skill development and entrepreneurship for youth.
- ✓ 'HAUSLA- inspiring her growth' scheme for empowering women by providing them entrepreneurial opportunities
- ✓ Jammu and Kashmir Entrepreneurship Development Institute (JKEDI)
- ✓ Public Private Partnership (PPP) Scheme to provide the quality vocational training to the youth, ensuring that the graduates are more employable.

Problems of Vocational Education in India

- Course irrelevance, resulting in a mismatch between labour market needs and training competencies
- There is a lack of focus on emerging industrial growth regions.
- Inadequate syllabi in schools, as well as the usage of outdated equipment
- Inadequate practical training abilities acquired by pupils that are not applicable in real-life situations
- Incompetent and untrained teachers for teaching skill-based courses.
- In schools, there is overall low enrolment in the vocational stream.
- Lack of social acceptability as well as low employment chances.
- Low access to skill-based education
- Preferences for general education and degrees.

Suggestions

Following suggestions will help to somehow meet the challenges and problems associated with vocational education:

- Curriculum needs to be revised in the light of skill-gap analysis in secondary vocational schools and also the demand-driven principle needs be applied to the development of vocational education.
- Moreover, the education implementation in vocational institutions should be carried out in aggregation with manufacturing units, and approaches dealing with strengthening vocational education both in and out of school.
- Proper orientation of the teachers regarding the VET should be done.
- A safe and supportive learning environment should be created where vocational training and internship to the students' will be provided on the basis of their need and interest.
- Adequate funding should be provided to run vocational courses.
- Create awareness among the general public regarding the different apprenticeships available for them. This is not only for conventional trades, but also with the advancements, opportunities in the fields like software development, law, and financial services should be looked for.
- The curriculum for vocational education must be designed for to meet the needs of the community.
- As a result, society must be considered when designing the curriculum's content. Industrial society and the demanded industrial skills should be taken care while formulating the course content for teaching.





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CONCLUSION

Overall, viewing the skill-based education as the need of an hour, it can be asserted that the learning of various skills becomes necessary in order to promote a culture where entrepreneurship and vocational education is seen as a part of life as well as a new economic world. Based on above discussion, it is true to say that the skill is the most valuable asset a person can possess. Moreover, VET improves people's ability to benefit from entrepreneurial activities and motivates them to get involved in them and emerge as self- reliant individuals. Vocational courses are a kind of surprise since they provide students with the opportunity to enhance and refine their innate talents and skills. They achieve great success in their industry and other sectors. Furthermore, both the government and the private sector have a significant demand for highly qualified workers. It benefits students, society, the nation, and employers most of all. Thus, there is a great need that all the stakeholders should carefully analyse and seriously consider the strategies for skill-based education and training in order to yield and improve the quality of human capital of the country. Provided with special support, the path of VET, will obviously generate graduates who are competent and skilled enough of competing in the present world of demand, where technical education will be seen as the key factor for success in career. Likewise, a great emphasis has been laid down from time to time by different policies of the government but the dearth is at the proper implementation of those policies. Now it is up to all interested shareholders to guarantee that the plans and the policies should be properly implemented.

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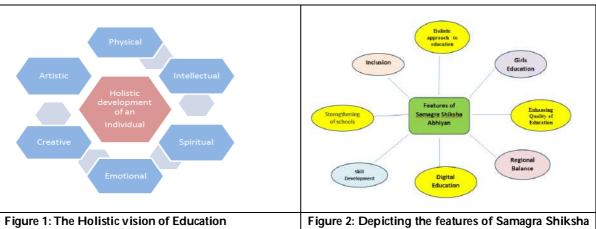


Figure 2: Depicting the features of Samagra Shiksha Abhiyan

 Teaching vocational course right from class 6th through different activities. Practicum based curriculum Vocational courses should also be made available through open and distance learning mode. Knowledge devloped in India should be made available to students and mapping of local opportunities and skill-gap analysis should be done. Lok Vidya NSQF (National Skill Qualification Framework) Should be detailed more over for each discipline enabling students to acquire desired competency in different skills. Skill · Internship opportunities from grade 6 to 8 for students by experts like artists, carpenters, etc. Vocational Craft •[Text] Collaboration between ITIs, polytechnics, local industry, and secondary schools. And that of higher education with NGOs and private Collaboration industry has been suggested in NEP 2020 Institutions must innovate in order to build vocational education models and various apprenticeships. Apprenticeship and vocational Incubation centres can be established in collaboration between higher education institutions and industries.







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ISSN: 0976 – 0997 **RESEARCH ARTICLE**

Area Efficient Arithmetic Block using Reversible Logic for DSP **Applications**

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ABSTRACT

Digital Signal Processing is a new revelation that has implementations data such as mp4, mobile Phones, medical analysis that have been digitized and manipulated according to the usage. In recent technology, all the portable devices require reduced area and high speed operation. The study uses reversible logic to minimalize the area of an arithmetic block. The study uses reversible logic to minimalize the area of an arithmetic block. With parity preservation, an efficient architecture of reversible 2-bit & 4-bit parity preserving carry select adder was provided. Reversible Logic Gates is another sort of reversible logic gate that uses only one of them. Such gates have a power efficiency and a fast response. The chosen gates can be used as also an adder and a subtractor. Carry Select Adder is used for fast data processing which achieves less gate count and garbage outputs. This adder reduces the area, increasing the speed of operation. Verilog is used for coding, and ModelSim 5.7g is used for simulation. Xilinx 14.2 ISE is used to synthesis the result.

Keywords: Carry Look Ahead adder, Array Multiplier, P2RG, Reversible Logic, Low Power Design, Xilinx.





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INTRODUCTION

The method of constructing electronic components by merging dozens of transistors into a single chip is known as VLSI. Newer advance signal processing technology has been developed in VLSI device since level for assembly continues to expand. Such digital signal systems also require a lot of computing power, but they also use a lot of energy. As energy usage becomes a primary issue of history's VLSI design process, as well as the requirement of reduced VLSI circuits derives through 2 main drivers, power and area seem to be the 2 main issues to consider. Initially, when operating frequencies and computational power on each chip increase, big currents must have been delivered, and heat generated by high energy usage must be evacuated using active cooling methods. Second, wearable digital gadget battery capacity was restricted. Within those gadgets, the reduced energy architecture immediately correlates to a longer operating duration. At microprocessors, digital signal processors, & especially electronic computers, addition seems to be a more known and easily utilised mathematical function. It also acts as a foundation for the creation of all subsequent mathematical functions. Because of it's simple construction of an arithmetic unit, binary adder designs seem to be very important hardware component. Since there is zero data bit deficit while processing, reversible logic seems extremely essential since it leads to reduced energy waste. Furthermore, to identify the incidence of defects, reversible circuits must be converted into fault resistant reversible circuits. Within present context, the architecture of reversible logic was gaining popularity with the help of smaller area. On construction of smaller circuits, reversible logic is critical.

Background Study

Palak Sharma (2017) in today's information age, advances in nanotechnology are reducing the power usage of logic circuits. Because of its reduced power usage and minimal heat loss, reversible logic architecture are some of the most key techniques. This study proposed reversible 4 bit & 2 bit parity preserving carry look ahead adder that uses only two reversible gates, the F2G and MFRG gates, and consumes low power. Reversible logic synthesis was performed including all 2 bit and 4 bit architectures in this paper. Ragunath G(2016) This redesigned XOR gate was utilised in traditional Square Root Conventional Carry Select Adder, Binary to Excess 1 Converter based Square Root Conventional Carry Select Adder & optimized Logic Based Square Root Conventional Carry Select Adder to achieve improved outcomes whenever the adder circuit includes additional XOR gates. The circuits were synthesised using a revised XOR-gate that is employed with traditional Square Root Conventional Carry Select Adder, Binary to Excess 1 Converter based Binary to Excess 1 Converter and optimized Logic Based Square Root Conventional Carry Select Adder. Performance outcomes reveal the ADP can be lowered in the suggested circuits in traditional SQRT CSLA. Manoj Kumar K (2015) came up with a design. Reversible logic had received much interest over latest days for this capacity to decrease power usage, that was big worry on electonic architecture. These characteristic architecture offers a low figure of garbage outputs, constant inputs, quantum costs, and gates. Eric Clapten J (2015) provided a new conceptual design for the CSLA based on a VLSI implementation of a reduced power area optimized fast carry choose adder that eliminates all duplicated logic functions of the conventional CSLA. As contrasted to a conventional CSLA, it improved SQRT CSLA minimizes area and power while increasing delay due to the usage of a Binary to Excess-I converter. Soniya (2013) presented various types of multipliers such as booth multipliers, combinational multipliers, Wallace tree multipliers, array multipliers, and sequential multipliers, that all provide better speed of operation, while SPST and block enabling methods are better aspects of low power dissipation & area.

Allipeera K (2012) On digital adders, the addition operating speed was reduced with time involved for transmit a carry through the adder. Delay used for transferring a carry on a digital adder limits pace of addition, according to Allipeera K (2012). CSLA was speed adder found on several information processing devices. It is evident from the CSLA's structure that there is potential for improvement in terms of area and time. The updated SQRT CSLA has a somewhat bigger area for lower order bits, which decreases further for higher order bits, according to the results.V Veena Nair (2010) The CSLA architecture has been built with the help of a Binary to Excess-1 converter (BEC). This research provides a cost-effective approach for replacing the BEC with a D latch. A system design delivers the





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threefold benefits of power, area &delay with respect to experimental investigation. This study proposes a simple method for reducing the size & power for Square Root Conventional Carry Select Adder architecture. As contrast to a conventional CSLA, the modified CSLA uses BEC for reducing power & area while increasing delay. This study suggests a technique that decreases the delay, area, and cost of the system. All of the previous projects has a high area, power, and delay, as well as a higher gate count and garbage output. So it operates in combinational circuits with reversible logic gates. Various multipliers revealed the sequential multipliers outperform combinational multipliers with respect of speed and area for big binary number multiplication.

Implementation of reversible logic using CLA

A reversible logic gate is an n-input n-output logic device with 1-1 mapping. Because only one concept isn't really reversible, direct fan-out isn't really permitted with in construction for reversible circuits. Fan-out is achieved in reversible circuits, though introducing gates. A reversible circuit must have the fewest possible reversible logic gates. This avoids data loss, that was the primary source of power dissipation on non-reversible logic circuits. This circuit is a 2-bit parity-preserving CLA.Figure 1 shows the CLA circuit. A architecture contains x(0-1) and y(0-1) are inputs, as well as (s0,s1)are outputs, (c1,c2),& 4 constant inputs. The F2G and MFRG gates are used in an existing P2RG Conventional Carry Select Adder architecture circuit. Current design contains 5 F2G reversible gates and 3 MFRG reversible gates, as well as the capacity to parity preservation.

The existing system has a flaw in the architecture for CSLA 2 bit, which necessitates the use of 4F2G and 4MFRG gates, as well as 6 garbage outputs.

Implementation of reversible logic using arithmetic unit

Parity Preserving Logic

In order to maintain parity, In logic, the parity of inputs and outputs must identical, a 4*4 reversible gate, for example, should fulfill the equation below in order to preserve parity.

 $A \oplus B \oplus C \oplus D = P \oplus Q \oplus R \oplus S$

The most significant aspect of the system, which acts as a full adder and subtractor with one P2RG and Fredkin gate, can benefit from parity preservation.

Fredkin Gate

I(A, B, C) is the input vector, while 0 is the output vector (P, Q, R). Figure 2 shows the Fredkin gate. The result was stated with P=A, Q=A'B+AC & R=AB+A'C. Fred kin gate quantum cost was five.

P2RG GATE

In Figure 3, an unique 5*5 P2RG is developed. Output is same as an input in this gate. Because of performing NOR operation, this proposed gate is said to be universal.

P2RG And Fredkin Gate Based Half Adder And Half Subractor

A parity preserving reversible gate & a Fredkin gate are used to create parity preserving half adder / subtractor. Basic building blocks for designing a full adder and sub-tractor are the half adder and sub-tractor. Requires two inputs, A and B for constructing a half adder & subtractor. In this case, no preceding carry or borrow is required. So, this circuit contains A,B as input and ctrl as a control line that controls the function. If Ctrl has 0 logic ,half adder executes else 1 logic means half subtractor executes. The following are some Boolean expressions that can be used to implement an operation for half subtractor & adder:





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Difference & Sum= A ^ B Carry = AB Borrow = A`B

4 garbage bits & 3 stable input will be provided. In Fig. 4.3, one P2RGgate and one Fred kin gate are used to create a parity preserving half subtractor & adder.

Arithmetic unit (au)

Arithmetic is a discipline of mathematics concerned with the examination of numbers and, in particular, the properties of the conventional operations of addition, subtraction, multiplication, and division on them. The block diagram of Arithmetic Unit shown in Fig 5. The Arithmetic Unit will use reversible logic to design a circuit that will boost speed while lowering area. The selection line influences the construction of the arithmetic unit. The addition operation should be conducted if the selection line is 00, and the same operation can be performed if the selection line is 01, 10, or 11. In addition, the AU must be resistant to errors that may occur during operation.

SQRT CSA

For improve the structure, you must first identify a critical path. Take, for example, a 16-bit linear carry select adder. Suppose if a full-adder and multiplexer cells have the same propagation delays, which are equivalent to a standardized quantity of 1. There is one evident chance. Examine data from the multiplexer for last steps of the adder. The signal from the preceding phase of the multiplexer is sent into this preceding the multiplexer adds extra bits to the next step. There is a significant disparity between the signal arrival times. The carry chains produce a reliable lengthy adder that requires longer time to generate the carrier signal. Reversible logic based gates must been used to create the square root carry select adder circuit. The CTRL line should be used to control the adder. The adder operation is done if the CTRL line is set to 0.

SQRT CSS

A Ripple carry adder is built using parity-preserving gates. The based parity preserving Reversible logic subtractor component should be built using the Ripple carry adder block. Reversible logic based gates should be used to create the Square root carry choose sub tractor circuit. The CTRL line should be used for check subtraction function. Procedure of subtraction is executed if one is in the Control line. Block diagram of square root carries select subtractor. Reversible logic based gates should be used to create the Square Root Carry Select Sub tractor circuit. The CTRL line should be used to control the subtraction operation. The subtraction procedure is executed if the CTRL line is set to 1.

Array Multiplier

Because of its regular construction, the array multiplier seems to be well. The add and shift algorithm is used in the multiplier circuit. For obtaining each PP, multiplicand is multiplies with one multiplier. PP are then added and moved as per their bit ordering. An array multiplier is implemented using full adder and half adder, which is based on reversible logic. Half adder and full adder circuits make up a typical array multiplier circuit. Where N is the multiplier, N-1 adders needed. The Array multiplier will be designed Using reversible logic based gates the Array multiplier will be designed in innovative world. Figure 6 shows the Array Multiplier block diagram. The add and shift algorithm is a basic multiplication method. However, as parallelism increases, shift degree between PP & intermediary sums are inserted it expands, potentially resulting in slower processing, enhanced silicon area due to irregular structure, and increased power consumption due to increased interconnect due to complex routing.

RESULTS AND DISCUSSION

P2RG And Fredkin Gate Based Adder and Subractor Output

Fig 7. represents Adder and subtractor output based on P2RG and Fredkin. The SD and C-out outputs were established using the control line and inputs a and b.





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Ripple Carry Adder Output

Fig 8. A 4-bit RCA output is shown. The result sum and carry have been calculated using c-in and inputs a and b. **SQRT CSA Output**

Fig 9. Result of the SQRT CSA is shown. The outputs s and c-out were created using c-in and inputs a and b.

SQUARE-Root Carry Select Subractor Output

Fig 10. shows the output of Square-root Carry select subtractor. Based on c-in and inputs a and b, the outputs s and c-out were produced..

Array Multiplier Output

Fig 11. Shows the output of Array Multiplier. Based on input x and y, the output z was produced.

Arithmetic Unit Output

The MODELSIM 5.7g software is used to compute the simulation results for a and b input and output 8-bit. Timing analyser review, RTL view, and design summary are all obtained using the Xilinx 12.1 software.

Device utilization for arithmetic block

Table 1. Device Utilization for Arithmetic Block

Comparison of delay, garbage output, gate count

Table 2. Comparison for Gate Count, Garbage Output and Delay

Comparison Chart

Figure 13. Comparison Chart

CONCLUSION

The gate count is decreased from 8 to 2, the garbage output is reduced from 6 to 4, and the delay is reduced from 5.28 to 4 in this paper. The low area is a major concern in VLSI design. The Arithmetic Block's size is decreased, decreasing the gate count from 8 to 2 for a 2-bit design and garbage output from 6 to 4. In addition, the design's delay is decreased by 15.238ns.

Future Work

In the future, this will work on reversible logic gates for combinational circuits, and then it'll be able to expand these to implement sequential circuits as well. According to the results, sequential multipliers outperform combinational multipliers in terms of speed and area for large binary number multiplication.

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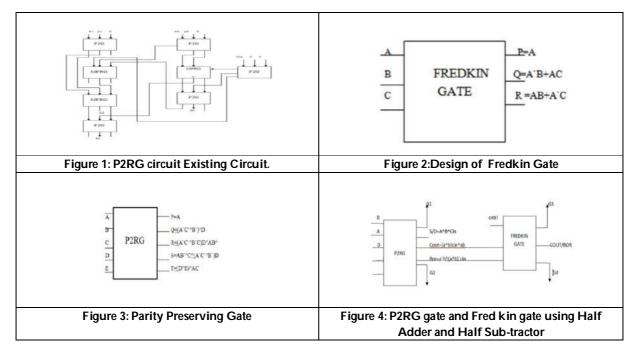
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Table.1: Device Utilization for Arithmetic Block

Summary of Device usage			
Logic Utilization	Used	Available	Utilization
4 Input LUT count	98	12,288	1%
Number of occupied Slices	50	6,144	1%
Related Logic slice count.	50	50	100%
unrelated Logic slice cc count	0	50	0%
Bonded IOBs count	27	240	11%

Table.2: Comparison for Gate Count, Garbage Output and Delay

Logic	Gate Count	Garbage Output	Delay
Reversible Logic using CSA	8	6	5.278
Reversible Logic using AU	2	4	4.009





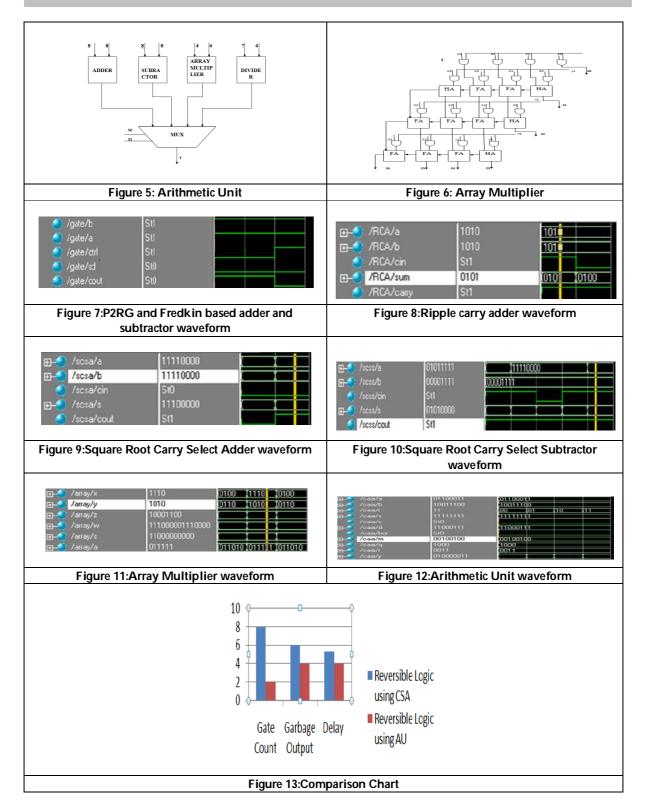


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RESEARCH ARTICLE

Emotional Intelligence, **Spiritual** Intelligence **Predictors** as Psychological well being

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ABSTRACT

The present study aims to analyse the emotional intelligence and spiritual intelligence as the predictors of psychological wellbeing in adolescents. Adolescence is characterised by significant physical, cognitive, and socio-emotional changes; mood swings and emotionally charged occurrences are prevalent at this stage of development of life. Psychological wellbeing is a sense that depends on how a person organizes their stream of consciousness into creation and development. The sample of the study consists of 300 adolescents between 15-19 years of age. The instruments used were Emotional Intelligence scale by Singh and Narayan, 2004, Spiritual Intelligence self-report inventory by David, 2008, Riff's Psychological Well Being Scale, 2007. The results of the current study shows that there is significant relationship between the variables and emotional and spiritual intelligence predicts the psychological wellbeing in an individual positively. There is the need of implementing techniques that develop teenagers' emotional intelligence and spiritual intelligence, which will contribute in improving their mental health and making them more competent. With higher psychological wellbeing they will be more capable to deal with the difficulties of life.

Keywords: Emotional Intelligence, Spiritual Intelligence, Psychological Well-being, Adolescents, Positive Psychology, Coping





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INTRODUCTION

Adolescents can be an age of overwhelming emotions, and all at once kind of feeling is very common in this age because of all different kind of changes taking place all at once like changes in body, social life, changed relationships with friends and family, identity crises, anxiety to stand out, also to fit in well, develop into better and productive adult and much more, and all these emotions bring out the frequent change in attitude, feelings and behaviour of adolescents, that could impact the overall working and wellbeing of this age group and confuse the people connected to them, they sometimes find it very difficult to manage and balance all these emotions at once. They sometimes need the outer support in the form of different mentors who could be teachers, parents or anyone who has more influence on them and sometimes they need to depend on their inner strengths. As people find it difficult to understand what is going on inside others and it makes it difficult for them to comprehend their emotions truly it becomes difficult to provide outer help and support all the time. Adolescence is the age when children face many different complex issues all at once, there are many changes taking place in their body and outside where they are dealing with finding the stable identity and role in the society along with all the other distractions that could be very tempting. They are faced with the seriousness of the age challenges, but also the excitement of this period which is the main reason that we need to focus on them and keep directing them. But many times, teenagers find it difficult to follow orders, so, the decisions of their life should come with their own mind. So, that is why we don't need to direct them to do things but we need help them in understanding their inner strengths that could help them with the appreciation of self and provide them with the internal positive tools that they can use in times of need like emotional intelligence and spiritual intelligence which could help them in attaining overall psychological wellbeing.

Achieving wellbeing is not a one step process and is not permanent, it takes time and continuity. So, it cannot be just given, it needs to be developed with time. What one can do is make it easy for adolescents to understand its importance and help them in the process of becoming the better versions of themselves, and then become a more fulfilling and productive individual. Psychological wellbeing is a sense that depends on how a person organizes their stream of consciousness into creation and development. This is possible only when a person possesses certain resources and strengths and uses them to channelize energy in a constructive and positive manner. Individuals who are high on psychological wellbeing function better and more towards the gathering of the positives in their life than the one who are low on this variable. This variable strengthens the overall mental health and increases the feelings of positive mood, love, gratitude, interest, satisfaction and helps to decrease the negative feelings like envy, anxiety, anger, dissatisfaction and fight with the challenging situations in life. With just the development of this variable of positive mental health we can help in increasing the other positive aspects of life because person who is high on psychological wellbeing will find it easy to comprehend all the other important areas of life and become selfsufficient and more virtuous, they become internally motivated to move towards becoming the better versions of themselves. Researcher Carol Ryff (1995) explained this with the help of six different factors that contribute to psychological well being, the factors are self-acceptance, purpose in life, environmental mastery, personal growth, positive relationships with others and autonomy. When we focus on adolescents age group the impact and importance of this variable increases as teenagers face the changes in all the above-mentioned factors all at once, as they are growing biologically it will affect their relationships with others, their education is at very crucial point, they need to more positive about their developing body and mind to find the balance.

So, we need to educate the adolescents with the positive psychology and help them to develop psychological wellbeing and the different strengths that comes along with it, this kind of education will provide them with all the integral tools that help them in maintaining and integrating the practicality of life along with the other internal aspects of the human experience of life. In adolescents it is important to pay attention to areas of emotional and spiritual intelligence to make them more effective in their performance towards different challenges. Emotional intelligence is the positive strength that develops the ability of understanding, recognising and managing the emotions of self, it also helps the people in influencing the emotions of others. Emotional intelligence makes people more aware of their emotions and their impact on their cognition and their behaviour and attitude which could be





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either negative or positive. A more emotionally intelligent adolescent becomes a more experienced young adult who will manage the different difficulties of life all the more efficiently and they will act fuller grown and will be better fitted in the general public. They will know the significance of acting sensibly and they will be more lenient. Their decisions and the approach to managing different circumstances will likewise be different then the understudy who have lesser EQ. In this way, the ability to appreciate anyone at their core could assume a significant part in the better change and better execution of the adolescents. When an individual becomes more emotionally intelligent, they understand their inner selves more clearly and this then helps in development of spiritual intelligence, which then further in combination with the other aspects of self helps in building the various abilities of individuals. The idea of spiritual intelligence is of more recent origin by Danah Zohar who coined this term in 1997 in her book.

Danah explained the concept of spiritual intelligence by the help of twelve different principles. The principle are Self-awareness, Spontaneity, Holism, being vision and value led, Compassion, Field independence, Celebration of diversity, Humility, Ability to reframe, Sense of vocation, Tendency to ask fundamental 'why' questions, Positive use of adversity According to researcher Richard (2012), Spiritual intelligence is one of the higher components of the internal knowledge that enables an individual to attain a credible self which is a kind of higher inner self that provide them the abilities like empathy, optimistic nature, altruism, love, gratitude, etc, it brings about the feelings of profound significance and meaning in life it gives life a wider inner scope to work with inner strengths and fundamental abilities. Spiritual Intelligence and Emotional Intelligence are those internal strengths that develop over time in our life and when we grow, we face different situations and deal with them they develop even more. But if it is possible to learn it somehow during the start of most of the challenges in life that is adolescence then it helps the young ones in achieving a higher psychological wellbeing. This is important to consider because till now most of the training and education provided to the students is focused on general Intelligence, that helps them grow academically, but it will be much more productive for students if their education could be more focused on the positive aspects of being.

Review of Literature

Walter & Routray (2022) conducted a study to find out the impact of the body and mind practice on the subjective well-being, emotional intelligence and compassion of 186 Indian college students. The results of their study showed a positive correlation of compassion and EI, but there was no significant correlation of EI and subjective wellbeing. Gonzales (2022) discussed in a review paper about how the school and its environment can help their students in increasing their levels of social emotional intelligence with the help of EI models and its combinations. They explained how emotional intelligence could help students in dealing with various mental health issues. Dhami, Sharma & Kang (2021) conducted research to assess the mental health of youth from two different states of India (Punjab and Uttrakhand) on 360 youth aged between 20-22 by using mental health battery and spiritual intelligence scale. The results of their study showed that the mental health of Punjab youth was better in comparison. A significant correlation was found in spiritual intelligence and mental health. They suggested some spiritual intelligence interventions. Balluerka, Gorostiaga *et.al.* (2016) conducted research to analyze the peer attachment and class emotional intelligence as predictors of adolescents' psychological well-being, on 2182 school students. The results of their multi-level approach showed a decrease in well-being when adolescents grow older, and they get engaged in higher peer attachment. Ahmadi, Azar *et.al* (2014) conducted a study on 800 employees of a bank in Teharan, Iran to study the effect of emotional intelligence on psychological well being.

Results of their study showed the positive relationship between the two variables. James, Bore & Zito (2012) conducted a study to examine the relationship of Big five personality traits and emotional intelligence with the psychological wellbeing in a sample of 150 law students. The results showed the positive relationship between the 3 of the traits with wellbeing and emotional intelligence. Davis and Humphrey (2012) conducted research to find out the influence of emotional intelligence on coping and mental health in adolescents, they analyzed 784 adolescents by using mediational analysis. The result showed that the ability emotional intelligence model has a significant relationship with coping and mental health. They gave the implications for the interventions. Salami & Samuel (2011) studied the link between the Big Five personality characteristics and teenage psychological well-being, as well as the





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moderating influence of emotional intelligence, for this they selected 400 students of secondary schools located in south-western Nigeria. The results showed that the emotional intelligence and personality factors are significantly correlated with variable psychological well-being and Emotional intelligence also plays a role in moderating the relationship among extraversion, neuroticism, and psychological well-being. Carmeli, Halevy & Weisberg (2009) conducted a study to examine the relationship emotional intelligence and four different aspects of psychological well being, on a sample of employees, with the help of administration of surveys that were structured, there were two surveys taken. Results showed a positive relationship between the four aspects of psychological wellbeing and the variable emotional intelligence.

METHODS

Objectives

- 1 To explore the relationship between Emotional Intelligence, Spiritual Intelligence and Psychological Well Being.
- 2 To study the predictive role of Spiritual and Emotional Intelligence on Psychological Well Being.

Hypothesis

H1: There will be a positive relationship between Emotional Intelligence, Spiritual Intelligence and Psychological Well Being.

H2: Emotional Intelligence and Spiritual Intelligence will significantly predict the Psychological Wellbeing of adolescents.

Sample

The sample consists of 300 students, between the age of 15-19 years. The data is collected from the students of eight different schools by the help of questionnaires.

Inclusion criteria

- Students within the age 15-19 years.
- Students who belong to urban areas.
- Students enrolled in mainstream private schools.

Exclusion criteria

- Adolescents with a chronic or life-threatening illness, physical or psychological disorder.
- Dropouts or ex-students.
- Adolescents from dilapidated houses / Single parents

Instruments

Emotional intelligence scale (Singh and Narayan, 2004)

This test is a kind of self-report measure that analyses emotional intelligence. It has 31 items. Its responses are 'yes' or 'no'. The emotional intelligence will be interpreted as high when the score is above 27. The test has 0.86 test-retest reliability. And it is valid at .01 significance level.

Spiritual Intelligence self-report inventory (David, 2008)

This test uses a scale of 24 items, the items measure the spiritual intelligence of a teste. It consists of a five-point Likert scale. The higher level of spiritual intelligence is measured by the higher level of score. The test has 0.95 test-retest reliability and 0.94 split half reliability. The validity of the test is significant at .01 level.

Ryff's Psychological Well Being Scale (Ryff, 2007)

This test measures psychological wellbeing of an individual by the help of 18 items on a 7-point Likert scale. The levels of psychological well being are estimated by the higher levels of scores. Higher the score more the





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psychological wellbeing. It has 0.82 test-retest reliability. And its construct and concurrent validity is significant at .01 level.

Procedure

The sample is collected by the purposive sampling, the students were selected randomly. The ethics were taken into consideration by taking their consent and informing them about the confidentiality of their responses. Brief instructions were given, and administration was done by using the questionnaires. Analysis was run through SPSS.

Research Design

Causal research design will be used

RESULT

Table 1 shows that Psychological Wellbeing scores correlated positively with Emotional Intelligence (r=0.290; p<0.001) and Spiritual Intelligence (r=0.374; p<0.001). Emotional Intelligence score is positively correlated with Spiritual Intelligence (r=0.216; p=0.001). Results in Table 2 presented that the two independent variables (Emotional Intelligence and Spiritual Intelligence) yielded a joint coefficient of multiple regression (R) of 0.499. In Table 4 regression analysis showed that the two variables independently significantly predict Psychological Wellbeing. Emotional Intelligence was predictive of Psychological Well Being (β =0.219; p<0.001), and Spiritual Intelligence was also predictive of Psychological Well Being (β =0.326; p<0.001).

DISCUSSION

The purpose of the present study was to gain a better understanding of the relationship of variables Emotional Intelligence, Spiritual Intelligence and Psychological Well Being. The results of the present study shows that the variables were positively and significantly correlated. Also, both the independent variables Emotional and Spiritual Intelligence significantly predicts Psychological Wellbeing. The results support both the hypothesis that were proposed in the study, that Emotional Intelligence, Spiritual Intelligence and Psychological Wellbeing are positively correlated and that variables Emotional and Spiritual Intelligence significantly predicts Psychological Wellbeing. The study was done to develop a new understanding towards the mental health of adolescents and gain a new perspective towards them. The study also shows the levels of psychological wellbeing in adolescents. It validates the links among the variables and the benefits of their development in adolescents. The both variables Emotional and Spiritual Intelligence are dependent on time and various life situations for development and they keep on increasing when the people deal with various issues. In adolescents it will be very fruitful to develop these variables as they provide internal positive strengths to deal with the difficult situations and according to the results of this study it will also be helpful in developing and increasing there Psychological Wellbeing which will help in improving the mental health and wellness of adolescents as they are at a very sensitive and challenging age and come across with various issues on daily basis, and the development of the emotional abilities that comes with emotional intelligence and spiritual strengths that includes understanding of higher inner self, which will help adolescents in dealing with various thoughts related to existence and meaning of life.

This pattern of results in relation of emotional intelligence with adolescents is consistent with the previous literature by Walter & Routray (2022) who found the similar positive effect of emotional intelligence in students wellbeing, and the similar positive correlation and prediction shows that why is it important to introduce the concept of emotional intelligence with the general traditional intelligence in students, also if we try to develop emotional intelligence it will be productive as it will make the students more healthier mentally and help them in becoming more productive adults. The present results are also consistent with Gonzales (2022) work that emotional intelligence provides students with various abilities to deal with different mental health issues that arise at this age. These results are





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consistent with the claim that emotional intelligence is positively correlated with coping and mental health and could help in the development of various interventions (Davis and Humphrey, 2012).

The susceptivity of this age group, and the transitioning and the developmental phase, challenges the students with very difficult situations like decision making, role identification, peer involvement, career goals, better performance, physical body changes, involvement and status in the society, participation in productivity of the society, etc, and with the help of introduction and understanding of emotional intelligence at this developmental stage will be very efficacious, favourable and fruitful. The result of this study provides supporting evidence that when we accommodate and provide students with the inner strengths like emotional and spiritual intelligence at the earliest by the help of some designed interventions in their schools, it will result into their positive development and help them in improving their overall psychological well being. Finding of the study highlights the need of the development of the interventions. The study provides the links for the better understanding of the positive strengths in adolescents and increase the theoretical basis of positive psychology and its utility in the field of education, because even today majority of the schools focuses on the career development by providing fine education to their students, and they judge or categorize these students on the basis of their performance in the traditional methods of testing and that categorisation leads to the presumption of the success of their children in future. They tend to ignore the fact that the success and productivity in the outer world is not only the result of the traditional education but an outcome of the overall personality of an individual. The personality development includes development of overall wellbeing which will be the result of the development of the internal strengths that helps students in dealing with the unpredictable, challenging and difficult situations.

Limitations

The present study was conducted by the help of self-report measures; it is therefore reasonable that social expectations may have influenced the responses, especially considering that respondents who were students filled the scales in an academic setting. Also, the variables for students' socioeconomic status who are deprived and belong to more or less affluent classes which are not considered in the selection of sample may influence certain risk behaviours. There are certain limitations in this study that could be addressed and dealt with in future research, like gender comparison. Despite the limitations of the study, the results suggest a number of theoretical and practical implications, especially the data that provide the potential interventions implications. The current study brings together two areas of research, namely emotional intelligence and spiritual intelligence. Both areas of study receive their own attention, but they are rarely analysed together, especially with this particular age group. The contribution of research can be that they enhance many interesting questions for future research.

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Conflicts of interest

There are no conflicts of interest

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Table 1: Mean and Standard Deviations

	Mean	Standard Deviation
Emotional Intelligence	18.5	3.2
Spiritual Intelligence	55.9	13.1
Psychological Well Being	84.6	13.1

Table 2: Correlations

		Spiritual Intelligence [SQ]	Psychological Wellbeing [PWB]	Emotional Intelligence [EQ]
Coiritual	Pearson Correlation	1	.374**	.216**
Spiritual Intelligence [SQ]	Sig. (2-tailed)		<.001	<.001
interrigence [SQ]	N	299	299	299
Dovebalagical	Pearson Correlation	.374**	1	.290**
Psychological Wellbeing [PWB]	Sig. (2-tailed)	<.001		<.001
vvenbering [F vvb]	N	299	299	299
Emotional	Pearson Correlation	.216**	.290**	1
2	Sig. (2-tailed)	<.001	<.001	
Intelligence [EQ]	N	299	299	299

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





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Table.3: Regression Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.499a	.249	.241	11.437

Note. Predictors: (Constant), Emotional Intelligence [EQ], Spiritual Intelligence [SQ]

Table 4: ANOVA

Model		Sum of Squares	Df	Mean Square	F	Sig.
	Regression	9521.335	2	4760.668	33.685	<.001b
1	Residual	41833.655	296	141.330		
	Total	51354.990	298			

Note. a. Dependent Variable: Psychological Well Being [PWB] b. Predictors: (Constant), Spiritual Intelligence [SQ], Emotional Intelligence [EQ]

Table 5: Coefficient

Model		Unstandardized Coefficients		Standardized Coefficients	Т	Sig.
		В	Std. Error	Beta		
1	(Constant)	49.907	4.547		10.976	<.001
	Emotional Intelligence [EQ]	.891	.219	.219	4.077	<.001
	Spiritual Intelligence [SQ]	.325	.053	.326	6.075	<.001

Note. a. Dependent Variable: Psychological Well Being [PWB]

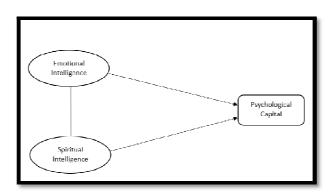


Figure.1: Statistical Analysis
Correlation and Multiple Linear Regressions



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RESEARCH ARTICLE

A Geo-Location based Browser for Secure Internet Banking

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ABSTRACT

With banks arriving at its clients by means of versatile banking, it is getting to be one of the fundamental highlights that are requested by pretty much every advanced mobile phone client. Versatile banking by means of a portable program is like web banking. Observation-based threats to PDAs are similar only to those for PCs, raising the need to focus on portable security. Among the few validation plans, geo area verification is picking up significance as it is discovered most reasonable for cell phones. In this paper, GeoMoB, a dedicated secure portable program for multifaceted financials using multidimensional confirmation, is structured and created. GeoMoB highlights a geolocation based verification plot that guarantees the security of portable exchanges dependent on the client region. Irrespective of the current two factor verification scheme using client ID, password and OTP, mobile number and geolocation are used for customer verification. Geolocation refers to the area of the banks from where the exchange will take place hence helping the banks to guarantee secure exchanges. The geolocation of the client is obtained through the system supplier and subsequently the need to use GSM is settled. The multifaceted confirmation utilized in Geo MoB guarantees security while performing portable exchange and keeps clients from different assaults.

Keywords: GeoMob, Banking, OTP, geolocation, portable security





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INTRODUCTION

Cell phones offer a few different ways to get to an administration which may incorporate portable applications, programs and even as gadgets. It goes to the choice of the business to pick how to arrive at their clients. Despite the fact that web applications are in effect ordinarily utilized among the clients, programs locate their own significance. Programs are the ones that empower the client to see sites on their hand-held gadgets while web applications or applications are the ones that are to be downloaded on the client's work area/PC so that once downloaded it might be utilized whenever. In spite of the fact that web applications are the most effortless path in getting to an administration, programs are favored in getting to different administrations as they have certain focal points when contrasted with the web Applications. A program called a smaller than expected program, miniaturized scale program or remote Internet program (WIB), is an internet browser intended for work area, workstation and tablets. They are uniquely planned in order to show web content for little screens. Program programming must be little and productive to suit the low memory limit. Some regular portable programs are Google Chrome, iris, Mozilla Firefox, fuel, Apple Safari, Opera, Internet Explorer, Maxthon, Blackberry, UC program, and so on.

As indicated by current insights, it very well may be seen that the pattern of portable web is developing immensely over work area web. Since 2013, a bigger number of tablets and advanced cells were sold than PC's., acquiring the requirement for versatile programs. Presently a days, world is tending towards ruled web. One out of each ten costumers is going to a site utilizing their work area, PC gadgets. More individuals in Africa have a workstation, work area than access to power. Programs make the different sites in a split second accessible dissimilar to PC, work area applications that should be downloaded for getting to administrations. The internet browsers are equipped for rendering sites in a typical manner while if there should arise an occurrence of applications, the working framework must be considered. The favorable circumstances that programs are that there is no requirement for continuous updates and makes the sites in a split second accessible. The financial situation has a few dangers related with it. Untouched network to the web in the work area, workstation gadgets have cleared route to a few assaults incorporating man in the center assault, phishing assaults and so forth making security a significant factor to be viewed as when giving administrations to the client. At the point when the business chooses offer their administrations through work area, PC programs, there comes the requirement for picking the proper program. A few open and private segment banks have propelled work area, PC applications to fulfill the client requests.

Yet, the downside with such applications is that the continuous updates and the unsurpassed network have represented a few dangers. Subsequently getting to work area, workstation banking administrations through versatile programs is substantially more critical. A few versatile programs have been developed since the approach of work area, workstation, yet they are looked with a few weaknesses. In the underlying days when the work area, workstation programs were into the PDAs, rendering the sites on the handheld gadgets was one of the serious issues. In any case, today the test is as far as keeping from different assaults. Despite the fact that internet browsers are furnished with advanced highlights regarding security, work area, workstation programs are yet to think of such changes. The issues in the current work area, workstation program have prompted the requirement for advancement of a safe work area, PC program for banking based exchanges.

Problem Statement

Presently days web banking isn't completely protected, part of security risk is there to break the security or attempting to abuse client information. Presently day's program not excessively much progressed to deal with all sort of security issues essentially for web banking.

Motivation

Program applications are the most straightforward route in getting to an administration; programs are favored in getting to different administrations as they have certain focal points when contrasted with the versatile applications. A portable program called a minibrowser, smaller scale program or remote Internet program (WIB), is an internet





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browser intended for cell phone and tablets. They are uniquely planned in order to show web content for little screens. Program programming should be short and efficient to oblige the low memory limit.

Literature Survey

This part is considered based on a comprehensive write-up study identified with Geo Location Based Browser for Secure Internet Banking. Gorde, 2016 A PC actualized technique for utilizing geo-area data in touchy Internet exchanges is revealed. In one model, such a technique may include: 1) getting, from a customer gadget, a solicitation to direct an Internet exchange, 2) requiring geo-area data from the customer gadget so as to lead the Internet exchange, 3) accepting the geo-area data from the customer gadget, 4) confirming the legitimacy of the geo-area data, and after that 5) leading the Internet exchange. Different strategies, frameworks, and PC decipherable media are likewise unveiled. Gupta, 2016 the web advancement in the present situation is encountering an abundance of phenomenal changes. Today, web progression methodology is driven by magnificent capable get-togethers, yet they don't have genuine planning and contribution in information structure plan in view of which diverse specific instruments bears extraordinary issues. For application architect, web development speaks to another method of programming designing with new mechanical assemblies, new frameworks and new designs. Hence, there's a need to find a fitting way to deal with adjust up to these troubles of web application improvement. This paper focuses on the differing estimation methods and the contraptions that are used for web progression. This paper in like manner means the diverse genuine and genuine issues and troubles that should be taken under idea while developing broad web applications.

Tsuchiya, 2016 Man-in-the-Browser (MITB) assaults are brought about by malware that taints an internet browser; thus, ordinary secure correspondence channels between a machine (bank server) and a machine (internet browser, for example, SSL can't avert the assaults. In this paper, we propose a way to deal with avoiding MITB assaults by building secure correspondence channels between a machine (bank server) and a human (end client). Our methodology utilizes the client as a computational asset and solicitations the client to process an end side of the channel. Building up a test and reaction convention that accomplishes the proposed channel, we directed a wellbeing assessment of the convention. The outcome demonstrates that the convention works securely under the supposition that the bank server can send a "challenge that malware in the program can't see" to the client. We additionally demonstrate that sending the test is possible by applying CAPTCHA innovation. Xi, Kai, 2011 With quick advancement of cell phones and portable system, the need of ensuring client touchy data locally and performing secure client verification remotely become ever more expanding. Bio-cryptography is developing as an amazing arrangement which can consolidate the benefits of traditional cryptography and biometric security. In this paper, we present a productive bio-cryptographic security convention intended for customer/server verification in current portable figuring condition, with a sensible suspicion that server is secure. In this convention, unique finger impression biometric is utilized in client check, ensured by a computationally effective Public Key Infrastructure (PKI) conspire, Elliptic Curve Cryptography (ECC). The certifiable unique mark data is covered up in the component vault which is the blend of real and refuse highlights. Unique finger impression highlights are utilized for biometric check as well as for cryptographic key age. Our security investigation demonstrates that the proposed convention can give a safe and reliable validation of remote versatile clients over unreliable system.

Exploratory outcomes on open space database demonstrate a worthy confirmation execution. We likewise tried the computational expenses and effectiveness of our convention on the CLDC emulator utilizing Java ME (past J2ME) programming innovation. The reenactment results demonstrate that the proposed convention suits current versatile condition. Grier, 2008, Current internet browsers are tormented with vulnerabilities, giving programmers simple access to PC frameworks through program based assaults. Program Security Efforts In the light of the fact that the planning of existing programs is very fundamentally incomplete, redeployment of existing programs has hindered achievement. To empower progressively secure web perusing, we plan and execute another program, called the OP internet browser that endeavors to improve the cutting edge in program security. Our general plan approach is to consolidate working framework structure standards with formal strategies to structure a progressively secure internet browser by drawing on the skill of the two networks. Our general structure reasoning is to segment the





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program into littler subsystems and make all correspondence between subsystems basic and unequivocal. At the center of our plan is a little program piece that deals with the program subsystems and intervenes on all interchanges between them to implement our new program security highlights.

System Analysis

Existing System

With banks reaching out to their customers through diversified banking, this is becoming one of the basic features that are requested by each advanced mobile phone customer. Banking through a program is like web banking. Observation-based threats to advanced mobile phones are the same as for PCs only, indicating the need to focus on portable security.

Proposed System

The proposed Geo Secure Banking is planned so that it is devoted for utilizing just secure banking. Geo Secure Banking gives an interface to access bank sites where the client has accounts just as guarantees secure verification systems to approve the client.

RESULTS

The proposed Geo Secure Banking program utilizes the geo area based verification alongside a few other confirmation systems making it a successful multifaceted validation plot for versatile financial situation. Validation in portable banking depends on three sorts information based, object based and Biometric based. Aside from the current verification instruments, this theory recommends the utilization of area based data for validating a client in secure financial situation. Secure banking encourages the client to perform exchanges in any area, consequently area based verification can be considered for confirming a substantial client. The accompanying table I utilizes four components for contrasting verification systems accessible for secure banking to be specific unpredictability, misrepresentation anticipation, client trust and cost. Unpredictability alludes to the execution of the confirmation system to the protected financial situation. It very well may be seen that however biometric based confirmation plan has an abnormal state of client trust and extortion anticipation, it has high intricacy in executing and coordinating to the safe financial situation. This is on the grounds that gadget support as far as designs and sensor exactness is the way to verifying the correct person. The above table features the advantages of utilizing proposed framework geo area verification when contrasted with existing confirmation systems that are connected for guaranteeing verified validation in secure banking. It tends to be seen that however the misrepresentation avoidance and client trust is high, it might be upgraded by utilizing multifaceted validation. Its low multifaceted nature and cost makes it simple to execute to verify banking. Secure banking in the present situation utilizes two-factor confirmation conspire and is moving towards the usage of multifaceted validation plot. For this situation the selection of area based verification makes it an important expansion to perform secure money related exchange through secure program.

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Table.1:Comparison of Authentication Mechanisms in Secure Banking

Authentication	Examples	Complexity	Fraud prevention	Customer trust	Cost
Knowledge based	Username, password, security questions, Images	Low	Low	Medium	Low
Object based	Credit/Debit cards	Low	Low	Low	Medium
Biometric based	Fingerprint	High	High	High	High
Proposed System (Location Based)	Geo location, security questions, Images capcha	Low	High	High	Low





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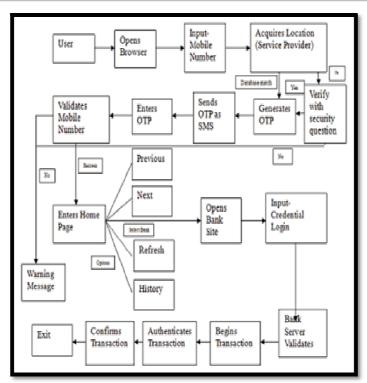


Figure 1. System Architecture





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ISSN: 0976 – 0997 **RESEARCH ARTICLE**

Tomato Plant Leaf Disease Classification by using Morphological **Operations and Machine Learning Techniques**

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ABSTRACT

Simple, rapid and accurate prediction methods have great importance in disease management of crops, since, they can give a scope to take early necessary disease control measures. The proposed work is to classify the diseases of tomato, such as bacterial spot and mosaic using Morphological Operations and Machine Learning techniques. To the leaf symptoms of tomato, a series of methods like Image acquisition, Pre-processing, Segmentation, Feature extraction and Classification in Python platform. The classifiers such as KNN, Logistic regression, Naive bayes, Decision Tree, Random Forest classifier (RFC) and Gradient boost were applied to predict the bacterial spot and mosaic diseases of tomato. A range from 10 to 50% test sample size applied and compared for disease prediction. Among the all the classifiers, Gradient boost has given best fit prediction for diseases of tomato with an accuracy of 98% at 10% of test sample size.

Keywords: Histogram equalization, GLCM, Decision Tree, RFC and Gradient boost classifier.

INTRODUCTION

Globally agriculture is severely suffering from biotic and abiotic factors. Phytopathogens are the major biotic factors which infects crops and account for significant yield loss. It is estimated that phytopathogens are involved in crop loss up to 20-30% annually (Kashyap et al., 2017). Agriculture is the major economic source of India; however, it is severely suffering from enormous diseases by pest and pathogens. Hence, there is a significant yield loss has been





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reported in every year. Stringent disease management practices are essential to control and spread of diseases. The very first step in disease control is early and accurate detection of disease, which requires specific diagnostic tools and experts. There are some effective and reliable laboratory-based tools like PCR, ELISA, Western blot, etc. developed for accurate disease detection. However, they have their own constraints and limitations like expensive. time consuming, labour intensive and requirement of scientific experts. Further, lack of correct disease diagnosis leads to usage of non-specific pesticides an adequate level for agriculture, which is toxic to the environment as well as human health. In view of these limitations of diagnostic there is a need to develop an alternative and effective approach with rapid and reliable detection of diseases. In recent years machine learning based approaches have gained attention for prediction of plant diseases based on the characteristic features of the diseases. Tomato is one of the highly consuming vegetable crops in the world, susceptible for many diseases and account for significant yield loss. In this study we have classified two major economically important diseases of tomato including tomato bacterial spot and tomato mosaic caused by Xanthomonas vesicatoria and Tomato mosaic virus, respectively. Classification of diseases has done based on the characteristic symptoms of the infected plant leaves, which are available in Kaggle-Plant Village. The common symptoms of the bacterial spot including circular or round dark brown or black spots on the leaves and extension of multiple lesions leads to the necrosis. Similarly, the mosaic disease with light and dark green spots on the leaves, leaf curling, yellowing and fern-lime in appearance (Basavaiah and Anthony, 2020).

Literature survey

Pallathadka et al., (2021) proposed that SVM classifier has high accuracy over Naïve bayes and CNN for detection and classification of rice leaf diseases like bacterial leaf blight, brown spot and leaf smut. In their study they have applied resize, histogram equalization, K- means clustering, PCA and the classifiers SVM, Naïve bayes and CNN. These three classifiers have shown accuracy of 96.2%, 78.8% and 91.3% for detection of diseases of rice, respectively. Basavaiah and Anthony, (2020) proposed that RFC has high accuracy than Decision Tree for detection of tomato leaf diseases like bacterial spot, septoria spot, mosaicand yellow curl. In their study they have applied resize, multiple feature extraction methods (colour histograms, hu moments, haralick and LBP) and the classifiers Decision Tree and RFC. These two classifiers have shown accuracy of 90% and 94% for detection of diseases of tomato, respectively. Harakannanavar et al., (2022) proposed that CNN classification has superior accuracy than SVM and KNN for identification of tomato disorders. In their study they have applied resize, RGB to Gray conversion, histogram equalization, K-means clustering, contour tracing and multiple descriptors (DWT, PCA and GLCM) and the classifiers SVM, KNN and CNN. These three classifiers have shown accuracy of 88%, 97% and 99.6% for identification of disordered samples of tomato, respectively. Hatuwal et al., (2020) proposed that CNN is the best classifier than SVM, KNN and RFC for detection of various diseases in apple, cherry, grape, peach, bell pepper and strawberry. In their study they have applied conversion of input image to gray scale, gaussian noise, haralick texture algorithm of GLCM and the classifiers SVM, KNN, RFC and CNN. These four classifiers have shown accuracy of 78.61%, 76.969%, 87.436% and 97.89% for detection of diseases of above plants, respectively.

Bhangea and Hingoliwala, (2015) proposed that morphology has given best results for detection of blight disease in Pomegranate fruit. In their study they have applied resize, colour, morphology, CCV feature vectors, K-means clustering and the classifier SVM to both leaf and fruit images. The SVM classifier have shown accuracy of 82% for detection of diseases of pomegranate. Lofstedt *et al.*, (2019) used invariant haralick texture features in different classification steps and obtained superior results over the original definitions suggesting the features are reproducible even when different graylevel quantization's are used. Patil and Burkpalli, (2021) proposed that colour features extraction better than texture features extraction based on the evaluation of performance parameters of the classifiers (precision, recall, F-measure and matthews correlation coefficient). In their study they have applied resize, MFACM, colour and texture features and the classifier multilayer perceptron, SVM, Naive Bayes, RFC, Adaboost and KNN. These six classifiers have shown accuracy of 96.69%, 93.38%, 90.91%, 95.86%, 92.56%, and 94.21% for cotton leaf classification, respectively. It suggests that colour features are enough to classify between healthy and unhealthy cotton images using weka tool. Panchal *et al.*, (2019) proposed that HSV alteration method proved to be very efficient for detecting the diseased part of the leaf, adding GLCM improved the accuracy for detect and classify





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bacterial spot of pepper bell and tomato and early and late blight of potato and tomato. In their study, the image resized, unwanted noise removed, converted BGR to gray scale as well as HSV format and used smoothing filter, K-means clustering or HSV value alteration, GLCM and the classifier RFC. The RFC classifier have shown accuracy 98% for detection and classification of diseases of pepper bell, tomato and potato. Zamani *et al.*, (2022) proposed that RBF-SVM classification shown better performs than SVM, RFC and ID3 for accurate detection of rice leaf diseases like leaf smut, leaf blight and brown spot. In this study they have applied mean filter, histogram equalization, K-means algorithm, PCA and the classifiers RBF-SVM, SVM, RFC and ID3. The evaluation of performance parameters of the classifiers such as accuracy, sensitivity and specificity have used for detection of diseases of rice.

PROPOSED METHODOLOGY

The proposed methodology of this study consists of a series of steps including Image Acquisition, Image Preprocessing (Resize, convert to Gray and Histogram equalization), Segmentation (Morphological operations), Feature extraction (GLCM) and Classification (KNN, Logistic Regression, Naïve Bayes, Decision Tree, RFC and Gradient boost) for prediction of disease in tomato leaves.



Flowchart: Work plan of proposed methodology

Image Acquisition

The Tomato leaf image dataset of this study has taken from the Kaggle-Plantvillage. The dataset with 2500 JPG images of diseases of tomato including Tomato_bacterial_spot (2127) and Tomato_mosaic (373).

Image Pre-processing

It is a method to remove non-essential distortions and improve the quality of the image. Images of this study have resized into 128×128 followed by conversion intogray colour and histogram equalization.

Image Segmentation

Morphology generally concerned with shape and properties of objects. In this study we used Opening, Gradient and Closing operation. (Gonzalez and Woods.,2004, Zhao *et al.*, 2006, Said *et al.*,2016, Bhangea and Hingoliwala., 2015, Nagaraju *et al.*,2011)

Opening

The major steps in opening are erosion followed by dilation. It removes the noise from the image. The mathematical expression for the Opening is

 $f \circ b = (f \ominus b) \oplus b$

In OpenCV the syntax of the morphological Opening is cv2. morphologyEx(image, cv2.MORPH_OPEN, kernel)





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Morphological Gradient

The major criteria of morphological gradient is to know the difference between dilation and erosion of an image. It highlights sharp gray level transitions in the input image and the result will look like the outline of the object. The mathematical expression for the morphological gradient is

$$g = (f \oplus b) - (f \ominus b)$$

In OpenCV the syntax of the Morphological Gradient is cv2.morphologyEx(image, cv2.MORPH_GRADIENT, kernel)

Closing

The major steps in closing are dilation followed by erosion. It is reverse to the opening. The mathematical expression for the Closing is

$$f \cdot b = (f \oplus b) \ominus b$$

In OpenCV the syntax of the Closing is cv2.morphologyEx(image, cv2.MORPH_CLOSE, kernel)

Feature Extraction (GLCM)

In this study, we have used GLCM statistical features such as contrast, dissimilarity, homogeneity, ASM, energy and correlation. (Mohanaiah *et al.*,2013,PS and VS.,2016, Lofstedt *et al.*, 2019)

$$\begin{aligned} & \text{Contrast} = \sum_{i,j=0}^{N-1} P_{i,j} (i-j)^2 \\ & \text{Dissimilarity} = \sum_{i,j=0}^{N-1} P_{i,j} | i-j| \\ & \text{Homogeneity} = \sum_{i,j=0}^{N-1} \frac{P(i,j)}{1+(i-j)^2} \\ & \text{ASM} = \sum_{i,j=0}^{N-1} \frac{P(i,j)}{1+(i-j)^2} \end{aligned} \qquad \begin{aligned} & \text{N - Number of Gray levels,} \\ & P(i,j) = \text{Gray scale normalized value at positions } i \text{ and } j, \\ & \mu = \text{Mean, and } \sigma = \text{Standard deviation.} \end{aligned}$$

$$& \text{Energy} = \sqrt{\text{ASM}}$$

$$& \text{Correlation} = \sum_{i,j=0}^{N-1} P_{i,j} \left[\frac{(i-\mu_i)(j-\mu_i)}{\sqrt{(\sigma_i^2)(\sigma_i^2)}} \right]$$

- Contrast measures the variance between the high and lowest intensity values in the adjacent pixels
- Dissimilarity measures the assessing local variations in the image
- Homogeneity measures the uniformity in intensity values of the image
- Angular Second Moment (ASM) measures the uniformity in the distribution of intensity values within the image
- Energy measures the uniformity in the distribution of gray tones.
- Correlation measures the linear dependencies of intensity values in the image.

Classification

In this study we have used the KNN, Logistic regression, Naïve bayes, Decision Tree, RFC and Gradient boost classifiers for prediction of tomato leaf diseases. The dataset which is taken for the study having the infected leaf images of bacterial spot (2127) and mosaic (373) caused by *Xanthomonas vesicatoria* and *Tomato mosaic virus* respectively.





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Evaluation of performance parameters of the classifiers (Pujari., 2001)

 $=\frac{}{TP + FP}$ F1-score = $\frac{2 * Precision * Recall}{Precision}$ TP + FN

Precision + Recall

Here, TP=True Positive, TN=True Negative, FP=False Positive and FN=False Negative

RESULTS

Plant disease control is an uphill task, since, an array of pathogens has involved for many diseases. However, there are approaches available for effective control and management of diseases under integrated pest management. Tomato is the second most largest cultivating important horticulture crop in the world. It is cultivating as a major vegitable crop in India, however, it is severely suffering from various plant pathogen infections and account for significant yield loss. Among the plant pathogens, bacterial and viral infections have negative impact on crop production. The major economically important diseases of tomato are Tomato_bacterial_spot and Tomato_mosaic caused by Xanthomonas vesicatoria and Tomato mosaic virus respectively. In recent years, Image processing and Machine learning based approaches have gained an attention in agriculture for prediction and classification of plant, fruits, seed, pest and diseases.(Fu et al., 2019, Khatri et al., 2022, Selvaraj et al., 2019, Murali and Nagaraju., 2022, Liu et al.,2018, Phadikar et al., 2012, Parmar et al.,2018, Liu et al.,2019, Mokhtar et al., 2015, Jayswal HS and Chaudhari JP, 2020, Sorte et al., 2019, Hatuwal et al., 2020). In this study, applied morphological operations and machine learning techniques for prediction of tomato disease like bacterial spot and tomato mosaic disease. The training dataset for this study having infected tomato images of bacterial spot (2127) and tomato mosaic (373) caused by Xanthomonas vesicatoria and Tomato Mosaic virus, respectively. The input image (256 × 256) was resized into (128 × 128) followed by conversion into gray colour and histogram equalize (Fig1). Later to the image morphological operations like Opening, Gradient and Closing were applied (Fig1) and subjected for feature extraction with GLCM statistical parameters like contrast, dissimilarity, homogeneity, ASM, energy, correlation (Fig. 2). As a final step classification was done based on the feature extraction. From the results, the KNN, Logistic regression, Naïve bayes, Decision Tree, RFC and Gradient boost classifiers have shown an accuracy of 88.4%, 89.6%, 92.8%, 93.2%, 96.8 and 98% for classification of tomato diseases, respectively. Out of the comparison of the test size, the best fit of 98% was obtained with 90% training and 10% test size with Gradient boost classifier (Table 1 & Graph 1). To the above classifiers applied evaluation of performance parameters such as Accuracy, Precision, Recall and F1-score. In this study, the highest accuracy obtained Gradient boost classifier with 98%.

The Precision, Recall and F1-score of the Gradient boost classifier shown 98%,100% & 99% with class '0' (bacterial spot) and 97%, 89% & 93% with class '1' (mosaic), respectively (Table 2 & Graph 2). The Precision, Recall and F1-score of the RFC shown 97%,100% & 98% with class '0' (bacterial spot) and 97%, 81% & 88% with class '1' (mosaic), respectively. (Table 2 & Graph 2). The Precision, Recall and F1-score of the Decision tree classifier shown 96%, 96% & 96% with class '0' (bacterial spot) and 77%,75% & 76% with class '1' (mosaic), respectively. (Table 2 & Graph 2). The Precision, Recall and F1-score of the Naïve bayes classifier shown 97%,95% & 96%with class '0' (bacterial spot) and 72%, 81% & 76% with class '1' (mosaic), respectively. (Table 2 & Graph 2). The Precision, Recall and F1-score of the Logistic regression classifier shown 92%,96% & 94% with class '0' (bacterial spot) and 69%, 50% & 58% with class '1'(mosaic), respectively. (Table 2 & Graph 2). The Precision, Recall and F1-score of the KNN classifier shown 92%, 95% & 93% with class '0' (bacterial spot) and 62%,50% & 55% with class '1'(mosaic), respectively. (Table 2 & Graph 2). (Liu G et al. 2019, Zamani AS et al. 2022). Further, applied Confusion matrix to all the above Classifiers with 10% test size (Fig3). The classifiers, KNN, Logistic regression, Naïve bayes, Decision tree, RFC and Gradient boost have obtained the best and poor accuracy of (88.4%, 89.8%, 92.8%, 96%, 96.8% & 98%) and (86%, 88%, 90.6%, 93.2%, 94.6% & 95.3%) with test sample size (10%, 20%, 10%, 20%, 10% &10%) and (30%, 30%, 30%, 10%, 30% & 30%), respectively. (Graph 3).





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DISCUSSION

In recent years there are many reports have been published on application of machine learning and image processing for prediction of various diseases of economically important crops. Most of the studies have used pre-processing, segmentation, feature extraction and classification for disease accuracy prediction. For example, pre-processing like resize, convert to gray and histogram equalization used for detection of diseases of tomato (bacterial spot, early blight and late blight), rice (bacterial leaf blight, brown spot and leaf smut), potato (early blight and late blight), bell pepper (bacterial spot) (Panchal *et al.*, 2019, Pallathadka *et al.*, 2021). Similarly, for segmentation k-means clustering have used for the above plant diseases, however some variations have noticed in feature extraction in rice (PCA), tomato (PCA and GLCM) (Harakannanavar *et al.*, 2022, Pallathadka *et al.*, 2021). In classification majorly the CNN, SVM and KNN have used to classify the diseases based on the feature extraction. In the present study, we have also followed the above methods for pre-processing, however for segmentation used morphological operations which has been used in some reported studies for detection of (Zhao *et al.*, 2006, Said *et al.*, 2016, Bhangea and Hingoliwala., 2015, Khirade and Patil., 2015). Further feature extraction was done as similar to the above studies with GLCM in this study. The classifiers like KNN, Logistic regression, Naive bayes, Decision Tree, RFC and Gradient boost used and obtained 98 percent accuracy in disease prediction.

CONCLUSION

This study is aimed to predict the diseases of tomato like bacterial spot, mosaic by using morphological operations and machine learning techniques. Application of histogram equalization, morphological operations (opening, gradient and closing), GLCM and classifiers like KNN, Logistic regression, Naive bayes, Decision Tree, RFC and Gradient boost for prediction of diseases of tomato have shown 98 percent of accuracy with gradient boost classifier.

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Table 1: Different classifiers accuracy with the test sample sizes

Sample Size	KNN	Logistic regression	Naive bayes	Decision Tree	RFC	Gradient boosting
0.1	88.4	89.6	92.8	93.2	96.8	98
0.15	88	88.8	91.4	95.7	96.2	97.3
0.2	88	89.8	92.4	96	96.4	97
0.25	86.7	88.3	91.8	95.3	95.6	95.8
0.3	86	88	90.6	94.1	94.6	95.3
0.35	86.6	88.3	91.5	94.4	95.3	95.6
0.4	86.9	88.6	91.9	94	95.5	95.7
0.45	87	88.5	92	95.2	95.4	96.1
0.5	88	88.1	91.8	94.4	95.6	95.9





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Table 2: Evaluation of performance parameters of the classifiers with 10% of test sample size.

	KNN		
	precission	recall	f1-score
class 0	92	95	93
class 1	62	50	55

D			
	precission	recall	f1-score
class 0	96	96	96
class 1	77	75	76

Log			
precission recall			f1-score
class 0	92	96	94
class 1	69	50	58

	RFC		
	precission	recall	f1-score
class 0	97	100	98
class 1	97	81	88

	Naïve bayes		
	precission	recall	f1-score
class 0	97	95	96
class 1	72	81	76

Gradient Boost			
	precission	recall	f1-score
class 0	98	100	99
class 1	97	89	93

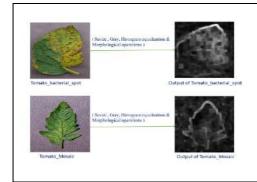


Fig. 1: Input image of the diseases of tomato and their outputs

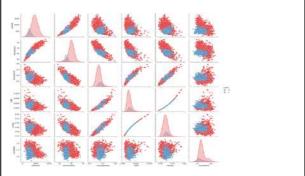


Fig. 2: GLCM features (Label '0' is 'Tomato_bacterial_spot' and Label '1' is 'Tomato _mosaic')

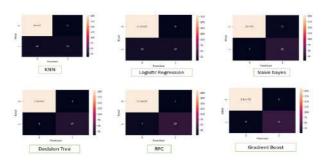


Fig. 3: Confusion matrix of the Different Classifiers with 10% test size

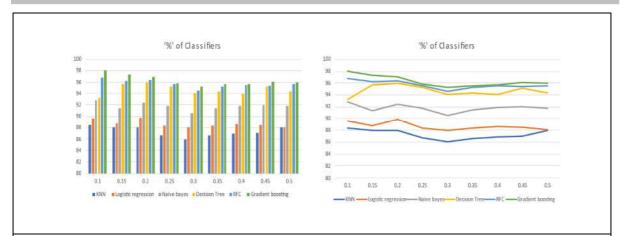


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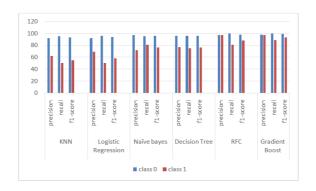
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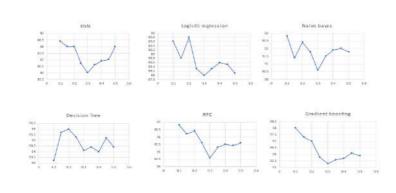
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Graph 1: Different classifiers accuracy with the test sample sizes graphs



Graph 2: Evaluation of performance parameters of the classifiers with 10% of test sample size(Class 0 is 'Tomato_bacterial_spot' & Class 1 is 'Tomato_mosaic')



Graph 3: X-axis is test sample_ sizes and Y-axis is Accuracy of the test sample_ sizes





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REVIEW ARTICLE

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A Brief Overview on Potential Biomedical and Pharmaceutical Application of Naturally Synthesized Chitosan

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ABSTRACT

Chitosan, a natural macromolecule is obtained by the alkaline deacetylation of chitin, which is the major component of cell wall in fungi, the exoskeletons of arthropods, such as crustaceans and insects, the radulae of molluscs. Adequate work has been done on the chitosan to explore its chemical and physico-chemical properties, biological activities, and pharmaceutical and biomedical application. The chitosan is biodegradable, biocompatible, non-toxic, and has different biological activity such as antimicrobial, antioxidant, anti-inflammatory, anticancer etc. Consequently, it has considerable use in the pharmaceutical and biomedical field such as drug delivery, tissue engineering, wound healing, wound care dressing, gene carrier, dialysis membrane, artificial skin, cardiovascular disease treatment, obesity treatment etc. The current review gives the comprehensive knowledge about chitosan synthesis, properties and application in pharmaceutical and biomedical field.

Keywords: Chitosan, Antimicrobial, Antioxidant, biomedical, pharmaceutical

INTRODUCTION

In recent years, scientists have been trying hard to replace petrochemical products by renewable, biosourced components to reduce the dependence on fossil fuels. Chitosan is an absolutely distinctive bio-based polymer and its inherent properties are very eminent and valuable, accordingly, chitosan possesses no actual petrochemical analogous. Thus, the intrinsic characteristics of chitosan make it employable for different uses [1]. Chitosan is a native polycationic linear polysaccharide derived from chitin. Chitin is the most abundant naturally occurring





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polysaccharide materials, which is the principal constituent of cell wall in fungi, the exoskeletons of arthropods, such as crustaceans and insects, the radulae of molluscs, and is the second most happening biopolymer in nature after cellulose [2]. The name "chitin" was derived from the Greek word "chiton", which means a coat of mail. The use of chitin was first reported by the French chemist. Henri Braconnot in 1811 [3]. It has high molecular weight and is chemically composed of N-acetyl-2-amino-2-deoxy-D-glucose units associated with glycoside bonds β (1 \rightarrow 4), establishing a linear chain with some of the deacetylated monomer units. Chitosan, consisting of 2-amino-2-deoxy-Dglycopyranose units joined by glycosidic bonds β (1 \rightarrow 4) is obtained by chitin deacetylation reaction through alkaline hydrolysis and subsequent treatment with acid solutions [4]. If the reaction pathway is allowed to go to completion (complete deacetylation), it yields up to 98% product [5]. The chitosan molecule can be considered as predominantly an ampholyte or predominantly a polyelectrolyte because of the presence of charged unit [6]. The structure of chitosan is depicted in figure 1. Among the biopolymers derived from natural sources, chitosan has acquired considerable observation because of their distinctive biomedical and physicochemical properties such as biodegradability, biocompatibility, non-toxicity, renewability, and ready availability. Because of their high existence in nature chitosan is preferred compared to synthetic polymers [7]. They are also hydrating agents, antimicrobial, non-antigenic, non-toxic, and biological active, with a haemostatic effect[8]. Due to its unique chemical nature, positive charge, presence of reactive hydroxyl (-OH), and amino -(NH₂) group chitosan has wide biological application. It has various inherent properties such as bactericidal, fungicidal activity, and wound healing potential [9]. The major focus of this review is to summarize the recent findings on the synthesis, characterization of chitosan and for biomedical applications including antimicrobial, anticancer, anti- diabetic, and wound healing activities.

Extraction of chitosan from chitin

Chitosan is obtained by deacetylation of chitin to varying degree. The main sources of chitin are the shells of crustaceans, mainly crabs, lobster, krill cuttlefish and shrimps along with insects, bacteria and algae. The production of chitosan requires various steps such as preparation of the chitin from biological material followed by the deacetylation. The protocol for chitosan preparation is divided into four basic steps such as demineralization, deproteinization, decolouration and deacetylation steps which can be carried out using chemical or biological (enzymatic treatment or fermentation) treatments [10]. Chemical deacetylation is more commonly used for commercial preparation as it is inexpensive and feasible for mass production. Demineralization is carried out by acid treatment using HCI, HNO₃, H₂SO₄, CH₃COOH and HCOOH, more preferably dilute hydrochloric acid of different concentrations at room temperature. It consists of the removal of minerals, primarily calcium carbonate. An expansive range of chemicals have been used as deproteinization reagents inclusive of NaOH, Na₂CO₃, NaHCO₃, KOH, K2CO₃, Ca(OH)₂, Na₂SO₃, NaHSO₃, CaHSO₃, Na₃PO₄ and Na₂S. NaOH is the privileged reagent. The deacetylation step is performed by treating with a hot concentrated solution of NaOH. Chemical deacetylation has some drawback like energy consumption, waste of concentrated alkaline solution, consequently an increase of environmental pollution, etc. In order to defeat these stumbling blocks in the chitosan preparation, a surrogate enzymatic method exploiting chitin deacetylases has been investigated. The enzyme, chitin deacetylase (EC 3.5.1.41) is a member of carbohydrate esterase family which hydrolyses theacetamido group of N-acetyl glucosamine units of chitin, and generates glucosamine units and acetic acid. Enzymatic deacetylation of chitin was explored with deacetylase isolated from several organisms like fungi (A. niger, F. velutipes, C. lindemuthianum, M. racemosus, etc.), insects (Apis mellifera, Drosophila melanogaster, Helicoverpa armigera, etc.) and bacteria (V. cholera and other bacteria of Vibrionaceae family [11]. The flow chart of synthesis of chitosan is given in fig 2. Different sources of chitin produce different amount of chitosan. The percentage of yield from different sources are discussed in the table 1 [5].

Properties

Chemical properties of chitosan

Unlike other naturally occurring polysaccharides such as cellulose, dextran, pectin, alginic acid, agar, agarose and carrageenan, which are neutral or acidic in nature, chitosan are highly basic polysaccharides [8]. Because of the presence of reactive groups as amino (NH₃) and hydroxyl (-OH) groups, chitosan can bind with other substances. Chitosan is a linear polyamine and has chelating ability for several transitional metal ions [12]. Chitosan is Insoluble in organic solvents and water and soluble in dilute hydrous acidic solutions. It has many functional properties, such





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as ability to form films, poly oxy salt formation and optical structural characteristics. It can also serve as polyelectrolytes (at acidic pH). Because of the ability to form film; Chitosan can act as adhesive materials for isolation of biomolecules. The entrapment and adsorption properties of chitosan can be used for separation and filtration. It consisting of large reactive groups for chemical activation and cross-linking. Chitosan is able to form hydrogen bonds intermolecularly, which raise its viscosity [13].

Physico-chemical properties of chitosan

Chitosan has enticed increasing attention within the past few years because of its distinctive properties together with non toxicity, biocompatibility, and biodegradation including several others. The structure of chitosan is incredibly like that of cellulose and is the second most plethoric natural polymer after cellulose. The solubility, biodegradability and reactivity of chitosan and adsorption of substrates are based on the degree of protonated amino groups in the chain of polymer [14]. The solubility of chitosan can be distinguished by not solely the fraction of 2-acetamido-2deoxy-D-glucose units within the molecule however conjointly by the N-acetyl group arrangement. Chitosan obtained from partial deacetylation of chitin becomes soluble in aqueous acidic solutions once the average degree of deacetylation is above 0.5, but not at a basic physiological pH [15]. In fact, this limit depends on the diffusion of acetyl groups across the chains. The physical properties of chitosan in solution are built strongly upon the degree of deacetylation and the acetyl group distribution across the polymer chain [11]. Irregular distribution of acetyl group can lower its solubility and allow them construct aggregates easily. Modification of chitosan at the molecular level increases its solubility and stability and thus makes it much versatile as a biopolymer. Chitosan is weak base and insoluble in water and organic solvents. However, soluble in nearly all diluted acids such as acetic, lactic, malic, formic and succinic acids at pH < 6 [16]. Chitosan is thermoelastic and decomposes at 280°C. Chitosan is polycationic at pH < 6 and it readily reacts with negatively charged molecules, such as proteins, anionic polysaccharides (e.g., alginate and carrageenan), fatty acids, bile acids and phospholipids [9]. Chitosan is a pseudo plastic material and is an excellent viscosity-enhancing agent in acidic environments.

Various factors that affect the viscosity of chitosan solution are the molecular weight, degree of deacetylation, pH, ionic strength, concentration, and the temperature. Generally, the magnitude of intrinsic viscosity of chitosan is higher than that of other biopolymers having the comparable molecular weight[17]. With increase in molecular weight of chitosan its intrinsic viscosity also increases. There is a decrease in the viscosity of the solution on increasing the temperature and increases with an increase in chitosan concentration. The effect of pH on the viscosity depends on the type of acid used. The viscosity of chitosan also impacts the biological properties such as wound-healing properties along with biodegradation by lysozyme [14]. There are various other factors which may affect the physicochemical properties of chitosan such as molecular weight, crystallinity and degree of deacetylation. The molecular weight (MW) and degree of deacetylation (DA) of chitosan define several properties of this polymer. The molecular weight of chitosan has considerable impact on its biomedical properties. Moreover, the degree of deacetylation strongly influences its antimicrobial properties by increasing its solubility and its positive charge. Some physico-chemical properties such as solubility, viscosity, and biocompatibility are inversely proportional to the degree of acetylation. Biological properties such as antimicrobial, analgesic, antioxidant, homeostatic, and mucoadhesive abilities increase once the degree of deacetylation decreases [18].

Biological Activity

Chitosan acts as a promising biomaterialdue to their different biological activity like antibacterial, anti-fungal, antioxidant, anti-inflammatory, anticancer and anti-tumour activity.

Antimicrobial properties

Chitosan has been explored as an antimicrobial material against an extended radius of target organisms like algae, bacteria, yeasts and fungi in investigations involving *In vivo* and *In vitro* interactions with chitosan in different forms such as solutions, films and composites. In these researches the chitosan is mainly considered as a bactericidal (kills





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the live bacteria or some fraction in that) or bacteriostatic (inhibit the growth of bacteria but does not imply whether the bacteria are killed or not).

Recent information has the tendency to characterize chitosan as bacteriostatic rather than bactericidal [19].Two mechanisms can explain the antibacterial and antifungal activities of chitosan. The first proposed mechanism is associated with the interaction of negatively charged groups at the microbial cell surface with positively charged chitosan, changing its permeability, which would keep away the entry of essential materials into the cells or the discharging of fundamental solutes out of it [20]. At a lower concentration (<0.2 mg/ml), the polycationic chitosan combine with the negatively charged bacterial surface to form agglutination, while at higher concentrations, the larger number of positive charges transmit a net positive charge to the bacterial surfaces to keep them in suspension [21]. The second mechanism uses the association of chitosan's protonated amino groups with the DNA of cell, leading to the obstruction of microbial RNA synthesis. This antibacterial or antifungal properties of chitosan explain its use in commercial germicide, where it hinders the development of an adequate variety of fungi and bacteria[20]. The antimicrobial effects of chitosan are due to the polycationic nature of chitosan in acidic medium (pH < 6). When the degree of deacetylation (DD) of chitosan is more, the amount of positively charged amine groups also increases, which affects antimicrobial activity [22]. The mode of antimicrobial action of chitosan is highly dependent on the type of targeted microorganism. Different factors affect the antimicrobial properties of chitosan, these are pH, molecular weight, degree of deacetylation along with their different derivatives or forms like films, hydrogels, coatings etc., [23]. The antimicrobial property of chitosan can be used in food preservation, manufacture of wound dressing and antimicrobial finished textile [24].

Antioxidant property

Trang Si Trung and Huynh Nguyen Duy Bao assessed the antioxidant potency of chitosan by several different in vitro procedure, including 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging, total reducing power, and inhibition of lipid peroxidation. The DPPH free radical scavenging, total reducing power, and lipid peroxidation inhibition activities of chitosan at varying concentration (0.125 to 1.0 mg/mL) range from 3.7 to 16.8%, 0.05 to 0.15, and 1.7 to 15.1%, respectively [25]. According to Xia et al., the nitrogen of amino groups of chitosan has a lone pair of electrons; it can attach to a proton released from acidic solution to form ammonium (NH³+) groups. The free radicals can react with the hydrogen ion from the NH³+ to form a stable molecule [26]. According to several scientists, the scavenging mechanism of chitosan is because of the reaction of hydroxyl and superoxide anion radicals with active hydrogen atoms in chitosan to form a stable macromolecule radical. As degree of deacetylation results in chitosan with better antioxidant properties, the number of free amino groups is important to a good antioxidant performance. Chitosan is also reported as significant chelator. The generation of radicals can be retarded by chelation of ferrous ions. Because of the presence of antioxidant property, chitosan be used as a replacement for synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxy-anisole (BHA), and tert-butylhydroquinone (TBHQ) [27].

According to previous studies, a high molecular weight chitosan has a lower mobility than a low-Mw chitosan. Consequently, this would increase the possibility of inter- and intramolecular bonding of the high molecular weight chitosan molecules; thus, the chance of exposure of their amine groups might be restricted which results in decrease in scavenging activity[28]. Although the antioxidant activity of chitosan has been proven through many researches, the level of activity is not very satisfactory due to the lack of a H-atom donor to serve as a good chain-breaking antioxidant[29]. The scavenging capacity of free radicals is related to bond dissociation energy of O–H or N–H and the stability of the formed radicals. Because of the presence of strong intramolecular and intermolecular hydrogen bonds in chitosan molecules, the OH and NH 2 groups are difficult to dissociate and react with hydroxyl radicals[30]. Various modifications of chitosan molecules are done to improve the antioxidant activity of chitosan. These are accomplished by grafting functional groups into molecular structure, among which the grafting of polyphenols onto chitosan is mostly studied. Most of polyphenols are obtained from natural sources and considered safe and environment friendly substance. The grafting reaction of chitosan and polyphenols is primarily helped by enzymes. In the enzyme-catalysed reaction, phenolic compounds are oxidized to o-quinones which are highly





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reactive electrophilic compounds, and then covalently graft to nucleophilic amine groups of chitosan through Schiff-base or Michael-type addition reaction. After modification of chitosan by grafting poly-phenols, the antioxidant activity is exceptionally increased due to the synergetic effects obtained from both chitosan and polyphenols [31]. Scavenging ability on hydroxyl radicals and chelating abilities on ferrous ions and can be used as a source of antioxidants, as a possible food supplement or constituent in the pharmaceutical industry [32].

Anti-inflammatory activity

Inflammation is the first protective response to infection or injury of human body operated in a tissue compartment by a particular set of immune and inflammatory cells to restore its structural and functional integrity after exposure to an adverse stimulus[33]. A great many researchers have worked on the anti-inflammatory properties of chitosan and its derivatives and they found that the main contribution to anti-inflammatory activity of chitosan is because of the structural elements comprising its molecule, but not depending on its molecular weight [31]. The anti-inflammatory property of chitosan is linked with the presence of N-Acetyl-D-glucosamine, which stimulates inflammatory cells such as macrophages, fibroblasts and polymorphonuclear neutrophils[34]. Water-soluble chitosan can repress the production and expression of proinflammatory cytokines (e.g., tumour necrosis factor- α and interleukin-6) and inducible nitric oxide synthase in astrocytes, the predominant neuroglia cells in the central nervous system, and by this way is actively associated with cytokine-mediated inflammatory events. Chitosan can throw anti-inflammatory effects by inhibiting prostaglandin E2 and cyclooxygenase-2 protein expression and attenuating the pro-inflammatory cytokines (e.g., tumour necrosis factor- α and interleukin-1 β). However, chitosan treatment increases the expression of the anti-inflammatory cytokine, interleukin-10 [9].

Anti-Cancer Activity

Chitosan has been reported to selectively permeate through the cancer cell membranes and show anticancer activity through the cellular enzymatic, permeation enhancing, antiangiogenic, immuno enhancing, antioxidant defence mechanism, and apoptotic pathways. They become concealed from noncancerous cells and furnish their intensified bioavailability in cancer cells in a sustained release manner [35]. According to permeation enhancing mechanism, the amino groups in chitosan leads to protonation in acidic to neutral medium. The positive charge produces in this cationic polysaccharide (pKa ~6.5) makes it water soluble and bio adhesive to combine with and enhance permeation through negatively charged surfaces such as mucosal and basement membranes [36]. Y. Xu and co-workers revealed that chitosan nanoparticles (CNP) could inhibit the growth of human hepatocellular carcinoma by a mechanism of CNP-mediated inhibition of tumor angiogenesis that was correlated to defective quantity of vascular endothelial growth factor receptor 2 (VEGFR2) [37]. A mechanism of anticancer activity of chitosan is associated with its capacity to improve the biodistribution level and gathering of drug in tumor cells. Zhang *et al.* through pharmacokinetic study *in vivo* have shown that mifepristone (MIF) loaded chitosan nanoparticles (MCNS) ensure controlled drug delivery in a sustained release manner and intensify the oral bioavailability and anticancer activity of the drug [38].

Antitumor activity of oligo chitosan is related to activation of intestinal immune functions due to increase in NK activity in intraepithelial lymphocytes (IELs) or splenic lymphocytes [39]. Immuno enhancing molecular mechanisms of chitosan could precede either with elimination of pathogenic microorganisms or tumour cells because of an immune response or with rise of cytotoxic activity to inhibit the production of tumour cells by activation of T-cells and NK-cells with the help of IL-1 and TNF- α cytokines[40]. Anticancer activity of chitosan in different cell lines is established owing to apoptosis, that is initiated by activation of procaspase triggered from outside the cell to speed up the cleavage of cascade to boost the death signals [41]. The antitumor effect of chitosan and its derivatives is due to the increase in secretion of interleukin-1 and 2 which causes maturation and infiltration of cytolytic T-lymphocytes. It is further supported that chitosan promotes lymphokine production and proliferation of cytolytic T-lymphocytes. Other investigations exhibit that chitosan is involved in direct killing of tumor cells by inducing apoptosis [11]. Low molecular weight water-soluble chitosan (21 kDa and 46 kDa) exhibits anti proliferative activity towards sarcoma 180-tumor bearing mice, while high molecular weight water-soluble chitosan of 130 kDa has no antitumor activity. The 21 and 46 kDa chitosan can trigger macrophages via cytokine (e.g., IFN- γ , IL-12, and IL-18) synthesis from the intestinal intraepithelial lymphocytes. Low molecular weight chitosan seizes cancer cells at the





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G1/S phase generating apoptosis. Moreover, chitosan can manage cell cycle-related genes (e.g., Cdc25A, p21/Cip, and p27/Kip), upregulate transcriptional growth factor β (TGF- β), and induce apoptosis by controlling nuclear factor k-light-chain enhancer of activated B cells (NF-kB) mediated signalling pathways. Additionally, the anticancer activities of chitosan and chitosan derivatives can be improved by incorporation with nanocomposite or other chemical agents [42]. The deacetylation degree (DDA), molecular weight (MW) and antimicrobial and antioxidant activities are the major deciding factors of antitumor activity [43]. Antitumor activity of chitosanalso depends on its source. Different studies have revealed that chitosan samples from crab and mushroom exhibits different activities against IMR32 and HepG2 cell lines, while mushroom chitosan is more effective than crab chitosan. Furthermore, low MW chitosan from mayfly exhibits a similar cytotoxic activity to low MW chitosan from shrimp, despite of having a similar deacetylation degree [44].

Pharmaceutical And Biomedical Application

Since 1970, Chitosan has been used in different scientific and industrial areas because of its peculiar macromolecular structure, biocompatibility, biodegradability and other inherent functional properties. Chitosan and its derivatives have empirical demand in the food industry, agriculture, pharmacy, medicine, cosmetology, textile and paper industries, and in chemistry. In the past few years, chitosan has got recognition in dentistry, ophthalmology, biomedicine and bio imaging, hygiene and personal care, veterinary medicine, packaging industry, agro chemistry, aquaculture, functional textiles and cosmetotextiles, catalysis, chromatography, beverage industry, photography, wastewater treatment and sludge dewatering, and biotechnology [45]. In this review we will focus only on the pharmaceutical and biomedical application of chitosan.

Drug Delivery

Drug delivery system refers to different approaches for delivering a pharmaceutical compound in the human body to accomplish or optimize the desired therapeutic effects, while minimizing its adverse effects if possible by affecting absorption, distribution, metabolism, and elimination of a drug compound[46]. Because of the presence of cationic group (amine group), chitosan can bind to negatively charged biological surface (mucosal glycoproteins) as mucoadhesive and can act as in situ gelling system and efflux pump inhibitory system. Moreover, chitosan is biocompatible, biodegradable, bioactive, and non-toxic. It contains a great controlled drug releasing ability property. Because of these extraordinary properties, chitosan can be considered as an encouraging material in developing drug delivery system. Chitosan drugs can be delivered through the parenteral route and different non-invasive routes like oral, nasal, and ocular mucosa routes along with intravesical mucosa via chitosan nanoparticles in a proper way [47]. Chitosan is a biological polymer having miscibility with clay and can easily intercalate in interlamellar spaces. The polycationic and hydrophilic nature of this polymer in an acidic medium results in this intercalation. This hybrid material favours the permeability and bioavailability of drugs by the body [48, 49]. The hydrolysis of chitosan generates a low molecular weight product which is extremely soluble in water (more than 50% (w/v), called low molecular weight chitosan (LMC). LMC got ample recognition from the pharmaceutical industry, as it can enhance the absorption rate of a drug with low water solubility by enhancing the dissolution rate of the drug, making the drug's surface hydrophilic by dispersing it into chitosan[50]. The micro flora, which is colon-available, can degrade chitosan. Because of this, chitosan can be considered a polymeric matrix specific for colon drug release. Specific oral release of sodium diclofenac into the colon by chitosan succinate and chitosan phthalate matrices has also been well utilized [51].

Tissue engineering

Chitosan has been used in tissue engineering and regenerative medicine in the past few years because of its properties, such as hydrophilicity, biocompatibility, biodegradability, and antimicrobial activities. Chitosan can be developed in distinct forms, such as gels, micro and nanoparticles, nanofibers, and scaffolds, and moreover, chitosan has an excellent ability to form porous structures for use in tissue regeneration and cell transplantation. A scaffold with specific pore size and mechanical properties desirable for several tissue engineering areas can be produced by using various compositions [20]. Chitosan porous scaffolds can be attained by freeze-drying of chitosan solution or by procedure such as an "internal bubbling process (IBP)," with the use of porogenic materials [52]. The highly porous





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structures with interconnected pores can enhance in vitro and in vivo cell proliferation[53]. Chitosan is used in tissue engineering for repair or regeneration of different tissues including skin, bone, cartilage, nerves, liver, and muscle. Different chitosan scaffold used in tissue engineering are fibre, film membrane, hydrogel, porous membrane etc. Chitosan can act as a scaffolding material or as an analogue or extracellular matrix (ECMD), thus, assist for the renewal of damaged tissue. The use of chitosan for tissue repair and regeneration is because of the fact that it can be easily processed and manufactured in different forms including fibres, films, sponges, and hydrogels. This furnish the ability to imitate the shape of the receiving tissue or biomaterial tissue interface. Moreover, chitosan has the ability to regenerate primary tissue cells and even stem cells[54]. Hybrid chitosan based scaffolds are evolved by a combination of chitosan with Nano ceramics, such as hydroxyapatite (HAp), silicon dioxide (SiO₂), titanium dioxide (TiO₂), bioactive glass-ceramic (BGC), and zirconium oxide (ZrO₂) [55]. On implantation, chitosan induces a minimum foreign body reaction, furthering cell adhesion, proliferation, and differentiation.

These hybrids combine each material's preferable characteristics, taking advantage of the organic part's flexibility and adequate molding capacity, and the inorganic part's properties, such as thermal stability and chemical resistance [5]. Hydrogels are used to partially imitate the stromatolytic structures and anisotropic compositions of the cartilage matrix, encouraging their repair [57]. Thus, the extensive use of chitosan-based hydrogels stands out for the regeneration of cartilaginous tissue, since this polymer has a structure similar to glycosaminoglycan (GAG), a lead component in the cartilage matrix, besides biocompatibility, biodegradability, bioadhesion, cell affinity, and intrinsic antibacterial, chondro-conductive, and chondro-integrative properties [58]. However, unmodified chitosan-based hydrogels are normally unsuitable for cartilage repair because of their low strength and elasticity, fast in vivo degradation, and limited capacity for tissue adhesion, which can be attributed to weak interactions with tissues without the formation of mutually intertwined chains between the two contact interfaces [57]. In that case, the conjugated catechol groups in the chitosan backbone assist covalent bonds between oxidized catechol groups and amine or thiol groups present in the proteoglycan structure, providing additional adhesion strength to tissue surfaces [59].

Wound Healing Activity

Wound healing may be defined as a dynamic process that involves various molecules and cells, such as mediators, natural extracellular matrix (ECM), blood, and parenchymal cells [60]. Properties such as antimicrobial action and reduced wound healing time makes chitosan the show stopper amongst the most frequently used natural polymers for skin regeneration[61]. Chitosan promotes surface-induced thrombosis and blood clotting, and thereby accelerates coagulation in vivo. Also, it affects platelet activation, the most significant component in blood clotting, and releases cytokines to improve the healing process [56]. Chitosan is also used to treat burn infections because of its antimicrobial properties and the capacity to transport extrinsic antimicrobial agents. Moreover, chitosan is utilized as a slow-release drug-delivery vehicle for growth factors to improve wound healing. There are a lot of evidences that chitosan can control effectively every single stage of wound healing process. Chitosan and its derivatives accelerate wound healing by enhancing the functions of inflammatory cells, such as polymorph nuclear leukocytes (PMN), macrophages, and fibroblasts or osteoclasts. Chitosan can also increase the tensile strength of wounds. The woundhealing effect of chitosan is affected by different factors like molecular weight, deacetylation degree, as well as the state of chitosan [62].

Chitosan can generate analgesia by providing a cool, pleasant and soothing effect when applied to an open wound. It can also give excellent pain relief when applied to open wounds, such as burns, skin abrasions, skin ulcers and skin grafted are as topical agent. Due to anti-inflammatory effects of chitosan, it is useful for the treatment of prolonged inflammation at the wound site [9]. In the treatment of open wound also chitosan shows effective results. Inas *et al.* studied chitosan powder as a stimulation of healing of full thickness skin wounds and found that it can promote hyperactive fibroblast proliferation within the first week of application. Two weeks post wounding showed advanced granulation tissue formation associated with formation of new blood vessels. Blood platelets were also activated with redundant effects and superior performance which is necessary to support physiologically ordered tissue formation. Three weeks post incision showed early proliferation of epidermal cells and reepithelization with





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different mitotic figures and dermal granulation tissue formation. After four weeks, chitosan treated wounds showed complete regeneration of epidermal cells with keratin layer similar to the normal skin associated with dermal connective tissue proliferation [63]. The main biochemical activities of chitosan based materials in wound healing are polymorph nuclear cell activation, fibroblast activation, cytokine production, giant cell migration and simulation of type IV collagen synthesis. Chitosan have been used as nano-fibres, gels, scaffolds, membranes, filaments, powders, granules, sponges or as a composite in the wound healing processes [64].

Wound care dressing

As a consequence of excellent hemostatic properties, antimicrobial activities, and anti-inflammatory responses chitosan can be used as dressings for traumatic or chronic wounds [62]. When a wound occurs, platelets become activated and aggregate themselves at the wound site to stop bleeding as the initial response to wound healing, known as the haemostasis stage. During the haemostasis stage, inflammatory cells, including macrophages and neutrophils, are engaged in the wound site to release a variety of mediators and cytokines that signal the following wound healing events of angiogenesis, thrombosis, fibroblast proliferation, and re-epithelialization. At typical wound conditions (pH < 7), chitosan-based membranes are capable of absorbing large amounts of wound exudate[65]. It has been shown that chitosan can regulate cellular activity to enable the release pro-inflammatory cytokines and growth factors and to promote the inflammatory microenvironment conducive for healing, such as interleukin 8, prostaglandin E, interleukin 1B, and others [67]. Clinical study on chitosan as wound dressing material revealed its ability to induce thrombosis at the tissue surface and stimulation of blood coagulation through platelet activation [66]. In addition, chitosan is an anti-microbial agent. It is universally accepted that the protonated chitosan molecules attach with the negatively charged microbial membrane walls leading to the membrane wall disassociation subsequently leakage of intracellular electrolytes, which promotes the death of microorganisms [62]. Furthermore, chitosan can increase the paracellular permeability by loosening the cellular tight junctions of the epithelium to enable the delivery of macromolecules (e.g., proteins / peptides and growth factors) to deep tissue for wound healing [68]. Different chitosan derivatives such as chitosan fibres, chitosan hydrogels, chitosan membranes, chitosan films, chitosan sponges, Chitosan Hydrocolloids are used as wound healing material [18].

The Nano-sized pores within the chitosan fibres promote penetrability of the fibre dressings, that intensifies the interchange of oxygen and nutrients with outside environment. Also, the porous fibre facilitates the incorporation of wound exudates while reducing the possibility of bacterial infections [69]. Chitosan based hydrogels are regarded as important wound dressing material. They furnish a moist wound environment, offer protection from secondary infections, remove wound exudate, be biocompatible, induce faster wound healing, and produce smoother scarring. Because of their anti-inflammatory and antibacterial properties, they provide a suitable microenvironment for healing, inhibit the inflammatory reaction in the wound and control the infection [70]. Chitosan membrane, prepared with a 75% degree of deacetylation and a thickness of 10 microns, is a potential substitute for human wound dressing [71]. Chitosan films have the capability to inhibit bacteria in vitro and are much more effective in wound healing activity than commercial product. They have good swelling properties and do not cause any unwilling symptoms like allergy or irritation and therefore are good wound dressing material [72]. Chitosan sponge can also be used as a wound dressing material. The porous structure of the sponge layer improves the process of blood coagulation and swelling [73]. Owing to the properties like water adsorption, effective antibacterial activity and biocompatibility chitosan hydrocolloids can be exploited in medical fields as wound dressings for wound healing [74].

Gene carrier

Gene therapy has emerged very promptly as it can be immensely used to cure numerous genetic diseases by the insertion of new genes (DNA and RNA) into target cells with expression of the transgene. There is a basic need for gene therapy to development safe and efficient gene carrier or delivery system [75]. Most of the effective non-viral DNA delivery systems are based on synthetic cationic polymers such as polyamidoamine dendrimers and polyethylenimine. As these polymers are not biodegradable, they can lead to potential toxicity [76]. Chitosan, because of its cationic nature, biodegradability and biocompatibility, can be used as a non-viral vector material for gene delivery. Different cationic polymers have been comprehensively explored as gene delivery systems, primarily on





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the grounds that they inherently expand immense gene packaging capacities and permit considerable modifications [77]. It has been established that chitosan-based carriers might be used as a secure delivery system for gene materials including plasmid DNA (pDNA), oligonucleotides and siRNA. Unlike other cationic polymers, chitosan has several advantages such as low toxicity (LD50:16 g/kg, while LD50 for NaCl is 3 g/kg), low immunogenicity, excellent biocompatibility as well as a high positive charge[78]. Also, chitosan can form complexes with negatively charged genes easily due to its abundant amine groups. However, due to several challenges like poor water solubility, charge deduction at physiological pH and poor targeting capability, clinical translation of chitosan-based gene delivery carriers is still unsatisfactory [75].

Dialysis membrane

Chitosan has high mechanical strength, permeability to urea, amino acids and creatinine and is able to reject compounds of high molecular weight. It is also impermeable to serum proteins which indicate the prevention of toxic metals into blood stream [79]. However, Mallete *et al.* reported that chitosan solution formed a coagulum when in contact with blood and its haemostatic property involved agglutination of red blood cells [80]. This problem was improved by blending chitosan with polyhexamethylene adipamide in 99% formic acid solution [81]. Other chitosan blends had also been developed such as chitosan polyvinyl alcohol blend membranes, chitosan-albumin blend membranes and chitosan-poly (ethylene oxide) blend membranes. Chitosan-polyethylene oxide (PEO) blend membranes can be used in dialysis. Different molecular weights of PEO can be used for improved permeability and blood compatibility. Chitosan-PEO blend membranes can reduce platelet adhesion and activation and therefore, seem to be beneficial in improving the permeability of toxic metabolites and reducing the thrombogenicity for haemodialysis [82]. Chitosan can be graft copolymerized with HEMA (2-Hydroxyethylmethacrylate) to make blood-compatible dialysis membranes. The grafted film CH-12.5 composition (425% grafting) can achieve very high permeation to creatinine by reaching the equilibrium within 45 min [83]. Enzyme immobilization: Enzyme immobilisation is primarily employed for biotechnological applications.

Enzyme immobilization enhances the availability of enzyme to the substrate with greater turnover for a significant period of time. Several natural and synthetic supports have been assessed for their efficiency for enzyme immobilization [84]. Chitosan have been used as supports for immobilization of enzymes. The protein or carbohydrate moieties of enzymes are used for binding them to chitosan [85]. Chitosan can also be combined with another substance to increase their efficacy. Chitosan can be blended with alginate in which chitosan-coated enzymes had less leaching effect compared to alginate owing to the physical and ionic interactions between the enzyme and support [86]. Likewise, a wet composite of chitosan and clay is reliable for enzyme trapping, as it has hydroxyl and amino groups, which easily link with enzymes, together with good hydrophilicity and high porosity. Chitosan in the form of beads can entrap twice as much of the enzymes [87]. Chitosan is biocompatible, biodegradable, non-toxic and has multiple functional groups. Chitosan and its different derivatives such as chitosan film, chitosan nanoparticle and chitosan nanocomposite are employed for enzyme immobilization. Chitosan bound enzymes, as compared to free enzymes, have improved the bio catalytic functions due to surprisingly high operational stability and reusability [88]. Gamze D.A et al studied immobilization of pepsin into chitosan beads where the beads were prepared using a cross linking agent and these were used in the immobilisation process. Immobilised pepsin showed higher thermal and storage stability [89].

Artificial skin

Chitosan is broadly used in biomedical field due to its characteristics like hem compatibility, haemostatic and biodegradability properties and diverse source for extraction. Because of the presence of functional groups like amino, hydroxyl and carboxyl groups, chitosan can form composite system with other natural and synthetic materials. This enhances the biological and mechanical properties which is exploited for the treatment of acute and chronic wounds and other skin tissue engineering applications [90]. An absorbable scaffold of chitosan with gelatine can be manufactured by freezing and lyophilizing methods, which is suitable for preparing a bilayer skin substitute. Different methods were employed to confirm the applicability of this chitosan-gelatine scaffold as an ideal skin substitute such as water uptake ability test, in vitro fibroblast proliferation, and scaffold tests in which fibroblasts





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were co-cultured with keratinocytes. Keratinocytes can be co-cultured with fibroblasts in chitosan-gelatine scaffolds to construct a flexible artificial bilayer skin in vitro with good mechanical properties [91]. Hyaluronic acid is also combined with chitosan–gelatine scaffold freezing and lyophilizing methods to prepare artificial skin. It has higher water uptake and retention abilities than chitosan-gelatine scaffolds. Fibroblasts cultured in chitosan-gelatin-hyaluronic acid scaffolds grew and proliferated effectively, and exhibited a strong potentiality. Keratinocytes were co-cultured with fibroblasts in chitosan-gelatin-hyaluronic acid scaffolds to establish an artificial bilayer skin in vitro. The artificial skin obtained was flexible and had good mechanical properties and suitable for preparing a bilayer skin substitute [92].

Cardiovascular disease treatment

Coronary heart disease (CHD) is mainly caused by circulating cholesterol accumulation on the artery walls, narrow arteries, and reduced blood flow to the heart [93]. Chitosan are effective anti atherosclerosis potential biological products [94]. Chitosan can also prevent intestinal lipid absorption and improve liver lipid biosynthesis and accumulation [95]. Additionally, antioxidant property of chitosan be used in the treatment of CHD, since long-term hyperglycemia can result in the enhancement of oxidative stress [96]. Intestinal flora disorder and disturbance increase the CHD risk and affect lipid metabolism [97, 98], which can be treated by chitosan consumption that influence fecal microbiota and metabolites of humans [99]. Chitosan oligosaccharides utilization increases the types and numbers of probiotic species of intestinal flora, and this upgrades the lipid profiles and antioxidant properties of coronary heart disease patients. Therefore, chitosan oligosaccharides ameliorate the symptoms of coronary heart disease patients by improving antioxidant capacities via the increase of probiotics in intestinal flora. Chitosan has also been substantially utilized for coronary heart disease therapy as biomaterials of the drug delivery system and coronary artery bypass graft [100]. There is also evidence that daily chitosan consumption remarkably decreases diastolic blood pressure in higher-dosage (>2.4 g/day) and shorter-term (<12 weeks) interventions, although chitosan has no significant effects on systolic blood pressure [101].

Obesity Treatment

Obesity is a chronic disease in which there is over accumulation of fat in adipocytes and is frequently linked with type 2 diabetes, inflammation, hypertension and cardiovascular diseases [102, 103]. It has been found that the antiobesity effects of chitosan generally arise from its unique fat-binding properties, which hamper the absorption of dietary lipids from the gastrointestinal tract. Dietary supplementation of chitosan hinders the intestinal absorption of dietary fat. It has been proposed that chitosan dissolves in the stomach, emulsifying fat and forming a gel, which binds with the fat in the intestine. This insoluble complex then passes undigested through the large intestine and is naturally excreted, hence, interfering with the absorption of fat in the intestine. However, more recent research has suggested a more complex mode of action for chitosan. Exposure of preadipocytes to chitosan stimulates adipokine secretion and inhibits adipogenesis in vitro. These adipokines, through their local and systemic actions, regulate energy metabolism, inflammation and insulin sensitivity. The potential anti-obesity effects of chitosan can be explained by modulation of adipokines [104]. Obesity lowering activity of chitosan was studied on 3T3-L1 cells, and it showed that chitosan has significant anti-obesity activity. The 3T3-L1 cell line was transformed into adipocytes and treated with chitosan. This treatment decreased the lipid accumulation activity of the adipocytes and downregulated adipogenic marker proteins such as leptin, resistin, etc. Chitosan mediated the anti-obesity activities by interfering with the adipocyte differentiation and inhibiting the adipogenic transcription factors and genes. Chitosan also has the capability of lowering cholesterol and low-density lipoprotein (LDL), and thereby helps in obesity treatment [105].





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CONCLUSION

Abundant research work has been done on chitosan and its derivatives. Chitosan is a biodegradable, biocompatible, inexpensive and easily accessible natural biopolymer. Different biological properties such as antimicrobial, antioxidant, anti-inflammatory, anticancer and antitumor activity make chitosan a useful component in the field of medical and pharmaceutical science. It has extensive applications in drug delivery, tissue engineering, wound healing, wound care dressing, gene carrier, dialysis membrane, artificial skin, cardiovascular disease treatment and obesity treatment.

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Table.1: Percentage of yield from different sources

Sources	Yield (%)	
Prawn	57.69	
Shrimp	34	
Fish	7.72	
Crab	41	
Bacteria, Bacillus sp/ Serratiasp	16 / 10	

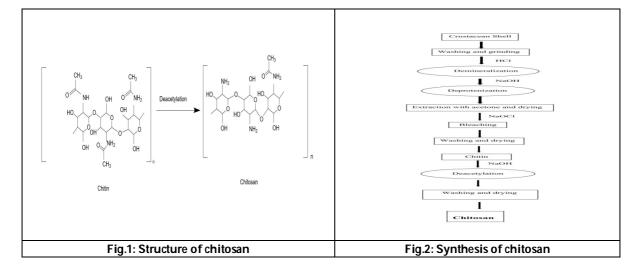




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RESEARCH ARTICLE

The Six Primitive Tribes and Other Major Tribes of Nilgiris, Coimbatore, Erode and Tiruppur Districts; Their Infrangible Asset of Relationship with Ecology, Their Food Basket, Way of Life and Traditional Medicinal Knowledge

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ABSTRACT

Despite the world's abundance of technological breakthroughs and inventions, certain communities throughout the globe continue to rely on forest resources and their output. Because they view nature as their deity, tribal groups revere the forest and the food it produces. Since they complement one another, tribal people's relationship with nature is always one that cannot be broken. They live in peace with nature and rely entirely on it, as evidenced by their style of life, their beliefs, and their natural environment. Tribal people do not abuse or mistreat the forest or its resources in any way, unlike other societies. Tribal people constantly work to safeguard the forest for both the sake of preserving nature and their ability to live sustainably considering future generations. This study deals with the six primitive tribes and other major tribes of Tamil Nadu and their traditional medicinal practices followed in their places. This study also highlights the geographical conditions and their food basket that contribute for their good health and sustainable life. Apart from mainstream medicine which is commonly known as Allopathy, there exist a variety of alternative therapies that are still practised around the globe. Many of these practices are commonly found in tribal and rural regions as compared to the urban regions. The practise of Allopathy in urban areas has overshadowed that of alternative and complementary therapies and hence, these therapies thrive mainly in the tribal and rural areas.

Keywords: Six Primitive tribes, Traditional medicinal practices, sustainable life, alternative therapies





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INTRODUCTION

The Latin word "Tribus" is the source of the English term "tribe". It denotes something noteworthy or one-third of the entire population. Next to Africa, India has the second-largest number of tribal people in the world. India is hence viewed as a blending pot of native races. Tribes have a few distinctive characteristics that set them apart from other religious communities. Tribes are very simple, distinct, and have their customs, traditions, and practices. They live a lonely life within the boundaries of their geographical area. For hundreds of years, India's native tribes have lived in forests and mountains without ever mingling with city dwellers. They may be referred to by several names, including tribes, indigenous, the oppressed classes, or Adivasis. An Indian Imperial Gazetteer describes a tribe as a collection of families that share a common name, a common language, and a common vocation while residing in an area. The majority of tribes adopt "endogamous" relationships, which forbid strangers from living together and only permit marriages between members of the same social group. People in the country are divided politically, but they also have a lot in common culturally. The population of this community is limited and its geographic area is constrained. The majority of tribal languages are unwritten, which means they frequently lack a written form. As a result, even the scope of their contact in terms of both space and time is extremely limited.

Tribes have a fascinating economic structure and always live compactly in a world of ravenous people. They have self-sufficiency, which is something many individuals in today's culture lack. The tribal communities in India are referred to as "Scheduled Tribes" or ST under Article 366 of Indian law, and the president of India grants them this designation. Depending on where they live, they speak a variety of languages. They coexist together with the forest, and both benefit from each other's existence. The overall tribal population in India was estimated to be 84.51 million individuals or 8.14% of the total tribal population, according to a survey conducted in 2011 by the Government of Tamil Nadu Forest Department. According to surveys, India's diverse regions are home to 449 distinct tribes and sub-tribes. They are mostly dependent on the forest and its products for their economy of life, with half of the tribal people living in or near the forest. According to the same census from 2011, Tamil Nadu has a total of 7.21 lakh tribal people, of which 1.10% were recognised. There are 36 tribes and sub-tribes that live in Tamil Nadu. Out of the 7.21 lakh indigenous people that make up Tamil Nadu's total population, or 27.9%, are literate. This number is rising as society advances daily. The majority of tribal people in India work as agricultural labourers and cultivators and the agricultural labourers are dependent on nature for their livelihood.

Primitive tribes, their geographical location and their relationship with Nature

Six of the 36 tribal groups have been classified as being primitive tribes. It's interesting to note that each of the six nomadic tribes originated in the Nilgiris. In Tamil Nadu, these savage tribes may be found in practically every district, in various places. Primitive tribes have made a considerable contribution to the management of the forest and its resources, doing it with the highest consideration for both their well-being and the sustainability of the forest. The six primitive tribal groups are Irular, Todas, Kotas, Kurumbas, Paniyan, and Kattunayaka, and they are dispersed throughout Tamil Nadu. A region or territory is designated as an "Integrated Tribal Development Programme Area" if it contains a larger proportion of tribal people than 5% of the overall population. It is known as a "tribal block" when just one tribe resides in such a region. "Wood nymphs" refers to the majority of the tribes that inhabit protected forest regions. They reside close to the periphery, and from a social and cultural standpoint, their way of life is entirely dependent on nature and her by-products. They constantly take the utmost precautions to protect natural resources. Reduced resource availability would result from any sort of deterioration. As a result, it affects their capacity to survive and work in the forest, as well as the availability of food and better possibilities for a living. As a result, people take greater care to protect the natural forest resources for their quality of life.

Kota Tribes

The Kota tribes, like the other agrarian tribes indigenous to the Nilgiris region of South India, number about 2000 and reside in the Queen of the Hills' tribal settlements. The Kota tribe speaks a unique tongue known as "Kormant" Despite having their language, they also speak Tamil, Badga, and several other languages. Some of the Kota tribes'





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literate members also use English to interact with outsiders as a result of the ongoing improvements of tribal people. Other words they use to refer to themselves are "Adivasis," "malaivasi," or "tribes". The Kotas consistently assert that they have a supernatural relationship with fate and nature through their supernatural abilities, and they even use these abilities to heal sick people. They consider themselves to be the ghosts of their deceased ancestors. The Kota tribes are skilled musicians and songwriters, and they present their works in a variety of forms. They create the words for songs of devotion and lamentation. They also write in general terms about how they view the world today. Agriculture is essentially the Kota tribes' primary industry. All of the tribes in their neighbourhood either work in agriculture full- or part-time. They worship their own God, "Aynor," and Goddess, "Amnor," and they follow different religious and cultural ideals. Although they are devoted to their gods, they also attend their Hindu neighbours' festivals, therefore they have accepted and adhered to some ceremonial customs.

Kattunayakan tribes

One of the primitive tribal groups residing in various regions of Tamil Nadu is the Kattunayakas. They were originally from Thangamalai, where their cave-dwelling forefathers dwelt. Since then, they have made great progress and are now residing in concrete homes. Only approximately 10 people reside in Thangamalai, which has only about three dwellings. They converse in a variety of languages, including Kannada, Tamil, and Malayalam. The people that make up this group are quite low in stature, seem like coloured people, and have a prominent forehead. These folks practice black magic in secrecy. Because of this, these tribal people's main source of income is working in tea gardens during the summer, along with gathering honey and firewood. They engage in hunting and consume forest products. Despite not being literate, the Kattunayakas send their kids to school. These youngsters represent the first generation of learners in their neighbourhood.

Paniya Tribes

The Malayalam word "Pani," which means "labour," is where the name "Paniya" originates. Paniyas are dependable employees, as the name implies. In essence, paniyas are agricultural labourers. They may be found in large numbers in the Gudalur and Pandalur taluks, but they have also established communities in other areas. They converse in the Dravidian language known as "Paniya." Their skin tone is dark, and they have big lips and curling hair. These people have a very basic understanding of religion; "Kalli" is their main god, and they also worship banyan trees. The only things that Paniyas care about are the essentials of life, such as food, clothes, and shelter. The practice of taking many wives is common in this tribal group, and it is permissible if the man or woman can afford to do so. They are unfamiliar with the basics of their way of life, including schooling and the health care system. Since they lack literacy, they are essentially disorganized and unable to even demand the minimal pay that the government and landlords have set for them. Of all the tribal groups, the Paniyas are said to be the poorest of the impoverished.

Irula Tribes

The Irulas are the second-largest scheduled tribe of all the tribes in Tamil Nadu, after the Badges. The Tamil word "Irul," which means "darkness" or "nighttime," is the source of the name "Irulas." Depending on where they live, some of its members speak the Tamil dialect, while others speak various Tamil Nadu dialects. They go by a variety of names, including kadupujari, illiga, and iruliga. They are locals of Coimbatore's the Western Ghats. The Irulas build their homes out of bamboo tree branches, and they cover them with grass and fried coconut tree leaves for roofs. They are collecting little amounts of forest products. Snatching snakes and rats are their primary source of income. Every home has a separate room for raising goats and cows, and they are also responsible for caring for the herds of livestock. Irulas are experts in the use of ethno medicine and its therapeutic techniques.

Kurumba Tribes

The Kurumbas are one of the six primitive tribes that inhabit the Nilgiris Hills in Tamil Nadu's west central region. Particularly, they reside in Gudalur block's Mutimoola village. They share a genus and species with the Pallavas. The Kurumbas' ancestors have been scattered since the end of the Pallava monarchy and currently reside in various parts of South India. Kurumbas mostly engage in hunting and harvesting foraged goods. Kurumbas, who reside in mountainous locations, work as labourers picking tea and are paid for their efforts. Additionally, they perform





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menial agricultural tasks on farms or serve as slaves in the landowner's fields. As indigenous groups have developed, they now sell the produce of the jungle by working as field labourers and hunters. They still hunt, and they occasionally use a bow and arrow. The Kurumba tribes are noted for having exceptionally sharp eyes. They manage to accomplish this by spending a lot of time observing honeycomb while they gather honey from hives. Black magic and traditional paintings are the Kurumba tribes' specialities. Plains kurumbas vary from hill kurumbas in that they work as sheep herders and have given up some of their traditional practices. People used to live without suitable housing in the wild and jungle in the past. They began residing in masonry homes with separate toilets and power facilities with the involvement of NGOs.

In addition, solar energy and biogas units are readily accessible. These folks reside in homes with brick walls. They employ ethnomedicine to cure their ailments and preserve their general health because there are no nearby medical facilities or hospitals within 2 or 3 kilometres of their homes in the community. They consume prepared foods three times each day along with various nutrient-dense foods like samai, noorai, spinach, capsicum, etc. The Kurumba tribes continue to employ some of the popular herbs for a variety of diseases today. These ancient medical practices were passed down orally from generation to generation as a legacy from their ancestors. Diseases were traditionally treated by Au-Kurumbas with the assistance of their local deities. They use the spirits of their ancestors to assist them to heal illnesses as well. The names of their ancestors are spelt out and recited as part of the healing procedure to eradicate any illness. Some people practice dark magic with an enigmatic air. They primarily employ medicinal herbs, spiritual ceremonies, and religious scriptures to cure illnesses while the healing process is taking place.

Toda Tribes

The Todas, an exotic race that is often not dark and one of the six tribal groups, are constantly exposed to the sun's rays. The majority of them have bright eyes that range in hue from brown to grey. South India's Nilgiris are home to the pastoral Toda tribes. Additionally, they are renowned for their superb needlework abilities. Although they had a relatively small population in the 1960s, they currently appear to have grown in number over time. They converse in the Dravidian language of Dravidian. The Toda tribe inhabited a scattering of thatched homes on grazing hillsides. It is supported by a wooden framework and has an arching roof, which is similar to the shape of a half-barrel. Their primary source of income comes from trading dairy products, exporting cane, and supplying forest goods. Todas appear to possess a thorough understanding of herbal remedies and traditional medicines. They were familiar with a wide range of species found in the Western Ghats. About 30 of them are very valuable for treating various illnesses. To ensure that their future generations receive the most possible advantages from these medicinal plants, they have also implemented a variety of conservation initiatives.

MahaMalasars

One of the first tribes to live in the Western Ghats is the Malasar Tribe. The southern Dravidian language is spoken by the Malasar tribes. These tribes had big lips, blunt noses, and a dark skin tone. The name Malasar derives from the Arabic phrase "Malai Arasar," which means "the king of hills." They reside in Poondi, which is located in the Bandipur Reserve Forest at the base of the Velliangiri Mountains. Since they have settled into both, they may also be found in a variety of locations, including Annamalai, Navamalai, Amaravathi Hills, Thirumoorthy Hills, and Anaikatti. They may be recognised by their habitation regions since they are clustered in various locations, such as caverns and hilly terrain. They reside in residences made of "Kalvazhai" and bamboo. Malasars are farmers who work in the forest, and their main food sources are honey and tubers. Tubers are constantly accessible, but they periodically harvest honey and store it for their daily diet. The Annamalai hills' forest is home to groups known as malasars, which often gather food. The Malasar people are well-versed in medicinal herbs. By smelling the herb's aroma, they can determine which parts of the plant are therapeutic. Even just by looking at the plant, they can forecast its medical qualities.

Urali Tribes

The Urali tribes are found in the Kadambur hills, in the Sathyamangalam taluk of Erode district. They live in the hills at an altitude of 1800 feet in the jungle of the Dimnhu area of Tamil Nadu. They are said to be the inhabitants of the





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Erode district. The Urali tribes live only in the deep forest areas and get used to the sounds of wild animals. They would be able to sense the smell of wild animals and make their community people alert if they approached them. They also make money by selling their harvest, like wild fruits, milk, dairy products, wild honey, etc. The Urali tribes mostly build their houses using soil and stones, and for terraces, they use straw. They are skilled at the collection of honey, and harvesting food, and are well versed in Indigenous medicinal practices. They are especially known for the preparation of medicines. They are highly knowledgeable about the places where medicinal plants are found. They go deep into the woods and collect medicinal plants, which they use to prepare medicines efficiently. They are well knowledgeable in the data of the vernacular name of the medicinal plant, its effective usage, the diseases for which it is treated, etc. They have been using only eco-friendly and bio-friendly plant-based products for their everyday use.

Traditional medicinal practitioners diagnose the disease based on the symptoms of the patients, but sometimes they associate it with spirits, and therefore the preparation of medicines, as well as the treatment of patients, are always accompanied by some rituals. They use several plants to serve as remedies to cure a single disease. The Urali tribes have a strong tendency to keep their knowledge secret. The Kadambur hills in the Sathyamangalam taluk of the Erode district are home to the Urali tribes. They occupy a hillside at an elevation of 1800 feet in the dimnhu region of Tamil Nadu. They are supposedly from the Erode neighbourhood. The Urali tribes are accustomed to the noises of wild creatures because they exclusively dwell in the forest. If wild creatures arrived, they would be able to detect their odour and inform the locals. Selling their crop, such as wild fruits, milk, dairy products, wild honey, etc., is another way they generate money.

The Urali tribes mostly employ earth, stones, and straw to construct their homes. Straw is also used to construct terraces. They are adept in gathering honey, gathering food, and practising Indigenous medical techniques. They are renowned for making medications in particular. They have extensive knowledge of the locations where medicinal plants may be discovered. They travel far into the forest to gather medicinal herbs, which they then utilise to make remedies quickly. They have a thorough knowledge of the information pertaining to the common name of the medicinal plant, its practical use, the illnesses it is used to cure, etc. They have only ever used plant-based goods that are safe for the environment and the environment's biosphere. The creation of medications and the treatment of patients are always accompanied by various rituals. Traditional medical practitioners diagnose the sickness based on the symptoms of the patients, although they occasionally correlate it with spirits. To treat a single ailment, they combine the medicinal properties of multiple herbs. The Urali tribes are notorious for keeping their knowledge to themselves.

Pulaya Tribes

The Annamalai hills in Udumalpet Taluk, in the Tiruppur district of Tamil Nadu, are home to the Pulaya tribes. They are difficult to communicate with since they live in steep terrain. Their homes are constructed with thatched roofs and bamboo walls. The Pulaya tribes address a variety of health issues, including diabetes, jaundice, asthma, infertility, etc. with traditional herbal remedies. People created and employed indigenous herbal remedies to cure infectious infections. It has the ability to both prevent and treat infectious and non-infectious illnesses. They appear to be better knowledgeable about the medicinal plants used for treating a variety of diseases. Due to their remote position from the town, they are accommodated in the adjacent mountain villages. Traditional male and female healers are knowledgeable about the use of medicinal herbs and their preparations. In addition to treating wounds and body injuries, male TMPs were also better knowledgeable at treating bites from scorpions and snakes. They are well knowledgeable about the herb's medical characteristics, preparation process, local name, etc.

Soliga Tribes

The Soliga tribes are located in Tamil Nadu's Erode district's Bargur hills. They are a group of mountainous tribes that live in the Eastern Ghats of the Erode district. Soligas dwell in 33 settlements that are sporadically spaced out among a protected forest. Since they typically cultivate and harvest forest products and perform labour on the land, the forest and land are their primary sources of resources. Additionally, they gather honey and sell it to make a





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living. They resided in thatched homes, however, some of the more educated males also resided in tiled homes. They utilize mud, bamboo, wood, and grass to construct their homes. They build their storehouse in the rural yard or a little granary that is built inside each of their homes. These tribes still hold onto their traditional medical expertise, which has a well-known genesis tale. They are well-versed in the therapeutic benefits of each plant as well as animal byproducts. Herbal medications are also sold there.

Summation

Tribal people inherit a wealth of traditional knowledge that they use in their daily practice of medicine. The majority of the tribes still rely on the plants for medical treatment, thus they are quite worried about how the forest and its byproducts are being damaged. They have a strong bond with nature and actively promote its sustainable development, making it an essential aspect of their way of life. They have valued forests as their homes for many generations. They enjoy a mutually sustainable relationship with nature since they have a close connection to it and its surroundings. The tribes' long-standing contact with nature is the source of their ecological knowledge.

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Table.1 District Wise Tribal Population (As per 2011 Census) of Tamil Forest Department

S. No	Districts	Total	Scheduled Tribes	Scheduled Tribes	Total Percentage of
		Population	in 2001	in 2011	tribes in the district
1	Coimbatore	3458045	29103	28342	0.82%
2	Erode	2251744	17693	21880	0.97%
3	The Nilgiris	735394	28373	32813	4.46%
4	Tiruppur	2479052	-	5458	0.22%





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RESEARCH ARTICLE

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Anti-Shoulder Surfing Graphical Password Authentication System

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ABSTRACT

Nowadays, textual password-based authentication systems are widely used in order to prevent security breaches and unauthorized access. But these textual passwords are easy to crack through various types of attacks. Authorized users should access the system or information. Authorization can't occur without authentication. There are various techniques available for this type of authentication. Graphical password is one of them, which has already been studied as a reliable authentication scheme over textual passwords. This paper proposes a new graphical password authentication technique that will provide maximum security with a minimalistic design and with a highly secure database. It is a recognitionbased authentication system where a user is given a set of images and must identify the image sequence selected during registration. During the login phase, all the images are blurred when the user hovers over the image palette and visible when the cursor is moved away from the images. The image grid gets shuffled every time the user clicks on an image. This method prevents shoulder surfing attacks. The proposed technique is also resistant to other types of possible attacks namely dictionary attacks and brute force attacks. It is easy for everyone to use over multiple sites on various platforms for their protection.

Keywords: Authentication; Authorization, Graphical password, Randomization, Privacy





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INTRODUCTION

For Authentication and Authorization, passwords are required. Authentication is the process of determining whether someone or something is, in fact, who or what it says it is [1] [2]. Only authenticated users are permitted to access and edit data; hence authorization is a method used to assure this. While there are different forms of user authentication methods, textual passwords are the most prevalent. They are adaptable and simple to deploy and use. These textual passwords are required to meet two necessary conditions. They must be simple for a user to remember and challenging for an imposter to guess. Users frequently select passwords that are quick to guess or short, making them vulnerable to brute-force and dictionary assaults. Furthermore, these passwords are vulnerable to hidden cameras, spyware, social engineering, key-loggers, and shoulder-surfing attacks. To overcome limitations in existing authorization methods, a graphical password technique is introduced. As the name suggests, in this technique images are used as a password instead of text. Additionally, psychological research indicates that people may recall images more quickly than text. Graphical passwords are therefore simple to remember and challenging to guess or hack[3-6]. The security of graphical passwords may be higher than that of text-based passwords. A dictionary search can quickly enable a hacker to access a system, but if a sequence of photos is chosen, the hacker must randomly test each conceivable grouping. If there are 100 photos on each of the 8 pages in an 8-image password, there are 1008 potential combinations that might make a Graphical Password. If the system just has a 0.1 second delay built in between choosing each image and displaying the next image, it would take millions of years to break into the system by bombarding it with random visual sequences [1].

Graphical passwords are more resilient to brute-force attacks since the search space can be unlimited. However, they are primarily exposed to shoulder surfing. Passwords have a lot of beneficial characteristics and are widely used in legacy systems, so we may anticipate their continued use in the near future. Unfortunately, a range of attacks based on observation, from simple eavesdropping (shoulder surfing) to more advanced techniques, can be made against the common password entry methods used today. When employing direct observation methods, like peering over someone's shoulder, to obtain passwords, PINs, and other sensitive personal information, this is known as a shoulder-surfing assault. When a person uses a traditional input device, such as a keyboard, mouse, touch screen, or any other sort of traditional input device, a malevolent observer may be able to acquire the user's password credentials. This paper proposes a method to solve this issue.

Related Work

Before designing a new graphical password system, it is imperative to analyse existing systems and observe their downfalls to overcome them in the new proposed system. A brief study of different graphical password algorithms and systems is explored in this section.

Recognition based algorithms

In recognition-based algorithms, users are required to choose images from a collection of images. During the authentication process, users are supposed to identify their registration choice among a set of images. Research shows that 90% of users can remember their passwords after one or two months [7]. This section summarizes the present recognition-based graphical password schemes. In the Passface scheme [8], users must choose four faces from the database as their passwords during the registration phase. During the login phase, users must choose a human face from a set of nine faces in which only one face is known to them and the rest act as a decoy. See figure 1. This stage is repeated four times. The order of faces displayed each time is randomized and different sets of images are shown each time. These features help secure a user's Passface combination against detection through shoulder-surfing and packet-sniffing. A study that was carried out on the Passfaces password scheme found that it was easier to recall Passfacess rather than text-based passwords and the users were highly influenced by the gender, attractiveness and race of the faces used [9].





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In the Déjà vu Scheme, the users select and recall an image subset from a larger sample to prepare the portfolio [10]. The pictures are developed using random art. See figure 2. Therefore, it is difficult for the user to disclose their password to others through image portrayal. But the login phase in this method takes longer for the user compared to textual password. On the other hand, the process of selecting a picture from the database can be tedious and consuming for the user. Another drawback could be the need for saving the seeds in the portfolio images of each user in plain text. [11-12]. In the Triangle Scheme [9], a collection of N items are displayed on the screen at random. There is a subset of K items that the user has already selected. The user must locate three of his password objects and click within the invisible triangle formed by those three objects during the login process, alternatively, they can click inside the convex hull of the shown pass items. See figure 3. Besides, this task is performed several times after each login while utilizing a different set of N items. Therefore, the probability of randomly clicking in the correct region in each challenge is very low [19].

In the Moveable Frame Scheme [13], three pass objects are only present. The movable frame will be targeted by one of the pass-objects. To authenticate, users only need to shift the frame by rotating it until all of the pass-objects are arranged in a straight line. See figure 4. To reduce the likelihood of login, the process is repeated a few times by randomly spinning or clicking it. To minimize the likelihood of randomly moving the frame, the procedure is repeated a few times [9]. The drawback of this algorithm is that the process is unpleasant, confusing and timeconsuming since there are too many objects. In the Picture Password Scheme [14], mainly designed for mobile interfaces, before creating a password, users must first select the theme, which consists of thumbnail photographs. The users then save a series of the selected thumbnail photo to generate a password. See figure 5. They must use a stylus to touch the appropriate photographs in the correct order and identify previously seen images in order to be authenticated. However, this scheme will make the memorability of the created password become more complex and difficult. In the Story Scheme [17], the user selects a series of images for their portfolio. In order to log in, the user is provided with an image panel to utilize to distinguish their portfolio photographs from other dummy images. The photographs show people, locations, or commonplace items. The story also included a sequential element by requiring users to select their photographs in the correct sequence. To help them remember the scheme, users were instructed to mentally create a tale that connected the photos in their set. Surveys convey that the users found it difficult to remember their Story passwords, with an 85% success rate [15].

In WYSWYE Scheme [16], the users must copy picture-based password patterns they find on one picture grid onto another. The idea of tabular-based reductions and pattern recognition forms the foundation of this simple technique. The MxM grid is used to identify the pattern of N password pictures, and an NxN grid is used to map the password image pattern that has been detected. While signing in, the system creates an empty, random picture grid and arranges them side by side on the screen. The challenge grid with N password photos and the M2-N images for a decoy is known as the MxM picture grid on the left. This grid is not directly used by the users. Instead, a unique NxN grid called the Response grid is used for input entry. See Figure 6. Users must precisely copy their responses into the answer grid and detect patterns in the password image patterns in order to log in. This is resistant to shoulder-surfing attacks. In S-Passface Scheme, the Passface scheme is enhanced and the selection of decoy pictures is done using visual similarity with the password face. The findings of the research revealed that Passface can be utilized by accurate decoy selection which lowers this method's vulnerability to description attacks. Therefore, it would be challenging for users to explain the password to another person because the fake images do not contain any features linked with the people or their faces. See Figure 7. The research reveals that moving the configuration from mouse-based input to keyboard input, lowers the possibility of being attacked using the shoulder surfing method. [16].

In a study conducted in 2016, Touraj *et. al.* [18] evaluated different recognition-based graphical password schemes, including the schemes mentioned above. Their paper highlighted the importance and the dichotomy of importance and security with graphical passwords – how when security is strong, usability is weak, and vice versa. They concluded that all shoulder-surfing resistant schemes lack ease of usability with users, with regards to login time and memorability, which are major weaknesses to tackle with future recognition-based models.





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Recall based algorithms

In recall-based algorithms, users are required to reproduce a pattern that they created or selected earlier during the registration process [19]. This approach is sometimes called the draw metric, as the user is recalling and reproducing a secret drawing only known to them. Recall-based algorithms can further be divided into two subcategories: Pure recall and cued recall-based algorithms.

Pure recall based algorithms

Pass doodle [20] approach involves the use of doodles drawn using a stylus or unique finger traces as the graphical password. Figure 8. shows an example of a doodle. Even though theoretically, the number of doodle possibilities is larger than textual passwords [20-21], users found it difficult to remember the exact order in which the doodle was produced. Draw a Secret (DAS) approach was proposed by Jermyn et. al. [21] in 1999. In this approach, the drawing interface is a rectangular G x G grid and each section of the grid is represented a pair of coordinates $(x,y) \in [1..G] \times [1..G]$. The user's drawing using their finger or a stylus is recorded as a sequence of coordinate pairs. For figure 9. shows a 4x4 grid DAS, the sequence would be (2,2), (3,2), (3,3), (2,3), (2,2), (2,1), (5,5). With reference to [22], Dunphy's survey shows that most users forgot their stroke order. The encoding represents a one-to-many relationship, therefore there may exist many drawings for a single sequence of coordinates. Such grid methodology also had common "hotspots" or "Points-of-interest" for users, using which a graphical dictionary attack can be initiated to guess users' passwords[23].

Cued recall algorithms

The Blonder approach proposed by Greg E. Blonder in 1996 involves a user selecting one or more predetermined points on an image displayed to them in a predetermined sequence, which will be recorded as a form of authorization. Figure 10 shows an example of a Blonder password. The main drawback of Blonder was that the number of predetermined clickable regions were relatively small and images used for this approach were usually simple and not real life images[23-24]. Pass points method was introduced to overcome the image limitations of Blonder. It was proposed by Wiedenbeck *et. al.* [25] in 2005, which required the user to select several points on a complex real-world image. This provided flexibility of not having predetermined click regions and well-defined boundaries like the Blonder algorithm. Figure 11 shows an example of a Pass points method. Even though Passpoints provided a complex password to crack, it was also a complex password for users to remember. It required more trial time and more time to complete compared to textual passwords. [26] A survey conducted by Habibi Lashkari *et. al.*[26] explores recall-based graphical password algorithms to a greater extent. They have analyzed many other algorithms such as Syukri Algorithm (pure recall), Passmap algorithms are not resistant to shoulder surfing attacks, which further solidifies the need for more approaches that are shoulder surfing attack resistant.

Hybrid Algorithms

Hybrid Based Approaches combine various existing schemes such as recognition or recall-based algorithms as shown in Fig. 12. These approaches give the user options to combine the advantageous aspects of various approaches. Hybrid Based Approaches may increase security, reliability or user-friendliness. In 2011 Wazir Zada Khan *et al.*[27] suggested a hybrid approach that combines the recognition and recall-based approaches. Initially, the system asks the user to enter their name followed by a textual password. The system then prompts the user to select a group of objects from the screen. At least three objects must be selected and any object can be chosen any number of times. The user then uses a mouse or a stylus to draw these objects on their screen. A database stores these templates with the username. During authentication, the user enters their username, and textual password and then draws the objects as their password. The drawn objects are then compared to the objects selected during registration. If the objects are recognised then the user is verified and if not the user is denied. The suggested application uses methods like stroke merging, pre-processing, sketch simplification, hierarchy construction, feature extraction, and hierarchical matching which Wing Ho Leung and Tsuhan Chen proposed [28]





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This system is more convenient than the grid-based techniques and eliminates the need to remember coordinates. It is also a system that visually challenged users may use. The drawback of this approach is that the user must recall and draw the object very similar to what was drawn during registration. It is also inconvenient for most phone users as it requires hardware components such as a mouse or a stylus. Sundari S. and Kuppusamy Karunakaran [29] proposed the VerCube model, a hybrid cryptographic technique for graphical password authentication. The model initially uses a conventional login system. After the login, the scheme generates a secret code that is encrypted using the TDES algorithm. The hidden code is split into two parts and the model loads a pair of images. The LSB scheme is used to encode the two pieces of the hidden code into each image's pixels. The encoded images are sent to the user via email and are downloaded. For authentication, the downloaded images are uploaded with a decryption key. The model then uses the key to decode the image and obtain the secret code. The decrypted code is verified with the actual secret code and the user is authenticated. The VerCube model is efficient with fast performance and very high security.

METHODOLOGY

We propose a recognition-based graphical password system called GP- ASS (Graphical Password - Anti Shoulder Surfing) which consists of several categories, and each category consists of a grid of 25 images. There are two phases in our GP-ASS system [32].

Registration Phase

- 1. As the first step of registration, we collect the user's email ID. Then, we use this data to identify each user in our database uniquely.
- 2. One Time Password (OTP) will be sent to the user's email id; the user will have to enter this 6-digit OTP correctly to proceed to the next step.
- 3. Users will be asked to choose one image set of their choice from given 8 sets, each themed with unique classes of images such as animals, flags, fruits, food, traffic signs, emojis, and musical instruments. Each of these sets will contain 25 images which will be shown to the users on the next screen based on the type of set they choose.
- 4. From the given 25 images, users will have to choose a sequence of images which can contain a minimum of four images and there is no upper bound. Repetition of images is allowed. This formed sequence will be the password of the user. Users who find it difficult to remember their passwords can save a screenshot of their password sequence at this stage using the screenshot option that is provided.
- 5. To confirm the password, users will be asked to re-enter their password. This time the screen will be enabled with our Anti Shoulder Surfing technique 3.3.
- 6. The registration will be successful if the passwords entered in the above two steps match.

Login Phase

- 1. Users will be entering their email ID, and if the email ID is present in the database, they will be redirected to the category-choosing page. If the email ID is not found in the database, they will be redirected to 3.1.
- 2. Users will be asked to choose what category their image belongs to. If the category selected by the user is the same as that chosen during registration, then the user will proceed to the next step. If the category selected is not the same as the category selected during registration, then the system will throw a warning and the user will have two more tries to re-enter the correct category.
- 3. Users will then enter the images from the given image set in the same order as they did during registration. But this time, our Anti Shoulder Surfing technique 3.3 will be enabled, and every time the user clicks on an image from the palette, all 25 images will be shuffled randomly within the palette to prevent automated brute force 3.4 attacks. Dictionary attacks 3.5 are also not possible.
- 4. If the user enters the correct sequence of images, they will be successfully logged into the system. If not, they are given 2 more tries to re-enter both the category AND the sequence of images.





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Anti-shoulder surfing technique

A shoulder surfing attack is when the attacker identifies the password by looking over a person's shoulder. This kind of attack is prevalent in crowded areas where it is not uncommon for people to stand behind each other like in a coffee shop or in the queue for an ATM [30]. Both recognition and recall-based graphical passwords are vulnerable to shoulder surfing attacks as the attacker looking over the shoulder can remember the image pattern, or the image drawn by the user. To tackle this problem in our recognition-based system, we implemented an anti-shoulder surfing technique where every time the user hovers over the image grid, all the images in the grid get blurred out. Thus, only the user knows which image they are clicking, and the shoulder surfer sees only a blurred image. Additionally, every time the user clicks on an image, the image palette will be shuffled randomly to ensure that the shoulder surfer does not memorize the location of each image in the grid.

Brute force prevention

Brute force attacks in graphical password authentication systems are generally carried out by automating the mouse clicks to try out every possible permutation from the given images. However, this automation is only feasible when the positions of the given images on the screen remain the same, which is necessary for programming the automation script, which takes the input of the coordinates of each image present on the screen. However, in our approach, we shuffle the images within the palette after every click. This will ensure that each image's positions change dynamically, making it difficult for the automation script to keep track of the tried permutations and making the automation task difficult or impossible.

Dictionary attack prevention

In alphanumeric-based password systems, a dictionary attack attempts to break into the system by constructing a list of strings that are commonly used as passwords and trying each member of the list until a matching string is found[31]. Recognition-based graphical passwords involve mouse input rather than a keyboard where the password image appears on the screen and trying all instances until the matching password is found is not feasible [32].

Drawbacks

- 1. The difficulty in remembering the password sequence is a major drawback of our system. A large portion of people prefer alphanumeric passwords over graphical passwords as alphanumeric passwords are easier to remember and to safely store somewhere.
- 2. The registration and login process in the Graphical Password Authentication System is usually time-consuming. Users may find it difficult to log in to the system for quick use and it may cause inconvenience to the people who will have to log in and log out of the system frequently.
- 3. Using Graphical Authentication System in web applications will cause increased loading times due to the relatively large size of the image data.
- 4. Our system is not suitable for people with visual impairments such as complete or partial blindness, color blindness, etc.

RESULTS

With our proposed idea we take care of all the possible attacks. It provides strong security against Brute force attacks, Dictionary attacks and Shoulder surfing. For instance, when we investigate one of the problems Shoulder surfing, our model ensures that there is no way for the person behind the user to know what is being clicked on the screen by blurring the images when being hovered. On every click, the picture palette is shuffled which makes sure even if the shoulder surfer tries to look after your click, he will end up seeing the wrong image. We provide 10 categories of 25 pictures each. The number of images a password can contain is a minimum of 4 with no upper bound. So, assuming the user sets up to 25 images, the total number of combinations results in 254+255+256+...+2525 which is of the order 1034 which is exponentially higher than a traditional alphanumeric password. On top of that, for every 3 wrong attempts, we block the account for 10 minutes. When an attacker tries to brute force the password





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of 4 images, let us assume it takes 33 seconds for an attempt. After 3 failed attempts he must wait 10 minutes. Approximately it takes 11 minutes for three attempts. The total combinations of 4 picture passwords are 254 which is 390625. It will take him (390625/3) *11=429,6875 minutes which is 23,871 hours which is 2.7 years for a 4-picture password in one category. The model provides 10 categories and provisions for up to 25 pictures if the user can remember which made brute force attack practically impossible. The application makes the user change their password every month. This makes sure that dictionary attacks are impossible. On top of that, the system will change the image palette every six months which will make sure the previously collected passwords are not a threat anymore for the user. Because of these two features previously collected dictionary data will become useless. If any user forgets their password, we will send them a mail to their registered email address for them to reset their account. This is possible only when they enter the OTP sent in the mail. This prevents any unauthorized logins.

CONCLUSION

Graphical passwords have proven to be more secure and authorized than traditional alphanumeric passwords. When people find it difficult to remember complex alphanumeric passwords, our model provides them easy to remember passwords with more security. People even found it easy to remember passwords because it's of the category they choose. We have provided a straightforward user interface which will help a lot of people. In the future, on one hand, we will improve the interface for creating passwords and on the other hand, we will further improve the security and privacy features. We aim at doing further research and making the application more robust.

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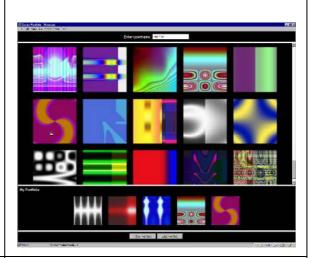


Figure 1. Passface scheme interface

Figure 2. Déjà vu scheme login

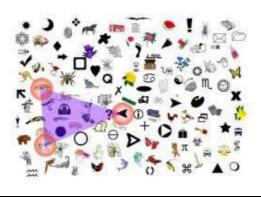
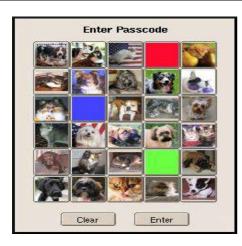




Figure 3. The convex hull for Triangle scheme.

Figure 4. Aligned frame in Movable frame scheme.





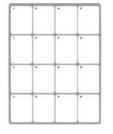


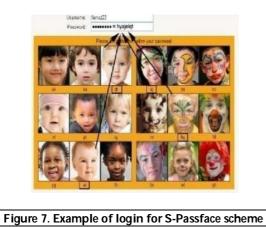
Figure 5. Login interface of Picture password scheme.

Figure 6. MxM picture grid (left) and NxN response grid (right)



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1 2 3

Figure 8. Doodle

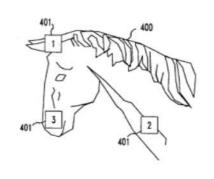


Figure 9. Draw a Secret

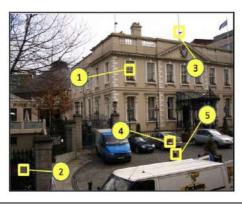


Figure 10. Blonder Password

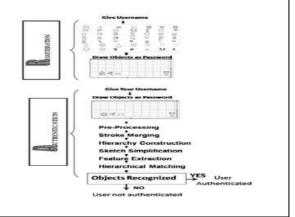


Figure 11. Passpoints

Figure 12. Recognition and Recall Method



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Figure 13. The user must choose one of the given categories



Figure 14 Images from selected category

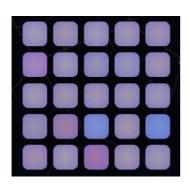


Figure 15. Images blurred on mouse hover



Figure 16. Images shuffled after selecting an image





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RESEARCH ARTICLE

Chemical Characterization of the Linga Chenduram Preparation Process as per Siddha Literature through Instrumental Analysis

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ABSTRACT

The Siddha medical system had described the usage of single metal and mineral in the form of Parpam and Chenduram prepared with the help of herbs and incineration processes. These prepared medicines were used for the treatment of several disease conditions. Linga Chenduram were Lingam (Cinnabar) is the primary constituent, this medicine was selected to this study and the change of particle size, chemical properties and functional group changes were identified via the analysis of step by step procedure of Linga Chenduram through FTIR, SEM and EDAX. The study results showed that Acidic group, Alcohol, Ester and Sulfoxide compounds were present in PL and PLL samples. Carboxylic acids compounds were present in PLL and LC samples. Alkyne, Nitro and Disulfide compounds were present only in PL sample. Lactam and Phenol compounds were present in only PLL. Compounds, Ammonium, Thiol, Arenes, Disulfide and Alkyl halides were present only in LC sample. In each process of the preparation of Linga Chenduram there were significant changes in the functional compounds. The particle size of *Linga Chenduram* (LC) ranged from 500 nm to 5µm. Before incineration process the particle size ranged from 1µm to 10µm. This showed that after incineration the particle size were reduced to Nano size. EDAX results indicated the presence of lead in sample PL only. Also less quantity of Sulphur was present in the sample PL and absent in the end product of the sample LC. Concluded from this study in each steps of Linga Chenduram preparation there were changes in the chemical properties, functional groups and end product had Nano size particles. This proves the importance of every process of Medicine preparation as per the siddha literature.

Keywords: Linga Chenduram, FTIR, SEM, EDAX, Lingam, Cinnabar





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INTRODUCTION

Siddha medicine is one of the ancient and precious medical systems in Southern part of India and Northern part of Sri Lanka [1]. This system was built up by Siddhars [2], where they had described the usage of single metal and mineral in the form of *Parpam* and *Chenduram* prepared with the help of herbs. These prepared medicines were used for the treatment of several disease conditions. According to *Anuboga Vaithiya Navaneetham*, Volume 4, *Linga Chenduram* were *Lingam* (Cinnabar) is the primary constituent, prepared by grinding with the latex of *Thirugukalli(Euphorbiatortilis)* and covered with *karkam*(paste) of the *Utthamani(Pergulariadaemia)* and *Vellarukkam* (*calotropisprocera*) flowers, then incinerated with cow dung cake is the main process of preparation[3]. Thus prepared medicine had been subjected to Fourier Transform Infra-Red Spectroscopy (FTIR), Scanning Electron Microscope (SEM) and Energy Dispersive X-Ray (EDAX) analysis. The change of particle size, chemical properties and functional group changes were identified through the analysis. The obtained results helps to provide the importance of step by step procedure of *Linga Chenduram* and as a proving it scientifically is the main aim of the study.

Aim

To evaluate the change in partial size, Chemical properties and functional group on the process of *Linga Chenduram* preparation.

Objectives

Preparation of *Linga Chenduram*as per literature.

Analysis of *Linga Chenduram* using FTIR, SEM and EDAX Instruments.

METHODOLOGY

Purification of Lingam (Cinnabar)

Placed the desired quantity of the Lingam sample on the stone motor, added sufficient quantity of lemon juice little by little and ground well for about 6 hours. Then collected the sample, made them as à single round disc and covered in a thin muslin cloth. It is then suspended in a mud pot 4 inches from the base without touching the bottom, added desired amount of lemon juice then ignited the pot in slow flame and burnt well until the lemon juice got dried off. Finally the sample was dried, ground and stored in an airtight glass jar and label as PL (Sample - PL)

Preparation Process

Purified lingam was measured and made into powder form with mortar and pestle. *Euphorbiatortilis(Thirugukalli)* latex poured into it and ground well by stone motor and pestle for 12 hours (*4 saamam*). After these steps the sample was then stored in an airtight glass jar and label as PLL (Sample - PLL) The mixture of *Lingam*was then made into small disc (*villai*) and spread in a suitable pot for drying in sun light. Flowers of *Pergulariadaemia (Utthamani)* and *calotropisprocera* (*Vellarukkam*)were ground together and made into paste (*karkam*). Dried disc of Lingam was covered with prepared *karkam* then placed into pot with lid and sealed with clay smeared cloth (*seelaimann*). Weight of clay pot with lid containing mixture was measured. Then it was subjected into incineration process (*Pudam*) by cow dung cake (4 times the weight of the measured clay pot weight). After the incineration process clay pot was allowed to cool itself. Processed medicine was taken from the clay pot and ground into fine powder. Finally, it was stored in an airtight glass container and labelled as **LC** (Sample - LC). Procedure of FTIR analysis. The Perkins Elmer Spectrum One Fourier Transform Infrared Spectrometer (FTIR) was used. The Samples PL, PLL and LC placed in Potassium Bromide (KBr) discs with scan rate of 5 scan perminute at the resolution 4cm-1 in the wave number region 450 - 4000cm-1. The samples were grounded to fine powder using agate motor and pestle and mixed with KBr. They were then Pelletized by applying pressure to prepare the specimen (The size of specimen was about 13 mm diameter and 0.3 mm in thickness) to record the FTIR Spectrum under Standard condition.





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Procedure of SEM and EDX analysis

The surfaces of the samples were analyzed through TESCAN CLARA, Ultra-High Resolution Scanning Electron Microscope (SEM). The Samples PL, PLL and LC were placed separately on a carbon tape inside an airtight chamber. High-energy electron beam was focused through a probe towards the samples. Variety of signals was produced on interaction with the surface of the samples. This results in the emission of electrons or photons, and it was collected by an appropriate detector. The EDX under ultra-high vacuum was used to examine the elemental composition.

RESULTS

The infrared spectrum of PL sample (as shown in Figure No.1) was in the wave length range of 596 cm-1 to 3411 cm-1 and there were 13 types of vibrations found and expected functional compounds were identified (Table No 1 and Figure No 1). The results of FTIR spectroscopy confirmed the presence of various chemical constituents such as Alkyne (CEC) and Aromatic (C- H) which were identified by following weak characteristic absorption bands exhibited at 2135 cm⁻¹ and 783 cm⁻¹. The medium instance peaks were identified at 596 cm⁻¹ which were assigned to Aromatic groups. The strong characteristic absorption bands were exhibited at 3411cm⁻¹, 2926 cm⁻¹, 2854 cm⁻¹, 1628 cm-1, 1514 cm-1, 1446 cm-1, 1364 cm-1, 1206 cm-1, 1163 cm-1 and 1035 cm-1 which was assigned to Alcohol (OH stretching), Alkane (CH₃ stretching), Alkane (C - H stretching), Alkene (C = C stretching), Nitro compound (N - 0 stretching), Acidic group (O - C - H), Sulfonate (S = O stretching), Tertiary alcohol (C - O stretching), Ester (C - O) and Sulfoxide (S = O stretching). The infrared spectrum of PLL sample (as shown in Figure No.2) was in the wave length range of 714 cm⁻¹ to 3364 cm⁻¹ and there were 14types of vibrations found and expected functional compounds were identified (Table No 2 and Figure No 2). The results of FTIR spectroscopy confirmed the presence of various chemical constituents such as Aromatic (C- H) which were identified by following weak characteristic absorption band exhibited at 714 cm⁻¹. The medium instance peaks were identified at 1546 cm⁻¹, 1318,cm⁻¹ and 778cm⁻¹ which were assigned to Alkane (C=C), Phenol (O – H bend) and Aromatic groups (C – H bend). The strong characteristic absorption bands were exhibited at 3364cm⁻¹, 3009cm⁻¹, 2925 cm⁻¹, 2854 cm⁻¹, 1708 cm⁻¹, 1650 cm⁻¹, 1455 cm⁻¹, 1412 cm⁻¹, 1281 cm⁻¹ and 1053 cm⁻¹ which was assigned to Alcohol (OH stretching), Alkane (C - H stretching), Alkane (CH₃ stretching), Alkane (C - H stretching), Carboxylic acid (C = 0 stretching), Lactam (C = O), Acidic Group (O - C -H), Sulfonate(S = O stretching), Aromatic ester(C - O stretching) and Sulfoxide (S = O). The infrared spectrum of LC (as shown in Figure No.3) was in the wave length range of 463 cm⁻¹ to 3429 cm⁻¹ and there were 9 types of vibrations found and expected functional compounds were identified (Table No 3 and Figure No 3). The results of FTIR spectroscopy confirmed the presence of various chemical constituents such as Carboxyl groups (OH stretching), Ammonium (N - H), Thiol(S-H stretching), Disulfide (S - S) and Anhydride (C-I) which were identified by following weak characteristic absorption bands exhibited at 3429 cm⁻¹, 2871 cm⁻¹, 2513 cm⁻¹, 514 cm⁻¹ and 463 cm⁻¹. The medium instance peaks were identified at 1797 cm⁻¹ and 712 cm⁻¹ which were assigned to Carboxylic acids and Alkenes groups. The strong characteristic absorption bands were exhibited at 1430 cm⁻¹and 875 cm⁻¹ which was assigned to Aromatic compound and Arenes.

Scanning Electron Microscope (SEM)

The SEM analysis photographs revealed that the particle size ranged from $1\mu m$ to $10\mu m$ in different batches of PL sample. So the particles in Purified Lingam sample were micrometers in size. (Figure No 4). The SEM analysis photographs of sample PPL revealed that particle sizes rangedfrom $1\mu m$ to $10\mu m$ (Figure No 5). in different batches. The sample Lingam ground with Euphorbiatortilis(Thirugukalli) latex showed that most of the particles present were micrometers in size. The SEM analysis photographs of sample LC revealed that the particle size ranged from 500 nm to $5\mu m$ (Figure No 6) in different batches. The final sample of Linga Chenduram showed that most of the particles were in Nano size.

EDAX analysis shows the elements present in the Purified *Lingam as* shown in Figure No 7. and the Table No 4.It was found that mass percentage of Carbon, Oxygen, Magnesium, Mercury, Sulphurand Lead were 9.1, 2.97, 2.23, 78.81, 3.14 and 3.74 percentages respectively. The result showed that Mercury (78.81) was present in larger amount





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compared to others. EDAX results of *Lingam* ground with *Euphorbiatortilis*(*Thirugukalli*) latex Sample (PLL) as shown in Figure No.8 and the Table No. 5 it was found that mass percentage of Carbon, Oxygen, Magnesium, Mercury and Sulphur were 19.93, 7.63, 1.58, 69.14 and 1.71 percentages respectively. The results showed that Mercury (69.14) and Carbon (19.93) were present in larger amount compared to others. EDAX analysis shows the elements present in the *Linga Chenduram as* shown in Figure No. 9 and the Table No. 6 It was found that mass percentage of Carbon, Oxygen, Magnesium and Mercury were 43.63, 4.01, 1.15 and 51.2 percentages respectively. The results showed that Mercury (51.21) and Carbon (43.63) were present in larger amount compared to Oxygen and Magnesium.

DISCUSSION

FTIR results shows that Alkenes and Aromatic compounds were present in three samples. Acidic group, Alcohol, Ester and Sulfoxide compounds were present in PL and PLL samples. Carboxylic acids compounds were present in PLL and LC samples. Alkyne, Nitro and Disulfide compounds were present only in PL sample. Lactam and Phenol compounds were present in PLL. Compounds such as Ammonium, Thiol, Arenes, Disulfide and Alkyl halides were present only in LC sample. In each process of the preparation of *Linga Chenduram* there were significant changes in the functional group of the compounds. The results of SEM analysis revealed that particle size of the samples PL and PLL ranged from 1μ m to 10μ m and end product of *Linga Chenduram* (LC) ranged from 500 nm to 5μ m. This showed that after incineration the particle size were reduced to Nano size. EDAX results indicated that Mercury, Magnesium, Oxygen and Carbon were present in all the samples but Lead was present in sample PL only. Also less quantity of Sulphur was present in the sample PL and absent in the end product of the sample LC.

CONCLUSION

In each steps of *Linga Chenduram* preparation there were changes in the chemical properties and functional groups also after incineration process the particle size were reduced to Nano size. Further study had to be conducted in large number of samples using more modern equipment for scientific evaluation.

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Table. 1: FTIR interpretation of PL

S.No	Wave numbers (cm-1)	Appearance	Vibration type	Functional compound
1	3411	Strong, Broad	OH stretching	Alcohol
2	2926	Strong	CH₃ stretching	Alkane
3	2854	Strong	C - H stretching	Alkane
4	2135	Weak	CEC stretching	Alkyne
5	1628	Strong	C = C stretching	Alkene
6	1514	Strong	N – 0 stretching	Nitro compound
7	1446	Strong	O - C - H	Acidic group
8	1364	Strong	S = O stretching	Sulfonate
9	1206	Strong	C - O stretching	Tertiary alcohol
10	1163	Strong	C - O	Ester
11	1035	Strong	S = O stretching	Sulfoxide
12	783	Weak	C - Hbend	Aromatic
13	596	Medium	S – S	Disulfiram

Table. 2: FTIR interpretation of PLL

S.No	Wave numbers(cm-1)	Appearance	Vibration type	Functional compound
1	3364	Strong, Broad	OH stretching	Alcohol
2	3009	Strong	C - H stretching	Alkane
3	2925	Strong	CH₃ stretching	Alkane
4	2854	Strong	C - H stretching	Alkane
5	1708	Strong	C = O stretching	Carboxylic acids
6	1650	Strong	C = O	Lactam
7	1546	Medium	C = C stretching	Alkane
8	1455	Strong	O - C - H	Acidic group
9	1412	Strong	S = O stretching	Sulfonate
10	1318	Medium	O -H bend	Phenol
11	1281	Strong	C – O stretching	Aromatic ester
12	1053	Strong	S = O stretching	Sulfoxide
13	778	Medium	C - H bend	Aromatic
14	714	Weak	C - H bend	Aromatic

Table.3: FTIR interpretation of LC

S.No	Wave numbers(cm-1)	Appearance	Vibration type	Functional compound
1	3429	Weak, Broad	OH stretching	Carboxyl groups
2	2871	Weak	N – H	Ammonium
3	2513	Weak	S-H stretching	Thiol
4	1797	Medium	C=0	Carboxylic acids
5	1430	Strong	C–C stretch (in-ring)	Aromatic compound
6	875	Strong	C-H bending	Arenes
7	712	Medium	CIS-RCH = CHR	Alkenes
8	514	Weak	S – S stretching	Disulfide
9	463	Weak	C-I	Alkyl halide





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Table. 4: Weight and atomic percentage of PL

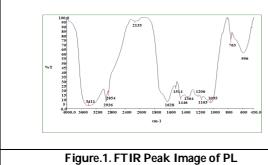
Element	Weight %	Atomic %	Net Int
CK	9.1	49.07	119.02
OK	2.97	12.03	50.72
MgK	2.23	5.95	65.25
HgM	78.81	25.44	677.26
SK	3.14	6.34	65.29
PbM	3.74	1.17	27.64

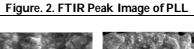
Table.5: Weight and atomic percentage of PLL

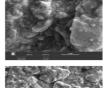
Element	Weight %	Atomic %	Net Int.
CK	19.93	63.84	122.88
OK	7.63	18.35	53.53
MgK	1.58	2.49	18.63
HgM	69.14	13.26	233.71
SK	1.71	2.06	14.01

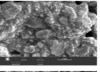
Table. 6: Weight and atomic percentage of LC

	22.0. 0. 1.0.g a.i.a a.to por our angle or = 0					
Element	Weight %	Atomic %	Net Int.			
CK	43.63	86.78	776.13			
OK	4.01	5.99	58.26			
MgK	1.15	1.13	31.95			
HgM	51.21	6.1	390.23			
SK	0	0	0.03			









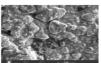




Figure.3. FTIR Peak Interpretation of LC

Figure. 4: Scanning Electron Microscope images of PL

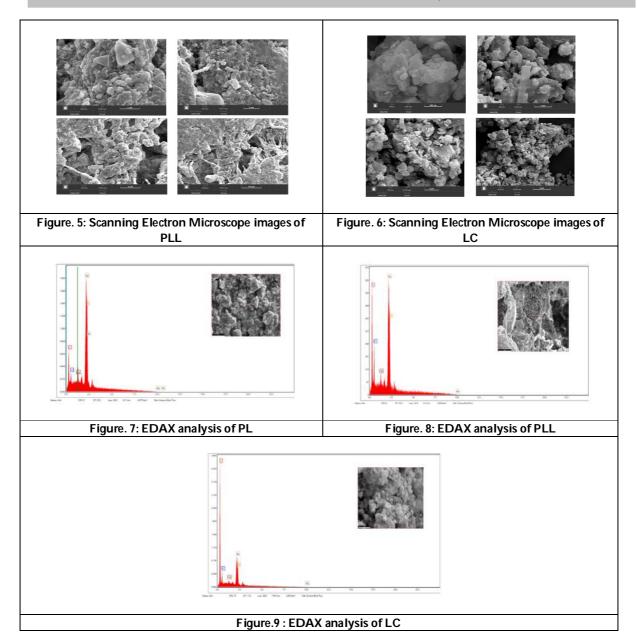




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RESEARCH ARTICLE

Perspectives on Chemometrics in Comprehensive 2D-Chromatography-Strategies and Importance in Pharmaceutical Research

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ABSTRACT

The modern pharmaceutical industry is constantly innovating faster and more accurate techniques of drug discovery, development, delivery, and monitoring; thus, the creation of quick chromatographic procedures is of paramount importance to analytical labs. Comprehensive two-dimensional chromatography (2D) is still being developed as a tool to address challenges in the examination of complex materials. Analysis of complicated samples might provide data that is difficult to process quickly and properly. Chemometrics, a type of computational statistical approach, is crucial in pharmaceutical research and should be used extensively, particularly in the development of 2D chromatography. Here, chemometric analysis of data has shown to be the most fruitful and illuminating method. This article explains the need for chemometric tools and reviews the most recent developments in 2D-chromatography techniques for pre-processing and interpreting data as well as obstacles. The focus of this review's last section is on the use of chemometrics in pharmaceutical research platforms and earlier analytical studies that used chemometrics as a basis.

Keywords: Chemometrics, 2D-liquid chromatography, PCA-principal compound analysis, ANNartificial neural networks.





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INTRODUCTION

There has been a discernible increase in the selection of multidimensional separation platforms for the analysis of extremely complicated data during the last two decades [1]. They are able to characterize difficult mixes in greater detail than mono-dimensional techniques due to their larger peak capacities and, subsequently, stronger separation powers [2]. To achieve this goal, 2D-chromatography data analysis is the best option moving ahead. As a part of the comprehensive 2D chromatographic process, two columns were linked together in series, with a modulator between them, and a spectral detector is located at the end of the second column. Complementary and not unduly correlated separations should be produced by the two columns to take full use of 2D chromatography's two-dimensional peak capacity [3]. For target chromatographic resolving, analytical peak capacity, and sample run time is multiplied by the median peak width at the base. That's why a 2D separation's optimal peak capacity is a total of its peak capacities for its first and second column separations [4]. The ability of new analytical instruments to generate more complicated data makes it challenging to extract relevant information and derive straightforward and accurate conclusions, especially when analysing samples with several components. To optimize something chromatography referred to as a "tsunami of data" or, more widely, "Big Data," effective data-analysis approaches are needed [5]. Multidimensional liquid chromatography methods have become more widely used and well-liked in recent years [6]. To deal with this type of data, certain chemometric algorithms have also been proposed [7]. As they presented several ways to assess the structure of this data and to carry out the quantification of the target analytes, most of the work so far has been on the analysis of data obtained by chromatography [8]. Overall, it might be said that there was some ambiguity about the data's trilinear behaviour, notably when the second chromatographic dimension was given a gradient.

Two-Dimensional Liquid Chromatography (2d-Lc)

In a 2D-LC study, two distinct liquid chromatography studies are combined into a single data analysis. In the late 1970s and early 1980s, two-dimensional liquid chromatography was first developed. This period saw trials and more theoretical and conceptual work proving the theorized principles of 2D-LC. When compared to more traditional onedimensional liquid chromatography methods, it was found that 2D-LC had a higher resolving power. Proteomics and polymer research relied heavily on the 2D-LC approach in the 1990s for the separation of very complex chemicals and materials [9]. Whenever it comes to analysing data, the approach had a big drawback. Due to the lengthy analysis time of 2D-LC, early research was restricted to a tiny fraction of liquid phase separations. Due to recent advancements in 2D-LC technology, this once debilitating flaw has been considerably curtailed. Highresolution separations may be performed in less than an hour using modern 2D-LC [10]. The evolution of 2D-LC is being pushed ahead because of the rising need for instruments that can analyse compounds of increasing complexity with greater detection limits. Since they've grown so common in the business, getting your hands on an instrument component is now a lot simpler than it used to be. 2D-LC was previously done utilizing components from 1D-LC equipment, and the outcomes were variable in terms of precision and accuracy. 2D-LC pioneering work has been possible because of a reduction in the burden on instrumentation engineers. Because one-dimensional liquid chromatography can't efficiently separate some mixes, this approach is used to accomplish the task. Liquid chromatography in two dimensions is more suitable for the analysis of complicated sample mixes like blood and other biomedical materials [11].

Compounds with many distinct effluents have a difficult time being separated. One reason for this is that it is impossible to separate them all. Dissolving closely related substances is another issue that might arise when using one-dimensional liquid chromatography. Similar chemical qualities may make it difficult to identify closely related compounds based on polarity, charge, or other characteristics. Liquid chromatography may be used to separate substances depending on more than just chemical or physical characteristics. The basicity of peptides may be used to separate a mixture; however, comparable peptides may also not elute efficiently. The same basicity of the peptides may be further differentiated by using variations in apolars character in a subsequent LC procedure [12]. As a consequence, a second LC analysis must have a separation selectivity considerably different from the first column if it is to separate the mixture more effectively [13]. Additionally,2D liquid chromatography must be used with highly





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orthogonal procedures, which implies that its two separation processes must be as distinct as feasible [14]. 2D liquid chromatography may be divided into two broad categories. 2D liquid chromatography (LCxLC) & heart-cutting 2D liquid chromatography (LCxLC) are two examples (LC-LC). Although the peaks of elution are completely sampled in comprehensive 2D-LC, the whole sample has been judged unneeded for transmission to the second column. A part of the sample is discarded, while the remainder is delivered towards the sampling valve for further analysis. In heart-cutting 2D-LC, just a tiny fraction of the peak is injected into a second column to target certain peaks. 2D-LC with heart-cutting has shown to be fairly effective for the sample analysis of chemicals that are not particularly complicated, assuming they exhibit identical retention behaviour. 2D-LC. With less system setup and lower running costs than complete 2D-LC, heart-cutting 2D-LC is an effective technology. To sample many peaks from the first-dimensional analysis, for instance, without running the risk of brief overlap with the second-dimensional analysis, multiple heart-cutting may be utilized. MLC-LC The "multiple heart-cutting" approaches makes use of a variety of sampling loops (MLC-LC) [15].

When it comes to 2D-LC, peak capacity matters a lot. For this, gradient elution separation is substantially more efficient than that of an isocratic separation provided an acceptable period. However, whereas isocratic extraction is more convenient in the short term, gradient extraction is more desirable in the second level. From a weaker eluent formulation to a stronger eluent formulation, the mobile phase concentration is changed. The link between retention time, instrument variables, and solute parameters in reversed-phase chromatography is shown here using linear solvent strength theory (LSST). In other words, the time constant is equal to the time constant multiplied by the time constant [2]. Since 2D-LC became a prominent analytical chromatographic method, considerable of pioneer work has been done, however, there are still many current issues to be addressed. The method is continually evolving, with a large number of experimental variables yet to be settled upon.

Chemometrics

In recent years, the majority of research analysis has used Chemometrics to understand the differences or variations in the data matrix. Chemometrics uses statistical and mathematical methods to analyse data in order to maximise the amount of information that may be collected and extracted for use. In separation science, the chemometrics technique is frequently used to forecast and examine peak asymmetry, peak overlaps, and peak optimizations. Due to interference brought on by improper technique optimization, co-elution of numerous analytes in chromatography greatly complicates quantification and identification of the target analyte. Principal components analysis (PCA), a commonly used chemometrics technique at the moment, has been expanded to include LC-HRMS analysis for proteomics and metabolomics. Additionally, factorial design (FD), cluster analysis (CA), partial least squares (PLS), and artificial neural networks (ANN) are also in use. [16, 17]

Chemometrics in one- and two-dimensional chromatography

Total first-dimension (1D) effluent is split up into several fractions in the development of 2D-chromatography, and each fraction is then subjected to 2D separation. Combining the outcomes of liquid chromatography separations results in 2D chromatography (LCxLC). While the intensities of the spots offer quantitative information, the spots' locations offer qualitative data. However, it can be challenging to extract information from exceedingly complex compounds such as protein digests, metabolic extracts, and oil mixtures. Even with today's high-resolution chromatography, it is still challenging to properly extract the information from a complex matrix. The effectiveness of chemometric data processing techniques is continually being improved by several studies. Chemometric is a useful pre- and post-data analysis method in chromatography for unwanted background signals, resolving baseline drift, unresolved peaks shifting and retention durations. Chemometrics-based information extraction, pre-data processing, and data interpretation can greatly improve an existing technique's analytical performance. Chemometrics techniques used in chromatography include penalised least squares (PLS) methods, Background erasure via multivariate curve resolution and orthogonal subspace projection, the local minimum value method, baseline estimation and denoising using scarcity, retention-time-alignment techniques, peak clustering, and principal





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component analysis (PCA). These approaches demonstrate that the largest advancements in spectroscopy, 1D and 2D chromatography, are being made by chemometric techniques. [18]

Chemometric Classifications

Unsupervised and supervised Techniques

Unsupervised approaches like PCA and its robust variation, independent compound analysis (ICA), and cluster analysis (CA) are utilised to comprehend the dissimilarity or variance in the data matrix. As a consequence, the unsupervised methods from the "calibration set" may be used to create loading vectors and project unknown data. Supervisory techniques, such as multivariate calibration methods, are used when data does not cluster in accordance with any objective criterion. Principal component regression is the term used to describe the process of creating a regression model using a large number of PCA variables. The primary basis for the PCR analysis of the data matrix is variance. In the linear supervised approach, the partial least squares (PLS) technique, also known as a projection to latent structures, is frequently employed. In order to develop a regression model using several of these paths as its initial possibilities, it first calculates the path through the data matrix that maximises the covariance between the matrix and the predicted variable [19].

ICA- Independent Component Analysis

It was the powerful tool for improving signal-to-noise ratio, but it frequently reveals differences in scalp topography between patients, making it impossible to consistently apply conventional statistical analysis methods like ANOVA. We describe an ICA technique that maintains the improved signal-to-noise ratio while restoring consistency [20].

CLS-Classical least squares

An efficient modelling technique for hyper spectral image identification and classification. The fact that target spectra may be known, but reference values for each pixel are infrequently available, makes CLS amenable to modelling pictures. Another reason is that clutter signals (interferences and noise) may be muted and weak target signals can be boosted by combining CLS with weighting algorithms. The four varieties of CLS provided are weighted least squares (WLS), generalised least squares (GLS), and extended least squares (ELS) [21].

PCA-Principal component Analysis

Principle component analysis (PCA) is a technique used to reduce the number of interrelated variables in a data collection while retaining as much of the data set's variation as is practical. To do this, Principal components, a fresh collection of uncorrelated variables, are employed. PCA loading plots were used to evaluate the two-dimensional high-performance liquid chromatography (2D-HPLC) fingerprint data from samples obtained at various stages of an industrial process chemical workflow to highlight significant differences. Data reduction was carried out using a 2D-HPLC peak selection procedure before chemometric analysis.

PCR-Principal component regression

PCR type of regression analysis approach related to principal component analysis, is used in statistics. In a conventional linear regression model, this especially employed to estimate the unknown regression coefficients.

PLS-partial least squares regression

PLS maximises the correlation between these variables and the response variable while minimising the variance of the explanatory factors. PLS regression is a compromise between multiple linear regression and principal component analysis. When there are several variables with multicollinearity or missing data, it might be helpful. PLS appears to have replaced other 2D-chromatography methods as the technique of choice among chemists, according to the majority of reports. among the most successful results These include the ease of interpreting PLS loadings, the fact that PLS should handle nonlinearities better than PCR, and the fact that PLS requires fewer latent variables than PCR and should thus be more economical. Because correlations with the y variable are sought after while calculating the





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scores, PLS ought to predict more accurately [22]. PARAFAC-Parallel factor analysis In that it decreases the dimensionality of the data collection, factor analysis is comparable to PCA. A subspace and the vector orientations are discovered using PCA, which is merely a dimension-reduction technique, in contrast to factor analysis, this also makes an underlying model assumption [23]. Parallel factor analysis (PARAFAC), which can also handle minor changes in retention time, is used to see data as trilinear and consisting of three modes, namely concentrations, chromatograms, and spectra [24].

MCR-ALS- Multivariate curve resolution

One of the most well-known techniques for 2-way data processing is MCR-ALS. It enables to recovery of a mixture's component count, response profiles, and estimated concentrations [25]. MCR is so frequently used for quantitative objectives. It may also be used to fix the backdrop, though. The requirement of bi-linearity in MCR must be met by the data. Using MCR, a matrix is broken down into pure chromatographic and spectral profiles, along with any noise or mistake.

MCR-Multivariate curve resolution

MCR is a useful pre-processing technique and may also be used to retrieve data from chromatograms [26]. By separating the data into response profiles and peak regions, MCR can ascertain the mixture's constituent parts. To extract unique chromatographic and spectral patterns for each analyte, MCR and ALS make an excellent combination.

SVM- Support-vector machines

The classification of various chromatographic data using support-vector machines is another way. SVM is a machine learning technique that can resolve classification and regression problems.SVM chooses the extreme vectors and points that help the hyperplane evolve. Due to these unusual circumstances, the method is known as a "support vector machine".

MLR- Multiple linear regression

Multiple linear regression, sometimes referred to as multiple regression, is a statistical technique that incorporates various explanatory factors. Simulate the linear relationship between the independent (explanatory) and dependent (response) variables using multiple linear regression.

ANN-Artificial neural networks

ANNs are software programs that learn to carry out tasks by taking into account multiple cases. As long as enough input is given, an ANN can detect traits and patterns in data. Then, predictions are made in novel conditions using these traits and patterns. ANNs have scarcely ever been utilised to improve 2D separations up until now.

Chemometrics Strategies and Methodology

Raw data acquisition

Data acquisition is the process of taking measurements of real-world physical occurrences using signals and digitising them so that a computer and software may alter them. This task is made easier by the input instrumentation subsystem, between the process and the control subsystem, which serves as the interface. It collects data from process variables in physical form, converts it into an equivalent electronic form without losing any information, and then sends it to controllers. The procedure consists of the subsequent steps. Process survey, Process studies, Data conversion, Data display, Data logging and history production, Data analysis and decision making[27].





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Pre-processing

One may generally agree that (1) denoising and smoothing, (2) baseline (drift) correction, (3) retention time alignment, (4) peak deconvolution and resolution improvement, and (5) data compression are the five basic data preprocessing techniques. For the specific targeting and, especially, quantification of analytes, steps (1) and (2) combined are known as background correction. Low-amplitude signals are initially eliminated during the denoising and smoothing processes, regardless of their frequency spectrum, and then high-frequency signals are eliminated, regardless of their amplitude. The next step is baseline (drift) correction, which aims to identify the baseline shape and deduct it from the measurement. Retention time changes between experiments are fixed using step three, retention time alignment. This is necessary to compare chromatogram series and make it possible to identify the true differences between comparable samples. Resolution enhancement (4) and peak deconvolution are used to separate two or more (partially) overlapping signals. Finally, data compression (5), which both reduces the amount of computer resources needed and speeds up data processing, is typically necessary for big datasets. It's important to remember that all pre-processing methods occasionally depend on premises or assumptions, which might possibly lead to incorrect inferences. One example is background adjustment, which may result in the elimination of real signals. This is more likely to happen when it is difficult to tell actual peaks apart from background noise.

Analysis of chromatographic data

After pre-processing, the focus is on turning complicated data into information that can be applied to a sample. Over the past few decades, a variety of information extraction techniques have been created[28, 29]The data-analysis procedure in this context might be broken down into many layers. First, it is necessary to find the peaks in the sample that correspond to the (partially separated) chemicals. In thorough two-dimensional chromatography, the number of modulations per first-dimension peak must be taken into account while grouping the detected peaks. The following stage is to gather generic information in one or two dimensions regarding each identified peak (e.g., area, statistical moments). The attributes can then be transformed into useful information. We will cover the most recent advancements for each of these processes in this section.

Optimization and validation

The creation and improvement of chromatographic techniques is a different area of chemometrics in chromatography. Such a technique necessitates a number of phases. Particularly, creating a two-dimensional chromatographic approach might be difficult and time-consuming [30, 31]. A trial-and-error optimization is time-consuming and impracticable due to the vast number of elements that must be taken into account [32]. The outcomes of these can then be utilised as a platform for more research, as input for chemometric modelling techniques like principal compound analysis, to develop retention models for the analytes, or both. Quality descriptors must be chosen following the establishment of retention parameters or PCA. These give the separation's quality an objective value.

Method validation

Only the appropriate method and parameters will be fixed and recorded for future examination when the optimised method is finished and reported utilising the final method validation.

Data prediction and results

Data were anticipated for known or unknown compounds of the targeted analytes after the technique was validated, and the data were displayed graphically.

Analytical Reports Based on Chemometric Analysis

Challenges and advantages

It is crucial to incorporate spectroscopic methods and chemometric modelling strategies into quantification since this enables the extraction of crucial data for a product or process's design, monitoring, and management. Greatly increasing the likelihood of developing and speedup the 2D-chromatography process. While it might be





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challenging to quantify complicated material in sample preparation and integrations of 2D-Chromatograpgy, particularly in bioanalytical, nano-preparation. Moreover, specially to apply the chemometric methods such as PLS and PCR in wide range in multi component of data analysis from the chromatography system, but the MLR method failed to develop a QSPR (Qualitative structural properties relationship) because of the over fitting the data leading to bias in the results.

CONCLUSION

Two-dimensional chromatography separations, which combine exceptional resolution & peak capacity with flexibility and surpass the limitations of any specific chromatographic method, have thus far proven to be among the most impactful and promising techniques for the fractionation and identification of complex sample compositions in various property coordinates. A barrier to identifying errors and incorrect predictions and gathering reliable data from a thorough chromatographic process is the current review of the many chemometrics approaches that have been widely employed in the 2D-chromatography method. We advised employing chemometrics in a variety of studies. Among other methods, ANN and PCA are the most effective. Only when employing chemometrics in the 2D chromatography system are there minimal limitations.

Conflict of Interest

The paper contains no conflict of interest.

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Table.1:Analytical method reports based on chemometrics

S.no	Analyte	Dosage form/Sample	Quantification method	Chemometrics method	Ref
1	Caffeine anhydrate	Tablets	NIR	PLS	[33]
2	Atenolol, Amiloride hydrochloride, and Chlorthalidone	Tablets	HPLC	PLS and PCR	[34]
3	Pseudoephedrine hydrochloride and Ibuprofen	Tablets	UV-Visible	PLS	[35]





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4	Acetaminophen and Ascorbic acid	Tablets	UV-Visible	PLS and PCR	[36]
5	Melatonin and Pyridoxine	Tablets	spectrofluorimetric and spectrophotometric	PLS and PCR	[37]
6	Amiloride, Propranolol and Dipyridamole	Tablets	Spectrofluorometric	PARAFAC	[38]
7	MELOXICAM AND EXCIPIENTS Meloxicam and its excipients	Tablets	NIR, HPLC	SNV, PLS and PCR	[39]
8	Antipyrine	Granules	NIR	PCR	[40]
9	Naproxen and Diflunisal	Suppositories	HPLC	CLS, PCR and PLS	[41]
10	Drotaverine hydrochloride and Nifuroxazide.	Capsules	UV-Visible	PCR, CLS, and PLS	[42]
11	β-blockers (Bisoprolol, carvedilol,propranolol, atenolol, and nebivolol).	Tablets and capsules	HPLC	GA-PLS, PCR, PLS	[43]
12	Daphnia magna metabolites	Natural samples	GC-MS	MCR-ALS	[6]
13	Theophylline, Methylparaben, Diphenhydramine, Guaiphenesin, Propylparaben and Sodium benzoate.	syrup	UV-Visible, HPLC	PLS and PCR	[44]
14	Selamectin	Topical formulations	NIR	PLS	[45]
15	Lansoprazoleand Clarithromycin	Commercial preparation	HPLC	PCR and PLS.	[46]
16	Miconazole nitrate and Nystatin	Tablets	HPLC, UV-Visible	CLS, PLS, and PCR	[47]

(PLS-Partial least square test, PCR- Principal compound regression, PARAFAC-Parallel factor analysis, SNV-Standard normal variance MCR-ALS- Multivariate curve resolution-alternating least squares, GA-PLS-Genetic algorithm Partial least square)

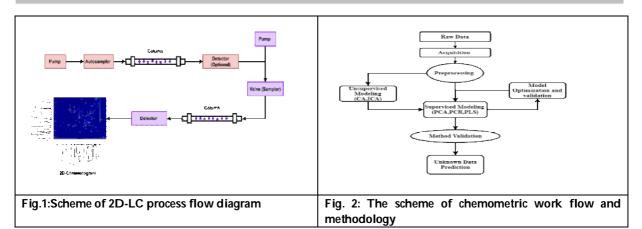




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RESEARCH ARTICLE

Design and Development of High Strength Hybrid Fiber Reinforced SCC (HSHBFRSCC) as per IS: 10262:2019

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ABSTRACT

A unique construction material considered as self compacting concrete (SCC) which may be poured and compacted without the necessity of external or internal vibration. Through IS 10262: 2019 revision, the BIS has established a recommendation for SCC mixes for the first time. The present investigation aims to cultivate high strength SCC mixes of M55 grade using Fly Ash, Micro Silica (White and Gray), GGBS, coarse and fine aggregates along with the addition of hybrid fibre (polypropylene and steel Fibers) which was kept constant. The design mix was created by considering the following material such as cement = 455.6 kg/m³, powder = 574 kg/m³ and water = 162 kg/m³, respectively. The designed SCC mixes satisfy the properties of fresh and hardened property requirements. Findings suggest that silica fumes and GGBS in combination may be used in designing high strength SCC mixes.

Keywords: IS: 10262:2019 High strength SCC, Fly-ash, Micro-Silica, GGBS, Polypropylene and Steel fibers, VMA, PCE.





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INTRODUCTION

India is the world's second-most populous country, accounting for more than 1.3 billion people, or more than one-sixth of the global population. As a result, the need for infrastructure is experiencing exponential growth. To reconcile our current growth rate with the resource needs of future generations, we must consider the sustainability credentials of special concretes such as Self-Compacting Concrete (SCC), Fiber-Reinforced (FR-SCC), and High-Strength Concrete (HSC). Therefore, the goal of this study is to provide a uniform method for using micro silica, GGBS and fibers (i.e. Steel and polypropylene) in the production of high strength SCC as per new IS: 10262:2019 and establishing the mix ratios for the high strength SCC from M₅₅ grade of concrete. Mohammad et al. (2011). claim that silica fume has an impact on the calcium oxide (CH) crystals' degree of orientation as well as the thickness of the transition phase in mortars This could be due to the inducing of fumed silica, This significantly reduces the number of pores, or the pozzolanic process, which takes place simultaneously with cement hydration, which causes larger holes to become smaller pores. Lian et al. (2011) used silica fume to study the relationship between porosity and strength in porous concrete. The porosity of porous concrete's internal structure had a significant impact on its strength across numerous porous media. Pratik Deshmukh's (2014) research focused on SCC strengthening utilising GGBS to improve concrete's cost effectiveness. Concrete is utilised to create long-lasting structures when GGBS is used in conjunction with regular Portland cement and other components. In the SCC mix, the amount of ground granulated blast furnace slag varies between 20% and 40%. To assess the SCC mix's qualities, several experiments are run. It has been found that using GGBS as a filler lowers the overall void content of SCC. At 30% GGBS addition, they calculated a 1.74 percent improvement in compressive strength.

The potential uses of industrial byproducts were investigated by Pai et al. in 2014. Self-compacting concrete is produced using silica and GGBS gases. The properties of GGBS and SF-based SCC concrete compositions were compared. For the creation of SCC, it was suggested to use mineral admixtures instead of cement, such as GGBS and SF, by including the mix design. The results showed that the GGBS-based SCC mixes worked effectively. Comparing a material's tensile qualities to an SF-based SCC blend, compressive, split-tensile and flexural strength should all be considered. Self-compacting concrete is produced using silica and GGBS gases. Fly ash and colloidal nano silica were used in hybrid fibre reinforced SCC, according to research by Chinmaya Kumar Mahapatra et al (2018). The characteristics of hybrid fibre reinforced self compacting concrete (HyFRSCC), which contains class F fly ash (FA), colloidal-nano-silica, steel fibres i.e. Crimped (CSF), and poly-propylene fibres (PPF), were investigated in this work. Aydin (2007) investigated how self-compactable high volume hybrid fibre reinforced concrete. The impacts of fibre inclusion on the compaction of hybrid fibre reinforced concrete were investigated using a mixture of carbon and steel fibres. Consider their volume and intended use when calculating the impact of fibres. According to research by Pons et al. from 2007, sliding PP fibres prevented catastrophic breaking while high-bond, high-modulus amorphous metal fibres postponed and effectively suppressed cracks. Fiber integration was found to be feasible in SCC by Ravi & Prakash (2008), and flow characteristics may be further enhanced by modifying VMA or SP doses. Fibers responded synergistically in HFRSCC. Metallic fibres like steel and Galvanized Iron (GI) demonstrated higher strength values when compared to nonmetallic fibres like PP and waste plastic fibres. Due to its strong bonding with the matrix, Rashid Hameed et al. (2010) found that adding amorphous stainless metallic fibre was extremely effective in controlling the micro-cracking process and led to fewer crack openings at peak resistance.

The Purpose of the Investigation and Its Scope

Beyond this analysis, it is intended to produce a HS-SCC with incorporation of hybrid fibres and assess viability of using different powder materials. The study's objective was to create M55 grade high strength hybrid fibre reinforced self-compacting concrete (HSHFRSCC) by using GGBS to partially substitute cement, and silica fume in varying amounts, adding hybrid fibres (polypropylene and crimped steel fibres) at a rate of 1% of cement weight, maintaining 1% of super-plasticizer, and evaluating the results of various properties of materials.





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MATERIALS AND PROPERTIES

In order to reduce the amount of cement, fine fillers, and sand that are needed in SCC, mineral admixtures are added to concrete (Okamura 1995). Concrete with condensed silica fume has the following qualities due to the lower grain size: Increases the cohesion of the concrete mix. Flow ability improves as segregation and mixing are minimized. To improve durability, decrease permeability. Improvements in frost resistance. Increases pozzolanic reactivity of silica fume, which improves strength and increases chemical resistance. It is less likely to creep or contract. (B C Shantappa 17). Hajime Okamura and Ouchi (2003) investigated the influence of Super Plasticizers [SP] on the flow ability and viscosity of SCC. The researchers provided an approach for employing Super Plasticizer to increase self-compact ability, flow ability, and viscosity in order to achieve self-compact ability based on their findings. By validating the standard as per IS 269:2015, Cement of OPC 53 grade was brought from the Kalaburagi market. To aid in the experiment, GGBS and Silica fumes was obtained from Aastra chemicals, Chennai. VMA and PCE chemical admixtures (super plasticizer) were procured from Astra Chemicals, Chennai. 4.75 mm or smaller fine particles were taken into consideration. From a nearby quarry in the Kalaburagi industrial area, crushed stone (angular) in sizes as small as 20 mm was purchased for the project. In accordance with acceptable test protocols, physical characteristics of binders, including OPC and GGBS, Silica-fumes etc, were investigated. Polypropylene and crimped Steel fiber were used.

Concrete Mix Design

Various concrete mixes, including control concrete, must be constructed in order to meet the goal of manufacturing high strength concrete utilizing GGBs and Silica Fume. The control concrete mix, which was developed in accordance with IS: 10262:2019, contained OPC, aggregates (fine and Coarse), and water. Despite the addition of super plasticizer to maintain the slump within an acceptable range, the effective w/c ratio was maintained at 0.32. To make mixtures, GGBS, Silica-fumes, and OPC were substituted up to 10% by mass. By increasing the super-plasticizer content with powder while keeping an even effective 0.32 w/b ratio, the workability remained intact.

Requirements for Proportioning

The OPC 53 grade, which conforms to IS 269:2015, was utilized. 20mm nominal maximum size of aggregate was considered, exposure of severe (RCC) was adopted as per table 3 and 5 of IS 456, aggregate type of crushed angular was taken, water absorption of 0.5 and 1% was taken for coarse and fine aggregate respectively. Fine aggregate of zone II confirming as per IS 383 was considered. Characteristics considered for SCC are SF3-slump flow class was considered which range from 760-850mm; passing ability (L-box) is taken to be 0.9, Class V1 and SR1 less than 15% was considered for Viscosity and segregation resistance respectively.

Mix Proportions for Trial

a) Cement $= 455.4(kg/m^3)$ b) Silica fume $= 50.6(kg/m^3)$ c) Water $= 162(kg/m^3)$ d) Fine-aggregate in SSD $= 850(kg/m^3)$ e) Coarse-aggregate in SSD $= 938.4(kg/m^3)$ f) Admixture-chemical $= 2.73(kg/m^3)$ g) Powder content $= 574(kg/m^3)$ h) Water to powder ratio = 0.86

i) Steel and Polypropylene = 1% by weight of cement





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RESULTS AND DISCUSSIONS

The purpose of the research was to examine the properties of an M55 grade concrete mix that included GGBS, slila fume, and super plasticizer at a w/b ratio of 0.32. Numerous tests must be performed to determine a substance's flow ability (slump flow test), passing capacity (L box test), viscosity (V funnel) and resistance to sieve segregation, and the outcomes must be evaluated in light of the available information.

CONCLUSION

The updated version provides the new method to produce SCC and it includes instructions for producing modern concrete grades of high strength concrete and because it is compatible with the current kind of demands. The high strength concrete cannot be created without admixtures, correct guidelines, relationships with other constituents, and how other values fluctuate with the use of admixture have been provided clearly. The change of the IS code has increased the standard and quality of mix design. The upgraded revision is superior and can be used to produce high-quality concrete.

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- 14. IS 269: 2015 Ordinary Portland Cement Specification (Sixth Revision)
- 15. IS 383: 2016 (Reaffirmed 2021)- Coarse and Fine Aggregate for Concrete Specification (Third Revision).
- 16. Indian Standard IS: 10262-2019- Concrete Mix Proportioning Guidelines (Second Revision).
- 17. Indian Standard Plain and Reinforced Concrete Code of Practice (fourth- revision), IS 456:2000.
- 18. IS 9103:1999 Concrete Admixtures Specification (First Revision) (Re-affirmed 2004)

Table 1: The Chemical Components of OPC Cement

S.NO	Components	Concentration (%)
1)	Si O ₂	28.67
2)	$Al_2 O_3$	8.57
3)	Fe ₂ O ₃	3.81
4)	Mg O	1.87
5)	SO_3	2.29
6)	CaO	41.94
7)	Na ₂ O	0.29
8)	K ₂ O	0.83

Table 2: Utilized Cement's Qualities

SI.NO.		Results	
1)	Normal	30.6	
2)	Satting	Initial (min)	165min
	Setting	Final (min)	240min
3)	The Finene	225min	
4)	Compressiv	58MPa	

Table 3: Properties and Chemical Composition of GGBS

SI.NO.	Characteristics	Results
1)	Fineness (m²/kg)	390min
2)	Specific-gravity	2.85
3)	Cumulative Particle size (45 micron %)	97.1
4)	Magnesia (%)	7.73
5)	Sulphide sulphur (%)	0.50
6)	Sulphite (%)	0.38
7)	Manganese (%)	0.12
8)	Chloride (%)	0.009
9)	Chemical referance (a). CaO+MgO+SiO ₂ (b). (CaO+MgO)/SiO ₂ (c). CaO/SiO ₂	76.01 1.32 1.07
10)	LoI (%)	0.26
11)	Moisture (%)	0.10

Table 4: Properties and Chemical Composition of Micro silica

SI.NO.	Characteristics	Results
1)	Physical State	Micronized powder
2)	Odour	odorless
3)	Appearance and colour	white
4)	Packing density gm/cc	0.76
5)	Specific-gravity	2.63
6)	Moisture (%)	0.058





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7)	Si O ₂ (%)	99.89
8)	Al ₂ O ₃ (%)	0.043
9)	Fe ₂ O ₃ (%)	0.040
10)	Mg O (%)	0
11)	TiO ₂ (%)	0.001
12)	Ca O (%)	0.001
13)	Na ₂ O (%)	0.003
14)	K ₂ O (%)	0.001
15)	LoI (%)	0.015

Table 5: Properties of VMA

SL.NO. Particulars		Remarks					
1	Product type	Polycarbolic superplasticizers					
2	Appearance and color	Colorless to light lazy liquid					
3	pН	6.00					
4	Specific-gravity	1.10					
5	Total Solids	45%					







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RESEARCH ARTICLE

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Antioxidant Properties of the Peptide from Vigna aconitifolia L.

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ABSTRACT

The antioxidative characteristics and total phenolic contents of Vigna aconitifolia L. were evaluated using the moth bean seed protein isolates which were extracted with phosphate buffer (pH 6.5). The crude peptide was screened for their potential antioxidant activities using, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3- ethylbenzothiazoline-6-sulfonic acid) (ABTS+), Ferric reducing/antioxidant power (FRAP) and Fe2+ chelating systems. The extract was found to possess an appreciable quantity of phenolic substances (245±0.01µg gallic acid equivalents/mg extract). A dose-dependent scavenging activity in the DPPH and ABTS assay with an IC50 value of 4.38±0.20µg/ml and 3.61±0.10µg/ml was observed. The reducing power assay exhibited that the peptides had a high affinity to donate electrons, which was involved in their antioxidant potential. The extract showed concentration-dependent reducing activity as revealed by an increase in the absorbance of the reaction mixture. The observed activity of seed extract could be related to the presence of phenolic substances.

Keywords: Antioxidant, DPPH, Ferric reducing, Folin-Ciocalteau, Radical scavenging

INTRODUCTION

Numerous chronic and degenerative diseases, including gastric ulcers, cardiovascular disease, diabetes mellitus, neurological problems, and ageing, are recognised to be primarily brought on by reactive oxygen species and free radicals [1]. Synthetic and natural antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene, flavonoids, polysaccharide, and peptide, have drawn increasing attention for their ability to suppress them both





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inside the body and in food [2]. However, due to potential health hazards, such as carcinogenic activity, the usage of common synthetic antioxidants is coming under closer examination [3-5]. Enzymatic proteolysis of many animal and plant proteins can produce bioactive peptides, which are typically made up of 3–20 amino acid residues. They are crucial in biological processes that depend on the content, sequence, and molecular weight of amino acids, such as those that have antioxidative, antihypertensive, and immunomodulatory effects [6,7]. There is growing evidence, in particular, that several types of peptides have a powerful ability to stop oxidative damage caused by free radical scavenging in living organisms [8-10]. Numerous investigations revealed that the high concentration of hydrophobic and aromatic amino acids in peptides may contribute to their antioxidant activities, even though the processes behind their anti-oxidant capabilities were not clearly understood [11]. Additionally, some branched-chain amino acids, such as leucine, isoleucine, and phenylalanine, have been found to have specific antioxidant action [12]. Protein and natural antioxidants are both found in large quantities in legume seeds.

Flavonoids, phenolic acids, and tannins are only a few of the phenolic chemicals found in legumes. Due to their significance in the treatment and/or prevention of numerous diseases, natural phytochemicals and antioxidants from plants are of great interest. Trypsin inhibitors have powerful anti-inflammatory properties and reduce the incidence of several malignancies. Tannins have been shown to have antioxidant and antiradical properties in numerous investigations. As a result, a substantial amount of information about seed proteins, nutritional effects, and phytochemical compositions has been included [6]. In India's dry and semi-arid regions, the moth bean (Vigna aconitifolia L.), a breeze-resistant legume of the Fabaceae family, is a widespread crop. It is a legume that is unusually strong and goes by a number of different names, such as mat bean, matki, Turkish gram, or dew bean. Rajasthan, a state in dehydration in India, contributes over 86% of the country's land to moth bean cultivation. A traditional remedy for paralysis, weight loss, rheumatism, cough, fever, and liver diseases is reported to be Vigna aconitifolia L. [15]. The extraction, phytochemical, functional, and biological characteristics of moth bean seed protein are therefore the subject of significant investigation at the moment [14]. Moth bean seed protein exhibits good functional qualities, such as solubility, emulsifying and foaming activities, and nutritional quality, such as digestion and absorbability, as was previously demonstrated [15]. The antioxidant activities of enzymatic hydrolysates from soy protein, chickpea protein, alfalfa leaf protein, whey protein, buckwheat protein, and horse gramme (Macrotyloma uniflorum) protein have all been extensively investigated by antioxidant evaluation systems in-vitro and in-vivo. However, to my knowledge, studying the peptide from moth bean seed is still uncommon [16]. The antioxidant potential of peptides from Vigna aconitifolia L. was not previously known, according to a survey of the literature. As a result, we assessed the antioxidant activity and total phenol content of the peptides from Vigna aconitifolia L.

MATERIALS AND METHODS

Sample collection

The *Vigna aconitifolia* L. seeds were obtained from the local market in Dharwad district, Karnataka. The seeds collected were identified and authenticated in the Department of Studies and Research in Botany, Sahyadri Science College, Shivamogga, Karnataka. India.

Extraction and purification of the moth bean seeds

The procedure as described was slightly modified to create moth bean seed protein isolate (MBSPI) from moth bean seed [17]. 100 ml of 0.1 M Phosphate buffer was used to homogenize30 g of seed flour (pH 7). The homogenate was then centrifuged for 15 minutes at 4°C at 6000 rpm. The supernatant served as the raw peptide extract. The crude extract was saturated with solid ammonium sulphate (12.6 g) with continuous stirring at 4 °C with 40% and 80% relative ammonium sulphate precipitation. This solution was incubated overnight at 4°C. The precipitate was lyophilized and used for further work.





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Desalting of Peptide

A Sephadex G-25 gel chromatography column that had already been equilibrated with deionized water was loaded with MBSPI hydrolysate. The 0.5 mL/min of deionized water was then used to elute the column. These fractions were subjected to antioxidant activity.

Total phenolic content of seed extract

The Folin-Ciocalteu technique was used to ascertain the MBSPI's total phenolic content. Sodium carbonate (4 mL) and a 1:1 diluted Folin-Ciocalteu reagent (0.5 mL) were combined with a 0.5ml of extract. The total phenol content was calculated calorimetrically at 765 nm. An increasing gallic acid concentration in methanol was used to create a standard curve. With varying amounts of gallic acid (standard, 0-1000g/mL), a standard curve was produced. Total phenolic content was measured in terms of gallic acid equivalents (GAE) per gram of dry sample (mgGAE/g) [12].

Antioxidant activity of seed extract

DPPH free radical scavenging assay

Based on the radical scavenging effect on the DPPH free radical, the radical scavenging capacity of MBSPI and Ascorbic acid (standard) was evaluated. 2 mL of the DPPH solution (0.002% in methanol) was combined with 2 mL of the extract and standard at various concentrations (ranging from 10 to 400 g/mL of methanol). The tubes were incubated at room temperature in the dark for 30 minutes and OD was measured at 517nm and scavenging activity was calculated using the formula,

Scavenging activity (%) = $[(A - B) / A] \times 100$,

where A is the absorbance of DPPH and B is the absorbance of DPPH and extract/standard combination.[13] The amount of extract needed to scavenge 50% of DPPH free radicals or the IC_{50} , was estimated.

ABTS radical scavenging assay

Based on the radical scavenging impact on the (ABTS) 2,2'-azinobis(3-ethylbenzonthiazoline-6-sulfonic acid) free radical, the ABTS radical scavenging activity of MBSPI and Ascorbic acid (standard) was assessed. A final concentration of 7mM ABTS+ and 2.45mM potassium per sulfate was used to prepare the ABTS radical cation (ABTS+). The resulting combination was kept at room temperature and kept in the dark for 16 hours. The ABTS+ solution was diluted to an absorbance of 0.70 at 734 nm in 5 mM phosphate buffered saline (PBS), pH 7.4. 20 ml of MBSPI were combined with 2 ml of diluted ABTS+ solution. The resulting mixture was forcefully agitated for 1 minute, and for a total of 10 minutes, the absorbance was measured at 1-minute intervals. The blank was made up of an equivalent volume of distilled water. By mixing 2 ml of diluted ABTS+ solution with 20µl of various ascorbic acid concentrations, a standard curve was created. Ascorbic acid (used as a standard reference) orantioxidant sample concentrations were measured and plotted as a function of the percentage of the absorbance reduction at 734 nm. The scavenging activity was calculated using the formula,

scavenging activity (%) = $[(A - B) / A] \times 100$,

Where A is the absorbance of ABTS and B is the absorbance of ABTS and the extract/standard combination. The ABTS scavenging activity of the samples was expressed as (mol ascorbic acid/mg protein) value. The amount of extract needed to scavenge 50% of ABTS free radicals or the IC₅₀, was estimated [24].

Ferric reducing assay

In separate tubes, different quantities of MBSPI and Tannic acid (standard), ranging from 10 to 400 g/mL, were combined with 2.5 mL of phosphate buffer (200 mM, pH 6.6), 2.5 mL of 1% potassium ferricyanide, and 1 mL of methanol. To the tubes, 2.5 mL of 10% trichloroacetic acid and 0.5 mL of 0.1% ferric chloride were added after they had been kept in a water bath for 20 minutes at $50\,^{\circ}$ C. After 10 minutes, the production of Perl's Prussian blue colour





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was measured at 700 nm to establish how much iron (II)-ferricyanide complex had been produced. The reaction mixture with greater absorbance is a sign of their improved reducing power [18].

Metal ion chelating activity

Ferrous ions were tested for MBSPI's capacity to chelate them. 200 μ l of MBSPI (0.3 mg/ml), 10 μ l of FeCl2 (2 mM), and 600 μ l of double-distilled water were combined for the chelation test. 20 μ l of ferrozine solution (5 mM) was then added, and the mixture was vigorously mixed for 2 min [25]. The combination was then held at room temperature for 10 minutes. The absorbance at 562 nm was then measured, and the colour reduction brought on by the chelation of Fe2+ was noted. The control sample comprised 20 μ l of ferrozine solution, 10 μ l of FeCl2, and 800 μ l of double-distilled water (5 mM).

The chelating activity (%) = $[(A - B) / A] \times 100$.

Where A is the absorbance of negative control and B is the absorbance of extract/standard combination. Plotting the percentage of chelating activity with the various peptide concentrations (0-1.5 mg/ml), the IC_{50} value (required concentration of peptide for 50% chelating activity) was calculated.

Statistical analysis

All the experiments were carried out in triplicates (n=3). The statistical analysis of the data was carried out by analysis of the variance (ANOVA). Results were considered significant when p<0.05.

RESULTS AND DISCUSSION

Total phenolic content of seed extract

The number of total phenols in the seed extract was estimated by the Folin-Ciocalteu method. The content of total phenols is expressed as GAE. The phenolic content of seed extract was found to be $245\pm0.01\mu g$ GAE/mg extract. The antioxidant activity of the seed extracts mainly depends on the concentration of phenolic content. The higher the phenolic content higher will be the antioxidant activity. The selected moth bean belongs to legumes, known to contain flavonoids, phenolic acids and tannins [6].

Antioxidant activity of seed extract

DPPH free radical scavenging assay

The DPPH free radical assay was used to assess the antioxidant activity of different doses of MBSPI and ascorbic acid in terms of their capacity to scavenge free radicals (Figure 1). By scavenging DPPH* (a free radical) and converting it into DPPHH, the MBSPI demonstrated significant antioxidant activity. It was observed that the action was dose-dependent. Ascorbic acid has a higher scavenging activity than MBSPI. DPPH readily takes an electron or hydrogen atom to transform into a stable diamagnetic molecule [19]. When the appropriate reducing agents interact with DPPH radicals, the electrons become paired off and the matching hydrazine is created. As a result, the number of electrons taken up determines how many colours the solution loses [20]. The reduction in the absorbance of methanolic DPPH solution at 517 nm in the extract's presence was used to gauge the seed extract's capacity to scavenge free radicals. The more extract there was in a given volume of solution, the more the DPPH solution bleached. The amount of extract needed to remove 50% of DPPH or the IC50 value was4.38 g/ml. The presence of phenolic compounds is primarily responsible for the bleaching activity ofour seed extract. The ability to scavenge radicals improves with decreasing IC50 [14].

ABTS radical scavenging assay

ABTS radical scavenging activity (Figure 2) of different concentrations of MBSPI and ascorbic acid was determined. This assay is based on the ability of antioxidants to scavenge the 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic





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acid (ABTS \bullet +) radicalcation produced by reacting ABTS solution with a strong oxidizing agent such as potassium permanganate or potassium persulfate. The IC $_{50}$ value for the extract was found to be 3.61 \pm 0.15 μ g/ml. The oxidation of ABTS with potassium persulfate produces an ABTS free radical, which is then reduced by the hydrogen-donating ability of the extract [24]. Similar to this, the nitrogen atom quenches the hydrogen atom and decolorizes the solution when it is in the presence of a hydrogen-donating antioxidant molecule of the peptide extract. Measurement of the decrease in the absorbance spectrum during the blue-green ABTS radical reaction was used to assess the hydrogen-donating potential of various extracts.

Ferric reducing assay

To examine the reducing power of extract, the reduction of Fe3+ to Fe2+ was investigated in the presence of MBSPI and standard (tannic acid). The result of reducing the power of different concentrations of MBSPI and tannic acid is presented in (Figure 3). The absorbance at 700 nm was found to increase with the concentration and is indicating reduced power of MBSPI and standard. The reducing power assay exhibited that the MBSPI with a high affinity to donate electrons, which shows high antioxidant potential. A compound's ability to reduce substances may be a useful predictor of its potential antioxidant activity [21]. The Fe3+/ferricyanide complex is reduced to the ferrous form in the presence of reductants, such as antioxidant compounds in antioxidant samples. As a result, the production of Perl's Prussian blue at 700 nm can be used to monitor Fe2+. Tannic acid and seed extract were present when researchers examined the ability to reduce ferric to ferrous. After the extract concentration was raised from 50 g/ml, a considerable change in reduction power was noticed. When compared to tannic acid, the extract's reducing power was less potent.

Metal ion chelating activity

The metal chelating activity of different concentrations of MBSPI is presented in (Figure 4). The IC50 values for the MBSPI were found to be $0.89\pm0.15\mu g/ml$. The IC50 value for 50% chelating effect on ferrous ions, the effect of the MBSPI on ferrous ion chelating was compared with that of EDTA. Radical scavenging activity was evaluated in the peptide concentration range of 0–1.5 mg/ml. The results indicate MBSPI with significant metal chelating activity and it was also found that chelating activity increases with the increase in concentration. By converting lipid hydroperoxides into cell-harming peroxyl and alkoxyl radicals, iron can accelerate lipid peroxidation. By chelating metals, the antioxidant activity stops the catalysis of hydroperoxide breakdown. Chelating substances present in the seed extracts prevent the production of metal hydroperoxide complexes, which reduces colour development [26]. The chelating ability of the peptide extract is determined by the measurement of colour decrease. An increase in the chelating power of the extract is shown by a reduction in absorbance. The maximum metal chelating activity is demonstrated by the data shown in (Figure 4). Additionally, it was observed that peptides may chelate Fe²⁺ ions. The findings demonstrated that colour generation decreases as concentration increases.

CONCLUSION

An innovative tool to examine the correlation between antioxidant characteristics and oxidative stress is the measurement of total antioxidant capacity (TAC) utilising several antioxidant techniques. Our study focused on evaluating the antioxidant potential of various quantities of peptides isolated from *Vigna aconitifolia* L. utilising five different *in-vitro* antioxidant methods. The investigation turned up peptides from *Vigna aconitifolia* L.have substantial dose-dependent scavenging activity in antioxidant techniques. This means that *Vigna aconitifolia* L. could be a novel source of naturally occurring antioxidants that are valuable for industrial applications. Our findings needs further research on characterization and purification of the phytochemicals in charge of the antioxidant properties of peptides obtained from seed extracts.





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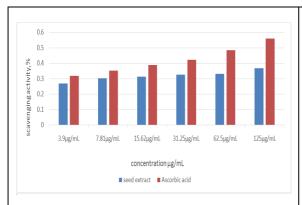


Fig. 1. Effect of different concentrationsof peptides from Vigna aconitifolia L. on DPPH inhibition. Reference standard: Ascorbic acid Bars in each column with a common letter indicating a significant difference (p < 0.05)Experimental results are mean \pm SD (n=3).

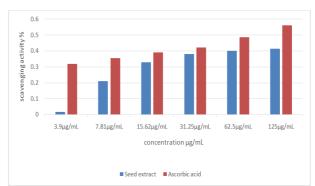


Fig. 2. Effect of different concentrations of peptides from Vigna aconitifolia L. on ABTS inhibition. Reference standard: Ascorbic acid Bars in each column with a common letter indicating a significant difference (p < 0.05). Experimental results are mean \pm SD (n=3).

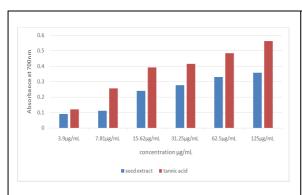




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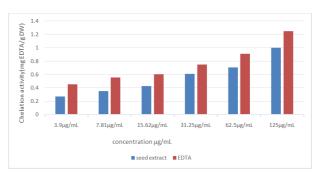


Fig. 3. Effect of different concentrations of peptides from *Vigna aconitifolia* L. on FRAP inhibition. Reference standard: Tannic acid Bars in each column with a common letter indicating a significant difference (p < 0.05). Experimental results are mean ± SD (n=3).

Fig. 4. Effect of different concentrations of peptides from Vigna aconitifolia L. on metal chelating activity. Reference standard: EDTA. Bars in each column with common letters indicate significant differences (p < 0.05). Experimental results are mean \pm SD (n=3).





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RESEARCH ARTICLE

Evaluation of Phytochemical Screening and Phytoanalytical Studies of Lead Compounds from Gloriosa superba Seed Extract

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ABSTRACT

The present investigations are phytochemical screening of methanolic seed extract of Gloriosa superba and analytical study using UV, TLC, HPLC, and FTIR. The role of its lead chemical colchicine was studied. All experiments were conducted following standard procedures. The results revealed that the presence of various secondary metabolites like Alkaloids, Phenols, Saponins, Flavonoids, Steroids, Tannins and Glycosides. A simple rapid and accurate high performance liquid chromatography (HPLC) method has been developed for the estimation of colchicine using of a mixture of chloroform: methanol (95:5 v/v) as a mobile phase. The pH of the mobile phase was adjusted to 5.2 among phosphoric acid. A C₁₈ column (5µm, 25 cm x 4.6 mm) was used for the elution. The flow rate of the mobile phase was set to 1.2 ml/min. Injection volume was set at 20 µl and the detection of the analyte was done at 254 nm. In addition to that the UV-vis (800 -200 nm), Thin Layer Chromatography and FTIR (4000-400 cm⁻¹) spectra were also recorded in methanolic seed extract of Gloriosa superba.

Keywords: Gloriosa superba seed, phytochemical, UV, TLC, HPLC, FT-IR.

INTRODUCTION

Gloriosa superba of the family Colchicaceae is an ornamental climbing herb native of tropical Asia and Africa. The plant species contains rich amount of colchicines, which is a toxic alkaloid compound. The seeds of Gloriosa superba are an important source for colchicine extraction (Nadkarni KM, 1996). The accumulation of colchicine occurs in different plant parts of Gloriosa including roots, stem, seeds and tubers. But, the seeds and tuber contain higher amounts of colchicine than other parts. In addition to G. superba, the colchicine content is found in other species of Gloriosa in





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smaller amounts (Bharathi *et al.*, 2006). The plant is endangered due to its immature harvesting from wild for its colchicine content. Medicinal plants are major sources of new drug entities in Indian traditional and modern medicine (Mukherjee and Wahil, 2006). According to current WHO estimates 80% of the people use traditional medicine for their day to day ailments. In traditional Indian medicine Gloriosa superba is used for the treatment of arthritis, gout, inflammation, leprosy, and skin diseases (Nirmala K, 2012). The aim of the present study was to develop a simple, sensitive, and reproducible TLC, HPLC, FT-IR, and UV spectrophotometery method, capable of detecting colchicine from Gloriosa superba with higher precision.

MATERIALS AND METHODS

The fresh seeds of *Gloriosa superba* were collected from the cultivating fields of Dharmapuri, Tamil Nadu. The seeds were cleaned physically to take out adhering foreign matter and were powdered with the help of grinder. The powdered material was then kept in an air-tight container until use. Organic solvent used in the present study for extraction were methanol, acetic acid were purchased from Sigma Aldrich.

Soxhlet extraction

Hot extraction procedure was carried out using Soxhlet apparatus. 5g of finely powdered plant material was packed and placed in thimble. 300 ml methanol was taken in round bottomed flask. The heating mantle was set at 65°C. The extraction process was constant for around 7 cycles. The sample packet was then removed from thimble and the solvent was recovered. Remaining organic phase in the extract was evaporated to dryness and the final yield was measured.

Qualitative evaluation of Phytochemicals

The preliminary phytochemical screening test were performed for the presence of following secondary metabolites such as alkaloids, phenols, saponins, tannins, steroids, glycosides and flavonoids (Trease and Evan, 1989). Alkaloids, phenols and flavonoids were estimated by (Harborne, 1998; Thangarai, 2016).

Determination of total phenolics

The quantitative estimation of phenolics in the methanolic seed extract of *Gloriosa superba* was determined based on the standardized method (Siddhuraju and Becker, 2003). About 0.5 ml of 1N Folin-Ciocalteu reagent and 2.5 ml of 20% sodium carbonate solution were added, and then the volume was made up to 10 ml with water. Followed by 40min dark incubation and the absorbance were recorded at 725nm against blank for the estimation of phenolics. The results were based on the calibration curve: y = 0.974x + 0.272, $R^2 = 0.999$ where x was the absorbance and y was the Gallic acid equivalents (mg/g) and were expressed in terms of milligrams Gallic acid equivalents (GAE) per gram of extract.

Determination of total flavonoids

The total flavonoid in the extracts is estimated by the general procedure (Zhishen *et al.*, 1999). To each 300 μ l methanolic seed extracts of *Gloriosa superba*, 2 ml of distilled water was added followed by 150 μ l of NaNO2. The tubes were subjected to incubation for 6 min at room temperature. After incubation 150 μ l of AlCl3 (10%) was added and incubated again for 6 min at room temperature. Then 2 ml of 4% NaOH was added, vortexed well, and kept at room temperature for another 15 min. The absorbance of pink colour thus developed was read spectrophotometrically at 510 nm. The results were based on the calibration curve: y = 1.095x - 1.267, R² = 0.999 where x was the absorbance and y was the rutin equivalents (mg/g) and the results were expressed in terms of milligrams rutin equivalents per gram of extract.

UV spectrophotometer

The Gloriosa superba seed extraction and its optical behavior were examined by UV spectrophotometer.





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High- performance liquid chromatography (HPLC)

The quantitative estimation of colchicine in the *Gloriosa superba* extract was performed by High Performance Liquid Chromatography (HPLC) - 1100 LC system (Hewlett-Packard, Waldbronn, Germany). Separation was achieved by using C₁₈ Reverse Phase Column, (250 x 4.6 mm) with a particle size 5.0 µm; temperature was set at 30°C. The flow rate was 0.5 ml/ min⁻¹. The mobile phase for chromatographic analysis as an isocratic system was with methanol and 0.1% acetic acid solution (40:60 v/v) with a flow rate of 1.0 ml/min⁻¹. Ultraviolet detection was set at 243 nm (Bodoki *et al.*, 2005). Standard colchicine from Sigma Chemical was used as authentic sample. The sample was injected into the HPLC using 25 µl capacity syringe into the loop via the injection valve. In mobile phase the rotation of valve rotor closed the valve and opened the loop in order to inject the sample into the stream.

Thin Layer Chromatography (TLC)

Presence of colchicine in the seed extract of *Gloriosa superba* was examined using Thin Layer Chromatography (TLC). Approximately $5.0~\mu$ L of raw methanolic extract of *Gloriosa superba* seed extract were loaded on to a 20~x~20~cm, 0.25~cm mthick, silica gel analytical TLC plate (Merck), using fine glass capillary tubes of $20~\mu$ L capacity and the plate was thoroughly air dried. Standard colchicine was used as authentic sample. The solvent mobile phase was developed using a mixture of chloroform/methanol (95:5 V/V) and poured in a TLC jar. The separation was performed at ambient temperature and humidity (20-24°C and 46-56%) avoiding a much as possible exposure to light.

Fourier transform infrared spectrum (FT-IR)

The functional groups presents in the methanolic seed extract of *Gloriosa superba* was studied by FT-IR spectrum in KBr medium in the range of 400-4000 cm⁻¹ using Perkin Elmer RX1 infrared scanner.

RESULTS AND DISCUSSION

Qualitative Phytochemical analysis of seed extract of Gloriosa superba

Positive results were shown for the presence of alkaloids, phenols, tannins, steroids, saponins and carbohydrates are present except glycosides, cardiac glycosides, proteins and amino acids are shown in the (Table 1). The performance of the seed extracts when reacted to chemical reagents shows the quality individuality for detecting the phytoconstituents. The active phytochemicals are collectively to provide protection from severe medical emerging issues (Craig, 1997). The bioactive compounds contribute to offering profound health benefits (Dilas *et al.*, 2009). Alkaloids are naturally occurring as chemical compounds in plant parts that have been pharmacological effects. Phenolic and flavonoid compounds are capable of scavenging free radicals and also possess antibacterial, antifungal and anti tumor properties (Middleton, 1998). Tannins are high molecular weight phenolics that precipitate protein (Hagerman *et al.*, 1998). Terpenoids are the plant based compounds that have been traditionally used in foods, chemical and pharmaceutical industries (Tholl, 2015). Steroids are antibacterial properties; they have medicinal and pharmaceutical activities to improve the immune response (Savjani, 2015). The presence of these biological active compounds in the seed extract of *Gloriosa superba* has several properties like anticancer activity, antioxidant capacity by scavenging the free radicals etc.

Quantitative phytochemical analysis of methanolic seed extract of Gloriosa superba

The Quantitative phytochemical screening of total phenolics and flavonoid content was analysed in the methanolic seed extract of *Gloriosa superba* (Table 2). From the analysis, it's clear that the extract shows better results for the polyphenolic contents. The seed extract showed maximum value for the total phenolics (33.574 % of extract). Flavonoids are (104.56 % of extract).

UV spectrophotometer

The UV-visible spectrum of methanolic seed extract of *Gloriosa superba* exhibits absorption band at 352 nm which can be assigned to $n \to \pi^*$ transition of carbonyl group (Fig 1). This gives an idea about the saturated compounds





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containing hetero atoms such as nitrogen, oxygen, etc, having non-bonding electrons in addition to σ electrons. According to Beer's Lambert law was obeyed in the concentration range of 2-12 μ g/ml for 352 nm respectively.

HPLC-High performance Liquid Chromatography

This HPLC method was accepted to determination of colchicine in seeds of *Gloriosa superba*. The HPLC chromatogram of referent standard colchicine and the seed extracts of *Gloriosa superba* showed in (Fig 2). The retention time of colchicine appeared at 3.276 min. The position of colchicine in HPLC chromatogram of seed extract was identified by comparison of retention time. The retention time of control extract appeared at 3.276 min respectively. Quantification of colchicine was determined using 1100 LC system. The detector was photodiode array detector mobile phase was methanol and 0.1% acetic acid solution (40:60 v/v) with a flow rate of 1.0 ml/min⁻¹. The chromatogram of seed extract of *Gloriosa superba* yields one single peak. The retention time of 3.276 minutes corresponds to colchicine. It has the area of 63675435 Au. The height of the peak obtained was 3129821 Au as shown in the (Table 3). The colchicine extracted from *Gloriosa superba* by hot extraction method was eluted at 2.5 to 5 min.

Thin Layer Chromatography (TLC)

The methanolic seed extract of *Gloriosa superba* was analyzed by using TLC method to determine the presence or absence of colchicine. The extract that has been on silica plate then elution by using the solvent chloroform and methanol (95:5). The elution results are more clearly seen using UV light to know the compounds that are found. The Rf value and color of the compound look same as the standard colchicine. Spraying silica plate elution using vanillin sulphate is also done to see clearly the color of extract sample and standard colchicine are formed. The results showed that the compound colchicine with blue color and the same Rf value between samples with standard colchicine by using spraying vanillin sulphate. To calculate the Rr value comparing with that of authentic sample, presence of colchicine in the ample was detected. The Rr values of the spots of authentic colchicine and that of extract was found to be same as 0.58 thus confirming the presence of colchicine in the seed extract of *Gloriosa superba* (Fig. 3).

Fourier Transform Infrared Spectrophotometer (FT-IR)

The functional groups of phytocompounds were detected by using FT-IR spectroscopy analysis is shown in (Fig. 4) and the data are presented in (Table 4). Infrared spectroscopic analysis given in the information about the possible functional groups of active principles between the wave number 4500-500 cm⁻¹. Solid FTIR spectroscopic analysis using KBR disc method was employed to analyze the functional groups of the seed in *G. superba*. The result of different peaks obtained in the functional group region (4000 to 1300 cm⁻¹), it is inferred that the broad band at 3000 – 3500 cm⁻¹ may be due to OH group (involved in inter molecular hydrogen bonding). The peak at 2937 cm⁻¹, 2840 cm⁻¹, 2272 cm⁻¹ may be due to N-H bond. The peak at 1733 cm⁻¹, 1655 cm⁻¹, 1433 cm⁻¹ may be due to C=O, C=C, C-C bond respectively. The FT-IR spectrum confirmed the presence of colchicines in methanolic seed extract of *Gloriosa superba*, significant peaks were found at 2937 cm⁻¹ corresponding to CH₂ group, 1655 cm⁻¹ attributed to carbonyl groups, and 1558 cm⁻¹ corresponding to amino acid groups, all of which confirms the presence of colchicine.

CONCLUSION

Since ancient times *Gloriosa superba* has been used to treat various kind of diseases with its high therapeutic potentials. The pharmacokinetic and pharmacodynamic principle of the plant *Gloriosa superba* is always unique for the management of chronic ailments especially dermatological issues. Colchicine has been detected from seeds through Thin Layer Chromatography (TLC) using a mixture of chloroform/methanol (95:5 v/v). The final quantification of colchicine has been carried out through High Performance Liquid Chromatography (HPLC). The colchicines content has been found to be variable in different species of *Gloriosa* ranging from 0.16 % (DW), in *Gloriosa carsonil* 0.26% (DW) in *Gloriosa superba*. In comparison the colchicine level of *Colchicum autumnale* has been found to be around 0.2 % (Bellet and Gaignault, 1985).





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AUTHORS CONTRIBUTIONS

Each author has given considerable and equal contributions to this research

CONFLICTS OF INTEREST

The authors have given considerable and equal contributions to this research.

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Table.1: Preliminary phytochemical screening of Gloriosa superba seed extract

S.No	Phytochemical constituents	Presence or absence
1	Alkaloids	++
2	Phenolic compounds	++
3	Tannins	+
4	Flavonoids	++
5	Terpenoids	+
6	Steroids	+
7	Glycosides	-
8	Flavanol glycosides	+
9	Cardiac glycosides	-
10	Saponins	-
11	Phytosterol	+
12	Fixed oils and fats	+
13	Carbohydrates	+
14	Proteins	-
15	Amino acids	-

^{(+):} presence of chemicals, (-): absence of chemicals or not detectable concentration, (+) < (++) < (+++): based on the intensity of characteristic.

Table.2: Quantitative phytochemical analysis of Gloriosa superba seed extract

Quantitative phytochemical analysis					
Phytoconstituents Percentage (%)					
Phenols	33.574				
Flavonoids	104.56				

Table.3: HPLC peak details of seed extract of Gloriosa superba

Peak ≠	RT	Area	Height	Conc	unit	Mark	Name
1	3.276	63675435	3129821	0.000	Mg/L	М	Colchicine

Table.4: Assignment of FTIR absorption bands in the spectra of Gloriosa superba

Absorption frequency cm-1	Vibrational assignments
3243	OH stretching
2937	N-H bond
1733	C=O
1655	C=C
1433	C-C
2937	CH₂ group
1558	NH₂ group

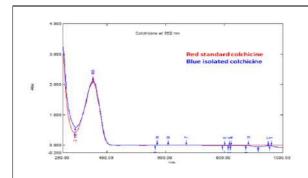




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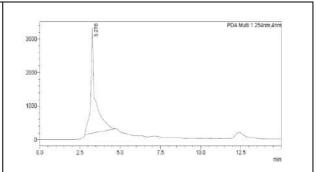
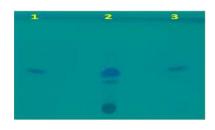
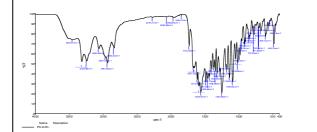


Fig 1. UV spectrum of methanolic seed extract of *Gloriosa superba*

Fig 2. HPLC chromatogram of methanolic seed extract of *Gloriosa superba*





(1.std colchicines, 2. Methanolic seed extract, 3. Isolated colchicines)

Fig 3. TLC analysis of methanolic seed extract of *Gloriosa superba*

Fig.4: FTIR spectrum of methanolic seed extract of *Gloriosa superba*





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ISSN: 0976 – 0997 RESEARCH ARTICLE

Comparative Phytochemical Analysis of Selected Seaweeds against **Different Solvents**

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ABSTRACT

Seaweeds are abundant in coastal areas which are the source of renewable resources. The biologically active compounds present in plants are called phytochemicals. These phytochemicals derived from seaweeds are biologically important and have health benefits. The present study investigated the presence of active compounds from Sargassum wightii and Amphiroa fragilissima. They were collected from Rameshwaram coast and Tuticorin coast, Tamil Nadu, India. They were dried and extracted with different solvents like acetone, ethanol, diethyl ether, benzene and distilled water. Standard procedures were followed to check the presence of active compounds. Out of 50 qualitative tests, selected solvent extracts of Sargassum wightii showed 40 positive tests to different bioactive compounds and the chosen





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solvent extracts of *Amphiroa fragilissima* showed 26 positive results to different compounds. The present study concluded that the number of bioactive compounds was higher in *Sargassum wightii* compared to *Amphiroa fragilissima* in different solvent systems.

Keywords: Sargassum wightii, Amphiroa fragilissima, phytochemical, solvents.

INTRODUCTION

Seaweeds belong to a group of plants known as algae. Seaweeds are classified as Rhodophyta (red algae), Phaeophyta (brown algae) and Chlorophyta (green algae) depending on their nutrient and chemical composition. Like other plants, seaweeds contain various inorganic and organic substances which can benefit human health [1]. Seaweeds are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities. Compounds with antioxidant, antiviral, antifungal and antimicrobial activities have been detected in brown, red and green algae [2,3,4]. Algae are the source of amino acids, terpenoids, phlorotannins, steroids, phenolic compounds, halogenated ketones and alkanes and cyclic polysulphides[5]. The inhibitory substances biosynthesized by the seaweeds were noted as early as in 1917 [6]. The first observation regarding antibiotic activities of seaweeds was reported (Pratt et al., 1944) [7]. Recent findings evidenced that seaweeds contained antibacterial [8], antiviral [9,10], antifungal [11,12], cytotoxic [13] and larvicidal potentials [14]. The secondary metabolites synthesized by seaweeds demonstrate a broad spectrum of bioactivity varying from neurologically active in humans to algicidal, nematicidal, insecticidal and ichthyotoxicity in lower form of animals [15]. Seaweed's antioxidant properties make it specific for prevention and treatment of cancer, supporting the immune system in eliminating the proliferation of cancer cells, says Tierra. Seaweed is considered a medicinal substance with wet, softening properties, which, according to traditional Chinese medicine, Tierra explains, enables it to dissolve hard nodules and tumors and to reduce swelling of the thyroid and lymph glands. Seaweed helps decongest swollen or inflamed lymph nodes; it can be consumed as a treatment for autoimmune illnesses, including chronic fatigue, HIV, arthritis and chronic allergies [16].

Seaweeds constitute a vital part of marine ecosystems. It was estimated that about 90% of the species of marine plant are algae and about 50% of the global photosynthesis is contributed from algae [17]. Over the past several decades seaweeds have been used by humans as medicine and food and their extracts have generated an enormous amount of interest in the pharmaceutical industry as a fresh source of bioactive compounds with immense medicinal potential. Seaweeds are reservoirs of carotenoids, pigments, polyphenols, enzymes, diverse functional polysaccharides. Seaweeds are excellent source of vitamin A, B1, B12, C, D and E [18]. The Southern Coast of India bears luxuriant growth of seaweeds [19]. These vast varieties of seaweeds were potential reservoirs of biochemical compounds, which might be a potential source of drug discovery in the future[20]. More than 2400 marine natural products have been isolated from seaweeds [21,22]. These natural products, are known as secondary metabolites which posses a broad range of biological activity [23]. From the findings it is well known that seaweeds contained antibacterial [24,25], antiviral [26,27] antifungal [28,29] cytotoxic [30] and larvicidal potentials [31]. The secondary metabolites synthesized by seaweeds demonstrate a broad spectrum of bioactivity[32] with this background, the present study was aimed to explore the bioactive potential of two major seaweeds collected from the Coast Tuticorin, Tamil Nadu,India as a potential source of marine bioprospecting.

MATERIALS AND METHODS

Sample collection and preparation

The samples of *Amphiroa fragilissima* (Red seaweeds) and *Sargassum wightii* (Brown seaweed), were collected along the Coast of Tuticorin, Tamil Nadu during low tides. Then the seaweeds were washed thoroughly with seawater to





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remove epiphytes, sand particles, and shells. They were brought to the laboratory in plastic bags containing water to prevent desiccation. Then the seaweeds were washed with running tap water, followed by distilled water to remove all the salt on the surface. The water was drained off and the seaweed was spread on blotting paper to remove excess water. Samples were shade dried at room temperature. The resulting dried material was pulverization to get coarse powder. The powdered samples subsequently stored in refrigerator.

Preparation of algal extracts

The seaweed powder was successively extracted using solvents of increasing polarity according to Arokiyaraj *et al.*, 2009[33] with some modifications. 15 g powder was initially soaked in 60 ml of acetone in air tight conical flask for two days. The flask was periodically subjected to shaking on an electronic shaker and then it was first filtered through double layered muslin cloth and then filtered through Whatman no 1 filter paper and filtrate was collected into sterile air tight bottle. Likewise, the above methods were repeated using ethanol, benzene, diethyl ether and distilled water.

Qualitative Phytochemical analysis of algal extracts

The phytochemical screening of different algal extracts was assessed by standard method as described by Savithramma *et. al.* 2011 [34]. Phytochemical screening was carried out to identify the major natural chemical groups such as alkaloids, terpenoids, steroids, tannins, saponins, flavonoids, phenols, coumarins, quinones and anthroquinone,. General reactions in these analyses revealed the presence or absence of these compounds in the algal extracts tested.

RESULTS

In the present study, the qualitative phytochemical analysis was performed with Acetone, Ethanol, Benzene, Diethyl Ether and Distilled water extracts of *Sargassum wightii*. Different kinds of phytochemicals like alkaloids, terpenoids, steroids, coumarins, tannins, saponins, flavonoids, quinone, anthraquinone and phenols were analyzed for their presence or absence in the solvent extracts of *S. wightii*. The results were presented in the Table 1. Totally 50 qualitative tests were done to prove the presence or absence of the above said compounds in the solvent extracts. 40 positive and 10 negative results for the phytochemical compounds were observed. The positive results showed the presence of alkaloids, terpenoids, coumarin, tannin, saponin, flavonoid, quinone, anthraquinone and phenols. The compounds, alkaloids, terpenoids, coumarins, tannins and quinone were reported in all the solvent extracts. Acetone extracts of *S. wightii* showed positive results for all the compounds except flavonoids. Ethanol extracts showed the presence of seven compounds other than steroids, saponins and flavonois. Flavonoids and phenolics compounds were absent in benzene extracts. Saponins, antraquinone and phenols were not reported positive in diethyl ether. Whereas distilled water showed the presence of all compounds except phenols.

Acetone and distilled water showed highest positive results 9 out of 10 each. As far as the results of the qualitative analysis of different solvent extracts of *Amphiroafragilissima* were concerned, anthraquinone and phenols were the two compounds which were present in all the solvent extracts. Presence of alkaloids was observed in benzene extract and distilled water only. Terpenoids were found in benzene extract, diethyl ether extract and distilled water. Steroids were absent in all the solvent extracts. The presence of coumarins were reported in ethanol extracts and diethyl ether extracts. Tannins were reported in acetone extract, benzene extract and diethyl ether extract. Saponins were not reported in acetone extract and diethyl ether extracts. Flavonoids were reported positive in 2 solvent extracts (Ethanol and Diethyl ether) and quinone was reported positive in distilled water only. Maximum number of 6 positive results were seen in benzene, diethyl ether and distilled water. Presence of tannin, anthraquinone and phenols were reported in acetone extract. Ethanol extract showed the presence of coumarin, saponin, flavonoids, anthraquinone and phenols. Out of the 50 tests only 26 tests showed positive results and the remaining 24 tests showed negative tests. The results of the qualitative analysis of phytochemicals in *Amphiroafragilissima* were presented in the Table 2.





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DISCUSSION

The present study revealed that the phytochemical properties of two marine algae *Sargassun wightii* and *Amphiroa fragilissima*. All solvent extracts of *S.wightiis* howed the presence of alkaloids whereas both benzene extract and distilled water only showed positive results in *Amphiroa fragilissima*. Presence of alkaloids make the seaweed *S.wightiio* great healing andit has been broadly used in the drug and pharmaceutical industry [35]. Terpenoids were found in all the solvent extracts in *S.wightii* whereas benzene, diethyl ether and distilled water only showed its presence in *Amphiroa fragilissima* .since terpenoids from seaweeds displayed wide spectrum of cytotoxic, nematicidal and anti-tumor activity [36,37,38]. Steroids are playing an important role in nutrition, herbal medicine and cosmetics. It has insecticidal, anti-microbial and anti-paracitic properties [39].steroids were present in all solvents except ethanol in the case of *S.wightii*. Whereas it was absent in all solvent extracts in *Amphiroa fragilissima*. Coumarins have been used as anti-coagulants. This bioactive compound was reported in all the solvent extracts in *S.wightii*. In *Amphiroa fragilissima* only 2 solvents (Ethanol and Diethyl ether) showed the presence of coumarins. Tannins were found in all the solvents in *S.wightii* and only 3 solvents (Acetone, Benzene and Diethyl ether) showed the presence of this compound in *Amphiroa fragilissima*. These compounds are used as antiviral, antibacterial, antiulcer and anti oxidant agents [40]. Saponin is used to treat hyper-cholestrolemia, hyper glycemia, antioxidant and they have anti cancer, anti inflammatory and antifungal properties [41,42].

In S.wightii, acetone extract, benzene extract and distilled water showed positivity to this compound. But in Amphiroa fragilissima ethanol extract, benzene extract and distilled water showed the presence of this compound. Flavonoids are the major group of phenolic compounds that are being used in medicinal fields for their antimicrobial, antiviral and spasmolytic activity. It may reduce the risk of various cancers and it is used to suppress the menopausal symptoms [43,44]. Diethyl ether extract and distilled water reported the presence of flavonoids in and S.wightii ethanol extract and diethyl ether extract showed its presence in Amphiroa fragilissima. All solvents reported the presence of guinone in S.wightii and distilled water only showed its presence in Amphiroa fragilissima. Quinones render cytotoxic activity through interfering the replication of DNA and RNA and mitochondrial oxidative pathways [45]. In S. wightii anthraquinone was reported in all solvent except diethyl ether whereas in all Amphiroa fragilissima solvents showed the presence of this compound. Phenolic compound have specific physical, chemical and biological activities that make them as useful drugs. They are responsible for the antimicrobial, anti inflammatory, antifeedant, antiviral, anticancer and vasodilatory actions [46]. Acetone and ethanol extract reported phenolic compound in S.wightii and all the solvent extracts showed the presence of the same in Amphiroa fragilissima. Out of 50 qualitative tests selected solvent extracts of S.wightii showed 40 positive tests to different bioactive compounds and the chosen solvent extracts of Amphiroa fragilissima showed 26 positive results to different compounds. The present study concluded that the number of bioactive compounds are higher in S.wightii compared to Amphiroa fragilissima in different solvent systems.

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Table.1: Results of Preliminary phytochemical studies on Sargassum wightii.

S.NO	Phyto chemical	Acetone	Ethanol	Benzene	Diethyl Ether	Distilled Water
1	Alkaloid	+	+	+	+	+
2	Terpenoid	+	+	+	+	+
3	Steroid	+	-	+	+	+
4	Coumarin	+	+	+	+	+
5	Tannin	+	+	+	+	+
6	Saponin	+	-	+	-	+
7	Flavonoid	-	-	-	+	+
8	Quinone	+	+	+	+	+
9	Anthraquinone	+	+	+	-	+
10	Phenol	+	+	-	-	-

^{+ (}indicate) presence, - (indicate) absence of phytochemical

Table 2. Results of Preliminary phytochemical studies on Amphiroa fragilissima.

S.NO	Phyto chemical	Acetone	Ethanol	Benzene	Diethyl Ether	Distilled Water	
1	Alkaloid	-	-	+	-	+	
2	Terpenoid	-	1	+	+	+	
3	Steroid	П	ı	ı	-	=	
4	Coumarin	=	+	-	+	=	
5	Tannin	+	ı	+	+	=	
6	Saponin	П	+	+	-	+	
7	Flavonoid	=	+	-	+	=	
8	Quinone	-	ı	ı	=	+	
9	Anthraquinone	+	+	+	+	+	
10	Phenol	+	+	+	+	+	

^{+ (}indicate)presence, - (indicate) absence of phytochemical.





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RESEARCH ARTICLE

Biosorption of Divalent Nickel by Immobilized Mixed Biosorbent; **Equilibrium Kinetic Modelling**

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ABSTRACT

Removal of heavy metal ions from waste water is important due to their extreme toxicity towards aquatic life and humans. Microorganisms (Aspergillus niger) and low cost natural biosorbents (Annona squamosa peel powder) are being increasingly studied for the removal of heavy metal ions from aqueous solution. In the present study, batch shaking sorption experiments were performed to investigate the sorption capacity of mixed biosorbents (Aspergillus niger & Annona squamosa peel powder) at different initial Nickel concentration, initial pH , temperature and biomass loading. Equilibrium was reached after 24 h of contact time. The optimum values of initial Nickel concentration, initial pH, temperature and biomass loading were found to be 100mg/l, 6,30°C and 6g/l. Under this optimised condition a maximum percentage removal of 97% and specific uptake of 7mg/g was obtained for Ni(II) sorption. Equilibrium model fits well with Langmiur and Freundlich adsorption isotherms. The kinetic study showed that the sorption process was very fast at the initial stage and equilibrium was reached after 24 h of contact time. Sorption of Nickel on mixed biosorbents proceeds by Lagergren second order kinetic model. The overall sorption process was governed by intraparticle and external diffusion mechanisms.

Keywords: Biosorption, mixed biosorbent, adsorption isotherms, equilibrium model, kineticmodel





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INTRODUCTION

The intensification of industrial activity during the recent years is greatly contributing to the increase of the heavy metal levels in the environment, mainly in the aquatic systems (Da Costa et al., 1996). Nickel (II) is widely used in industrial processes such as electronics manufacturing and metal plating and is found in waste water from oil refineries and mines. While nickel is an essential element at low concentrations for many organisms, it is toxic at higher concentrations. Because of its toxicity, drinking water standards mandate nickel concentrations below 0.1 mg/I (Magyarosyet al., 2002). Many physic-chemical methods have been proposed for their removal from industrial effluents. However, these methods are often inefficient and/or cost prohibitive when used for the removal of heavy metal ions at low concentrations (Wilde and Benemann, 1993). Therefore, there is a need for the development of low cost, easily available materials, which could adsorb Nickel. Biosorption is a process that utilizes inexpensive dead biomass to sequester toxic heavy metals and to remove contaminants from industrial effluents. Fungal cell walls and their components have major role in the biosorption. Fungal biomass can also take up considerable quantities of heavy metals from aqueous solution by adsorption or a related process, even in the absence of physiological activity. Many fungal species such as Rhizopus arrhizus and Penicilium spinulum have been extensively studied for heavy metals biosorption and the process mechanism seems to be dependent upon species (Hafez et al., 1997; Kapoor and Viraraghavan, 1997). Previous research shows that there is growing interest of searching for a variety of materials as low cost adsorbents including sawdust, cocoa shell, rice husk, modified sawdust of walnut, papaya wood, maize leaf, rice husk ash and neem bark, fly ash and tea-industry waste (Siti et al., 2013). Hence, the conversion of these materials as low cost adsorbents is recognized as a potential and economic application for wastewater treatment.

Chemical Equilibrium based kinetic modeling

Sorption equilibrium is usually described by an isotherm equation whose parameters express the surface properties and affinity of the sorbent, at a fixed temperature, pH and initial metal concentration. The simplest forms of these isotherms are Freundlich and Langmuir isotherms which in most cases are used to obtain maximum biosorption capacity of the biosorbent. The Langmuir model suggests, as a hypothesis, that uptake occurs on a homogeneous surface by monolayer sorption without interaction between sorbed molecules. The basic assumptions on which the model is based are: 1) metal ions are chemically adsorbed at a fixed number of well- defined sites, 2) each site can hold one sorbate ion, 3) all sites are energetically equivalent and 4) there is no interaction between ions adsorbed on neighbouring sites. This model is described by the equation (Zumriye Aksu, et al., 1997; Zumriye Aksu, 2001):

$$q_{eq} = \frac{Q^{o}bC_{eq}}{1 + bC_{eq}} \qquad \dots (1)$$

where q_{eq} and C_{eq} are the amount of adsorbed metal per unit weight of biosorbent and unadsorbed metal concentration in solution at equilibrium, respectively. Q_0 is the maximum amount of metal per unit weight of biomass to form a complete monolayer on the surface bound and b is a constant related to the affinity of the binding sites. Q_0 and b can be determined from a plot of 1/qeq and 1/Ceq. The Freundlich model proposes a monolayer sorption with a heterogeneous energetic distribution of active sites, and with interactions between sorbed molecules, as described by the equation:

where K_F and n are the Freundlich constants characteristics of the system. K_F and nare indicators of adsorption capacity and adsorption intensity, respectively. Eq. (2)can be linearized in logarithmic form and Freundlich constants can be determined. Both models are developed for a single-layer metal sorption (Zumriye Aksu, *et al.*,1997; Zumriye Aksu, 2001).





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Sorption kinetic data and Modeling

The kinetics of sorption is important as it controls the process efficiency and the prediction of sorption rate gives important information for designing batch sorption systems. The kinetics of biosorption is studied by using Chemical reaction-based kinetic model and Diffusion based kinetic model.

Chemical reaction - based kinetic modeling

The Lagergren first- order and second- order kinetic models are the most popular model used to study the biosorption kinetics of heavy metals and to quantify the extent of uptake in biosorption kinetics. These models are tried with experimental data and are used to predict the rate of biosorption of heavy metals during the course of biosorption (Zumriye Aksu, 2001). In order to investigate the mechanism of biosorption and potential rate controlling steps the first – and second – order kinetic models have been used. The first – order rate expression of Lagergren is

$$\log (q_{eq} - q) = \log q_{eq} - \frac{K_{1,ad} t}{2.303} \dots (3)$$

Where $K_{1,ad}$ is the rate constant of first- order biosorption (min⁻¹), q_{eq} is the adsorbed quantity of the metal ion per g of dried fungal biomass at equilibrium(mg/g) and q, is the amount of metal adsorbed by sorbent (mg/g) at any time and is calculated from the following equation:

$$q = \frac{\left(C_o - C_o\right)V}{W} \qquad \dots (4)$$

Where C_0 is the initial concentration of metal ions (mg/l), V is the volume of metal solution (l), W is the weight of biosorbent (g) and C is the final concentration of metal ions (mg/l). Linear plots of log (q_{eq} - q) versus time indicate the applicability of this model Eq. (3). The integrated form of second order kinetic model is expressed as

$$\frac{t}{q} = \frac{1}{K_{2,ad} q_{eq}^2} + \frac{t}{q_{eq}}$$
 ... (5)

Where $K_{2,ad}$ is the rate constant of second – order biosorption (g/mg/min). If the second order kinetics is applicable, the plot of t/q versus time should give a linear relationship (Guven Ozdemir *et al.*, 2003).

Diffusion - based kinetic modeling

Diffustion of sorbate has a significant effect on sorption. Sorption of any sorbate molecule on sorbent particles necessarily involves the following steps: (i) bulk diffusion, (ii) external mass transfer of sorbate molecules across the external liquid film around the sorbent particles, (iii) binding of sorbate molecules on the active sites distributed on the outer surface of the sorbent particles, (iv) intra-particle diffusion of sorbate molecules into macro, meso and micropore, and (v) sorption of sorbate molecules onto active sites distributed within the sorbent particles. The steps (iii) and (v) are very rapid and hence they do not have much role in determination of the rate of sorption process. Thus, only external mass transfer and intra-particle diffusion play important role in rate determination (Hameed *et al.*, 2008). Weber and Morris established that, if the intra-particle diffusion is the control step of the process, sorption capacity will vary with the square root of time.





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$$q_t = K_{id}t^{1/2} + I$$
 ... (6)

Where K_{Id} is the intra-particle diffusion rate constant and I is the intercept. Plot of q_1 versus $t^{1/2}$ should be straight line when sorption mechanism follows the intra-particle diffusion process (shah *et al.*, 2009). The external mass transfer model is represented by Eq. (7).

$$\left[\begin{array}{c} \frac{d\left(\begin{array}{c} C \\ \end{array}\right)}{dt} \right]_{t=0} = -K _{ES}$$
 ... (7)

Where C_1 and C_1 , respectively represent the concentration in the beginning and at time (mg/l). The rate constant of external mass transfer model, K_{ES} (min⁻¹), is calculated by the slope of the plot of C_1/C_1 versus time.

Boyd plot

Boyd's model determines whether the main resistance to mass transfer is in the thin film (boundary layer) surrounding the adsorbent particle, or in the resistance to diffusion inside the pores. The Boyd plot predicts the actual slow step involved in the adsorption process. The Boyd kinetic expression is given by (Vadivelan and Vasanth kumar., 2005).

$$F = q_t / q_o \qquad ...(8)$$

Where q_0 is the amount of adsorbates adsorbed at infinite time (mg/g) and q_t represents the amount of metal adsorbed at any time t (min), F represents the fraction of solute adsorbed at any time t, and B_t is a mathematical function of F and is expressed by the following equation. The Boyd plot is obtained by plotting B_t versus time.

$$B_{t} = -0.4977 - \ln(1 - F)$$
... (9)

The objective of the present work was the development of an efficient and cheap, biotechnological system for the removal of Nickel using immobilized mixed biosorbent. In the present study, batch shaking sorption experiments were performed to investigate the sorption capacity of immobilized mixed biosorbents at different initial Nickel concentration, initial pH, temperature and biomass loading.

MATERIALS AND METHODS

Preparation of sorbate solution

A 1000 mg/l stock solution of Nickel was prepared by dissolving 4.47 g of Nickel sulphate in double distilled water. The required concentrations of Nickel ions were prepared from the stock solution by dilution method.

Preparation of the biosorbent

Annona squamosa (custard apple) peel powder was used in this study. The custard apple peel was obtained from local market; materials were washed, dried, and crushed in primary crusher and air dried in sun for several days until its weight remains constant. After drying, it was crushed in roll crusher and hammer mills. The material obtained





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through crushing and grinding was screened through BSS meshes. Finally the products obtained were stored in glass bottles for further use. *Aspergillus nigerMTCC-132*, a filamentous fungus obtained from the Institute of Microbial Technology, Chandigarh, was also used in this study. The culture was grown at 30°C in an agitated liquid media containing potato extract (200 g/l) and dextrose (20 g/l). The pH of the medium was adjusted to 5.5 with dilute sulphuric acid before sterilization. The cell suspension was then separated, dried, homogenized and stored for subsequent biosorption studies.

Preparation of immobilized mixed biomass beads

Immobilized mixed biomass beads are prepared using 8% (w/v) sodium alginate. A known equal amount of biomass (peel powder & *A.niger*) is mixed with sodium alginate and the mixtures are constantly stirred under warm condition until the alginate gets dissolved. The suspension is dripped into 2% (w/v) calcium chloride solution through a syringe. The beads are stored in calcium chloride solution for about 30 min before being rinsed in double distilled water.

Batch biosorption studies

Batch experiments were carried out in Erlenmeyer flasks by adding known amount of immobilized mixed biomass beads in 100 ml aqueous Nickel sulphate solution. The flasks were gently agitated on a shaker with a constant shaking rate at 150 rpm for 240 min until equilibrium sorption was obtained. Samples were taken from the solution at regular time intervals for the residual metal ion concentration in the solution. The residual concentration of Nickel ions in the solutions was determined spectrophotometrically at 445 nm using Dimethylglyoxime as the complexing agent (APHA, 1994). The effect of initial Nickel ion concentration on percentage removal of Nickel is studied by conducting experiments with different initial Nickel ion concentrations namely 100 mg/l, 200 mg/l, 300 mg/l, 400 mg/l and 500 mg/l under identical conditions of temperature, pH and biomass loading and the experiment was carried out as described above. The effect of initial pH on percentage removal of Nickel is studied by conducting experiments with different initial pH namely 3,4,5,6 and 7 underidentical conditions of initial concentration, temperature and biomass loading and the experiment was carried out as described above. The effect of temperature on percentage removal of Nickel is studied by conducting experiments with different temperature namely25°C, 30°C, 35°C and 40°Cunder identical conditions of initial concentration, initial pH and biomass loading and the experiment was carried out as described above. The effect of biomass loading on percentage removal of Nickel is studied by conducting experiments with different namely 2g/l, 4g/l, 6g/l, 8g/l and 10g/l under identical conditions of initial concentration, initial pH and temperature and the experiment was carried out as described above.

RESULTS AND DISCUSSION

The biosorption of metals using immobilized mixed biosorbent in a batch process depends on both contact time between the adsorbate and adsorbent particles and initial metal ion concentration. The effect of initial metal ion concentration on contact time, percentage removal and specific uptake of Nickel was given in Fig. 1 and Fig. 2 respectively. Fig. 1 shows that equilibrium was attained in 24 h, also the sorption of Nickel on immobilized mixed biosorbent increases with increasing contact time. The Nickel removal efficiency was affected by the initial metal ion concentration, with decreasing removal percentages as concentration increases from 100 mg/l to 500 mg/l, the percentage removal of Nickel decreases from 97 % to 74 % and the specific uptake of Nickel increases from 7 mg/g to 26 mg/g respectively. At lower initial metal ion concentrations, sufficient adsorption sites are available for adsorption of metal ions. However, at higher concentrations the number of metal ions relatively higher compared to availability of adsorption sites. The pH dependency of biosorption efficiency could be explained by the functional groups involved in metal uptake and the metal chemistry. The percentage of metal sorption vary with pH of the medium which is given in Fig.3. The percentage removal of Nickel increases from 76% to 98% as the pH increased from 4.0 to 6.0 and thereafter the percentage removal of Nickel decreased to 73%. Biosorption of Nickel was low at alkaline condition. The maximum percentage removal was found to be 98% at pH 6.0 and selected as the optimum pH. The percentage removal of





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Nickel by immobilized mixed biosorbent is very less at low pH, because a high concentration of hydrogen ion (H⁺) competed with cationic Ni²⁺. The percentage removal of Nickel decreases at pH higher than 6.0 may be due to the formation of soluble hydroxylated complexes of the metal ions and their competition with the active sites.

Similar results are obtained for the other adsorbent systems (Gupta and Rastogi, 2008). The effect of temperature on percentage removal of Nickel was studied in Erlenmeyer flasks with 100 ml of aqueous Nickel solution at different controlled temperatures namely 25°C, 30°C, 35°C and 40°C. The effect of temperature on percentage removal of Nickel by immobilized mixed biosorbent was given in the Fig.4. A maximum Nickel removal of 91% was obtained at 30°C because the number of binding sites is more at that temperature. The percentage removal of Nickel by immobilized mixed biosorbent was higher at room temperature and it decreases with further increase in temperature due to the destruction of the cell walls expected, and a reduction in Nickel removal was observed. It may also due to the precipitation of metal ions into its hydroxides at high temperatures from 30°C to 45°C. The effect of biomass loading on percentage removal of Nickel was studied by conducting the experiments in Erlenmeyer flasks with 100 ml of aqueous Nickel solution with different biomass loading namely 2g/l, 4g/l, 6g/l, 8g/l and 10g/l. The results of effect of biomass loading on contact time and percentage removal of Nickel during the biosorption process was given in Fig.5. It was observed that the percentage removal of Nickel increased from 79 to 91% as the biomass loading increased from 2 g/l to 6 g/l thereafter the removal efficiency decreases with further increase in biomass loading upto 10 g/l. A maximum Nickel removal of 95% was observed at a biomass loading of 6 g/l. The linearized Langmuir and Freundlich adsorption isotherm of Nickel was given in Fig.6 and Fig.7. The higher correlation coefficients showed that the biosobent was very suitable for describing the biosorption equilibrium in the studied concentration ranges. The Langmuir and Freundlich constants were used to compare the biosorptive capacity of the biosorbent for Nickel. The Constants were evaluated and tabulated in Table1.

The sorption of Nickel onto immobilized mixed biosorbent data were plotted according to Eq.3 and the results are shown in Fig. 8. The first- order kinetic model does not provide a good fit to the experimental data for the sorption of Nickel also the theoretical qe values found from the kinetic model did not give reasonable values. The plot of t/q against t of Eq.5 is shown in Fig.9. For the second- order sorption model, the linearity is good with the correlation coefficients shown in Table 2. The correlation coefficients are extremely high (>0.99) and also the theoretical qe values were found to be in good agreement with the experimental qe values for the second-order model. The second- order kinetics predicts the behaviour over the whole range of studies supporting a second- order equation and is in agreement with chemisorption (diffusion) being the rate-controlling step. The prediction of sorption rate-limiting step is an important aspect for the study of sorption process. Generally, in batch mode sorption experiments, the sorption can be governed by film diffusion and /or intraparticle diffusion processes. In order to evaluate the relative importance of these two processes, time-course metal sorption data are processes and proceeds through one or more than one steps or combination of two steps. The role of pore diffusion as the single rate controlling steps is tested by the aid of 'Weber and Morris' intraparticle diffusion model. Table 4 shows the kinetic parameter data obtained for the sorption time period that involved intraparticle diffusion process. Fig. 10 shows the plot of specific uptake versus t_{1/2} for five different initial concentrations of Nickel. The first linear portion related to the film diffusion for the sorption period of 0-60 min, the second linear portion represented the intraparticle diffusion for the sorption period of 60-900 min and the last linear portion indicated sorption-desorption equilibrium for the period of 900-1440 min. A high regression coefficient value suggests a significant relationship between q and ti/2. The linearity of the plot demonstrates that the intraparticle diffusion plays a significant role in uptake of metals. The value of intercept I from Table 3, increased with increasing concentration of metal ions in the solution. The values of intraparticle diffusion rate constant Kid decreases with increase in Nickel concentration.

Higher concentrations of metal ions have provided better driving force to external mass transfer process. It is clear from Fig. 10, the biosorption process is jointly controlled by film (outer) diffusion and intraparticle (inner) diffusion for the sorption of Chromium and Nickel by immobilized mixed biosorbent respectively. Similar results are reported by Al-Degs et al., 2006 and Gupta et al., 2009. Other researchers have also reported this non-linear relationship and considered that there were both boundary diffusion and intraparticle diffusion (krim et al., 2006). Fig 11 shows the





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external diffusion plot of Nickel sorption on immobilized mixed biosorbent for the initial rapid phase. Table 4 shows the external mass transfer rate constant for the initial rapid phase at different initial concentration of Nickel. The external diffusion model shows excellent correlation with the sorption data for an external mass transfer period of 60 min, with high correlation coefficients ($R^2 = 0.952 - 0.959$). The external mass transfer rate constant decreased with increasing metal ion concentration in the solution. This would indicate that at high metal concentration, the sorption of metal is probably a surface process occurring on the exterior of the sorbent particle which in turn reduces the external mass transfer resistance for the sorption of Nickel. Similar trends have also reported by earlier workers for the adsorption of lauryl benzyl sulfonate on algae (Fernadez et al., 1995). The film diffusion and intraparticle diffusion are differentiated by employing the plots of B_1 versus t and are presented in Fig. 12 for different initial Nickel concentrations. Boyd plot show the linearity for external mass transfer time period (0-60 min) for all the tested concentration of Nickel. In all the cases, the plot did not pass through the origin, thereby confirming that external particle film diffusion is a dominant rate governing sorption process and also the obtained results are similar to the earlier report (Shah et al., 2009).

CONCLUSION

This study indicated that the immobilized mixed biosorbent (*Annona squamosa + Aspergillus niger*) could be used as an efficient biosorbent for the removal of Nickel from aqueous solution in a batch reactor. Biosorption was influenced by initial Nickel ion concentrations and it was found that as the initial Nickel concentration increases from 100 mg/l to 500 mg/l, the percentage removal of Nickel decreases from 97 % to 74 % and the specific uptake of Nickel increases from 7 mg/g to 26 mg/g respectively. The kinetics of the sorption process is governed by a second-order mechanism. The uptake phenomenon of Nickel undergoes the external (bulk to solid surface mass transfer) and intraparticle diffusion processes. It was found that for a short time period the rate of sorption is controlled by film diffusion and pore diffusion controls the rate of sorption at longer sorption times.

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Table1.Langmuir and Freundlich Constants-Biosorption of Nickel by immobilized mixed biosorbent

Langmuir constants				
Q₀(mg/g)	b (I/mg)	R ²		
20.83	0.166	0.9426		
Freundlich constants				
K _F	n	R ²		
4.795	2.961	0.9952		

Table 2: Comparison of first- and second- order sorption rate constants obtained at different initial Nickel concentrations

Initial Concentration of Nickel (mg/l)	q _{eq} (exp) (mg/g)	First order rate constant K _{1,ad} (min ⁻¹)	զ _{։զ} (theo) (mg/g)	R²	Second order rate constant K _{2,ad} (g/ mg-min)	զ _{eq} (theo) (mg/g)	R ²
100	7	0.0023	2.35	0.737	0.005	6.99	1.000
200	13	0.0023	4.99	0.604	0.072	13.89	0.999
300	19	0.0023	11.48	0.953	0.050	20.0	0.998
400	24	0.0023	12.65	0.846	0.040	25.0	0.998
500	29	0.0023	14.99	0.848	0.034	28.1	0.998

Table 3: Kinetic parameters of the intraparticle diffusion model for the sorption of Nickel by immobilized mixed biosorbent.

S. No	Initial Nickel	Kinetic parameters		
3. NO	concentration (mg/l)	I (mg/g)	Kid (mg/g-min)	R ²
1	100	4.831	0.083	0.903
2	200	5.251	0.335	0.89
3	300	7.797	0.401	0.871
4	400	10.47	0.477	0.865
5	500	10.67	0.659	0.929

Table 4: Estimated external mass transfer rate constant for the initial rapid phase at different initial concentration of Nickel.

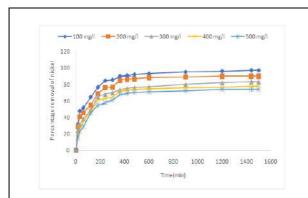
S. No	Initial Nickel concentration (mg/l)	Kes (min ⁻¹)
1	100	0.010
2	200	0.007
3	300	0.005
4	400	0.006
5	500	0.003



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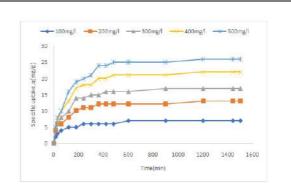
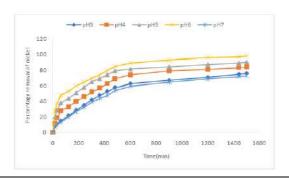


Fig.1. Effect of initial Nickel concentration on Percentage Removal of Nickel by Immobilized mixed biosorbent

Fig.2. Effect of initial Nickel concentration on Specific uptake of Nickel by Immobilized mixed biosorbent



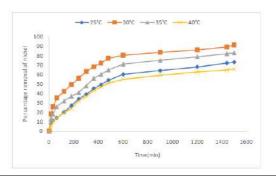
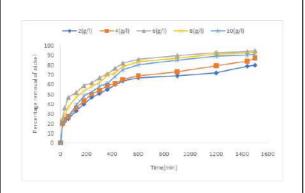


Fig.3. Effect of initial pH on Percentage Removal of Nickel by Immobilized mixed biosorbent

Fig.4. Effect of Temperature on Percentage Removal of Nickel by Immobilized mixed biosorbent



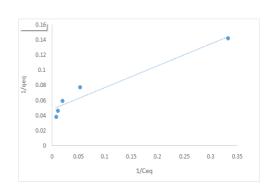


Fig.5. Effect of Biomass loading on Percentage Removal of Nickel by Immobilized mixed biosorbent

Fig.6.Langmuir Adsorption Isotherm for the Biosorption of Nickel by Immobilized mixed biosorbent

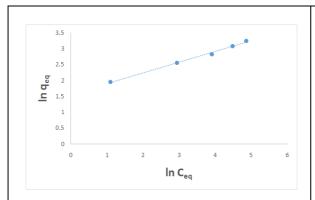




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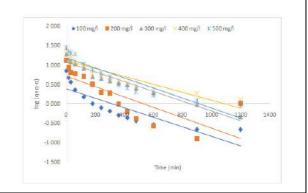
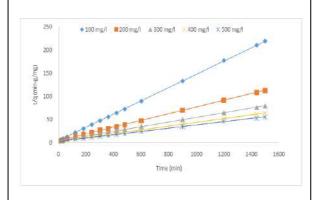


Fig.7.Freundlich Adsorption Isotherm for the Biosorption of Nickel by Immobilized mixed biosorbent

Fig 8First-order Lagergren Plot for the biosorption of Nickel by immobilized mixed biosorbent



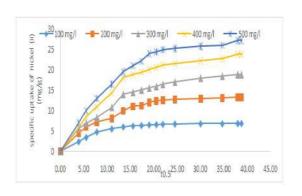
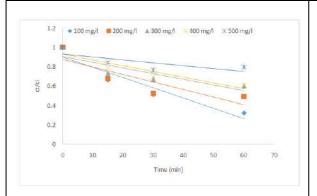


Fig 9 Second-order Lagergren Plot for the biosorption of Nickel by immobilized mixed biosorbent

Fig.10 Intraparticle diffusion plot for the sorption of Nickel by immobilized mixed biosorbent



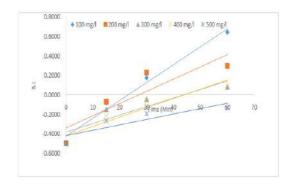


Fig 11External diffusion plot of Nickel sorption on immobilized mixed biosorbent for the initial rapid phase

Fig 12Boyd plot for the sorption of Nickel by immobilized mixed biosorbent





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RESEARCH ARTICLE

Impact Deep-Learning Approaches Identification An for and Classification of the Plant Leaf Diseases: A Survey

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ABSTRACT

From planting through harvesting, there are several challenges in the agricultural industry. Infections with different diseases are a serious source of panic. Using excessive pesticides leads to a significant decrease in production and ecological degradation. There has been a massive increase in demand for food as the global populace has grown at an exponential rate. One of the most devastating risks to the agricultural business is the spread of diseases from diseased to healthier plants. If an infection is not diagnosed early, it has the potential to infect the entire plantation. Methods for detecting plant diseases assist identify contaminated plants at a preliminary phase, and they may also be used to recognize a wide range of plants together in a cost-efficient way. Farmers' traditional methods are not enough to accommodate the increasing demand, but they also damage the land via the excessive use of harmful pesticides. An "Artificial Intelligence (AI)" has shown several benefits over traditional disease diagnosis methods. Many ways have been devised to solve a wide range of agricultural challenges. However, the development of datasets and automated judgment of AI tools has also been extensive. In recent days some of the best outcomes have been achieved via the use of "Deep Learning (DL)" methods. To further assist in the discovery of the complexities of each illness, researchers were able to identify and propose a remedy that may be a great match. The capacity of DL algorithms to understand crucial representations from data input without the requirement for human specialists to define task-specific features is one of the fundamental elements of their success. This paper reviews all those DL approaches that have been developed and utilized in agriculture for the identification of plant leaf diseases and explores the future of precision farming via the application of DL techniques.





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INTRODUCTION

Nearly 30.7 percent of the world's populace is actively engaged in agricultural activities, comprising "2,781mha" of cultivation. Pest and insect administration is a significant issue, apart from weeding, pesticide and insecticide usage, and storage and irrigation management from seeding to harvests. Diseases epidemics and pest and insect control are two of the most challenging problems. For smallholder farmers, leaf infections pose a serious threat to their livelihoods, as well as to worldwide food security [1]. Several biotic residing species, including fungus, viral infections, bacterial, and parasites, as well as abiotic climatic circumstances, including elevated heat, salt, droughts, an iron deficit, poor soil quality, and pH issues, have influenced the development of various plant leaf pathogen. Biotic variables are the most destructive, resulting in the greatest declines in production [2]. It is important to distinguish between the many types of microorganisms. Around 8,000 different kinds of fungi have been found to generate illness in plants, making them the leading cause of leaf degeneration. In addition, a wide range of bacteria may generate leaf illness in plants. Infections caused by bacteria in leaves may be distinguished by their characteristics from those of other infections. When a virus infects a leaf, there is no way to treat it chemically and the entire plant must be thrown away to prevent the virus from spreading further. Whereas bacteria may transit by sprinkling water, fungus particles are transmitted with the air. Aside from that, virus transmission is carried out via a particular insect carrier [3].

The circumstances in the complex field of agriculture couldn't be oversimplified to suggest a particular strategy. Computers were used for the first occasion in agriculture in 1983, according to reports. Other serious issues in agriculture, such as infections of plants' leaves, are being addressed using a multitude of approaches [4]. As a result, there had been a light possibility for solving complicated problems. The consumption of agricultural products would inevitably increase with the ever-increasing demographic. It is now more necessary than ever to use agrotechnology as well as precise agriculture in today's society [5]. A considerable of investment is being spent at the moment by South Korea, the United States, and China to expand the use of computerized agricultural technologies. Among the most important sectors in India, agriculture has the power to promote almost all disciplines and increase its importance in many further regions. To minimize damages and guarantee optimum output, timely and correct diagnosis and categorization of leaf disease are essential [6].

Problem Statement

The categorization of plant leaf disease has typically done by experienced professionals visually inspecting parts of the plant. Such an approach has a difficult challenge since it is expensive, long time, and inaccurate. Computerized "Leaf Disease Detection (LDD)" has been proved to be a viable solution to this issue. The LDD technology has recently been automated using conventional "Machine Learning (ML)" methods in image processing. However these ML methods, on the other hand, are strongly dependent on the features that users deliver. Such strategies are costly and time intensive since the professional retrieves those features through the hard and guessing process [7].

Contribution

The limitations of classic ML algorithms may be effectively overcome through DL assisted extraction of features. While classical ML is still extensively deployed for LDD, the DL has surpassed it in terms of performance. This research contributed to the evaluation of the current condition of DL development and the discovery of a research problem that might contribute to a greater LDD strategy that focuses on DL.

Paper Organization

Section 2 provides a brief overview of some recent works in LDD employing DL models, Section 3 details the methodologies involved in LDD and a survey of some existing DL models, and Section 4 includes a summary of the surveyed approaches, challenges, and suggestions, and Section 5 comes to the conclusion of this comprehensive review with future scope.





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Related Works

For tomato leaves, the authors in [9] suggested a hybridized LDD. Pretrained network "AlexNet" has been employed to extract features, while "KNN" was applied for disease categorization. This model's accuracy was 76.1%. For big dimensionality datasets, their approach performs poorly in terms of computing time and efficiency. A "CNN" framework for tomato LDD focusing on "Transfer Learning" alongside fine tuned "MobileNetV2" was used by the authors in [10]. The outcomes demonstrated that fine tuning the "MobileNetV2" system could diagnose the condition with above 90% accuracy. Resulting in additional fine tuning procedures, their approach is less precise and has a higher computation complexity. According to authors in [11], they have developed a stronger "CNN" framework that can detect 14 distinct agricultural diseases by using the "Inception Module" and "Dilated Convolution". Leveraging the "Plant Village" database, the suggested CNN framework attained a 99.37% overall accuracy. Although this model's accuracy is higher, the databases that they examined are limited. Using the "Plant Village" database and a "Lightweight CNN" framework with "8-hidden layers", the authors in [12] were able to correctly detect tomato pathogens images including precision of98.4%. Mostly in a single disease dataset was their model shown to be more accurate, and it wasn't evaluated for multi-class efficiency at all. Using the "Plant Village" database, authors in [13] created a "9-layer CNN" framework that could distinguish 39 different types of plant disease. Supervised learning techniques have been used to boost the training set's size. The suggested technique has a 96.46 % overall precision. Their method is more accurate, however, the "9-layer" calculation consumes a lot of time. The authors in [14] developed a "Transfer Learning" technique for LDD insisting on a pretrained "ResNet-50" framework. Predictive performance of 97 % had been achieved using the pretrained "ResNet-50" framework. There seems to be a multitude of trained mistakes in this model, even though it is more accurate.

METHODOLOGY

General Methodology for Autonomous LDD

Autonomous LDD has relatively generic needs comparable to common classifying, identification, and segmentation operations in pattern recognition. To be more precise, its specifications may be split down into 3 categories: "What", "Where", and "How". The standard LDD automation process is shown in Figure 1 [15].

Stage 1

In pattern recognition, "what" refers to the categorization problem. In Figure 1, the tag for the class with which it corresponds is indicated. During this stage, the process is referred to as categorization since all it provides is data about the image's class.

Stage 2

Here "Where" refers to the pattern recognition problem of determining a specific place, and the placement here is based on a specific perception of detection. During this stage, we learn not only what kinds of illnesses and infestations are present throughout the image, but we also learn where they are located. The plaque region of the "Grey Mold" is designated by a rectangular area as illustrated in Figure 1.

Stage 3

During this stage, the question of "how" relates to the pattern recognition problem of segmentation. Figure 1 shows that the lesions of "Grey molds" appear pixels by pixels distinct from the backdrop. The next step is to collect a variety of data, including the size, volume, and placement of the "Grey Mold" lesions, intended to aid in the assessment of illness and pests' seriousness at a greater position. Classification provides a holistic description of the image via the representation of feature characteristics. After that, a classification process decides whether the image contains a specific type of disease entity. Disease identification, on the other hand, concentrates on the image as a whole, i.e., determining what entities are there and where they are located. To begin, there is a clear distinction between disease identification and disease categorization based on the nature of the images. Such that, disease categorization relies on feature representation, whereas disease identification relies on structural learning. The3 stages of autonomous LDD have various functions and purposes, but in reality, they are all closely interdependent and re-configurable. As an instance, the "Where" of the second stage incorporates the first stage's "What" procedure, and the third stage's "How" might complete the second stage's "Where". The first stage "what" may also be used to





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accomplish 2nd and 3rd stages' objectives. As a result, the issue in this research is addressed as autonomous LDD as conventional in the coming sections, and the nomenclature distinguishes only after alternative network architectures and functionalities are employed.

Conceptual of DL

In an attempt to analyze data and discover features, "Neural Network (NN)" is used as the foundation of DL. Numerous hidden layers retrieve the data's features, and each of these hidden layers may be compared to a perceptron. Local minima may be greatly reduced by using the perceptron as a tool for extracting and combining lower-level information into higher-level information. Since conventional methods depend on artificial characteristics, the DL has become more popular among academics. Feature extraction, reinforcement learning, voice identification, and computational linguistics have all been effectively implemented using this technology. Traditional ML has been compared with DL to further explain the properties of DL, as per available literature. Table 1 provides an overview of the comparative findings. Manual designing features can be classified and recognized using conventional image categorization and identification techniques, however, these approaches are limited in their ability to retrieve the more sophisticated and fundamental aspects. This snag can be eliminated using the DL approach. Multi-level image characteristic data, including lower, medium, and higher level contextual characteristics may be extracted from the source image using unlabeled data. Conventional image processing methods are based on the challenging and luck-based process of manually constructed features, that are unable to autonomously train and retrieve the features from an image's source content. The DL, on the other hand, could extract features from vast datasets without the need for user intervention.

A multi-layered model with high self-learning and feature interpretation capabilities could derive visual features autonomously for the recognition and classification of images. Autonomous LDD will benefit greatly from the DL's contributions. The "Deep Belief Network (DBN)", "Deep Boltzmann Machine (DBM)", "Stack Denoising Auto Encoder (SDAE)", and "Deep Convolutional Neural Network (DCNN)" are some of the well-known deep NN architectures generated via DL approaches. Conventional manual techniques of extracting features have substantial disadvantages in the field of image classification, where deep NN architectures are used to automate the extraction of features from the higher dimensional feature set. Since deep NNs are trained on a larger training data set and have more computing resources to work with, their characterization abilities are being enhanced further. Enterprise and academics alike are seeing an unprecedented surge in the use of DL. The Deep NN approaches all outperform standard models in terms of reliability. The DCNN has become the most widely used DL architecture in the latest days.

LDD based on DL methods

An outline of autonomous LDD relying on DL modeling techniques is provided in this section. To look at it another way, the autonomous LDD using DL may be interpreted as being an agricultural application of applicable conventional networks. The different DL techniques for autonomous LDD are shown in Figure 2. Figure 2 illustrates how well the network could be progressively separated into classifying, detecting, and segmenting networks based on the various network configurations.

(i)Network for Classification

Plant Leaf pests and pathogens are hard to ascertain in their natural habitat because of the wide range of variances in their appearance, structure, textures, color, backdrop, arrangement, and photographic lighting. As a result of the powerful extraction of features capabilities of CNN, CNN relied on LDD has been the most popular strategy. Following the "Cascaded Convolution and Pooling layer", the CNN classifying networks often use a "Full Connection layer (or an Average Pooling layer) and Softmax Structure" for extracting the features and categorization. The object recognition networks like as "AlexNet", "GoogleLeNet", "VGGNet", "ResNet", "Inception V4", "DenseNets", "MobileNet", and "SqueezeNet" are often used to classify leaf infections and diseases. In other research, network architectures have been developed in response to real-world issues. The network examines the image and delivers the appropriate label for the image's categorization when a test is entered.





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(ii) Network for Detection

Computational vision's simplest fundamental duty is locating objects in an image. In the classical view, it may be the easiest function for the recognition of plant pests and diseases. Obtaining precise position and categorization data is its primary goal. There is a constant stream of new object-detection systems that use DL. The "Faster R-CNN" is the most often used two-stage plant pathogen and insecticide identification network relying on DL, whereas "SSD" and "YOLO" are the most commonly used one-stage networks. There's also a significant difference between these 2 networks except that the two-stage structure must first construct a candidate box that could comprise the lesions before executing the object identification procedure. Instead, the one-stage structure employs the retrieved network properties immediately to forecast the position and classification of lesions.

(iii)Network of Segmentation

The plant infection and disease identification problem is transformed into semantics and maybe even actual segmentation of diseased and healthy regions by the use of segmentation networks. The Plaque region is carefully segmented, as well as the position, categorization, and associated geometric qualities such as height and breadth and the contour and center of the plaque region.

Current DL approaches for LDD

In thissurvey study, well-known current five pre-trained DL based CNN's "VGG16, VGG19, ResNet50, Inception V3, and Xception" for automated LDD techniques weresurveyed and compared.

(i)Two VGGs (16) and (19) Network

The "VGG network", which came in second through the "2014-ILSVRC competition", has been designed by experts in [16]. The "VGG network" comprises "3x3 convolutional filters" stacked on the upper edge of one another and expanding deeper from "64 to 512" layers. It is simply the frequency of weighted layers that differs between the two "2-VGG networks (VGG-16 and VGG-19)": the "VGG-16 network" contains 16 and the "VGG-19 network" contains 19. The depths of the network are implemented to minimize the dimensionality of the source image by using maximal pooling layers of dimension "2x2". After connecting to a Softmax Classification, that generates probability for every class label, the network contains "4,096nodes" in every one of the closings "2-fully connected layers". The designs are quite large in respect of disc storage "533 MB for VGG-16" and "574 MB for VGG-19" because of the deeper of the "2-VGG networks" with its numerous linked nodes, rendering it hard to train and implement. Figure 3 depicts the VGG-16 network in diagrammatic form.

(ii) Inception-V3 Network

Researchers in [17] came up with the "Inception-v3 model," an improvement on "GoogleNet". Employing datasets from the "2012-ImageNet ILSVRC competition", these are given the training to categorize images into each of a thousand different categories. Stacking the outcome among those filters anywhere along multichannel band length and feeding it towards the subsequent layers, the "Inception module" performs "1*1", "3*3", and "5*5" convolutions inside every network component. Despite its "48-layers", the new "Inception-v3" is optimized for better efficiency and easier scalability. For instance, the computational cost of constructing the network is just 2.5 times greater than the computational cost of "GoogleNet", which still contains a depth of "22-layers". This model comprises 96MB in size. Figure 4 depicts this network's structure.

(iii) Res Net50 Network

The experts presented the "ResNet (residual network)" framework in [18] and this topped the "2015-ILSVRC competition". "Residue learning" is often a micro-architectural structural block module that consists of layers such as "convolutional and pooling". Residue modules have been successfully used for training a deep neural network. As opposed to "Fully Connected Layers", the "Res Net50" architecture in the Keras package employs "50-weight layers" and "Global Average Pooling" alternatively. Once compared to the "VGG16" and "VGG19" models, the model's size is "102 Mb". Even though "ResNet50" has substantially more depth than "VGG networks". Figure 5 depicts this network's architecture.





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(iv) Xception Network

Experts in [19] suggested the "Xception network", which has been developed by the "Keras Python API" designer. By substituting separate convolutions all along network depths for the usual inception modules, they modify the "Inception" design. The model comprises 91 MB in size. Figure 6 depicts this network's design.

DISCUSSION OF THE STUDY

Performance comparison of existing algorithms

Table 2 shows the evaluation measures outcomes from the DL approaches examined for autonomous LDD. In diagnosing various disease classes across all kinds of plant leaf images individually and collectively, the models have been trained mostly on the "VGG16" network and surpassed all others. This is evident except few, which had an accuracy rate of 99.11% when utilizing the testing database, comparable to 99.98% for the "VGG16" model. There was no difference between the 2 models when it came to other evaluation criteria, which both had a value of 0.99. The "ResNet50" has been the worst network, with the least impact among all training sets. A massive incline in training and testing sets levels of accuracy was seen for all "VGG16 models" throughout training. It's a sign also that models match the dataset effectively and might be extrapolated to other situations. Validating and training losses including all "VGG16 models" indicate a gradual decline in final loss values, which finally stabilize after model training. There was neither "under-fitting" nor "over-fitting" of the model, which implies that the learning process was performed successfully. As a result, the validation and accuracy rate contours for the "ResNet50 models" indicate a constant rise in accuracy during training and a reduction in accuracy during validation. Thus, the models also couldn't generalize to new data because they were "over-fitted". Whereas "ResNet50 models" training losses were steadily decreasing, validation losses climbed marginally or were fairly unchanged. As a result of their inability to generalize to new data, the "ResNet50 models" were "over-fitting" the training data.

Present DL Techniques Gaps

Many different leaf diseases are now being studied for their ability to be automatically identified using DL. All of these tasks were performed, including more complicated activities like determining the severity of an illness and classifying it. For the time being, the majority of existing DL approaches for autonomous LDD were only applicable to a small subset of accessible datasets. All methods can't be contrasted since there isn't a unique publicly accessible and sufficient database. The efficiency of several common methods on various databases has increased over time as a result of the growth of DL algorithms. This survey found that the highest functioning LDD models have been those trained on the "VGG16 network," whereas those trained on the "ResNet50 network" performed the poorest. Astonishing advancements have been made in recent research. There seems to be a gap between both the intricacy of contagious pest and disease images in existing experiments and actual field pest and disease identification based on portable gadgets, etc. Finding discoveries in bigger, higher complicated, and much more authentic data repositories will be a challenge for future research. The image processing system that relies on DL doesn't even need the extraction of particular features, unlike other standard image processing approaches. Using continuous learning, researchers can locate the features needed, that can also obtain regional and contextualize characteristics of images, as well as stronger resilience and greater detection rates.

Challenges

(i)Problem with Smaller Datasets

Aside from its usage in agriculture, LDD has been considered specialized applicability of DL approaches for other problems in computing images nowadays. A lack of agricultural plant leaf disease collections is hampering our ability to conduct research. The self-collected sets of data are smaller and more time-consuming to classify than open platform resources. The issue of smaller samples in LDD is even more problematic than those in "Image-Net datasets", which include over "14million samples". In the real world, certain leaf illnesses are uncommon, but acquiring an image of such a condition is expensive. Only because a small or a handful of trained data are obtained, DL approaches cannot be used in the area of LDD because of this limitation.





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(ii)Problems with Detection Rate

While the DL approaches provide better outcomes than conventional approaches, they are often more computationally intensive. There must be an escalation in computing load if you want an accurate prediction model to discover an image's features. Real-time demands necessitate a faster detection rate, however, this is impossible to achieve. To guarantee that the detecting performance is maintained, it is frequently required to limit the number of calculations. This, on the other hand, will lead to inadequate training and erroneous or missing detection. Hence, it's also essential to create an approach that is both accurate and fast in its identification. Information labeling, model construction, and modeling inferences are the three essential linkages in the autonomous LDD relying on DL approaches. Modeling inferences receives widespread interest in actual agriculture contexts. At the moment, the overwhelming of LDD approaches concentrate on accuracy rate. The effectiveness of modeling inferences receives less consideration.

Solution

Design of a logical network structure

It is possible to decrease the number of samples needed by developing a logical network topology. The "3-color components" were combined to create a "3-channel CNN" framework for LDD by certain studies. The "3-color RGB" plant leaf images make up every channel's "3-CNN" component. In certain cases, a better CNN approach for diagnosing leaf diseases was shown. To avoid "over fitting" and restrict the number of variables, the model made advantage of a deep separated convolution rather than a regular convolution [20]. The model's capacity to retrieve multi-scale features was improved by applying the original structure to different-sized leaf diseases. Better converging and improved precision was achieved throughout training compared to the traditional "GoogLeNet" and "ResNet" architectures. However, this approach has a recognition rate of 97.22%.

CONCLUSION

The existing DL techniques for autonomous LDD were reviewed in this study. In addition, a variety of visualization methods and mappings have been explored to identify various leaf diseases. There has been a lot of development in the recent 3 to 4 years, yet the following shortages remain to research:

- Mostly the "Plant Village" had been employed in almost all of the preceding articles to measure the reliability and effectiveness of the different DL models. Despite the large number of images depicting various species of plants infected with various illnesses, the dataset's backdrop is quite flat. However, when it comes to a real-world situation, the surroundings must be taken into account.
- In recent years, hyper spectral imagery has already been applied in a multitude of scientific fields. When professionals want to identify leaf illnesses earlier their indications become obvious, professionals must employ DL models with high efficiency.
- Introducing a more effective method of identifying disease patches in plants can save money by preventing needless biocides treatment.
- Leaf severity of symptoms fluctuates over time, hence DL approaches need to be developed so that they can identify and categorize illnesses throughout their lifecycle.
- The DL approach must be able to handle a wide range of lighting situations, hence the databases should include images collected in a diversity of field circumstances.

We'll need further research in the future to figure out how things like database types and quantity, training frequency, and lighting all play a role in detecting plant illnesses.





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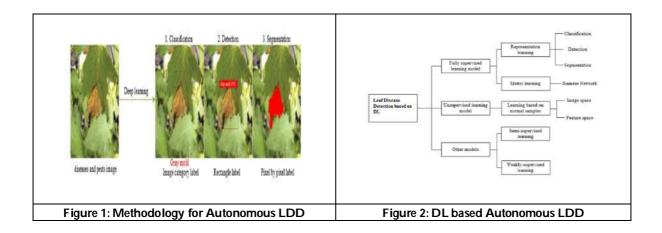
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Table 1: Comparison of DL and Conventional ML techniques

Technology	Traditional image processing methods	Deep learning methods
Essence	Manual design features + classifiers (or rules)	Automatic learning of features from large amounts of data
Method	Image segmentation method: Threshold segmentation; Rob- erts, Prewitt, Sabel, I aplace and Kirsh edge detection; region segmentation Feature extraction method: SIFT, HOG, LBP, shape, color and texture foature extraction method Classification method: SVM, BP, Bayesian	CNN
Required conditions	Relatively harsh imaging environment requirements, high contrast between lesion and non-lesion areas, less noise	Adequate learning data and high-performance computing units
Applicable scenarios	It is often necessary to change the threshold or redesign the algorithm when imaging environment or plant diseases and pests class changes, which has poor recognition effect in real complex natural environment	It has ability to cope with certain real and complex natural environment changes

Table 2: Results of Training Models

Disease Type	Pre-trained Network (Model)	Training Accuracy (%)	Validation Accuracy (%)	Testing Accuracy (%)	Average Precision	Average Recall	Average F1 Score
	VGG16	98.10	98.34	99.01	0.99	0.99	0.99
	VGG19	97.41	97.02	98.18	0.98	0.98	0.98
dternaria Alternata	ResNet50	98.62	48.84	50.41	0.25	0.50	0.33
	Inception	96.72	63.41	62.98	0.72	0.62	0.55
	Xception	98.15	69.87	68.76	0.78	0.69	0.65
	VGG16	98.68	98.59	98.98	0.99	0.99	0.99
	VGG19	98.35	98.85	99.11	0.99	0.99	0.99
Anthracnose	ResNet50	97.62	12.53	12.64	0.04	0.12	0.04
	Inception	96.17	65.73	62.20	0.83	0.64	0.60
	Xception	97.94	58.31	51.60	0.78	0.53	0.47
	VGG16	97.35	96.77	96.84	0.97	0.97	0.97
	VGG19	96.39	96.30	95.68	0.96	0.96	0.96
Bacterial Blight	ResNet50	98.18	25.12	29.40	0.32	0.29	0.13
	Inception	94.85	81.81	83.96	0.86	0.84	0.84
	Xception	96.99	87.80	85.11	0.88	0.85	0.83
	VGG16	97.03	98.38	98.25	0.98	0.98	0.98
rcospora Leaf Spot	VGG19	96.46	97.68	97.37	0.97	0.97	0.97
	ResNet50	98.22	11.97	10.93	0.01	0.11	0.02
	Inception	94.18	72.31	71.17	0.84	0.71	0.72
	Xcention	97.53	75 99	74.67	0.88	0.75	0.77



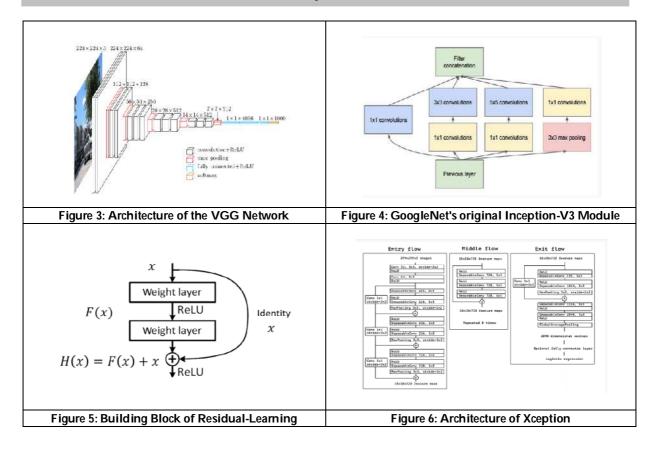




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RESEARCH ARTICLE

Determination of Casein in Milk Samples Available in Guwahati, Assam, India

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ABSTRACT

Milk contains a protein called casein. The filtration process did the determination of casein in milk. For this experiment, various samples of milk were taken, like buffalo, goat, cow, and dairy (Amul, Sudha, and Purabi milk) collected from the local market of Guwahati, Kamrup Metro district, Assam. The milk was poured into a beaker, and saturated ammonium sulfate was added with continuous stirring with a glass rod's help until it precipitated. It was filtered with a filter paper, and the residue was collected in another beaker to which 30 ml water was added and heated in the water bath at 40°C checked by a thermometer frequently to which 1% acetic acid was added drop-wise; the beaker had been withdrawn when it reached the desired temperature. It is then again filtered using a filter paper, and the residue left in the filter paper is collected as casein which is then dried and weighed.

Keywords: Casein, Milk, Precipitate, Protein.

INTRODUCTION

For at least 10,000 years, humankind has consumed dairy products from cows, sheep, and goats. We consume milk because it has proved a positive result in human health as it provides the nutrition required for human growth and development. Scientists have uncovered four distinct instances of human genetic adaptation to digest milk. Drinking





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milk reduces chronic diseases like deficiency of calcium in the body. Heated milk plays a vital role as prebiotics to feed healthy gut bacteria to help reduce the risk of chronic disease [1]. As there is a high demand for nutritious food resources, milk is a good preference to contribute to a healthy food habit. Milk is a nutritionally liquid food produced by the mammary glands. Milk contains (3.4%) protein, lactose (4.8%), fats (3.6%), water (87.5%) vitamin and minerals (0.7%). India is the world's greatest producer of milk. Milk makes cream, butter, yogurt, kefir, ice cream, and cheese, among other dairy products [2]. Colostrum is a type of early lactation milk containing antibodies that aid in preventing various diseases. Dairy milk is a farm product that is gathered from farm animals. India is the largest exporter of skimmed milk powder in the world. The top milk exporters are New Zealand, Germany, and the Netherlands. Milk is taken in two ways: as a source of nutrition for all young mammals and as a food product derived from other mammals such as cows, goats, and sheep for consumption by people of all ages [3].

The experiment was mainly done to determine which milk sample (buffalo milk, cow milk, goat milk, dairy milk like Amul milk, Sudha milk, and Purabi milk) gives the highest casein content and which milk is best for human consumption in terms of protein requirement. Casein is a long-digesting dairy protein that is widely used as a supplement. Bodybuilders mostly take casein powder as it gives a good source of protein required for muscle building. Casein micelles are formed by calcium phosphate and casein from colloidal particles. Casein is the essential component of milk, accounting for 80% of milk protein and being a necessary component of cheese. Casein, along with phosphor-protein, is the most common protein found in milk. It has an isoelectric pH of around 4.7, which allows it to be easily separated. It dissolves well in dilute acids and alkalies [4]. Casein is responsible for half of the overall acidity in the milk. Calcium caseinate, found in casein, can be extracted from milk by curdling it with rennet. The amount of calcium bonded to casein determines the acidity of the milk; the higher the acidity, the less calcium in the milk. Casein can be determined using milk acidity and whey acidity by rapidly precipitating calcium caseinate with rennet, which is proportional to the difference between total milk acidity and whey acidity. However, it should be affected by microflora development in the milk because amino and carboxyl groups are liberated equally [5].

The quality, quantity, and fat content of various samples of milk vary depending on the type of mammal and their food, and the composition of the milk also varies to ensure that the growth rate and development of young species are accurate. Milk composition changes according to age, location, and breed. Due to the presence of protein, milk is colloidal. The protein and non-protein content of milk are 6%, and the nitrogen content of casein is 76 percent. A polypeptide chain of amino acids is joined together by peptide bonds to form protein. Milk and milk products are consumed by almost six billion people, with 750 million people living in dairy farming homes. It is also utilized as a water-soluble, fast-drying medium in paints. Casein, water, hydrated lime, and sodium hydroxide can all be used to make glues. The main function of protein is to promote growth and maintenance for a good healthy body [6]. Milk contains globular proteins of three different from that is casein, lactalbumins, and Lacto globulins. Globular protein does not interact with themselves, resulting in the formation of colloidal suspension more simply than fibrous protein. It also contains a variety of protein combinations, including alpha, beta, kappa, and gamma caseins. The quantity of phosphate groups in the protein defines its structure. Electrophoresis can separate the primary protein in cow's milk into four major components: alpha casein, beta-casein, gamma casein, and kappa casein. Casein is a class of phosphoproteins with a molecular weight of more than 20,000 that can be precipitated at pH 4.6 (in cow's milk) or by the action of the enzyme chymosin (renin) [7].

The purified protein is insoluble in water. It's also insoluble in neutral salt solutions, but it dissolves readily in dilute alkalis and salt solutions such as aqueous sodium oxalate and sodium acetate. It can also operate as an organic adhesive with the help of the enzyme trypsin, which hydrolyses phosphate-containing peptone [8]. The gel form of casein in our stomach gives a steady supply of amino acids to muscles throughout the night that helps in muscle recovery and growth [9]. The downside of casein is that it can act as an allergen to people with milk allergies, which is why it is advised not to take milk protein including casein and whey protein powder, for people withmilk allergies. It is also advised not to take casein powder if the individual is allergic to soy, as many casein products contain soy. People having lactose intolerance can safely takecasein since most of the lactose is removed during the





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manufacturing process of casein. Casein also contains less of the branched-chain amino acid known as leucine, which triggers the muscle-building process that, if compared to whey protein, contains 26% more than casein [10].

METHODOLOGY

Materials

Ammonium Sulphate Acetic acid Distilled water Thermometer.

Sample and collection

A milk sample was collected from the local market in Guwahati city, Assam, India. Collection of different milk like buffalo milk, cow milk, and dairy milk was done in a beaker and labeled (Table 1 and Figure 1).

Method

The filtration method was used to determine casein in milk. 20 ml milk was poured into a beaker, and saturated ammonium sulfate was added with continuous stirring until the fat and casein precipitated. Then it was filtered with filter paper, and the residue was collected in another beaker, into which 30ml of water was measured and poured using a measuring cylinder. It was then heated in a water bath at 40°C, using a thermometer to keep track of the temperature. Once it reached the temperature of 40°C, it was taken out from the water bath, and 1% acetic acid was added drop-wise with continuous stirring till the casein get precipitated. It was again filtered in a filter paper and the residue left was then dried and weighed in a weighing balance, and the amount of casein was noted.

The calculation for standard deviation is expressed as:

Where,

$$s = \sqrt{\frac{1}{N-1} \sum_{i=1}^{N} (x_i - \overline{x})^2},$$

xi is one sample value $x\bar{}$ is the sample mean N is the sample size

RESULTS AND DISCUSSION

Milk with a higher percentage of casein is better for health than milk with a lower concentration of casein. Alpha, beta, kappa, and gamma caseins make up casein, which is a protein mixture. To estimate the concentration of milk, 6 samples (cow milk, goat milk, buffalo milk, Sudha milk, Purabi milk, and Amul milk) of milk were taken, including tetra pack milk samples. Before conducting the experiment, all the milk samples were stored in a cool place and filtered to remove suspended impurities in the milk. Casein concentration ranges from 70-80 percent of the total protein in milk, but it varies from sample to sample. Casein is a white amorphous solid that, after the collection, was dried. The result showed (Table 2, Table 3, Figure 2, Figure 3, and Figure 4) that buffalo's milk contains the highest concentration of casein, with 1.97 gm in 100 ml of milk having a 1.91 casein percentage. Similarly, cow's milk is beneficial, with a case in concentration of 1.88 gm in 100 ml of milk having a 1.81 casein percentage. Goat's milk contains the lowest casein concentration, i.e. 1.88 gm in 100 ml of milk having a 0.81 casein percentage. Purabi milk contains the highest casein concentration, i.e., 2.61 gm in 100 ml of milk, having a 2.53 casein percentage, among dairy milk. Sudha milk contains a case in concentration of 2.26 gm in 100 ml of milk, having a 2.19 casein percentage. From these results, we can conclude that Purabi milk contains the highest concentration of casein which is 2.61 gm in 100 ml of milk, having a 2.09 casein percentage among all the other milk samples.





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These experiments were performed in triplicate, and the percentage of casein was calculated using the equation given below:

Percentage yield of casein = $\frac{\text{Weight of casein }(g)}{\text{Weight of milk }(g)}$ x100

Density of milk=1.029gm/L, SD=Standard deviation

Percentage of casein in all milk samples can be expressed as follows:

Purabi milk>Amul milk>Sudha milk> Buffalo's milk> Cow's milk> Goat's milk.

CONCLUSION

Casein is the most abundant protein in milk containing 80 percent of protein in milk; it contains all of the essential amino acids for human growth and development. We have taken 6 samples of milk that is cow's milk, goat's milk, buffalo's milk, Amul milk, Purabi milk, and Sudha milk. From the experiment, we can conclude that Purabi dairy milk is more beneficial as it has the highest Percentage of casein compared to locally available cow's milk, buffalo's milk, goat's milk, and other dairy milk like Amul milk and Sudha milk. Purabi milk contains a case in concentration of 2.61 gm in 100 ml with a casein percentage of 2.53. The lowest concentration of casein was seen in goat's milk, which is 1.88 gm in 100 ml of milk, having a casein percentage of 1.81. As a result, we can say that Purabi milk is appropriate for good muscle growth and bodybuilding. Goat's milk was discovered to contain a minor quantity of casein, according to the analysis.

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Table 1. Milk Samples used for casein determination

Composition	Price
Nutritional information	Rs- 50/litre
Amount per 100g	
Energy, kcal: 58.2	
Total Fat: 3.0g	
Saturated Fat: 2.0g	
Trans Fat: 0g	
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Protein: 3.0g	
Nutritional information per 100 ml	Rs- 40/litre
Energy Value: 72.39(Kcal)	
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	Rs- 25/500 ml
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	Rs- 44/litre
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Protein: 3.5 g	
Vitamin A: 55.3 mcg	
Calcium: 134.4 mg	
Phosphorus: 70 mg	
Nutritional value	Rs- 150/litre
Fat (%): 3.8	
Solids-non-fat (%): 8.9	
Lactose (%): 4.1	
, ,	
	İ
Casein (%): 2.4	
Casein (%): 2.4 Calories/100 ml: 70	
Calories/100 ml: 70	Rs- 60/litre
	Rs- 60/litre
	Nutritional information Amount per 100g Energy, kcal: 58.2 Total Fat: 3.0g Saturated Fat: 2.0g Trans Fat: 0g Total Carbohydrate: 4.8g Added Sugar: 0.0g Protein: 3.0g Nutritional information per 100 ml Energy Value: 72.39(Kcal) Carbohydrates: 4.95g Fat: 4.5g Protein: 3.00g Mineral: 0.7g Cholesterol: 0.015g Fatty Acid: 0. 015g Added Vitamin A: 27µg RE Added Vitamin D: 0.5µg Nutritional Information per 100gm (Approx, Value) Energy: 71.3 Kcal Total fat: 4.5 g Saturated fat: 4.5 g Trans fat: 0 g Total carbohydrates: 4.5 g Added sugar: 0 g Protein: 4.0 g Nutritional value per 100 ml Total Fat: 3.91 g Saturated Fat: 2.7 g Cholesterol: 7.3 mg Total Carbohydrates: 5.35 g Protein: 3.5 g Vitamin A: 55.3 mcg Calcium: 134.4 mg Phosphorus: 70 mg Nutritional value Fat (%): 3.8 Solids-non-fat (%): 8.9





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Carbs: 12g	
Protein: 9g	
Fat: 17g Lactose: 13g	

Table 2. Weight of casein (g) in different Milk samples.

Serial No.	Milk Sample	Sample (1) in 100 ml	Sample (2)	Sample (3)	Mean SD
1.	Cow milk	1.99	1.95	1.97	1.97±0.020
2.	Buffalo milk	2.00	1.97	2.02	1.99±0.025
3.	Goat milk	1.89	1.86	1.90	1.88±0.020
4.	Amul milk	2.26	2.24	2.28	2.26±0.02
5.	Purabi milk	2.61	2.60	2.68	2.61±0.01
6.	Sudha milk	2.16	2.14	2.18	02.16±0.02

Table 3.Percentage of casein in different Milk samples.

Serial No.	Milk Sample	Sample (1) in 100 ml	Sample(2)	Sample (3)	Mean SD
1.	Cow milk	1.93	1.89	1.91	1.91±0.020
2.	Buffalo milk	1.94	1.91	1.96	1.96±0.025
3.	Goat milk	1.83	1.77	1.84	1.81±0.037
4.	Amul milk	2.19	2.17	2.21	2.19±0.02
5.	Purabi milk	2.53	2.52	2.54	2.53±0.01
6.	Sudha milk	2.09	2.07	2.11	02.09±0.02



Figure 1. Different milk samples used for casein determination

Figure 2.Yield of casein from different milk samples
(a) Amul milk (b) Sudha milk (c) Purabi milk (d)
Cow milk (e) Goat milk (f) Buffalo milk

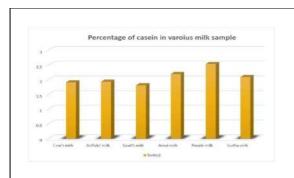




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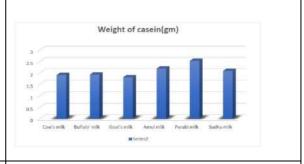


Figure 3.Graphical representation of the amount of casein (g) in different samples of milk

Figure 4.Graphical representation of the Percentage of casein in different samples of milk.





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ISSN: 0976 – 0997 **RESEARCH ARTICLE**

Chemo-Profiling, Antioxidant and Anti-Inflammatory Activity of Endophytic Bacteria, Brevundimonas vesicularis JAP Isolated from Morinda citrifolia L.

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ABSTRACT

Endophytic bacteria reside in the host plants are beneficial rather causing any harm to them. Numerous bioactive compounds have been reported from these bacteria reflecting the host's metabolism they invade. In our study, we have isolated endophytic bacterial strain Brevundimonas vesicularis JAP from the leaves of medicinal plant Morinda citrifolia L. Since this plant is well-known for anticancer activity, endophytic bacteria in it may have active compounds showing similar property. The present research is focused on profiling the active compounds present in the ethyl alcohol extract of endophytic bacteria to investigate the antioxidant and in vivo anti-inflammatory activity of the active compounds present in the sample. VOCs of the ethyl alcohol extract of this bacterium were categorized using GC-MS analysis. Isolation and molecular identification of endophytic bacteria reveal the strain was Brevundimonas vesicular is JAP. The extraction and compound identification detected the primary compound were phthalates, synthesized by this isolate. The total phenolic content of extract calibrated from the gallic acid standard graph was 12.5 ±0.92 µg/mL. The sample exhibited high antioxidant activity in FRAP assay with IC50 (46.04 ±1.42 µg/mL) when compared with DPPH (227.134 ±0.45 µg/mL) and for superoxide radical scavenging activity (170.26 ±0.78 µg/mL). Regarding anti-inflammatory activity, a higher dose depicted the reduction of paw edema followed by a lower amount compared to the diclofenac standard. Our findings reveal that active compounds in the sample labeled antioxidant and anti-inflammatory effects, justifying the chemical constituents present in the endophytic bacteria, may meet the criteria for the drug discovery of pharmaceutical interest in the future.

Keywords: GC-MS, endophytic bacteria, ethyl alcohol extract, antioxidant anti-inflammatory





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INTRODUCTION

Endophytic bacteria have been identified as depots of novel secondary metabolites for immense therapeutic use (Tan and Zou, 2001). Bacteria produce a wide array of volatile organic compounds, which develop metabolic products or by-products such as hydrocarbons, aliphatic alcohols, ketones, and in dole (Audrain *et al.*, 2015). To extract intracellular and extracellular secondary metabolites, we can use organic solvents like chloroform, ethyl acetate, and ethanol to add solvent to the culture medium (Khanna *et al.*, 2011). From the microbial cells, extracellular metabolites can be separated using simple techniques like filtration or centrifugation, whereas intracellular metabolites achieved by breaking the cell, which is a complex process (Villas-Boas and Bruheim, 2007; Tredwell *et al.*, 2011). Various analytical techniques are introduced to identify and quantify exometabolome present in the spent microbial culture media. These metabolites formed due to secretion in different growth phases during cell uptake of nutrients from the external culture medium (Villas-Boas et al., 2007).Gas chromatography-mass spectrometry (GC-MS) is the most appropriate technique for finding several components from a complex mixture of volatile active compounds (Stashenko *et al.*, 2012). Oxidative damage of human cells or tissues pathologically leads to cancer, arthritis, atherosclerosis, cirrhosis, and emphysema (Halliwell and Gutteridge, 1984).

Among the microorganisms, bacteria stand as one of the copious sources of natural antioxidants and antimicrobials products. Antioxidants play a significant role in human nutrition due to more lipid-free radicals in diet and after food ingestion in vivo (Evans, 1997). Free radical scavenging (in vitro and in vivo) of endophytic bacteria has been reported in the culture supernatant, in cells, and in cell-free extracts (Shen *et al.*, 2011). The compounds showing efficient antioxidant activity may also exhibit anti-inflammatory activity. Inflammation is the initial response of the immune system to infection. Surface membrane components such as lipopolysaccharide and lipoteichoic acid of Gram-negative and Gram-positive bacteria, respectively, triggers inflammation (Chien *et al.*, 2012; Draing *et al.*, 2008). Many natural microbial compounds such as omega-3 fatty acid, cyclic peptide, peptide derivatives, acetyl derivatives, oligosaccharides, and polysaccharides can reduce inflammation (Berdy, 2005; Marinelli *et al.*, 2015). *Brevundimonas vesicularis* belongs to phylum proteobacteria is a gram-negative, non-fermenting, aerobic bacilli. Our study aims to extract compounds from the isolated endophytic bacterial strain *Brevundimonas vesicularis* JAP from the anticancer plant *Morinda citrifolia*, commonly known as noni, to investigate the antioxidant and anti-inflammatory activity of the bioactive compounds present in it.

MATERIAL AND METHODS

Plant material

The leaves of medicinal plant *Morinda citrifolia* L. were collected from Thrissur district (Lat 10°37'06"N and Lon 76°12'17"E), Kerala, India. A voucher specimen of *M. citrifolia* (15785) was maintained in Kerala Forest Research Institute, Peechi, Thrissur.

Endophytic bacteria isolation

The leaves of the plant were employed as the source material for the isolation of endophytic bacteria. Fresh and healthy leaves were surface sterilized in 1 % sodium hypochlorite (10 min), 70 % ethanol (1 min), and washed thrice in sterile double distilled water. With a clean and sterile mortar and pestle, the material was macerated in 0.85% NaCl and serially diluted (10^{-1} to 10^{-7} dilution factors). 100 μ L of it spread plated onto nutrient agar (HiMedia) incubated at 37°C for five days and observed periodically for bacterial growth (Fahy and Persley, 1983).

Molecular identification by 16S rDNA sequencing

The 16S rRNA gene of the isolate was amplified using primers 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492R (5'- GGT TAC CTT GTT ACG ACT T-3') (Jose et al., 2011). The genomic DNA of the strain was amplified using a PCR machine (Thermo fisher scientific, U.S.A). 50 μ L of reaction mixture containing 1 μ L genomic DNA, 1 μ L forward and reverse primer each, 38 μ L sterile water, 1 μ L Taq DNA polymerase (HiMedia), 5 μ L 10X assay buffer,





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and 3 μ L 10mM dNTP mix was used. The DNA amplicon was sequenced using BigDyeTM Terminator v3.1 Cycle sequencing kit on ABI 3730 XL cycle Sequencer. The data was used to carry out BLAST with the NCBI GenBank non-redundant (nr) database (Zhang et al., 2000).

Extraction of the culture filtrate

The bacterial isolates were inoculated into the LB broth separately and incubated at 37 °C for ten days in an orbital shaker. After incubation, centrifuge the culture broth at 3000 rpm for 10 min, and the cell-free supernatant was collected. The culture filtrate (100 mL) was extracted twice with ethyl acetate solvent in the ratio of 1:1 (v/v) using a separating funnel. The ethyl acetate fraction was concentrated using a lyophilizer, and the residue was dissolved in an ethyl alcohol solvent (Ahmed, 2007).

Experimental animals

Male Swiss Albino mice weighing 26 ± 5.2 gm (Mean \pm S.D) were used in this study. Animal studies were conducted following Institutional Animal Ethics Committee (IAEC) regulations approved by CPCSEA (ACRC/IAEC/20(1)-P13) and conducted humanely. The mice were obtained from Small Animal Breeding Station (IAEC Reg. no. 328/PO/c/01/CPCSEA), Kerala Veterinary and Animal Science University (KVASU), Mannuthy, Thrissur, Kerala.

GC-MS analysis

The chemical constituents present in the sample extract were identified using combined gas chromatography (Shimadzu QP2010S) and a mass spectrophotometer. The GC-MS parameters as follows- ion-source temperature, 200 °C; interface temperature, 280 °C; pressure, 61.5kPa and one μL injection mode split less with injection temperature of 260 °C. The column temperature started at 70 °C for 2 min and changed to 200 °C at the rate of 10 °C/min. The temperature was increased to 280 °C at 5 °C/min and held for 15min. The total flow was 54.0 mL/min. Comparing the average peak to the entire area of each component can calculate the relative percent amount. GCMS solution software provided by the supplier uses the Real-Time Analysis module for system control and data acquisition. Volatile Organic Compounds (VOCs) were identified based on the m/z peaks representing mass to charge ratio. Recorded chromatogram and mass spectra of the compounds obtained were analyzed following the standard mass spectrum of the National Institute for Standards and Technology library and WILEY 8 library of the corresponding organic compound.

Total phenolic content estimation

The quantity of total phenolic compounds in the extracts was estimated using the Folin-Ciocalteu method Nabavi *et al.*, 2008. Briefly, 50µL of ethanol extract (0.5 mg/mL), 2.5 mL of one-tenth dilution of Folin-Ciocalteu's reagent, 2 mL of sodium carbonate solution (7.5% W/V) was taken. The mixture was incubated at 45°C for 15 mins, and the absorbance was measured against a blank sample at 765 nm. The total phenolic content was measured from the standard graph of gallic acid prepared using different concentrations, and the experiment was conducted in triplicates.

DPPH free radical scavenging activity

The extract of the sample was tested for their scavenging activity against the stable free radical DPPH by the method of Aquino et al., 2001. The reaction mixture contained 1mL of freshly prepared DPPH (0.0025g in 10mL methanol) solution, different concentrations of sample extract (0, 25, 50, 75, 100, 125, 150, 175, 200, 225 and 250 μ g/mL) and the volume was made up to 3mL with methanol. After 20 minutes of incubation at room temperature, the absorbance was measured at 515 nm. Ascorbic acid was kept as the standard reference.

Superoxide free radical scavenging activity

Superoxide radical scavenging activity was analyzed by the NBT reduction method of Mc Cord and Fridovich, 1969. Different concentration (0, 50, 100, 150, 200, 250, and 300 μ g/ml) of the extract was added to the reaction mixture contained 0.12mM riboflavin (50 μ L), 1.5mM NBT (100 μ L), 0.003 mg NaCN in 0.1M EDTA (200 μ L), and 0.6 M SOD buffer (pH 7.8) in to final volume of 3 ml. The tubes containing the reaction mixture were uniformly illuminated for





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20 minutes using an incandescent lamp (Philips, 40W). The optical density was measured at 560nm against the blank before and after illumination.

FRAP assay

The extract of the sample was analyzed for their antioxidant activity against the stable free radical FRAP reagent by the method Benzie et al., 1996. The reaction mixture contained 900 μ L of FRAP reagent (75 μ L ferric chloride (20mM), 75 μ L TPTZ (10mM), 750 μ L acetate buffer of pH 3.6) and different concentration (0, 20, 40, 60, 80 and 100 μ g/mL) of the sample extract. Incubate for 15 min at 37 °C, and optical density reading was monitored at 593 nm. The value obtained from a FeSO₄.7H₂O standard graph is expressed.

Acute toxicity Study

For toxicity study, two groups of male Swiss albino mice (6mice/group) were taken. First group was kept as control, and Group II was administered with a single dose drug (300 mg/Kg b.wt) orally as per the OECD guidelines. The experimental animals were monitored for 14 days, and the mortality rate of the mice was noted. After completion of in vivo studies, carbon dioxide chamber was chosen as euthanasia method to dispose the mice taken. In vivo anti-inflammatory study

Carrageenan paw edema model

The anti-inflammatory activity of the extract was analyzed by acute inflammation induced by carrageenan according to the method by Winter et al., 1962. The experimental animal male Swiss albino mice were divided into normal and control group treated received saline water alone, vehicle control group treated with 1% propylene glycol, the standard group received reference drug diclofenac 10 mg/kg body wt., and test drug groups received doses of 25 and 50 mg/kg body wt. The sample size (n) of animals is sixin each group, which is the minimum number required to meet a statistical conclusion of acceptable standards (Erb, 1990). The animals of the respective group were pretreated with the drug for 5 consecutive days. The edema was induced in the right hind paw of mice by injecting 0.02 mL freshly prepared 1% suspension of carrageenan. The paw edema was measured before and after the administration of phlogistic agents using digital vernier calipers and recorded every hour up to the 6th hour. The inhibition was calculated by the formula [(tCn-tC0) - (tTn-tT0)]/(tCn-tC0) x 100 where tC0, tCn represents paw edema of control animal and tT0, tTn paw edema of treated animal at respective hours before and after injection of phlogistic agents.

Formalin inflammation model

The chronic anti-inflammatory activity was evaluated by formalin-induced paw edema according to the method by Chau, 1989. The experimental mice were separated into five groups containing six animals (n=6) each. Normal and control group 1 received saline water alone, vehicle control group 2 treated with 1% propylene glycol, standard group 3 given reference drug diclofenac 10 mg/kg b. wt, test drug groups (4 and 5) received doses of 25 and 50 mg/kg b. wt. respectively. The drug was administrated orally for 5 consecutive days. On the fifth day, chronic inflammation was induced by sub-plantar injection of freshly prepared 0.02 ml of 2% formalin on the right hind paw in all animals. The inflammation was measured using digital vernier calipers before and after injection of formalin for six consecutive days. An increase in paw thickness as a measure of inflammatory edema was calculated using the formula of, Pt-P0 where the initial thickness was before induction. The inhibition percentage was found using the formula Pc - Pt/Pc×100, where Pt increases the thickness of the treated. Pc is the control.

Statistical analysis

All the experiments were performed in triplicates (n=3), and the values were expressed in Mean ± SE. The data were checked for statistical significance (P<0.001, P<0.01, and P<0.05) using one-way analysis of variance (ANOVA) followed by an appropriate post hoc test (Tukey's multiple comparison test) using Prism 9.0 Graph Pad software Inc. USA.





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RESULTS AND DISCUSSION

GC-MS analysis

GC-MS analysis is a widely recognized technique to identify chemical constituents especially volatile organic compounds. Seven compounds were identified from ethanol extract of endophytic bacteria *Brevundimonas vesicularis* JAP isolated from anticancer plant *Morinda citrifolia* L. as shown in Fig. 1 and Table. 1. The compounds detected using GC-MS were isobutyl phthalate (MW= 221.28), Butyl isobutyl phthalate (MW= 278.34), Dibutyl phthalate (MW= 278.34), Bis methyl glycol (MW=90.18), Diamyl phthalate (MW= 306.40), Oxiraneoctanoic acid, 3-methyl ester (MW=312.49), and Dicarpyl phthalate (MW= 394.59) based on retention time, peak area and molecular weight. The significant compounds identified from NMC15 extract are phthalates. Studies reveal that this compound has anticancer, antioxidant, and antitumor properties (Gao and Wen, 2016). Usually, phthalates are plasticizers, classified as environmental pollutants with some risk for humans. However, in recent years this compound is produced by plants, fungi, and bacteria. Many aerobic bacteria degrade phthalates forming key intermediates such as dicarpylphthalic acid and monoester phthalate in anaerobic mineralization of phthalate esters (Ortiz and Sansinenea, 2018). The isolated endophyte, *Brevundimonas vesicularis*, an aerobic bacterium, produces compounds such as phthalate derivatives as secondary metabolites with biological activity.

Antioxidant capacity

The total phenol content of the sample NMC15 was calibrated 12.5 $\pm 0.92~\mu g/mL$ from the standard graph of Gallic acid. The antioxidant activity for different free radical scavenging was studied using ethyl alcohol extract of the sample as described in table 2 and Fig 2. The sample exhibit a potent antioxidant activity with IC50= 227.134 $\pm 0.45~\mu g/mL$ for DPPH assay, IC50= 170.26 $\pm 0.78~\mu g/mL$ for superoxide radical scavenging activity, and IC50= 46.04 $\pm 1.42~\mu g/mL$ for ferric reducing antioxidant power assay. The activity increases in a concentration-dependent manner when compared to ascorbic acid (standard) with IC50 value 19.02 $\pm 1.11~\mu g/mL$, 23.02 $\pm 1.02~\mu g/mL$, and 0.45 $\pm 1.27~\mu g/mL$ for DPPH, superoxide scavenging, and FRAP assay respectively with significant difference (p < 0.05).

Toxicity study

The administration of propylene glycol extract of the sample (single dose) in mice reported no observable toxicity such as weight loss, hair loss, skin color change, food, and water intake difference. Also, no mortality was recorded, assuming the drug is safe for in vivo studies.

In vivo anti-inflammatory activity

Carrageenan induced study

When carrageenan was used as an inflammatory agent, the control and vehicle control group showed maximum paw thickness 1.07 ± 0.10 and 1.02 ± 0.05 mm respectively at the 3rd h. Treatment groups with low dose (25 mg/Kg b.wt) and high dose (50 mg/Kg b.wt) produced a maximum reduction in paw edema at 3rd h with 32.71 and 34.57 % of inhibition respectively when compared to diclofenac (standard) with 45.79 % as given in table 3 and fig. 3.

Formalin induced study

Formalin-induced chronic inflammation study in mice reports that the control and vehicle control group have no inhibitory activity. In contrast, treated mice with a low dose and high dose showed inhibition at the 7th day with 20.23 and 32.14 % compared with standard (58.33%) of statistical significance as shown in table 4 and fig. 4. Various studies on acute and chronic inflammation in mice have been reported in many plants and fungi extracts. But a handful of literature was found on bacterial components showing anti-inflammatory activity. In searching of the endophytic bacteria for novel anti-inflammatory agents, *Streptomyces aureofaciens*, a root endophyte isolated from Zingibero fficinale have been investigated for this property (Taechowisan *et al.*, 2007). There are no reports on the findings of an in-vivo anti-inflammatory study of phlogistic agents present in the isolated endophytic bacteria *Brevundimonas vesicularis*, which our work pointed out the activity.





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CONCLUSION

Endophytic bacteria produce innumerable bioactive compounds relative to the secondary metabolites of the host plant they inhabit. In the current study, the bacteria *Brevibacterium vesicularis* JAP strain isolated from an important medicinal plant *Morinda citrifolia* L. have biologically active metabolites profiled using GC-MS. The findings of our work disclose antioxidant activity on scavenging different free radicals, and in vivo anti-inflammatory activity that marked these compounds can be used in microbiology pharmaceuticals. This work represents an essential primary step in understanding the endophytic bacterial compounds and their biological activity to benefit the medical field in further investigation.

Ethics approval and consent to participate

This research involves animal studies which were approved by Institutional Animal Ethics Committee (IAEC) following regulations of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) under reference number (ACRC/IAEC/20(1)-P13). The experiment was conducted in biochemistry department, Amala Cancer Research Centre, Thrissur, Kerala, India.

Conflicts of interest

The authors declare that we have no conflict of interest.

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Authors' contributions

We declare that this research work was done by the authors named in this article Neenu A Santhosh (NAS) and Anto P.V (PVA). NAS carried out the laboratory work and data collection and writing of the manuscript. PVA supervised the work and assisted in the data analysis and all authors read and approved the final manuscript.

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Abbreviations

VOCs: Volatile Organic Compounds; CPCSEA: Committee for the Purpose of Control and Supervision of Experiments on Animals; GC-MS: Gas chromatography-mass spectroscopy; DPPH: 2, 2-diphenyl-1-picryl hydrazyl; FRAP: Ferric Reducing Antioxidant Power; IC: Inhibition Concentration.

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Table.1: GC-MS analysis of ethyl alcohol of extract NMC15

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RT(mins)	Peak area %	Name of the Compound Molecular Formu		Property References				
28.001	4.73	Isobutyl phthalate	C12H13O4 ⁻	Antitumor Gao and Wen, 2016				
29.009	15.07	Butyl isobutyl phthalate	C16H22O4	AntioxidantBenzidiaet al., 2019				
29.339	9.86	Dibutyl phthalate	C16H22O4	AntibacterialShobi and Viswanathan, 2018				
30.808	6.99	Bismethylglycol	C4H10O2	Anticancer Adhikari and Yadav, 2018				
31.105	2.05	Diamyl phthalate	C18H26O4	Cytotoxicity Rajamanikyam <i>et</i> al., 2017				
35.122	2.56	Oxiraneoctanoic acid, 3 methyl ester,	C19H36O3	Antimicrobial Kumariet al., 2019				
39.111	58.74	Dicarpyl phthalate	C24H42O4	AntimicrobialGarba, 2016				
100								

Table.2: Showing IC₅o value of sample and ascorbic acid (standard) of different assay

ASSAY	SAMPLE IC50	ASCORBIC ACID IC50
DPPH	227.134 ±0.45 μg/mL	19.02 ±1.11 μg/mL
Superoxide radical scavenging assay	170.26 ±0.78 μg/mL	23.02 ±1.02 μg/mL
FRAP	46.04 ±1.42 μg/mL	0.45 ±1.27 μg/mL

Table.3: Inhibition of carrageenan induced acute inflammation by NMC15 extract

Group (N=6)	Drug Dosage (mg/Kg b.wt.)	Mean Paw thickness (mm)		Increase in paw thickness(mm)	% of inhibition
		0 th h	3 rd h		
Control	-	1.83±0.11	2.9±0.10	1.07±0.10	
Vehicle control	=	1.84±0.07	2.86 ±0.10	1.02±0.05	4.60
Standard(Diclofenac)	10	1.88±0.05	2.46±0.05	0.58±0.05***45.79	
Lower dose	25	2.00±0.08	2.72±0.04	0.72±0.04**32.71	
Higher dose	50	1.95±0.08	2.55±0.05	0.70±0.05***	34.57

Values are expressed as mean ± SD for 6 animals in each group. P<0.001***, P<0.01***, ns - non significant.





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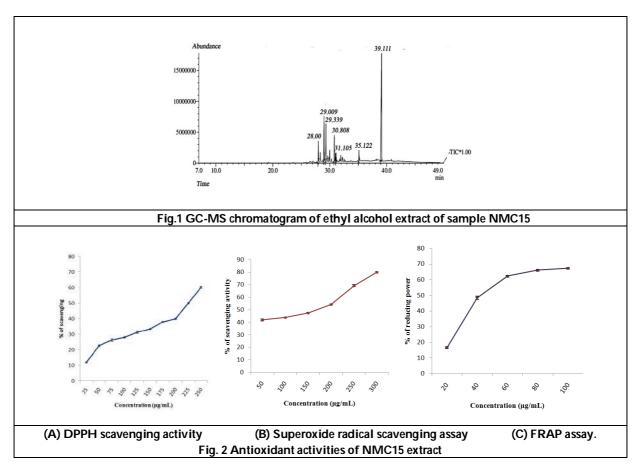
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Table.4 Inhibition of chronic induced acute inflammation by NMC15 extract.

Group (N=6)	Drug Dosage (mg/Kg b.wt.)	Mean Paw thickness(mm)		Increase in Paw thickness(mm)	% of Inhibition
		Initial	7 th day		
Control		1.84±0.33	2.68±0.21	0.84±0.20	
Vehicle control		1.96±0.33	2.76 ±0.30	0.80±0.25	4.76
Standard (Diclofenac)	10	1.92±0.09	2.27±0.12	0.35±0.14***	58.33
Lower dose	25	1.77±0.29	2.44±0.25	0.67±0.33**	20.23
Higher dose	50	1.75±0.12	2.32±0.12	0.57±0.25***	32.14

Values are expressed as mean ± SD for 6 animals in each group. P<0.001***, P<0.01***, ns – non significant







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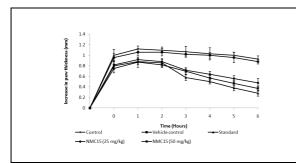


Fig. 3 Effect of sample NMC15 on carrageenan induced acute inflammation in reducing the paw edema in Swiss albino mice.

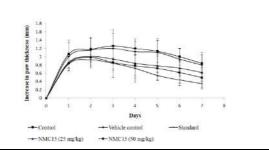


Fig.4 Effect of sample NMC15 on Formalin induced chronic inflammation in reducing the paw edema in Swiss albino mice.





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RESEARCH ARTICLE

Quality Assurance and Quality Control of MAPs in India

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ABSTRACT

The demand for Medicinal and aromatic plants (MAPs) increases with the global acceptance by the people because of the inadequate availability, cost prohibitive disease treatments, unwanted toxic effects, and important development of resistance against infectious diseases by the Allopathic medicines. Therefore, in current research the most important focus toward the determination of potency, efficacy and toxicity of MAPs which are broadly ensured with the scale of quality assurance and quality control of MAPs but still standardization of herbal and its products through quality control based on the internationally recognized guidelines by the World Health Organization is the utmost concern for global acceptability as a prudent form in the modern herbal medicine. Of late, quality control implies the identity, purity, and maintaining a healthy quality of the raw herbals as well as herbal products by devoid of adulteration that ensures the safety and clinically effectiveness as per standard specifications.

Keywords: Herbal plants; Quality assurance; Quality control; Standard guidelines.

INTRODUCTION

People relied on the healing properties of medicinal plants from an ancient time before synthetic drugs came into the market due to their non-toxic nature and easy availability with very minimal cost. According to the World Health Organization (WHO), more than 80 to 85 % of people in the world have accepted plant-based traditional medicines in their daily life for primary health care management[1]. Worldwide, more than 2000 ethnic groups live worldwide,





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and all of them are well versed with their specific traditional herbal-based knowledge and its utilization as Herbalism[2]. Of late, India is home to several indigenous people with a hub of natural plant-based sources along the bountiful heritage of knowledge on the uses of MAPs from time immemorial. Being a diverse country, India has varied climates and geographical regions that have caused a wide distribution of natural MAPs species as resources. MAPs in India serve such an immense role in every moment so that herbal plants become the strength of the medical system of India. The abundant sources of MAPs in India are due to the geographical location on the globe. The Southwest part, Southern East part, and the Northern East part of India are the main hub of natural forests, and the presence of the vast MAPs, among which many are, still unexplored. In India, forests are the main sources for the MAPs and the proper utilization is possible through the cultivation by the proper identification of the plants as per the GAP (Good agricultural practices). Therefore, the need for the time is to abide by standard protocols lied for assuring the quality of the MAPs through the standardization and quality control of herbal products followed by an assurance of safety and efficacy[3]. Not only that, herbal-based market products are generally complex mixtures of different plant species, and it is evident that a single plant contains a number of chemical compounds which show therapeutic efficacy. The composition and number of chemical compounds are vary based on several exogenous (e.g. climate, soil composition, and altitude) and endogenous factors (e.g. genetics, epigenetics) (4). The current article delves into the standardization of MAPs and provides an in-depth look at the quality control and quality assurance standard protocol of various herbal drugs in India, including WHO, Ayurvedic, and ICH protocols.

The authentication and identification of herbal drugs in India

The Ministry of AYUSH, under Govt. of India, New Delhi was developed the quality assurance parameters for many herbal formulations and published different volumes of The Ayurvedic Pharmacopoeia of India. The publication of the Ayurvedic Formulary of India and the Ayurvedic Pharmacopoeia of India helps the Government to implement the Drugs and Cosmetic Act, 1940 in respect of quality control for the Ayurvedic, Siddha, Unani drug manufacturers, their license, distribution, and sale in India. Various tests for heavy metals, microbial content, therapeutic uses, dose, administration, and storage are included. In India, the guidelines and regulations are described under the Drug and Cosmetic Act (1940) under the Ministry of Health and Family Welfare and The Indian Council of Medical Research (ICMR), New Delhi. Therefore, when the medicinal plants are used as modern medicine by the herbal industries, a systematic approach is required for a plant from authentication, systemic cultivation, ethno pharmacology, utilization, isolation, and identification of active constituents to efficacy evaluation, pharmacology, safety, standardization, formulation and to clinical evaluations(5). Several challenges are there in the quality control of herbal drug preparations which should be considered for further steps (Fig. 1). The quality issues such as Good Agricultural and Collection Practices (GACP), provided by WHO, are a vital step for improving the quality of herbal medicines. On another hand, Good Manufacturing Practices (GMP) is a standard protocol in processing and/or manufacturing herbal products as well as Good Supply Practices (GSP) is also an effective regulation for marketing of herbal medicines(6). Therefore, it is worthwhile to discuss in detail about quality assurance and quality control of MAPs in India.

Quality Assurance (QA) and Quality Control (QC) of MAPs

Quality control is the processes that involve maintaining the quality of raw herbals in terms of identity, purity, content or assay, physical, chemical, and biological properties, and validity of the marketed products based on the batch to batch consistency, and stability studies. Botanical identity is carried out with macroscopic and microscopic evaluations, and taxonomical parameters. Not only are that, marker-based standards more popular for the identification or authentication of herbal drugs. Purity is based on the safe use of drugs, and other factors like ash values, the content of foreign matters, heavy metals, microbial content, Aflatoxins, pesticide residues, and radioactivity and various analytical methods for estimation of plant chemicals. Finally, the quality of the herbal medicines broadly depends on the implementation of Good Agricultural and Collection Practices (GACP), provided by WHO, the processing and manufacturing of herbal products as per Good Manufacturing Practices (GMP), and marketing of herbal medicines in accordance with Good Supply Practices (GSP) which are the part of quality assurance.





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Standard guidelines for quality of herbals

Identification and quality control are essential for the quality assurance of MAPs. The first step in quality assurance is Good agricultural and collection practices because the safety and efficacy of herbal medicinal products are directly dependent on it. These practices are also significant in the conservation and protection of MAPs for sustainable use. Many other international guidelines are also published viz. Good Laboratory Practice (GLP), Good Manufacturing Practice (GMP), and Good Clinical Trial Practice (GCTP), ICH guidelines, etc(7). WHO has recommended the two divisions of guidelines that are a) quality of herbal medicinal products and b) Concept of GMP. The herbal products are covered in different Pharmacopoeias, the specifications should contain all analysis of authenticity and impurity limits. Good Manufacturing Practices (GMP) for herbals is framed under Schedule-T to ensure the quality of the drug products which will be uniform throughout life. Good collection practices ensure the long-term endurance of MAPs. All the collection practices are dependent on several factors, such as geographical location, age of the plant, cultural conditions, collection source, the influence of temperature, light, and water etc. WHO broadly described the parameters in four aspects such as assessment of quality, assessment of safety, assessment of efficacy, and intended use(8).

Assessment of quality

Pharmaceutical assessment

This should cover all important aspects of the quality assessment of herbal medicines. It should be sufficient to make reference to a Pharmacopeial monograph if one exists. If no such monograph is available, a monograph must be supplied and should be set out as in an official pharmacopoeia. All procedures should be in accordance with good manufacturing practices.

Identification of Crude plant material

The botanical definition, including genus, species, and authority, should be given to ensure correct identification of a plant. A definition and description of the part of the plant should be provided, together with an indication of whether fresh, dried, or traditionally processed material is used. The active and characteristic constituents should be specified and if possible, content limits should be defined. Foreign matter, impurities, and microbial content should be defined or limited. Voucher specimens, representing each lot of plant material processed, should be authenticated by a qualified botanist and should be stored for at least a 10-year period.

Plant preparations

Plant preparations include comminuted or powdered plant materials, extracts, tinctures, fatty or essential oils, expressed juices, and preparations whose production involves fractionation, purification, or concentration. The manufacturing procedure should be described in detail. A method for identification and assay of the plant preparation should be added. If identification of an active principle is not possible, it should be sufficient to identify a characteristic substance or mixture of substances (e.g. "chromatographic fingerprint") to ensure consistent quality of the preparation.

Finished products

The manufacturing procedure and formula, including the number of excipients, should be described in detail. A finished product specification should be defined. A method of identification and, where possible, quantification of the plant material in the finished product should be defined. If the identification of an active principle is not possible, it should be sufficient to identify a characteristic substance or mixture of substances (e.g. "chromatographic fingerprint") to ensure consistent quality of the product.

Stability

The physical and chemical stability of the product in the container in which it is to be marketed should be tested under defined storage conditions and the shelf-life should be established.





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Assessment of safety

This should cover all relevant aspects of the safety assessment of a medicinal product and reported side-effects should be documented according to normal pharmacovigilance practices.

Toxicological studies

Toxicological studies, if available, should be part of the assessment as per the guideline.

Documentations of safety based on experience

For assessing safety, documentation of a long period of use should be taken into consideration. If a toxicological risk is known, toxicity data must be submitted. The assessment of risk, whether independent of dose or related to dose, should be documented. An explanation of the risks should be given, if possible. Potential for misuse, abuse or dependence must be documented.

Assessment of efficacy

This should cover all important aspects of efficacy assessment.

Activity

The pharmacological and clinical effects of the active ingredients and, if known, their constituents with therapeutic activity should be specified or described.

Evidences required to supports indications

The indication(s) for the use of the medicine should be specified. In the case of traditional medicines, the requirements for proof of efficacy should depend on the kind of indication. For treatment of minor disorders and for non-specific indications, some relaxation in requirements for proof of efficacy may be justified, taking into account the extent of traditional use. Where traditional use has not been established, appropriate clinical evidence should be required.

Combination products

As many herbal remedies consist of a combination of several active ingredients, and as the experience of the use of traditional remedies is often based on combination products, assessment should differentiate between old and new combination products. Identical requirements for the assessment of old and new combinations would result in the inappropriate assessment of certain traditional medicines. In the case of traditionally used combination products, the documentation of traditional use (Ayurveda, traditional Chinese medicine, Unani, Siddha) and experience may serve as evidence.

Finished product related information

Product information for the consumer

Product labels and package inserts should be understandable to the consumer or patient. The package information should include all necessary information on the proper use of the product. The elements of information are required for products such as the name of the product, quantitative list of active ingredient(s), dosage form, indications, dosage (if appropriate, specified for children and the elderly), mode of administration, duration of use, major adverse effects, if any, over dosage information, contraindication, warnings, precautions, and major drug during interactions, use pregnancy and lactation, expiry date, lot number and holder of the marketing authorization. Identification of the active ingredient(s) by the Latin botanical name, in addition to the common name in the language of preference of the national regulatory authority, is recommended.

Promotion

Advertisements and other promotional material directed to health personnel and the general public should be fully consistent with the approved package information. As per WHO, evaluation of crude drug or standardization of crude drug is carried out as per Standard Operating Procedures (SOPs) given for respective drugs. SOP is a set of





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written instructions that document a routine or repetitive activity which is followed by employees in an organization. The development and use of SOPs are an integral part of a successful quality system. It provides information to perform a job properly, and consistently in order to achieve pre-determined specification and quality end-result. SOPs allow the continual improvement of standards of service and provide evidence of commitment towards protecting patients. It also helps to assure quality and consistency of service, ensure achievement of good practice, help to avoid confusion, provides advice and guidance to locums and part-time staff, useful tools for training new members of staff and provide a contribution to the audit process.

WHO Guidelines

Based on the various above parameters, the WHO guideline is classified broadly divided into following categories (Fig. 2). Reference to the identity of the drug. Botanical evaluation – sensory characters, foreign organic matter, microscopical, histological, his to chemical evaluation, quantitative measurements, etc. Reference to the physiochemical character of the drug. Chromatographic profiles, ash values, extractive values, refractive index, polarimetric readings, moisture content, volatile oil content, etc. Reference to the pharmacological parameters. Biological activity profiles, bitterness values, haemolytic index, astringency, swelling factor, foaming index, etc.

Toxicity details

heavy metals like cadmium, lead, arsenic, mercury, etc which are determined with Atomic Absorption Spectrophotometer.

Determination of pesticides

Pesticides should not be more than 1%, an ARL (in mg of pesticide per kg of plant material) is calculated on the basis of the maximum acceptable daily intake of the pesticide for humans (ADI), as, recommended by WHO.

Microbial contamination

Total viable aerobic count, pathogenic bacteria like *Enterobacter* sp., *Escherichia coli*, *Salmonella*, *Pseudomonous aeruginosa*, *Staphylococcus aureus*, etc. and presence of aflatoxins etc. Aflatoxins are natural occurring mycotoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus*. The presence of Aflatoxins is determined by chromatographic methods using standard aflatoxins B1, B2, G1, G2 etc.

Radioactive contamination

The range of radionuclides are released into the environment as the result of a nuclear accident might include long-lived and short lived fission products, actinides, and activation products. The radioactivity of the plant samples should be checked accordingly to the guidelines of the International Atomic Energy Agency (IAEA) in Vienna, Austria.

ICH guidelines

The International Council for Harmonisation (ICH) of Technical Requirements for Pharmaceuticals for Human Use is established in 1990. It is a joint regulatory project that brings together the regulatory authorities and pharmaceutical industry to discuss scientific and technical aspects of drug registration. The ICH's mission is to achieve greater harmonisation worldwide to ensure the safe, effective, and high quality medicines that are developed and registered in the most resource-efficient manner. Therefore, only the six parties of ICH represent the regulatory bodies and research based industry in the three regions viz. Europe, Japan and USA where the maximum number of new medicines are developed. The six parties are European Commission- European Union (EU), European Federation of Pharmaceutical Industries and Associations (EFPIA), Ministry of Health, Labour and Welfare-Japan (MHLW), Japan Pharmaceutical Manufacturers Association (JPMA), US Food and Drug Administration (FDA), and Pharmaceutical Research and Manufacturers of America (PhRMA).





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Stability testing of herbal drugs

Stability testing is an important component of the herbal drugs and product development processes. Drugs regulatory agencies across the globe have recommended guidelines for the conduct of stability studies on these herbal drugs and products. The most important aspect in the evaluation of the stability study of a product and drugs is its storage conditions. The purpose of a stability testing is to provide proof on how the quality of the herbal products varies with the time under the influence of environmental factors such as temperature, light, oxygen, moisture, other ingredient or excipients in the dosage form, particle size of drug, microbial contamination, trace metal contamination, leaching from the container and to establish a recommended storage condition and shelf-life (Fig 3).

Good agricultural practices (GAP)(9)

This section includes some specific parameters such as, a) Selection of medicinal plants where plant authentication is most essential as per the standard guideline followed for the identification followed by cultivation, b) selection of propagated materials mainly seeds that must be well identified and all necessary information relating to identity, quality and performance should be certified from the authentic government registered organizations, c) Site selection on which the quality and other specific plant secondary metabolites are varied for the MAPs, d) Cultivation, ultimately for healthy growth and quality biomass yield of the MAPs cultivation plays a significant role that requires intensive care and effective management, e) Irrigation and drainage system, which is required to control and well managed and maximum plant growth is depended on these systems but the irrigation depends on the needs of the individual medicinal plant species in their every growth stages, f) Organic production, that is most essential in recent era for the organic production of medicinal plants. The harmless and easily degradable pesticides, eco-friendly biocontrol agents and effective control measures are taken care of for the healthy and disease free cultivation of MAPs. The organic cultivated raw plant materials are effective to cure many diseases which the retained quality of its chemicals, q) Harvest management for MAPs which is carried out with two objectives, viz., an economic point which needs to focus on the type of plants that to be harvested and the Pharmacopeial standards of which it needs to procure. Further, the most important step is the storage of the finished herbal materials of the better desired quality. Some examples are like some plants having a maximum accumulation of phytoconstituents in particular stage and position of leaves (Digitalis, Mentha); some during the particular developmental stage of leaves (Andrographis); some during specific growth period (Taxus, Uragoga) etc.

Good Manufacturing Practice (GMP)

It is the most essential component to a pharmaceutical manufacture which includes issues like quality management, documentation, and audit procedures. Some essential steps are like, Primary processing, in which visual inspection to examine any cross-contamination by untargeted medicinal plants as well as the content of foreign matter, imparts quality based on appearances, damage, size, colour, odour, taste of the plant products are essential. Drying conditions, harvesting and collection time, proper storage facilities to avoid microbial contamination of the plant materials are also important parameters under GMP. The drying is possible either naturally or artificially which greatly depends on the nature of phytoconstituents. Various techniques like the open-air system, placed in a thin layer on drying frames, wire screened rooms, oven dry, solar dry etc. It is important to understand that the temperature and humidity should be controlled specifically for the soft plant parts as well as avoid damage to the therapeutically effective plant constituents(10). The stored room for the harvested raw materials should be well ventilated and clean to avoid microbial contamination. Packaging is the next step for the transportation of the raw materials as well as commercial herbal products. Packing materials should be nontoxic, cleaned, and packed in well hygienic conditions for the quality materials with proper labelling (as per the European Union and national labelling regulations) to avoid admix of the products or raw materials. The next step is storage and transport on which the quality of raw-plant based materials and herbal-based products impart their quality for effective therapeutic actions. Finally, the most vital step is a quality guarantee between producers and buyers with regards to all the quality aspects in terms of the monograph of the products should be well explained and documented as written form (11).





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Good laboratory practices (GLP)

It is one of the most important laboratory-based measures, which includes proper identification of plant products, consistency, and purity determination to ensure uniformity, and specifications to obtain commercial values. Various laboratory-based experiments such as organoleptic evaluation, phytochemical screenings, and pharmacognostic evaluation methods are most essential. Organoleptic methods include visual observation of the plant parts for identification such as colour, odour, taste, size, and shape, etc. Thereafter, the phytochemical screening method helps in the identification of phytochemicals present in the plants through various chemical tests. Various chromatographic techniques such as TLC, HPLC, HPTLC, GC, some modern hyphenated techniques such as LC-MS, GC-MS, LC-MS/MS, etc. and spectroscopic methods such as UV, IR, MASS, NMR, etc. are used for isolation, identification, estimation, and structural characterization of the plant constituents⁽¹²⁾. Botanical identity is most essential to identify the pure plant products and also to identify the adulteration that is carried out with microscopic as well as morphological evaluations(13). Thereafter, vernacular names of the herbals play an immense role in the avoidance of confusion and inconsistent uses that affect the transportation of the MAPs products. Not only that, various physical evaluation parameters especially moisture content, extractive matters, ash values determination, loss on drying, determination of foreign matters, etc. are most important(14).

Hereditary evaluation for quality assessment of herbals

For standardization of any MAPs, the first and foremost requirement is the identification of medicinal plants which is carried out by the taxonomists or botanists by the correct authenticity. Improper or wrong identification of MAPs especially species, subspecies, or different varieties leads to widespread of adulterated raw materials or products related to herbals in the market, not only that, but it also adversely affects therapeutic activities or sometimes show very toxic effects with the presence of unwanted materials or toxic substances(15). In general, various DNA-based technologies such as non PCR based (RFLP) and PCR based markers, hybridization and microarrays are applied for reliable authentication and selection of genuine plants(16). Restriction Fragment Length Polymorphism (RFLP) is a type of molecular marker which is used to detect species-specific variation in the number and size of digested DNA fragments in MAPs(17). Most importantly, genetic diversity among the MAPs are determined by some dominant markers namely, Random Amplified Polymorphic DNA (RAPD), DNA amplification fingerprinting (DAF), Arbitrarily primed polymerase chain reaction (APPCR), Inter-simple sequence repeat (ISSR), Amplified Fragment Length Polymorphism (AFLP), and some co-dominant markers such as Simple sequence repeats (SSRs), Sequence characterised amplified regions (SCARs), Cleaved amplified polymorphic sequence (CAPS), Expressed sequence tags (ESTs), Single Nucleotide Polymorphisms (SNPs) and sequence tagged sites (STSs) such as Microsatellites, Simple Sequence Repeat (SSR), Short Tandem Repeat (STR), Sequence Tagged Microsatellite (STMS) or Simple Sequence Length Polymorphism (SSLP) etc(18). These molecular markers are utilized for the DNA fingerprinting as well as analysis in the genetic diversity of the herbal plants(19). Thereafter, the authentication of medicinal plants is carried out by a recent approach of the DNA barcode technique. The technique is reliable, uses a short DNA sequence from the standard genome, and also not affected by external factors (like, climates, age, parts of plant etc.)(20). Initially, chloroplast and nuclear regions are selected as a standard barcode for species identification in the MAPs but a multilocus barcode is identified as a requisite for plant DNA barcode(21). It is a method based on the detection of variable sites of the rDNA internal transcribed spacer (ITS) of the plant gene.

Chromatographic fingerprinting as quality assessment

The plant phytochemicals are most essential for the therapeutic action, but the content of the same is varied based on several factors such as climatic conditions, geographical location, soil nature, harvesting time etc.(22, 23). A fingerprint is a characteristic pattern that chemically represents herbal extracts. The combination of quality control with respect to fingerprinting and multi-component quantification in combination contributes to a quantitative quality evaluation(24). Fingerprinting technology is the most suitable process for the identification of unknown phytochemicals or bioactive components in a mixed preparation of several raw drugs or extracts and batch to batch consistency for the herbal formulations. Some standard analytical techniques used in fingerprinting method include thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), high-performance thin layer chromatography (HPTLC)and capillary electrophoresis (CE), Ultra High-Performance Liquid Chromatography





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(UHPLC), Gas Chromatography (GC), Pressurized Capillary Electro Chromatography (pCEC), or electrophoretic (Capillary Electrophoresis (CE) techniques. Some other novel techniques are like infrared spectroscopy, metabolic fingerprinting and quantitative determinations based on nuclear magnetic resonance spectra, mass spectroscopy etc. are used for Chemo-profiling of MAPs. In the modern era, hyphenated instrumentations such as LC-MS, GC-MS, GC-MS/MS, LC-MS/MS etc. are used for quantitative analysis of various plant constituents(25).

Use of Systems biology in quality assessment

In recent era, systems biology is a new established method after the establishment of standard molecular biological techniques. An omics concept is an advance technology that widely acceptable in the scientific world. It is required entire profiles of molecules in whole cells, organs or organisms. Traditional medicines generally follow holistic therapeutic approaches without any scientific evidences but the modern herbal medicines are established with lot of scientific evidences especially with molecular mechanisms of MAPs(26). Newer approaches such as Microarray hybridization, measure changes in the genome (genomics), transcriptome (transcriptomics), proteome (proteomics), metabolome (metabolomics) technologies through hyphenated instrumentation methods such as LC-MS/MS are widely applied for herbal raw materials and their products(27). Thereafter, Bioinformatical methods such as hierarchical cluster analysis and principal component analysis are employed in omics technology for statistical analysis of huge data process(28). Systems biological research may also facilitate to understand synergistic interactions of herbal mixtures.

Preclinical evaluation in quality assessment

Preclinical tests facilitate the total safety, quality and efficacy data prior to commencement of clinical trials that are depends on numerous pre- and postharvest factors. In the preclinical investigations, generally *in vitro* and *in vivo* models are used. With the help of the reverse pharmacology, effective and safer drugs from natural plant sources are developed from the combined knowledge of traditional medicine and the modern technology(29).

Animal experimentation as precondition in quality assessment

Any therapeutic investigations of plant based extracts or products, initially animal experiments are carried out through *in vitro* test models. The experiment is easy to perform, and used for either crude extracts or any isolated phytochemicals from the plant extracts. Generally, animal models are selected for carried out of any human diseases as well as to find toxicity. Rodents, like rats and mice, are most commonly used animals in scientific research. Hence, identification of bioactivity in animal experiments is a precondition to perform clinical trials in human patients.

Clinical Evidence in quality assessment

A clinical research is started only after collection of relevant preclinical data and further grant of due approval from the relevant Health Authority/Ethics Committee with the design and objective of the experiment (30). The trial then only be initiated after the respective national regulatory authority is satisfied based on the provided quality data such as (i) The safety and efficacy of the drug; (ii) the requirement for the trial; (iii) steps followed for the trial based on protocol and (iv) the skilled investigators/sponsor. In 2005, World Health Organization (WHO) issued operational guidelines regarding regulatory requirements needed to support clinical trials of herbal products(31).

Toxicological evaluation in quality assessment

Toxicity determination is the most challengeable task in quality control of MAPs that resulted intrinsic and extrinsic effects. The intrinsic toxicity in herbals resulted from the innate active compounds, over dosage and interaction of herbal drugs with other drugs(32). The toxicity is also resulted from foreign substance present in the herbal preparation, heavy metal contaminations, pesticide content, microbial contaminations, misidentification of plant species and fallacious dose selection which are considered as extrinsic toxicities. Test organisms are used in toxicity testing (acute, subacute, and chronic studies) with animal brine shrimp, and with other live animals like mice, rats, guinea pigs and rabbits etc.(33). Recently, various animals and human cell lines, *in silico* methods, molecular biological methods are adopted to study of various toxicological parameters of herbal materials and their products. Furthermore, a novel approach, organ-on-achip model (transparent 3D polymeric micro channels) is also developed





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to simulate the cellular physiology in an artificial environment as high throughput toxicity screening tool in herbal drug industry (34).

Labelling of herbal products

Labelling of the finished herbal products is an important parameter for quality of consumer information about the authentication of the product details. Label should provide all detail information including warnings in the red boxes and expiry date on the label helps to reduce the risk adverse reactions. The prime source on herbal products information is the product label. The most important thing, herbal remedy labels often cannot be trusted to reveal what is in the container because there is no organization or government body to certify the herb as being labelled correctly.

CONCLUSION

Quality is the first concern for the healthy life of a human. So, it is of utmost importance to use quality products that are authentic and standardized, as followed with a standard protocol documented by the Government bodies. Even it is necessary to maintain quality, efficacy and batch to batch consistency of the MAPs and their market products through the standard guideline for maintaining the safety and efficacy of the herbal related products. Many advanced technologies are adopted such as DNA-based technologies for the authentication of plant species, good practice guidelines for maintaining standard, various analytical techniques for chemo-profiling of medicinal herbs, as well as various toxicological parameters to detect contaminations etc. for the quality assurance and quality control of the MAPs in India.

Ethical approval

The conducted research is not related to either human or animal use.

Conflict of interest

Authors declare no conflict of interest.

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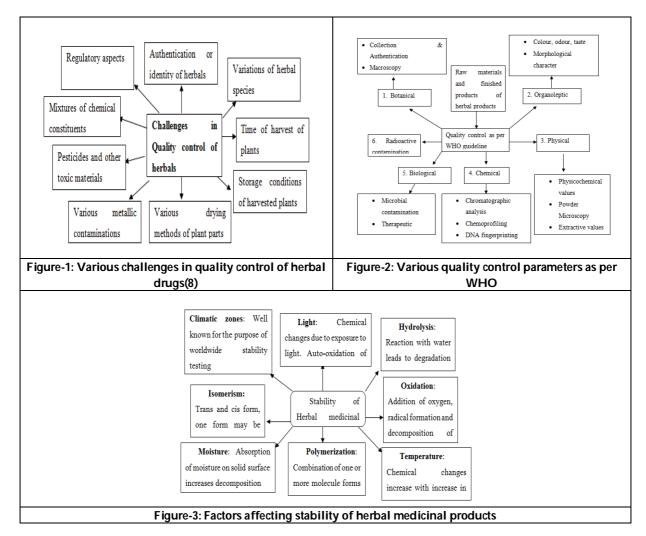


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RESEARCH ARTICLE

Construction and Design of Safer Braking System for Automatic **Transmission**

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ABSTRACT

An automobile moped vehicle has a front tyre brake and an accelerator on the right side, so when braking, the accelerator remains stuck and the accelerator is not fully zero, making braking difficult. Our paper considers the reduction in road accidents and that during braking time the accelerator assembly is disconnected, meaning the accelerator is zero, therefore an increase in safety. Because our hands can't do three things at once, it's impossible to fully engage the front brake lever and twist the throttle to neutral position at high speeds under panic or emergency braking conditions. i.e. (exert brake lever Pressure, release throttle in fraction of second, maintain grip on accelerator handle). This engages front and/or rear brakes partially and doesn't let go of throttle action fully. As a result, braking distance considerably increases, causing more road accidents. Our idea is that when the front and/or rear tyre brake is applied, the accelerator is zeroed automatically. This is possible when the brake is applied and the accelerator assembly is disconnected from the engine.

Keywords: Coupling, Helical spring, Brake lever, Throttle Handle, Bearing

INTRODUCTION

There has been an increase in vehicle accidents on the road in the past few years with the aggrandisement of motor vehicles in India. Injuries to humans and fatalities have come up as a major public concern as it is one of the main causes of death and permanent disability in this country. According to the National Transportation Planning and Research Centre, one road accident takes place every four minutes in India. Almost 97% of all road accidents are caused by hasty or negligent driving [1]. Road traffic crashes are a leading cause of death globally and the main





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cause of death among those aged 15–29 years. Generally, powered two-wheel bikes and mopeds (PTWs) are dynamically unstable vehicles, so the possibility of an accident involving a PTW is higher than for another automobile on the road[5]. Research on road crashes has shown that two-wheel drivers specifically have a greater risk of being sacrificial (fatalities or injuries) in a traffic crash compared with any other vehicle. Reports show that PTW drivers are over 34 times more likely to have a fatality in a traffic crash than other types of motor vehicles. In India, young vehicle users still constitute a higher risk group with regard to road traffic accidents. The accident rate of a moped is four times higher than that of a motorcycle, and the likelihood of being injured in a road traffic accident is 10 to 20 times higher among moped drivers compared to car drivers. In the moped, mostly at high speed and in emergency situations, drivers don't take proper decisions and braking distance is increased, so accidents occur[6].

Literature Review

The role of sensation seeking and attitudes as mediators of driver age and risky driving of powered two-wheelers [01]. The author's analysis of the effect of the age of the driver on risky driving of mopeds includes an analysis of safety attitude and risky driving of the driver. From research they analysed, road traffic crashes are the leading cause of death globally and the main cause of death among those aged 15-19 years. After analysis, they note that because of the instability of two-wheelers, there are more accidents by two-wheelers than four-wheelers. Two-wheeler accidents occur more frequently because of risky driving behaviours at high speeds, rule violations, drunk driving, and cell phone use while driving. They also analyzed of accident of two wheeler age wise. They also analysed the accidents of two-wheelers age-wise. After study, they suggested that accidents could be reduced for youngsters by providing knowledge of rules and regulations for safely driving and speed limitation at school level[7]. NiccolòBaldanzini Rider behavioural patterns in braking manoeuvres [2]. While driving, different riders have different presences of mind and achieve different goals. This author is considering different driver parameters and their behaviour when driving. He considers different parameters like gender, age, riding experience, type of vehicle, and riding distance. In this analysis, we will not use any brake assist systems like ABS or combined braking systems. In this paper, the following signals were considered: longitudinal velocity, braking pressure of the front and rear, and longitudinal deceleration, analysis of braking event type, duration, braking pattern, maximum pressure, maximum pressure position, velocity, deceleration, and maximum deceleration position. The main objective of the paper is that, by this analysis, they can find new technology by analysing driver behaviour for braking. Selma Marie Siddiqui Injury patterns among motorised two-wheeler pillion riders in New Delhi, India[3]. This paper's research is about the injury of pillions on mopeds. According to research, the majority of female Pilon injuries and deaths are caused by not wearing helmets, and the majority of head and neck injuries are caused by not wearing helmets. Research concludes that pilons that do not wear helmets are highly injured or killed compared to helmet-wearing pilons. They also analysed injury types of two-wheeler pillion like head and neck injury, face injury, chest injury, and abdominal injury[4].

Objectives of The Study

- 1.To decrease accidents on mopeds, braking distance should be increased when braking.
- 2.To reduce wear on the brake lining and increase the life of the brake lining.
- 3.To decrease the adverse effect on clutch lining material.

Component Selection

Coil Spring

A helical spring, also known as a coil spring as shown in figure 1, is a mechanical device which is typically used to store energy and subsequently release it, to absorb shock, or to maintain a force between contacting surfaces. They are made of an elastic material in the shape of a helix, which returns to its natural length when not loaded. Under tension or compression, the material (wire) of a coil spring tends to toss under torsion. The spring characteristics therefore depend on the shear modulus, not the young's modulus. A coil spring may also be used as a torsion spring; in this case, the spring as a whole tends to torsion about its helical axis. The spring material thereby subjected to a bending moment, either reducing or increasing the helical radii[8].





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Coupling

A square jaw type is used where engagement and disengagement in motion and under load is not necessary. This type of coupling will transmit power in either direction of rotation. The spiral jaws may be left-handed or right-handed because the power transmitted by them is in one direction only. This type of coupling is occasionally used where the coupling must be engaged and disengaged while in motion.

Brake Lever

Abstract Hand brake levers as shown in figure 3 are widely used in all automotive, which acts as a linkage between occupant and brake mechanism. So here helical spring attached to come in original position and set to zero position of accelerator.

METHODOLOGY

When we rotate the throttle handle, the coupling which is connected to the throttle handle also rotates, and therefore the coil spring is twisted at that time, the coupling is in the engaged position. When we apply the brake by using front brake lever, the mechanism is displaced and also displaced bearings with the linkages that bearings push the coupling. Therefore, disengagement of the coupling occurs. Due to disengagement of coupling, the reverse rotation of the throttle mechanism with the help of twisted spring force and rapidly accelerator zeros automatically. There is a linkage connected with the left brake lever through a cable and the other end of the linkage is connected with a spring which is connected with the coupling. When we press the left brake lever, the linkage pulls the coil spring, therefore the coupling is disengaged. When we apply both brakes at the same time, the right-side brake lever and the pulling action of the linkage at the left side brake lever, when we apply both brakes, we combine the mechanisms of both side role play.

RESULT AND ANALYSIS

Spring Calculation

Maximum force on brake lever 50-70N. Maximum linear displacement of spring X=10-15 mm. Angle between brake lever and coupling Θ =20 degree.

(1) Take max. Force F=50N

Horizontal force or effective force is Fcos20 Fcos20=50cos20=47N

Calculation for spring stiffness K

K=Fcos20/X.....[1]

Taking X is 10 mm.

K=47/10

K=4.7 N/mm

(2) Take max. Force F=60N
Horizontal force or effective force is Fcos20
Fcos20=60cos20=56N
Calculation for spring stiffness K
K=Fcos20/X
Taking X is 10 mm.
K=56/10
K=5.6 N/mm

(3) Take max. Force F=70N





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Horizontal force or effective force is Fcos20

Fcos20=70cos20=65N

Calculation for spring stiffness K

K=Fcos20/X

Take X is 10 mm.

K=65/10

K=6.5 N/mm

Stopping Distance Calculation

Speed: 40 km/hr

Theoretical Calculation

(11) V=11.11 m/s t-t0 =2.58 sec Putting in equation 2 $a = -4.30 \text{ m/s}^2$ And putting in equation 1 x=14.35 m.

RESULT AND DISCUSSION

Graph: 1 Simple Braking vs. Safer Braking (Both Brake Lever Pressed)

From practical reading and theoretical calculation it can be shown in graph for both brake lever pressed stopping distance for safer braking system (practical) is 9.48 m and stopping time is 2.08 sec. from theoretical calculation for safer braking system stopping distance is 11.55 m. From practical reading and theoretical calculation it can be shown in graph, for left brake lever pressed stopping distance for safer braking system(practical) is 11.68 m in 2.08 sec. For safer braking system (theoretical) stopping distance is 11.55 m. For simple braking (practical) stopping distance is 14.80 m in 2.56 sec. From practical reading and theoretical calculation, it can be shown in the graph that the right brake lever pressed stopping distance is for a safer braking system (practical) of 11.85m in 2.37 sec. The theoretical stopping distance for a safer braking system is 13.18 m. For simple braking (practical), the stopping distance is 16.04 in 3.01 sec.

CONCLUSION

After practically measurement we find that, it can be reduced approximately 4 – 4.5 meter stopping distance and reaction time reduced approximately 0.50 sec by Safer Braking System setup. So, our objective to reduce accidents by optimum use of the brake and accelerator is fulfilled.





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Table 1: Practically Measure Reading

<u> </u>								
	Stop	oing Dista	nce (m)	7				
	Both	left	Right	Both	Left	right		
Simple(theo)	14.35	16.72	14.22	2.58	3.01	2.56		
Simple(prac)	13.98	16.04	14.80	2.58	3.01	2.56		
Safer (theo)	11.55	11.55	13.18	2.08	2.08	2.37		
Safer (prac)	9.48	11.68	11.85	2.08	2.08	2.37		

Table 2. Comparison simple and safer braking

	Sin	nple brak	ing	Safer braking		
	Both	Left	Right	Both	Left	Right
Stopping	13.60	19.64	17.43	10.39	12.41	10.53
distance	14.33	16.12	12.55	8.49	10.83	13.98
(m)	14.02	12.36	14.43	9.56	11.69	11.05
Avg	13.98	16.04	14.80	9.48	11.64	11.85
	2.36	3.51	2.62	2.56	2.46	2.34
Time(s)	2.78	3.31	2.51	1.63	1.82	2.41
	2.60	2.23	2.56	2.05	1.96	2.38
Avg	2.58	3.01	2.56	2.08	2.08	2.37





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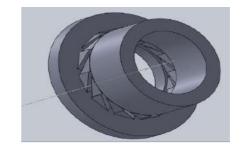
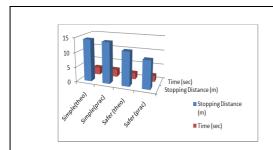


Figure -1 Coil spring attached with accelerator

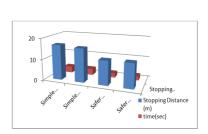
Figure 2: Coupling



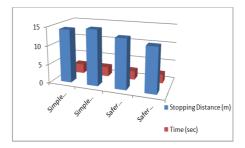
Figure 3: brake lever



Graph: 1 Simple Braking vs. Safer Braking (Both Brake Lever Pressed)



Graph: 2 Simple Braking vs. Safer Braking (Left Brake Lever Pressed)



Graph: 3 Simple Braking vs. Safer Braking (Right Brake Lever Pressed)





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RESEARCH ARTICLE

Neutrosophic Pre - Baire Spaces

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ABSTRACT

The purpose of this paper is to introduce Neutrosophic Pre - Baire Spaces and its characterizations are discussed. Examples are given to explain the concept in this paper.

Keywords: Neutrosophic pre-open set, Neutrosophic pre-dense set, Neutrosophic pre-nowhere dense set, Neutrosophic pre-first category, Neutrosophic pre-Baire spaces.

INTRODUCTION

The notion of fuzzy set introduced by L.A.Zadeh[1965], had degree of membership for each element[8]. The concept of fuzzy topological space was introduced and developed by C.L.Chang in 1968[2]. The notion of "intuitionistic fuzzy set" was introduced by K.Atanassov[1]. The concept of Neutrosophic set was introduced by F.Smarandache[5]. Neutrosophicset is a generalisation of Intuitionistic fuzzy set. A.A.Salama and S.A.Elbowi introduced the concept of Neutrosophic Topological Spaces[4]. The idea of Neutrosophic Baire spaces was introduced and its characterizations were studied by R.Dhavaseelan, et.al [3]. The idea of fuzzy pre-Baire space was defined by G.Thangaraj and S.Anjalmose[6]. The concept of Neutrosophic pre-open sets and pre-closed sets was studied by V.VenkateswaraRao and Y.Srinivasa Rao[7].

Preliminaries

In Neutrosophic Topological space, then non-empty set \mathcal{H} together with Neutrosophic topology \mathfrak{F} is denoted by $(\mathcal{H}, \mathfrak{I}).$





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Definition 1.1[3].

A neutrosophic topology on a nonempty set \mathcal{H} is a family \mathfrak{F} of $\mathcal{N}eu_S$ in \mathcal{H} satisfying the following axioms:

(i) $0_N, 1_N \in \mathfrak{J}$.

 $(ii)\mathcal{K}_1 \cap \mathcal{K}_2 \in \mathfrak{J}.$

(iii) $\bigcup \mathcal{K}_i \in \mathfrak{I}$ for arbitrary family $\{\mathcal{K}_i / i \in \Lambda\} \subseteq \mathfrak{I}$.

In this case the ordered pair $(\mathcal{H}, \mathfrak{F})$ is called a neutrosophic topological space (briefly $\mathcal{N}eu_{TS}$) and each $\mathcal{N}eu_{S}$ in \mathfrak{F} is called a neutrosophic open set (briefly $\mathcal{N}eu_{OS}$). The complement $\bar{\mathcal{C}}$ of a $\mathcal{N}eu_{OS}$ \mathcal{C} in \mathfrak{F} is called a neutrosophic closed set (briefly $\mathcal{N}eu_{CS}$).

Definition 1.2[3]

Let C be a Neu_S in $(\mathcal{H}, \mathfrak{F})$. Then

 $\mathcal{N}eu_{int}(\mathcal{C}) = \bigcup \{\mathcal{K}/\mathcal{K} \text{ is a } \mathcal{N}eu_{OS} \text{in } \mathcal{H} \text{and } \mathcal{K} \subseteq \mathcal{C}\}.$

 $\mathcal{N}eu_{cl}(\mathcal{C}) = \bigcap \{\mathcal{L}/\mathcal{L} \text{ is a } \mathcal{N}eu_{CS} \text{ in } \mathcal{H} \text{ and } \mathcal{L} \supseteq \mathcal{C}\}.$

It can also be shown that $\mathcal{N}eu_{int}(\mathcal{C})$ is a $\mathcal{N}eu_{\mathit{OS}}$ and $\mathcal{N}eu_{\mathit{cl}}(\mathcal{C})$ is $\mathcal{N}eu_{\mathit{cS}}$ in \mathcal{H} .

a) \mathcal{C} is a $\mathcal{N}eu_{OS}$ if and only if $\mathcal{C} = \mathcal{N}eu_{int}(\mathcal{C})$

b) \mathcal{C} is a $\mathcal{N}eu_{\mathcal{C}S}$ if and only if $\mathcal{C} = \mathcal{N}eu_{\mathcal{C}l}(\mathcal{C})$

Definition 1.3[3].

A $\mathcal{N}eu_S$ \mathcal{C} in a $\mathcal{N}eu_{TS}(\mathcal{H},\mathfrak{J})$ is called a *neutrosophic dense set*($\mathcal{N}eu_{DS}$) if there exists no $\mathcal{N}eu_{CS}\mathcal{D}$ in $(\mathcal{H},\mathfrak{J})$ such that $\mathcal{C} \subset \mathcal{D} \subset 1_N$.

Definition 1.4[3].

A $\mathcal{N}eu_S \mathcal{C}$ in a $\mathcal{N}eu_{TS}(\mathcal{H}, \mathfrak{F})$ is called *neutrosophic nowhere dense set*($\mathcal{N}eu_{NDS}$) if there exists no $\mathcal{N}eu_{OS} \mathcal{D}$ in $(\mathcal{H}, \mathfrak{F})$ such that $\mathcal{D} \subset \mathcal{N}eu_{cl}(\mathcal{C})$ that is $\mathcal{N}eu_{int}\mathcal{N}eu_{cl}(\mathcal{C}) = 0_N$.

Proposition 1.1.

If C is a $\mathcal{N}eu_{NDS}$ in $(\mathcal{H}, \mathfrak{F})$ then \bar{C} is a $\mathcal{N}eu_{DS}$ in $(\mathcal{H}, \mathfrak{F})$.

Definition 1.5[7].

A $\mathcal{N}eu_S\mathcal{C}$ in a $\mathcal{N}eu_{TS}$ (\mathcal{H} , \mathfrak{J}) is called $\mathcal{N}eutrosophic\ pre-openset(\mathcal{N}eu_{OS}^P)$ if $\mathcal{C} \subseteq \mathcal{N}eu_{int}\mathcal{N}eu_{cl}(\mathcal{C})$ and $\mathcal{N}eutrosophic\ Pre-closedset(\mathcal{N}eu_{OS}^P)$ if $\mathcal{N}eu_{cl}\mathcal{N}eu_{int}(\mathcal{C}) \subseteq \mathcal{C}$.

Definition 1.6.

If \mathcal{C} is a $\mathcal{N}eu_{\mathcal{S}}$ in $(\mathcal{H}, \mathfrak{F})$. Then

 $\mathcal{N}eu_{int}^{P}(\mathcal{C}) = \bigcup \{\mathcal{K}/\mathcal{K} \text{ is a } \mathcal{N}eu_{OS}^{P} \text{ in } \mathcal{H} \text{ and } \mathcal{K} \subseteq \mathcal{C} \}$

 $\mathcal{N}eu_{cl}^{P}(\mathcal{C}) = \bigcap \{\mathcal{M}/\mathcal{M} \text{ is a } \mathcal{N}eu_{cs}^{P} \text{ in } \mathcal{H} \text{ and } \mathcal{M} \supseteq \mathcal{C}\}$

Theorem 1.1.

In $(\mathcal{H}, \mathfrak{F})$. the following are valid.

- a) C is $\mathcal{N}eu_{OS}^{P}$ if and only if $\mathcal{N}eu_{int}^{P}(C) = C$.
- b) \mathcal{C} is $\mathcal{N}eu_{CS}^P$ if and only if $\mathcal{N}eu_{Cl}^P(\mathcal{C}) = \mathcal{C}$.

Theorem 1.2.

Let \mathcal{C} be a $\mathcal{N}eu_{\mathcal{S}}$ in $(\mathcal{H}, \mathfrak{F})$. Then,

- 1. $\mathcal{N}eu_{cl}^{P}(\mathcal{C}) \supseteq \mathcal{C} \cup \mathcal{N}eu_{cl}\mathcal{N}eu_{int}(\mathcal{C})$.
- 2. $\mathcal{N}eu_{int}^{P}(\mathcal{C}) \subseteq \mathcal{C} \cap \mathcal{N}eu_{int}\mathcal{N}eu_{cl}(\mathcal{C})$.

Proposition 1.2.

Let \mathcal{C} be a $\mathcal{N}eu_S$ in $(\mathcal{H}, \mathfrak{F})$.

Then $\mathcal{N}eu_{int}(\mathcal{C}) \subseteq \mathcal{N}eu_{int}^{P}(\mathcal{C}) \subseteq \mathcal{C} \subseteq \mathcal{N}eu_{cl}^{P}(\mathcal{C}) \subseteq \mathcal{N}eu_{cl}(\mathcal{C})$.





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2. Neutrosophic Pre –nowhere dense sets Definition: 2.1.

A $\mathcal{N}eu_{\mathcal{S}}\mathcal{C}$ in $(\mathcal{H},\mathfrak{J})$ is a neutrosophic pre - dense set $(\mathcal{N}eu_{\mathcal{D}\mathcal{S}}^{P})$ if there exists no $\mathcal{N}eu_{\mathcal{C}\mathcal{S}}^{P}\mathcal{D}$ in $(\mathcal{H},\mathfrak{J})$ such that $\mathcal{C} \subset \mathcal{D} \subset 1_{N}$. That is $\mathcal{N}eu_{\mathcal{C}\mathcal{S}}^{P}(\mathcal{C}) = 1_{N}$.

Example 2.1

Let $\mathcal{H} = \{u, v\}$. Define the neutrosophic set $\mathcal{C}_{i}\mathcal{D}$ as follows:

$$\begin{split} \mathcal{C} = & \langle \hbar, \left(\frac{u}{0.6}, \frac{v}{0.6}\right), \left(\frac{u}{0.6}, \frac{v}{0.5}\right), \left(\frac{u}{0.3}, \frac{v}{0.5}\right) \rangle \\ \mathcal{D} = & \langle \hbar, \left(\frac{u}{0.6}, \frac{v}{0.5}\right), \left(\frac{u}{0.6}, \frac{v}{0.5}\right), \left(\frac{u}{0.4}, \frac{v}{0.5}\right) \rangle \end{split}$$

Then $N_{\tau} = \{0_N, 1_N, C, D\}$. Thus $(\mathcal{H}, \mathfrak{J})$ is $a\mathcal{N}eu_{TS}$. C, D are $\mathcal{N}eu_{DS}^P$.

Definition 2.2

A $\mathcal{N}eu_{\mathcal{S}}\mathcal{C}$ in $(\mathcal{H},\mathfrak{F})$ is a neutrosophic pre-nowhere dense set($\mathcal{N}eu_{NDS}^{P}$) if there exists no non-zero $\mathcal{N}eu_{OS}^{P}\mathcal{D}$ in $(\mathcal{H},\mathfrak{F})$ such that $\mathcal{D} \subset \mathcal{N}eu_{cl}^{P}(\mathcal{C})$. That is $\mathcal{N}eu_{int}^{P}\mathcal{N}eu_{cl}^{P}(\mathcal{C}) = 0_{N}$.

Example2.2:

Let $\mathcal{H} = \{u, v\}$. Define the neutrosophic set \mathcal{C}, \mathcal{D} as follows:

$$\mathcal{C} = \langle \hbar, \left(\frac{u}{0.6}, \frac{v}{0.6}\right), \left(\frac{u}{0.6}, \frac{v}{0.5}\right), \left(\frac{u}{0.3}, \frac{v}{0.5}\right) \rangle$$

$$\mathcal{D} = \langle \hbar, \left(\frac{u}{0.6}, \frac{v}{0.5}\right), \left(\frac{u}{0.6}, \frac{v}{0.5}\right), \left(\frac{u}{0.4}, \frac{v}{0.5}\right) \rangle$$

Then $N_{\tau} = \{0_{N_{\tau}} 1_{N_{\tau}} C_{\tau} D\}$. Thus $(\mathcal{H}, \mathfrak{F})$ is a $\mathcal{N}eu_{TS}$. \overline{C}_{τ} \overline{D} are $\mathcal{N}eu_{NDS}^{P}$.

Proposition 2.1

If \mathcal{C} is a $\mathcal{N}eu_{NDS}$ in a $\mathcal{N}eu_{TS}(\mathcal{H}, \mathfrak{F})$, then $\mathcal{N}eu_{int}^{P}(\mathcal{C}) = 0_{N}$.

Proof:

Let \mathcal{C} be a $\mathcal{N}eu_{NDS}$ in $(\mathcal{H},\mathfrak{F})$. Then we have $\mathcal{N}eu_{int}\mathcal{N}eu_{cl}(\mathcal{C})=0_N$. $\mathcal{N}eu_{int}^P(\mathcal{C})\subseteq \mathcal{C}\cap \mathcal{N}eu_{int}\mathcal{N}eu_{cl}(\mathcal{C})$. Then $\mathcal{N}eu_{int}^P(\mathcal{C})\subseteq \mathcal{C}\cap 0_N=0_N$. That is $\mathcal{N}eu_{int}^P(\mathcal{C})=0_N$.

Proposition 2.2

If \mathcal{C} is a $\mathcal{N}eu_{NDS}$ in $(\mathcal{H}, \mathfrak{I})$, then $\mathcal{N}eu_{int}(\mathcal{N}eu_{cl}^{P}(\mathcal{C})) = 0_{N}$.

Proof:

Let \mathcal{C} be a $\mathcal{N}eu_{NDS}$ in $(\mathcal{H},\mathfrak{F})$. Then $\mathcal{N}eu_{int}\mathcal{N}eu_{cl}(\mathcal{C})=0_N$. By proposition 1.2 $\mathcal{N}eu_{int}(\mathcal{N}eu_{cl}^P(\mathcal{C}))\subseteq \mathcal{N}eu_{int}\mathcal{N}eu_{cl}(\mathcal{C})$. Then, $\mathcal{N}eu_{int}(\mathcal{N}eu_{cl}^P(\mathcal{C}))\subseteq 0_N$. That is $\mathcal{N}eu_{int}(\mathcal{N}eu_{cl}^P(\mathcal{C}))=0_N$.

Proposition2.3

If the $\mathcal{N}eu_S \mathcal{C}$ and \mathcal{D} are $\mathcal{N}eu^p_{NDS}$ in $(\mathcal{H}, \mathfrak{F})$, then $\mathcal{C} \cap \mathcal{D}$ is a $\mathcal{N}eu^p_{NDS}$ in $(\mathcal{H}, \mathfrak{F})$.

Proof:

Let the $\mathcal{N}eu_{S}$ \mathcal{C} and \mathcal{D} be $\mathcal{N}eu_{NDS}^{P}$ in $(\mathcal{H},\mathfrak{F})$. Now, $\mathcal{N}eu_{int}^{P}(\mathcal{N}eu_{cl}^{P}(\mathcal{C}\cap\mathcal{D}))\subseteq \mathcal{N}eu_{int}^{P}(\mathcal{N}eu_{cl}^{P}(\mathcal{C})\cap\mathcal{N}eu_{cl}^{P}(\mathcal{D}))\subseteq \mathcal{N}eu_{int}^{P}(\mathcal{N}eu_{cl}^{P}(\mathcal{C}))\cap \mathcal{N}eu_{int}^{P}(\mathcal{N}eu_{cl}^{P}(\mathcal{D}))\subseteq 0_{N}\cap 0_{N}=0_{N}.$ [Since $\mathcal{N}eu_{int}^{P}\mathcal{N}eu_{cl}^{P}(\mathcal{C})=0_{N}$ and $\mathcal{N}eu_{int}^{P}\mathcal{N}eu_{cl}^{P}(\mathcal{D})=0_{N}$]. That is $\mathcal{N}eu_{int}^{P}\mathcal{N}eu_{cl}^{P}(\mathcal{C}\cap\mathcal{D})=0_{N}.$ Hence, $\mathcal{C}\cap\mathcal{D}$ is a $\mathcal{N}eu_{NDS}^{P}$ in $(\mathcal{H},\mathfrak{F})$.





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Proposition 2.4

If \mathcal{C} is a $\mathcal{N}eu_{NDS}^{P}$ and \mathcal{D} is a $\mathcal{N}eu_{S}$ in $(\mathcal{H}, \mathfrak{F})$, then $\mathcal{C} \cap \mathcal{D}$ is a $\mathcal{N}eu_{NDS}^{P}$ in $(\mathcal{H}, \mathfrak{F})$.

Proof

Let \mathcal{C} be a $\mathcal{N}eu^{p}_{NDS}$ in $(\mathcal{H},\mathfrak{F})$. Then, $\mathcal{N}eu^{p}_{int}\mathcal{N}eu^{p}_{cl}(\mathcal{C})=0_{N}$. Now, $\mathcal{N}eu^{p}_{int}(\mathcal{N}eu^{p}_{cl}(\mathcal{C}\cap\mathcal{D}))\subseteq \mathcal{N}eu^{p}_{int}(\mathcal{N}eu^{p}_{cl}(\mathcal{C})\cap\mathcal{N}eu^{p}_{cl}(\mathcal{D}))\subseteq \mathcal{N}eu^{p}_{int}(\mathcal{N}eu^{p}_{cl}(\mathcal{C}))\cap \mathcal{N}eu^{p}_{int}(\mathcal{N}eu^{p}_{cl}(\mathcal{C}))\subseteq 0_{N}$. That is $\mathcal{N}eu^{p}_{int}\mathcal{N}eu^{p}_{cl}(\mathcal{C}\cap\mathcal{D})=0_{N}$. Hence, $\mathcal{C}\cap\mathcal{D}$ is a $\mathcal{N}eu^{p}_{NDS}$ in $(\mathcal{H},\mathfrak{F})$.

Proposition 2.5

If a $\mathcal{N}eu_{NDS}$ \mathcal{C} in a $\mathcal{N}eu_{TS}(\mathcal{H}, \mathfrak{F})$ is a $\mathcal{N}eu_{CS}^P$, then \mathcal{C} is a $\mathcal{N}eu_{NDS}^P$ in $(\mathcal{H}, \mathfrak{F})$.

Proof

Let \mathcal{C} be a $\mathcal{N}eu_{NDS}$ in $(\mathcal{H},\mathfrak{F})$. Then, $\mathcal{N}eu_{int}\mathcal{N}eu_{cl}(\mathcal{C})=0_N$. Then by proposition 2.1, we have $\mathcal{N}eu_{int}^P(\mathcal{C})=0_N$. Since, \mathcal{C} is a $\mathcal{N}eu_{CS}^P$ in $(\mathcal{H},\mathfrak{F})$, then $\mathcal{N}eu_{cl}^P(\mathcal{C})=\mathcal{C}$. Then $\mathcal{N}eu_{int}^P\mathcal{N}eu_{cl}^P(\mathcal{C})=0_N$. Hence, \mathcal{C} is a $\mathcal{N}eu_{NDS}^P$ set in $(\mathcal{H},\mathfrak{F})$.

Proposition 2.6

If a $\mathcal{N}eu_{NDS}^{P}\mathcal{C}$ in $(\mathcal{H}, \mathfrak{F})$ is a $\mathcal{N}eu_{cs}$, then \mathcal{C} is a $\mathcal{N}eu_{NDS}$ in $(\mathcal{H}, \mathfrak{F})$.

Proof

Let \mathcal{C} be a $\mathcal{N}eu^p_{NDS}$ in $(\mathcal{H},\mathfrak{J})$. Then, we have $\mathcal{N}eu^p_{int}\mathcal{N}eu^p_{cl}(\mathcal{C})=0_N$. Now, $\mathcal{C}\subseteq \mathcal{N}eu^p_{cl}(\mathcal{C})$ implies that $\mathcal{N}eu^p_{int}(\mathcal{C})\subseteq \mathcal{N}eu^p_{int}\mathcal{N}eu^p_{cl}(\mathcal{C})$. Then $\mathcal{N}eu^p_{int}(\mathcal{C})\subseteq 0_N$. Now, $\mathcal{N}eu^p_{int}(\mathcal{C})\subseteq \mathcal{N}eu^p_{int}(\mathcal{C})$, implies that $\mathcal{N}eu_{int}(\mathcal{C})\subseteq 0_N$. Now, $\mathcal{N}eu_{cl}(\mathcal{C})=\mathcal{C}$ [Since, \mathcal{C} is a $\mathcal{N}eu_{cl}(\mathcal{H},\mathfrak{J})$]. $\mathcal{N}eu_{int}(\mathcal{C})=0_N$ implies that $\mathcal{N}eu_{int}(\mathcal{C})=0_N$. Hence, \mathcal{C} is a $\mathcal{N}eu_{NDS}$ in $(\mathcal{H},\mathfrak{J})$.

Proposition 2.7

If \mathcal{C} is a $\mathcal{N}eu_{DS}^P$ and $\mathcal{N}eu_{OS}^P$ in $(\mathcal{H}, \mathfrak{J})$ and if $\mathcal{D} \subseteq 1 - \mathcal{C}$, then \mathcal{D} is a $\mathcal{N}eu_{NDS}^P$ in $(\mathcal{H}, \mathfrak{J})$.

Proof:

Let \mathcal{C} be a $\mathcal{N}eu^p_{DS}$ and $\mathcal{N}eu^p_{OS}$ in $(\mathcal{H},\mathfrak{F})$. Then, we have $\mathcal{N}eu^p_{cl}(\mathcal{C})=1_N$ and $\mathcal{N}eu^p_{int}(\mathcal{C})=\mathcal{C}$. Now, $\mathcal{D}\subseteq 1-\mathcal{C}$ implies that $\mathcal{N}eu^p_{cl}(1-\mathcal{C})$. Then, $\mathcal{N}eu^p_{cl}(\mathcal{D})\subseteq 1-\mathcal{N}eu^p_{int}(\mathcal{C})=1-\mathcal{C}$. Hence, $\mathcal{N}eu^p_{cl}(\mathcal{D})\subseteq 1-\mathcal{C}$. Then, $\mathcal{N}eu^p_{int}(\mathcal{D})\subseteq 1-\mathcal{N}eu^p_{int}(1-\mathcal{C})=1-\mathcal{N}eu^p_{cl}(\mathcal{C})=1-1=0$. That is, $\mathcal{N}eu_{int}\mathcal{N}eu_{cl}(\mathcal{D})=0_N$. Hence, \mathcal{D} is a $\mathcal{N}eu^p_{NDS}$ in $(\mathcal{H},\mathfrak{F})$.

Proposition 2.8

If \mathcal{C} is a $\mathcal{N}eu_{NDS}^P$ in $(\mathcal{H}, \mathfrak{J})$, then $1 - \mathcal{C}$ is a $\mathcal{N}eu_{DS}^P$ in $(\mathcal{H}, \mathfrak{J})$.

Proof

Let \mathcal{C} be a $\mathcal{N}eu^P_{NDS}$ in $(\mathcal{H},\mathfrak{F})$. Then $\mathcal{N}eu^P_{int}\mathcal{N}eu^P_{cl}(\mathcal{C})=0_N$. Now $\mathcal{C}\subseteq\mathcal{N}eu^P_{cl}(\mathcal{C})$ implies that $\mathcal{N}eu^P_{int}(\mathcal{C})\subseteq\mathcal{N}eu^P_{int}\mathcal{N}eu^P_{cl}(\mathcal{C})=0_N$. Then $\mathcal{N}eu^P_{int}(\mathcal{C})=0_N$ and $\mathcal{N}eu^P_{cl}(1-\mathcal{C})=1-\mathcal{N}eu^P_{int}(\mathcal{C})=1-0=1_N$ and hence $1-\mathcal{C}$ is a $\mathcal{N}eu^P_{DS}$ in $(\mathcal{H},\mathfrak{F})$.

Proposition 2.9

If \mathcal{C} is a $\mathcal{N}eu_{NDS}^{P}$ in $(\mathcal{H}, \mathfrak{F})$, then $1 - \mathcal{C}$ is a $\mathcal{N}eu_{DS}$ in $(\mathcal{H}, \mathfrak{F})$.





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Proof

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Let \mathcal{C} be a \mathcal{N}eu^P_{NDS} in (\mathcal{H},\mathfrak{F}). Then by proposition 2.8 1-\mathcal{C} is a \mathcal{N}eu^P_{DS} in (Z,N_\tau) and \mathcal{N}eu^P_{cl}(\mathcal{C})=1_N. Since \mathcal{N}eu^P_{cl}(1-\mathcal{C})\subseteq \mathcal{N}eu_{cl}(1-\mathcal{C}), we have 1_N\subseteq \mathcal{N}eu_{cl}(1-\mathcal{C}), That is, \mathcal{N}eu_{cl}(1-\mathcal{C})=1_N. Hence 1-\mathcal{C} is a \mathcal{N}eu_{DS} in (\mathcal{H},\mathfrak{F}).
```

Proposition 2.10

If \mathcal{C} is a $\mathcal{N}eu^P_{NDS}$ in $(\mathcal{H}, \mathfrak{F})$, then $\mathcal{N}eu^P_{Cl}(\mathcal{C})$ is also a $\mathcal{N}eu^P_{NDS}$ in $(\mathcal{H}, \mathfrak{F})$.

Proof.

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 \begin{array}{l} \operatorname{Let} \mathcal{C} \operatorname{bea} \mathcal{N}eu^P_{NDS} \operatorname{in} \left(\mathcal{H}, \mathfrak{J}\right). \operatorname{Then}, \mathcal{N}eu^P_{int} \mathcal{N}eu^P_{cl}(\mathcal{C}) = 0_N. \\ \operatorname{Now} \mathcal{N}eu^P_{cl} \mathcal{N}eu^P_{cl}(\mathcal{C}) = \mathcal{N}eu^P_{cl}(\mathcal{C}). \operatorname{Hence}, \mathcal{N}eu^P_{int} \left(\mathcal{N}eu^P_{cl}(\mathcal{N}eu^P_{cl}(\mathcal{C}))\right) = \\ \mathcal{N}eu^P_{int} \mathcal{N}eu^P_{cl}(\mathcal{C}) = 0_N. \operatorname{Therefore}, \mathcal{N}eu^P_{cl}(\mathcal{C}) = 0_N \operatorname{which is a} \mathcal{N}eu^P_{NDS} \operatorname{in} \left(\mathcal{H}, \mathfrak{J}\right). \end{array}
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Proposition 2.11

If \mathcal{C} is a $\mathcal{N}eu_{NDS}^{P}$ in $(\mathcal{H}, \mathfrak{F})$, then $1 - \mathcal{N}eu_{cl}^{P}(\mathcal{C})$ is also a $\mathcal{N}eu_{DS}^{P}$ in $(\mathcal{H}, \mathfrak{F})$.

Proof

Let \mathcal{C} be a $\mathcal{N}eu^P_{NDS}$ in $(\mathcal{H}, \mathfrak{J})$. Then by proposition 2.10 $\mathcal{N}eu^P_{cl}(\mathcal{C})$ is a $\mathcal{N}eu^P_{NDS}$ in $(\mathcal{H}, \mathfrak{J})$. Also by proposition 2.8, $1 - \mathcal{N}eu^P_{cl}(\mathcal{C})$ is also a $\mathcal{N}eu^P_{DS}$ in $(\mathcal{H}, \mathfrak{J})$.

Proposition 2.12

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Let \mathcal C be a \mathcal Neu^P_{NDS} in (\mathcal H, \mathfrak J). If \mathcal D is any \mathcal Neu_S in (\mathcal H, \mathfrak J), then \mathcal D is a \mathcal Neu^P_{NDS} in (\mathcal H, \mathfrak J), iff (\mathcal C \cap \mathcal D) is a \mathcal Neu^P_{NDS} in (\mathcal H, \mathfrak J).
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Proof

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Let \mathcal{D} be a \mathcal{N}eu^P_{NDS} in (\mathcal{H},\mathfrak{F}). Then, \mathcal{N}eu^P_{int}\mathcal{N}eu^P_{cl}(\mathcal{D}) = 0_N.

Now, \mathcal{N}eu^P_{int}\mathcal{N}eu^P_{cl}(\mathcal{C}\cap\mathcal{D})\subseteq \mathcal{N}eu^P_{int}(\mathcal{N}eu^P_{cl}(\mathcal{C})\cap \mathcal{N}eu^P_{cl}(\mathcal{D}))\subseteq \mathcal{N}eu^P_{int}(\mathcal{N}eu^P_{cl}(\mathcal{C}))\cap \mathcal{N}eu^P_{int}(\mathcal{N}eu^P_{cl}(\mathcal{C}))\cap \mathcal{N}eu^P_{int}(\mathcal{N}eu^P_{cl}(\mathcal{C}))\cap \mathcal{N}eu^P_{int}(\mathcal{N}eu^P_{cl}(\mathcal{C}))\cap \mathcal{N}eu^P_{int}(\mathcal{N}eu^P_{cl}(\mathcal{C}))\cap \mathcal{N}eu^P_{int}(\mathcal{N}eu^P_{cl}(\mathcal{C}))=0_N.

That is, \mathcal{N}eu^P_{int}(\mathcal{N}eu^P_{cl}(\mathcal{C}\cap\mathcal{D}))=0_N. Hence, (\mathcal{C}\cap\mathcal{D}) is a \mathcal{N}eu^P_{NDS} in (\mathcal{H},\mathfrak{F}).

Conversely, let \mathcal{C}\cap\mathcal{D} be a \mathcal{N}eu^P_{NDS} in (\mathcal{H},\mathfrak{F}). Then, \mathcal{N}eu^P_{int}(\mathcal{N}eu^P_{cl}(\mathcal{C}\cap\mathcal{D}))=0_N. Since, \mathcal{C} is a \mathcal{N}eu^P_{DS} in (\mathcal{H},\mathfrak{F}) then \mathcal{N}eu^P_{int}(\mathcal{N}eu^P_{cl}(\mathcal{C})\cap \mathcal{N}eu^P_{int}(\mathcal{N}eu^P_{cl}(\mathcal{C}))=0_N.

(i.e) , \mathcal{N}eu^P_{int}(1_N)\cap \mathcal{N}eu^P_{int}\mathcal{N}eu^P_{cl}(\mathcal{D})=0_N. (i.e) 1_N\cap \mathcal{N}eu^P_{int}\mathcal{N}eu^P_{cl}(\mathcal{D})=0_N.

Hence, \mathcal{N}eu^P_{int}\mathcal{N}eu^P_{cl}(\mathcal{D})=0_N. which means that \mathcal{D} is a \mathcal{N}eu^P_{NDS} in (\mathcal{H},\mathfrak{F}).
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Proposition 2.13

If \mathcal{C} is a $\mathcal{N}eu_{NDS}^{p}$ in $(\mathcal{H}, \mathfrak{F})$, then $\mathcal{N}eu_{int}^{p}(\mathcal{C}) = 0_{N}$.

Proof

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Let \mathcal{C} be a \mathcal{N}eu^{P}_{NDS} in (\mathcal{H}, \mathfrak{J}). Then, \mathcal{N}eu^{P}_{int}\mathcal{N}eu^{P}_{cl}(\mathcal{C}) = 0_{N}.
Now, \mathcal{C} \subseteq \mathcal{N}eu^{P}_{cl}(\mathcal{C}) implies that \mathcal{N}eu^{P}_{int}(\mathcal{C}) \subseteq \mathcal{N}eu^{P}_{int}\mathcal{N}eu^{P}_{cl}(\mathcal{C}). Hence \mathcal{N}eu^{P}_{int}(\mathcal{C}) \subseteq 0_{N}. That is, \mathcal{N}eu^{P}_{int}(\mathcal{C}) = 0_{N}.
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Proposition2.14

If \mathcal{C} is a $\mathcal{N}eu_{CS}^P$ in $(\mathcal{H}, \mathfrak{F})$ and if $\mathcal{N}eu_{int}^P(\mathcal{C}) = 0_N$, then \mathcal{C} is a $\mathcal{N}eu_{NDS}^P$ in $(\mathcal{H}, \mathfrak{F})$.

Proof

Let \mathcal{C} bea $\mathcal{N}eu^P_{CS}$ in $(\mathcal{H},\mathfrak{J})$. Then, we have $\mathcal{N}eu^P_{cl}(\mathcal{C})=\mathcal{C}$. Now, $\mathcal{N}eu^P_{int}\mathcal{N}eu^P_{cl}(\mathcal{C})=\mathcal{N}eu^P_{int}(\mathcal{C})$ and $\mathcal{N}eu^P_{int}(\mathcal{C})=0_N$. Therefore, $\mathcal{N}eu^P_{int}\mathcal{N}eu^P_{cl}(\mathcal{C})=0_N$. Hence, \mathcal{C} is a $\mathcal{N}eu^P_{NDS}$ in $(\mathcal{H},\mathfrak{J})$.





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3. Neutrosophic Pre—Baire space Definition3.1

Let $(\mathcal{H}, \mathfrak{F})$ be a $\mathcal{N}eu_{TS}$. A $\mathcal{N}eu_{S}$ \mathcal{C} in $(\mathcal{H}, \mathfrak{F})$ is called neutrosophic pre-first category set $(\mathcal{N}eu_{FCS}^{p})$ if $\mathcal{C} = \bigcup_{k=1}^{\infty} \mathcal{C}_{k}$, where \mathcal{C}_{k} 's are $\mathcal{N}eu_{NDS}^{p}$ in $(\mathcal{H}, \mathfrak{F})$. Anyother $\mathcal{N}eu_{S}(\mathcal{H}, \mathfrak{F})$ is called a neutrosophic pre-second category set $(\mathcal{N}eu_{FCS}^{p})$.

Definition 3.2

Let \mathcal{C} be a $\mathcal{N}eu^p_{FCS}$ in $(\mathcal{H}, \mathfrak{I})$. Then $1-\mathcal{C}$ is called a Neutrosophic pre-residual set $(\mathcal{N}eu^p_{RS})$.

Proposition 3.1

If \mathcal{C} is a $\mathcal{N}eu_{FCS}^P$ in $(\mathcal{H},\mathfrak{F})$, then $1-\mathcal{C}=\bigcap_{k=1}^\infty \mathcal{D}_k$, where $\mathcal{N}eu_{cl}^P(\mathcal{D}_k)=1_N$.

Proof

Let \mathcal{C} be a $\mathcal{N}eu^P_{FCS}$ in $(\mathcal{H},\mathfrak{J})$. Then, we have $\mathcal{C}=\bigcup_{k=1}^\infty \mathcal{C}_k$, where \mathcal{C}_k 's are $\mathcal{N}eu^P_{NDS}$ in $(\mathcal{H},\mathfrak{J})$. Now, $1-\mathcal{C}=1-\bigcup_{k=1}^\infty (\mathcal{C}_k)=\bigcap_{k=1}^\infty (1-\mathcal{C}_k)$. Let $\mathcal{D}_k=1-\mathcal{C}_k$. Then $1-\mathcal{C}=\bigcap_{k=1}^\infty (\mathcal{D}_k)$. Since, \mathcal{C}_k 's are $\mathcal{N}eu^P_{NDS}$ in $(\mathcal{H},\mathfrak{J})$. By proposition 2.8, we have $(1-\mathcal{C}_k)$'s are $\mathcal{N}eu^P_{DS}$ in $(\mathcal{H},\mathfrak{J})$. Hence, $\mathcal{N}eu^P_{cl}(\mathcal{D}_k)=\mathcal{N}eu^P_{cl}(1-\mathcal{C}_k)=1_N$. Therefore, we have $1-\mathcal{C}=\bigcap_{k=1}^\infty \mathcal{D}_k$, where $\mathcal{N}eu^P_{cl}(\mathcal{D}_k)=1_N$.

Definition 3.3

A $\mathcal{N}eu_{TS}(\mathcal{H}, \mathfrak{J})$ is called a Neutrosophic Pre-First Category Space, if the Neu.Set 1_X is a $\mathcal{N}eu_{FCS}^P$ in $(\mathcal{H}, \mathfrak{J})$. That is, $1_X = \bigcup_{k=1}^{\infty} \mathcal{C}_k$, where \mathcal{C}_k 's are $\mathcal{N}eu_{NDS}^P$ in $(\mathcal{H}, \mathfrak{J})$. Otherwise $(\mathcal{H}, \mathfrak{J})$ is a neutrosophic pre-second category space.

Example3.1:

Let \mathcal{H} ={u,v}. Define the neutrosophic set \mathcal{C} , \mathcal{D} on \mathcal{H} as follows:

$$C = \langle \hbar, \left(\frac{u}{0.7}, \frac{v}{0.4}\right), \left(\frac{u}{0.6}, \frac{v}{0.5}\right), \left(\frac{u}{0.4}, \frac{v}{0.5}\right) \rangle$$

$$D = \langle \hbar, \left(\frac{u}{0.6}, \frac{v}{0.6}\right), \left(\frac{u}{0.6}, \frac{v}{0.5}\right), \left(\frac{u}{0.4}, \frac{v}{0.5}\right) \rangle$$

Then $\mathcal{N}eu_T = \{0_N, 1_N, \mathcal{C}, \mathcal{D}, \mathcal{C} \cup \mathcal{D}, \mathcal{C} \cap \mathcal{D}\}$. Thus $(\mathcal{H}, \mathfrak{F})$ is a $\mathcal{N}eu_{TS}$ $.\bar{\mathcal{C}}$, $\overline{\mathcal{D}}$, $\overline{\mathcal{C}} \cup \overline{\mathcal{D}}$ are $\mathcal{N}eu^p_{NDS}$ and $[\bar{\mathcal{C}} \cup \overline{\mathcal{D}} \cup \overline{\mathcal{C}} \cap \mathcal{D}] = \overline{\mathcal{C}} \cap \overline{\mathcal{D}}$ is a $\mathcal{N}eu^p_{FCS}$.

Definition 3.4

Let $(\mathcal{H}, \mathfrak{J})$ be a $\mathcal{N}eu_{TS}$. Then $(\mathcal{H}, \mathfrak{J})$ is called a *neutrosophic pre-Baire Space* $(\mathcal{N}eu_{BS}^P)$, if $\mathcal{N}eu_{int}^P(\bigcup_{k=1}^\infty \mathcal{C}_k) = 0_N$, where \mathcal{C}_k 's are $\mathcal{N}eu_{NDS}^P$ in $(\mathcal{H}, \mathfrak{J})$.

Example 3.2

Let $\mathcal{H} = \{u, v\}$. Define the neutrosophic set \mathcal{C}, \mathcal{D} on \mathcal{H} as follows:

$$C = \langle h, \left(\frac{u}{0.7}, \frac{v}{0.4}\right), \left(\frac{u}{0.6}, \frac{v}{0.5}\right), \left(\frac{u}{0.4}, \frac{v}{0.5}\right) \rangle$$

$$D = \langle h, \left(\frac{u}{0.6}, \frac{v}{0.6}\right), \left(\frac{u}{0.6}, \frac{v}{0.5}\right), \left(\frac{u}{0.4}, \frac{v}{0.5}\right) \rangle$$

Then $\mathcal{N}eu_T = \{0_N, 1_N, \mathcal{C}, \mathcal{D}, \mathcal{C} \cup \mathcal{D}, \mathcal{C} \cap \mathcal{D}\}$. Thus $(\mathcal{H}, \mathfrak{F})$ is a $\mathcal{N}eu_{TS}$. $\overline{\mathcal{C}}, \overline{\mathcal{D}}, \overline{\mathcal{C}} \cup \overline{\mathcal{D}}$ are $\mathcal{N}eu_{NDS}^P$ and $[\overline{\mathcal{C}} \cup \overline{\mathcal{D}} \cup \overline{\mathcal{C}} \cup \overline{\mathcal{D}} \cup \overline{\mathcal{C}} \cap \overline{\mathcal{D}}] = \overline{\mathcal{C}} \cap \overline{\mathcal{D}}$ is a $\mathcal{N}eu_{FCS}^P$ and $\mathcal{N}eu_{int}^P(\overline{\mathcal{C}} \cap \overline{\mathcal{D}}) = 0_N$. Therefore, $(\mathcal{H}, \mathfrak{F})$ is a $\mathcal{N}eu_{BS}^P$.

Example3.3

Let $\mathcal{H} = \{u, v, w\}$. Define the neutrosophic set $\mathcal{C}, \mathcal{D}, \mathcal{E}, \mathcal{F}$ on \mathcal{H} as follows:

$$\begin{split} &\mathcal{C} \!=\! \langle \hbar, \left(\frac{u}{0.7}, \frac{v}{0.7}, \frac{w}{0.5}\right), \left(\frac{u}{0.7}, \frac{v}{0.7}, \frac{w}{0.5}\right), \left(\frac{u}{0.4}, \frac{v}{0.4}, \frac{w}{0.5}\right) \rangle \\ &\mathcal{D} \!=\! \langle \hbar, \left(\frac{u}{0.7}, \frac{v}{0.7}, \frac{w}{0.5}\right), \left(\frac{u}{0.7}, \frac{v}{0.7}, \frac{w}{0.6}\right), \left(\frac{u}{0.4}, \frac{v}{0.3}, \frac{w}{0.5}\right) \rangle \\ &\mathcal{E} \!=\! \langle \hbar, \left(\frac{u}{0.4}, \frac{v}{0.4}, \frac{w}{0.5}\right), \left(\frac{u}{0.4}, \frac{v}{0.4}, \frac{w}{0.4}\right), \left(\frac{u}{0.8}, \frac{v}{0.8}, \frac{w}{0.5}\right) \rangle \end{split}$$





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 $\mathcal{F} = \langle \text{\it h}, \left(\frac{u}{0.4}, \frac{v}{0.4}, \frac{u}{0.5}\right), \left(\frac{u}{0.4}, \frac{v}{0.4}, \frac{u}{0.4}\right), \left(\frac{u}{0.8}, \frac{v}{0.8}, \frac{u}{0.8}\right) \rangle$

Then $\mathcal{N}eu_T = \{0_N, 1_N, C, \mathcal{D}\}$. Thus $(\mathcal{H}, \mathfrak{J})$ is a $\mathcal{N}eu_{TS}$. $\bar{\mathcal{C}}$, $\bar{\mathcal{D}}$, \mathcal{E} , \mathcal{F} are $\mathcal{N}eu_{NDS}^P$ and also $\mathcal{N}eu_{int}^P[\bar{\mathcal{C}} \cup \bar{\mathcal{D}} \cup \mathcal{E} \cup \mathcal{F}] = 0_N$. Therefore, $(\mathcal{H}, \mathfrak{J})$ is a $\mathcal{N}eu_{BS}^P$.

Proposition 3.2

If $\mathcal{N}eu_{int}^P(\bigcup_{k=1}^\infty \mathcal{C}_k) = 0_N$, where $\mathcal{N}eu_{int}^P(\mathcal{C}_k) = 0_N$, and \mathcal{C}_k 's are $\mathcal{N}eu_{CS}^P$ in $(\mathcal{H}, \mathfrak{F})$, then $(\mathcal{H}, \mathfrak{F})$ is a $\mathcal{N}eu_{BS}^P$.

Proof

Let $\mathcal{C}_{\mathbf{k}}$'s be the $\mathcal{N}eu^P_{CS}$ in $(\mathcal{H},\mathfrak{J})$. Since $\mathcal{N}eu^P_{int}(\mathcal{C}_{\mathbf{k}})=0_N$, by proposition 2.13, $\mathcal{C}_{\mathbf{k}}$'s are $\mathcal{N}eu^P_{NDS}$ in $(\mathcal{H},\mathfrak{J})$. Therefore, we have $\mathcal{N}eu^P_{int}(\bigcup_{k=1}^\infty \mathcal{C}_k)=0_N$, where $\mathcal{C}_{\mathbf{k}}$'s are $\mathcal{N}eu^P_{NDS}$ in $(\mathcal{H},\mathfrak{J})$. Hence $(\mathcal{H},\mathfrak{J})$ is a $\mathcal{N}eu^P_{RS}$.

Proposition 3.3

If $\mathcal{N}eu_{cl}^{P}(\bigcap_{k=1}^{\infty}\mathcal{C}_{k})=1_{N_{+}}$ where \mathcal{C}_{k} 's are $\mathcal{N}eu_{DS}^{P}$ and $\mathcal{N}eu_{OS}^{P}$ in $(\mathcal{H},\mathfrak{F})$. Then $(\mathcal{H},\mathfrak{F})$ is a $\mathcal{N}eu_{BS}^{P}$.

Proof

Let $\mathcal{N}eu^P_{cl}(\cap_{k=1}^\infty \mathcal{C}_k) = 1_N$, where \mathcal{C}_k 's are $\mathcal{N}eu^P_{DS}$ and $\mathcal{N}eu^P_{OS}$ in $(\mathcal{H},\mathfrak{F})$. Now $1 - \mathcal{N}eu^P_{cl}(\cap_{k=1}^\infty \mathcal{C}_k) = 1 - 1 = 0_N$. Then, we have $\mathcal{N}eu^P_{int}(1 - \cap_{k=1}^\infty \mathcal{C}_k) = 0_N$ in $(\mathcal{H},\mathfrak{F})$. This implies that $\mathcal{N}eu^P_{int}(\bigcup_{k=1}^\infty (1 - \mathcal{C}_k)) = 0_N$. Since \mathcal{C}_k 's are $\mathcal{N}eu^P_{DS}$ in $(\mathcal{H},\mathfrak{F})$, $\mathcal{N}eu^P_{int}(\mathcal{C}_k) = 1_N$ and $\mathcal{N}eu^P_{int}(1 - \mathcal{C}_k) = 1 - \mathcal{N}eu^P_{cl}(\mathcal{C}_k) = 1 - 1 = 0_N$ and $(1 - \mathcal{C}_k)$'s are $\mathcal{N}eu^P_{CS}$ in $(\mathcal{H},\mathfrak{F})$. Then by proposition 3.2, $(\mathcal{H},\mathfrak{F})$ is a $\mathcal{N}eu^P_{BS}$.

Proposition 3.4

Let $(\mathcal{H}, \mathfrak{F})$ be a $\mathcal{N}eu_{TS}$. Then the following results are equivalent.

- (1) $(\mathcal{H}, \mathfrak{J})$ is a $\mathcal{N}eu_{BS}^{P}$.
- $(2)\mathcal{N}eu_{int}^{P}(\mathcal{C}) = 0_{N}$, for every $\mathcal{N}eu_{FCS}^{P}$ in $(\mathcal{H}, \mathfrak{F})$.
- (3) $\mathcal{N}eu_{cl}^{P}(\mathcal{D}) = 1_{N_{1}}$ for every $\mathcal{N}eu_{RS}^{P}\mathcal{D}$ in $(\mathcal{H}, \mathfrak{J})$.

Proof

(1) \Rightarrow (2),Let \mathcal{C} be a $\mathcal{N}eu^P_{FCS}$ in $(\mathcal{H},\mathfrak{F})$. Then, $\mathcal{C}=\bigcup_{k=1}^{\infty}\mathcal{C}_k$, where \mathcal{C}_k 's are $\mathcal{N}eu^P_{NDS}$ in $(\mathcal{H},\mathfrak{F})$. Now $\mathcal{N}eu^P_{int}(\mathcal{C})=\mathcal{N}eu^P_{int}(\bigcup_{k=1}^{\infty}\mathcal{C}_k)=0_N$. (since $(\mathcal{H},\mathfrak{F})$) is a $\mathcal{N}eu^P_{BS}$.). Therefore, $\mathcal{N}eu^P_{int}(\mathcal{C})=0_N$ in $(\mathcal{H},\mathfrak{F})$.

(2) \Rightarrow (3), Let \mathcal{D} be a $\mathcal{N}eu^P_{RS}$ in $(\mathcal{H},\mathfrak{F})$. Then $1-\mathcal{D}$ is a $\mathcal{N}eu^P_{FCS}$ in $(\mathcal{H},\mathfrak{F})$. By hypothesis, $\mathcal{N}eu^P_{int}(1-\mathcal{D})=0_N$ in $(\mathcal{H},\mathfrak{F})$. This implies that $1-\mathcal{N}eu^P_{cl}(\mathcal{D})=0_N$ and hence $\mathcal{N}eu^P_{cl}(\mathcal{D})=1_N$ in $(\mathcal{H},\mathfrak{F})$.

Proposition 3.5

If $(\mathcal{H}, \mathfrak{F})$ is a $\mathcal{N}eu_{RS}^p$ and if every $\mathcal{N}eu_{NDS}^p$ \mathcal{C} in $(\mathcal{H}, \mathfrak{F})$ is a $\mathcal{N}eu_{CS}$ in $(\mathcal{H}, \mathfrak{F})$, then $(\mathcal{H}, \mathfrak{F})$ is a $\mathcal{N}eu_{RS}$.

Proof

Let $(\mathcal{H},\mathfrak{J})$ be a $\mathcal{N}eu^P_{BS}$ such that every $\mathcal{N}eu^P_{NDS}$ in $(\mathcal{H},\mathfrak{J})$ is a $\mathcal{N}eu_{CS}$ in $(\mathcal{H},\mathfrak{J})$. Since, $(\mathcal{H},\mathfrak{J})$ is a $\mathcal{N}eu^P_{BS}$ then $\mathcal{N}eu^P_{int}(\bigcup_{k=1}^\infty \mathcal{C}_k)=0_N$, where \mathcal{C}_k 's are $\mathcal{N}eu^P_{NDS}$ in $(\mathcal{H},\mathfrak{J})$. Since the $\mathcal{N}eu^P_{NDS}$ \mathcal{C}_k 's in $(\mathcal{H},\mathfrak{J})$ are $\mathcal{N}eu_{CS}$ in $(\mathcal{H},\mathfrak{J})$, by proposition 2.6, \mathcal{C}_k 's are $\mathcal{N}eu_{NDS}$ in $(\mathcal{H},\mathfrak{J})$. Now, $\mathcal{N}eu_{int}(\bigcup_{k=1}^\infty \mathcal{C}_k)\subseteq \mathcal{N}eu^P_{int}(\bigcup_{k=1}^\infty \mathcal{C}_k)$ and $\mathcal{N}eu^P_{int}(\bigcup_{k=1}^\infty \mathcal{C}_k)=0_N$, implies that $\mathcal{N}eu_{int}(\bigcup_{k=1}^\infty \mathcal{C}_k)=0_N$ in $(\mathcal{H},\mathfrak{J})$. Thus $\mathcal{N}eu_{int}(\bigcup_{k=1}^\infty \mathcal{C}_k)=0_N$, where \mathcal{C}_k 's are $\mathcal{N}eu_{NDS}$ in $(\mathcal{H},\mathfrak{J})$ implies that $(\mathcal{H},\mathfrak{J})$ is a $\mathcal{N}eu_{BS}$.





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CONCLUSION

In this paper, we introduced the concept of neutrosophic pre-baire spaces and discussed some of its characterizations using examples. This shall be extended in the future research with some applications.

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RESEARCH ARTICLE

Depression, Anxiety and Stress among Physiotherapy Students of Surat **District - A Questionnaire-Based Survey**

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ABSTRACT

Stress, Anxiety and Depression are unavoidable in Physiotherapy Education, so it becomes essential to have an idea about presence of stress, anxiety and depression among Physiotherapy students. The objectives of the present study were to find out severity of stress, anxiety and depression among physiotherapy students of surat, Gujarat. For the present study 480 undergraduate physiotherapy students from various Physiotherapy colleges in Surat district, who have been volunteered to participate for the study were recruited. Questionnaire based survey study was done, where participants were givenDASS21questionnaire and the aim of the study was explained to them. Duly completed questionnaire was taken away for further research study purpose. Among all of total 480 students; Depression 62(12.92%) were reported under normal category, 349(72.71%) of the min mild, 53(11.04%) in moderate level, 4(0.83%) in severe category and 2 (0.42%) had fallen in the extremely severe category. The Anxiety scale reported 63(13.13%)in normal,336(70%)in mild,59(12.29%)in moderate level, 13(2.71%)in severe and 9(1.88%) of them have been under extremely severe category. The study reported 56(11.67%) normal cases for Stress scale, 338(70.42%) were in mild,63(13.13%) in moderate, 14(2.92%) in severe and 9 (1.88%) case in extremely severe score. This study concludes that majority of the students fall under mild and moderate category of stress, anxiety and depression and few under severe and very severe, which suggests the urgent needs to carryout psychological health related interventions to control this growing issue among Physiotherapy students.

Keywords: Stress, Anxiety, Depression, DASS 21, physiotherapy students, undergraduate





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INTRODUCTION

There are 12 Physiotherapy colleges in Surat district, Gujarat with good number of intake capacity for Physiotherapy students per annum. The course duration is of 4 years with six months of compulsory rotatory internship to urge the best knowledge and acquire skill. These four and half year may be stressful for college going students to successfully complete the course and realize their dream. Any quite change that puts physical, mental, or psychological strain on an individual is taken into account to be stress. The body's reaction to anything that demands focus or action is stress. Everyone goes through periods of stress. However, how an individual handle stress incorporates a significant impact on his/her general wellbeing. Fear, worry, difficulty in relaxing, increased heart rate, breathing problems, disruption of sleep, change in eating habits, difficulty concentrating, worsening of pre-existing health conditions (physical and mental), and increasing use of alcohol, tobacco, and other substances are all signs of stress[1]. Anxiety may be a feeling that's amid tense feelings, concerned thoughts, and bodily changes like elevated pressure. Typically, intrusive thoughts or worries repeat throughout the lives of these with anxiety disorders. They might stand back from specific circumstances out of fear. They may even have physical side effects as sweating, shaking, nausea, or an accelerated heartbeat. Fear could be a proper, in the moment reaction to a clearly recognized and precise threat, but anxiety could be a long-lasting, broadly focused, future-oriented response to a diffuse threat[2]. Depression may be a prevalent psychopathy. In step with estimates, 5% of adults worldwide experience depression.

The biggest explanation for disability within the world today is depression, which also significantly contributes to the burden of sickness on the earth. Depression affects more women than males. Suicide may end up from depression. Depression is effectively treated in the least stages—mild, moderate, and severe[3]. Depression has afflicted mankind ever since its inception. It's attracted public interest rather recently[4]. WHO (World Health Organization) rates depression to be the world's fourth most disabling health problems[5]. WHO has projected that emotional disorder are ranked as second most disabling disorders, unless appropriate measures are taken for early diagnosis, prompt and effective treatment and prevention of depression. As per the National Depressive and Manic-Depressive Association, depression is an under treated disorder[6]. Untreated depression leads to high morbidity and mortality. Proper diagnosis and treatment cannot be over emphasized. Physiotherapy Students are Exposed to stressors across clinical practice due to multiple reasons[8], constant stress may affect academic performance of students negatively[9]. College students experience high stress levels at predictable times each semester due to academic commitments, financial pressures, lack of time management skills, high parental expectations, staying in hostels, peer pressure, vastness of syllabus and lack of facilities for entertainment. When stress is perceived negatively or becomes excessive, students experience physical and psychological impairments[10]. There is a lack of knowledge about depression, anxiety, and stress among undergraduate physiotherapy students in Surat's various physiotherapy colleges. The current study's objective was to determine the severity of stress, anxiety, and depression among physiotherapy students.

METHODOLOGY

A questionnaire-based survey was conducted among students of physiotherapy in various physiotherapy colleges in Surat district, Gujarat. The self-administered DASS21 questionnaire[11] was administered. There are 21 questions in the DASS-21 questionnaire, and it is a set of three self-reported scales designed to measure the emotional states of depression, anxiety, and stress. Each of the three DASS21 scale contains 7 items, divided into subscales with similar content. The DASS 21 rating scale is as follows: where 0=did not apply to me at all. 1 = applied to me to some extent or at another time, 2 = applied to me to a significant extent or for a significant portion of the time, 3 = very much applied to me or for the majority of the time. The questionnaire was used to evaluate depression, anxiety, and stress in undergraduate physiotherapy students and interns at various physiotherapy colleges in the Surat district. The questionnaire was distributed among all the students for their response. Informed consent and demographic details were taken from all the subjects. Ethical clearance was obtained from the ethical committee of Nirmal's Children's Hospital, Surat. All the responses were analysed in Excel. The participants included were as follows: (i) Age group





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between 18–23 years old (ii) both male and female students (iii) students from Surat District's various physiotherapy colleges The exclusion criteria were as follows: (i) students who are not studying physiotherapy (ii) students who are doing postgraduate studies (iii) students who are unwilling to participate. In the classroom, a briefing on the study was held. In the Surat district of Gujarat, 480 physiotherapy students from several physiotherapy institutes participated in a survey. Data was gathered at the beginning of the academic year from B.P.T. interns and all four-year students at these colleges. The study's purpose was explained to the participating students, who were also assured of the confidentiality of their information and its safety. The students were given a DASS21 Questionnaire, which was returned for analysis once it had been properly completed by the participants. Baseline scores were gathered when everyone was at ease and there were no exams to avoid exam related bias.

RESULTS

A total of 480 Physiotherapy students (432females & 32 males) participated in this study forming a response rate of 92.31%. Among the respondents 100 were first BPT students (20.83%), 98 was second BPT students (20.42%), 100 were third BPT students (20.83%), 17.08% (82) were in terns. Out of 480 physiotherapy students: 398 were going through their academics while rest 82 of them were managing their new experiences according to internship schedule. Among all of total 480 students; Depression 62 (12.92%) were reported under normal category,349 (72.71%) of the min mild ,53 (11.04%) in moderate level, 4 (0.83%) in severe category and 2 (0.42%) had fallen in the extremely severe category (Table 2). The Anxiety scale reported 63 (13.13%) in normal, 336 (70%) in mild, 59 (12.29%) in moderate level, 13(2.71%) in severe and 9 (1.88%) of them have been under extremely severe category. The study reported 56 (11.67%) normal cases for Stress scale, 338 (70.42%) were in mild, 63 (13.13%) in moderate, 14 (2.92%) in severe and 9 (1.88%) case in extremely severe score (Graph1).

DISCUSSION

This survey provided information regarding stress, anxiety and depression among physiotherapy students. In this study 480 undergraduates and interns from various physiotherapy colleges in Surat were included. The findings of this study revealed that 349 subjects (72.71%) were under mild depression. Which depicts they might have mild symptoms as well.53 subjects (11.04%) were in a moderate level ,4(0.83%) in severe category and 2(0.42%) had fallen in the extremely severe category. In the present study, for anxiety component 336 Subjects (70%) in mild ,59 subjects (12.29%) in moderate ,13 subjects (2.71%) in severe and 9 (1.88%) in extremely severe category. For the stress 338 (70.42%) were in mild ,63(13.13%) in moderate and 14 (2.92%) in severe and 9 (1.88%) case in extremely severe cases. So according to the finding of this study institution should find the causative factors for the anxiety, stress and depression. Common interventions like change in curriculum like include relaxation technique in syllabus. Frequent screening to find out occurrence and advised the students in mild category to practice relaxation or any stress management exercises and/or techniques. This study was the first to assess stress, anxiety and depression among Physiotherapy students in various colleges of Surat, Gujarat. Through this study future researchers can get benefit by finding the reason for the present finding and can find out effect of different interventions and/or techniques to reduce stress, anxiety or depression. Apart from this, there are certain limitations of this study like could have included a greater number of samples.

CONCLUSION

After analyzing DASS 21, which were collected from various physiotherapy colleges of surat district, data indicates that maximum number of students are in mild, moderate category of stress, anxiety and depression irrespective of their year of study or internship. Consequently, it is proposed that this Physiotherapy institutes could take appropriate steps for prevention of Stress, anxiety, depression and its related problems among the Physiotherapy Students.





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Table 1: Baseline scores

Severity	Depression	Anxiety	Stress
Normal	0-9	0-7	0-14
Mild	10-13	8-9	15-18
Moderate	14-20	10-14	19-25
Severe	21-27	15-19	26-33
Extremely severe	28+	20+	34+

Table 2: Severity Distribution of DASS21 Scoring among Physiotherapy Students.

Sub Scale		Normal	Mild	Moderate	Severe	Extremely severe
	Final year	14	64	16	4	2
	Third Year	11	70	13	4	2
	Second year	10	76	10	1	1
Depression	First year	15	79	6	0	0
	Internship	12	60	8	1	1
	Final year	12	62	20	3	3
	Third Year	14	66	14	4	2





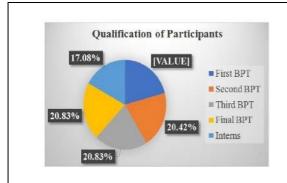
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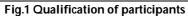
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Anxiety	Second Year	12	71	10	3	2
	First Year	15	75	8	1	1
	Internship	10	62	7	2	1
	Final Year	12	64	17	4	3
	Third Year	10	71	15	4	0
	Second Year	10	70	13	2	3
Stress	First Year	14	71	11	2	2
	Internship	10	62	7	2	1





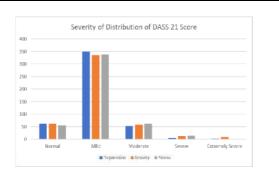


Fig 2: Severity of distribution of DASS 21 score.
Shows Severity of DASS21 Scoring among
undergraduate and interns of Physiotherapy
Students (n=480).N=Number of undergraduate and
interns of Physiotherapy Students.





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RESEARCH ARTICLE

A Preliminary Screening on Biodegradability of Face Mask and Their Impact on Plant Growth of Hibiscus sabdariffa, Andhra Pradesh, India

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ABSTRACT

The outbreak of the COVID-19 pandemic led to a tremendous increase in the production of facemasks across the world. Face masks are for monitoring origins to avoid transmission from infected persons. The purpose of the facemask is like air filters that protect air born infectious pathogens. Filtering facemasks are either pharmacologically or non-pharmacologically used. There are many types of facemasks that are in use nowadays. They include surgical masks, N95, and cloth masks which have three-layered structures. The primary raw materials for the manufacturing of the surgical and N95 facemasks are nonbiodegradable synthetic polymers made of polypropylene fibres. Disposal of these synthetic facemasks is increase solid waste load in the environment causing damage to natural flora and fauna. The present study aimed to analyse the biodegradability of two different kinds of face masks by using three different soil types to know the natural degradation of facemasks and also by the artificial pure microbial plate method. The degradation of 2 different face masks was not observed in both the mentioned methods during the study period from the Month of March 2022 to the end of August 2022. But a good sign in our identification that there is quick biodegradability when it was converted into ash form. The time of biodegradability was about 1-2days/ 3gms of ash in both the natural and artificial methods. The use of reusable cloth masks is recommended to avoid soil pollution. The present study concluded that cloth masks have more potential degradation capability when compared with surgical masks within a short period of time. Further research should focus on assessing the soil profile status, and efficacy of cloth





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masks and surgical masks when dumped in the soil directly. The present literature review aims to evaluate these determinants and provide a framework for future interventions directed at increasing facemask usage as an effective public health measure to curb airborne infectious disease outbreaks.

Keywords: Covid-19, Face masks, Biodegradability, polymers, and Ash

INTRODUCTION

Epidemiological investigations have helped quantify the benefit of mask-wearing to prevent the spread of COVID-19. Face masks are the ubiquitous symbol of a pandemic that has sickened 35 million people and killed more than 1 million. Wearing a mask can become uncomfortable, particularly for long periods in warm environments, and covering the nose and mouth may inhibit verbal and nonverbal communication. Various varieties of masks are in use by the public. Droplets can shoot through the air and land on a nearby person's eyes, nose, or mouth to cause infection. Aerosols, by contrast, can float in the air for minutes to hours, spreading through an unventilated room like cigarette smoke. As a great alternative, homemade fabric masks have become very popular in several affected countries, mainly in Brazil [2]. Pharmaceutical and non pharmaceutical measures against respiratory infections are available. Pharmaceuticals such as vaccines and antiviral medications are highly effective in eradicating respiratory infections, as evidenced in the case of smallpox. Aside from hand washing, the use of facemasks is also valuable in infectious disease control, especially in circumventing droplet transmission [3]. Increased usage of masks led to increased dumping of masks everywhere, littering our environment and polluting the soil. Moreover, the plastic fragments or microplastics derived from masks can further cause many other environmental problems. Microplastics (less than 5 mm) even nano-size plastic particles (less than 100 nm) are widely present in marine [1], sediments [8], soil [5], [9] freshwater [7], [6] the atmosphere [12] and other environmental matrices [6], [10], [11]. The arrival of COVID-19brought a wide range of masks made of different fabrics and materials into the market. Some of the most common masks used in India were cloth masks, N95 masks, surgical masks, FFP1 masks, and activated carbon masks. A cloth mask is made from several layers of tightly woven fabric and fits well over your nose and mouth to be an effective filter. In non-healthcare settings, multiple-layer fabric cloth masks are excellent barriers for containing respiratory droplets and interrupting viral transmission if they are worn consistently and properly, covering the nose and mouth. However, the explosive growth in the use of masks has introduced numerous issues related to the solid waste management

MATERIAL AND METHODS

Processing of facemasks

Both surgical and cloth masks were purchased from a nearby medical store. The elastic ear loop and nose bridge were first removed from the surgical mask and the rest of the mask was then cut into strips (1cm × 1cm) whereas cloth masks were directly made into small pieces (1cm x 1cm). The mask strips had an outer layer, middle layer, and inner layer which were submitted for biodegradation using natural and artificial methods. Fig:1

Biodegradation by Natural method:

Three different soil types were taken into Petri plates from natural environment. Some pieces of surgical and cloth masks were placed in the Petri plates by using sterile forceps in the presence of three different types of soil samples (20 g) separately. The types of soil samples taken for the study were concrete sand, moisture red soil, and dry soil. Fig:2

Biodegradation by Artificial pure microbial method

Nutrient and potato dextrose broths were prepared separately under sterile condition. The microbial cultures used for this study were 2 bacterial *E. coli*, *Staphylococcus aureus*, and 2 fungal cells *Aspergillus niger* and *Trichoderma*. Each





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type of mask piece was placed into the Erlenmeyer flask containing microbial broths using sterile forceps and incubated for study on the artificial degradation of masks. The flasks were placed in a NEO- LAB incubator shaker (CAT: 051/263- NEO-LAB instruments Mumbai) with 300 rpm and 37 °C for 24 h. Fig:3

Conversion of mask into ash form

Cloth masks were taken without making it into small pieces and submitted to direct incineration to convert them into ash form. The direct incineration of surgical masks and the indirect method of processing of surgical masks before incineration were performed. The processing of the surgical mask was done by soaking the mask in a bleaching solution for 3-4days. After this, the soaked mask material was carefully taken and dried. Finally, dried mask material was submitted for incineration. Fig: 4,5&6

Effect of mask on plant growth

Both mask pieces and ash form of two different kinds of masks were used to check their effect on plant growth. The leafy vegetable selected for this study was *Hibiscus sabdariffa*. Three different pots were taken, and soils in the first two pots were mixed with small mask pieces and ash form of the mask respectively along with a control pot without any mask products. The 6-8 seeds of *Hibiscus sabdariffa* were planted into the three pots and observed for their germination and measured the length of plants during the study period of 2weeks. Fig:7

RESULTS

Biodegradation of both the masks were not observed in both natural and artificial methods. The mask pieces remained as it is during the study period from March 2022 to August 2022. The processing of surgical masks in bleaching solution made the mask lose its polysynthetic nature where it got converted into ash form easily when compared with the direct incineration of surgical masks. Direct incineration of the mask is also one of the good practices, but it won't lose its polysynthetic nature and can disrupt the soil flora and fauna. The effect of the mask pieces on the plant growth was observed where the growth was slow when compared with ash-mixed soil plant. The growth parameters are mentioned in Table:1. The maximum plant growth rate was observed in ash-mixed soil of 15 cm where the minimum growth rate was 5cm during the study period. Pathogenic microorganisms present on the used mask will be killed up on processing the surgical mask in bleaching solution.

DISCUSSION

A surgical mask was used mainly after 1960 in different countries. These masks are prepared from nonwoven fabrics through the melt-blowing process. Surgical masks have three layers; the outer layer was made from nonwoven fabric. As a great alternative, homemade fabric masks have become very popular in several affected countries, mainly in Brazil [2]. Through our research work, we found that the masks i.e. both synthetic and surgical masks could not be degraded either in soil or in artificial media in a period of 5 months. If small mask pieces are not remaining as it is without any degradation, then amount of time taken for a normal mask dumped in soil to get degraded becomes a matter of great concern. Direct dumping of masks could also pollute soil and thereby cause environmental pollution. Microplastics (less than 5 mm) even Nano-size plastic particles (less than 100 nm) are widely present in marine [1], sediments [8], soil [5], [9] freshwater [7], [6] the atmosphere [12] and other environmental matrices [6], [10], [11]. The present study has suggested burning synthetic masks before their disposal is beneficial as it turns into Ash which is a good soil fertilizer for plants. But, masks should be processed in bleaching powder before incineration for better result. When these were crushed using mortar and pestle turned into a powder form. Natural cellulosic fibers like cotton mask has good degradation potentiality when compared with surgical masks. Processing the mask in bleaching solution will make the used surgical mask free from pathogenic microorganism that are present on it. Direct dumping of used masks may pollute the natural hygienic surrounding soil environment. So, It's better to process the used masks before their disposal into the soil. Further research should be done to know the interaction between mask material and soil microorganisms.





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Table 1: The growth parameters are mentioned

S. No	SOIL TYPE	GROWTH RATE (cm)
1	Control soil	13cm
2	Soil with mask ash	15cm
3	Soil with mask pieces	5cm



Fig-1: Processing of surgical Mask

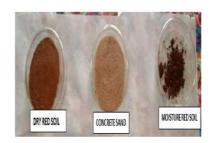


Fig-2: Biodegradation of mask using 3 natural soil types





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Fig-3: Biodegradation of mask using artificial microbial method







Fig-5: Grinding of surgical mask pellet to powder form



Fig-6: Processing the surgical mask to make into ash form



Fig:7: Effect of mask on plant growth of *Hibiscus* sabdariffa





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ISSN: 0976 – 0997 **RESEARCH ARTICLE**

Teacher Educators' Attitude towards using New Technology and the Application of Digital Technologies in Classroom Instruction

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ABSTRACT

The study aims to investigate the teacher educators' attitude towards using new technology and the application of digital technologies in classroom instruction. Survey method of research has been used in the present study. For the purpose of this study teacher educators' attitude towards using new technology scale and the application of digital technologies in classroom instruction scale was constructed and standardized by the Researchers. The investigator randomly selected two hundred and fifty two teacher educators in and around Trichy, Thanjavur, Namakkal, Karur and Ariyalur Districts of Tamil Nadu. The data was analyzed using mean, standard deviation, 't'- test, F-test and Duncan Multiple Range Test. The result of the study reveals that there is no significant difference exists in the attitude towards using new technology and their application of digital technology in classroom instruction among teacher educators except in the dimensions of using hardware and e-content in the classroom instruction with reference gender. The finding of the study reveals that there is a significant difference in the application of digital technology in classroom instruction and its dimensions (VAI, CAI, WBI, Online/Offline, Hardware and E-content)among teacher educators and also the study reveals that there is no significant difference in the attitude towards using new technology among teacher educators with respect to age.





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Keywords: Teacher Educators' Attitude, E-content, New Technology, Application of Digital Technologies, Classroom Instruction, Digital Technology, Teacher Educators, Prospective Teacher Educators, Web Based Instruction, ICT, Digital Resources.

INTRODUCTION

Teacher Education is an important professional course of study where the future teachers and teacher educators are trained adequately in order to accomplish the educational aims and objectives of school education. The world now, has innumerable advancement in all areas of life such as industry, agriculture, transport and communication, army, medicine, engineering, governance, administration and education etc. The system of education is acting as the soul for the development of the nation. The classroom is holding the base for all developments of the nation. The curriculum is the centrifugal point of the quality of education. Today the world approaches towards digital revolution and learners have become digital natives and the teacher's role becomes a facilitator, instructor and director to their students in their search for knowledge. The transformation of the society is possible when learners are exposed to a new platform for learning which offers opportunities to pursue knowledge unlimited, independent and proceed at their own interest and pace and also which fosters creative thinking and critical enquiry among the students. Mishra, Koehler & Henriksen, (2011) digital technologies have revolutionized practically every aspect of our lives and work, 'we live in an exponential time'.

Review of Literature

Fatima Islahi&Nasrin(2019) examined the Exploring Teacher Attitude towards Information Technology with a Gender Perspective. Results reveal that there is no gender specific differences in attitude towards information technology were found with regard to different factors viz., training, location of schools, medium of instruction and marital status. The study concluded that the effective use of technology in classrooms should be expected from all teachers irrespective of their gender. NighatBasu and Gawher Ahmad (2016)studied Attitude towards Using New Technology among Higher Secondary School Teachers in District Budgam. The results reveal the Govt. secondary school teachers have altogether favorable attitude towards ICT than the private secondary school teachers. Ana Luísa Rodrigues(2020) investigated digital technologies integration in teacher education the active teacher training model. Findings of this study reveal that the participating teachers were able to develop skills and integrate digital technologies in their own teaching-learning process and could change their teaching practices, which will support the development of online education in the future.

Statement of the Problem

Now a day's Technology and digital tools plays an important role to facilitate the teaching learning process more effective. Hence, the study would find out the teacher educators attitude towards using new technology and the application of digital technologies in their classroom instructions and also to find whether the integration of technology in classroom instruction would facilitate the student teachers to acquire knowledge on ICT. Therefore, the researchers intended to study the problem specifically stated as "Teacher Educators' Attitude towards using New Technology and the Application of Digital Technologies in Classroom Instruction".

Need and Importance the Study

There is a paradigm shift from the conventional method of administering and managing of educational process to the new and innovative strategy of the integration of technology, many researches were conducted and reported with evidence that the digital technological tools are effective in many activities of education and in the process of instruction and facilitating learning and research among students in the educational system. It has been now analyzed and recognized the need for incorporating the digital technologies in enhancing quality in school education in general and higher education in particular. This study is aimed to find out, whether the attitude of teacher educators towards the digital technology contribute to the application of digital technologies in the classroom





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instruction and if the application of digital technologies in classroom instruction facilitates significantly in improving the achievement in ICT among student-teachers in colleges of education.

Research Questions

- ❖ Is there any significant difference exist among Teacher Educators in their Application of Digital Technologies in Classroom Instruction and its Dimensions of VAI, CAI, WBI, Online/Offline, Hardware, and E-content and Attitude towards using New Technology with reference to Gender?
- ❖ Is there any significant difference exist among Teacher Educators in their Application of Digital Technologies in Classroom Instruction and its Dimensions of VAI, CAI, WBI, Online/Offline, Hardware, and E-content and Attitude towards using New Technology with reference to Age?

Objectives of the Study

- ❖ To find out if there is any significant difference exists among Teacher Educators in their Application of Digital Technologies in Classroom Instruction and its Dimensions of VAI, CAI, WBI, Online/Offline, Hardware, and Econtent and Attitude towards using New Technology with reference to Gender.
- ❖ To find out if there is any significant difference exists among Teacher Educators in their Application of Digital Technologies in Classroom Instruction and its Dimensions of VAI, CAI, WBI, Online/Offline, Hardware, and Econtent and Attitude towards using New Technology with reference to Age.

Hypotheses of the Study

In this research the researcher has formulated hypothesis in null form where the literature review for the particular variable is absent or scanty with reference to the demographic variables.

- There is no significant difference exist among Teacher Educators in their Application of Digital Technologies in Classroom Instruction and its Dimensions of VAI, CAI, WBI, Online / Offline, Hardware, and E-content and Attitude towards using New Technology with reference to Gender.
- ❖ There is no significant difference exist among Teacher Educators in their Application of Digital Technologies in Classroom Instruction and its Dimensions of VAI, CAI, WBI, Online/Offline, Hardware, and E-content and Attitude towards Using New Technology with Reference to Age.

METHODOLOGY OF THE STUDY

Method of the Study

The researcher adopted the survey method to collect the relevant data from desired areas.

Population

A population is any group of individuals that have one or more characteristics in common. The population for the study includes all the Teacher Educators worked in Trichy, Thanjavur, Namakkal, Karur and Ariyalur Districts of Tamil Nadu.

Sample

The sample for the present study includes the Teacher Educators from Government, Government-aided and Private Colleges of Education.

Sample size

In the present study 252 Teacher Educators were randomly selected on the basis of the random criteria.

Sampling Technique

The researcher adopted stratified random sampling technique for selecting the sample.





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Research Tools used

The following research tools used for collection of data.

- 1. Application of Digital Technologies in Classroom Instruction Scale developed and standardized by the Researcher
- 2. Attitude towards Using New Technology Scale developed and standardized by Rajasekar, S (2011).

Statistical Techniques used

The following statistical techniques used for analyze the data:

- Mean and Standard Deviation.
- t-Test, ANOVA and Duncan Multiple Range Test: to know the difference between the means of variables.

Data Analysis and Interpretation

The obtained t-value shows that there is no significant difference exists among teacher educators in their application of digital technology in classroom instruction except in the dimensions of using hardware and e-content and their attitude towards using new technology with reference gender. Hence null Hypotheses formulated were accepted with regard to VAI, CAI, WBI, Online/Offline, Total and Teacher Educators' Attitude towards using new technology but the null hypothesis on the dimensions of using Hardware and e-content among teacher educators are rejected. Hence there is significant difference exists among teacher educators in their application of digital technology in classroom instruction of using hardware and e-content with reference to gender. The obtained F-value in the table reads that there is a significant difference exists among teachers educators in their application of digital technologies in classroom instruction in total and in all dimensions of VAI, CAI, WBI, Online/Offline, Hardware and e-Content with respect to age. There is no significant difference exists among teacher educators in their attitude towards using new technology in relation to age and so the null hypothesis is accepted. The null hypothesis is rejected with regard to Teacher Educators' Application of Digital Technology in Classroom Instruction in relation to their Age and there is no significant difference exists in teacher educators attitude towards using new technology with reference to age is retained. Duncan Multiple Range Test reads that Teacher Educators whose age group belongs to 41-50 have significantly differ from their counter parts in their application of digital technology of CAI, WBI, and Online/Offline and in total in classroom construction, and 50 years of age group in VAI and up to 30 years in E-content application.

RESULTS AND DISCUSSIONS

The result of the study reveals that there is no significant difference exists in the attitude towards using new technology and their application of digital technology in classroom instruction among teacher educators except in the dimensions of using hardware and e-content in the classroom instruction with reference gender. Male and female teachers are equally applied digital technology in classroom instruction and they are not significantly differed each other. There is significant difference exists only in the dimension of application digital technology of hardware and econtent only in this study. Gender is not a significant factor to differentiate significantly in the application of digital technology. The finding of the study reveals that there is a significant difference in the application of digital technology in classroom instruction and its dimensions (VAI, CAI, WBI, Online/Offline, Hardware and e-content) among teacher educators. The study reveals that there is no significant difference in the attitude towards using new technology among teacher educators with respect to age. Analysis of the data with appropriate statistical techniques have facilitated the investigator to verify the hypothesis and answered research questions formed in the start of research endeavour. Analysis of variance has put forth results pertaining to the significance of difference exists among teacher educators in their attitude and application of digital technology facilitated the investigator to trace the existence of difference among variables with respect to personal factors provided the necessary results to arrive inference to make generalization from the study. The Duncan Multiple Range Test has facilitated the investigator to find the categories of the variable whose significance of difference with their counter parts has explained many insightful finding to express through the study.





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Educational Implications

- The study highlights the various applications of Digital Technologies in Classroom Instruction in the knowledge era
- Special efforts should be made in order to develop Application of Digital Technologies in Classroom instruction and to develop positive attitude towards new technology among teacher educators.
- The study will be immense use of educational policy makers and curriculum planners in the field of teacher education.
- ❖ In the Colleges of Education, All the Teacher Educators are highly motivated to actively participate in various training and professional development programmes to excel digital technologies and digital tools.
- The study paves the way to establish educational technology cell in all teacher education institutions then only teacher educators master various technological skills.

CONCLUSION

The role of the teacher is expected to direct and lead students towards self-learning and thinking which is most assured through the application of digital technology in classroom instruction. The quality of education depends upon the quality of teaching and the quality of teaching depends upon the quality of infrastructure and appropriate utilization of digital tools and devices in teaching. Digitalization of educational process of teaching would enable the successful academic achievement. Teacher educators' attitude towards new technology and their application of digital technology in classroom instruction is significantly plays a major role in transacting the knowledge and curriculum in digital era. Therefore the study gives interesting results and the findings of the study very useful for the educational planners and policy makers in the field of teacher education

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Table 1. Significance of Difference exists among Teacher Educators in their Application of Digital Technologies in Classroom Instruction as whole and its Dimensions and Attitude towards Using New Technology with reference to Gender

Dimensions of		Gender					
Digital Technology	Ma	Male		Female		Level of Sig	
Application & Attitude	Mean	SD	Mean	SD			
VAI	2.633	1.151	2.553	1.110	0.560	NS	
CAI	20.267	2.978	20.809	2.381	1.603	NS	
WBI	30.816	5.050	30.950	4.609	0.220	NS	
Online/Offline	47.290	6.000	47.760	4.985	0.679	NS	
Hardware	28.175	3.864	29.396	2.984	2.819**	0.01	
E-Content	18.687	3.448	19.578	3.305	2.095*	0.05	
Total	1.478	17.167	1.510	13.823	1.625	NS	
Attitude	93.938	11.934	92.619	10.044	0.342	NS	

^{**} denotes 1% level of significance * denotes 5% level of significance

Table 2. Significance of Difference exists among Teacher Educators in their Application of Digital Technologies in Classroom Instruction as whole and it's Dimensions & Attitude towards Using New Technology with reference to Age

Dimensions of Digital Technology Application &	Source of Variation	Sum of Squares	Deg. Freedom	Mean Square	F-Value	Level of Sig
	Between Groups	18.987	3	6.329		
VAI	Within Groups	301.727	248	1.217	5.202**	0.001
	Total	320.714	251	-		
	Between Groups	327.566	3	109.189		
CAI	Within Groups	1525.239	248	6.15	17.754**	0.001
	Total	1852.806	251	-		
	Between Groups	230.13	3	76.71		
WBI	Within Groups	5636.299	248	22.727	3.375*	0.05
	Total	5866.429	251	-		
Online/	Between Groups	433.019	3	144.34	4.942**	0.01
Offline Offline	Within Groups	7243.918	248	29.209		
Offilite	Total	7676.937	251	-		
	Between Groups	334.207	3	111.402		
Hardware	Within Groups	2769.507	248	11.167	9.976**	0.001
	Total	3103.714	251	-		
	Between Groups	121.229	3	40.41		
E-content	Within Groups	2786.434	248	11.236	3.597**	0.01
	Total	2907.663	251	-		
	Between Groups	5947.026	3	1982.342		
Total	Within Groups	55933.292	248	225.537	8.789**	0.001
	Total	61880.317	251	-		
	Between Groups	469.687	3	156.562		
Attitude	Within Groups	30263.785	248	122.031	1.283	NS
	Total	30733.472	251	-		

 $^{^{\}star\star}$ denotes 1% level of significance * denotes 5% level of significance





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Table 2.1.Post Hoc Tests of Duncan Multiple Range Test of Homogeneous Sub Sets Highlights the Specific Group Influence on the Application of Digital Technologies in Classroom Instruction with reference to Age

Application of Digital			Subset f	for alpha
Technologies in Classroom Instructions and its Dimensions	Age Number		1	2
	Up to 30 Years	59	2.644	
	31- 40 years	147	2.455	
VAI	41-50 years	33	2.697	
	50 yrs above	13		3.692
	Sig.		0.425	1
	Up to 30 Years	59		20.644
	31- 40 years	147		20.693
CAI	41-50 years	33		21.454
	50 yrs above	13	15.769	
	Sig.		1	0.232
	Up to 30 Years	59		30.406
	31- 40 years	147		31.102
WBI	41-50 years	33		32.121
	above 50 years	13	27.384	
	Sig.		1	0.187
	Up to 30 Years	59		47.389
	31- 40 years	147		47.598
Online/offline	41-50 years	33		49.333
	above 50 years	13	42.538	
	Sig.		1	0.187
	Up to 30 Years	59		28.644
	31- 40 years	147		28.932
Hardware	41-50 years	33		30.030
	above 50 years	13	24.153	
	Sig.		1	0.127
	Up to 30 Years	59		19.491
	31- 40 years	147		19.265
E- content	41-50 years	33		18.909
	above 50 years	13	16.230	
	Sig.		1	0.527
	Up to 30 Years	59		149.220
	31- 40 years	147		150.047
Total	41-50 years	33		154.545
	above 50 years	13	129.769	
	Sig.		1	0.194



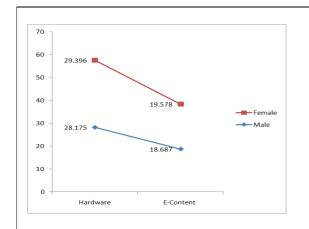


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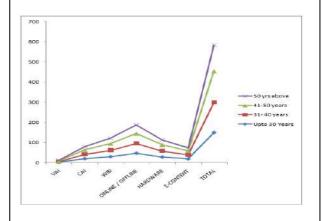


Figure:1. Significance of Difference exists among Teacher Educators in their Application of Digital Technologies in Classroom Instruction and its Dimensions of Hardware and E-content with reference to Gender.

Figure:2. Significance of Difference exists among Teacher Educators in their Application of Digital Technologies in Classroom Instruction as whole and its Dimensions with reference to Age





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RESEARCH ARTICLE

Assessment and Awareness of Occupational Hazards in Fireworks Industry

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ABSTRACT

Sivakasi, a town in the Virudhunagar district of the Indian state of Tamil Nadu, is where the vast majority of India's firework products are produced. In the firework industries, many different chemicals are handled by hand during the production process. Consequently, the firework industry is widely recognized as a particularly risky occupation. The purpose of this article is to examine the risks inherent in the fireworks manufacturing, including the factors that contribute to accidents and the negative impacts that fireworks have on air quality. Human error has been cited as the primary cause of accidents in the vast majority of prior studies, which have focused on unsafe behaviours and environments. The fireworks industries are struggling with the problem of improperly handling dangerous chemicals including the risk of unanticipated fires and explosions, exposure to toxic chemicals, and an environment that is not properly maintained, which has resulted in an unpleasant circumstance for everyone. Explosion caused by the self-oxidation of chemicals, other parameters analyzed in this study include explosion caused by friction, explosion triggered by temperature, explosion caused by human error, and accumulated static electricity.

Keywords: Explosion, Chemical hazards, Risk factor, Occupation.





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INTRODUCTION

After China, India is the world's second largest producer of firecrackers, with almost all of its output being consumed domestically. Sivakasi, which is located in the Virudhunagar District of Tamilnadu in South India, is home to the world's largest manufacturer of matches and firecrackers. Nearly every resident and worker in the Sivakasi and Virudhunagar areas is directly or indirectly involved in the fireworks and cracker manufacturing and retailing industries. This includes match manufacturers. There are approximately 1050 factories, and the labor force totals 1 Lakh and above [1]. The fireworks industry is regarded as a harmful and hazardous industry, as the risk of loss of life and property was extremely high during the early stages of development of transportation and storage of fireworks in garages. The possibility of fiery incidents occurring was the production process loading and treatment, which are important risk factors [2]. Women work in the fireworks industry as well. This fireworks business generates revenue for the economy. The majority of employees in the fireworks industry are female; manufacturing of crackers, in particular, falls into the hands of girls. The preferred way of life of women workers' families is dependent on the earnings of the women in their circle of relatives. The increase in output in such industries is largely due to their sense of accomplishment [3]. In India, Sivakasi produces 90% of the fireworks, with a large number of women working there. Every process must be completed manually by laborers, who are still unfamiliar with handling hazardous chemicals. As a result, the incident rate of accidents is high in comparison to other units [4]. Handling chemicals in this business poses numerous risks, including disease transmission, bronchial asthma, accidents-chemical burns, hypersensitive reactions, allergic contact dermatitis, irritant touch dermatitis, pores and skin contamination, skin injuries, pores and skin cancer, reproductive issues, and even death. The health effects of fireworks are numerous, including inhalation of smoke from the fireworks, which causes asthma, eosinophilic pneumonia, and perchlorate damage to thyroid glands [5]. The primary goal of industrial health and security is to protect workers from various hazards in the workplace and to provide effective precautions to reduce danger that may arise in the factory [6]. Despite this, each firework factory must follow the health protection protocol outlined in the Factory Act, and the government should take appropriate steps to review the industry's safety policies and procedures [7]. As new firework industries emerge, existing industries expand, and new technologies are implemented in this field, the fireworks environment is increasingly threatened, posing threats to human health. The "sternness" of the danger is defined by experience, and the consequences predict the level of contact that causes harm to the workers' health [8].

Hazards in Firework Industries

All fireworks chemicals are dangerous. Fireworks combine fuel and oxidizer. Continuous chemical exposure may harm workers. Toxic release, fire, and explosion are firework hazards. Fire is the main hazard, causing human illness and property loss [9]. Static electricity is another firework hazard. When solids and chemicals come into contact, these hazards can occur. Wooden plate manual blending of fuels, oxidizers, and igniters. Static electricity, friction, impact, and human error are chemical blending hazards. Careless handling, impact loading, improper stacking, and dragging cause accidents during product transport [10]. Firecrackers emit pollutants like sulphur dioxide, carbon dioxide, carbon monoxide, nitrogen oxide, and other suspended particles. Cracker use at festivals increases environmental toxicity [11]. The chemical combinations to reduce industrial hazards. Burning extra chemicals reduces risk. Immediate safe disposal of unused chemical mixture. This should be burned far from the industry, and friction should be avoided when transporting unwanted chemicals. Sridhar et al., [12]. cited chemical ignorance, lack of infrastructure, unmaintained shed floors, and lack of training as causes of firework accidents.

Impact of Hazardous Chemicals

Fireworks are composed of a pyrotechnic element that, when ignited, can release heat, light, sound, and gas. In addition, inappropriate mixing of hazardous and non-hazardous chemicals, such as oxidizer, fuel, sand, igniter, and unique impact chemical compounds on wood trays, must take place. This chemical can either be considered hazardous or non-hazardous, according to the [13] classification system. The manufacturing process for entertainment uses a variety of chemicals, which simultaneously creates health problems due to the manufacturing process. When inhaled, potassium nitrate can cause irritation to the respiratory tract, specifically the nose and throat.





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When potassium nitrate is present in the blood at levels that are high enough to interfere with its normal function, the capacity of the blood to carry oxygen is reduced, which can lead to a variety of serious health problems [14]. Potassium perchlorate is known to be a substance that can irritate both the skin and the eyes. Long-term contact has been shown to be disruptive to the thyroid, white blood cells, and kidneys [15]. Aluminum compounds – The skin and eyes are more likely to become irritated due to the fact that these compounds cause metal fume fever. Metal fume fever is an infection that manifests itself similarly to the flu and is characterized by a taste of metal in the mouth, fever, headache, cough, and a tightness in the chest in addition to skin irritation. A history of exposure to dirt of a particularly high quality will leave a stain on the lungs and will also bring on a persistent cough and shortness of breath [16]. Barium Nitrate: Barium nitrate causes irritation to the respiratory tract, specifically the lungs, throat, and nasal passages. An extended period of exposure to it can result in cancer, paralysis, a point of weakness in the muscles, diarrhea, abnormal heartbeat, and occasionally even death. Kidney function can become impaired as a result. When there is consistent contact, unusual chest X-ray findings can be the result[17]. Copper chloride - The stomach ache, nausea, salivation, belly ache, vomiting, and diarrhea are all the result of the copper chloride, which is the cause of the health issues. The irritation that is caused in the bone that divides the inner nostril can be attributed to repeated contact with the allergen [18]. This can also lead to a reduction in the thickness of the internal lining of the stomach, which can result in an ulcer or a hollow in the stomach.

Damage caused by inhalation Taking a deep breath of the hot smoke produced by the combustion of hazardous materials could result in an unhealthy level of carbon monoxide inhalation. The development of carboxyhaemoglobin is a potential side effect of carbon monoxide exposure that takes place in the bloodstream. If carboxyhaemoglobin levels continue to rise, the patient may experience headaches, the condition will be detrimental to their nervous system, and ultimately, the patient will pass away [19]. PM2.5 - A wide variety of health warnings have been distributed on the worldwide web to vulnerable individuals, warning them to stay away from the materials used in the production of fireworks and also to stay away from the fumes and airborne PM that may be released as a result of the combustion of those materials. There are a lot of papers that have marked the associations between airborne debris and breathing significances ranging from signs and symptoms to humanity [20]. Copper and lead Lead has been shown to inhibit mind function and may also have an effect on the haematological profile of an individual, in addition to the relevant terrified device. Exposure can also lead to behavioral consequences, learning delays, developmental delays, and growth retardation. It can also delay mastery of skills. Copper dust fumes, when breathed in, have the potential to irritate the respiratory system and cause inflammation[21]. Because of the greater amount of contact and usage of chemicals throughout the industry, laborers have reported cases of persistent dizziness, ulcers, and headaches. These symptoms are caused by the chemicals. Because workers do not wear gloves or masks while running, the respiration tract is exposed to the entry of metallic substances inside the body [22]. Since workers do not wear gloves or masks, the respiration tract is subjected to the entry of metallic substances inside the body.

Safety Measures for avoid Accidents

The following is a list of the various safety precautions that can be taken to reduce the risk of accidents occurring:

- Always wear appropriate personal protective equipment.
- Doors must always face outward.
- Rubber mats should be placed on the floor.
- It is required that there be a distance of 18 meters between each mixing shed and each filling shed.
- Drying should take place on the platform, and the materials should under no circumstances be dragged inside the factory.
- In the workplace, there should be no more than the minimum amount of the chemical mixture required.
- A prescribed ix minimum qualification level ought to be in place for the foreman.
- The number of supervisors is to be proportional to the number of workers, with one supervisor for every twenty employees x.
- The bare minimum of qualifications is required of supervisors.





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- It is necessary to have industrial safety in order to check everything that could go wrong.
- chances of accidents, with the goal of minimizing human casualties and material damage.
- It is required to educate all members regarding the subject matter.
- the application of safety principles with the goal of preventing industrial accidents.
- It is essential to accomplish this in order to improve the morale of the industrial workers.
- It is essential to have improved relations with other people within the organization.
- In order to boost production means to a higher level, it is necessary to: the average annual income of a population.

DISCUSSION

- Every single step of the fireworks manufacturing process is carried out by hand. Powdered forms of various chemicals are being handled by workers. The majority of accidents are brought about by carelessness on the part of workers in addition to improper handling of chemicals.
- •It is possible for there to be an accident or an explosion if there is any kind of friction that takes place. In the fireworks industry, the vast majority of accidents take place during the production of fancy type crackers. This is due to the fact that in this type of cracker, the chemicals are mixed with water to make slurry, and this slurry will eventually decompose.
- Therefore, the appropriate amount of drying time and ventilation must be maintained in order to prevent heat accumulation. Accidents can be caused by the smallest amount of carelessness.
- According to a statistic, out of thirty accidents, twenty are caused by unsafe acts committed by workers, while the remaining ten are caused by unsafe conditions within the factory.
- 99 workers lost their lives as a result of unsafe acts, and 28 workers lost their lives as a result of unsafe conditions in the factory. It demonstrates that the workers have not been educated about the safe method of work.
- It is important to note that out of 127 fatalities, 63 fatalities occurred while the victim was filling out paperwork. It is clear from this that the employees are not aware of the risks involved in working with the chemicals.

CONCLUSION

Every age group of the human population enjoys the joy and happiness that fireworks represent on a variety of occasions. Fireworks are a symbol of joy and happiness. On the other hand, the manufacture of fireworks posed a number of dangers to the people who worked in the relevant industries. However, accidents do not occur very frequently in those industries, and when they do, they are typically the result of human error or one of the many unknown factors that have not yet been identified. We decided to conduct this survey in order to evaluate the fundamental requirements of the industry as well as to gain a better understanding of the potential risks factors that are associated with these industries. Wherever the risk factors are high and our recommendations are given for the safe operation of these industries, the industries in question should adhere to the safety measures as closely as is practically possible and replace unsuitable machinery with more appropriate options. This will help to reduce the risk factors associated with these industries.

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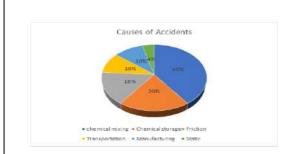


Fig 1: Causes of Accidents



Fig 2: Explosion at firework industries





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RESEARCH ARTICLE

Development and Evaluation of Cyclosporine a Inserts Prepared using Sodium Hyaluronate and Badam Gum for Treating Dry Eye Disease

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ABSTRACT

The purpose of this research involves development and characterization of novel drug delivery system for sustained-release cyclosporine A (CsA) by sodium hyluronate (SH) and badam gum (BG) to cure dry eye disease. CsA polymer inserts were designed using solvent casting technique with 23 complete factorial design to test the size, folding durability, in vitro drug release and wettability effects of SH, BG ratios and product quality. Inserts have also been tested for product consistency, absorption of moisture, degradation, and pH. Designed CsA filled SH inserts have been subjected to sterilization by Ultra Violet radiation and assessment of morphology. By adding BG, drug load and thickness uniformity. Formulation F4 of 1% w / v SH and 0.25% w / v BG shows high folding endurance and continued release of CsA for a period of 20 hours. Sterility tests for F4 on plate and direct inoculation have been checked and the formulation sterility process have been validated. For a total of 3 months the formulations were stable at 4° C, 25° C and 40° C. The ocular insert is an innovative tool in the treatment of eye disease. The design and development of an ocular insert is a challenge that pharmaceutical researchers or manufacturers have ever faced. In ophthalmology; eye drop has ever proven to be an effective solution from the point of view of administration. The quick precorneal loss of the medication has been a major difficulty in the case of traditional dosage types. The researcher has always made efforts to release the drug at controlled rate by means of an ocular insert to avoid repeated drug administration.

Keywords: Polymeric ocular insert; Sodium Hyaluronate: Dry eye disease: 23 full factorial design





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INTRODUCTION

Dry eye syndrome (DES) is a troubling tear film condition which causes damage to the eye surface and is associated with eye pain symptoms. Dry eye disease is marked by tear film instability which may be due to insufficient tear output or low tear film consistency resulting in increased tear evaporation. So, the dry eye can be split primarily into two categories, namely

- 1.) evaporative dry eye disease.;
- 2.) aqueous production deficient dry eye disease

Insufficient tears cause damage to the interpalpebral eye surface and are associated with malaise symptoms 1. The International Dry Eye Workshop (2007) described dry eye as a multifactorial tear-and- eye surface disease that results in symptoms of pain, visual disruption, and tear film instability with possible eye surface damage. It is accompanied by decreased tear film osmolarity, and eye surface inflammation. DES is associated with a diminished ability to perform such tasks such as reading, driving and computer-based work involving visual attention (1). DES is associated with a diminished ability to perform such tasks such as reading, driving and computer based work involving visual attention (2). Cyclosporine A (CsA) is used for the diagnosis of inflammatory ocular diseases like corneal repair and dry eye disease cyclosporine A acts as the partial immunomodulatorCsA can be used in treatment of inflammation of both the lacrimal gland and ocular surface and keratoconjunctivitis sicca, commonly referred to as dry eye disease. The conventional dosage form containing CsA when applied topically in the form of sprays are quickly washed off from the precorneal surface due to certain protective mechanisms, this result in poor bioavailability(3)(4). In order to overcome the loss of drug by these mechanisms, CsAis formulated as ocular insert. This approach enhances the residence time of CsA in the eye and also increases the patients' compliance. PEG 400 was used as a plasticizer, Badam gum as a gelling agent and sodium hyluronate as a lubricating and film forming agent. The prepared ocular inserts were evaluated by the measurement of pH, folding endurance, wettability, moisture absorption. In vitro release studies were carried out to check the drug release rate by the use of Franz diffusion cell.

MATERIALS AND METHODS

MATERIALS

Cyclosporine A was procured by ACE Rasayan, Mysuru-570002, Sodium hylorunate was procured by Sisco Research Laboratories (SRL), Polyethylene glycol was obtained by LOBA CHEMIE and Badam gum was obtained as a gift sample from Mysuru, Karnataka, India.

Methods

Pre-formulation study

Fourier-transform infrared spectroscopy (FT-IR)

The drug excipient compatibility study was determined using 0.1 mm KBr pellets using FT-IR (Fourier Transformer Infrared Spectroscopy). The pure drug IR spectrum (bromfenac sodium) is compared with the combination IR spectrum of MoxifloxacinHCI and all the excipients to check the interaction.

Differential scanning calorimetry (DSC) characterization

Pure drug and physical mixture thermal characterisation was performed with calorimeter. Samples were placed in aluminum pans which were sealed. The specimens were scanned from 20 ° C to 300 ° C at 20 ° C / min.

Proton nuclear magnetic resonance (1HNMR)

¹HNMR was done for pure cyclosporine A and inclusion complex were recorded using VNMRS-400 (Agilent- NMR) and they were recorded.





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Method of preparation

Preparation of blank and CsA loaded ophthalmic insert

The method of evaporation from the solvent casting was used to prepare ophthalmic CsA inserts. SH 1% w / v solutions are prepared through absence or presence of BG (0.25% w / v) using a distilled-water magnetic stirrer (40 ml). The blend was subjected to stirring for 1 hour. CsA has been liquified in ethyl alcohol and transferred into mixture, contributing to 0.024 percent w / v concentrations. PEG 400 was then used as a plasticizer (30 per cent w / w comparative to polymer's dry weight) and mixture was blended 3 hours in order to dissipate all air bubbles for 30 minutes. The films were cast onto plates made of polystyrene (100 x 15 mm) enabled to dry up for 48 hours at room temperature. Punched out (\sim 50% µgCsA / insert) inserts of 6 mm diameter were put in a dark sealed container. Blank inserts were also made without use of CsA employing the same tool.

Evaluation Studies

Thickness measurement

Insert thickness was measured by means of digital micrometer, and the mean value for 10 independent measurements was reported. To measure thickness the inserts were chosen at random.

Folding Endurance

The count by which a insert is subjected to folding without any break, is endurance. Folding durability was introduced for the evaluation of the mechanical properties of the inserts.

Wettability study

The measurement of the contact angle is useful for assess surface wettability, and hydrophilicity / lipophilicity of surface energy. Simulated tear fluid (STF), consisting of NaCl(0.670 g), sodium bicarbonate (0.2 g), CaCl₂ dehydrates (0.8 g), was used in 100 ml of deionized distilled water. The contact angle of the inserts was assessed using a contact angle meter employing Half-Angle TM technique.

Moisture absorption

Checking CsA integrates physical stability and integrity in wet environments, percent moisture absorption was performed. Weighed and put the inserts in a saturated aluminum chloride solution (80 per cent RH) desiccators. Three days later the inserts were cut, and weighed. The triplicate check had been carried out. Using the equation below, the percent absorption of moisture was determined.

Percent moisture absorption = Final weight - Initial weight * 100 Initial weight

Moisture loss

A % loss of moisture was achieved in dry conditions to test physical stability and integrity of CsA charged SH inserts. Inserts containing anhydrous calcium Chloride have been weighed and dedicated. The inserts were cut and measured three days later. There has been a triple test

Percent moisture loss <u>= Initial weight</u> x 100 Initial weight

Surface pH

Before usage the pH meter was calibrated with normal pH 4, 7, and 10 buffer solutions. Inserts were put into vials which contained distilled water. They allowed inserts for swelling to about 30 minutes. pH meter was placed near insert surface, and the pH values were noted for 1 minute after equilibrium (5). This test was carried out in triplicate

Drug content

CsA inserts were thoroughly dissolved in an Eppendorf tube and vortex in 1 ml of ethanol. The content of the drug has been analysed using SH(5). The content of the drug was measured in triplicate.





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In vitro drug release study

To extract CsA from in vitro inserts, a dialysis membrane and two chamber-side diffusion cells were used. The dialysis membrane had been sandwiched with an insert side by side between the two demi-cells of a two-chamber diffusion cell. Temperature was held to model eye temperature by circulating water jacket at $34\pm0.5\,^{\circ}$ C. $300\,\mu$ l of the sample shall be removed at definite time periods and replaced by corresponding fresh buffer volume. Triplicate analysis was performed in the bath, and samples were tested through HPLC (7).

Sterility Test

Formulation F4 subjected to sterilization through exposure to both sides to Ultra Violet radiation for 10 minutes and sterility was verified to test suitability of sterilization process to create microbe free insert. Sterilized inserts were tested before and after sterilization for any difference in surface morphology using SEM and in vitro drug releases(8). Sterility checking was carried out through methods of plate and direct / tube inoculation, according to existing protocol (9).TSB has been used for direct / tube inoculation process. Concentration was measured through Spectronic 20 Genesys spectrophotometer employing sterile water as blank in comparison to 1 McFarland norm with CFU / ml (3/108) concentration. Samples of direct inoculation comprised negative, positive, positive sample controls, and sterile vials. There were 9 ml sterile water and 1 ml uninoculated fluid in negative control vial. Positive control vial held 9 ml uninoculated fluid and 1 ml 102 CFU / ml vapor. Those samples were consistently distributed on agar plates of Mueller Hinton (MH), incubation done at 37 ° C for 24h, and visually tested for growth of bacteria at room temp. Work performed under sterile environments on a laminar air-flow platform.

RESULTS AND DISCUSSION

Fourier-transform infrared spectroscopy

IR spectroscopic studies performed and it confirms that drug is compatible with all the excipients. Characteristics peaks of Cyclosporine A pure drug also exist in physical mixture, hence concluded that there was no interaction between the drug and excipient.

Differential scanning Calorimetery (DSC)

The physical condition and thermal characteristics of the CsA, SH, BG, CsA-loaded SH insert (F4) with their corresponding blankness (F3 formulation) were defined using a Differential Scan Calorimeter (DSC). Samples weighing 5 mg were placed into an aluminum pan with microbalance and sealed. Heating of samples was done through steady rate by 10 ° C / min over 30-300 ° C the Cyclosporine A DSC thermo gram and the actual mixture of Cyclosporine A with all the excipients. Cyclosporine A had a long and distinctive endothermic peak at 134.02 ° C. Cyclosporine A physical mixture of SH + BG displayed characteristic peak at 134.02 ° C, 84.16 ° C, and 64.30 ° C. It follows from this, that there is no interaction between Cyclosporine A and excipients [15](10). The characteristic sharp endothermic peak of CsA was completely absent in drug loaded insert. This indicated the absence of any undissolved CsA in the formulation

¹H Nuclear Magnetic Resonance

The analysed proton NMR of cyclosporineA compared with the physical mixture, showed no chemical interaction with the structure and chemical shift values nearly matches to the pure drug. Hence, there is no interaction.

Statistical Analysis

Data obtained were shown as means and standard deviations with data replicate number in every sample. ANOVA has been used for creating statistical significance. A meaning p < 0.05 was deemed important. Experimental design and data analysis was carried out through version Design expert version 12.





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Evaluation Studies

Comparison of Post-Compression Evaluation Parameter

The releaseprofilesofCsAfrominsertsareshowninFig.3.7.After12h,Formulations F2, F4, F6 and F8 produced approximately 65%, 37%, 76%, 78%, respectively, containing CsA after 12 h. Post 16hrs cyclosporine A was released 100% from the inserts, with the exception of F4, which held CsA release for up to 20 hours.

CONCLUSION

Within this study, we discuss preparations for DED treatment of CsA loaded SH inserts and physicochemical evaluations. The inserts CsA loaded with SH (1% w / v) and BG (0.25% w / v) presented significant structural characteristics, wett ability, thickness so that no psychological damage to the eyes during application. CsA was dispersed evenly in polymer matrix. In vitro release studies suggested that up to 20 hours of drug release was maintained. BG showed important implications for the maintenance of CsA release and development of the folding strength. The inserts were sterilized with success using UV light. To conclude, successfully prepared CsA inserts using SH, BG, and PEG 400. Radical combination therapy with SH and CsA might potentially achieve lubrication and anti-inflammatory effects as well as a possible one-day DED formulation.

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Conflict of Interest

None

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None

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Table 1: Comparing the post compression Evaluation Parameter of Cyclosporine Ainserts:

Formulation	Drug content (µg/insert)	Film thickness (mm)	Folding endurance	Contact angle (⁰)	Percent moisture absorption %	Percent moisture loss%	Surface pH
F1		0.072 ± 0.009	485.7±12.5	56.0 ± 4.32	1.01 ±0.18	2.17 ±0.11	6.82 ± 0.075
F2	50 ± 2.60	0.073 ± 0.007	480.3±1.50	62.4 ± 2.95	1.00 ±0.14	2.25 ±0.31	7 ± 0.021
F3		0.086 ± 0.007	593.3±7.60	62.6 ± 1.90	1.63 ±0.19	2.09 ±0.11	6.73 ± 0.208
F4	50.7 ± 1.46	0.088 ± 0.008	591.3±1.50	62.0 ± 1.33	1.78 ±0.40	2.03 ±0.52	6.98 ± 0.056
F5		0.146 ± 0.005	69.7±4.60	56.8 ± 2.35	1.08 ±0.08	1.68 ±0.16	6.86 ± 0.04
F6	52.5 ± 2.58	0.139 ± 0.024	67 ±6.2	60.0 ± 1.33	1.00 ±0.08	1.64 ±0.54	7.03 ± 0.026
F7		0.165 ± 0.014	216±5.30	59.6 ± 2.80	1.06 ±0.09	1.76 ±0.09	6.68 ± 0.176
F8	50.2 ± 3.72	0.154 ± 0.005	229± 25.4	63.4± 3.41	1.02 ±0.4 0	1.77 ±0.33	6.99 ± 0.032

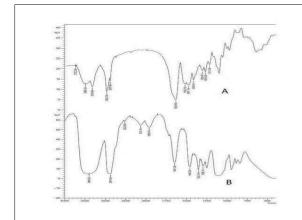


Figure 01: FTIR Spectra of (a) CsA (b) Physical mixture

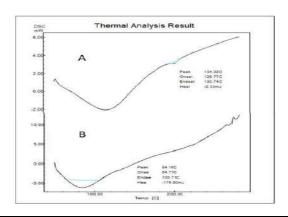


Figure 02: Differential scanning thermo grams of (A)
Pure drug, (B) (1:1) Physical mixture



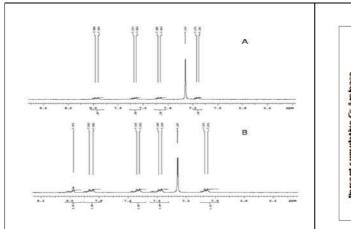


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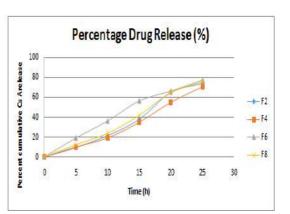


Figure 03: 1HNMR of pure Cyclosporine A (A) and (1:1)
Physical mixture (B)

Figure 04:Percentage Drug release of all batches





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RESEARCH ARTICLE

Information Security in Distributed Computing Utilizing a Novel Compelling Light Weight Homomorphic Cryptographic Calculation

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ABSTRACT

Distributed computing is an innovation where the assets are conveyed as administrations. Clients can get to them anyplace, whenever through the Web with practically no need to know the framework information, experience, or even power that offers such types of assistance. It has turned into a significant vehicle for endeavors to foster their organizations due to these assets. With the developing requirement for distributed computing, security is becoming significant for the two people and business needs. Numerous scientists have concentrated on the security of distributed computing. In any case, security holes or dangers are expanding as the interest for distributed computing availability increments. Since an outsider generally gives cloud stages to the cloud client, information security in the cloud is the essential issue. This paper presents a novel, powerful lightweight homomorphic cryptographic calculation which contains two layers of encryption. The principal layer utilizes the new successful, light-weight cryptographic calculation and the second layer multiplicative homomorphic plans considered for further developing security information in distributed computing. This approach offers both symmetric and lopsided cryptography highlights. The proposed approach's presentation is assessed utilizing various measurements, including memory, computational time and (key responsiveness), factual investigation, picture histogram, and entropy change examination. The proposed calculation's trial discoveries showed an elevated degree of safety and a clear improvement in encryption execution time, memory use, and throughput. When contrasted with the cryptographic frameworks generally utilized in distributed computing.





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Keywords: Cloud computing Light-weight cryptographic Multiplicative homomorphic RSA cloud base IoT

INTRODUCTION

In the beyond couple of many years, correspondence has been growing quickly among clients by means of the Web. Moreover, information utilization has been progressively developing among various clients. Transmission of important and private data has been at a more elevated level. Security of classified data is an unquestionable requirement for secure change. One of the areas for putting away classified data is the cloud, and information security in the cloud is one of the issues that should be tended to. Distributed computing is a pattern in the field of data current advances. Subsequently, numerous business applications require new advancements to be applied to store or handle huge information. Distributed computing is a state of the art technique for putting away a lot of information and running applications [1]. It permits clients to have almost boundless handling limit and offers potential benefits regarding simple openness, adaptability, and asset sharing. The cloud administrations include online document stockpiling, webmail, long range interpersonal communication stages, and online business applications. On-request self-support, complete organization network, asset pooling, quick adaptability, and controlled application are fundamental elements of distributed computing. Clients (individuals or associations) can request and control their machine administrations by means of on-request self-administration. The appropriation of administrations across the Web or confidential organizations is made conceivable by admittance to pooled assets. Bunch administrations imply that clients are gotten from different figuring assets, normally in far off server farms. Quick adaptability guarantees that projects can be changed pretty much. Item use is evaluated, and customers are charged appropriately [2]. Distributed storage has turned into a high level model of administration, the use of its advancements by clients (organizations, people, and so on) are limited because of worries over the absence of security of their confidential information.

To safeguard the entirety of the administrations and advantages presented by distributed computing and the Web, information security is fundamental. Information classification can be accomplished across the organization utilizing encryption innovation, which is encryption and unscrambling. Cryptography expects to give a security capability group that can guarantee the secrecy of the framework. These goals can be sorted into the five classifications recorded underneath [3]. Validation: The personality of the source and beneficiary should be checked prior to sending the message. Secrecy: Just approved clients can decipher the message, and no other person can utilize it. Trustworthiness: guaranteeing that the substance of the communicated information contains no sort of alteration. Administration dependability and accessibility: As gatecrashers impact administration accessibility to clients. The innovation ought to give clients the help quality they expect [4]. Non-renouncement: This capability shows that neither the shipper nor the beneficiary can reject that a specific message has been sent. As indicated by the connected work, present encryption strategies and the plan of cryptographic cycles offer the two benefits and impediments. Existing methods utilize a similar sort of generator to produce keys. A similar key is utilized for all information types. There is no example randomization for message encryption. Data is uninhibitedly open to outsider clients. Examples should be physically refreshed. To address these issues, this article work mean to plan another Lightweight Homomorphic Cryptographic Strategy for upgraded the security in cloud by involving the irregular example age for key and Sbox.

Related work

Distributed computing is a change in PC innovation. It tends to different regular figuring difficulties, including overseeing top burdens, downloading program redesigns, and utilizing repetitive processing spans. Distributed computing significantly affects each part of our lives as well as the market structure. In a distributed computing setting, information security is a central issue. A few exploration drives have been proposed to safeguard cloud information. The creator of [13] introduced a half and half cryptography approach for cloud security and protection





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that utilized both symmetric and uneven key procedures. This procedure will be utilized for encryption and unscrambling tasks with key creation. Very Circular Bend Cryptography has been utilized to upgrade information encryption in the cloud (HECC.). The designers of [14], made a half and half encryption hashing technique for distributed computing information stockpiling that forestalls information robbery. They utilized half and half calculation RSA, AES and hash capabilities to safeguard cloud information capacity utilizing a different information input size (34, 67, and 93 kb). The creators of [10] recommended a strategy for getting distributed computing client information utilizing encryption methods, they utilized a few calculations. They recommended various calculations to resolve issues connected with information misfortune, separation, and security. They assessed the exactness of R.S.A., D.E.S., A.E.S., and Blowfish calculations to information encode and unscramble in the cloud. The creators of [5] fostered a cryptographic technique to increment security information in distributed storage. As indicated by the method, information may be encoded prior to being sent to the server. The secrecy of the information given by the client is guaranteed in two ways the calculation encodes the information and just allows dynamic verification to get to it. In this calculation, the gave document will be encoded utilizing the A.E.S. calculation. The A.E.S. key is encoded utilizing the R.S.A. calculation. The made calculation encodes and stores the record in the cloud, though the suggested approach scrambles and stores the text in the cloud. The creators of [15] fostered a technique to increment cloud information security. They fostered a basic encryption technique. The techniques work in three stages. To start with, trade the keys. This stage is partitioned into two sections: key creation and key trade. The encoded information is saved in the cloud in the second information stockpiling. The client's third admittance to information is the point at which they demand information from distributed storage.

The proposed algorithm

This segment examines the proposed procedure, an original variety of cryptography calculation that consolidates two degrees of encryption to improve distributed computing security. The principal layer is another successful light-weight cryptographic calculation, in particular (N.E.L.C.) and the second layer multiplicative homomorphic property of the R.S.A. calculation. This approach offers both symmetric and deviated cryptography highlights, which further develops distributed computing security and protection. The accompanying subsections depict this calculation.

Description

Two degrees of encryption are converged in the proposed strategy. As represented in Fig. 3, the main layer is based on a symmetric-key calculation and (replacement/stage) underlying methodologies motivated by Feistal blended in with Shannon's hypothesis (dissemination/disarray) by the cooperation of coherent tasks, for example, (XOR, XNOR, moving, draining). The subsequent layer depends on deviated cryptography. This sort of encryption permits explicit types of calculations to be performed on scrambled text to come by an encoded outcome that matches the item when decoded tasks are finished on the plain text. The RSA calculation's multiplicative homomorphic highlight is created to increment information security in distributed computing. Also, the second layer technique adjusts RSA with homomorphic encryption calculation execution. The security of untrusted applications or frameworks is improved by utilizing a homomorphic encryption method. It changes over the information into figure text, which is then unscrambled [36]. It capabilities like it were still in its unique state. It empowers the execution of complex numerical procedure on encoded information. The development and calculated work process of the proposed calculation are introduced in Fig (1). Two cycles are accommodated the calculated showing, for example transferring and downloading of information from distributed storage; the main information input is scrambled and downloaded to the cloud through the encryption framework. Downloading information from distributed storage switches a comparable strategy. There are three stages for the proposed encryption calculation. 1. Key Age Interaction • First Layer • Second Layer 2. Encryption Interaction • First Layer • Second Layer 3.2. Encryption layer 1: the new viable lightweight cryptographic calculation To further develop distributed computing soundness while keeping up with superior execution and low handling. The new proposed calculation has carried out the fundamental Boolean tasks like XOR, Ex-NOR activities and sequencing. The calculation consolidates the Feistel structure highlights with Organization replacement change (S.P.) elements to expand the disarray and dispersion, further developing the encryption intricacy. The center thought of this calculation is to utilize a 8-16 byte (64/128-bit) block encryption and a 8-16-byte (64/128 cycle) key to encode the information in a symmetric key method, crypto adjusts are required; each





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round relies upon numerical capabilities to create dispersion and disarray. To guarantee the encryption interaction sufficiently able to satisfy gadgets norms, encryption methods are frequently intended to take 10 to 20 rounds overall. The proposed approach is limited to 7 rounds to further develop energy productivity, with each cycle requiring crypto including 32 pieces of information to execute. The security is high because of the utilization of both the S.P and Feistel designs. Besides, the blended activities in this calculation are given in a few logarithmic capabilities, including XOR, XNOR, F.function and expansion tasks to make intricacy for the aggressors. The methodology's itemized advances are illustrated underneath. 1) Key Development Block. 2) Encryption Block. 3) Decoding Block.

Key Expansion Block

The key is the basic part of the calculation during the time spent encryption and decoding. The dispersion and disarray methods used to create the proposed calculation fortify the key. The strength of key age brings about expanded security, better encryption intricacy, and diminished information on the key by aggressors. Two keys will be produced from the Key. The main will be utilized to unscramble the information, while the subsequent will be utilized as a section key in the key extension process. The key extension requires a 128-bit length, and the key is parted into seven sub-keys (K1, K2, K3, K4, KK1, KK2, SK). The extension key cycle is displayed in pseudo code calculation (1) and Fig. 2.

Encryption block process

The method utilized for changing over information from a recognizable kind into a justifiable structure utilizing dispersion and disarray systems is named cryptography. Unscrambling is switching information from a new organization over completely to a structure that clients can comprehend with power to do as such. The encryption block process is displayed in pseudo code (2) and Fig. 4. The calculation deals with seven rounds to decrease energy utilization. Each round depends on essential math to build the security rate. Each round's result is the contribution for the following round to get the code text (C.T).

F-function

The F-Capability approach comprises of various non-straight and direct activities that guarantee the complicated reliance of result bits on input bits [22,37]. This system, alluded to as confusion and dispersion, relies upon the P and Q values seen in found in Tables 2 and 3 and Fig. 3.

Decryption

Block process layer 1 The cryptographic calculation decodes the Ci code text block, which is like the cryptography methodology in Mi block. The Ci Block 128-bit is first partitioned into four sub-blocks, which are then modified utilizing a mix of XOR and Sub tasks with a similar working keys. Also, on the grounds that they are the backwards of the encryption system, it isn't crucial for spread out the particular stages for the interaction. The unscrambling block technique is portrayed in Fig. 5. The Pseudo code calculation (2) make sense of the Encryption cycle steps.

Encryption layer 2

Multiplicative homomorphic property of RSA calculation Homomorphic encryption is one smart thought that performs erratic calculations on information while never unscrambling it. In 1978, performing calculations on scrambled information was proposed by Ron Rivest, Adleman and Dertouzos to advance concealing the information. The limit of performing calculations brought about a few valuable applications. This likewise included reappropriating erratic qualities secretly in the cloud to store the resultant encoded text, convey calculations on encoded information and decode just when important. Homomorphic encryptions are of two kinds: completely homomorphic and somewhat or "to some degree homomorphic conspire". A totally homomorphic encryption plot takes into consideration possibly refined procedure on encoded information. It performs both expansion and augmentation procedure on encoded information. Fairly or to some extent homomorphic encryption conspire permits either duplication or expansion procedure on scrambled information, yet not both. In the field of cloud security, the utilization of homomorphic encryption is a significant achievement. It is feasible to re-appropriate





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delicate information with calculations on the cloud server by maintaining the mystery key for decoding [38]. A multiplicative homomorphic framework's encryption capability has the accompanying qualities: M1 and M2 are plaintext messages, consequently E(M1) *E(M2) = E(M1*M2). Information handling, distributed computing, electronic democratic, and monetary exchanges are only two or three minor applications for homomorphic encryption [7]. The RSA calculation is portrayed as an outline for duplicating homomorphic calculations in the accompanying depiction [39]. Encryption/Decoding cycle of Multiplicative RSA Calculation. • Age of the Key • Pick two enormous primes, p and q, to such an extent that $(p \neq n = p * q, \varphi(n) = (p-1) *(q-1)$, where φ is Euler's totient capability. • Select a number e to such an extent that $1 < e < \varphi(n)$ and gcd $(e, \varphi(n)) = 1$ (co prime). • d = e - 1 mod φ (n) • Public key ((e,n)) • Confidential key d • Encryption c = me mod n • Unscrambling M = Cd mod n The RSA calculation's multiplicative homomorphic trademark is as per the following [19]: Surrendered (1)

```
C1 = M_1^E M.O.D. N,

C2 = M_2^E MOD N

C1.C2 = E_{PK} (M_2). E_{PK} (M_2) = M_1^E M_2^E (MOD N) = (M_1.M_2)<sup>E</sup> (MOD N) = E_{PK} (M1.M2).
```

Simulation and implementation environment

Omnet C++ v6 was created to carry out a proposed calculation made in a confidential cloud Omnet introduced on the DELL Inspiron 13 7000 series on an Intel CoreTM i7-3120 M processor, 2.50 GHz 16G.B. Smash. Three gatherings are used to create mists with sufficient hub numbers (proprietors/clients) to show every hub. In iCanCloud few classes have been altered, specifically Power have, VMScheduler, VM, and Cloudlet Scheduler for recreation purposes to acknowledge dynamic asset assignment and class dynamic memory. Dynamic memory was recently added to figure the necessary asset. This class additionally refreshes virtual machine assets after cloudlet execution. Calculations have been carried out in MAT.LAB. what's more, applied in C++ utilizing the Dev. C++ program, as delineated in Figs. 6 and 7. The Various boundaries have been assessed, for example (execution time, throughput, key size encryption/decoding, length Code text, and security standard. In the accompanying section, an exhibition productivity examination is likewise completed for correlation.

RESULTS AND DISCUSSION

A few security tests are performed to confirm the calculation's quality, contrasted with other lightweight encryption calculations and current symmetric and unevenly key encryption strategies like A.E.S., D.E.S. also, Blowfish R.S.A., E.G.A.M.A.L. The examination of the two calculations is underneath. We tried different things with various document sizes in kilobytes (K.B.) as well as time gauges for encryption and decoding (milliseconds).

Evaluation parameters

Avalanche test

An essentially significant boundary for dissecting the encryption calculation's security (haphazardness). The torrential slide try is utilized to check the cryptography plan's awareness and to modify the underlying circumstances. This shows that a minor change in the encryption key ought to bring about an alternate code text. For this situation, the evaluation is thought of as ideal if half of the pieces are changed because of a solitary piece of change as indicated by the Severe Torrential slide Standards (S.A.C.) [40]. We decode the picture utilizing a vital that varies by the slightest bit from the right one to outwardly distinguish this impact. As found in Fig. 6 over, the proposed calculation can make countless pieces move. This might be credited to moving a solitary piece of picture/text or key pieces like an avalanche. The proposed calculation 128-cycle torrential slide is 51.55%.

Time complex

With a 128-digit key size, the aggressor would have to track down 2128 potential keys. Subsequently, the time intricacy of 2128 for getting the appropriate key is O by and large (1). As a general rule, the proposed calculation has a period intricacy similar to AES, yet it is more proficient since there are no a greater number of reiterations than





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A.E.S. also, the other comparable calculations. Execution time One of the most essential angles to consider while building cryptography is execution time. The general time taken to encode/unscramble the exceptional information is known as the encryption cryptographic execution time. This cryptography calculation was carried out in dev C++ and MAT. LAB. R2016a. The assessment test has done in the text in various size and on the grayscale picture "Peppers", "panda" of size 128*128, 256 × 256 The trial was performed multiple times as plain pictures Table 4 shows the typical opportunity to execute the encryption and decoding process for text and Table 5 for the picture. The tables demonstrate the execution time in milliseconds of identical calculations with different document sizes. Clearly the proposed calculation takes less time than different calculations. Throughput The throughput rate can be utilized to assess the calculation's viability. The calculation's throughput is straightforwardly connected with its presentation; the higher the exhibition/the higher the throughput. The recipe for working out the exchange pace of encoder

innovation is as per the following. Throughput = Plain Text Size/Encoding Time.

Statistical analysis

Picture Entropy Data entropy examination: the encryption calculation applies additional data to the subtleties, for example, the error between the first information and the calculation utilized, which is muddled for the aggressor. The entropy of a picture It's a clear boundary to assess the haphazardness of the scrambled picture. This boundary figures the distinction in entropy between the first and encoded picture. The higher the entropy adjustment, the better the encryption. The accompanying condition can be utilized to register the entropy. $E = \sum N \cdot 1 \cdot I = 1 \cdot Xi(\log 2(Xi))$ (1) Where "E" represents picture entropy, "X" represents the likelihood of every level of power in the picture, and "N" represents the all out number of levels of force in the picture. Picture Histogram lately, histogram investigation has used a boundary that portrays the haphazardness of the dissemination of encoded picture information. To fathom the typical change in the strength of the encoded data, the histogram of scrambled and decoded photographs relates with this action. The fundamental object of the histogram examination is to show the properties of encoded information vulnerability and dissemination. Fig. 8. Show the investigation of the histograms. Connection examination Relationship is a successful technique to assess out areas of strength for how cryptographic calculation is. In this review, the association between the encoded information and the first information is cs assessed. A powerful encryption approach will in a perfect world outcome in encoded information with 0% cross-over. The examination of relationships found in Table 5 and Fig. 9.

Comparative analysis

In this segment, relative examinations are directed to introduce and confirm the proposed calculation's practicality and depict the examination of the distinction between the proposed calculation, to be specific (N.E.L.H.C.) with a homomorphic, light-weight, symmetric and hilter kilter encryption calculation that is normally utilized for distributed computing security. The similar examinations separated into two areas portray the accompanying.

Comparative analysis of the proposed algorithm with a light-weight cryptographic algorithm

The primary segment was led, considering a few assessed boundaries ordinarily utilized for assessed the encoding and deciphering processes, for example, Block Size, execution time, Key Length, Conceivable Key, Numerical Tasks, Code Type and Security Power boundaries, as displayed in Table.

Comparative study of some symmetric and asymmetric algorithm

The proposed calculation was contrasted with a few symmetric and lopsided encryption calculations oftentimes utilized for security data in distributed computing in the second section. The similar review in light of assessed boundaries regularly utilized for assessed the Enc/Dec processes, for example, Construction, Key size, Block Size, Conceivable Key, Execution Time, Code Type and Security Power boundaries, as displayed in Table 7.

Security analysis and C.I.A. Achievements

The strength of any cryptographic interaction relies upon its manageability. The assailant generally endeavors to start various potential types of assault to think twice about data's secrecy. Specifically, it endeavors to with savage power assault, Frail Key Assaults, and Square Assault for figure parsing.





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Brute force attack

A beast force assault is the point at which a programmer endeavors to track down keys by using an elective space. As the space used by the switches is in a 16-cycle block multi-block structure, n is the space of n*2128-bit with n being the quantity of blocks. The keys can't be numerically estimated. In addition, regardless of whether one of these keys is trusted, the heaviness of 2128 expected to fabricate the leftover keys can't be assessed. Subsequently, the proposed cryptographic plan forestalls a savage attack forcibly.

Linear and differential cryptanalysis

Straight and differential assaults are totally ineffective when totally encoded. That is, the new f-capability works exactly the same thing in Ref. [30]. Assuming you utilize the straight guess for two adjusts; the proportion of info and result is major areas of strength for exceptionally. Round change is frequently kept up with consistently, equivalently treating any piece and contradicting differential assaults.

Weak Key Attacks

Encryption is viewed areas of strength for as the calculation produces solid encryption keys. The real key gives the encryption key. The genuine key to XORing safeguards against this assault before it is utilized. A similar applies to S.I.T [30]. also, figure [8], which has shown protection from feeble keys. Along these lines, the calculation utilizes F-Capability and afterward utilizes tasks (XNOR, XOR) to amplify intricacy (disarray and dissemination). The calculation, consequently, upsets this kind of assault.

C.I.A. Achievement

Secrecy This shows that unapproved people are involved when data is shared. The recommended arrangement lays out classification by encoding every single communicated substance and boundaries. Information respectability guarantees that no changes are made to the client because of addition, cancellation, or adjustment. All in all, albeit the information has been controlled, the recipient ought to offer explicit cycles to guarantee that new data is acquired. Information trustworthiness was accomplished in this approach by means of division. Accessibility This means to guarantee accessibility and access information to clients any place the need emerges. The proposed cryptographic calculations are speculative procedures and are in this manner useable consistently. It likewise upholds text designs for broadband information encryption and decoding without loss of data, without losing any plan where not lost any piece during transmission. The plan additionally tests the proposed calculation encryption utilizing the blank areas and unique characters sizes up to 10,000 plain text character.

The conclusions and further recommendations

Distributed computing has filled in ubiquity among organizations because of its far off availability, lower expenses, and quick re-provisioning. Despite the fact that clients are energetic about this new processing model, they are likewise stressed over the security dangers related with the cloud. There are as yet numerous obstructions to information security and protection that should be survived. Since the cloud gives adaptable and simple to-oversee admittance to information capacity, there is as yet the chance of unapproved assaults and pernicious exercises. As a result, new encryption procedures and strategies for upgrading distributed storage security should be gotten to the next level. In the proposed research, an Original Successful Lightweight Homomorphic Cryptographic Calculation contains two layers of encryption to improve information mystery. The main layer An original 128-cycle Lightweight cryptography strategy relies upon feistal and change/replacement structural cycle with Shannon's hypothesis of dissemination/disarray in view of the including of coherent tasks, for example, (XNOR, XOR, trading, moving) to work on the intricacy of the encryption which use in the primary layer of encryption and the subsequent layer is the multiplicative homomorphic property of the R.S.A. calculation for upgrading information security. The consequence of the proposed calculation outflanks another Lightweight Cryptographic Calculation. (HIGHT, Ocean, Drove., RC6, and NLCA) and a portion of the symmetric and hilter kilter calculation (AES, BLOWFISH, SIT, HOMOMORPHIC RSA, HOMOMORPHIC ElGamal) concerning the size of the ciphertext, encryption time, throughput, and security level. The proposed encryption calculation has tentatively approved for animal power, scrambled text just, knownplaintext assaults and differential cryptanalysis assaults. It's been placed to the test on various information, including





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whitespace and exceptional characters. The C.I.A. guideline is additionally met by the proposed encryption procedure. The proposed method may be carried out in equipment (cloud-based IoT) later on, bringing about altogether improved results.

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Table 1: common lightweight cryptographic algorithm







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Table 2 and 3: Relies upon the P and Q values

Table 2 (P) Table.																
Ke	0	1	2	3	4	5	6	7	8	9	A	1	Ċ	E	D	F
P(RC)	-3	Ŧ	E	0	- 5	4	В	0	D	A:	0	6	: 7	-8	2	1
Table 3 (P) Table. Er	0	i	2	3	4	5	6	7	8	9	A	В	c	ı	D	,

Table 4: Comparative analysis of the proposed algorithm with a light-weight cryptographic algorithm

Alpositim	HOTES	SEA [41]	TID let1	RC6 [72]	NLCA (25)	PROPOSED ALGORITHM
	symmetric	symmetric	symmetric	agranitis	Symmetric	symmetric segments
Sautur	Peirid	Petial	Feetal	Pettal	Pertial + SP	Festial + 50 + Multiplicative Homomorphic
Leve	1	1	1	1	1	2
Elect time	64 box	48, 96, 144 (6)	64 or 128	128 hes	126, or 256	32,64,128
Key star	125 bin	48, 96, 144 hi	5H or 128	128, 192, 256 bits	128,256	64.118
No. of Record	32	Verable:	Verselse	20	4	4
Penthir lay	2 th bits	2" 2" 0: 2" bis	2" 2" line	2 ¹³⁸ 2 to bin	2" 2" bits	272 200 2120
(krange Exc/Dec Erne (5)	24	42	29	2,63	1.89	166
Nodemous	Addition, subtraction, XXX, Surfreq. (8 bin)	30R, retation, 2n med publicon, substitution (8	XXX, potations, 2n mod addition,	Addition (2's exempl).	Shifting, Substitution (4	Stalling, Substitution (3) lists)
		hin)	abstatic (6-bts)	Variable function, 2006, (25 bits)	DID) NOR, SNOR,	NOR, MACR,
Security rate	Sear	Secare	Securi	Secure	Secure	Highly Secure

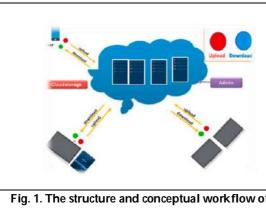


Fig. 1. The structure and conceptual workflow of the proposed model

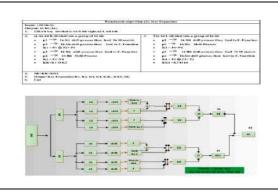


Fig. 2. Key expansion block process.



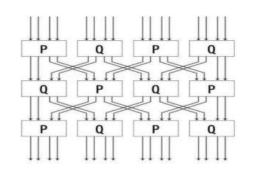


Fig 3. F function





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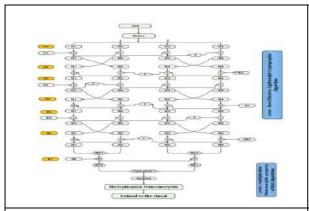
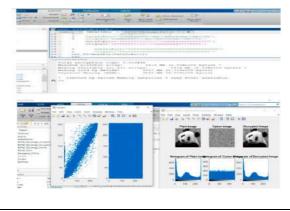


Fig 4. Encryption process

Fig 5: Comparative analysis with a light-weight cryptographic technique



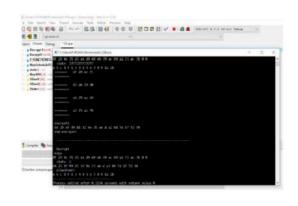


Fig 6: Implemented process in MATLAB

Fig 7: Implemented process in DEV C++



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RESEARCH ARTICLE

Embedded Technology based Automatic Agriculture Assistance System for Indian Farmers

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ABSTRACT

Prediction of paddy leaf disease is a pivotal aspect to obviate severe outbursts. The dominant diseases in paddies originated from fungi, bacteria, and viruses. Traditionally farmer visually checks the disease. Farmers by default can predict the diverse change in crops through inspection by perceptible recognition by means of prolonged agronomy encounters. But it is a complex assignment for the farmers to communicate their wide knowledge in agronomy to their ensuing engenders. The proposed work grants a perspective for accurate observation of diseases and hugely assists in preventing the paddy crop from diseases and loss to the farmers. Occurrence of diseases in paddy results in reducing the yield and income for the farmers. The major diseases in paddy occur in the leaves of paddy than in the other parts in paddy crop. Almost all the diseases roughly 95 percentage of diseases exists on the leaves of paddy inclusive of Brown spot, bacterial blight, Blast disease and Yellow dwarf on the Leaf. The proposed system elaborates the development of an Electronic gadget that proffers beneficiaries or farmers to recognize the paddy leaf diseases from the images of paddy leaves extracted from the electronic device. Recognizing diseases on the leaves of paddy crops at premature phases offers courage to conquer and assists, farmers, by providing the particulars of the diseases to the farmer so that they can take preventive measures to prevent the paddy crop from further attacking diseases. The disease detection system initially captures the image of the infected leaf and performs the pre-processing to extract features from the diseased crop. The system employs a K-means clustering algorithm for the segmentation of images which classifies the images based on the extracted features into K number of classes followed by





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classification performed with the help of an SVM classifier (Support Vector Machine) for accurate prediction of disease in crops.

Keywords: Paddy Leaf Diseases, Brown spot, bacterial blight, Blast disease, K-means clustering, SVM classifier.

INTRODUCTION

Paddy Leaf Diseases

Rice is the major cash and food crops worldwide, especially in India and in the countries belongs to Asian continent. Numerous aspects create immense impacting the production and yield of paddy crop [1]. These aspects result in less production and loss to the farmers who greatly demands on paddy as their major source of income. Out of which it felts difficult for the farmers to harvest the minimum target of about 08 tons per hectare. The maximum yield that a farmer can get is approximately about ten hectares from their farm fields. The causative agents like bacteria, fungus is responsible for diseases and less productivity. The disease in general is an anomalous growth that causes damage to the paddy crop. The diseases can be detected by the symptoms that are visible to the naked eyes of the farmers. In some cases, the disease occurs can be predicted by the farmers in the earlier stage as they occur in very few numbers and can spread hastily in minimum period of time and causes immense loss to the farmers [4]. These diseases can be predicted by farmers only by rapid keen inspection of farm fields. In reality there are large number of diseases that are occurring in paddy crops. The proposed work insists on six crucial diseases that greatly effects the productivity of paddy. Out of the six diseases the three diseases of paddy have more or less same symptoms. The diseases are Blast Disease (BD), Brown Spot Disease (BSD), and Narrow Brown Spot Disease (NBSD) and Bacterial Blight, Yellow dwarf and finally Sheath Blight. These diseases are responsible for great impact occurred in paddy [2].

Blast disease is the crucial disease occurs in growing stage of crops. The blast is a fungal disease. This disease occurrence rate is higher in the eastern part of the Asian continent. It also affects the paddy crops cultivated in the regions of high temperature worldwide. Blast disease is an aggressive plant disease caused by *Bipolaris oryzaes home* (Helminthosporium oryzae) (padwick 1950; Rice Doctor, 2003). NBSD, caused by the fungus Cercospora janseana, the disease becomes severe by years that is once the paddy reaches its full growth the disease will shows its severity. Most of the diseases effects the crop as soon as it reaches its mature state. The symptoms of blast disease include patches that are uncurled and Venetian red. For endangered variety of paddy, the contusion is extended, appears on the surface of leaves and is in mild brown with gray color with center part is in decayed state. Bacterial blight is because of Xanthomonas oryzae pv. oryzae (Rice Doctor,2003; OU 1985). It causes the seeds to droop and in inclusion the seed reaches its yellowing stage, the leaves are dried as a result of bacterial flight. The crop is unable to attain its normal growth and become yellowish at tits earlier stages are the symptoms of P. oryzaeare general chlorosis, pronounced as the one responsible for reducing the growth in crops and leads to adundant farming. Sheath blight is caused by the fungus Rhizoctonia solani, (OU, 1985; Sreenivasaprasad and Johnson 2001). In earlier stages patches are smaller in size, oval or in the shape of ellipse, the color of leaves turned from green color to grey and appears like immersed in water.

Harshal Waghmare *et al.*, [8]in the proposed work inspects the paddy and detect the diseases occurs in paddy. The images of the diseased paddy crop are captured by the farmers with the help of their mobile phone. The farmer has to take the detailed photograph of diseased paddy leaf and sent it to the experts so that they can detect whether the crop is effected by the disease. If the crop is effected by disease, then predict the type of the disease by which the paddy crop get infected. The image of paddy leaf requires to undergone the preprocessing to extract the features from the diseased leaf. Once the feature of the image is predicted then differentiating the diseased and normal paddy crop becomes easier. The proposed work makes use of decision support system for accurate classification of diseased crop. The features that are taken from the diseased leaf of paddy and the range in which the diseased leaf varies from the normal leaf are feed as an input to the decision support system [8]. The system can detect the leave based on the





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color shape and texture of the paddy leaf. The proposed work is designed to proffer input to the decision support system and the system can detect the disease and inform it to the farmer concerned to prevent he outbreak that occurs and loss to the farmers. The proposed work will inform the details of disease to the farmers through the GSM and mobile phone. H.M. Deshmukh *et al.*, design a system in [8] that will be able to detect the disease in paddy accurately and hasty and at the low cost from the symptoms that occurs in the earlier stages to prevent the losses of grains from paddy [6]. The proposed work employs machine vision techniques for gaining accuracy in prediction. The system can detect the disease automatically in the absence of human. The machine vision techniques in which the real time images of paddy leaves from the farm fields are fed as an input to it. The techniques employ deep learning to extract features from the various layers of deep neural networks and with the help of activation factors the detection is made with appropriate accuracy [6]. The proposed work employs the algorithms of deep learning to detect the diseased leaves of paddy. The details acquired from the convolution and hidden layers of deep learning to gain precision in detection of diseases in paddy.

Prakash M. Mainkar proposed a technical solution [6] for detecting and classify the various diseases in leaves of crops. The proposed work utilizes deep learning techniques especially neural network to categorizethe numerous diseases and in inclusion proffers suggestions for the recovery of diseases without huge loss [6]. The proposed work with algorithms of neural networks assists in improving crop yields. John William orillo in [10] work employs artificial neural networks for the effective detection of diseases in the leaves of paddy crop. The system employed uses back propagation techniques which in turn supports accurate classification of disease by adjusting the weight and bias. The artificial neural networks play a vital role in our routine [10]. The artificial neural network uses various layers like convolutional layers, hidden layers for classify the normal and diseased leaves with accurate results. P. Revathi M. Hemalathaet.al., employs edge detection techniques to predict the occurrence of disease in cotton leaf by segmentation used called homogeneous segmentation [3]. The work detects eight different types of disease that may occur in cotton plant. The diseases occur in cotton crops are Fusarium wilt, Verticillium wilt, Rootrot, Bollrot, Grey mildew, Leaf blight, Bacterial blight, Leaf curl. In this system the images of diseased cotton plant are initially captured with the assistance of neural networks the image attributes are extracted. The classification is made with the location of pixels especially the center pixel with the other pixel and the distance between them. The pixels and the distance between them are taken care by the homogeneity detector [3]. The edge detection and homogeneity segmentation can be used to classify the leaf as diseased or normal one along with the type of the disease in the eight diseases.

The results from the edge detection system is extracted and is used to get the homogeneity from the homogeneity detector. The system is more accurate and better one to detect the eight different diseases [3]. The proposed work is implemented in real time and its computational speed is high. It proffers low cost and instant result so as to prevent the losses that may occur due to the diseases in crops. In addition to this the proposed work offers suggestions to the farmers in inclusion to the type of the disease that occurs in paddy crops through the mobile phone of the person concerned. The farmers from the agricultural family can easily predict the diseases occurs in crops with the changes that occurs in crop when it is affected by the disease. Then the type of the image and the growth stages of crops in which the particular disease occur and the traditional ways by which they can prevent the losses. They are able to get high yield. In case if they went on gathers the details of the disease or the pest required to overcome the losses from disease and the techniques to increase the crop yield it is difficult because of the following. Finding the way to identify person practicing agriculture, in-depth experience in agriculture for a long time is difficult and at the same time they may available from the long distances. The pest required for better cultivation may or may not be known at that instant. But for the future agronomists the disease detection through perception is not much possible. It is taken care by the deep learning and the artificial neural networks. The proposed work employs K means clustering for extracting the attributes from the image captured from real time and the classified as normal or diseased one is made by Support Vector Machine (SVM) classifier.





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Proposed Work

The traditional methods of farming and prediction of diseases by human with experience in that is still practicing in farm fields. The innovative methods of farming and the usage of modern tools are also practicing in some areas. The disease detection is done by observing the crops on a regular basic. The farmers need to inspect the farm fields. The new generation farmers practicing agriculture has no knowledge of detecting the type of the disease that occurred. But the deep learning technique offering better results and accuracy in prediction of diseases is not yet implemented in a full-fledged manner. The one possible way is there in which the agronomist can sent the diseased crop for testing in the agriculture and research department for analysis the type of disease. But it is a time consuming process. Within that time there may be possibility for that disease to spread and cause huge loss. At present there are large number of diseases affecting paddy crops. Visiting the agriculture department for each and every diseases and pest is a waste of time and requires labor and causes transportation costs. Finding the reason for low productivity in farm fields by analyzing each and every factor is not possible at all. The proposed work will assist in overcome the problem of low productivity and detection of diseases with accurate results. It reduces the need for man power to detect the diseases and results in increasing the yields from farm fields.

METHODOLOGY

Image Acquisition

Image acquisition in which the real time image is extracted from the farm field with the assistance of mobile phone or camera. In the proposed work the image is gathered from the agriculture university, Coimbatore in Tamil Nadu, from the paddy farm in Dharmapuri district Tamil Nadu. The images from the farm field includes the normal and diseased images from the fields. The dataset extracted from the agriculture university consists of about 200 images including the normal and diseased images of all varieties. The gathered images are in jpg format.

Block Diagram

The block diagram for extracting the attributes from the images are shown in Figure 1. To draw out the attributes from the images, the images should be taken from the farm fields and the images takes out from farm fields are collected in large number and is fed as a database to the proposed work. The pretreatment in which the various attributes are taken out from the images as it is required to differentiate the normal and diseased images and to classify them based on the attributes obtained from the pretreatment process of images. The image preprocessing is not only to extract the attributes but also to intensify the image and to segment the image. Once all these process are done then the image get predicted as diseased or normal one. Initially the image is in RGB format that is red, green, blue format. The image is converted into grey scale image from RGB image to initiate the process of extraction of images. The proposed work employs K means clustering for takeout the attributes from images. The clustering method in which it curtains the green pixels so as to remove the green color pixels then segmentation is made by employing K means clustering. The features after getting the attributes of image and segmentation the classification is made by SVM classifier.

Image Preprocessing Resizing

To make the computation easier the size of the images are reduced to the required format by the process of resizing. The original image size is large and it requires more computational time. Resizing is pivotal because the images are not necessary to be in standard format. Once the resizing is done all the images are in the same size to make the classification accurate. The standard size will be 256X256.

Image Enhancement

Contradiction is a crucial factor in any form of intuitive assessment of quality of an image. Contradiction is important when going for human explanation. Contradiction is what it creates difference in brightness echoed in the adjoining areas. In addition, contradiction is the variation in perception that makes the difference between the





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images and from its background. From human perception the contradiction is nothing but the variation in color, radiance of images from other images. Naked eyes of humans are very delicate to contradiction than the normal luminance. To make the system over delicate to contradiction similar to human eye it is required to convert the image in RGB to HSV format. The image preprocessing is done after the conversion of image to HSV format. This format is preferred because it presents colors similar to human eyes.

Image Segmentation

Image segmentation in which the image is partitioned into several segments. The segmentation is required to make the computation easier. In computer vision, segmentation is the technique of dividing an image into numerous segments. The requirement of segmentation is to alter the presentation of an image into one that is to make the process of analyzing simple. Image segmentation to identify the locale of images and their boundaries. In other words, image partition is to provide a label for individual's pixels of an image so as to cluster them and to gather important attributes from an image. The image partition technique creates certain clusters from the entire image to get the extract attributes from an image. The individual cluster offers us a pivotal parameter from an image. The images are clustered by various attributes like color, texture and intensity. The different methods to segment an images are as follows.

- Thresholding methods such as Ostu's Method
- Color-based Segmentation such as K-means Clustering
- Transform methods such a Watershed Segmentation
- Texture methods such as Texture Filters

Color based segmentation

K-means clustering

The K-means clustering is employed for recognition of image upon the attributes extracted and clustered into K number of clusters. Categorizing the image is made possible by reducing the addition of the squares of the distance between each pixel and the corresponding cluster. The algorithm for K –means Clustering:

- Find the cluster centre for K number of clusters, based on its locale.
- Group the nearby pixels to the K cluster near to it so that the distance between the centre of K cluster and the pixel will be minimum.
- By iterative process, the cluster centers get updated after every iterative process by finding the average of all clusters.

Clustering is the process of separating each pixel into the clusters. After choosing the cluster center K-means clustering consider each pixel has a locale in that particular cluster. The segmentation partitions the image into multiple parts from which the clusters are identified based on the similarity and distance between them. The partitioned pixels are too close to its cluster center and are far away from the other clusters.

K-means clustering employed in our proposed work is in need of number of the clusters from the user. We need to mention the number of clusters and in inclusion the distance between the cluster center and the other objects in the cluster required from K Means Clustering.

Advantages Of K-Means Clustering

The proposed work employs the Lloyd's algorithm as it is the easiest algorithm to implement. The Lloyd's algorithm is powerful because it is able to work with the large number of datasets rather than its easy implementation. The algorithm used in the proposed work has numerous applications ranging from segmentation for market, embedded visions, and agriculture applications. Lloyd's algorithm is mostly used as a preprocessing before going for other algorithms. The various algorithm other than Lloyd's algorithms are edge detection, merging and split methods, compression, histogram, partial differential equations.





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Curtain Green Pixels

The proposed system employs curtaining of green pixels to infer the mostly green colored pixels. After which it is proceeded upon peculiar decided range which is evaluated for all pixels present in the image. The available green pixels are curtained as follows: The intensity of the green pixel in an image if it is lower than the prior mentioned decided range then the pixels of blue, green and red are considered to be zero. The reason beyond curtaining the green color pixel is that the green color pixel represents the healthy portion or the healthy leaves so which is not required while detecting the diseased portion that is why the green color pixels are curtained before identifying the diseased portion. The process of curtaining the green pixels assists in enhancing the computational speed.

Removing Green Pixels

Initially the image is in RGB format. For accurate segmentation and to obtain the attributes similar to human perception it is better to convert the image into HSV format. Next will be the masking of colors and removal of pixels of the colors includes the blue, red, green and zero value pixels from the image for accurate classification and in turn greatly reducing the computational time.

Attributes Extraction

Attributes draws out implies clarifying the aggregate of man oeuvre requisite for elaborating a huge set of data preciously. Investing a huge numerals of data are normally requiring a wide range of memory and computation speed and a suitable classification algorithm for recognizing the type of the disease. The algorithm employed for classification should coherence with the training dataset which discern imperfectly to latest dataset. Attributes draws out is an extensive word for procedure of profitable amalgam of the dataset to resolve the inaccuracy when going with the dataset with inadequate accuracy.

Glcm Methodology

GLCM Gray Level Co-occurrence matrix (GLCM) an acronym of GLCM is executed for every object map for HSV images of infected cluster.

Texture Analysis

For the effective calculation of hue of an image in the dataset the numerous textures of image inclusive of Homogeneity among the images, Contradiction in the image, Energy, shades of the clusters and Cluster distinction.

Color Feature Extraction

Mean

To find the average value of brightness of an image. In general, the image total brightness value can be predicted using the mean value of brightness of an image. The brightest image is assumed to have large mean value. On the other hand, the darkest image has least value of mean.

Variance

The reduction of noise in an image is the pivotal task to perform in case of image processing techniques. The variance deals with the contradiction of the image. Computation of variance is required for the effective classification of image.

Standard deviation

The standard deviation of image processing is nothing but the square root of the variance computed. The standard deviation deals with the contradiction of the image. It investigates the wide spread of data in image processing. The image with huge contradiction is predicted to have a high range of variance rather than the image with the low contradictions.





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Image Classification

The classification of paddy diseases as a normal or disease one is made by SVM classifier. If the paddy crop is diseased then the type of the disease is predicted by the proposed work. The paddy crop is classified as disease effected or normal from the leaf is paddy upon its wide range of outmost structure of the paddy crop.

SVM Classifier

The proposed work employs SVM (Support Vector Machine) for accurate classification of image as normal or diseases and in inclusion the type of disease which effects the paddy crop. In the proposed work it detects six different diseases that occurs in paddy crop. The input fed to the proposed work will be attribute vectors of the trained dataset and its comparable classes. The output from the proposed image processing technique will be the decision made by the Support Vector Machine to predict the type of the image that occurred in paddy. The selection of suitable kernel for classification of diseases in paddy is a crucial factor. The other important term will be the SVM classifier and its way of getting trained and tested with the assistance of kernel functions. The kernel function inclusive of Cauchy kernel, Laplacian Kernel and Invmult Kernel so as to distinguish the various result obtained from the different kernels. In order to investigate the execution of a Support Vector Machine as a classifier, transverse substantiate, the machine learning techniques with small numbers of training datasets are preferred. The transverse substantiate method is to segment the input datasets into large numerals of small datasets in order to train the classifier larger number of times to predict the better and accurate results. After every iteration of training the dataset the dataset removed from the iteration are used for the validation phase of dataset.

In order to evaluate the performance of the classifier, K means clustering in inclusion with K fold transverse substantiate is considered. Initially the dataset preferred for the classification of disease in paddy crop is split into k number of clusters. Then the transverse substantiate is take place with k-1 clustering for training the clusters and 1 of the subset is employed for testing the performance of the classifier. The process is repeated iteratively until the classifier performance is found satisfactory. The iteration is made K times to employ all the combinations of training testing and validation of datasets. The performance of the classifier is predicted by considering the aggregate of efficiency of Iterations involved. The algorithm execution estimate can be computed as the aggregate of the execution of fifty iterations. Preference of optimum specification of kernel functions is the pivotal factor to execute the better Support Vector Machine classifier. The accurate combination of excitable specifications for the dataset for classification is generally evaluated with a technique named grid search. The mentioned technique can be practiced by training, validating, with the assistance of SVM classifier for several iterations assisting inner k fold cross validation scheme within each training set. At the end of this step, a set of hyper parameters with the best validation results are selected. The supremacy of SVM includes, its forecast reliability is high. The operation of the SVM classifier is robust even if the training examples contain errors. Its simple geometric interpretation and a sparse solution like neural networks the computational complexity of SVMs does not depend on the dimensionality of the input space.

RESULTS AND DISCUSSION

The figure 2. shows the input image given for paddy disease identification. It is a image collected from Agriculture University, Coimbatore and also some from Dharmapuri paddy field. The image here is affected by Blast disease which is given as sample.

Recognition Result

The figure 3 shows the identified result for a given input image.

Performance Evaluation Of Svm Classifier

Table 5.1 shows the cluster shade and cluster prominence values for all the diseases mentioned above. From the table it is inferred that if the cluster shade range is between 0.525 to 0.628 it belongs to bacterial blight disease and it range





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is between 0.1002 to 0.1481 it belongs to blast disease and it is between 0.21 to 0.2835 it belongs to brown spot disease and it is between 0.11 to 0.201 it is for healthy leaf of paddy and finally if the range is between 0.302 to 0.412 it belongs to yellow dwarf and for the cluster prominence, if range is between 0.939 to 1.190 it belongs to bacterial blight disease and it range is between 0.200 to 0.380 it belongs to blast disease and it is between 0.4021 to 0.5082 it belongs to brown spot disease and it is between 0.982 to 3.457 it belongs to healthy leaf of paddy and finally if the range is between 0.829 to 0.999 it belongs to yellow dwarf.

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Table 1: performance evaluation of SVM classifier

PADDY DISEASE	CLUSTER SHADE (MIN)	CLUSTER SHADE (MAX)	CLUSTER PROMINENCE(MIN)	CLUSTER PROMINENCE(MAX)
BACTERIAL BLIGHT	0.525	0.628	0.939	1.190
BROWN SPOT	0.1002	0.1481	0.200	0.380
BLAST DISEASE	0.21	0.2835	0.4021	0.5082
HEALTHY	0.11	0.201	0.982	3.457
YELLOW DWARF	0.302	0.412	0.829	0.999







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RESEARCH ARTICLE

Understanding The Need, Content, Structure and Feasibility of Wearable Device in Knee Osteoarthritis: A Qualitative Study of Clinician's Perspective using Focus Group Discussion

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ABSTRACT

Knee is one of the most commonly affected joints in osteoarthritis of the lower limb. It is evident that several potential biomechanical alterations may occur due to changes in joint structure leading to altered weightbearing patterns and may contribute to further progression of the disease and vice versa. This study aimed to understand the clinician's perspective on the needs, content, structure, and feasibility of a wearable device for assessment, training, and monitoring of biomechanical deviations in knee osteoarthritis using a qualitative exploratory study design. Twenty-four physiotherapists having a minimum clinical experience of three years participated in one of the three focus group discussions. Participants who expressed their willingness to participate by filing the e-consent form were included in the discussion. Participants were in favor of using the wearable device for assessment, telemonitoring, feedback, activity tracking, and adherence to exercise. The appearance, comfort, and ease to use, cost of the device, and easy-to-understand user interface with local and cloud storage were the primary factors responsible for the acceptance of wearable technology among the participants. The device should assess and give real-time feedback to users for asymmetries in limb loading, plantar pressure, and spatiotemporal gait parameters. The study recommends for development of wearable technology to support assessment and training not only for osteoarthritis but also for other conditions where gait and balance are affected. It is essential to take into account clinicians' views to identify the clinical issues, features, functionality, and applicability for developing such devices.

Keywords: wearable, knee osteoarthritis, focus group discussion, design, structure.





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INTRODUCTION

Standing and walking is two important primary functional positions for performing activities of daily living, but are complex tasks for human as it requires a well-coordinated muscle activity. Bipedal motion in humans also known as walking requires a high amount of balance and stability along with the complex synchronized oscillatory movement of different joints of the body [1, 2]. The stability, balance, and motion of the body are facilitated by the oscillatory movement of the joints [3]. The synchronized activity of the neuronal system and the musculoskeletal system with the environment is required for a good standing balance and stable gait [4]. The gait pattern can get affected by various medical conditions. Any change in musculoskeletal structures of the lower limb and trunk results in abnormal limb load distribution that leads to the development of abnormal standing posture and gait pattern. Having a good static and dynamic posture, that is standing and walking respectively does not only reduce the burden on foot, ankle, knee, and hip but also enhances one's appearance. There can be several potential biomechanical alterations that may occur due to the changes in joint structure resulting in altered weight-bearing patterns and may contribute to development as well as further progression of osteoarthritis (Vincent et al., 2013). Occupation-related physical activities that involve frequent sitting and standing, prolonged standing or walking, and vigorous physical activity like in sports and athletic events predispose the individual for developing KOA at an early age [6]. Occupations that require prolonged standing or walking up and downstairs, put the knee joint under continuous compression which causes early degeneration of joint cartilage; whereas weight lifting puts additional load on the knee joints that further compresses the meniscus and may potentially damage the ACL and MCL which may predispose the knee joint to adapt to an altered limb loading pattern [7,8] that may further lead to development or progression of KOA [9]. The asymmetrical limb loading can further lead to altered kinetic and kinematic of lower limb which further enhances joint degeneration [9]. The increased tibiofemoral rotation and peak knee abduction moment during the weight-bearing positions are two important factors that are of significant importance (Zhao et al., 2007) along with limb load asymmetry and kinetic and kinematic assessment in hip and KOA [9] or any other disease and injury of lower limb.

Wearable devices that can assess patients' performance based on these parameters could potentially be used for providing feedback on their rehabilitation goals to them and their healthcare provider and maximize the benefit of care accordingly. The wearable devices being portable would allow patient monitoring and guidance while performing their rehabilitation program in their chosen environment reducing organizational barriers to exercise adherence. Although numerous wearable devices introduced in researches, the clinical adoption of such devices remains poor [11, 12]. Most of the studies conducted in this direction so far have primarily focused on the validation and use of wearable technology to analyze and compare the movement pattern among healthy and diseased population with only few provided feedback to patients based on acquired data. Researchers have primarily focused the technological aspects of such devices with lesser emphasis on clinician and patient preferences resulting in poor adaptation of such technologies in clinical practice [13]. For better implementation and adaptation of wearable devices in clinical practice, certain questions need to be addressed from a clinician's point of view like challenges with the current technologies, knowledge, awareness, attitude, likes, and dislikes, about wearable devices, interest in using wearable technology for monitoring their patient's rehabilitation from comfort of your smart phone at your home or clinic, features and parameters they would like to include in such a device for KOA, appearance, and duration of usage of such a device by their patient, and the expected cost of such technology.

Few studies have explored patients' preferences and identified that they would prefer to use a device that is small, easy to use and does not interfere with their activities of daily living (Papi et al., 2015). The preferences of both patients and healthcare professionals should be integrated into the design and development process of the technology for its adaptation into clinical practice that will ultimately benefit the patient. The questions stated above should be extensively explored during the early stage of the designing process. Therefore, this study adopted a user-centered approach for developing a wearable device to assess and guide patients with KOA in their rehabilitation training. For the better adaptation of the developed device in clinical practice, we discussed with physiotherapists





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about their views, preferences, and expected use of such technology. This study further explores the need, content, and structure for our prototype development.

MATERIAL AND METHODS

This study follows a qualitative study design using focus group discussion (FGD) to understand the needs from the clinician's perspective. Due to the prevailing COVID-19 pandemic, the FGD was conducted in the online mode via cloud meeting. The potential participants were purposely approached via email and personalized text message with an invitation to participate in one of the three FGDs along with an information sheet (Supplementary 1) containing all the information related to the project- investigator details, aims, and objectives of the discussion. All the participants provided their consent via Google form before taking part in the study. A total of 29 physiotherapists had consented to participation in the FGD, out of which 4 physiotherapists could not make it to the FGD, and the remaining 25 physiotherapists participated in one of the three FGD sessions conducted via online video-conferencing. Ultimately, 25 physiotherapists with a minimum experience of three years in treating KOA with good English communication skills volunteered to participate in this study. The duration of each discussion was between 90 and 120 minutes. Two moderators (AA and SR) facilitated the discussion based on a semi-structured topic guide pilot tested before the discussion session (Table 1), where AA moderated the discussion and SR took the field note and summarized the discussion.

Each FGD began with an introduction, clarifying the ground rules and format of the discussion followed by confidentiality assurance of the information exchanged. The aims of the discussion were thoroughly explained; the definition of KOA and wearable technology were provided. The discussion was then articulated following a semi-structured guide in Table 1. Each FGD session was videotaped and verbatim transcribed for analysis in later stage. Thematic analysis for each FGD was conducted at participant level using framework method for qualitative analysis [15]. Data were analyzed separately by the two moderators for cross-validating the outputs from each FGD before categorizing the results. Key thematic areas were identified from which concepts could be derived. These themes were used for comparing the responses among focus groups, and for data mapping and interpretation. The responses of physiotherapists were categorized in the different themes and concepts identified using Microsoft Excel spreadsheets.

Ethical Approval

All procedures were approved by the institutional ethical review committee of Paramedical and Applied Health Science, Lovely Professional University (LPU/IEC/2019/03/18). The study is reported in accordance with the COREQ (Consolidated Criteria for Reporting Qualitative research) Checklist (Supplementary 2).

RESULTS

A total of 25 physiotherapists participated in one of the three FGD sessions to reach data saturation. The participants included 15 male and 10 female physiotherapists with a minimum clinical experience of three years. However, their years of experience range from a minimum of 3 years to a maximum of 29 years, with a mean of 11.8 years of experience. The majority (23 out of 25) of the participants had master's degree in different specialties of physiotherapy, and two participants had a doctorate degree in physiotherapy (Figure 1). All participants were aware of the topic of discussion and were able to provide feedback, and explanations related to various questions raised in the discussion. The discussion with the focus group revealed certain repeated concepts as expressed by participants' views. The findings suggested three major interlinked themes associated with wearable technology in KOA: clinical issues, applicability, and features, and functionality is summarized in the coding tree (Figure 2). As per the aim of this study, that assesses the clinicians need of a wearable device in KOA all the themes were discussed in detail. The participant's quotes are reported along with the acronym FG followed by numbers 1–3 indicating the focus group's number, and P1-P8 indicates the participant number of the respective focus group they attended.





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Clinical issues

Physiotherapists associated various clinical issues related to KOA and its impact on prognosis. Their view expresses concern regarding the prevalence rate, economic burden, scarcity of assessment technology, delayed diagnosis, lack of preventive measures, and non-adherence to exercise related to KOA as summarized in Figure 3. All physiotherapists expressed a supportive attitude towards the need for wearable technology, although different views emerged on the issues they face clinically. For instance, some physiotherapists believed that although the

Prevalence rate is very high most of the patients visit their clinic at a later stage of KOA

- "Overall prevalence of OA in India is 20 to 30% as per National Health Portal of India." (FG1, P2)
- "With the changing lifestyle as we can understand, the prevalence is gradually increasing. The prevalence of OA range is between 22 to 30% of the population that is affected after around 60 years of age." (FG3, P4)

This creates additional economic burden on the patients as patients rely mostly on medications for their condition rather than exercises which ultimately results in total knee arthroplasty surgery

- "Around 303 million people suffer from OA globally and out of that 263 million are affected by KOA. And in Indian context, it's about 27- 28% of elderly have KOA. And in global population, around 4% of total population are affected by KOA globally." (FG2, P4)
- "There are some psychomotor component of this problem, women tend to comes out, seek any type of medication only when their sleep disturbances come because of the pain. When that level of issues raises, only then they come forward." (FG1, P6)
- "Actually, the prevalence rate is more among women than men, and they continue on self-managing their activities of daily living with the pain killer and all other modalities that are available in their house like with moist heat or everything." (FG1, P7)

On the other hand, few physiotherapists suggested that some of the patients do visit their clinic at an early stage of KOA as soon as symptoms sets in. However, these patients do not adhere to the exercises prescribed to them. When discussing about gait changes among the patients, assessment of kinematic and kinetic of gait emerged to be very crucial part of their assessment. However, different views emerged in terms of assessment of gait changes in clinical practice. Many suggested that due to unavailability of gait and motion labs in almost all clinical practice. In clinical practice, they mostly rely on observational gait analysis.

- "We are not giving importance to how much weight they are giving on the particular joint based on observation, we are not treating based on load. And the reason behind, why we cannot assess these things is the lack of the device, the labs in our clinics or hospitals due to its cost." (FG1, P1)
- "We assess while observing, but we cannot quantify. We do not check for limb load asymmetry in clinical practice." (FG2, P2)

All physiotherapists stated that only few government and private hospitals, and research organizations have such facility due to its cost, infrastructure and manpower requirements.

While discussing the major challenges they face in clinical practice, most of the physiotherapist suggested that the

major reason behind poor outcome in such patients is lack of awareness and preventive measure and scarcity of advanced assessment technology

"Even if the facility we are implemented, we have to get charges from the patient only. So, the patients here are also not ready to pay that much for doing the proper assessment. So, they want only symptomatic treatment. And, the reason for unavailability regarding the gait lab or the force platform is that it's too costly." (FG1, P7)

Applicability and features

All physiotherapists expressed a supportive attitude towards the need of wearable technology, however different views emerged on the applicability and features they wish to see in wearable technology as summarized in Figure 4. For instance, most of them suggested that the device should have real-time assessment and feedback capability.





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"It should be used for better assessments as well as the diagnostic and for therapeutic purpose." (FG1, P8)

"If patient know the progression of their activity, they will be more active, motivated and involved into active participation. Direct implication of this will be that it will give feedback to the patients in the way the patient follows the exercise." (FG2, P6)

Whereas, some suggested that the device should have activity tracking feature, and some suggested that it should have both activity tracking and Tele-monitoring feature.

"These things should have storage facility and linkage with their healthcare provider. So, if he is my patient, I would like to see his progression over the laptop or phone and it will be easy for us, especially for patients living in remote areas." (FG3, P2)

Some physiotherapists also suggested that the device should have its own smart phone application with user-friendly interface.

- "The result should be such that both an educated and uneducated people can also interpret and can use it for the biofeedback or self-assessment or to improve their limb loading." (FG1, P4)
- "The device can be a single user device or multi-user device. It should take less time for getting the correct measurements. And application should be easy." (FG2, P7)

A few physiotherapists also suggested that the device should have storage capability so that it can work as standalone device.

"It should be affordable for everyone. If any such type of device is developed, it should be affordable and should not be dependent on the internet. It can be used anywhere by everyone." (FG2, P6)

In addition, all the physiotherapists suggested that the device should be lightweight, portable, and cosmetically sound.

- "The device should be, I think, comfortable and should be small in size and lightweight." (FG2, P1)
- "The device should have qualities like it should be a small size and looks good cosmetically. Also, people are attracted to this type devices so, it should be easy to use." (FG1, P4)
- "I think design of these devices should be compact, cost-effective and can be easily charged." (FG3, P2)

Functionality

All physiotherapist expressed their views in terms of functionality of such wearable devices, however different views emerged on the functions they wish to see in wearable technology as summarized in Figure 5.

For instance, majority of physiotherapists suggested foot mapping and plantar pressure measurement as a key function they wish to see in such wearable devices as it will allow them to assess patient's foot arch and plantar pressure distribution within the foot and between the foot.

- "There should be foot mapping assessment, so that you can identify which area is getting more pressure- anterior or posterior, medial or lateral, and arch type." (FG 1, P3)
- "We can have something like heat mapping similar to what we have seen in other systems." (FG 2, P2)

In addition, some of the physiotherapists suggested that the device should have the ability to assess limb loading patterns which will allow them to understand how the load is being distributed on the foot during different gait phases.

"The device should have the ability to assess limb load asymmetry as asymmetrical limb loading is the primary response to pain in lower limb which leads to altered gait pattern." (FG 3, P4)

When discussing gait changes among the patients, assessment of kinematic and kinetic of gait emerged to be very crucial part of their assessment. However, different views emerged in terms of assessment of gait changes in clinical practice. Many suggested that due to unavailability of gait lab, they are not assessing it.





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"Assessment of joint angles, joint alignment, joint line, symmetry index, should be incorporated in the device." (FG2, P3)

However, one physiotherapist agreed that it is practically impossible to incorporate, as it requires 3-dimensional force data ideally from force plate and accurate motion analysis using reflective markers and motion analysis camera or wearable sensors. The assessment of spatial and temporal parameters of gait was an important area of concern among the physiotherapist. They suggested that the device should also have daily activity tracking and inactivity reminder function for fitness enthusiast.

"It should assess step length, stride length, cadence, joint range of motion." (FG 1, P2)

However, only one physiotherapist suggested that electromyography should also be considered for evaluating the muscle activation pattern during different phases of gait.

"It would be great if we see muscle activity or muscle activation pattern during movement." (FG 1, P5)

Many physiotherapists pointed towards having telemonitoring function in such wearable device if developed. It will be very useful feature for patients living in remote location, with poor connectivity, longer travel time to reach healthcare facility, weather, and lack of transport.

"For patients who live in remote areas, who cannot access physiotherapy services on daily basis, it will be helpful as most of India lives in rural areas. So, having tele function will allow us to monitor the effectiveness and efficacy of the treatment or intervention plan." (FG 2, P5)

When discussing the training feature, all the physiotherapists agreed on having feedback-based training function in such device for their patients. However, the type of feedback varied among them, some suggested that there should be auditory feedback, some suggested for visual feedback, and a few suggested for both auditory and visual feedback function in such wearable device.

- "It will enhance patient involvement, active participation, performance tracking, and self-awareness." (FG 3, P6)
- "Having real-time data will guide them whether they are going the right way, or is any change is required in the treatment." (FG 2, P2)
- "It will allow to monitor the effectiveness and efficacy of the treatment or intervention plan." (FG 2, P5)
- "The data can be utilized for future studies or research. It would be like evidence-based practice and it will give good feedback to the patients." (FG 2, P4)
- "Based on the usage, this device will help in all three aspects- diagnosis, prognosis, and treatment or intervention plan." (FG 2, P8)

In addition, a few physiotherapists suggested for incorporating artificial intelligence and machine learning modeling for predicting the disease development, risk of fall, and track their improvement over time. While discussing the usage of such wearable device in their clinical practice, all physiotherapists agreed on having two variants of device-one for the patients with lesser feature and one for clinicians with advanced features.

"I think you should develop two models- one for the patients' with limited features or features relevant to them, and one for clinician with more features with higher cost." (FG 1, P6)

Different views emerged among the physiotherapist in terms of cost of such wearable device, as some suggested that the price should be under Rs. 5000 and some suggested upto Rs. 10000 for patient variant with lesser features. For clinician variant with advanced features, some of the physiotherapist suggested that the price should be upto Rs. 50000, and only a few were willing to pay upto Rs. 100000 for such device when developed.



[&]quot;It should assess all spatial and temporal parameter of gait." (FG 1, P7)



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- "It depends on who is going to buy this device, for a clinician it can range between Rs. 10000 to Rs. 20000. But for the patient, it should be pocket friendly like Rs. 3000 to Rs. 4000, that is also too high for patients from villages who would prefer painkillers." (FG 1, P3)
- "I think on an average patient can pay Rs. 10000 to Rs. 12000. And for clinicians, it can be up to Rs. 30000 to Rs. 40000." (FG 2, P1)
- "For patients the cost should be really low with very limited features, and for clinicians it can range anywhere between Rs. 50000 to Rs. 100000 with all advanced features." (FG 2, P4)
- "For institutions like hospitals, clinics and academic institutions, the price can be Rs. 100000 to Rs. 200000. From patient's perspective it should be low because it bears additional cost of mobile plus Internet also. So, Rs. 5000 is maximum." (FG 3, P4)

DISCUSSION

The wearable sensors technology being portable and cost-effective and can be used inside or outside the laboratory setup and has gained popularity in recent times. For analyzing gait using wearable sensors, inertial motion sensors and pressure sensors are worn or attached to different body parts. These sensors may be a gyroscope, magnetometer, accelerometer, force-sensitive resistor, inclinometer, etc. to measure various parameters of gait [16]. This study suggests that the primary factors responsible for acceptance of wearable technology among the participants were appearance, comfort, and ease of use (Bergmann et al., 2012; Papi et al., 2015), cost of the device, and easy to understand user interface with local and cloud storage [19]. The clinicians suggested that these wearable devices should be small, lightweight, portable, discrete, and cosmetically sound such that it does not reveal the user's identity and allow them to use them with their usual outfit (Papi et al., 2016; Papi et al., 2015). In terms of duration of usage, the clinicians agreed to use of clinician variant of the wearable device for assessment and training purposes whereas the patient's variant can be used by the patients throughout the day for activity tracking while exercising or consulting with their clinician remotely. It aligns with clinicians' as well as patients' perspectives on real-time and real-life scenario data for assessment and the need for quantifiable data for rehabilitation and ongoing optimization of patient outcomes (Campbell et al., 2001; Papi et al., 2015). Depending on who, when, and how the device will be used, the assessment parameters will vary.

The usage of wearable technology as recommended by the participants would allow them in improving their clinical practice by supporting their rehabilitation goals. Further, the technology will be useful in the assessment, training, and telemonitoring of their patients during different phases of their rehabilitation in both their clinical setting and patients' living environment. In addition, they suggested that the real-time assessment and feedback will be useful in telemonitoring their patients' movement patterns, daily activity, adherence to exercise, active participation, prognosis, and guiding them virtually in real-time. These factors encourage the patients to actively participate in their rehabilitation and adhere to the rehabilitation program as prescribed by their clinician in reaching desired goals [21, 22]. The inclusion of telemonitoring has added benefits of saving time and money involved with frequent visits to the clinics. Also, the information derived from the system can be utilized for designing and modifying the rehabilitation plan, patient prognosis, identifying challenges or problems being faced by the patients, research, and evidence-based practice by the clinicians; and for tracking daily performance and improvement overtime by the patients.

The recommendation from this study has identified the need for wearable technology for clinical practice in terms of real-time assessment, training, and telemonitoring of patients. The design, content, structure, and features of such wearable technology depend on the perceived usefulness of the technology, stage of the clinical condition being treated, and clinical outcome measures to be considered; hence it should be considered while developing the technology. The transmission and storage of data on local devices and cloud servers should be addressed adequately while considering data privacy, to provide accurate real-time feedback and track user performance overtime based on parameters assessed. In addition, few physiotherapists suggested that incorporating electromyography would be





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very useful for assessing the muscle activation pattern during the activity; however, it may add challenges for the developers to incorporate all features in a single device. Electromyography has shown to be effective for assessing muscle activity and providing muscle retraining and biofeedback to the user in the rehabilitation phase in various conditions [23]. Hence, the use of wearable technology has great potential not only in enhancing patient performance and clinical outcomes in KOA, but also for improving clinical practices in terms of assessment, exercise prescription, rehabilitation, and patient prognosis.

The adaptation of wearable technology in clinical rehabilitation has been suggested to develop a patient-centric healthcare model in which the data related to patient performance will not only encourage their active participation but will also impact the delivery of treatment in the clinician practice and the development of new guidelines and recommendations based on the information generated through the technology [24, 25]. The design of wearable technology as proposed in this study by the clinician lies in line with the patients' perspectives as identified in previous studies (Papi et al., 2015). This study further addresses the need for literature for identifying the clinician's perspective towards wearable technology in clinical rehabilitation in terms of design, structure, features, and applicability. The previous studies have reported the use of wearable and portable sensors for acquiring patient health-related data in different health conditions without considering clinicians' and patients' perspectives to understand the clinical issues with the existing technologies. This focus group-based study further identified the clinical issues with the existing assessment technology as well as the clinician's preference for wearable technology in terms of features, functions, application, perceived usefulness, and cost of the proposed technology. Considering the economical background, the cost of the device was identified as major factor for non-adaptation of technology by clinicians as most of the clinician's work in independent clinical practice and they cannot afford it. The same stands true for the patients as well as majority of Indians live in rural areas and have poor economic status. The commercially available wearable technologies have failed to consider these issues despite having a well-structured product in addressing the challenges faced by the clinical in terms of assessment, rehabilitation and telemonitoring, and bridging the gap between patient's and clinician's need.

The proposed technology would not only allow the clinicians to assess their patient and plan and perform their rehabilitation with real-time feedback but also to diagnose the limb loading and gait changes at an early stage thereby preventing the development and further progression of the condition. In addition, the same technology can be used by the patients while performing their rehabilitation program based on real-time feedback provided via mobile computing application for correction and during daily activity tracking purposes as performed by most of the commercially available devices. It would also allow the patient to be actively involved in their rehabilitation, adhere to their rehabilitation program, and track their performance overtime [26]. Telemonitoring function would have an additional advantage over the commercially available technologies in situations like COVID pandemic, remote locations, etc. where traveling is not possible as it allows clinicians to monitor and provide feedback to their patient's rehabilitation in real-time remotely via cloud computing. The findings of this study are applicable for KOA or any other conditions in which gait and balance are affected for improving clinical rehabilitation including both assessment and training. Although the concepts are similar, the clinical outcomes may change; therefore, the technology should be developed based on the clinical presentation of each condition, and follow the clinical guidelines for the assessment of patient performance.

Unlike any other study, this study also had some limitations like, the FGDs were conducted via video conferencing which brings additional challenges of active participation, technical issues, and communication difficulty. The discussion could have been better they were conducted physically. There were no restrictions on participants' recruitment with regards to their clinical specialization. The participants in this study only represent the perspective of physiotherapists working in clinical setup in India except one from Malaysia, with 3 or more years of experience with a majority of them being male. Although the recommendations from the discussion were relevant to the objective of the study, future research should include physiotherapists as well as orthopedics, and physical medicine rehabilitation specialists working across the globe.





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CONCLUSION

This study identifies the clinicians' perspective towards the design, content, structure, features, and clinical application of the wearable device for KOA. The participants showed a positive and supportive attitude towards the use of wearable technology as a rehabilitation tool for assessment and feedback-based training. However, the recommendation from this study applies to any other health condition in which gait and balance are affected. The recommendation from this focus group-based study should provide guidelines to the researchers working in this direction to identify the need, function, features, design, and application of the wearable technology and maximize user acceptance. Hence, these recommendations should be taken into consideration in the clinical environment, and encourage developers and researchers to address patient's requirements to accelerate the clinical translation and enhance patients' benefits.

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Table 1. Semi-structured topic guide for focus group discussion

1. Introduction

- Moderators and participants introduce themselves
- Setting the ground rules and format of discussion and its aim
- Confidentiality Assurance

2. Knee Osteoarthritis

- Overview
- What is their opinion about knee osteoarthritis and its prevalence in our society?
- What is the most common complaint of patients with KOA from their perspective?
- Do they think the assessment of limb load asymmetry is important in KOA?
- How do they assess limb load asymmetry?
- What are the challenges with the current technologies?

3. Wearable technology

- Overview
- Ask if they know or use any wearable devices or have seen the demonstration of any developed prototype?
- Ask if they like wearable technology and if so why:
 - o Would you use it?
 - o How often would you like to wear it?
- Ask what they do not like about wearable technology and if so, why?
 - o What would put you off from using such technologies?
- Ask if they know of any alternative to wearable sensor technology?

4. Feelings about wearable medical technology

- How are they doing in general in dealing with KOA?
- Do they think wearable technology would help their patient's situations? If so, how?
- How do they view this technology in comparison to conventional forms of treatment? And why?
- Do they see themself using this kind of technology? If so, how?
- Would they use this technology for monitoring their patient's rehabilitation practice from their home rather than going to clinical practice?

5. Application





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- What features they would like to include in such a device for knee osteoarthritis?
- What parameters would you like to include in such a device for knee osteoarthritis?

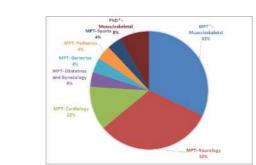
6. Impact on relationships

- If they did decide to use this technology, how do they think it would impact their clinical practice?
- Do they think it would change how they interact with patients?
- What are their views on data privacy?

7. Closing

- Is there anything else they would like to say about what we have discussed?
- What are their expectations from the new technology and its cost?
- Summarize the discussion
- Thank everyone for their time and participation

Note: This guide was followed in all three focus group discussions to identify the key thematic areas.



Master of Physiotherapy [‡]Doctor of Philosophy

Figure 1. Participant distribution based on area of expertise: This figure shows the level and area of expertise of the participants who attended the focus group discussion where 92 % of the participants have postgraduate degree in one of the seven specialties of

physiotherapy, whereas 8% of the participants have

doctorate degree in physiotherapy.

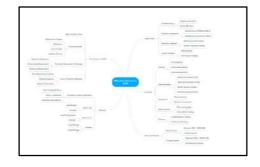


Figure 2.Coding tree for focus group discussions

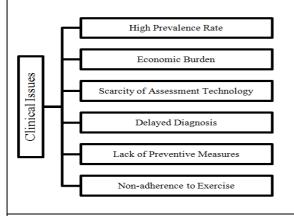


Figure 3. Clinical issues associated with knee osteoarthritis reported in focus group discussion

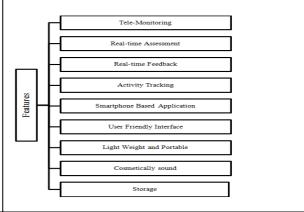


Figure 4. Features of wearable device identified in focus group discussion

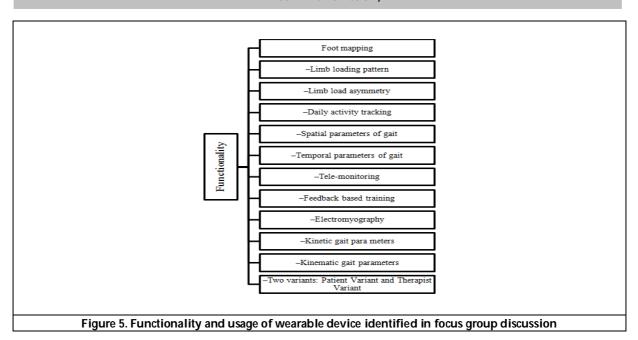




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RESEARCH ARTICLE

A Light-Weight Secure Authentication Model for Wireless Sensor **Network**

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ABSTRACT

A wireless sensor network is a self-organizing, distributed network used to sense and recognize the physical world. An appropriate safeguard should be implemented to secure data against unauthorized access, unlawful eavesdropping, and tampering. An authentication protocol is a widely used security mechanism that establishes a session key for communication parties to achieve safe data exchange by providing identity authentication and establishing a session key. Designing a functional authentication protocol for resource-constrained WSNs is a difficult challenge. This paper proposes a lightweight, secure authentication and data access model proposed for a WSN-based health environment. This method utilizes a pseudo-identity-based approach. It provides integrity and privacy for multiple patients with lower interaction and processing costs. The proposed method evaluated using communication and computation cost. The results show that the proposed method has low communication and computation cost.

Keywords: lightweight, security in WSN, cost

INTRODUCTION

WSN usually has several sensor nodes, one or more gateway nodes and a significant number of external users. The sensor nodes have limited computing capability and space for storage. They deployed to collect valuable information in unattended environments and to transmit the information to the gateways. External users can use the gateway to access the collected data. Data transmitted through the wireless channel is not secured. Consequently





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,an appropriate security mechanism would be taken to protect the data from unwanted entry, unlawful eavesdropping and exploitation. An authentication protocol is a widely accepted security mechanism [1] that offers identity verification and maintains a session key to ensure safe data sharing for the contact parties. Nonetheless, developing a functional authentication mechanism for resource-constrained WSNs is not an easy task. The hash function-based systems have high performance, but the reliability of the session key is difficult to guarantee. Sensor nodes are computing capable and have

limited storage space. They are gathering useful information and bringing it over the bus. Users may use the portal to view the data collected. Because data is transmitted through an unsecured and unprotected source, the transmitted data must be protected against threats such as unauthorized entry, illegal eaves-dropping and successful action tampering. The sensor network should be omnipresent in the future to make new technology or the atmosphere or systems safer. These include health insurance, sensor smart houses, environmental control, and more. Under these settings, anonymity, message confidentiality, and user authentication are essential since enemy messages can be detected, censored, or diverted. Due to resource limitation constructions, it carries several security threats in the wireless sensor structure, such as hardware manipulation, eavesdropping, injecting false messages, etc. Thus, it transmits to the network more efficient security mechanisms that conform to specific WSN functions. Symmetric cryptography is the most common encryption method able to provide encryption features[2]. When two nodes wish to communicate with each other in such security mechanisms, they use a shared key for encryption and decryption. The nodes have already chosen and exchanged this symmetric keys are produced is called key management[3].

Traditional communications are one-to-one forms of correspondence comprising only two people. The bulk of current app authentication schemes[4] only require two people: the prover and the other being the verifier. To verify the prover's identity, the verifier deals with the prover. A new authentication method is introduced, called group authentication[5], that can be used to discourage whether or not all members belong to the same party. The community authentication is very useful because all participants can be authenticated at one time. Nonetheless, group authentication can only be used as a pre-processing of user authentication, as group authentication can not decide who is not a member if there are non-members. The detection of non-members requires extra one-to-one user authentications. Many authentication protocols [1] [4] has been proposed to solve the security problems in WSN. Due to the minimal resources available, enforcing protection in WSN can be difficult, as resources highly consumed during data transmission. The following security specifications must be considered when developing stable and lightweight authentication and key agreement protocols [6]. Three-factor security: To protect the legitimate user's privacy, the protocol must satisfy three-factor authentication. Defending against well-known attacks: WSN's protocol must be protected against various threats, including smart card theft, masquerade, privileged insider, and MITM attacks. Mitigating sensor node capture attacks: Even though a malicious attacker captures specific sensors, it is difficult for the adversary to impersonate other sensors. Offline password guessing attack prevention: If a disruptive intruder intercepts transmitted messages or approaches smart card contents, the protocol must avoid thinking about the legitimate user's actual password. Prohibiting a stolen smart card attack: In this attack, it is believed that a malicious adversary will obtain the stored secret parameters on the smart card; hence, knowing the obtained parameters should not be enough for the malicious adversary to get helpful information to impersonate a legal user. Prohibiting vulnerable insider attacks: The protocol must be protected against privileged insider attacks, in which an insider with database privileges can access and misuse the hidden credentials. Anonymity and untraceability A destructive attacker cannot discover or monitor a genuine user's true identity. User authentication and key distribution: The method must successfully create a protected session key and mutually authenticate entities. Secrecy All messages sent between participants must be sent securely using a secret credential, allowing only approved participants to validate the message. The rest of the paper is organized as follows: Section 2 reviews the related work of various congestion control scheme. The proposed congestion aware network scheme is explained in section 3 and section 4 analyze the performance of the proposed work. Finally section 5 conclude the paper.





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Related Work

User authentication is a crucial security problem in wireless sensor networks because of their unattended and aggressive application in the field. Given that the sensor nodes fitted with minimal processing capacity, storage, and communication devices, authenticating remote users in such a resource-constrained environment are a major security issue. Impressive efforts were made to design user-anonymous authentication schemes using only the lightweight cryptographic primitives, such as symmetric key encryption/decryption, and hash functions. This section explains various authentication scheme in WSN. A lightweight and secure user authentication protocol based on the proposed Rabin cryptosystem in [7] which has the computational asymmetry characteristic. ProVerif is used to conduct a formal verification to show that our scheme meets the required security properties. This protocol is secure against all possible attacks and offers security features. Improved anonymous user authentication based on biometrics and the key agreement scheme introduced in [8], which also loaded with symmetric cryptosystem for WSNs. The benefit of using biometric authentication is to ensure a secure login from a legitimate person. A lightweight and privacy-conserving shared user authentication protocol suggested in [9] that only the user with a trusted computer is allowed to access the Industrial wireless sensor network. This scheme provides security, even if an adversary captures a sensor node. This protocol uses the lightweight primitives of cryptography, such as one way cryptographic hash function, physically unclonable function, and bitwise exclusive operations.

In [10], a selective authentication-based geographic opportunistic routing (SelGOR) proposed to defend against DoS attacks, meeting the authenticity and reliability requirements in WSNs. By analyzing wireless connection statistical state information (SSI), SelGOR leverages a confidence model focused on SSI to boost data transmission performance. SelGOR ensures data integrity by developing a selective authentication algorithm based on entropy and can isolate DoS attackers and reduce the cost of computation. A collaborative, cooperative authentication system intended to accelerate off offender isolation. This scheme, therefore, prevents repeated transmission of data and additional authentication of signatures arising from opportunistic routing. A TinySet-equipped Multiuser Dynamic Cipher Puzzle (M-DCP) suggested in [11]. This new approach ensures anonymity in the authentication of the multiuser WSN and the lightweight DoS resistance. The M-DCP, paired with the elliptical curve digital signature algorithm (ECDSA), used RC5 encryption. In [12], a blockchain-based IoT Multi-WSN authentication scheme suggested. Depending on their capability differences, the IoT nodes divided into base stations, cluster head nodes and ordinary nodes which formed into a hierarchical network. A blockchain network designed to build a hybrid blockchain platform from various types of nodes, including the local chain and the public chain. In this hybrid model, nodes identity mutual authentication is performed in different communication scenarios, ordinary node identity authentication operation performed by local blockchain, and authentication of cluster head node identity performed in the public blockchain. Lightweight anonymous authentication techniques for solving the black-hole attack issue associated with WSNs presented in [13].

The Medium Access Control (MAC) address is used in this scheme to register each node in WSNs with its nearest cluster head (CH) or base station module (s). The registration process is carried out in an offline step to ensure the validity of valid nodes as well as the base station. This approach solves the problem of black-hole attack because it is not feasible to register an attacker node for all gateway and adjacent nodes. Also, a hybrid data encryption scheme, integrated elliptic curve encryption standard and elliptic curve Diffie-hellman problem, is used to improve the authenticity, confidentiality and integrity of the data collected. A secure and efficient authentication protocol proposed in [14] focused on three-factor authentication, using biometrics. This protocol uses a technique called honey list to protect against brute force and stolen smartcard attacks. This protocol can provide security through the use of the honey list technique and three factors, even if two of the three factors are compromised. Anonymous access authentication scheme suggested in [15] to achieve competitive security capabilities for wireless sensor networks in Big Data Environments (AAA-WSN). The AAA-WSN scheme is not only capable of delivering solid security features such as user anonymity and shared authentication, but also has the ultimate forward confidentiality functionality with high efficiency. An effective and reliable authentication protocol proposed in WSN [16] for military applications with a context. The custom digital certificate used to demonstrate the legitimacy of the sensor nodes. In WSN, at the time of deployment, a node is preloaded with the public key of the base station; it requests and





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acquires its certificate from the base station and is then used by sensor node for shared authentication. Robust authentication scheme for WSNs, designed in [17] to provide security against known active and passive attacks. A new protocol for secure and efficient authentication proposed for multi-gateway communication in [18]. The protocol adds biological knowledge, which involves calculations for hash which XOR only. BAN-logic is used to demonstrate the security of the session key between users, sensors and gateway nodes and Real-or-Random model is used to formalize the new protocol 's security proof.

Proposed Methodology

This section explains the proposed secure authentication and data access model for WSN. The proposed work contains four entities: Sink/Base Station (BS), Gateway Nodes (GWN=GWN1, GWN2,...,GWNg), Sensor Nodes (SN=SN1,SN2,...,SNn) and Users (U=U1,U2,...,Um) Figure 1 shows the proposed WSN model.

Registration Phase

User Registration

The user registers their information with a remote server (Base Station) over a secure channel in this process. The user Ui chooses his or her own UIDi and PWDi at first. The Server looks to see if the UID has already been saved in a database. The registration process will begin if it does not occur in a database; otherwise, it will be stopped. The detailed steps involved in a user registration process as follows:

User Registration

Step 1: User Ui select User Identity UIDi

Step 2: Select Password PWDi

Step 3: Select Pin Number UPINi

Step 4: Select random nonce r

Step 5: Compute $UH_1 = h(r | PWD_i)$ and $UH_2 = h(r | UPIN_i)$

Step 6: Send [UIDiUH1 UH2] to BS

Step 7: BS receives [UID $_1$ UH $_1$ UH $_2$] and checks whether the UID $_1$ already exists or not. If it exits, send a rejection notification to U $_1$. Otherwise, BS store UID $_1$, UH $_1$ and UH $_2$

Step 8: BS select secret pseudo-identity UPID for User Ui

Step 9: Compute BH₁ = h(UID_i⊕UPID_i)

Step 10: Compute $BH_2 = h(UID_i | UH_1 | UH_2)$

Step 11: Compute $BH_3 = h((UH_1 \mid | UPID_i) \oplus (UH_2) \mid | UPID_i))$

Step 12: BS sends UPIDi, BH1, BH2, BH3 to User Ui

Step 13: The following parameters are stored in the smart card or mobile device by Ui [UID $_i$, UPID $_i$, BH $_1$,

BH₂, BH₃]

Sensor Registration

Sensor Nodes are randomly deployed in a grid environment. The sensor node is registered with the nearest Gateway node.

Sensor Registration

Step 1: Randomly generate unique SID and nonce sr

Step 2: Generate SH₁ = h(SIDj⊕sr)

Step 3: Compute $SH_2 = h(SH_1 \mid \mid sr)$

Step 4: Compute $SH_3 = h(SID_j \mid \mid sr)$

Step 5: Sensor SN_j send $[SID_jsr\ SH_1\ SH_3]$ to nearest $GWID_k$

Step 6: GWIDkselect secret pseudo-identity SPID for Sensor SNj

Step 7: GWID_k compute $GH_1 = SH_1 \oplus h(SID_j \mid \mid SPID_j)$

Step 8: GWIDksend [GH1SPIDj] to sensor





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Gateway Registration

The steps for Gateway registration

Gateway Registration

- Step 1: Randomly generate unique GWID and nonce gr
- Step 2: Generate GH₁= h(GWID_j | | gr)
- Step 3: Gateway GWNk send [GWIDkgr GH1] to BS
- Step 4: BSselect secret pseudo-identity GPID for Gateway GWNk
- Step 5: BS compute $BH_1 = GH_1 \oplus h(GWID_k \mid \mid GPID_k)$
- Step 6: BSsend [BH1GPIDk] to Gateway GWIDk

User Authentication Phase

When U_i wants to gain access to WSN, the user login process is used to verify the user's credentials and prevent a denial of service attack. U_i first inserts a smart card into the card reader and enters UID_i and PWD_i. The user computes the authentication information and then compares it to the previously stored data. After authentication, the legitimate user is granted access.

User Login

- Step1: Uinserts smart card to the authentication device and provides the UIDi, PWDi and UPINi
- Step 2: Compute $UH_1^* = h(r | PWD_i)$ and $UH_2^* = h(r | UPIN_i)$
- Step 3: $BH_3^* = h((UH_1^* | UPID_i) \oplus (UH_2^*) | UPID_i))$
- Step 4: Verify UH₁≈ UH₁* and BH₃≈ BH₃*
- Step 5: Check if the BH1 matches the BH_3 that the BS has saved in the SC. If the SC is not fulfilled, the login request is terminated. Otherwise, the verification stage is successful, and the SC considers the U_1 to be a genuine user.

User Password Change

When U_i wishes to change his or her old password to a new one, this procedure is initiated. U_i places the smart card in the card reader and enters UID_i and PWD_i to change the password. The first step in this process is to use the user authentication process, and the second step is to change the old PWD_i to the newPWD_i, which the verified Ui can do.

User Password Change

- Step 1: U_i sends the request to BS
- Step 2: Uperform the user authentication process
- Step 3: Uiinput new password newPWDi and newUPINi
- Step 4: Select newurand recompute newUH₁= h(newur | | newPWD_i) and newUH₂= h(newur | | newUPIN_i)
- Step 5: Regenerate newBH₁, newBH₂, newBH₃
- Step 6: Store newly generated values in SC.

Mutual authentication is a function that ensures that all authentications are successful. To create a secure communication channel for transmitting information between them, entities should authenticate each other. The BS represents the trustworthy entity and the link bridge that allows the GWN_k and SN_j entities to authenticate one another in this scheme. The term "user anonymity" refers to two distinct features: (1) user identity protection, which means hiding the user's identity to prevent an unauthorized entity from learning the user's true identity; and (2) user untraceability, which means the unauthorized entity cannot tell that the user is who requests access to the sensor node or which sensor node the user has accessed. As a result, an unauthorized party would be unable to establish if the same person carried out the two authentication sessions. Sensor node anonymity is a critical security feature for a variety of applications. In healthcare applications, for example, the sensor node identity is used to represent the patient's identity.





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SIMULATION RESULT

This section describes the proposed work's efficiency in terms of communication and computing costs. With the suggested experimental setup, the proposed work was simulated and tested using MATLAB (R2014b). The time spent in the network by the user and the Server is referred to as computational costs. The number of messages sent by the user during the process is known as the communication cost. Table 2 shows the bit length of authentication parameters

Communications cost

The cost of communications is determined by the size of the authentication messages exchanged between entities during authentication. Table 3 shows the communication cost comparison.

The following are the comparison results in terms of communications cost

- o Both Xiong *et al.* [21] and Nashwan [22] have five authentication messages, while Lu *et al.* [19] and Jung *et al.* [20] schemes have four, and the proposed work has only three messages.
- o The scheme Lu et al. [19] have a high total communication cost compared to other methods.
- o Among other authentication schemes, the proposed method has the lowest communications cost.

Computational Cost

In terms of computing costs, this section compares the proposed scheme to the authentication schemes proposed by Lu *et al.* [19], Jung *et al.* [20], Xiong *et al.* [21], and Nashwan [22]. According to the operations performed in each authentication entity, this function is computed for all authentication schemes.

The following notations are used to simplify the computations cost analysis:

- o Th denotes the hash value computation time;
- \circ $T_{\text{E/D}}$ denotes the running time of encryption/decryption functions
- o Th is ≈ 0.00032 s, and the Te/D is ≈ 0.0056 s, as stated in Xiong et al. [21].

The results of the computations cost analysis for the proposed as well as related authentication schemes are summarized in Tables 4 and 5. Table 4 depicts the costs of computations in each authentication entity of authentication schemes, while Table 5 depicts the overall costs of computations of all authentication method. The results show that, compared to other authentication schemes, the proposed scheme has the lowest overall computations cost.

CONCLUSION

This paper explains an effective authentication scheme for WSNs to provide appealing and efficient security services. Robust security features of the proposed method include anonymity for both the user and sensor node entities, complete mutual authentication between all authentication entities, and absolute forward secrecy in all authentication stages. This paper uses a light weight one-time hash chain and pseudonym identity for authentication. Compared to recent WSN authentication schemes, the performance analysis in terms of total computations cost and total communications cost shows that the proposed method achieves a high degree of security while maintaining a reasonable efficiency level.

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Table 1 shows the notations and symbols used in this chapter

Notation	Description	
Ui	i th User	
SNj	j th Sensor Node	
GWNk	k th Gateway Node	





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UIDi	Identity of ith User	
PWDi	The password of ithUser	
UPINi	Secret Pin number of ithUser	
UPIDi	Pseudo-identity of ithUser	
SIDj	Identity of j th sensor	
SPIDj	Pseudo-identity of jthSenor	
GWIDk	Identity of kth Gateway Node	
GPID _k	Pseudo-identity of kth Gateway Node	
h(.)	Cryptographic hash function	
	Concatenation Operator	
\oplus	Exclusive-OR Operator	

Table 2 Authentication Parameter bit length

Parameter	Length in bits	
Identity	32	
Password	32	
Random nonce	256	
Hash value	160	
Encryption block size	128	
Encryption function	128	
Decryption function	128	

Table 3 Communication Cost Comparison

Method	No of message	No of Bits
Lu et al. [19]	4	3840
Jung et al. [20]	4	2624
Xiong et al. [21]	5	2208
Nashwan [22]	5	2592
Proposed	3	1440

Table 4 Computation Of authentication entities

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Method	User	GWN	Sensor Node
Lu et al. [19]	7Th+2TE/D	8Th+4TE/D	4Th+2TE/D
Jung et al. [20]	5Th+2TE/D	5Th+2TE/D	4T _h
Xiong et al. [21]	9Th+2TE/D	11Th+2TE/D	4T _h
Nashwan [22]	4Th+2TE/D	10Th+2TE/D	4T _h
Proposed	2Th+2TE/D	2Th+2TE/D	-

Table 5 Total computational cost analysis

Method	Total Function	Cost/s	
Lu et al. [19]	19Th+8TE/D	0.05088	
Jung et al. [20]	14Th+4TE/D	0.02688	
Xiong et al. [21]	24Th+4TE/D	0.03008	
Nashwan [22]	18Th+4TE/D	0.02816	
Proposed	4Th+4TE/D	0.02368	

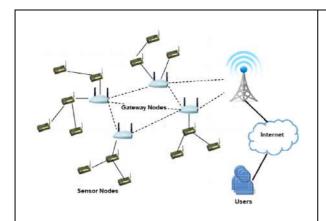




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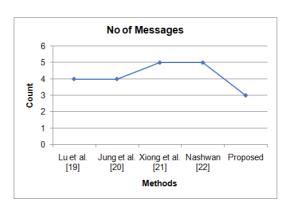
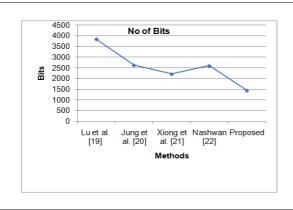


Figure 6 General WSN Model

Figure 2 shows the number of communication message comparison of different methods.



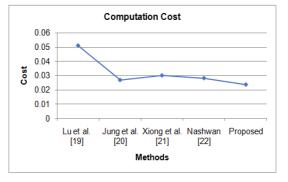


Figure 3: shows the comparison of the number of bits required for different methods

Figure 4: shows the comparison of computation cost.





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RESEARCH ARTICLE

Evaluation of Insecticidal Activity and Phytochemical Screening of Azadirachta indica Leaves Extract

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ABSTRACT

This work is aimed at evaluating the phytochemicals and insecticidal activity of Azadirachta indica leaves. The qualitative phytochemical tests exhibited the presences of common phytocompounds including tannin, saponins, flavonoids, steroids, terpenoids, anthroquinone, polyphenol, glycosides, coumarins and alkaloids were present in both extract aqueous and ethanol of Azadirachta indica leaves. Significant amount of flavonoids (17.85±1.54 mg/gm) and phenol (185.41±5.39 mg/gm) were presented in Azadirachta indica leaves. The insecticidal activity clearly revealed that maximum (85% in 100mg/kg) and minimum (15% in 20mg/kg) insecticidal activity was recorded in Azadirachta indica leaves extract in 24 hours.

Keywords: Azadirachta indica, qualitative, quantitative and insecticidal activity.

INTRODUCTION

The protection of stored grain and seeds against insect pests has been a major problem from the development of agriculture. Plant products have been successfully exploited as insecticides, insect repellents and insect antifeedants. Several plant extracts, volatile oils and compounds have been reported as effective fumigants and repellents against many stored product pests (Jahromi et al., 2012). The use of chemical agents to prevent or control insect infestations has been the main method of grain protection, since it is the simplest and most cost-effective means of dealing with stored product pests. Thus, there is an urgent need to develop safe alternatives to conventional insecticides and fumigants for the protection of grain products against insect infestations. There are increasing efforts to understand





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indigenous pest control strategies, with a view to reviving and modernizing their use (Belmain *et al* 2001). Plants are a rich source of secondary metabolites with interesting insecticidal activities (Dubey *et al* 2008; de-Fátima *et al* 2006).

MATERIALS AND METHOD

Collection of plant materials

The leaves powder of *Azadirachta indica* were purchased in April - 2022 from Country Medical Shop, Thanjavur district, Tamil Nadu, India.

Preparation of extract

1 gram of the powder of Azadirachta indica leaves were transferred in to different conical flask (250ml). The conical flasks containing 50ml of different solution (ethanol and water). The conical flask containing Azadirachta indica leaves were shaken well for 30 minutes by free hand. After 24 hrs, the extracts were filtered using Whatman filter paper No.1 and filtrate is used for further analysis.

Phytochemical screening

Chemical tests were carried out on the extract using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973 and 1984). Total phenols estimated by the method of Edeoga *et al.*, (2005). Flavonoid determine by the method of Bohm and Kocipai-Abyazan (1994).

Insecticidal activity

Collection and authentication of insect

The unsexed adult red flour beetles (*Tribolium castaneum*) were collected from grain storage room at Thanjavur, the insect was identified and authenticated by Dr. M. Sukumaran, Assistant professor department of zoology, Rajah Serfoji government college (Autonomous), Thanjavur - 613 005. The adult beetles were used for the bioassays.

Insecticidal activity against red flour beetle (Tribolium castaneum)

Insecticidal activity assayed by Sadeghi *et al.* (2016) with mild modification. The *Azadirachta indica* leaves *extract* were applied (spiked) to 10g of wheat flour, by mixing to give 20, 40, 60, 80 and 100 mg/kg. Controls for each set of treatments consisted of flour treated without sample. 20 unsexed adult *Tribolium castaneum* was introduced into the petri plate containing the treated and untreated flour. The petri plate was covered with cotton cloths held with rubber bands. The number of dead insects in each petri plate was counted after 24 hours and the percentage insect mortality was calculated by following formula. % of mortality = Number of Observed mortality / Total exposed \times 100

RESULTS AND DISCUSSION

In developing countries, the traditional use of plants and plant derivatives for the protection of stored products is long established. There are major opportunities for the development and use of less refined botanical insecticides for domestic and agriculture use in these countries (Fischer *et al.*, 2013). A number of plant substances have been considered for use as insecticides, antifeedants or repellents, which include terpenes, flavonoids, alkaloids, phenols, and other related compounds (Adeyemi, 2010). Plants are naturally gifted at the synthesis of medicinal compounds, whose characterization has led to the discovery of new, cheap drugs with high therapeutic potential (Ukwuani *et al.*, 2013). In the present study, the phytochemical characteristics of the *Azadirachta indica* leaves investigated tannin, saponins, flavonoids, steroids, terpenoids, triterpeniods, alkaloids, anthroquinone, polyphenol, glycosides and coumarins were present in both aqueous and ethanol extract. A significant quantity of flavonoids (17.85±1.54mg/gm) and total phenol (185.41±5.39mg/gm) are present in *Azadirachta indica* leaves (Table 1 and 2).





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Insecticidal activity

Insecticidal activity of ethanol extract of *Azadirachta indica* leaves were studied at different concentrations in 20, 40, 60, 80 and 100 mg/kg. Insecticidal activity of solvent extracts was calculated based on adult mortality after treatment. High adult mortality normally indicates potential insecticidal activity of plant extracts. In the present study irrespective of concentrations used for the insecticidal activity varied significantly. Data pertaining to the insecticidal activity clearly revealed that maximum (85% in 100mg/kg) and minimum (15% in 20mg/kg) insecticidal activity was recorded in *Azadirachta indica* leaves extract in 24 hours (Table 3). The LC50 value were 58.85 mg/kg for *Azadirachta indica* leaves extract respectively (Figure 1). Foster *et al.* (2002) determined an LC50 value of 0.42 to 2.8 mg/l towards first instars of *M. persicae* after 96 h, when tested at a range of 0.080 to 30 mg/l in a leaf disk dipping bioassay. The respective LC50 values for pymetrozine were 2.3 and 27 mg/l for the BC12-01 and WA19 clones of *Aphis pomi*, when tested in a leaf disk test. It should be marked that the latter values were somewhat higher than our results; however, toxicity may depend on the aphid species clone used (Lowery *et al.*, 2006). Similarly, our study reported LC50 value was 58.85 mg/kg for *Azadirachta indica* leaves extract.

CONCLUSION

The experimental studies demonstrated that the grain protection potential of *Azadirachta indica* leaves extract in rice against *T. castaneum* proved by insecticidal activity. The potential insecticidal activity of *Azadirachta indica* leaves extracts might be present in bioactive compounds. *Azadirachta indica* could be considered an ideal grain protectant from the point of view of seed viability and safety to mammals.

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Table 1: Qualitative phytochemical analysis of Azadirachta indica leaves extract

S. No	Phytochemicals	Ethanol extract	Aqueous extract
1	Tannin	++	++
2	Saponin	++	+
3	Flavonoids	++	++
4	Steroids	++	+
5	Terpenoids	++	+
6	Triterpeniods	++	+
7	Alkaloids	+	+
8	Antroquinone	++	+
9	Polyphenol	++	+
10	Glycosides	++	+
11	Coumarins	++	+

⁽⁺⁾ Presence, (++) High concentrations and (-) Absences

Table 2: Quantitative analysis of Azadirachta indica leaves

S. No	Phytochemicals	Results (mg/gm)
1	Flavonoids	17.85±1.54
2	Total phenols	185.41±5.39

Value expressed as Mean ± SD (N=3)

Table 3: Insecticidal activity of Azadirachta indica leaves extract against red flour beetle (Tribolium castaneum)

Concentration	Total exposed 10 insect	24	Hours
(mg/kg)	in 20g flour	# of mortality	% of mortality
20	20	3	15
40	20	7	35
60	20	10	50
80	20	14	70
100	20	17	85
Control	20	Nil	Nil
LC₅ (mg/kg)	58.85		



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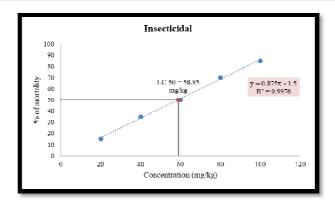


Figure 1: Insecticidal activity of Azadirachta indica leaves extract against red flour beetle (Tribolium castaneum)





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RESEARCH ARTICLE

An E-Learning Platform for Medical Education based on Internet of Things and Agent Based Systems

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ABSTRACT

E-learning is a formalized teaching-based learning system that makes use of electronic resources. This can be done by the use of computers and the Internet, even if instruction can take place in or outside of classrooms. Intelligent based learning systems have become a more realistic choice in this technological age for a variety of users from beginners to gain information to specialists to stay current in learning. In this paper, intelligent agents and Internet of Things were combined in order to improve the medical education. Specifically, all the advantages of particular technical fields and elements of learning methods such as IoT and Agent based systems in E-Learning, application of E-Learning in medical education, IoT based classroom, Agent based systems for Examinations and its Evaluations were described in detail. This paper also explores the applications of agent and IoT based techniques for providing insights to the users and enabling them to plan, using the resources especially for the specific challenges in medical education.

Keywords: Intelligent agents, Medical education, Internet of Things (IoT), learning methodologies

INTRODUCTION

One of the key goals of learning environments is to improve student achievement and happiness. Instructors must accurately assess the many competences of learners, which might obviously vary in terms of depth of awareness, motivation, cultural status, and level of enthusiasm in order to be able to personalize the classroom instruction based on the requirements and preferences of each individual [2]. A skilled teacher in a regular classroom is cognizant of





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the pupils' varied personalities and aptitudes for learning. Furthermore, due to classroom size and the accuracy of the teacher's assessment process, there are restrictions on how much a teacher may modify the educational atmosphere to concurrently instruct every learner as best as possible [3]. A reduced classroom sizes also would help educators to concentrate on the requirements and preferences of each student individually, which might improve the precision of understanding and analyzing such traits [4]. One-on-one teaching is more likely to lead to a better teaching and learning efficiency than group instruction, according to studies [5-10]. It could be challenging to give such care and instruction in conventional schools, though. E-learning [1] can be thought of as an E-learning approach in which directions are created or prepared to assist learning and then distributed to the recipient via electronic devices, which typically take the form of laptops or smart phones [11]. There are two different ways that e-learning may be created. One way is as a teacher - directed method of instruction called as synchronous e-learning, and the second is as a self-paced independent study called as asynchronous e-learning. The educators in adaptive e-learning have the freedom to choose the date and place where they wish to complete one's own teaching in addition to the work rate at that they do so [11-18]. This is because the curriculum research uses being spoken published writings which are presented as drawings, images, animations, or videos as learning resources. Real-time teacher-led teaching, sometimes referred to as live e-learning, is the other teaching strategy that is intended for teaching to be provided or guided by a teacher to take occur in real time [19], [21]. This kind of e-learning material is typically supplied in real time, primarily over the Web, and uses a variety of communication techniques. In order to communicate with the lecturers, individuals who are participating in the training typically log on at a predetermined time [20]. Due to the lack of communication, it seems that all these e-learning contexts might be online or offline the same issues identified in classroom learning, therefore, this indicates that the evaluating procedure cannot be properly incorporated between the lecturers and the students. Additionally, the medical based learning courses are provided and created for all students without taking into account their particular requirements and skills [21–24].

Following are the provocations that are still under consideration from the perspective of E-Learning platform for medical education

- 1. A deeper comprehension of how agent- and Internet of Things (IoT)based learning techniques is applied in medical based learning.
- 2. Useful learning management strategies based on IoT and agent application. In order to determine the best learning method, this should be done.

Related Works

A known effective tool for overcoming IoT issues is the Agent-Based Simulation Model (ABSM) [16]. It stands for a suitable and reliable simulation methodology to successfully address these issues and help the development of the IoT paradigm [17]. The incorporation of ABSM with cloud infrastructure in an IoT environment was proposed by Fortino and Guerrieri [18]. Decentralized multi-agent platforms were supported by ABSM, and the cloud architecture improved IoT devices by giving them access to large amounts of information and sophisticated computational capabilities [18]. A modified ARM Microprocessor is used in the IoT-based Smart Education Environment system. This approach is employed for managing instructors, resources, and participation. The learner's or visitor's position was monitored using ID cards and bracelets. The automatic parking technology, adaptive message queue, etc. are also covered by this smart class framework. [5]. A smart seat in a smart classroom was acquired through an RFID surveillance system using a different technology that uses a touch-based interface and cloud-based architecture backup system. These platforms are offered in each seat in the classroom so that students can engage with a notepad and take notes while attending to the lesson. It also makes it simpler for them to comprehend and manage resources. This recent technical development caused changes to the educational system. [6]. In order to maximize instructional utilization, the application of IoT and Intelligent systems was documented in a Smart Campus. The system comprises sensor technologies for tracking class attendance for all campus lecture rooms. The system's functions include live occupancy tracking, gathering attendance data for 250 programs spread across individual hours, identifying completed exams, and canceling lecture hours. For predicting attendance, it also uses All approaches. The system uses a mechanism to determine the best distribution of students throughout classrooms





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IoT and Agent based systems in E-Learning

User experience, authentication, user interaction, voice system apps and its classification, regulating the presence that used an enrollment program, hand motion instructions, etc. are all included in IoT and agent-based e-learning. Through the portal, Al-based systems handle interaction between various devices in a smart classroom as well as between the user and the consumer [34]. The placement of a gateway node between the gadgets illustrates how the architecture of the AI-based technology in education is built on an IP network, requiring that each classroom utilize its own private IP system in order to manage the equipment. Different researchers have produced a variety of works in the area of learning based. Item Response Theory (IRT) and IoT were combined to create a unique IoT based E-Learning architecture [35]. They displayed adaptive tests and personalized IoT devises in their platform. These architecture function as a instructor which leads the students through a well-organized and individualized online based learning strategy, adding intelligence and adaptability to the learning environment. An ANN technique was proposed by authors in [36] for determining the learning preferences of students [37]. In order to obtain scoring from previous datasets and define advanced functionality using agent based systems, were proposed in [38]. This model is used to measure the output of student grades using intelligent agents. Authors in [39] created a methodology by anticipating the new students in an e-learning plat using a success predictor and employing the ANN and Agent based systems. According to their technique, the students were evaluated electronically with a structured questions consisting of 25 questions, each of which had a level of difficulty consistent with the pedagogical approach. At the conclusion, each evaluation was recorded in a special registry. Their approach gives each learner, for the lifetime of the online class, statistical information related to the assessment process.

E-Learning in Medical Education

E-learning is a cutting-edge method of instruction. It outlines a new framework for education that enables students to learn in settings that are more productive [4, 31–33]. Additionally, it provides a customized technical learning atmosphere through the use of computer-based solutions. Overall structure of E-learning in medical environment is shown in **figure1**.

Figure1.

This learning based on offers the following features for the medical education.

- (i) It focuses on content of medical education and its learners based on advanced computing-based technologies
- (ii) It is an intelligent based effective and tailored learning methodology based on advanced infrastructure of IT.

Internet of Things

A recent Internet breakthrough that links the virtual and real worlds is known as the "Internet of Things." With the help of any network, the IoT enables connections between anything and everyone as depicted in Figure 2. In addition to the time, distance, and system aspects that enable everyone to interact at any moment and from anyplace over any system, it has added a new perspective to information and communication system. We now have a new direction of methods that enables us to communicate to anything that can be networked, tracked, and labeled.

Figure 2 Proposed Methodology IoT based Classroom

The smart workplace, confidentiality, and preservation systems offered by IoT-based cloud computing methodology support teaching, scientific research, employee and student governance, attendance monitoring, class work or assessment monitoring, online invoicing, and the recovery of misplaced books, laptops, or required objects. Smartphones and PDAs can be used by teachers and students to facilitate learning. Offline vs. online videos If a student skips a class, they can still participate in lectures online. The system's equipment is made up of numerous sensor modules with wired and wireless connection and the software system processes data from the sensor and stores it in the cloud. The foundation of a cloud network is made up of smart networking devices like gateways, routers, switches, and WiFi routers.





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Agent based systems for Examinations

In order to develop questions for each of the enrolled students, the examination agents are in charge of creating and overseeing exams. After the student has provided his response or the allotted time has expired, each examination Agent responds to the server node with the student's response. Each answer modules are created by the assessment agent and sent to the assessment server.

Agent based systems for Evaluations

The evaluation agent is in charge of assessing the responses, compiling the data, and publishing the findings. The evaluation server looks at the sort of replies provided by the response agent to determine if they are accurate or qualitative. If it is the correct response to the subjective question, the answer agent advances to the computer-based mechanism that evaluates the objective inquiry. If the question is of the subjective variety, the answer representative will speak with the relevant authorities who will evaluate the subjective issue until all responses have been evaluated. The response agent then supplies the scores to the outcome system after analyzing the response.

Proposed model based on the IoT and Agent based systems based Smart Learning in medical education

The proposed model includes three key components, including user learning data from various devices, Al and agent-based frameworks and smart learning-based tools. **Fig 3** displays the overall architecture of the proposed framework.

Figure 3.

The whole schema of assessing various formats information derived from different learning system. The fusion of the AI and NN-based systems processes this data. Two components part of the AI-based system were information management module and the document management tool. Main aim of the proposed system is to employ the AI based technology for processing the user's inputs. It is also used as a platform for the input management, report management, and data analysis tool. The AI based report management module obtains, analyze, perform and trigger the action by, classifying the user input using the free text approach. In the learning database, all the analyzed sources have been stored and modified. This module comprises of multiple features, such as learning-based information storage and a tool for data processing. The data processing tool collects the input information, processes it into datasets and sends it to the report management module based on AI. AII the queries and sources that were handled are provided as feedback to the smart learning system. This model improves the overall accuracy of intelligent learning because the AI and learning methodologies were combined.

Limitations

In addition to the special advantages of this agent and IoT-based medical learning, there were also a few drawbacks that needed to be considered: a sizable portion of the population, the framework really cannot be too accurate, it had to rely solely on technology, and there were numerous confidentiality and security problems. Access to an intelligent learning-based architecture is denied in regards to a sizeable percentage of the populace as well as due to the size, their difficulty to obtain it, and their sluggish expertise and skills to apply it. The agent-based system can't be too exact because that would leave individuals and its presumptions behind. These applications have simplified the learning process, but they cannot take the place of instructors. Additionally, no program, no matter how advanced the technology, could ever be 100% accurate. These clever learning techniques frequently enable a user to become totally dependent on them. If the user forgets their user ID or password, all of their data could be lost temporarily or permanently. It's possible that there will be issues with both the secrecy and security of the data it contains. It is possible for malevolent users to alter the information in such circumstances.

CONCLUSION

E-learning can be characterized as an incorporated learning-based technology system that combines techniques for delivering instructional contents to medical students with software functionalities that communicate through





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computer-mediated means. A similar accomplishment for the intelligent-based system of education is the deployment of agent-based systems and IoT in medical education. Agent-based platforms and IoT-based learning techniques can be used to enhance the current medical instruction methods. These surroundings offer personalized services to enhance the learner and faculty expertise by engaging with the learning system and the medical student. In this paper, a detailed review of various applications of intelligent agents and IoT based learning methods used for medical student is done. Specifically, all the advantages of particular technical fields and elements of learning methods such as IoT and Agent based systems in E-Learning, application of E-Learning in medical education, IoT based classroom, Agent based systems for Examinations and its Evaluations were described in detail. Applications of various models intelligent agent based systems and IoT in medical education is presented. A novel agent and IoT based architecture to provide intelligent teaching strategy for medical education is also proposed.

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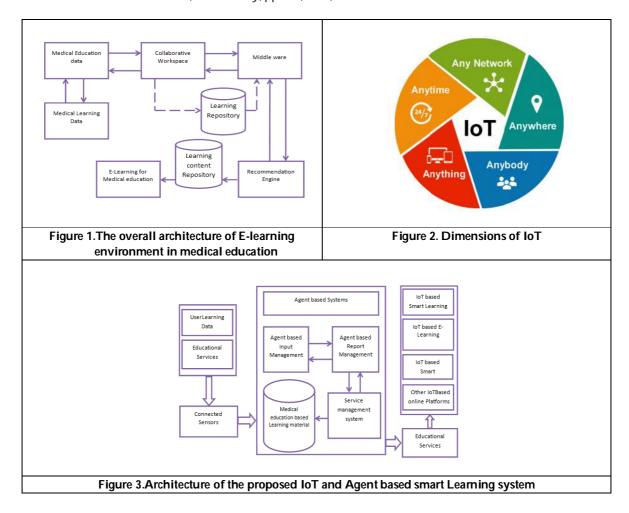


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RESEARCH ARTICLE



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Microwave Synthesis of 2,3-Diphenylquinoxaline using O-Phenylene Diamine and Benzil and Evaluation of Antioxidant Antibacterial Activity

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ABSTRACT

Microwave technique is a new, simple and efficient technology which opens new prospects to the chemists, organic synthesis opens up new opportunities to the synthetic chemist in the form of new reaction that are not possible by conventional heating and serve a flexible platform for chemical reaction. In this aim of present study synthesis of microwave mediated 2,3-Diphenylquinoxaline using o-Phenylene diamine and Benzil, to evaluate the anti-oxidant and antibacterial activity. The antioxidant activity of 2,3-Diphenylquinoxaline was based on concentration depended (R2 = 0.99) and potential antibacterial agent in our study concluded, on the advances in the developing of innovative application.

Keywords: Microwave technique, 2,3-Diphenylquinoxaline, antioxidant, antibacterial.

INTRODUCTION

Organic synthesis is one of the most important driving forces towards life support and sustainable development. It provides the essential tools for building up all the molecules that help humanity to preserve health, to produce and protect crops and foods, and to improve the quality of life. Organic compounds give rise to all forms of life, and understanding the nature of their transformations is essential for the interpretation of natural phenomena and to work out life-supporting systems. As we shall see organic synthesis offers powerful and continuously evolving





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methods that act in this direction. As the population raises and health troubles also increases and requirement to investigate new drugs development is more desire. The heterocyclic organic compounds are extensively disseminated in natural and synthetic medicinal chemistry and are vital for human life. There are various biologically active organic compounds are clinically active and several of which are in usual clinical practices (Finar, 2009). Arrival of microwaves (MWs), the magnetron an unexpected appliance for forming fixed-frequency MWs, was considered by Randall and Booth (Harvey *et al.*, 2001).

Microwave (MW) irradiation method is providing fairly booming in the development of a diversity of hetero organic compounds. It is a process for the synthesis of various organic compounds. It is an essential tool in the directions of green chemistry, which is well- known as an existing guide in preparation of various organic compounds. The conventional methods of synthesis require more heating time, complicated and tiresome equipment set-up, which outcome in more cost and also cause ecological pollution. The modern transform taken place to make standard chemistry tests, eco-friendly and with growing alertness about green chemistry, developed simple methods for synthesis of various organic compound using MW oven as a heating resource (Singh and Kapoor, 2008). In the aim of present study synthesis of microwave mediated 2,3-Diphenylquinoxaline using o-Phenylene diamine and Benzil, to evaluate the anti-oxidant and antibacterial activity of 2,3-Diphenylquinoxaline.

MATERIALS AND METHODS

Synthesis of 2,3-diphenylquinoxaline compounds using Microwave Technology 2,3-diphenylquinoxaline synthesized broom temperature and the product the method of Shrinivas *et al.*, (2013). A mixture of o- phenylene diamine (1.1gm; 0.01M), Benzil (2.1gm; 0.01M) and ethanol (16ml) were taken in a 100ml conical flask. After covering with a funnel, the mixture was irradiated with microwaves at 60% (540W) intensity for 55 seconds. A beaker containing water was placed in the oven next to reaction vessel to serve as a heating sink. Then reaction product was cooled to room temperature. Separated product was filtered and recrystallized from ethanol. Synthesized 2,3-diphenylquinoxaline further application used for anti-oxidant and anti-antibacterial activity.

In vitro antioxidant activity using DPPH radical-scavenging activity

DPPH radical-scavenging activity was determined by the method of Shimada, et al., (1992).

Determination of antibacterial activity

The antibacterial activity was performed by disc diffusion method (NCCLS, 1993; Awoyinka et al., 2007).

Microorganisms

The microbial strains employed in the biological assays was *Escherichia coli*, (MTCC 732) for bacteria. Obtained from Microbial type culture collection (MTCC) at the institute of Microbial Technology (IMTECH), Chandigarh, India.

RESULTS AND DISCCUSSION

The use of microwaves in organic synthesis was initially hampered by a lack of understanding of the basic principal of MW heating and the inability to obtain reproducible results with domestic microwave oven. With microwave heating, the energy can be applied directly to the sample rather than conductively, via the vessel. Heating can be started or stopped instantly, or the power level can be adjusted to match the required (Jain and Singla, 2011). The interest in the microwave assisted organic synthesis has been growing during the recent years. Drug companies are exploiting microwave in the area of organic/pharmaceutical synthesis for drug screening and discovery. Microwave heating is also called as green chemistry and the development of cleaner technologies is a major emphasis in green chemistry. Among the several aspects of green chemistry, using efficient and less hazardous energy sources such as microwave energy is recommended (Barchin *et al.*, 2002). In the present study to synthesis the 2,3-Diphenylquinoxaline. A mixture of o-phenylene diamine (1.1gm; 0.01M), Benzil (2.1gm; 0.01M) and ethanol (16ml)





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were taken in a 100ml conical flask. After covering with a funnel, the mixture was irradiated with microwaves at 60% (540W) intensity for 55 seconds. A beaker containing water was placed in the oven next to reaction vessel to serve as a heating sink. Then reaction product was cooled to room temperature. Separated product was filtered and recrystallized from ethanol and collected in 2,3-Diphenylquinoxaline (figure 1) to evaluation of antioxidant and bacterial activity. 2,3-Diphenylquinoxaline are an important class of nitrogen containing heterocycles with variety of biological activities. It is a low melting solid and is miscible in water. It is a weak base and can form salts with acids. The synthesis of 2,3-Diphenylquinoxaline has been extensively studied for the last two decades. A very primitive and effective method to derive quinoxaline is the condensation reaction between ortho phenylenediamine and dicarbonyl compounds (Irfan *et al.*, 2017; Pereira *et al.*, 2015). Microwave heating produces heat in entire material in the same rate and the same time at a high speed and at a high rate of reaction. Microwave assisted synthesis has become an important tool to the medicinal chemist for rapid organic synthesis (Rajput *et al.*, 2020).

In vitro antioxidants activity of 2,3-diphenylquinoxaline using DPPH radical scavenging activity method

Antioxidants play a significant role in several important biological processes such as immunity, protect ion against tissue damage, reproduction and growth or development. They preserve adequate function of cells against homeostatic disturbances such as those caused by septic shock, aging and, in general, processes involving oxidative stress (Prakash et al., 2011). DPPH, 2,2-Diphenyl-1-picrylhydrasyl, is a dark-coloured compound composed of stable free radical molecules. The purple colour of DPPH decays in the presence of antioxidants. The change in absorbance at 517 nm in the presence of antioxidants can be equated with the antioxidant potential of the compound. DPPH radical scavenging assays are based on the reduction of DPPH molecule by a hydrogen donor antioxidant and the colour of the solutions change from purple to yellow (Brand-Williams et al., 1995). The 2,3-diphenylquinoxaline maximum observation was 100 μg/ml in 78.31±0.56 of inhibitions (Table 1). The half inhibition concentration (IC₅₀) of Ascorbic acid (48.64 µg/ml) and 2,3-diphenylquinoxaline (66.86 µg/ml). The reducing abilities of the examined compounds were determined by their interaction with the free stable radical DPPH at three different concentrations at 20, 40, 60, 80 and 100 µg/ml, respectively the 2,3-diphenylquinoxaline exhibited a significant dose dependent (R² = 0.99) inhibition of DPPH assay activity (Figure 2). Prakash et al. (2011) studying novel Schiff bases of isatin were synthesized by condensation of imesatin with different aromatic aldehydes. The imesatins were synthesized by reaction of isatin with p-phenylenediamine. These compounds were screened for antioxidant activity by DPPH radical scavenging activity. In this method, the compound 3-(4-(4-dimethylaminobenzylideneamino) phenylimino) indoline-2-one (5c) showed highest antioxidant activity because of the presence of electron donating group. Similarly synthesized compound of 2,3-diphenylquinoxaline exhibited a significant dose dependent in % of inhibition DPPH assay method.

Antibacterial Activity of synthesized 2,3-diphenylquinoxaline

The *in vitro* antimicrobial activity of the 2,3-diphenylquinoxaline against these *E. coli* bacteria strains was qualitatively assessed by the presence in zones of inhibition (mm: millimeter) represented in the photographic figure 3. The inhibitory activity in culture media of the 2,3-diphenylquinoxaline reported in 50µl (2.65 mm), 100µl (5.10 mm) and 150µl (9.80 mm) while comparable with standard antimicrobiotic viz. chloramphenicol (12.10 mm). Similarly, Saeed *et al.* (2008) reported in synthesis of some new 1-aroyl-3-(substituted-2-benzothiazolyl)-thioureas. The synthesized compounds were assayed *in vitro* for their antimicrobial activity against Gram positive and Gram negative bacteria and were found to exhibit moderate to potent activity towards the tested microorganisms, as compared to the standard drugs.

CONCLUSION

The 2,3-diphenylquinoxaline is a heterocyclic aromatic organic compound, synthesized by condensation of ophenylene diamine and Benzil. It is an important pharmacophore and a privileged structure in medicinal chemistry. There is reduction in time and ultimately cost in the use of microwave procedure of synthesis of 2,3-





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diphenylquinoxaline also a potential antioxidant and antibacterial agent and advances in the developing of innovative application.

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Table 1: In vitro ant-oxidant of 2,3-diphenylquinoxaline using DPPH method

Concentration	% of inhibitions		
(µg/ml)	2,3-diphenylquinoxaline	Std. (Ascorbic acid)	
20	10.53±0.86	23.38±0.28	
40	24.10±0.10	40.72±0.75	
60	41.71±0.74	63.25±0.37	
80	65.04±1.09	79.17±1.47	
100	78.31±0.56	95.08±1.89	
IC50 (µg/ml)	66.86	48.64	

Value were expressed as Mean ± SD for triplicates

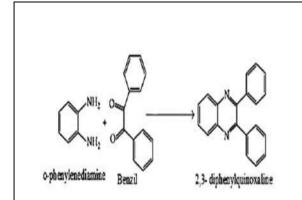




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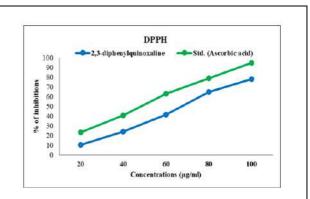


Figure 1: Mechanism of 2,3-Diphenylquinoxaline formation

Figure 2: In vitro ant-oxidant of 2,3diphenylquinoxaline using DPPH method

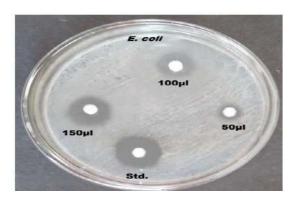


Figure 3: Antibacterial activity of 2,3-diphenylquinoxaline against Escherichia coli strains





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REVIEW ARTICLE

A Comprehensive Review on A Novel Nutraceutical Krill Oil

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ABSTRACT

Overfishing and pollution have reduced fish stocks in recent years. Thus, krill may become a new marine resource with several applications. Its potential as a novel food additive hasn't been extensively investigated, though. This review discusses krill oil's source, chemical makeup, isolation methods, health advantages, and existing uses. The krill oil is a rich source of phospholipids (40-80%) and its main constituents include long-chain fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DPA). Krill oil also contains biologically active minor constituents such as the powerful antioxidant astaxanthin, vitamins A and E, flavonoids, sterols, and traces of minerals. The key difference between fish oil and krill oil is that in krill oil, omega-3 fatty acids are linked to phospholipids, whereas in fish oil they are linked to triglycerides, which makes krill oil unique from fish oil In addition to this The omega 3-fatty acids in krill oil are also connected with choline. Krill oil has several documented health applications, including anti-inflammatory activity, in treating rheumatoid arthritis, ulcerative colitis, cardiovascular disorders, myocardial infraction, obesity, diabetes, postmenopausal health problems, and neurological disorders.

Keywords: Krill oil, Components, Astaxanthine, Omega 3-fattyacids, Health benefits.

INTRODUCTION

Scientists from all around the world are interested in Antarctic krill. Krill from Antarctica, known scientifically as Euphausia superba. Because the vast majority of the larger marine species that reside in the Antarctic derive their nutrition from the shrimp-like krill, this marine crustacean organism is vitally crucial to the food web in that region





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[1]. It has been calculated that the volume of Antarctic krill is around 379 million metric tonnes, and that the annual post-larval output ranges from 342 million to 536 million metric tonnes [2]. These days, krill is mostly used in aquaculture and the sport fishing industry. Many attempts have been undertaken to make human-safe krill products. Due to its abundant nutrients, krill oil is becoming a popular source of healthy lipids[3]. krill are bioluminescent, oxygen molecules are used in their internal chemical reactions that result in the production of light. These chemical reactions take place in photocytes, which are arranged into light organs and are luminous cells. By tensing or relaxing their muscles, or both, krill may alter the brightness of the light. The light is thought to be emitted whenever the particular closure muscles relax because more oxygenated blood is thought to be reaching the photocytes as a result. This light emission is thought to be essential for distinguishing members of the same species and keeping them together in a swarm [4].Krill oil is distinctive because it contains vital nutrients. It supplies the body with EPA/DHA, phospholipids, and choline, all of which work in the body both independently and in conjunction with one another to achieve the following: I as essential components in the formation and operation of cells; ii) as a means of contributing to the maintenance of a state of internal equilibrium; and iii) help improve in cardiovascular, brain, inflammatory, immunity, and liver health. The US FDA conferred the GRAS (Generally Recognized as Safe) certification to krill oil in the year 2008. (FDA). In the European Community in 2009, they gave their stamp of approval for it to be put on the market as a new food additive. In China in 2014, they gave their stamp of approval as well. More recently, the European Community came to the conclusion that pregnant women and nursing mothers should be allowed to consume krill oil for its permitted purposes. It is projected that the market for krill oil will develop more in the years to come[3].Krill oil has been ignored. This paper summaries krill oil's source, chemical makeup, Isolation methods, health advantages, and existing uses to produce it and develop new products.

Source of krill oil

Antarctic krill is known by its scientific name, *Euphausia superba* (*E. superba*). There are 86 different species of the shrimp-like crustaceans in the family Euphausiacea, which are collectively known as 'krill. Known colloquially as "Antarctic krill," the *Euphausia superba* (*E. superba*) krill population is the largest in the pristine waters that surround Antarctica. Antarctic krill has one of the greatest biomasses, at roughly 500 million metric tonnes, of any multicellular animal species on Earth [5]. The Antarctic seas of the Southern Ocean are home to a kind of swimming crustaceans known as krill. The Southern Ocean is considered to be one of the most inaccessible regions on our world; as a result, it is free from the majority of the globe's environmental pollutants. Because it consumes plankton and is consequently near to the base of the food chain, Antarctic krill is devoid of any buildup of toxins such as heavy metals, which are often present in many fishes. This is because Antarctic krill feeds on the plankton[6].Krill has Earth's strongest digesting enzymes [7]. By contracting their bodies and storing energy in their own biomaterial as fat, krill are able to last up to 200 days without food.

Composition of krill oil

Krill oil consists of two types of constituents: major constituents and minor constituents. Major constituents include lipids and fatty acids, whereas minors include astaxanthin, sterols, vitamins, flavonoids and minerals.

Major components

Lipid types in krill oil

Phospholipids (PLs), triacylglycerols (TAGs), diacylglycerols (DAGs), monoacylglycerols (MAGs), and free fatty acids (FFAs) are the most prevalent lipid classes in krill oil, but the majority of common edible oils consist of triacylglycerols (TAGs) [8].

Fatty acids types in krill oil

The major Fatty acids present in krill oil are eicosapentaenoic (EPA) and docosahexaenoic (DHA). It is well known that EPA and DHA, n-3 polyunsaturated fatty acids (PUFAs) generated from dietary fat, are beneficial to the human body. Fish oils are an ideal source of n-3 polyunsaturated fatty acids for diet supplements due to their high EPA and DHA content. Krill oil has comparable EPA and DHA amounts to fish oil. Unlike fish oil, krill oil's EPA and DHA are mostly attached to Phospholipids(PLs) rather than TAGs in fish oil [9]. Recent research has demonstrated that PLs





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have a substantially higher bioavailability than TAGs. Krill oil may deliver more EPA and DHA than fish oil [10]. Lipid nutrition, absorption, and metabolism depend on FA composition and positional distributions on TAG and PL backbones. TAG and PL form lipid bilayers. The majority of the phospholipids in krill oil are choline-containing phospholipids[11]. There is growing evidence that omega-3 fatty acid molecular structures (triglycerides and ethylesters in fish oil and phospholipids in krill oil) impact Phospholipids from krill oil help tissues absorb omega-3 fatty acids better than triglycerides and ethylesters (from fish oil). Thus, krill oil may require fewer omega-3 fatty acids than fish oil for the same health advantages. Phospholipids with choline also have significant health benefits [12].

Minor components

Astaxanthin

The primary kind of carotenoid found in certain marine organisms and plants is called astaxanthin. It has a powerful antioxidant capacity and many other beneficial bioactivities [13]. Astaxanthin has 10 times the antioxidant capacity of zeaxanthin, lutein, canthaxantin, and -carotene, according to Miki (1991). It has 100 times greater than the antioxidant power of -tocopherol. The pigment known as astaxanthin is responsible for giving krill oil its characteristic dark red colour[14]. One of the most powerful antioxidants is astaxanthin [15], it aids in the stability of the omega-3 fatty acids present in krill oillt protects cells from free radicals and regulates oxidative stress in smokers and obese people. Thus, astaxanthin has been linked to anti-inflammatory and pain-relieving abilities, faster workout recovery, and skin UV radiation protection [16], age-related illnesses, liver, heart, eye, joint, and prostate wellness [17]. According to several studies, krill oil may contain 40–45 thousand (mg/kg) astaxanthin, depending on the krill material, extraction process, and testing procedures [18].

Sterols

Krill oil, primarily cholesterol and desmosterol, contains 2.3% to 3.9% sterols. Cholesterol makes up 81.33% to 82.34% of total sterols at 18.95 to 31.96 mg/g of oil. Desmosterol, the precursor of cholesterol, makes up only 1.70 to 18.63 percent of total sterols [19].

Vitamins

Vitamin E

Vitamin E includes tocopherols and tocotrienols. Four homologous pairs of tocopherols and tocotrienols are labelled α -, β , γ and δ .All of them combat free radicals and offer biological benefits, but -tocopherol is strongest. Krill oil contains about 90% a-tocopherol. Tocopherols may help krill oil fight free radicals and work synergistically with other bioactive components [20].

Vitamin A

Humans need vitamin A, which is linked to immune system function and infectious diseases [21]. Frozen krill offers 0.11 milligrammes of vitamin A per 100 grammes of wet weight. Krill oil provides more vitamin A than other marine fish oils [22].

Flavonoids.

Antioxidant, antibacterial, immunomodulatory, anticancer, anti-inflammatory, and antithrombotic effects have been shown for flavonoids [23]. According to reports, Fruits, vegetables, and grains contain most flavonoids, research say. A novel flavonoid compound similar to 6,8-di-C-glucosyl luteolin was identified in krill oil and analysed [24].

Minerals

Whole krill contains calcium (1322 mg/100 g), phosphorus (1140 mg/100 g), and magnesium (360 mg/100 g), which meet adult RDAs [25].

Krill oil extraction process

Different krill biomasses, such as fresh krill and dried krill, are used to make krill oil. Extremely high amounts of active proteolytic enzymes found in krill cause it to rapidly autolyze after being caught. Thus, krill oil must be





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produced soon fresh krill after harvesting. There are several methods for extracting krill oil, each with its own benefits and drawbacks, including solvent extraction, non-solvent extraction, super/subcritical fluid extraction, and enzyme-assisted extraction.

Solvent extraction

This method used most frequently to extract krill oil is solvent extraction. For the efficient extraction of desired compounds from biomass, polarity similarity is crucial [26].

Single solvent method

Different solvents like ethanol, isopropanol, acetone, ethyl acetate, isohexane, n-hexane were used for extraction from krill meal. It is a simple method. The solvent used to extract krill lipids affects product composition [27].

Folch method

This method uses a 2:1 chloroform-to-methanol extraction solvents. Effective method in the reported studies. Use of a huge quantity of hazardous solvents is drawback of this method[28].

Two-step successive solvent method

Two step successive solvents acetone and alcohol are use extraction. High lipid yield. Requires a lengthy period of time and several steps, as well as a significant number of solvents[29].

Mixed solvents extraction method

Ratio of acetone to ethanol (1:1; v: v); hexane to ethanol (9:1; v:v) First, the krill meal is extruded, and then it is extracted using n-hexane. High lipid extraction, and the two-step extraction process is simplified in comparison. Greater efficiency than the extraction method that did not involve extrusion pre-treatment are advantages of method. High solvent use and low PLs in extracted oil are drawbacks of this method[30].

Non-solvent extraction

Non-solvent extraction removes krill oil without organic solvents. Sunflower oil (40–42%) and sesame oil (50–57%) are commonly extracted by mechanical pressing, the earliest nonsolvent extraction process. Both oils are sesame-based.

Cooking and grinding; centrifuging process

It is a Solvent-free method. Easily emulsified throughout the production process; requires a significant financial investment in equipment; inefficient are the drawbacks of this method [31].

Cooking; centrifuging; membrane filtration process

It is a Solvent-free method. Possible emulsification during the manufacturing process; high initial investment required for equipment; inefficient are the drawbacks of this method [32].

Cooking at high temperature; decanting; pressing; centrifuging process

Obtaining PLs-enriched krill oil as well as neutral lipid-enriched krill oil without the need of a solvent while doing so concurrently. High temperature; substantial financial investment required for equipment; potential for oxidation of items are draw backs of this method [33].

Super/subcritical fluid extraction

Supercritical fluid extraction for lipid extraction is popular because it extracts lipids without a solvent, is gentle, and is environmentally friendly. SC-CO2, the most widely utilised supercritical solvent, is safe, nontoxic, chemically inert, and moderately critical [34].





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SC-CO2 extraction process

Extract made without the use of solvents; settings that aren't very harsh; a low impact on the environment; functional proteins of a better grade and stability at higher temperatures are advantages of this method. Extraction of PLs that is inefficient, costly, and difficult to scale up are drawbacks [35].

SC-CO2 extraction with added co-solvent ethanol process

In this method Conditions are mild, which is good for the environment, and PLs can be extracted well. Expensive, difficult to scale up, and fraught with challenges in ethanol removal are drawbacks [36].

Subcritical butane extraction process

This method is Solvent-free method and uses low temperature. Expensive; hard to scale up are drawbacks[37].

Enzyme-assisted extraction

Dominguez, Nunez, and Lema (1994) found that enzyme retreatment is a unique and successful way to release bound molecules and increase lipid yield in extracted oil. Enzymes like amylase, glucanase, protease, cellulase, and pectinase can remove lipid bodies, hydrolyze structural polysaccharides, and break down cell walls, making oil extraction easier [38].

Method -I

The process begins with pulverising the krill, followed by protease pre-treatment in an ultra-high-pressure reactor, followed by filtering, centrifugation, and ethanol extraction. Mild circumstances; concurrently producing both the oil and meal with improved quality; high lipid yield; obtaining a useful by-product krill peptides are advantages of this method. It is difficult to scale up, it is expensive, and it requires assistance from other extraction techniques. The hydrolysis process takes a long time are drawbacks[39].

Method -II

Disintegrating, adding water, hydrolysing with heat and enzymes, deactivating enzymes, centrifuging to remove particles, separating and drying the PLs-protein complex, and then extracting it using solvents are the steps in the process. Low levels of fluoride in the final products; the ability to extract oil and protein hydrolysates at the same time; high lipid yield. It is difficult to scale up, it is expensive, and it requires assistance from other extraction techniques. The hydrolysis process takes a longer period are drawbacks [40].

Health Benefits of Krill Oil

Krill oil is rich in healthful nutrients like EPA, DHA, PLs, tocopherols, vitamin A, and astaxanthin. Krill oil's anti-inflammatory, cardiovascular disease-preventing, anti-obesity, anti-diabetic, neuroprotective, and anti-cancer actions have been studied extensively.

Role of krill oil in inflammation

Inflammatory bowel disease, psoriasis, asthma, rheumatoid arthritis, and chronic obstructive pulmonary disease are all closely related to chronic inflammation [41]. In addition to this, Systemic inflammation may also contribute to obesity, atherosclerosis, cachexia, anorexia, and osteoporosis [42]. Krill oil at 12.5 and 50 g/mL blocked LPS binding to TLR4, reducing TNF- α production in RPMI-1640 medium[43]. Krill oil at 250 mg/L in DMEM or RPMI 1640 reduced inflammation in Caco2, HT29, and RAW 264.7 cells. This treatment restored intestinal barrier integrity, regulated bacterial adhesion and invasion of epithelial cells, and lowered TNF- and IL-8 mRNA expression [44].LPS reduced TNF- α release from the peritoneal macrophages of obese Zucker rats fed krill oil (0.44 g EPA+DHA per 100 g diet) for four weeks [45].

Krill oil role in Rheumatoid arthritis

lerna et al. examined krill oil's anti-inflammatory effects in collagen-induced arthritis-susceptible DBA/1 mice. The mice had collagen-induced arthritis. Krill oil at 0.44 g EPA+DHA per 100 g diet for two months improved the





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histology in arthritic mice. this was accomplished by reduced Cell influx, thickened synovial membrane, eroded cartilage, and reducing the inflammatory cells infiltrated the joint. Inflammation raises CRP levels [46].300 mg krill oil daily improved arthritic symptoms and CRP in 7–14 days [47].

Krill oil role in ulcerative colitis

Krill oil reduced inflammation in a rat model of dextran sulphate sodium-induced ulcerative colitis, a good therapeutic outcome. For four weeks, rats supplemented their diets with 5% krill oil, which retained colon length and reduced inflammation-related ILs and PGs. Further research showed that krill oil inhibited peroxisome proliferator-activated receptor gamma (Pparg) mRNA expression, which lowered NF-kB transcriptional activity and cytokine production [48].

Krill oil role in cardiovascular diseases and Myocardial Infarction

The fundamental underlying cause of cardiovascular disease (commonly known as CVD) is atherosclerosis, which is characterised by abnormal accumulations of lipids, cholesterol, and other substances in arteries [49]. Maintaining healthy levels of total-adipose-glycoprotein (TAG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) and avoiding excessive blood pressure and thrombotic events are crucial to cardiovascular health [50]. In an 8week feeding study of high-fat-fed mice, diets supplemented with 1.25%, 2.50%, or 5.00% krill oil significantly reduced hepatic TG, TC, and serum TG [51]. Krill oil increased serum adiponectin in mice, supporting its antiatherogenic properties [52]. Hals et al. (2017) found that krill oil improved cardiovascular disease risk markers such total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, apolipoprotein B100, and A1 in dyslipidemic nonhuman primates with type 2 diabetes [53]. Bunea et al. (2004) examined the effects of commercial krill oil on 120 patients with hyperlipidemia. These individuals had moderately to highly high blood TC (193.9-347.9 mg/dL) and TAG (203.8-354.4 mg/dL). Patients had hypertriglyceridemias. Krill oil at doses of 1 to 3 grammes per day for 12 weeks increased HDL levels and lowered blood glucose, total cholesterol, triglycerides, and LDL compared to a placebo [54]. Berge et al. (2014) found that krill oil capsules reduced cardiovascular disease risk variables in 300 persons with fasting blood TG levels of 150-499 mg/dL. (CVD). After 12 weeks of krill oil treatment at 0.5 to 4.0 grammes per day, serum TAG levels reduced by 10% compared to the placebo group [55].KO also affected cardiac remodelling and function in a rat model of experimental myocardial infarction (MI) [56].

Krill oil role in obesity and diabetes

Type-2 diabetes often develops fat-induced insulin resistance. Ivanova et al. (2015) showed that obese New Zealand white rabbits fed 600 mg n-3 PUFAs per day of krill oil for 60 days enhanced glucose tolerance and decreased blood glucose while fasting. In addition, krill oil's n-3 PUFAs were thought to increase insulin sensitivity, insulin secretion, and gene expression of beta-oxidation and lipogenesis enzymes in liver and skeletal muscle [57]. After 8 weeks of krill oil (4 g/day) consumption by healthy participants, fasting blood glucose dropped significantly. Omega-3 fatty acids in krill oil supported this outcome [58].

Krill oil role in women's health

PMS affects many young and middle-aged women. Emotional, behavioural, and psychological problems arise during the luteal phase of menstruation [59]. Krill oil surpassed fish oil in premenstrual syndrome and dysmenorrhea tests. Krill oil's unique biomolecular makeup of n-3 PUFAs, PLs, and antioxidant chemicals may explain its superior performance. These findings suggest postmenopausal women may benefit from krill oil as a supplement [60].

Krill oil role in body and tissue weights

A four-week high-fat diet plus KO resulted in significantly lower body weights than the controls who were fed merely the diet [61]. In another trial, rats were given a regular, high-fat, or high-fat diet with 2.5% krill oil for 12 weeks. KO supplementation prevented fat-fed rats from gaining weight [62].





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Krill oil role in neuroprotection, cognitive function and depression

The Aversive Light Stimulus Avoidance Test, Unavoidable Aversive Light Stimulus Test, and Forced Swimming Test showed that krill oil at 1.25 grammes per 100 grammes of diet for seven weeks improved learning and memory in rats. Krill oil-fed rats had higher Bdnf mRNA levels. The hippocampus's Bdnf gene develops and differentiates neurons. KO and Imipramine groups had lower immobility durations than the control group, suggesting antidepressant effects. These data suggest that KO's active components support memory, learning, and antidepressant qualities [63]. These findings match Tome-Carneiro et al (2018). Krill oil intake and brain proteome changes in D-galactose-induced aged mice were linked. They found that feeding aged rats dietary krill oil at 100 to 600 mg/kg/day for 40 days drastically affected the expression levels of 29 brain proteins [64]. Krill oil supplementation (1% in diet) for 12 weeks improved learning, memory, and anxiety in SAMP8 mice in the Morris water maze, Barnes maze, and open-field tests. A β accumulation affects AD pathogenesis and cognitive decline. Krill oil also reduces hippocampal Aβ and two indicators of oxidative stress, MDA and 7,8-dihydro-8-oxoguanine (8-oxo-G)[65]. Krill oil improved cognitive performance in human studies. Since cerebral blood flow is linked to oxyhemoglobin concentration, it may be a partial predictor of regional brain function activation during cognitive activities [66]. Konagai et al. (2013) studied the effects of krill oil on memory and calculation in 45 healthy elderly men. After ingesting krill oil (1.98 g/day for 12 weeks), the elderly showed a significant change in oxyhemoglobin concentrations during the working memory task and a decreased differential value of P300 delay during the calculating task [67].

Krill oil role as antioxidant

oxidative stress indicators in serum that were evaluated, such as superoxide dismutase (SOD), glutathione per oxidase (GSH-Px), and malondialdehyde, showed improvements after being treated with krill oil (MDA) [65].

Krill oil role in cancer

Cancer has quickly become one of the top causes of death worldwide, in both industrialised and developing nations. As the population ages and accepts cancer-causing lifestyle choices like smoking, lack of exercise, and "westernised" diets, the global cancer rate continues to grow. Colon cancer kills the second-most Americans. Krill oil had time- and dosage-dependent antiproliferative effects on colon cancer cells (SW480) [68]. In human osteosarcoma cells, krill FFA extract showed anti-cancer action, and 1.89 M of FFA extract from krill oil was similar to 0.5 to 1.0 M of doxorubicine, a cancer drug [69].

Krill oil role in endocannabinoidss

Mood, hunger, pain perception, and memory are some of the physiological processes that are affected by lipid signalling molecules, which are formed from omega-6 fatty acids. An hyperactive endocannabinoid system is characteristic of obese people. Endocannabinoids were lowered in obese people who took krill oil[70].

Krill oil role in immune function

Over prolonged training, natural killer (NK) cells and interleukin-2 (IL-2), an immune response signalling molecule, may decrease, down regulating the immune system. After intense exercise, krill oil increased NK cell and IL-2 levels, boosting immunity[71].Krill rowing recovery reduces red blood cell oxidative damage in national team rowers [72].

Side Effects and Safety of krill oil

Maki et al. investigated how krill, fish, and olive oils affected plasma EPA and DHA levels (the control). They monitored adverse effects at this time. 32–40% of patients in each treatment group had an adverse event after 4 weeks of supplementation. The krill oil group had 32% of people afflicted, whereas the olive oil control group had 40%. The most common side effects were gas or bloating, flatulence, diarrhoea, nausea, constipation, or gastrointestinal tract pains [73]. Marine fatty acids inhibit platelet aggregation and blood thinning (Saravanan *et al.*, 2010). Krill and fish oils have same fatty acids. Even though fatty acids from krill oil have not been studied for their blood-thinning effects, they may cause this negative effect. Using krill oil with blood clot or platelet-prevention drugs may increase bleeding risk [74].





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Dose

The typical therapeutic dose of krill oil is between two and three capsules, or milligrammes, taken daily. The typical daily maintenance dose is 500 milligrammes (1 capsule) For optimal benefits to the cardiovascular system, the suggested daily doses are between 1000 mg and 1500 mg (2-3 capsules) According to some sources, it is preferable to begin with a greater intake and then gradually lower it after approximately two months, provided that the advantages can be preserved.

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Table 6: Composition of krill oil

Composition	Krill oil
Protein	-
Total lipids	89
Triglycerides	34
Total PLs	43
Total omega-3	25
EPA	13
DHA	7
Total omega-6	2
Saturated FA	23
Saturated FA	15
Choline	5

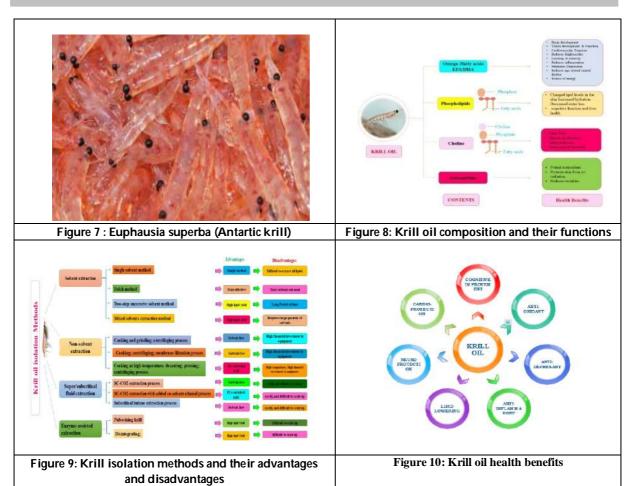




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ISSN: 0976 – 0997 RESEARCH ARTICLE

Estimation of Survival Probability and Analysis in Breast Cancer

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ABSTRACT

Breast cancer is the leading cause of death worldwide and the fifth among cause death from all types of cancer; it is the most common cause of death from cancer women in both developing and developed countries. Comparably, the last three decades the Breast cancer is one of the most diagnosed cancer in India. The prognostic variables are playing vital role in this paper like, Age group, Area, Medical History, Tumor Grade and Stage, Treatment...etc.... This paper focuses on the study is to find out the survival probability, Rate and final outcome of the Breast Cancer patients by the method of Kaplan-Meier, Minimum Survival probability, Maximum Survival Probability.

Keywords: Breast Cancer, Descriptive Statistics, Kaplan-Meier, Log Rank Test, Minimum Survival probability, Maximum Survival Probability.

INTRODUCTION

Breast cancer (BC)is the non-communicable diseases and that begins in the cells of the breast. BC is one of the leading cancer among Indian women, with over 1.5 lakh new BC patients recorded in India in 2018. It accounts for 14 percent of all cancers among women BC is not common in men, with 1 in 400 men getting BC. This is most common in Indian women 1 in 28 can develop BC at some point in their lives. Unfortunately, the number of BC cases reported each year is increasing faster than ever. The BC accounts for more than 27 percent of all new cancer cases. There is an increase in the trend of new cancer patients and comparably the risk is higher in urban areas as 1 in 22 women and lower in rural areas as 1 in 60 women. In India, the average age of the high-risk group is 40-55 years are more prone to BC.





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The overall numbers in India are better compared to the number for developed countries like US/UK is less where in 1 in 8 women are diagnosed annually. However, as the awareness level about the disease in developed countries is quite high and there is a lot of government funding which promotes timely detection, most cases are detected and treated at early stages leading to better survival rates. In India on the other hand, has a very low survival rate due to its large population and low awareness. 1 in 2 women diagnosed with BC will die within the next five year. One of the main reasons for high mortality rates is lack of awareness, late diagnosis and absence of proper BC screening programme. Most of the BCs are diagnosed at advanced stage. Many patients in the urban area are diagnosed at stage-2and most of the cases from rural areas, these lesions are diagnosed only after they transform to metastatic tumours. The exact cause of BC is still unknown, but years of medical research have identified several risk factors. It is still unclear why some women at very high risk do not develop BC, while some women without risk factors may develop BC. The risk factors for BC include genetics and heritage, late pregnancy, use of oral contraceptives, early onset of menstruation, late menopause, excessive alcohol intake, smoking, adolescent obesity, increases stress and poor eating habits-these factors are due to the increased incidence of BC. Through cancer, especially BC is a very dangerous disease that is widespread all over the world (Torre et al., 2015). Cancer is a group of diseases that causes by the uncontrolled growth and spread of abnormal cells anywhere in the body (Diabate et al., 2018). BC is so expensive that it has received a lot of attention from doctors and statisticians. Mortality with unstable mortality with many different prognostic (Pg) factors (Parkin et al., 2014). American joint committee on cancer (AJCC)breast cancer staging is associated with survival prognosis (American Cancer Society,, 2017). This situation is indicated by reduced survival from stage-1 90%, stage-2 65%, stage-3 20% and stage-4 (Sinaga et al., 2017).

To determine why BC survival rates are low, it is necessary to identify that predict survival rates (Alvarez-Banuelos et al., 2016). The main prognostic factors of BC involve several possible diagnostic methods, including clinical staging (Saadatmand et al., 2015), and histopathological grade (Azim and Partridge, (2014), Klauber-DeMore and Nancy (2006)). Survival rate also depend on the type diagnosis based on the existence of metastases (Elston et al., (1999), Chang et al., (2003) and Kuru et al., (2008)). Radiologic figure of mammography (Lee et al., 2014). BC is a type of malignant tumor that begins in the cells of breast tissue and consists of glands that connect the lobules to the nipple. The majority of BC cases are classified as either invasive or non-invasive. Invasive BC has spread throughout the body, but non-invasive not spread to the body(Abay et al., 2018). Caner is a major burden increasingly underdeveloped countries. Based on global cancer statistics, there were approximately 14 million newly diagnosed cancer cases and 8.2 million deaths worldwide in 2012 (Torre et al., 2015). Age has a significant effect on women getting BC. He points out that the mortality rate from BC increases with increasing age (Rezaianzadeh et al., 2009). Drinking alcohol increases the risk of dying from BC in women is about 7% to 12% for every 10g (about one glass) of alcohol consumed per day (Desantis et al., 2013). A study conducted by Addis Ababa University on the impact of several risk factors on BC and survival showed that stage and disease type had a significant effect on survival of BC (Kantelhardt et al., 2014). The goal of this study is to look into the survival and risk of death from the Adayar Cancer institute in 2013. Specifically, 1) To investigate the relationship between factor variable and survival time. 2) To determine the influence of factor variable and/or covariates that affect breast cancer. 3) To find out the overall survival time for the breast cancer patients. 4) To examine the Pg variables that are linked to the survival rate of breast cancer patients. Based on stage of breast cancer, particularly in determining crucial Pg variables. This breast cancer survival analysis implemented using, which includes various models, was employed. The K-M with log rank test is most commonly utilized models (Lee and Wang (2003)). In addition, we looked at the minimum survival probability and maximum survival probability (Felix and Kannan (2007)).

STATISTICAL METHODS

The Survival Function

Individual opportunities to survive for time x are expressed by S(x) = P(X > x). Let X be the continuous random variables, then the survival function is the complement of the Cumulative Distribution function S(x) = 1-F(X) where $F(X) = P(X \le x)$. The survival function is the integral of the probability density function f(x):





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$$\hat{S}(x) = P(X > x) = \int_{x}^{\infty} f(t)dt$$
$$f(x) = -\frac{dS(x)}{dx}$$

Then if X is the discrete random variables, and can be obtained x_j , j=1,2,3,... with the probability mass function (p.m.f) $p(x_j) = P(X = x_j)$, j=1,2,3,... where $X_1 < X_2 < X_3 < X_4$...then the survival function for the discrete variables X is given by:

$$\hat{S}(x) = P(X > x) = \sum_{x_j > x} p(x_j)$$

Kaplan-Meier and Log Rank Test

Estimated survival function for Kaplan-Meier. Expressed as:

$$\widehat{S}(x_{(j)}) = \widehat{S}(x_{(j-i)})\widehat{P}(X > x_{(j)}|X \ge x_j)$$

In general, log rank is used to compare kaplan-Meier survival curves formed by the following hypothesis:

 H_0 : There is no difference between the survival curves:

 H_1 : At least one difference between the survival curves:

Log Rank Test =
$$\frac{(O_i - E_i)^2}{Var(O_i - E_i)}$$

$$O_i - E_i = \sum_{i=1}^n m_{ij} - e_{ij}$$

 m_{ij} denotes the number of individuals who experience the event at time x_{j} , and e_{ij} is the value of hope. The null hypothesis will be rejected if log rank statistics $\geq \chi^2_{\alpha}$ with n-1 degrees of freedom (df) = 1 or p-value $< \alpha$.

Minimum Survival Probability (MISP)

Survival probabilities are calculated on the assumption that all those that are censored, the result of interest occurred. Then, for any interval i, D_i denotes the number of deaths during i, W_i denotes the number of censored observation during i and N_i denotes the number of subjects at the beginning of i. Then MISP for time interval i is expressed by

$$MISP=1-(D_i-W_i)/N_i$$

Maximum Survival Probability (MASP)

The survival probabilities are calculated by assuming that all those who are censored at time *i*are alive till the end of time interval *i*. Hence the notations of MASP is,

$$MASP=1-(D_i/N_i)$$

Source of Breast Cancer Data

The data we used in our research were obtained from the Adayar Cancer Institute in Chennai. These data are the newly diagnosed breast cancer for 2013 and where we used the number 257 breast cancer patients for our research. Using these data we calculated through this research how patients with breast cancer survive or die for the next five years from the day the diagnosed. The data provided by the cancer center for this research: Gender, Age, Medical History, Date of Diagnosis, laterality of the breast cancer, Grade, Stages, Treatments, follow-up details with dates and Alive Status.

RESULT AND DISCUSSION

Descriptive Statistics

There were 1 (0.4%) male, 256 (99.6%) females, the mean age of the BC sample was 51.4 years, median age was 50 years and standard deviation of the age was 11 and the people over the age of 31 are more likely to be affected by





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this BC ie., 252 (98.1%). 83 (32.3%) Rural, 174 (67.7%) Urban, 126 (49%) left BC, 131 (51%) right BC, 4 (1.6%) patients with stage-1, 98 (38.1%) patients with stage-2, 118 (45.9%) patients with stage-3, 37 (14.4%) patients with stage-4, 10 (3.9%) patients with recurrence, 247 (96.1%) patients with no recurrence, 52(20.2%) patients with metastasis, 205(79.8%) patients with no metastasis, 31(12.1%) patients without surgical treatment, 226(87.9%) patients with surgical treatment, 26(10.1%) patients without chemotherapy, 231(89.9%) patients with chemotherapy, 90 (35%) patients without radiotherapy, 167 (65%) patients with radiotherapy, 59(23%) patients without Hormone therapy, 198 (77%) patients with Hormone therapy, 247(96.1%) patients survived in End of 1st year, 226(87.9%) patients survived in End of 3rd year, 193(75.1%) patients survived in End of 4th year, 183(71.2%) patients survived in End of 5th year. The comparison of survived and died patients with Pg variables are presented by probability proportion in above Table-1 and the comparison of Pg variables by stage wise details are presented in Table-2. The stage are the most important factor variable in this breast cancer and only these can determine the conditions of the cancer patients. In Table-2 the Pg variables are compared by the cancer stage. Its indicates stage-1 and stage-2 BC patients survived or died outcome has much better than compared to stage-3 and stage-4. These will happened for all Pg variables.

Analysis Of Kaplan-Meier Estimates

Survival probability of newly diagnosed breast cancer at Adayar Cancer Center in 2013 was indicated by months (0-80) as revealed in following figures, In figure-1 shown that the age of patients with a BC and clearly seen which age group was mostly survived or died in these BC. survival probability was more useful to compared the age of these BC patients with others, it's indicate that the BC is common to all ages and most prevalent in patients over 30 year of age. The Kaplan-Meier estimated the number of months patients with BC survives by age group ie., survival months of the age group 1-30 is 69 months, 31-40 age group is 70 months, 41-50 age group is 68 months, 51-60 age group is 66 months and above 60 age group is 64 months. In figure-2 survival probability shown that the cancer Stages of patient with BC and clearly seen which stage is mostly survived or died in these BC. The survival rate of stage-1 and stage-2 BC patients was very high compared to stage-3 and stage-4 and risk rate of BC patients in stage-1 and stage-2 was very low when compared to other stages. The KM estimated the survival months of stage-1 and stage-2 was 70 months and stage-3 and stage-4 was 65 months.

Analyses of K-M Log Rank Test

Than used the log rank test to determine if there is a difference between the survivals curves. The log rank test of significant or not significant in Pg variables are given Table-3. Based on the Log Rank Test in Table-3, the equality of survival distribution of the breast cancer variables Cancer Stages, Recurrence, Treatment of Surgery were statistically recorded a p-value <0.05 makes a significant difference and other variables have statistically no significant difference.

Comparison of MISP, MASP and Kaplan-Meier Method

Table-4 shows the cumulative survival probabilities at the end of each year from the date of completion of treatment through different methods. These estimates are obtained by using MISP, MASP and KM methods. In general, by all the methods estimates of the cumulative probabilities have been decreased as the survival period has increased. The higher probabilities have been estimated by MASP. i.e. the estimates of MISP and MASP provide the two extreme values of the survival band within which the true survival probability lies. The three estimates are similar but not identical. The overall five-year survival probability (%) for the breast cancer patients has been found to be 69%, which is very much similar to other methods. However, this overall survival probability may not be an appropriate one, since the stage of the disease at diagnosis is one of the significant factors associated with the number of deaths occurred.

Case Description for Kaplan-Meier Survival Analysis BC for Gender

According to the Kaplan-Meier survival analysis for BC, the survival months of gender was 69 and 32 months for patients female and male, respectively. The male BC is a rare cancer that forms in the breast tissue of men and most common in older men. The survival analysis of BC for gender indicated statistically significant difference in the K-M





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survival curve, whereas the log-rank test = 0.000 (<0.05) indicated statistically significant difference between the gender and survival time.

Diagnosis Age for BC

Survival analysis of Age group for BC, survival months at age of diagnosis was69 months, 70 months, 68 months, 70 months and 69 months for the patients Age group <30, 31-40, 41-50, 51-60, >60 years, respectively. The survival analysis of BC at the age of diagnosis indicated no significant difference in the number of months of survival at the age of diagnosis, whereas the log-rank test = 0.205(>0.05) indicated no statistically significant difference between the survival time.

BC for Area Wise

The Kaplan-Meier survival months for Area wise BC was 69 months of both rural and urban area. Although the survival analysis of area wise BC showed that there was no significant difference in the number of months, the log-rank test = 0.617 (>0.05) indicated that there was a statistically no significant difference in the survival time.

Medical History for BC

The survival months for co morbidity with or without BC was68 months and 69 months. While the survival analysis of with or without medical history of BC showed that there was no significant difference in the number of months, the log-rank test = 0.796 (>0.05) indicated that there was a statistically no significant difference in the survival time.

Laterality for BC

The Kaplan-Meier survival analysis for BC, the survival months of laterality (Left Breast and Right Breast) cancer patients was 68 months and 70 months, respectively. The survival analysis of BC for laterality indicated statistically no significant difference in the K-M survival curve, whereas the log-rank test = .372 (>0.05) indicated statistically no significant difference between the survival time.

Stages for BC

The Kaplan–Meier survival analysis for BC Stages differentiation had a mean survival time was 70 months for the Stage-1 and Stage-2 and 69 months, 62months for Stage-3 and Stage-4, respectively. As shown in the graph, there was a significant difference in the number of months of survival for BC by the stage of differentiation, and the log-rank test = 0.000(<0.05) indicated that there was a statistically significant difference in the survival time by the Stage of differentiation.

Recurrence for BC

The survival months for Recurrence (Yes or No) of BC was 66 months and 69 months. While the survival analysis of with or without recurrence of BC showed that there was significant difference in the number of months, the log-rank test = 0.026(<0.05) indicated that there was a statistically significant difference in the survival time.

Metastasis for BC

The Kaplan-Meier survival months for Metastasis (Yes or No) BC was64 months and 69 months. Although the survival analysis of BC Metastasis showed that there was significant difference in the number of months, the logrank test = 0.043 (<0.05) indicated that there was a statistically significant difference in the survival time.

Surgery for BC

Survival analysis of surgical treatment for BC was 69 months and 64 months with and without surgery, respectively. Survival analysis of the surgical treatment of BC indicated a significant difference in the number of months of survival with or without surgical treatment of BC, whereas the log-rank test = 0.003 (<0.05) indicated a statistically significant difference in the survival time with or without surgical treatment of BC.





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Chemotherapy for BC

Survival analysis of chemotherapy for BC was 69 months and 68 months with and without chemotherapy, respectively. A significant difference was detected in the number of months survived with or without chemotherapy for BC, while the log-rank test for chemotherapy = 0.144 (>0.05)indicated a statistically no significant difference in survival time with or without chemotherapy for BC.

Radiation Therapy for BC

Survival analysis of radiation therapy for BC was 69 months and 68 months with and without radiation therapy, respectively. Survival analysis of BC radiation therapy indicated no significant difference in the number of months of survival with or without BC radiation therapy, whereas the log-rank test for radiation therapy = 0.155 (>0.05)indicated no statistically significant difference in the survival time with or without BC radiation therapy.

Hormone Therapy

Survival analysis of hormonal treatment for BC was 69 months and 68 months with and without hormonal treatment. Survival analysis of hormone therapy for BC indicated no significant difference in the number of months of survival with or without hormone therapy for BC, whereas the log-rank test = 0.711 (>0.05) for hormone therapy indicated a statistically no significant difference in survival time with or without hormone therapy for BC.

CONCLUSION

The Kaplan-Meier survival results of the study showed that age, medical history, resident, laterality of breast, stage, recurrence, metastasis, surgery, chemo therapy, radiation therapy and hormone therapy affected the time to death of breast cancer patients 2013 at Adayar Cancer Hospital. The Kaplan-Meier estimated the survival month of the breast cancer is 70 months. The main factor behind the poor survival time is that the treated patients is already in the advanced stage.

RECOMMENDATION

Health professionals, governments and NGO should raise awareness of early cancer screening and should also encourage women to be diagnosed at an early stage to improve mortality risk, and cancer screening facilitation and scheduling should be planned and scheduled in rural areas of this region to elucidated mortality risk.

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Table-1:Basic attributes of Breast Cancer Patients.

PgVariable /Alive	Number	Of Cases with		Proportions (%)			
Status							
Row Labels	No Of BC Patients	Alive	Dead	Alive	Dead		
Male	1(0.4)	0(0.0)	1(0.4)	0.0	100		
Female	256 (99.6)	183(71.2)	73(28.4)	71.5	28.5		
		Age Gro	up				
1-30	5(1.9)	5(1.9)	0(0.0)	100.0	0.0		
31-40	45(17.5)	33(12.8)	12(4.7)	73.3	26.7		
41-50	80(31.1)	58((22.6)	22(8.6)	72.5	27.5		
51-60	75(29.2)	52((20.2)	23(8.9)	69.3	30.7		
61 and Above	52(20.2)	35(13.6)	17(6.6)	67.3	32.7		
		Area					
Rural	83(32.3)	56(21.8)	27(10.5)	67.5	32.5		
Urban	174(67.7)	127(49.4)	47(18.3)	73.0	27.0		
		Medical Hi	story				
No	142(55.3)	102(39.7)	40(15.6)	71.8	28.2		
Yes	115(44.7)	81(31.5)	34(13.2)	70.4	29.6		
Laterality Of BC							
Left Breast	126(49.0)	91(35.4)	35(13.6)	72.2	27.8		
Right Breast	131(51.0)	92(35.8)	39(15.2)	70.2	29.8		





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• •				0.0
• •	` '		91.8	8.2
118 (45.9)	` '	38(14.8)	67.8	32.2
37(14.4)	9(3.5)	28(10.9)	24.3	75.7
	Recurrence (Of BC		
247 (96.1)	178(69.3)	69(26.8)	72.1	27.9
10(3.9)	5(1.9)	5(1.9)	50.0	50.0
	Metastatic C	Of BC		
205 (79.8)	173(67.3)	32(12.5)	84.4	15.6
52(20.2)	10(3.9)	42(16.3)	19.2	80.8
	Surgery	/	1	
31(12.1)			22.6	77.4
• •			77.9	22.1
•			1	
26(10.1)	19(7.4)		73.1	26.9
231 (89.9)	164(63.8)	67(26.1)	71.0	29.0
, ,	Radiother	apy	ı	
90(35.0)			80.0	20.0
, ,		` '	66.5	33.5
(***)				
59(23.0)			64.4	35.6
, ,	` ,	` '	73.2	26.8
	, ,		ı	
257(100)	247(96.1)	10(3.9)	96.1	3.9
• •	` ,	` '	91.5	8.5
		• •	92.9	7.1
210(81.7)	, ,		91.9	8.1
, ,		• •	94.8	5.2
		` '	71.2	28.8
		,		
	247(96.1) 10(3.9) 205(79.8) 52(20.2) 31(12.1) 226(87.9) 26(10.1) 231(89.9) 90(35.0) 167(65.0) 59(23.0) 198(77.0) Year 257(100) 247(96.1) 226(87.9)	98(38.1) 90(35.0) 118(45.9) 80(31.1) 37(14.4) 9(3.5) Recurrence (1) 247(96.1) 178(69.3) 10(3.9) 5(1.9) Metastatic (2) 205(79.8) 173(67.3) 52(20.2) 10(3.9) Surgery 31(12.1) 7(2.7) 226(87.9) 176(68.5) Chemother 26(10.1) 19(7.4) 231(89.9) 164(63.8) Radiother 90(35.0) 72(28.0) 167(65.0) 111(43.2) Hormone Th 59(23.0) 38(14.8) 198(77.0) 145(56.4) Year Wise Patient S 257(100) 247(96.1) 247(96.1) 226(87.9) 226(87.9) 210(81.7) 210(81.7) 193(75.1) 193(75.1) 183(71.2)	4(1.6) 4(1.6) 0(0.4) 98(38.1) 90(35.0) 8(3.1) 118(45.9) 80(31.1) 38(14.8) 37(14.4) 9(3.5) 28(10.9) Recurrence Of BC 247(96.1) 178(69.3) 69(26.8) 10(3.9) 5(1.9) 5(1.9) Metastatic Of BC 205(79.8) 173(67.3) 32(12.5) 52(20.2) 10(3.9) 42(16.3) Surgery 31(12.1) 7(2.7) 24(9.3) 226(87.9) 176(68.5) 50(19.5) Chemotherapy 26(10.1) 19(7.4) 7(2.7) 231(89.9) 164(63.8) 67(26.1) Radiotherapy 90(35.0) 72(28.0) 18(7.0) 167(65.0) 111(43.2) 56(21.8) Hormone Therapy 59(23.0) 38(14.8) 21(8.2) 198(77.0) 145(56.4) 53(20.6) Year Wise Patient Survival Time 257(100) 247(96.1) 10(3.9) 247(96.1) 226(4(1.6) 4(1.6) 0(0.4) 100.0 98(38.1) 90(35.0) 8(3.1) 91.8 118(45.9) 80(31.1) 38(14.8) 67.8 37(14.4) 9(3.5) 28(10.9) 24.3 Recurrence Of BC 247(96.1) 178(69.3) 69(26.8) 72.1 10(3.9) 5(1.9) 5(1.9) 50.0 Metastatic Of BC 205(79.8) 173(67.3) 32(12.5) 84.4 52(20.2) 10(3.9) 42(16.3) 19.2 Surgery 31(12.1) 7(2.7) 24(9.3) 22.6 226(87.9) 176(68.5) 50(19.5) 77.9 Chemotherapy 26(10.1) 19(7.4) 7(2.7) 73.1 231(89.9) 164(63.8) 67(26.1) 71.0 Radiotherapy 90(35.0) 72(28.0) 18(7.0) 80.0 167(65.0) 111(43.2) 56(21.8) 66.5 Hormone Therapy 59(23.0) 38(14.8) 21(8.2) 64.4 198(77.0) 145(56.4) 53(20.6) 73.2 Year Wise Patient Survival Time 257(100) 247(96.1) 10(3.9) 96.1 247(96.1) 226(87.9) 21(8.2) 91.5 226(87.9) 210(81.7) 16(6.2) 92.9 210(81.7) 193(75.1) 17(6.6) 91.9 193(75.1) 183(71.2) 10(3.9) 94.8

Table-2: Breast Cancer Alive Status of PgVariables Compared by Stages

PgVariable/ Stages	Stag	Stage-1 Stage-2		e-2	Stage-3		Stage-4	
Gender	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
Female	4(1.6)	0(0.0)	90(35.0)	8(3.1)	80(31.1)	38(14.8)	9(3.5)	27(10.5)
Male	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.4)
			A	ge Group				
1-30	0(0.0)	0(0.0)	4(1.6)	0(0.0)	1(0.4)	0(0.0)	0(0.0)	0(0.0)
31-40	3(1.2)	0(0.0)	13(5.1)	2(0.8)	15(5.8)	8(3.1)	2(0.8)	2(0.8)
41-50	1(0.4)	0(0.0)	27(10.5)	1(0.4)	27(10.5)	11(4.3)	3(1.2)	10(3.9)
51-60	0(0.0)	0(0.0)	25(9.7)	1(0.4)	25(9.7)	10(3.9)	2(0.8)	12(4.7)
61 and Above	0(0.0)	0(0.0)	21(8.2)	4(1.6)	12(4.7)	9(3.5)	2(0.8)	4(1.6)
	-			Area				
Rural	0(0.0)	0(0.0)	30(11.7)	4(1.6)	23(8.9)	16(6.2)	3(1.2)	7(2.7)
Urban	4(1.6)	0(0.0)	60(23.3)	4(1.6)	57(22.2)	22(8.6)	6(2.3)	21(8.2)
			Med	dical Histo	ry			





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	1			1				
No	4(1.6)	0(0.0)	47(18.3)	6(2.3)	48(18.7)	18(7.0)	3(1.2)	16(6.2)
Yes	0(0.0)	0(0.0)	43(16.7)	2(0.8)	32(12.5)	20(7.8)	6(2.3)	12(4.7)
			Late	rality Of B	C			
Left Breast	1(0.4)	0(0.0)	42(16.3)	3(1.2)	45 (17.5)	19(7.4)	3(1.2)	13(5.1)
Right Breast	3(1.2)	0(0.0)	48(18.7)	5(1.9)	35(13.6)	19(7.4)	6(2.3)	15(5.8)
			Recu	rrence Of	ВС			
No	4(1.6)	0(0.0)	88(34.2)	8(3.1)	78(30.4)	35(13.6)	8(3.1)	26(10.1)
Yes	0(0.0)	0(0.0)	2(0.8)	0(0.0)	2(0.8)	3(1.2)	1(0.4)	2(0.8)
			Meta	astatic Of E	3C			
No	3(1.2)	0(0.0)	88(34.2)	4(1.6)	75(29.2)	11(4.3)	7(2.7)	17(6.6)
Yes	1(0.4)	0(0.0)	2(0.8)	4(1.6)	5(1.9)	27(10.5)	2(0.8)	11(4.3)
				Surgery				
No	0(0.0)	0(0.0)	3(1.2)	1(0.4)	0(0.0)	4(1.6)	4(1.6)	19(7.4)
Yes	4(1.6)	0(0.0)	87(33.9)	7(2.7)	80(31.1)	34(13.2)	5(1.9)	9(3.5)
			Ch	emotherap	у			
No	0(0.0)	0(0.0)	14(5.4)	3(1.2)	4(1.6)	3(1.2)	1(0.4)	1(0.4)
Yes	4(1.6)	0(0.0)	76(29.6)	5(1.9)	76(29.6)	35(13.6)	8(3.1)	27(10.5)
			Rad	dio Therap	у			
No	2(0.8)	0(0.0)	61(23.7)	4(1.6)	6(2.3)	5(1.9)	3(1.2)	9(3.5)
Yes	2(0.8)	0(0.0)	29(11.3)	4(1.6)	74(28.8)	33(12.8)	6(2.3)	19(7.4)
			Horn	none Thera	ру			
No	0(0.0)	0(0.0)	22(8.6)	0(0.0)	15(5.8)	14(5.4)	1(0.4)	7(2.7)
Yes	4(1.6)	0(0.0)	68(26.5)	8(3.1)	65(25.3)	24(9.3)	8(3.1)	21(8.2)
	Year Wise Patient Survival Time							
End of 1st year	0(0.0)	0(0.0)	1(0.4)	0(0.0)	0(0.0)	6(2.3)	0(0.0)	5(1.9)
End of 2nd year	0(0.0)	0(0.0)	1(0.4)	0(0.0)	0(0.0)	10(3.9)	1(0.4)	11(4.3)
End of 3rd year	0(0.0)	0(0.0)	1(0.4)	2(0.8)	1(0.4)	9(3.5)	0(0.0)	5(1.9)
End of 4th year	0(0.0)	0(0.0)	0(0.0)	4(1.6)	2(0.8)	8(3.1)	0(0.0)	5(1.9)
End of 5th year	4(1.6)	0(0.0)	87(33.9)	2(0.8)	77 (30.0)	5(1.9)	8(3.1)	2(0.8)

Table-3: Log rank test for the breast cancer Pg variables

Pg Variable	Chi-Square	Df	p-value
Gender	18.303	1	0.000
Age Group	5.917	4	0.205
Area	.250	1	0.617
Medical History	.067	1	0.796
Laterality	.798	1	0.372
Stages	25.353	3	0.000
Recurrence	14.988	1	0.026
Metastasis	12.299	1	0.043
Surgery	15.713	1	0.003
Chemo Therapy	2.132	1	0.144
Radio Therapy	2.482	1	0.115
Hormone Therapy	.137	1	0.711





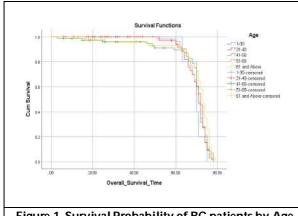
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Table-4. Cumulative Survival Rate by MISP, MASP, KM Methods

	IstYear	II nd Year	III rd Year	IV th Year	VthYear
MISP	95.3%	86.3%	79.2%	71.4%	66%
MASP	97.2%	88.4%	82.3%	75%	71.2%
Kaplan-Meier	96.2%	87%	81.3%	73.4%	69%



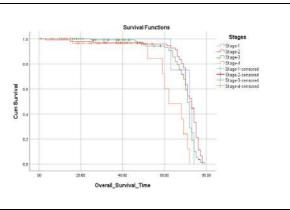


Figure 1. Survival Probability of BC patients by Age Group

Figure 2. Survival Probability of breast cancer patients by Stages





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ISSN: 0976 – 0997 **RESEARCH ARTICLE**

Using the Mean Value as a Fuzzy Ranking Approach for Randomized **Block Designs in a Field of Crops**

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ABSTRACT

The Two way classification Randomized Block Designs (RBD) has been generally utilized in agricultural to represent randomness with fuzziness, because uncertainty is common to all real-life problems. In such cases, statistical RBD analysis using ambiguous observations is unavoidable. The statistical analysis of RBD is proposed in this study utilizing a fuzzy ranking method formed on the position of the mean value in support of Hexagonal Fuzzy Numbers (HFNs). The proposed approach's numerical examples would be more precise.

Keywords: Hexagonal Fuzzy Numbers, Ranking Method. Mean Value, Randomized Block Designs, Paddy crop yield.

INTRODUCTION

Prof. R.A. Fisher first proposed the Analysis of Variance (ANOVA) in the 1920 to solve problems in agricultural and biological investigations. For significance testing, the analysis of variance is a useful statistical tool. For agricultural experiments, the RCB is the conventional design. To accommodate for any variations in the field, the field or orchard is divided into units. The participants in the blocks are then assigned treatments at random, once in each block. Within blocks of adjacent subjects, treatments are assigned at random, with each treatment occurring once per block.





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The number of replications is equal to the number of blocks. Any treatment can be next to another treatment within the block, but not the same treatment. Used to account for spatial effects in an experiment to control variation. RBD are used in two-way categorization. Experimentation is a must for new ideas and technologies. Replication, randomization, and local control are the three essential elements of experimental designs and they are used to identify cause and effect correlations. The most basic of all the principles-based designs of randomization and replication is Completely Randomized Designs (CRD). When the testing tools are not homogeneous in some circumstances. RBD divides the experimental area into smaller homogeneous blocks, with treatments applied at random to each block and reproduced across all blocks. Any treatment's inaccuracy can be separated, and any number of treatments can be eliminated without complicating the study. The data in this study is ambiguous, necessitating the use of an wide view of the RBD to analyze this ambiguous evaluations. Fuzzy set theory introduced by Lotfi A. Zadeh [1] in 1965. For integrating membership values that represent ambiguous knowledge, this approach provides appealing coordination bonds. The term "fuzzy number" refers to a generalization of a normal number and a real number that isn't associated with a specific value but rather than a collection of related alternative values, each with its own membership functions between 0 and 1. Where each conceivable value has its own membership function between 0 and 1.

A new membership function on hexagonal fuzzy numbers was introduced by Dhurai K, Karpagam A [2]. Jain [3] was proposed ranking fuzzy numbers method for making decisions in uncertain situations. Fuzzy interval and fuzzy number can be considered as real number by according to Dubois and Prade [4]. Bodjanova [5] established the concept of a fuzzy number. Shaun-Hu Chen [6] has demonstrated an rankings and distances of exponential fuzzy number. According to Sanjib Kumar Behera and Dhyan Singh [7] manganese should be applied continuously. The randomised block designs were immediately affected by fertilizer in the soil as a portion of a dose. Generalized fuzzy integers with different left and right heights has been proposed by Shyi-Ming Chen [8]. Using two illustrations with trapezoidal fuzzy numbers, Parthiban and Gajivarathan [9] discovered a ANOVA two-way method under distinct forms of fuzzy trapezoidal numbers. Rajarajeswari P, Sudha AS[10] was proposed using rank, mode, divergence and spread ordering hexagonal fuzzy numbers. Gnanapriya [11] proposed a incomplete block design based on the cut interval method for determining decision level. Rajarajeshwari P, Sudha AS, Karthika[12] introduced the hexagonal fuzzy number for use in a ranking technique for solving multi-objective Fuzzy Linear Programming Problems. Cochran and Cox, John Wiley & Sons[13] Complete Randomized Block Designs to adapted from Experimental Designs. DIPA&Dutt S and FAO [14,15,16] to proposed the facts and role of Sustainability Fertilization Handbook.

Application

Primary data was used to collect paddy yields in the Thanjavur district. Six paddy crop varieties [IR20, IR50, CO43, ADT 38, TPS3, White Ponni] with three kinds of fertilizers are [N,P,K] investigated in the Randomized Block Designs examination. The Randomized Block Designs is used in the study were approximations. As a result, fuzzy observations are required to calculate yield. As a result, fuzzy observations are necessary to assess the yield's fuzziness. The data shown here is situated on HFNs in kg per hectare. Test whether the significant difference between the paddy crop yield varieties and fertilizers. Find the mean for each and every set of values.

F-Ratio for Varieties

$$F_{B} = \frac{MSS(B)}{MSS(E)} = 1.20$$
 at $F_{t(Table \, Value)} = 3.20$ Where $F_{B} < F_{t}$

The null hypothesis H0 is accepted. There is not that much of distinction between the varieties. The six varieties do not differ significantly with respect to the paddy crops yield.

F-Ratio for Fertilizers

$$F_T = \frac{MSS~(T)}{MSS~(E)} = 3.23$$
 at $F_{t(Table~Value)} = 3.98$ Where $F_T < F_t$





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The null hypothesis H0 is accepted. There is not that much of distinction between the fertilizers. The three fertilizers do not differ significantly with respect to the paddycrops yield.

R Programming

```
v1 = c(67.5, 49.3, 8)
V2 = c(28.7, 37.7, 36.5)
V3 = c(28.3, 18.8, 28.7)
v4 = c(51, 17, 18.8)
v5 = c(33.2, 28, 26.2)
v6 = c(48, 35.3, 31.2)
y = c(v1, v2, v3, v4, v5, v6)
block = c(rep("1", 3), rep("2", 3), rep("3", 3), rep("4", 3), rep("5", 3), rep("6", 3))
tr = rep(c("1", "2", "3", "4", "5", "6"), 3)
n = rep(c(-1, 1, -1), 6)
p = rep(c(-1, -1, 1), 6)
k = rep(c(-1, -1, -1), 6)
mode11 = aov(y~block+tr)
summary(mode11)
mode12 = aov(y\sim n+p+k)
summary(mode12)
```

CONCLUSION

In Six paddy crop varieties [IR20, IR50, CO43, ADT 38, TPS3, White Ponni] with three kinds of fertilizers are [N,P,K] investigated in the Randomized Block Designs examination. The Randomized Block Designs is used in the study were approximations. As a result, fuzzy observations are required to calculate yield. There is not that much of distinction between the varieties. There is not that much of distinction between the fertilizers. Therefore, the six varieties do not differ significantly with respect to the paddy crops yield and also the three fertilizers do not differ significantly with respect to the paddy crops yield.

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Table 1: Table based on HFNs of Paddy harvest produce

Fertilizers	Varieties								
	IR20	IR50	CO43	ADT 38	TPS3	White Ponni			
NI	(58,63,67,69,72,76)	(24,25,27,29,32,	(24,25,28,29,3	(45,47,50,52	(26,29,33,35,3	(40,45,47,49,50,			
N		35)	0,34)	,55,57)	6,40)	57)			
D	(44.47.40.50.52.54)	(33,35,37,39,40,	(13,16,17,20,2	(12,15,16,17	(20,25,27,29,3	(30,33,35,36,38,			
Р	(44,47,49,50,52,54)	42)	2,25)	,20,22)	2,35)	40)			
V	(10.15.17.20.22.24)	(30,32,35,37,40,	(23,25,27,30,3	(4,6,7,20,22,	(20,23,25,27,3	(26,28,30,32,35,			
K	(10,15,17,20,22,24)	45)	2,35)	24)	0,32)	36)			

Table 2: RBD values using Mean Value concept

Fertilizers		_	\	/arieties		
reitilizers	IR20	IR50	CO43	ADT 38	TPS3	White Ponni
N	67.5	28.7	28.3	51	33.2	48
Р	49.3	37.7	18.8	17	28	35.3
K	8	36.5	28.7	18.8	26.2	31.2

(i) TSS = 3071.58

(ii) BSS = 785.23

(iii)TrSS = 846.61

(iv)ESS = 1439.74

Table 3: F-Ratio for Varieties

SS	df	SS	MSS	F -Ratio
Blocks	5	785.23	157.05	1.20
Treatment	2	846.61	423.31	3.23
Remainder	11	1439.74	130.89	





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Figure.1: R Programming





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RESEARCH ARTICLE

An In silico Evaluation of Indigofera tinctoria Leaf Extract Efficacy against Breast Cancer

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ABSTRACT

Breast cancer is one of the principal causes of death among women and there is a pressing need to develop novel and effective anti-cancer agents. Natural plant products have shown promising results as anti-cancer agents. Their effectiveness is reported as decreased toxicity in usage, along with safety and less recurrent resistances compared with hormonal targeting anti-cancer agents. The objective of the study is to assess the cytotoxic potential of *I. tinctoria* and to provide a rationale as anticancer drug with aid of insilico molecular docking studies.

Keywords: Breast cancer, *Indigofera tinctoria*, COX2, Docking, *Insilico* Studies

INTRODUCTION

Cancer is the second major cause of death world-wide, accounting for 9.6 million deaths in 2018, or one of every six deaths. According to the survey every year there are more than 14 million people are diagnosed with cancer majority of them live in lower middle- income countries. Lung, Prostate, Colorectal, Stomach and liver diseases are the most well-known kinds of cancer in Men, while Breast, Colorectal, Lung, Cervical and Thyroid cancer are the widely recognised cancer among women. Cancer occurs when damaged cells grow, divide and spread abnormally instead of self-destructing as they should. Cancer form a subset of neoplasms or tumor. A neoplasm or tumor is a group of cells that have undergone unregulated growth and will often form a mass or lumps, but may be distributed diffusely. The common environmental factors that contribute to cancer death include tobacco (25-30%), diet and obesity (30-35%), infections (15-20%), radiation (both ionizing and non-ionizing, up to 10%), stress, lack of physical activity and environmental pollutants (Anand et al., 2005). It is not generally possible to prove what caused a particular cancer





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because the various causes do not have specific fingerprints[1]. Breast cancer is the most commonly occurring cancer in women, comprising almost one third of all malignancies in female and it is the leading cause of death for women between the ages of 40 and 55. The lifetime risk of a woman developing invasive breast cancer is 12.6% - one out of 8 females are prone to develop breast cancer at some point in her life. The majority of breast cancers begin in the ducts (ductal cancer). A small number start in the sacs or lobules (lobular cancers), Within these two groups, there are different subtypes of breast cancer. Some grow very slowly, while others grow rapidly. Breast cancer can spread to lymph glands and to other parts of the body, such as the bones and liver. Breast cancer is typically detected either during a screening examination, before symptoms have developed, or after a woman notices a lump Screening mammograms are administered to detect breast cancer in women who have no apparent symptoms. In India Breast cancer counts rank one with 1,78,361 of new cases in 2020. Even in mortality rate cases are increasing compared to other cancer rates [2,3]. Medicinal plants are a very important a part of our natural wealth. They function vital therapeutic agents also as valuable raw materials for producing various ancient and modern medicines. The history of medicinal plant use for treating diseases and ailments is probably dates back to from the start of human civilization. Since past, plants with therapeutic properties have secured a very important place in the healing practices and treatment of diseases. In several developing countries, ancient drugs continues to be the mainstay of health-care, and most of the medication and cures come from natural sources, such as, plants.

Even in developed countries, the raw materials for producing essential medication are extracted from medicinal plants, harnessing its natural properties of healing. progressively. a lot of individuals are turning to flavouring remedies, particularly for treating minor ailments. Medicinal plants naturally synthesize and accumulate some secondary metabolites, like alkaloids, sterols, terpenes, flavonoids, saponins, glycosides, cyanogenics, tannins, resins, lactones, quinines, volatile oils etc[4]. Computer-aided drug design (CADD) methodologies are taking part in an ever-increasing role in drug discovery that are essential within the cost-efficient identification of promising drug candidates. These computational strategies are relevant in limiting the utilization of animal models in pharmacological research, for aiding the rational design of novel and safe drug candidates, and for locating marketed drugs, supporting medicative chemists and pharmacologists throughout the drug discovery trajectory[5]. In the recent research on the drug discovery increasing concentrating on the modern medicinal chemistry methods, especially molecular docking, dynamics[6]. Through this pharmacodynamics and pharmacokinetics properties has been clearly through these methodologies. Molecular dynamics are the most commonly used strategies for the wide range as binding energetics, molecular interaction and confirmations changes. The present study delineates the efficacy of the components of leaf extract of *Indigofera tinctoria* – a lesser known medicinal plant as a potent antibreast cancer agent using *insilico* models.

MATERIALS AND METHODS

Insilico Approach

Analysis of active sites

The active amino acid residues that may be attributed to interaction with the ligands are determined by predicting the active site glycoprotein using Auto Dock Tool. The protein is selected as surface, and the active sites are predicted in association with hydrogen bond donor and acceptor region with specific amino acid residues. The active sites are used in the subsequent stage of fixing the grid box so as to select the actives site for docking with the ligands.

Preparation of ligands

The ligand structures were retrieved from PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The saved SDF files were converted to PDB using Molegro visualisation tool and used as input in PyRx software (http://PyRx.sourceforge.net/).





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Preparation of targets

The crystal structure breast cancer receptors p53(PDB code = 10LG) and COX2 (PDB code = 5IKR), were retrieved from the Protein Data Bank (http://www.rcsb.org/). All bound waters, ligands and cofactors were removed from the proteins using Molegro visulaization tool and then hydrogen atoms were for optimization.

Molecular docking

Intermediary steps, such as pdbat files for protein and ligands preparation and grid box creation were completed using Graphical User Interface program AutoDock Tools (ADT) (Baxter *et. al.*, 1981).ADT assigned polar hydrogens, united atom Kollman charges, solvation parameters and fragmental volumes to the protein. AutoDock saved the prepared file in PDBQT format. AutoGrid was used for the preparation of the grid map using a grid box. The grid size was set to 25.0 x 70.4148954936 x 21.7439301415 xyz points with grid spacing of 0.375A and grid centre was designed at dimensions (-18.842941559, 25.2143325921, 1.13120869254)

p53

center_X = 0.589893701429, center_y = -1.31303979622, center_z = -0.2328 size_x = 46.2684184589, size_y = 36.3466183384, size_z = 25.0

Cox2

center_x = 22.3663509584,center_y = 30.8607376516, center_z = 46.2085759084 size_x = 44.5236483831, size_y = 58.1304192049, size_z = 36.0093795567.

A scoring grid is calculated from the ligand structure to minimize the computation time. Docking using protein and ligand information along with grid box properties in the configuration file were carried out in PyRx using AutoDock/Vina option. AutoDock/Vina employs literated local search global optimizer. During the docking procedure, both the protein and ligands are considered as rigid. The results less than 1.0 A in positional root-mean-square deviation (RMSD) was clustered together and represented by the result with the most favorable free energy of binding. The pose with lowest energy of binding or binding affinity was extracted and aligned with receptor structure for further analysis.

RESULTS

Docking result of anticancer protein with lead compounds from ethanolic extract of *Indigofera tinctoria* Linn. Leaves

To study the docking for anticancer property of 2 active compounds from *I.tinctoria Linn*. was taken. p53 and COX2 are the proteins used here for docking analysis. The p53 (1 olg) was docked with the 2 ligands. From the results compound 1, and 2 2-pentyne-1,4-diol,1-(2-furanyl)-4-methyl and 4,8,12-Tetradecatrien-1-ol,5,9,13-trimethyl by forming 2 H-bonding with the amino acids in compound 1 (Glu349, Asp352, Arg337, Asg353) and compound 2 (Arg 337, Arg 333, Glu349). Binding energy was -4.9and -5.2 kcal/m. The COX2 (5IKR)was docked with the 3 ligands. From the results compound 1, 2 and 3, [2-pentyne-1,4-diol,1-(2-furanyl)-4-methyl-] and [4,8,12-Tetradecatrien-1-ol,5,9,13-trimethyl], [2(4H)- Benzofuran one,5,6,7,7a-tetrahydro-4,4,7a-trimethyl] by forming two H-bonding with the amino acids in compound 1 (Pro142, Pro139, Cys21, Cys22, Cys26, Pro 25, Leu138, Arg 29 and compound 2 (Arg 455, aleu138, Glu451, Ltd 454, Pro139, Gln 447). Binding energy was -6.4,-6.2 and -5.3 kcal/mol.

DISCUSSION

On account of chemical, physiological, and clinical studies, numerous forgotten plants and drugs obtained thereof were restored to pharmacy. The active components of medicinal plants are a product of the natural, most seamless laboratory. Plants have formed the basis of sophisticated traditional medicine systems among which are Ayurvedic, Unani, Chinese amongst others. These systems of medicine have given rise to some important drugs still in use





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today. The search for new molecules, nowadays, has taken a slightly different route where the science of ethnobotany and ethnopharmacognosy are being used as guide to lead the chemist towards different sources and classes of compounds[7]. Anticancer property of *Indigo tinctoria* was validated for breast cancer receptors p53. The lead components possess similar function when compared to that of standard drug. In the present study, the docking scores against selected natural compounds showed higher binding affinity towards p53, which is evident from the hybrid Chemgauss 4 score. Collectively, our results suggest that selected natural compounds from *Indigo tinctoria* may inhibit the function of p53 and the lead components can be considered as potential bioactive molecules to treat various p53 associated diseases[8].

CONCLUSION

Molecular docking studies and explores the interaction mechanism between ligands and receptors. Interactions between the compound and the receptor play a crucial role in the discovery of drugs. All these molecules were docked against the target enzyme p53 and ranked based on their dock scores. These molecules could be utilized for further innovation and development of anticancer compounds against breast cancer. However, further researches warranted to investigate the potential uses of the medicinal plants containing these compounds[9].

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Table 1: Anticancer Protein With Lead Compounds I

Target	Compound ID	Binding energy	Interacting amino acid	H-bond
p53	4912963 2-pentyne-1,4-diol,1-(2- furanyl)-4-methyl	-4.9	Glu349,Asp352,Arg337,Asg353	2
p53	5362831 4,8,12-Tetradecatrien-1- ol,5,9,13-trimethyl	-5.2	Arg337, Arg333,Glu349	2

Table 2: anticancer protein with lead compounds II

Target	Compound Id	Binding energy	Interacting Amino acid	H-Bond formed
COX 2	5362831 [2-pentyne-1,4-diol,1-(2- furanyl)-4-methyl-]	-6.4	Pro142,Pro139,Cys21,Cys32, Cys26,Pro25,Leu138,Arg29	2
COX 2	4912963 [4,8,12-Tetradecatrien-1- ol,5,9,13-trimethyl]	-6.2	Arg455, Leu138, Glu451,Lys454,Pro139,Gln447	2
COX 2	[2(4H)- Benzofuranone,5,6,7,7a- tetrahydro-4,4,7a-trimethyl	6	His200, Val277,Asn368,Thr198	2





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RESEARCH ARTICLE

Isolation, Identification and Antibacterial Activity of Pigmented Bacteria **Isolated from Marine Soil**

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ABSTRACT

Pigment from natural source have been obtained since long time ago, and synthetic pigments caused by the toxicity problems. Pigment from microbial source are a good alternative. As the present trend throughout the world is shifting towards the use of eco friendly and biodegradable commodities, the demand for natural colorants is increasing day by day. In the present study, Pigmented marine bacteria were isolated from soil samples in five different places of Cuddalore district. Out of 20 distinct pigmented bacterial isolates, high intensity of yellow pigmentation were observed in one bacterial strain. It was performed to explore the optimize various parameters for the production of pigment. Among the test isolates, Kocuria flava (AP2) which was identified using 16S rRNA gene sequencing analysis was found to be the most promising. Evaluation of different parameters aspect of the pigmentation processes such as NaCl concentration(2 %) influenced the pigment yield, incubation temperature(30°C) and pH-7, found to be a favorable for growth and pigmentation of production medium is required to maximize the metabolic yield. Among the carbon and nitrogen sources tested, supplementation of lactose and beef extract(1%) enhanced production respectively. This extracted yellow pigment screened for antibacterial activity for bacterial pathogens. The zone of inhibition was determined by agar well diffusion method of pigment showed highest activity Enterobacter sp(22) and least activity of Escherichia coli (16) in the 300 µl of concentrations.

Keywords: Marine soil, *Kocuria flava*, optimization, Antibacterial activity





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INTRODUCTION

Pigmentation is a characteristic nature of most of the bacterial species. Pigments are chemical components with lightabsorbing character. Natural colours have an advantage over chemically manufactured colours, particularly in their utility spectra in industrial sectors, owing to their biocompatibility, safety of usage, and eco-friendliness, as well as the safety risks associated with many artificial synthetic colourants. Because of their biocompatibility and growing awareness of environmental degradation, natural colours are in high demand in the food, pharmaceutical, cosmetics, textile, printing, and dye industries (Kim et al., 1999). Many natural pigments, in besides providing colour, are known as important bioactive substance with potential health advantages. (Venil and colleagues, 2020b). There have been numerous discoveries of microbial bioactive pigments, many of which exhibit antioxidant, anti-inflammatory, and/or antibacterial characteristics (Ramesh et al., 2019). Marine microorganisms have evolved special metabolic and physiologic skills that allow them to survive in high pressure, salinity, and temperature changes. It also offers the potential in the production of metabolites, which may be different from terrestrial microorganisms (Fenical, W. and Jensen, P.R.1993; Fenical, W.1993). A main characteristic feature of marine bacteria is that a large proportion of them are pigmented (Zobell, C.E.1946). It has previously been observed that some colours produced by microbes can inhibit the growth of other bacteria. Microbial pigments are of great interest due to their stability, ease of availability, and simple cultivation and extraction procedures (L. Selvameenal, et al.,2009; J. S. Yaradoddiet al.,2021). Many bioactive substances are produced by microorganisms, including carotenoids, melanins, flavins, and guinones. One of these natural substances is derived from microbe pigments, which have recently received a lot of attention. Overall, microorganism-produced pigments contain medical features such as anti-oxidant, cytotoxic, antileishmanial, anti-ulcer, anti-viral, anti-tumor, and anti-bacterial effects (Gupta et al., 2011; Joshi &Attri, 2006). The current study was carried out to optimise several factors for the generation of K. flava pigment generating bacteria as well as the antibacterial activity of bacterial pathogens.

MATERIALS AND METHODS

Sample collection

The Marine soil sample were collected from Aiyampettai, Killai, Veliangripettai, Parangipettai, Samiyarpettai places of Cuddalore district ,Tamil Nadua depth of 6-10 cm, from the wetland transferred into sterile bags and transported immediately to the laboratory and maintained at 4°C.

Bacterial Pathogens

The following microorganisms were procured from RMMCH, Department of Microbiology laboratory, Annamalai University, Chidambaram, Cuddalore district. Pathogens are *Escherichia coli*, *Streptococcus pyogenes*, *Enterococcus faecalis* and *Enterobacter sp*, *Serratia marcescens and Staphylococcus aureus*. All the tested bacteria were maintained on Mueller-Hinton agar(MHA) and 24 h old culture were used for the assay.

Isolation and Identification of bacterial pigment

One gram of soil was dissolved in 100 mL of sterile saline in an Erlenmeyer flask and mixed by overtaxing. The sample was serially diluted from 10-1 to 10-8. 100 l of each was spread on a Zobel marine agar plate and incubated at 37° C for 24-72 hours. Morphologically distinct colonies with distinct pigments were selected and streaked on Zobel marine agar plate to obtain pure culture for further studies. The bacterial isolates were identified using classification schemes published in Bergey's (1994) manual of systematic bacteriology. To identify and characterize phylogenetically, a small yellow bacterial colony was chosen and identified using 16s rRNA gene sequencing analysis, and a phylogenetic tree was generated by the programme.

Optimization of bacterial pigment

Optimization of growth conditions particularly physical and nutritional parameters are of prime importance in the development of any pigment production process. The optimization for pigment production from isolates was carried out in zobell broth. Optimization was performed to improve pigment production and isolate growth rates.





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Temperatures (25°C,30°C,37°C,40°C,45°C, and 50°C), pH (4,5,6,7,8,9, and 10) and NaCl concentration (1%,2%,3%,4%,5%,6%,7%,8%,9%,10%) were varied. At 1% (w/v) concentration, different carbon sources (glucose, maltose, sucrose, lactose, fructose, mannose, glycerol, xylose) and nitrogen sources (yeast extract, peptone, beef extract, malt extract, and urea) were tested to determine the best carbon and nitrogen sources for optimum growth and pigment production.

Production and Extraction of the bacterial pigment

In a 500 ml Erlenmeyer flask containing 200 ml of Zobell broth inoculated with 1 ml of a 24 hour culture suspension, growth and pigment production were observed. For 72 hours, the flasks were shaken on a rotary shaker at 180 rpm. The pigment-producing bacteria were extracted by centrifuging them at 10,000 rpm for 20 minutes. After the supernatants were discarded, the pellets were resuspended in methanol. The mixture was vortexed, and the suspension was centrifuged for 10 minutes at 10,000 rpm to collect the supernatant. The pigment containing solvent was then tested for antibacterial activity against bacterial pathogens. The absorbance of the filtrates was measured using a UV-visible spectrophotometer in the 300-700nm range.

Antibacterial activity of crude pigment

Screening of antibacterial activity of crude pigment extract was determined by agar well diffusion technique described by Kirby-bauer on Mueller Hinton agar. Each bacterial pathogenic microbe were swabbed on the surface of the agar, 20 ml of MHA was added to the Petri dish, and various concentrations of crude pigment were applied to the wells. For 24 hours, plates are incubated at 37°C. By evaluating the growth inhibition in the area surrounding the well, antibacterial activity were determined.

RESULTAND DISCUSSION

Isolation of pigment producing bacteria from marine soil:

Marine Soil sample from Aiyampettai, Cuddalore district was showed for yellow pigment producing organisms. The colony morphology and cell characteristics of the bacterial isolates in zobell agar shown in fig-1,within 3-7 days of incubation the bacterial isolate grew to form intense yellow colonies having entire margin and smooth consistency. Gram staining of bacterial cells revealed the presence of Gram positive. Bacterial isolation produced a variety of coloured isolates, the majority of which were orange in colour. Yellow, red, pink, blue-green, and violet were next in popularity. The majority of bacteria are pigmented, and the carotenoid, flexirubin, xanthomonadine, and prodigiosin pigments discovered in marine heterotrophic bacteria are among these. (Kim *et al.*, 2007).

Identification of bacterial strains

The isolated colonies were identified based on 16s rRNA sequence. Based on the gram staining, morphological characteristics and 16S rRNA gene sequences (AP2), the pigment producing stain was identified as *Kocuria flava* (AP2). Isolated strain exhibited maximum similarity show in Fig.2, and sequence has been submitted to the NCBI/Nucleotide database under accession no. ON651728.

Assembled data

>Seq1[Kocuria flava]16SrRNApartialsequence

TTAACACATGCAAGTCGAACGCTGAAGCTCCAGCTTGCTGGGGTGGATGAGTGGCGAACGGGTGAGTAATA
CGTGAGTAACCTGCCCTTGACTCTGGGATAAGCCTGGGAAACCGGGTCTAATACTGGATACGACGTCCTACC
GCATGGTGGGGGGTGGAAAGGGTTTTATTGGTTTTGGATGGGCTCACGGCCTATCAGCTTGTTGGTGGGGTA
ATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGTGACCGGCCACACTGGGACTGAGACACGG
CCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCGACGCCG
CGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCCACAAGTGACGGTACCTGC
AGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTA
TTGGGCGTAAAGAGCTCGTAGGCGGTTTGTCGCGTCTGCTGTGAAAGCCCGGGGCTCAACCCCGGGTCTGCA
GTGGGTACCGGCCAGACTAGAGTGCAGTAGGGGAAACCTGGAATTCCTGGTGTAACCGCGTGAAATGCGCAGATA
TCAGGAGGAACACCGATGGCGAAGGCAGGTCTCTGGGCTGTTACTGACGCTGAGGAGCGAAAGCATGGGG





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Maximum Likelihood tree

Optimization of pigment

The results for the optimization of parameters for pigment production are represented the effect of pH value of the liquid medium on growth and pigment production by isolated marine bacterial strain was studied by evaluate the bacterial growth in Zobell marine broth prepared with different pH values 4-10. Optimum for pigment production at pH value of 7 increasing pigment yield. I R Booth (1985) also showed maximum microbial growth is effective when it maintain a cytoplasmic pH in order to compatible with optimal functional and structural integrity of the cytoplasmic proteins that support growth. Though most non-extremophilic microbes grow over a wide range of external pH values ranging from 5.5-9.0, however maintain a cytoplasmic pH that lies within the narrow range of pH 7.4-7.8. Palanichamy V, (2011) reported the Streptomyces species were isolated from soil and the cultures were screened for actinorhodin production. The culture showed a narrow range of incubation temperature and pH for relatively good growth and pigment production; where maximum growth, as well as pigment intensity, was observed at 30°C and pH7.6-8.0.Optimum for pigment production at different temperature (25°C,30°C,37° C,40°C,45°C and 50°C). The result effect of temperature on pigment production shown that highest pigmentation were observed by K. flava 30°C.Similar study observed that the Optimum effect of pH and temperature on pigment production had been also observed by (Zhou, G., 2008). Streptomyces flavofuscusARITM02 isolated from rhizophere soil also showed optimum pigment production at 35°C, pH7.5(Parmaret al.,2017). Choubey, et al.,(2021) reported that the yellow pigmented colony grows at 30°C and 37°C.

The optimized culture condition for pigment production by *K. flava* was achieved when it was grown in Zobell medium supplement with lactose(1%) and beef extract (1%) and incubated at 30°C for 48h in the presence of light under shaker conditions 120rpm.In a similar study observed that the pigment was produced best when supplied by 1% lactose as a carbon source and 1% beef extract as a nitrogen source.(Kim, Y. S., & Park, J. S. 2010). Different NaCl concentration prepared with Zobell marine broth(1%,2%,4%, and 6%,8%,9%,10%) show that highest pigment production were observed(2%). Another species of *Kocuria* sp. K70, have exhibited pigment production at 2% NaCl(Kim, Y. S., & Park, J. S. 2010). NaCl both reduces water activity and osmotic stress in microbial cells, the cellular stress imposed triggers increase in various types of metabolites .The reduction in water activity is pertinent to the current study as this is the parameter which stimulates an increase in secondary metabolites.

Extraction of the pigment producing bacteria

The isolated strain were grown in Zobell marine broth for 72 hrs of incubation extensive growth of pigment producing bacteria were seen in the flasks fig-3. For the extraction of pigment producing bacteria, various methods were used such as centrifugation, addition of methanol so that cells get lysed and intracellular pigments can extracted. Fig-4 show that UV-vis absorption from 400-500 nm.

Antibacterial activity of crude pigment

The findings of this study point to the coastal region's potential as a source of marine bacteria that can produce antibacterial activity that is beneficial for numerous medicinal applications. In order to assess the antibacterial





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activity against bacterial pathogens using the Agar well diffusion method, extract pigments were dissolved in DMSO. Antibacterial activity of the extracted pigments against bacterial pathogens result are shown in table 1. Present results are consistent with a previous study that showed the inhibitory activity of pigment produced by Micrococcus species on gram-positive bacteria (Mohana DC, et al., 2013). The results of antibacterial activity of bacterial pathogens against pigments, the maximum zone of inhibition in Staphylococcus aureus (21mm), Escherichia coli (16 mm) followed by Streptococcus pyogenes (18mm), Enterococcus faecalis (18 mm) and Enterobacter sp (22 mm) Serratia marcescens (17 mm). In a similar study show that Carotenoid pigment caused inhibition on bacterial pathogens (Rostami, H et al., 2016; Majeed, H. Z. 2017).

CONCLUSION

The present study concluded that the isolation of pigment producing bacteria from marine soil. Optimization of the culture to enhance the pigment production. The extracted pigments showed antibacterial activity against bacterial pathogens. The isolate *Kocuria flava* (AP2)were selected for further studiesanti-biofilm and anticancer. In this study suggested the potential of this marine microbes for industrial application especially in the development of promising drug.

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Table-1: Antibacterial Activity of Bacterial pathogens

S.no	Bacterial Pathogens	Zone of inhibition(mm)			Positive
		100 µI	200 µI	300 µI	control(ciprofloxacin)
1	Escherichia coli	11	14	16	10
2	Streptococcus pyogenes	12	16	18	19





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3	Enterococcus faecalis	-	16	18	20
4	Enterobacter sp	15	19	22	20
5	Staphylococcus aureus	16	18	21	10
6.	Serratia marcescens	12	15	17	10

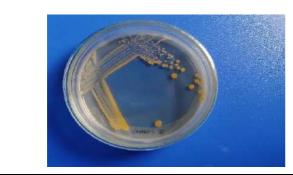
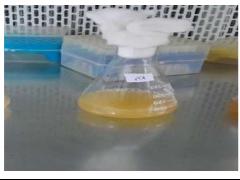


Fig.1: Plate show the Pigmented bacterial colonies after streaking

Fig.2: Phylogenetic tree analysis of the isolated strain Kocuria flava AP2



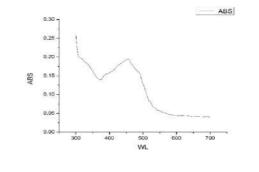


Fig.3: Production of pigment from AP2 isolates

Fig. 4: UV-Vis spectrophotometer of pigment





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RESEARCH ARTICLE

Design, Formulation and Evaluations of Sustained Release Matrix Tablets of Selected Antihypertensive Drug

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ABSTRACT

In the present study, Nifedipine (30mg) was chosen as a model drug which is an Antihypertensive agent. Because of its short life (2hr) and its high water solubility it was chosen as a suitable candidate for sustained release matrix tablet formulation. The aim of this present work is to design and formulate sustained release matrix tablets of Nifedipine using different combinations of polymers like HPMC K100M, guar gum, karaya gum which act as the release rate retardants. All the precompressional parameters were found to be within the standard limits, which indicates that granules used for the preparation of tablets were free flowing. The tablets were prepared by direct compression method. The prepared tablets were evaluated for various post compressional evaluations and found to be within the acceptable limits. Matrix tablet containing a blend of HPMC K100 M and guar gum (1:3) successfully sustained the release of Nifedipine for a period of 12hrs. Highest swelling index (97.97±0.28%) was found for tablets of formulation F18 of Nifedipine. The formulation F18 showed better sustained drug release (71.45%) up to 7hrs as compared to the remaining formulations. All the formulations of the prepared sustained release matrix tablets of Nifedipine follows first order kinetics and diffusion as the main mechanism of drug release. The optimized formulations were kept for the stability study at short term conditions as per the ICH quidelines (40° C ± 2°C at 75% RH ± 5% RH). The parameters of the tablets after stability study were found to be satisfactory and no considerable changes were observed. Thus, the formulation F18 is considered as the stable formulation containing drug: polymer (guar gum and HPMC K100M) in 1:3 ratio.





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Keywords: Sustained; Antihypertensive; Drug Nifedipine; Swelling index; Dissolution; Drug content

uniformity; Diffusion.

INTRODUCTION

Oral delivery of drugs is the most preferable route of drug delivery due to its ease of administration, patient compliance and flexibility in formulation.¹ It does not cause any sterility problem and may have minimal risk of damage at the site of administration.² Tablets are the most accepted drug delivery systems among the oral administration. They are convenient to manufacture on a large scale with reproducibility, stability and high patient acceptability[2,3]. Pharmaceutical dosage forms have been developed to release active substances in modified manner as compared with conventional formulations[7,8]. Modification in the release of active substances may have a number of objectives, but the main intention is to maintain therapeutic activity without frequent dosing, reducing the toxic effect and to reduce the work load of the patient. The European Pharmacopoeia defines modified release in terms of the rate or the site at which the active ingredient is released. A modified release dosage form is defined as "A formulation of medicinal drug taken orally releases the active ingredients over several hours in order to maintain a relatively constant plasma concentration of the drug" [3,4,5,6]. The goals of sustained release drug delivery systems are to conserve and to maintain an effective drug concentration and uniform blood level of drug, to improve the patient compliance, to improve the efficiency of drug and to decrease the side effects. Release rate from sustained release dosage form is controlled mainly by the type and proportion of various natural and synthetic polymers used in the formulation. Hydrophilic polymer matrix is widely used for formulating the sustained release dosage forms.

Physicochemical nature of the drug generally decides the pharmacokinetic profile of the drug. Sustained release drug delivery systems are formulated by decreasing the rate of absorption or by modifying the structure of the drug.⁶ In the matrix system, a solid drug is dispersed in an insoluble matrix system. The release of the drug is controlled by dissolution as well as diffusion method. Among the various methods used to control drug release from pharmaceutical dosage form, the matrix system is the most frequently applied. Nifedipine is the selected antihypertensive drug for this study. It is a selective calcium channel blocker which is poorly water soluble drug that belongs to BCS class II. It is a peripheral arterial vasodilator which acts directly on vascular smooth muscles. Nifedipine is widely used in the treatment of angina pectoris and in systemic hypertension. Its absorption from GIT is limited by the dissolution rate. It has a short biological half-life (4 hrs). Absorption of nifedipine is poor following administration orally via immediate release dosage forms. It exhibits 45-65% oral bioavailability due to hepatic first pass metabolism[4,7]. Immediate release formulations of nifedipine clearly shows fluctuation in drug plasma concentration results in specific side effects like increase in heart rate. The design of sustained matrix drug delivery systems should be primarily aimed to achieve the more predictability and reproducibility to control the drug release, drug concentration in the target tissue and optimization of the therapeutic effect of a drug by controlling its release in the body with lower and less frequent dosing with better patient compliance[1,6].

MATERIAL AND METHODS

Identification of drug[7]

Identification of the drug Nifedipine was done by Infrared spectroscopy.

Drug- Excipient compatibility study[10]

FTIR spectra of drug samples were recorded using potassium bromide (KBr) pellet method at resolution of 4cm⁻¹ for its authentication and to study the principle peaks using FTIR spectrophotometer. Dry samples of the drug and potassium bromide were mixed uniformly and filled into the die cavity of sample holder and an IR spectrum was





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recorded. The identified peaks were compared with the principle peaks of reported IR spectrum. Thus the samples were authenticated.

Preparation Of Sustained Release Matrix Tablet Of Nifedipine[14,15]

The sustained release matrix tablets were prepared by direct compression and the composition were shown in **Table 1 and Table 2**. Accurately weighed quantity of Nifedipine, polymers and MCC were added in geometrical order into a mortar and pestle. Then it is passed through sieve no.60. The magnesium stearate and talc were added as the lubricant and glidant. The total powder blend is weighed individually for fifty tablets. Then it was compressed into tablets by using 10 station rotary tablet punching machine having 8mm internal diameter.

Post compression evaluations

General Appearance[11]

The tablets were visually observed for capping, chipping and cracking. There should be uniformity in colour, size and shape of the tablets.

Thickness[10,14]

Thickness of the tablets were determined using Vernier calliper. Three tablets were selected randomly from each batch and were used for thickness determination. It was expressed in mm. The mean and SD were calculated.

Diameter[10]

Three tablets were selected randomly from each batch and were used for diameter determination. The diameter of each tablet was measured using a Vernier calliper and their values were reported in mm. The mean and SD were calculated.

Hardness [10]

Hardness of tablets were determined using a validated Monsanto hardness tester. It is expressed in kg/cm². Three tablets from each batch were selected and evaluated and the average value with SD was recorded.

Friability [16,17]

20 tablets were preweighed and placed in Roche friabilator which was then operated for 100 revolutions. It is a plastic chamber which revolves at a speed of 25rpm for 4 minutes, dropping the tablets to a distance of 6 inches in each revolution. The tablets were then deducted and reweighed. And the percentage weight loss in tablets was determined.

Weight Variation [10,18]

The USP weight variation test is done by weighing 20 tablets individually, then calculating the average weight and comparing the individual weights to the average weights. The tablets must meet the IP specifications. Weight variation specifications as per IP.

Drug content uniformity [19]

Five tablets were weighed and powdered. Powder equivalent to 30mg of Nifedipine was dissolved in 10ml of 0.1N HCI, then made up to 100ml with 0.1N HCI in a 100ml standard flask. From this 10 μ g/ml, equivalent solution was prepared and analysed at 274nm using UV-Visible spectrophotometer.

Swelling Index [18]

To determine the extent of matrix swelling, three tablets from each batch were weighed and placed in a petridish containing 25ml of 0.1N HCl. After specific time intervals, the tablets were removed from the media, excess of media was wiped off by using a filter paper and weighed again upto 7hrs. The swelling index was calculated using the following formula:

 $S.I(\%) = W_{t-w0}$





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Where.

Wt is the weight of tablet at time 't'. Wo is the initial weight of the tablet

Dissolution Study [20,21]

The dissolution assessment was performed using dissolution USP type II (paddle method) apparatus. Dissolution rate was determined using 900ml of 0.1N HCl as the dissolution medium for the complete release profile of Nifedipine. Each batch of tablets was placed in each bowl of dissolution apparatus. The temperature of 37 ± 0.5 °C was maintained during the test and the paddle speed was fixed at 50rpm. The samples of 1ml were withdrawn at specific time intervals up to 7hrs and were replaced with the same volume (1ml) of fresh dissolution medium (0.1N HCl) after each withdrawal. Then it was made up to 100ml. The collected filtrate was filtered using Whatman filter paper (0.45 μ m pore size). Each sample was evaluated for drug contents by measuring absorbance at 274nm using UV-Visible spectrophotometer. The % release of drug was calculated.

Comparison With Marketed Formulation [22]

The optimized formulation of Nifedipine sustained release matrix tablet was compared with commercially available tablets. Nicardia CD Retard 30 (Nifedipine sustained release tablets 30 mg) was selected as a choice. Physical appearance, thickness, diameter, hardness, drug content uniformity and *invitro* drug release of optimized formulation of prepared Nifedipine sustained release matrix tablets was determined and compared with the marketed Nifedipine sustained release tablets in table 3.

Stability study [19]

Stability studies were carried out at short term conditions as per the ICH guidelines (40° C \pm 2° C at 75% RH \pm 5% RH) for the optimized formulation. The optimized formulations were packed in amber coloured bottles, tightly covered with aluminium foil. Then they were stored at 40° C \pm 2° C at 75% RH \pm 5% RH for 60 days. The samples were withdrawn at an interval of 15 days. The samples were analyzed for its physical appearance, hardness and drug content.

RESULTS AND DISCUSSIONS

The drug was identified by using IR spectroscopy. The obtained peaks were found to be similar to that of standard spectrum of Nifedipine. The obtained peaks are 3182.35cm-1, 1730cm-1, 1404cm-1, 1647cm-1 for NH, C=O, N=O and C=C respectively. The compatibility of Nifedipine with the polymers and excipients such as guar gum, karaya gum, HPMC K100M, magnesium stearate, microcrystalline cellulose and talc were established by FTIR absorption spectral analysis. Any changes in the chemical composition after combination with polymers were investigated. The IR characteristics of the drug with the polymers almost resemble the IR structural characteristics of the pure drug (Nifedipine). The IR spectrum of drug in combination with polymers and excipients were shown in Figure 1. All the characteristics peaks of Nifedipine were found in the spectra of Nifedipine with polymers without the appearance of new peaks, disappearance and/or shift in the frequency of bands. This revealed that Nifedipine is compatible with all the tested polymers and excipients. So they can be used for this study without any interactions. The sustained release matrix tablets were prepared using guar gum, HPMC K100M, karaya gum as rate retarding polymers. They were prepared by direct compression method, where the drug and polymers were selected in the ratios 1:1, 1:2 and 1:3 alone and in combinations in figure 2. The total weight of the prepared tablets were approximately 200mg. The prepared sustained release matrix tablets of Nifedipine were subjected to various evaluation parameters like thickness, diameter, hardness, friability, weight variation, uniformity of drug content, swelling index, invitro drug release study and stability study. The mean hardness values were measured for all the formulations using Monsanto hardness tester. The hardness value ranges from 6.13±0.13 to 6.33±0.35 kg/cm². The results indicates that the prepared tablets ensure perfect compactness during shipping, packaging and to get proper shape and design.





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Friability was determined by using Roche friabilator. The % friability ranges from 0.50 to 0.89 for all formulations. These values are within the acceptable limits implying good compactness and strength. It also implies the ability of tablet to withstand physical and mechanical stress conditions. 20 tablets of each formulation were subjected to weight variation test as per the Pharmacopeial specifications. The average weight of tablets were in the range of 196.35±0.01 to 204.41±0.02 mg. The values indicates that the tablets of all formulations were within the Pharmacopeial specifications. The drug content uniformity was calculated for all the formulations of sustained release matrix tablets of Nifedipine. The average drug content of tablets of Nifedipine was found to be between 90.80±0.13 and 108.69±0.02%. These values were found to be satisfactory and meet the requirements of IP. The low values of standard deviation indicates the uniform distribution of the drug within the prepared sustained release matrix tablets. Percentage swelling of all the sustained release matrix tablets of Nifedipine values represents the mean of three replicate determination. The swelling indices for the drug Nifedipine were determined in the formulation containing the polymers like HPMC K100M, guar gum, karaya gum alone and in combinations after 7 hours were determined. The data obtained from *invitro* drug release study performed up to 7hrs gives a clear indication that prepared tablets showed necessary sustained release profile. The formulation F18 showed better sustained drug release (71.45%) upto 7hrs as compared to the remaining formulations. So F18 was found to be the optimized formulation containing guar gum and HPMC K100M as the polymers, where the drug: polymer ratio is 1:3 (higher concentration). Invitro drug release studies showed that release rate of drug decreases with increase in the concentration of polymers. So it provides the sustained release of drug which is shown in table 3. After the comparison of the % CDR of both the optimized formulation and the marketed formulation, it was found that the difference factor is greater than the similarity factor. Two products are said to be similar if the similarity factor exceeds 50%. The % CDR of the optimized formulation is 71.45% after 7hrs and that of the marketed formulation is 99.11% after same time. The stability of the optimized sustained release matrix tablets in humidity chamber was performed. The following parameters like physical appearance, hardness and drug content were determined for an interval of 15 days. The parameters were found to be satisfactory and no considerable changes were observed. Thus, the formulation F18 can be considered as a stable formulation.

DISCUSSION

In the present study a successful attempt has been made to develop sustained release matrix therapeutic system for Nifedipine in the form of tablets to improve its bioavailability and to overcome its inherent drawbacks. Hypertension, also referred to as high blood pressure, HTN or HPN, is a medical condition in which the blood pressure is chronically elevated or an abnormal rise in diastolic pressure and/or systolic blood pressure. Antihypertensive agents are the drugs which lowers the blood pressure in hypertensive patients. A sustained release dosage form will provide a therapeutic concentration of the drug in the blood that is maintained throughout the dosing interval with a reduction in peak concentration ratio. Matrix tablets are the type of controlled drug delivery systems, which release the drug in continuous manner by dissolution controlled as well as diffusion controlled mechanisms. Nifedipine is a group of compounds thought to act by blocking the transmembrane inward movement of calcium. Nifedipine being antihypertensive agent act by blocking Ca²⁺ channel and interfere with the working action of Ca2+ in blood vessel constriction and heart muscle contraction and nerve conduction in the heart. Sustained release formulations are most efficient for routine hypertension therapy with Nifedipine. The SR dosage forms should primarily reduce the occurrence of steep rises in plasma concentration of the drug. Another important therapeutic goal that can be achieved with SR formulations is the improvement of chronic therapy compliance by prolongation of the dosing intervals. The sustained release formulations may maintain the therapeutic concentrations over prolonged periods. Preformulation studies are carried out in order to evaluate the physical and chemical properties of the drug alone and in the combined form with the excipients. The melting point of Nifedipine was determined by capillary tube method and it was found to be 171-175°C. The melting point was determined to be within the specified official limits and it indicates the purity of the drug. Nifedipine was found to be good soluble in 0.1N HCI, insoluble in water, sparingly soluble in alcohol and freely soluble in acetone. Nifedipine was identified by using IR spectroscopy. The obtained peaks were found to be similar to that of standard spectrum of Nifedipine. The





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obtained peaks are 3182.35cm⁻¹, 1730cm⁻¹, 1404cm⁻¹, 1647cm⁻¹ for NH, C=O, N=O and C=C respectively. The compatibility of Nifedipine with the polymers and excipients were established by FTIR absorption spectral analysis. This revealed that Nifedipine is compatible with all the tested polymers and excipients and can be used for this study without any interactions, λ_{max} of Nifedipine was found to 274nm by using UV-Visible spectrophotometer and this complies with the IP standards, indicating the purity of the drug. From the standard calibration curve of Nifedipine in 0.1N HCI, it was clear that the drug obeys Beer's law in the range 2 to 10µg/ml. The results of precompression parameters such as angle of repose, bulk density, tapped density, Hausner's ratio and Carr's compressibility index showed excellent to passable flow property of the powder blends. The sustained release matrix tablets of Nifedipine was prepared by direct compression method. The selected dose of Nifedipine was 30mg. The tablets are formulated by using different natural and synthetic polymers such as karaya gum, HPMC K100M and guar gum. The selected drug:polymer ratios are 1:1, 1:2 and 1:3 alone and in combinations. The total weight of the prepared tablets were 200mg. The other excipients are MCC, magnesium stearate and talc. 18 batches of tablets each containing 50 tablets were prepared. The prepared sustained release matrix tablets of Nifedipine were subjected to various post compression evaluations and the thickness, diameter and hardness were found to be satisfactory for all the formulations. The friability of the prepared tablets was within the acceptable limits implying good compactness and strength.

Weight variation indicates that the tablets of all formulations were within the Pharmacopeial specifications. The drug content uniformity values of the tablets were found to be satisfactory and meet the requirements of IP. As the polymer concentration increases, the swelling also increases. For formulation containing the combination of HPMC K100M and guar gum polymers, the swelling index increased considerably when compared to the formulations containing other polymers. Highest swelling index (97.97±0.28%) was found for tablets of formulation F18 of Nifedipine. The formulation F18 showed better sustained drug release (71.45%) up to 7hrs as compared to the remaining formulations. So F18 was found to be the optimized formulation containing guar gum and HPMC K100M as the polymers, where the drug: polymer ratio is 1:3 (higher concentration). All the formulations of the prepared sustained release matrix tablets of Nifedipine follows first order kinetics and diffusion as the main mechanism of drug release. The similarity factor of the marketed formulation and the optimized formulation was found to be less than 50%. The parameters of the tablets after stability study were found to be satisfactory and no considerable changes were observed. Thus, the formulation F18 can be considered as a stable formulation.

CONCLUSION

The present study has been a satisfactory attempt to design, formulate and evaluate the sustained release matrix tablets of a selected antihypertensive drug by using different combination of polymers. It mainly aims to extend the duration of action of the drug, to reduce the frequency of dosing, to minimize the fluctuations in plasma level, improved drug utilization with less adverse effects and enhanced patient compliance. Nifedipine is selected as the antihypertensive agent. The polymers used are karaya gum, HPMC K100M and guar gum in the ratio 1:1, 1:2, 1:3 (drug: polymer). The formulation F18 containing the combination of HPMC K100M and guar gum in the ratio 1:3 is selected as the optimized formulation. The results of the evaluation parameters of F18 like thickness, diameter, hardness, friability, weight variation, drug content uniformity, swelling index and *invitro* dissolution studies were found to be satisfactory. The swelling index was found to be more (97.97±0.28%) and the drug release showed better sustained (71.45%) release up to 7hrs as compared to remaining formulations. So, F18 was selected as the best formulation which follows the first order kinetics and diffusion as the main mechanism of drug release.

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Table 1: Formulation of sustained release matrix tablets of Nifedipine

Ingredients (mg)	F1	F2	F3	F4	F5	F6	F7	F8	F9
Nifedipine	30	30	30	30	30	30	30	30	30
Karaya gum	30	60	90	-	-	-	-	-	-
Guar gum	-	•	-	30	60	90	-	-	-
HPMC K100M	-	ı	-	-	-	-	30	60	90
Microcrystalline cellulose	128	98	68	128	98	68	128	98	68
Talc	2	2	2	2	2	2	2	2	2
Magnesium stearate	10	10	10	10	10	10	10	10	10
Total weight	200	200	200	200	200	200	200	200	200

Table 2: Formulation of sustained release matrix tablet of Nifedipine using combination of polymers

Ingredients (mg)	F10	F11	F12	F13	F14	F15	F16	F17	F18
Nifedipine	30	30	30	30	30	30	30	30	30
Karaya gum	15	30	45	15	30	45	-	-	-
Guar gum	15	30	45	-	-	-	15	30	45
HPMC K100M	-		-	15	30	45	15	30	45
Microcrystalline cellulose	128	98	68	128	98	68	128	98	68
Talc	2	2	2	2	2	2	2	2	2
Magnesium stearate	10	10	10	10	10	10	10	10	10
Total weight	200	200	200	200	200	200	200	200	200

Table 3: Invitro drug release profiles of F18 and marketed formulation

Times (h	Percentage cumulative drug release (%)			
Time (hours)	F18			
0.5	2.54			
1	16.01			
2	22.32			
3	32.79			
4	43.11			
5	54.16			
6	63.67			
7	71.45			

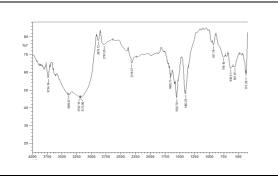


Figure 1: IR spectrum of Nifedipine + HPMC K100M + Guar gum + Magnesium stearate +MCC +Talc



Figure 2: Sustained release matrix tablets of Nifedipine

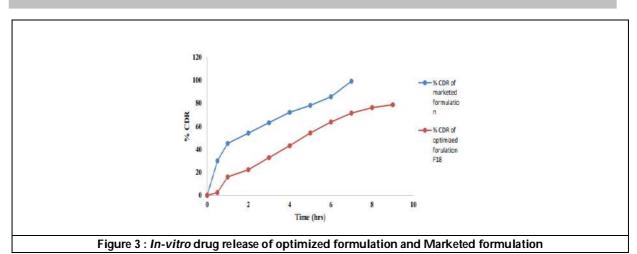




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RESEARCH ARTICLE

Artificial Intelligence in Education: Research and Development

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ABSTRACT

Artificial intelligence technology has advanced significantly in recent years, resulting in significant changes in people's lives. In every sector of life, new technologies are rapidly evolving. Artificial intelligence and education was created, and education has been altered on a regular basis. This article discusses the meaning, characteristics, and key technologies of artificial intelligence education applications, based on an analysis of the current state of artificial intelligence education in India, in order to sort out and reflect on the advancement strategies of artificial intelligence education applications in the intelligent era, and to provide development ideas for artificial intelligence education applications.

Keywords: Artificial intelligence, Parallel Education, Smart Education, social networks, Virtual Teachers

INTRODUCTION

Examining the Evolution of Smart Education

In an era when technology is changing education, smart education is leading the way in terms of education informatization and has emerged as a major theme in educational progress. Intelligent Tutoring System (ITS), smart campus, Big Data in Education (BDE), knowledge graph, educational robots, virtual teachers, and personalised education are the seven primary branches of smart education. This paper provides a complete review of the study and application of smart education from the following seven perspectives, and recommends its development based





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on the current state of smart education development, based on a literature survey and market research. Smart education is the process of promoting educational reform and progress via the use of current information technologies (for example, educational management, educational teaching, and educational research). Informatization of education is a critical component of national informatization. Changing education views and ideas, deepening education reform, improving education quality, and cultivating innovative skills are all important. Realizing the leap-forward progress of education is an unavoidable choice. A review of the historical process is required. This paper examines research on smart education with the goal of determining the current state of smart education and its many fields, as well as summarising its progress and existing challenges in order to make recommendations for future development at both the academic and application levels.

Outline Of Smart Education Research

Five road signs of smart education were presented, which include learners' technical immersion, personalised and diverse learning paths, global integration of knowledge, skills, systems, culture, and resources, and the critical role of economic development in the twenty-first century. E-learning[1][2], big data[3], learning analytics[4][5], intelligent tutoring system[6], knowledge graph[7], and so on are some of the main research directions in smart education. Some universities have developed various types of smart teaching systems in response to the need for scientific research and application, such as the University of Scholars in India, on the other hand, have made some efforts toward the development of smart education, including research into its concept and characteristics, system design, technical growth path, learning environment, and implementation of new methods [1]. Information application, which is supported by educational big data technology and uses the Internet + technology platform to study smart courses and smart classrooms; [2] system structure, which focuses on the fusion of artificial intelligence and educational science; and [4] system structure, which focuses on the fusion of artificial intelligence and educational science [3] future classroom, which investigates the design of a smart learning environment, calculates emotional state changes during the learning process, and offers timely instructional intervention; [4] customised, adaptive, and self-directed learning, which makes use of artificial intelligence to deliver greater assistance and services to students.

Smart Education Analysis Branch Fields

In this section, we'll cover everything from smart campus infrastructure to key technologies like Big Data in Education, Knowledge Graph, and personalised education, as well as application systems like Intelligent Tutoring System, Virtual Teachers, Educational Robots, and new ways to explore education like Parallel Education and Social Education.

Campus of the Future

In the educational process, a smart campus was proposed based on the building of a digital campus informatization. The term "smart" comes from the term "smart planet." Smart campus connotation features primarily include five aspects: [1] fast and ubiquitous broadband internet access; [2] real-time and comprehensive awareness of intelligent environment; [3] real-time and comprehensive awareness of intelligent environment; and [4] real-time and comprehensive awareness of intelligent environment, [3] business application and intelligence integration; [4] enormous data intelligent mining analysis; [5] intelligent and friendly service and tailored convenience[9]. The purpose of a smart campus is to provide assistance. Smart campus design and building aims to increase education's modernization, intelligence, innovation, leadership, and socialization[10]. Cloud computing, the Internet of Things, big data, mobile internet, and social networks are all used in the creation of smart campuses. Currently, some Indian schools have established smart campuses, while others are in the planning or development stages. The construction content, however, may be problematic due to the lack of a common definition of smart campus. Also, because there are so many departments involved, the planning and building process takes a long time. The network environment, the view of the Internet of Things, and the integrated information service platform are all covered by each school's priority. The smart campus, built on the foundation of the original digital campus, must continue to improve in terms of concepts, technology, and specific construction measures. clever planet's reference The five main characteristics of a smart campus are: (1) fast and ubiquitous broadband internet access; (2) real-time and





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comprehensive awareness of the intelligent environment; (3) integration of business applications and intelligence; (4) massive data intelligent mining analysis; and (5) smart and friendly service and personalised convenience[9]. The purpose of a smart campus is to provide assistance. Smart campus design and building aims to increase education's modernization, intelligence, innovation, leadership, and socialization[10]. The smart campus, built on the foundation of the original digital campus, must continue to improve in terms of concepts, technology, and specific construction measures. Computing, the Internet of Things, big data, mobile internet, and social networks are all examples of new technologies. Some schools in India have established smart campuses, while others are in the planning or construction stages. The construction content, however, may be problematic due to the lack of a common definition of smart campus. Also, because there are so many departments involved, the planning and building process takes a long time. The network environment, the view of the Internet of Things, and the integrated information service platform are all covered by each school's priority.

Smart Education and Learning Systems: Knowledge Engineering Paradigms

Artificial intelligence (AI) has recently gotten a lot of press in the realm of building smart digital education. Researchers have developed a smart tutoring system using computational intelligence (CI) and machine learning technologies (STSs). On the other hand, the confluence of artificial intelligence (AI), data science, and the Internet of Things (IoT) is enabling the development of a new generation of web-based smart solutions for all educational and learning tasks. The CI and knowledge engineering paradigms are discussed in this study for designing smart educational and learning systems. Case-based reasoning and ontological engineering, two popular CI paradigms, are addressed and investigated in this work. The major goal of this research is to identify and investigate the benefits and advantages of such intelligent paradigms in order to improve the efficacy and efficiency of smart tutoring systems. The study also discusses the difficulties that application developers and knowledge engineers confront while designing and deploying such systems. The large-scale digitalization of all spheres of economic activity is currently manifested not only in the widespread adoption of information and communication technologies (ICT) in a variety of human activities, but also in the convergence and integration of the digital and physical worlds through the use of smart digital solutions. The digital economy holds a considerable amount of potential for improving the lives of people in diverse places throughout the world. However, taking advantage of the potential of smart technologies in the digital economy is only achievable if there are enough people with the necessary skills to develop and apply such technologies. This raises new demands on the educational system, first, in terms of developing new areas of training in universities in order to prepare highly qualified personnel for the ICT sector, and second, in terms of integrating ICT competencies into all areas of training in order to expand the skills of applying smart technologies in all areas of economic activity, and third, in terms of developing new educational models that are adequate to the requirements. It is critical to discuss the formation of Smart education in this scenario. Smart education (SE) refers to a set of eservices that use digital media and information technology to aid educational operations.

SE is a multidisciplinary field that encompasses a wide range of educational technologies, including instruction, training, teaching, learning, pedagogy, communication, and collaboration. Furthermore, in recent years, the field of artificial intelligence in education has become the most complex. The field's purpose is to develop knowledge-based software that may be utilised in real-world teaching, learning, and training scenarios. The convergence of three interrelated areas is associated with theoretical research in the subject field and the development of the concept of Smart education: the design of new smart technologies (engineering paradigm), the development of the digital economy (technological paradigm), and the transformation of the education system, university models, and labour market (innovative, economic, organisational and management paradigms. In this scenario, we're discussing both educational model and process change as well as technological and teaching method modernization. In recent years, a number of scholarly studies [6,7] have attempted to define the research framework for smart education. The studies look at the educational process from an engineering perspective [8] as well as organisational and managerial difficulties [9,10]. Simultaneously, there is definitely an insufficient number of works that consider these two aspects of the construction of the smart education system.





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Conclusions And Next Steps

We list some of the important open concerns that must be addressed in order to assure the success of constructing strong intelligent e-learning systems in this final section. To summarise, developing intelligent e-Learning/educational systems is a challenging and complex process that presents numerous technological and research difficulties that must be tackled collaboratively. Many of the technical obstacles and difficulties in creating new generation of intelligent e-Learning/educational systems are now solved by combining computational intelligence and machine learning techniques with knowledge acquisition approaches. However, more study is needed to combine knowledge engineering, artificial intelligence, machine learning, and educational technology with current Internet of Things (IoT) technology trends. As a result of this convergence, a new generation of web-based intelligent e learning and tutoring systems will emerge. Such web-based technologies can help to improve online education learning and training processes. As a result, combining these methodologies with educational technology can help designers create more efficient, robust, and intelligent e-learning systems. Furthermore, assuring the success of such systems when they migrate to the cloud is a significant problem.

Future Management of Education in Healthcare Communities

Building a customised curriculum in continuous education is difficult since there are numerous educational institutions that provide diverse courses, and the content of the courses is not presented in a consistent manner. Furthermore, in many firms, it is critical that personal curriculums match the skills and competencies required by the organisation. We demonstrate how a healthcare community can use modern ICT technology to manage continuous education in this study. Our developed Education ontology is the cornerstone of our solution. Employee Ontology, Competence Ontology, Business Rule Ontology, and Learning Object Ontology are all combined in one ontology. Such an integrated ontology allows us to process a wide range of questions that would be impossible to process on a single ontology, allowing us to accomplish results that would be impossible to achieve if we worked independently. The applications in our cloud-based architecture are built on a shared Education ontology. As a result, anyone with a good internet connection and a normal browser can use the cloud applications.

CONCLUSION

In today's digital society, information integration and sharing are two fundamental principles. We examined their role in a healthcare community where information sharing among members is essential and where the implementation of a contemporary data infrastructure can considerably increase information sharing quality. The rapid evolution of medication treatments and technologies in healthcare necessitates specific skills and knowledge that must be renewed on a regular basis. As a result, the importance of continuing education is growing, and communities of interest are playing an increasingly vital role in promoting it. Building a tailored curriculum in continuing education can be difficult since it must align with the skills and competencies required in the learner's organisation. We looked at this issue in the context of a healthcare community made up of people from various organisations. Our developed Education ontology is the cornerstone of our solution. Employee Ontology, Competence Ontology, Business Rule Ontology, and Learning Object Ontology are all combined in one ontology. An integrated ontology like this can handle a wide range of questions that can't be handled by a single ontology. The fundamental difficulty in developing shared ontologies is that there are so many different factors and parties to consider. However, in some domains, such as a healthcare community, it has shown to be simpler because the domain is relatively narrow and all stakeholders have common concerns. Our main purpose in this project was to show that cloud-based education management can be implemented from a technological standpoint. However, there are issues that could compromise the system's success. The introduction of new technology, in particular, necessitates training: wrong use of a new technology, as a result of a lack of sufficient training, may jeopardise the entire system. In order to reduce the impact of the system's implementation on healthcare workers, we'll look into whether education management systems may be connected into employees' day-to-day work patterns in the future. We'll look at how the system's features can be combined with other e-health solutions that education providers utilise.





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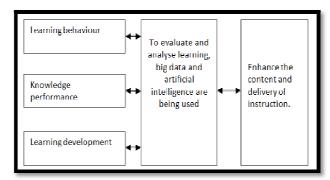


Fig.1: Architecture of Smart education Systems





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RESEARCH ARTICLE

Covering based Soft Rough Finite State Automata and their Properties

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ABSTRACT

In this proposed article the core of automata theory extended to soft set theory with the aid of covering. By the definition of covering based soft set we designed the Covering based Soft Rough Finite State Automata (CSRFSA) by which one can generate languages which is a subset of rough finite state automaton. After this deep examine, we proved some of the properties by some of the suitable examples.

Keywords: Automata (CSRFSA), Covering based, automata theory

INTRODUCTION

In 19th century researchers designed finite state machines. In between these 10 years, Automaton has gained popularity among the researchers due to its applications in various subjects/ areas[1][2][4][7]. Massive number of publications was written during the period. Some of the researchers linked rough set theory to automaton[5][10]. Like roughest theory, soft set theory is a another tool to develop real world problems which is dealing with uncertain and not clearly defined objects. Though many authors extended finite automaton to soft set theory, its properties, the links between soft finite state automaton and rough finite state automaton is left aside. In [3], the authors introduced soft successor and soft immediate successor concepts and the studied the concept of direct product of soft machines. The defined the soft FSM and also studied the concept of soft sub systems and soft sub machines. They also proved some of the properties of the SFSM by proving some theorem. Finite machine also extended to cubic structure, a cubic set $A = (\delta_A, w_A)$ is defined as a subset in $S \times X \times S$, where S-states, X- input symbols and also the properties of cubic FSM is defined and also they clubbed 2 cubic FSM using some of the operations like Cartesian composition, direct product etc.,. In this purpose we defined SFSM in a different manned in such a way that it can also be named as covering based soft finite state machine. As we already defined RFSA(Rough Finite State Automaton) and non-





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homogeneous rough finite state automata[8][9], we also extended this paper to the concept of Rough set theory, which is named as covering based soft rough finite state automaton, the propertied of this Covering based Soft Rough Finite Automaton (CSRFSA) is also defined by theorems and also that the properties of the language is also discussed in detail. Section 2 deals with basic definitions which will be useful to study the rest of the article. In Section 3 we defined Covering based Soft Rough Finite State Automaton and their properties followed by a conclusion.

Preliminaries

In this section we review some of the basic definition and properties of RFSA and Covering based Soft Rough set, which will be required to study the later sections.

Definition 2.1

Consider an information system I=(U,A) which contains rows and columns and any set $X\subseteq U$, then the lower approximation space $P_-(X)=\{x\in U\big|[x]_p\subseteq X\}$ and the upper approximation space $P^-(X)=\{x\in U\big|[x]_p\cap X\neq \phi$. Then the roughest corresponding to X in the approximation space P is the ordered pair $RS(X)=(P_-(X),P^-(X))$.

Definition 2.2[9]

RFSA is a five tuple $M = (Q, \Sigma, \delta, q_0, F)$ where Q is the set of states Σ is the finite set of input symbols, δ is the rough transition function defined by $\delta: Q \times \Sigma \to \mathcal{P}(Q) \times \mathcal{P}(Q)$, q_0 is the initial state of Q, $F \subseteq Q$ is the final state/states.

Definition 2.3

Let U is an initial universe set and E is a set of parameters, $A \subseteq E$ then (F, A) a pair called soft set over U, F is a mapping $F: A \to \mathcal{P}(U)$.

Definition 2.4

Let U is an universe and C is the family of subset of U. If the 2 conditions holds (ie) if all subsets of C are non empty and union of C equal to U (ie) $\bigcup C = U$ then C is a covering of U and the covering approximation space is the ordered pair (U,C).

Definition 2.4 Covering Based Soft Rough set[10]

As already defined in , let (F, A) be a soft cover on U and (U, C_G) is a covering based approximation space, then any set $X \subseteq U, CRS(X) = (CR_-(X), CR^-(X))$ is a Covering Based Soft Rough set.

3. Covering Based Soft Rough Finite State Automaton

Let U is an initial universe set and E is a set of parameter and C be covering based soft sets. Then Covering Based Soft Finite State Automaton (SFSA) is a five tuple $M = \langle Q, \Sigma, \delta, q_0, F \rangle$ (which is same as RFSA) where Q is the finite set of states (U), Σ is a finite set of input symbols, q_0 is the initial state belongs to Q and F is the final state/states. The only difference between RFSA and SFSA is a transition map. The Soft transition map (δ) is defined by δ : $Q \times \Sigma \to \mathcal{P}(Q) \times \mathcal{P}(Q)$.

Definition 3.1 Covering based Soft Rough Finite State Automaton

Without loss of generality one can consider that (F, A) is a parameterized covering based soft set over U and (U, C_G) is a covering based soft approximation space and $CRS(X) = (CR_-(X), CR^-(X))$ is a covering based soft roughest. By this using RFSA defined model one can find the machine where $M = (CR_-(M), CR^-(M))$ and the language $L(CR(M)) = (L(CR_-(M)), L(CR^-(M)))$

Example.3.1

Consider the covering based soft set $C_1=\{q_0,q_1,q_2\},C_2=\{q_2,q_3\}$ Let $\delta_a=0.25$ and $\delta_b=0.3$





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\begin{array}{l} \eta_a(q_0) = \{q_0,q_1,q_2\}\eta_b(q_0) = \{q_0,q_1,q_2\}\\ \eta_a(q_1) = \{q_0,q_1\}\eta_b(q_1) = \{q_0,q_1\}\\ \eta_a(q_2) = \{q_0,q_1,q_2,q_3\}\eta_b(q_2) = \{q_0,q_2,q_3\}\\ \eta_a(q_3) = \{q_2,q_3\}\eta_b(q_0) = \{q_2,q_3\}\\ RS(\eta_a(q_0)) = \{C_1,C_1\cup C_2\}\\ RS(\eta_a(q_1)) = \{\phi,C_1\}\\ RS(\eta_a(q_2)) = \{C_1\cup C_2,C_1\cup C_2\}\\ RS(\eta_a(q_3)) = \{C_2,C_1\cup C_2\}\\ RS(\eta_a(q_3)) = \{C_2,C_1\cup C_2\}\\ RS(\eta_b(q_1)) = \{C_2,U\}\\ RS(\eta_b(q_1)) = \{C_
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Example 3.2

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Consider the covering based soft set C_1 = \{q_0, q_1\}, C_2 = \{q_2\} and C_3 = \{q_3\}. Let the threshold value be \delta_a = 0.3 and \delta_b = 0.4 RS(\eta_a(q_0)) = \{X_1 \cup X_2, X_1 \cup X_2\} \qquad RS(\eta_b(q_0)) = \{X_1 \cup X_2, X_1 \cup X_2\} \qquad RS(\eta_b(q_1)) = \{X_1, X_1\} \qquad RS(\eta_a(q_1)) = \{X_1 \cup X_2 \cup X_3, X_1 \cup X_2 \cup X_3\} \qquad RS(\eta_b(q_2)) = \{X_2 \cup X_3, X_1 \cup X_2 \cup X_3\} \qquad RS(\eta_b(q_1)) = \{X_2 \cup X_3, X_2 \cup X_3\} \qquad RS(\eta_b(q_1)) = \{X_2 \cup X_3, X_2 \cup X_3\}
```

Theorem 3.1

In which case the language of the lower\upper approximation of soft set and rough set will be same\different.

Proof

The lower Approximation of the soft set and Rough set will be different in the case that, if there are k-covering (ie) $c_i, c_j, ..., c_k$ and $c_i \cap c_j = x_k \neq \emptyset$ and in the equivalence class of Rough set if x_k is a only element presented in one of the equivalence class. Without loss of generality the language of lower approximation of the CSRFSA and RFSA will be different and upper approximation of CSRFSA and RFSA may be different but not in all the time.

Proposition 3.1

For every covering based soft rough finite state automata $CR_{-}(M) \subseteq CR^{-}(M)$. If $CR_{-}(M) = CR^{-}(M)$ then such CSRFSA is said to be definable.

Proposition 3.2

If $\exists m_i, m_i'$ and $\delta_{m_i} < \delta_{m_i}'$ then $CR_-(M) \subseteq CR_-(M')$ and $CR_-(M) \subseteq CR_-(M')$ and also $L(CR_-(M)) \subseteq L(CR_-(M'))$ and $L(CR_-(M)) \subseteq L(CR_-(M'))$

CONCLUSION

The core of automata theory extended to the parameterized family of sets namely soft set. Since its boundary of the set depends on the parameters, the one who wish to deal with parameters can make use of the covering based rough finite state automaton. We discussed the Covering based Soft Rough Finite State Automaton(CSRFSA) and the language of such automaton discussed in details. Some of the properties of CSRFSA were also defined.

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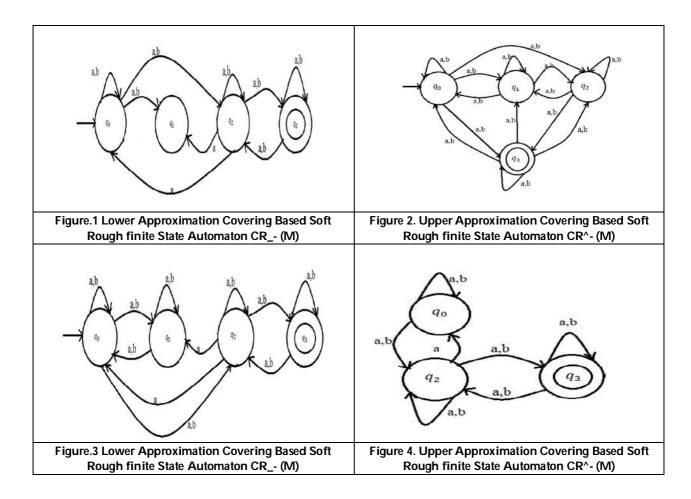


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ISSN: 0976 – 0997 **RESEARCH ARTICLE**

Effective Automatic Monitoring **System** An for Aquaculture **Applications**

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ABSTRACT

The chemical, physical, biological, and radioactive properties of water are together referred to as its radiological properties. In order to ensure the survival and development of aquatic life, the water quality must be properly maintained. From this point forward, an autonomous system should be used to monitor aquaculture using IoT technology, as suggested in this study, in order to preserve the quality of the water. The planned system will include a variety of sensors, including temperature, water level, and PH sensors. The quality of the water will be greatly influenced by temperature, PH, and water level. The quality of the water and aquatic life may suffer from changes in these characteristics. To communicate with the user's specific mobile phone, GSM is used. The primary purpose of a temperature sensor is to monitor temperature changes, and if the temperature increases above a normal level, the DC Motor will turn on. The PH Sensor is used to continuously check the soil's pH level. Water level sensors are used to keep track of the water level, and the motor is activated if it rises above the typical level. The collected data will subsequently be sent through GSM to the appropriate mobile device. When the sensors detect any abnormal value, the GSM will send a notification to the mobile device. All sensor values are shown on an LCD.

Keywords: IOT, Sensors, mobile device, Aquaculture





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INTRODUCTION

The quality of water as determined by its chemical, physical, and biological sensors is referred to as having radiological properties. To ensure the survival and development of aquatic life, the quality of the water must be maintained. Therefore, a real-time water quality monitoring system is created for aquaculture applications employing IOT technology in order to preserve the quality of the water. The water quality is always maintained by this technique. All sensor data will be displayed on the LCD utilized in this operation. The current system introduces the procedure for monitoring sea water, which assesses the crucial instrument for assessing water quality and offers assistance in validating the extreme environmental circumstances. In this system, all operations are carried out by the microcontroller [1]. To establish a mobile communication, the data is transmitted to the Global System using the Zigbee transceiver. The usage of sensors, such as conductivity and temperature sensors, is common. The sensors will interpret the readings made by the various sensors and send the information to a Zigbee receiver through a Zigbee transmitter. The Zigbee transceiver can only connect with each other when they are in close proximity to one another and operating at the same frequency. The Zigbee transceiver might not always operate in the same frequency band. If so, neither sending nor receiving the values is possible. If data are transferred, a UART cable will be linked to a computer so that all sensor readings may be seen.

Aquaculture culture

A proposal one of the most important aspects of aquaculture culture will continue to be system quality [2-4]. Additionally, it will be significant in practically every area. Consequently, an aquaculture real-time water quality monitoring system utilizing IOT technology is needed to maintain the water quality to a larger level. Different sensors, including the Sensor Temperature, Sensor PH, and the Sensor Water Level, are employed in this suggested system to monitor the water quality. Additionally, the message for the mobile phone is sent using the Global System for Mobile communication (GSM) [5]. The temperature sensor keeps track of temperature variations. The DC Motor is ON if the Temperature Sensor notices changes in the temperature. The pH of the water is checked using the PH Sensor. The DC Motor turns on if the water level reaches its level, which is determined by the Water Level Sensor. When these sensors detect an abnormal value, the GSM sends a message to the mobile phone. As a result, the water quality is routinely checked, which contributes to the expansion of aquaculture.

Architecture of Systems

The degree of heat associated with the body is the system employed in this instance to assess temperature and the amount of heat present. A precision IC temperature sensor is the output that is proportional to the LM35's temperature [6-10]. Along with the LM35, a thermistor will be utilised to measure the temperature more precisely. A pH metre is a scientific tool that evaluates the alkalinity or acidity of water-based solutions by indicating their hydrogen-ion activity, which is represented as pH. The pH metre calculates the difference between the pH electrode and the reference electrode. The term "potentiometric pHmeter" is used to describe this instrument [11]. The differences in electrical potential are related to the acidity and pH of a solution. The pH metre is used in numerous applications, including laboratory research. A liquid level sensor is used to measure the liquid level in tanks and reservoirs without any moving parts in the surrounding area [12]. The element that is electrically shielded and isolated from the liquid will be regarded as the sensor [13]. Unlike previous sensors, the measuring range will be changeable from a few centimetres to over several metres [14]. The reading is presented as an analogue voltage with a range of 0 to 3 volts, where 0 volts correspond to the sensor that is not submerged and 3 volts correspond to the highest water level.

Development of Aquaculture Environment Monitoring Systems Functions Performed

- The operator can control environmental indicators in breeding farms thanks to a combination of a control system for the farm and the internet of things, as well as the operating mode remote King view interface;
- Keep track of environmental and animal breeding farm expansion throughout time to ensure farm quality;





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- Recognize that the remote control can be used anywhere in the globe as long as the control system is connected to the same network:
- The number of nodes and wireless connectivity between nodes can both be decreased by using serial communication nodes;
- Each node uses solar energy, promoting sustainable agriculture.
- Sufficient daytime conversion of solar energy into electrical energy for an all-weather system;
- Monitoring animal and bird waste to improve warnings and other features.

Response and Conversation

Finally, the output can be seen on the user's mobile phone and LCD monitor. CONCLUSION As a result, the proposed method is employed to keep an eye on the water's quality. Different types of sensors, such as temperature, pH, and water level sensors, are employed to keep track of these variables. The GSM will send a message to the mobile phone if the sensor detects an abnormal value for any sensor, such as a temperature sensor, pH sensor, or water level sensor. As a result, this system will continue to be more cost- and time-effective for monitoring aquaculture activities. 5. Information-Measuring System for Pond Farm Aquaculture Monitoring and Control. The essay discusses the issues with pond farms employing automated process control systems. The current automated pond farm equipment as well as potential future deployment and use scenarios are examined. The block diagram of the pond farm's aquaculture management system, which enables both continuous pond condition monitoring and automatic feeding. India has the greatest water reserve in the world, consisting of coastal oceans and interior reservoirs, and its use is intricate and cross-sectoral. Aquaculture is the only dependable way to increase the production of food fish products at a time when ocean fish and other seafood catches are declining and fish stocks in inland reservoirs are in critical condition and primarily sustained through artificial reproduction. Aquaculture is a type of activity used to breed, maintain, and cultivate fish, other aquatic animals, plants, and algae under full or partial human control in order to produce products for sale, to replenish commercial stocks of aguatic biological resources, to conserve their biodiversity, and for recreational purposes. In order to support aquaculture, the Ministry of Agriculture has developed a plan for the growth of Indian aquaculture up to 2020, the main goal of which is to consistently supply the population of the nation with a variety of fish products from indigenous aquaculture at costs that are affordable for people of all income levels .Pond farming, industrial farming, and pasture farming are the three primary methods of growing freshwater aguaculture in India.

The automation of every step in pond fish farming, from setting up the pond for fish stocking to receiving commodity fish, would greatly improve the economics of fish breeding, lower costs, and ultimately raise competitiveness. As a result, the fish productivity of the utilized reservoirs is greatly increased: lakes can reach 400-500 kg per ha, ponds can reach 2.5-5 t per ha, etc. Seventy to eighty percent of the fish produced at pond farms are fed artificially. Complete automation of this process from feed receipt to feeding enables properly timed feeding of growing fish while accounting for planting density, water temperature, dissolved oxygen concentration, and feed palatability. In this instance, a single automation system unifies the ordering of ready-made feed or its components for in-house production, storage in the central warehouse, creation of feeding maps, shipment from the central warehouse, distribution by ponds, accounting for the introduced feed, control of feed eat ability, and monitoring of water and environmental parameters. This system uses pre-balanced fish feed, therefore just the application will be used to create feed purchases. Given the technologies of the fourth industrial revolution, or Industry 4.0, such as the industrial Internet of things and cloud technologies, the complicated automation system of any manufacturing firm is currently performed more logically. It will make it possible to increase profit margins and the enterprise's overall performance. Automated pond farm aquaculture process control system. The system is made up of the following components a unit for buying fish feed or for making it; a unit for central storage; a unit for formation feeding maps; a unit for automated feed accounting at ponds; a unit for automatic feed; a unit for monitoring water and environmental parameters; a unit for additional functions; and a unit for radio communication. Making fish feed units or purchasing formation feed. This module may be an application for accounting and forecasting feed consumption, or it may be a completely independent automated feed manufacturing line that takes feed





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consumption into consideration, depending on the type of feed and the accessibility of the feed preparation shop. The automatic accounting and storage of the forages that arrive and are released from the central storage are implemented by the central storage unit. The device uses automated product accounting, and it can be expanded to include an automated feed storage management system that takes climate into consideration. Maps of formation feeding. The amount of feed that will be supplied to the reservoir today is decided upon in accordance with the data collected from the equipment that monitors water and environmental conditions, which regulates water temperature and composition. The type of fish, season, and illness prevention are all taken into account when creating the feeding map. It is suggested that the unit be developed as an application.

The amount of feed transferred from the central store to the bunker that is situated on the pond and the distribution from this bunker to the automated feeders are both considered in the automated feed accounting unit at ponds. a robotic complex with a feed tracking system from the central storage to automatic feeders was proposed as a way to make it a reality. Automated feed unit. This unit regulates the operation of automated feeders while taking the unit's formation feeding map's suggestions into consideration. Granular, crumbly, and dough-like feeds can be added to ponds from the shore (dam) as well as with the aid of floating or fixed feeders. Fish can eat food at any time according on their physiological needs, which is known as feeding. The floating automatic feeder with a selfsufficient power source from solar batteries was suggested in this research. Delivering feed is a fully automated operation. The unit for monitoring water and environmental factors is made to measure things like air and water temperatures, dissolved oxygen levels in water, air velocity, water pH, and others. Such machines can be installed on either fixed platforms or floating automatic feeders. It is suggested that a floating feeder be fitted with a monitoring device. It allows for the collection of water parameters across the entire pond while reducing the need for additional measuring devices. Based on the data it receives from the blocks, the central control unit directs their work. Additionally, the unit offers remote access to the system and storage of the received data on a cloud server. A unit with extra features can operate equipment for attracting flying insects at night, driving away fish-eating birds during the day, and deoxidizing ponds. It is suggested that this module be mounted on a floating automatic feeder. All modules are interconnected by a single information system that communicates via wireless radio technology. As a result, the automatic floating feeder serves as the system's primary building block, the functionality of which will determine its design and functional aspects. Floating automatic fish feeders with solar battery power are currently used in fish farms. On the catamaran where the feeder is mounted, field tests of its capabilities have been done. Additionally, studies on the development of a fish-eating system using flying insects and the defense of fish against fish-eating birds have been conducted .Additionally; several components are implemented as applications, providing a more adaptable solution for various fish farm requirements.

CONCLUSION

Thus, it is established that the agro-industrial complex's (AIC) growth of aquaculture, particularly in the Southern federal area, is a key responsibility. At the same time, the material and technical foundation of pond farms prevents further development of aquaculture. To automate the control systems of fish breeding businesses, a new strategy is required that takes into consideration cutting-edge technologies like the industrial Internet of things, big data banks, and a single system of data storage and processing. Only by combining all of these technologies into a single automation system can assurances be made. the ability of domestic businesses to compete with overseas fish producers, which will attract investment in fish farms. It is suggested to develop robotic aquaculture control systems based on autonomous floating feeders to provide the best possible feeding for fish in pond farms. All of these actions will assist in achieving the primary objective of the aquaculture development strategy in India, which is to consistently supply the population of the nation with a variety of domestic aquaculture-produced fish products at costs that are affordable for people of all income levels.



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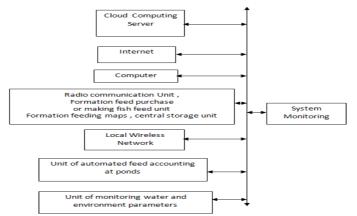


Fig 1: Effective Automatic Monitoring System for Aquaculture Applications





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RESEARCH ARTICLE

Impact of Farmer Producers Company (FPC) on Production Cost of Paddy in Cuddalore District of Tamil Nadu

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ABSTRACT

Paddy is a major food crop cultivated in coastal areas due to the soil condition. Recently the rice cultivating farmer's income is significantly lower than the national average owing to many reasons like seed quality, labour cost, and lack of machinery, marketing costs and marketing margin. The present study was conducted in Tamil Nadu Irrigated Modernization Project (TNIMP) - Phase II Lower Pennaiyaru Farmer Producer Company (FPO) in Cuddalore district of Tamil Nadu With the sample size of 240 respondents. It is a comparative study conducted between FPO registered farmers and FPO Nonregistered farmers in the study area. The results are observed that the FPO registered farmers cost of rice production is reduced due to the FPO interference in reducing the input cost than Non FPO registered farmers. The FPO Farmers produce relatively higher yield when compared to Non-FPO Farmers. The Net income of FPO Farmers is Rs. 38320.24, which is 11.56 per cent higher than the Non - FPO Farmers (Rs. 30683.36). The benefit cost ratio was higher for FPO registered farmers 1.82 as compared to FPO Nonregistered Farmers 1.57.

Keywords: Farmers Producers Organization, Cost of Production, Gross Income, Net Income, Marketing.





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INTRODUCTION

A producer company is defined as formal rural groups whose members came together to increase farm income through better production, marketing, and regional processing. (Rondot and Collion, 2011). Producer Company work to enhance agricultural production techniques, provide access to inputs and services, including agricultural loans, market agricultural production, process it locally, and market it locally. These are just a few of the policies that Producer Company deal with. A producer Company is a group that was formed to advance the interests of farmers. It could be an association, society, cooperative, union, federation, or even a company. (Bijman and Wollni 2008). The producer Company's principal objective was to offer services to help farmers with their farming operations, including the sale of their output. (Shylendra 2009). The farmers in this FPO in the Cuddalore district of Tamil Nadu exclusively grow rice due to the high rainfall and poor soil conditions in this coastal area. Production, harvesting, processing, procurement, grading, handling, marketing, and selling are all duties that FPO helps this farmer with. Additionally, it supports the import of goods and services for the benefit of the members as well as the export of their main products. The issues facing the agriculture sector are fast filling up the basket. Population has grown by 3.5 times, from 350 million in 1947 to 1.22 billion in 2012, increasing food consumption but reducing total farmland. From 0.22 ha in 1950 to 0.10 ha in 2010, the grain area per person has been continuously decreasing over a number of decades, and in 2050, it is expected to be as low as 0.06 ha per person (Larsen 2003). Thus, the crucial, worrisome issue of food and economic security has been brought up.

Due to current problems with food security, which are partially the result of a long-standing lack of proper technological dissemination, agriculture has come to the attention of the world. Globally, there is an increasing need for better food, both in terms of quantity and quality. It is possible to find a solution in this situation by integrating the farmers, particularly the small farmers, with the value chain. This will guarantee that the farmers receive a sufficient net return to support their continued involvement in agriculture. To incorporate farmers into the production and value chain, a range of institutional forms, such as Farmers' Cooperatives, Self Help Groups, and Farmers' Interest Groups, have been formed in India. A more recent example that enables farmers to band together, provides them with a business perspective on agriculture, and connects them to the market is Farmers Producers' Company.

MATERIAL AND METHODS

The present study was conducted in Tamil Nadu Irrigated Modernization Project (TNIMP) – Phase II Lower Pennaiyaru Farmer Producer Company (FPO). Cuddalore district of Tamil Nadu, Purposive sampling was adopted to select the sample farmers. Cuddalore district was purposively selected for this study because Cuddalore district comes under efficient cropping zone for paddy production in Tamil Nadu. Sample farmers were randomly selected from the selected Farmer Producer company in Cuddalore District of Tamil Nadu. The present study aimed at estimation of cost of cultivation of paddy in FPO Farmers and Non – FPO farmers. Primary data on costs and returns in paddy cultivation were collected from 40 sample farmers of selected FPO. A random sample of 240 farmers was drawn from Chinnapettai, Kaalaiyur, Solavalli and Melkumaramanagalam villages, including the following number of farmers belonging to different categories.

i. Data collection

Primary Data

Primary information was obtained from selected farmers through private interviews using a pre-tested and well-structured schedule for research purposes.

Secondary data

Secondary data were obtained from various sources such as the District Statistical Office, Deputy Director of Agriculture, District Food Supply and Consumer Protection Office, Block Development Office.





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ii. Cost Concepts

In farm management, cost concept approach is widely used in India for evaluating crop profitability in production. The cost concepts in brief, are Cost A₁, A₂, B₁, B₂, C₁, C₂, and cost C₃.

Cost A1: This gives the total cash expenses incurred by the owner or operator. It included the following terms of costs.

- 1. Value of hired human labour
- 2. Value of bullock labour.
- 3. Value of machinery charges (except depreciation).
- 4. Value of fertilizers and manures.
- 5. Value of seeds.
- 6. Value of insecticides, pesticides and weedicides
- 7. Irrigation charges
- 8. Depreciation on farm implements
- 9. Interest on working capital.
- 10. Land revenue paid to government.

Cost A₂ = Cost A₁+ Rent paid for leased in land, if any

Cost B_1 = Cost A_{1+} Interest on value of owned fixed capital assets.

Cost B₂ = Cost B₁+ Rental value of owned land less land revenue

Cost C₁ = Cost B₁+ Imputed value of family labour

Cost C_2 = Cost B_2 + Imputed value of family labour.

Cost C_3 = Cost C_2 +10% of Cost C_2 on account of managerial functions performed by farmer.

In the present study, the rent paid for leased in land was zero, as none of the sample farmers took land on lease. Hence, cost A_1 and cost A_2 are similar.

iii. Rates of Returns over Different Cost Concepts

Gross Income: Yield of main product (in kg/ha x their prices (Rs.) + Yield of by Product (in kg/ha) and their prices (Rs.)

Net Income: Gross Income - Cost C.

Farm Business Income: Gross Income - Cost A2

Farm Investment Income: Farm business income- wages of family labour

Family Labour Income: Gross Income - Cost B.

RESULT AND DISCUSSION

i. Cost of cultivation of Paddy

The cost of cultivation calculated per hectare by FPO Farmers and Non - FPO Farmers Farmers are presented in Table 2. The various costs incurred by the sample farmers are presented in Table 2. The variable cost incurred was higher among Non - FPO farmers with Rs. 43142.19 per hectare than among the FPO Farmers with Rs. 38774.91 per hectare. Cost of human labour accounted for the largest share in total variable costs is 24.18 per cent and 25.09 per cent for FPO farmers and Non – FPO farmers respectively. The cost of machine labour constituted for Non – FPO farmers Rs. 8106.98 is higher than FPO farmers (Rs. 6276.16). The total cost of cultivation of paddy per hectare for Non – FPO Farmers was comparatively high because of greater use of human labourers, seeds, fertilizers and plant protection chemicals. In fact, the total cost of cultivation of paddy incurred by Non – FPO Farmers was 8.91 per cent higher than FPO Farmers.

ii. Profitability Aspect of Paddy Cultivation

Table 3 shows that the FPO Farmers produce relatively higher yield when compared to Non-FPO Farmers. The Net income of FPO Farmers is Rs. 38320.24, which is 11.56 per cent higher than the Non - FPO Farmers (Rs. 30683.36). The cost of cultivation was computed separately for the two categories, viz. FPO Farmers and Non - FPO Farmers. It could be observed from Table 3 that the gross income was estimated to be Rs. 82750.00 and Rs. 79500.00 per hectare





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respectively for FPO Farmers and Non FPO Farmers. Thus gross income is 3.93 per cent higher for FPO Farmers as compared to Non - FPO Farmers. Further, it could be seen that the net income was higher for FPO Farmers than Non-FPO Farmers. It was mainly due to the low rental cost of machinery available for the FPO registered farmers by FPO subsidies. The cost seed and fertilizer also lower for FPO Farmers than Non - FPO Farmers of rice cultivation.

CONCLUSION

The major expenditure was incurred on machine labour, fertilizer and plant protection measures and the cost of seeds occupied a major amount because there was a significant reduction in seed rate and affordable price of fertilizer and lowest rental value of machine labour for FPO farmers. The cost of cultivation of paddy under FPO registered farmers was found to be 8.91 per cent lower than the Non – FPO farmers. The net returns were higher for FPO farmers due prevention of intermediates. It was also observed that the benefit-cost ratio was higher for FPO farmers (1.82) as compared to Non – FPO farmers (1.57). In the FPO registered farmers easily get inputs on adequate time and price and prevention of intermediates farmers can get maximum price of their products. The study shows the cost of cultivation of paddy for FPO farmers was lower than Non – FPO farmers and the net income of FPO farmers also higher than Non – FPOI farmers.

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Table 1. Sample distribution of farmers among different Villages

SI. No	Category	No. of Sample Farmers	FPO Farmers	Non-FPO Farmers	Percentage
1	Chinnapettai	60	30	30	25
2	Kaalaiyur	60	30	30	25
3	Solavalli	60	30	30	25
4	Melkumaramanag alam	60	30	30	25
5	Total	240	120	120	100

Table 2. Shows the Cost of Cultivation of Paddy (In ha)

SI. No.	Particulars	Non – FPO Farmers	FPO Farmers
1	Seed	3209.51(6.57)	2710.56(6.10)
2	Fertilizers and manures	7111.43(14.57)	5607.98(12.62)
3	Human labour	11801.46(24.18)	11148.43(25.09)
4	Bullock labour	3702.65 (7.58)	3655.69(8.23)
5	Machine labour	8106.98(16.61)	6276.16(14.13)
6	Irrigation	3580.44(7.33)	3658.87(8.24)
7	Plant protection chemicals	1445.22(2.96)	1534.56(3.45)
8	Depreciation on farm implements	368.67(0.76)	370.76(0.83)
9	Interest on working capital @ 7%	3803.33(7.79)	3799.4(8.55)
10	Land revenue	12.5(0.03)	12.5(0.03)
	Cost A1	43142.19(88.29)	38774.91(87.27)
11	Interest on own fixed capital @12%	445.56(0.91)	453.87(1.02)
	Cost B1	43587.75 (89.29)	39228.78(88.29)
12	Rent value of own land	3180(6.51)	3201(7.20)
	Cost B2	46767.75 (95.80)	42429.78(95.50)
13	Imputed value of family labour	1448.89(2.97)	1449.98(3.26)
	Cost C1	45036.64(92.26)	40678.76(91.56)
	Cost C2	48216.64(98.77)	43879.76(98.76)
14	Managerial cost	600(1.23)	550(1.24)
	Cost C3	48816.64(100)	44429.76(100)

Table 3. Shows the Return of Paddy Cultivation (In Rs.)

able of briows the Retain of Faday Californian (III RS.)							
SI. No.	Particulars	Non – FPO Farmers	FPO Farmers				
1	Gross Income	79500.00	82750.00				
2	Net Income	30683.36	38320.24				
3	Farm Business Income	36357.81	43975.09				
4	Farm Investment Income	34908.92	42525.11				
5	Family Labour Income	32732.25	40320.22				





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RESEARCH ARTICLE

Binary Generalized Star Closed Set in Binary Topological Spaces

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ABSTRACT

In this paper, we introduce a new class of sets namely binary g^* -closed sets in binary topological spaces. This class lies between the class of binary closed sets and the class of binary q^* -closed sets. The complement of an binary g^* -closed set is called an binary g^* -open set. Moreover, we introduce two new spaces namely, binary T_g^{\star} -spaces and binary $_gT_g^{\star}$ -spaces.

Keywords: binary g^* -closed set, binary g^*_a -closed set, binary T^*_a -spaces and binary T^*_a -spaces

INTRODUCTION AND PRELIMINARIES

In 1970 Levine [6] gives the concept and properties of generalized closed (briefly g-closed) sets and the complement of g-closed set is said to be g-open set. Njasted [15] introduced and studied the concept of α -sets. Later these sets are called as α -open sets in 1983. Mashhours et.al [9] introduced and studied the concept of α -closed sets, α -closure of set, α -continuous functions, α -open functions and α -closed functions in topological spaces. Maki et.al [7, 8] introduced and studied generalized α -closed sets and α -generalized closed sets. In 2011, S.Nithyanantha Jothi and P.Thangavelu [10] introduced topology between two sets and also studied some of their properties. Topology between two sets is the binary structure from X to Y which is defined to be the ordered pairs (A, B) where $A \subseteq X$ and $B \subseteq Y$. In this paper, we introduce a new class of sets namely binary g^* -closed sets in binary topological spaces. This class lies between the class of binary closed sets and the class of binary g^* -closed sets. The complement of an binary g^* -closed set is called an binary g^* -open set. Moreover, we introduce two new spaces namely, binary T_a^* -spaces and binary ${}_{q}T_{q}^{\star}$ -spaces.

Throughout this paper, (X, Y) denote binary topological spaces (X, Y, \mathcal{M}) .





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Let X and Y be any two nonempty sets. A binary topology [10] from X to Y is a binary structure $\mathcal{M} \subseteq \mathbb{P}(X) \times \mathbb{P}(Y)$ that satisfies the axioms namely

- 1. (ϕ, ϕ) and $(X, Y) \in \mathcal{M}$
- 2. $(A_1 \cap A_2, B_1 \cap B_2) \in \mathcal{M}$ whenever $(A_1, B_1) \in \mathcal{M}$ and $(A_2, B_2) \in \mathcal{M}$, and
- 3. If $\{(A_{\alpha}, B_{\alpha}): \alpha \in \delta\}$ is a family of members of \mathcal{M} , then $(\bigcup_{\alpha \in \delta} A_{\alpha}, \bigcup_{\alpha \in \delta} B_{\alpha}) \in \mathcal{M}$.

If \mathcal{M} is a binary topology from X to Y then the triplet (X,Y,\mathcal{M}) is called a binary topological space and the members of \mathcal{M} are called the binary open subsets of the binary topological space (X,Y,\mathcal{M}) . The elements of $X \times Y$ are called the binary points of the binary topological space (X,Y,\mathcal{M}) . If Y=X then \mathcal{M} is called a binary topology on X in which case we write (X,\mathcal{M}) as a binary topological space.

Definition 1.1 [10]

Let X and Y be any two nonempty sets and let (A, B) and $(C, D) \in \mathbb{P}(X) \times \mathbb{P}(Y)$. We say that $(A, B) \subseteq (C, D)$ if $A \subseteq C$ and $B \subseteq D$.

Definition 1.2 [10]

Let (X,Y,\mathcal{M}) be a binary topological space and $A\subseteq X$, $B\subseteq Y$. Then (A,B) is called binary closed in (X,Y,\mathcal{M}) if $(X\setminus A,Y\setminus B)\in \mathcal{M}$.

Proposition 1.3 [10]

Let (X, Y, \mathcal{M}) be a binary topological space and $(A, B) \subseteq (X, Y)$.

Let $(A, B)^{1*} = \bigcap \{A_{\alpha}: (A_{\alpha}, B_{\alpha}) \text{ is binary closed and } (A, B) \subseteq (A_{\alpha}, B_{\alpha}) \}$ and $(A, B)^{2*} = \bigcap \{B_{\alpha}: (A_{\alpha}, B_{\alpha}) \text{ is binary closed and } (A, B) \subseteq (A_{\alpha}, B_{\alpha}) \}$. Then $((A, B)^{1*}, (A, B)^{2*})$ is binary closed and $(A, B) \subseteq ((A, B)^{1*}, (A, B)^{2*})$.

Proposition 1.4 [10]

Let (X,Y,\mathcal{M}) be a binary topological space and $(A,B)\subseteq (X,Y)$. Let $(A,B)^{1*}=\cup\{A_\alpha:(A_\alpha,B_\alpha)\text{ is binary open and }(A_\alpha,B_\alpha)\subseteq (A,B)\}$ and $(A,B)^{2*}=\cup\{B_\alpha:(A_\alpha,B_\alpha)\text{ is binary open and }(A_\alpha,B_\alpha)\subseteq (A,B)\}$.

Definition 1.5 [10]

The ordered pair $((A,B)^{1*},(A,B)^{2*})$ is called the binary closure of (A,B), denoted by b-cl(A,B) in the binary space (X,Y,\mathcal{M}) where $(A,B)\subseteq (X,Y)$.

Definition 1.6 [10]

The ordered pair $((A,B)^{1*},(A,B)^{2*})$ defined in proposition 1.4 is called the binary interior of (A,B), denoted by bint(A,B). Here $((A,B)^{1*},(A,B)^{2*})$ is binary open and $((A,B)^{1*},(A,B)^{2*})\subseteq (A,B)$.

Definition 1.7 [10]

Let (X,Y,\mathcal{M}) be a binary topological space and let $(x,y) \subseteq (X,Y)$. The binary open set (A,B) is said to be a binary neighbourhood of (x,y) if $x \in A$ and $y \in B$.

Proposition 1.8 [10]

Let $(A, B) \subseteq (C, D) \subseteq (X, Y)$ and (X, Y, \mathcal{M}) be a binary topological space. Then, the following statements hold:

- 1. b-int(A,B) \subseteq (A,B).
- 2. If (A, B) is binary open, then b-int(A, B) = (A, B).
- 3. b-int(A, B) \subseteq b-int(C, D).
- 4. b-int(b-int(A,B)) = b-int(A,B).
- 5. $(A, B) \subseteq b\text{-cl}(A, B)$.
- 6. If (A,B) is binary closed, then b-cl(A,B) = (A,B).
- 7. $b-cl(A, B) \subseteq b-cl(C, D)$.
- 8. b-cl(b-cl(A, B)) = b-cl(A, B).





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Definition 1.9 A subset (A, B) of a binary topological space (X, Y, \mathcal{M}) is called

- 1. a binary semi open set [14] if $(A, B) \subseteq b\text{-cl}(b\text{-int}(A, B))$.
- 2. a binary pre open set [4] if $(A,B) \subseteq b$ -int(b-cl(A,B)),
- 3. a binary regular open set [13] if (A, B) = b-int(b-cl(A, B)).

Definition 1.10

A subset (A, B) of a binary topological space (X, Y, \mathcal{M}) is called

- 1. a binary g-closed set [11] if $b cl(A, B) \subseteq (U, V)$ whenever $(A, B) \subseteq (U, V)$ and (U, V) is binary open.
- 2. a binary gs-closed set [16] if b-scl $(A,B) \subseteq (U,V)$ whenever $(A,B) \subseteq (U,V)$ and (U,V) is binary open.
- 3. a binary sg-closed set [16] if b- $scl(A,B) \subseteq (U,V)$ whenever $(A,B) \subseteq (U,V)$ and (U,V) is binary semi open.
- 4. a binary gr-closed set [13] if b- $rcl(A, B) \subseteq (U, V)$ whenever $(A, B) \subseteq (U, V)$ and (U, V) is binary open.
- 5. a binary gsp-closed set [5] if b- $\beta cl(A, B) \subseteq (U, V)$ whenever $(A, B) \subseteq (U, V)$ and (U, V) is binary open.
- 6. a binary gp-closed set [5] if b- $pcl(A, B) \subseteq (U, V)$ whenever $(A, B) \subseteq (U, V)$ and (U, V) is binary open.
- 7. a binary gpr-closed set [13] if b- $pcl(A, B) \subseteq (U, V)$ whenever $(A, B) \subseteq (U, V)$ and (U, V) is binary regular open.

Definition 1.11

A subset (A, B) of a binary topological space (X, Y, \mathcal{M}) is called

- 1. a binary α -open [2] if $(A, B) \subseteq b$ -int(b-cl(b-int(A, B))).
- 2. a binary β -open [4] if $(A, B) \subseteq b\text{-}cl(b\text{-}int(b\text{-}cl(A, B)))$.

Definition 1.12 [1]

A subset (A, B) of a binary topological space (X, Y, \mathcal{M}) is called

- 1. a binary $g\alpha$ -closed if $b \cdot cl(A, B) \subseteq (U, V)$ whenever $(A, B) \subseteq (U, V)$ and (U, V) is binary α -open.
- 2. a binary αg -closed if $b \alpha cl(A, B) \subseteq (U, V)$ whenever $(A, B) \subseteq (U, V)$ and (U, V) is binary open.

Definition 1.13 A binary topological space (X,Y,\mathcal{M}) is called

- 1. binary $T_{1/2}$ -space [12] if every binary g-closed set in it is binary closed.
- 2. binary T_h -space [3] if every binary gs-closed set in it is binary closed.
- 3. binary T_{α} -space [2] if every α -closed set in it is binary closed.

2 Binary g^* -closed sets

We introduce the following definitions.

Definition 2.1

Let (A, B) be a subset of a binary topological space (X, Y). Then (A, B) is called

1. binary g*-closed set if b-cl(A, B) \subseteq (P, Q) whenever (A, B) \subseteq (P, Q) and (P, Q) is binary g-open in (X, Y).

The complement of binary g*-closed set is called binary g*-open.

The family of all binary g^* -closed sets in (X,Y) is denoted by binary $G^*C(X,Y)$.

2. binary g^*r -closed if b-rcl(A, B) \subseteq (P,Q) whenever (A, B) \subseteq (P,Q) and (P,Q) is binary g-open in (X,Y).

The family of all binary g^* -closed sets in (X, Y) is denoted by binary $G^*RC(X, Y)$.

3. binary g_{α}^* -closed set if b- α cl(A, B) \subseteq (P, Q) whenever (A, B) \subseteq (P, Q) and (P, Q) is binary g-open in (X, Y).

The family of all binary g_{α}^{\star} -closed sets in (X,Y) is denoted by binary $G_{\alpha}^{\star}C(X,Y)$.

Theorem 2.2

Every binary closed set is binary g*-closed.

Proof

If (A, B) is any binary closed set in (X, Y) and (G, H) is any binary g-open set containing (A, B), then $(G, H) \supseteq (A, B) = b - cl(A, B)$. Hence (A, B) is binary g^* -closed.

The converse of Theorem 2.2 need not be true as seen from the following example.





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Example 2.3

Let $X = \{a, b\}$, $Y = \{1,2\}$ and $\mathcal{M} = \{(\phi, \phi), (\phi, \{2\}), (\{a\}, \{1\}), (X, \{1\}), (X, Y)\}$. Then $(\{b\}, \{1\})$ is binary g^* -closed set but not binary closed.

Theorem 2.4

Every binary g^* -closed set is binary g^*_{α} -closed.

Proof.

If (A,B) is a binary g^* -closed subset of (X,Y) and (G,H) is any binary g-open set containing (A,B), then $(G,H) \supseteq b - cl(A,B) \supseteq b - \alpha cl(A,B)$. Hence (A,B) is binary g_{α}^* -closed.

The converse of Theorem 2.4 need not be true as seen from the following example.

Example 2.5 Let $X = \{a, b\}$, $Y = \{1,2\}$ and $\mathcal{M} = \{(\varphi, \varphi), (\{a\}, \{1\}), (\varphi, \{1\}), (X, Y)\}$. Then $(\{b\}, \varphi)$ is binary g^* -closed but not binary g^* -closed.

Remark 2.6

The following examples show that binary g^* -closed sets are independent of binary α -closed sets.

Example 2.7

In Example 2.5, ({b}, Y) is binary g^* -closed but not binary α -closed and ({a}, {2}) is binary α -closed but not binary g^* -closed.

Remark 2.8

The following examples show that binary g*-closed sets are independent of binary semi-closed sets.

Example 2.9

Let $X = \{1,2\}$, $Y = \{a,b\}$ and $\mathcal{M} = \{(\phi,\phi), (\{1\}\{a\}), (\phi,\{b\}), (\{1\},Y), (X,Y)\}$, Then $(\{2\},\{a\})$ is binary g^* -closed but not binary semi-closed and $(\{1\},\{a\})$ is binary semi-closed but not binary g^* -closed in (X,Y).

Remark 2.10

The following examples show that binary g*-closed sets are independent of binary pre-closed sets.

Example 2.11

In Example 2.5, ($\{b\}$, Y) is binary g^* -closed but not binary pre-closed and (ϕ , $\{2\}$) is binary pre-closed but not binary g^* -closed in (X, Y).

Remark 2.12

The following examples show that binary g^* -closed sets are independent of binary β -closed sets.

Example 2.13

Let $X = \{1,2\}$, $Y = \{a,b\}$ and $\mathcal{M} = \{(\phi,\phi), (\{1\},\{a\}), (\{2\},\{a\}), (\{1\},\{b\}), (X,\{a\}), (\{1\},Y), (\phi,\{a\}), (\{1\},\phi), (X,Y)\}$. Then ($\{2\},\{b\}$) is binary g^* -closed but not binary g^* -closed in (X,Y).

Remark 2.14

The following examples show that binary g*-closed sets are independent of binary sg-closed sets.

Example 2.15

In Example 2.5, ($\{b\}$, Y) is binary g^* -closed but not binary sg-closed and ($\{a\}$, ϕ) is binary sg-closed but not binary g^* -closed in (X, Y).





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Theorem 2.16

If (A, B) and (C, D) be binary g^* -closed, then $(A, B) \cup (C, D)$ is binary g^* -closed.

Proof. Let (A, B) and (C, D) be binary g^* -closed sets in (X, Y, \mathcal{M}) . Let (P, Q) is binary g-open sets in (X, Y) and b- $cl(C, D) \subseteq (U, V)$ where $(C, D) \subseteq (U, V)$ and (U, V) is binary g-open. Since (A, B) and (C, D) are subsets of (P, Q) and (U, V). Then $(A, B) \cup (C, D)$ is a subset of $(P, Q) \cup (U, V)$ and $(P, Q) \otimes (U, V)$ is binary g-open. Then b- $cl((A, B) \cup (C, D)) = b$ - $cl((A, B) \cup (C, D)) \subseteq (P, Q) \cup (U, V)$, which implies that $(A, B) \cup (C, D)$ is binary g^* -closed.

Example 2.17

In Example 2.13, take $(A,B) = (\{1\},\{b\})$ and $(C,D) = (\{2\},\varphi)$. Then (A,B) and (C,D) are binary g^* -closed, then $(A,B) \cap (C,D) = (X,\{b\})$ is also g^* -closed set.

Theorem 2 18

The intersection of any two binary g^* -closed sets in (X, Y, \mathcal{M}) is binary g^* -closed in (X, Y, \mathcal{M}) .

Proof.

Let (A, B) and (C, D) are any two binary g^* -closed sets. $(A, B) \subseteq (P, Q)$ and $(C, D) \subseteq (P, Q)$ where (P, Q) is binary gopen. Then b-cl $(A, B) \subseteq (P, Q)$ and b-cl $(C, D) \subseteq (P, Q)$. Therefore b-cl $(A, B) \cap (C, D) \subseteq (P, Q)$, (P, Q) is binary g-open. Since (A, B) and (C, D) are binary g-closed. Hence $(A, B) \cap (C, D)$ is a binary g*-closed set.

Example 2.19

In Example 2.13, take $(A,B) = (\{2\},\{a\})$ and $(C,D) = (\{2\},\{b\})$. Then (A,B) and (C,D) are binary g^* -closed, then $(A,B) \cap (C,D) = (\{2\},\varphi)$ is also g^* -closed set.

Theorem 2.20

Every binary g*-closed set is binary g-closed.

Proof.

If (A,B) is a binary g^* -closed subset of (X,Y) and (G,H) is any binary open set containing (A,B), since every binary open set is binary g-open, we have $(G,H) \supseteq b\text{-cl}(A,B)$. Hence (A,B) is binary g-closed in (X,Y). The converse of Theorem 2.20 need not be true as seen from the following example.

Example 2.21

In Example 2.5, ($\{a\}$, $\{2\}$) is binary g-closed but not binary g^* -closed set in (X, Y).

Theorem 2.22

Every binary g*-closed set is binary gs-closed.

Proof.

If (A, B) is a binary g^* -closed subset of (X, Y) and (G, H) is any binary open set containing (A, B), since every binary open set is binary g-open, we have $(G, H) \supseteq b\text{-cl}(A, B) \supseteq b\text{-scl}(A, B)$. Hence (A, B) is binary gs-closed in (X, Y). The converse of Theorem 2.22 need not be true as seen from the following example.

Example 2.23

In Example 2.5, ({a}, Y) is binary gs-closed but not binary g*-closed set in (X, Y).

Theorem 2.24

Every binary g*-closed set is binary gr-closed.





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Proof

Let (A, B) be binary g^* -closed in (X, Y). Let (P, Q) be binary open set such that $(A, B) \subseteq (P, Q)$. Since every binary open set is binary g-open. We have b-rcl $(A, B) \subseteq (P, Q)$ implies b-rcl $(A, B) \subseteq (P, Q)$. Therefore (A, B) is binary gr-closed. The converse of Theorem 2.24 need not be true as seen from the following example.

Example 2.25

Let $X = \{a, b\}$, $Y = \{1\}$ and $\mathcal{M} = \{(\phi, \phi), (\{a\}, \phi), (X, Y)\}$. Then (ϕ, Y) is binary gr-closed but not binary g^* -closed set in (X, Y).

Theorem 2.26

Every binary g^* -closed set is binary αg -closed.

Proof

If (A,B) is a binary g^* -closed subset of (X,Y) and (G,H) is any binary open set containing (A,B), since every binary open set is binary g-open, we have $(G,H) \supseteq b\text{-cl}(A,B) \supseteq b\text{-}\alpha\text{cl}(A,B)$. Hence (A,B) is binary $\alpha g\text{-closed}$ in (X,Y). The converse of Theorem 2.26 need not be true as seen from the following example.

Example 2.27

In Example 2.5, ($\{b\}$, $\{1\}$) is binary αg -closed but not binary g^* -closed set in (X, Y).

Theorem 2.28

Every binary g*-closed set is binary gsp-closed.

Proof

If (A,B) is a binary g^* -closed subset of (X,Y) and (G,H) is any binary regular open set containing (A,B), since every binary regular set is binary g-open, we have $(G,H) \supseteq b\text{-cl}(A,B) \supseteq b\text{-gcl}(A,B)$. Hence (A,B) is binary gsp-closed in (X,Y). The converse of Theorem 2.28 need not be true as seen from the following example.

Example 2.29

In Example 2.3, $(\phi, \{1\})$ is binary gsp-closed but not binary g^* -closed set in (X, Y).

Theorem 2.30

Every binary g*-closed set is binary gp-closed.

Proof.

If (A,B) is a binary g^* -closed subset of (X,Y) and (G,H) is any binary open set containing (A,B), since every binary open set is binary g-open, we have $(G,H) \supseteq b\text{-cl}(A,B) \supseteq b\text{-pcl}(A,B)$. Hence (A,B) is binary gp-closed in (X,Y). The converse of Theorem 2.30 need not be true as seen from the following example.

Example 2.31

In Example 2.3, $({a}, {2})$ is binary gp-closed but not binary g^* -closed in (X, Y).

Theorem 2.32

Every binary g*-closed set is binary gpr-closed.

Proof.

If (A,B) is a binary g^* -closed subset of (X,Y) and (G,H) is any binary regular open set containing (A,B), since every binary regular open set is binary g-open, we have $(G,H) \supseteq b\text{-cl}(A,B) \supseteq b\text{-pcl}(A,B)$. Hence (A,B) is binary gpr-closed in (X,Y). The converse of Theorem 2.32 need not be true as seen from the following example.





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Example 2.33

In Example 2.3, ({a}, Y) is binary gpr-closed but not binary g*-closed in (X, Y).

Theorem 2.34

Every binary g*-closed set is binary g*r-closed set.

Droof

Let (A, B) be a binary g^* -closed set of (P, Q) and $(A, B) \subseteq (P, Q)$, (P, Q) is binary g-open in (X, Y). Since (A, B) is binary g^* -closed, b-cl $(A, B) = (A, B) \subseteq (P, Q)$. That is b-cl $(A, B) \subseteq (P, Q)$. Also b-rcl $(A, B) \subseteq (P, Q)$, since (P, Q) is binary g-open in (X, Y). Hence b-rcl $(A, B) \subseteq (P, Q)$. Therefore (A, B) is binary g^* -closed.

Remark 2.35

From the above discussions and known results in [11, 14, 2, 3, 1, 4] we obtain the following diagrams, where $A \rightarrow B$ (resp. $A \not\leftarrow B$) represents A implies B but not conversely (resp. A and B are independent of each other).

3 Properties of binary g^* -closed sets

Definition 3.1

The intersection of all binary g-open subsets of (X,Y) containing (A,B) is called the binary g-kernel of (A,B) and denoted by binary g-ker(A,B).

Lemma 3.2

A subset (A, B) of a binary topological space (X, Y) is binary g^* -closed if and only if $b - cl(A, B) \subseteq binary g - ker(A, B)$.

Proof.

Suppose that (A,B) is binary g^* -closed. Then $b\text{-}cl(A,B) \subseteq (P,Q)$ whenever $(A,B) \subseteq (P,Q)$ and (P,Q) is binary g-open. Let $\{i,j\} \in b\text{-}cl(A,B)$. If $\{i,j\} \not\in b\text{-}cl(A,B)$, then there is a binary g-open set (P,Q) containing (A,B) such that $\{i,j\} \not\in (P,Q)$. Since (P,Q) is a binary g-open set containing (A,B), we have $\{i,j\} \not\in b\text{-}cl(A,B)$ and this is a contradiction. Conversely, let $b\text{-}cl(A,B) \subseteq b\text{-}i\text{-}i\text{-}j$. If (P,Q) is any binary g-open set containing (A,B), then $b\text{-}cl(A,B) \subseteq b\text{-}i\text{-}j$. Therefore, (A,B) is binary g^* -closed.

Theorem 3.3 If a set (A, B) is binary g^* -closed in (X, Y), then b-cl(A, B) contains no nonempty binary g-closed set in (X, Y).

Proof.

Suppose that (A,B) is binary g^* -closed. Let (G,H) be a binary g-closed subset of b-cl(A,B) - A. Then $(A,B) \subseteq (G,H)^c$. But (A,B) is binary g^* -closed, therefore $b\text{-}cl(A,B) \subseteq (G,H)^c$. Consequently, $(G,H) \subseteq (b\text{-}cl(A,B))^c$. We already have $(G,H) \subseteq b\text{-}cl(A,B)$. Thus $(G,H) \subseteq b\text{-}cl(A,B) \cap (b\text{-}cl(A,B))^c$ and hence (G,H) is empty.

Theorem 3.4

If (A, B) is binary q^* -closed in (X, Y) and $(A, B) \subseteq (C, D) \subseteq b$ -cl(A, B), then (C, D) is binary q^* -closed in (X, Y).

Proof

Let (P,Q) be binary g-open set in (X,Y) such that $(C,D) \subseteq (P,Q)$. Since (A,B) is binary g^* -closed, b-cl $(A,B) \subseteq (P,Q)$. Since b-cl $(C,D) \subseteq b$ -cl(A,B), we have b-cl $(C,D) \subseteq (P,Q)$. Hence (C,D) is binary g^* -closed set.

Theorem 3.5

If (A, B) is both binary g-open and binary g*-closed in (X, Y), then (A, B) is binary closed in (X, Y).

Proof.

Since (A, B) is binary g-open and binary g^* -closed, b-cl $(A, B) \subseteq (A, B)$ and hence (A, B) is binary closed in (X, Y).





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Theorem 3.6

For each $\{i,j\} \in (X,Y)$, either $\{i,j\}$ is binary g-closed or $\{i,j\}^c$ is binary g^* -closed in (X,Y).

Proof

Suppose that $\{i,j\}$ is not binary g-closed in (X,Y). Then $\{i,j\}^c$ is not binary g-open and the only binary g-open set containing $\{i,j\}^c$ is the space (X,Y) itself. Therefore b-cl $(\{i,j\}^c) \subseteq (X,Y)$ and so $\{i,j\}^c$ is binary g^* -closed in (X,Y).

Theorem 3.7

Let (A, B) be a binary g*-closed set of a binary topological space (X, Y). Then,

- 1. If (A,B) is binary regular open, then b-pint(A,B) and b-scl(A,B) are also binary g*-closed sets.
- 2. If (A,B) is binary regular closed, then b-pcl(A,B) is also binary g^* -closed set.

Proof

- (1) Since (A,B) is binary regular open in (X,Y), (A,B) = b-int(b-cl(A,B)). Then $b\text{-scl}(A,B) = (A,B) \cup b\text{-int}(b\text{-cl}(A,B)) = (A,B)$. Thus, b-scl(A,B) is binary $g^*\text{-closed}$ in (X,Y). Since $b\text{-pint}(A,B) = (A,B) \cap b\text{-int}(b\text{-cl}(A,B)) = (A,B)$, b-pint(A,B) is binary $g^*\text{-closed}$.
- (2) Since (A, B) is binary regular closed in (X, Y), (A, B) = b-cl(b-int(A, B)). Then $b\text{-pcl}(A, B) = (A, B) \cup b\text{-cl}(b\text{-int}(A, B)) = (A, B)$. Thus, b-pcl(A, B) is binary g^* -closed in (X, Y).

The converses of the statements in the Theorem 3.7 are not true as we can see in the following examples.

Example 3.8

In Example 2.3, binary $G^*C(X,Y) = \{(\phi,\phi), (\phi,\{2\}), (\{b\},\phi), (\{b\},\{1\}), (\{b\},\{2\}), (\{b\},Y), (X,\phi), (X,\{2\}), (X,Y)\}$. Then the set $(A,B) = (\{b\},\{1\})$ is not binary regular open. However (A,B) is binary g^* -closed and b-scl $(A,B) = (X,\{1\})$ is a binary g^* -closed and b-pint $(A,B) = (\{b\},\{1\})$ is also binary g^* -closed.

Example 3.9

In Example 2.3, binary $G^*C(X,Y) = \{(\varphi,\varphi), (\varphi,\{2\}), (\{b\},\varphi), (\{b\},\{1\}), (\{b\},\{2\}), (\{b\},Y), (X,\varphi), (X,\{2\}), (X,Y)\}$. Then the set $(A,B) = (X,\{2\})$ is not binary regular closed. However (A,B) is a binary g^* -closed and b-pcl $(A,B) = (X,\{2\})$ is binary g^* -closed.

4 Binary g^* -open sets

Theorem 4.1

- 1. Every binary open set is binary g^* -open.
- 2. Every binary g^* -open set is binary g-open.

Proof.: Proof follows from the Theorems 2.2 & 2.20.

Theorem 4.2

A subset (A,B) of (X,Y) is binary g^* -open if and only if (G,H) \subseteq b-int(A,B)whenever (G,H) is binary g-closed and (G,H) \subseteq (A,B).

Proof.

Suppose that $(G,H) \subseteq b\text{-int}(A,B)$ such that (G,H) is binary g-closed and $(G,H) \subseteq (A,B)$. Let $(A,B)^c \subseteq (P,Q)$ where (P,Q) is binary g-open. Then $(P,Q)^c \subseteq (A,B)$ and $(P,Q)^c$ is binary g-closed. Therefore $(P,Q)^c \subseteq b\text{-int}(A,B)$ by hypothesis. Since $(P,Q)^c \subseteq b\text{-int}(A,B)$, we have $(b\text{-int}(A,B))^c \subseteq (P,Q)$. i.e., $b\text{-cl}((A,B)^c) \subseteq (P,Q)$, since $b\text{-cl}((A,B)^c) = (b\text{-int}((A,B)))^c$. Thus $(A,B)^c$ is binary $g^*\text{-closed}$. i.e., (A,B) is binary $g^*\text{-open}$.

Conversely, suppose that (A, B) is binary g^* -open such that $(G, H) \subseteq (A, B)$ and (G, H) is binary g-closed. Then $(G, H)^c$ is binary g-open and $(A, B)^c \subseteq (G, H)^c$. Therefore, b-cl($(A, B)^c \subseteq (G, H)^c$ by definition of binary g^* -closedness and so $(G, H) \subseteq b$ -int((A, B), since b-cl($(A, B)^c \subseteq (B, H)^c$) = $(B, H)^c$ by definition of binary $(B, H)^c \subseteq (B, H)^c$ by defi





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Theorem 4.3

If b-int(A, B) \subseteq (C, D) \subseteq (A, B) and if (A, B) is binary g^* -open, then (C, D) is binary g^* -open.

Proof

Let b-int(A,B) \subseteq (C,D) \subseteq (A,B), then (A,B)^c \subseteq (C,D)^c \subseteq b-cl((A,B)^c), where (A,B)^c is binary g*-closed and hence (C,D)^c is also binary g*-closed by Theorem 3.4. Therefore, (C,D) is binary g*-open.

Theorem 4.4

If (A, B) is binary g^* -closed, then b-cl(A, B) – (A, B) is binary g^* -open.

Proof

Let (A, B) be binary g^* -closed. Let (G, H) be binary g-closed such that $(G, H) \subseteq b$ -cl(A, B) - (A, B). Then $(G, H) = \phi$. Since b-cl(A, B) - (A, B) cannot have any non-empty binary g-closed set. Therefore, $(G, H) \subseteq b$ -int(b-cl(A, B) - (A, B)). Hence b-cl(A, B) - (A, B) is binary g^* -open.

5 Applications

As applications of binary g^* -closed sets, we introduce the notions called binary T $_g^*$ -spaces and binary $_g$ T $_g^*$ -spaces and obtain their properties and characterizations.

Definition 5.1

A space (X, Y) is called a binary T_g^* -space if every binary g^* -closed set in it is binary closed.

Example 5.2

In Example 2.13, the sets in $\{(\varphi, \varphi), (\varphi, \{b\}), (\{1\}, \{b\}), (\{2\}, \varphi), (\{2\}, \{a\}), (\{2\}, \{b\}), (\{2\}, Y), (X, \{b\}), (X, Y)\}$ are called binary closed. Then binary $G^*C(X, Y) = \{(\varphi, \varphi), (\varphi, \{b\}), (\{1\}, \{b\}), (\{2\}, \varphi), (\{2\}, \{a\}), (\{2\}, \{b\}), (\{2\}, Y), (X, \{b\}), (X, Y)\}$. Thus (X, Y) is a binary T_{σ}^* -space.

Example 5.3

In Example 2.9, the sets in $\{(\varphi, \varphi), (\{2\}, \varphi), (\{2\}, \{b\}), (X, \{a\}), (X, Y)\}$ are called binary closed. Then binary $G^*C(X, Y) = \{(\varphi, \varphi), (\{2\}, \varphi), (\{2\}, \{a\}), (\{2\}, \{b\}), (X, \{a\}), (X, \{a\}), (X, \{b\}), (X, Y)\}$. Thus (X, Y) is not a binary

Theorem 5.4

Every binary $T_{1/2}$ -space is binary T_g^* -space but not conversely.

Proof.

Follows from Theorem 2.20. The converse of Theorem 5.4 need not be true as seen from the following example.

Example 5.5

Let $X = \{a, b\}$, $Y = \{1\}$ and $\mathcal{M} = \{(\varphi, \varphi), (\{a\}, \varphi), (X, Y)\}$. Then the sets $\mathcal{M}^c = \{(\varphi, \varphi), (\{b\}, Y), (X, Y)\}$ are called binary closed. Then binary $G^*C(X, Y) = \{(\varphi, \varphi), (\{b\}, Y), (X, Y)\}$ and we have binary $GC(X, Y) = \{(\varphi, \varphi), (\varphi, Y), (\{a\}, Y), (\{b\}, \varphi), (\{b\}, Y), (X, \varphi), (X, Y)\}$. Thus (X, Y) is not a binary $T_{1/2}$ -space.

Theorem 5.6

Every binary T_b -space is binary T_g^{\star} -space but not conversely.

Proof.

Follows from Theorem 2.22. The converse of Theorem 5.6 need not be true as seen from the following example.





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Example 5.7

In Example 5.5. Then we have binary $GSC(X,Y) = \{(\phi,\phi), (\phi,Y), (\{a\},Y), (\{b\},\phi), (\{b\},Y), (X,\phi), (X,Y)\}$. Thus (X,Y) is not a binary T_h -space.

Remark 5.8

We conclude from the next two examples that binary T_g^* -spaces and binary α -spaces are independent.

Example 5.9

In Example 5.5. Then we have binary $\alpha C(X,Y) = \{(\varphi,\varphi), (\{b\},\varphi), (\{b\},Y), (X,Y)\}$. Thus (X,Y) is a binary T_g^* -space but not an binary T_{α} -space.

Example 5.10

Let $X = \{a,b\}$, $Y = \{1\}$ and $\mathcal{M} = \{(\varphi,\varphi), (\{a\},Y), (X,Y)\}$. Then $\mathcal{M}^c = \{(\varphi,\varphi), (\{b\},\varphi), (X,Y)\}$ are called binary closed. Then we have binary $\alpha C(X,Y) = \{(\varphi,\varphi), (\{b\},\varphi), (X,Y)\}$ and binary $G^*C(X,Y) = \{(\varphi,\varphi), (\{b\},\varphi), (X,Y)\}$. Thus (X,Y) is an binary T_{α} -space but not a binary T_g^* -space.

Theorem 5.11

For a binary topological space (X, Y), the following properties are equivalent:

- 1. (X, Y) is a binary T_g*-space.
- 2. Every singleton subset of (X, Y) is either binary g-closed or binary open.

Proof.

- (1) \Rightarrow (2). Assume that for some $\{i,j\} \in (X,Y)$, the set $\{i,j\}$ is not a binary g-closed in (X,Y). Then the only binary g-open set containing $\{i,j\}^c$ is (X,Y) and so $\{i,j\}^c$ is binary g^* -closed in (X,Y). By assumption $\{i,j\}^c$ is binary closed in (X,Y) or equivalently $\{i,j\}$ is binary open.
- (2) \Rightarrow (1). Let (A, B) be a binary g^* -closed subset of (X, Y) and let $\{i,j\} \in b\text{-cl}(A,B)$. By assumption $\{i,j\}$ is either binary g-closed or binary open.
- Case (a): Suppose that $\{i,j\}$ is binary g-closed. If $\{i,j\} \notin (A,B)$, then b-cl(A,B) (A,B) contains a nonempty binary g-closed set $\{i,j\}$, which is a contradiction.
- Case (b): Suppose that $\{i,j\}$ is binary open. Since $\{i,j\} \in b\text{-cl}(A,B)$, $\{i,j\} \cap (A,B) \neq \varphi$ and so $\{i,j\} \in (A,B)$. Thus in both case, $\{i,j\} \in (A,B)$ and therefore $b\text{-cl}(A,B) \subseteq (A,B)$ or equivalently (A,B) is a binary closed set of (X,Y).

Definition 5.12

The space (X, Y) is called a binary ${}_{g}T_{g}^{*}$ -space if every binary g-closed set in it is binary g^{*} -closed.

Example 5.13

In Example 2.13. Then (X,Y) is a binary ${}_gT_g^{\star}$ -space and the space (X,Y) in the Example 2.9 is not a binary ${}_gT_g^{\star}$ -space.

Theorem 5.14

A space (X, Y) is binary $T_{1/2}$ if and only if it is both binary T_g^* and binary $_gT_g^*$.

Proof

Necessity. Follows from Theorem 5.4. Sufficiency. Assume that (X,Y) is both binary T_g^* and binary g^* . Let (A,B) be a binary g-closed set of (X,Y). Then (A,B) is binary g^* -closed, since (X,Y) is a binary g^* . Again since (X,Y) is a binary T_g^* , (A,B) is a binary closed set in (X,Y) and so (X,Y) is a binary $T_{1/2}$.





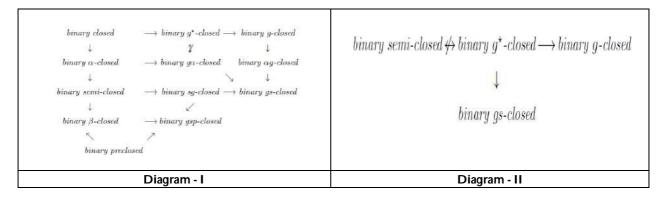
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RESEARCH ARTICLE

Prime Labeling of Tutte-Coxeter Graph

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ABSTRACT

Let G=(V(G),E(G)) be graph with P vertices. A bijection $f:V(G)\to\{1,2,\ldots,|V|\}$ is called a prime labeling if for each edgee = uv, gcd(f(u), f(v)) = 1. A graph which admits prime labeling is called prime graph. Here we proved that prime labeling of Tutte-Coxeter graph. The graph admits Prime labeling, the Duplication of any vertex of degree 3 admits Prime labeling and the Switching of a vertex v_1 in a Tutte-Coxeter graph admits Prime labeling.

Keywords: Tutte-Coxeter graph, Graph labeling, Prime labeling, Duplication, Switching.

INTRODUCTION

In the Mathematical field of graph theory, labeled graph is a wide range. Nowadays, research in graph labeling is increasingly expanding. The Tutte-Coxeter graph or Tutte eight-cage graph is a 3-regular graph with 30 vertices and 45 edges. All graphs considered here are finite, simple, undirected, connected and non - trivial graph. Here we consider the Franklin graph FG has vertex set V=V(FG) and the edge set E=E(FG). The number of elements of V, denoted as |V| called the order of the graph while the number of elements of E, denoted as |E| called the size of the graph. G.Prabhakaran, S.Vijayaraj, and V.Ganesan, Proved the Prime Labeling of Franklin graph [5]. S.Lavanya and V.Ganesan [3] Introduced Some Results on Grotzsch Graph.[4] J.A.Gallian, "A dynamic survey of Graph labeling "[7]. Dr.V.Ganesan, Proved the Prime labeling of split graph of star K I,n. S.K.Vaidya and K.K.Kanmani [10] Prime labeling of cycle related graphs. Dr.V.Ganesan [8] Prime labeling of split graph of cycle C_n .A Tout A.N.Dabboucy and K.Howalla Prime labeling of graphs[1]. Here we will give brief summary of definitions and other information which are useful for the present task.





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PRELIMINARY DEFINITIONS

Definition 1

Let G=(V(G),E(G)) be graph with P vertices. A bijection $f:V(G)\to \{1,2,\ldots,|V|\}$ is called a prime labeling if for each edge $e=uv,\gcd(f(u),f(v))=1$. A graph which admits prime labeling is called prime graph.

Definition 2

Duplication of a vertex v_k of a graph G produces a new graph G_1 by adding a vertex $v_k^{'}$ with $N(v_k^{'}) = N(v_k)$. In other words a vertex $v_k^{'}$ is said to be a duplication of v_k if all the vertices which are adjacent to v_k are now adjacent to $v_k^{'}$.

Definition 3

A Vertex Switching G_v of a graph G is obtained by taking a vertex v of G, removing the entire edges incident with v and adding edges joining v to every vertex which are not adjacent to v in G.

Definition 4 (Tutte Coxeter graph)

In the Mathematical field of graph theory, the Tutte Coxeter graph or Tutte eight-cage graph is a 3-regular graph with 30 vertices and 45 edges.

Illustration1

MAIN RESULTS

Theorem 1

The Tutte-Coxeter graph admits Prime Labeling

Proof

Let TC be the Tutte-Coxeter graph

Tutte-Coxeter graph contains 30 vertices and 45 edges

The graph is 3-regular

$$V(TC) = \{v_1, v_2, v_3, v_4, \dots, v_{30}\}$$

$$E(TC) = \{v_i v_{i+1} / 1 \le i \le 30\} \cup \{v_{30} v_1\} \cup \{v_i v_{5i+5} / 1 \le i \le 4\} \cup \{v_5 v_{12}\} \cup \{v_6 v_{29}\} \cup \{v_{10} v_{10} + v_{10} v_{10} + v_{10} v_{10} + v_{10} v_{10}\} \cup \{v_{10} v_{10} + v_{10} v_{$$

$$\{v_7v_{16}\} \cup \{v_8v_{21}\} \cup \{v_9v_{26}\} \cup \{v_{11}v_{18}\} \cup \{v_{13}v_{22}\} \cup \{v_{14}v_{27}\} \cup \{v_{17}v_{24}\} \cup \{v_{17}v_{24}\} \cup \{v_{18}v_{27}\} \cup \{v_{18}v_{21}\} \cup \{v_{18}v_{21}$$

$$\{v_{19}v_{28}\} \cup \{v_{23}v_{30}\}$$

$$|V(TC)| = 30$$
and $|E(TC)| = 45$

Now define a labeling

$$f: V(TC) \rightarrow \{1, 2, 3, 4, 5, \dots, 30\}$$

$$f(v_i) = i$$
 for $1 \le i \le 30$

We have to verify the relative prime of adjacent vertices

We consider the following type of edges Namely

$$\gcd(f(v_i), f(v_{i+i})) = 1$$
 for $1 \le i \le 30$

$$\gcd(f(v_i), f(v_{5i+5})) = 1$$
 for $1 \le i \le 4$

$$\gcd(f(v_5), f(v_{12})) = 1$$

$$\gcd(f(v_6), f(v_{29})) = 1$$

$$gcd(f(v_7), f(v_{16})) = 1$$

$$\gcd(f(v_8), f(v_{21})) = 1$$

$$\gcd(f(v_9), f(v_{26})) = 1$$





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\begin{split} &\gcd(f(v_{11}),f(v_{18})) = 1 \\ &\gcd(f(v_{13}),f(v_{22})) = 1 \\ &\gcd(f(v_{14}),f(v_{27})) = 1 \\ &\gcd(f(v_{17}),f(v_{24})) = 1 \\ &\gcd(f(v_{19}),f(v_{28})) = 1 \\ &\gcd(f(v_{23}),f(v_{30})) = 1 \\ &\operatorname{Therefore} f \operatorname{Satisfy} \text{ the condition of Prime labeling} \\ &\operatorname{TC} \operatorname{admits} \operatorname{prime labeling} \end{split}
```

Theorem 2

In Tutte-Coxeter graph the duplication of any vertex of degree 3 admits Prime labeling

Proof

Tutte-Coxeter graph be TC

Hence TC is a prime graph

The graph contains 30 vertices and 45 edges

Tutte-Coxeter graph is 3-regular

Let G be the graph obtained from TC by duplicating any vertex of degree 3

Take the vertex v_1 , the vertex v_1 to be the duplicating vertex and let $v_1^{'}$ be the duplication vertex of v_1

$$V(G) = \{v_1, v_2, v_3, v_4, \dots, v_{30}\}$$

$$E(G) = \{v_i v_{i+1} / 1 \le i \le 30\} \cup \{v_{30} v_1\} \cup \{v_i v_{5i+5} / 1 \le i \le 4\} \cup \{v_5 v_{12}\} \cup \{v_6 v_{29}\} \cup \{v_{10} v_{10} + v_{10} v_{10} + v_{10} v_{10} + v_{10} v_{10}\} \cup \{v_{10} v_{10} + v_{10} v_{1$$

$$\{v_7v_{16}\} \cup \{v_8v_{21}\} \cup \{v_9v_{26}\} \cup \{v_{11}v_{18}\} \cup \{v_{13}v_{22}\} \cup \{v_{14}v_{27}\} \cup \{v_{17}v_{24}\} \cup \{v_{17}v_{24}\} \cup \{v_{18}v_{27}\} \cup \{v_{18}v_{27}$$

$$\{v_{19}v_{28}\} \cup \{v_{23}v_{30}\}$$

$$|V(G)| = 30$$
 and $|E(G)| = 45$

A function is defined $f:V(G) \rightarrow \{1,2,3,4,5,\dots,31\}$

Take
$$f(v_i) = i$$
 for $1 \le i \le 30 f(v_i) = 31$

We have verify the relative prime of adjacent vertices

$$\gcd(f(v_i), f(v_{i+i})) = 1$$
 for $1 \le i \le 30$

$$\gcd(f(v_i), f(v_{5i+5})) = 1 \text{ for } 1 \le i \le 4$$

$$\gcd(f(v_5), f(v_{12})) = 1$$

$$\gcd(f(v_6), f(v_{29})) = 1$$

$$gcd(f(v_7), f(v_{16})) = 1$$

$$\gcd(f(v_8), f(v_{21})) = 1$$

$$\gcd(f(v_9), f(v_{26})) = 1$$

$$\gcd(f(v_{11}), f(v_{18})) = 1$$

$$\gcd(f(v_{13}), f(v_{22})) = 1$$

$$\gcd(f(v_{14}), f(v_{27})) = 1$$

$$\gcd(f(v_{17}), f(v_{24})) = 1$$

$$\gcd(f(v_{19}), f(v_{28})) = 1$$

$$\gcd(f(v_{23}), f(v_{30})) = 1$$

$$\gcd(f(v_{30}), f(v_1)) = 1$$

$$\gcd(f(v_2), f(v_1)) = 1$$





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\gcd(f(v_{10}), f(v_1)) = 1
f fulfill the prime labeling condition \therefore G admits prime labeling
Hence G is a prime graph
```

Theorem 3

The Switching of a vertex v_1 in a Tutte-Coxeter graph admits prime labeling

Proof

```
Tutte-Coxeter graph be TC
The graph contains 30 vertices and 45 edges
Tutte-Coxeter graph is 3-regular graph
The graph obtained by vertex switching of TC with respect to the vertex v_1 is denoted as G_u
V(G_u) = \{v_1, v_2, v_3, v_4, \dots, v_{30}\}
E(G_u) = \{v_i v_{i+1}/2 \le i \le 30\} \cup \{v_{30} v_1\} \cup \{v_i v_{5i+5}/2 \le i \le 4\} \cup \{v_5 v_{12}\} \cup \{v_6 v_{29}\} \cup \{
\{v_7v_{16}\} \cup \{v_8v_{21}\} \cup \{v_9v_{26}\} \cup \{v_{11}v_{18}\} \cup \{v_{13}v_{22}\} \cup \{v_{14}v_{27}\} \cup \{v_{17}v_{24}\} \cup \{v_{17}v_{24}\} \cup \{v_{18}v_{27}\} \cup \{v_{18}v_{21}\} \cup \{v_{18}v_{21}
\{v_{19}v_{28}\} \cup \{v_{23}v_{30}\} \cup \{v_1v_{2+i}/1 \le i \le 7\} \cup \{v_1v_{10+i}/1 \le i \le 19\}
Then |V(G_u)| = 30 and |E(G_u)| = 68
Labeling is defined by f: V(G_n) \rightarrow \{1,2,3,\dots,30\}
f(v_i) = i for 1 \le i \le 30
We have verify the relative prime of adjacent vertices
\gcd(f(v_i), f(v_{i+i})) = 1 for 1 \le i \le 30
\gcd(f(v_i), f(v_{5i+5})) = 1 \text{ for } 1 \le i \le 4
\gcd(f(v_5), f(v_{12})) = 1
\gcd(f(v_6), f(v_{29})) = 1
\gcd(f(v_7), f(v_{16})) = 1
\gcd(f(v_8), f(v_{21})) = 1
\gcd(f(v_9), f(v_{26})) = 1
\gcd(f(v_{11}), f(v_{18})) = 1
\gcd(f(v_{13}), f(v_{22})) = 1
\gcd(f(v_{14}), f(v_{27})) = 1
\gcd(f(v_{17}), f(v_{24})) = 1
\gcd(f(v_{19}), f(v_{28})) = 1
gcd(f(v_{23}), f(v_{30})) = 1
\gcd(f(v_1), f(v_{2i+1})) = 1
                                                                                                                                                                     for 1 \le i \le 7
\gcd(f(v_1), f(v_{10+i})) = 1
                                                                                                                                                                        for 1 \le i \le 19
```

Thus f is a prime labeling and consequently G_u is a Prime graph. Therefore the Switching of a vertex v_1 in a Tutte-Coxeter graph admits prime labeling

CONCLUSION

In this paper we discussed about the Prime labeling of Tutte Coxeter graph, Duplication of any vertex of degree 3 admits prime labeling, Switching of a vertex v_1 in Tutte Coxeter graph admits prime labeling. In future, this may helpful to prove the prime labeling for some more standard graphs.





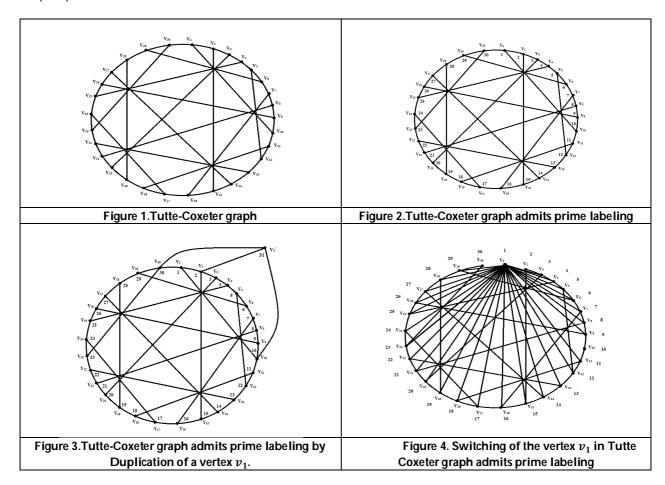
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REVIEW ARTICLE

Microplastics Prevalence in Aquatic Environment: An Integrative Review on its Sources, Pathways, and Mitigation

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ABSTRACT

This paper aims to discuss the prevalence of microplastics in the aquatic environment, specifically the sources, pathways, and mitigation using a computerized check on the relevant journals using Boolean operators AND, OR, & NOT with associated keywords. A five-step integrative review method was employed. Results revealed that the accumulation of microplastic particles in the aquatic environment is undeniably attributed to domestic and industrial sources. It was found out that the abundant microplastic particles from identified sources of microplastics were polyolefins, polyester, polystyrene, polypropylene terephthalate, acrylic, and polyamide. Moreover, the sizes of these microplastics particles range from 3 µm to 2.03 mm. The pathways of these microplastic particles are rivers, effluents, and runoff coming from numerous sources. Hence, the present study recommends (a) community and industrial-wide assessment approaches using a standardized method to minimize microplastic contamination, (b) bioremediation as a potential strategy for microplastics removal in the aquatic environment, and (c) Perform more studies on the environmental effects of microplastics and viable alternative for plastics.

Keywords: anthropogenic activities, aquatic environment, integrative review, microplastics, mitigation, transport





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INTRODUCTION

Plastic is an organic polymer derived from petroleum sources through polymerization and polycondensation process (Anderson et al., 2016; Karbalaei et al., 2018). The material properties of plastic have many appealing characteristics to both consumers and manufacturers (Horton and Dixon, 2018), particularly in its durability, versatility, lightness, and corrosion resistance making it suitable for a broad range of applications (Lusher et al., 2017; Yang et al., 2021; Zeng, 2018; Zhang et al., 2020;). The annual global production of plastics has enormously increased in the last 50 years (Plastics Europe 2017), with production rising from 1.5 million metric tons in the 1950s to 368 million metric tons in 2019; however, due to COVID – 19's industrial impacts, it dropped to 367 million metric tons in 2020 (Tiseo, 2020). Trends of global plastic production, consumer usage habits, improper plastic waste disposal directly point out an increase in plastic use in the future. However, these trends also led to the surge of plastics bits and pieces discharged into the environment, whether intentional or unintentional losses (Jambeck et al., 2015). Plastics are manufactured in all shapes and sizes depending on their range of applications and utilizations, which will eventually fall into various categories of degradations leading to embrittlement and fragmentation into smaller particles known as microplastics (Andrady, 2017). Microplastics are tiny plastic debris with < 5mm in size (Horton and Dixon, 2018; Mason et al. 2016; Murphy et al. 2016) originating from two major sources: (i) primary microplastics that is purposefully manufactured for industrial and domestic applications (Halfar et al., 2021; Nizzetto et al., 2016), and (ii) secondary microplastics which are the result of the breakdown of larger plastic litter items (Horton & Dixon, 2018) due to weathering and aging processes such as UV radiation, mechanical strains, hydrolysis, and microbial degradations (Xu et al., 2020).

These microscopic plastic particles reach the aquatic environment through a variety of land-based and marine-based activities. For example, microplastic beads found in facial cleansers, synthetic garments, toothpaste, and scrubs enter the aquatic ecology via drainage systems used at home and wastewater treatment plants for industrial settings. (Murphy et al., 2016). Similarly, larger plastic particles that have been broken down into smaller bits from waste dumps can be transferred into seas, resulting in microplastic pollution (Alomar et al., 2016). Microplastics are dispersed throughout the world (Vandermeersch et al., 2015), and have been detected in every level of the aquatic environment (Auta et al., 2017). These microplastics are found in shorelines, sediments, water columns (Gallagher et al., 2016), as well as in beaches and wastewater effluents (Fahrenfeld et al., 2019; Paler et al., 2019; Ziajahromi et al., 2016). Microplastics' presence in the aquatic environment has raised major concerns in the scientific community because of its potential threat to the aquatic ecosystem and human health (Ziajahromi et al., 2016). The small sizes of microplastics' are often mistaken for food and vulnerably ingested by various aquatic animals with numerous consequences (Cortes & Otadoy, 2020; Roch et al., 2020). Reports on microplastics ingestion by aquatic species has been linked to reproductive issues, growth rate reduction, false satiation, enzyme production blockage, pathological stress, and oxidative stress (Barboza et al., 2018; De Sá et al., 2018; Qiao et al., 2019).

As a global pollutant, several studies have been conducted and documented regarding the prevalence of microplastics in the environment. Most of these review studies are precisely focused on the distribution of microplastics in the marine environment (Auta et al., 2017; Coyle et al., 2020) and the freshwater environment (Parker et al., 2021; Sarijan et al., 2021). Common themes/ topics reflected on these microplastics studies are in the areas of occurrence, impacts, sources, and possible solutions. However, these papers do not directly point out the specific sources and are only limited to categorizing them as primary or secondary sources of microplastics but were not able to discuss the sizes, microplastic types and methodology of identifying the microplastic particles. In addition, viable alternative for plastics products is not yet taken into account as an efficient strategy in minimizing microplastic particles in the environment. In view of these, this integrative review paper aims to point out the direct sources of microplastics, discuss the sizes, methods, and pathways from sources to an aquatic environment, and mitigation of microplastics.





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METHODOLOGY

There is no central repository for empirical research on microplastics in the aquatic environment. Therefore, the current study conducted manual and computerized checks on relevant journals to document the study's objective as mentioned above and organize them. Boolean operators AND and OR were associated with the descriptors search keywords that includes microplastics sources, marine debris, freshwater debris, microplastics transport, pollutions, and mitigation measures. Additionally, web search in Google Scholar using publish and perish were conducted in the early stages of the review. The search so far yielded 15 studies from reputable journals which qualify for the standards set in the inclusion criteria.

Inclusion Criteria

The studies included in this study are based on the following criteria.

- 1. Microplastic sizes ranging from 1 um 5 mm.
- 2. Report on the microplastics abundance with identified sources, transports, and migration measures.
- 3.Research on microplastics that undergone peer review process and published in respectable journal and conducted from 2011 2021. These papers are listed in the references.

The researcher developed the search keywords and criteria to establish guidelines in choosing studies to be included. Finally, the tabulation of the important findings through a research matrix helps the researcher identify the pattern and content of the review during the data analysis and synthesis. This process resulted in this article's development, which clearly and concretely presents the integrative review described by Whittemore and Knaf (2005), identified in Figure 1.

RESULTS AND DISCUSSION

Microplastic particles in the aquatic environment are made up of heterogeneous aggregates of particle shapes, sizes, polymer types, and environmental microplastics' physical and chemical properties (Lambert et al., 2017) table 1. They are found in everyday use products (primary microplastics) or from the breakdown of larger macroplastics debris under environmental conditions (secondary microplastics).

Personal care products (PCPs)

Plastic is not listed in every PCP's ingredient list, but that does not imply they are free of microplastics (Carr et al., 2016). Plastic micro beads are used as an additive in a variety of personal care products (PCPs), such as cleaning products, makeup cosmetics, shower gel, facial cleanser, hand sanitizer, soap, toothpaste, shaving cream, bubble bath, sunscreen, and shampoo, are sources of microplastics in the environment (Nizzetto et al., 2016). Microplastic in PCPs are determined through simulations of actual use processes wherein physical - chemical features are established. The most common type of polymer present in PCPs is polyethylene (PE), followed by polypropylene (PP), polystyrene (PS), and ethylene, consecutively (Table 1). This characterization is mostly accomplished through Fourier Transform Infrared Spectroscopy (FTIR) that can be applied in phases of production cycle and failure analysis (Baysal, 2019; Godoy et al., 2019; Praveena et al., 2018; Renner et al., 2021; Suardy et al., 2020; Ustabasi et al., 2019). In addition, High-Temperature Gel-Permeation Chromatography (HT-GPC) using Infrared Radiation (IR) is also a capable method of distinguishing polymers; however, this method is more efficient in identifying polyolefin to all aqueous and hydrocarbon-based matrices (Hintersteiner et al., 2015). In can be noted also that the presence of microplastic particles in these personal care products varies in sizes with the minimum of 3 µm and maximum of 551.39 µm depending on its brand and product applications. Among PCP's, facial cleanser/ scrub is the most studied sample; other PCPs, such as moisturizer, nail polish, sun creams, toothpaste, and shower gel, have received less attention. This difference is attributed to the high abundance of the large mass of microplastics contained in facial cleansers and toothpaste (Sun et al., 2020). Overall, polyethylene is the main polymer in personal care products based on the empirical research available, which sheds light on the idea that PCPs are one of the major contributors to microplastics in the environment.





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Blasting abrasive and cleaning products

Microplastics can exert their abrasive function in the sector of blasting abrasive for cleansing of surfaces. Microplastics are primarily used as an alternative or in mixture with blasting agents (Bergmann et al., 2015; Galafassi et al., 2016), cleaning products (Verschoor et al., 2016), as well as cosmetics products (Lei et al., 2017; Sharma et al., 2017). For instance, when a gentler action is needed during blasting, microplastics are alternatively used instead of sand, corundum, and steel grit. Studies (Bergmann et al., 2015; Galafassi et al., 2016; Lei et al., 2017; Sharma et al., 2017; Verschoor et al., 2016) revealed that different classifications of polymers are found in blasting abrasive and cleaning products (Table 1). Each of these polymers has unique characteristics depending upon its wide array of applications. Methods employed in identification of these sources are Scanning Electron Microscopy, Micro Fourier transform infrared spectroscopy (µ-FTIR) and Fourier transform infrared spectrometry (FT-IR). These methods are utilized to obtain information from a homogenized sample and localized information on a particular point of the sample such as compounded microplastics. The average size to which these polymers are used alternatively as a blasting agent or mixed in other products such as cleansing, and cosmetics ranges from 50 µm to 2.03 mm. This implies that size and polymer classification blended with the product plays an important role in efficiency as an abrasive cleaning agent. For instance, an abrasive that is too hard or coarse can remove too much material or leave undesired scratch marks. While finer or softer abrasives will tend to leave much finer scratch marks. However, the softer abrasive may become less effective more quickly as the abrasive is itself abraded.

Synthetic textile

The efficient textile fabrication, domestic and commercial laundry of synthetic textiles have an immense contribution to the environmental concern (Henry et al., 2019). Microfibers are an active component in garments production and are classed as primary microfibers. In contrast, fragmented textiles of large microfibers during the distribution of the textile and garment industries to the consumers are considered secondary microfibers (Liu et al., 2019). Meanwhile, microfibers tend to be detached due to their sloppy-structured synthetic clothing textiles or through their laundry process. Microfiber's abundance can be attributed to the production and cleansing of textile products. Based on micro plastic's empirical research, synthetic textile products have been found to have particles of microfibers of <1mm to maintain their appealing characteristics and material properties (Table 1). This identification is accomplished through FTIR, and SEM. Polymers present in textiles include but are not limited to polyester, acrylic, polypropylene, polyethylene, and polyamide. The most prevalent microfibers released in the aquatic environment from the textile industry and washing of synthetic garments are polyester (Browne et al., 2011) accounting for 56% of the identified microfibers. However, among the five identified polymers, polyamide is distinguished to be less widespread with about 3% (Almroth et al., 2018) in the aquatic environment. These results can be attributed to the consumer's rate of synthetic textile utilization, geographical location, and weather condition. For instance, polyamide textile is unsuitable to wear during cold winters. Hence, it is not very insulating, and if it gets wet, it tends to trap the water for guite some time. In comparison, polyester is a kind of polymer with a hydrophobic nature (Browne et al., 2011).

Paints and coatings

Intentional manufacturing of paints and coatings embedded with micro beads is a significant contributor to the emerging issues on microplastics (Hale *et al.*, 2020). Paints contain polymers that give matting effects and act as a color amplifier, improving longevity, hardening, and abrasion resistance (Mehra *et al.*, 2020). This persistence of microplastic paint particles in the aquatic environment is heavily attributed to its polymer's compositions and various applications. Hence, paints and coatings particles play an increasingly important understanding of microplastic pollution of our aquatic environment. Microplastics paint particles are detected in the aquatic environment using Fourier Transform Spectroscopy which identifies the abundance of paints microplastics particles such as Alkyd resins, Polyacrylate, Polystyrene, and Polypropene (Table 1). These microplastics paint particles have the sizes between < 50 µm and 1000 µm, with the identified smallest particles of 4 µm. Most of these paint particles are coming from ships paints and coatings, and fiber glasses which were more concentrated at the aquatic environment surface (Chae *et al.*, 2015; Song *et al.*, 2015). This implies that painted and coated surfaces are prone to deterioration mechanisms such as binding polymer degradation upon exposure to UV radiation and intentional or unintentional mechanical disturbance (e.g., maintenance or removal, damage, wear, and tear). These suggest that the





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manifestation of microplastics paint particles in the aquatic environment are dependent on factors (e.g., brittleness, size, density, and polymers). For instance, low-density polymers are expected to float (e.g., polystyrene, polyacrylate, and alkyd resins), while high-density polymers are predicted to sink in the aquatic environment.

Microplastic pathways in the aquatic environment

Microplastics are becoming a bigger problem; with global plastic output expected to exceed 56 gigatons by 2050 (Shen et al., 2020). However, these plastics are important for innovation and development in various fields, including health care, energy generation, aerospace, automobile industries, constructions, electronics, packaging, textiles, cosmetics, etc. (Ivleva et al., 2017). With all these anthropogenic activities, microplastics can enter the aquatic environment mainly through rivers, wind, industrial and urban effluents (Lebreton, 2017). For instance, cosmetics such as scrubs, toothpaste, air-blasting media, and garments contain microplastic beads that can reach the aquatic environment via industrial or home drainage systems. Similarly, clothing made of synthetic fibers produces microplastic effluents from sheds that are washed into water or wastewater treatment plants (Murphy et al., 2016). Figure 2 shows the significant pathways of microplastics in the aquatic environment. Rivers, effluents, and runoff all contribute to the transfer behavior of microplastics into the aquatic environment (Leslie et al., 2017; Murphy et al., 2016). Also, a study by Andrady (2011) emphasized that the disintegration of most plastics via weathering resulted in embrittlement, yielding microplastics that are carried into the bodies of water by the wind. Microplastics size of (<5 mm) and low-density are extensively distributed by currents across longer distances (Eerkes-Medrano et al., 2015). Meanwhile, Cole et al. (2016) reported that biological transport of microplastics coming from the excrement of zooplankton after its consumption of microplastic particles is a potential pathway for microplastics in the aquatic environment. When exposed to microplastics, the organisms readily consumed them as they went through the gut, were encapsulated in feces, and were egested. Following egestion, the feces settled to the bottom of the exposure vessel and were eaten by the other organisms (larger copepod). The study indicated that microplastics could be swallowed indirectly via fecal pellet intake, indicating that fecal pellets are a source of microplastics in the marine environment. Thus, these transport pathways have led to the pervasiveness of microplastics throughout the world'saquatic environment. Moreover, microplastics account for 92% of the world's 269 million tons of 5.25 trillion particles, and these microplastics are a hundred times less on the aquatic surfaces than expected, supporting the theory that most microplastics sink to sediments (Eriksen et al., 2014). Results of the gathered works of the literature revealed that the transport of microplastics particles in the aquatic environment is not only limited to natural environmental transport but also includes biological transports.

Strategic mitigation on the prevalence of microplastic

Remediation must be implemented to prevent additional increases of microplastics waste (Löhr et al., 2017), along with solutions centered on the source of pollution. Most microplastic pollution originates from the degradation of macroplastic products. An initiative aimed at reducing plastic usage or discharge to the environment can be of help to alleviate the pollution on microplastic. Regulations and enforcement, a fee on plastic items, increased awareness, recycling, and the adoption of a circular economy are examples of preventive approaches. According to Kershaw (2015), the successful problem reduction will necessitate coordinated measures from multiple public and business sectors at the global, regional, and local levels. The attitudes and beliefs of the people regarding littering can be changed thorough education awareness programs on the environmental impacts of microplastics. With this Hartley et al., 2018, further highlighted the need for a robust program to elicit deep - rooted modification of behavior. The reduction of pollution from the source as a strategy of restoration has demonstrated to be successful (Jambeck et al., 2015; Wagner and Lambert, 2018). Schneider and Ragossnig, (2015) highlighted that source reduction must put emphasis on reduce, reuse, recycle and recover in the optimization of waste disposal management. Among these source reduction strategies, recycling is the most favored approach due to its efficiency on energy and resources, emission curtailing of pollutants, decrease amount of waste that needs to be disposed of, and aids in the economics and environmental protection. Meanwhile, the least favored approach is the landfill mainly because of the danger it posed such as contamination and its impact on the environment (Liu et al. 2018). The shifting of circular economy from linear led to profound sustainable solutions of waste reduction, increase of material worth, and intensive support on plastic economy. Moreover, zero-waste production can only be realized through the continuous practice





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of circular economy (Wagner and Lambert, 2018) which necessitates eradication of waste landfilling. For instance, in circular economy, landfilled plastics can be recovered through landfill mining. Moreover, biobased plastics are a viable alternative to traditional plastics since they are renewable, biodegradable, and have fewer environmental consequences (Ruinić-Sokele & Pilipović 2017). Bioplastics, for example, can be used as alternatives to reduce the use of plastic. As seen in the study of Liu et al., 2018 another feasible preference in reducing plastic waste accumulation is through an extended producer responsibility program operation on upgrading the rate of recycling on single use of plastics, as well as control and regulation of plastics. Microbeads an essential component of personal care products have been banned in several countries including United Kingdom, New Zealand, United States and Canada (Dauvergne, 2018). Also, numerous companies of the European Union have expressed their commitment to the phasing out cosmetics and personal care goods containing microplastics. There is a 50% chance that these deliberate actions may minimize microplastic pollution in the environment (Hirst and Bennett 2017). During the United Nations Environment Assembly, approximately 170 countries across the world have pledge to minimize the consumption of plastic by the year 2030 (BBC New 2019). Due to its degree of being an emerging global pollutant different treaties and guidelines have been crafted and implemented internationally such as Convention on Biological Diversity (Gabriel et al., 2018) and Montreal Protocol (Raubenheimer & McIlgorm 2017) with the goal to protect the ecosystem from the adverse effects of microplastic pollution in the environment.

CONCLUSION

The accumulation of microplastics particles in the aquatic environment nowadays are undeniably attributed to the domestic and industrial sources. The study found out that the abundant microplastic particles from identified sources of microplastics were polyolefins, polyester, polystyrene, polypropylene terephthalate, acrylic, and polyamide. Identified sizes of these polymers has the range of 3 µm to 2.03 mm. The pathways of these microplastics particles to the aquatic environment are through rivers, effluents and runoff coming numerous sources. Microplastics size of (<5 mm) and low-density are extensively distributed by currents across longer distances. In addition, biological transport of microplastics coming from the excrement of zooplankton after its consumption of microplastic particles is a potential pathway for microplastics in the aquatic environment. Result of the study suggests a knowledge gap on the riverine systems since there are only few literature studies conducted on the prevalence of microplastics. Meanwhile, rivers are major transport systems of microplastics entering the marine environment. Literatures also revealed strategic mitigation approaches on microplastics in aquatic environment which directly point to its reduction from its sources. Such approaches lie on the introduction of circular economy and biobased plastics as an alternative for polymer-based plastics. Moreover, different treaties and guidelines have been crafted to address the emerging environmental problems on microplastics. Future research should focus on the strategic mitigation approaches other than the highlighted approaches. Such as:

- a) Community and industrial-wide assessment approaches using standardized method to minimize microplastic contamination
- b) Bioremediation as potential strategy for microplastics removal in aquatic environment.
- c) Perform more studies on the environmental effects of microplastics and viable alternative for plastics.

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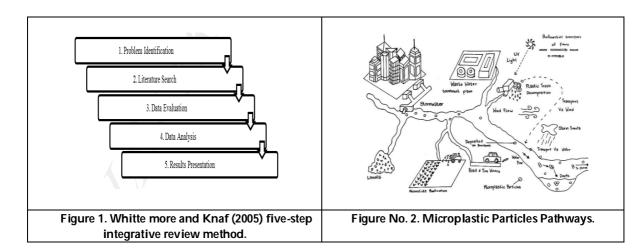


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Table 1. Microplastic Sources in the Aquatic Environment

Sources		Method	Microplastic Identified	Size	Reference	
	Toothpastes and Facial scrubs	Micro Fourier transform infrared spectroscopy (μ- FTIR	Polyethylene	$5-483~\mu m$	Renner et al., 2021	
	Shower gels, and Facial cleaners	High-Temperature Gel-Permeation Chromatography (HT-GPC) using Infrared Radiation (IR)	Polyolefin (polypropylene (PP) and polyethylene (PE)	100 – 300 μm	Hintersteiner et al., 2015	
Personal care products	Facial Scrubs, bath gels, nail polishes or sun creams	Fourier Transform Infrared Spectroscopy (FTIR)	Polyethylene	264.19 – 551.39 μm	Godoy et al., 201	
	Toothpaste, Facial scrubs, and Body Scrubs	Fourier Transform Infrared Spectroscopy (FTIR)	Polyethylene, Ethylene, polystyrene	200-500 μm	Suardy et al., 2020	
	Facial cleanser/ scrub, Body Scrub, and Moisturizer	Fourier Transform Infrared Spectroscopy (FTIR)	LDPE (low density polyethylene) and polypropylene,	3 to 178 μm	Praveena et al., 2018	
	Toothpastes	Fourier Transform Infrared Spectroscopy (FTIR)	Polyethylene	$4-20~\mu m$	Ustabasi and Baysal, 2019	
Blasting abrasive and cleaning products	Detergents (laundry detergents, dishwasher detergents, bathroom cleaners, bleaching cleaners and surface cleaners)	Scanning Electron Microscopy	Polypropylene terephthalate	50 and 1000 μm	Verschoor et al., 2016	
	Plastic media blasting	Micro Fourier transform infrared spectroscopy (µ- FTIR)	Acrylic, Polyester, Polyamide	0.012 to 2.03 mm	Galafassi et al., 2016; Bergmann et al., 2015	
	Cosmetics Products	Fourier transform infrared spectrometry (FT-IR)	Polyethylene	74–420 μm	Lei et al., 2017; Sharma et al., 2017	
		Fourier Transform Infrared Radiation	Polyester (56 %)	<1mm	Brown et al., 201	
		Scanning EM	Acrylic (23%)	1.2µm	Almroth et al., 2018	
Synthetic textile	Textile industry, and Washing of all type of Synthetic garments	Fourier Transform Infrared Radiation	Polypropylene (7%)	<1mm	Brown et al., 201	
		Fourier Transform Infrared Radiation	Polyethylene (6%)	<1mm	Brown et al., 201	
		Scanning EM	Polyamide (3%)	1.2 µm	Almroth et al., 2018	
Paints and coatings		Fourier transform infrared spectroscopy (FTIR)	Alkyd resins, Polyacrylate, and Polystyrene	<50 and 1000 μm	Song et al. (2015	
	Paints and coatings	Fourier transform infrared spectroscopy (FTIR)	Polypropylene, Polyethylene, and Expanded Polystyrene (e.g., Styrofoam)	50–300 μm	Chae et al., 2015	





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RESEARCH ARTICLE

Development and Quality Evaluation of Papaya Fruit Bar

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ABSTRACT

The present investigation on the "Development and quality evaluation of Papaya fruit bar" was carried out at Post Harvest Lab, Department of Horticulture, Faculty of Agriculture, Annamalai University, Annamalai Nagar during 2020-2022. The experiment was carried out a in completely randomized design with four formulations and five replications. Papaya was processed into fruit bar and the effect of processing on the nutritional and sensory quality of fruit bar was determined. The sensory evaluation was carried out at monthly intervals for three months during storage. Papaya fruit bar was developed with variation in forms of sugar (T1-Sugar, T2-Honey, T3-Jaggery, T4- Palm candy). Among the formulations, Jaggery (50 °B of jaggery) was considered as the best treatment with a moisture content of 16.83 %, titratable acidity (16.83 %), vitamin C (43.87 mg/100g), total sugar (69.82 %). The maximum sensory scores for organoleptic evaluation were obtained by T2 (50 °B of honey) for taste, colour, flavour and overall acceptability followed by T₄ (palm candy). The cost economics for the production of papaya fruit bar was 1.78.

Keywords: Papaya fruit bar, Sugar, Honey, Jaggery, Palm candy.

INTRODUCTION

Papaya scientifically called as Carica papaya L. is an important tropical and subtropical fruit crop native to the central and South American region. Papaya fruit is known for its nutrient content such as vitamin – A, C, and D, minerals viz., magnesium and potassium. Papaya fruit is consumed as raw fruit and also in value-added product. Papaya





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fruits are processed into different value-added products such as papaya candy, papaya pickles, Osmo dried papaya sweet, and papaya shake by involving different preservation methods such as drying, preservation, vacuum packing, freezing, pasteurization and canning. The shelf life of papaya fruits is enhanced by value addition technology. Hence an attempt was made to process papaya into papaya fruit bar with variation in forms of sugar.

MATERIALS AND METHODS

This present investigation was carried out at Post Harvest Lab, Department of Horticulture, Faculty of Agriculture, Annamalai University, Annamalai Nagar during 2020-2022. The experiment was carried out in a Completely Randomized Design with four formulations and five replications. Papaya was processed into fruit bar and the effect of processing on the nutritional quality and sensory quality of fruit bar were determined. The sensory evaluation was carried out at monthly intervals for three months during storage. Papaya fruit bar was prepared with four formulations viz., T₁-Sugar, T₂-Honey, T₃-Jaggery, T₄- Palm candy(Fig. 1). Prepared fruit bar was stored up to three months. For the preparation of papaya fruit bar, whole ripe papaya fruits were selected. The fruits were peeled and then whole fruit was cut in to pieces. Then pieces were ground to pulp and kept in a bowl. Pulp was transferred to pan and started cooking. After 10-12 minutes, various forms of sugar according to the formulations mixed with pulp and stir the mixture continuously till it reached to 50° Brix. And then cooked mixture was transferred to tray and kept in hot air oven for drying at 65 °C for five hours. Then fruit bars were allowed to cool down. Then they are sliced into rectangular pieces and stored in a polythene pack. The organoleptic quality of papaya processed products was evaluated by the panellists for sensory attributes such as colour, taste, flavour, and also overall acceptability. As explained by Wichchukit and Mahony (2014) a nine-point hedonic scale was used ranging from like extremely (9) to dislike extremely (1). All samples were presented before the panellists at ambient temperature under normal lighting conditions. Nutritional quality such as pH, TSS (oBrix), total sugars (%), protein (%) and moisture (%) were determined. Total sugars (%) and protein (%) were analyzed by the method of AOAC (2004).

RESULTS AND DISCUSSION

In the present study, papaya fruit bar was formulated with variations in forms of sugar (T₁-Sugar, T₂-Honey, T₃-Jaggery, T₄-Palm Candy). Fruit bar prepared with T₃ (Jaggery) considered as the best formulation over others (Table 1) with low amount of moisture content (16.83%), higher amount of ascorbic acid (143.87%) and total sugar (69.82%). When compared to fresh fruit (nearly 90% moisture), there was a huge reduction in moisture content in the prepared papaya fruit bar. The reduction in moisture content might due to evaporation of water from the pulp during processing at high temperature. These findings are also in conformity with observations made by other workers in guava leather by Jain and Nema (2007), Sreemathi et al. (2008), Safdar et al. (2014). Sugar is commonly used for preparing and preserving candy which reduce the water activity of the food to the point where microbial growth is impossible. In this study different forms of sugar were used in the preparation of papaya fruit bar. It might have raised the osmotic pressure of the solution and caused plasmolysis of microbial cells, thus the water activity of the fruit bar was reduced and the growth of micro-orgainsms was checked (Uma Maheswari et al. 2019). Total soluble solids ranged from 72.31°Brix (T₃) to 75.82°Brix (T₁) among the formulations (Fig. 1). The highest total soluble solids 75.82°Brix was recorded in fruit bar with Jaggery (T₃) followed by 74.89°Brix in palm candy (T₄). The significant increase in total soluble solids in fruit bar was noted when compared to fresh fruit (10.52°Brix) by Asiama et al. (2020). Increase in sugar might be due to inversion of sugar reported by Roy and Singh (1979). Increasing trend in TSS content in fruit bar was correlated with the investigations in papaya and guava pulp by Jain et al. (2011), guava bar by Khan et al. (2014) and guava jelly bar by Kuchi et al. (2014).

The highest titratable acidity of 0.95% was recorded in fruit bar with sugar (T_1) followed by 0.92% in palm candy (T_4), 0.85% in honey (T_2) and 0.83% in jaggery (T_3). The titratable acidity of fruit bar increased when compared with fresh fruit (0.34%) (Falaha *et al.* 2015). Similar results were recorded on apricot fruit bar by Sharma *et al.* (2013) and papaya toffee and leather by Attri *et al.* (2014). The highest value for vitamin C content recorded in treatment T_3 with jaggery





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(43.87 mg/100g), followed by T₂ with honey (41.67 mg/100g), T₄ with palm candy (38.62 mg/100g) and the lowest vitamin C content recorded in T₁ with sugar (35.78 mg/100g). The fresh papaya fruit had the vitamin C of 60.9 mg (Sudhakar and vidhya, 2014) which was higher than prepared Fruit bar. Vitamin C was probably the most unstable vitamin and it's readily oxidized by many non-enzymatic processes. It was easily oxidized and will be drastically reduced in short period of time (Uma Maheswari *et al.* 2020). The results are also in conformity with the report on guava nectar by Karanjalker *et al.* (2013). Among the formulations of fruit bar, the highest total sugars of 69.82 per cent was recorded in fruit bar made by 50°B jaggery (T₃) and followed by other formulations. The increase in total sugars per cent of the fruit bar samples were noted among the different formulations (Fig. 1). Due to additional sucrose, the product showed higher total sugar. The higher sugar content might be due to infusion of sucrose, due to addition of sugar in different forms as reported by Uma Maheswari *et al.* (2021). These results are in conformity with the findings on apricot fruit bar by Sharma *et al.* (2013) and papaya toffee and papaya leather by Attri *et al.* (2014).

The average sensory scores for taste (8.2), colour (8.5), flavour (8.1) and overall acceptability (7.8) of papaya fruit bar were initially maximum in T₂ formulated with honey followed by T₄ formulated with palm candy had a sensory score of taste (7.7), colour (8.0), flavour (7.7) and overall acceptability (7.7)(Table 2). Results are in accordance with the report of Dalanache *et al.* (2015). The organoleptic quality of papaya fruit bar gradually decreased during storage. In papaya bar there was a gradual decline in score for colour and appearance might be due to change in colour attributed to maillard, enzymatic browning and polymerization of anthocyanins with other phenolics (Garcia *et al.*, 1999). Score for flavour got decreased significantly during storage might be due to various chemical changes and loss of volatiles (Anju *et al.*, 2014). There was a gradual decline in the score for taste in fortified papaya guava fruit bar during storage might be due to fluctuations in acids, pH and sugar acid ratio as reported by Safdar *et al.* (2014). From the microbial analysis, it is clearly observed that the lesser count of bacteria and fungi were observed in papaya fruit bar(Table 3). It might due to higher acidity and high sugar content in papaya fruit bar might have prevented the growth of micro-organism and it had good microbial storage stability during storage period. These results are similarly matching with the Pawase *et al.*(2018). Benefit cost ratio obtained through production of papaya fruit bar was found to be 1.78. Since this BCR provides the person who engages in value addition with considerable returns processing of papaya into papaya fruit bar can be used for commercial exploitation.

CONCLUSION

Finally, it is concluded that among the formulations, T_3 (50 °B of jaggery) was considered as the best treatment with a moisture content of 16.83 %, titratable acidity (16.83 %), vitamin C (43.87 mg/100g), total sugar (69.82 %). The maximum sensory scores for organoleptic evaluation were obtained by T_2 (50 °B of honey)for taste, colour, flavour and overall acceptability followed by T_4 (palm candy). The cost economics for the production of papaya fruit bar was 1.78. Hence processing of papaya into papaya fruit bar can be commercialized.

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Table 1: Effect of variation in forms of sugar on nutritional quality of papaya fruit bar

		TSS	Titratable	Vitamin C	T	
Treatment	Moisture (%)	(°B)	acidity (%)	(mg/100g)	Total sugar (%)	
T ₁ (Sugar)	20.54	75.82	0.95	35.78	58.31	
T ₂ (Honey)	17.39	74.38	0.85	41.67	68.52	
T₃ (Jaggery)	16.83	72.31	0.83	43.87	69.82	
T ₄ (Palm candy)	18.21	74.89	0.92	38.62	61.83	
S. Ed.	0.203	0.188	0.004	0.911	0.416	
C.D.(P=0.05)	0.41	0.38	0.01	1.84	0.84	





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Table 2: Mean sensory scoring for papaya fruit bar

	1st month				2 nd month			3 rd month				
Treatment	Taste	Colour	Flavour	Overall acceptabi	Taste	Colour	Flavour	Overall acceptabi lity	Taste	Colour	Flavour	Overall acceptabi lity
T ₁ (SUGAR)	6.6	6.7	6.6	6.9	6.4	6.3	6.5	6.7	6.1	6.0	6.1	6.5
T ₂ (HONEY)	8.2	8.5	8.1	7.8	7.9	8.1	7.6	7.5	7.6	7.8	7.4	7.2
T ₃ (JAGGERY)	7.2	7.4	7.1	7.2	6.8	7.0	6.8	6.9	6.5	6.7	6.6	6.7
T ₄ (PALM CANDY)	7.7	8.0	7.7	7.7	7.4	7.7	7.4	7.3	7.1	7.4	7.2	7.0
S. Ed.	0.208	0.213	0.218	0.203	0.159	0.179	0.079	0.069	0.169	0.184	0.069	0.054
C.D.(P=0.05)	0.42	0.43	0.44	0.41	0.32	0.36	0.16	0.14	0.34	0.37	0.14	0.11

Table 3: Microbial analysis for papaya fruit bar

				Replication	าร	Mean =	
S.No	Organism	Medium	Dilution	R1	R2	R3	R1 + R2 + R3 3
01	Bacteria	NA	10-6	4	6	5	5
02	Fungi	RBA	10-4	1	Nil	Nil	0.3
03	Actinomycetes	KKA	10-5	Nil	Nil	Nil	Nil



Fig. 1 Prepared Papaya Fruit Bar





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REVIEWARTICLE

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Reusable Medical Device Reprocessing: A Critical Overview in the **United States**

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ABSTRACT

Minimally invasive surgical and diagnostic methods have spurred the development of new and better medical equipment. Engineering and material science advancements have made this feasible. In order to provide healthcare that is both affordable and effective, devices must be reusable. Many patients may be diagnosed and treated using reusable medical devices. Stethoscopes, endoscopes, and surgical forceps are examples of reusable medical equipment. Reusable devices become dirty and microbially contaminated when used on patients. To prevent infection, reusable equipment is "reprocessed," or cleaned, disinfected, and sterilized. When the reprocessing instructions are followed fully and properly, a medical device may be safely used more than once on the same patient, or more than one patient. Reusable medical equipment reprocessing is critical to patient safety. Preventing the spread of infection through reprocessed reusable medical equipment is a top priority for the FDA. We are uniquely positioned to assist manufacturers and reprocessors alike in understanding and complying with FDA regulations, as well as addressing public health issues that may arise after a product has hit the market by reviewing premarket and postmarket data from a variety of sources. FDA, as a public health agency, works to raise public health awareness and foster collaboration among all stakeholders, including manufacturers, health care institutions and personnel, accrediting organizations, professional organizations, standards-setting organizations, and government agencies. There is a current trend toward reusing more electronics, including those that were originally intended to be thrown away. When both the user and the manufacturer are involved in the design process, the greatest results may be attained.

Keywords: Medical devices, USFDA, Reusable, Device Design, Premarket submissions





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INTRODUCTION

Medical devices that can be used repeatedly for patient diagnosis and treatment are known as reusable medical devices. Medical instruments that can be reused include stethoscopes, endoscopes, and surgical forceps[1]. Reusable equipment becomes dirty and infected with bacteria when they are used on patients. Reusable medical equipment is subjected to "reprocessing," a multi-step cleaning and disinfection/sterilization procedure that ensures there is no danger of infection from a contaminated item. It is possible to reprocess medical devices so that they can use on more than one patient, as long as the reprocessing labeling requirements are followed exactly and precisely after each usage. Reusable medical equipment must be properly reprocessed to ensure patient safety[2]. Biological material (dirt) can accumulate in reusable medical equipment if good cleaning procedures are not followed between patients. As a result, germs can survive the subsequent cleaning or sterilizing procedure, increasing the risk of HAIs (HAIs). Chemical sanitizers, which might remain in leftover reprocessing components, can cause tissue inflammation in patients if the reprocessing isn't done properly[3]. Considering the enormous number of medical equipment in use, the chance of contracting this illness from an improperly reprocessed one is rather low. However, the danger of infection outbreaks connected with their usage should not be underestimated. In addition, illnesses caused by improperly reprocessed medical equipment are seldom been reported to the Food and Drug Administration (FDA). We don't know how many HAIs are caused by subpar gadget reprocessing since it is rarely studied as a possible cause of HAI[2,3].

Reusable equipment is being investigated by the FDA as a potential source of infection. By analyzing premarket and post-market data from a variety of sources, we are uniquely positioned to help manufacturers and reprocessors alike understand and comply with FDA regulations as well as address public health concerns that may surface after a product has hit the market. As a public health organization, FDA works to promote cooperation among all stakeholders, including manufacturers, healthcare providers, institutions, and staff, as well as industry associations, standard-setting organizations, governmental organizations, and accrediting bodies[4]. Patients' safety is dependent on proper infection management. An estimated 90,000 people die every year as a result of HAIs in the US (Stone, 2009). As a result, the number of hospital-acquired infections (HAIs) has decreased. Sadly, not all devices can be thrown away. Making endoscopes, for example, disposable would be prohibitively expensive due to the complexity and high cost of the instrument. Equipment like endoscopes must be cleaned and sterilized after each usage to avoid cross-contamination. Because many HAIs have been traced back to reprocessing problems, the issue is one of contamination. To keep viruses like HIV, hepatitis C, and hepatitis B from being spread, medical equipment must be properly sterilized. This includes things like sponges[5].

MATERIALS AND METHODS

This review article's content was primarily sourced from FDA official websites. The information in the review article that follows was combined using data from several sources and provided by FDA guidance documents.

What are Reusable Medical Devices?

Equipment makers' instructions for use (IFUs) and healthcare facility rules and procedures all play a role in medical equipment reprocessing. To categorize medical equipment according to the degree of reprocessing severity they pose, the Spaulding classification was developed. A device's processing requirements can be determined using this tool[6].





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CLASSIFICATION:[7]

Device Type	Description	Examples
Non-Critical Devices	Devices that contact intact skin	Blood pressure cuffs,
	but not mucous membranes	stethoscope, external sensor,
Semi-Critical Devices	Devices that encounter mucous	Noninvasive flexible and rigid
	membranes or non-intact skin	fiber optic endoscopes,
		Aspirator tubes, Respiratory
		therapy equipment
Critical Devices	Devices that enter sterile tissue	Surgical instruments, needles,
	or the vascular system	Catheters, Implants, Invasive
		endoscopes

Non-Critical Medical Device Reprocessing

If a device touches the skin but not mucous membranes, it is a non-critical device. Stretchers, stethoscopes, and blood pressure cuffs are all non-critical medical devices. Cleaning and low- or intermediate-level disinfection are required for devices in this category if they are transferred between patients[8].

Semi-Critical Medical Device Reprocessing

Semi-critical machinery may come into contact with mucous membranes or skin that isn't intact during a process. For example, cystoscopes, anesthetic equipment, laryngoscopes, and a few endoscopes fall under the category of semi-critical medical devices. Semi-critical equipment should be devoid of microorganisms; however, a tiny number of bacterial spores may still be present in very small quantities in some cases. The cleaning and sanitation of semi-critical equipment are mandatory, and sterilization is recommended. When it comes to gadgets that cannot be sterilized, high-level disinfection is permissible.

Critical Medical Device Reprocessing

Critical equipment, such as pacemakers, can come into direct touch with the vascular, which is sterilized. These include surgical forceps, scalpels, implants, biopsy tools, and urine catheters. Cleansing and sterilization are must-do for critical devices. Steam sterilization, vaporized hydrogen peroxide sterilization, and liquid chemical sterilization are all common means of sterilization[7,8].

These components are designed to be recycled between patients, which is why they are thoroughly cleaned before being disinfected or sterilized to a high degree. Reprocessing, including brushing by hand and chemical treatment, is no problem for the materials used to make them.

Reusable medical devices include

- Surgical instruments, like forceps and clamps
- Endoscopes, such as duodenoscopes, bronchoscopes and colonoscopes, used to see inside the body
- Endoscopes, such as duodenoscopes, bronchoscopes and colonoscopes, used to see inside the body
- Accessories for laparoscopic surgery, like arthroscopic shavers [9].

What Can Be Reprocessed?

Hundreds of thousands of devices from a wide range of manufacturers can be compressed. There is several non-invasive medical equipment that may be recycled, such as patient monitoring and compression sleeves. Guide wires, electrophysiology catheters, imaging catheters, femoral compression devices, heart stabilizers and positioners, hot and cold biopsy forceps, as well as apparatus for balloon inflation, are additional surgical tools that may be reprocessed. It is now being investigated whether Class III equipment, such as implanted infusion pumps, may be reused. The device is cleaned with an enzymatic solution at the hospital immediately after usage and then stored in a designated container. The reprocess or—if necessary—up to several times a week, they are gathered. In the reprocessing industry, the loop is closed. To guarantee that each device is returned to its rightful owner, used gadgets are electronically recorded (usually through a Data Matrix code) when they are picked up[10].





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DISCUSSION

How are processable medical devices made??

The proper reprocessing of reusable medical equipment is an essential step in ensuring patient safety. Medical equipment must be reprocessed to remove blood and tissue, as well as infectious bacteria, so that they may be used again. Reprocessing is time-consuming and labor-intensive. The reprocessing protocol for each reusable medical equipment is unique and must be followed to the letter. Reprocessing is carried out by trained personnel in the following settings:

- Large medical facilities
- Limited in-outpatient services
- Relatively small medical office buildings
- Facilities with independent providers, such as ambulatory surgery centers
- Independent reprocessing service facilities

There are three main phases to reprocessing reusable medical equipment:

- 1.To prevent the drying of blood, tissue, and other biological material and pathogens on operating room instruments, they are first cleaned and disinfected.
- 2. The device is then brought into the reprocessing area, where it receives a thorough cleaning and sanitization.
- 3. After disinfection or sterilization, the device is kept or returned to use, depending on its intended purpose and the materials from which it was constructed[11].

Process Overview

How It's Done

Reprocessing plants sort the contents of collecting containers to separate the devices that can be reconditioned from the ones that can't be. Clear rejections, and extremely dirty or non-processable goods are weeded out using sorters. The OEM part numbers and descriptions are electronically tallied and documented for easier traceability of processable devices. It is the goal of the recycling process to disassemble and recycle as much equipments as feasible. Afterward, the gadgets are disinfected and cleaned using enzymatic solutions, steam, and automated and manual equipment employing these cleaning methods. Device-specific and typically proprietary cleaning techniques are required for each device. Pneumatic tools spray sodium carbonate, a mild abrasive, over hard-to-clean features like jaws or hinges. This solution is used to clean these parts. After cleaning, the gadgets are dried with compressed, filtered air. The number of reprocessing cycles is recorded on each device once it has been thoroughly cleaned. The FDA sets limits on how many times a gadget may be recycled before it must be discarded. After then, it must be thrown away. There are a lot of gadgets that need to be functionally repaired. Laparoscopic equipment, for example, may need to be painstakingly cleaned of burrs and dull edges. Whenever possible, this work is performed by automated equipment, but skilled technicians also perform a significant amount of work manually. Reprocessors must ensure that the gadgets function as designed when they have been restored. Electrical, mechanical, leak-testing, high-speed rotation examination, sharpness or curvature measurement, and other device-specific functional tests can all be part of this process. The settings under which the gadget will be utilized can even be replicated using anatomical models by some reprocessors" [12]. Equipment is washed with filtered, deionized water to eliminate any residuals that may have been generated during the restoration and testing procedure. The penultimate step is a microscopical examination of each gadget by the technicians. Mylar or Tyvek pouches are then used to package, seal, and label the devices. At least twice as fast as in a regular operating room, the air in the packing area is purified and circulated. Reprocessing facilities include airlocks to prevent cross. After packaging, the devices are sterilized with ethylene oxide by ISO 11135-1994. Or, to put it another way, the likelihood that a disease could have survived sterilization using this method is one in a million. The devices are guarantined for at least 72 hours after their final evaluation. The packaging used to ship back devices to the hospital makes it easy to tell them apart from brand-new ones. The entire procedure can take two to three weeks, depending on the processor[11,12].





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Design for Reprocessing

The rise of recycling has not always been welcomed by OEMs who have made their fortunes by generating an unending supply of so-called single-use products. If something goes wrong when using reprocessed equipment, many OEMs fear that they might be held responsible for any infections that may result from improper cleaning and sterilization. Moreover, they object to the thought that a rival firm is benefitting from their products without having invested in them. Therefore, some original equipment manufacturers (OEMs) don't work with reprocessors very well. A few OEMs have been unwilling to publish sterilization requirements for their products, as an example Reprocessing has become more challenging for other original equipment manufacturers (OEMs). Ultrasonic welders, for example, have replaced threaded fasteners and snap-fits in the assembly of plastic pieces, resulting in a junction that cannot be dismantled. Some OEMs are adopting the "if you can't beat them, join them" approach to this issue. Ethicon Endo-Surgery, a division of Johnson & Johnson that has opposed reprocessing in the past, just bought SterilMed, the second biggest reprocessor in the United States, for example, back in November of 2018. Ascent Healthcare Solutions, the biggest U.S. reprocessor, was purchased by Stryker in 2010. (Stryker Sustainability Solutions is the new name of the resulting company.). A more integrated supply chain is being promoted by both firms, which includes both remanufactured and OEM items[13]. To help with cleaning, disinfection, and sterilization, the FDA has recognized certain design elements for reusable medical equipment.

These features include

- smooth Surfaces, including interior surfaces for lengthy, narrow interior channels. Channels ought to have a width that allows a brush to pass through.
- The capability of disassembling complex devices.
- Connectors for essential parts that cannot be changed. For instance, it is not advisable to interchange tubes used with endoscopes for direct patient contact and waste drainage tubes.
- Clearly label all connecting components, such as drainage tubing.
- Components with clear labels that must be discarded after use and cannot be processed or used again.
- Disposable components for hard-to-clean areas.
- Designs that consider how fluid flows through the device and potential debris accumulation points.

The following characteristics make cleaning more difficult:

- Internal channels that are long and narrow.
- Hinges.
- Covers for the inserters, activators, and blades.
- Surfaces of adjacent devices where debris may be forced or caught while being used.
- O-rings.
- Valves used to control a device's fluid flow.
- components that can't be taken apart.

The FDA is also studying the movement of liquids and debris within devices using computer modeling. To assist OEMs in better understanding exactly how much design factors render a device susceptible to debris retention, such modeling might be used. The FDA is also looking at the link between the design of a device and the buildup and retention of debris across several washing cycles. As part of their research to see which cleaning procedures are most successful, FDA scientists are also working on a method for measuring the amount of particle biological material that remains in reusable medical equipment. Using traditional testing methods, the number of debris, such as tissue, cartilage, and bone, may be overestimated. These underestimations may lead to the design of reusable devices that are susceptible to debris retention[14].





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Reprocessing of Reusable Medical Devices Labelling[15].

Reprocessing instructions for general device labeling considerations

A Summary of Reprocessing

According to the manufacturer, precleaning, also known as point-of-use cleaning, takes place while the device is still in the operating room, immediately after a patient operation. Its objective is to clean the endoscope of major impurities before it dries. It does this by washing the outside and, if necessary, flushing water or a cleaning solution through the interior passages.

Leak Testing

Before the endoscope is manually cleaned, the damage is found using this technique. The endoscope's mechanical integrity is crucial for the cleaning procedure to be successful.

Manual Cleaning

Following a thorough cleaning with detergent solution, any leftover gross impurities are manually removed by wiping, flushing, and washing the endoscope's interior and external channels, as well as the device's interior and exterior channels.

Disinfection or Sterilization

The manufacturer's guidelines guide the decision on whether or not to execute high-level disinfection or sterilization. Sterilizing an endoscope relies on its materials and capacity to withstand various sterilization procedures. The endoscope can be manually disinfected to a high level by immersing it in a germicidal solution and flushing it. This can be done manually, however, an automated endoscope reprocess or (AER) can also be employed.

Drying

The endoscope is washed to eliminate any remaining cleaning chemicals after it has been disinfected to a high level. Then, the endoscopes flushed with air and, if necessary, flushed with a disinfectant to speed up the process of drying up.

Storage

To guarantee that pollutants are not reintroduced to clean the endoscope, proper storage is necessary. When storing endoscopes, they should be hung vertically in places that are free of dirt, moisture, and humidity[16].

Human Factors in Developing Reprocessing Instructions

When creating your reprocessing instructions, keep the following considerations in mind:

Your reprocessing instructions should be uniform across all of your product lines. Users' understanding and compliance with instructions may be improved by using labeling that uses the same document and layout across all devices of a kind. There may be post-market human factors difficulties for your item or comparable technologies that you should address. For example, acts requiring a high degree of dexterity or strength, excellent eye acuity, or expertise with unusual procedures are all examples of human factors concerns. Information on post-market issues can be found in the Medical Device Reporting (MDR) system, published materials, FDA Safety Alerts and Public Health Notifications, as well as internal user complaint files. You should validate your reprocessing instructions for devices that are subject to design controls under 21 CFR 820.30 to make sure that users will be able to comprehend and adhere to them. FDA advises taking into account the following.

- a) Participants in your validation studies should be drawn from the pool of qualified personnel who will carry out the reprocessing operations in the field. Participants in the validation research should wear the same PPE that users would, such as full-length face shields, goggles, heavy-duty utility gloves, or liquid-resistant covering.
- b) It's up to the students whether they utilize the instructions to complete a real or simulated reprocessing operation or vocally describe their actions as they go through the steps of the operation.
- c) The investigation should include any environmental factors that may influence the usage of the instructions or reprocessing of the device.





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d) By watching and documenting participant behavior during testing, you can examine compliance with the rules and identify and analyze any errors or problems..[17]s

Instructions issued by the Food and Drug Administration for reprocessing

Users will be able to follow your reprocessing instructions more easily if you address the following six requirements for clear reprocessing instructions.

Criteria 1: The device's intended use should be reflected in the labeling.

Your labeling must include reprocessing instructions that are appropriate for the device's physical construction, intended use, and potential soiled and contaminated conditions during clinical use. The instructions for reprocessing the device are dependent on whether it:

- Only intact skin should be touched.,
- Mucosal surface contact with an intact;
- Come into contact with normally sterile bodily fluids like aqueous humor, cerebrospinal fluid, peritoneal fluid, or blood.
- The closeness to the patient makes it susceptible to spatter or splashing of bodily fluids or blood, even if it is not in touch with the patient directly.
- Become contaminated during use by patients' or caregivers' dirty hands; (notice that both unwashed and gloved hands may transmit organic soil and germs to the surfaces they touch);
- patient bleeding, incontinence, vomit, and wounds spilling through bandages may all lead to contamination.

Criterion 2. As part of the reprocessing process, users of reusable devices should be instructed on how to properly clean the device.

Pre-cleaning is an important aspect of reprocessing and should be included in the overall guidelines. Sterilization and disinfection effectiveness are directly related to the level of cleanliness achieved during cleaning. The user's manual should include step-by-by-step instructions on how to do a complete cleaning. Depending on the device's complexity, the cleaning procedure's specifics will change. Unless the manufacturer can certify successful cleaning without it, devices with characteristics that could lead to dirt retention or features that make them difficult to clean may need to be disassembled (i.e., data should be obtained from testing soiled devices cleaned with and without disassembly for comparison). The cleaning instructions for such devices should include instructions and/or diagrams for proper disassembly. (For more information, see Criteria 5.C.)

Criterion 3. Detailed instructions for reprocessing devices should include information on the suitable microbicidal technique for the device.

Instructions given should adhere to current infection control guidelines. If the equipment is to be sterilized or disinfected (at a high, moderate, or low level) the microbicidal method indicated is sterilization or disinfection[18].

Criterion 4. There should be no gadgets or accessories that are illegally sold in the reprocessing instructions.

The site where the reprocessing instructions are to be implemented must be able to support them (e.g., health care setting or home use). Detailed descriptions and component numbers, if relevant, should be provided enabling users to easily get the equipment and accessories required to carry out the instructions. In addition to the sterilizer type, users should have access to the manufacturer-validated sterilization cycle settings and accessories. Radiation sterilization, for example, is often reserved for industrial settings. When it comes to sterilization in healthcare facilities, steam sterilization is the most often used procedure. In some healthcare facilities, there are also liquid chemical sterilizing methods like EO, H2O2, and O3. Less frequently used techniques for infection control include chemical vapor sterilisation and dry heat sterilization.

Criterion 5. Instructions for reprocessing should be detailed.

The user will be able to securely and successfully carry out the complete reprocessing operation with detailed instructions. Instructions may be presented in a variety of ways. All of the items listed below should be included in





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the reprocessing instructions to make sure they are complete. You should explain in your premarket application if any of these requirements do not apply to your device.

Criterion 6. Instructions for reprocessing should be clear and understandable.

From the initial processing stage through the final processing stage, concise, visible (i.e., appropriate font size) reprocessing instructions should be provided. Reprocessing recommendations (e.g., point-of-use processing, disassembly, cleaning, rinsing, reassembly, disinfection or sterilization, final rinsing after disinfection or liquid chemical sterilization, and post-process handling). Instructions should be provided in the most straightforward manner possible. They should also be appropriately comprehensive to guarantee that every action is properly explained. To ensure proper reprocessing, workstations must have charts, diagrams, and/or instructions on how to reprocess devices with illustrations. When responding to customer inquiries directly or through customer service personnel, posted images, and diagrams of equipment on the internet may be useful[19].

FDA premarket submissions

Before submitting their FDA applications for reusable medical equipment, manufacturers should complete all validation of reprocessing procedures and provide instructions with the rest of the application. This information is required by several FDA market channels for reprocessing instructions and labeling:

- Applications under 510(k) As part of the 510(k)-The device description, intended use, and use instructions, including reprocessing instructions, must all be included on the proposed device labels during the application process. The labeling for the device that the registrant has submitted will be compared to one for a predicate device that is already available in the US.
- **Pre-market Acceptance (PMA)** For registrants to confirm reprocessing instructions, the manufacturing and design part of the PMA should provide techniques and test results.
- Exemption for investigational devices (IDE) An IDE application should contain details of all prior preclinical, clinical, and laboratory device testing, including summaries of validation testing of reprocessing guidelines. According to the FDA, applicants should validate these guidelines before submitting an IDE application[20].

Factors Affecting Reprocessing Quality

Based on reports, we've received and research we've seen, the FDA has recognized the following examples as potential safety hazards:

- > Device design
- > reprocessing procedures
- > techniques for verifying cleaning, high-level disinfection, and sterilization instructions are all important[21].

Reprocessing Methodology

- The process of reprocessing is complex, time-consuming, labor-intensive, and prone to mistakes. The reprocessing of each reusable medical equipment necessitates a unique set of actions or techniques. When reprocessing reusable medical equipment, several elements might affect their performance.
- Reprocessing difficulties at specific facilities, such as:
- o Personnel in charge of various stages of the procedure
- o Staff can take advantage of available training.
- o Accessible equipment, such as brushes of the proper size.
- The manufacturer's reprocessing instructions' quality and comprehensiveness.
- Having access to the product's user guide.

To keep up with the changing needs of the medical device industry, these variables must be regularly updated and re-trained to ensure employee competency[21,22].

Cleaning and High-Level Disinfection or Sterilization Validation Techniques

Washing, disinfection, or sterilization procedures prescribed by a manufacturer must provide consistently sufficiently reconditioned equipment, as documented by the manufacturer. As a rule, cleaning validation





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investigations begin with soiling the devices (either from actual clinical usage or via the use of artificial test dirt), continue with cleaning the devices and conclude with some technique of measuring the amount of test soil residue that remains on the devices. It seems that many tests conducted by manufacturers to certify the cleanliness of their products were conducted under settings that did not meet FDA standards. The use of clinically relevant test soil, such as blood, tissue, or bone, may have resulted in cleaning instructions that do not sufficiently remove soils such as these from the surface of the product. Manufacturers frequently use a bacterial-spore suspension solution to verify cleaning procedures, but it's unclear whether getting rid of bacteria is the same as getting rid of patient materials like blood and tissue. As a result, the FDA does not recommend using spore log reduction testing as a method to determine how effective cleaning is. The FDA also examined situations in which the capability of cleaning internal device components was not considered as part of the design validation and evaluation of cleaning instructions. For proper validation of cleaning procedures, the worst-case clinical dirt, the worst-case soiling conditions, and an appropriate component of residual soil should be used. A device's disassembly for reprocessing is also a consideration. If these procedures are not followed, cleaning instructions may not indicate how to remove remaining patient dirt[23].

Collaboration to Improve Reusable Medical Devices Reprocessing

Reprocessing medical equipment properly is a crucial step in providing high-quality treatment for patients. Some reusable medical devices may retain blood, tissue, and other biological material (soil) if they are not properly reprocessed in between patient usage. Health Care-Associated Infections (HAIs) may occur if microorganisms escape the disinfection or sterilizing procedure because of the debris (HAIs). Tissue irritation by remaining reprocessing materials, such as chemical disinfectants, may also be a consequence of insufficient reprocessing. Reducing the danger of exposure to medical devices that have been incorrectly reprocessed is a shared responsibility among numerous stakeholders. Companies are also expected to provide user-friendly and reliable instructions for reprocessing equipment. Reusable medical gadgets shouldn't collect trash in the first place. We can get to the bottom of this issue by involving all parties involved in the reprocessing procedure[24].

Three areas of focus are

- 1. The Design of the Device
- 2. Development of Regulatory Science
- 3. Increasing Cooperation

The Design of the Device

Based on its extensive experience reviewing reprocessed products and research conducted by the agency and others, the FDA has highlighted designs that foster innovation in next-generation reusable medical devices. These structural elements facilitate easier cleaning, disinfection, and sterilization while reducing the accumulation of dirt.

These elements of the design must include:

- Smooth surfaces, such as the inner smooth surfaces of the long, narrow interior channels (lumens);
- Clearly labeling connecting components, such as drainage tubing
- Components that must be discarded after patient use and cannot be processed again or used should be indicated and identified.
- Disposable elements for the dirtiest areas
- Device designs that take into account the way fluid flows through them and the places where debris accumulates. Additionally, the FDA is working with organizations that set standards, such as the Association for the Advancement of Medical Instrumentation and ASTM International, to reach a consensus on a set of best practices. Manufacturers can find recommendations in standard and TIR documents for creating processable equipment, as well as test methods and standards for approving reprocessing procedures for disposable medical devices[25].





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Development of Regulatory Science

The FDA is also working to develop reprocessing science. To help manufacturers improve their premarket submissions for cutting-edge reusable medical devices, the FDA has released the final guidance on "Reprocessing Medical Devices in Health Care Settings: Validation Methods and Labeling."There have been significant breakthroughs in medical device reprocessing techniques over the years, and this publication is an attempt to keep pace with these developments. The suggestions in this document are aimed at enhancing the security and efficacy of medical devices that have been reprocessed. Among the medical devices included in Appendix E of the advice paper is a subgroup that has a larger risk of microbial transmission and illness if not properly reprocessed. These devices' 510(k) submissions should contain data to verify the reprocessing procedures and instructions. Protocols and test reports that verify the reliability and consistency of reprocessing instructions and their ability to be followed exactly as intended are examples of data used to validate these procedures and instructions. This guidance outlines guidelines for adequate labeling and scientific validation (demonstrating the appropriateness) of cleaning, disinfection, and sterilization processes to guarantee that reusable medical equipment can be cleaned, disinfected, and sterilized properly and that health care facilities have clear instructions on how to do so. Given the resources at the disposal of medical institutions, sterilization and cleaning procedures must be effective and practical. Our understanding of reprocessing is being furthered by FDA researchers. To advance regulatory science in this area and use it to further improve the design of reprocessed devices, the FDA is researching the use of computer modeling to examine the movement of fluid and debris inside devices. Manufacturers may use this type of modeling to alter their designs before production to determine how much a device is susceptible to debris retention as a result of specific design elements. According to FDA experts, the design of the device influences debris buildup and retention throughout several cycles of cleaning and soiling. To establish whether different cleaning procedures are successful, they are developing a way to measure the amount of particle biological material retained in reusable medical equipment. The presence of debris such as tissue, cartilage, and bone may be overestimated using conventional testing techniques. These underestimates might lead to the design and production of reusable devices that are prone to debris retention[26].

Increasing cooperation

By involving all relevant parties, including manufacturers, healthcare institutions and their personnel, accreditation bodies, and governmental authorities, we may make progress in tackling the issue of reprocessing medical devices. There is a lot of information that the FDA gets from companies and healthcare providers on many sorts of medical devices, including product submissions. It is in the FDA's best interest to communicate with all the parties involved in a successful reprocessing program so that they can work together. On June 8-9, 2011, the FDA organized a workshop to bring together manufacturers, medical facilities, standards organizations, accreditation organisations for the healthcare industry, governmental organisations, and professional societies to share experiences, work toward resolving current problems, and create innovative designs for new devices. A comprehensive Quality Assurance procedure for reprocessing medical devices must be implemented by healthcare institutions, even if FDA quideline materials and explicit instructions are available[27]. As a crucial step in quality control, healthcare organizations should ensure that their staff adheres to the manufacturer's instructions and reprocessing specifications. Some statistics indicate that a large number of facilities do not consistently adhere to reprocessing criteria. Single External Link Disclaimer In 2009, the FDA released a joint safety communication with the CDC and the Veterans Administration that alerted medical facilities to the dangers endoscopes and their accessories posed to patients and offered suggestions for mitigating those dangers. The CDC and Veterans Administration worked together to release this communication. The Information for Health Care Institutions page was developed by the FDA to provide access to resources that offer reprocessing guidance and best practices to help healthcare facilities improve their reprocessing Quality Assurance procedures. As far back as the autumn of 2013, a possible link between multi-drug-resistant bacteria and duodenoscopes was initially raised by the CDC. It was discovered via additional inquiry and consultation with federal partners that these infections were happening even though users had followed manufacturer cleaning, disinfection, and sterilization guidelines. To further understand how to effectively prevent these illnesses, since then, the FDA has collaborated with federal and other stakeholders. Visit the website Infections Associated with Reprocessed Duodenoscopes to learn more about these infections. The FDA is





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interested in finding out more about the difficulties hospitals have reprocessing medical devices because it is a complicated process. FDA is collaborating with professional organisations and healthcare organisations, including Med Sun partner facilities, to better assess the effectiveness of reprocessing procedures used in healthcare settings[28].

Duodenoscope Infections Linked to Reprocessed Devices

Flexible, illuminated tubes called duodenoscopes are inserted into the small intestine from the mouth, throat, and stomach (duodenum). These tools are used in an operation called endoscopic retrograde cholangiopancreatography (ERCP), which has the potential to save lives. Every year, more than 500,000 endoscopic retrograde cholangiopancreatographies (ERCP) procedures are performed in the US using duodenoscopes. A duodenoscope is a challenging piece of medical gear with numerous tiny moving parts. If a duodenoscope is not thoroughly cleaned and disinfected before being used on another patient, tissue or fluid from the previous patient may remain inside. Rarely, this could cause an infection to spread from one patient to another. In the fall of 2013, the CDC informed the FDA that there may be a link between duodenoscopes and bacteria that are multi-drug resistant. Although it had been established that the users had followed the manufacturer's cleaning, disinfecting, or sterilizing instructions, it soon became apparent that these infections were still happening[29].

Ongoing Activities of FDA

Duodenoscopes are necessary to identify and treat life-threatening conditions. The FDA takes the risk of infection very seriously and is making great efforts to address it, even though the vast majority of procedures involving these devices are safe and successful. The FDA, other federal agencies, public health systems, state and local health departments, medical device manufacturers, healthcare facilities, professional societies, and others are all jointly responsible for the safety of reprocessed medical devices used in multiple patients. To better understand how infectious agents, spread and how to lower patient exposure to such agents, the FDA must collaborate closely with a variety of stakeholders. The FDA required Olympus, Fujifilm, and Pentax to carry out post-market surveillance studies ("522 study") to learn more about how these devices are reprocessed in actual environments and their effects on duodenoscope-transmitted infections. The studies were to be completed by the end of the year. Studying a device after it is on the market may provide valuable information, such as the incidence of adverse events that were not predicted or the actual pace at which they occurred, that can help safeguard the public's health[30].

Reprocessing of Used Medical Equipment: Patient Information

You may have heard of cases when reusable medical gadgets were improperly reprocessed and used on patients. The FDA advises against delaying or canceling any planned procedures if you are worried about the possibility of infection due to insufficient reprocessing without first speaking with your doctor.

Common medical procedures that utilize reusable medical equipment include[31]

Several common medical procedures use reusable medical equipment, such as:

- Colonoscopy
- Bronchoscopy
- > Esophagogastroduodenoscopy (EGD)
- > Endoscopic retrograde cholangiopancreatography (ERCP)

Reporting Problems with Reusable Medical Devices

The FDA can better detect and understand the hazards associated with medical devices if adverse occurrences are promptly reported. Please contact the FDA if you believe that reusable medical equipment or its reprocessing methods may be defective.

Reporting Serious Problems to FDA

You can report a suspected major adverse event, a product quality issue, a usage or medication error, or therapeutic in equivalence/failure for any FDA-regulated drug, biologic, medical device, or dietary supplement on the





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MedWatch website. Another way to inform the FDA of potential fake medical products is through MedWatch. Through MedWatch, there is yet another way to alert the FDA to potentially fraudulent medical products. Both consumers and healthcare professionals who wish to submit voluntary reports as well as those organizations required by FDA requirements to submit reports may find the information on this website helpful.

Voluntary Reporting by Consumers, Patients, and Health Professionals

The FDA depends on self-reporting of adverse occurrences to maintain safe and effective medical items on the market. The FDA relies on these statistics to keep an eye on the items in question. Your report might be the catalyst for a change in the product's usage or design that enhances the product's safety profile and increases patient safety.

What Should Be in a Report

Please include the following information, if available, in your reports of adverse events or difficulties relating to reusable medical devices or reusable medical device reprocessing to help us learn as much as we can about these incidents or problems:

Information about the event, such as but not limited to:

- ✓ model and manufacturer of the gadget
- ✓ in addition to the catalog and serial numbers, if applicable
- ✓ A detailed account of the incident, including the patient's result
- ✓ The medical professional's account of the incident and their assessment of the role played by the device
- ✓ Errors in the design or labeling of the device
- ✓ Exposure of the patient to accumulated waste
- ✓ Disclosure to the patient including infection control input

It is important to know whether the gadget has been examined and, if so, what the conclusions were [32].

CONCLUSION

This study showed that medical devices may be safely reprocessed and reused several times in the same patient or across multiple patients with proper and comprehensive follow-up procedures after each time they are utilized. To ensure patient safety, reprocessing reusable medical equipment properly is essential. Certain reusable medical equipment may retain blood, tissue, and other biological waste if they are not adequately cleaned in between patient usage. Due to a lack of investigation, the number of HAIs that may be attributable to poor device reprocessing is not known. Preventing the spread of infection through reprocessed reusable medical equipment is a top priority for the FDA. For the safety of the next patient, devices undergo reprocessing to remove blood, tissue, and other biological waste, as well as to inactivate infectious bacteria. During the first decontamination and cleaning of medical equipment, such as in the operating room, measures are made to avoid the drying out of blood, tissue, and other biological waste and pollutants on the instruments. In the end, depending on its intended function and the materials it is constructed of, the equipment is disinfected or sterilized before being stored or sent back into service. What's Involved in the Process Devices that can be reconditioned are separated from those that can't through a reprocessing plant's sorting process. Dismantling and recycling as much equipment as possible is a primary objective of the recycling process. Using the same document style across all devices of the same kind may help users better comprehend and adhere to instructions.

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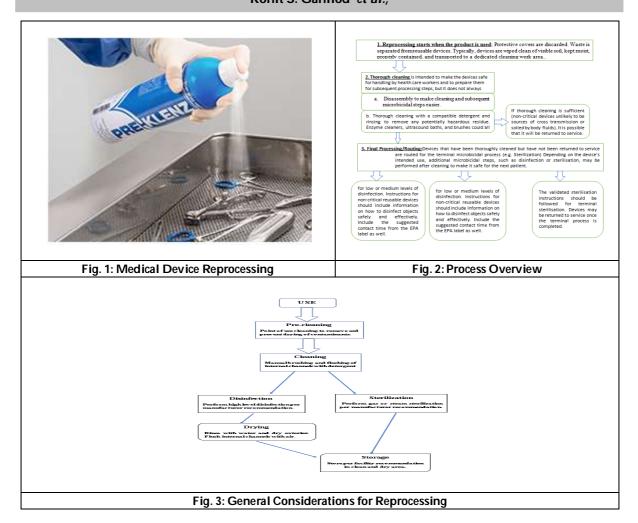




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RESEARCH ARTICLE

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A New Generalization of Two Parameter Odoma Distribution with **Applications of Engineering Sciences**

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ABSTRACT

In this paper, we have introduced a new extension of two parameter Odoma distribution known as weighted three parameter Odoma distribution by using the weighted technique. The different statistical properties of the proposed distribution as the moments, order statistics, reliability measures, bonferroni and Lorenz curves have been derived and discussed. The technique of maximum likelihood estimator have been employed for estimating the parameters of the proposed distribution and also its Fisher's Information matrix have been discussed. Finally, the two real data sets are executed to illustrate the supremacy and usefulness of newly proposed distribution.

Keywords: Weighted distribution, two parameter Odoma distribution, Reliability measures, Order statistics, Maximum likelihood estimator.

INTRODUCTION

The two parameter Odoma distribution is a newly executed lifetime distribution proposed by Enogwe et al. (2020). Some statistical properties of the proposed distribution such as raw and central moments, skewness, kurtosis, coefficient of variation, index of dispersion, moment generating function, characteristic function, order statistics, stochastic ordering, mean deviation, Bonferroni and Lorenz curves, Renyi entropy, reliability measures, quantile function and mean residual life function have been obtained and presented. The parameters of the two parameter Odoma distribution are estimated by using the technique of maximum likelihood estimator. The two parameter Odoma distribution is an extension of one parameter Odoma distribution. The one parameter Odoma distribution





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was introduced by C.C.Odom and M.A. Ijomah (2019) and discuss its various statistical properties. The technique of maximum likelihood estimator is also used to estimate the parameters of Odoma distribution and also its goodness of fit have been discussed. The two parameter Odoma distribution enjoys a close form distributional expression which is more flexible than that of the one parameter Odoma distribution. The application of the proposed two parameter Odoma distribution was illustrated with two real data sets and its goodness of fit was compared with that of the one parameter Odoma, two parameter Lindley, two parameter Akash, two parameter Sujatha, two parameter Rama and two parameter Pranav distributions by using some model selection criteria and it is found from the results that the two parameter Odoma distribution is the best selection for modelling the data sets. The probability density function of two parameter Odoma distribution (TPOD) with parameters α and β is given by

$$f(x;\alpha,\beta) = \frac{\alpha^5}{2(\alpha^5\beta + \alpha^3 + 24)} \left(2x^4 + \alpha x^2 + 2\alpha\beta\right) e^{-\alpha x}; \ x > 0, \alpha > 0, \beta > 0$$
 (1)

and the cumulative distribution function of two parameter Odoma distribution is given by

$$F(x;\alpha,\beta) = 1 - \left(1 + \frac{2\alpha^2 x^2 \left(\alpha^2 x^2 + 4\alpha x + 12\right) + \alpha x^2 \left(\alpha^4 x + 2\alpha^3 + 48\right)}{2\left(\alpha^5 \beta + \alpha^3 + 24\right)}\right) e^{-\alpha x}; x > 0, \alpha > 0, \beta > 0$$
 (2)

Weighted Three Parameter Odoma Distribution

The weighted distributions plays a key role in studying the lifetime data and is considered to be important in various research areas related to reliability, biomedical sciences, engineering and environmental sciences. The weighted distributions are useful in distribution theory because it provides an approach to deal with model specification and data interpretation problems. Weighted distributions also takes into account the method of ascertainment, by adjusting the probabilities of the actual occurrence of events to arrive at a specification of the probabilities of those events as observed and recorded. The study of weighted distributions are also useful in distribution theory, because it provides an understanding of the existing standard probability distributions and it provides methods for extending existing standard probability distributions for modeling lifetime data due to introduction of additional parameter in the model which creates flexibility in their nature. The weighted distributions occur frequently in the studies related to reliability, analysis of family data, meta analysis and analysis of the intervention data, biomedicine, ecology and other areas for the improvement of proper statistical models. The concept of weighted distributions was firstly introduced by Fisher (1934) to study how the method of ascertainment can influence the form of distribution of recorded observations and then Rao (1965) introduced and formulated in general terms in connection with modeling statistical data when the usual practice of using standard distributions was found to be unsuitable. Much work has been carried out in this direction. Dar, Ahmad and Reshi (2020) discussed the weighted gamma-pareto distribution and its application. Hassan et al. (2020) discussed on weighted quasi Xgamma distribution and its application to survival times. Anthony and Elangovan (2020) obtained the weighted version of quasi Aradhana distribution and its application to survival times.

Rather and Subramanian (2019) discussed on weighted Sushila distribution with properties and its applications. Rather and Ozel (2020) also discussed the weighted power Lindley distribution with application on the lifetime data. Para and Jan (2018) introduced three parameter weighted Pareto Type II distribution as a new life time distribution with applications in medical sciences. Hassan et al. (2018a, 2018b, 2019) introduced three new weighted probability models with application to handle life time data in engineering and medical sciences. Ganaie, Rajagopalan and Rather (2020) discussed on weighted two parameter quasi Shanker distribution with properties and its applications. Recently, Ganaie and Rajagopalan (2021) discussed on the length biased two parameter Pranav distribution, which shows more flexible and reliable than the classical distribution.





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To introduce the weighted distribution, Let us consider the non-negative random variable X with its probability density function f(x). Let w(x) be the non-negative weight function, then the probability density function of the weighted random variable X_w is given by

$$f_W(x) = \frac{w(x)f(x)}{E(w(x))}, x > 0.$$

assuming that $E(w(x)) = \int w(x) f(x) dx < \infty$.i.e. the first moment of w(x) exists

In this paper, we have to obtain the weighted version of two parameter Odoma distribution known as weighted three parameter Odoma distribution. Note that the different choices of weight function w(x) gives different weighted distributions. Consequently for $w(x) = x^c$, the resulting distribution is called weighted distribution with probability density function given by

$$f_W(x) = \frac{x^C f(x)}{E(x^C)} \tag{3}$$

Where $E(x^C) = \int_{0}^{\infty} x^C f(x) dx$

$$E\left(x^{c}\right) = \frac{2\Gamma(c+5) + \alpha^{3}\Gamma(c+3) + 2\beta\alpha^{6}\Gamma(c+1)}{\alpha^{c}\left(2\left(\alpha^{5}\beta + \alpha^{3} + 24\right)\right)} \tag{4}$$

Substitute equations (1) and (4) in equation (3), we will obtain the probability density function of weighted three parameter Odoma distribution

$$f_{W}(x) = \frac{\alpha^{c+5}}{2\Gamma(c+5) + \alpha^{3}\Gamma(c+3) + 2\beta\alpha^{6}\Gamma(c+1)} x^{c} \left(2x^{4} + \alpha x^{2} + 2\alpha\beta\right) e^{-\alpha x}$$
 (5)

and the cumulative distribution function of weighted three parameter Odoma distribution is obtained as

$$F_W(x) = \int_0^x f_W(x) dx$$

$$F_{W}(x) = \int_{0}^{x} \frac{\alpha^{c+5}}{2\Gamma(c+5) + \alpha^{3} \Gamma(c+3) + 2\beta\alpha^{6} \Gamma(c+1)} x^{c} \left(2x^{4} + \alpha x^{2} + 2\alpha\beta\right) e^{-\alpha x} dx$$

$$F_W(x) = \frac{\alpha^{c+5}}{2\Gamma(c+5) + \alpha^3 \Gamma(c+3) + 2\beta \alpha^6 \Gamma(c+1)} \int_0^x x^c \left(2x^4 + \alpha x^2 + 2\alpha\beta\right) e^{-\alpha x} dx$$





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Put
$$\alpha x = t \Rightarrow \alpha dx = dt \Rightarrow dx = \frac{dt}{\alpha}$$

When
$$x \to x, t \to \alpha x$$
 and When $x \to 0, t \to 0$, Also $x = \frac{t}{\alpha}$

After simplification, we will obtain the cumulative distribution function of weighted three parameter Odoma distribution as

$$F_{W}(x) = \frac{1}{2\Gamma(c+5) + \alpha^{3}\Gamma(c+3) + 2\beta\alpha^{6}\Gamma(c+1)} \left(2\gamma(c+5,\alpha x) + \alpha^{3}\gamma(c+3,\alpha x) + 2\beta\alpha^{5}\gamma(c+1,\alpha x) \right)$$
(6)

Reliability Measures

In this section, we will discuss the reliability function, hazard rate and Reverse hazard rate functions of the weighted three parameter Odoma distribution.

3.1 Reliability function

The reliability function or the survival function of weighted three parameter Odoma distribution can be obtained as $R(x) = 1 - F_W(x)$

$$R(x) = 1 - \frac{1}{2\Gamma(c+5) + \alpha^{3}\Gamma(c+3) + 2\beta\alpha^{6}\Gamma(c+1)} \left(2\gamma(c+5,\alpha x) + \alpha^{3}\gamma(c+3,\alpha x) + 2\beta\alpha^{5}\gamma(c+1,\alpha x) \right)$$

3.2 Hazard function

The hazard function is also known as hazard rate, failure rate or force of mortality and is obtained as

$$h(x) = \frac{f_W(x)}{1 - F_W(x)}$$

$$h(x) = \frac{\alpha^{c+5} x^{c} \left(2x^{4} + \alpha x^{2} + 2\alpha\beta\right) e^{-\alpha x}}{\left(2\Gamma(c+5) + \alpha^{3}\Gamma(c+3) + 2\beta\alpha^{6}\Gamma(c+1)\right) - \left(2\gamma(c+5,\alpha x) + \alpha^{3}\gamma(c+3,\alpha x) + 2\beta\alpha^{5}\gamma(c+1,\alpha x)\right)}$$

3.3 Reverse hazard function

The reverse hazard function of weighted three parameter Odoma distribution can be obtained as

$$h_r(x) = \frac{f_W(x)}{F_W(x)}$$

$$h_r(x) = \frac{\alpha^{c+5} x^c \left(2x^4 + \alpha x^2 + 2\alpha\beta\right) e^{-\alpha x}}{\left(2\gamma(c+5,\alpha x) + \alpha^3 \gamma(c+3,\alpha x) + 2\beta\alpha^5 \gamma(c+1,\alpha x)\right)}$$

4. Statistical properties

In this section, we will discuss different statistical properties of weighted three parameter Odoma distribution especially its moments, harmonic mean, moment generating function and Characteristic function.





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4.1 Moments

Let X denotes the random variable following weighted three parameter Odoma distribution with parameters α , β and c, then the r^{th} order moment $E(X^r)$ of X is obtained as

$$E(X^r) = \mu_r' = \int_0^\infty x^r f_W(x) dx$$

$$= \int_{0}^{\infty} x^{r} \frac{\alpha^{c+5}}{2\Gamma(c+5) + \alpha^{3}\Gamma(c+3) + 2\beta\alpha^{6}\Gamma(c+1)} x^{c} \left(2x^{4} + \alpha x^{2} + 2\alpha\beta\right) e^{-\alpha x} dx$$

$$= \frac{\alpha^{c+5}}{2\Gamma(c+5) + \alpha^3 \Gamma(c+3) + 2\beta \alpha^6 \Gamma(c+1)} \int_0^\infty x^{c+r} \left(2x^4 + \alpha x^2 + 2\alpha\beta\right) e^{-\alpha x} dx$$

$$= \frac{\alpha^{c+5}}{2\Gamma(c+5) + \alpha^{3}\Gamma(c+3) + 2\beta\alpha^{6}\Gamma(c+1)} \left(2\int_{0}^{\infty} \int_{0}^{(c+r+5)-1} e^{-\alpha x} dx + \alpha \int_{0}^{\infty} \int_{0}^{(c+r+3)-1} e^{-\alpha x} dx + 2\alpha\beta \int_{0}^{\infty} \int_{0}^{(c+r+1)-1} e^{-\alpha x} dx \right)$$

After simplification, we obtain

$$E(X^{r}) = \frac{2\Gamma(c+r+5) + \alpha^{3}\Gamma(c+r+3) + 2\beta\alpha^{6}\Gamma(c+r+1)}{\alpha^{r} \left(2\Gamma(c+5) + \alpha^{3}\Gamma(c+3) + 2\beta\alpha^{6}\Gamma(c+1)\right)}$$
(7)

By putting r = 1, 2, 3 and 4 in equation (7), we will obtain the first four moments of weighted three parameter Odoma distribution.

$$E(X) = \frac{2\Gamma(c+6) + \alpha^3\Gamma(c+4) + 2\beta\alpha^6\Gamma(c+2)}{\alpha\left(2\Gamma(c+5) + \alpha^3\Gamma(c+3) + 2\beta\alpha^6\Gamma(c+1)\right)}$$

$$E(X^2) = \frac{2\Gamma(c+7) + \alpha^3 \Gamma(c+5) + 2\beta \alpha^6 \Gamma(c+3)}{\alpha^2 \left(2\Gamma(c+5) + \alpha^3 \Gamma(c+3) + 2\beta \alpha^6 \Gamma(c+1)\right)}$$

$$E(X^{3}) = \frac{2\Gamma(c+8) + \alpha^{3}\Gamma(c+6) + 2\beta\alpha^{6}\Gamma(c+4)}{\alpha^{3}\left(2\Gamma(c+5) + \alpha^{3}\Gamma(c+3) + 2\beta\alpha^{6}\Gamma(c+1)\right)}$$

$$E(X^{4}) = \frac{2\Gamma(c+9) + \alpha^{3}\Gamma(c+7) + 2\beta\alpha^{6}\Gamma(c+5)}{\alpha^{4}\left(2\Gamma(c+5) + \alpha^{3}\Gamma(c+3) + 2\beta\alpha^{6}\Gamma(c+1)\right)}$$





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$$Variance = \frac{2\Gamma(c+7) + \alpha^{3}\Gamma(c+5) + 2\beta\alpha^{6}\Gamma(c+3)}{\alpha^{2}\left(2\Gamma(c+5) + \alpha^{3}\Gamma(c+3) + 2\beta\alpha^{6}\Gamma(c+1)\right)} - \left(\frac{2\Gamma(c+6) + \alpha^{3}\Gamma(c+4) + 2\beta\alpha^{6}\Gamma(c+2)}{\alpha\left(2\Gamma(c+5) + \alpha^{3}\Gamma(c+3) + 2\beta\alpha^{6}\Gamma(c+1)\right)}\right)^{2}$$

$$S.D(\sigma) = \sqrt{\frac{2\Gamma(c+7) + \alpha^3\Gamma(c+5) + 2\beta\alpha^6\Gamma(c+3)}{\alpha^2\left(2\Gamma(c+5) + \alpha^3\Gamma(c+3) + 2\beta\alpha^6\Gamma(c+1)\right)} - \left(\frac{2\Gamma(c+6) + \alpha^3\Gamma(c+4) + 2\beta\alpha^6\Gamma(c+2)}{\alpha\left(2\Gamma(c+5) + \alpha^3\Gamma(c+3) + 2\beta\alpha^6\Gamma(c+1)\right)}\right)^2}$$

4.2 Harmonic mean

The harmonic mean for the proposed weighted three parameter Odoma distribution can be obtained as

$$H.M = E\left(\frac{1}{x}\right) = \int_{0}^{\infty} \frac{1}{x} f_{W}(x) dx$$

$$= \int_{0}^{\infty} \frac{\alpha^{c+5}}{2\Gamma(c+5) + \alpha^{3}\Gamma(c+3) + 2\beta\alpha^{6}\Gamma(c+1)} x^{c-1} \left(2x^{4} + \alpha x^{2} + 2\alpha\beta\right) e^{-\alpha x} dx$$

$$=\frac{\alpha^{c+5}}{2\Gamma(c+5)+\alpha^3\Gamma(c+3)+2\beta\alpha^6\Gamma(c+1)}\int_0^\infty x^{c-1}\left(2x^4+\alpha x^2+2\alpha\beta\right)e^{-\alpha x}dx$$

$$= \frac{\alpha^{c+5}}{2\Gamma(c+5) + \alpha^{3}\Gamma(c+3) + 2\beta\alpha^{6}\Gamma(c+1)} \left(2\int_{0}^{\infty} x^{c+3} e^{-\alpha x} dx + \alpha \int_{0}^{\infty} x^{c+1} e^{-\alpha x} dx + 2\alpha\beta \int_{0}^{\infty} x^{c-1} e^{-\alpha x} dx \right)$$

$$= \frac{\alpha^{c+5}}{2\Gamma(c+5) + \alpha^{3}\Gamma(c+3) + 2\beta\alpha^{6}\Gamma(c+1)} \left(2\int_{0}^{\infty} x^{(c+4)-1} e^{-\alpha x} dx + \alpha \int_{0}^{\infty} x^{(c+2)-1} e^{-\alpha x} dx + 2\alpha\beta \int_{0}^{\infty} x^{(c+1)-2} e^{-\alpha x} dx \right)$$

After simplification, we obtain

$$H.M = \frac{\alpha^{c+5}}{\alpha^{c+4}(2\Gamma(c+5) + \alpha^3\Gamma(c+3) + 2\beta\alpha^6\Gamma(c+1))} \left(2\Gamma(c+4) + \alpha^3\Gamma(c+2) + 2\beta\alpha^5\Gamma(c+1)\right)$$

4.3 Moment Generating Function and Characteristic Function

In probability theory and statistics, moment generating function is the alternative specification of a real valued random variable of its probability distribution. Let X denotes the random variable following weighted three parameter Odoma distribution, then the moment generating function of X can be obtained as

$$M_X(t) = E(e^{tx}) = \int_0^\infty e^{tx} f_w(x) dx$$

Using Taylor's series, we obtain





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$$= \int_{0}^{\infty} \left(1 + tx + \frac{(tx)^2}{2!} + \dots\right) f_w(x) dx$$

$$= \int_{0}^{\infty} \sum_{j=0}^{\infty} \frac{t^{j}}{j!} x^{j} f_{w}(x) dx$$

$$=\sum_{j=0}^{\infty}\frac{t^{j}}{j!}\mu_{j}'$$

$$= \sum_{j=0}^{\infty} \frac{t^{j}}{j!} \left(\frac{2\Gamma(c+j+5) + \alpha^{3}\Gamma(c+j+3) + 2\beta\alpha^{6}\Gamma(c+j+1)}{\alpha^{j} \left(2\Gamma(c+5) + \alpha^{3}\Gamma(c+3) + 2\beta\alpha^{6}\Gamma(c+1) \right)} \right)$$

$$M_{X}(t) = \frac{1}{2\Gamma(c+5) + \alpha^{3}\Gamma(c+3) + 2\beta\alpha^{6}\Gamma(c+1)} \sum_{j=0}^{\infty} \frac{t^{j}}{j!\alpha^{j}} \left(2\Gamma(c+j+5) + \alpha^{3}\Gamma(c+j+3) + 2\beta\alpha^{6}\Gamma(c+j+1) \right)$$

Similarly the characteristic function of weighted three parameter Odoma distribution can be obtained as

$$\varphi_X(t) = M_X(it)$$

$$M_X(it) = \frac{1}{2\Gamma(c+5) + \alpha^3\Gamma(c+3) + 2\beta\alpha^6\Gamma(c+1)} \sum_{j=0}^{\infty} \frac{it^j}{j!\alpha^j} \left(2\Gamma(c+j+5) + \alpha^3\Gamma(c+j+3) + 2\beta\alpha^6\Gamma(c+j+1) \right)$$

5. Order Statistics

Order statistics deals with the application of ordered sample values and their functions. Order statistics has been widely used in reliability and life testing. Suppose $X_{(1)}$, $X_{(2)}$,..., $X_{(n)}$ denotes the order statistics of a random sample X_1 , X_2 ,..., X_n drawn from a continuous population with probability density function $f_X(x)$ and cumulative distribution function $F_X(x)$, then the probability density function of r^{th} order statistics $X_{(n)}$ is given by

$$f_{X(r)}(x) = \frac{n!}{(r-1)!(n-r)!} f_X(x) \left(F_X(x)\right)^{r-1} \left(1 - F_X(x)\right)^{n-r}$$
(8)

substitute the values of equation (5) and (6) in equation (8), we will obtain the probability density function of r^{th} order statistics of weighted three parameter Odoma distribution





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$$f_{x(r)}(x) = \frac{n!}{(r-1)!(n-r)!} \left(\frac{\alpha^{c+5}}{2\Gamma(c+5) + \alpha^{3}\Gamma(c+3) + 2\beta\alpha^{6}\Gamma(c+1)} x^{c} \left(2x^{4} + \alpha x^{2} + 2\alpha\beta \right) e^{-\alpha x} \right)$$

$$\times \left(\frac{1}{2\Gamma(c+5) + \alpha^{3}\Gamma(c+3) + 2\beta\alpha^{6}\Gamma(c+1)} \left(2\gamma(c+5,\alpha x) + \alpha^{3}\gamma(c+3,\alpha x) + 2\beta\alpha^{5}\gamma(c+1,\alpha x)\right)\right)^{r-1}$$

$$\times \left(1 - \frac{1}{2\Gamma(c+5) + \alpha^3\Gamma(c+3) + 2\beta\alpha^6\Gamma(c+1)} \left(2\gamma(c+5,\alpha x) + \alpha^3\gamma(c+3,\alpha x) + 2\beta\alpha^5\gamma(c+1,\alpha x)\right)\right)^{n-r}$$

Therefore, the probability density function of higher order statistic $X_{(n)}$ of weighted three parameter Odoma distribution can be obtained as

$$\begin{split} f_{x(n)}(x) &= \frac{n\alpha^{c+5}}{2\Gamma(c+5) + \alpha^{3}\Gamma(c+3) + 2\beta\alpha^{6}\Gamma(c+1)} x^{c} \bigg(2x^{4} + \alpha x^{2} + 2\alpha\beta \bigg) e^{-\alpha x} \\ &\times \left(\frac{1}{2\Gamma(c+5) + \alpha^{3}\Gamma(c+3) + 2\beta\alpha^{6}\Gamma(c+1)} \bigg(2\gamma(c+5, \alpha x) + \alpha^{3}\gamma(c+3, \alpha x) + 2\beta\alpha^{5}\gamma(c+1, \alpha x) \bigg) \right)^{n-1} \end{split}$$

and the probability density function of first order statistic $X_{(l)}$ of weighted three parameter Odoma distribution can be obtained as

$$\begin{split} f_{x(1)}\left(x\right) &= \frac{n\alpha^{c+5}}{2\Gamma(c+5) + \alpha^{3}\Gamma(c+3) + 2\beta\alpha^{6}\Gamma(c+1)} x^{c} \bigg(2x^{4} + \alpha x^{2} + 2\alpha\beta\bigg) e^{-\alpha x} \\ &\times \left(1 - \frac{1}{2\Gamma(c+5) + \alpha^{3}\Gamma(c+3) + 2\beta\alpha^{6}\Gamma(c+1)} \bigg(2\gamma(c+5,\,\alpha x) + \alpha^{3}\gamma(c+3,\,\alpha x) + 2\beta\alpha^{5}\gamma(c+1,\alpha x)\bigg)\right)^{n-1} \mathbf{6}. \end{split}$$

Likelihood Ratio Test

Suppose the random sample X_1, X_2, \dots, X_n of size n drawn from the weighted three parameter Odoma distribution. We set up the hypothesis for testing.

$$H_O: f(x) = f(x; \alpha, \beta)$$
 against $H_1: f(x) = f_W(x; \alpha, \beta, c)$

For testing, whether the random sample of size n comes from the two parameter Odoma distribution or weighted three parameter Odoma distribution, the following test statistic is used.

$$\Delta = \frac{L_1}{L_0} = \prod_{i=1}^{n} \frac{f_W(x; \alpha, \beta, c)}{f(x; \alpha, \beta)}$$





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$$\Delta = \frac{L_1}{L_o} = \prod_{i=1}^{n} \left(\frac{\alpha^c x_i^c \left(2\left(\alpha^5 \beta + \alpha^3 + 24\right) \right)}{2\Gamma(c+5) + \alpha^3 \Gamma(c+3) + 2\beta \alpha^6 \Gamma(c+1)} \right)$$

$$\Delta = \frac{L_1}{L_o} = \left(\frac{\alpha^c \left(2\left(\alpha^5 \beta + \alpha^3 + 24\right) \right)}{2\Gamma(c+5) + \alpha^3 \Gamma(c+3) + 2\beta \alpha^6 \Gamma(c+1)} \right)^n \prod_{i=1}^{n} x_i^c \left(\frac{\alpha^5 \beta + \alpha^3 + 24}{2\Gamma(c+5) + \alpha^3 \Gamma(c+3) + 2\beta \alpha^6 \Gamma(c+1)} \right)^n \prod_{i=1}^{n} x_i^c \left(\frac{\alpha^5 \beta + \alpha^3 + 24}{2\Gamma(c+5) + \alpha^3 \Gamma(c+3) + 2\beta \alpha^6 \Gamma(c+1)} \right)^n \prod_{i=1}^{n} x_i^c \left(\frac{\alpha^5 \beta + \alpha^3 + 24}{2\Gamma(c+5) + \alpha^3 \Gamma(c+3) + 2\beta \alpha^6 \Gamma(c+1)} \right)^n \prod_{i=1}^{n} x_i^c \left(\frac{\alpha^5 \beta + \alpha^3 + 24}{2\Gamma(c+5) + \alpha^3 \Gamma(c+3) + 2\beta \alpha^6 \Gamma(c+1)} \right)^n \prod_{i=1}^{n} x_i^c \left(\frac{\alpha^5 \beta + \alpha^3 + 24}{2\Gamma(c+5) + \alpha^3 \Gamma(c+3) + 2\beta \alpha^6 \Gamma(c+1)} \right)^n \prod_{i=1}^{n} x_i^c \left(\frac{\alpha^5 \beta + \alpha^3 + 24}{2\Gamma(c+5) + \alpha^3 \Gamma(c+3) + 2\beta \alpha^6 \Gamma(c+1)} \right)^n \prod_{i=1}^{n} x_i^c \left(\frac{\alpha^5 \beta + \alpha^3 + 24}{2\Gamma(c+5) + \alpha^3 \Gamma(c+3) + 2\beta \alpha^6 \Gamma(c+1)} \right)^n \prod_{i=1}^{n} x_i^c \left(\frac{\alpha^5 \beta + \alpha^3 + 24}{2\Gamma(c+5) + \alpha^3 \Gamma(c+3) + 2\beta \alpha^6 \Gamma(c+1)} \right)^n \prod_{i=1}^{n} x_i^c \left(\frac{\alpha^5 \beta + \alpha^3 + 24}{2\Gamma(c+5) + \alpha^3 \Gamma(c+3) + 2\beta \alpha^6 \Gamma(c+1)} \right)^n \prod_{i=1}^{n} x_i^c \left(\frac{\alpha^5 \beta + \alpha^3 + 24}{2\Gamma(c+5) + \alpha^3 \Gamma(c+3) + 2\beta \alpha^6 \Gamma(c+1)} \right)^n \prod_{i=1}^{n} x_i^c \left(\frac{\alpha^5 \beta + \alpha^3 + 24}{2\Gamma(c+5) + \alpha^3 \Gamma(c+3) + 2\beta \alpha^6 \Gamma(c+1)} \right)^n \prod_{i=1}^{n} x_i^c \left(\frac{\alpha^5 \beta + \alpha^3 + 24}{2\Gamma(c+5) + \alpha^3 \Gamma(c+3) + 2\beta \alpha^6 \Gamma(c+1)} \right)^n \prod_{i=1}^{n} x_i^c \left(\frac{\alpha^5 \beta + \alpha^3 + 24}{2\Gamma(c+5) + \alpha^3 \Gamma(c+3) + 2\beta \alpha^6 \Gamma(c+1)} \right)^n \prod_{i=1}^{n} x_i^c \left(\frac{\alpha^5 \beta + \alpha^3 + 24}{2\Gamma(c+5) + \alpha^3 \Gamma(c+3) + 2\beta \alpha^6 \Gamma(c+1)} \right)^n \prod_{i=1}^{n} x_i^c \left(\frac{\alpha^5 \beta + \alpha^3 + 24}{2\Gamma(c+5) + \alpha^3 \Gamma(c+3) + 2\beta \alpha^6 \Gamma(c+1)} \right)^n \prod_{i=1}^{n} x_i^c \left(\frac{\alpha^5 \beta + \alpha^3 + 24}{2\Gamma(c+5) + \alpha^3 \Gamma(c+3) + 2\beta \alpha^6 \Gamma(c+1)} \right)^n \prod_{i=1}^{n} x_i^c \left(\frac{\alpha^5 \beta + \alpha^3 + 24}{2\Gamma(c+5) + \alpha^3 \Gamma(c+3) + 2\beta \alpha^6 \Gamma(c+1)} \right)^n \prod_{i=1}^{n} x_i^c \left(\frac{\alpha^5 \beta + \alpha^5 + \alpha^5 + 2\beta \alpha^6 \Gamma(c+1)}{2\Gamma(c+5) + \alpha^5 \Gamma(c+3) + 2\beta \alpha^6 \Gamma(c+1)} \right)^n \prod_{i=1}^{n} x_i^c \left(\frac{\alpha^5 \beta + \alpha^5 + \alpha^5 + 2\beta \alpha^5 \Gamma(c+1)}{2\Gamma(c+5) + 2\beta \alpha^5 \Gamma(c+1)} \right)^n \prod_{i=1}^{n} x_i^c \left(\frac{\alpha^5 \beta + \alpha^5 + 2\beta \alpha^5 \Gamma(c+1)}{2\Gamma(c+5) + 2\beta \alpha^5 \Gamma(c+1)} \right)^n \prod_{i=1}^{n} x_i^c \left(\frac{\alpha^5 \beta + \alpha^5 + 2\beta \alpha^5 \Gamma(c+1)}{2\Gamma(c+5) + 2\beta \alpha^5 \Gamma(c+1)} \right)^n \prod_{i=1}^{n} x_i^c \left(\frac{\alpha^5 \beta + \alpha^5 \Gamma(c+1)}{2\Gamma(c$$

We refuse to accept the null hypothesis if

$$\Delta = \left(\frac{\alpha^{c} \left(2\left(\alpha^{5}\beta + \alpha^{3} + 24\right)\right)}{2\Gamma(c+5) + \alpha^{3}\Gamma(c+3) + 2\beta\alpha^{6}\Gamma(c+1)}\right)^{n} \prod_{i=1}^{n} x_{i}^{c} > k$$

Or equivalently, we reject the null hypothesis if

$$\Delta^* = \prod_{i=1}^n x_i^c > k \left(\frac{2\Gamma(c+5) + \alpha^3 \Gamma(c+3) + 2\beta \alpha^6 \Gamma(c+1)}{\alpha^c \left(2\left(\alpha^5 \beta + \alpha^3 + 24\right) \right)} \right)^n$$

$$\Delta^* = \prod_{i=1}^n x_i^c > k^*, \text{ Where } k^* = k \left(\frac{2\Gamma(c+5) + \alpha^3 \Gamma(c+3) + 2\beta \alpha^6 \Gamma(c+1)}{\alpha^c \left(2\left(\alpha^5 \beta + \alpha^3 + 24\right) \right)} \right)^n$$

For large sample of size n, $2log \Delta$ is distributed as chi-square distribution with one degree of freedom and also p-value is obtained from the chi-square distribution. Also, we reject the null hypothesis, when the probability value is given by

$$p\left(\Delta^* > \lambda^*\right)$$
. Where $\lambda^* = \prod_{i=1}^n x_i^c$ is less than a specified level of significance and $\prod_{i=1}^n x_i^c$ is the observed value of the statistic Δ^* .

7. Bonferroni and Lorenz Curves

The Bonferroni and Lorenz curves are known as income distribution curves and are applied in various areas like reliability, medicine, insurance and demography, but mostly it is also being used to study the distribution of income of inequality or poverty. The bonferroni and Lorenz curves are given by

$$B(p) = \frac{1}{p\mu_1} \int_0^q x f_W(x) dx$$

and
$$L(p) = pB(p) = \frac{1}{\mu_1} \int_{0}^{q} x f_w(x) dx$$





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Where
$$\mu_1' = \frac{2\Gamma(c+6) + \alpha^3\Gamma(c+4) + 2\beta\alpha^6\Gamma(c+2)}{\alpha\left(2\Gamma(c+5) + \alpha^3\Gamma(c+3) + 2\beta\alpha^6\Gamma(c+1)\right)}$$
 and $q = F^{-1}(p)$

$$B(p) = \frac{\alpha \left(2\Gamma(c+5) + \alpha^{3}\Gamma(c+3) + 2\beta\alpha^{6}\Gamma(c+1)\right)q}{p\left(2\Gamma(c+6) + \alpha^{3}\Gamma(c+4) + 2\beta\alpha^{6}\Gamma(c+2)\right)^{0}} \frac{q}{2\Gamma(c+5) + \alpha^{3}\Gamma(c+3) + 2\beta\alpha^{6}\Gamma(c+1)} x^{c+1} \left(2x^{4} + \alpha x^{2} + 2\alpha\beta\right) e^{-\alpha x} dx$$

$$B(p) = \frac{\alpha^{c+6}}{p\bigg(2\Gamma(c+6) + \alpha^3\Gamma(c+4) + 2\beta\alpha^6\Gamma(c+2)\bigg)^0} \int\limits_0^q x^{c+1} \bigg(2x^4 + \alpha x^2 + 2\alpha\beta\bigg) e^{-\alpha x} dx$$

$$B(p) = \frac{\alpha^{c+6}}{p \left(2\Gamma(c+6) + \alpha^{3}\Gamma(c+4) + 2\beta\alpha^{6}\Gamma(c+2)\right)} \begin{pmatrix} q \\ 2\int_{0}^{1} x (c+6) - 1 \\ e^{-\alpha x} dx + \alpha\int_{0}^{1} x (c+4) - 1 \\ e^{-\alpha x} dx + 2\alpha\beta\int_{0}^{1} x (c+2) - 1 \\ e^{-\alpha x} dx \end{pmatrix}$$

After simplification, we get

$$B(p) = \frac{\alpha^{c+6}}{p\bigg(2\Gamma(c+6) + \alpha^3\Gamma(c+4) + 2\beta\alpha^6\Gamma(c+2)\bigg)} \bigg(2\gamma(c+6,\alpha q) + \alpha\gamma(c+4,\alpha q) + 2\alpha\beta\gamma(c+2,\alpha q)\bigg)$$

$$L(p) = \frac{\alpha^{c+6}}{\left(2\Gamma(c+6) + \alpha^3\Gamma(c+4) + 2\beta\alpha^6\Gamma(c+2)\right)} \left(2\gamma(c+6,\alpha q) + \alpha\gamma(c+4,\alpha q) + 2\alpha\beta\gamma(c+2,\alpha q)\right)$$

8. Maximum Likelihood Estimator and Fisher's Information Matrix

In this section, we will discuss the parameter estimation of weighted three parameter Odoma distribution by using the method of maximum likelihood estimator and also its Fisher's information matrix have been discussed. Let X_1 , X_2 ,..., X_n be a random sample of size n from the weighted three parameter Odoma distribution, then the corresponding likelihood function is given by

$$L(x) = \prod_{i=1}^{n} f_{W}(x)$$

$$L(x) = \prod_{i=1}^{n} \left(\frac{\alpha^{c+5}}{2\Gamma(c+5) + \alpha^{3}\Gamma(c+3) + 2\beta\alpha^{6}\Gamma(c+1)} x_{i}^{c} \left(2x_{i}^{4} + \alpha x_{i}^{2} + 2\alpha\beta \right) e^{-\alpha x_{i}} \right)$$

$$L(x) = \frac{\alpha^{n(c+5)}}{\left(2\Gamma(c+5) + \alpha^3\Gamma(c+3) + 2\beta\alpha^6\Gamma(c+1)\right)^n \prod_{i=1}^n \left(x_i^c \left(2x_i^4 + \alpha x_i^2 + 2\alpha\beta\right)e^{-\alpha x_i}\right)}$$

The log likelihood function is given by

$$\log L = n(c+5)\log \alpha - n\log \left(2\Gamma(c+5) + \alpha^{3}\Gamma(c+3) + 2\beta\alpha^{6}\Gamma(c+1)\right) + c\sum_{i=1}^{n}\log x_{i} + \sum_{i=1}^{n}\log \left(2x_{i}^{4} + \alpha x_{i}^{2} + 2\alpha\beta\right) - \alpha\sum_{i=1}^{n}x_{i}$$
(11)





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Themaximum likelihood estimate of α , β and c can be obtained by differentiating the log likelihood equation (11)

with respect to parameter α , β and c. We obtain the normal equations as

$$\frac{\partial \log L}{\partial \alpha} = \frac{n(c+5)}{\alpha} - n \left(\frac{3\alpha^2 \Gamma(c+3) + 12\beta\alpha^5 \Gamma(c+1)}{2\Gamma(c+5) + \alpha^3 \Gamma(c+3) + 2\beta\alpha^6 \Gamma(c+1)} \right) + \sum_{i=1}^{n} \left(\frac{\left(x_i^2 + 2\beta\right)}{\left(2x_i^4 + \alpha x_i^2 + 2\alpha\beta\right)} \right) - \sum_{i=1}^{n} x_i = 0$$

$$\frac{\partial \log L}{\partial \beta} = -n \left(\frac{2\alpha^6 \Gamma(c+1)}{2\Gamma(c+5) + \alpha^3 \Gamma(c+3) + 2\beta\alpha^6 \Gamma(c+1)} \right) + \sum_{i=1}^{n} \left(\frac{2\alpha}{\left(2x_i^4 + \alpha x_i^2 + 2\alpha\beta\right)} \right) = 0$$

$$\frac{\partial \log L}{\partial c} = n \log \alpha - n \psi \left(2\Gamma(c+5) + \alpha^3 \Gamma(c+3) + 2\beta \alpha^6 \Gamma(c+1) \right) + \sum_{i=1}^{n} \log x_i = 0$$

Where $\psi(.)$ is the digamma function

It is important to mention here that the above likelihood equations are too complicated to solve it algebraically. Therefore, we use numerical technique like Newton-Raphson method for estimating the parameters of the proposed distribution. We use the asymptotic normality results to obtain the confidence interval. We have that if $\hat{\theta} = (\hat{\alpha}, \hat{\beta}, \hat{c})$ denoted the MLE of $\theta = (\alpha, \beta, c)$. We can state the result as follows:

$$\sqrt{n}(\hat{\theta} - \theta) \to N_3(0, I^{-1}(\theta))$$

Where $I(\theta)$ is the Fisher's Information matrix. i.e.

$$I(\theta) = -\frac{1}{n} \begin{bmatrix} E\left(\frac{\partial^2 \log L}{\partial \alpha^2}\right) & E\left(\frac{\partial^2 \log L}{\partial \alpha \partial \beta}\right) & E\left(\frac{\partial^2 \log L}{\partial \alpha \partial c}\right) \\ E\left(\frac{\partial^2 \log L}{\partial \beta \partial \alpha}\right) & E\left(\frac{\partial^2 \log L}{\partial \beta^2}\right) & E\left(\frac{\partial^2 \log L}{\partial \beta \partial c}\right) \\ E\left(\frac{\partial^2 \log L}{\partial c \partial \alpha}\right) & E\left(\frac{\partial^2 \log L}{\partial c \partial \beta}\right) & E\left(\frac{\partial^2 \log L}{\partial c^2}\right) \end{bmatrix}$$

Here, we define





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$$E\left(\frac{\partial^{2} \log L}{\partial \alpha^{2}}\right) = -\frac{n(c+5)}{\alpha^{2}}$$

$$-n\left(\frac{\left(2\Gamma(c+5) + \alpha^{3}\Gamma(c+3) + 2\beta\alpha^{6}\Gamma(c+1)\right)\left(6\alpha\Gamma(c+3) + 60\beta\alpha^{4}\Gamma(c+1)\right)}{-\left(3\alpha^{2}\Gamma(c+3) + 12\beta\alpha^{5}\Gamma(c+1)\right)\left(3\alpha^{2}\Gamma(c+3) + 12\beta\alpha^{5}\Gamma(c+1)\right)}\right) - \sum_{i=1}^{n} \left(\frac{\left(x_{i}^{2} + 2\beta\right)^{2}}{\left(2\Gamma(c+5) + \alpha^{3}\Gamma(c+3) + 2\beta\alpha^{6}\Gamma(c+1)\right)^{2}}\right)$$

$$E\left(\frac{\partial^2 \log L}{\partial \beta^2}\right) = n\left(\frac{\left(2\alpha^6 \Gamma(c+1)\right)^2}{\left(2\Gamma(c+5) + \alpha^3 \Gamma(c+3) + 2\beta\alpha^6 \Gamma(c+1)\right)^2}\right) - \sum_{i=1}^n \left(\frac{4\alpha^2}{\left(2x_i^4 + \alpha x_i^2 + 2\alpha\beta\right)^2}\right)$$

$$E\left(\frac{\partial^2 \log L}{\partial c^2}\right) = -n\psi'\left(2\Gamma(c+5) + \alpha^3\Gamma(c+3) + 2\beta\alpha^6\Gamma(c+1)\right)$$

$$E\left(\frac{\partial^2 \log L}{\partial \alpha \, \partial \beta}\right) = -n \left(\frac{\left(2\Gamma(c+5) + \alpha^3\Gamma(c+3) + 2\beta\alpha^6\Gamma(c+1)\right)\left(12\alpha^5\Gamma(c+1)\right)}{-\left(2\alpha^6\Gamma(c+1)\right)\left(3\alpha^2\Gamma(c+3) + 12\beta\alpha^5\Gamma(c+1)\right)} - \frac{\left(2\Gamma(c+5) + \alpha^3\Gamma(c+3) + 2\beta\alpha^6\Gamma(c+1)\right)^2}{\left(2\Gamma(c+5) + \alpha^3\Gamma(c+3) + 2\beta\alpha^6\Gamma(c+1)\right)^2}\right)$$

$$+\sum_{i=1}^{n} \left(\frac{2(2x_{i}^{4} + \alpha x_{i}^{2} + 2\alpha\beta) - 2\alpha(x_{i}^{2} + 2\beta)}{(2x_{i}^{4} + \alpha x_{i}^{2} + 2\alpha\beta)^{2}} \right)$$

$$E\left(\frac{\partial^2 \log L}{\partial \alpha \partial c}\right) = \frac{n}{\alpha} - n\psi \left(\frac{3\alpha^2 \Gamma(c+3) + 12\beta\alpha^5 \Gamma(c+1)}{2\Gamma(c+5) + \alpha^3 \Gamma(c+3) + 2\beta\alpha^6 \Gamma(c+1)}\right)$$

$$E\!\!\left(\frac{\partial^2 \log L}{\partial \beta \, \partial c}\right) = -n \psi\!\!\left(\frac{2\alpha^6 \Gamma(c+1)}{2\Gamma(c+5) + \alpha^3 \Gamma(c+3) + 2\beta \alpha^6 \Gamma(c+1)}\right)$$





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Since θ being unknown, we estimate $I^{-1}(\theta)$ by $I^{-1}(\hat{\theta})$ and this can be used to obtain asymptotic confidence intervals for α , β and c.

Application

In this section, we have fitted two real data sets in weighted three parameter Odoma distribution to show that the weighted three parameter Odoma distribution fits better over two parameter Odoma, one parameter Odoma, exponential and lindley distributions. The two real data sets are given below: The first data set represents the breaking stress of carbon fibres of 50 mm length (GPa) and has been already used by Al-Aqtash et al. (2014) to demonstrate the appropriateness of Gumbell-weibull distribution and the data set is presented below in table 1. The second real data set represents the strength of 1.5cm glass fibres measured at the National physical laboratory England used by smith and Naylor (1987) and is executed below in table 2. In order to estimate the model comparison criterion values, the unknown parameters are also estimated through the R software. In order to compare the weighted three parameter Odoma distribution with two parameter Odoma, one parameter Odoma, exponential and Lindley distributions, we are using the criterion values AIC (Akaike Information Criterion), BIC (Bayesian Information Criterion), AICC (Akaike Information Criterion) and -2logL. The better distribution is which corresponds to lesser values of AIC, BIC, AICC and -2logL. The following formulas are applied for the estimation of criterion values.

$$AIC = 2k - 2\log L$$
, $BIC = k\log n - 2\log L$ and $AICC = AIC + \frac{2k(k+1)}{n-k-1}$

Where k is the number of parameters in the statistical model, n is the sample size and -2logL is the maximized value of the log-likelihood function under the considered model. From table 3, it has been observed from the results that the weighted three parameter Odoma distribution have the lesser AIC, BIC, AICC and -2logL values as compared to the two parameter Odoma, one parameter Odoma, exponential and Lindley distributions, which concluded that the weighted three parameter Odoma distribution fits better than the two parameter Odoma, one parameter Odoma, exponential and Lindley distributions.

CONCLUSION

In the present study, we have introduced a new generalization of two parameter Odoma distribution known as weighted three parameter Odoma distribution. The subject distribution is generated by using the weighted technique and taking the two parameter Odoma distribution as the base distribution. The different statistical properties of the proposed distribution as the moments, order statistics, reliability measures, harmonic mean, bonferroni and lorenz curves have been derived and discussed. The technique of maximum likelihood estimator have been employed for estimating the parameters of proposed distribution and also its Fisher's information matrix have been discussed. Finally, the application of new distribution in real life is illustrated by taking the two real data sets and the results are compared over two parameter Odoma, one parameter Odoma, exponential and lindley distributions. Hence, it can be concluded from the results that the weighted three parameter Odoma distribution fits better as compared to two parameter Odoma, one parameter Odoma, exponential and lindley distributions.

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Table 1: Data regarding breaking stress of carbon fibres of 50 mm length (GPa) represented by Al-Aqtash et al. (2014)

0.39	0.85	1.08	1.25	1.47	1.57	1.61	1.61	1.69	1.80
1.84	1.87	1.89	2.03	2.03	2.05	2.12	2.35	2.41	2.43
2.48	2.50	2.53	2.55	2.55	2.56	2.59	2.67	2.73	2.74
2.79	2.81	2.82	2.85	2.87	2.88	2.93	2.95	2.96	2.97
3.09	3.11	3.11	3.15	3.15	3.19	3.22	3.22	3.27	3.28
3.31	3.31	3.33	3.39	3.39	3.56	3.60	3.65	3.68	3.70
3.75	4.20	4.38	4.42	4.70	4.90				





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Table 2: Data consists of strength of 1.5cm glass fibres measured at National laboratory England reported by Smith and Naylor (1987)

	,	` '									
0.55	2	1.82	1.76	1.7	1.7	0.93	0.74	2.01	1.84.	1.77	1.78
1.25	1.04	0.77	2.24	1.84	1.89	1.36	1.27	1.11	0.81	0.84	1.49
1.39	1.28	1.13	1.24	1.52	1.49	1.42	1.29	1.3	1.61	1.59	1.54
1.5	1.51	1.64	1.61	1.6	1.55	1.55	1.68	1.66	1.62	1.61	1.61
1.73	1.68	1.66	1.62	1.63	1.81	1.76	1.69	1.66	1.67	1.58	1.53
1.5	1.48	1.48									

Table 3: Performance of distributions for data set 1 and data set 2

Data sets	Distribution	MLE	S.E	-2logL	AIC	BIC	AICC
	Weighted Three Parameter Odoma	$\hat{\alpha} = 2.9645543$ $\hat{\beta} = 0.0010000$ $\hat{c} = 3.5976798$	$\hat{\alpha} = 0.4947380$ $\hat{\beta} = 0.0997418$ $\hat{c} = 1.3406833$	180.4219	186.4219	192.9908	186.8089
	Two Parameter Odoma	$\hat{\alpha} = 1.6807361$ $\hat{\beta} = 0.0010000$	$\hat{\alpha} = 0.1131248$ $\hat{\beta} = 0.1253191$	191.0669	195.0669	199.4462	195.2573
	Odoma	$\hat{\alpha} = 1.43737746$	$\hat{\alpha} = 0.06306653$	213.9102	215.9102	218.0998	215.9727
	Exponential	$\hat{\theta} = 0.36237943$	$\hat{\theta} = 0.04460548$	265.9887	267.9887	270.1784	268.0512
1	Lindley	$\hat{\theta} = 0.59025384$	$\hat{\theta} = 0.05324128$	244.7681	246.7681	248.9578	246.8306
	Weighted Three Parameter Odoma	$\hat{\alpha} = 11.834922$ $\hat{\beta} = 0.001000$ $\hat{c} = 14.316711$	$\hat{\alpha} = 1.311928$ $\hat{\beta} = 0.023466$ $\hat{c} = 2.214752$	49.0224	55.0224	61.45181	55.42917
2	Two Parameter Odoma	$\hat{\alpha} = 2.68113079$ $\hat{\beta} = 0.00100000$	$\hat{\alpha} = 0.07527934$ $\hat{\beta} = 0.00547623$	105.4957	109.4957	113.7819	109.6957
	Odoma	$\hat{\alpha} = 1.90028478$	$\hat{\alpha} = 0.08390026$	181.4835	183.4835	185.6266	183.5490
	Exponential	$\hat{\theta} = 0.66364740$	$\hat{\theta} = 0.08361152$	177.6606	179.6606	181.8038	179.7261
	Lindley	$\hat{\theta} = 0.99611639$	$\hat{\theta} = 0.09484179$	162.5569	164.5569	166.7	164.6224

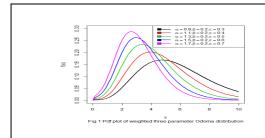


Fig 1: Pdf point of weighted three parameter Odoma
Distribution

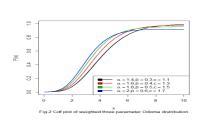


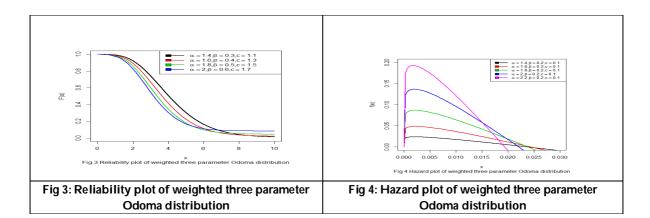
Fig 2: Cdf plot of weighted three parameter Odoma distribution





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RESEARCH ARTICLE

Innovation and Intellectual Property of Indian Rights Law

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ABSTRACT

An overview of India's legislation governing innovation and intellectual property rights is given in this article. Additionally, it provides details on remedies for these rights violations and information on patent, copyright, trademark, and design laws. Many features of intellectual property were covered by India's Act, which was take on by the parliament. Industry and politicians alike are concentrating on methods for boosting innovation capacity as they consider the requirements for India's next phase of growth. In the end, India will succeed ultimately if it supports a strong intellectual property environs and safeguards of its own businesses. This article includes an evaluation of the current status and also the general framework of India's current intellectual property policy.

Keywords: Patent law, copyright, trademark, remedies.

INTRODUCTION

The Indian constitution guarantees a number of fundamental rights, including the right to life [1]. (which includes the majesty, seclusion, livelihood, protection, access to crisis, health maintenance and habitat), equity [2], nondiscrimination [3], basic education [4], liberty of speech, association, development, company, trade, and commerce [5]. In 1978, property rights were dropped from the chapter on basic entitlement and relocated to a new section of the constitution. Intellectual property is the world's largest and fastest-growing sectors. Consequently, handling IPRs at national and international borders requires the expertise of experts with a strong IP background. Industry and government need to concentrate on ways to develop potential for innovation as they consider India's needs for the following phase of development. India falls 11 spots short of its ambitions, placing 71st out of 144 nations in the World Economics Forum's Global Competitiveness Reports 2014–2015. In the global innovation index published in





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2014 by WIPO, Cornell University, and the Institute for European Administrative Affairs, India was rated 76th out of 143 nations [6]. To address this issue, it is important to concede the connection between intellectual property and innovation, where today's creation becomes future innovation. As a result, India has the 1994 General Agreement on Tariffs and Trade (GATT) agreement on Trade Related Aspects of Intellectual Property Rights (TRIPS), later known as the World Trade Organization. India's IP system and businesses were able to conform to international IP standards as a result. The Indian courts must evaluate of its constitution, which upholds the essential tenet of policy, when legal interpretations or issues arise that violate of a cross-section of the population. Health-related issues raised by Section 39(e) and (f) should be taken into account by Indian legislators when drafting laws. The legislation is essential in striking a balance between the public interest in promoting innovation, revolution and the privilege of IPR owners.

Types of Protected Intellectual Property and how Indian Law Addresses Them

Under Indian law, innovations fall into six different broad categories that are eligible for IP protection.

Patent Law

India's lack of adequate patent protection in one area brought to light domestic problems with food security and drug access, particularly in the context of the manufacture of food and pharmaceutical products covered by patents. The patent law in India is a statute from that year. In order to comply with India's commitments and journeys under the agreement creating the World Trade Organization, the law has been modified. The 1800s saw the process industry promoted as the initial stage in building India's generic pharmaceutical sector. For innovations in food, medicine, and healthcare, the process protection term is only five years long. The Patent Act was modified three times, following India's accession to TRIPS. The thinking (from January 1995) enabling patent claims for an object was made legislation in 1999. The 2002 Amendment established industrial application and according to innovation and specified innovation as a new product. In 2005 the Amendment reinstated the current Section of the Act, which makes the patent necessary if invented, and permitted the product to be patented for pharmaceuticals. Neither at a reasonable cost nor in an effort to meet the demands of the broader people. The Indian Patent Office is held liable in matters involving patent administration. Cases of this nature involve disputes over patent grants, licence requirements, upholding, and patent invalidation.

Cases of patent infringement

A patent infringement case in India cannot be established in the District Court, which was clearly explained in the Patent Act. Depending totally on the product, the Supreme Court's ultimate decision in Novartis AG13 section 3 (d) may be interpreted differently.

Copyright Law

During the course of time, the power is granted to the creator only. The Supreme Court equate the several rights—to the words, tones, teleplay, images, and other blueprints, among others—as a collection of entire rights, each of which is capable of independent exploitation in the early portion of the ruling^[7]. Based on the British Act of 1911, the Copyright Act of India was drafted in 1914. Following independence, the Copyright Terms of India were substantially revised, and the Copyright Act of India was eventually passed. 50 years of copyright protection are also included. The most current revisions, five total, were made in 2012. Although the term original does not need creative or original ideas, the work should not be plagiarised and must come from the author. The set of original content, according to the court, required effort, expertise, or expense. The foreseeing of the transformative use of privileged works was one of the prior instances of reasonable use, which permits the usage of secured works for investigation, teaching and typically trading motive. Utilization of a privileged material as part of reasonanable use (later clarified by Campbell17 (1994) by the US Supreme Court in a different context). There will be no copyright infringement issues even if the theme is the same, but the following work will be entirely new if it is presented in a different way. The Chancellors and academics of the University of Oxford follow the philosophy of expressive integration in India. Copying a piece of content for a necessary purpose is not illegal and is regarded as fair use.





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Trademark

Since it started at 92,251, the overall number of trademark applications has increased by double. As part of India's accession into TRIPS, the laws governing trademarks were altered, and Indian courts acknowledged the notion of trademarks in related with merchandise or favour unrelated to the prosecutors merchandise or services[8]. The trademark legislation of 1999analyses this recognition legally[9]. When the owner's claims are verified, they are accepted. According to the ruling of the Indian Supreme Court, distinctive and well-known trademarks are not required to be restricted within national borders, and Indian courts have acknowledged the reputation of such markers outside of national borders in order to provide relief from unethical attempts at their allotment. Similar to trademarks, domain names are acknowledged and given legal effect. If a field name exhibits all the features of a trademark, a go-off legal action may result, the court ruled in its Satyam Infoway decision (2004). Through promotional efforts, the original field name had misstate its products or services, costing the first field owner its reputation.

Remedies

Solutions for intellectual property infringement are frequently said to include barriers and harm. Infringement of a trademark or copyright may also result in penalties. India adheres to international legal norms for granting temporary relief, including the core case study, irreparable difficulties, and facility balance. The Delhi High Court granted Yahoo because time relief after learning that the defendants planned to create a features film with the trademarked name "YAHOO" as its title. Similar to this, the Supreme Court ruled in Fast rack Communications that the plaintiff has the power to foreclose the issue and bring a time action. John Doe orders give the plaintiff the ability to make anonymous or unidentifiable defendants comply with an injunction. The specific characteristics of copyright piracy served as inspiration for the implementation of this approach.

CONCLUSION

Intellectual property law has seen substantial developments in recent years. In the present, the legal profession and courts are more aware. IPR is one of the most important factors in the growth of our economy. Younger lawyers are aware of the complex concerns surrounding intellectual property law and India's commitment to international IPR protection. Additionally, because it does not provide intellectual property rights protection, the Indian court rejected approval for the remedy on the decision regarding life-saving medications. Despite these challenges, the Indian judiciary has acknowledged the advancement of technology. As a result, Indian courts have approved a variety of remedies to address various kinds of infractions. It is reasonable to assume that the legal framework protecting intellectual property rights. It is impossible to overstate the influence Indian courts have on shaping the direction of IPR law at a time when India is still in its infancy.

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REVIEW ARTICLE

Analyzing Urban Heat Island Effect in India: Literature Review

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ABSTRACT

In the last few decades, cities around the world have seen significant urbanization marked by the increase conversion of natural landscape into anthropogenic structures resulting in change of local atmosphere concomitant with elevated land surface temperature compared to the surrounding open areas. Elevated temperature phenomenon widely known as Urban Heat Island (UHI) became an area of concern for Urban Designers and Planners as the communities living in urban areas got affected by increased temperature bringing the notion of comfort at risk. The UHI has been the subject of intensive research in various countries including India since the 2000s. Computer application has assisted researchers in generating thermal image of an area by linking urban database with the simulation tools which provides detailed analysis of impact of anthropogenic activity on urban climate. This paper carries out a literature review of the UHI studies conducted in last two decades in different cities of India with main focus on description of reasons for UHI formation and also to trace the transition of UHI mapping approach from manual, mobile observatory to computer aided images.

Keywords: Urban Heat Island, urbanization, unplanned growth, temperature, thermal mapping, GIS, etc.,





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INTRODUCTION

With rapid urbanization, cities have turn out to be growth engines for development, prosperity, innovation and hope followed by the employment opportunities surfing out making them home to two thirds of the global population. This count encompasses one billion poor people living in slums or marginal and informal settlements. Due to intensified urbanization and population expansion the available green covers and open spaces are transformed into anthropogenic structures which results in formation of a dome of stagnant warm air over the heavily built-up areas of the city. This phenomenon termed as Urban Heat Island indicates the tendency of an urban area to have significantly high temperatures as compared to their rural counterparts. The concentration of human activities in urban areas creates an "island" of heat surrounded by a "sea" of cooler rural areas, called the urban heat island [1]. Heat urban island was first observed in London by Luke Howard in 1833[2]. Today, it is the most studied climate effect of cities[3]. IPCC (Intergovernmental Panel on Climate Change) 2014, had one chapter dedicated on UHI. The report recognizes the presence of UHI due to urban densification, reduction in vegetation cover, and increase in anthropogenic heat. UHI can be of two types- surface urban heat island (SUHIs) and atmospheric urban heat island (AUHIs)[4] [5] . Further, AUHIs can be sub classified as urban canopy layer and urban boundary layer. Basically, there are two main ways to evaluate the UHI phenomenon: conventional weather station (automatic temperature measurement) or the Land Surface Temperature (LST) measurements, given by Thermal Infrared (TIR) remote sensing data [6]. With the advancement in computers, Remote sensing emerged as a new technique of obtaining information about an area from a distance, typically through remote sensors installed on satellites or aircrafts. The remote sensors detect the energy reflected from the earth's surface in form of different color bands. Various satellite and air borne sensors have been developed to collect TIR from the earth, such as HCMM, Land sat TM/ETM+, AVHRR (Advanced Very High-Resolution Radiometer), MODIS (Moderate Resolution Imaging Spectroradiometer), ASTER (Advanced Spaceborne Thermal Emission and Reflection), and TIMS [7].

The convention weather station is used to measure Atmospheric Urban Heat Island Intensity (AUHII) while for measuring Surface Urban Heat Island Intensity (SUHII) LST data retrieved from remote sensing is majorly used. The research conducted worldwide focused on deriving Satellite derived LST data making it evident that LST is one of the significant parameters employed in the study of UHI. Monitoring UHI via remote sensing has become a research hotspot in recent years [8]. According to Decheng Zhou et al., the studies on Urban heat Island have dramatically increased, especially after 2010; the reason being the usage of Remote Sensors for mapping temperature data. This paper uses a literature-based study to analyze the Urban Heat Island studies done in different cities of India with varied climate and also to explore the tools used by the researchers for mapping Urban Heat Island in different regions of the country. While reviewing the paper, the author's main objective was to: (a) understand factors responsible for UHI formation (with special emphasis on role played by unplanned growth), (b) determine importance of role played by computer technology (usage of sensors, software etc.) in measuring UHI effect, and (c) outline the key research findings and potential future directions for conducting research on UHI in Lucknow in India. The work published by researchers published over the variety of sources such as IEEE, Science Direct and individual publications over websites were studied and analyzed.

Climate And Urban Development In India Climate

India is home to an extraordinary variety of climatic regions, ranging from tropical in the south to temperate and alpine in the Himalayan north, where elevated regions receive sustained winter snowfall. Indian climate has been categorized into the following five parts in National Building code (2005): Hot and Dry, Warm Humid, Composite, Temperate and Cold. The climatic map of India is shown in figure 1. Due to existence of wide variety of climatic zones it is necessary for the researchers to analyze the impact of urbanization in each climatic zone as the study approach will differ depending upon the climate. Given the large geographic biases of past literature, there is a clear need for more SUHI research in Africa, South America, and India due to their high urbanization potentials and/ or climate sensitivity [8].





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Urban Development Scenario in India

Cities in India contribute to two-thirds of country's GDP which is expected to rise to 75% by 2031. At present in India, there are 59 urban agglomerations of more than I million people in the country which is expected to inflate to 78 by 2035. The increase GDP will produce more job opportunities, accommodating the growing urban work force will require large investments in new urban spaces rather than unsustainable approach of accommodating them in the existing urban cores leading to increase in temperature due to anthropogenic activities. Progress of urbanization in India is threatened by climate change induced due to increased urban sprawl and unplanned growth of Indian cities. The unplanned growth demands more built environment obtained through elimination of open spaces and green covers which stimulate increase in anthropogenic emissions with reduced space for evaporative cooling leading to heat accumulation in atmosphere causing UHI. A country-based analysis indicates that China was the most actively-studied country of SUHI research (213), which was followed by the United States (106) and India (38)[8]. Rapid urbanization is resulting in dramatic land use change of which some examples of Metropolitan cities are listed below:

In last forty years urban area in Delhi increased by 30.6% while agriculture area reduced by 22.8% and dense forest by 5.3%.

- Mumbai has become almost paved and concretized in the span of 40 years
- Kolkata experienced decrease in vegetation area from 33.6% of the city area to 7.4% between 1980 and 2010.

In Chennai the urban built up area has tripled between 1991 and 2016 while the vegetation area has reduced by 12%. The above data makes it quite evident that UHI exists in Indian city and needs attention of researchers for understanding the causes of its formation and also the policy framework be given based on analysis so as to reduce the UHI effect. In figure 2, the temperature map for India derived at NASA GSFC from the GEOS-5 model plotted air temperature at 2.0m above the ground on 10 June, 2019 which clearly reports heat zones.

Literature Review

Due to urbanization cities around the world are warmer than their adjoining rural areas due to Urban Heat Island phenomenon. But Indian cities seem to be bucking the trend, courtesy an anomaly in 'evapotranspiration' patterns between urban and non-urban areas, new research suggests [9]. Urban Heat Island studies are gaining importance in the urban areas of tropical regions (Roth, 2007) and most of tropical cities are showing presence of heat islands throughout the year (Jonsson, 2004). The same effect has been observed in various metropolitan regions of India. Various methods like satellite-based SHI study, station survey and mobile traverses have been used to determine heat islands. The review on the studies conducted in India is discussed in the article so as to identify the research gap in UHI studies. In 1973 Daniels and Krishnamurthy [10] initiated mapping of urban temperatures in India over the industrial city of Pune and Mumbai. The study obtained data through portable meteorological instruments using a mobile vehicle in clear winter nights. After analyzing data collected, heat island phenomenon was confirmed over some parts of the city during winters with a temperature difference of 6°C in between the urban and rural areas. Mukherjee and Daniel (1976) also identified and recorded heat islands over city of Mumbai on certain specific occasions. In this study also the data was collected through mobile meteorological instruments in clear winter nights. The temperature difference recorded was 11°C between the urban and rural suburbs of Mumbai in January 1976 [11]. Bahl and Padmanabhamurthy (1977, 1979) mapped temperature of highly dense and industrialized city, Delhi through mobile observation. They identified hot pools over north and north east Delhi while cold pools were observed over West Delhi. The reported UHI intensity was 5°C during December and 7°C during January- March. The maximum heat island formation is reported in areas with high density-built mass and transportation [12] [13]. Jayanthi (1988) conducted first UHI study in Chennai which revealed the existence of UHI in three area of Chennai viz. Mambalam, Vepery and Ennore Industrial zone with coexistence of cooler island at Guindy Raj Bhavan areas on account of vegetation and open spaces available in the area [14]. S. David Sundersingh (1991) conducted mobile temperature mapping survey over urban areas of Madras city [15]. Surface temperature, humidity and wind data was collected from 77 points using Mobile observations. The difference between the heat islands and cool pocket observed varied from 2.5°C to 4°C. It also emphasized that vegetation can have a moderating influence on lowering temperature while the establishment of UHI is confirmed over densely populated and congested areas. The study also recommended usage of Remote sensing techniques as a powerful tool to be used for future climatic studies in





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combination with ground studies to give the right perspective of the study. Vrishali Deosthali (2000) studied horizontal structure of the heat and moisture islands in Pune city. The dry and wet bulb temperature was recorded from 170 locations on devised 9 routes through whirling psychrometers in a mobile survey conducted in April 1997 [16]. The results established that during day the core of Pune city appears as heat and moisture island whereas in the time of sunrise as heat and dry island. The core of Pune covering about 8% of the total area and accommodating 335 of the total population showed formation of large and highly intense UHI consisting of 3 more secondary warm pockets mainly due to residential development. The author established coexistence of heat and moisture island tends to reduce night comfort for which the planners should make use of prevailing westerly winds round the year for generating comfort.

Gopalakrishnan, Rama Krishna and Sharan (2002) has shown evidence of 'stress' in vegetation temperature index in regions of peninsular India partly due to expansion of Chennai, Bangalore and Hyderabad metropolitan cities [17]. A Three-dimensional model was used to understand UHI heat patch generation and its impact on regional scale meteorology. The study showed an elevated hot pool of about of 6°C at sunset around all the three major cities in Southern India during pre-monsoon, monsoon and winter period with strong influence found in Chennai. The reason identified was abrupt discontinuities in irrigated land, change in land use pattern leading to outgrowth of the city into its suburban areas. Badarinath, Kiran, Madhavilatha and Raghavaswamy (2005) analyzed day and night AATSR (Advanced Along Track Scanning Radiometer) satellite data for studying UHI and surface thermal inertia [18]. Field study was also conducted in synchronous with satellite data for validating surface temperature estimated from satellite data. The study displayed correlation between the density patterns of urban area and temperature variation during day and night. In addition, spatial variation exhibited positive correlation with the land use/ land cover in urban areas. The analysis showed that the surface temperature is minimum over water surface (18-21°C) and maximum over urban areas (36-40°C). The study suggested UHI studies to be conducted in urban areas so that recreational parks and vegetations could be planned for reducing heat island formation by urban planners. Lei, Niyogi, Kishtawal, Pielke, Beltran, Nobis and Vaidya (2008) conducted study on the role of urban land- atmosphere interactions in modulating the heavy rain event over the Indian monsoon region [19]. The study was conducted using Regional Atmosphere Modelling system (REMS 4.3) coupled with and without explicit urban energy balance model- town energy budget (TEB) for assessing the role of urban- atmosphere interactions in modulating heavy rain on 26 July 2005. It was found that even with the active monsoon, the representation of urbanization contributes to local heavy precipitation and mesoscale precipitation distribution over the Indian monsoon region.

In summary, the SSTs (sea surface temperature) and landscape pattern helped transport large amount of moisture over the Western Ghats region while the mesoscale boundaries created by urban land surface and sea surface temperature gradients caused heavy precipitation focus over Mumbai. After a span of almost 20 years, another study was conducted in Chennai by Devadas and Lilly Rose in May 2008 and January 2009 [20]. Air temperature was recorded in 30 stationary locations covering urban, suburban and rural areas of the city. Mobile survey was conducted for collecting data by using HOBO dataloggers installed over the car. The result indicates the existence of UHI in the city ranging from 2.48°C in summers and 3.35°C during winters. The study reinforces the relationship between the urban air temperature increase and the land use characteristics which comprise of densely built spaces, anthropogenic heat, building materials, transportation modes and vegetation. Ramachandra and Uttam Kumar (2010) studied UHI effect in Bangalore. In this study Land surface temperature has been measured using Remote Sensing to understand UHI effect. Landsat data of 1973, 1992 and 2000, IRS LISS-III data of 1999 and 2006 and MODIS data of 2002 and 2007 has been analyzed which make it evident that due to urban growth the temperature has increased by 2-2.5°C during the last decade [21].

Mohan, Kandya and Battiprolu (2011) studied temperature trends in four stations of the Delhi NCR region by analyzing seasonal and annual temperature trends through trend analysis. The annual mean max temperature did not show any specific trend however the annual mean minimum temperature indicates a warming trend in general over NCR indicating significant urbanization in past few decades [22]. Puneeta Pandey et al., (2012) studied day and night time thermal mapping of Delhi done with MODIS satellite data for the months of November and December from 2007-2010. The data analysis revealed the formation of day time cool island over central parts of Delhi which is





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found to be 4-6°C cooler than the surrounding rural areas and formation of warmer pockets exhibiting 4-7°C more temperature than its surrounding rural area during the night. The study suggests that urban areas behave more like moderators of diurnal temperature in low wind conditions [23]. Borthakur and Nath (2012) has done analysis of Landsat imageries which revealed subsequent decrease of land cover with a distinct spatial heterogeneity of Land surface temperature from 1991-2008 in Metropolitan Guwahati. The decrease in vegetation is associated with development of hot spots and thus this study established a correlation in temperature and land use type [24]. Bajaj, Inamdar and Vaibhav (2012) in their research mapped the UHI of Ahmedabad city using Thermal infrared Imagery of Landsat TM and ETM+. The UHI intensity varies from 0.4 to 0.8°C approximately. The study proved strong linear relationship between Land surface temperature (LST) and Normalized Vegetation Index (NDVI) and the regions having high NDVI showed lower temperature proving that built up areas are major contributors of UHI [25].

Thomas, Sherin, Ansar and Zachariah (2014) divided the city of Kochi into Local climatic Zones for studying UHI. Twelve mobile surveys were conducted to quantify UHI intensity from 2011-13. UHI intensity in winter was found more than summer ranging from 4.6-3.7°C in pre-dawn and early night respectively. Maximum intensity was seen in the central compact midrise areas of the city while the open and sparsely built areas were comparatively cooler [26]. Nidhi and Puneeta (2015) conducted a study to determine the temperature of Bhatinda city and existence of UHI. Temperatures were recorded using Mextech Digital Thermometer at five rural areas and three urban areas of the city from Feb-April, 2015. ArcGIS 10.3 software was used to generate surface temperature maps of Bathinda city. The study exhibited strong correlation between the urban areas and UHI [27]. Another study done by Grover and Babu Singh (2015) compared UHI in two cities- Delhi and Mumbai by using Landsat 5TM image of 5 May, 2010 for Delhi and 17 April, 2010 image for Mumbai. The study revealed that built up and fallow lands recorded high temperatures compared to vegetated areas and water bodies. In Delhi max temperature recorded in west Delhi was 35°C while minimum of 24°C was recorded in the east at River Yamuna. In Mumbai the temperature recorded was: Coastal areas- 28-31°C; Bandra Kurla Complex- 31-36°C; Chhatrapati Shivaji International Airport- 38°C [28]. Goswami, Mohammad and Sattar (2016) did temporal assessment of remote sensing data for mapping UHI in Ahmedabad. LST data derived from MODIS data from 2003-2014 was analyzed which showed increase of temperature from 2.96-4.04°C in winter & in summer it varied from 1.27 -1.93°C. The study justified inverse relationship between the UHI and rural temperature observed during the study period [29].

Singh, Kikon and Verma (2017) studied the negative impact of urbanization on Lucknow city from 2002-2014 by using Landsat thermal data and field survey. The exhibited increased temperature and degradation of urban ecology are some of the urbanization aftereffects. Results indicated rise in temperature (0.75°C) due to land use change and anthropogenic causes mostly in the central portion of the city [30]. Kortharkar and Bagade (2018) measured canopy layer heat island in Nagpur city using local climate zone classification. The methodology described LCZs mapping, data collection technique using fixed station points and mobile traverse survey conducted from Dec 2015- February 2016 of winter season. Temperature due to UHI ranges from 1.76-4.09°C in compact low urban areas of Nagpur [31]. Mehrotra, Bardhan and Ramamritham (2018) in their study linkage between informal urban settlements such as slum urban form and surface urban heat island has been analyzed by spatial statistical analysis. LST is measured of the Mumbai city [32]. Sultana and Satyanarayana (2018) conducted a study which showed spatial relationship of land use and land cover changes and land surface temperature using remote sensing and geographical information techniques over 10 metropolitan areas of India. Landsat 7 Enhanced Thematic Mapper Plus images of these cities during winters from 2001-13 were analyzed which established existence of multiple UHIs and their increase in number in all cities due to increase in built up areas mostly in Lucknow, Jaipur and Nagpur, indicating rapid development in these cities. The authors suggested that UHI studies should be done for developing cities like Lucknow which will help urban planners in mitigating UHI effect through effective urban planning [33]. Chandramathy and Kitchley (2018) through their study have observed that areas with high density development typically have higher LST, whereas areas with more green cover typically have lower LST. This study further investigates the efficiency of various green cover types (trees and turfs) and the optimal green plot ratio to lower air temperature using Envi-met simulation software for the particular situation [34]. Soumendu Chatterjee et al (2019) in their study evaluated the performance of four different mitigation strategies to mitigate the effects of UHI





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phenomena. The study sites, which were selected from three different urban morphologies of open low-rise, compact low-rise, and mid-rise residential areas, were replicated by simulating the effects of various mitigation strategies, including cool pavement, cool roof, added urban vegetation, and cool city (a combination of the three former strategies). As per study, vegetation covers plays an important role in mitigating UHI [35].

The macro- and micro-level UHI study of Mumbai city was carried out by Aparna (2019). Higher LST values were observed in high-built regions compared to lower LST values in water and vegetation-covered areas. This study was successful in identifying the hot spots in the city and the main causes of these hot spots [36]. Ziaul and Pal (2020) conducted a study to assess the impact of the town's green infrastructure on improving microclimatic conditions and to assess the most effective mitigation method by modelling cool city with ENVI-met software [37]. Kalia (2021) studied the UHI phenomena in urban public space and suggested mitigation measures to mitigate it and make the place more comfortable. She has done comparative study of various cases that can be designed in an urban open space and simulated the designs to identify the best options which can improve thermal comfort [38]. Sood and Patil (2021) based on Literature review has created hypothetical built environment adopting UHI mitigation design strategies on a site. This design is tested through simulation which supports that through effective design strategies such as vegetation, cool roofs, green roofs, green walls etc. the negative impact of UHI can be minimized [39]. Rajan and Amirtham (2021) conducted a study validating presence of heat pockets in Chennai. The study suggested that to improve the comfort condition, the new developments should conside developing large canopy spaces to mitigate thermal heat stress [40]. The phenomenon of informal growth is associated with urbanization which leads to increase in anthropogenic activities for which the area was not planned leading to unplanned anthropogenic emissions causing rise in temperature, an attribute of Urban Heat Island as evident from the studies analyzed. Consequently, informal growth represents a defect in urban development in cities which is very significant in old cores of a city as unplanned growth has led to increased population area when compared to a newly planned area.

The literature review summarizes the nature of the studies conducted in India along with the description of data collection methodology which shows a shift from manual (Mobile Observation) approach to computer aided (Remote Sensing) approach. Remote sensing approach as evident from the studies analyzed gives an alternative, easy and most updated way against the traditional empirical analysis using the available data for climatic studies. Through computer support high resolution imagery is generated of land use/ land cover which gives more accurate and precise results. Further, with the use of simulation software the base model (existing condition on a site) can be compared with the hypothetical models (implementing UHI mitigation design strategies) of same site which will help urban designers, policy makers and architects in implementing the UHI mitigation strategies for achieving comfort. Also, through various studies it has been established that developing metropolitan cities such as Lucknow is experiencing multiple UHI effect on account of rapid urbanization, unplanned/informal growth, and increase in population density in the central core of the city accompanied with reduction in vegetation and open area [30] [33]. Accordingly, future research should be conducted in developing cities which will definitely contribute in confronting the issue of UHI in India. Thus, it can be concluded that developing country like India should focus on conducting research for analyzing the gravity of UHI phenomenon as the available research done in the country is not at par with the research conducted worldwide. All these efforts are worthy, as this will contribute in enhancing sustainable development in urban areas of a country.

CONCLUSION

This review examined the UHI studies in India along with its intensity with special emphasis on the methods adopted for its mapping. As established through the studies analyzed, the unplanned/informal urban growth is a prime factor responsible for increased urban temperature causing UHI effect, trailed by the thermal image. The UHI effect varied in different areas depending on city size, land use/land cover, topographic factors, vegetation, urbanization, atmospheric pollutants, anthropogenic heat, seasons of the year and also the prevalent meteorological conditions. Of this the more concerned is the relation between increased populace and city size which will become





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more severe with increasing urbanization. Most of the UHI studies are site specific and the extrapolation of the results to other climatic zones and geographical areas might be challenging. Leal Filho et al. (2017) stated that there is a need to better understand the UHI phenomenon and also how it affects individual regions and that there is a need to consider mitigation and adaptation strategies which take the particularities of each city into account so as to make them more UHI resilient [41]. Therefore, it is necessary to conduct research in each of the climatic zones of the country and also a uniform methodology for collecting data should be established for analyzing UHI formation and its intensity so that urban development norms and building regulations incorporate UHI mitigation measures to minimize its impact. Further simulation research should be used to establish relative contribution between the varied factors responsible for increased land temperature. The spatial structure of UHI is obtained through satellite data which should be paralleled with the value of ground-based measurements and involvement of numeric modelling for obtaining better results. Further, for assessing the impact of mitigation strategies on a selected urban space or site simulation of the existing model and the proposed model should be done for comparative analysis and thereby suggesting UHI mitigation strategies for adoption by the designers and planners.

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Table 1: Summary of UHI studies conducted in India

Author	Year	City & Year	Variable studied	Methods used
Mukherjee and Daniel [10]	1973	Pune and Mumbai	AT	Mobile observations
Mukherjee and Daniel [11]	1976	Mumbai AT		Mobile observations
Padmanabhamurthy and Bahl [12] [13]	1977 1979	Delhi	AT	Mobile observations
David Sundersingh [15]	1991	Madras	AT	Mobile observations
Vrishali Deosthali [16]	2000	Pune	AT	Mobile observations
Gopalakrishnan et al [17]	2002	Chennai, Bangalore and Hyderabad	AT ST	Manual
Chand et al [18]	2005	Hyderabad	LST	AATSR satellite data Field measurements
Lei et al [19]	2008	Mumbai	LST	Regional Atmosphere Modeling system
Devdas et al [20]	2008	Chennai	AT	Mobile survey
Ramachandra and Kumar [21]	2010	Bangalore	AT, LST	Remote Sensing- Landsat
Mohan et al [22]	2011	Delhi NCR	AT	Temperature Trend
Puneeta Pandey et al [23]	2012	Delhi	ST	MODIS satellite data
Borthakur et al [24]	2012	Guwahati	LST	Landsat imageries
Bajaj et al [25]	2012	Ahmedabad	LST	Landsat TM and ETM+
Thomas et al [26]	2014	Kochi	AT	LCZ approach
Sharma et al[27]	2015	Bathinda	ST	Field observation and ArcGIS 10.3 software
Grover et al Singh [28]	2015	Delhi and Mumbai	LST	Landsat 5TM image
Goswami et al [29]	2016	Ahmedabad	ST	MODIS satellite data
Prafull Singh, Noyingbeni Kikon, Pradipika Verma [30]	2017	Lucknow	LST	Landsat thermal data and field survey
Rajashree Kortharkar,	2018	Nagpur	AT	LCZ mapping and mobile





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Anurag Bagade [31]				traverse survey
Mehrotra et al [32]	2018	Mumbai ST LS1		LST measured
Sultana et al [33]	2018	Metropolitan cities	LST	Remote Sensing and GIS
I. Chandramathy and Jinu	2018	Madurai	LST	Satellite Data
Louishidha Kitchley [34]	2010		LSI	ENVI-met simulation
Soumendu Chatterjee, Ansar	2019	Kolkata	AT	Field study
Khan etal [35]	2019		Ai	ENVI-met simulation
Aparna Dwivedi [36]	2019	Mumbai	LST	Satellite Data
Apartia Dwrvedi [30]	2019		LST	ENVI-met simulation
Sk Ziaul, Swades Pal [37]	2020	West Bengal	LST	Satellite Data
	2020		LJI	ENVI-met simulation
Aastha Kalia [38]	2021	Murthal, Haryana	AT	Field study
	2021		Ai	ENVI-met simulation
Rishika Sood, Dr. Anjali S.	2021	Taleigao, near	AT	Weather Data
Patil [39]	2021	Panaji, Goa	Ai	ENVI-met simulation
Ebin Horrison Salal Rajan,	2021	Chennai	AT	Field study
Lilly Rose Amirtham [40]	2021		AI	ENVI-met simulation

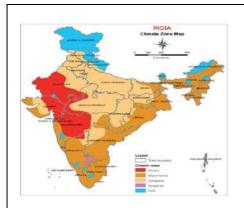


Figure 1: Map showing climatic zones in India. Source: Nation Building Code of India (2005)

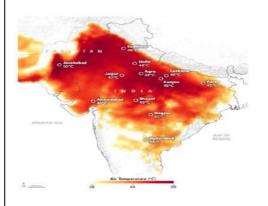


Figure 2: Image derived from GEOS shows temperature across India on June 10, 2019. Delhi reached 480 Celsius. Source:https://earthobservatory.nasa.gov/images/145167/heatwave-in-india





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RESEARCH ARTICLE

An Insight into the Therapeutic Potential of *Tinospora cordifolia* for **Antihyperlipidemic Activity**

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ABSTRACT

Current hyperlipidaemia treatments have a lot of drawbacks, thus researchers are looking for alternatives from medicinal herbs with antihyperlipidemic property. Natural medications derived from medicinal plants are believed to be effective and safer therapeutic option for hyperlipidaemia. There has been some evidence that Tinospora cordifolia (T. cordifolia) has hypolipidemic abilities. It is a perennial shrub found throughout India and it is considered as a pool of chemical phytoconstituents possessing medicinal values. T.cordifolia has a wide range of uses in ayurvedic and folk medicine for treatment and cure of several ailments. The isoquinoline alkaloid berberine, which is found in T. cordifolia root and stem bark, has an antihyperlipidemic action. The aim of this review is to probe into the accessible experimental studies on animals and human volunteering subjects eliciting antihyperlipidemic effect of Tinospora





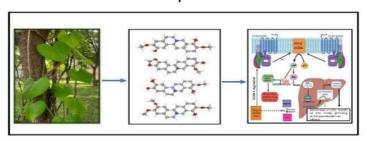
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cordifolia. Interpretation on the feasible mechanism of action of T.cordifolia and its extracts restraining hyperlipidaemia and its associated medical complications. The chemical components of T.cordifolia such as tetrahydro jatrorrhizine, tetrahydro columbamine, tetrahydropalmatine, and palmatine serves as an agonists of dopamine D1 receptors (DDR1), which play a crucial role in the activation of genes involved in lipid metabolising. Genes involved in fatty acid oxidation are controlled by the nuclear receptor Peroxisome proliferator-activated receptor alpha (PPAR- α).Preclinical and clinical studies showed that there is a notable reduction in Low Density Lipoprotien, Very Low Density Lipoprotien and triglyceride levels and increase in High Density Lipoprotien levels.

Pictorial Graphical Abstract



Keywords: Tinospora cordifolia, Hyperlipidaemia, Clinical trials, Natural Chemistry, Biological activity

INTRODUCTION

Hyperlipidaemia is a condition in which the blood lipid levels are abnormally high. Hyperlipidaemia is a more common condition, especially in the western hemisphere, but its occurrence is throughout the world. The elevation of plasma lipid levels is considered as the major risk factor associated with cardiovascular diseases. People with hyperlipidaemia have roughly twice the risk of developing cardiovascular diseases as compared with people having normal lipid levels. Cardiovascular diseases account for one third of the total deaths around the world. It is understood that it will be a major cause of death and disability worldwide in the upcoming years[1]. The National Cholesterol Education Panel's (NCEP) Adult program-3 (ATP-3) recommendations are currently being used to classify and determine therapy levels for hyperlipidaemia. In the research done by the students of Universidad Santiago de Cali, Colombia, the findings revealed that 33.8% of population who took part in the survey had hyperlipidaemia in one form or another, with males (50.6%) being more prone than women to have hyperlipidaemia (28.8%) [2]. Although statins are being used as hypolipidemic drugs, they produce significant side effects [3]. Ayurvedic herbs can serve as a preferable alternative, owing to their cost efficiency and fewer side effects. Tinospora cordifolia is one of the widely acknowledged and geographically isolated traditional ayurvedic plants. Tinospora cordifolia is a fresh perennial vine belonging to the family Menispermaceae. This glamorous wild vine is found throughout the tropical and subtropical zones of India at an altitude of 600 metres. Tinospora cordifolia is also described as Amrutaballi in Kannada, Seendil kodi in Tamil, Chittamrutu in Malayalam, Gurcha in Hindi and Giloy in English. Tinospora cordifolia contains a variety of phytoconstituents, including glycosides, steroids, aliphatic compounds, essential oils, fatty acid mixtures, calcium, phosphorus, proteins, palmatine, polysaccharides, tinocordifolioside, columbin, tinosporaside, berberine, tembeterine, jatrorhizine, phenylpropene disaccharides Alkaloids and terpenes abound in Tinospora cordifolia[4]. And this perennial shrub is mainly known for its pharmacological activities such as immunomodulator, cardio protective, antiulcer, antihyperlipidemic activity, antioxidant, anti-depressant, anticancer, anti-diabetic [5], antihyperlipidemic activity, antimalarial, antibacterial, allergic rhinitis, antipsychotic activity, nootropic effect, hepatoprotective, antidiarrheal, gastro protective and antifertility effect. Ayurvedic literatures describes various parts of guduchi as constituents of various medicinal





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preparations. According to Ayurveda, a substance's activity is determined by five characteristics: *rasa* (taste), *vipaka* (intestinal digestion and tissue metabolism), *guna* (substance property), virya (potency), and *prabhav* (specific action through specialised receptors). The five characteristics of *T.cordifolia* are Rasa – Tikta, kasaya; Guna – Laghu; Virya - Usna; Vipaka- Madhura; Karma-Balya, Dipana, Rasayana, Sangrahi, Tridosasamaka, Raktasodhaka, Jvaraghna[4]. *T.cordifolia* is mentioned in old Ayurvedic texts such as Charaka, Sushruta, Ashtang Sangraha, Bhava Prakash, and Dhanvantri Nighantu for treating fever, asthma, jaundice, leprosy, diabetes, dysentery, and other ailments. For generations, several tribes in India have employed Tinospora cordifolia as a traditional remedy.

METHODOLOGY

A search was conducted from 2002 to 2022 using PubMed and Google Scholar to gather information on the relationship between *Tinospora cordifolia* and its antihyperlipidemic potential. "*Tinospora cordifolia*," "antihyperlipidemic potential," pharmacological actions"," and "metabolites" are some of the terms utilised. Research and review articles were both included, although only works published in English were taken into consideration.

Hyperlipidaemia- A threat to human race

Hyperlipidemia is a set of disorders characterised by unusually high lipid (fat) levels in the blood. Hyperlipidemia is a condition in which the level or amount of cholesterol or triglycerides in plasma carrying lipoproteins exceeds the usual limit[8]. When these lipoproteins accumulate in the interarterial gaps of arteries emerging from the aorta, blood circulation from the heart is restricted. Atherosclerosis is the term for this phenomenon. Angina or chest pain is caused when these lipids deposit on the interarterial space of coronary artery (the artery that feeds blood to the heart). Myocardial Infarction, often known as heart attack, occurs when this artery becomes fully blocked due to increased lipoprotein deposition. The Lipoproteins have been divided into 6 classes namely chylomicrons, chylomicron, Very Low Density Lipoproteins (VLDL), Intermediate Density Lipoproteins (IDL), High Density Lipoproteins (HDL)[8]. Chylomicrons- They carry dietary triglyceride contents and are larger in size and density. VLDL- Very low density lipoproteins are tiny particles that transport triglycerides and cholesterol from the liver to the body's organs and tissues. IDL- VLDL when degraded by lipase enzymes give rise to Intermediate density lipoproteins. LDL- They are produced in the intestinal chyle in part and after VLDL lipolysis. It is known to be the primary cause of coronary heart disease (CHD)[9].

HDL- High density lipoproteins is commonly termed as good cholesterol since they carry cholesterol and other lipids from the tissues and organs to the liver. Thus, the raised levels of VLDL, IDL and LDL have the ability to cause atherosclerosis, while HDL provides protective function since HDL facilitates removal of cholesterol from tissues [8]. The other complications associated with hyperlipidaemia include Coronary Artery Disease (CAD), myocardial infarctions (MI), Angina pectoris, Cerebrovascular Accident (CVA). Recent treatment suggestions for managing hyperlipidemia include multi-target treatment and using medications based on the anticipated response. Statins are frequently used as the first line of treatment for hyperlipidemia. However, many patients, especially those with Familial hypercholesterolemia (FH), may not achieve the desired low-density lipoprotein-cholesterol (LDL-C) goals, necessitating the use of alternative multi-targeted therapy. Adverse reactions to statin medication, like myalgias and the less common rhabdomyolysis, can make it worse. For patients who are statin-resistant, statin-intolerant, or non-adherence patients, it may be beneficial to utilise multi-targeted plant-based alternatives due to these treatment-related problems.

Characteristics of T.cordifolia:

Taxonomical Classification [11]

Kingdom: Plantae, Division: Magnoliophyta, Class: Magnoliopsida,Order: Ranunculale, Family: Menispermeaceae, Genus: *Tinospora*, species: *cordifolia*.





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Morphological Features of *Tinospora cordifolia* Leaves

The leaves of *Tinospora cordifolia* are simple, membranous, alternate, chordate with broad sinus, acute at the apex. The fresh leaves look intensively green when seen in bulk whereas the matured leaves are yellowish in colour [12,13]. The top portion of the leaf appears bright green, while the lower part is pale or dull in appearance. And the leaves are bitter in taste [14].

Stem

The stems are long, glabrous, succulent climbing and filiform in nature. Whereas the *T.cordifolia* has its succulent green bark covered by a brown thin bark. Because of the longitudinal fractures along the rows of lentickes, the dried stem is cylindrical with a rough surface [4,12,13 and 14].

Flowers

T.cordifolia produces separate male and female flowers. Male flowers are found in the axils of little subulate bracts, while female flowers are solitary. The flowers are slightly yellowish green and consist of 6 sepals in which 3 inner sepals are larger and 3 outer sepals are tiny oblong in shape. And the flowers usually grow during summer season. [12,13,14]

Root

The roots of *T.cordifolia* are aerial in nature which are formed by matured branches and they are long, squarish, filiform and threadlike and the dried roots are bitter in taste and creamy white or greyish brown in colour[14,15].

Fruits

The fruits of *Tinospora cordifolia* are usually stalked red subglobose drupes, smooth, oval in shape and plumpy in nature and the flowers are formed during the winter [13].

Microscopic characteristics of T.cordifolia

l eaf

A dorsiventral arrangement with distinct palisade and spongy mesophyll can be seen in the cross section of the leaf's lamina. In the surface view epidermal cells are angular and consist of unicellular trichomes. Multicostate reticulate venation is observed. Veins are notable on the dorsal side. The cross section of the midrib portion of the leaf shows matured collateral vascular bundle at the centre[14,16].

Stem

The transverse section of the *T.cordifolia* stem reveals multi-layered cork, with thick lined brownish and compacted cells in the outer zone and the innermost zone consists of 3 to 4 rows of tangentially oriented colourless thinly lined cells. The cortex is fractured in some regions due to the opening of lenticels. The cortex is a broad parenchymatous region with columnar mucilage cells. The outer row of cortical cells are smaller than the inner one. Three to five rows of irregularly aligned, tangentially lengthened chlorenchymatous cells comprise the outer region of the cortex. The polygonal shaped inner zone of cortex consists of plenty of simple, ovoid, irregularly ovoid starch grains [4,14]. The transverse cut of the stem shows secondary anomalous growth. The xylem is united at the centre and stellate in nature. The stellate appearance of xylem with the phloem at the radii is due to the decimation of the pith and phloem consists of sieve tubes [14,16]. The stem consists of bicollateral vascular bundle with outwardly encircled pericyclic fibres surrounding the semi-circular phloem strips [4,14,16].

Root

The transverse section of aerial roots is characterized by arch shaped circular outline and consists of irregular multilayered exodermis cells. The cortex consists of a thick walled outer zone and parenchymatous inner zone. Xylem is arranged at the radii and phloem is present in four groups and the inner parenchymatous zone consists of mucilage cells and tannin containing cells. The caretenchyma is present above the phloem towards the peripheral [14,16].





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Phytoconstituents of T.cordifolia

T.cordifolia has been shown to have a number of phytochemical compounds that belong to various classes such as alkaloids, glycosides, diterpene lactones, sesquiterpenoid, phenolics, polysaccharides, steroids and aliphatic compounds. Several key phytoconstituents and their classes were mentioned in the Table 1 [17-36].

Contribution of T.cordifolia as tribal and folk medicine

India is one of the world's most biodiversity-rich countries. Various geographical regions and climatic conditions play a vital role in the growth of several medicinal plants in different parts of India. And there is several notable evidence that the tribes and folks of various regions of India use T.cordifolia for different medicinal preparations for curing several diseases. The tribal of Gujarat's Khedbrahma range used T.cordifolia decoction to treat dysentery and the Baiga tribes of Varanasi made fever-treating pills from the stem of T.cordifolia. [6,7]. The Nepal folks of the Himalayan range use Guduchi stem juice to cure diabetes, dysentery and genital disorders [37]. The native people belonging to different tribes of Mizoram are encountered with the usage of stem extracts of T.cordifolia for the therapy of diabetes, stomach ache, skin diseases and inflammation and fruit tonic prepared from T.codifolia is used as antirheumatic[38]. The tribal taluka of Thane district is detected with the usage of root extracts of *T.cordifolia* to cure fever and diabetes[39]. The usage of T.cordifolia for treating jaundice, rheumatoid arthritis, diabetes has been observed among the tribes of Rajouri district of Jammu and Kashmir[40]. The stem decoction of *T.cordifolia* is used to treat fever by the tribes of the Bigwada region of Rajasthan. The local people of Punjab's Patiala region used to prepare decoction from the leaves of *T.cordifolia* for treating fever. For the treatment of asthma, the villagers of Badala (U.P.) consume the juice of the stem orally with honey. The residents of Bhuvneshwar (Orissa) take warm juice of T.cordifolia root orally for the treatment of fever [41]. The in-dwellers of Yercaud hills consume the leaf extract of Tinospora cordifolia as a roborant for rejuvenation of health [42]. Tinospora cordifolia leaf extract is taken three to four times a day with black pepper by the Bagata tribe of Vishakapatnam as an antidote for snakebites[43].

Experimental studies

Preclinical studies

The experimental pharmacological studies have proven *in-vivo* antihyperlipidemic potential of different extracts of *T.cordifolia* Table 2[44-55]. Most of the animal studies were done in Albino rats of wistar strain which has shown a significant antihyperlipidemic effect. In a study conducted by Stanley *et al.* alcoholic and aqueous root extract of *T.cordifolia* were used which claimed to possess antihyperlipidemic effect[44,55]. According to the research of *Sparshadeep et al, T.cordifolia* extract (*T.C* Ext) has shown greater elevation of HDL-C (good cholesterol as it transports lipids to the liver for metabolism) compared to rosuvastatin. *Venkata et al.* reported that an ayurvedic polyherbal formulation containing *T.cordifolia* as one of the constituents was given at a dose of 400mg/kg over 7 days, preventing the elevation of serum total cholesterol, triglycerides, Low density lipoprotein-C, Very Low Density Lipoprotiens-C, and decrease of serum High Density Lipoprotien-C.[56]. *Ramalingam et al*, studied the antihyperlipidemic and antiper oxidative effect of diasulin which is a multi-herbal drug made up of ethanolic extracts comprising 10 medicinal plants, one of which is *T.cordifolia* and they reported that diasulin may help in preventing the progression of cardiovascular heart diseases(CHD)[57]. All this research has provided a valid scientific basis for consuming *T.cordifolia* for further clinical studies in humans which will be helpful in treatment of CHD.

Clinical studies

For a period of 14 days, patients suffering from hypertriglyceridemia were administered an aqueous extract of *T.Cordifolia* stem and their blood samples are obtained for the analysis of lipid profiles. After 14 days' time interval of administration of *T.cordifolia* extract there was a substantial reduction in Triglycerides, Very Low Density Lipoprotein and Low - density lipoprotein levels as well as a considerable increase in High Density Lipoprotein levels in hypertriglyceridemia patients. And it also implies that the interference *Tinospora cordifolia* led to decreased excretion of branched-chain amino acids (BCAA) and also in minimization of abnormal lipid biomes in hypertriglyceridemia patients[58]. In the study of *A.Shirolkar et al.*, implicates the treatment of alcohol induced hyperlipidemia using water extract of *Tinospora cordifolia* in alcoholic patients. *T.cordifolia* extract was given to the alcoholic patients about a period of 14 days. On the first and last day of the study the fasting blood and urine





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samples of the alcoholic patients were collected and analysed. The Guduchi sawras establishes antihyperlipidemic activity by depleting the triglycerides and LDL levels and increasing the HDL levels in the alcoholic patients and healthy individuals [59].

Proposed mechanisms indicating hypolipidemic potential:[59,60]

The chemical constituents such as $Tetrahydro\ Jatrorrhizine$, $Tetrahydro\ Columbamine$, $Tetrahydro\ palmatine$ and Palmatine of T.cordifolia acts as the agonist of Dopamine D1 receptor (DDR1) which has a significant role in activation of lipid metabolising genes. The nuclear receptor PPAR- α governs the expression of genes engaged in fatty acid oxidation. Therefore DDR1 agonist on binding with the receptor, leads to increase incyclic adenosine monophosphate(cAMP) levels that cause activation of protein kinase which in turn causes phosphorylation of PPAR α . The phosphorylation of PPAR α causes binding of phosphorylated PPAR α to Peroxisome Proliferator Activated Receptor Elements (PPARE) which leads to activation of transcription of multiple genes involved in lipid metabolism[60]. Another protein cAMP Response Element Binding (CREB) protein that controls the expression of carnitine transferases (which is crucial for mitochondrial β oxidation of long chain fatty acids) is regulated by the secondary messenger cAMP. It is also stated that T.cordifolia increases lipid metabolism by inhibiting dopamine metabolism. The mechanism of T.cordifolia is illustrated in Figure 1.

Phytoconstituents With Antihyperlipidemic Virtues

Tinospora cordifolia has numerous secondary phytoconstituents that possess antihyperlipidemic properties and other medicinal properties Figure 2. Berberine, an isoquinoline alkaloid present in the root and stem bark of *T.cordifolia* elicits antihyperlipidemic effect. It is accounted in the research of *Weigia Kong et al.* This research reveals that there is an increase in hepatic LDLR (Low Density Lipoprotein Receptor) function by mRNA expression which directly results in the elevation of plasma LDL clearance through receptor mediated endocytosis. The hepatic LDLR is mainly involved in plasma low density lipoprotein homeostasis. The main working mechanism underlying the effect of berberine on liver LDLR expression appears to be post-transcriptional regulation of LDLR mRNA[61]. The phytoconstituent berberine was extracted from *T.cordifolia* leaves of by HPLC analysis in a research conducted by *Prajwala et al.* The extraction was performed using methanolic extract of *T.cordifolia* leaves[62]. Palmatine is a protoberberine alkaloid present in abundance in the stem and roots of *T.cordifolia*, which does have antihyperlipidemic potential. The mechanism of action of palmatine and other secondary metabolites in antihyperlipidemic effect is represented in Figure 2. Palmatine is also claimed to possess antidiabetic potential [63].In the research of *Huma Ali et al.*, the secondary metabolite plamatine was extracted from *T.cordifolia* stem [64].

Salutary roles of *T.cordifolia* in Hyperlipidaemia complications and associated conditions Cardiovascular Heart Diseases

Arrhythmia is one of the common types of cardiovascular diseases serving as an important cause for mortality throughout the world, it is due to disruption in the heart's normal rhythm. *Ashish et al*, investigated the antiarrhythmic efficacy of alcoholic concentrate of *T.cordifolia* by generating arrhythmia in rats with calcium chloride [65]. They concluded that it showed a dose-dependent antiarrhythmic activity being compared with verapamil and they also suggested that it can be used in treatment of ventricular tachyarrhythmias.

Atherosclerosis

Atherosclerosis is one of the common CVD (Cardiovascular Disease) characterised by the hardening and constriction of arteries over time due to the formation of lesions by deposition of low density lipoprotein cholesterol [66,67]. And the atherosclerotic plaque blocks the proper flow of blood and results in causing stroke, heart attack, carotid artery stenosis, angina pectoris and myocardial infarction. Administration of aqueous extract of stems and root of *T.cordifolia* on diabetic induced rats resulted in the decreased levels of LDL-c level in their body and also there is an increased HDL levels in the blood[48,53,55]. Therefore, preventing LDL retention or accumulation on the arterial walls forming plaques (atherosclerotic plaques).





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Cardioprotective activity

Oxidative stress is caused by the uninhibited generation of oxygen-derived free radicals. Oxidation of by-products derived from normal metabolism can cause sizeable damages to proteins, DNA and lipids. The oxidative damage of lipids causes oxidative stress on the heart, which results in causing cardiovascular diseases [68]. The methanolic extract of *T.cordifolia* roots suppressed the intensity of myocardial infarction biomarkers like LDH, AST, CK-MB, ALT and TROPONIN in serum of isoprenaline treated rats and thereby establishing cardio protective activity [69]. In rats with myocardial injury caused by ischemia-reperfusion, the alcoholic decoction of the whole plant of *T.cordifolia* displays a concenteration -dependent cardioprotective action [70].

Transient Ischaemic Attack (TIA)

TIA is an acute loss of neurological function that arise when the brain's blood supply is temporarily blocked by blood clot or decreased flow which may be due to the block in blood vessels that supply blood to the brain. The symptoms last less than 24 hours. *Anshuman et al.* investigated the efficacy of an ethanolic concenterate of *T.cordifolia* upon acute cerebral ischaemia reperfusion in rats. The findings reveals that *T.cordifolia* has a protective role in cerebral ischemia reperfusion injury which could be related to its free radical scavenging property [71].

Obesity

According to WHO, obesity is described as an abnormal or excessive build-up of fat in the body that can be harmful to one's health. Obesity in adults is scientifically defined as the BMI greater than or equal to 25. Excessive calorie consumption, variations in dietary framework, sedentary behavior and alterations in the gut microbiome are some of the possible factors which can lead to obesity [72]. The administration of petroleum ether concenterate of *T.cordifolia* stem in cafeteria diet-prompted obese rats and sulpiride-induced obese rats resulted in significant weight loss, a decrease in serum cholesterol and TG levels, and increased High Density Lipoprotien levels in the body [73]. The dry stem powder of *T.cordifolia* when mixed with high fat diet feed and fed to Wister albino female rats shows a consistent weight loss. The *T.cordifolia* stem powder supplement reduces the accumulation of fat in the body and promotes lipolysis in rats [74].

Type 2 Diabetes

Diabetes Mellitus is most often accompanied with hyperglycaemia and hyperlipidaemia. By acting on vital metabolic enzymes implicated in glucose metabolism, aqueous root extract of *T.cordifolia* produced a hyperglycaemic effect in rats [75].

Hypercholesterolemia

Hypercholesterolemia is a susceptible factor for cardiovascular diseases. Familial Hypercholesterolemia is one of the inherited dyslipidemic conditions which increases the threat for cardiovascular diseases in children[76]. Administration of *Dihar* (a polyherbal formulation containing several herbs including *T.cordifolia*) to the stz instigated diabetic rats resulted in lowering the high cholesterol level in the body[47]. The administration of ethanolic extract of dried powder of roots, stems and leaves of *T.cordifolia* in male albino wistar rats showed lowering the serum LDL-c levels thereby resulting in Anti Hypercholesterolemia activity [56].

Other potential activities of *Tinospora cordifolia*Anticancer activity

In mice transplanted with EAC (Ehrlich ascites carcinoma), various doses of dichloromethane extract of *Tinospora cordifolia* elicit a dose-dependent attenuation of tumour development via inhibiting tumour cell proliferation. Which in turn results in retardation of increase in body weight due to EAC cells during early stage of carcinoma. *Tinospora cordifolia*'s anticancer potential is related to the presence of berberine, a vital phytoconstituent [77]. The metabolic extract of *Tinospora cordifolia* seemed to repress SNA12 and TIMP1 genes which is involved in differentiation, augmentation of HAC-7 colon cancer cells. Where the antitumor activity of *Tinospora cordifolia* extract was also compared with that of pure berberine [78].





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Hepatoprotective activity

Tinospora cordifoliasatwa, a polysaccharide preparation, exhibits hepatoprotective action in mature albino rats intoxicated with carbon tetrachloride. Repeated administration of CCI4 in rats causes liver toxicity which can be rectified by *Tinospora cordifolia* treatment which results in enzyme modulation by enhancing the tight bonding between transaminase and intermediate which is formed by pyridoxal phosphate resulting in Hepatoprotective activity [79]. *Tinospora cordifolia satwa* helps in regeneration of liver cells resulting in hepatoprotective activity in repeated acetaminophen dosed rats. The rats are routinely dosed with acetaminophen while also being administered with *Tinospora cordifoliasatwa*. Acetaminophen causes degradation of hepatocytes causing liver toxicity due to repeated administration. *Tinospora cordifolia* helps in reparating liver marker enzymes and bilirubin content [80].

Anti-oxidant activity

The methanolic concenterate of *Tinospora cordifolia* stem possess anti-oxidant activity which was proved using 1,1-diphenyl-1-picryl-hydrazyl-hydratefree radical screening activity by forming non radical DPPH (1,1-diphenyl-1-picryl-hydrazyl-hydrate) due to free radical scavenging, Superoxide radical scavenging action by destructing superoxide anions and also by reducing power assay in the in vitro models [81]. The antiradical action of *Tinospora cordifolia* was also proved by scavenging reactive oxygen species in several in vitro models such as DPPH scavenging activity, FRAP with the help of methanolic quintessence of leaves and acetic ether concenterate of stems of *Tinospora cordifolia*. The presence of important phytoconstituents including tannins and polyphenols in *T.cordifolia* contributes to the antioxidant action [82].

Anti-osteoporotic activity

The anti-osteoporotic ability of *Tinospora cordifolia* was established by the treatment of *Tinospora cordifolia* ethanolic extract on in vitro models such as human osteoblast cells, MG-63 and primary osteoblast cells isolated from the femur of rats which in turn stimulates osteogenesis and enhances the mineralization of a matrix similar to bone. Where the osteoblast growth was indicated by biomarkers [83]. The ethanolic extract of *Tinospora cordifolia* also increases the activity of serum alkaline phosphate which is a biomarker for osteoblast growth which successively indicates the prevention of bone loss in bone demineralized Sprague-dawley rats by ovariectomy or sham operated [84].

Immunomodulatory activity

Isolated aqueous extract of *Tinospora cordifolia* showed a dose dependent cytotoxic effect in B16F10 mouse melanoma cells by supervising the production of Interleukin-6 and also by increasing the productivity of nitrous oxide. *Tinospora cordifolia* plays a critical role in cytokinin network and it also enhances phagocytic proficiency of macrophages[87]. The water and hot water extracts of *Tinospora cordifolia* appeared to improve neutrophil phagocytic ability thereby resulting in enhancement of immunostimulatory activity in PMN phagocytic function studies. This immunomodulatory action of *Tinospora cordifolia* is due to the existance of certain phytoconstituents such as Cordifolioside A and Syringin [85].

CONCLUSION

In conclusion, the present review helps to support the traditionally claimed hypolipidemic, cardio protective and anti-atherosclerosis potential of *Tinospora cordifolia*. The synthetic drugs in the market which were used in the treatment if hyperlipidaemia is quite expensive and has side effects. Therefore *T.cordifolia* and their analogues hold promise as better alternatives and seems to be less toxic and economical. Among the natural materials, the discovery of novel medications relies mainly on medicinal plants. Even though evidence from preclinical and clinical studies supports the antihyperlipidemic claims of *T.cordifolia*, detailed investigation of active phytoconstituents is more important in revealing its complete mechanism of action, therapeutic efficacy, evaluation of its safety and its interaction with conventional drugs in order to propose *Tinospora cordifolia* as antihyperlipidemic agent. Hope this review will be helpful and endorse further research studies.





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Summary

Tinospora cordifolia is a potent herb with a wide range of therapeutic applications. Commonly addressed as Guduchi and Giloy has several clinical evidences to prove its medicinal activities against diabetes, ulcer, cancer, fertility problems, inflammatory responses, osteoporosis etc. Its extracts are rich in alkaloid content that is responsible for its preventive and curative management of hyperlipidaemia. On the basis of this review, it can be summarised that T.cordifolia predominantly helps in decreasing the lipid levels in hyperlipidemic patients and it also justifies the investigation of the bioactive phytoconstituents, traditional uses and exploring its effects against hyperlipidaemia and associated medical conditions. Because of the diverse formulations, complicated phytochemical compositions, and extrinsic noxious substances, evaluating the safety of botanical products for human use has been challenging. Preclinical studies showed that there is a notable reduction in LDL, VLDL and triglyceride levels and increase in HDL levels. Most of the animal studies proving its antihyperlipidemic effect were done in animals in which diabetes and hyperlipidaemia were in coexisting condition. Experimentation on humans illustrated its possible mechanism of action by regulating lipid metabolism. In addition to that it also indirectly reduces the activity of cyclic adenosine monophosphate (cAMP)-responsive element-binding protein H (CREBH) which regulates the expression of apolipoprotein B, an essential protein in VLDL and also regulates the expression sterol regulatory element-binding protein 1c(SREBP-1c) protein involved in lipid production. In the published studies there is no specific information on adverse events. No mortality and side effects were recorded. This is so far the best evidence available proving its anti-hyperlipidemic property claiming its constructive role in hyperlipidaemia treatment.

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Abbreviations

PPAR- α : Peroxisome proliferator-activated receptor alpha; NCEP: National Cholesterol Education Panel ;VLDL: Very Low Density Lipoproteins ;IDL: Intermediate Density Lipoproteins ;HDL: High Density Lipoproteins ;CHD: Coronary heart disease; CAD: Coronary Artery Disease ;MI: Myocardial infarctions; CVA: Cerebrovascular Accident ;FH: Familial hypercholesterolemia.

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Table 1: Secondary Phytoconstituents of Tinospora Cordifolia

Secondary Metabolite	Chemical Constituents	References
	Furanolactone diterpene, Furanolactone	
Terpenoids	clerodane diterpene, Furanoid diterpene,	17,18,19,20,21,22,23,24,25,
	Tinosporon ,cordifolioside A, B and C,	26
	cordifolioside D and E, Sesquiterpene	





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	tinocordifolin, Tinocordioside, Tinosporaside, Sesquiterpene glucoside tinocordifolioside, columbin	
Alkaloids	Magnoflorine, choline, Jatrorrhizine, Berberine, Palmitine, Tembetarine	27,28,29
Steroids	20a-Hydroxy ecdysone, ß-Sitosterol, d- sitosterol, g-sitosterol, makisterone,	30,31
Lignans	3 (a, 4-dihydroxy-3-methoxybenzyl)-4-(4- hydroxy-3-methoxybenzyl)	32
Others	Heptocosanal, Tinosporic acid, Arabinogalactan, Tinosponone, Tinocordioside, Giloin	33,34,35,36

Table 2: In vivo study of antihyperlipidemic potential of different extracts of T. cordifolia

Part	Extract	Animal model	Dosage	Period	Effect	Remarks/Results	Reference
Root	Aqueous	Alloxan	5g/kg	6 weeks	Нуро	Showed highest	
		induced			lipide	hypolipidemic effect at 5g /kg	[44]
		diabetic albino			mic	even though it is done for	
		male rats				7.5g/kg	
	Alcoholic						
		Alloxan	100mg/	6 weeks	Нуро		[45]
		induced	kg		lipide	Lowered the cholesterol,	
		diabetic albino			mic	phospholipids and free fatty	
	Aqueous	male rats				acids. Weight loss was	
						decreased.	
		Cholesterol		30days			
		induced	5ml/kg	Treatm			[46]
		hyperlipidemia		ent	Antih		
		in albino male		period	yperli	Markedly decreased the	
	Dihar	rats			pide	triglyceride level and Elevated	
	(Polyher				mic	the HDL level. No side effects	
	bal prep.)	Streptozotocin	_	6 weeks		and mortality of rats.	[47]
		induced					
		diabetic male					
		wistar rats.			Antih	Produced significant decrease	
Stem	Aqueous				yperli	in Hypercholesterolemia and	
					pide	Hypertriglyceridemia in	
			200mg/	14 days	mic	diabetic rats. It had a role in	[48]
		Streptozotocin	kg			lipid metabolism.	
		induced					
		diabetic and					
	Aqueous,	diet induced				Increased HDL-c level,	
	Alcoholic	hyperlipidemia			Antih	lowered LDL-c level,	
		rats(male		30days	yperli	decreased serum creatine	
		albino wistar	200,	Treatm	pide	kinase and free fatty acid level.	[49]
		rats)	400mg/	ent	mic	It has the potential to control	
			kg	period		the diabetes associated	
	Aqueous	Streptozotocin				hyperlipidemic conditions	
		induced				effectively.	[50]





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		11 1 11 11 1	l	(0.1	I	T	
		diabetic albino	400 /	60 days		Top	
		rats.	400mg/			TCErt ameliorate the	
	Methanol		kg		Antih	metabolic derangements in	r=
	ic	Fructose diet			yperli	lipid metabolism.	[51]
		induced		30 days	pide		
		diabetic albino	200mg/		mic		
		rats	kg,			Lipid metabolism is influenced	
	Aqueous		400mg/			by extract. Total lipids,	
		Hyperlipidemi	kg		Нуро	cholesterol, triglycerides, and	[52]
		a induced		_	lipide	free fatty acids in the liver	
		Sprague			mic	were all decreased.	
	Powder	Dawley rats				Reduction in serum cholesterol	
	form					level which may be due to	[53]
			_	60 days	Нуро	interference of cholesterol with	
					lipide	residues at the absorption site	
Whole	Aqueous	Fructose			mic	in GIT. HDL levels not	[54]
Plant	·	induced				affected by TCErt.	
		diabetic Male		50 days		Significant HDL level increase,	
		wistar rats	_			decreased LDL, VLDL,	
					Antih	Cholesterol and triglycerides.	
		Jersey			yperli		
Dried	Ethanolic	crossbred	3g/kg,6		pide	Powder along with fenugreek	[55]
Root,	Ethanono	lactating cows.	g/kg		mic	was effective in lowering the	[00]
Stem,		lactating covvs.	9/10	14 days	11110	total cholesterol and LDL	
Leaves				Treatm		plasma levels	
extract		WR339 induced		ent	Нуро	pidsiria ieveis	
CATIACT		Hyperlipidemi		period	lipide	Significantly reduced LDL,	
		a cholesterol	100mg/	period	mic	VLDL and triglyceride level	
		male albino	_		TITIC	and enhanced HDL level.	
		wistar rats	kg 200mg/			and enhanced FIDE level.	
		Wistai Tats	_		Antih		
			kg		· ·		
		A.II			yperli		
		Alloxan			pide	Lowers hypertriglyceridemia	
		induced			mic,	and hypercholesterolemia.	
		diabetic rats			cardi		
					oprot		
					ective		
					, anti-		
					ather		
					oscler		
					osis		
					Нуро		
					lipide		
					mic		



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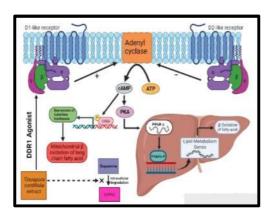


Figure 1: Diagrammatic representation of mechanism of action of phytoconstituents in T.C extract responsible for eliciting antihyperlipidemic activity [58, 59].

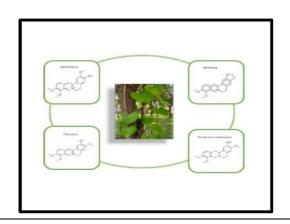


Figure 2: Secondary metabolites of *Tinospora* cordifolia possessing antihyperlipidemic virtues.

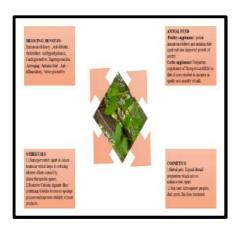


Figure 3: Schematic format indicating the uses of Tinospora cordifola.





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RESEARCH ARTICLE

Effect of Different Levels of Pruning and Growth Regulators on Growth and Yield of Ixora (Ixora coccinea L.) cv. Red

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ABSTRACT

A field experiment was conducted to study the "Effect of different levels of pruning and growth regulators on growth and yield of Ixora (Ixora coccinea L.) Cv. Red" at Manickapuram village, Thiruchirappalli District, Tamil Nadu during the year 2020 - 21. The treatment comprises of three different levels of pruning at 30 cm, 45 cm and 60 cm along with three different growth regulators viz., GA₃ @ 150 ppm, NAA @ 150 ppm and Ethrel @ 150 ppm along and without application of growth regulator to pruned plants (Control). The experiment was laid out in Factorial Randomized Block Design with three replications comprising of twelve treatments. Observation on various vegetative and flower yield parameters were assessed. Among the various treatments, it was significantly increased all the parameters in the treatment (T₅) when pruning was done at 45 cm height above the ground level along with foliar application of GA₃ @ 150 ppm recorded maximum in all growth parameters viz., plant height (79.95 cm), number of branches (75.86), canopy spread N-S (112.44 cm) and E-W (109.24 cm), number of leaves (2752.59) and leaf area index (2.64 cm²) at 180 days after pruning. With regard to yield parameters viz., number of flower heads plant-1 (36.73), number of florets flower head-1 (54.18), fresh weight of flower head (9.01 g) and flower yield (330.93 g plant⁻¹). From the above studies, it is revealed that pruning at 45 cm along with foliar application of GA₃ @ 150 ppm could be recommended for enhanced growth and higher yield in Ixora coccinea.

Keywords: Ixora, Pruning, Growth regulators.





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INTRODUCTION

Ixora (Flame of woods, Flame of the forest, Jungle flame, Burning love, Scarlet Ixora, Jungle gerenium) botanically named as Ixora coccinea belongs to the Rubiaceae family. Now, it is identified as one of the commercial loose flower crop grown in India. The word 'Ixora' is a Portuguese version of Iswari, name of Goddes Parvati to which the flowers of Ixora coccinea are offered, while the word 'coccinea' is a Latin word meaning Scarlet coloured (Kulawe et al., 2019). Ixora coccinea L. is a perennial hedging plant common in the most parts of tropical and subtropical regions. The plants are notable for its bright coloured flowers which are composed of small blooms massed together into dense, flat topped flower heads. It bears flowers which are numerous having bright scarlet colour in dense, sessile corymbiform cymes (Baliga and Kurian, 2012). They have tiny conspicuous and colorful shades of white, yellow, pink and scarlet orange. Though flowers are not fragrant and they are very popular due to its attractive color and good keeping quality. In Tamilnadu, among the growers community, traditional Ixora flowers are commercially grown as "loose flower" to multipurpose use especially garland makeovers for spiritual and religious offerings, decoration purpose and in landscape gardening which are mainly used for making hedges, foundation planting, potted plants, massed in flowering beds or grown as a specimen plant. It also has a good export potential and popularity on account of wide adaptability and increasing demand during the festival occasions. Pruning, it is an essential method since antique times for restarting growth of plants. Pruning is an invigorating process which promotes growth by releasing a plant's internal chemical substances it allows new branches to grow. Moreover it drives energy towards to sprout multiple new shoots to get increased, as a resulted in maximized canopy spread and flower production by activation of physiological activity (James Sellmer et al., 2004). Plant growths are regulated by phytohormones that presumably exert their influence on particular metabolic reactions in the target tissue via receptor molecules. Besides those natural phyto-hormones, the group of growth regulators that modify a plant in its growth and developmental behavior without inducing phyto-toxic or malformative effects includes synthetic substances such as the growth regulators. According to the PGR's have quicker effect on flower plants to modify growth, foliage colour as well as flower yield (Mukesh kumar et al., 2021). At the same time concluding the right level of pruning is very important in maximizing more number of branches hitherto production of flowers is enhanced due to pruning along with appropriate quantity of growth regulators as foliar application which modifies the flower yield. Regulation of flowering in Ixora has immense practical value. In these aspect pruning with appropriate height in addition to this utilization of plant growth regulators with right quantity for maximizing the growth and yield of Ixora. Keeping this in view, the present investigation was therefore taken to study the effect of different levels of pruning and foliar application of growth regulators on growth and yield of Ixora (Ixora coccinea L.).

MATERIALS AND METHODS

The present study was formulated with three levels of pruning heights along with three growth regulators *viz.*, GA₃, NAA and Ethrel were taken up for enhancing growth and yield of Ixora (*Ixora coccinea* L.) cv. Red. This experiment was carried out in the farmer's field at Manickapuram village, Mannachanallur Taluk, Thiruchirappalli District, Tamil Nadu during the period of 2020-21. The field is geographically located at (10° 92′) North Latitude and (78° 75′) East Longitude at an altitude. The maximum temperature of the location reaches up to 34-36°C and minimum is 22-20°C. The experiment was laid out in Factorial Randomized Block Design (FRBD) with 12 treatments and replicated thrice. Two years old *Ixora* plants with the spacing of 2 m × 2 m were taken for this experimental study. Conventional pruning at different heights *viz.*, 30 cm, 45 cm and 60 cm is carried out during second fortnight of April, 2020. After pruning, when the new shoots were developed with sufficient number of leaves, the freshly prepared Gibberllic acid (GA₃), Naphthalene acetic acid (NAA), Ethrel each @ 150 ppm were sprayed and without application of growth regulator to pruned plants (Control) is followed as per the treatment schedule. The data on vegetative and flower yield parameters were taken at periodic intervals. Observations are recorded on the selected tagged plants for each treatment in each replication and the mean data is statistically analyzed by adopting the standard procedure given by Panse and Sukhatme (1985). The treatment details are presented in Table 1.





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RESULT AND DISCUSSION

The findings of the investigation undertaken with a view to study the effect of different levels of pruning height and foliar application of certain growth regulators on growth and yield parameters of Ixora (*Ixora coccinea* L.) are presented in Table 2 and Table 3.

Effect of different pruning levels

The factor responsible for growth in a plant is depending upon climate, soil, cultural manipulations and their interactions. On that way optimum pruning height play a prime role in deciding the growth and yield of Ixora. Acquiring Knowledge on this aspect, it's very necessary to manipulate the plants physiology, which may eventually lead to the maximum production. In Table 2, among the different levels of pruning heights clearly shows that the maximum growth performed in P2 in all vegetative parameters viz., Plant height (77.39 cm at 180 DAP), number of branches (57.22), canopy spread (104.37 cm in North - South) and (100.96 cm in East - West), number of leaves (2032.40) and leaf area index (2.09 cm²) at 180 DAP respectively. This increase in growth might be due to higher accumulation of polysaccharide content, reduced apical dominance, increased light intensity, good aeration thus results in improved plant growth parameters. It was reported by Raghuram Pawar et al., (2019) in Jasmine and Zekavati (2013) in Rose. In Table 3, Yield parameters viz., number of flower heads (34.50 plant-1), number of florets (43.99 flower head-1), fresh weight of single flower head (7.45 g) and flower yield (257.02 g plant-1) was recorded as superior when Pruning has been carried out at 45 cm height respectively. Pruning height significantly increased the number of branches which may be due to accumulation of cytokinin which are the source to produce new shoots, which might in turn leads to more number of flowers. This might be due to the accelerated photosynthetic mobility from the source to the sink as influenced by growth hormones are diverting its energy for production of new shoots and that leads to produce more nodes resulted from maximizing the number of flower heads. This increase in flower heads could be due to the fact that pruning helps to broaden the uptake of C:N ratio, thus stimulates the flowering and increasing the vigor of plant. These results are coinciding with Ghulam et al., (2004) in Rose, Sushree Choudhury et al., (2019) in Jasmine and Vijai ananth and Rameshkumar (2012) in Nerium.

Effect of growth regulators

It is explicit from the data in (Table 2 and 3), clearly shows that increase in growth and yield of *Ixora* were obtained in the treatment G1 with the foliar application of gibberellic acid (GA₃) @ 150 ppm. The increase in plant height (79.60 cm), number of branches (71.71), canopy spread (110.63 cm in North - South and 107.40 cm in East - West), number of leaves (2592.54) and leaf area index (2.51 cm²) at 180 DAP. When compare to other growth harmones like NAA and Etheral application of gibberellic acid significantly increased in terms of all the growth parameters. These increases in growth parameters are due to the early production of florigin in gibberellins. GA3 is component of florigin which initiates the growth in various ways. This, results are in the conformity with the findings of Dhanasekaran (2019) in Jasmine and Anil K Singh et al., (2019) in Rose. The maximum plant height, number of branches, number of leaves and leaf area might be attributed to the enhanced vegetative growth in early phase attributed by exogenous application of GA3 which would have favoured the increased photosynthesis and CO2 fixation ultimately it increased the number of leaves and increased photosynthetic efficiency of the plant. These results are coinciding with Rakshana et al., (2020) in Carnation and Kadam et al., (2020) in Gaillardia. Yield parameters viz., number of flower heads (35.38 plant-1), number of florets (50.33 flower head-1), fresh weight of single flower head (8.47 g) and flower yield (299.66 g plant⁻¹) was noticed with the increased dose of foliar application of GA₃ at 150 ppm. These findings are coincided with the results of Pragnya Paramita Mishra et al., (2018) in China aster. The increase in number of flower heads, fresh weight of flowerhead, number of florets and flower yield plant-1 were obtained with the foliar application of Gibberellic acid (GA₃) @ 150 ppm. The maximum yield were obtained due to the production of more number of secondary shoots at early stage, with this foliar application of GA3 @ 150 ppm profusely increased all the yield parameters. These findings are coinciding with Shobana (2014) and Sridhar et al., (2013) in Jasmine.





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Interaction effect of pruning levels and growth regulators

The individual effect of different levels of pruning and growth regulators significantly influenced the growth and yield parameters of Ixora (Table 2 and 3). This influence was reflected in interaction also. In the present study revealed that the increasing in vegetative parameters was occurred in the treatment when pruning is carried out at 45 cm height along with foliar application of GA₃ @ 150 ppm. Whereas minimum performance in growth was significantly ineffectiveness is observed in untreated of growth regulators in different levels of pruning. The treatment T₅ (P₂ G₁) with the combination effect of different levels of pruning at 45 cm along with foliar spray of gibberellic acid @ 150 ppm was revealed as the best treatment in terms of growth and flower yield characters over the rest of other treatment combinations. Table 2 and 3 shows the result of interaction effect of different levels of pruning and foliar application of GA3, increases the plant height (79.95 cm), maximum number of branches (75.86), canopy spread (112.44 cm in North to South) and (109.24 cm in East to West), number of leaves (2752.59) and leaf area index (2.64 cm²) at 180 DAP. Among the different cultural operations pruning is one of the fruitful horticulture technique which is being followed in many flower crops. It is a useful method since antique times for restarting growth of plants (Kumaresan et al., 2017). As a result of pruning, number of new shoots get increased which resulted in increased flower yield by activation of physiological activity. When the developing new shoots appears from the plants which are simultaneously sprayed with application of growth regulators especially. Pruning at 45 cm height enhanced maximum growth in Ixora which might be due to the accelerated mobility of photosynthetic from the source to the sink as influenced by growth hormone released or synthesized due to higher plant growth and its allocating energy for production of new shoots and due to increase in nodes resulted from cell elongation and cell division. Similarly these findings are coincided with Vijai ananth and Rameshkumar (2012) in Nerium and Maske et al., (2018) in Mogra.

These increased vegetative characters are significantly influenced due to the foliar application of plant growth regulators were absorbed by the leaves and these are immediately translocated into xylem and phloem tissues in which it is distributed to all parts of the plants. Among all the growth regulators viz., GA3, NAA and Ethrel, the effect of GA3 increased a significant role in all the characters, when compared to foliar application of NAA and Ethrel. GA3, it is a perfect plant growth regulator has been used to change the entire plant growth and its specific plant responses to growth and yield. These are evolved by the way of application of GA3 at various right intervals. Gibberllic acid also play an important role in modifying the growth and flowering pattern in Ixora plants and its utilization of optimum quantity of GA₃ can promote, inhibits or quantitatively modifies growth and development in plants. Application of gibberellic acid induce active cell division and cell elongation increase the auxin level of tissues and enhances the conversion of tryptophan to IAA, which in turn cause active cell division and cell elongation. Growth might also be increased due to osmotic uptake of water and nutrients under the influence of gibberellins which maintain swelling force against the softening of cell wall and thereby increasing the maximum of growth in all vegetative characters. Similar findings are revealed by Nomita Laishram et al., (2020) in Marigold and Sendhilnathan et al., (2020) in Carnation. Yield parameters viz., number of flower heads (36.73 plant-1), number of florets (54.18 flower head-1), fresh weight of single flower head (9.01 g) and flower yield (330.93 g plant-1) are recorded as superior than all other treatments with respect to optimum pruning height along with application of gibberellic acid which enhances the yield parameters. The increase in yield might be due to accumulation of initial higher polysaccharide content in plants. On the other hand gibberellic acid caused rapid cell elongation and improved source sink relation resulting in enhanced vigorous vegetation in Ixora. Maximum number of flower heads and number of florets increased might be due to the application of gibberellic acid stimulates the endogenous gibberellins level in the plants. The major changes in flower yield can be explained by the fact that gibberellic acid reducing the juvenile period of plants because of its higher capacity of cell division and cell elongation which caused early maturity in plants. Similar results were reported by Harkulkar et al., (2022) in Jasmine and Sendhilnathan et al., (2019) in Rose.





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CONCLUSION

Based on the present investigation findings, it can be concluded that, Pruning at 45 cm height along with foliar application of gibberellic acid (GA_3) @ 150 ppm concentration served as a best treatment with respect to performance of all growth and yield parameters in Ixora (Ixora coccinea L.) Cv. Red.

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Table 1: The Details of the treatments are furnished below.

Treatments	Details
T ₁ (P ₁ G ₁)	Pruning at 30 cm height + GA3@150 ppm
T ₂ (P ₁ G ₂)	Pruning at 30 cm height + NAA @ 150 ppm
T ₃ (P ₁ G ₃)	Pruning at 30 cm height + Ethrel @ 150 ppm
T ₄ (P ₁ G ₄)	Pruning at 30 cm height + Control
T ₅ (P ₂ G ₁)	Pruning at 45 cm height + GA3@150 ppm
T ₆ (P ₂ G ₂)	Pruning at 45 cm height + NAA @ 150 ppm
T ₇ (P ₂ G ₃)	Pruning at 45 cm height + Ethrel @ 150 ppm
T ₈ (P ₂ G ₄)	Pruning at 45cm height + Control
T ₉ (P ₃ G ₁)	Pruning at 60 cm height + GA3@150 ppm
T ₁₀ (P ₃ G ₂)	Pruning at 60 cm height + NAA @ 150 ppm
T ₁₁ (P ₃ G ₃)	Pruning at 60 cm height + Ethrel @ 150 ppm
T ₁₂ (P ₃ G ₄)	Pruning at 60 cm height + Control

Table 2: Effect of different levels of pruning height and growth regulators on growth parameters in *Ixora (Ixora coccinea* L.). Cv. Red

Plant Treatments Height (cm 180 (DAP)		Number of branches plant ⁻¹	Canopy spread (cm) 180 (DAP)		Number of leaves 180 (DAP)	Leaf area index (cm²)
	100 (DA1)	180 (DAP)	N - S	E – W	100 (DA1)	180 (DAP)
			Pruning height (F	P)		
P ₁	62.76	47.95	98.36	94.89	1656.85	1.81
P_2	77.39	57.22	104.37	100.96	2032.40	2.09
P ₃	92.54	53.08	102.58	99.12	1872.36	1.97
S.Ed	0.64	0.50	0.95	0.90	62.59	0.02
C.D (p=0.05)	1.27	1.00	1.88	1.81	125.18	0.03





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	Growth regulators (G)							
G ₁	79.60	71.71	110.63	107.40	2592.54	2.51		
G ₂	78.10	46.87	99.89	96.36	1632.30	1.79		
G₃	77.09	59.29	105.27	101.88	2112.42	2.15		
G ₄	75.46	33.13	91.28	87.66	1078.21	1.38		
S.Ed	0.82	0.57	1.09	1.04	189.29	0.02		
C.D (p=0.05)	1.64	1.15	2.17	2.09	378.58	0.04		
			Interaction (P×G)				
T ₁ (P ₁ G ₁)	65.39	67.58	108.84	105.56	2432.51	2.40		
T ₂ (P ₁ G ₂)	63.60	42.73	98.12	94.52	1472.26	1.67		
T ₃ (P ₁ G ₃)	61.35	55.16	103.49	100.04	1952.39	2.04		
T4 (P1 G4)	60.70	26.34	82.99	79.45	770.24	1.15		
T ₅ (P ₂ G ₁)	79.95	75.86	112.44	109.24	2752.59	2.64		
T ₆ (P ₂ G ₂)	78.23	51.00	101.68	98.22	1792.33	1.90		
T ₇ (P ₂ G ₃)	76.59	63.43	107.07	103.72	2272.46	2.27		
T ₈ (P ₂ G ₄)	74.78	38.59	96.32	92.69	1312.22	1.55		
T9 (P3 G1)	93.47	71.70	110.63	107.42	2592.53	2.50		
T ₁₀ (P ₃ G ₂)	92.46	46.88	99.89	96.36	1632.31	1.80		
T ₁₁ (P ₃ G ₃)	93.34	59.29	105.27	101.89	2112.42	2.15		
T ₁₂ (P ₃ G ₄)	90.91	34.46	94.54	90.84	1152.19	1.44		
S.Ed	1.43	1.00	1.89	1.81	82.59	0.04		
C.D (p=0.05)	2.85	2.00	3.76	3.63	165.19	0.07		

Table 3: Effect of different levels of pruning height and growth regulators on yield parameters in *Ixora (Ixora coccinea* L.). Cv. Red

Treatment	Number of flower	Number of florets	Fresh weight of single	Flower yield plant-1					
Heatment	heads (plant-1)	(flower head-1)	flower head (g)	(g)					
	Pruning height (P)								
P ₁	33.64	39.68	6.77	227.74					
P ₂	34.50	43.99	7.45	257.02					
P ₃	33.87	41.46	7.05	238.78					
S.Ed	0.12	0.37	0.03	2.23					
C.D (p=0.05)	0.21	0.76	0.08	4.47					
		Growth regulators (0	<u>a</u>)						
G ₁	35.38	50.33	8.47	299.66					
G ₂	33.54	38.83	6.63	222.37					
G₃	34.17	44.07	7.52	256.95					
G ₄	32.92	33.62	5.75	189.29					
S.Ed	0.13	0.42	0.04	2.58					
C.D (p=0.05)	0.24	0.87	0.09	5.16					
		Interaction (P×G)							
T ₁ (P ₁ G ₁)	34.59	47.53	8.10	280.17					
$T_2(P_1 G_2)$	33.32	37.02	6.33	210.91					
T ₃ (P ₁ G ₃)	33.95	42.33	7.22	245.11					
T4 (P1 G4)	32.70	31.87	5.45	178.21					
T5 (P2 G1)	36.73	54.18	9.01	330.93					
T ₆ (P ₂ G ₂)	33.76	40.61	6.94	234.29					
T ₇ (P ₂ G ₃)	34.40	45.81	7.82	269.00					





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T ₈ (P ₂ G ₄)	33.13	35.38	6.05	200.43				
T9 (P3 G1)	34.82	49.29	8.31	289.35				
T ₁₀ (P ₃ G ₂)	33.55	38.87	6.64	222.77				
T ₁₁ (P ₃ G ₃)	34.18	44.08	7.53	257.37				
T ₁₂ (P ₃ G ₄)	32.93	33.63	5.75	189.34				
S.Ed	0.22	0.75	0.08	4.47				
C.D (p=0.05)	0.42	1.52	0.17	8.95				





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REVIEW ARTICLE

Herbal Plant Supplements used As Aphrodisiacs - A Systemic Review

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ABSTRACT

Despite progress made in modern bioscience herbal drugs constitute a significant share of all the officially recognized systems of health in India viz. Ayurveda, Yoga, Unani, Siddha, Homeopathy and Naturopathy. An aphrodisiac could be a substance that increases concupiscence, sexual attraction, pleasure, or sexual behavior. Various synthetic drugs like Sildenafil citrate, Tadalafil citrate, Vardenafil, etc are comfortable with treat ED but these drugs even have fatal side effects. Male sexual problems include libido, erection, ejaculation and orgasm Other side effects include facial flushing, stomach upset, blurred vision and sensitivity to light which usually occur at higher doses. This review contains an assortment of in excess of 50 therapeutically significant plants which are sexual enhancer in nature as well as used to treat Erectile Dysfunction. All the herbal plants during this review are verified from the Scientific Literatures and also as traditional book.

Keywords: Herbal Drugs, Aphrodisiacs, concupiscence, Erectile Dysfunction (ED), libido

INTRODUCTION

Herbal plants are the main part of our life. Any plant organ like root, stem, leaves, blossoms and natural products are utilized in some restorative, culinary or fragrant properties. A spice is a plant that is esteemed for characteristics like restorative properties, flavor, fragrance or the concentrates and like. Customary medication or society medication rehearses depend on the utilization of plants and plant extricates. Natural plants might be annuals, biennials and





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perennials[1]. Yearly herbaceous plants life period is half-one year just, that is the plant pass on totally toward the finish of the developing season or when they have blossomed and fruited. Biennial home grown plants life period is 1 to 2 years.

Role of Herbal Drugs

Herbal medications establish a significant portion of the multitude of formally perceived frameworks of wellbeing in India. Presently, there is no different classification of natural medications or dietary enhancements, according to the Indian Drugs Act. Proof based herbals are broadly utilized in the different frameworks and produced, according to the pharmacopoeial rules, by an efficient industry. Huge fundamental and clinical examination has been completed on the restorative plants and their details, with the cutting edge strategies in various Institutes/Universities. Consequently, the worldwide information about Ayurveda and Indian herbals will ideally be improved by data on the proof base of these plants.

Therapeutic Plants and Disease Prevention

Therapeutic plants have been utilized in medical services since days of yore. Studies have been completed around the world to confirm their viability and a portion of the discoveries have prompted the creation of plant-based prescriptions. The worldwide market worth of therapeutic plant items surpasses \$100 billion for every annum[2]. A correlation is drawn between the 'entire populace' and 'high-hazard' methodologies. The helpfulness of the normal factor approach as a technique for drawing in other wellbeing advertisers in proliferate the beliefs of restorative plants is featured.

Aphrodisiacs

An Aphrodisiacs is a substance that increments sexual longing, physical allure, sexual joy, or sexual conduct. "Aphrodisiacs" comes from the name of Aphrodite, the Greek goddess of adoration. Aphrodisiacs have been utilized and pursued for millennia and have regularly been produced using everything from minerals to food to plants[3]. Substances range from an assortment of plants, flavors, food sources, and engineered synthetics. In this way, they can be ordered by their synthetic properties. Regular aphrodisiacs like liquor are additionally ordered into plant-based and non-plant-based substances. Unnatural aphrodisiacs like delight or methamphetamine are named those that are produced to mimic a characteristic substance.

Sexual Problems

Male sexual issues incorporate moxie, erection, discharge and climax. Male sexual reaction cycle is called typical if every one of the means are opportune and consecutively if any of coming up next isn't in arrangement or deferred than it leads sexual brokenness in people. Male sexual brokenness (MSD) could be brought about by different variables[4].

These include

Mental disorders (performance uneasiness, stressed relationship, despondency, stress, responsibility and dread of sexual disappointment), androgen insufficiencies (testosterone inadequacy, hyper supportive of lactinemia), persistent ailments (diabetes, hypertension, vascular in sufficiency (atherosclerosis, venous spillage), penile Disease (peyronie's, priapism, phinosis, smooth muscle brokenness), pelvic medical procedure (to address blood vessel or in stream problem), Neurological disorders (Parkinson's sickness, stroke, cerebral injury Alzheimer's spinal rope or nerve injury), drugs (side effects)(anti-hypertensive's, focal specialists, mental meds, antiulcer, antidepressants, and enemies of androgens), way of life (persistent liquor misuse, cigarette smoking), maturing (decline in hormonal level with age) and fundamental diseases (cardiac, hepatic, renal aspiratory, malignancy, metabolic, post-organ relocate).

History of Aphrodisiacs

The name comes from the Greek aphrodisiakon, for example "sexual, aphrodisiacs", from aphrodisios, for example "relating to Aphrodite", the Greek goddess of adoration. All through mankind's set of experiences, food, beverages, and practices have gained notoriety for making sex more feasible as well as pleasurable. Notwithstanding, from a





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recorded and logical angle, the supposed outcomes might have been primarily because of simple conviction by their clients that they would be compelling (self-influenced consequence)[5]. Similarly, many meds are accounted for to influence charisma in conflicting oridiopathic ways: upgrading or lessening generally sexual longing relying upon the circumstance of the subject Antiquated human advancements like Chinese, Indian, Egyptian, Roman, and Greek societies accepted that specific substances could give the way to working on sexual craving, sexual delight, and additionally sexual conduct. This was significant on the grounds that a few men experienced erectile dysfunction and couldn't repeat.

Need of Aphrodisiac Drugs

The course of human origination is ridiculously wasteful and totally relies upon possibility. There is just a single sperm out of billion found the opportunity to experience with egg. What's more, on the off chance that one sperm does at last finishing the excursion, it might have the energy left for preparation[6]. There are many types of male infertility, incorporates erectile dysfunction, sexual insufficiency, decrease in sperm thickness and semen volume and unusual sperm morphology. There are confirmations to show that sperm counts have been declining in the course of the most recent 50 years, with a resulting expansion in male infertility. The worldwide frequency of couple barrenness is assessed at 10 to 15 %. Of men matured 40-70 years, an expected 34.8% have moderate to finish erectile dysfunction.

Uses of Aphrodisiacs

Aphrodisiacs ordinarily can be categorized as one of three unique classes. Such substances are regularly suspected to libido, power, or delight.

- **Libido**: Low longing is the most common sexual issue for moderately aged ladies, influencing almost 70% of ladies during midlife. However low libido is something that can influence people, all things considered. Enhancements are regularly showcased to build moxie, albeit the viability of these substances stays sketchy[7].
- **Potency**: Aphrodisiacs are frequently additionally suspected to increment sexual strength and execution. A few substances are showcased to further develop endurance, grease, and perseverance.
- Sexual Pleasure: Finally, a few aphrodisiacs are promoted as having the option to work on by and large sexual joy. Such items are thought to make sex more pleasant. Indeed, even individuals who partake in a solid sexual coexistence might discover the bait of more charming sex a valid justification to attempt an aphrodisiac[8].

Impact of Aphrodisiacs

The unavoidable issue is accomplish aphrodisiacs really work some proof backings the potential impacts of some regular enhancements, for example, horny goat weed, yet note that a considerable lot of these discoveries come from creature studies.

Types of Aphrodisiacs

Aphrodisiacs will in general fall into a couple of various classifications as far as how they should function. For instance

- A. Spicy substances, for example, hot bean stew peppers, are at times considered aphrodisiacs to actuate sensations of excitement since they increment internal heat level[9].
- B. Conceptive organs of specific creatures, like eggs or creature gonads, are some of the time accepted to increment sexual power or execution.
- C. Food varieties that bring out the faculties, including sights, smell, and taste, are frequently expected to have sexual enhancer properties. By stimulating the faculties, such substances are thought to assist individuals with feeling all the more physically excited[10].
- D. Uncommon and intriguing food sources or flavors are frequently seen as having sexual enhancer impacts.
- E. Food varieties that take after sexual organs are now and again accepted to be invigorating, including certain products of the soil[11].





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Others Ways

There are some things that you just can do this can help:

- a. Eat a healthy diet. Examination has shown that after an eating routine that underlines lean meat, vegetables, organic product, and entire grains can further develop chemical levels, blood stream, and nerve working [12].
- b. Get ordinary exercise. Studies have shown that actual work gainfully affects sexual wellbeing. Indeed, even little explosions of movement can assist with by and large sexual working.
- c. Control your feelings of anxiety. Stress can truly affect drive, so discover approaches to oversee feelings of anxiety viably. Great self-care and methods like reflection, care, profound breathing, and representation can be useful for bringing down pressure[13].

Causes For Impotence

Sexual dysfunction is a genuine clinical and social manifestation that happens in 10-52% of men and 25-63% of ladies. ED, the primary explanation of male feebleness, is considered as quite possibly the main general medical condition, since it influences an incredible level of men. ED is characterized as the steady failure to get or keep an erection for palatable sexual relations. An expected 20-30 million men experience the ill effects of some level of sexual brokenness. It happens generally in moderately aged and more established men. Feebleness happens in half of men with diabetes mellitus. Atherosclerosis is the reason for around 40% of ED in men more established than 50 years.

Mechanism of Sexual Behavior: Modern Perspective

Our comprehension of the interaction and commencement of sexual excitement is tracking down a clearer premise, which comes from confirmations in both preclinical and clinical investigations. Sexual excitement is reliant upon neural (tactile and intellectual), hormonal, and hereditary elements, something characterized in Ayurveda also however utilizing a logical language appropriate to this age[14].

Cerebrum and Neurochemical Basis of Sexual Behavior

Drugs influencing sexuality can either follow up on the central nervous system (Brain) or potentially on the peripheral nervous system. Medications influencing the cerebrum and probably sex focuses are by and large credited with an expansion or lessening in sexual excitement. Medications that influence fringe nerves won't influence excitement straightforwardly yet may influence sexual capacity[15].

Nitric Oxide-Based Mechanism of Sexual Behavior

Nitric oxide (NO) is an abnormal administrative particle playing the double part as an auxiliary courier/synapse. It has been involved in different physiological capacities. Discoveries so far demonstrate that NO may likewise be a significant neuronal courier[16]. Recent studies recommend that NO is a significant physiological boost for unwinding of penile vasculature and trabecular smooth muscle, fundamental for penile erection. Unwinding of the trabecular smooth muscle of the corpus cavernosa prompts a diminished vascular opposition and expanded blood stream to the penis.

Androgen-Based Mechanism of Sexual Behavior

Androgens assume an essential part in the advancement of optional male sexual organs like the epididymis, vas deferens, original vesicle, prostate, and the penis. Besides, androgens are required for pubescence, male fruitfulness, and male sexual capacity[17]. Testosterone is the essential androgen emitted by the testicles. Testosterone is blended in the Leydig cells of the testicles, invigorated by luteinizing chemical (LH). One of the chief impacts of testosterone inside the testicles is the incitement of spermatogenesis in seminiferous tubules. The testosterone-or dihydrotestosterone-receptor complex next crosses the atomic film, ties to DNA, and animates new mRNA combination and, during this way, new protein blend. The impact of testosterone on drive might require transformation of testosterone to estradiol within the nerve center.





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Ayurveda And Concept of Aphrodisiacs

Conventional Ayurvedic composition arranged aphrodisiac within the accompanying five classifications; some of plants are given as references to each kind of the helpful class characterized[18].

- 1. Medications which enhancement the quantity of semen or vitalize the formation of semen for example, Microstylis wallichii, Roscoea procera, Polygonatum verticillatum, cowitch, and Asparagus racemosus.
- 2. Drugs which decontaminate and work on the character of semen for instance, costusroot, Myrica nagi, sesame, Vetiveria zizanioides, and Anthocephalus cadamba.
- 3. Drugs which improve ejaculatory works as an example, Strychnos nuxvomica, marijuana, nutmeg, and mogdad coffee.
- 4. Drugs deferring the hour of discharge or improving ejaculatory execution for instance, Sida cordifolia, Asparagus racemosus, Cinnamomum tamala, anacyclus pyrethrum, Mucuna pruriens, and Cannabis sativum.
- 5. Medications should provoke sexual need, to be determine, ashwagandha, Asparagus racemosus, datura stramonium, anacyclus pyrethrum, Hibiscus abelmoschus, and along with Opium.

Medicines that cause Damage due to Self-Medication -

Issues will generally happen when personal attempts to self-analyze and self-cure without legitimate interview. As in case with the Sildenafil Citrate, more normally known as Viagra and different names, which was initially expected to be used among heart patients. Nonetheless, it unintended affected the blood stream to the penis and hence clad to be exceptionally accepted for treating erectile dysfunction. Be that because it may, on the off chance that somebody self sedate, it would really harm his/her veins and finish up harming the heart and other crucial organs. A variety of botanicals is known to have potential effects the sexual functions, supporting more established cases and offering more current expectations. This survey, while assessing different elements that control sexual function, recognizes an assortment of botanicals that might be possibly valuable in treating sexual dysfunction. The investigation of this study can be to understand the aphrodisiac class of medicines or any food that excites the sexual impulse prompts veneral want and builds delight and execution.

Study Review

The systemic study was done in a basic appraisal and assessment of all exploration concentrates on that address a particular issue and the articles were chosen from Pubmed, NCBI, Researchgate, Sciencedirect, etc., with the Inclusion critetia as following,

- Guideline for including the utilization of Aphrodisiacs.
- To assess the potential and regenerative wellbeing of Aphrodisiac Drugs for people.
- Methods and Equipments are utilized to break down the aphrodisiac potential and action of home grown medications.
- Aphrodisiacs are utilized for treating/relieving reason or supporting/further developing body capacities.
- Invivo studies are fundamental to provide the capacity to assess a medication's attributes, including physiological and biochemical cycles.
- Models can remember reads for human models. Aphrodisiacs for human utilize as it were.

Natural Supplements as Aphrodisiacs

There are various regular substances that are frequently included as fixings in supplements that are promoted as aphrodisiacs[19].

- Ambrien, a substance emitted from the stomach related arrangement of sperm whales, has been found in creature studies to expand testosterone levels, bringing about expanded sexual interest and conduct.
- Horny goat weed, a kind of blooming plant frequently utilized in Chinese medication, has been utilized as a love potion to expand drive and joy.
- Yohimbe could be a substance that comes from the bark of trees that fill in Africa and India. It is accessible by remedy within the U.S. to treat sexual brokenness, yet it's likewise accessible in some over-the-counter enhancements. Be that as it may, yohimbe can likewise prompt incidental effects including sporadic heartbeat and uneasiness.





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- **Ginseng**, the foundation of a plant that is frequently utilized in correlative medication and as a dietary enhancement, is likewise at times utilized as a sexual enhancer, in spite of the fact that its viability for this intention is hazy.
- **Bufotenin** is found in the skin and organs of Bufo frogs. It is usually utilized in West Indian and Chinese societies. West Indian societies use it as an Aphrodisiacs called 'Love Stone'. Chinese societies use bufotenin as heart medicine called Chan su. Exploration shows that it can negatively affect pulse.

Pot reports are blended in with half of clients asserting an expansion in sexual longing and sexual joy while the other half reports no impact. Weed strain, utilization, and individual affectability are known elements that influence results[20].

Food as Aphrodisiacs

Food sources are frequently promoted for their aphrodisiac properties also. Such food sources have been utilized all through mankind's set of experiences to increment sexual delight, execution, and moxie[21]. Food varieties utilized as aphrodisiacs can incorporate all the more ordinarily discovered food sources or uncommon food varieties that are substantially harder to obtain. Different substances presumed to go about as aphrodisiacs may include #Pomegranate, #Figs, #Pine nuts, #Almonds, pecans #Maca, #Pumpkin, #Watermelon, #Celery, #Bananas, #Garlic, #Salmon, #Coffee, #Asparagus, #Saffron, #Avocados, #Honey, #Strawberries etc., While logical proof to show that these food varieties really impact sexual longing is missing, many are suspected to work due to things like potassium, zinc, and phytochemicals that can further develop prosperity.

Medicinal Plants with Aphrodisiac Potential

A portion of the therapeutic plants have demonstrated to have a conventional just as logically demonstrated Aphrodisiacs that can upgrade energy, increment moxie, improve sexual execution and help to expand the power of lovemaking [22]. A concise report of plants has been tried for sexual enhancer potential is recorded [23] as given in the Table 01.

Current Statuses of Aphrodisiacs

Modern life vogue and bound natural openings have brought about male infertility. The activating factors turn out varying kinds of disturbances that straight forwardly or in a roundabout way cause sexual dysfunctions. Male impotence conjointly known as erectile dysfunction (ED) might be a typical ailment that influences the sexual lifetime of abundant men around the world. ED is illustrated in light of the fact that the powerlessness of an individual to acknowledge and keep an erection sufficient for normally agreeable intercourse. This literary appraisal discusses regarding aphrodisiac potential of plants, its biological science name, Common name, family, parts used and chemical constituents, which are useful for investigator to development new aphrodisiac formulations.

Current Scenario of Aphrodisiacs and Its Marketed Product Status

Several PDE5 inhibitors have been authorized by the FDA as physician endorsed medications to treat ED, for example, Avanafil (Stendra), sildenafil (Viagra), tadalafil (Cialis), and vardenafil (Levitra, Staxyn). These medications work by loosening up muscles and boosting blood stream in the penis, making it simpler to get and keep up with [25]. The most normal symptoms of PDE5 inhibitors are retinal deformities, cerebral pains, flushing, dazedness, visual unsettling influences, nasal clog and different problems brought about by restraint of PDE6. The utilization of natural drugs with high selectivity might be rotating. Some notable home grown aphrodisiacs are family Allium sativum, Alpinia galangal, Anacardium occidentale, Anacyclus pyrethrum, Butea frondosa, Caesalpinia benthamiana, Cannabis sativa, Chlorophylum borivilianum, Citrullus lanatus, Eurycoma longifolia, Ginkgo biloba, Hibiscus sabdariffa, and so forth Many ayurvedic details have been professed to have a sex-animating impact in Indian medication framework. A couple of models are Vita-ex Capsules and Stay-On Capsules. Some hormonal arrangements likewise guarantee sex-invigorating impact like testosterone, androgen, aptoge. As per World Health Organization (WHO) Sexual wellbeing is prime to the physical or enthusiastic wellbeing and prosperity of individuals, couples and families and to the socio-monetarily advancement of networks and nations.





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Aphrodisiac Activity Evaluation Techniques

The remedial worth, adequacy and poisonousness of medication might be assessed in creatures tentatively, trailed by clinical preliminaries. Both in-vivo and in-vitro creature models are utilized to get to the sexual enhancer action in trial creature (rodent, mice, guinea pig, and so on[24].

Mating Behavior Test

This test would be completed by KOLODNY and GOVIER, and altered by WALSH.

- **a. Mount Frequency** Mount recurrence is characterized as the quantity of mounts without intromission from the hour of presentation of the female until discharge.
- **b. Mount Latency** Mount inactivity is the time stretch between the presentation of the female and the principal mount by the male.
- **c. Intromission Frequency** Intromission recurrence is the quantity of intromission from the hour of presentation of female until discharge.
- **d. Intromission Latency** Intromission dormancy is the time span from the hour of acquaintance of the female with the main intromission by the male.
- **e. Post Ejaculatory Interval** Post ejaculatory span is the time stretch among discharge and the main intromission of the accompanying series.
- **f. Copulatory Rate** Copulatory rate measured as the (No. of mounts+No. of Intromissions/time from first mount till discharge)
- g. Record of Libido % Index of Libido measured as the (number of mated/number of matched)*100

Penile Microcirculation Study

Penile ultrasound and duplex doppler, empowers portrayal of blood vessel and venous blood stream inside the erect penis. It also takes into consideration an enumerate study of the corpora cavernosa recognizing calcification or scarring inside the erectile tissue of the penis. Laser Doppler Flow Meter may utilize for assurance of penile microcirculation.

Intra Cavernosal Pressure (ICP) Study

Erectile work in rodents can be assessed by estimating the intra cavernosal pressure (ICP). Practically speaking, ICP can be checked after electrical incitement of the enormous nerves (CNs). The blood vessel pressing factor of the carotid supply route (the mean blood vessel pressure) is utilized as the reference for ICP.

Orientation Behavior

Orientation in Behaviorism is intellectually coordinating consideration or truly coordinating the body towards a boost or some likeness thereof, like light or a commotion. Orientation can basically be depicted as a reaction to an improvement.

Tests for Potency

A power test is a clinical trial, the consequences of which can set up whether an individual (the denounced for this situation) is fit for taking part in sexual demonstrations or not and regardless of whether might have submitted the offense he has been accused of.

Biochemical Test

Assurance of the,

a. Testicular and Serum Cholesterol (Chod PAP) Method

Cholesterol (CHOD-PAP): Enzymatic Colorimetric approach to determine Serum Cholesterol. NS Biotec cholesterol reagent is expected for the in vitro quantitative assurance of absolute cholesterol in serum and plasma on both robotized and manual frameworks.





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b. Hormonal Determination Assay

A blood test is one of the most widely recognized approaches to test chemical levels. This test can recognize testosterone, estrogen, cortisol, and thyroid levels. You should arrange a test that is explicit to your sex, as a ladies' chemical test will search for various degrees of sex chemicals than a men's test.

In-vitro Sperm Preservation

Semen cryopreservation (usually called sperm banking or sperm freezing) is a method to protect sperm cells. Semen can be utilized effectively endlessly after cryopreservation. For human sperm, the longest announced effective stockpiling is 24 years.

In-vivo Sperm Count

Normal semen contains 40 million to 300 million sperm for each milliliter. A low sperm count is viewed as anything somewhere within the range of 10 and 20 million sperm for every milliliter. Twenty million sperm for each milliliter might be sufficient for pregnancy if the sperm are sound.

Fructose Content in Seminal Vesicles

Fructose in semen is the wellspring of energy for all sperm exercises. The higher of sperm focus, and imperativeness and motility requested more energy, so fructose is lower. Normal original fructose focus affirms the job of testosterone and the capacity of vesicles and vas deferens is ordinary.

DISCUSSIONS AND CONCLUSION

In modern time a few variables like weight, uneasiness, stress conditions, different illness conditions and extreme utilization of meds of manufactured beginning has expanded the danger of erectile dysfunction. Sexual issues are identified with sexual longing and male erectile dysfunction. Successful treatment of sexual dysfunction may work on sexual connections, yet in addition the general personal satisfaction. Writing overview of the referred to plants affirmed that strong aphrodisiac capability of above mentioned plants. The manufactured plans accessible in market, however they are showing great action in sexual dysfunction yet they have huge antagonistic impact subsequently natural medications are liked over engineered medication to stay away from serious side effects. Plants, since ancient occasions, have been utilized globally across variety of societies all through the referred to civic establishments as a significant and safe regular wellspring of prescriptions and as specialists of remedial, mechanical and natural utilities. Sexual capacity is a significant part of personal satisfaction and subject for prosperity in people. Current interest in traditional medication has prompted the fast turn of events and investigations of numerous home grown cures utilized for sexual dysfunction. Novel data accumulated from the current information is significant in protecting people native information just as in the revelation of novel possible mixtures with promising aphrodisiac potential. Therefore, this survey has been arranged to furnish another aggregation of plants with explicit use as aphrodisiacs just in various nations.

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Table 1: List of few Medicinal plants with Aphrodisiac potential

Name of the Plant	Common Names	Family	Parts used	Chemical Constituents	References
Abelmoschus	Bhindi, Okra,	Malvaceae	Root	Isoguereitrin	[22]
esculantus	Gumbo	iviatvaceae	Root	Isoquercitrin	[22]
Allium sativum	Garlic	Liliaceae	Bulb	Allicin	[23]
Anacyclus pyrethrum	Akarakarabha	Compositae	Root	Sapathulenol	[25]
Argyreia nervosa	Adhoguda	Convolvulaceae	Root	Homogenate	[23]
Asparagus racemosus	Asparagus	Liliaceae	Root	Shatavarin	[22][20]
Arachis hypogaea	Peanut	Fabaceae	Seeds	Arachin, Conarachin	[22]
Dianharia adulia	Litangan / Chilchi	A conthococo	Coodo	Blepharin,	[10]
Blepharis edulis	Utangan/ Shikhi	Acanthaceae	Seeds	Benoxazolone	[18]
Butea frondonsa	Flame-of-the-	Papilionaceae	Whole	Palasonin,	[20]





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	forest		plant	Monospermin	
Chenopodium album	White goosefoot	Chenopadiaceae	Seed	Apocortinoids, xyloside	[21]
Chlorophytum borivilianum	Safed Musli, white musli	Liliaceae	Roots	Sapogenin	[22][25]
Crossandra infundibuliformis	Firecracker Flower	Acanthaceae	Leaf	-	[23]
Cucurbita pepo	Pumpkin	Cucurbetaceae	Seed	Linolenic acid, Palmitic acid	[22]
Dactylorhiza hatagirea	Marsh Orchis	Orchidaceae	Root	-	[20]
Durio zibenthinus	Durian Fruit	Bombacaceae	Fresh fruit	β-sitosterol	[18]
Glycyrrhiza glabra	Liquorice	Papilionaceae/L eguminoaceae	Root	Glycyrrhizin	[22]
Abrus preacatorium	Ganja	Fabaceae	Seed	Triterpene glycosides, abrin	[18]
Abrus precatorius	Crab's Eye	Paplionaceae	Seed	Triterpene glycosides, pinitol	[20]
Abutilon indicum	Thuthi	Malvaceae	Seed, root,bark, leaf	β-sitosterol	[22][23]
Acacia catechu	Catechu	Mimosaceae	Heartwood		[93]
Acorus calamus	Sweet flag	Araceae	Rhizome	Acorine, pinene and camphene	[17]
Aconitum heterophyllum	Attesh	Ranunculaceae	Root	Streptozotocin	[18]
Achyranthes aspera	Apamarg, Latjeera	Amaranthaceae	Root	Achyranthine	[20]
Bombax ceiba	Silk-Cotton Tree	Bombacaceae	Bark	Steroids, saponins	[22][23]
Boesenbergia rotunda	Temu kunci	Zingeberaceae	Rhizomes	Boesenbergin, krachaizin, panduratin, and pinostrobin	[19]
Amaranthus spinosus	Chaulai	Amaranthaceae	Leaves, Whole plant		[20]
Bauhinia tomentosa	Manja Mandaram	Caesalpiniaceae	Seed	Alpha tocopherol	[20]
Bussea occidentalis	Kpayeli	Caesalpiniaceae	Bark, seed		[22]
Carica papaya	Papita	Caricaceae	Fruit	Oleanolic acid	[21][25]
Cannabis indica	Indian hemp	Cannabinaceae	Leaf		[22]
Celastrus paniculatus	black oil, climbing staff, intellect	Celastraceae	Whole plant		[18]
Cola nitida	Kola nut	Sterculiaceae	Seed		[20]
Cucumis callosus	Melon, Muskmelon, Cantaloupe, Honeydew, Sugar melon	Cucurbitaceae	Fruit		[19]
	Mussii	Hypoxidaceae (or)	Rhizome	Curculigoside	[17]
Curculigo orchioides	Musali	Amaryllidaceae	11111201110	o an ourngoon as	





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Daucus carota	Carrot	Umbelliferae	Root		[21][22]
Emblica officinalis	Emblic	Euphorbiaceae	Fruit	Quercetin, vitamin C	[22]
Euadenia eminens	Dinsinkro	Capparidaceae	Root		[20]
Euphorbia hirta	Asthma weed, Cat's hair, Egele, Nonan kurchiya, Odane inenmili	Euphorbiaceae	Whole plant	Glycosides	[20][22]
Eurycoma longifolia	Tongkat Ali	Simarubaceae	Whole plant	Eurycomanone, Quassionoids	[18]
Fadogia agrestis	Black aphrodisiac	Rubiaceae	Stem	Phenolics compounds, anthraquinones	[22]
Ficus arnottiana	Paras Pipal	Moraceae	Bark	β-sitosterol, tannins	[22]
Flueggea virosa	White berry bush	Euphorbiaceae	Whole Plant	Bergenin	[23]
Garcinia afzelii	Bitter kola	Guttiferae	Bark		[22]
Gmelina arborea	Coomb teak	Verbenaceae	Fruit	β- sitosterol, quercetin	[20]
Hibiscus rosa-sinesis	China rose	Malvaceae	Leaf	Ethanimidic acid	[23]
Hygrophila auriculata	Gokulakanta, Neeramulli, Marsh Barbel	Acanthaceae	Seeds	Lupeol, butelin	[23]
Ipomoea mauritiana	Giant potato	Convolvulaceae	Root		[17]
Jatropha curcas	Purging Nut, Barbados Nut, Physic Nut	Euphorbiaceae	Leaves		[22][23]
Linum usitatissimum	Alsi	Linaceae	Seed	Berberine	[20]
Mallotus philippensis	Kamala Tree, Red Kamala, Kumkum Tree	Euphorbiaceae	Fruit	Isorottlerin, Acetyl rottelerine	[19]
Mangifera indica	Mango	Anacardiaceae	Bark	Vitamin C, carotenoids,	[18]
Mezoneuron benthamianum	Senegal	Caesalpiniaceae	Twig or Stem	Deoxycaesaldekarin C	[25]
Orchis latifolia	Munjaataka	Orchidaceae	Roots	Saponins, tannins	[22]
Panax ginseng	Ginseng	Araliaceae	Root	Ginsenosides	[23]
Papaver somniferum	Poppy plant	Papaveraceae	Flower	Noscapine	[17]
Punica granatum	Anar	Punicaceae	Fruit	Methyl pelletierine, pseudo-pelletierine	[23]
Psoralea corylifolia	Bavaci	Fabaceae	Fruit	Psoralen, Isopsoralen	[22]
Saccharum spontaneum	Kasa	Poaceae	Root stock		[22]
Santalum album	Sandal wood	Santalaceae	Heart wood	Santene, Teresantalol	[19]
Scindapsus officinalis	Gajapipali	Arecaceae	Fruit		[23]
Sida cordifolia	Countary mallow	Malvaceae	Root, seed	Sidasterone A, sidasterone B	[22]
Solanum nigrum	Aguaragua	Solanaceae	Berries	Solasonine, alpha- & beta-solanigrine	[23]
Tamarindus indica	Tamarind	Fabaceae	Bark	Tartaric acid	[22]
Terminalia arjuna	Arjuna	Combretaceae	Bark	Arjunic acid, arjunolic acid, arjungenin	[23]





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Turrea heterophylla	Ahunanyakwa	Meliaceae	Root, Seed, bark		[19]
Valeriana jatamansi	Jatamansi	Valerianaceae	Root		[18]
Withania somnifera	Ashwagandha	Solanaceae	Root	Withanolides, Withaferin A	[18][19]
Wrightia tinctoria	Ivory tree	Apocynaceae	Leaf, Seed, bark	Indigotin, indirubin	[22]
Ziziphusabyssin ica.	Magarya	Rhamnaceae	Leaves		[18]
Zingiber officinale.	Gingembre	Zingeberaceae	Rhizome	Zingiberene, β- bisabolene	[23]





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RESEARCH ARTICLE

Serious Cardiovascular Adverse **Events Associated** Hydroxychloroguine / Chloroguine Alone or with Azithromycin in Patients with Covid -19: A Pharmacovigilance Analysis of the FDA Adverse Event Reporting System (FAERS)

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ABSTRACT

Study has shown that the combination of azithromycin and hydroxychloroquine (HCQ) are effective for COVID 19. However, FDA has already issued an EUA for the use of these drugs on covid-19 patients. In this study, we have reviewed the reported cases of serious cardiovascular events associated with HCQ/CQ and azithromycin in the FAERS database to summarize the case characteristics and to estimate SCAE reporting associated with combination therapy for COVID-19 patients. The study found that composite SCAEs, TdP/QTc prolongation, and ventricular arrhythmia were reported evenly more often in adverse event reports listing HCQ/CQ than in reports listing other alternative therapies. Therefore HCQ/CQ monotherapy and HCQ/CQ + azithromycin combination therapies are likely associated with an increase risk of SCAEs, specifically ventricular arrhythmia and TdP. This study has assessed that the potential cardiovascular adverse events associated with the use of HCQ/CQ, with or without azithromycin, in patients with COVID-19 by analyzing adverse events reported in the FAERS database.





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Keywords: COVID-19, FDA Event Reporting System (FAERS), cardiovascular adverse effects, hydroxychloroquine, chloroquine, azithromycin.

INTRODUCTION

The severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) [COVID-19] pandemic has lasted for more than 2 years, and the number of confirmed cases continues to grow, with an increasing in emergency. In early March 2022, a total of 435,501,000 confirmed COVID-19 cases had been diagnosed and more than 5,950,000 deaths had occurred worldwide. Since the publication of French study suggesting that the combination of hydroxychloroquine (HCQ) and azithromycin may be effective for COVID-19, this regimen has been widely used despite controversy over its safety and efficacy. The US FDA issued an Emergency Use Authorization (EUA) for the use of HCQ and chloroquine (CQ) for the treatment of COVID based on only limited results, which contributed to the use of these drugs during the early months of the pandemic in March and April 2020. Trials and observational studies have reported that HCQ/CQ monotherapy or HCQ/CQ-azithromycin combination therapy was not fully associated with improved clinical outcomes for COVID-19 and that it has posed serious cardiovascular safety risks, especially QTc prolongation and torsade de pointes (TdP)[2]. FDA issued an alert in april 2020 cautioning against the use of HCQ outside hospital or clinical trial settings, revoked EUA on June 15, 2020, and issued COVID-19 treatment guidelines recommending against the use of HCQ/CQ with or without azithromycin for the treatment of COVID-19, although prescribing of these agents remained elevated through October 2020 [3]. Similarly, current evidence on cardiovascular adverse effects is conflicting across studies, pharmacovigilance studies may be valuable for early evidence generation in real-world populations [4]. One pharmacovigilance study using the USFDA Event Reporting System (FAERS) evaluated that the safety of HCQ/CQ + azithromycin in case reports from before the COVID-19 pandemic through the first quarter of 2020. In this study, case reports of HCQ-induced SCAEs during and before COVID-19 were found to be largly different mainly because of the changes in patient characteristics, comorbid conditions, effects of drug interactions, and possible modifications to drug pharmacokinetics in patients with COVID-19 [5]. In this study, we reviewed the reported cases of serious cardiovascular events associated with HCQ/CQ and azithromycin in the FAERS database[6].

MEDHODS

Data Sources and Study Design

We used the FAERS data files from Q1/2020 through Q4/2020, using both generic and brand names (Table S1 in the electronic supplementary material [ESM]) to identify intervention and comparator drugs. We studied only case reports with a COVID-19 diagnosis [7]. The primary outcome was SCAEs, which were defined as a composite endpoint based on previous literature including cardiac arrest, ventricular arrhythmia, bradyarrhythmia, QTc prolongation, myocardial infarction, stroke, coronary ischemia, and TdP, atrial fibrillation, cardiac failure. We designed eight comparison groups to evaluate the SCAEs associated with HCQ in patients with COVID-19:

- 1.HCQ/CQ + azithromycin versus HCQ/CQ + amoxicillin
- 2.HCQ/CQ + azithromycin versus HCQ/CQ
- 3.HCQ/CQ + azithromycin versus lopinavir/ritonavir
- 4.HCQ/CQ + azithromycin versus remdesivir
- 5.HCQ/CQ + azithromycin versus all other drugs except for HCQ/CQ, azithromycin, lopinavir/ritonavir, and remdesivir (e.g., insulin, simvastatin, etc.)
- 6.HCQ/CQ versus lopinavir/ritonavir
- 7.HCQ/CQ versus remdesivir





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8.HCQ/CQ versus all other drugs.

Therapeutic alternatives were selected as comparison drugs [8]. Amoxicillin was taken as a comparator antibiotic because of its shared indications with azithromycin and because it has not been shown to have any adverse cardiovascular effects. Lopinavir/ritonavir and remdesivir were chosen as comparators because they are likely used to treat patients with COVID-19 [9]. Comparison 2 and comparisons 6–8 was designed to evaluate the hypothesis that combined therapy is associated with increased SCAE reporting compared to the monotherapy of HCQ/CQ. Performed subgroup analyses for (1) a subpopulation aged > 65 years and (2) a subpopulation with other concomitant QTc-prolonging drugs (as shown in Table1) for two comparisons: (1) HCQ/CQ + azithromycin versus remdesivir and (2) HCQ/CQ + azithromycin versus lopinavir/ritonavir[10].

DISCUSSION

This study has found that composite SCAEs, TdP/QTc prolongation, and ventricular arrhythmia were reported more in adverse event reports listing HCQ/CQ than that in reports listing alternative therapies. RORs for cardiac arrest include number of estimates close to or on the other side of null value, suggesting no signals; but, these analyses were imprecise compared to the other outcome analyses, mainly because of the small number of events[11] .Further, increases in adverse event reports listing the combination use of HCQ/CQ and azithromycin may be associated with increased reporting of SCAE compared to the events reported with HCQ/CQ monotherapy, and the potential signal was greater among reports in older adults[12]. This is the most extensive pharmacovigilance study to assess between HCQ/CQ with or without azithromycin and SCAEs in the patients with COVID. Our study provides additional information for the safety profiles of HCQ/CQ in the patients with COVID-19[13]. Previous studies have suggesting that HCQ/CQ monotherapy is associated with increased risk of QTc prolongation and cardiovascular events and that contineous use may further increase this risk [14]. A case series report of 84 patients with COVID-19 treated within 5 days of HCQ + azithromycin found that QTc increased greatly from a mean ± standard deviation of 435 ± 24 ms to a maximum of 463 ± 32 ms (P < 0.001) on day 3.6 ± 1.6 of therapy. Nine out of 84 (11%) patients recorded QTc > 500 ms (of which five of the nine patients had a normal QTc at baseline), however none of them developed TdP or cardiovascular death. Knowing that both HCQ/CQ and azithromycin have a long half-life (approximately 40 days and 68 h, respectively), cardiovascular events could occur even after discharge[15]. If such risk associated with HCQ/CQ + azithromycin combination therapy truly exists, it could be possibily explained by the hypothesis that HCQ/CQ and azithromycin used regularly may simultaneously block the hERG/Kv11.1 potassium channel and increasing the risk of QTc prolongation, TdP, and other SCAEs [16].

Similarly, regular use of meds that induce QTc prolongation may have a additive effect in QTc prolongation risk, and almost one-third of the reports of HCQ/CQ monotherapy or HCQ/CQ + azithromycin included that the concomitant use of other QTc-prolonging agents in the study[17]. In addition, another point worth considering is the pharmacokinetic interaction between HCQ/CQ and these concomitant drugs, which can inhibit drug metabolism and further lead to increase the systemic exposure of HCQ/CQ, increasing the cardiotoxicity. Both the drugs; HCQ and CQ are metabolized by cytochrome P450 (CYP) enzymes, and the concomitant use of CYP3A4 inhibitors, including lopinavir/ritonavir, antifungals, and macrolides may increase the risk of SCAEs[18]. Azithromycin, is a newgeneration macrolide, a weaker CYP3A4 inhibitor, but the risk of QTc prolongation might increase when used in combination with HCQ/CQ for treatment in COVID. This further explains why HCQ/CQ + azithromycin combination therapy has a more significant QT prolongation effect . This may also explain why cases reporting the concomitant use of other QTc-prolonging agents and HCQ/CQ + azithromycin were more common to report SCAEs than cases reporting the use of lopinavir/ritonavir[19]. 111 (19.30%) case reports with COVID-19 listed HCQ/CQ ± azithromycin combined with lopinavir/ritonavir, suggesting that it is more important to monitor QTc prolongation and ventricular arrhythmia in this setting. Our finding that HCQ/CQ + azithromycin was associated with increased reporting of SCAEs in older patients is consistent with a retrospective cohort study that found that age > 65 years was a big risk factor associated with QTc prolongation ≥ 500 ms in hospitalized patients with COVID . Special





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attention should be paid in this regards since age has been to be a key risk factor of mortality in such patients .HCQ/CQ monotherapy and HCQ/CQ + azithromycin combination therapies are likely to be associated with an increased risk of SCAEs, specifically TdP and ventricular arrhythmia. A study found that cardiovascular diseases, including coronary artery disease and congestive heart disease, were also associated with an increased risk of fatal cardiac complications, such as TdP, cardiac arrest, and ventricular arrhythmia, specially in patients receiving HCQ + azithromycin[20] . Also the use of HCQ and azithromycin has proven to have little impact on QTc duration in most patients with COVID, and does not induce any prone to arrhythmia , patients with COVID-19 and primary moderate hypokalemia, tachycardia, and subclinical-to-mild long QT syndrome have a very high risk of developing drug-induced arrhythmias than patients without these comorbidities . More importantly, when treating patients with COVID-19, such therapy should associated with a higher risk of cardiovascular events as such patients are prone to QTc prolongation[21]. The American College of Cardiology has already been recommended for a close monitoring of QTc prolongation and arrhythmia in patients with COVID-19 treated with HCQ + azithromycin .

Extra caution is needed in patients with QTc > 480 ms at baseline. Atrial fibrillation is also an observed serious cardiac adverse event in this study, [22] One recent study shows up with 416 patients hospitalized with COVID-19 found that high-sensitivity troponin I was elevated in 82 (19.7%) patients. Another similar study was reported 52 cases (27.8%) of troponin T elevation out of 187 patients hospitalized with COVID-19. Both studies reported a significant increase in mortality of more than 50% among patients with myocardial injury. It should be noted that the concurrent QTc agents which are listed on case reports of QTc prolongation events which includes quinolones and azole antifungals; this should be especially noted for severe COVID-19 cases as they may develop nosocomial infection after longterm hospitalization and broad-spectrum antibiotic coverage.[23]. Also, patients under ICU often develop electrolyte disturbances, such as hypokalemia and hypomagnesemia, which would further increase the risk of QTc prolongation/TdP. The results of the study support an increased cardiovascular risk in patients treated with HCQ/CQ and azithromycin particularly when treating patients with COVID-19[24]. First, we assessed the cardiovascular safety of HCQ/CQ + azithromycin in patients with COVID-19. The availability and lag time of FAERS data updates meant that previous studies were based on patients with rheumatoid arthritis, lupus, or malaria.[25] when treating rheumatoid arthritis or lupus, HCQ/CQ is used for longer than the typical 5-day course which is used for the treatment of COVID-19. Given that patients with COVID-19 are more prone to QTc prolongation and myocardial injuries , our results are more focused to the COVID-19 patient population than were those prior studies[26]. Additionally, we also extracted detailed information of the cases by diging FAERS data, and we have summarized clinical features associated with SCAEs.

CONCLUSION

This study shows the potential cardiovascular adverse events associated with use of HCQ/CQ, with or without azithromycin, in patients with COVID by analyzing the adverse events reported in FAERS database[27]. Compared with the case reports listing comparison drugs, case reports of patients treated with HCQ/CQ were associated with the increased reporting of the composite endpoint SCAEs and fatal QTc prolongation-related cardiovascular events (majorly TdP and ventricular arrhythmia), especially when azithromycin was also listed. When treating with concomitant use of both other QTc-prolonging agents and CYP3A4 inhibitors, the benefits need to be weighed against the potential harms[28]. Given the scarcity of evidence demonstrating benefits from HCQ/CQ treatment for patients with COVID-19, the evidence for harm is crucial for clinical decision making[29]. Together with evidence, the data provide further evidence to caution against the widespread use of the combination regimen in preventing or treating COVID-19[30].

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Table 1: Descriptive characteristics of serious cardiovascular adverse events case reports listing the use of hydroxychloroquine/chloroquine monotherapy and hydroxychloroquine/chloroquine + azithromycin combination therapy in COVID-19 cases reported to FAERS from January 1, 2020, to December 31, 2020

ombination therapy in C	COVID-19 cases reported to FAERS t	rom January 1, 2020, to December 3	1, 2020
Characteristics	HCQ/CQ + azithromycin (N = 312)	HCQ/CQ monotherapy (N = 263)	Total (N = 575)
Age, years	229 reported	208 reported	437 reported
Mean ± SD	64.1 ± 14.7	63.7 ± 20.1	63.9 ± 17.7
Sex	230 reported	213 reported	443 reported
Female	65 (28.3)	70 (32.9)	135 (30.5)
Weight, kg	70 reported	78 reported	148 reported
Mean ± SD	84.15 ± 16.8	85.93 ± 19.7	85.09 ± 17.5
	Precipitating fa	ctorsa	
Hypertension	13 (4.2)	23 (8.8)	36 (6.3)
Heart failure	2 (0.6)	6 (2.3)	8 (1.4)
Diabetes	4 (1.3)	13 (4.9)	17 (3.0)
Sepsis	0	1 (0.4)	1 (0.2)
Female sex	65 (28.3)	70 (32.9)	135 (30.5)
Advanced age >65	135/229 (58.0)	132/208 (63.5)	267 (61.1)
years	• •		
(Concurrent QTc-prolonging medication	ons that may induce SCAEs ^b	
Lopinavir/ritonavir	67 (21.5)	44 (16.7)	111 (19.3)
Quinolones	5 (1.6)	12 (4.6)	17 (3.0)
Macrolides	0	2 (0.7)	2 (0.4)
Azole antifungals	0	5 (1.9)	5 (0.9)
Tricyclics	0	7 (2.7)	7 (1.2)
SSRIs	5 (1.6)	1 (0.4)	6 (1.0)
5-HT3 antagonist	0	6 (2.28)	6 (1.04)
Antipsychotics	4 (1.3)	3 (1.1)	7 (1.2)
Loop diuretics	15 (4.8)	19 (7.2)	34 (5.9)





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Class III						
antiarrhythmics	15 (4.8)	7 (2.7)	22 (3.8)			
Donepezil	0	1 (0.4)	1 (0.2)			
Serious cardiac adverse events ^c						
QTc prolongation	195 (62.5)	158 (60.1)	353 (61.4)			
Ventricular arrhythmia	48 (15.4)	21 (8.0)	69 (12.0)			
Atrial fibrillation	24 (7.7)	23 (8.8)	47 (8.2)			
Torsade de pointes	14 (4.5)	14 (5.3)	28 (4.9)			
Cardiac arrest	12 (3.9)	13 (4.9)	25 (4.4)			
Heart failure	9 (2.9)	13 (4.9)	22 (3.8)			
Stroke	3 (1.0)	8 (1.0)	11 (1.9)			
Myocardial infarction	2 (0.6)	8 (3.0)	10 (1.7)			
Bradyarrhythmia	5 (1.6)	5 (1.9)	10 (1.7)			
Serious outcomes						
Number reported	312 reported	256 reported	568 reported			
Hospitalization	46 (14.7)	42 (16.0)	88 (15.3)			
Life threatening	46 (14.7)	37 (14.1)	87 (15.1)			
Death	46 (14.7)	33 (12.6)	79 (13.7)			
Other serious events	177 (56.7)	144 (54.8)	321 (55.8)			

Data is presented as n (%) of events or mean ± standard deviations unless otherwise to be noted COVID-19 coronavirus disease 2019, HCQ hydroxychloroquine CQ chloroquine, serious cardiovascular adverse event (SCAE), (SD) standard deviation, SSRIs selective serotonin reuptake inhibitors a Precipitating factor included- heart failure, hypertension, hyperlipidemia, left ventricular hypertrophy, severe renal disease, sepsis, hypokalemia, hypomagnesemia, hypocalcemia, and obesity, diabetes, female sex, advanced age >65 years. Those with zero or missing values were not been listed b The individual drugs of each group is shown in the Table S5 in electronic supplementary material c SCAEs was predefined as a composite endpoint including cardiac arrest, ventricular arrhythmia, atrial fibrillation, stroke, cardiac failure, coronary ischemia, and torsade de pointes, bradyarrhythmia, QTc prolongation, myocardial infarction.





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RESEARCH ARTICLE

Farmer Producer Companies and Broad-based Extension Services: A Case Study of FPC in Dharmapuri District of Tamil Nadu

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ABSTRACT

Farmer Producer Organizations (FPOs) have been identified by the Indian government as a new type of collectivization for producers, particularly small and marginal farmers. India had 569 FPOs registered, as well as 5.83 lakh farmers, who had been identified and organised into 34,345 Farmer Interest Groups (FIGs). The present study was conducted in Dharmapuri district of Tamil Nadu and 40 farmers were selected from the registered Farmer Producer Company (FPC) located in Dharmapuri district of Tamil Nadu. The study is mainly based on personal interview with the management of FPCs and hence the research is descriptive and empirical. It was about seventeen FPCs officially functioning in this district. The CEOs, Managing Directors and the farmers in the FPCs were interviewed and data has been collected during October - November, 2021. The findings of the study indicated that the member farmers of the FPCs perceived that the FPCs had provided several services to the farmers to improve the profit and there is a need to establish a knowledge based management framework for small and emerging agricultural enterprises/organizations, so that they can use to improve their performance without having to spend a lot of money.

Keywords: Farmer Producer Organization, Production, Agricultural Marketing, Management and Agribusiness





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INTRODUCTION

Farmer Producer Organizations (FPOs) have been identified by the Indian government as a new type of collectivization for producers, particularly small and marginal farmers (FPOs). The FPOs were established under the rules of the Companies Act of 1956 and address the issues that small and marginal farmers experiences, primarily those relating to increasing access to investments, technology, efficient inputs, and markets. Producer Organisations(POs) are defined in a variety of ways. Producer groups are formal rural organisations, whose goal is to increase the farm income through better production, marketing, and local processing. The Government of India has approved and launched the Central Sector Scheme of "Formation and Promotion of 10,000 Farmer Producer Organizations (FPOs)", to form and promote 10,000 new FPOs till 2027-28 with a total budgetary outlay of Rs.6865 Cr. Under the scheme, the formation and promotion of FPO is based on Produce Cluster Area approach and specialized commodity based approach. While adopting cluster based approach, formation of FPOs will be focused on "One District One Product" for development of product specialization. Initially one FPO is allocated per block. So far, a total of 4,465 new FPOs produce clusters have been allocated to Implementing Agencies for formation of FPOs, of which a total of 632 FPOs have been registered. (Ministry of Agriculture & Farmers Welfare, 2021). Twenty farmers from the surrounding area who are interested in forming a groups can get together to form the Farmer Interested Group (FIG). A Farmer Help Group (FHG) of five FIG can be formed, with a total of 100 members. Finally, a Farmer Producer Company can be formed from a group of 10 FHG. People with professional talents are more likely to be hired as FPC workers, with the title of Chief Executive Officer (CEO). The CEO will carry out his tasks and responsibilities as directed by the Board of Directors, the Resource Institute (RI), the Producer Organization Promoting Institution (POPI), and other government agencies such as NABARD, SFAC, and others.

There are 500 FPCs in the State. As most of the FPCs in the state are at start-up stage of not more than five years, they face various challenges. The biggest challenge faced by the FPCs is the lack of access to institutional credit as they embark to take up or scale up their business activities. To address this issue, the Government formulated the following three schemes to assist the FPCs, viz., 1. Mezzanine Capital Assistance (MCA) to enhance the quantum of credit, 2. Credit Guarantee Scheme (CGS) to access credit 3. Revolving Fund Support (RFS) to avail concessional credit. This scheme will be jointly implemented by the Department of Agricultural Marketing and Agri Business, Government of Tamil Nadu and NABKISAN (Subsidiary of NABARD). The scheme envisages to benefit more than 10 lakh farmers by covering more than 1000 FPCs in the next four years. Additionally, provision of 20 SPUs to 20 FPCs is under process. Taking into consideration, the growing number of FPO Government of Tamil Nadu formulated "Farmer Producer Organisation Policy" exclusively for the FPOs. This FPO policy was unveiled by Hon'ble Chief Minister on 09.02.2020. An attempt has been made to understand the reach and initial challenges faced by FPCs in the region. For this, an in-depth interview was conducted with CEOs, BODs and farmers of the FPCs in the study area with the specific objectives (a) to explore the current status of the FPCs running in the Dharmapuri district of Tamil Nadu. (b) to analyse the details of sales by FPCs during Covid 19 lockdown Period and (c) to analyse the problems faced by the Farmers Producer Companies in Dharmapuri District. Farmer Producer Organizations (FPOs) are legal entities that bring together farmers from the same geographical cluster who are producing similar crops in order to achieve economies of scale and share the benefit/profit (NABARD, 2015). Financial assistance, government policy, and a favourable market environment for farmers are all being used by the government to solve these concerns. The government could set the criteria for selecting the FPO based on an objective business strategy for possible growth and policy implications for assisting those in need (Michalek et al., 2018). Farmer Company faces a number of problems that could prohibit it from keeping competitive in the local market(Jain and Narnaware, 2018). As a result, for greater performance and sustainability in India, FPOs require resource institute help (Singhet al., 2018). Since September 30th 2019, the Small Farmers Agribusiness Consortium (SFAC) was aiding 902 FPOs, benefiting 8.84 lakh farmers (GoI, 2019).





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MATERIALS AND METHODS

This study is based on Farmers Producer Companies operated in Dharmapuri district, which has a total of 2,10,300 farmers, with 1,90,000 of them being small and marginal farmers. Agriculture is the most important sector of the district economy, with 70 per cent of the population dependent on agriculture and related businesses for a living. The district covers 4,49,777 hectares, with a net cultivated area of roughly 1,95,740 hectares. Millets and pulses are the main crops grown in this district, which is rain-fed. The government's policies and goals have been to maintain agricultural output stability, while simultaneously increasing agricultural production in a sustainable manner to fulfil the growing population's food needs, thereby providing employment opportunities to the rural population. Dharmapuri district has always been one of the state's agriculturally productive districts, with farmers who are more adaptable to changing technologies and market factors. The Agriculture Department has risen to the challenge of achieving a faster rate of agricultural growth by adopting a number of development plans as well as the dissemination of essential technologies to boost production. There are seventeen Farmer Producer Companies registered under Companies Act by Department of Agricultural Marketing and Agribusiness with a financial outlay of Rs.191.90 lakhs benefitting 10,310 farmer members in Dharmapuri district. As the establishment of FPCs in the Dharmapuri district has commenced only from the year 2014, it is still in the nascent stage. This study is mainly based on personal interview with the management of FPC and hence the research is descriptive and empirical. It was about sixteen FPCs officially functioning in this district. The CEOs, Managing Directors and farmers in the FPCs were interviewed and data were collected during October to November 2021. The secondary data was collected from the official websites of NABARD and SFAC and government reports during the period 2015-2020.

RESULTS AND DISCUSSION

Status of the Farmer Producer Companies

All the Farmer Producer Companies in the selected district were registered under the Companies Act, 2013. The authorized capital of FPCs documented in the Memorandum of Association (MoA) was Rs. 10,00,000 only. However, management can increase it by passing a special resolution in the meeting and communicating with the Registrar of Companies (RoC). The average equity capital contributed by the producers(farmers) is Rs. 10,00,000 (Rs.1000/farmer). The establishment of FPCs in Dharmapuri district was started in 2014. During 2014 and 2015, only one FPC was formed Simultaneously, in 2016, three FPC were formed, in 2018 five FPCs were formed and in 2019 four FPCs were started and in 2020 three more FPCs were started successfully. The majority of FPC deals with more than two crops such as millets, pulses and oil seeds. The details of the selected FPCs is presented in Table1. Only farmers are eligible to join the FPCs, and interested farmers must invest Rs.1000 in the form of a share capital (10 shares @ Rs.100). It was declared that each member should contribute to ten shares. From the above table, it is observed that Thagadur Uzhavar Producer Company Limited has the highest turnover of around 120.15 lakhs with the help of 1288 members, followed by Navathaniya Farmer Producer Company Limited, which has the turnover of about Rs.30lakhs with the help of 1000 members, Subramaniyasiva Collective Farming Farmer Producer Company Limited which produces a turnover of about Rs.25.50 lakhs with the membership of 700 farmers, Minor Millet Farmer Producer Company Limited, which has the turnover of about Rs.15.75 lakhs, with1021 members of the FPC and so on. From the selected FPCs in Dharmapuri District eight FPCs were promoted by Tamil Nadu Small Farmers Agri- Business Consortium (TNSFAC), four FPCs were promoted by National Bank for Agriculture and Rural Development (NABARD), two FPCs were promoted by National Agricultural Development Programme (NADP), two FPCs were promoted by Tamil Nadu Irrigated Agriculture Modernization Project (TNIAMP) and only one FPCs was promoted by National Mission for Sustainable Agriculture (NMSA). From the Table 1, it is also observed that the NABARD is the promoting agency for Adhiyaman Vegetables and Millets Producer Company Limited with 1000 farmers as members, Harur Farmers Producer Company Limited with 700 farmers as members, Navadhanya Farmers Producer Company Limited with 1001 farmers, and Sittilingi Valley Organic Farmers Producer Company Limited with 500.





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Sales by FPCs

The details of sales of vegetables, fruits and groceries during Covid – 19 lockdown were collected from the FPCs involved in these activities. From the Table 2, it is observed that Hogenakkal CFFPC Limited has the highest quantity sales of about 10,452 kgs of vegetablesand1500 kgs of groceries and obtained Rs.3,26,550, followed by Muthamizh CFFPC Limited with a sales quantity of 9,294 kgs of vegetables and1500 kgs of groceries and obtained Rs.2,95,478, which is followed by Kurinji CFFPC Limited sold 9,000 kgs of vegetables and 4,500 kgs of groceries and obtained 3,40,000, followed by Palacode CFFPC Limited has sold 2,550 kgs of vegetables and 2000 kgs of groceries and obtained Rs.1,10,500, finally TNIAMP Pambar FPC Limited with 2500 kgs of vegetables and500 kgs of groceries and realised only Rs.90,809.

Support to FPCs by Department of Agricultural Marketing and Agri - Business

The support given by the Department of Agricultural Marketing and Agri - Business to FPCs were collected and presented in Table.3, it is seen that the highest subsidy has been allocated to Nellikani CFFPC and Adhiyaman CFFPC with Rs. 60 lakhs and Rs.5lakh rupees for SPU and Start up grant, followed by TNIAMP Pambar FPC Ltd with Rs10 and 20 lakhs for Startup grant and Productive investment. The Mezzanine capital assistance has been allocated Subramaniyasiva CFFPC and Navathaniya FPC Ltd, with Rs, 7 lakhs and 9.9, lakhs respectively.

Advantages of FPCs in Dharmapuri District

The FPCs will take over the responsibility of any one or more activities in the value chain of the produce right from procurement of raw material to delivery of the final product at the ultimate consumers' doorstep. The FPCs will basically bridge this gap.

In brief, the FPCs will undertake the following activities

1. Procurement of inputs, 2. Disseminating market information, 3. Dissemination of technology and innovations, 4. Facilitating finance for inputs, 5. Aggregation and storage of produce,6. Primary processing like drying, cleaning and grading, 7. Brand building, Packaging, Labelling and Standardization, 8. Quality control, 9. Marketing to institutional buyers, 10. Participation in commodity exchanges and 11. Export facilities. The FPCs will support the members in getting more income by undertaking all of these activities mentioned above. By aggregating the demand for inputs, the FPCs can buy in bulk, thus procuring at cheaper price compared to individual purchase. Besides, by transporting in bulk, cost of transportation is reduced, thus, reducing the overall cost of production. Similarly, the FPC may aggregate the produce of all members and market in bulk, thus, fetching better price per unit of produce. The FPC can also provide market information to the producers to enable them hold on to their produce till the market price become favourable. All these interventions will result in more income to the farmers.

Problems Faced by FPCs

The details regarding the problems faced by the FPCs were collected and analysed using Garrett ranking and is presented in Table.4From Table4, it is observed that the problems during allotment of profits among shareholders in FPCs ranked first, followed by low level of input procurement of the companies, which ranked second, followed by less remunerative prices of the produce ranked third by Time lag between sale of output and price realisationis ranked as fourth, followed by high operational and maintenance challenges.

CONCLUSION

The findings of the study indicated that the member farmers of the FPCs in Dharmapuri District perceived that the FPO had provided several services to the farmers to improve the profit. FPCs can also immediately supply inputs and purchase produce at a more remunerative price in order to maintain their trustworthiness and grow their membership. There is a need to launch producer-focused teaching efforts regarding the benefits of producer organisations. Government should use broadcasts to encourage more people to join producer organisations. There is a need to establish a knowledge management framework for small and emerging agricultural enterprises/organizations in India that they can use to improve their performance.





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Table.1 Details of the Selected FPCs in the Dharmapuri District of Tamil Nadu.

SI. No	Name of the FPCs	Year of Establishment	Promoting Agency	No. of Share Holders	Activities of the FPC	Turnover (in Lakhs)
1	Minor Millet Farmer Producer Company Limited.	2014 -15	TNSFAC	1021	Minor Millet Processing	15.75
2	Thagadur Uzhavar Producer Company Limited.	2015 -16	TNSFAC	1288	1.Oil Extraction Unit 2. Input Shop	120.15
3	Nellikani Collective Farming Farmer Producer Company Limited.	2018 -19	TNSFAC	1000	1.Seed Processing Unit 2. MSAD Value addition for Oilseed	5.30
4	Adhiyaman Collective Farming Farmer Producer	2018 -19	TNSFAC	1000	1.Seed Processing Unit	2.68





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	Company Limited.				2. MSAD	
	Company Limited.				Value	
					addition	
					for Oilseed	
					1.Flower	
					Business	
					2.	
	Subramaniyasiva				Tamarind	
5	Collective Farming	2018 - 19	TNSFAC	700	Trading,	25.50
	Farmer Producer				3.Groundn	
	Company Limited.				ut Business	
					4.Input	
					Shop	
	Aavai Collective				Dulasa	
4	Farming Farmer	2010 10	TNICEAC	700	Pulses	3.50
6	Producer Company	2018 - 19	TNSFAC	700	Processing	3.50
	Limited.				Unit	
	Palacode Collective				Oil	
7	Farming Farmer	2019 - 20	TNSFAC	800	Extraction	3.45
,	Producer Company	2017 20	11431740	000	Unit	5.45
	Limited.					
	Kurinji Collective				Vegetables	
8	Farming Farmer	2019 - 20	TNSFAC	842	and	3.69
	Producer Company				Grocery	
	Limited.				items Sales	
0	TNIAMP- Pambar	2010 20	TALLANAD	1000	Vegetables	7.40
9	Farmer Producer	2019 - 20	TNIAMP	1000	and Maize	7.60
	Company Limited. Harur Farmer				Sales	
10	Producer Company	2016 - 17	NABARD	700	Rice Sales	4.50
10	Limited.	2010 - 17	NADARD	700	Rice Sales	4.50
	Adhiyaman					
	Vegetables and				Cattle Feed	
11	Millets Producer	2016 - 17	NABARD	1000	Sales	0.75
	Company Limited.				30103	
	-				Millets,	
4.5	Navathaniya Farmer	0044 1-		4.555	Pulses -	00.55
12	Producer Company	2016 - 17	NABARD	1000	Value	30.00
	Limited.				Addition	
	Sittiling Valley				Millets,	
12	Organic Farmer	2010 10	NADADO	700	Pulses -	15 50
13	Producer Company	2018 - 19	NABARD	700	Value	15.50
	Limited.				Addition	
	Hogenakkal CFFPC				Vegetables,	
14	Limited.	2020 - 21	NADP	500	Millets and	0.48
	Ziiiitod.				Oil Seeds.	
	Muthamizh CFFPC			_	Vegetables	_
15	Limited.	2020 - 20	NADP	500	and oil	0.60
					Seeds.	





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16	Theerthamalai CFFPC Limited.	2019 - 20	NMSA	500	Millets, Pulses and oilseeds	-
17	TNIAMP II Vaniyar FPC Limited.	2020 - 21	TNIAMP Phase-I	500	Pulses, Oilseeds & Maize	-

Table.2 Details of Sales by FPCs During Covid -19 Lockdown Period in Dharmapuri District

SI. No	Name of the FPC	Quantity of Fruits and Vegetables sold (Kgs)	Groceries (Kgs)	Value (Rs)	No of vehicles utilised (Nos)
1	Palacode CFFPC LTD, Karimangalam.	2,550	2,000	1,10,500	2
2.	Muthamizh CFFPC Ltd, Palacode.	9,294	1,500	2,95,478	2
3.	Hogenakkal CFFPC Ltd, Dharmapuri	10,452	1,500	3,26,550	2
4.	Kurinji CFFPC Ltd Ramaiyanahalli.	9,000	4,500	3,40,000	2
5.	TNIAMP Pambar FPC Ltd Kambailnallur.	2,500	500	90,809	2
	Total	33,796	10,000	11,63,337	10

Table.3 Support to FPCs by the Department of Agricultural Marketing and Agri Business

				Amo	ount (Rs.lakh)	
S. No	Name of the FPC	Scheme	Business activity/Infrastruc ture	Allocated subsidy (Rs.lakhs)	Subsidy released (Rs.lakhs)	Balance subsidy (Rs.lakhs
1	Nellikani	NADP/18-19	SPU	60	54	6
ı	CFFPC	NADI / 10-17	Start up grant	5	5	-
2	Adhiyaman	NADP/18-19	SPU	60	54	6
Z	CFFPC	NADP/10-19	Start up grant	5	5	-
3	Subramaniyasiv a CFFPC	NADP/18-19	Mezzanine capital assistance	7	7	-
	a CFFPC		Start up grant	5	5	-
4.	Aavai CFFPC	NADP/18-19	Start up grant	5	5	-
	TNUANAD	TNUANAD	Start up grant	10	5	5
5.	TNIAMP Pambar FPC Ltd	TNIAMP Phase I	Productive investment	20	10	10
6	TNIAMP II Vaniyar FPC Ltd	TNIAMP Phase II	Start up grant	5	-	5
7	Navathaniya FPC Ltd	NABARD	Mezzanine capital assistance	9.9	9.9	-
		Total		191.90	159.90	32.00





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Table.4 Problems Faced by FPCs in Dharmapuri District of Tamil Nadu

S. No	Problems of FPCs	Ranking
1.	Problems during allotment of profits among share holders	I
2.	Low level of Input procurement	П
3.	Less remunerative prices of produce	III
4.	Time lag between sale of output and price realisation	IV
5.	High operational and maintenance challenges	V





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RESEARCH ARTICLE

Grade Wise Detection and Classification between Iron-Ore and Pellets using YOLOv5 Algorithm

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ABSTRACT

Iron is the major mineral widely used throughout the world. The Iron-ore is the main raw source for the production of many alloys like steel etc. Iron-ore in oxide form is convenient to extract iron. Based on the quality there are four grades in classifying the iron-ore. Pelletization process is done for fining and exportation of iron-ore. The pellets are also classified based on the grades. The classification of Iron-ore is based on physical properties, chemical properties, image processing methods. This paper proposes a novel method of categorization of grades of iron-ore and pellets using deep learning algorithms. Classification of pellets by other learning algorithms will not be much accurate as pellets are small in size. Here a novel YOLOv5 algorithm is implemented to differentiate the grades of iron-ore collectively with fine pellets in an image or instant video by utilizing the bounding boxes and differentiation between the probability of each class. The results indicate more accurate prediction of the pellets.

Keywords: YOLOv5 algorithm, Pellets, Grades of Iron-ore, Magnetite, Haematite, Object detection, Classification





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INTRODUCTION

Iron is extracted from the sources like rocks and minerals. The Iron content in minerals mainly appear as Silicates, Carbonates and Oxides. Out of which oxide type is the most appropriate category for the extraction of iron, as it uses simple technique with reducing agent for reduction purpose. Among the available source the Magnetite and Hematite reflect on to be the natural and direct shipping ore as they together be readily used in the blast furnaces for iron production. The 98% of this raw material moreover as pig iron is utilized in the production of steel. The main advantage for utilizing the hematite than magnetite is mining of hematite is cheaper compared to magnetite. Hematite constitutes nearly 70% of Fe and the other composition includes elements like phosphorus, water content, aluminium clays. Alloys of Iron is possible by mixing with various elements. This result in producing various grades of iron alloys with the properties required for various applications. Therefore, pelletization is significant method that yields pellets which is of high grades to less grades and can be exported. The pelletization is done by binders. The pellets are also distinguished by their quality based on physical, chemical and metallurgical specifications [1]. The Pelletizing process are of multi stage, where the concentrated iron-ore is added to the additives, anthracite, dolomite along with binders are fed to pelletizing plant. Elicited from the mixture of these additional components the size, chemical specifications are changed accordingly and quality of pellets also differs. Pellets are of spherical in shape and is of 8-16mm diameter.

Literature Review

The classification is determined by analysing the texture of the ore. The texture properties like porosity, physical properties, mineral properties, mineral association of ores are observed. The image classification is performed by the texture modelling and it shows better prediction [2]. Characterisation of iron-ore to determine their grade based on experiments using X-ray diffractometer, X-ray florescence spectrometer, scanning electron microscope and petrographic microscope is performed [3]. Characterisation helps in identification of grade quality, density, shape and phase compositional analysis. The chemical composition and microstructure of raw iron-ore is evaluated [4]. The classification of grades of ore are identified by experimental procedures where, samples are taken and detailed studies were conducted to establish the composition and properties of the 5 samples [5]. Methods based on X-ray Diffraction (XRD), X-ray Fluorescence (XRF), Atomic Absorption Spectrometry (AAS) and Petrography microscopy techniques were utilized. Various classification based on image processing has been stated based on the colour difference between the ores. A machine learning algorithm using SVM regression algorithm to predict the grades [12]. The YOLOv5 algorithm for the detection of small objects in remote sensing with faster region CNN algorithm but the detection speed is relatively slow [14]. Using YOLO v5 algorithm the bacterial spot in bell pepper leaf is detected with better accuracy and speed [15]. [16-19] uses algorithms like SIFT, HAAR, HOG, Convolutional features for detection. Our paper proposes a novel YOLOv5 algorithm in accurate prediction based on the image that can be captured through the conveyor. Each small particle can be identified by the YOLOv5 algorithm.

PROPOSED METHODOLOGY

In the proposed algorithm the input fire image is divided into M x M grids. If the target is detected then the grid that constitutes the detection is identified as target. Nearby three bounding boxes are then detected and identifies the confidence score. The confidence score is given by,

Confidence= $P_{\gamma} \times IoU_{truth}^{Pred}$, $P_{\gamma}(object) \in [0,1]$

 P_{γ} = 1, for the target in grid and P_{γ} =0, for the target not in the grid. IoU is the coincidence between the true bounding box and predicted bounding box. The confidence level denotes the precision accuracy that the object in the grid is true. For the algorithm detecting the multiple boxes at same instant then Non-Maximum Suppression (NMS) strategy is used in selecting the suitable bounding box. The proposed network architecture is illustrated in Figure. 1. and Figure. 2.





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This model scales the input image to 416 x 416 pixels and then uses efficient net feature extraction algorithm. Here three scale bounding boxes like 13x13, 26x26, 52x52 are used. The depth wise convolution is executed by combining two processes, one is channel by channel convolution and the other is step by step point convolution. This two-step process extracts small target as fine and different granules. By channel-by-channel method the convolution is performed in one channel that has no impact with the true feature of other channels. For the D_f x D_f x M pixel size, the channel by channel convolution is performed by Dk x Dk x 1 convolution kernels for M number of times separately independent of each other. This channel-by-channel convolution will lack in the transfer of information to other layers so point wise convolution operation is performed by a 1x1 kernel. The information of one layer is transferred to the consecutive layers. This enables the advantage of feature extraction at the fine level with a smaller number of computations with the necessary information for feature extraction is also transferred. As the ores are need to be examined in real time while the conveyor belt is moving along the camera that captures the real time data, the speed and accuracy is very much important to be considered. And this model gives better result in classification of the ores. Further the speed can be achieved by providing less calculation and lesser computations. This further reduces the hardware cost implementation. The Algorithm uses another important blocks Squeeze and Expand blocks. Three dimensions of the true feature map is H x W x C where H is the height of feature map, W denotes the width of the feature map and C denotes the number of independent channels i.e. depth of the feature map. Compression to 1x1xC from true feature map is performed by squeeze block. Height and Width are confined to onedimensional vector. The excitation block predicts the primacy of fully connected layer. The smoothening of function is performed by sigmoid activation function.

 $f(x)=x \times sigmoid(\beta x)$

where β is trained constant.

RESULTS AND DISCUSSION

Simulation of the algorithm is performed by python codes with YOLO v5 algorithm. The classes used in this paper are pellets, magnetite, siderite, haematite, limonite. The train samples in the dataset are labelled. The images to train and test are taken from internet sources. Real time images can give much accurate and precision. The Figure. 8. Confusion matrix shows very much highest accuracy of 1 in distinguishing the pellets and the accuracy is much affected by the background.

The precision value and recall values can be acquired by the equation (1) and (2).

$$Precision(P) = \frac{TP}{TP+FP}$$
(1)
$$Pocall(P) = \frac{TP}{TP}$$
(2)

$$Recall(R) = \frac{TP}{TP + FN}$$
 (2)

Where, TP is the true positive and FP is the false positive. By the calculation of precision and recall and optimization of the model perhaps identified by the F1 curve.

The F1 curve is calculated by the equation (3)

$$F1 = \frac{2}{\frac{1}{\text{precision}} + \frac{1}{\text{recall}}} \tag{3}$$

The Figure. 3. F1confidence curve shows better accuracy for pellets and limonite classification. The overall class F1 curve value is 0.65 at 0.113. As the paper contributes mainly for the classification of the pellets the instances for pellets are given higher than the other minerals. The centre of the bounding boxes are x and y. And the width and height of the bounding boxes are w and h respectively. The Figure. 4. shows the precision of 0.824 and Figure 5. Shows there call curve has roll off at 0.97. The Figure 6. shows the mean average precision graph values. With the dataset of 170 training images the classification shows better accuracy for pellet classification. Increasing the dataset in training with real time images the precision can be more accurate for other grade classification. Figure. 7. Correlogram shows the overall data analysis for the whole data set. Dataset is created for the types of iron-ore and pellets. The training dataset contains 170 images from sources. The data is labelled for the true identification. The dataset is randomly divided into test set, validation set and training set. The dataset contains the pellets along with types of ores. The samples are trained with two set of batches by batch normalization. The Figure 10. shows the train batch1 set of samples with pellets and other types of iron-ore that are to be identified and classified. The figure 11.





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Shows the validation results of identifying the pellets, magnetite, limonite, siderite, haematite. Labelling is performed manually for small targets. The classification is performed for magnetite, hematite, siderite, limonite and pellets. Fine tuning by YOLOv5 enables to predict the number of pellets. Figure 8. shows the confusion matrix. Figure 9. Shows the labels of the dataset. Around 75% accuracy is obtained that realizes the small targets like pellets accurately. Our proposed model imparts high accuracy with a lesser number of computation complexity. And can efficiently perform under natural light.

CONCLUSION

This paper brings forth that YOLOv5 the efficient algorithm can be utilized in classifying of ore and pellet than the conventional deep learning algorithms. The efficient classification for the smaller targets is also quite possible with less computational complexity and of greater accuracy. The future scope is enabling an optimized pre-processing of videos with types of ores and improve the algorithm by transfer learning. Where the successive frames can be learned and thus enables the lesser computational time in video processing.

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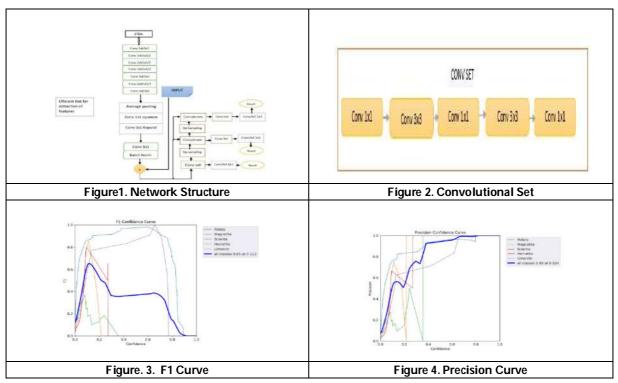
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Table, 1. Categories of Iron-ore

Type of Ore	Chemical Combination	Percentage of Iron (Fe)	Colour and properties
Magnetite	Fe ₃ O ₄	72.4 %	Reddish in colour
Hematite	Fe ₂ O ₃	69.9 %	Black in colour
Goethite	FeO(OH)	62.9 %	Dark reddish or dark brown
Limonite	FeO(OH).n(H ₂ O)	55 %	Yellowish in colour
Siderite	FeCO₃	48.2 %	Yellowish brown with presence of lime





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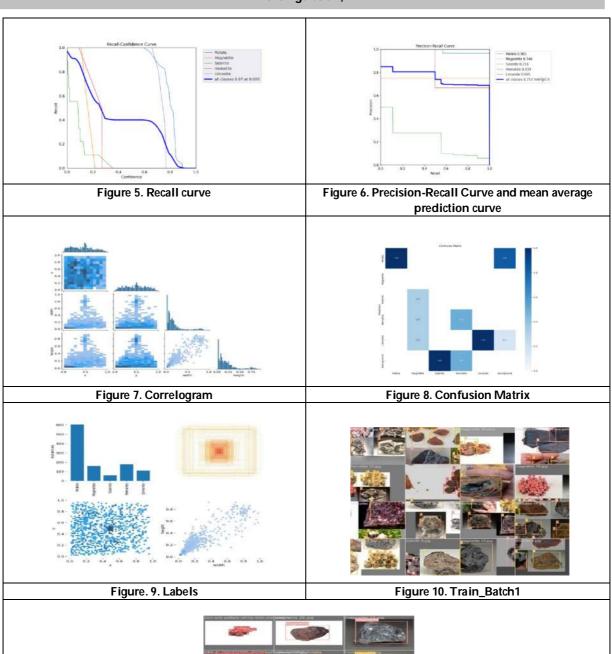




Figure 11. Validation of BATCH0 labels





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RESEARCH ARTICLE

Analysis of Seasonal Variation of Some Physico-Chemical Parameters of Subansiri River, North-East India

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ABSTRACT

Physico-chemical parameter analysis is one of the major indicators to determine river health. In the upper Brahmaputra basin of India Subansiri is one of the major rivers. One of the major regulatory components of the aquatic ecosystem is temperature. In the case of the Subansiri river, the temperature varies significantly with respect to the season. The dissolved oxygen of the Subansiri river is highest during winter and lowest in monsoon. Similarly, the maximum (17.65±1.11) and minimum (11.07±1.43) value of free CO₂ was recorded in the monsoon and winter season respectively. During monsoon, the pH of the Subansiri was closer to neutral (7.11 ±0.31) which was turned to more alkaline (8.28±0.22) during winter. Both TDS and TSS of Subansiri river are moderate for all the seasons. The data so far analyzed was satisfactory to some extent in context to pH, FCO₂, water temperature and total solid concerns. However, the DO₂ level was found to be in a slightly lower range.

Keywords: Dissolved oxygen, Free Co₂, Physico-chemical parameter, River health, Temperature.

INTRODUCTION

Water is an unavoidable competent to sustain life and it exists in three states gases, and liquid [1]. Not only human beings almost all living organisms depend on water for different means. Aquatic productivity is directly related to water quality [2,3]. Rivers are a complex ecosystem and their health is extremely important for the flora and fauna connected to them. A river is a flowing water body, which follows downhill movement towards a bigger river, sea or ocean. River One of the richest fresh water sources used for human consumption and utilization is river water. Rivers play a vital role in meeting the demands of local water supplies, industries, agriculture, etc. The quality and quantity of river water are determined by different factors like the amount of rainfall, temperature, and weathering





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of rocks in that surface area [4]. River health is analogous to human health which is depending upon different parameters of water. Measurement of Physico-chemical parameters is one of the major indicators of river health. The measurement of dissolved oxygen, free carbon dioxide, pH, transparency, dissolved solid etc. can be used to measure the health of a river body at a particular period of time. As nowadays due to pollution, global warming or human exploitation the environment is changing rapidly, therefore, by measuring these parameters the health of a river can be predicted and necessary steps can be taken if there is any critical situation.

By taking this point into consideration work has been done aiming to fulfil the following objectives-

- 1. Evaluating water quality by using physical and chemical analysis.
- 2. Evaluating the seasonal variation of Physico-chemical parameters.

MATERIALS AND METHODS

Study Area

Subansiri is one of the major trans-Himalayan rivers and the biggest tributary of the river Brahmaputra in the upper basin. The river flows through Tibet's Lhünzê County, Shannan Prefecture and the Indian states of Arunachal Pradesh and Assam. The river is 442 km long with a drainage basin of 32,640 square kilometres. The latitude of Subansiri River, Assam, India is 27.730566, and the longitude is 94.329559. The Lower Subansiri Dam or the Lower Subansiri Hydro-Electric Project is an under-construction dam gravity dam on the river. The dam is seen as a problem and many organizations for the environment and is protesting against it. Therefore, it is necessary to see if the river is in good health or not. All the selected parameters for this project will be studied on a seasonal basis viz., pre-monsoon (Mar-May), monsoon (Jun-Aug), post-monsoon (Sept-Nov) and winter (Dec-Feb). Five random sample sites were selected for the sampling of water.

Water Physico-chemical analysis

Water quality

To find out the relevant parameters concerned with the present study and to have a complete picture of the river, qualitative assessment was made by following standard protocols. For the study of water quality parameters, five random samplings were made in the river. Sampling was made on monthly basis (Thrice in a month) and data were presented seasonally i.e. four study seasons was considered, namely pre monsoon, monsoon, post monsoon and winter. In the present investigation, data had been collected from March, 2021 to February 2022.

The methodology adopted for the analysis of water samples are:

- 1. Water temperature: Using mercury thermometer graduated up to 110°C
- 2. Air temperature: Using mercury thermometer graduated up to 110°C
- 3. **Transparency**: By using secchi disc of 18 cm diameter[5].
- 4.**pH**:-Using digital pen type pH meter.
- 5. **Total dissolved solid** (TDS), **total suspended solid** (TSS) and **total solid** (TS) were estimated as per Trivedy and Goel, 1987[5].
- 6. Dissolvedoxygen (DO₂): Winkler's modified method was followed to measure dissolved oxygen[6].
- 7. Freecarbondioxide (FCO₂): Titration method is using phenolphthalein as indicator[7].

Statistical analysis

All the qualitative data were collected and descriptive statistics were done by following Biswas, 1998and analytical statistics were done by using the software Microsoft Office Excel 2007[8].





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RESULTS AND DISCUSSION

Seasonal variation of physico-chemical parameters of Subansiri River is depicted in the Table I. The mean air temperature was highest in monsoon (37.14±1.51) and lowest (10.94±1.88) in winter. The mean water temperature was also following the same trend (Fig.2.). Temperature is one of the most important physical factors influencing aquatic life. Water temperature, a regulatory factor for various physicochemical as well as biological activities in ecosystems, was found to fluctuate markedly with the variation in air temperature[9]. There are several factors which influence the water temperature in the river basin. Some of these factors are basin morphology, altitude, topography and vegetation [10] Air and water temperature play important role in the physicochemical and physiological behaviour of biotic components of aquatic ecosystems [11]. The mean value of DO2 was maximum (7.21±1.10) in winter season and minimum (3.71±1,02) in monsoon (Fig.3.). This was however lower than the desired range. Dissolved oxygen is the most important parameter which can be used as an index of water quality, primary production and pollution[12]. Dissolved oxygen content is the most significant factor regulating metabolic processes of the organism and also the community as a whole. Dissolved oxygen in general affects the solubility and activity of various nutrients and therefore, the productivity of an aquatic ecosystem [13]. A marked variation of dissolved oxygen content in water bodies of India was observed by various investigators [14-17], and it varies greatly from one water body to the other in the same area. Rodgi and Nimbergi (1978) viewed that disposal of domestic sewage and other oxygen demanding wastes reduce the dissolved oxygen of the receiving water body.

The present finding is in agreement of Rodgi and Nimbergi[18]. The free CO₂ concentration in water indicates the presence of decomposable organic matter, bacterial action on organic matter and physiological activities of biotic components[10]. In the present study, the maximum (17.80±3.28) and minimum (10.55±1.53) value of free CO₂ was recorded in the monsoon and winter season respectively (Fig.3.). Lower level of free CO2 during winter is mainly due to high photosynthetic activity utilizing free CO₂, which is in agreement with the work of Yousuf et al. 1986[19]. pH of natural waters is due to available hydrogen ion concentration, Webber and Stumm (1963) concluded that the pH of the raw water sources mostly lies within the range of 6.5 to 8.5[20]. The pH of the Subansiri was closer to neutral (7.21 ±0.21) during monsoon which was turned to more alkaline (8.37±0.19) during winter (Fig.4.). Higher pH value is normally associated with the high photosynthetic activity in water [21]. The lowering of pH in monsoon may be due to higher runoff from the adjacent catchment area which is having slightly acidic soil [22]. Although the tolerance of individual species varies, pH values between 6.5 and 8.5 usually indicate good water quality and this range is typical of most major drainage basins of the world [23]. Water has the capacity to dissolved salts those are in contact. So, variety of salts will be there in water those were actually the component of rocks and soil of flood plain. In freshwater ecosystem, dissolved solids originate from natural sources and depend upon location, geological basins of water body, drainage, rainfall bottom deposits and inflowing water. Dissolved salts and minerals are necessary components of good quality water as they help maintain the health and vitality of the organisms that rely on this ecosystem service[24]. The dissolved solids are in fact more diverse in nature and apart from its natural sources of its input; sewage becomes the most important source [25].

The quantity, quality, intensity and duration of light influence the life of organisms in different ways[26]. Transparency or light penetration was found to fluctuate according to season. An inverse relationship between transparency and suspended sediment load was observed. The rain water brought large amounts of dissolved and suspended inorganic and organic materials from upper catchment areas as well as from lower floodplain zone during rainy season that made water turbid and cause lower transparency. Similar observation was also made by Timms and Midgley [27]. The TSS of Subansiri was found to be in moderate range (78.9-105.0 mg/L) and the TDS in also in slightly moderate range (5.5-15.7) (Fig.5.). The year-round monthly as well as seasonal variation of physicochemical parameters of Subansiri is proving a vivid picture of ecological status at present. The data so far analyzed was satisfactory to some extent in context to pH, FCO₂, water temperature and total solid is concerned. However, the DO₂ level was found to be in slightly lower range. Formation of pit by decomposed organic matter should be managed along with excessive growth of floating aquatic vegetations. Disposal of domestic sewage, agricultural





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wastage, along with cow dung from nearby area must be checked. Awareness among the people of nearby areas is utmost necessary.

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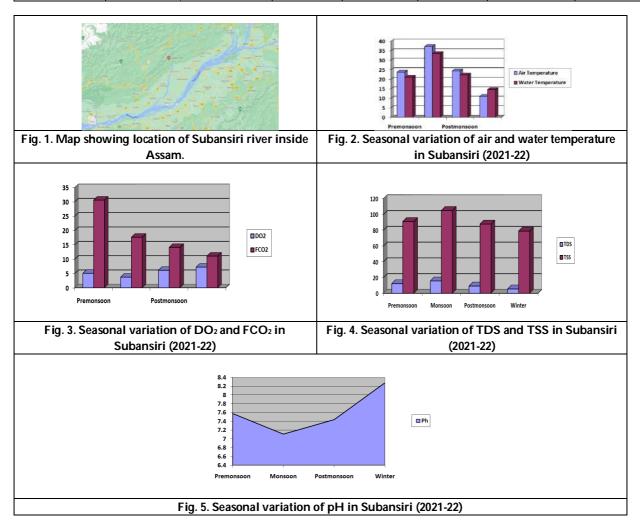
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Table. 1: Seasonal variation of physico-chemical parameters of Ranganadi River (2021-2022)

Seasons	Air temp (°C)	Water temp (°C)	DO ₂ mg.L- ¹	Free CO2 mg.L ⁻¹	рН	TDS mg.L-1	TSS mg.L-1
Preemonsoon	23.71±1.51	20.96±2.92	5.07±1.32	16.17±1.11	7.58±0.90	12.2±3.31	91.5±2.86
Monsoon	37.14±1.66	33.51±1.22	3.71±1.02	17.65±3.48	7.11±0.31	15.7±6.26	105.0±3.43
postmonsoon	24.30±3.44	22.19±4.75	6.11±1.36	14.11±2.09	7.44±0.13	9.2±2.72	87.8±4.14
Winter	10.94±1.88	14.51±1.21	7.21±1.10	11.07±1.43	8.28±0.22	5.5±1.91	79.9±3.56







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RESEARCH ARTICLE

Psychometric Properties of Tamil Version of the Caregiver Burden Scale in Caregivers of Patients with Psychiatric Illness

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ABSTRACT

Caregiver Burden Scale is considered as the most sensitive tool to assess the burden of the inflicted on caregivers of chronic patients, especially people with psychiatric illnesses. However, there is no study carried out to translate such valuable tool in Tamil Language and assessed its psychometric properties. The purpose of this study is to investigate the psychometric properties of the Tamil version of Zarit Caregiver Burden Scale (ZBS/CBS) in caregivers of patients with psychiatric patients. This is a crosssectional study utilized back-translation method. The CBS was administered to 201 caregivers of patients with psychiatric illness. The internal consistency and test-retest reliability of the CBS were examined using Cronbach's α and the intraclass correlation coefficient. The results revealed that a good internal reliability was obtained with the Cronbach's alpha coefficient of 0.769. The CBS revealed acceptable internal consistency showed that adequate test-retest reliability of the scale (0.824). All subscales of CBS significantly correlated with BDI which confirmed that the CBS's construct validity. This Tamil version of the CBS is a reliable and valid measure for assessing burden of care in caregivers of patients with





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psychiatric illness and is suitable for both clinical and research use. Its properties are similar to those reported in the existing literature with CBS translation in other languages.

Keywords: Zarit Scale, Burden, Caregiver, internal consistency and reliability.

INTRODUCTION

The Caregiver Burden Scale (CBS) is also known as Zarit Burden Interview (ZBI) have been translated in various languages and validated in different population worldwide. The original version of CBS is 29-item published in 1980 (Zarit, Reever, & Bach-Peterson., 1980). However, most researchers used the full revised version of the burden scale which is 22-item. Caregiver burden is refers to a psychological state that ensues from the combination of the physical work, emotional and social pressure, like the economic restriction that arise of taking care of the patients (Dillehay& Sandys, 1990). It can be examined within the range of social, cognitive, and behavioural models. Distress and dissatisfaction are also termed as perceived losses in terms of both in their own life and through empathy with the patient. Reviewing the literature showed that Caregiver Burden Scale (CBS) appears to be the most effective tool to assess the burden experienced on caregivers of chronic patients, especially psychiatric cases. Chronic dysfunctional behaviours impair the emotional, thought and intellectual competences of people; also change personal and individual characteristics and cause social and economic losses (Gultekin, 2010; Buldukoglu, Bademli, Karakaya, Goral, &Keser, 2011; Yuku, &Derleme, 2017). Of individuals with chronic psychological maladjustments, ten percent of people only need to care in the long term hospitalization and rest of them mostly live with their families (Duman, &Bademli, 2013; Yuku, &Derleme, 2017). Hence, at least one family member needs to take care of the affected person and the member is distanced from social life as well as experience physical, mental, emotional, social and economic problems (Magliano, Fiorillo, Malangone, De Rosa, &Maj, 2006; Duman, &Bademli, 2013; Yuku, &Derleme, 2017).

After extensive review of the literature, found that Caregiver Burden Scale was translated into many languages throughout the world. Martin-Carrasco et al. (2010) validated the CBS tool in Spanish version. Whereas, Ai-Rawashdeh et al. (2016) conducted a study to validate and reliability of the tool with caregivers of the patients with Heart failure. Furthermore, Hsueh-Fen & Acton (2006) studied the psychometric properties of the caregiver burden scale with Taiwanese caregiver. Although various studies conducted to establish the reliability and validity of the CBS tool in various languages, no study conducted with the translated version of CBS tool in Tamil Language, particularly in Tamil-speaking caregivers. Considering the lack of a validated tool to measure the burden imposed on Tamil-speaking caregivers of patients with psychiatric illness, CBS seems to be an appropriate choice in this scenario. Hence, the present study designed to translate and culturally adapt the CBS for Tamil-speaking caregivers of individuals with psychiatric illness, and to test the psychometric properties of this Tamil version of Caregiver Burden Scale as well.

MATERIALS AND METHODS

Caregivers of caring for patients with psychiatric illnesses who attended Government Mohan Kumaramangalam Hospital, Outpatient Psychiatric ward in the city of Salem, Tamil Nadu, were approached for participation in the study. Participants were living along with a patient with psychiatric illnesses were selected for the present study. Caregivers for patients with psychiatric illnesses were evaluated at the Psychiatric Outpatient ward after obtained from the hospital dean and the consent with the participants. The first author explained the aim of the evaluation and invited the caregivers of the patients to take part of the study. After providing consent to participate in the study, caregivers were assessed individually in a separate room. The assessment was taken around 25 min. Participants were administered Tamil version of the Zarit Caregiver Burden Scale. The Zarit Caregiver Burden Scale was developed to measure the Burden of caregivers. Zarit(Zarit, Reever, & Bach-Peterson, 1980) and team was





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developed this instrument in English to study the burden of the English-speaking caregivers. The original tool was translated into several languages and used in a variety of cultures. However, there was no Tamil translation for the generic instrument available when the researcher wanted to use it with Tamil-speaking caregivers handling with psychiatric illness. Therefore, the idea of translating this tool into Tamil Language was started and translated by the Principal Author and content was validated through experts with the approach of back to back translation method. In the translation process, six independent translations of the CBS tool from English into Tamil were done – two by bilingual nursing tutors, two by physician from Government Mohan Kumaramangalam Hospital, Salem and two by psychology research scholars at Periyar University, Salem, Tamil Nadu. The importance of independent translation was explained to each translator.

The principal author collected the translations and compared the translated versions with each other and with the original English version. Following this, two bilingual nurse educators and an English teacher from the Government Nursing College, Salem reviewed the translations. Each translation was compared and double-checked for accuracy of the Tamil meaning of the words. As the translation of each question was reviewed, the meaning, clarity and appropriateness to the cultural values of the intended subjects were determined. After careful review of the translations, the principal author synthesized them into one form. The back translation was reviewed again by two additional English teachers and finalized by the principal author. The tool is a self-administered tool consists of 22 items, which constitutes negative effects on the caregiver in relation to physical and mental health, social activities, and economic resources. Each question is evaluated on a Likert scale with five possible responses from "never" (0) to "almost always" (4). Total scores range from 0–88.

Data Analysis

Data analyses were performed using the Jamovi software (Jamovi, 2021). Internal Internal consistency reliability was assessed using Cronbach's alpha coefficient. A coefficient of greater than 0.70 was considered indicative of acceptable internal consistency. Item-total and item-item correlations were used to demonstrate homogeneity of the items as a basis of internal consistency. 29 Item-total correlations of more than 0.20 were considered acceptable. 29 Item-item correlations were considered acceptable if they ranged between . 30 and . 70.

Convergent validity was evaluated by Pearson correlation between Zarit Burden Inventory (ZBI) scores and Beck's Depression Scale (BDS) scores. Evidence of construct validity was demonstrated through hypothesis testing that depressive symptoms would be higher in caregivers with higher burden scores. Also, test-retest reliability was established. For the test-retest reliability, after six weeks, a total of 64 caregivers were selected randomly through lottery method from the total of 214 study participants to administer the CBS once again.

RESULTS

Sample characteristics

A total of 228 caregivers take care of psychiatric illness patients in their family completed baseline assessment in the parent study, but we included only those 214 family caregivers of patients with psychiatric illness who had no missing data on study variables. As shown in Table 1, the mean age of caregivers was 41.3 years. Most of the caregivers were female (115), studied up to 5th grade (30.37%), and helping patients on a daily basis. About one third of the caregivers (30.37%) was Jobless and takes care of the illness person on fulltime basis. However, half of the caregivers were managed to be employed full time. Figure 1 stated that the caregiver scores were normally distributed with the entire sample. Nearly 70% of the study sample from the locality of rural area (150).

Reliability of Zarit Caregiver Burden Scale (Tamil Version)

Test-retest reliability data were collected from a subsample of 66 caregivers tested after six weeks after the first test. The test-retest reliability was calculated using Carl Pearson Correlation coefficient for the overall scale. The rest-retest value of Tamil version of Zarit Caregiver Burden Scale (CBS) is high enough (Intraclass Correlation Coefficient [ICCs] = 0.824, p = 0.001), which indicates good test-retest reliability over time.





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Internal consistency reliability of Tamil version of Zarit Caregiver Burden Scale

Internal consistency reliability of the CBS tool was estimated using Cronbach's α . Item reliability statistics for internal consistency of CBS instrument is shown in Table 2. The Cronbach's alpha value of 0.7 to 0.9 was considered as supportive evidence to good test. The consistency of the CBS (Tamil version) was high, yielding a Cronbach's alpha of 0.769 indicating good internal consistency.

DISCUSSION

A combination of translation procedures is perfect for fruitful translation. Researchers utilize a combination strategy including the back-translation strategy, or they apply single back-translation strategy to get fitting disobedient for their considerations. In this way, they frequently report distinctive psychometric properties such as internal consistency from the initial ponders utilizing the measures. The issue is whether true contrasts exist between the initial measures and the measures utilized in cross-cultural research. The most commonly used tool to measure caregiver burden is the Zarit Caregiver Burden Scale, which was translated into various languages and used in different countries and cultures. In this study, the short form of ZBI/CBS was translated into Tamil Language and showed a good psychometric property of the scale within the sample, specifically caregivers of psychiatric illness. The results of this study showed that this tool to be a very reliable and showed good validity. The Cronbach's alpha coefficient is much similar to other studies with translated in other languages in different countries (Bedard *et al.*, 2001; Yap, 2010; Ankri *et al.*, 2005; Chan *et al.*, 2005; Bachner&O'rourke, 2007; Hebert *et al.*, 2000; Hagell *et al.*, 2017). Furthermore, the participants within the study not varied means that the sample not heterogeneous nature. However, it is much reliable to when applied to caregivers who provide care to various groups of care recipients, including patients with elderly care, dementia, chronic illness, and brain injury.

These research findings showed that the short form of Tamil version of Zarit Burden Scale can be applied to caregivers who provide care to wide range of participants. This findings is much more consistent with various other similar studies used the tool of Zarit Burden scale. There are several limitations that should be considered in this study; there is need to learn more about the validity and reliability of the measure by further exploration of split-half reliability, factor analysis, and divergent validity. Secondly, the generalization of this findings is limited because of the sample was non-randomized and the sampling procedures was carried out in a hospital, and in a district only, not covered all the districts of Tamil Nadu, that Tamil—speaking caregivers. Further study should be include random sampling procedures with wide range of entire Tamil-speaking caregivers and that will be better represent the entire districts of the caregivers in Tamil Nadu.

CONCLUSION

In spite of the study's confinements, the discoveries appeared great psychometric qualities of the Tamil ZaritBurden Inventory, this short adaptation in terms of unwavering quality and legitimacy and can be utilized for different subgroups of caregivers involved in psychiatric illnesses. Furthermore, the think about illustrated that the instrument is additionally appropriate for caregivers of other beneficiaries. It can offer assistance to distinguish caregivers who are in trouble and require proficient help in adapting with the caregiver burden. It can moreover be used for encourage investigate within the field of care giving in an period of social changes in family structures and parts on the one hand, and expanding life anticipation and developing care needs of the other population on the other hand.

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Table 1: Socio demographic characteristics

	Number	%	Total	CBS-Score
Age				
25-40	111	51.86		42.6
41-60	99	46.26		48.1
61 & Above	4	1.86	214	53.2
Gender				
Male	99	46.26		44.0
Female	115	53.73	214	46.5
Education				
Illiteracy	53	24.76		51.2
Up to 5th grade	65	30.37		46.5
6 th to 10 th grade	47	21.96		42.6
11 th grade and above	49	22.90	214	40.5
Employability				
Full time	122	57.01		48.6
Part-time	27	12.62		42.3
Jobless	65	30.37	214	43.5
Locality				
Rural	150	70.09		45.2
Urban	64	29.90	214	45.9

Table 2: Item reliability statistics for internal consistency of Caregiver Burden Scale of Tamil Version

Item	Mean (SD)	Item-rest correlation	Cronbach's α , If item dropped
CBS_1	2.627 (0.88)	0.580	0.717
CBS_2	2.211 (0.89)	0.456	0.723
CBS_3	1.193 (0.92)	0.543	0.616
CBS_4	2.495 (0.88)	0.352	0.730
CBS_5	1.279 (0.97)	0.585	0.715
CBS_6	2.684 (0.91)	0.291	0.735
CBS_7	1.798 (0.86)	0.643	0.806
CBS_8	3.343 (1.01)	0.583	0.717
CBS_9	2.642 (0.78)	0.580	0.718
CBS_10	1.923 (0.92)	0.432	0.713
CBS_11	2.684 (1.35)	0.506	0.723
CBS_12	2.541 (1.15)	0.302	0.732
CBS_13	2.297 (0.87)	0.243	0.615
CBS_14	2.358 (0.72)	0.352	0.733
CBS_15	1.529 (1.06)	0.286	0.612
CBS_16	2.568 (1.25)	0.191	0.631
CBS_17	2.618 (1.25)	0.443	0.607
CBS_18	1.223 (1.13)	0.382	0.615
CBS_19	2.722 (1.11)	0.481	0.719
CBS_20	2.563 (1.09)	0.533	0.818
CBS_21	2.224 (1.28)	0.406	0.724
CBS_22	2.472 (0.71)	0.385	0.615
	45.1 (5.47)		0.769



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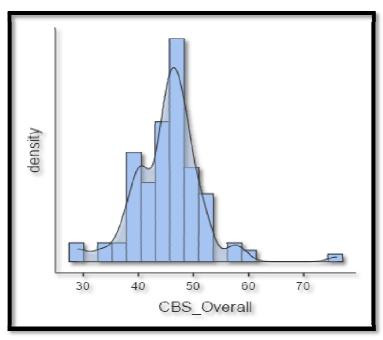


Figure 1: Normal distribution Curve of Caregiver Burden Scale Scores





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RESEARCH ARTICLE

A Study on Heavy Metals (Ni, Cd, Cr, Cu, Pb) Concentration in the Most Polluted Soil of Kalkulam Taluk and Its Removal by Phytoremediation Process using *Ananas comosus*

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ABSTRACT

The main purpose of this study is to help the people to purify their polluted land with phytoremediation technique. Phytoremediation being less cost effective and few side effects than physical and chemical approaches has gained increasing popularity in both academic and practical circles. A physicochemical study of soil is based on various parameters like soil pH, electrical conductivity (EC), organic carbon (OC), available nitrogen (N), phosphorus (P), potassium (K), and heavy metals. Adsorption atomic spectroscopy is used to find the heavy metal concentration in soil . Biotoxic effects of heavy metal in human biochemistry are of great concern.. It is also important to know their sources, leaching processes, chemical conversations and their modes of deposition to pollute the environment.. In this study Ananas comosus (pineapple) is used for the absorption of heavy metals. The concentration of heavy metals before and after phytoremediation of the pineapple were recorded. 10 soil samples from different areas of Kalkulam thaluk was taken. The results indicates there is a gradual adsorption of heavy metal by the plant. After the ten month study the concentration of heavy metals reaches below the permissible limit. At the end of the study the Ananas comosus fruit was taken, its juice is subjected to AAS. It is recorded that the heavy metals concentration is nil. So the fruit is edible to human being.

Keywords: biotoxic effects, phytoremediation, *Ananas comosus*, heavy metals.





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Helen and Cini Roach

INTRODUCTION

The pollution of soils by heavy metals from automobile sources is a serious environmental issue. These metals are released during different operations of the road transport such as combustion, component wear, fluid leakage and corrosion of metals. Lead, cadmium, copper, and arsenic are the major metal pollutants of the roadside environments and are released from fuel burning, wear out of tyres, leakage of oils, and corrosion of batteries and metallic parts such as radiators etc. The majority of the heavy metals are toxic to the living organisms and even those considered as essential can be toxic if present in excess. The heavy metals can impair important biochemical processes posing a threat to human health, plant growth and animal life. The distribution of these metals in the roadside soils is strongly but inversely correlated with the increase in the distance from road. Soil is the critically environmental medium, which is subject to a number of pollutants due to different human activities. The ongoing rapid economic boost has put a great burden on soil. With the increasing demand for metals during the course of industrialization and urbanization, more and more pollutants containing heavy metals has become widespread. Though studies on several sources of these heavy metals accumulation in the soils are well documented, little attention has been focused on vicinities of other human activities such as wet market, mechanic workshop, dumping site, paddy field and car wash which have profound influence on the soils.

The activities at the mechanic workshop generate wide varieties of wastes that are indiscriminately dumped on soils toxic metals in food stuffs, and ultimately can endanger human health. Because of its environmental significance, studies to determine risk caused by metal levels in soil on human health and forest ecosystem have attracted attention in recent years. Heavy metals may be derived from many different sources to the urbanized area. One of the most important heavy metals sources is vehicle emission. Three main factors known to influence the levels in soil samples, which have been reported, are traffic, industry and weathered materials. Top soil and dusts in urban areas are indicators of heavy metal contamination from atmospheric deposition. It has been noted that location close to roads are severally polluted by heavy metals such as Pb, As, Cu, Cd, etc, from traffic. These metals are toxic to human beings. Generally, the distribution of these metals is influenced by the nature of parent materials, climate and their relative mobility depending on soil parameter, such as mineralogy, texture and classification of soil. These waste ranging from petrol, grease, oils, suspended solid, organic solvents, junked car parts contain heavy metals that may be phototoxic to plants and harmful to animals. The objective of this study was to determine the concentration of six heavy metals (Cd, As, Cu, Cr, Pb and Ni) in soil.

Chromium

Among the elements present in the earth crust Chromium ranks the 21th and it exists in different oxidation states of which Chromium (III) and Chromium (VI) are the most water soluble and so can easily enter into the living organisms.[Determined by the National Toxicology program (NTP) the international Agency for Research on Cancer (IARC)]. Hexavalent chromium introduced into the environment through industrial discharges effluents from electro plating, leather tanning, glass, ceramic, paint and scanning industries .Chromium is used in tanneries and textile mills. Wastes from tanneries are reported to have a Chromium Concentration range from 10-50 mg/L. Textile mills also produce wastes containing Chromium. These wastes are produced during the khaki dyeing process where complex salts of Chromium are fixed in the textile and then unfixed salts Chromium get into the waste solution. Text





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mills are reported to contain Chromium range from 5- 20 mg/L. The chromium concentration in industrial wastewater ranges from 0.5 to 270 mg/l. The permissible limit of Chromium (VI) for discharge into surface water is 0.1mg/L and Chromium (VI) concentration permissible limit in drinking water is 0.005 mg/L. It is considered one of the top 16 toxic pollutants because of its carcinogenic and teratogenic characteristics. It has become a serious health concern. At higher concentrations of Chromium is toxic to plants and animals causes perforations, bronchiogenic, carcinoma etc.

Nickel

The presence of trace amounts of Nickel (II) ions in water causes great problems to the environment because of its toxicity and non- biodegradable nature. Nickel is largely present in wastewater from various industries such as electroplating, Cadmium–Nickel batteries, air-craft, phosphate fertilizers, pigments and alloys discharge into environment. Acute nickel ions poisoning can causes dizziness, headache, vomiting, chest pain, cough and cyanosis. Lungs and nasal cancers are associated with sulphide and oxide forms of nickel.

Cadmium

Cadmium discharged in the mining, metallurgical operations and electroplating. The cadmium, zinc ratio in minerals and soils being 1: 100 to 1: 1000, Cadmium is obtained as the by-product from the refining of zinc and copper, lead. Cadmium concentration in commercial phosphate fertilizers used in Wisconsin, USA have been reported to be in the range of 1-91 mg/kg. Cadmium is linked with pulmonary empbysema and lung cancer. Hypertension patients have more Cd in urine and low molecular weight protein, known as tubular proteinurea, Aminoaciduria, gulucoseurea and phosphateurea. A disease ""itai-itai" found in Japan was traced to Cd contamination in water.

Lead

Lead exist in many forms in the natural sources throughout the world and is now one of the widely and evenly distributed trace metals. Soil can be contaminated with Pb from several sources such as industrial sites, from leaded fuels, old lead plumbing pipes. Since Pb is not biodegradable, once soil has become contaminated, it remains a long term source of Pb exposure. It has an ability to cause behavioural and cognitive defects in children. The dose response of Pb affect the intelligent quotient (IQ) of children.

Copper

Copper has been described by Alloway (1990) as an important pollutant of air and agricultural soil. Intensive use of fungicides and herbicides as well as sludge and manure application has been identified as the main cause of agricultural soil contamination by Cu. Ingestion of elevated levels of copper causes gastro-intestinal distress, while long term exposure to high copper concentration causes liver and kidney damage.

MATERIALS AND METHODS

Collection of soil

The soils were collected from various villages of Kalkulam taluk. The soil was taken about 15cm below the ground level. After the collection of soil, it was subjected for sieving to remove unwanted materials and about 20 kg were transfer to each drum. Initial pH and EC were recorded before planting *Ananas*





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comosus (pineapple). Plants were brought from the nursery and planted in the drum. Inside the drum cocopeat and organic manure were added with the soil. Before plantation the soil parameters were studied. pH, EC, the concentration of heavy metals Ni, Cd, Cr were found out. Heavy metals. The soil sample was initially digested by nitric acid digestion method and then subjected to Atomic Absorption Spectroscopy (AAS). The concentration of heavy metals was recorded in terms of ppm.

RESULTS AND DISCUSSION

Present study was conducted in order to assess the heavy metal contamination of soil of villages in Kalkulam taluk namely Alanvilai, Muttom, Kappiyarai, Katimancode, Vellimalai, Manavalakurichi, Aathivilai, Kothanalloor, Thickanamcode, Colachel.. As soil pollution is dangerous for both aquatic and human health so it is the need of hour to assess the soil quality as this is a very important issue related to human and environment. For this purpose 10 soil samples were collected from the surface. Soil samples were subjected to heavy metal analysis and also for chemical parameters including pH, electrical conductivity and hardness while plants were only analyzed for heavy metals. Results were presented in the form of tables. When soil samples were collected and analyzed for heavy metals (Cu, Cd, Cr, Ni and Pb) it was found that the concentrations of heavy metals i.e. cadmium, chromium, copper and lead were beyond the maximum permissible limits set by WHO. The study revealed the heavy metal content in the soil in the villages of Kalkulam taluk is beyond the normal range and is extremely dangerous for human consumption. Analysis of chemical parameters of soil concluded that pH of soil is within the normal range set by WHO while electrical conductivity and hardness of water were recorded above the normal range set by WHO. Similarly in all soil samples concentration of all heavy metals was recorded above the permissible limits set by WHO. The reason for this extremity in values is might be due to the addition of civic wastes and industrial effluents as the sewage of the village, usage of chemical fertilizers, pesticides, insecticides is directly discharged into the soil . This is in agreement with the other studies reported that the level of heavy metals increasing in the agricultural soil due to discharge of industrial effluents and civic pollution of various kinds. This is in turn deteriorating the soil quality making it unsuitable for both aquatic and human life. Phytoremediation is a new innovative technique used to remove the heavy metals in the soil by adsorption. In this study plant Ananas comosus is planted in 10 different soil and recorded the heavy metal concentration by AAS method. The study reveals that there is a gradual decrease in the concentataion of heavy metals in every month. It is understood that the plant take the heavy metals and purify the soil.

CONCLUSION

A total of 10 soil samples were collected and were analyzed for heavy metals (Cr, Cd, Pb, Cu and Ni) using standard procedures. The results show that all the collected soil samples have heavy metals extremely higher than permissible limits set by WHO. It is phytoremediated by the plant *Ananas comosus*. After phytoremediation the heavy metals concentration values falls below the permissible limit.

Recommendations

- 1. People should minimize the use of chemical fertilizers, insecticides, pesticides
- 2. Research work should be carried out to study the accumulation of heavy metals in the crops .
- 3. Research work should be carried out to study the effects of heavy metals





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4. Government should pay attention to improve soil quality which should also consider heavy metals. So that soil is be exposed to minimum amount of heavy metals.

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Table 1.NICKEL (Concentration in ppm)

STAT	ION	NAME OF THE PLACE	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR	APR	MA
1		ALANVILAI	214.80	204.02	185.24	164.04	158.07	146.56	138.42	127.25	116.45	105.90	94.29	78.2
2		MUTTOM	216.09	207.23	184.25	173.74	162.57	151.55	140.47	129.54	107.42	96.25	85.63	74.0
3		KAPPIYARAI	215.74	204.02	183.65	172.58	161.20	150.65	149.65	128.23	116.23	105.58	84.25	73.6
4		KATIMANCODE	204.60	193.23	182.25	171.80	160.32	149.65	138.52	127.54	116.34	105.58	84.42	73.5
5		VELLIMALAI	212.98	205.02	180.23	169.63	158.54	147.45	136.65	125.42	114.52	103.25	82.24	71.2
6		MANALVALAKRURICHI	195.64	184.25	173.25	162.54	151.04	140.25	129.65	118.56	107.45	96.23	85.54	74.2
7		AATHIVILAI	183.55	171.25	160.25	149.65	138.52	127.56	116.54	105.42	94.13	83.57	72.89	61.4
8		KOTHANALLOOR	184.91	171.42	162.52	151.23	145.27	137.54	125.05	112.02	104.25	98.65	77.86	66.7
9		THICKANAMCODE	176.39	164.53	152.55	146.25	129.35	118.87	109.24	96.65	84.11	75.41	67.54	59.7
10).	COLACHEL	185.24	174.27	163.74	152.54	141.08	130.24	119.54	108.45	97.95	86.48	75.21	64.3

Table 2. CADMIUM (Concentration in ppm)

STATION	NAME OF THE PLACE	JUN	JUL	AUG	SEP	ОСТ	NOV	DEC	JAN	FEB	MAR	APR	MAY
1.	ALANVILAI	14.80	14.02	13.24	12.04	11.07	10.56	8.42	7.25	6.45	5.90	4.29	3.00
2.	MUTTOM	16.09	15.23	14.25	13.74	12.57	11.55	9.47	7.54	6.42	5.25	4.63	3.02
3.	KAPPIYARAI	15.74	14.02	13.65	12.58	11.20	10.65	9.65	8.23	6.23	5.58	4.25	3.68
4.	KATIMANCODE	14.60	13.23	12.25	11.80	10.32	9.65	8.52	7.54	6.23	4.58	3.42	2.56
5.	VELLIMALAI	12.98	11.02	10.23	9.63	8.54	7.45	6.65	5.42	4.52	3.25	2.24	1.23
6.	MANALVALAKRURICHI	15.64	14.25	13.25	12.54	11.04	10.25	9.65	8.56	6.45	5.23	4.54	2.83
7.	AATHIVILAI	13.55	11.25	10.25	9.65	8.52	7.56	6.54	5.42	4.13	3.57	2.89	1.47
8.	KOTHANALLOOR	18.91	16.42	15.52	14.23	13.27	11.54	10.05	8.02	6.25	4.65	3.86	2.75
9.	THICKANAMCODE	17.39	16.53	15.55	14.25	12.35	10.87	9.24	7.65	5.11	4.41	3.54	2.76
10.	COLACHEL	15.24	14.27	13.74	12.54	10.08	9.24	7.54	6.45	5.95	4.48	3.21	2.30

Table 3.CHROMIUM (Concentration in ppm)

STATION	NAME OF THE PLACE	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR	APR
1.	ALANVILAI	213.54	202.02	190.58	178.23	169.63	148.65	136.56	125.32	114.23	103.31	92.17
2.	MUTTOM	211.99	190.23	179.63	168.56	157.52	146.63	135.23	124.58	113.21	102.54	81.60
3.	KAPPIYARAI	215.46	194.25	182.35	161.20	150.48	139.54	128.25	117.80	106.25	95.14	84.23
4.	KATIMANCODE	213.52	181.20	170.23	159.63	148.52	137.54	126.32	115.24	104.17	93.54	82.04
5.	VELLIMALAI	212.80	189.26	179.65	168.54	157.45	146.98	135.47	124.25	113.25	102.47	91.20
6.	MANALVALAKRURICHI	214.43	187.54	162.23	151.02	149.54	138.87	127.64	116.98	105.25	94.50	83.20
7.	AATHIVILAI	211.96	192.24	189.65	178.54	167.47	156.21	145.40	134.58	103.54	92.25	82.20
8.	KOTHANALLOOR	219.53	185.56	176.89	165.54	153.32	141.44	139.88	128.54	117.66	106.44	95.02
9.	THICKANAMCODE	242.59	222.25	210.47	190.68	178.54	167.48	156.77	145.23	134.25	113.25	92.20
10.	COLACHEL	283.62	263.89	245.66	210.65	186.33	175.28	164.12	143.77	132.25	121.80	100.02

Table 4. COPPER (Concentration in ppm)

STATION	NAME OF THE PLACE	JUN	JUL	AUG	SEP	ОСТ	NOV	DEC	JAN	FEB	MAR	APR	MAY
1.	ALANVILAI	125.24	118.07	102.45	91.20	85.52	78.63	66.54	55.42	44.48	33.52	22.5	16.8
2.	MUTTOM	110.79	99.69	85.65	76.72	69.68	57.54	48.99	35.85	29.85	21.85	18.4	18.4
3.	KAPPIYARAI	100.86	95.84	88.54	74.04	68.85	56.90	48.50	36.90	29.50	21.90	18.7	15.7
4.	KATIMANCODE	152.32	135.30	120.25	99.65	86.52	77.45	63.58	55.25	47.25	38.68	26.2	18.8
5.	VELLIMALAI	136.80	128.60	110.47	91.24	87.41	76.53	65.87	54.75	43.02	31.78	28.65	22.5
6.	MANALVALAKRURICHI	125.43	111.41	100.21	98.54	85.40	73.80	60.40	58.35	46.07	34.08	22.54	21.27
7.	AATHIVILAI	122.32	110.43	98.65	88.22	78.72	67.25	52.32	41.21	39.22	28.99	22.41	21.8
8.	KOTHANALLOOR	108.59	102.57	96.53	85.47	74.95	63.65	53.03	42.95	39.50	32.02	26.08	21.05
9.	THICKANAMCODE	92.69	81.57	70.57	69.45	61.54	57.24	46.24	35.39	31.17	28.11	22.45	19.02
10.	COLACHEL	100.92	98.01	87.20	70.28	68.65	61.36	56.54	51.28	42.23	38.54	27.54	20.27





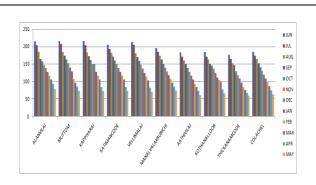
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Table 5. LEAD (Concentration in ppm)

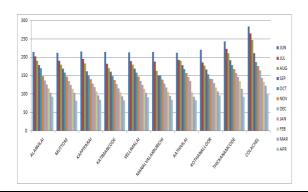
STATION	NAME OF THE PLACE	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR
1.	ALANVILAI	213.90	200.53	186.59	175.35	137.23	111.65	95.23	75.54	65.63	50.42
2.	MUTTOM	219.19	198.94	174.54	165.54	155.56	148.25	123.23	111.04	100.25	90.02
3.	KAPPIYARAI	213.94	185.45	171.22	160.23	139.53	118.85	92.58	85.50	79.02	65.08
4.	KATIMANCODE	212.20	204.01	178.10	154.02	147.63	138.23	124.99	110.31	94.7	87.21
5.	VELLIMALAI	211.98	201.72	171.32	154.52	134.54	127.45	118.92	95.23	87.21	73.65
6.	MANALVALAKRURICHI	210.74	198.68	179.68	158.52	147.54	126.54	105.58	94.23	83.20	62.54
7.	AATHIVILAI	211.15	191.09	169.52	148.22	137.54	116.22	105.89	84.23	73.25	52.54
8.	KOTHANALLOOR	111.71	181.40	170.23	159.65	138.56	127.59	106.68	95.95	84.49	73.65
9.	THICKANAMCODE	211.29	181.02	165.88	149.45	128.23	117.45	106.65	85.02	74.25	63.88
10.	COLACHEL	212.21	186.10	175.74	163.23	159.65	138.52	127.52	106.63	95.54	84.45



ig 2: The permissible limit of cadmium in soil i

Fig 1: The permissible limit of nickel in soil is 80 ppm. In all the 10 selected stations the concentration of nickel is high. After the plantation of *Ananans comosus* there is a gradual decrease in the concentration of nickel. At the month of may the concentration falls below the permissible limit value.

Fig 2: The permissible limit of cadmium in soil is 3.5 ppm. In all the 10 selected stations the concentration of cadmium is high. After the plantation of *Ananans comosus* there is a gradual decrease in the concentration of cadmium. At the month of may the concentration falls below the permissible limit value.



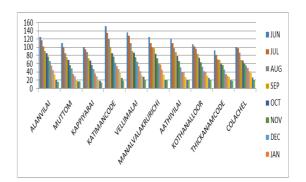


Fig 3: The permissible limit of chromium in soil is 100 ppm. In all the 10 selected stations the concentration of chromium is above the permissible limit. After the plantation of *Ananans comosus* there is a gradual decrease in the concentration of chromium. At the month of may the concentration falls below the permissible limit value.

Fig 4: The permissible limit of copper in soil is below 36 ppm. In all the 10 selected stations the concentration of copper is high. After the plantation of *Ananans comosus* there is a gradual decrease in the concentration of copper. At the end the concentration falls below the permissible limit value.



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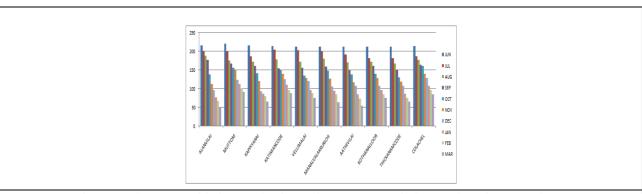


Fig 5: As per the WHO the permissible limit of lead in soil is 90 ppm. In all the 10 selected stations the concentration of lead is high. After the plantation of *Ananans comosus* there is a gradual decrease in the concentration of lead. At the end the concentration falls below the permissible limit value.





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REVIEW ARTICLE

A Study and Review of Classical, Machine Learning and Deep Learning Methods of Software Reliability Estimation for Safety-Critical Systems

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ABSTRACT

The estimation of software reliability is a crucial component for critical systems as safety is the main concern. As most of the systems are digitized, it is highly required to employ a precise method of computing the reliability. Failure of digital systems may be due to software faults which may occur either at the design phase or at the implementation/ maintenance. This paper briefly provides an insight into the various software reliability prediction methods suitable for essential systems. Most of the methods discussed in the literature demand the history of failure data and results in prediction with less accuracy. Therefore, it is the need of the hour to review the current methods of reliability applied for software and suggest an advanced method with better accuracy under uncertain circumstances. This work scrutinizes the classical techniques featuring their pros and cons with a detailed literature review. To stride over the setbacks of the classical methods, this paper also explores machine learning, and deep learning techniques. A comparative study of the above-mentioned methods is discussed in detail which is useful for the researcher. From the study, it is inferred that the machine learning and deep learning algorithms lead to accurate prediction of software reliability compared to the classical methods. Compared to the conventional methods, the Bayesian belief network and the deep learning algorithms culminate in the accurate prediction of software reliability.

Keywords: Reliability, safety critical system, classical, machine learning, deep learning





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INTRODUCTION

With the development of advanced techniques and applications, the need for complex software becomes indispensable. In most software systems, the term reliability is an important evaluation parameter. It is essential to maintain the software for critical systems fault-free and ensure its reliability [1]. To maintain the reliability of the software, estimation and prediction the software faults are necessary during the different stages of software development. The estimation of reliability and design of reliability models is extensively discussed in the literature [2-3] and these models are of paramount importance for certain applications. Assessing reliability right from the beginning stage is indispensable for most safety-critical systems [4]. Software reliability focuses on all types of errors and failures that prove to be a barrier in the process of providing expected solutions or is usually referred to as the 'probability of failure-free operation of the software. Especially for safety-critical systems[5], usage of bug-free and thoroughly tested software is to be emphasized heavily, given the fact that any minor issue could lead to loss of lives or several damages to the environment and surroundings. This paper reviews the significant reliability estimation models and methods employed in sensitive utility areas like software governing the subsystems of nuclear power plants[6-8].

Software reliability can be measured in three ways:

- 1. Estimation based on failure data obtained during the testing or operation.
- 2. Static analysis using software metrics
- 3. Evaluating the reliability of the components and its system architecture

Software reliability relies on software defects that may lead to the failure of the software. Software defects can be predicted by constructing machine learning classifiers to predict the defects in the code snippets and in the change records via the historical information and the code metrics respectively. The software fails during the design phase or at the maintenance phase; hence reliability prediction and estimation[9] are required. Normally, reliability measurement based on historical data is adopted by most companies and organizations. Software reliability prediction using historical data is computed either at the design phase or at the validation phase[10-11]. But the software for critical systems is designed following the standards compared to the other software. When there is insufficient data with respect to failure and unrealistic assumptions, the reliability models reported in the literature [12] fail to accurately predict the software reliability. Most of the researchers have developed reliability models supported by failure data with better accuracy, but this cannot be applied to safety-critical systems. Software reliability by estimating and predicting software defects can be performed via several techniques that can be broadly classified into classical, machine learning, and deep learning approaches. The most popularly used method for predicting reliability is the fault tree, Markov model, Petri nets, Bayesian belief network, and deep learning algorithms are extensively reported in the literature.

The safety of Nuclear power plant (NPP) depends mainly on the reliability of nuclear application software. To ensure the quality of nuclear application software, independent software verification and validation (V&V) work has to be carried out meticulously throughout the development life cycle of the software. The standards and regulations for Validation and Verification (V&V) of nuclear application software are developed by the international organizations, like IEEE [13-17], and IEC [18]. These standards and regulations provide the various V&V activities to be carried out and benchmark for the performance requirements of the software. They do not carry any system implementation process. Hence the nature of the test type that ensures software reliability becomes a challenging task during the software implementation phase V&V. Various regulations that are governing the implementation of V & V activity are presented in [19]. This paper also presents various principles to be followed by the testing person and key points that are to be considered for the test case. The paper also documents the various tests like logical test, function test, regulatory compliance test, reliability test, performance test etc along with the requirement specification to be followed for performing each test. For Nuclear power plants to have a sustainable development, the improvement of the safety and reliability aspects of the software being used becomes more significant. The Digital Control System





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(DCS) used in nuclear power plant is a very important device to maintain the safety of nuclear power station. Authors in [20] has presented software development project by the evaluation of V & V activity. The paper presents failure mode effect analysis (FMEA) pertaining to implementation of V & V activity. In [21], the authors report three key V & V activities like traceability analysis, hazard analysis and safety testing related to computer-based Instrumentation and control systems. The association between this analysis is explored, and their implementation techniques are discussed.

Survey On Software Reliability Estimation Methods

Instrumentation and control (I&C) systems are extremely important in nuclear power plants (NPPs) and are now integrated into the operation and reliability of nuclear power plants[22]. Because these systems constitute the first and most crucial layer of safety, reliability, and stability in a nuclear power plant, it is critical to assure the system's reliability. With the emergence of digital control systems[23] in recent decades, the digital I&C's reliability must not be jeopardized. The shift from analog to digital I&C safety systems posed new hurdles for researchers and software developers in terms of delivering accurate software reliability. It is critical for I&C systems[24] to be secure and reliable in order to avoid severe mishaps caused by attacks. Various methodologies, including as fault tree analysis (FTA), reliability block diagram (RBD), Bayesian network, and others, have been used in the literature[25] to address the reliability analysis of such systems. This paper describes the reliability analysis based on classical, machine learning and deep learning methods for critical systems as depicted in Fig.1.

Among the classical methods, Fault Tree Analysis (FTA) [26] is a well-known modelling approach applied for estimating the reliability of software that graphically depicts parallel and/or sequential fault events that may cause the system to fail. A top event in the tree is characterized as a system failure. Using Boolean gates, possible failure scenarios could be rationally illustrated through a fault tree. A quantitative analysis is performed based on those probable failure scenarios to predict the failure probability of the top event. Fault Tree Analysis can give designers useful information about the reliability of their systems, such as how likely their system is to fail and how to make it safer in the most effective way possible. The major setback of this analysis is that the reliabilities of basic events are evaluated probabilistically using past failure data and moreover, they are time and resource intensive. The Markov model is based on the probability of transitioning from one state to the next in a Markov chain[27]. It is used for a particular system consisting of a list of the system's possible states, transition paths between those states, and rate parameters for those transitions. Failures and repairs are the most common transitions in reliability analysis. Each state of a Markov model is commonly represented visually as a circle, with arrows signifying the transition paths between states for a single component with only two states: success (no noticeable damage) and failure. However, the Markov chain has some drawbacks, such as transition rates between states following an exponential distribution, the inability to describe concurrent operations, and so on. This is overcome with the Petri net method [28]where modules are represented independently according to system function and finally the integration of the system is done. A Petri Net (PN) is a directed bipartite graph with places and transitions.

The nominal behaviour of systems is simulated using Timed Petri nets, and the model is then expanded by including failures and faults. This extended model is examined for system safety and criticality analysis. PNs have been employed both as standalone methodologies and as part of model-to-model transformation approaches. More on classical techniques are elaborated in Section 3. For safety-critical systems, machine learning (ML) approaches are utilized to improve the accuracy of software reliability prediction. ML plays a critical role in the discovery of knowledge and the assessment of software reliability. Various machine learning algorithms[29] such as Bayesian Belief network, decision tree, support vector machine, random forest, logistic regression, and K-nearest neighbours are investigated for estimating the reliability of software employed for I & C units in critical systems. An elaborate review of these algorithms is discussed in Section 4. One of the major challenges in developing a software system is its quality. High quality ensures high reliability and can be affected by the occurrence of faults. Advancements in technologies have provided support for the development of computationally intensive algorithms. Deep learning is a part of machine learning that has been developed on artificial neural networks with a complex architecture and to model nonlinear relationships. Deep learning algorithms are widely used in image processing, text processing,





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speech processing[30], and other related areas. Its main advantage is the automatic extraction of features from the data, making it an easy choice for various application domains. Deep learning architectures have several layers stacked on top of each that help to learn the representations and patterns from vast amounts of data. Apart from the listed domains, deep learning approaches have also been applied in software engineering, especially in software fault predictions[31]. Convolutional Neural Networks (CNN), Deep Belief Networks (DBN), Recurrent Neural Networks (RNN), Long Short Term Memory (LSTM), Bi-directional LSTMs, transformer architectures are a few of the noticeable algorithms that help to predict the presence of faults in the software systems. Section 5 elaborates on various such algorithms available in the literature.

Classical Methods

Fault Tree Analysis is an event-oriented method used for the identification of dependence of top-level events (or root nodes) on its lower-level software events (or branches). The tree-like structure is visualized to have the undesirable event as the root node and the undesirable events that are likely to cause the top event to occur as branches. Using the failure data and repair data of the system components the Fault Tree analysis predicts the probability or frequency of failure of a system. Fault Tree Analysis is a graphical tree-like representation of the system as shown in Fig.2 with some of the causes of failure responsible for the top event to occur. The various steps involved in the fault tree analysis are system definition, construction of fault tree, qualitative evaluation, and quantitative evaluation. The step-by-step procedure for implementing the fault tree is as follows:

The system must be analysed, the top event must be determined, and the objectives must be stated.

- Prepare the fault tree,
- Identify the Cut sets (probability of an event occurring
- Fault tree analysis, quantitative interpretation, and fault tree qualitative analysis
- · Recording the outcomes and generating reports
- Assessing the implications of the failure modes, i.e. mitigating the risk

The following authors have investigated the applications of the fault tree method for nuclear power plants and the detailed literature review is discussed below: The fault tree method-based reliability evaluation has been discussed in [32]. The reliability evaluation is done for the 220 kV Kerala Power System. Open FTA and ETAP software are used to obtain the numerical probability of failure and for the simulation studies of the substation respectively. Reliability indices have been derived from this software. With the help of neural networks reliability prediction is carried out and a Neural lab has been used for this purpose. The minimal cut sets prediction was carried out using fault tree analysis. This yields the dependency of the whole system's failure upon a minimum set of components' failure. The reliability indices obtained predicted the off supply for a period of time. The number of occurrences of interruptions caused in the supply, average outage duration was also observed. The probabilistic modelling of fault tree gates and their composition as Markov Decision Processes have been discussed in [33]. The proposed technique is extended to perform an iterative probabilistic analysis of Triple Modular Redundancy architectures. The proposed methodology is more scalable, faster, and has better quality results in comparison with contemporary techniques. Reliability studies for critical software used for satellite functionalities using Software Fault tree analysis (SFTA) have been investigated in [34]. The SFTA is used to power safety logic for on-board software.

The safety requirements depending upon its security levels are categorized into catastrophic, major, and minor based with the help of Preliminary Hazard Analysis for power safety logic. Using SFTA the probability of basic events, middle event and top event are calculated, and the top event's probability is observed to be 4.2 E-7. With SFTA, the failure modes and qualitative and quantitative analysis aids early analysis of the system. However, there are certain limitations such as including the component dependency in the model are difficult. Also, the static nature of fault trees makes the modelling of redundancies, time delay conditions and other dynamic behaviour complex. The fault – tree approach is incapable of evaluating systems that can be reconfigured. Hence, it is essential to model those systems with a strong method such as the Markov model. Markov modelling is an example of analytical state space modelling techniques, used for the analysis of safety-critical systems quantitatively. The following authors have





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investigated the applications of the Markov model for nuclear power plants and detailed literature review is discussed below: The safety sensitivity analysis approaches have been developed by [35]. The sensitivity analysis is a combination of both acyclic Markov reliability model and Markov Chain Modular Approach. The modular approach enables to decompose the system into modules that can be further split into components. The sensitivity analysis in carried on a sensor system. The prediction of failure state can be easily achieved using modular approach. Application of Markov modelling for the analysis of safety of industrial Instrumentation and Control systems operating on Nuclear Power Plant have been reviewed by [36]. The model consists of deep testing features and the control system is based on programmable FPGA and also, self-diagnostic in nature. The study is based on the Reactor Trip System architecture with Markov state-space modelling used for the analysis of its safety parameters. The architecture constructed on an FPGA-based digital platform complies with the safety level "SIL 2". Markov-based chain models for the safety and reliability analysis of a digital feed water control system have been developed by [37]. A Digital Feed-Water Control System is used to control the input water level of the steam generator. A fault tolerant Digital Feed-Water Control System (DFWCS) designed using the Markov Model. Using WinSURE and the simulation results of modelling, a probability of 0.99 was achieved when the digital feed water control system was in complete operation during six months' time period.

The major setback of the Markov model is that it can be used for systems with exponentially distributed lifetimes. Petri Net is a scientific tool that is executed for modelling the information flow and applied for controlling any type of system. Visually, it is a directed bipartite graph consisting of places and transitions. The following authors have investigated the applications of the Petri Nets for nuclear power plants and detailed literature review is discussed below: Dynamic models for the test facility associated with the safety critical system in a Nuclear Power Plant have been proposed by [38]. Stochastic Petri net uses timed transition with exponentially distributed firing time associating each transition. The evaluation of stochastic Petri net using Time NET tool. This method is used for early phase performance identification. Petri nets for validation of the design of safety critical systems of Nuclear Power Plant have been used by [39]. 17 different safety critical, control, and monitoring systems were validated using the proposed model. The limitations of the exponential distribution, concurrent task modelling and performability issues have been addressed. A modular approach for the reactor protection system modelling using Petri nets have been proposed by [40]. This includes separate modelling of various modules of the protection system categorised based on their functions and integrating them together. Through modularization the system becomes more flexible, understandable, robust as well as the modelling time is reduced. The traditional Petri nets can be complex for large scale projects hence with modular Petri nets, the simulation and collaboration become easier aiding to the upgrade of it in future. The Reactor Protection system is considered to have two subsystems physically isolated. The Petri nets are modelled as modules depending on their functions as input, decision and output. GPenSim tool has been used for the module simulations. The Petri net can adapt various probability distributions and is feasible to model as modules. The major disadvantage of Petri nets is their complicated computation which makes the modelling cumbersome. The major drawbacks of traditional dependability prediction approaches are highlighted in Fig. 3.

Machine Learning Methods

Machine learning is a subpart of Artificial Intelligence. In ML, the data that is collected from application is first cleaned and then made to fit into data models. These models mimic the real time problem, and it helps us to understand the utilization of data for a particular purpose. Hence, Machine learning finds its suitability in the estimation of software reliability for dynamic models. In a dynamic model, the software reliability is estimated by cumulative failure profile or the at which failures are discovered in the past. In this literature, a few of the machine learning algorithms that have been applied for software reliability are presented. Xing, Guo, and Lyu, used Support Vector Machines (SVM) to predict Medical Imaging Software reliability [41]. The reliability was studied using 11 method-level metrics. Type I and Type II errors were evaluated to understand the performance of the proposed model. From this study authors concluded that SVM was superior to quadratic discriminant analysis and classification trees. In [42] Challagulla *et al*, evaluated the performance of various regression techniques namely linear regression, pace regression, etc. based on NASA datasets for software fault prediction that were available in public. They used eleven metrics for evaluation and the performance of the regression technique was assessed using





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average absolute error. In this study, it was concluded that there was no regression method to provide best performance for the NASA data set that was available in public. They do observe that to improve the fault prediction the size and complexity metrics were not sufficient and hence suggested to include few new metrics into the regression techniques. Koru and Liu employed K-star and random forests methods for software fault prediction model [43]. They employed the same data set used by Challagulla et al. They computed F-measure from the NASA data set and studied the performance of the proposed fault detection method. The operations like maximum, minimum, average etc were employed to convert the method level metric of KC1 dataset into class level metrics. It was observed that model performance of the proposed method was improved at class level metrics and the ability to detect the faults was much better at model level. Similar research work was carried out by Ma, Guo, and Cukic in [43]. They studied the behaviour of various machine learning algorithms on public NASA datasets. They computed method-level metrics like G-mean1, G-mean2, and F-measure to rank the performance of these machine learning algorithms. The performance of Random and Balanced Random Forests were examined by comparing the area under the region of convergence. The authors further extended an exhaustive investigation on the performance measurement for Logistic regression, discriminant analysis, classification tree, etc. From this study, they concluded that for software fault prediction problem on large dataset, Balanced Random Forests was more reliable and robust algorithm. They further concluded that the performance of boosting, ruleset, single tree classifiers were not up to the acceptable level of software reliability. For an unbalanced dataset Ma, Guo et al proposed the computation of Gmean and F-measure parameters suitable for evaluating the performance of software reliability. Khoshqoftaar, Seliya, et al employed embedded software of wireless product to enumerate the software quality in [45].

For this they used classification techniques like C4.5 decision tree, discriminant analysis, case-based reasoning, and logistic regression. They used a five file-level metrics for evaluating the software reliability. The cost of misclassification by the software was considered as performance evaluation metrics. From this study it was reported that the proposed method showed encouraging and promising performance by classifying the fault prediction with labels High, Medium and Low with much better accuracy and consistency. In [46] Wang, Yu, and Zhu, used ANN technique based on Clustering Genetic Algorithm (CGA) using rule sets and neural network-based model for improving the quality prediction on a large telecommunication system for software quality prediction. They concluded from this research that, CGA with proposed rule set was able to predict the quality of software with better accuracy, but Neural network-based prediction model provided results that were more meaningful and understandable. The prediction model for determining the high-risk modules using machine learning techniques and software complexity metrics have been discussed by [47]. Highly risky software module's reliability prediction has been carried out using the SVM technique with the help of the software metrics. The prediction was carried out with software metrics data of two NASA software projects; CM1 and PC1. The major setback of this method was the unavailability of fault data. Bayesian Network based reliability analysis for a multi-state system. The association of the system and components are brought out clearly using graphical Bayesian network and conditional probability diagram have been discussed by [48].

The Bayesian network can provide system reliability both qualitatively and quantitatively. The network was studied for how likely the fault would occur, its causes and its diagnosis and, the Bayesian network is found to work with both forward and backward inference function. GO-FLOW and dynamic Bayesian network combination approach-based reliability evaluation with uncertainty have been proposed by [49]. For evaluation, the Auxiliary Power Supply System has been considered in a Nuclear Power Plant. It has been observed that the proposed approach identifies the risk associated with the system. The integrated GO-FLOW dynamic Bayesian network approach is found to enhance the ability and range of GO – FLOW methodology in reliability evaluation and the sensitive components obtained using dynamic Bayesian network eases the evaluation. Bayesian Belief Network (BBN) based reliability prediction for computer-based systems in safety critical systems used for atomic power plants, space, medical and other systems have been discussed by [50]during the design phase itself. The software needs to be safe and reliable which is a vital one for critical systems. Software based reliability growth models have been reviewed extensively in the literature for predicting and estimating the reliability. Due to insufficiency of failure data, these models fail to predict the reliability in an accurate manner. But this work focused on BBN which resulted in an





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accurate estimation as it accounts each phase of the software development cycle. BBN approach has been formulated accommodating the errors of each phase and, propagating them to the next phase in SDLC. BBN model includes evidence collected from the knowledge team, experts, available resources and various other constraints. The methodology presented in this work has been demonstrated for a computer-based safety critical system in NPP. The accuracy of the predicted reliability model is about 97.45%. Software reliability analysis for safety-critical and control systems have been examined by [51]. This paper has focused on the application of Bayesian update methodology for twelve safety critical systems of nuclear plants. Baye's theorem and conditional probability of various evidence of safety systems have been employed to estimate the reliability. Using BBN, three steps have been carried out namely: 1. Prior estimation 2. Determining likelihood 3. Posterior estimation. Using prior estimation, with the knowledge of the system, the probability values are obtained. With real time data, the consequence is predicted. With the help of prior estimation and likelihood, the updated value of reliability estimation is found out. These three steps have been employed for all the phases of the software for 12 systems. The accuracy obtained was about 98.006% which is higher in comparison with the methods existing in literature.

The major benefits of applying BBN for software reliability estimation are as follows:

- The complexity in software, its design, verification and validation quality, and test coverage can be included in the BBN model easily.
- Likelihood function is integrated with the model, thereby making it easier to characterize and analyze uncertainty.
- BBN can include even failure-free program executions observed in the operational environment.
- BBN includes both prior probability (prediction before the event) and posterior probability (prediction after the event).

Deep Learning Methods

The different deep learning methods used in literature along with the steps involved in software reliability via fault detection is shown in figure 4, Software defects can be identified at four levels namely at the package level, modulelevel [52], method level and change level. The probability/ number of defects can be computed using two methods, static fault prediction or dynamic fault prediction. Static defect prediction is done with the help of static software metrics and the dynamic defect prediction is done over a period of time, containing the information about when the defect is generated. Syntactic and semantic features of the code can be obtained by two means. The first one is the Control flow graph (CFG) and the second one is Abstract Syntax Trees (AST) [53]. AST gives a visual representation of the code that provides top level correlation between different segments of the software components where CFG represents the code during execution. With these static/dynamic, semantic/syntactic features are used for classifying the code as faulty or not faulty ones. Conventional machine learning approaches have been widely used in the literature. Due to the advances in technology and devices, deep learning has also gained momentum and found its application in software reliability. Deep learning is one of the learning algorithms, which model the data using complex architectures via several non-linear transformations. Neural network serves as the basic building block, when stacked up to form a deep neural network. In general, deep learning architecture such as Convolutional Neural Network (CNN), Deep Belief Network (DBN), variants of Recurrent Neural Network (RNN) (54)have been used so far to detect the faulty code. To predict the defects in the code both CFG and AST have been used by extracting features from either of these two representations.

The features can then be given to traditional machine learning classifiers or the deep learning networks for software defect prediction [53]. The metrics of the nodes are computed, which can be external or internal linear metrics. The features in this work are given to attention based Bi-LSTM and Gated Recurrent Unit (GRU) to perform the statement-level defect prediction. This deep learning-based prediction system is evaluated using Code4Bench for C/C++ code dataset. Performance metrics namely accuracy, precision, recall and F1-score are calculated to measure the effectiveness of the defect prediction system. Among the deep learning approaches used for software fault prediction, LSTM is observed to perform better, since it can handle tasks [54] of indefinite sequence and long-distance dependencies. In order to capture past, future data, BI-LSTM is used, and the syntactic data is captured by Tree-LSTM. Therefore, to conserve the structure of AST, a two-channel framework that combines both BI-LSTM and





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Tree-LSTM is used in this work. AST is parsed into a matrix using an embedding layer that represents the semantic features concealed by the AST nodes. Embedding matrix has vectors, whose Euclidean distance between them provides the semantic difference between the nodes. Logistic regression serves as the final component in this architecture after the two different LSTMs to predict the software faults. The evaluation of the proposed system was done on Java open-source projects dataset. SDP at statement level is proposed with the help of a deep learning framework named SLDeep [55] that leverages the LSTM model. 32 external and internal linear software metrics that capture the static features, complexity aspects of the code were used, which were also able to distinguish similar statements of code. LSTM models a long sequence of data and its relationship, used here as a binary classifier to detect the presence of fault in a statement of source code. A matrix of metrics along with a token of statement is fed to LSTM. Dataset comprises C/C++ code from Code4Bench. The model had two layers of Bi-LSTM and followed by 6 fully connected layers, the last being the binary classification layer. The model performance was compared against Random Forest classifier on four performance metrics namely, precision, recall, F1- measure and accuracy and observed that SLDeep model is effective. A variant of Recurrent Neural Network (RNN), DP-ARNN (defect prediction using attention-based recurrent neural network was used to predict the faults in the source code [56], by learning the essential features that contain syntactic and semantic information.

The source code is parsed into Abstract Syntax Trees, a hierarchical representation that leverages on the syntactic and semantic characteristics of the code. Method invocations, declarations and control flow constructs are considered as the nodes of AST. ASTs are encoded into vectors using an index dictionary, where the frequency of occurrence of each token is considered. These encoded vectors are fed to Bi-LSTM followed by an attention layer and 2 fully connected layers and a classification layer. Attention layer is used to give proper and greater weight age to the discriminating/ essential features instead of giving equal weights to all features. This framework was also compared for its efficiency against Random Forest, restricted Boltzmann machine, Deep belief Network, Convolutional Neural Network. The other networks taken for comparison are given with the software metrics to show the significance of ASTs over the static code metrics. Predicting the number of defects present in the source code will help to estimate the faults present in the software. A deep learning neural network-based defect prediction (DPNN) system was used to predict the number of faults in a module . Datasets with software metrics were collected, pre-processed for data distribution, and normalised. The pre-processed labelled data is fed to the neural network with one input, one output and two hidden layers. The final layer predicts the number of faults in the source code. Mean squared error (MSE) and the coefficient of determination (R2) were used to evaluate the performance of the model. 10-fold cross validation is also applied, where in each fold of k-fold, one-fold is used for testing and remaining 9 folds will be used for training. However, this work has predicted the number of faults at the module level but not at the change level.

Source code is parsed in the form of ASTs. ASTs can represent the code at character-level, token-level, AST-nodelevel, tree-level, graph-level, path-level, among which the authors used node-level representations. ASTs are converted using Skip-gram based embedding layers into a vector. The embedding vector is now fed to a CNN [57]. Model over fitting is handled using a dropout mechanism, which is employed between the dense layers of CNN with L2 regularization. The model has three convolutional layers followed by a logistic regression layer to identify the fault in the source code. CNN method is evaluated on the PSC dataset for its performance and Friedman test and post-hoc Holm-Bonferroni test were conducted to show the significance of their model with other models. Authors have observed that the CNN model is significantly different from the baseline machine learning classifiers but no different from deep learning-based classifiers like DBN and other variants of CNN. The type of defect cannot be predicted by their approach, which may help in handling the defects. NASA Metrics Data Program (MDP) dataset with software measurement data and associated error data has been used [58] to evaluate the proposed CNN model for software fault prediction (SFP). The attributes of the software were normalized to convert the data instances having Gaussian Distribution and differing means and distribution. CNN was trained with several varying hyper parameters to choose the best classifier for SFP along with a Multilayer Perceptron (MLP). Afile level software defect detection approach using LSTM is proposed, since this architecture has the capability to capture the dependencies in the code in the long term [59]. A deep tree-based LSTM is used to model the AST of the source code in order to syntactic and semantic information of the source code for defects prediction. The attention mechanism used, helps to





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locate the segment of the code that caused the fault. The proposed approach parses the source code into ASTs and is again converted into embedding vectors to be fed to tree based LSTMs, which in turn generates a vector that represents ASTs, followed by a traditional classifier. Authors used both logistic regression and random forest to detect the defects. Tizen, an open-source project by Samsung and PROMISE are the two datasets used for the evaluation of the proposed work. The main limitation of this work is that they have not identified the defects at method level and defects are not predicted at code level. Two deep learning-based systems are designed for software fault prediction where the first deep neural network model proposed is DBN [60], a generative graphical model that is built on the Belief network and restricted Boltzmann machine. The first deep neural network model used by this author is DBN, a generative graphical model that is built on the Belief network and restricted Boltzmann machine. DBN is an unsupervised algorithm that has the ability to reconstruct the input with varying probabilities that is built by stacking the RBMs with a final softmax layer. The second deep learning architecture used here is Stack Sparse Auto-Encoder (SSAE) which is built using a stack of auto encoders. Authors have used 14 datasets of NASA project for fault prediction that undergo Feature scaling as part of pre-processing steps before they are fed to the two deep learning networks. A 10-fold cross validation is performed on the datasets to allow the learning models to learn from all training samples. The models are evaluated with respect to performance metrics; the authors have observed that both models did not handle the class imbalance problem well. A RNN encoder-decoder [61] deep learning model for predicting software reliability is designed where model encodes the input data into a vector and decodes them into another sequence with the help of several hidden states. This network is designed with one input and output layer and 2 hidden layers in between them. The input values are scaled to have normalized data for training.

This model considers the time of the fault as input and number of faults as target variable. 14 datasets were used to evaluate the performance of the proposed deep learning network and compared against 4 neural network models namely Feed-forward network (FFN)-Generalization, FFN-Prediction, Jordan Net-Generalization, and Jordan Net-Prediction. Average error and average bias were used as the metrics for evaluation and the authors observed that the proposed model has the lowest prediction error. Also, it was noted that the proposed model was affected by under fitting issues, which can only be handled by providing more training data samples.

Following are the characteristics observed from the deep learning models used for software reliability:

- Deep learning framework automatically captures the semantic and syntactic features of the code to discriminate between the faulty and non-faulty ones, thereby increasing the performance of the defect prediction.
- To give significance to the discriminative features of the software that possess the crucial syntaxes and semantics, attention mechanisms can be employed to capture such essential features by giving larger weights to the crucial units of the source code.
- Intrinsic nature of the LSTM framework can capture the dependency among the statements of the source code which can be considered as a sequence data.
- Deep learning supports the scalability of the system since it requires vast amounts of data for the system to learn during training.
- Efficient in learning the inherent nature of the data

Comparititive Study Of Software Reliability Estimation Techniques

A summary of classical, machine learning and deep learning methods reviewed in this work for software reliability has been tabulated in Table 1.

CONCLUSION

A comprehensive review of the different state of the art techniques to ensure software reliability is presented in this article. This survey has consolidated the different approaches used and identified the potential gaps and limitations of each of the algorithms. It is crucial to choose the appropriate technique for software reliability, with respect to the underlying application. Critical systems do not have any tolerance towards software faults. This leads to the





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development of many such new algorithms to ensure software reliability. From the literature reviewed, it is inferred that Bayesian belief network and attention based deep learning methods are observed to be prospective solutions to ensure software reliability in safety critical systems. This area of software engineering has a long history and will tend to evolve and grow with the demands. This research has given a peripheral view of the existing approaches and will surely serve as a comprehensive guide to software reliability in the future.

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Table 1. Survey of Classical, machine learning and deep learning methods for software Reliability

Tuble 1: Survey of Oil		icarriing and ac	ep learning methods for softw	vare iteriability
Author name	Type of Classification CL/ML/DL	Technique Employed	System details undertaken for the study	Observations
Abdul et al, 201	CL	Fault Tree	220 kV Kerala Power System software	Computed the probability of failure of the software employed. Computed the information on the number of interruptions caused in the supply and average outage duration
Marwan et al, 2016	CL	Fault Tree	Triple Modular Redundancy (TMR)	Scalable Less time consuming
Santhoshkumar et al, 2020	CL	Fault tree	Satellite Functionalities	Component dependency in the model is difficult. System can be analyzed at early stage Increases the complexity it is applied for a bigger system
Yangyang Yu et al, 2012	CL	Markov	Sensor system	Includes a safety sensitivity analysis approach Modular approach is employed
Valentyna <i>et al,</i> 2016	CL	Markov	I & C systems in Nuclear power plants	Self-diagnostic in nature Employed FPGA for implementing the controller
Shawkat et al, 2020	CL	Markov	Digital feed water system	A fault tolerant structure is built for the proposed system. Probability of 0.9 is obtained for fully operating states for 6 months' time
Lalit <i>et al</i> , 201	CL	Petri net	Nuclear Power Plants	Suggested for reliability prediction at the early stage
Pooja et al, 2019	CL	Petri net	Safety-critical and control systems of atomic power plants	Proposed a technique to determine the cycle time in order to process a task of the system. 17 systems were considered
Dongliang <i>et al,</i> 2021	CL	Petri Net	Reactor protection system	Modular petri nets is suggested method involves too much complexity
Young-Mi Kim and Hyeon Soo Kimas 2005	ML	SVM	NASA software Projects	SVM was employed for predicting the reliability of high-risk software modules that gave better erformance compared to other ML algorithms. Fault data and relevant complexity data set were not available.
Xing, Guo, and Lyu	ML	SVM	Medical Imaging Software	SVM provided superior results compared to that of quadratic discriminant analysis and classification trees
Challagulla et al	ML	Logistic	Public NASA datasets	Suggested to include new metrics for





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		Regression		fault prediction
Koru and Liu	ML	Random Forests	Public NASA datasets	Model performance of the proposed method was improved at class level metrics Ability to detect the faults was much better at model level.
Ma, Guo, and Cukic	ML	Random Forest	Public NASA datasets	Suggested computation of G-mean and F-measure parameters for evaluating the software reliability performance
Khoshgoftaar, Seliya, and Gao	ML	Decision Tree	Software for wireless products	Encouraging and promising performance was observed with faults being classified into 3 levels of label viz, High, Medium and Low.
Wang, Yu, and Zhu	ML	Genetic Algorithm	Software used in telecommunications system	CGA with proposed ruleset was able to predict the quality of software with better accuracy, Neural network-based prediction model provided results that were more meaningful and understandable.
Ying-Kui et al, 2013	ML	BBN	Multi-state system	Provided system reliability both qualitatively and quantitatively
Yi Ren <i>et al</i> , 2017	ML	BBN	Auxiliary Power supply system in NPP	Uses the integrated GO-FLOW dynamic Bayesian network approach Easy computation
Pramod kumar <i>et</i> al, 2021	ML	BBN	Safety-critical systems in NPP	Results in accurate prediction of software reliability even in uncertainty conditions where the history of failure data is not available. The accuracy obtained is 97%
Pramod kumar et al, 2019	ML	BBN	Safety-critical systems in NPP	Reliability prediction involved prior estimation, determining the likelihood, and posterior estimation. Accuracy obtained is 98%
Munir H <i>et al,</i> 2021	DL	GRU, Bi- LSTM	C/C++ programs in Code4Bench	Statement level fault detectionLimited to single language, No cross-project defect predictions
Zhou X <i>et al</i> , 2020	DL	BI-LSTM and Tree-LSTM with Logistic regression	Java open-source projects	Automaticallycaptures semantic and syntactic information. Will not support different programming languages
Majd A <i>et al</i> , 2020	DL	Bi-LSTM	C/C++ code from Code4Bench	Statement-level analysis, Cross-project and withinproject defect prediction
Fan G <i>et al</i> , 2020	DL	Attention- Based Recurrent Neural	Java projects in Apache	Superior features extracted No cross-project defect predictions

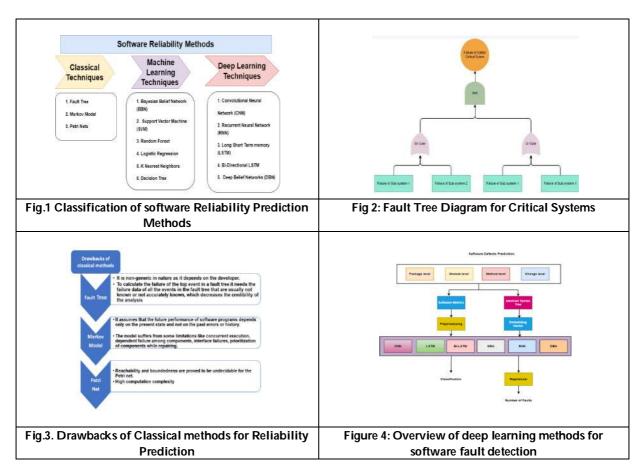




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		Network		
Qiao L <i>et al</i> , 2020	DL	DNN	MIS dataset and the KC2 dataset	Software Module Level Generates Number of Faults No Cross Project Defect Predictions
Pan C et al, 2019	DL	CNN	PROMISE repository	Within Project Defect Prediction No Cross Project Defect Predictions
AI Qasem O et al, 2020	DL	CNN, MLP	Four mission-critical NASA software projects datasets (PC1 and CM1, (KC1 and KC3)	Code level metrics used for prediction Analysis done with varying hyper- parameters
Khanh <i>et al</i> , 2018	DL	Deep tree- based LSTM	Tizen, an open-source project by Samsung and PROMISE datasets	Within-project and cross-project predictions Defects at method level, code level are not predicted.
Hasanpour <i>et al</i> , 2020	DL	DBN, Stack Sparse Auto- Encoder	14 datasets of NASA project	Did not handle the class imbalance problem well.
Wang et al, 2018	DL	RNN	14 fault data sets	Lowest Prediction Error Underfitting Issues

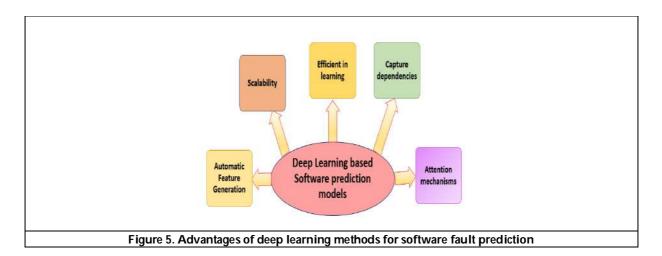






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RESEARCH ARTICLE

Analysis of Bio-Active Compounds from Cylohexane Extract of Sansevieria cylindrica Leaves by Gas **Chromatography-Mass** Spectrometry

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ABSTRACT

Sansevieria cylindrica is an Indian herb plant used for various ailments by traditional healers and it is a small tree plant. In the present investigation, there are ten phytochemical constituents have been identified and isolated from cyclohexane extract of Sansevieria cylindrica by Gas Chromatography-Mass Spectrometry. The ten phytochemical constituents were present in leaves parts are (z)6,(z)9-Pentadecadien-1-ol (44.4%), 7-Hexadecenoic acid, methyl ester,(z)- (17.8%), Pentadecanoic acid,14methyl-methyl ester (6.7%), n-Hexadecanoic acid (5.7%), BIS(2- Ethylhexy)phthalate (4.5%), Tricyclo[20.8.0.0(7,16)]1 (22),7(16)-diepoxy- (3.4%), Cyclopentane undecanoic acid, methyl ester (3.3%), Cyclopentane tridecanoic acid, methyl ester (1.3%), 3,7,11,15-tetramethyl-2- hexadecen-1-ol (1.3% & 1.0%). Out of ten phytochemical constituents, nine phytochemical constituents are bio-active compounds were identified by Gas Chromatography-Mass Spectrometry.

Keywords: Sansevieria cylindrica, Phytochemical constituents, Cyclohexane extract, Bio-active compounds, Gas Chromatography-Mass Spectrometry





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INTRODUCTION

Medicinal plants are considered as one of the main sources of biologically active compounds [1]. The plants have valuable medicinal properties due to the presence several phytochemical constituents such as tannins, alkaloids, flavonoids, terpenoids etc., compounds called secondary metabolites [2]. Among them some were act as synergistic and enhance the biological activity of other compounds. *Sansevieria cylindrica* is commonly referred to as Spear *Sansevieria* and it is cultivated in Egypt for ornamental purposes [3]. And it is also found in some part of India as an ornamental plant. *Sansevieria cylindrica* plant has different chemical constituents such as steroidal saponins, ester, cumarins, phenols, fatty acids etc.,[4]. The plant *Sansevieria* species was investigated for many pharmacological activities, such as antitumor, antimicrobial, antioxidant etc.,[5]. The many of medicinal plants and their purified chemical constituents have shown beneficial therapeutic potentials. GC-MS analysis is the best technique to identify and isolated bioactive phytochemical constituents [6]. With this background study were aimed to identify and isolated the bioactive constituents from *Sansevieria cylindrica* by using GC-MS study.

MATERIALS AND METHODS

Collection and Preparation of Plant Materials

The fresh plant leaves of *Sansevieria cylindrica* were collected from Puthnampatti Village in Trichirappalli district of Tamil Nadu, India during October to November 2021 and authenticated by director of the Rapinat Herbarium and Centre for Molecular Systematic, St. Joseph's college (campus), Trichirappalli, Tamil Nadu, India. Fresh Plants leaves of *Sansevieria cylindrica* were cleaned with running tap water and dried under the shade (sunlight). Then the dried plant leaves were ground to fine powder mechanically and preserved in containers for further analysis.

Extraction Procedure

The fine powdered of *Sansevieria cylindrica* leaves (200g) were extracted with cyclohexane (500ml, 6 h) at temperature between 50-60°C by using Soxhlet extractor. The solvents were evaporated by Rotary Vacuum Evaporator, (Model RE-801) to obtained semi solid. The semi solid dry cyclohexane crude extract were suspended in water and it analyzed by GC-MS.

GC-MS Analysis

The Gas Chromatography-Mass Spectrometry were performed on a combined GC-MS instrument (ITQ 900 Model of Thermo Fisher Scientific make) using a HP-5 fused silica gel capillary column. The method to perform the analysis were designed for both GC and MS. 1 μ L aliquot of sample were injected into the column using a PTV injector whose temperature were set at 275°C. The GC Program were initiated by a column temperature set at 60°C for 5 min, increased to 300°C at a rate of 8 C/min, held for 10 min Helium were used as the carrier gas (1.5 ML/min). The mass spectrometer was operated in EI mode with mass source were set at 200°C. The chromatogram and spectrum of the peaks were visualized. The particular phytochemical constituents present in the sample were identified and isolated by matching their mass spectral fragmentation of the respective peaks in the chromatogram with those stored in the National Institute of Standard and Technology Mass Spectral database library.

RESULT AND DISCUSSION

The results pertaining to Gas Chromatography-Mass Spectrometry studies led to the identified and isolation of number of phytochemical constituents from the Gas Chromatography fractionations of the cyclohexane extract of *Sansevieria cylindrica* leaves. These compounds were isolated and identified through mass spectrometry attached with Gas Chromatography. The compound prediction is based on NIST Database. The results of present investigation, 10 phytochemical constituents have been identified and isolated by Gas Chromatography-Mass Spectrometry (listed in Table 1). The ten phytochemical constituents were present in leaves parts are (z)6,(z)9-Pentadecadien-1-oI (44.4%), 7-Hexadecenoic acid, methyl ester,(z)- (17.8%), Pentadecanoic acid,14-methyl-methyl ester (6.7%), n-Hexadecanoic acid





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(5.7%), BIS (2-Ethylhexy) phthalate (4.5%), Tricyclo[20.8.0.0(7,16)]1 (22),7(16)-diepoxy- (3.4%), Cyclopentaneun decanoic acid, methyl ester (3.3%), Cyclopentane tridecanoic acid, methyl ester (1.3%), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (1.3% & 1.0%) (Table 1). The spectrum profile of GC-MS was confirmed the presence of ten major phytochemical constituents with the retention time 20.9, 26.44, 29.64 & 32.38, 30.04, 31.46, 34.54, 33.74, 35.05, 37.23 and 42.39 respectively (Fig: 1).The individual fragmentation of the Phytochemical constituents was illustrated in (Fig: 2A-2J). Out of 10 phytochemical constituents, 9 phytochemical constituents are bio-active compounds were identified and isolated by GC-MS. The various biological activities of bio-active compounds were indicated in the Table 2.

CONCLUSION

Sansevieria cylindrica extracts contains various phytochemical constituents with biological activity can be of valuable therapeutic key. The GC-MS analysis of cyclohexane extract of Sansevieria cylindrica reveals the presence of ten phytochemical constituents. The presence of such variety of phytochemical constituents may be attributed to the medicinal characteristics of this plant Sansevieria cylindrica. The presences of out of ten phytochemical constituents, nine phytochemical are bio-active reveals the importance of the plant as medicinally used. Recommended to further research, isolate the bio-active compounds individually and subjecting them biological activity.

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Table.1: Phytochemical Constituents Indentified and Isolated from Cyclohexane Extract of Sansevieria cylindrica Leaves

S.No	Compound Name	Molecular Formula	Molecular Weight	Retention Time	% Peak Area
1	Cyclopentanetridecanoic acid, methyl ester	C19H36O2	296	20.9	1.4
2	Cyclopentaneundecanoic acid, methyl ester	C17H32O2	268	26.44	3.354
3	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C20H40O	296	29.64&32.38	1.388 & 1.039
4	1-Heptatriacotanol	C37H76O	536	30.04	0.275
5	Pentadecanoic acid,14-methyl-methyl ester	C17H34O2	270	31.46	6.711
6	n-Hexadecanoic acid	C ₆ H ₂₂ O ₆	256	34.54	5.788
7	7-Hexadecenoic acid, methyl ester, (z)-	C17H32O2	268	33.74	17.841
8	Tricyclo[20.8.0.0(7,16)]1 (22),7(16)-diepoxy-	C30H52O2	444	35.05	3.402
9	(z)6,(z)9-Pentadecadien-1-ol	C15H28O	224	37.23	44.432
10	BIS(2-Ethylhexy)phthalate	C24H38O4	390	42.39	4.503

Table.2: Bio-Active Compounds Identified and Isolated from Cyclohexane Extract of Sansevieria cylindrica Leaves

S.No	Compound Name	Molecular Formula	Nature of the Compound	***Biological Activity
1	Cyclopentanetridecanoic acid, methyl ester	C19H36O2	Fatty acid	Antimicrobial, Anti-inflammatory, Anticancer, Dieruretic [7,8].
2	Cyclopentaneundecanoic acid, methyl ester	C17H32O2	Fatty acid	Antimicrobial[9].
3	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C20H40O	Terpenol	Antimicrobial, Anti-Oxidant, Anti-Inflammatory[6, 10,16].
4	1-Heptatriacotanol	C37H76O	-	Antimicrobial, antineoplastic, anticancer and anti-HIV [11,12]
5	Pentadecanoic acid,14-methyl-methyl ester	C17H34O2	Palmitic acid methyl ester	Antioxidant [13].





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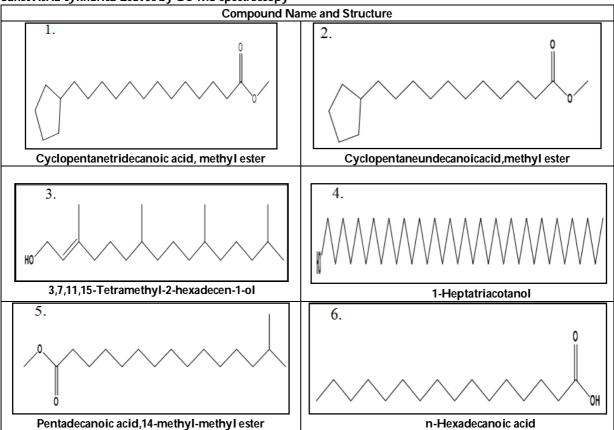
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				Antioxidant, Antitumor,
				Anti-Oxidant, Anti-
				inflammatory,
				Hypochloesterolemic,
6	n-Hexadecanoic acid	C6H22O6	Palmitric acid	Nematicide, Pesticide,
				Lubricant, Antiandrogenic,
				Hemolytic,
				5-Alpha reductase inhibitor
				[10, 13, 16].
7	7-Hexadecenoic acid, methyl ester,(z)-	C17H32O2	Palmitic acid methyl ester	Antimicrobial [14].
			Fatty	Antioxidant, Antimicrobial
8	(z)6,(z)9-Pentadecadien-1-ol	C ₁₅ H ₂₈ O	acid,alcohol	[15, 16].
			Diester of	r1
9	BIS(2-Ethylhexy)phthalate	C24H38O4	phthalic acid	Plasticizer[17].

^{***}Activity source: Dr. Duke's Phytochemical and Ethno botanical Database

Table 3: NIST data of Phytochemical Constituents structure Indentified and Isolated from Cyclohexane Extract of Sansevieria cylindrica Leaves by GC-MS spectroscopy



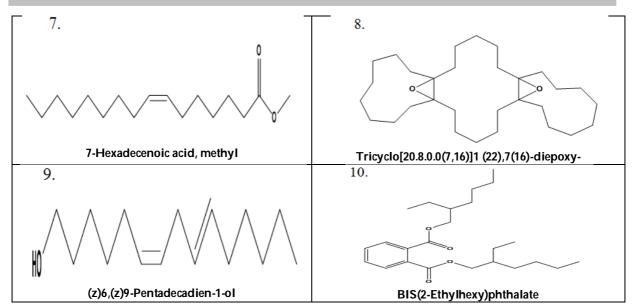




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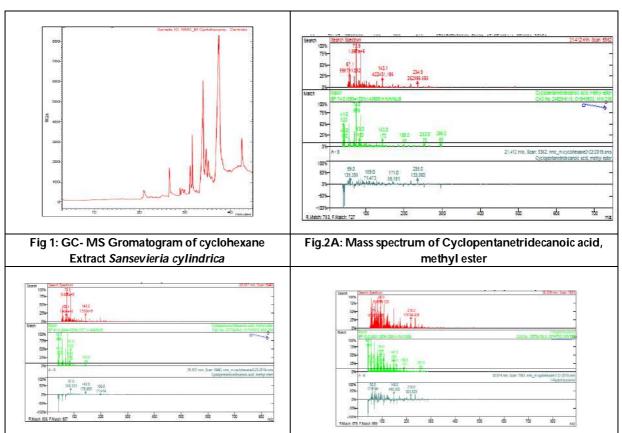




Fig.2 B: Mass spectrum of

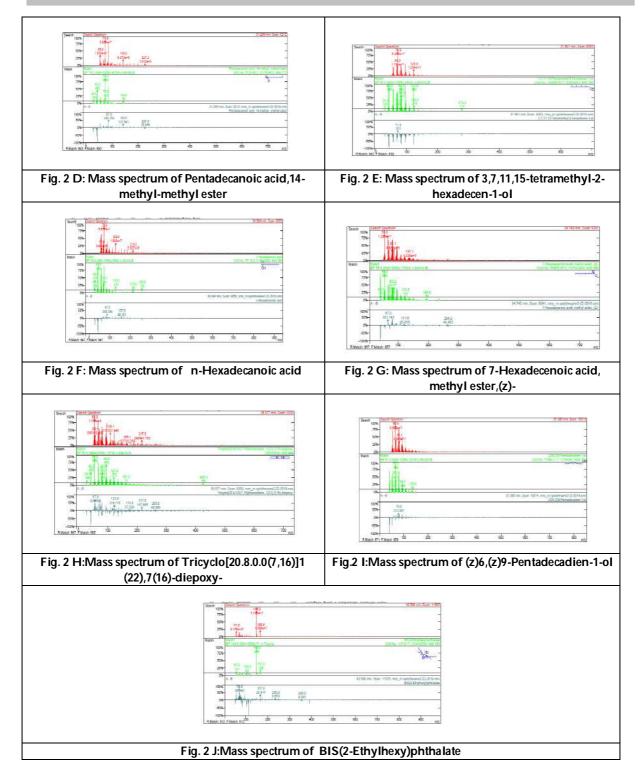
Cyclopentaneundecanoic acid, methyl ester

Fig.2C: Mass spectrum of 1-Heptatriacotanol

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RESEARCH ARTICLE

Variants of Labeling on Certain Chemical Graphs

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ABSTRACT

We call a mapping $f: V(G) \to \{1,2,...,k\}$, lucky labeling or vertex labeling by sum if for every two adjacent vertices u and v of G, $\sum_{(v,u)\in E(G)}f(v)\neq \sum_{(u,v)\in E(G)}f(u)$. The lucky number of a graph G, denoted by $\eta(G)$, is the least positive k such that G has a lucky labeling with $\{1,2,...,k\}$ as the set of labels. Let $I:V(G)\to N$ be a labeling of the vertices of a graph G by positive integers. Define $c(u) = \sum_{v \in N(u)} l(v) + d(u)$, where d(u)denotes the degree of u and N(u) denotes the open neighborhood of u. The labeling I is d-lucky if c(u) $\neq c(v)$, for every pair of adjacent vertices u and v in G. The d-lucky number of a graph G, denoted by $\eta_d(G)$, is the least positive k such that G has a d-lucky labeling with $\{1,2,...,k\}$ as the set of labels. In this paper we study the lucky labeling and d-lucky labeling problem as a vertex coloring problem. We obtain $\eta(G)$ for H-Naphtalenic [2m,2n] nanosheet, $C_6[m,n]$ nanosheet, $C_4C_8(R)[2m,2n]$ and $\eta_{dl}(G)$ for $C_4C_8(R)[2m,2n]$ and triangular graphs.

2010 AMS Classification: 05 C78

Keywords: d-lucky, tessellations, nanosheet, lucky, labeling

INTRODUCTION

A graph is a collection of objects and the binary relations between these objects. A chemical graph is a replica of a chemical system that is used to describe the relationship between chemical objects like atoms, bonds, group of atoms and molecules. Graph theory has magnificent use in chemistry, especially in designing chemical structures. A chemical structure is a carrier of chemical information in compressed form. The molecular formula of a chemical compound is a general parameter that broadly delimits the scope of structures to isomers in which stability plays a





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remarkable role. Carbon nanosheets are a new kind of two-dimensional polymeric material that is fabricated by cross-linking aromatic self-assembled monolayers with electrons. Due to their uniform thickness of only about one nanometer, as well as their high chemical, mechanical, and thermal stability, such materials are of high interest for a wide variety of applications [10]. As the nanosheet is stable under an electron beam, patterns can also be written by electron beam induced deposition (EBID). Because of their stability and flexibility, carbon nanosheets will likely find a multitude of applications, including potential use as sensors, filtration membranes, sample supports, and even conductive coatings [11]. Graph coloring is one of the most studied subjects in graph theory. Graph labeling was introduced by Rosa in 1967. Recently, Czerwinski et al. [1] have studied the concept of lucky labeling as a vertex coloring problem. Karonski, Luczak and Thomason [2] initiated the study of proper labeling. A proper labeling of a graph is an assignment of integers to some elements of the graph, which may be the vertices, the edges, or both of them, such that we obtain a proper vertex coloring via the labeling, subject to some conditions. Ahadi et al. [4] have proved that computation of lucky number of planar graphs is NP-hard. Lucky labeling is applied in real life situations such as transportation network, where pair wise connections are given some numerical values and each weight could represent the stations or city with certain expenses or costs etc. They are also applicable in computational biology to model protein structures.

Main Results

We now give the definition of lucky labeling. For a vertex u in a graph G, let $N(u) = \{v \in V \ (G)/(u,v) \in E(G)\}$ and $N[u] = N(u) \cup \{u\}$.

Definition 2.1 [1]

Let $I: V(G) \to N$ be a labeling of the vertices of a graph G by positive integers. Define lucky sum of a vertex u ass $(u) = \sum_{w \in N(u)} I(w)$. The labeling I is a lucky labeling if $s(u) \neq s(v)$ for every pair of adjacent vertices u and v in G. The lucky number of G, denoted by g(G), is the least positive integer g(G) such that G has a lucky labeling g(G) and g(G) are g(G) as a variant of lucky labeling g(G).

Definition 2.2 [3]

Let $I:V(G)\to N$ be a labeling of the vertices of a graph G by positive integers. Define the d-lucky sum of vertex u as $c(u)=\sum_{v\in N(u)}I(v)+d(u)$, where d(u) denotes the degree of u. The labeling I is a d-lucky labeling if $c(u)\ne c(v)$, for every pair of adjacent vertices u and v in G. The d-lucky number of a graph G, denoted by $\eta dI(G)$, is the least positive integer k such that G has a d-lucky labeling $I:V(G)\to \{1,2,...,k\}$.

In this paper, the lucky number is obtained for:

- i. H-Napthalenic [2m,2n] nanosheet
- ii. C₆[m,n] nanosheet ii. C₄C₈(R)[2m,2n] nanosheet and

d-lucky number is obtained for:

- i. $C_4C_8(R)[2m,2n]$ nanosheet and
- ii. triangular graphs.

H-Naphtalenic [2m,2n] nanosheet

Definition 2.3 [5, 6]

A H-Naphtalenic [2m,2n] nanosheet is a trivalent decoration made by alternating squares C_4 , pair of hexagons C_6 and octagons C_8 and it is a bi-regular graph with m number of rows and n number of columns. The H-Naphtalenic [2m,2n] nanosheet has 10mn vertices. See Fig.1.

2.1.1Algorithm

Lucky labeling of *H*-Naphtalenic [2*m*,2*n*] nanosheet

H-Naphtalenic [2m,2n] nanosheet





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Algorithm

Step 1. Removal of edge cuts $S_1, S_2, ..., S_m$ as shown in Fig.1(a) leave m+1 paths $P_1, P_2, ..., P_{m+1}$. See Fig.1(b).

Step 2. The vertices of P_i for i odd, $1 \le i \le m$ are labeled alternately with 1 and 2 from left to right, beginning with 1.

Step 3. The vertices of P_i for i even, $2 \le i \le m+1$ are labeled alternately with 2 and 1 from left to right, beginning with 2. See Fig.2.

Output: $\eta(H - Naphtalenic[2m,2n]) = 2$.

Proof of correctness

Let $E_1 = \{e = (u, v) \text{ in } E \text{ / degree of every vertex in } N[u] \cup N[v] \text{ is 3}\}$. By the algorithm, if I(u) = 1, then I(v) = 2. Further all neighbors of u are labeled 2 and all neighbors of v are labeled 1. Thus s(u) = 6 and s(v) = 3.

Let $E_2 = E \setminus E_1$. If e = (u, v) is an edge in E_2 and if I(u) = 1, then I(v) = 2. Further all neighbors of u are labeled 2 and the neighbors of v are labeled 1. Thus s(u) = 4 and s(v) = 2.

Theorem 2.4

Let G be a H-Naphtalenic [2m,2n] nanosheet. Then $\eta(G) = 2$.

C₆[m,n] nanosheet

Definition 2.5 [7, 6]

A $C_6[m,n]$ nanosheet is a trivalent decoration made by hexagon C_6 and it is a bi-regular graph with m number of rows and n number of columns. It has (4n + 2)m vertices. See Fig.3(a).

2.2.1Algorithm

Lucky labeling of C₆[m,n] nanosheet

Input: C₆[m,n] nanosheet Algorithm

Step 1. Removal of edge cuts $S_1, S_2, ..., S_m$ as shown in Fig.3(a) leave m+1 paths $P_1, P_2, ..., P_{m+1}$. See Fig.3(b).

Step 2. The vertices of Pifor i odd, $1 \le i \le m$ are labeled alternately with 1 and 2 from left to right, beginning with 1.

Step 3. The vertices of P_ifor i even, $2 \le i \le m+1$ are labeled alternately with 2 and 1 from left to right, beginning with 2. See Fig.4.

Output: $\eta(C_6[m,n]) = 2$.

Proof of correctness

Let $E_1 = \{e = (u,v) \text{ in } E \text{ / degree of every vertex in } N[u] \cup N[v] \text{ is } 3\}$. By the algorithm, if I(u) = 1, then I(v) = 2. Further all neighbors of u are labeled 2 and all neighbors of v are labeled 1. Thus s(u) = 6 and s(v) = 3.

Let $E_2 = E \setminus E_1$. If e = (u,v) is an edge in E_2 and if I(u) = 1, then I(v) = 2. Further all neighbors of u are labeled 2 and the neighbors of v are labeled 1. Thus s(u) = 4 and s(v) = 2.

Theorem 2.6

Let G be a $C_6[m,n]$ nanosheet. Then $\eta(G) = 2$.

C₄C₈(R)[2m,2n] nanosheet

Definition 2.7 [8, 9]

A $C_4C_8(R)[2m,2n]$ nanosheet is composed of alternating squares C_4 and octagons C_8 and it is a bi-regular graph with m number of rows and n number of columns. The $C_4C_8(R)[2m,2n]$ nanosheet has 4(m+1)(n+1) vertices. See Fig.5(a).

2.3.1Algorithm:

Lucky labeling of C₄C₈(R)[2m,2n] **Input:** C₄C₈(R)[2m,2n] nanosheet





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Algorithm

Step 1. Removal of edge cuts $S_1, S_2, ..., S_m$ as shown in Fig.5(a) leave m+1 chains $D_1, D_2, ..., D_{m+1}$ of alternating sequences of diamonds and edges, beginning and ending with diamonds. See Fig.5(b).

Step 2. Choose vertices of D_i inducing a path. Vertices of any longest path beginning with an unlabeled vertex of the first diamond of D_i and ending with an unlabeled vertex of the last diamond of D_i are labeled alternately with 1 and 2 from left to right, beginning with 1 if i is odd and beginning with 2 if i is even, $1 \le i \le m$. See Fig.6.

Output: $\eta(C_4C_8(R)[2m,2n]) = 2$.

Proof of correctness

Let $E_1 = \{e = (u,v) \text{ in } E \text{ / degree of every vertex in } N[u] \cup N[v] \text{ is } 3\}$. By the algorithm, if I(u) = 1, then I(v) = 2. Further all neighbors of u are labeled 2 and all neighbors of v are labeled 1. Thus s(u) = 6 and s(v) = 3.

Let $E_2 = E \setminus E_1$. If e = (u,v) is an edge in E_2 and if I(u) = 1, then I(v) = 2. Further all neighbors of u are labeled 2 and the neighbors of v are labeled 1. Thus s(u) = 6 and s(v) = 3.

Theorem 2.8

Let G be a $C_4C_8(R)[2m,2n]$ nanosheet. Then $\eta(G) = 2$.

Remark 2.9

The algorithm for lucky labeling of $C_4C_8(R)[2m,2n]$ nanosheet does not yeild a d-lucky labeling of the nanosheet. In Fig.6, s(u) = s(v) = 6.

2.3.2Algorithm

d-lucky labeling of C₄C₈(R)[2m,2n] **Input:** C₄C₈(R)[2m,2n] nanosheet

Algorithm

Step 1. Label all the vertices of C₄C₈(R)[2m,2n] of degree 2 as 2.

Step 2. Removal of edge cuts $S_1, S_2, ..., S_m$ as shown in Fig.5(a) leaves m+1 chains $D_1, D_2, ..., D_{m+1}$ of alternating sequences of diamonds and edges, beginning and ending with diamonds. See Fig.5(b).

- (i) The unlabeled vertices of D₁ in the nanosheet induce a path. Label vertices of this path alternately with 1 and 2 from left to right beginning with 2.
- (ii) Label the unlabeled vertices of $D_2,...,D_m$ as follows:

Vertices of any longest path beginning with an unlabeled vertex of the first diamond of D_i and ending with an unlabeled vertex of the last diamond of D_i are labeled alternately from left to right with 1 and 2, beginning with 1 if i is even and beginning with 2 if i is odd, $2 \le i \le m$.

(iii) The unlabeled vertices of D_{m+1} are labeled with 1 and 2 alternately, beginning with 1 if m is odd and beginning with 2 if m is even. See Fig.7.

Output: $\eta dl(C_4C_8(R)[2m,2n]) = 2.$

Proof of correctness

Let $E_1 = \{e = (u,v) \text{ in } E \text{ / degree of every vertex in } N[u] \cup N[v] \text{ is } 3\}$. By the algorithm, if I(u) = 1, then I(v) = 2. Further all neighbors of u are labeled 2 and all neighbors of v are labeled 1. Thus c(u) = 9 and c(v) = 6.

Let $E_2 = E \setminus E_1$. If e = (u,v) is an edge in E_2 and if I(u) = 1, then I(v) = 2. Further all neighbors of u are labeled 2 and the neighbors of v are labeled 1,1 and 2. Thus c(u) = 9 and c(v) = 7.

Theorem 2.10

Let G be a $C_4C_8(R)[2m,2n]$ nanosheet. Then $\eta_{dl}(G)=2$.





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Remark 2.11

The algorithm for d-lucky labeling of $C_4C_8(R)[2m,2n]$ nanosheet does not yield a lucky labeling of the nanosheet. In Fig.7, c(u) = c(v) = 4.

Triangular Graph

Definition 2.12

Let n be a non-negative integer. A triangular graph of order n, TG_n , is defined in the following way: TG_1 is a hexagon When $n \ge 2$, TG_n is built according to the following step: Draw n rows of regular hexagons of the same size within an equilateral triangle (which is called the framework of TG_n) so that the first row consists of one hexagon, the second row consists of two hexagons and the n^{th} row consists of n hexagons. Set all the vertices of these hexagons to be the vertices of TG_n and set all the sides of these hexagons to be the edges of TG_n .

2.4.1Algorithm

d-lucky labeling of triangular graph

 $\textbf{Input:} \ Triangular \ Graph, \ TG_n$

Algorithm

- Step 1. Removal of edge cuts S₁,S₂,...,S_m as shown in Fig.8(a) leave m+1 paths P₁,P₂,...,P_{m+1}. See Fig.8(b).
- **Step** 2. The vertices of P_i for $1 \le i \le m$ are labeled alternately with 1 and 2 from left to right, beginning with 2.
- Step 3. The vertices of P_{m+1} are labeled alternately with 1 and 2 from left to right, beginning with 1. See Fig.9.

Output: $\eta dI(TG_n) = 2$.

Proof of correctness

Let $E_1 = \{e = (u,v) \text{ in } E \text{ / degree of every vertex in } N[u] \cup N[v] \text{ is } 3\}$. By algorithm, if I(u) = 1, then I(v) = 2. Further all neighbors of u are labeled 2 and all neighbors of v are labeled 1. Thus c(u) = 9 and c(v) = 6. Let $E_2 = E \setminus E_1$. If e = (u,v) is an edge in E_2 and if I(u) = 1, then I(v) = 2. Further all neighbors of u are labeled 2 and the neighbors of v are labeled 1. Thus c(u) = 6 and c(v) = 4.

Theorem 2.13

Let G be a triangular graph. Then $\eta dI(G) = 2$.

CONCLUSION

In this paper, the lucky number is obtained for H-Napthalenic [2m,2n] nanosheet, $C_6[m,n]$ nanosheet, $C_4C_8(R)[2m,2n]$ nanosheet and the d-lucky number is obtained for $C_4C_8(R)[2m,2n]$ nanosheet and triangular graph. These problems are under investigation for certain other chemical structures.

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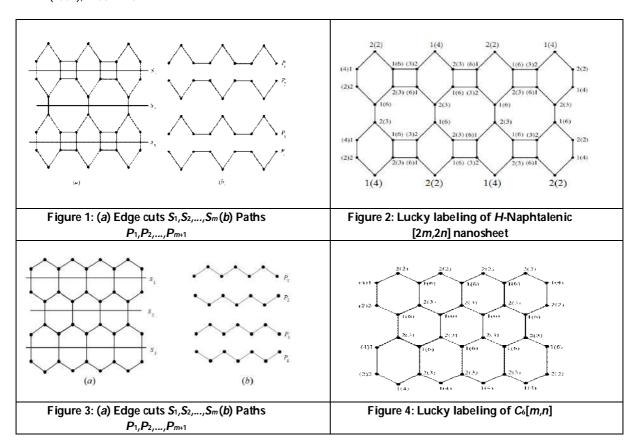


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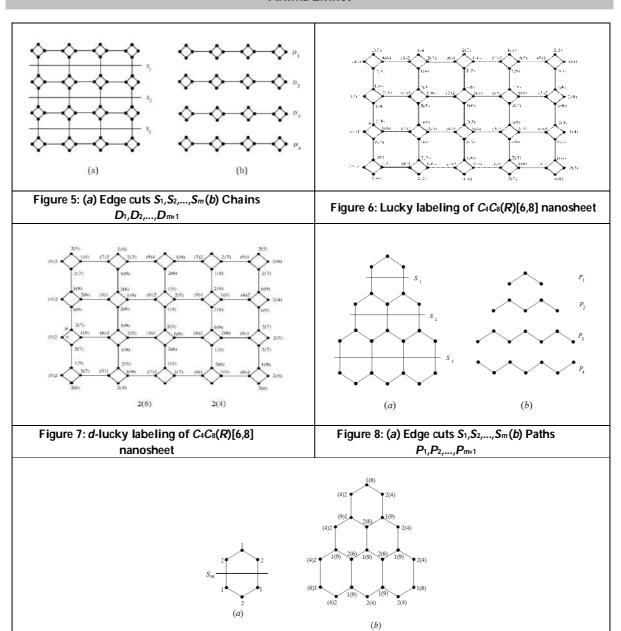




Figure 9: (a) d-lucky labeling of TG1 (b) d-lucky labeling of TG3



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REVIEW ARTICLE

Chronotherapeutics in COVID-19: A Systematic Review

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ABSTRACT

Circadian biological rhythms are precognizant rhythms that have evolved to benefit the host in assessing and perceiving environmental stressors. A ginormous wealth of information supports the role of the endogenous clock in governing many aspects of host responses, viral replication, and pathology. A novel corona virus, Severe Acute Respiratory Syndrome Corona virus 2 (SARS-CoV-2), was uncovered in late 2019 in the Wuhan city of central China, and has expeditiously spread around the world, ushering in a global pandemic. nCoV-2 infection affects metabolic processes, macrophage activity, cellular oxidant detoxification, and platelet degranulation. Many of these activities are time-dependent, which is in line with the disruption of circadian signalling caused by SARS-CoV-2. For pathologies including cardiovascular, rheumatologic, and oncologic disorders, time-of-day treatment regimens and chronomodulated medicine delivery may increase efficacy while minimizing detrimental consequences. As a result, chronotherapy refers to the process of restoring the robust circadian pattern of the sleepwake cycle through improved sleep schedule, controlled exposure to light, and the use of a chronobiotic drug as melatonin, that modulates the output phase of circadian cycle and thus controls the clock. Chronotherapy is used to prevent drug levels from reaching a steady-state, which, like anti-inflammatory





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therapy, would reduce the virus's inflammatory response. Its goal is to use the body's natural circadian rhythms to improve medical treatments.

Keywords: Circadian misalignment, melatonin, n-Cov19, Chronotherapeutics, sleep-wake rhythms.

INTRODUCTION

In December 2019, the novel corona virus - SARS-CoV-2 was asserted in Wuhan, China, causing the COVID-19 illness [1]. The outbreak was named "once in a century" [2] that promptly spread around the world, affecting over 200 nations [3]. This is an RNA virus [4, 5] that preferably, affects both humans and animals and the outcomes of this notable pandemic have been now no longer confined to the disorder particularly, breathing symptoms [6]. A complete image of the underlying mechanisms and instantaneously and overdue aspect consequences aren't completely understood yet. Serious outcomes affect now no longer the handiest inflamed and recovered patients, but additionally the overall populace beneath the current pandemic [1]. Infection with SARS-CoV-2 alters various physiological systems. After latching to the angiotensin-converting enzyme 2 (ACE2) receptor, it is an RNA-enveloped virus with a mono strand of RNA that targets cells via the viral structural spike protein [4, 5]. Lifestyle and intellectual fitness disruptions [7], social isolation [8], neuropsychiatric disturbances [9], behavioral problems [10, 11], emotional difficulties [11-14], and cognitive deficits [15, 16] are a number of the outcomes of the pandemic, affecting predominant factors of our existence as human beings (i.e., intellectual, cognitive, social, behavioral, and emotional) [1]. A predominant outcome of the COVID-19 is its impact on the sleep-wake cycle via lifestyle modifications [7]. Sleep is a key physiological occurrence that has a massive, pervasive, and cyclical influence on our basic physiological systems (e.g., metabolism, immune system), emotion, behaviour and cognition [1, 17].

Circadian rhythms synchronize the daily physiological activity, including brain functions, innate and adaptive immunological functions, and hence have a considerable influence on human health [18, 19]. House quarantine has been a key repercussion of the COVID-19 pandemic, resulting in massive swings in activity levels during the day. This has an effect on light exposure, which is a key regulator of circadian rhythms. Circadian rhythms may deviate from their optimum pattern as a result, a condition known as "circadian misalignment" [1]. The primary elements contributing to circadian disorganization is caused by the COVID-19 pandemic, according to the findings of the previous research, are light exposure, home confinement, daily activities, sleep time, and mental anguish [1]. Beyond stress, the consequences of confinement and unusual work hours are seen as the two variables associated with the COVID-19 pandemic syndrome. Homeostatic pressure (awake duration) and the circadian timing system, the latter of which is controlled by exposure to daylight and many environmental and social timekeepers, govern sleep-wake patterns. Daily habits such as waking up at a set time using an alarm clock, reaching work at a defined time, exercising, eating, and engaging in social and leisure activities at relatively regular periods all through the day and evening are examples of timekeepers. Several of those time cues are modified in constrained situations because there are fewer constraints on executing various jobs at specific times [20, 21]. Furthermore, because daylight exposure is a major component in managing the circadian timing system, receiving some sun exposure is essential for maintaining a healthy signal for the circadian timing system, especially because time spent indoors is maximized to full when in confinement [22].

COVID-19 infection has two stages: The first phase is of viral replication, during which people with the disease do not experience dyspnea and hydroxychloroquine may be of particular importance, and the second inflammatory phase (the cytokine storm) occurs, during which the virus is eradicated but lung lesions develop, and cortisol therapy appears to be the most effective treatment [23]. In inflammatory settings, the circadian clock is a biological timer that has been found to regulate the rhythmic synthesis and release of numerous cytokines. Chronotherapy is used to prevent the virus from reaching the steady-state of the drug levels, which would lessen the virus's inflammatory response, similar to anti-inflammatory therapy [24]. It has been demonstrated that there is a link between the date of the viral attack and the time of vaccination [24]. A malfunction in the body's multiple regulating





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functions leads to sleep difficulties. Insomnia is the everyday sleep-related complaint, is a complicated illness that reflects a person's physical and mental health. It is defined as a difficulty in initiating, maintaining, and consolidating sleep, as well as a decline in overall sleep quality, which causes physical and emotional suffering [17]. Insomnia might develop as a result of depression or a general increase in worry. Excessive worry about the pandemic's progression, their own or others' health, and financial problems, in addition to social restraints, lead to sleep disturbance, which, given the importance of sleep in emotional stability, can exacerbate mental health [25]. As a result, during the COVID-19 pandemic's societal restrictions, a deterioration in sleep quality may be envisaged on a global scale [26]. Even though determining the prevalence of insomnia in such situations is difficult, there will be a huge number of unreported cases of these public health concern [27]. Sleep, depression, immunity, and people with depression, who are naturally under a lot of stress, have higher levels of pro-inflammatory markers including C protein (CRP) and interleukin6. As a result, an escalation in inflammation exacerbates depressive symptoms. In addition, sleep disorders as insomnia have been linked to an increase in the occurrence of depression as well as promoting inflammation [28, 29]. Organizational and occupational issues have a big impact on mental health, especially when there's a global pandemic. As a result, efforts to manage mental health related problems linked to the pandemic must be focused on the workplace [30]. PTSD, suicidal thoughts, anxiety, sleep disorders, depression and drug and alcohol addiction are more likely to afflict healthcare personnel, especially those on the front liners, migrant workers, and public-facing professions such as law enforcement [31]. These challenges are linked to a high level of job stress, the fear of being sick and passing the disease on to family members and the possibility of discrimination and humiliation, a study concluded [32].

Biological Rhythms

Circadian rhythms aid organisms maintain homeostasis [33]. Core clock genes activate or repress downstream targets through transcriptional, translational, and posttranslational mechanisms. The molecular clock contains several redundancies [34]. Circadian clock genes (period -per 1,2,3-; cryptochrome -cry 1 and 2, Clock and Bmall) and their protein products are entrained to environmental conditions by light in suprachiasmatic nucleus (SCN) cells, resulting in the circadian rhythm of sleep [35]. Circadian and homeostatic sleep times and dates are combined in the anterior hypothalamic nuclei. [36, 37]. A circadian clock within the brain regulates the daily cycles of mammals. The periphery receives timing data to synchronize tissue clocks [38]. The hypothalamus' paired SCN are mammals' master pacemakers and body coordinators. Illumination on the retina resets the SCN daily, and certain monosynaptic projections terminate in it, initiating various signalling cascades such clock gene transcription and chromatin remodelling [39]. In mammals, the master transcription factors BMAL1/2 (brain and muscle Arnt-like protein) and CLOCK (circadian locomotor output cycles kaput) dimerize with NPAS2 (neuronal PAS domaincontaining protein 2) to regulate Per1-3 and Cry gene transcription via E-box promoter elements. The cytoplasmic PER-CRY complex migrates to the nucleus and inhibits BMAL and CLOCK, preventing PER and CRY transcription [13, 34-35]. The proteasome destroys the inhibitory PER/CRY complexes after CKIe phosphorylation and ubiquitination, lowering CLOCK and BMAL inhibition and resetting the feedback loop in 24 hours. Complex feedback loops fine-tune the process. REV-ERBa/b and RORa, orphan retinoic acid receptors, bind to Bmal1 promoter enhancer sites to inhibit or increase transcription. BMAL1/CLOCK offers an "accessory" loop by lowering reliance on the REV-ERBa gene, and oscillations in REV-Erba and RORa transcription induce rhythmic expression of BMAL1[38-40]. The molecular clock has multiple layoffs. Bmal1 is the sole nonredundant gene that induces circadian arrhythmia, a loss of rhythmicity in locomotor activity and body temperature, with a single deletion [41]. Bmal1 alteration is a popular circadian research model. Immune activity is becoming rhythmically controlled and pathogenresponsive [42]. SARS-CoV-2 replicates in the respiratory system, hence a circadian-regulated mechanism may promote COVID-19 development. Most treatments target individual stages of the viral replication life cycle or the host immune response, but the circadian system's integrative function in organismal homeostasis provides potential. [43].

SLEEP

Sleep is a mammalian universal with several biochemical levels. Sleep, unlike torpor and hibernation, is rescindable [17]. Sleep affects synaptic plasticity, memory, emotional regulation, metabolic function, energy balance,





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macromolecule biosynthesis, toxic chemical and metabolic waste clearance, and preventative cellular maintenance. It may also be associated with adaptive inactivity since it is a meta-regulatory process that involves several molecular, cellular, and network processes that work together to ensure efficient (adaptive) wakefulness[13]. A normal human sleep alternates between REM and NREM sleep states. Muscular tone and mental activity are minimal during NREM sleep, and the electroencephalogram shows K complexes, sleep spindles, and slow waves in synchrony. Dreaming, muscle atonia, and EEG desynchronization are all hallmarks of REM sleep [44]. The two-process framework is proposed to explain sleep regulation, in which a homeostatic process (Process S) interacts with the circadian pacemaker-controlled process (Process C), with time courses generated from the physiological and behavioral factors [45]. The nexus of the homeostatic process (depending on sleep or waking) with a mechanism mediated by the circadian pacemaker determines crucial variables of sleep regulation. REM Slow Wave Activity (SWA) represents Process S, whereas core body temperature and melatonin cycles indicate Process C [46]. Process S increases in amplitude and lowers in severity anytime sleep occurs; for example, a midday nap causes an exponential fall in process S to the point that it may interfere with sleep beginning at the usual bedtime. Process C modulates sleepy time based on natural circadian clocks, the hypothalamic SCN, mostly by activating and deactivating the mechanism that prompts awakening [47]. Sleep involves the withdrawal of passive afferent impulses and the activation of functioning brain regions [48]. It does not entail total absence of consciousness or perception or withdrawal of all senses. Sleep impacts mood, focus, memory, and body temperature, and is not a linear process [49]. Sleep deprivation can damage the immune system, making illness inevitable. Incongruence between the intrinsic circadian rhythm system and the 24-hour ambient cycle is a risk factor for cardiovascular and inflammatory diseases, elevated blood pressure, and inflammatory markers. In socially stressful situations, like as the COVID-19 pandemic, sleep disturbances would crop up, interfering with inflammatory and antiviral responses[50, 51].

Chronotherapy

The purpose of chronotherapy is to make medical analysis more effective by taking body's circadian rhythms into account [52]. Chronotherapy refers to and it is carried out in two ways: (1) adjusting patients' sleep-wake patterns to alleviate the effects of various illnesses; and (2) taking into patients' account of circadian rhythms to modulate therapies [53]. Excessive diurnal sleeplessness, an extension in sleep start latency, phase advances or delays in sleep initiation, increased periodic leg movements, delayed and reduced REM (rapid eye movement) sleep, multiple nocturnal awakenings, and reduced sleep are all symptoms of a biological clock failure [54, 55]. Excellent sleep hygiene, regulated light exposure, and the use of chronobiotic drugs like melatonin, which alter the output phase of circadian rhythms and so monitor the clock, are all used in chronotherapy to restore the appropriate circadian rhythmic pattern of the sleep-wake cycle. Treatment results, such as chemotherapy efficiency and tolerance, are connected to the time of day in the next use of chronotherapy. Living beings (humans), on the other hand, have a large range of interpersonal clock time[53]. Operational circadian clocks are important in changing organismal and cellular responses to physiological signals (e.g., exercise, food intake), pathological stressors (e.g., parasite infections and virus), and pharmaceutical remedies, according to animal and human model studies (e.g., medication). We can target circadian rhythms for the prevention of disease and therapy because we have a concrete understanding of the molecular and cellular mechanisms that cruise circadian physiology and pathology [56, 57]. Chronotherapy is a revolutionary concept in sleep medicine that is growing exponentially. Immunological responses to the vaccination of SARS-CoV-2 and the prospective effect of immunization timing in the 24-hour circadian rhythmic cycle are not well understood[58,59]. The antibody response to flu immunizations is powerful in the morning than in the arvo in certain cases. However, it is unknown whether the circadian rhythm has an impact on the immune response to SARS-CoV-2 vaccinations. In a recent study, researchers revealed that inactivated immunization triggered strong immune responses. The fraction of antibody-secreting cells (ASCs) among follicular helper T (Tfh) cells and B cells in the peripheral blood increased after inoculation. The kinematic changes in immune cell subsets in the peripheral blood demonstrated an increase in Tfh cells and increased HLA-DR interpretation on B cells, indicating that the vaccine induced a germinal centre response. The vaccine had about no effect on other T-cell subset [60-62]. Circadian cycles, intrinsic cellular oscillations with a periodic duration of 24 hours, control all physiological systems, including the immune system. Studies on the adaptive immune system have indicated a time-of-day variation, such as the trafficking of lymphocytes to lymph nodes and their amplification [63]. In addition to their involvement in





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homeostasis, circadian rhythms have been associated with the pathological-physiological processes as influenza infection and autoimmune disorders, where they play a essential role in modulating the response to inflammation. These insights have contributed to the creation of immunotherapies that employ the time of day to improve treatment success. Temporal oscillations in antibody production have been recorded in both mice and humans in response to timed antigen infusions in the setting of vaccination [64, 65]. The production of pro-inflammatory mediators as interleukin IL-1, IL-6, and IL-12, as well as the activity of macrophage and leukocyte, spikes in the morning, causing tissue damage. Anti-inflammatory mediators and other development or angiogenesis molecules spike during the resting phase, CD4 and CD8 T lymphocyte activity against viral antigens is at its peak, and natural killer cell cytotoxicity is at its crest at the start of the dynamic portion of the day. The time of day when a viral infection begins has an impact on survival [64].

Infections that occur at the beginning of the activite phase, for example, are more dangerous than pathogens at the beginning of the resting period. Because these spatiotemporal rhythms may be interrupted in the elderly, it's vital to be aware of circadian disorientation while utilizing immune modulators and anti-inflammatory drugs. Antiinflammatory medications administered at the right time of day (chronotherapy) may be able to target the harmful inflammatory process in COVID-19 patients while not meddling with the immune system's combat against the virus.[59, 61]. Doses of about 50-100 mg p.o. have been advocated for prophylaxis in vulnerable organisms, such as elderly, to improve therapeutic efficacy. The elderly is especially prone to circadian misalignment during this period of obligatory and widespread solitude and may benefit from using the chronobiotic medication such as melatonin to perpetuate an optimum sleep-wake cycle and circadian rhythms. The chronobiotic effects are maintained even with high melatonin concentrations and the use of a detachable preparation regulated at a certain time of day (bedtime). As a result, the case for utilizing melatonin as a COVID-19 pandemic prophylactic medicine is based not just on the immunoinflammatory disease, but on the overall improvement and avoidance of probable symptoms offered by maintaining healthy circadian rhythms also [66]. The design and implementation of pharmacological drugs that alter circadian protein activity have begun. CRY stabilizers that block BMAL1 expression and ROR modulators are illustrations. Because they compete for and bind with the same DNA response elements and control numerous same genes, ROR inverse agonists may have comparable effects on the viral replication as the REV-ERB agonists. These ligands may be useful in enhancing or inhibiting inflammatory and viral responses in particular situations. In people with HIV, a link is discovered between the genetic diversity in circadian clock genes and sleep habits.

Polymorphisms in the Clock and Per2 genes have been linked to the maintenance of poor sleep (wake after sleep initiation) and raised total sleep length in HIV-positive people; however, the underlying processes remain obscure. When mice were vaccinated with the dendritic cells that were primed with the ovalbumin peptide during the daytime rather than at night, their circadian clock altered their immune response, resulting in enhanced T-cell activation [67]. The morning influenza vaccination (9-11 a.m.) was more effective than the afternoon immunization (3-5 p.m.), with greater antibody responses measured against multiple influenza strains. In nations with seasonally dependent settings, the month of vaccination delivery can alter antibody responses and protection against a variety of viruses. Seasonal variations in B-cell maturation factor (BCMA or TNFRSF17) expression are linked to a favorable response to the trivalent influenza vaccine. Seasonality in PRR expression, which is higher in the winter, affects the quality of vaccination reactions and is associated with improved protection against the yellow fever virus. These basic timing approaches for medicine or vaccine administrations can improve clinical effectiveness while minimizing detrimental consequences. The link is anticipated to influence the viral infection and decoding it would need multidisciplinary approaches combining virology, immunology, circadian biology, and pharmacology. The convergence of external cycles with endogenous biology is a hallmark of evolution. Harnessing biological cycles might lead to innovative viral treatment strategies and pharmaceuticals in an era of shift work-related sleep disorders, global viral pandemics, social jet lag, and escalating viral drug resistance [68].





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CONCLUSION

COVID-19 is a global health crisis of the 21st century. Beyond physical and geographic obstacles, the novel coronavirus has had a substantial impact on people's daily lives. While measures to restrict viral infection should be prioritized, the implications of these actions must also be examined and remedied. Chronic stress damages the immune system, allowing infectious diseases to proliferate and degrading intellectual health and quality of life because sleep is linked to mental health and the immune system [66]. During the epidemic, health professionals' duties and sleep patterns fluctuate, causing sleep loss and stress. Stress and its avoidance enhance emotional disturbances, sleep disruptions, and immunological susceptibility [67]. According to studies, these features can be prevented by implementing measures before and after work to mitigate the aforementioned challenges and establish stronger survival mechanisms for both the COVID 19 pandemic and future issues and contingencies [69].

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Chart1: Tabular representation of the recent studies exploration for effective treatment against the coronavirus pandemic that is based on the idea of chronotherapeutics.

S.No	Major Idea	Time	Major Findings	References
1.	Efficacy of hydrochloroquine in COVID-19	2021	Hydrochloroquine used in primary infection and corticosteroid therapy in imflammatory phase of infection	https://www.ncbi.nlm.nih. gov/pmc/articles/PMC7997 534/
2.	Melatonin and its properties in regards to corona virus	2020	Melatonin therapeutic significance in the neurological context in association with the covid-19 infection.	https://pubmed.ncbi.nlm.ni h.gov/33256258/
3.	Sleep, Circadian Rhythms, and immunity relationships with respiration disadvantages	2021	Chronotherapy integration with the immunological rhythmicity in young and elderly.	https://pubmed.ncbi.nlm.ni h.gov/34220430/
4.	Timekeeping and Covid-	2021	Possible pathways of infection by the novel corona virus with respect to chronobiology	https://pubmed.ncbi.nlm.ni h.gov/33480287/





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5.	Respiratory distress enhances due to the immune regulatory treatment leading to Inflammatory infection	2020	ARDS (Acute respiratory distress syndrome) managed by Chronotherapy	https://pubmed.ncbi.nlm.ni h.gov/32442317/
6.	Clinical approach of chronotherapy	2021	Administration timings influence the effectiveness of the doses of vaccine	https://pubmed.ncbi.nlm.ni h.gov/32875944/
7.	Elderly most vulnerable to n-CoV infection	2020	Timing-based melatonin administration effects positively to the health of humans especially elderly	https://pubmed.ncbi.nlm.ni h.gov/33015537/
8.	Temporized schedules of administration	2020	Morning administration of drugs is more effective compared to late day timings.	https://pubmed.ncbi.nlm.ni h.gov/32767353/
9.	Cancer , Covid-19 and Circadian Rhythms	2021	Non-invasive monitoring system for easy approach towards oncology treatment	https://pubmed.ncbi.nlm.ni h.gov/33606562/
10.	Application- based Measurement of Circadian Rhythms	2021	Circadian biomarkers are desynchronized after the home quarantine during the pandemic.	https://pubmed.ncbi.nlm.ni h.gov/34870267/
11.	Immunological effects of Covid-19.	2021	Morning administration of vaccines mat be effective due to increased serological response.	https://pubmed.ncbi.nlm.ni h.gov/34341489/

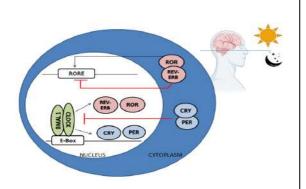


Figure 1: A diagrammatic representation of the molecular circuitry of the human/mammalian circadian rhythm depicting the self-regulatory transcriptional/translational feedback loop.

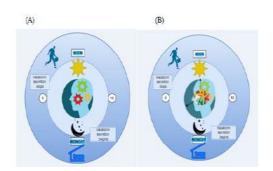


Figure 2: This figure represents the Circadian Cycle in

(A) aligned condition with the environmental conditions. (B) Aligned with the use of melatonin (prominent chronobiotic drug) depicting the importance of chronotherapy in the present lifestyle.





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RESEARCH ARTICLE

Photodegradation Competence of Zn Doped Ins Nano Structures on Methylene Blue under Natural Sunlight

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ABSTRACT

Zinc-doped indium sulfide (Zn- InS) was prepared using zinc chloride (ZnCl₂), indium chloride (InCl₃) as precursors, thiourea CS (NH)₂)₂ as sulfur source and oxalic acid as reducing agent by mild hydrothermal method. The ultimate aim of this work is to know the structural, morphological, optical, luminescence and photocatalytic properties of the nanostructures zinc doped indium sulfide analyses reveal that Zn doping changed the structure of Indium sulfide and formed new crystalline compounds XPS study suggests the existence of Zn2+. EDX &FE - SEM images show that the composition of elements and the grain size increases after Zn doping. The photoluminescence (PL) spectrum reveals the presence of welldefined band edges and emissions in the wavelength region around 400-800nm. Further, after doping Zn2+, the photocatalytic H2 evolution rate is increased and this work offers to develop efficient photocatalysts degradation efficacy of zinc-doped Indium sulfide nanoparticle on methylene blue under natural sunlight (86%).

Keywords: Zn doped Indium sulfide, mild hydrothermal, Photodegradation, Natural sunlight, structural properties





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INTRODUCTION

The anxiety over the reduction in the quality of drinking water, and the pollutants from industries and factories are a threat to the greatest issue for the humanity in future. In the aspect of photocatalysis, the nanostructured materials are the eco-friendly approach to the degradation of organic pollutants by electron-hole pair of the semiconductor catalyst [1-2]. Conservative waste water treating technique applications are on a large scale but the suitable semiconductor material which is crucial to the growth of cost-effective material for the treatment. Out of numerous different photocatalysts, zinc-dopedindium sulfide is studied because of its oxidizing ability, chemical stability, and low- cost [3-4]. Zinc-doped indium sulfide is the most hopeful in the semiconducting material family and is dependent on composition and deposition technique. The outstanding physical properties of zinc-doped Indium sulfide find widespread functional devices similar to optoelectronics. On a large scale at an inexpensive cost, the mild hydrothermal route is used to make zinc-doped nanoparticles at room temperature [5-6]. The prepared catalysts studied physical, optical and photocatalytic properties [7-8]. As per the result, Zn²+ doped nano samples are suitable material which shows very good optical reflectance and PL studies. In the current study, the synthesized material zinc doped indium sulfide nanoparticles were structurally characterized using XRD, UV- DRS, FE-SEM and XPS techniques [8-9]. The advanced photocatalytic activity and excellent stability gifted a promising potential for zinc doped indium sulfide nanoparticles to apply in energy storage applications.

MATERIALS AND METHODS

Reagents

Indium chloride (InCl₃), Thiourea (NH₂)₂, Oxalic Acid, Ammonia, from Sigma Aldrich, Zinc (II) Chloride tetrahydrate (Z_{11}), Thiourea (NH₂)₂, Oxalic Acid, Ammonia, from Sigma Aldrich, Zinc (II) Chloride tetrahydrate (Z_{11}), Thiourea (NH₂)₂, Oxalic Acid, Ammonia, from Sigma Aldrich, Zinc (II) Chloride tetrahydrate (Z_{11}), Thiourea (Z_{11}

Synthesis of Zinc doped Indium Sulfide - Nanoparticles

Indium sulfide nanoparticles were synthesized through the hydrothermal procedure. 25 mmol InCl3 and 350 mmol thiourea were, respectively, dissolved in 25 ml deionized water at room temperature for 10mins. After fully dissolved, 0.126g of oxalic acid used as a reducing agent and ammonia as a complex agent were added to the solution and stirred vigorously for 30 mins. For the preparation of zinc doped indium sulfide, zinc chloride (ZnCl2) was added to the mixture of InCl3 and CS(NH2)2 in different ratios of 0.01,0.07,0.1 in grams. Furthermore, zinc doped indium sulfide is prepared by adding ZnCl2 to the combined product of indium chloride and thiourea derived from constant magnetic mixing. The same concentration was transferred into a Teflon-lined autoclave, followed by a hydrothermal treatment method at 120°C for 5h. When the autoclave was cooled down the room temperature, the assynthesized zinc doped indium sulfide precipitate was separated from different concentrations collected and washed sequentially with deionized water and ethanol several times by centrifugation and filtration, and then obtained assynthesized precipitates were dried in a hot air oven at 80°C for 5h and then kept in a muffle furnace for 300°C for 1 hour to get resultant nanoparticles.

Characterization techniques

X-ray diffraction (XRD) investigated phase identification and crystal size in the range of 10° to 80° with the Cu K α radiation source (λ = 1.5406Å). By using FE-SEM, the surface morphology of pure, zinc-doped Indium sulfide and the grain size distribution of zinc-doped indium sulfide nanoparticles were determined. UV-DRS spectrophotometer (V-670-JASCO) studied the samples for optical absorption within the wavelength range of 300-1200 nm.

RESULT AND DISCUSSION

X-ray diffraction commonly has been used for the examination of crystallite structure materials, and the composition of chemical and physical properties of a material. it can be used to give relevant surface changes in suitable situations [10]. For phase identification and to find out the crystallinity structure of the samples XRD diffraction is the suitable





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technique. The figure shows the XRD pattern of Indium sulfide and for different concentrations of zinc-dopedindium sulfide nanoparticles [11]. The diffraction pattern of 2θ values at $21.66\,^{\circ}, 30.43\,^{\circ}, 35.27\,^{\circ}, 51.24\,^{\circ}$, and $60.42\,^{\circ}$ corresponding planes of (110) (101) (121) (112) and (250). The peak intensity is improved with increasing particle size and no impurities are found. The undoped and doped nanoparticles calcinated at $300\,^{\square}$ and also peaks are indexed using JCPDS (Card No.65-1472).

The average crystallite size (D) is determined from major peaks using

Debye -Scherer formula: $D = \frac{k\lambda}{\beta \cos \Theta}$

k- constant (0.9), λ – X-ray radiation wavelength, β – FWHM

The average crystallite size increases from 10 nm for pure and 19 to 21 nm for zinc-dopedindium sulfide. FE-SEM technique was adopted to examine the morphology of the synthesized sample. The figure (2) images were obtained at 300□ with agglomerated nanoparticles in the formation of irregular spherical shapes in orthorhombic structure[12]. Figure (2) indicates that the spherical grains spread consistently on the surface of the pure indium sulfide after zinc doping with the pure sample size of the spherical grain having been increased on the surface. The figure (3) EDX images confirm the elemental composition present in the pure and the dopant material such as In ,S, and Zn. The UV-DRS is a subset of the absorption spectrum so the reflectance and absorption techniques were carried out[13]. Optical absorption spectra are used to provide information on the electronic structure of semiconductors, it allows the compounds to be distinguished from one another in the mixture. The absorbance and reflectance spectra of indium sulfide and zinc-doped indium sulfide in the wavelength range of 300 − 1200nm are presented in Figures (4&5). The absorbance of prepared samples has comparable in the visible region and the absorbance increased when the dopants increased such as 380nm for pure InS and 404nm,407nm, and 420nm for doped material. It could be explained by the slight increase of dopants, forming an improvement in grain size. Reflectance spectra are a surface analytical technique, and the samples evaluated from reflection spectra and the values are 476nm,499nm,513nm and 535nm.

The reflectance is represented by R in the Kubelka-Munk formula, which is $[F(R)h] = A (h - E_g)$, $F(R) = (1-R)^2 / 2R$). using this equation, the energy band gap fluctuates in the range from 2.80eV, 3.57eV,3.51eV and 3.31eV in accordance with pure InS, Zn(0.01), Zn(0.07) and Zn(0.1) doped indium sulfide. Photoluminescence is a technique to measure the purity and semiconductor's crystalline quality and for quantifying the disorder amount present in the synthesized nanoparticles and also it is used to measure the intensity of emitted radiation of the exciting wavelength, photoluminescence spectra of nanoparticles exhibit an λ_{exc} 445nm as portrayed in figure three intense peaks were detected in the wavelength range between 445nm to 624nm. Here, compared to pure indium sulfide, the doped indium sulfide nanoparticles' emission intensity is a little higher, with increasing doping concentrations the electronhole pair recombination has been subdued and is resulting in stronger photocatalytic activity. X-ray photoelectron spectroscopy is the surface-sensitive quantitative and powerful measurement technique because it not only shows what elements are present but also what other elements are bonded to. The technique can be used for the elemental composition across the surface. The XPS spectrum is a plot of the number of electrons detected at a specific binding energy[14]. Each element produces a set of characteristic XPS peaks[15]. The In3d indicates the binding energy at 445.83eV and 453.40eV, and for S2p the binding energy level is at162.51eV and 170.55eV. The Zn²⁺ gives the peaks at approximately 1022.58eV and 1045.56eV showing higher binding energy shoulders, The figure showed the Zn 2p_{1/2} and Zn2p3/2 spectra of Zn-doped Indium sulfide powder with a doping concentration of 0.1g. Two peaks with binding energy of 1022.58 ±0.5 and 1045.56±0.5 were observed that could be accredited to Zn 2p_{1/2} and Zn2p₃/2. These peaks indicate the presence of Zn²-in the Zn-doped Indium sulfide, which proves the incorporation of Zn into the indium sulfide powder. Besides, since XPS analysis reflects the elemental compositions on the surface, XPS results from the indication of the Zn element has complete diffusion up to the surface. Photocatalytic is commonly used to investigate the function of semiconductor nanoparticles. Usually, methylene blue is brought into contact with the photocatalytic active surface of a test sample that is irradiated through the supernatant solution (320<<400nm). The solution is decolourized in the process. Throughout the measurement, the colour concentration of the solution is measured. The performance of the as-prepared photocatalysts in the Methylene blue solution under visible light





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irradiation is shown in figure(9). The bulk quantity of species including O₂ and OH was absorbed for the photocatalytic process. Zn doping helps to prevent the recombination of photogenerated electron-hole pairs, leading to a increase in photodegradation efficiency. Six different reaction times Zn doped sample was explored in an endeavour to reveal the mechanism involved in the photocatalytic process. As prepared Zn doped nano catalysts show a positive effect on the photocatalytic activity of methylene blue (MB) degradation. The possible mechanism of MB by zinc-doped indium sulfide nanoparticles was shown in Figure (9). In a photoactivity reaction, the electrons rose from lower to higher energy levels while leaving in the charge separation as a result of positively charged holes. But these electrons and holes recombine quickly, which leads to suppressing the photoactivity of the catalyst. It will be overcome using transition metal doping. The Zn doping extends light harvesting in both UV and visible regions. Which assists the charge separation and enhances the photoactivity performances. The efficient separation of photoinduced electron-hole pairs was possible due to induced energy levels by impurity doping. Hence, Zn-dopedInS nanoparticles show better photodegradation efficacy 86% after 120 mins on methylene blue dye. The degradation percentage of the methylene blue dye was evaluated from the following equation,

 $X = (C_0 - C_t) / C_0 X 100 \%$

C₀ = initial concentration of the dye

Ct = concentration of dye after irradiation in the selected time interval

The photodegradation efficiency of pure and metal (Zn) doped Indium sulfide nanoparticles are determined and presented in figure (10).

CONCLUSION

The structure, morphology, and optical aspects were investigated using the hydrothermal method's successful creation of zinc-doped indium sulfide nanoparticles. The prepared sample's crystallinity, grain size, and shift in lattice points are revealed by XRD and the elemental composition and refined grain size are confirmed by EDX and FE-SEM, respectively. Reflectance spectra show the energy band of naturally occurring, undoped, and doped substances. Eg values for the dopants, 3.57eV, 3.51eV, and 3.31eV, and 2.80eV for indium sulfide. The photoluminescence investigation identifies doping samples with better emission. When exposed to visible light over a shorter period of time (0-120 minutes) at 86% efficiency, the synthetic photocatalyst showed good performance. The maximum photocatalytic activity of zinc-doped indium sulfide is exploited solar energy for the whole breakdown of the pollutant methylene blue and it may be successfully used for water and wastewater treatment under solar light irradiation.

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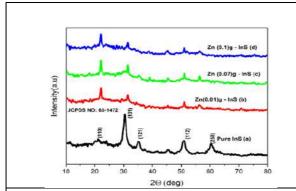


Figure 1. XRD pattern of (a) Pure Indium sulfide (b) Zn(0.01) (c) Zn(0.07) (d) Zn(0.1) doped Indium sulfide

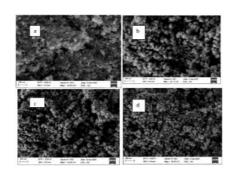


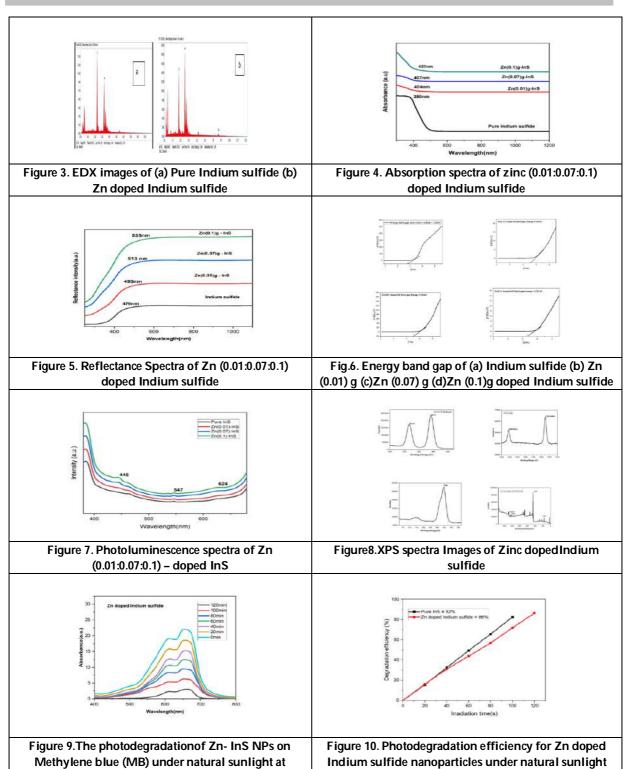
Figure 2. FE – SEM images of (a) Pure Indium sulfide (b) Zn (0.01) (c) Zn(0.07) (d) Zn(0.1) doped Indium sulfide nanostructures



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different intervals of time (0-120 min).



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RESEARCH ARTICLE

Impact of Blended Learning Strategies on Concept Attainment among Elementary School Students Studying Social Studies - An Experimental Study

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ABSTRACT

In the present world of remarkable developments in the field of science and education, the education system is confronted with pressure to adapt educational programs that reflect innovative methods of learning and develops 21st century skills in students. Blended Learning proved to be a boon in the time of pandemic which mediates distance learning by providing online learning supplemented with web based online methods, tools and methods employed in e-learning and a number of pedagogical approaches in both online and offline modes. The objective of the paper was to find out the impact of blended learning strategies on concept attainment of elementary school students. The two-group pre-test post-test design was used for the study. After the administration of pre-test, the experimental group was taught general concepts of social study by using ppts, audio clippings, video clippings and online test components, online discussions, educational games rightly blended with face-to-face components to make blended learning application module (BLAM) and the control group was taught the same concepts using conventional method. After that the post-test on concept attainment ability was administered to both the groups. The experiment lasted for two weeks. The results indicated that blended learning strategies have a positive impact to increase the concept attainment abilities of elementary school students. It is presumed that blended learning strategies would yield fruitful results in the hands of resourceful persons, teachers, administrators, curriculum planners and teacher educators who have a positive bend for making transformative changes in the teaching learning process.





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Keywords: Blended Learning Strategies, Innovative Methods of Learning, 21st Century Skills, Concept Attainment Ability, Pedagogical Strategies.

INTRODUCTION

It has been experienced that due to huge increase in the population and knowledge explosion, the pattern of human life has changed a lot which also impacted the patterns in the educational transaction. There is a turbulent revolution in science and technology which leads to the remarkable growth in the field of scientific knowledge and activities of mankind. All these technological advancements have pressurized the whole education system around the globe to adapt those educational activities that considers new paradigm of teaching and learning. In this 21st century of technological rise, the technology is integrated in every aspect of human society. The internet access has accelerated this growth and forced to transform the teaching learning scenario to meet the challenges of lifelong learning of 21st century. Rapid developments in the field of educational technology have also extended the educational facilities and upgraded the instructional methods in just a few years. There has been a rapid development of educational technology and its application in education at all levels with the purpose to extend educational facilities and upgrade instruction in recent years. This rapidly changing scenario of teaching landscape should be upgraded so as to reflect the incorporation of technology in the teaching learning modal. The technology has become the part and parcel of our life, it will keep on integrating into our society and there is need to espouse it for its various advantages. To survive and compete in this technological realm of education, there is indispensable need to generate, embrace and take advantage of innovative technologies so as to remain updated. Technology has the advantage of effectively and efficiently out flowing the information in a fraction of minutes. Blended learning can be considered as the interspersed amalgamation of conventional face-to-face teaching with web-based online methods, tools and media employed in e-learning and a number of pedagogical strategies.

As stated by Hofmann (2011) blended learning strategy is not a new notion as it has been evolving from the last four decades to blend face-to-face instruction with technological approaches. Collis and Monnen (2001)in their definition of blended learning described it as a hybrid of both online and traditional face-to-face learning so that instructions can be provided in both classroom and outside the classroom in blended mode where both these methods complement each other. Garrison and Vaughan (2008) considered blended learning as that strategy in which there is thoughtful fusion of conventional face-to-face learning and online learning thus stressing the need to contemplate on conventional approaches of teaching by redesigning the teaching learning in this new era. Blended learning approaches have amplified the need to improve personalization, collaboration and communication in teaching learning process by utilizing the benefits of possibilities provided by technologies as technology can reach to a wider community and has the capability of catering the individual needs of the diverse learners and also the need of special children. The integration of latest mobile technologies and online resource is successful in confronting the challenge of limited resources by providing educational support to institutions in the form of various technological resources of education.

Concepts are the furniture of our minds and relevant learning can be attained when pre-existing concepts and schemas or cognitive constructs will comprehend with the new knowledge or else rote learning will take place. Concept reduces the complexity of the environment. Conceptual learning is how the objects of environment are identified. Concept attainment refers to the activity of discovering which elements belong to the category and which do not belong. Akdeniz, Celal(2016) quoted Gunter et al (2003) to remark that in the process of concept attainment, concepts are defined by finding those features that are remarkably necessary to the meaning and disregarding those that are not necessary. It also means to discriminate between what is and what is not an example of the concept. Furthermore, Joyce et al (2014) expressed that in the process of concept attainment there is structured inquiry of concept through positive examples i.e., exemplars which describes characteristics, properties and distinctive attributes of the concept and negative examples i.e., non-exemplars which help to eliminate those characteristics of





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concept that do not apply to it. In the present time where rapid advancements are occurring in the field of technology, education cannot proceed without the knowledge of basic concepts that have enormous relevance in everyday life. In the past teaching paid more emphasis on just giving information to students. But today's students are digital natives who have access of internet to update their knowledge and so teacher of today need to transform teaching into comprehension and reflection-based teaching. To prepare the students of 21st century for life-long learning, innovative methods of teaching should be employed that help them to attain concepts in a better way and for longer duration of time. One of such method is blended learning strategies which provide flexibility to the students and caters to their varied demands and bring out expected outcomes. It has been endorsed that blended learning anticipated time to enrich understanding of the concepts due to the provision of in class activities and digital activities and thus deepens the conceptual side of learners (Sayed, 2013). Fatkhulloh and Haryanto (2020) expressed that blended learning helps in fulfillment of 21st century skills. It was also pointed out that within in the framework of blended learning students can explore and elaborate the previously owned concepts which ultimately helps in their concept attainment.

Review of Related Literature

Following research studies have been consulted while conducting the present research Fox and Ghezzi (2003) while examining the effect of computer-based fluency training on formation of concepts made four groups of study viz-fluency group or practice group with either examples group or definitions group. This study revealed that in computer-based training, examples group performed better than definition group. Whereas no clear perception can be made about fluency and practice group due to lack of evidences. It was further highlighted that that future researches in fluency training and concept attainment can be advantageous for exploring improved instructional practices. In another study by Harris et al (2004) on effect of e-learning on various attributes of learners like their participation, attainment, retention and progression in education. It was reported that e-learning creates a sense of engagement in learners and also increases their excitement and involvement. e-learning increases personalization in learning due to which there is increase in participation, attainment, retention and progression of learners in the learning interface.

Sood (2004) investigated the effect of strategies in mastery learning in attainment of concepts of geometry in high school students. In this research Bloom's Mastery Learning strategy and Keller's Personalized System was used and it was found that by using these strategies there is better attainment of concepts. It has been recommended that principles of mastery learning strategies should be applied by teachers with emphasis on identifying learning difficulties and providing corrective feedback. Kara (2008) determined the Computer Assisted Instruction's effect on retention and academic achievement of students in physics. It was determined through this research that CAI has improved the achievement of students in academics and also and their retention in physics. However, teachers need to be very careful while planning CAI for students and must take into account the suitability of program for students, its features and ability to motivate students etc. Sadasivan (2009) examined the impact of technology assisted instruction on students' exploration and understanding of concepts of mathematics. It was found that students' exploration and understanding of geometrical concepts has significantly improved through technology assisted learning. It has also been emphasized that the overall standard of mathematics teaching could be improved by the use of technology assisted learning, therefore at all levels of instructional process the curriculum and courses should include technology and technological tools. Kumar (2010) carried out a study to see the effect of the blended strategies on secondary school students learning retention and attitude. The students have better learning retention and positive attitude when taught through blended strategies. The study has also provided desirability of different teaching methods for immediate and long-term learning of students.

Krishnan (2011) studied higher order thinking skills and skills of learning science among secondary school students in blended learning situations and found that the critical thinking skills, problem solving skills, science process skills and achievement of students has significantly improved in blended learning situations. It was further emphasized that blended learning makes learning joyous and at the same time enhances its quality. Teachers should be provided sufficient pre-service and in-service training for effectively implementing blended learning strategies. Nair(2014) in





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his study on blended learning revealed that blended learning strategies are better in increasing achievement of students in biology. It was further pointed out that blended learning integrates the best elements of both in class teaching and teaching outside the class in digital mode with the key focus on enriching their learning experiences and expectations. Similarly, students have developed positive environmental and social attitude through blended learning strategies. Furthermore, Baweja (2015) reported strategies to improve concept development of students. These strategies included constructivist approach, cognitive development with the help of activities and firsthand experience, small group and whole class discussions, linking of new knowledge with prior knowledge, multidisciplinary approach that integrate technology with effective teaching learning practices, concept mapping, use of concept cartoons etc. Chaurasia(2015) studied science process skills of ninth grade students with respect to their concept attainment in science, their intelligence and learning style. The study results revealed that concept attainment in science is directly related with the science process skills, ability to perform experiments and ability to interpret data. It was also emphasized that science process skills play an important role in developing concept attainment and scientific thinking. It has also been recommended that teachers should emphasize on science teaching for developing science process skills. Paul and Richard (2017) carried out a case study of secondary school students of Air Force School in River State Nigeria to see effect of blended learning approach on academic achievement and retention. The results indicated that students taught using blended learning performed more and have higher retention than students taught by conventional method. Station rotation model of blended learning was implemented. It was further implicated that station rotation model of blended learning helps students in integrating their knowledge and also in collaborative learning which significantly enhances their learning outcomes. Anice (2019) manifested the variations in various cultural factors like region, religion, language, socio-economic status has significant impact on concept attainment ability in mathematics and also on problem solving ability.

It was further pointed out that culturally relevant mathematics curriculum should be developed and emphasis should be paid on new strategies of teaching which includes activities like group discussions, projects, discussions, self-discovery methods etc. Jayanthi (2019) while explaining the concept of blended learning stated that blended learning is a promising method of future education due to the availability of internet connection and technological advancements. Blended learning is very effective in developing the 21st century skills among teachers and students. However, there is a challenge of time requirement for its proper implementation in the higher education and efforts by teachers are needed for this. It has also been suggested that blended learning is under researched topic and further research is needed in this area. Fernandes (2020) examined the effect of computer-based concept mapping on concept attainment ability, reasoning ability and achievement of secondary school students in biology. The study results revealed that computer-based teaching is helpful in concept attainment of secondary school students. However more research needs to be conducted to see whether concepts are retained over a period of time or not. Suman (2022) scrutinized the effect of constructivist pedagogy on concept attainment ability and mathematical anxiety. It was pointed out that child centered methods of teaching like constructive pedagogy students can selflearn which will ultimately develop their concept attainment ability. Teachers should show dedication in organizing and implementing constructive pedagogy in the classroom. For this government should organize various programs for teachers for effectively implementing constructive pedagogy.

The review of related literature reflects that several studies have been conducted by researchers to provide the concept of blended learning and also to see its impact on various domains of learning. Blended learning has been shown to have increase the academic achievement of secondary school students (Kumar, 2010; Nair, 2014; Paul & Richard, 2017). It has also been revealed that the concept attainment and concept acquisition has been significantly enhanced using better methods of teaching and learning like technology assisted teaching, computer-based teaching (Sadasivan, 2009; Baweja, 2015; Fernandes, 2020). It has further been highlighted that child centered methods should be employed in class so as enrich the concept attainment ability of students as highlighted by Suman, 2022. The researches done previously are mostly conducted by taking secondary school students as sample of study. Therefore, this study has been particularly being done to see the impact of blended learning strategies on elementary school students with respect to their concept attainment ability.





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Rationale of the study

In the pastthe whole process of teaching and learning has been predominantly interpreted as teacher controlled. But today as a result of vast and explosive scientific and technological revolution the paradigm of learning has shifted from rote memorization of facts to knowledge construction. The present generation of students are born with internet connection so they can be regarded as digital natives. These students cannot be conceived as passive learners. There is need to integrate web-based technology in teaching so as to keep up with the changing times. There is need to evolve the teaching methods so as to make it more participatory, collaborative and interactive. It is imperative to maximize the learning experiences of students by providing variety of learning strategies. Some of the most effective establishments are using dynamic digital tools such as e-Learning along with traditional classroom practices to create blended learning models. After reviewing the literature, itcan be inferred that various domains of learning of students have been positively impacted by using blended learning strategies. The researches done by Barber (2009), Kumar (2010), Krishnan (2011), Nair (2014), Baweja (2015), Paul and Richard (2017) indicated that blended learning strategies have significantly improved the students' understanding, exploration, acquisition and retention of concepts. Blended learning strategies are also conceived as effective learning strategy that develops skill of critical thinking, skill of problem solving and science process skills etc. (Krishnan, 2011; Chaurasia, 2015).

Blended learning can be entrenched as an efficient learning model of the 21st century (Fatkhulloh& Haryanto, 2020) that expands and enriches the learning opportunities for students, personalizes instructions by offering innovative tools and techniques for use of teachers, effectively develops the professionalism in teachers, transforms recovery of credits and accelerates the whole academic programmes. The research evidences indicate that the objectives of formal education like concept attainment, acquisition and retention of stable, organized and meaningful learning can be considerably achieved if better methods of teaching like blended learning strategies, web-based learning, computer assisted learning, technology assisted learning etc. are used. However, it has been found that very few researches have been done to see the impact of blended learning strategies in Indian context. Besides there are very few researches done on concept attainment in blended learning environment. Therefore, the investigator attempted to do experimental research to see the impact of blended learning strategies on concept attainment through the present study. The present research will guide on formulating Government Policies to integrate technology in process of teaching and learning so as to bring improvement in the quality of education. The study may start deliberations in educational sector for bringing new initiatives so as to evolve pedagogical approaches to empower students so as to make them the life-long learners of 21st century.

Objectives of the Study

The investigator completed the study with the objectives given below:

- $1. To study the impact of blended \ learning \ strategies \ on \ concept \ attainment \ ability \ of \ elementary \ school \ students.$
- 2.To find out the difference in scores of concept attainment of experimental group and control group on pre-test.
- 3. To find out the difference in scores of concept attainment of control group on pre-test and post-test.
- 4.To find out the difference in scores of concept attainment of experimental group on pre-test and post-test.
- 5.To find out the difference in scores of concept attainment of experimental group and control group on post-test.
- 6.To suggest some educational implications based on outcomes of the study.

Hypotheses of the Study

On the basis of review of literature, the investigator formulated following hypotheses:

- 1. There will be no significant difference in scores of concept attainment of experimental group and control group on pre-test.
- 2. There will be no significant difference in scores of concept attainment of control group on pre-test and post-test.
- 3. There will be no significant difference in scores of concept attainment of experimental group on pre-test and posttest.
- 4. There will be no significant difference in scores of concept attainment of experimental group and control group on post-test.





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METHODOLOGY

Research Design

For the present study the investigator used the quasi-experimental research design. The two-group pre-test post-test design was used. This design will help to compare experimental and control group on concept attainment ability after implementation of experimental intervention.

Participants

For the present study the students studying at elementary level constituted the population. Elementary education refers to classes 1st to 8th. The present study was conducted on students of 6th class of Kendriya Vidyalaya, Rahya, Jammu and Kashmir. The participants comprise of 36 students out of which 18 were included in experimental group and other 18 were included in control group. In order to obtain two equivalent groups of students, level of academic performance was taken as variable and for that purpose information was collected from school records. After that the students were arranged in ascending order of their academic performance and divided into two groups viz. the control group and the experimental group by matched pairing.

Research Procedure

The present study was carried out in three phases. For the purpose of experimentation, blended learning application module was prepared which was related to the general concepts of social science. The module was prepared by using a variety of technological tools like MS Word documents, audio and video clippings, online discussions over WhatsApp along with online assessment adequately blended with traditional teaching in face-face setting. The duration of experiment was 2 weeks. The whole procedure of experiment has been depicted in figure 1 and done in three phases described below:

Phase I

The selected sample consisted of 36 students of class 6th of KendriyaVidyalayaRahya. The students were assigned to two groups viz. experimental and control group by equalizing them on the basis of their academic achievement. Thus 18 students were kept in experimental group and 18 students were kept in control group. In the first phase of experiment, pre-test on concept attainment was done on the students of both experimental group and control group separately.

Phase II

In this phase the treatment was given to both the groups. The same concepts were taught to both the groups by using two different strategies. The experimental group was taught using blended learning strategies and the control group was taught using traditional face-to-face method. The investigator prepared the blended learning application module (BLAM) for creating the blended learning environment. The major concepts covered are shown in table 1.

Phase III

In the third phase the concept attainment test was administered as post-test to both the groups. Proper instructions were given to the students of both the groups by the investigator for the successful administration of post-test.

Tool Used

Concept Attainment Test (CAT) by Dr. Anuradha Joshi (Indore) and Ms. Ratanmala Arya (Indore) was used to measure concept attainment ability of students. The concept attainment test was divided in four sections and each section had separate instructions according to its items. The test concepts were related with general subjects. The final draft of scale had 9 items in each section and overall, 36 items.

Statistical Techniques Applied

To compare the mean scores of the experimental and control group in the pre-test and post-test situation t test for repeated measures was employed.





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RESULTS

The results of the study can be provided by analyzing the data given in table 2. The different groups in different test situations can further be compared in the figure 2 and figure 3. It can be interpreted from the Table 2that for experimental and control group under pre-test situation, the computed value of t came out to be 0.51 which is less than 1.74(table value of t at df 17) at 0.05 level of significance. Hence the hypothesis no. 1 stating, "There will be no significant difference in scores of concept attainment of experimental and control group on pre-test" is accepted at said level. Thus, it can be inferred that there is no significant difference in scores of concept attainment of experimental and control group on pre-test. From the table it can further be elucidated that for the control group under pre-test and post-test situation, the computed value of t is 0.52 which is less than 1.74 (table value of t at df 17) at 0.05 level of significance. Hence the hypothesis no. 2 stating, "There will be no significant difference in scores of concept attainment of control group on pre-test and post-test" is accepted at said level. Thus, it can be said that there is no significant difference in scores of concept attainment of control group on pre-test and post-test. Furthermore, for experimental group under pre-test and post-test situation, the computed value of t is 3.60 is more than 2.57 (table value of t at df 17) at 0.01 level of significance. Hence the hypothesis no. 3 stating, "There will be no significant difference in scores of concept attainment of experimental group on pre-test and post-test" is rejected at said level.

Thus, it can be concluded that there is a significant difference in scores of concept attainment of experimental group on pre-test and post-test. At last, it can also be transcribed that for experimental and control group under post-test situation, the computed value of tis 2.32 which is more than 1.74 (table value of t at df 17) at 0.05 level of significance. Hence the hypothesis no. 4 stating, "There will be no significant difference in scores of concept attainment of experimental and control group on post-test" is rejected at said level. Thus, it can be remarked that there is a significant difference in scores of concept attainment of experimental and control group on post-test. The results can further be established in Figure 2 and Figure 3. From figure 2 the experimental and control group can be compared in pre-test and further in post-test situation which depicts a significant mean difference. Similarly, from the figure 3 experimental group can be compared in pre-test and post-test situation and further control group can be compared in pre-test and post-test situation. It can be expressed that that there is significant difference in the mean scores of experimental group as compared to control group.

FINDINGS AND DISCUSSION

The following findings have been revealed by analyzing and interpreting the data in the present study

- 1. There is no significant difference in scores of concept attainment of experimental group and control group on pretest. Thus, both the experimental and control group are equivalent in terms of the concept attainment ability before the commencement of the experimentation procedure.
- 2. There is no significant difference in scores of concept attainment of control group on pre-test and post-test. This result implicated that conventional method of teaching has no significant role in the development of concept attainment ability of elementary school students.
- 3. There is significant difference in scores of concept attainment of experimental group on pre-test and post-test. It can be pointed that the blended learning strategies have significantly improved the concept attainment ability of elementary school students. It could be because blended learning provides variety of activities and learning experiences to students which could help them to effectively attain concepts and ultimately better understanding of the concepts. This finding is in line with the study conducted by Hawi and Sudhira (2019) who reported that by the frequent use of blended learning in classroom the conceptual understanding of students can be increased.
- 4. There is significant difference in scores of concept attainment of experimental group and control group on post-test. Thus, it can be affirmed that blended learning strategies significantly improves the concept attainment ability of students however there is no such improvement during the conventional mode of teaching. This could be attributed to the fact that blended learning strategies provides lot of content in the form of visuals which maintain their attention and retention in the classroom as also suggested by Çiftçi (2020).





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Educational Implications

Following educational implications have been mentioned by the researcher in the present study:

- 1. The study presents a learning model where we can effectively integrate online learning with conventional face-to-face teaching at elementary level.
- 2.The study highlights positive impact of blended learning strategies over conventional method of teaching in developing concept attainment ability of elementary school students. Further studies can be done to strengthen this outcome
- 3. The current research also has implications on formulating Government Policies stressing integration of technology in education so as to bring qualitative improvement in the whole education sector.
- 4.The study may also start discussions in educational sector to bring new initiatives in pedagogical strategies so as to empower the students and make them life-long learners of 21st century.
- 5.Blended learning strategies carefully balances and recognizes the learning needs of learners, their preferred learning methods and the available resources. It significantly increases the effectiveness of learning experiences and facilitates them by transforming theory into practice.
- 6. The study recommends that blended learning provides a platform where learners and subject matter can come in close interaction in supportive manner. The students can learn the basic concepts in an efficient and effective manner.
- 7.An outcome of the study is that the experimental group taught using blended learning strategies attained higher scores in concept attainment test than the control group taught using conventional method of teaching. The reason of such an outcome may be that blended learning strategies ensures that the learner is actively engaged in learning process so that the unique learning styles of heterogenous group of learners are addressed and they effectively attain the concepts.
- 8.Blended learning strategies have changed the learning scenario. The teacher acts as facilitator of learning while using blended leaning strategies rather than an authoritarian individual as in traditional method.
- 9.It is presumed that blended learning strategies would yield fruitful results in the hands of resourceful persons, teachers, administrators, curriculum planners and teacher educators who have a positive bend for making transformative changes in the teaching learning process.

CONCLUSION

Blended learning strategies involve effective integration of conventional face-to-face teaching with web-based online learning. Blended learning strategies creates flexibility in the classroom as a variety of resources are used to provide diverse learning experiences to students. The present research indicated that the blended learning strategies have significantly increased the concept attainment ability of elementary school students in the subject social study. Through the present research it can be inferred that there is a need to bring innovation in the teaching methods so as to maximize the learning experiences of the learners. It has also been recommended that the educational institutions and educators should continuously strive towards making improvements in teaching learning process by effectively utilizing benefits of technology. Blended learning strategies appears to be improvement over conventional method however, it has also been suggested through present research that similar kind of researches can be conducted with different sample and different variables.

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Table.1: Showing Major Concepts of Social Studies Covered During the Experimental Phase

Day	Duration	Content	BLAM
Day 1	40 minutes		Formation of experimental and control group and administration of pre test
Day 2	40 minutes	Countries and their currencies, Continents of the world, Oceans	Use of ppt and video, Formation of Whatsapp group of students
Day 3	40 minutes	Major religions and their religious books, Difference between historical monuments, national monuments, palaces and museums	Face-to-face teaching, online discussion with students
Day 4	40 minutes	Difference between national parks, sanctuaries and parks, Fundamental rights	Face-to-face teaching and a video clipping on national parks was shown to students, online material was supplied to students
Day 5	40 minutes	Forms of government, types of sectors	Ppt was used while discussion with students, online discussion on WhatsApp group with students
Day 6	40 minutes	Natural Disasters	Video clippings on volcanoes, earthquake and landslide was posted on group
Day 7	40 minutes	Revision	Online test was taken
Day 8	40 minutes	Natural Disasters and parts of speech	Discussion by using video clippings and ppt
Day 9	40 minutes	Satellites, Missiles and aircrafts of India, Difference between comets, meteors and meteorite	Face-to-face teaching, online material on types of plants was posted
Day 10	40 minutes	Types of plants on the basis of their habitat, edible parts of a plant, Fertilizers and insecticides	Ppt was used while discussion with students, online test was taken
Day 11	40 minutes	Difference between antigens, antibodies and antibiotics; Difference between elements, compounds and minerals	Face-to-face teaching along with the use of charts for demonstration
Day 12	40 minutes		Administration of post test

Table.2:Showing Scores of Concept Attainment Test of Experimental and Control Group in Pre-Test and Post-Test Situations

Group	Test Situation	N	Mean	S.D	r	t-value	Level of Significance
Experimental Group	Pre-Test	18	16	3.45	0.14	0.51	Not Significant
Control Group	Pre-Test	18	15.28	4.27	-0.14	0.31	at 0.05 level
Control Group	Pre-Test	18	15.28	4.27			Not
Control Group	Post-Test	18	15.94	4.59	0.307	0.52	Significant at 0.05 level
Experimental Group	Pre-Test	18	16	3.45			Significant** at
Experimental Group	Post-Test	18	20.28	6.09	0.60	3.60**	0.01 level
Experimental Group	Post-Test	18	20.28	6.09	0.02	2.32**	Significant** at
Control Group	Post Test	18	15.94	4.59	-0.02	2.32	0.05 level

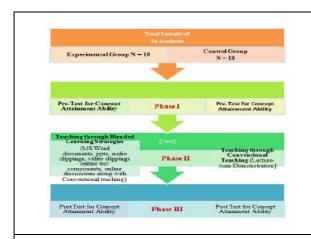




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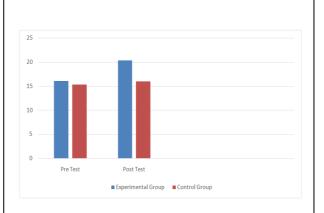


Figure 1 Showing Synoptic Presentation of the Experiment

Figure 2 Represents comparison of mean of experimental and control Group in pre-test and post-test situation



Figure 3 Represents comparison of experimental and control group on pre-test and post-test situation





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RESEARCH ARTICLE

Optimal Decisions on Business Analytics Tools using Combined **Plithogenic Hypersoft Sets**

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ABSTRACT

The data world is overwhelming with new outfits that transmutes the universe data-driven. The business sector needs a variety of data analytics tools to map out the best strategies in order to improve profits. The results of this study led to the formulation of an ideal decision-making method integrating TOPSIS with combined plithogenic hyper soft sets. The decision-making model put forward in this study is used to decide on information analytics technologies, and it includes 5 alternatives and 7 criteria. It is found that the suggested model uses plithogenic aggregate operators to obtain the agreement of the decision makers and also includes additional space for various representations of expert opinion at the level of satisfaction of a criterion through the alternatives. This paper strongly recommends the proposed model to the managerial decision makers for framing optimal decisions on different entities. The proposed model shall be compared with the existing integrated TOPSIS with Plithogenic Hyper soft sets as the future work of this paper.

Keywords: Business Analytics tools, Plithogeny, Hypersoft sets, decision making

INTRODUCTION

The present business scenario is sailing with the mammoth competitive waves. The managerial people require suitable strategies to reach their targets by making decisions on various entities. The mechanism of making optimal decisions demands scientific approach with the consideration of the alternatives and the attributes of criteria without





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the intervention of uncertainty. The theory of Plithogeny introduced by Smarandache is the generalization of varied sets such as crisp (C), fuzzy (F), intuitionistic (I) and neutrosophic (N) and has been extensively applied in making optimal decisions. A Plithogenic (P) set is a quintuple comprising of the extent of congruity and incongruity of the elements to the trait values of the primary traits. The nature of congruity classifies plithogenic sets into PC, PF,PI and PN [25,27]. A MCDM system consists of a number of criteria and alternatives with various characteristic types, as well as the expert's opinion describing the degree to which the alternatives satisfy the criteria as represented by various kinds of sets, including C,F,I and N Researchers at recent times use plithogenic representations of decision-making system to take into consideration of all the attributes and its attribute values together with degree of appurtenance and degree of contradiction. A plithogenic decision-making model was created by Abdel *et al.* [1,4] to facilitate the best possible supplier and supply chain metric selection. Abdel *et al.* [2] also framed another integrated plithogenic decision making model to make optimal decisions on performance of the industries. Gomathy *et al.* [9] discussed plithogenic decision-making models. Grida [10] formulated a plithogenic decision making model for performance evaluation. Priyadharshini and Irudayam [17] framed decision-making models using plithogenic refined sets. Plithogenic based MCDM was used to make optimal decisions on COVID scanrios. Smarandache [27] developed Hypersoft sets from soft sets and also introduced plithogenic Hypersoft sets.

Numerous academics have talked about hypersoft sets in the context of F, I and N thinking. Rana et al. [19] created a PF whole hypersoft set using new plithogenic operators to achieve the best ranking of the alternatives. Nivetha and Smarandache [14] introduced plithogenic combined Hypersoft sets in which the degree of appurtenance was not confined to a single kind of representation, but rather to various combined form of representations, such as a combination of C,F,I and N. Nivetha et al [14] created a model to make decision using the extended plithogenic Hypersoft sets. The literature on plithogenic Hypersoft sets reflect its significance and compatible nature in decisionmaking. TOPSIS (Technique for Order of Preference by Similarity to Ideal Solution) is one of the most commonly used MCDM Methods to rank the alternatives using positive and negative net flow [12]. Rahim et al. [18] has applied this method in crisp sense to make optimal selection on the employees. Behzadian [7] has presented the literature on the research works of TOPSIS in crisp environment. Fuzzy TOPSIS [30] is applied to handle the conditions of ambiguity in decision-making. Haddad [11] applied the method of Fuzzy TOPSIS in ranking the alternatives in a case study. The state of art of Fuzzy TOPSIS was discoursed by Palczewski et al [16]. Rouyendegh [21] developed the integrated Fuzzy TOPSIS with ANP method and also several hybrid methods were also developed. Intuitionistic theory by Atanassov [6]discusses about the sense of belonging and not. Tlig [29] developed Intuitionistic TOPSIS method for a specific case study on the airports in North Africa. Daneshvar et al [8] applied in the location selection of wind power plants. Roszkowska [20] applied to evaluate the socioeconomic aspects. The theory of Neutrosophy developed by Smarandache [24] deals with indeterminacy, an inevitable element in decision-making scenario. Nadaban et al [15] presented a generic view of Neutrosophic TOPSIS. Zulqarnain et al [31] discussed the application of neutrosophic TOPSIS in MCDM scenario.

Plithogenic TOPSIS is applied to make optimal decisions on COVID 19 issues by Sankar *et al* [22]. Nivetha [13] integrated the method of Plithogenic TOPSIS with SWARA to make optimal decisions on food processing methods. Ahmad *et al* [5] elaborated a modern genre of decision-making models by integrating the method of TOPSIS with plithogenic hyper soft sets. This has motivated the authors to develop a new decision-making model by integrating TOPSIS with Combined Plithogenic hyper soft sets. In the model developed by only a single expert's opinion is considered, but in the proposed model the opinion of two decision makers is taken into account and a consensus is determined using plithogenic aggregate operators with contradiction degree. The representations using combined plithogenic Hypersoft sets are more realistic in nature. The following is how the paper is set up into segments: the framework of the integrated TOPSIS with combined plithogenic Hypersoft sets is briefly explained in segment 2, the suggested method is employed in segment 3 to create an optimal ranking on the data analytics tools, the resultants are discussed in section 4, and finally the work is concluded.





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METHODOLOGY

The steps in the suggested method are presented in this segment.

Step 1. On the basis of the expert's recommendation, the alternatives and criterion are selected.

Step 2:The criteria's trade values are mapped out, and the degree to which they conflict with dominant trait value is identified.

Step 3: The initial combined plithogenic decision making matrix, which comprises the level of gratification of the trait values by the alternatives, is constructed using the expert's judgement.

Step 4: Using plithogenic representation and plithogenic aggregate operators, the aggregated plithogenic decision-making matrix is transformed into a regular plithogenic matrix with a fuzzy degree of congruity to get an expert consensus value using defuzzification techniques of imprecision.

Plithogenic Inter section Operators

$$(\alpha_1,\alpha_2,\alpha_3) \wedge p (\beta_1,\beta_2,\beta_3) = \{(\alpha_1 \wedge_P \beta_1), \frac{((\alpha_2 \wedge_P \beta_2) + (\alpha_2 \vee_P \beta_2))}{2}, (\alpha_3 \vee_P \beta_3)\}$$
 where, $\alpha_1 \wedge_P \beta_1 = [1 - c (v_D,v_1)]. tnorm (v_D,v_1) + c(v_D,v_1).tconorm(v_D,v_1)$ t-norm: $\alpha_1 \wedge_P \beta_1 = \alpha_1 \beta_1$, t-conorm: $\alpha_1 \vee p \beta_1 = \alpha_1 + \beta_1 - \alpha_1 \beta_1$

Defuzzification

Where

$$\mathbf{f}_{\mathcal{A}} = = \left\{ \begin{array}{ccc} \mathbf{F}_{\mathcal{A}} + \frac{[\mathbf{1} - \ \mathbf{F}_{\mathcal{A}} - \mathcal{I}_{\mathcal{A}}][\mathbf{1} - \ \mathbf{F}_{\mathcal{A}}]}{[\ \mathcal{F}_{\mathcal{A}} + \mathcal{I}_{\mathcal{A}}]} & if & \mathbf{F}_{\mathcal{A}} = \mathbf{0} \\ \\ \mathbf{F}_{\mathcal{A}} + \frac{[\mathbf{1} - \ \mathbf{F}_{\mathcal{A}} - \mathcal{I}_{\mathcal{A}}][\mathbf{F}_{\mathcal{A}}]}{[\ \mathbf{F}_{\mathcal{A}} + \mathcal{I}_{\mathcal{A}}]} & if & \mathbf{0} < & \mathbf{F}_{\mathcal{A}} \leq \mathbf{0}.5 \\ \\ \mathbf{F}_{\mathcal{A}} + [\mathbf{1} - \ \mathbf{F}_{\mathcal{A}} - \mathcal{I}_{\mathcal{A}}] \left[\mathbf{0}.5 + \frac{\mathbf{F}_{\mathcal{A}} - \mathbf{0}.5}{\mathbf{F}_{\mathcal{A}} + \mathcal{I}_{\mathcal{A}}}\right] & if & \mathbf{0}.5 < & \mathbf{F}_{\mathcal{A}} \leq \mathbf{1} \end{array} \right.$$

Step 5: The overall decision-making matrix is reduced to the matrix consisting of the degree of congruity of the selected trait values using plithogenic fuzzy accuracy function of the formS $^+$ S $_d$ × C $_d$, where S $_d$ is the degree of congruity of the dominant trait value, S is the degree of congruity of the other trait values and C $_d$ is the respective incongruity degree.

Step 6: The normalized matrixND_p = $\begin{bmatrix} k_{ij} \end{bmatrix}_{mxn}$ is obtained using $k_{ij} = \frac{d_{ij}}{\sqrt{\sum_{i=1}^{m} d_{ij}^2}}$, j=1,2,3....n.

Step 7: We obtain the weighted normalised matrix $V_{p} = [v_{ij}]_{mxn}$, using ND_pW_n

the criterion weights are $\omega_1, \omega_2, ... \omega_n$ and $\sum_{k=1}^n \omega_k = 1$

Step 8: The plithogenic positive and negative ideal result from the below method

$$V_p^+ = \left\{ max_{i=1}^m \left(v_{ij}\right) if \ a_j \in benifit \ criteria, \\ min_{i=1}^m \left(v_{ij}\right) if \ a_j \in cost \ criteria \ , \ j=1,2 \dots . n \right\}$$

$$V_p^- = \left\{ \min_{i=1}^m \left(v_{ij} \right) if \ a_j \ \in \ benifit \ criteria, \\ \max_{i=1}^m \left(v_{ij} \right) if \ a_j \ \in \ cost \ criteria \ , \ j=1,2 \dots . \ n \right\}$$

Step 9: The plithogenic positive distance (PPD) S_i^+ and plithogenic negative distance (PPN) S_i^- of every alternative from $V_p^+ \& V_p^-$ is calculated using





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$$S_i^+ = \sqrt{\sum_{j=1}^n (v_{ij} - v_i^+)^2}$$
, i=1,2....m.

$$S_i^- = \sqrt{\sum_{j=1}^n (v_{ij} - v_i^-)^2}$$
, i=1,2....m.

Step 10: The following relation is used to obtained the relative closeness coefficient C₁ of each possibility.

$$C_i = \frac{S_i^-}{S_i^+ + S_i^-}, i = 1, 2, \dots, m.$$

Step 11: Based on the values, the alternatives are ranked.

Application To Decision-Making On Ranking The Business Analytics Tools

The availability of business analytics tools are ample, it is vital to make the greatest choices possible when it comes to choosing the right tools that can replace the decision-making process. The criteria taken as the attributes together with the attribute values are given along with the respective contradiction degree with the dominant attribute values presented in Table.1.

Case (i)

Let P_{ch} = and taking the consideration of the attribute values (A_1^1 , A_2^1 , A_3^1 , A_4^1 , A_5^1 , A_6^3 , A_7^1), the corresponding matrix { $A_1 \times A_2 \times A_3 \times A_4 \times A_5 \times A_6 \times A_7$ } obtained is

0.37	0.52	0.65	0.6	0.385	
0.45	0.485	0.5	0.55	0.475	
0.535	0.54	0.55	8.0	0.7	
0.435	0.34	0.7	0.65	8.0	
0.415	0.45	0.7	0.55	0.59	
0.67	0.65	0.65	0.4	0.655	
0.44	0.435	0.6	0.6	0.57	,

The Normalized matrix is

0.319867	0.408106	0.377329	0.319187	0.337128	0.488268	0.36824
0.449543	0.439847	0.380856	0.24948	0.365561	0.473693	0.364055
0.561929	0.453451	0.387909	0.513634	0.56865	0.473693	0.502145
0.518703	0.498796	0.564231	0.476946	0.446797	0.291503	0.502145
0.332835	0.430778	0.493702	0.587011	0.479291	0.477337	0.477038

The weighted normalized matrix is

							3	
1	0.044781	0.057135	0.052826	0.044686	0.047198	0.07324	0.055236	
ı	0.062936	0.061579	0.05332	0.034927	0.051179	0.071054	0.054608	
ı	0.07867	0.063483	0.054307	0.071909	0.079611	0.071054	0.075322	
ı	0.072618	0.069831	0.078992	0.066772	0.062552	0.043725	0.075322	
l	0.046597	0.060309	0.069118	0.082182	0.067101	0.0716	0.071556	
١								,

Let us repeat the same procedure by considering another set of attribute values.





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Case (ii)

Let P_{ch} and taking the consideration of the attribute value $(A_{1^2}, A_{2^2}, A_{3^2}, A_{4^2}, A_{5^2}, A_{6^3}, A_{7^2})$, the corresponding matrix obtained $\{A_1 \times A_2 \times A_3 \times A_4 \times A_5 \times A_6 \times A_7\}$ is

0.54	0.5	0.6	0.45	0.67	1
0.67	0.65	0.65	0.4	0.655	
0.67	0.65	0.65	0.4	0.655	
0.45	0.485	0.5	0.55	0.475	
0.67	0.65	0.65	0.4	0.655	
0.67	0.65	0.65	0.4	0.655	
0.535	0.54	0.55	8.0	0.7	/

Thus, by varying the required attribute values, the alternatives are ranked respectively.

DISCUSSION

In the first case the dominant attribute values and the required dominant attribute values are same. In the second case they are not the same, they are different. In both the cases the ranking obtained is different.

CONCLUSION

This research work proposes a novel decision-making method integrating TOPSIS and combined plithgenic hyper soft sets. The proposed method is highly advantageous as it provides space for expressing expert's opinion in various forms, not confining to a single representation. The innovative features of the proposed model are the plithogenic aggregate operators applied to create a consensus. The model can be compared with the plithogenic decision-making model integrated with TOPSIS to test the efficacy of the proposed model.

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Table 1: presents Contradiction degree with the dominant attribute value as follows:

	Attributes	Attribute Values	Contradiction Degree
A 1	Veracity	{(high accuracy A ₁), (moderate accuracy	$C(A_{1^1}, A_{1^2}) = 1/3, C(A_{1^1}, A_{1^3}) = 2/3$
A ₂	Velocity	A ₁ ²), (less accuracy A ₁ ³)} {(high A ₂ ¹), (moderate A ₂ ²), (low A ₂ ³)}	$c(A_{2}^{1}, A_{2}^{2}) = 1/3, c(A_{2}^{1}, A_{2}^{3}) = 2/3$
A 3	Scalability	{(high A_3^1), (moderate A_3^2), (low A_3^3)}	$C(A_{3^1}, A_{3^2}) = 1/3, C(A_{3^1}, A_{3^3}) = 2/3$
A 4	Security	{(highly secure A_4^1), (moderately secure A_4^2), (less secure A_4^3)}	$C(A_4^1, A_4^2) = 1/3, C(A_4^1, A_4^3) = 2/3$
A 5	Compatibility	{(high A_{5}^{1}), (moderate A_{5}^{2}), (low A_{5}^{3})}	$C(A_{5^1}, A_{5^2}) = 1/3, C(A_{5^1}, A_{5^3}) = 2/3$
A 6	Costs	{(high A_{6}^{1}), (moderate A_{6}^{2}), (low A_{6}^{3})}	$C(A_{6^3}, A_{6^1}) = 1/3, C(A_{6^3}, A_{6^2}) = 2/3$
A 7	Time Efficiency	{(high A_{7}^{1}), (moderate A_{7}^{2}), (low A_{7}^{3})}	$C(A_{7}^{1}, A_{7}^{2}) = 1/3, C(A_{7}^{1}, A_{7}^{3}) = 2/3$

Table 2: states the initial combined plithogenic decision making matrix

/	ernatives	oa piidiogei	iic decision makir	-3 man m		
A	ttribute	T ₁	T_2	T ₃	T ₄	T ₅
\	/alues					
A ₁ ¹	Expert-I	(0.5,0.3)	(0.8,0.1,0.2)	0.8	(0.7,0.2)	(0.4,0.3)
A1'	Expert-II	(0.8,0.1,0.2)	(0.7,0.2)	(0.6,0.1)	0.7	(0.2,0.2,0.7)
A 1 ²	Expert-I	(0.7,0.1,0.3)	0.4	0.7	(0.4,0.2)	(0.9,0.1,0.1)
Al-	Expert-II	0.6	0.6	(0.7,0.2)	(0.8,0.1)	0.7
A 13	Expert-I	(0.6,0.2)	(0.6,0.1,0.2)	(0.5,0.1,0.1)	(0.2,0.5)	0.5
Αľ	Expert-II	0.6	0.6	(0.7,0.2)	(0.8,0.1)	0.7
A ₂ ¹	Expert-I	(0.7,0.2)	(0.5,0.1,0.1)	(0.7,0.2)	(0.4,0.3)	(0.5,0.2,0.3)
A 2'	Expert-II	(0.6,0.2)	(0.6,0.1,0.2)	(0.5,0.1,0.1)	(0.2,0.3,0.7)	0.5
A 2 ²	Expert-I	(0.5,0.3)	(0.8,0.1,0.2)	0.8	(0.7,0.2)	(0.4,0.3)
A 2 ²	Expert-II	(0.8,0.1,0.2)	(0.7,0.2)	(0.6,0.1)	0.7	(0.2,0.2,0.7)
A_2^3	Expert-I	(0.5,0.1,0.2)	(0.6,0.1)	(0.5,0.1,0.1)	0.6	(0.4,0.1,0.6)
A 2 ³	Expert-II	(0.5,0.1)	(0.5,0.1,0.3)	0.9	(0.6,0.1)	0.5
A ₃ ¹	Expert-I	(0.6,0.1,0.2)	(0.7,0.1,0.3)	(0.8,0.2	0.9	(0.8,0.1)
A 3.	Expert-II	0.6	0.6	(0.7,0.2)	(0.8,0.1)	0.7
A 3 ²	Expert-I	8.0	0.6	8.0	0.6	(0.8,0.1,0.1)
A3 ²	Expert-II	(0.8,0.1,0.2)	0.7	(0.6,0.1)	(0.50.3)	0.7
A_3 3	Expert-I	(0.6,0.2)	(0.6,0.1,0.2)	(0.5,0.1,0.1)	(0.2,0.3,0.7)	0.5
A3*	Expert-II	(0.5,0.3)	(0.8,0.1,0.2)	8.0	(0.7,0.2)	(0.4,0.3)
A_4^1	Expert-I	(0.6,0.1,0.2)	(0.7,0.1,0.3)	(0.8,0.2	0.9	(0.8,0.1)
A 4'	Expert-II	(0.6,0.2)	(0.50.3)	8.0	(0.5,0.1)	0.9
A_4^2	Expert-I	(0.7,0.2)	(0.5,0.1,0.1)	(0.7,0.2)	(0.4,0.3)	(0.5,0.2,0.3)
A42	Expert-II	(0.6,0.2)	(0.6,0.1,0.2)	(0.5,0.1,0.1)	(0.2,0.3,0.7)	0.5
A_4 ³	Expert-I	(0.7,0.1,0.3)	0.4	0.7	(0.4,0.2)	(0.9,0.1,0.1)
A 4°	Expert-II	(0.6,0.2)	(0.50.3)	8.0	(0.5,0.1)	0.9
A ₅ ¹	Expert-I	(0.5,0.1,0.2)	(0.6,0.1)	(0.5,0.1,0.1)	0.6	(0.4,0.1,0.6)
H 5'	Expert-II	(0.5,0.1)	(0.5,0.1,0.3)	0.9	(0.6,0.1)	0.5
A 5 ²	Expert-I	0.8	0.6	0.8	0.6	(0.8,0.1,0.1)
A 5*	Expert-II	(0.8,0.1,0.2)	0.7	(0.6,0.1)	(0.50.3)	0.7
Λ 2	Expert-I	(0.6,0.1,0.2)	(0.7,0.1,0.3)	(0.8,0.2	0.9	(0.8,0.1)
A_{5^3}	Expert-II	(0.6,0.2)	(0.50.3)	0.8	(0.5,0.1)	0.9
Λ 1	Expert-I	0.8	0.6	0.8	0.6	(0.8,0.1,0.1)
A_{6}^{1}	Expert-II	(0.8,0.1,0.2)	0.7	(0.6,0.1)	(0.50.3)	0.7
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Λ 2	Expert-I	(0.6,0.1,0.2)	(0.7,0.1,0.3)	(0.8,0.2	0.9	(0.8,0.1)
A 6 ²	Expert-II	(0.6,0.2)	(0.50.3)	0.8	(0.5,0.1)	0.9
A ₆ ³	Expert-I	0.8	0.6	0.8	0.6	(0.8,0.1,0.1)
A63	Expert-II	(0.8,0.1,0.2)	0.7	(0.6,0.1)	(0.50.3)	0.7
A ₇ 1	Expert-I	(0.7,0.1,0.3)	0.4	0.7	(0.4,0.2)	(0.9,0.1,0.1)
A/·	Expert-II	(0.6,0.2)	(0.6,0.1,0.2)	(0.5,0.1,0.1)	(0.2,0.3,0.7)	0.5
Λ 2	Expert-I	(0.6,0.1,0.2)	(0.7,0.1,0.3)	(0.8,0.2	0.9	(0.8,0.1)
A 7 ²	Expert-II	0.6	0.6	(0.7,0.2)	(0.8,0.1)	0.7
Λ 3	Expert-I	(0.5,0.3)	(0.8,0.1,0.2)	0.8	(0.7,0.2)	(0.4,0.3)
A ₇ 3	Expert-II	(0.8,0.1,0.2)	(0.7,0.2)	(0.6,0.1)	0.7	(0.2,0.3,0.7)

The combined plithogenic fuzzy decision matrix is given in Table 3

	ernatives					
	tribute	T 1	T ₂	T ₃	T ₄	T ₅
V	/alues					
A_{1}^{1}	Expert-I	0.2	0.54	0.8	0.5	0.1
Α,	Expert-II	0.54	0.5	0.5	0.7	0.67
A_{1^2}	Expert-I	0.48	0.4	0.7	0.2	0.64
A1-	Expert-II	0.6	0.6	0.5	0.7	0.7
Λ 3	Expert-I	0.4	0.47	0.5	0.64	0.5
A_{1}^{3}	Expert-II	0.6	0.6	0.5	0.7	0.7
A 1	Expert-I	0.5	0.5	0.5	0.1	0.45
A_{2}^{1}	Expert-II	0.4	0.47	0.5	1	0.5
Λ 2	Expert-I	0.2	0.54	0.8	0.5	0.1
A_2^2	Expert-II	0.54	0.5	0.5	0.7	0.67
A 2	Expert-I	0.43	0.5	0.5	0.6	0.68
$A_{2^{3}}$	Expert-II	0.4	0.4	0.9	0.5	0.5
Δ 1	Expert-I	0.47	0.48	0.6	0.9	0.7
A_{3}^{1}	Expert-II	0.6	0.6	0.5	0.7	0.7
Λ 3	Expert-I	0.8	0.6	0.8	0.6	0.61
A_{3^2}	Expert-II	0.54	0.7	0.5	0.2	0.7
Λ 3	Expert-I	0.48	0.4	0.7	0.2	0.64
A_3^3	Expert-II	0.4	0.47	0.5	1	0.5
Λ 1	Expert-I	0.47	0.48	0.6	0.9	0.7
A_4^1	Expert-II	0.4	0.2	0.8	0.4	0.9
A ₄ ²	Expert-I	0.5	0.5	0.5	0.1	0.45
A4 ²	Expert-II	0.4	0.47	0.5	1	0.5
A_4^3	Expert-I	0.48	0.4	0.7	0.2	0.64
A 4°	Expert-II	0.4	0.2	0.8	0.4	0.9
A_{5}^{1}	Expert-I	0.43	0.5	0.5	0.6	0.68
1 45'	Expert-II	0.4	0.4	0.9	0.5	0.5
A_{5^2}	Expert-I	8.0	0.6	8.0	0.6	0.61
~ 5⁻	Expert-II	0.54	0.7	0.5	0.2	0.7
$A_{5^{3}}$	Expert-I	0.47	0.48	0.6	0.9	0.7
~ 5⁻	Expert-II	0.4	0.2	8.0	0.4	0.9
A ₆ ¹	Expert-I	8.0	0.6	0.8	0.6	0.61
∠ 0.	Expert-II	0.54	0.7	0.5	0.2	0.7





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A ₆ ²	Expert-I	0.47	0.48	0.6	0.9	0.7
A6-	Expert-II	0.4	0.2	0.8	0.4	0.9
A_{6^3}	Expert-I	0.8	0.6	0.8	0.6	0.61
A63	Expert-II	0.54	0.7	0.5	0.2	0.7
A ₇ 1	Expert-I	0.48	0.4	0.7	0.2	0.64
A/·	Expert-II	0.4	0.47	0.5	1	0.5
A ₇ ²	Expert-I	0.47	0.48	0.6	0.9	0.7
A72	Expert-II	0.6	0.6	0.5	0.7	0.7
A ₇ 3	Expert-I	0.2	0.54	0.8	0.5	0.1
A 7°	Expert-II	0.54	0.5	0.5	0.7	1

Table 4. represents the combined plithogenic fuzzy decision matrix with the plithogenic aggregated values

Alternatives		<u> </u>			33 3 3
Attribute	T ₁	T ₂	T₃	T ₄	T ₅
Values	11	12	13	1 4	15
A ₁ ¹	0.37	0.52	0.65	0.6	0.385
A ₁ ²	0.54	0.5	0.6	0.45	0.67
A_{1}^{3}	0.5	0.535	0.5	0.67	0.6
A_{2}^{1}	0.45	0.485	0.5	0.55	0.475
A_2^2	0.37	0.52	0.65	0.6	0.385
A_{2}^{3}	0.415	0.45	0.7	0.55	0.59
A ₃ ¹	0.535	0.54	0.55	0.8	0.7
A ₃ ²	0.67	0.65	0.65	0.4	0.655
A_{3}^{3}	0.44	0.435	0.6	0.6	0.57
A_4^1	0.435	0.34	0.7	0.65	0.8
A_4^2	0.45	0.485	0.5	0.55	0.475
A_4 ³	0.44	0.3	0.75	0.3	0.77
A_{5}^{1}	0.415	0.45	0.7	0.55	0.59
A_{5}^{2}	0.67	0.65	0.65	0.4	0.655
A_{5}^{3}	0.435	0.34	0.7	0.65	8.0
A ₆ 1	0.67	0.65	0.65	0.4	0.655
A ₆ ²	0.435	0.34	0.7	0.65	8.0
A63	0.67	0.65	0.65	0.4	0.655
A ₇ 1	0.44	0.435	0.6	0.6	0.57
A ₇ ²	0.535	0.54	0.55	0.8	0.7
A ₇ 3	0.37	0.52	0.65	0.6	0.55

Table 5 is the final ranking of the alternatives

3						
Alternatives	S+	S-	Ci	Rank		
T ₁	0.075666	0.009779	0.11445	5		
T ₂	0.072056	0.019241	0.21075	4		
Тз	0.038756	0.063585	0.621304	2		
T ₄	0.023772	0.064582	0.730949	1		
T ₅	0.046525	0.056549	0.548626	3		





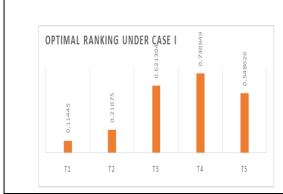
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Table 6 is the final ranking of the alternatives

Alternatives	S+	S-	Ci	Rank
T ₁	0.062572	0.042676	0.405479	4
T ₂	0.060484	0.04019	0.399208	5
T ₃	0.046962	0.055203	0.540331	2
T ₄	0.055507	0.0584	0.512698	3
T ₅	0.034531	0.06113	0.639026	1



11 T2 T3 T4 T5

Figure 1 Optimal Ranking under Case I

Figure 2 Optimal Ranking under Case II





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REVIEW ARTICLE

Six Sigma Applicability to Industrial Products - A Review

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ABSTRACT

The purpose of this review article is to discuss in detail about Six Sigma applicability to industrial products. The current review article highlights are the history, benefits, implementation, methodology and Applications of Six Sigma. The goal of this technique is to decrease unnecessary expenses, improving customer loyalty by offering exactly what the user wants. It is a process improvement strategy used in the pharmaceutical industry to reduce waste, enhance profitability, and enhance product standards and user needs.

Keywords: Six Sigma, Benefits, DMAIC, DMADV, Applications.

INTRODUCTION

In statistical terms the purpose of six sigma is to reduce process variation so that virtually all products or services provided to meet or exceed customer expectations. Six sigma is highly disciplined process that focuses on developing and delivering near perfect products and services. The range of six sigma applications is also growing from reduction of defects in a systematize processes. Six sigma approach to any process is focalized on target on improving understanding of customer requirements and fecundity, financial performances. It is all about gleaned on customer and data. Six sigma is a process which is a combination of good manufacturing practice that is man, machine, materials and methods for producing products or services. At six sigma level, there are 3.4 defects occurred





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per million opportunities. Six sigma find strategies to decrease internal cost and cycle times by imparting high quality services, through innovative designs & effective response to the unexpected increase in demand for certain products. Every organization aims at profit maximization and growth. Growth and profits are directly related to the level of satisfaction that is imparted by the product or the services to the customers. Customer wants value for money. He wants the best quality in the given cost. This quality is percolated in the product through right set of processes, activities with the use of right resources in terms of human and technology and ultimately right quality is achieved by reduction in defect in the product. Lesser is the tolerance limit for defect, better is the quality. This concept drives the organization towards the concept of least deviation in the products that are manufactured. This drives the organization towards Six Sigma. The world is experiencing unprecedented times since the beginning of 2020. COVID 19 has halted many manufacturing operations. The manufacturing facility where this case study was conducted continued to manufacture during the pandemic given its classification as an essential service and saw an unparalleled increase in volume demands. Paracetamol is used to treat mild cases of COVID 19 as it is effective in treating high temperatures. To benefit from the increase in demand and avoid potential lost sales, it was critical for the business to ensure a smooth, continuous supply of product to the customer. The researchers needed to deliver results in an environment where there was an unprecedented spike in demand without any risk of disruption to supply. Pharmaceutical and medical device manufacturers are looking in Lean manufacturing and Six sigma principles the way for significant improvement of operational efficiency and quality, while facilitating compliance. Today, manufacturers in these industries are focused as never before on reducing operational costs while ensuring compliance. To ensure a solid position on the market and competitive advantage they are looking to increase the efficiency of their operational and manufacturing processes optimizing resources, improving efficiency, reducing waste and controlling inventory. All previously presented elements and current developments in the pharmaceutical and medical device industry are in favour that it is now an ideal time to turn to the principles of Six Sigma.

History of Six Sigma

Carl Frederick Gauss (1777-1855) is known for inventing Six Sigma. He was the first to propose the concept of the normal curve. Six Sigma was invented as a measurement standard relates to the 1920s. Many measurement criteria followed, but a Motorola engineer named Bill Smith is credited by coining the word "Six Sigma" in 1986. Bill Smith spent lot of time convincing higher authorities that his new quality control system 'Six Sigma" would provide significant benefits for the company. On the advice of Bill, CEO Robert Galvin and his management team then looked at Japanese models for quality work then they realized that it would be crucial for the success of this new quality control system and the improvement work and then the implementation was very successful. Six Sigma is focused on six major key principles such as

- Critical to quality: what is important for the customer needs to be identified
- Defect: anything that is not deliver exactly what customer need
- Process Capability: processes should be deliver as what customer need
- Variation: it is experienced by customer
- Stable Operation: its goal is to secure reliable that improve customer's experience
- Design for Six Sigma: the design should meet the customer need and capability of process.

Benefits of Six Sigma

- > Ensuring long term success
- > Increases consumer value
- > It promotes learning and cross pollination
- > Number of defects is reduced in manufacturing process
- Process variability is reduced
- > In pharmaceutical sector, this approach is used to reduce defects and improve operating performance and quality of service.

Methodology of Six Sigma

Methodology includes





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DMAIC Process

DMAIC (Define, Measure, Analyze, Improve, and Control) is a systematic improvement framework for current processes that aren't meeting specifications. This approach is applied to existing business processes to enhance them. It involves the parameters mention on below chart.

DMADV Process

DMADV (Define, Measure, Analyze, Design, and Verify) is a Six Sigma quality management approach for building new processes or products. If a current procedure needs more than gradual enhancement, it can also be incorporated. This approach is used to design new products or processes in such a way that their performance is more consistent, effective, and defect-free. It shown a great impact following principles as mentioned on below chart.

Six Sigma Implementation

Six Sigma is made up of three essential components

- Process Improvement
- Process Development
- Process Management

Process Improvement

Process improvement aims to remove the causes of performance defects in existing processes in organization. These defects may cause serious problems for company from running effectively as it could.

Process Development

Process development must consider the process suitability and design requires a broad perspective of whole organization.

Process Management

Process management is a method of analyzing and managing the process that exist in a company. In general, process management entails Defining procedures, identifying customer's needs. Customer criteria and primary performance are used to evaluate performance. Analyzing data is used to improve measures and process management systems. Controlling process inputs and outputs are quickly react to problems and process variation

Applications Of Six Sigma Pharmaceutical Industry

In the pharmaceutical industry, this technique is used to minimize waste, make needed changes in manufacturing processes, and increase efficiency, all of which help to improve product quality and customer service. Process capability refers to the capability of the process to meet the desired specification and requirement of a product or service. The capability of a process to achieve consistent output under defined constraints is measured by its process capability index. In the pharmaceutical industry, several firms are attempting to transform their operations to increase profits. Many businesses are combining conventional Six Sigma and Lean Manufacturing to improve the quality and operational aspects of the manufacturing process. Both methodologies have had positive results in adjacent markets such as the automotive and electronics industries. The pharmaceutical industry places a high priority on reducing waste and defects in the manufacturing process, so Lean Manufacturing is a hot topic that should provide value to customers when involving management at all times.

Research and Development

The most significant phase in the pharmaceutical industry is research and development, which accounts for a large portion of costs. In this case, the Six Sigma principle is desirable to consider the essential processes in new drug production, as well as to study and streamline existing ones. These goals are necessary to minimize drug failures, enhance resource usage, and boost efficiency and staff and other resource usages.





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Cycle Times

Increased cycle times are a major factor influencing the manufacture, supply, and launch of new drugs on time. The ability of earlier industries to take advantage of this business situation inevitably determines whether a product succeeds or fails. Lean Manufacturing and Six Sigma principles such as value stream mapping and process modeling can help reduce cycle times and operating costs while also increasing the reliability of processes and personnel.

Defects

Any drug-related flaws will be a major setback for the pharmaceutical industry. Six Sigma principles can benefit because they rely on scientific and mathematical methods that have been shown to reduce the cost of human errors. The industry may use advanced methods to conduct quality analysis, yield analysis, job cost comparisons, risk analyses, and manufacturing process comparisons across several locations. Six Sigma is extremely beneficial to the pharmaceutical industry.

In Healthcare System

The assessment, evaluation, and knowledge of process variations is required for continuous improvement in healthcare systems. Wherever possible, process variation should be eliminated. Important process variables in healthcare include laboratory turnaround times, patient longer waits, patient satisfaction scores, adverse events (prescription, dispensing, and administration), emergency service response times, infection rates, mortality rates, patient falls, postoperative lengths of hospital stay, "door-to-needle" times, and counts of an adverse event. Monitoring, analyzing, and studying such variables with care can result in significant improvements in healthcare service quality. Visionary leadership, proper planning, education, and training, the availability of trained staff, productive resources, personnel, and process management, and cooperation and teamwork among service providers can all help to improve healthcare quality.

CONCLUSION

Six Sigma is a systematic approach to data collection and statistical analysis. The primary goal of improved quality is for the organization to earn more profit. Quality is defined as the degree of excellence of a product or service given to a customer in very simple terms. If the consumer is pleased with the product or service, it is of the required standard of quality. Six sigma is used in the pharmaceutical industry to reduce waste, make effective changes in the production process to enhance operating performance, and improve product quality and customer service.

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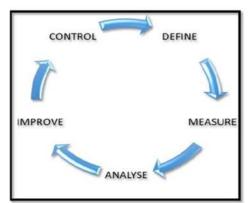


Fig: 1 DMAIC Process

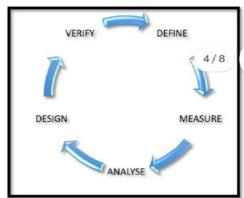


Fig: 2 DMADV Process





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RESEARCH ARTICLE

The Colonial Foundation of Scientific Forestry in Mikir Hills of Assam: Some Annotations

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ABSTRACT

This paper explores the colonial foundation of scientific forestry in Mikir Hills of Assam. It was one of the important forest areas during the British rule in Assam. It was one of the oldest Autonomous District Councils created under Article 244(A) under the sixth schedule to the Constitution of India. Before the present Karbi Anglong district was popularly known as Mikir Hills. This is the largest district of Assam with large forest coverage. In the academic discourse, there are two groups of scholars in the context of the management of forests in India. The first group argued that the colonial power not only exploited the natural resources but also neglected the forest user's rights over the resources. The second group defended the colonial foundation of scientific forest management for India and there is no exception to Mikir Hills of Assam too. This branch advocated a colonial sort of conservation of forest resources. This paper has been developed after compiling data from archival sources, the contemporary administrative reports which are still available from the forest territorial divisions of the present Karbi Anglong Autonomous Council (KAAC), and also used the primary data collected in the field. This paper concluded that the foresters of India who were in involved for preparation of the scientific working plans/schemes for resource management were highly influenced by the colonial forest officials which led to many loopholes in the contemporary forest management of Mikir Hills of Assam.

Keywords: Conservation of forest, Deforestation, Plantation, Ecology, Forest resources, Shifting cultivation





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INTRODUCTION

Forest management has been considered a key issue in the protection of forest resources which is essential to the state and the people as well. The importance of environmental protection and conservation of natural resources has been given wide attention at the state and national levels. As a result, various institutions with the emergence of forest departments have come forward to protect and maintain the forestry sector of the state. The various tribes of India since time immemorial have also depended upon the forests to meet their daily needs. It is a fact that the management of forest laws in the pre-independence period tried to address many issues of forest with the objectives of conservation, management, and regulation of forest by the colonial state which are largely state-centric. In the academic discourse, there are two schools of thought in the context of the management of forest resources in India. The first section criticized that the colonial power not only exploited the natural resources but also neglected the forest dweller's rights over the resources (Sen 1992, Gadgil and Guha 1994, Rangarajan 1994, Sivaramakrishnan 1995, Rangarajan 1996, Skaria 1999, Sivaramakrishnan 2000, Philip 2002, Saravanan 2004, Saikia 2005, Basar 2006, Handigue 2007, Kumar et al. 2011, Dhanaraju 2012, Dhanaraju 2014 a, Dhanaraju 2014 b, Dhanaraju 2016, Dhanaraju 2019, Dhanaraju 2021). The second school of thought opposes that though the commercialization of forests happened, simultaneous conservation of forests was also emphasized. Subsequently, the colonial historians defended the colonial forest policies of the British. (Powel 1882, Ribbentrop 1900, Stebbing 1921, Stokes 1959, Stein 1990, Cohn 1966, Washbrook 1981, Grove 1995, Mosse2003). This school argued that scientific forest management put an end to deforestation. This branch advocated a colonial sort of conservation of forest resources. Rapacious indigenous attitudes towards the forest and ecological degradation in India were the themes of the official version of the imperial scholars.

The perceptions of the state's hegemony towards forest resources had changed during the colonial period as the forest was increasingly viewed as a 'property of the state' with excessive commercial possibility. In this context, this paper is an attempt to explore the historical background of scientific forestry of Mikir Hills which was a very important forest area in colonial Assam. This paper has been developed after compiling data from various sources which are still available from the forest territorial divisions of the present Karbi Anglong Autonomous Council (KAAC). It is one of the oldest District Autonomous Councils created under Article 244(A) under the sixth schedule to the Constitution of India. Formerly Karbi Anglong district was popularly known as Mikir Hills. This is the largest district of Assam with large forest coverage. It is a vast storehouse of natural beauty with its endless variety of landscape hilly terrain, meandering streams, and a rich wealth of flora and fauna. Most of the people in this district depend on the forest. Forest play important role in the socio-economic life of the local population.

MATERIALS AND METHODS

Topographically, the Mikir Hills district can be broadly divided into two parts such as hills and plains. The Mikir Hills, blended with hills and plains, are situated in the central part of Assam. The total geographical area of the Mikir Hills district is 10,434 sq. km, which accounts for about 13.53% of the state's total geographical area of 78, 438 sq. km. It is the largest district of the state comprising 2633 villages. The district with dense tropical forest-covered hills and plains is situated between 25°33° and 26035° north latitudes and 92°10° and 93°50° east longitudes. The district is mostly rugged and hilly being part of an Archean plateau. The plateau in Mikir Hills comprises two parts: the east plateau and the west plateau. The forest area of the hill regions of Assam covers about 34% of the total geographical area of the region against the regulated forest coverage of 60% as fixed by the National Forestry Policy, 1952. As per the State of Forests Reports (SFR), 2001, published by the Forest Survey of India, the total area under forests in Mikir Hills district was 7,97,200 hectares constituting 76.4% of its geographical area. Historically, the population of Mikir Hills is predominantly tribes and Karbi is the major tribe. This paper is developed based on the data collected for Sponsored Research Project of the Indian Council of Social Science (ICSSR), New Delhi. The study was conducted in the Karbi Anglong district of Assam from 2016 to 2018. The secondary sources were collected from the relevant Government publications, including Annual Souvenirs of Karbi Anglong Autonomous Council (KAAC), Reports,





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Websites of Assam State, etc. The field observations were used in this paper while applying qualitative research techniques and mainly used the historical method.

RESULT AND DISCUSSION

Categories of Forest in Mikir Hills

The general status of Mikir Hills of wild fauna and flora is quite rich which could well be gauged from the fact that this district is considered one of the hot spots of mega bio-diversity of the northeastern region. As per the revised classification by Champion and Sheath (1968), the following kinds of forests are found in this district.

- 1. Moist Semi-Evergreen Forest
- 2. Moist Mixed Deciduous Forests
- 3. Crane Brakes
- 4. Wet Bamboo Brakes
- 5. Secondary Moist Bamboo Brakes
- 6. Assam sub-Tropical Pine Forests
- 7. Subtypes of moist mixed deciduous forests (bamboo mixed with tree and tree mixed with bamboo). This type of forest depends on rainfall.

The forest produce in the Mikir Hills is divided into two categories i.e timber and non-timber forest produce and other non-timber forest produce. The State Reserve Forest (RF), District Council Reserve Forests (DCRF), Proposed Reserve Forests (PRF), and Un-classed State Forests (USF) have been notified in 2015 by the KAAC. A total of 46 nos. are notified forest areas. The total forest area under these four categories is 6589.99 sq. km. It is mentioned here that the Un-classed State Forests (USF) in the forest area are under community forest management. But recently the KAAC has put some restrictions that the people are not allowed to use the USF for *jhum* cultivation without prior approval of the Council. It hampered the traditional community ownership of forest management.

Early Attempts of Forest Management (1850-1904)

The general history of the forest management of Mikir Hills is not different from the plain district of Assam. Before the establishment of the Mikir Hills district in 1951, the notified forest areas were under erstwhile Nagaon, Sibsagar, Khasi, and Jantia Hills. It was more difficult to explore the nature of forest resource management due to the unavoidable incidents that took place during World War-I & II. Therefore, the old record of the forest department was difficult to trace. The following section deals with the early attempts at forest management in the Mikir Hills of Assam. There was no record of forest management during the Ahom regime of 600 years in the Mikir Hills of precolonial Assam. During the early British period, there was no scientific management of forests in this region. But there was evidence of shifting cultivation being practiced by the Karbi tribe and other indigenous communities without any restrictions. The shifting cultivation essentially provides the bare requirements of tribes for survival rather than generating surplus. At that time population was very thin in comparison to the forest areas and damage by the human agency for timber or land was not at all a problem. On the other hand, it was considered indispensable to cut trees mercilessly and open land everywhere to spread settlement and grow paddy and other food crops. It was in the third quarter of the nineteenth century that preservation of the forest resources and systematic exploitation of the trees on a sustained yield basis was considered to be virtues (WPKAEHD, 2006).

It is noted that before 1850, there was no organized attempt in Assam to realize dues on felled timber. In 1850, a tax of Rs. 15 per 100 logs was imposed by the collector of a coupe with a check on the felling of a young tree (WPWD, 2005). This system was however abolished in 1852 by the Board of Revenue which led to the formation of a system of farming certain tracts of forests to the highest bidders for 5 years. In this system, there was no such restriction as regards the size of trees to be felled and the revenue was collected by fiscal Officers. These fiscal officers were replaced by *mouzadars* in 1868 with the change in the revenue system. The constitution of the reserve forest started





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during 1870-71 with the appointment of the first forest officer in Assam (Saikia, 2005). To save the remnants of the well-stocked valuable forests from destruction, some measures were taken that resulted in the formal constitution of reserve forests in 1873. While the forest department-controlled forest operation only in reserve forests, the control in other areas by the local authority through mouzadar. But the control of forest operations in all areas was transferred to the forest department by 1883-84. In 1876, W.R. Fisner, officer-in-charge of the Goalpara division was the first to introduce a management plan for the conservation of sidli forests. Saikia (2005) has described in his book about nature of forest management in colonial Assam. The process of establishing a forest department began in 1868 with the deputation of Gustav Mann, an assistant conservator of forest to inspect the forests of Assam. The department remained in an unorganized form till 1873. The province was divided into two divisions-Lower Assam and Upper Assam. The forest of the Guwahati and Goalpara sub-divisions were included in the Lower Assam division while the forests of the Lakhimpur, Sibsgar, and Naga Hills divisions were included in the other. Gustav Mann carried out an extensive survey of the forest tracts of Assam and estimated that approximately 4,565 sq. miles of forests could be brought under reserved forest. Accordingly, Gustav Mann was appointed Assistant Conservator of Forest in 1873 and marking the beginning of the career of a forest department. Immediately after the formation of the chief commissionership in 1874 the forest department was transferred to Assam and was placed under the jurisdiction of the chief commissioner of Assam. In the same year, the chief commissioner sanctioned five forest divisions comprising the Goalpara, Guwahati, Tezpur, Golaghat, and Cachar divisions.

The Golaghat forest division was comprised of the Nambor reserve in the Sibsagar and the Naga Hill districts and the Mikir Hills reserve forest in the eastern part of the Nagaon district in the present Assam state. The forest was managed in this division by an officer under the supervision of the political agent of the Naga Hill district. The Golaghat forest division had altered drastically in 1875. This alteration saw the transferring of some portions of the Nagaon and Sibsagar districts into the Naga Hill district. In addition, the Mikir Hills forest reserve which was formerly part of the Sibsagar district was transferred to the Naga Hill district. Subsequently, the forest conservator Dietrich Brandis had extensively traveled through the various forests in Assam in 1879. His visit gave a new orientation to the matters of forest administration. His suggestions stimulated forest conservation in Assam. This was the first-ever comprehensive report on the part of the forest administration in Assam. His suggestions for the forest administration soon become crucial in matters of policy decisions and classification of forest resources according to the standard forest knowledge. Brandis emphasized in his report that fundamental economic considerations must be kept in mind behind the establishment of the forest administration. It can be argued that forest management has to be understood in the wider context of the evolution of state power and its intervention in the day-to-day life. Sivaramakrishnan (1995) argued that forest management is a form of 'government rationality'. Skaria (1999) argues that penetration of the colonial state apparatus, especially the forest department, deprived the local communities by evolving a plan-based intermediaries' exploitation and at the same time, transformed their identity. This was the main motto for the formulation of forest management by the colonial rulers.

During 1878-1894 witnessed numerous activities in the preservation of forest areas. By 1894, the total area reserved was 9539 sq. km and, in the meantime, the Jhum Regulation of Sylhet was introduced in 1891 for the protection of catchment areas. The forests falling under the division are in the hilly tract and these hilly tracts were subjected to extensive *jhumming* from time immemorial upon the time of the constitution of the reserved forests. However, *jhumming* was allowed to continue for consideration of the prevalent system of livelihood. In the past, good Sal bearing areas were disposed of as wastelands without even charging for the standing crops. The compact block of timberland in the southern part of the Golaghat subdivision was the first area that was constituted into a reserve and designated as Nambor Reserve Forest. This belt of forest land was a virgin area with many valuable species. At that time, Nambor reserved forest extended from near Golaghat, the northern boundary being the Thorajan, southwards for a distance of about 40 miles in a compact block. At that time, the administration of Assam was a part of Bengal province and consequently, Nambor was constituted into a Reserved Forest area in 1872 as per government notification which also appeared in Calcutta Gazette. After the separation of the administration of Assam, renotification of this reserve was done in Assam Gazette to make it legally effective. The scale of fees which was earlier in force was revised with the establishment of an independent forest department.





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It was also identified that a system of selection of felling introduced over the reserves possessing trees of good timber value in 1892 helped in partial control over the felling. The sale of rubber, cane, agar, elephant, etc. was through *mahals* which were sold under the public auction system by the local authority. It is also seen that initial efforts for plantation were undertaken in 1886 to train the *jhumias* (*shifting cultivators*). They introduced the plantation technique of *taungya* system in 1890 which proved futile in absence of a positive response from the shifting cultivators. *Taungya* is a system of forest management in which land is cleared and planted initially for food crops.

Concept of Scientific Forest Management

Generally, a Working Plan is a tool for scientific forest management that was initiated by the colonial government in India. It is very useful for evaluating the status of forest resources of a division, assessing the impact of past management practices, and deciding about suitable management interventions for the future. Working plans were a detailed study of the quantitative data on forests. These written accounts were compiled to provide objective information before the beginning of various activities in the forest. In the silvicultural work the forest working plans played a crucial role. The preparation of the Working Plan is a highly technical and time-framed scheduled operation. Silviculture is the practice of controlling the growth, composition/structure, and quality of forests to meet values and needs, specifically timber production. The name comes from the Latin silvi (forest) and culture (growing). The study of forests and woods is termed silvology. The preparation of the Working Plan is based on stock maps which are prepared through ground surveys. Recently, the use of modern tools like Remote Sensing, Geographical Information System (GIS) and Global Positioning System (GPS) are being done for preparing the forest cover maps of divisions. Every Working Plan includes area-specific scientific prescriptions for the proper management of forests of a particular forest division. Accordingly, the working circles, felling series, and annual coupe area computations are done for the plan period for implementation of the Working Plan prescriptions. The first Working Plan in India was introduced in 1837 by U. V. Munro, the Superintendent of the Travancore Forest. The results of field studies were compiled in tables of measurements and calculations, organizing trees according to different categories and serving as an abacus for foresters in their silvicultural works. It was Dietrich Brandis, a German forester who suggested the preparation of Working Plans, and Schlich who followed it successfully. Brandis introduced liner surveys in the 1870s to estimate the growing stock of trees in the forests which became a basis of subsequent early working plans.

The scientific forest management in India was inaugurated by Dietrich Brandis (1824-1907) a German botanist at Bonn University. He was appointed the first Inspector General of Indian forests in 1864. It was with his help that the Imperial Forest Department was formally inaugurated in the same year. In south India, the department was known as Forest Conservancy Department. His objectives of forest management involved ensuring the protection of forests against natural and human destruction; regeneration of forests and a well-considered system of working. He laid down strict rules for the felling of trees and ensured the supervision of professional foresters at the time of the process of felling. Brandis recommended the establishment of commercial plantations of valuable timber like teak and *sal* when he realized the depletion of these species. As a part of forest management forest surveys were made across India and working plans and *silvicultural* operations were devised and implemented under the guidelines of Brandis. The government appointed competent personnel in the forest department who received training in professional forest management. The high-level administrators in the Indian Forest Service were trained in Britain. The forest personnel subordinate to Forest Service was trained in India especially at Forest Schools at Pune and Dehra Dun.

Scientific Forest Management (1904-1919)

After 1904 the territorial divisions of forest in Mikir Hill were managed under the prescriptions of working plans, which were generally prepared based on principles of sustainable forest resource management and innovative *silvicultural* practices. It is also important to mention here that the identification of the forest division whose normal working plan is to be prepared or revised is done by the Working Plan officers. The manager of the forest area must ensure the preparation of the working plan. While a detailed working plan is prepared for large areas such as forest division, working schemes are prepared for smaller areas for a specific purpose. The following sections discuss the history of working plans for the management of forests in the Mikir Hills district. The East





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Division of forest records mentioned that there were three reserved forests (Kalyani, Mikir Hills, and Nambor) and declared them as the oldest reserved forests in colonial Assam (WPKAEHD, 2006). The Number was the first reserved forest declared in 1872 as per Bengal Forest Rules (vide rule 7 of part II) and a notification appeared in Calcutta Gazette in 1872. Subsequently, after the enactment of Assam Forest Rules in 1876, it was again re-notified in Assam Gazette by rule 8 of Assam Forest Rules of 1876 vide notification No.5 dated.17th October 1878. Subsequently, Mikir Hills and Kalyani reserved forest was constituted on 7th October 1882 with vide notification No. 24, and the Kalyani reserved forest was constituted on 5th August 1887 with vide notification no. 47 respectively. Consequently, new forest areas such as Sildharampur and Jungthung were constituted.

A.R. Dicks was appointed to prepare the first working plan for Nambor Reserve Forest in 1904 (A. R Dick's Working Plan). Though the old record of Nambor and its management is available with the forest department of KAAC little information was available about the management of the Kalyani and Mikir Hills reserved forest. However, A.R. Dicks Working Plan (1904-1919) was the first document for the scientific management of forests for 15 years. It was identified that during the compilation of the Working Plan, there was no enumeration of growing stock carried out and no data was involved. The entire reserved forest was divided into 15 compartments of unequal size varying from 1.8 to 8.4 sq. miles. In the course of the reservation of forest *Nahar* treewas considered the most valuable species. This tree has many medicinal properties. The annual yield was fixed by area and trees of prescribed girth limit were allowed in one compartment earmarked for the year. The rotation for Nahar species was fixed at 90 years. To safeguard against severe exploitation, the plan restricted the felling of the 6' girth tree. Only one tree group of the poor stocked the remaining tree to be retained for a future crop. The reason for such restriction was that out of 15 compartments 7 compartments were already worked out before 1904. Thinning was not prescribed but a subsidiary silvicultural measure was advised. Due to the availability of good stock of natural seedlings, the artificial plantation was not prescribed. For the stocking of Nahar, peripheries of Nahar patches were prescribed. The result of this plan could not be known as there was no control prescribed. However, it is known from the administrative report that the removal of inferior spp. and climber cutting was resorted to.

First Interim Period (1919-1935)

Subsequently, the interim period had been appeared in 1919 due to World War-I. The interim period saw a shortage of officers for compiling the working plan. Since most of the officers were involved in World War-I the revision of the plan could not be affected after the expiry of E.M Coventry's Working Plan (1908-1919). In the pending preparations of the revised plan, some measures were adopted to manage and control the reserves bearing Sal and miscellaneous species separately. These measures were found to extend only to Dhansiri and Kaki Reserves. Under the purchase control system trees of reserved and un-reserved species were worked out and allotted to different contractors from time to time by dividing the reserves into several blocks. This system was in vogue from 1926 to 1932 when Sonaru posts and some first-class timbers were extracted from Lumding and Kaki reserved. From Dhansiri reserve, mostly Bonsum along with Gomari, Gonsoroi, Titasopa, Hollock, Bogipoma, Khokan, etc. were removed. This system was mostly responsible for the removal of almost all the large trees of valuable species particularly Bonsum as the lower girth class was very inadequately represented. Under the shade of shelterwood, some experimental plantations of Bonsum in lines were tried in Dhansiri Reserve during 1929-30 and 1930-31 by planting in about 69 acres. During 1924-25 regular plantation work was started and organized based on regular planting in 1930-31. During 1936-37, an area of 900 acres was planted. Thus, after the expiry of Dick's plan in 1919, there was no proper plan was prepared till 1931 for the reserved forests of the Sibsagar division. But, during this period some spectacularly important projects were initiated. There was a record that in 1905, the plantation of agar (Acquilaria agallocha) was taken up in 50 acres by planting 1900 saplings out of which 700 died in the very first year. The fate of this plantation was not known but again in 1920, 20 acres were planted. Soon after, an area of 4.5 acres was taken up for plantation in 1921.

Scientific Forest Management (1936-1945)

During this period one of the important developments in the management of forest resources was the establishment of the Mohanlal Working Plan (1936-1945). This Mohanlal's working scheme was the first scheme that extended to all





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the 24 reserve forests of the Nagaon division amongst which the reserve forests subsequently transferred to Mikir Hills West Division were included. The main objective of Mohanlal's working scheme was the conservation of trees of higher girth classes which are deficient in numbers and the improvement of existing stock with the realization of yield as *silvicultural* was available and replacement of the stock removed by compensatory planting in convention centers. The *silvicultural* marking for the benefit of the existing *Bonsum* trees, particularly those stands which are below exploitable girth and poles and saplings aiming at the sufficiency of exploitable size trees of this species for future working and removal of dead and wind fallen and badly top broken species of commercial value. The area under the plantation increased considerably during the period. During eight years a total area of 2502 acres of plantations was created, the annual average being 313 acres. On average annual yield of timber during the period was 473000 cft. Thus, when they are brought under artificial regeneration compared with the average annual yield removed, it is found to be much above the quota prescribed as a minimum in replacement of the yield removed.

To induce the traders to come to work in the poor type of miscellaneous forests, a new system was introduced in 1937-1938 in which blocks were leased out for 3 years on a monopoly basis reducing the royalty on inferior species and imposing surcharge on species. Like *Bonsum* and *Bola* which were in great demand but limited availability. According to the administrative report of 193940, the underplanting of *Hollock* and *Poma* plantation with *Bonsum* and *Amari* was tried and the plantations were doing well, but no subsequent record was made available. The establishment of natural regeneration of miscellaneous valuable species was started at Dhansiri reserve forest on an experimental basis in 1942-1943 (WPKAWD, 2005). It was also identified those ten years plantation scheme was drawn up by J.B Rowntree, D.C.F. and C.S Purkayastha, D.C.F. during 1937-1947. As per the scheme, it was allowed to take up the regeneration of 57 acres distributed over 12 centers annually in a convenient place. But it is seen from the plan of P.N. Bhattacharjee that was a shortfall of 191 acres regeneration during the 10 years ending in 1945-1946 after scrutinizing the relevant records. It is seen further that though there was provision for raising 1 acre of plantation for removal of outturn for every 2000 cft of commercial timbers in Mohanlal's scheme and through 2321 thousand cft of timbers in the round was removed within the 10 years of the scheme, the plantation raised was 809 acres against the extent of 1161 acres which ought to have been raised corresponding to the removal during the period.

The scheme was drawn up on comprehensive silvicultural principles of conservation and improvement. This has also got the advantage of the improved mark and thus helped in disposing of the yield coming out from the improvement feeling. But the scheme did not achieve the result aimed as it did not set up the marking rules precisely based on which the yield to be removed. The scheme aimed to conserve the stock of Bonsum for future yield and through the prescribed principle was to subordinate revenue consideration to those of silviculture, yet the actual position was found to be different as remarked by P.N. Bhattacharjee. On analysis of Bhattacharjee, it is pointed out that despite restrictions imposed on felling in the scheme Mohanlal's heavy depletion took place since the inception of the scheme and at the expiry of the scheme. He opined that windfalls and mortality may be partly responsible for this state of affairs but there must have been over-felling probably due to growing demand and oversupply under the Conservator's order. This silvicultural consideration was set aside, which he considered not possible to scrutinize in absence of records of felling. The Working Plan of Mohanlal has classified the reserve forest of the Sibsagar division as Bonsum Zone to distinguish it from the Sal Forest of Nagaon division. In the Bonsum areas above, 7 reserve forests were examined, and accordingly, four Working Circle has been formed. The annual yield was fixed by area only and the felling cycle of 20 years was fixed. A girth limit of 7' 6' was fixed for Bonsum and other valuable species. The results of Mohanlal's Scheme could not be found out but no major efforts were taken for artificial regeneration due to World War II.

The reserve forest of the Sibsagar division namely Nambor (south block), Nambor (west), Rengma, Doyang, Intaki, Diphu, and Rongapahar was covered by this plan. But, after the constitution of the Dhansiri Valley Division in 1949 the reserved forest except Doyang were transferred to Dhansiri Valley Division with its headquarters in Dimapur of the present Nagaland. During the years 1962-63, Dhansiri Valley Division was re-nomenclature as United Mikir and North Cachar Hills division, and Langting Mupa, Dhansiri RF, Disama, and Chelabor RF were entrusted to this





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division. Subsequently, after the creation of Meghalaya State in 1970, again the administrative control of Nambor (south), Nambor (west), and Rengma reserved forests were transferred to the Sibsagar division and Mikir Hills reserved forest and Kalyani reserved forest was given to Mikir Hills division with headquarter at Diphu and the KAAC authorities claimed absolute authority.

Second Interim Period (1946-1950)

There was no detailed plan was prepared during this period due to the break out of World War II. During the latter part of this interim period, new plantation centers were taken up in the Nambor reserve forest. After the expiry of Mohanlal's Scheme in 1945, an ambitious scheme for creating large-scale plantations of 138 acres of felled areas was taken up in 1946. But it could not be continued due to a shortage of labor and inadequacy of supervisory staff and ultimately it was reduced to 20 acres in the subsequent years to fill up the blank areas of the previous year's creation by refilling technique. From the above discussion, it can be argued that due to the intervention of colonialism in Mikir Hills the state authority has evolved various measures which tried to control the people's access to forest resources with the cooperation of forest officials who were appointed by them for the preparation of various working plans for the management of the forest. The colonial government, post-independent Assam state, and the Karbi Anglong Autonomous Council from its inception (1952) formulated and authorized to implement of various forest and land policies. Some of the important acts and regulations passed by the Council are as follows: the Assam Land and Revenue Regulation, 1886, the Assam Forest Regulations, 1891 (Regulation No. VII of 1891), the Mikir Hills (Land and Revenue) Act, 1953, the Mikir Hills District (Jhumming) Regulation Act, 1954, the Mikir Hills District (Forest) Acts, 1957, etc. All these acts were empowered to restrict the people to access forest resources and jhum cultivation in the present Karbi Anglong district. However, there are many amendments to these acts but all these acts failed to protect the traditional rights of the people over the natural resources. The colonial system of scientific forest management continued even after 1947 with little modifications, emphasizing revenue generational and commercial exploitation while its policy orientation excluded tribes who had the most longstanding claim on forest resources.

CONCLUSION

As mentioned, the scientific idea of forest management provides a rich source of information on forests including the species of trees, height and diameter, and the technical aspects of the forest policy that playedan important role in the formulation of the colonial forest policy. The working plans themselves were declared the most important job of the forest officers as the forest department quickly recognized them as the key to professional continuity in forest management. This paper observes that Indian foresters were appointed by the colonial state the preparation of various working plans for the small forest regions like Mikir Hills under the state authority of the respective major forest divisions. Many of these foresters were not from the forest regions but rather came from outside of the region having a colonial mindset as they were highly trained by the colonial officials. As a result, there were many loopholes in the formulation of scientific forest management due to a lack of knowledge of the policymakers, and non-familiarity with the social customs and traditions of the people. According to them, jhum cultivation is the most destructive form of cultivation in terms of erosion, forest degradation, low productivity, and enhancing flood situations that ultimately led to environmental imbalances. It is argued for total state control over all forest resources and all forest land belonged to the state with the ideas of scientific forestry. Such ideas defended the colonial intervention in the forest areas. They claimed that the so-called norms of community and access to forests were dependent on the 'sweet will of the ruler'. The idea of colonial control over forests was initially prompted more by commercial rather than conservation needs. This can be argued that the introduction of the concept of scientific forestry was a 'colonial watershed' that resulted in the commercialization of forest use and brought about unprecedented destruction in forested areas. There is no exception to Mikir Hills too.





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RESEARCH ARTICLE

Green Synthesis, Characterization and Antibacterial Activity of Aqueous **Betel Leaf Extract Assisted Silver Nanoparticles**

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ABSTRACT

Plant based synthesis of nanoparticles has gained lot of importance because it is cost effective and ecofriendly. The prime goal of this article is to focus green synthesis of betel leaf extract assisted silver nanoparticles. In this study, silver nanoparticles were synthesized from aqueous extracts of betel leaves (BL) and the biological activities evaluated. The silver nanoparticles (AgNPs) were green synthesized using aqueous BL extract and silver nitrate. The AgNPs were characterized using UV-visible spectroscopy (UV-Vis), Fourier transform infrared (FT-IR) spectroscopy, Powder X-ray diffraction (PXRD), Scanning electron microscopy (SEM) and Energy dispersive X-ray spectroscopy (EDX). The FT-IR spectra of AgNPs showed characteristic groups pertaining to active molecules of extract indicating their surface functionalization. The XRD pattern of AgNPs revealed diffraction peaks corresponding to the planes of face-centered cubic (FCC) crystalline structure. The particle sizes are in the range from 15 -The SEM images indicate that the particles are spherical in shape. The nanoparticles demonstrated substantial antimicrobial activity against Gram +ve and Gram -ve harmful bacteria species viz. Staphylococcus aureus (S. aureus) and Escherichia coli (E. coli). The current study shows that aqueous BL extract can be used as an effective reducing and capping agents for the green synthesis of silver nanoparticles (AgNPs).

Keywords: Green synthesis, Silver nanoparticles, Aqueous betel leaf extract, Powder XRD, SEM-EDX, and Antibacterial activity





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INTRODUCTION

Since the last decade, the exploration of new medicinal properties of various plant species has evoked the attention of the scientists towards the biologically active compounds. The reason behind this is that the bioactive compounds possess potent pharmacological activities and have low or no toxicity. This emerged interest in plant-derived medicines is mainly due to the resistance caused by indiscriminate use of synthetic medicines as well as the on-going perception that green medicines are safer than the synthetic drugs having severe adverse effects. Previous studies [1] revealed that many herbs possess varying degree of antimicrobial activities. Therefore, the natural medicinal plants may be a potent source of new antibacterial agents. Piper betel is a popular medicinal plant in Asia. Plant leaves have been used as a traditional medicine to treat various health conditions. It is highly abundant and inexpensive, therefore promoting further research and industrial development, including in the food and pharmaceutical industries. Piper betel is an indigenous climber plant of India. Although cultivation of betel Piper betel can be found in many tropical regions, the use of the betel leaf (BL) originated in India. It is also used in a few Asian countries as well, such as Malaysia. The leaves of Piper betel (locally known as Paan) have long been in use in the Indian local system of medicine for its antioxidant and antimicrobial properties. Betel leaves are also used in ayurvedic medicine to cure a variety of problems, and this branch of medicine also originated in India.

In India, betel leaves are consumed after the meal. People used to chew betel leaves to get the health benefits of it. It is also believed that betel leaf has an important role to detoxicate blood and providing antioxidant nutrients. The paste of betel leaves has been used as face wash. Betel leaves are known to prevent allergies and also heal wounds and also benefits digestive system. Betel leaf is widely used as a mouth freshener in India so it comes as no surprise that it improves oral health. Betel leaf (Fig.1) can help reduce the growth of bacteria in your mouth, preventing a wide range of oral infections and diseases. Betel leaves contain a variety of biologically active components (Fig. 2) like hydroxychavicol, chavicol, piperbetol, chavibetol, piperol A, methylpiperbetol, and piperol. Ethanol Extract of Betel Leaf (Piper betle L) was tested by Mahfuzul Hoque et al [2] against some food borne pathogens. Antimicrobial activity of ethanol extract of Piper betel leaves was evaluated by Singh et al [3] against human pathogenic bacteria. Silver nanoparticles have been a potent antibacterial, antifungal, anti-viral and anti-inflammatory agent. Silver nanoparticles are in high demand due to their widespread use. Among noble-metal nanomaterials silver nanoparticles have received considerable attention due to their attractive physico-chemical properties. The surface plasmon resonance and large effective scattering cross section of individual silver nanoparticles make them ideal candidates for potential biological labeling[4]. Ionic silver is highly toxic to most bacterial cells and has long been used as a potent bactericidal agent. Silver nanoparticles are used as antibacterial agent because of their high reactivity that is due to the large surface to volume ratio.

Antibacterial activity of the silver-containing materials can be used, for example, in medicine to reduce infections as well as to prevent bacteria colonization on prostheses [5], catheters [6, 7], vascular grafts[8], dental materials[9], stainless steel materials and human skin. In the present investigation, simple, eco-friendly, inexpensive biosynthetic methods were employed to synthesize silver nanoparticles using betel leaf extract. The present work gained importance as there is no report in the literature on the aqueous betel leaf (BL) extracts assisted synthesis of silver nanoparticles, their characterization and antibacterial activity. The main goal of this article is to focus green synthesis of silver nanoparticles using betel leaf extract that contain a variety of biologically active components (Fig.2). In this work, green synthesis of novel silver nanoparticles having low particle size (15 - 29 nm) may be considered as new contribution to the literature. In this article, green synthesis of new silver nanoparticles and their characterization using UV–visible (UV–Vis) spectroscopy, Fourier transform infrared (FT-IR) spectroscopy, Powder X-ray diffraction (PXRD), Scanning electron microscope (SEM) and Energy dispersive X-ray spectroscopy (EDX) and their antibacterial activity are presented.





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MATERIALS AND METHODS

Preparation of Betel Leaf (BL)aqueous extract

Betel leaves were collected from local market at Anantapuramu city in Andhra Pradesh state, India. They were first washed with tap water and dried at room temperature. About 10 g of Betel leaves were taken in a clean 250 mL beaker containing 50 mL of deionized water. They were boiled up to a temperature of 80 °C for about 1 hour and cooled to room temperature then filtered into a 50-mL standard flask and diluted to volume with distilled water. The extract was kept in refrigerator at 5° C when not in use.

Synthesis of silver nanoparticles (AgNPs)

Silver nitrate (1.73g; Merck) was taken in a clean 100 mL and dissolved in 20 mL of water. To this, a 25 mL of betel leaf aqueous extract was added and heated on water bath for 1 hour. During the formation of silver nanoparticles, the colourless silver nitrate solution changes its colour to brown and then to black colour. After cooling to room temperature, the material was collected by filtration, washed with hot water and then dried in oven at 100°C for 30 minutes. The percentage yield of AgNPs is about 65%.

Characterization of AgNPs

The silver nanoparticles were studied by using SL-210 Multipurpose UV-Visible spectrophotometer in the wavelength range, 190-1100 nm. All measurements were carried at room temperature; the graphs were plotted between absorbance and wavelength. FT-IR analysis was carried out by Perkin Elmer Spectrum 100 instrument in KBr discs to determine the properties of synthesized silver nanoparticles. The FT-IR spectra were recorded in the wave number region, $4000 - 400 \, \text{cm}^{-1}$. The silver nanoparticles were analyzed by using X-ray diffractometer (Japan Smart Lab SE) in the scan range from $5 - 100^{\circ} \, 20 \, \text{with}$ a scan speed of 10 degree per minute. The instrument was operated at 40kV voltage and 30mA current. The scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDX) analysis were carried using JEOL at a voltage of 20kV by placing AgNPS on the carbon tape. The magnification and size details were indicated on the SEM image.

In vitro antibacterial activity of green synthesized AgNPs

The silver nanoparticles were screened for antimicrobial activity by taking two bacterial species viz. *Staphylococcus aureus* (S. aureus, Gram-positive bacteria) and Escherichia coli (E. coli, Gram-negative bacteria) by agar disc diffusion method. A 20mL of nutrient agar medium was poured into sterilized petri plates. Cultured bacteria and fungi were spread on petri plates. The sterilized 6 mm disks were soaked in different concentration of nanoparticles in DMSO (100, 200, 300 & 500 μ g/mL) and dried in oven and those were placed in petri plates. Ciprofloxacin was taken as Positive control for bacterial species, and 10% DMSO was taken as negative control (blank). The Petri plates were incubated at 37 °C for 24 hours and their zones of inhibition were measured and expressed in mm.

RESULTS AND DISCUSSION

Synthesis of silver nanoparticles

The addition aqueous extract of BL to AgNO₃ solution, resulted in the formation of AgNPs@BL as observed by colour change of silver nitrate solution from colourless to brown and then to black due to the reduction of silver metal ions (Ag·) into silver nanoparticles (Fig. 3). Fig. 3. The images of reactants and reaction mixture during the formation of AgNPs: 1- Betel Leaf (BL) extract 2- AgNO₃ Solution; 3- Reaction mixture consisting of 1 & 2 after heating to 80°C for 30 minutes and under hot condition; Colour change was noticed from colour less to brown and to black; 4- Image of 3 after cooling to room temperature to give black coloured AgNPs at the bottom.

UV-Vis spectral analysis

The UV-Visible spectrum of the synthesized silver nanoparticles dispersed in methanol showed a strong SPR peak (Fig. 4) at 405 nm indicating the isotropic shapes silver nanoparticles. It was known that the position and shape of





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plasmon absorption of nanoparticle strongly dependent on particle size and surface adsorbed species according to Mie's hypothesis[10].

FT-IR spectral studies

Absorption bands at 3548, 1795, 1521, and 1261 cm $^{-1}$ in the IR spectrum of AgNPs@BL are respectively assigned to voh, vole0, vole0 and vole0 vibrations respectively, corresponding to the functional groups of ingredients (hydroxychavicol, piperbetol, chavibetol, piperol A, etc) of betel leaf functionalized on the surface of silver nanoparticles.

Powder X-ray diffraction (PXRD) analysis

The powder X-ray diffractogram of silver nanoparticles was recorded between 20 values, 20-100°. The PXRD pattern of AgNPs is shown in Figure 5. Careful examination indicates that the peaks with reference to silver nanoparticles (Standard JCPDS-04-0783) were observed with high intensities and along with some other peaks are observed and these match with silver oxide nanoparticles (Standard JCPDS-76-1393) with small intensities. The peaks were corresponded to h k I indexing (1 1 1), (1 1 1), (2 0 0), (2 2 0), (3 1 1) and (2 2 2). The results suggested that the silver nanoparticles (AgNPs and Ag₂ONPs) are in face centred cubic(FCC) phase. The average particle size of metal nanoparticles were calculated by Debye-Scherrer equation,

$$D = \frac{0.9 \,\lambda}{\beta \, Cos\theta} \qquad \dots \tag{1}$$

Where

 λ = x-ray wavelength (0.1541nm); β = Full width half maximum (rad); θ = diffraction angle.

On substituting the values in Eq. (1) and on calculation, the grain sizee (Table 1) of Ag_2ONPs and AgNPs are found in the range, 21.96-25.65nm and 15.24-29.12 nm respectively.

Scanning electron microscopy and energy dispersive X-ray spectrometry

SEM and EDX experiments indicated the formation of silver nanoparticles. The SEM micrographs (Fig. 6) showed spherical shaped and agglomerated silver nanoparticles. The EDX image of AgNPs is shown in Figure 7. Data indicated binding of some bioactive compounds in betel leaves extract on the surface of silver nanoparticles. The quantitative analysis using EDX showed high silver content as shown in Table 2.

Antibacterial studies

The antibacterial activity of silver nanoparticles were tested against Gram +ve S. aureus, Gram -ve E. coli using Agar disc diffusion method. The bacteria isolates were sub cultured on nutrient agar plates and incubated at 37 °C for 24 h. After incubation, the culture was diluted with fresh media and was added on to the plate and spread into agar lawn using a sterile glass spreader. In this medium, the silver nanoparticles showed significant antimicrobial activities with increase in concentration of nanoparticles. In the present case, AqNPs are more significant to S. aureus and E. coli bacteria. Figure 8 shows the images of inhibition zones by silver nanoparticles against the microbes and the detailed zone of inhibitions was presented in Table 3. (A) for E. coli and (B) for S. aureus by AgNPs S - standard (Ciprofloxacin); B - Blank; Nanoparticle concentration 1 - $100 \mu g$ /mL; 2 - $250 \mu g$ /mL; 3- $500 \mu g$ /mL. The antibacterial mechanism action of MNPs has been given in recent literature[11-15]. The mechanism suggested that the metal ion (Cu²⁺) was released in situ from the nanoparticles and then binds to bacterial cell wall which causes denaturation of protein coat and finally death of the cells[11]. Electro- static attraction between Cu²⁺ and plasma membrane and also membrane based reductases lead to reduction some part of them to Cu+ [11-15]. The Cu+ ions are energetically easier to move across a lipid bilayer and taken up by the cell, generating reactive oxygen species, leading to lipid peroxidation and protein oxidation Haung et al [12] suggested that the copper nanoparticles will bound to cell wall of E. coli and penetrate in to bacteria leading to the destruction of cell membrane. Some similar types of mechanisms were proposed by Line et al [13], Azam et al [14], Nawaz et al [15] for copper nanoparticles.





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SUMMARY AND CONCLUSIONS

The present study reports on the green synthesis of silver nanoparticles using betel leaf (BL) extract. The present biogenic method here is nontoxic, environmentally friendly, simple, and low cost and has no toxic chemicals. The results confirmed that the extract played an important role in the reduction and stabilization of silver nanoparticles. The UV-Visible absorption spectrum indicated the isotropic nature of nanoparticles. The powder XRD studies revealed that the nanoparticles were in face centered cubic(FCC) phase. The bio-produced AgNPs were characterized using SEM and EDX. The SEM images revealed that the particles are spherical in morphology and sizes were found in the range 67 - 128 nm. The green synthesized AgNPs screened for their antibacterial activity. The AgNPs may be useful in a wide variety of applications in pharmaceutical, biomedical fields, industrial appliances like bandage, food, and water storage. The study explains successfully the use of betel leaf extract as an effective reducing and capping agent for the green synthesis of AgNPs as well as their substantial antibacterial activity.

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Table 1: Peak position and grain size data of Silver nanoparticles

Tubio III ouit	able 1.1 car position and grain size data of onver handparticles							
	Peak position and grain size (nm)data of silver nanoparticles							
Peak position, 2θ								
JCPDS-76-	S-76- Ag ₂ ONPs hkl JCPDS-04-0783 AgNPs@ hk				hkl	Size of	7	
1393	@BL			BL		Ag ₂ ONPs@BL	AgNPs@BL	
27.94	27.91	110	38.11	38.19	110	23.44	25.92	
32.27	32.32	111	44.27	44.53	200	25.65	15.24	
46.34	46.38	211	64.42	64.55	220	24.67	29.12	
54.92	54.87	220	77.47	77.46	311	21.96	25.33	
67.48			81.53	81.53	222		19.50	

Table 2: Elemental composition of silver nanoparticles as revealed by EDX

Element	Line	Mass%	Atom%
С	K	9.50±0.14	34.38±0.51
0	K	9.87±0.40	26.82±1.09
Al	K	0.73±0.07	1.17±0.12
Cl	K	6.60±0.13	8.09±0.16
Ag	L	73.31±0.62	29.54±0.25
Total		100.00	100.00
Spc_002			Fitting ratio 0.0592

Table 3. Zone inhibition data showing Antimicrobial activity of Silver nanoparticles against pathogens

Samples	Treatment (μg/mL)	S aureus	E coli
Standard	100	20	27
Ciprofloxacin	100	30	21
AgNPs	100	14	7
	250	20	14
	500	24	16



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Fig.1: Image of Betel Leaves

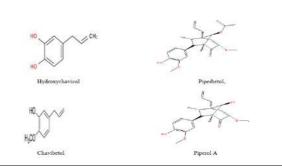


Fig. 2: Chemical structures of some biologically active ingredients of betel leaves



Fig. 3. The images of reactants and reaction mixture during the formation of AgNPs

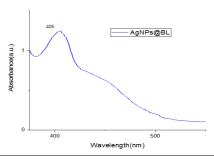


Fig. 4: UV-Visible spectrum of silver nanoparticles

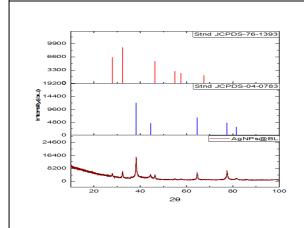


Fig. 5: PXRD pattern of standard JCPDS-04-0783 and AgNPs@BL

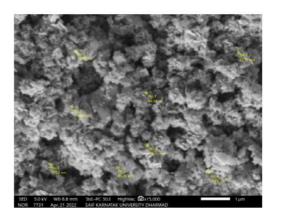


Fig. 6: SEM image of silver nanoparticles.



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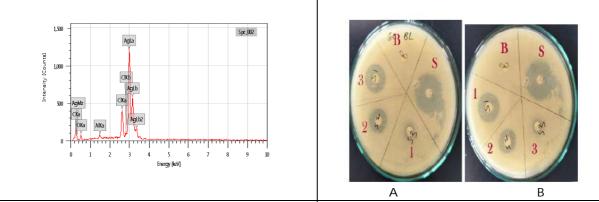


Fig. 7: EDX image of AgNPs

Fig. 8: Photographs showing inhibition zones in Petri dishes. (A) for *E. coli* and (B) for *S. aureus* by AgNPs S - standard (Ciprofloxacin); B - Blank; Nanoparticle concentration 1 - 100 μ g /mL; 2 - 250 μ g /mL; 3- 500 μ g /mL

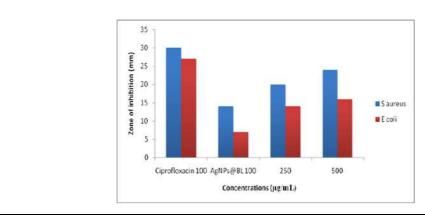


Fig. 9: Histogram showing Antimicrobial activity of Silver nanoparticles at different concentrations against pathogens based on zone of inhibitions.





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RESEARCH ARTICLE

Effect of Myofascial Release Technique Versus Instrument-Assisted Soft Tissue Mobilization on Pain in Cricket Players with Acute Hamstring Strain

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ABSTRACT

Cricket is one of the most popular games among men and women. It demands a high level of physical fitness and technical skills. Hamstring muscle injury is the third most common and the highest frequency injury after knee and ankle injuries in cricket. Hamstring muscle strain is due to eccentric contraction of the muscles. Myofascial release technique and instrument-assisted soft tissue mobilization techniques are sophisticated form of massage therapy which helps to reduce pain and improve function associated with soft tissue injuries. Institutional ethics approval was taken, and 30 participants were included in the study based on inclusion and exclusion criteria. Players were divided into two groups, Group A: Myofascial release group and Group B: Instrument-assisted soft tissue mobilization group. Pre-postintervention pain evaluation was measured via the use of a Numeric pain rating scale (NPRS). Withingroup analysis of NPRS in both the groups shows a statistically significant reduction in pain (p<0.05), between-group analysis shows group A: 1.5333 ± 0.9904 and for group B: 1.2000 ± 0.941, the significant value was 0.290 which is more than 0.05, thus between group analysis was not significant. Effect size estimation was done using Cohen's d which was 0.2341 which suggested a small effect size. Present study results conclude that the myofascial release technique is more effective to relieve pain compare to the instrument-assisted soft tissue mobilization technique.

Keywords: Myofascial release, Instrument-assisted soft tissue mobilization, hamstring strain, cricket injuries, massage therapy





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INTRODUCTION

Cricket is one of the most popular games in India played by men and women of all age groups. Cricket is a game of endurance as well as strength, which demands a high level of physical fitness and technical skills like upper extremity power, and hand grip strength (bat grip and ball grip) with coordinated movements of the shoulder, arms and wrists for overall performance[1]. Cricket is a multi-day game where both teams have 2 innings. 6 bowling deliveries per over, the bowlers must keep bowling until they have dismissed the opposite team. This leads to potentially high workloads in the bowling unit[2]. The increased physical demands on the players may be associated with an increased risk of injuries[3]. The incidence and nature of cricket injuries during a season have been reported in well-conducted studies by British sports counsel, the Australian cricket board and the South African cricket club.[4]the most common types of injuries are fractures, dislocations and contusions sustained during batting, fielding and catching, especially finger injuries (20.5%)[4, 5, 6, 7, 8]. The seasonal incidence of injuries in bowlers (42%) and fielders (40.9%) was higher than in batsmen (17.1%). Young players (<26.2 years) sustained more injuries than older players. The upper limb (34.1%) and lower limb (37.5%) the most common site of injuries were reported in many studies[9]. Hamstring muscle injuries are the third most common and the highest frequency injuries after the knee and ankle injuries in cricket[10,11]. The incidence of hamstring muscle injury is gradually increased since 2006[2]. Hamstring muscle strain involves sports activities such as sprinting, running, jumping and sudden weight shifting. Multiple factors are responsible for the occurrence of muscle strain such as muscle weakness, lack of flexibility, fatigue, inadequate warm-up and poor lumbar posture[12]. Hamstring muscle strain is due to eccentric contraction of the muscle where lengthening of the muscle occurs towards gravity, this kind of contraction causes damage to the muscle fibres[13, 14]. Multiple studies suggested that microscopic analysis of the eccentric exercises revealed non-uniform lengthening of sarcomeres in damaged muscles. Which affects the length-tension relationship[13].

EMG studies have found that the hamstring muscle is activated during the mid-swing phase to the terminal stance phase. The peak activation of the hamstring muscle occurs during the terminal swing. Furthermore, various studies also show hip extension component with knee flexion moment during the terminal swing which implies that during that phase hamstring muscle getsengaged[14,15]. Therefore, damage to the hamstring muscle during running or sprinting is more common than in walking. The myofascial release technique is an application of a minimal load, sustained stretching technique over a myofascial component. This technique is used to restore the length of the muscle, reduce pain and improve the functions of the tissue[16]. The application of the technique is direct or indirect. The direct technique works directly on the restricted fascia of the body. Elbow or knuckles or other specialized tools are used to slowly apply over the fascia and pressure is applied over the same fascia. The indirect technique uses gentle stretch and application of the pressure, hands follow the direction of the restriction and hold the stretch position[17]. Instrument-assisted soft tissue mobilization (IASTM) technique is used to reduce practisers' effort and maximize the delivery force to the tissue. This technique is using a variety of instruments to address musculoskeletal pathology and related impairments of the tissue and help the healing process of soft tissues[18]. Application of stimulus by specialized instrument over the injured soft tissue will activate localized inflammatory reaction which enhances several fibroblast activities. Local response leads to synthesis and realignment of collagen fibres[19]. Various studies have reported that IASTM can alleviate pain and improve functions of the soft tissues and also help to improve the flexibility of the local tissues[20]. The purpose of the study was to evaluate and compare the effect of the myofascial release technique and instrument-assisted soft tissue mobilization technique on pain in cricket players with an acute hamstring strain.

METHODOLOGY

An experimental study was conducted at the Sun Sports academy, Ahmedabad from January 2019 to March 2019 (Written consent was taken from the academy). Ethical clearance was taken from the Institutional Ethical Committee. Out of 34 cricket players, 30 players were included in the study based on inclusion criteria by convenience method.





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Written informed consent was taken from all players. Players of both gender with age groups 15-30 years, more than 1 year of playing experience (weekly 5 days), an acute hamstring muscle strain diagnosed by a clinical examination, site and type of pain and positive clinical tests (bent-knee stretch and modified bent knee stretch test) were included in the study. Players with any neurological symptoms such as tingling, numbness, disk prolapse, players with fever and fracture, or players playing cricket weekly 2 to 3 times were excluded[10,11,13]. Baseline Measurement before the treatment was conducted on for of pain intensity by a Numeric pain rating scale (NPRS)[21].

Group A: Myofascial Release Technique (MFR) (15 players in the group)

Participants were in a prone lying position. The hip and knee of the players remained straight and bend. The therapist stands on the affected side and places one hand over the gluteal fold area and the other hand placed near the first hand. Sliding from proximal to distal part (Hip to Knee) was performed by the therapist. While performing sliding movement slight pressure was applied over the hamstring and held it for at least a minute for 5 repetition[22].

Group B: Instrument-Assisted Soft Tissue Mobilization Technique (IASTM) (15 players in the group)

Participants were in a prone lying position. The moisturizer was applied before proceeding for manoeuvre, which worked as a medium for allowing a smooth and friction-free movement. Light pressure in the transverse direction was applied from proximal to distal over the hamstring muscle by edge tool for 1 minute for 3 repetitions[23]. The treatment was provided for 4 days a week for the 2 weeks of time duration, hence a total of 8 sessions were provided to all players. Players continue with their routine fitness protocol.

Data Analysis

Data were entered and analysed through Microsoft excel 2016 and SPSS version 26.Before the statistical tests data was screened for normal distribution. As the total sample size was 30, normality was checked using the Shapiro-Wilk test. Pre- and post-all outcomes were normally distributed. Analysis was done within each group and between the two groups using outcome measures taken before and after intervention. The level of significance was kept at 5% with a confidence interval (CI) of 95%. Paired t-test was applied for comparison of pre and post for all outcome measures as the data was normally distributed. The effect size was calculated to know the clinical effectiveness of two interventions and was done using Cohen's d method for all the outcome measures.

RESULTS

Table 1: In the present study, the mean age of participants undergoing the myofascial release technique was 25.2667 ± 2.0517 years and 24.8000 ± 1.6987 for instrument-assisted soft tissue mobilization. Table 2: There was no significant difference between pre-intervention NPRS measures in both the groups, hence the groups were comparable with each other. Table 3: Within-group comparison of NPRS, before and after the intervention was carried out by paired t-test, there was a statistically significant difference in the within-group analysis of NPRS in Group A and B. Table 4: Between-group comparisons for change in NPRS post-intervention suggested no statistically significant difference between group comparisons of NPRS. Effect size estimation was done using Cohen's d which was 0.2341 which suggested a small effect size. As Cohen's d value suggests only effect size and does not conclude which group has maximum effect clinically, hence mean difference of both the groups was compared and the group with the highest mean difference suggested the highest effect on the outcome measures clinically. Out of both the groups, the mean difference of group A (1.5333) is the more which suggests the more effect on function among all.

DISCUSSION

The present study was designed to find out the effectiveness of the myofascial release technique and instrument-assisted soft tissue mobilization technique on pain in acute hamstring strain in Cricket players. Baseline data comparison was made for the two groups for the age, gender and outcome measures. The groups were found to be





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similar in all aspects which suggested that the differences in the outcome measure in all groups after the interventions can be attributed solely to the given intervention to the particular group. The results from this study showed statistically significant improvements in terms of the Numeric pain rating scale (NPRS) post-intervention of the 8th session. Tissue healing occurs in three different stages. Stage I is a stage of degeneration or inflammation: this stage includes hematoma formation and initiation of inflammatory reactions. It will take 3 to 4 days. Stage II is a stage of Regeneration: the phagocytosis process takes place at the level of damaged tissue which is followed by the regeneration of muscle fibres. It will take 3 to 4 weeks. Stage III is a stage of Remodelling regenerated muscle fibres become mature and enhance muscle capacity. It will take 5 to 7 weeks[31]. According to Schleip,[32] fascia and connective tissues tend to move with minimal restrictions. Trauma, repetitive strain, and inflammation alter fascia and tissue length and elasticity which causes restriction. Schleip claims that compromising the sliding system of fascia between its layers causes by movement dysfunction which may be induced by alteration in connective tissue.

A study conducted by Meltzer et al[33]. Suggested that the myofascial release technique after repetitive strain injury resulted in normalization of cell breakdown rate, alteration in cell morphology, and fibroblast orientation. Myofascial release technique in hamstring strain players enhances the healing process. As with any massage technique, the analgesic effect can be attributed to the stimulation of afferent pathways and the excitation of afferent delta fibres causes pain modulation via descending pain suppression system or the pain gate theory[34]. Therefore, normalization in a length due to myofascial stretch helps to regain tissue properties which reduce pain. The dense connective tissue is highly innervated by autonomic fibres thus the re-establishment of the sliding system of the fascia's layers may inhibit nociception[32]. Instrument assisted soft tissue mobilization technique uses a special type of instrument which provide gradual mechanical pressure over the connective tissues, continuous action of an onand-off application of pressure during the loading and unloading phase creates tissue creeping and hysteresis which alters the viscoelastic nature of tissue[18]. The neurological phenomena of autogenic inhibition may relax the stretched muscle, which leads to a reduction in passive hamstring stiffness[19]. Based on pain gate theory, the instrument-assisted soft tissue mobilization technique worked on facilitating inhibitory interneurons in the dorsal horn of the spinal cord which reduces the activity of A-delta and C pain fibres. This continuous inhibitory stimulation of the tissue results in an increase in pain threshold[32, 34]. Result of the present study shows that within-group analysis of myofascial release technique and instrument-assisted soft tissue mobilization techniques shows a statistically significant improvement in pain (p<0.05) post-intervention. E Silva, D. C. C. M.[35] analysed the immediate effect of myofascial release after total knee replacement, the author looked for the immediate effect of the myofascial technique on hamstring and quadriceps muscles. The study concluded that the myofascial release technique enhances muscle activation, simultaneously helping for the reduction of pain and improving knee range of motion.

These findings are consistent with the present study on the possible mechanism for improvement in pain explained by Le-Bars et al.,[34] and Meltzer et al.,[33] Joshi D.G. et al.,[25] found that the myofascial release technique helps to reduce pain and improve the flexibility of the hamstring muscles this finding are similar with the present study the possible mechanism for that is given by Schleip et al.,[32]Zaghloul, H. M. S. et al.,[26] found a similar result for improvement in pain, fast recovery and healing rate in groin strain. The result of the present study was not showing A statistically significant effect between group analysis of myofascial release technique and instrument-assisted soft tissue mobilization techniques on improvement in pain (p>0.05) post-intervention. Davidson, C. J.et al.[19]. studied the effect of massage technique on marathon runners, the study concluded that there was no effect of massage therapy on pain. In the study, measurements were taken from the different durations such as post-intervention days 1, 4, 8, and 11. Thus probably the mechanism for not gaining improvement is continuous stress over the muscle for long durations of the marathon. Mendes, A. C ,et al.,[22] found that there was more effect of neural tissue mobilization technique compared to myofascial technique because the acute effect of static stretch reduces the activity of contractile tissues, while the dynamic movement of the nerve and muscles can help to restore the properties of the muscles and nerve. The present work findings suggest that facial release has a good impact on cricket players with an acute hamstring strain. Both groups show improvement in pain individually. However, the comparison of both technique they are not statistically significant because both techniques have different methods of





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application. Long-term follow-up was not taken, players continue with their regular fitness protocol and sports activities and were not provided with any other exercises or therapy which were the limitations of the study. However, the study can be performed with a non-athletic population, self-massage by a tennis ball or form roller can be used, and more exercises protocol can add and check for the combined effect.

CONCLUSION

Present study results conclude that independently the myofascial release technique and instrument-assisted soft tissue mobilization technique help to the reduction of pain in Cricket players with an acute hamstring strain. The myofascial release technique is clinically more effective to relieve pain compare to the instrument-assisted soft tissue mobilization technique.

CONFLICT OF INTEREST: Nil

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Table 1:Age and gender distribution of players (n=30)

	Group A (MFR)	Group B (IASTM)
Age (Mean <u>+</u> SD)	25.2667 <u>+</u> 2.0517	24.8000 <u>+</u> 1.6987
Gender (Male: Female)	10:5	10:5

Table 2: Baseline comparisons of pre-intervention NPRS in both groups:

		9 1			
Variables (Mean + SD)	Group A (MFR)	Group B (IASTM)	t value	p value	Significant
NPRS	5.9333 <u>+</u> 0.8837	5.8000 <u>+</u> 0.9411	0.397	0.698	No

Table 3: Comparison of NPRS within both the groups

Groups	Pre-Post	Mean <u>+</u> SD	T value	p value	Significant
Group A	Pre	5.9333 <u>+</u> 0.8837	18.721	<0.01	Yes
(MFR)	Post	1.5333 <u>+</u> 0.9904			, 02
Group B	Pre	5.8000 <u>+</u> 0.9411	19.572	<0.01	Yes
(IASTM)	Post	1.2000 <u>+</u> 0.9411		.5.01	. 03

Table 4: Between-group comparisons of change in NPRS post-intervention

Crounc	NF	PRS	tvoluo	n value	Cohen's d	Cianificant	
Groups	Mean	SD	t value	p value	Conen s a	Significant	
Α	1.5333	0.9904	1.099	0.290	0.2341	No	
В	1.2000	0.9411	1.099	0.290	0.2341	INO	

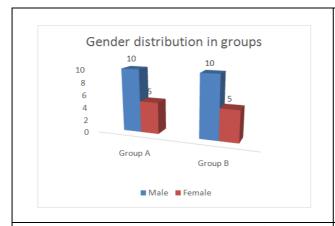




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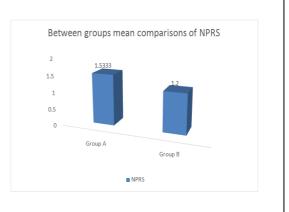


Chart 1: Gender distribution of players (n=30)

Chart 2: Between groups comparisons of NPRS





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RESEARCH ARTICLE

Product Cordial Labeling of Bistar Related Graphs

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ABSTRACT

A graph G is said to be a product cordial graph if there is a mapping g from V(G) to $\{0,1\}$ such that if each line rt is give the value g(r), g(t), then the cardinality of points with value 0 and the cardinality of points with value 1 differ at most by 1 and the cardinality of lines with value0 and the cardinality of lines with value 1 differ by atmost 1. In this case g is said to be a product cordial labelling of G. In this paper we investigate the product cordial labelling of bistar related graphs and we prove that the graphs such as Bistar $B_{\ell,\ell}$ the graph got by dividing the center line of the bistar $B_{\ell,\ell}$ the graph got by dividing the every pendant line of the bistar $B_{\ell,\ell}$, the subdivision graph $S(B_{\ell,\ell})$ of bistar, $S(B_{\ell,\ell}) \odot \overline{K_1}$, $S(B_{\ell,\ell}) \odot \overline{K_2}$ all are product cordial graphs.

Keywords: Product cordial labeling, Subdivision graph, Corona product, Bistar.

INTRODUCTION

Simple and finite graphs with p points and q lines are consider in this article. For entire survey of graph labelling we refer [2]. Several variations of graph labelling has been developed including prime labeling and product cordial labelling [3,4]. Many researchers have studied about product cordial graphs [5].

Definition 1.1

The corona product of two graphs P and Q is define as the graph got by take one copy of P and |V(P)| copies of Q and connecting the i^{th} point of Pto all point in the i^{th} copy of Q.





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Definition 1.2

A subdivision graph S(P) of a graph P is a graph got from P by dividing all lines of P exactly once.

Definition 1.3

Bistar is a graph obtained from a path P_2 by joining the root of stars S_ℓ and S_ℓ by P_2 . It is denoted by $B_{\ell,\ell}$.

II. MAIN RESULTS

The product cordial labelling of bistar related graphs were given in this paper.

Theorem 2.1

 $B_{\ell,\ell}$ is a product cordial graph.

Proof:

```
Let G = B_{\boldsymbol{\ell},\boldsymbol{\ell}}

Let V(G) = \{r_0, r_k, s_0, s_k/1 \leq k \leq \boldsymbol{\ell}\}

E(G) = \{r_0s_0\} \cup \{r_0r_k / 1 \leq k \leq \boldsymbol{\ell}\} \cup \{s_0s_k / 1 \leq k \leq \boldsymbol{\ell}\}

Here |V(G)| = 2\boldsymbol{\ell} + 2, |E(G)| = 2\boldsymbol{\ell} + 1

Give a labeling \boldsymbol{g} from V(G) to \{0,1\}by \boldsymbol{g}(r_0) = 1

\boldsymbol{g}(r_k) = 1 for 1 \leq k \leq \boldsymbol{\ell}

\boldsymbol{g}(s_0) = 0

\boldsymbol{g}(s_k) = 0 for 1 \leq k \leq \boldsymbol{\ell}

Here, v_{\boldsymbol{g}}(0) = \boldsymbol{\ell} + 1, where v_{\boldsymbol{g}}(0) is cardinality of points with value v_{\boldsymbol{g}}(1) = \boldsymbol{\ell} + 1, where v_{\boldsymbol{g}}(1) is cardinality of points with value v_{\boldsymbol{g}}(0) = \boldsymbol{\ell} + 1, where v_{\boldsymbol{g}}(0) is cardinality of lines with value v_{\boldsymbol{g}}(0) = \boldsymbol{\ell} + 1, where v_{\boldsymbol{g}}(0) is cardinality of lines with value v_{\boldsymbol{g}}(0) = \boldsymbol{\ell} + 1, where v_{\boldsymbol{g}}(0) is cardinality of lines with value v_{\boldsymbol{g}}(0) = \boldsymbol{\ell} + 1, where v_{\boldsymbol{g}}(0) is cardinality of lines with value v_{\boldsymbol{g}}(0) = \boldsymbol{\ell} + 1, where v_{\boldsymbol{g}}(0) is cardinality of lines with value v_{\boldsymbol{g}}(0) = \boldsymbol{\ell} + 1, where v_{\boldsymbol{g}}(0) is cardinality of lines with value v_{\boldsymbol{g}}(0) = \boldsymbol{\ell} + 1, where v_{\boldsymbol{g}}(0) is cardinality of lines with value v_{\boldsymbol{g}}(0) = \boldsymbol{\ell} + 1, where v_{\boldsymbol{g}}(0) is cardinality of lines with value v_{\boldsymbol{g}}(0) = \boldsymbol{\ell} + 1, where v_{\boldsymbol{g}}(0) is cardinality of lines with value v_{\boldsymbol{g}}(0) = \boldsymbol{\ell} + 1, where v_{\boldsymbol{g}}(0) is cardinality of lines with value v_{\boldsymbol{g}}(0) = \boldsymbol{\ell} + 1, where v_{\boldsymbol{g}}(0) is cardinality of lines with value v_{\boldsymbol{g}}(0) = \boldsymbol{\ell}
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 $e_{\mathcal{G}}(1) = \ell$, where $e_{\mathcal{G}}(1)$ is cardinality of lines with value 1 Thus the absolute difference of $v_{\mathcal{G}}(0)$ and $v_{\mathcal{G}}(1)$ is less than or equal to 1 and the absolute difference of $e_{\mathcal{G}}(0)$ and $e_{\mathcal{G}}(1)$ is less than or equal to 1.

Hence $B_{\ell,\ell}$ is a product cordial graph.

Theorem 2.2

The graph got by dividing the center line of the bistar $B_{\ell,\ell}$ is a product cordial graph.

Proof:

Let G be the graph got by dividing the center line of the bistar $B_{\ell,\ell}$ and let to be the new point.

```
Let V(G) = \{r_0, t_0, s_0, r_k, s_k/1 \le k \le \ell\} E(G) = \{r_0 t_0\} \cup \{t_0 s_0\} \cup \{r_0 r_k / 1 \le k \le \ell\} \cup \{s_0 s_k / 1 \le k \le \ell\} Here |V(G)| = 2\ell + 3, |E(G)| = 2\ell + 2 Give a labeling \mathcal G from V(G) to \{0,1\} by \mathcal G(t_0) = \mathcal G(r_0) = 1 \mathcal G(r_k) = 1 for 1 \le k \le \ell \mathcal G(s_0) = 0 \mathcal G(s_k) = 0 for 1 \le k \le \ell Here, v_{\mathcal G}(0) = \ell + 1, v_{\mathcal G}(1) = \ell + 2, e_{\mathcal G}(0) = \ell + 1, e_{\mathcal G}(1) = \ell + 1.
```

Thus the absolute difference of $v_{\mathcal{J}}(0)$ and $v_{\mathcal{J}}(1)$ is less than or equal to 1 and the absolute difference of $e_{\mathcal{J}}(0)$ and $e_{\mathcal{J}}(1)$ is less than or equal to 1.

Hence the graph got by dividing the center line of the bistar $B_{\ell,\ell}$ is a product cordial graph.

Theorem 2.3

The graph got by dividing the each pendant line of the bistar $B_{\ell,\ell}$ is a product cordial graph.





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Proof:

Let G be the graph got by dividing the each pendant line of the bistar $B_{\ell,\ell}$

Let
$$V(G) = \{r_0, s_0, r_k, r_k', s_k, s_k' / 1 \le k \le \ell\}$$
 $E(G) = \{r_0 s_0\} \cup \{r_0 r_k' / 1 \le k \le \ell\} \cup \{r_k r_k / 1 \le k \le \ell\}$
 $\cup \{s_0 s_k' / 1 \le k \le \ell\} \cup \{s_k' s_k / 1 \le k \le \ell\}$
Here $|V(G)| = 4\ell + 2$, $|E(G)| = 4\ell + 1$
Give a labeling g from $V(G)$ to $\{0,1\}$ by $g(r_0) = 1$
 $g(r_k) = g(r_k') = 1$ for $1 \le k \le \ell$
 $g(s_0) = 0$
 $g(s_k) = g(s_k') = 0$ for $1 \le k \le \ell$
Here, $v_g(0) = 2\ell + 1$, $v_g(1) = 2\ell + 1$, $e_g(0) = 2\ell + 1$, $e_g(1) = 2\ell$.

Thus the absolute difference of $v_{_{\mathcal{G}}}(0)$ and $v_{_{\mathcal{G}}}(1)$ is less than or equal to 1 and the absolute difference of $e_{_{\mathcal{G}}}(0)$ and $e_{_{\mathcal{G}}}(1)$ is less than or equal to 1.

Hence the graph got by dividing the each pendant line of the bistar $B_{\ell,\ell}$ is a product cordial graph.

Theorem 2.4

The subdivision graph $S(B_{\ell,\ell})$ of the bistar $B_{\ell,\ell}$ is a product cordial graph.

Proof:

```
Let G=S(B_{\ell,\ell}) be the subdivision graph of the bistar B_{\ell,\ell}. Let V(G)=\{r_0,t_0,s_0,r_k,r_k^{'},s_k,s_k^{'}/1\leq k\leq \ell\} E(G)=\{r_0t_0\}\cup\{t_0s_0\}\cup\{r_0r_k^{'}/1\leq k\leq \ell\}\cup\{r_k^{'}r_k/1\leq k\leq \ell\} \cup\{s_0s_k^{'}/1\leq k\leq \ell\}\cup\{s_k^{'}s_k/1\leq k\leq \ell\} Here |V(G)|=4\ell+3, |E(G)|=4\ell+2 Give a labeling {\mathcal G} from V(G) to \{0,1\} by {\mathcal G}(r_0)={\mathcal G}(t_0)=1 {\mathcal G}(r_k)={\mathcal G}(r_k^{'})=1 for 1\leq k\leq \ell {\mathcal G}(s_0)=0 {\mathcal G}(s_k)={\mathcal G}(s_k^{'})=0 for 1\leq k\leq \ell Here, v_{\mathcal G}(0)=2\ell+1, v_{\mathcal G}(1)=2\ell+1.
```

Thus the absolute difference of $v_{_{\mathcal{G}}}(0)$ and $v_{_{\mathcal{G}}}(1)$ is less than or equal to 1 and the absolute difference of $e_{_{\mathcal{G}}}(0)$ and $e_{_{\mathcal{G}}}(1)$ is less than or equal to 1.

Hence the subdivision graph $S(B_{\ell\ell})$ of the bistar $B_{\ell\ell}$ is a product coodial graph.

Theorem 2.5

The carona product $S(B_{\ell,\ell}) \odot \overline{K_1}$ is a product cordial graph.

Proof:

Let
$$G = S(B_{\ell,\ell}) \odot \overline{K_1}$$

Let $V(G) = \{r_0, r_0, t_0, t_0, s_0, s_0, r_k, r_k, s_k, s_k, u_k, u_k, v_k, v_k / 1 \le k \le \ell\}$
 $E(G) = \{r_0r_0\} \cup \{r_0t_0\} \cup \{t_0t_0\} \cup \{t_0s_0\} \cup \{s_0s_0\} \cup \{r_0r_k / 1 \le k \le \ell\}$
 $\cup \{r_kr_k / 1 \le k \le \ell\} \cup \{r_ku_k / 1 \le k \le \ell\} \cup \{u_ku_k / 1 \le k \le \ell\}$
 $\cup \{s_0s_k / 1 \le k \le \ell\} \cup \{s_ks_k / 1 \le k \le \ell\} \cup \{s_kv_k / 1 \le k \le \ell\}$
 $\cup \{v_kv_k / 1 \le k \le \ell\}$
Here $|V(G)| = 4\ell + 6$, $|E(G)| = 4\ell + 5$
Give a labeling $\mathcal G$ from $V(G)$ to $\{0,1\}$ by





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$$\begin{split} & g(r_0) = g(r_0') = g(t_0) = 1 \\ & g(r_k) = g(r_k') = 1 & \text{for } 1 \leq k \leq \ell \\ & g(u_k) = g(u_k') = 1 & \text{for } 1 \leq k \leq \ell \\ & g(s_0) = g(s_0) = g(t_0') = 0 \\ & g(s_k) = g(s_k') = 0 & \text{for } 1 \leq k \leq \ell \\ & g(v_k) = g(v_k') = 0 & \text{for } 1 \leq k \leq \ell \\ & \text{Here,} \quad v_g(0) = 2\ell + 3, v_g(1) = 2\ell + 3, \\ & e_g(0) = 2\ell + 3, e_g(1) = 2\ell + 2. \end{split}$$

Thus the absolute difference of $v_{_{\mathcal{G}}}(0)$ and $v_{_{\mathcal{G}}}(1)$ is less than or equal to 1 and the absolute difference of $e_{_{\mathcal{G}}}(0)$ and $e_{_{\mathcal{G}}}(1)$ is less than or equal to 1.

Hence $S(B_{\ell,\ell}) \odot \overline{K_1}$ is a product coodial graph.

Illustration 2.5.1

Theorem 2.6

The carona product $S(B_{\ell,\ell}) \odot \overline{K_2}$ is a product coodial graph.

Proof

Thus the absolute difference of $v_{\mathfrak{g}}(0)$ and $v_{\mathfrak{g}}(1)$ is less than or equal to 1 and the absolute difference of $e_{\mathfrak{g}}(0)$ and $e_{\mathfrak{g}}(1)$ is less than or equal to 1.

Hence $S(B_{\ell,\ell}) \odot \overline{K_2}$ is a product cordial graph.

Illustration 2.5.1

Product cordial labelling of $S(B_{4.4}) \odot \overline{K_2}$ (Figure 2)

CONCLUSION

The product cordial labelings of bistar related graphs were investigated. To derive analogous results for some other graph families and other graph labelings in an open area research.



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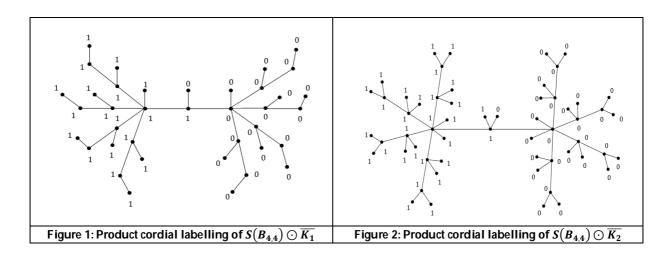
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RESEARCH ARTICLE

Assessment of Streptomyces rochei Isolated from Tannery Effluent as **Antibacterial and Antioxidant**

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ABSTRACT

The study was performed to analyse the potential of ethyl acetate extracts of actinomycetes isolated from tannery effluent collected from Pammal, Chennai, Tamil Nadu, India.A total of 35 actinomycetes were isolated to study their antibacterial activity against clinical isolates and antioxidant activity. 8 strains were found show good results in terms of zone of inhibition out of which one strain JA3 was found to show an increased activity. The phylogenetic analysis of 16s rRNA sequencing indicated that the isolate JA3 was found to be Streptomyces rochei JA246 and deposited in GenBank with accession number OP422332. JA3 showed increased activity against *E.coli* (22.13±0.15) followed by moderate activity against E.faecalis and S.typhii (18.83±0.05 and 17.1±0.1) and a minimum activity of 7.13±0.11 against P.putida. Isolate JA3 exhibited a dose dependent inhibition of DPPH activity with a maximum percentage of 81.399% at 100µg/ml when compared to that of standard, 81.41% of hydroxyl radical scavenging, 82.11% for hydrogen peroxide scavenging and 90.57% for nitric oxide scavenging at 100µg/ml. The Gas chromatography mass spectrometry (GC-MS) analysis revealed the presence of a major bioactive compound Actinomycin C2 \$\$ Actinomycin D, 2A-D-alloisoleucine.

Keywords: Tannery effluent, Streptomyces rochei, Antimicrobial activity, Antioxidant activity, GC-MS





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INTRODUCTION

The capability of the actinomycetes to produce microbial bioactive compounds makes it a unique feature to thrive on. The secondary metabolites play an important role in cellular, physiological and biological processes. Actinomycetes are widely populated in natural ecosystem such as soil, rhizosphere soil, hypersaline soil, freshwater, limestone, plants, marine, sponges, volcanic cave- hot spot, desert, air, insects gut, earthworm casting, goat faeces and endophytic actinomycetes. Among the microorganisms, actinomycetes produce a unique and novel secondary metabolite that showcases a wide range of activity. They also play a wide role in medical sciences and pharmaceutical industry with chemical structures and biological records diversely. In human, agriculture and veterinary sectors thousands of bioactive compounds are used to treat an expandable range of diseases[1-3]. Each actinomycetes has the genetic potential to produce 20-30 metabolites[4,5]. Proofs show that actinomycetes compile to 82% of the total antibiotic producers especially Streptomycessp[6,7]. They are producers of antifungals namely as amphotericin, antibacterialnamely tetracyclines, cephalosporins, anticancer drugs and immunosuppressants such as tacrolimus[8,9]. Streptomycetes and other related actinomycetes are still useful sources of novel secondary metabolites with a diverse range of biological activities that find applications as anti-infectives, anticancer agents, or other pharmaceutically useful compounds[10]. Hence screening, isolation, characterization and analysis of rare and promising actinomycetes with major antibiotic and as therapeutic applications[11,12]. Studies are concentrating on the antioxidant system of bacteria, which is useful in terms of biotechnology, such as Streptomyces which is able to grow in various stress conditions[13]. Nocardiopsis sp. may be good producers of pharmacological compounds which play important applications namely antibacterial, antioxidant, anti-inflammatory, antitumor and anticancer properties. Antioxidants play a major role in engulfing free radicals thus protecting humans from infectious and degenerative diseases[14]. An increase in free radical and decrease in antioxidant leads to oxidative stress which urges to find antioxidant agents. There are certain antioxidants that are naturally occurring, which are found to reduce the oxidative stress inducing damage to human cells[15]. There are a wider range of cause for pollution that occurs globally. Industries among them play a major role. Tannery industry is the main source of polluter to the environment and codes a great potential to cause soil, water, plants, vegetables, terrestrial and atmospheric systems owing to the discharge of untreated effluent[16]. These effluents are made up of nitrogen, ammonia, chromium, sulphideetc using which microbes grow pertaining to the environment. Isolating actinomycetes from these tannery effluents can pave way to clear and remove waste which are utilized by them. The manuscript focuses on isolation, characterization and production of bioactive compounds from actinomycetes for antibacterial and antioxidant activity.

MATERIALS AND METHODS

Collection of Sample

Tannery effluents were collected from various parts of Chennai namely, Ambur, Chromepet, Manali, Madhavaram, Pammal, Ranipet and Vellore. The samples were collected in sterile screw cap bottles and transported to the laboratory and processed within 2hours of collection (Fig1).

Isolation of Actinomycetes from Tannery Effluent Samples

The samples were serially diluted for the isolation of actinomycetes. Starch Casein Agar (SCA) was suspending Rifampicin, Actidione and Nystatin (30µg/ml) as antibacterial and antifungal antibiotics[17,18,19].

Screening for Bioactive compound producing Actinomycetes

35 strains were isolated and screened for the production of bioactive compounds. The isolates were inoculated in ISP4 medium followed by incubation for 7days in a rotatory shaker at 28°C. The broth obtained after incubation was filtered and used for antimicrobial activity. 24hour old cultures of *E.coli, Salmonella typhi, S.mutans, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas putida* and *Pseudomonas aerogenosa.*were used as test





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organisms. The test organisms were swabbed on nutrient agar plates that are bored using sterile borers prior to swabbing. The filtrates were added to the wells and the plates were incubated for 24 hours at 37° C.

Morphological and Taxonomic Identification of Isolated Strain

Based on the results obtained for antimicrobial screening 8 strains were found show good results in terms of zone of inhibition out of which one strain JA3 was found to show an increased activity. The isolate JA3 was studied for its aerial mycelium, substrate mycelium, spore chain morphology, diffusible pigment, reverse pigment and other cultural and biochemical characteristics. The isolate JA3 was identified taxonomically based on Bergey's Manual and Nonomura's key[20,21] and the strain was identified by 16s rRNA sequencing.

Production and Extraction of Bioactive compounds

Mass production of the selected isolateJA3 was performed by inoculating the isolates in 50ml production medium taken in 250ml conical flask and incubated in the shaker for 48hours for 120rpm maintained at 28° C. 10ml of the inoculum was transferred to 100ml production medium and kept in rotatory shaker for 7days at 28° C. After 7days of incubation the mycelial mass was separated from the supernatant by filtration and centrifuged for 20min for 10000rpm at 4°C. Bioactive compounds from the supernatant was extracted by liquid-liquid extraction separately using equal volumes of ethyl acetate, n-hexane and n-butanol and concentrated by Rota evaporation.

Antibacterial activity of actinomycetes

Antibacterial activity was carried using modified method of Kirby-Bauer [22]. Test cultures were prepared by inoculating 1ml of the cultured broth with 9ml of sterile nutrient broth completing McFarland solution (0.5) with the turbidity comprising to 108-110 cfu/ml. Muller –Hinton agar (MHA) plates were swabbed with test cultures using sterile cotton swabs and allowed to dry for 5min. crude extract of 1mg/ml concentration were dissolved in DMSO. Various concentrations of the extracts JA3 were loaded on a 6mm diameter sterile disc and allowed to dry for 5min. Commercially available Streptomycin discs were used as positive control. The discs were placed on the swabbed MHA plates aseptically and incubated at 28C for 18-24 hours.

Preliminary Screening of Antioxidant activity DPPH Activity

DPPH (1, 1 -diphenyl-2-picryl hydrazyl) radical scavenging activity of isolates JA1, JA3, JA13, JA19, JA23, JA32, JA33, JA35 were performed in accordance with the method proposed by Sanchez *et al.* (1998). The extracts and standard reference compounds were prepared with 99% ethanol at various concentrations (20-100µg/mL). 1 ml of various concentrations (20-100µg/mL) of the extracts and standard reference compounds were dissolved in 1 ml of 0.2 mM DPPH separately and made up using 99% ethanol in 10 ml test tube to achieve a final volume of 3 ml. The mixture was vortexed and incubated for 90 min at room temperature. The optical density was measured at 517 nm.

Hydroxyl radical scavenging assay

The ability of actinomycetes isolates extracts to scavenge the hydroxyl radical generated by the Fenton reaction was measured according to the modified method of Chung *et al.* (1997). The Fenton reaction mixture containing 200 μ L of 10 mM FeSO₄·7H₂O, 200 μ L of 10 mM EDTA and 200 μ L of 10 mM 2-deoxyribose was mixed with 1.2 ml of 0.1 M phosphate buffer (pH 7.4) containing 200 μ L of actinomycetes isolates JA1, JA3, JA13, JA19, JA23, JA33, JA35. Thereafter, 200 μ L of 10 mM H₂O₂ was added to the mixture and incubated for 4 hrs at 37 °C. After incubation, 1ml of 2.8 % TCA and 1ml of 1 % TBA were added and placed in a boiling water bath for 10 min. The resultant mixture was then allowed to cool to room temperature and centrifuged at 395 × g for 5 min. Absorbance was recorded at 532 nm in a UV-VIS spectrophotometer.

Hydrogen peroxide radical scavenging assay

The ability of the endophytic extracts to scavenge hydrogen peroxide was determined according to the method of Ruch *et al.* (1989). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Actinomycetes extracts of isolates JA1, JA3, JA19, JA23, JA32, JA33, JA35 (20-100 µg/mL) in distilled water were



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added to a hydrogen peroxide solution (0.6 mL, 40 mM). The absorbance of hydrogen peroxide at 230 nm was

determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide using a UV-VIS spectrophotometer (Jasco V-530, Japan Servo Co. Limited., Japan).

Nitric oxide radical scavenging assay

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which was measured by the Griess reaction (Green et al. 1982). The reaction mixture (3 ml) containing 10 mM sodium nitroprusside in phosphate-buffered saline and actinomycetes isolatesJA1, JA3, JA13, JA19, JA23, JA32, JA33, JA35 and the reference compounds at different concentrations (50, 100, 200, 400, 500 and 1000 µg/ml) were incubated at 25 °C for 150 min. A 0.5 ml aliquot of the incubated sample was removed at 30 min intervals and 0.5 ml Griess reagent (1 % sulfanilamide, 0.1% naphthylethylene diamine dihydrochloride in 2 % H₃PO₄) was added. The absorbance of the chromophore formed was measured at 546 nm. All the tests were performed in triplicates. Percentage inhibition of the nitric oxide generated was measured by comparing the absorbance values of control and test preparations. Butylated hydroxytoluene and Ascorbic acid were used as a positive control.

	OD control – OD test sample × 10
Radical scavenging (%) =	
	OD control

Determination of Biological activities of bioactive compounds

Based on the results, isolate JA3 was found to showan increased activity when compared to the other 7 isolates. The crude extract of isolate JA3 was subjected to Gas Chromatography- Mass Spectroscopy (GCMS) (Table3).

RESULTS AND DISCUSSION

Isolation and Screening of Actinomycetes

35isolates were screened for antimicrobial activity using starch casein culture broth containing the bioactive compound out of which 8 isolates showed promising results. Further screening of the 8 isolates by disc diffusion methodshowed that isolate JA3 showed an increased zone of inhibition (Table 1). The isolates JA1, JA3, JA13, JA19, JA23, JA32, JA33, and JA35 were also checked for antioxidant activity. Isolate JA3 showed maximum activity and hence was studied for its morphological, cultural, molecular characterization and bioactive compound production. The results of actinomycetes isolation from three caves located in the north of Spain, showed that majority of these were isolated at 28C and belonged to Sporoactinomycetes[23]. which corresponds to the results obtained in this work, JA3 was isolated ISP4, at 30°C.

Morphological and Biochemical Characterization

The isolate JA3 was found to be a Gram positive, aerobic organism (Fig3A). Colony colour was grey with aerial mycelium to be matte in texture. These morphologicalcharacters are closely agreed with the findings of Goodfellow[24] and Hoshino et al[25]. The slides showed aerial mycelium along with sporangium. The genus denotes aerobic aretes thatare more inclined to form acid fromcarbohydrates, and form extensive monopodialand aerial mycelia with a DNA basecomposition denoting high GC content [26]. The mycelial hyphae showed extensive branching and non-septate no spore bearing hyphae and is considered to be biverticillus. Spore silhouettes were hairy (Fig3B). Chromogenicity of aerial mycelium isconsidered an important character for grouping of actinomycetes[27]. In carbon and nitrogen assimiliation test, isolate JA3 grew abundantly utilising glucose and mannitol as the sole carbon source with well developed mycelium whereas the isolate grew poorly utilizing cellulose and lactose. These enzymes represent the largest groups ofindustrial enzymes[28], widelyutilized in soaps and detergent industries, medical and food processing industries. With nitrogen assimilation, isolate JA3 grew abundantly utilizing beef extract, yeast extract and peptone whereas they grew poorly with tryptone and ammonium sulphate. Actinomycetes are capable of degradingsimple and complex substances present in theirenvironment [29,30] mentioning their composite substances and their





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geneticmakeup[31] (*Streptomyces* genus)[32]. The isolate showed positive results hydrolysing starch, casein and gelatin as tabulated in Table1.

Identification of Actinomycetes.

The taxonomic identification of the isolate JA3 was based on 16s rDNA analysis. The16s rDNA sequence of the strain was compared with thesequences in GenBank using BLAST and aligned with thesequences retrieved from NCBI GenBank database using the ClustalWmethod. The phylogenetic tree was constructedbased on neighbor joining tree method and illustrated inFigure 4. The database was deposited in NCBI GenBankwith an accession number OP422232. Based on the cultural,morphological, physiological, and molecular analysis, the JA3 was identified as *Streptomyces rochei* JA246.

Antimicrobial activity of actinomycetes

Based on the colony morphology, staining, sub culturing stability, 35 suspected actinomycetes isolates were isolated and purified on ISP-4 slants. The isolates were primarily screened for their capability to produce antimicrobial compounds. In the initial stages of screening, 9% isolates showed good activity, 26% showed moderate and 24% weak activity against the test organisms. Disc Diffusion Technique was carried out for the 8 isolates, JA1, JA3, JA13, JA19, JA23, JA32, JA33, and JA35 and tested against E.coli, E. faecalis, Salmonella typhii, S.mutans, Enterobacter, Klebsiella pneumoniae, Pseudomonas putida and P.vulgaris(Table2). Ethyl acetate extracts of isolate JA3 showed the maximum activity against all the test organisms. Isolate JA3 showed increased activity against E.coli (22.13±0.15) followed by moderate activity against E.faecalis and S.typhii (18.83±0.05 and 17.1±0.1) and a minimum activity of 7.13±0.11 against P.putida(Fig.5(C)). Actinomyces are useful biological tools in the production of antimicrobials against bacteria and fungi[33]. Isolate JA32 showed minimum activity against E.coli, K.pneumoniae, P.vulgaris, P.putidaand S.aureus but did not show any activity against E.faecalis, S.mutans and S.typhii. These bacteria produce about 75% of commercially and medically useful antibiotics[34].Isolate JA1 and JA35 showed minimum activity against Klebsiella pneumoniae, Pseudomonas putida and P.vulgaris (10.13±0.11, 5.53±0.05 and 5.06±0.11).N-Hexane (HEA) and n-Butane extracts of actinomycetes (BEA) isolate JA3 were also tested for its activity against the test cultures. HEA showed maximum activity against E.coli (19.15±0.0057), whereas BEA showed its activity against S.typhii(18.98±0.005). Among the three different extracts of actinomycetes (EEA, HEA and BEA), EEA showed the maximum activity. The Most of the secondary metabolitesand antibiotics are extracellular in nature and extracellular products of actinomycetes show potent antimicrobial activities[35]. Experimental results are the mean ±standard deviation (SD). Statistical comparisons using one way analysis of variance (ANOVA). The results showed statistically significant.

Antioxidant activity of isolate JA3

Based on the results obtained, isolate JA3 was selected for further screening of antioxidant activity. The study explains the radical scavenging activity of actinomycetes isolates against DPPH, Hydroxyl, Hydrogen peroxide and Nitric oxide respectively.

DPPH radical scavenging assay

The crude actinomycete isolate JA3 exhibited a dose dependent inhibition of DPPH activity with a maximum percentage of 81.399% at 100µg/ml when compared to that of standard (BHT-99.03% and Ascorbic acid (99.11%). Isolate JA19 and JA23 exhibited the minimum scavenging percentage of 52.73 and 54.43% respectively. The results are presented in Fig. 6. Presence of phenolics is shown by Folin–Ciocalteau. This might have contributed to scavenging potential. DPPH is used to evaluate the free radical engulfing ability of the hydrogendonating antioxidant, which in turn can transfer hydrogenatoms or electrons to DPPH radicals[36].

Hydroxyl radical scavenging assay

The results of hydroxyl scavenging activity is depicted in Fig. 7. Highest scavenging percentage was shown in isolate JA3 and found to be 81.41% at 100µg/ml whereas the standard Catechin showed only 78.91% and ascorbic acid showed 84.08% at 100µg/ml. The interaction between the hydroxyl radicals with that of the DNA leading to breakdown of DNA and therefore plays a crucial role in cancer formation [37].





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Hydrogen peroxide radical scavenging assay

Hydrogen peroxide has the ability to react with metal ions and produce reactive oxygen species such as hydroxyl free radical which are loaded with toxic effects[38]. The results of hydrogen peroxide scavenging is exhibited in Fig. 8. Isolate JA3 showed highest scavenging activity of 82.11% at 100µg/ml. the standards showed activity that were close to the test values. It was reported that a marine *Streptomyces* sp. MAB18collected from mangrove soil, Andhra Pradesh produced extracellular protease which was found to exhibit antioxidant activity[39]. Likewise another isolate *Nocardiopsis alba*showed antioxidant activity at par with standard ascorbic acid [40].

Nitric oxide radical scavenging assay

Nitric oxide scavenging assay by actinomycetes isolate JA3 was increased in a dose-dependent manner as exhibited in Fig. 9. At concentration 100 μ g/ml the extract showed 90.57% inhibition which is higher than the standard VitaminC (68.83%) and Ascorbic acid (74.34%). JA3 inhibited nitrite formation by competing with oxygen to react with nitric oxide directly and also to inhibit its synthesis[41].

GC-MS analysis

The chemical composition of ethyl acetate extract of isolate JA3 was investigated using GC-MS analysis. In comparison with the mass spectra data of the constituents with the NIST library, fourteen compounds were identified based on the retention time, molecular formula and molecular weight. Out of the fourteen compounds mentioned in Table 3. Of the fourteen compounds identified, the important compoundwas actinomycin C2 which constituted for6.12%. The GC-MS has been widely considered as a "gold standard" for forensic substance identification. A specific test positively identifies the actual presence of a particular substance in a given sample. A non-specific test, however, merely indicates that a substance falls into a category of substances. Although a non-specific test could statistically suggest the identity of the substance, this could lead to false positive identification [42].

CONCLUSION

Tanning waste is generated in large amounts during the process of tanning by leather industries globally. Considered as one of the most polluted industrial waste with high amounts of metals which are in turn very toxic to the plants, animals and soil. The diverse ecological distribution of microorganisms make it possible for them to metabolize most organic compounds found in industrial waste hence constitute the basic biological units in tannery treatment effluent system. The ethyl acetate extract of actinomycetes isolate *Streptomyces rochei*JA246, exhibited significant biological activities against bacterial pathogens. It also showed antioxidant activities against DPPH, Hydroxyl, Hydrogen peroxide and Nitric oxide when compared to n-hexane and n-butane extracts. GC-MS analysis showed the presence of Actinomycin C2 \$\$ Actinomycin D, 2A-D-alloisoleucine (6.12%) which may be the active compound in showing positive results.

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Table1: Morphological and Biochemical Characterization of Isolate JA3

Characteristic features	Isolate JA3
Colony appearance	Chalky
Colony colour and texture	Greyish white, matte
Aerial mycelium	
Diffusible pigment	Absent
Spore chain	Biverticullus, no spirals
Motility	Non-motile
Starch hydrolysis	+
Casein hydrolysis	+
Gelatin Hydrolysis	+
Carbon util	ization
Glucose	+++
Sucrose	++
Maltose	++
Cellulose	+
Mannitol	+++
Lactose	+
Nitrogen uti	lization
Beef extract	+++
Yeast extract	+++
Peptone	++
Tryptone	+
Ammonium Sulphate	+
Ammonium Chloride	+
Ammonium Nitrate	++





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Table2: Antimicrobial activity of isolates JA1, JA3, JA13, JA19, JA23, JA32, JA33, JA35

Isolates	E.coli	E.faecalis	K.pneumoniae	P.vulgaris	P.putida	S.aureus	S.mutans	S.typhii
JA1	10.66±0.15	-	5.06±0.057	7.56±0.057	-	1	1	-
JA3	22.13±0.15	18.83±0.05	12.06±0.11	10.53±0.05	7.13±0.11	7.36±0.05	13.3±0.26	17.1±0.1
JA13	5.03±0.05	-	-	6.2±0.17	5.3±0.26	-	-	8.33±0.05
JA19	-	6.36±0.05	-	-	9.26±0.05	11.1±0.1	5.73±0.05	-
JA23	8.26±0.05	6.03±0.05	9.13±0.05	-	-	-	-	5.13±0.05
JA32	6.16±0.15	-	7.86±0.05	5.06±0.05	13.16±0.05	5.26±0.05	-	-
JA33	11.4±0.17	9.7±0.05	=	-	-	-	8.66±0.05	10.27±0.05
JA35	-	-	10.13±0.11	5.53±0.05	5.06±0.11	-	-	-

Table3: GC-MS Profile of Ethyl acetate extract of isolate JA3

Name of the chemical constituents	Retention time	% Area
1. 1,2-Benzenediol	9.693	10.03
2.2-Furaldehyde, 5-(hydroxymethyl)-	10.131	0.19
3. 1,4-Benzenediol	10.709	5.95
4. Resorcinol \$\$ 1,3-Benzenediol	10.820	4.04
5. Methacrylic acid, dodecyl ester	11.209	0.40
6. 4-Ethylcatechol	12.242	0.43
7. trans-Cinnamic acid	12.774	0.20
8. (1R,3R,4R,5R)-(-)-Quinic acid	16.608	1.90
9. Actinomycin C2 \$\$ Actinomycin D, 2A-D-alloisoleucine	17.684	6.12
10. Ethanone, 1-(3-methyl-2-nitrophenyl)	18.772	0.21
11. Oleic Acid	20.529	0.45
12.2,5-Piperazinedione, 3,6-bis(2-methylpropyl)-	20.893	0.38
13.Dihydroergotamine	22.417	0.47
14. Pentacosanoic acid, methyl ester	33.032	0.17



Fig.1: Collection of tannery effluent samples from various parts of Chennai

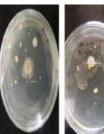








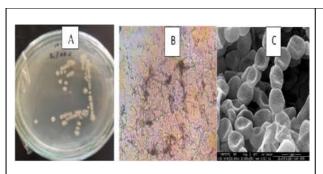
Fig. 2: Isolation of actinomycetes from tannery effluent



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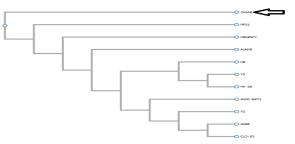
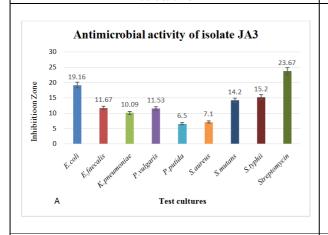


Fig. 3: (A) Pure isolate of JA3 (B) Gram staining of isolate JA3

Fig. 4: Phylogenetic tree of isolated actinomycetes JA3



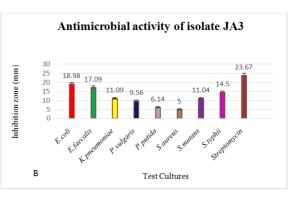


Fig.5: (A) Antimicrobial activity ofn-butane extract of JA3

Fig. 5: (B)Antimicrobial activity ofn-hexane extract of JA3

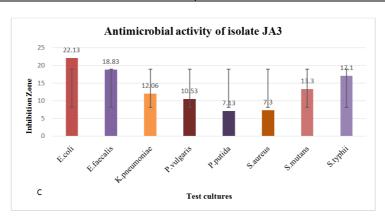


Fig.5: (C)Antimicrobial activity of ethyl acetate extract of JA3by Disc diffusion method

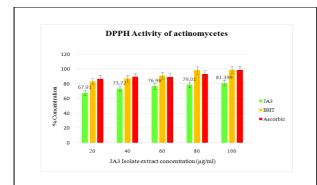




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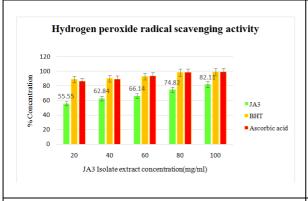
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Hydroxyl radical scavenging activity of actinomycetes

Fig.6: DPPH scavenged activity of actinomycetes isolate JA3

Fig. 7: Hydroxyl scavenged activity of actinomycetes isolate JA3



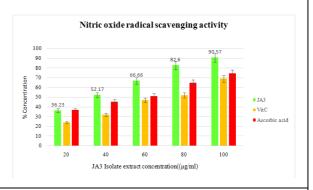


Fig.8: Hydrogen peroxide scavenged activity of actinomycetes isolate JA3

Fig.9: Nitric oxide scavenged activity of actinomycetes isolate JA3





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RESEARCH ARTICLE

Preparation and Characterization of Activated Charcoal from Kapok Tree, Coconut Shell, and Peanut Shell with Comparative Study

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ABSTRACT

Due to the wide applications of activated charcoal in many chemical and pharmaceutical, separation and purification industries. The bulk production requires to produce with sustainable approaches. For commercial applications mainly cellulosic precursors like various grades of coals, mainly peat & lignite are used for the preparation of activated charcoal. In order to make the production more sustainable usefulness of biomass is tested by many researchers. In this study we used three different biomass or biowaste for the preparation of activated charcoal viz. Kapok tree (Ceiba pentandra) Coconut Shell (Cocos nucifera) and Peanut Shell (Arachis hypogaea). Moreover, to produce low cost, moderate to highly porous and optimal activated carbons we used vacuumed lab scale method. Ultimate and proximate analysis was carried out to find out the characteristics and physico-chemical properties of the prepared materials. Activated carbon produced by this pyrolysis process and activation methods has higher surface area with the potential to be a promising precursor to produce activated carbon.

Keywords: Activated Charcoal, Biomass, Kapok tree, Coconut, Peanut

INTRODUCTION

Activated carbon (AC), is an important carbonaceous solid material used for the removal of hazardous components in exhaust gases, purification of drinking water, waste water treatment, chemical and pharmaceutical, processes, medicinal uses, gas storage, pollutant and odour removal, gas separations, catalysis, gas separation and purification, metal extraction, water purification, chromatographic separation, chemical purification, trapping mercury, fuel cells, spill clean-up, groundwater remediation, drinking water filtration, air purification, volatile organic compounds removal, gasoline dispensing operations and many more. It is mainly derived from coal or biomass via thermal or thermo chemical processes. It should be composed of a microporous, homogenous structure with high surface area





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and some specific properties like iodine index, molasses index, tannin index, methylene blue index, butane index, CCI4 index, de-chlorination half-value length, density, ash and moisture content and particle size distribution. Usually, the activated carbon in the market were made from conventional precursors e.g., coal, lignite, peat, petroleum residue like fossils and wood which are quite expensive and exhaustible too. Due to the environmental concerns, economic viability, non-renewability, they cannot be the permanent choices for the production of AC from conventional resources. In other side the environmental issues of abundant biomass waste around us are of top concern of many researchers, industries and environmental agencies. These agricultural residues and different types of plant-based biomass are one of the responsible reasons of greenhouse gases (GHG). Both these problems can become the solution to each other if the activated charcoal is prepared from the biomass waste. These biomasses or biowaste if becomes resources, will be cheap, renewable, easily available, and ecologically suitable for the possible solution to the pollution. Therefore, the interest has developed for utilization of AC made from agricultural, municipal, industrial and forestry bio-wastes is being increasing.

Several researches have been found including the production of activated carbon from waste biomass such as *Imperata cylindrical* [1] date stone [2]durian shell [3], Grape stalk[4], coconut shell [5], and Peanut shell [6], palm shell and coconut shell [7, 8], Bael fruit [9], rice husk [10], bamboo [11], guava seeds[12]. From the wide literature survey, it is found that there are many types of biowaste and agricultural wastes are used for preparation of AC although there are mainly three types of AC produced with different morphology viz. i) Powdered Activated Carbon (PAC) ii) Granular Activated Carbon (GAC) and iii) Extruded Activated Carbon (EAC). All these ACs are prepared with different temperature and pressure as per the materials and method given by different researchers, but the commonly used synthetic rout can be given with following general flow diagram (Fig.1). In this present study we have prepared ACs from three different types of biomass feed stocks viz. Kapok tree (*Ceiba pentandra*) Coconut Shell (*Cocos nucifera*) and Peanut Shell (*Arachis hypogaea*) with the help of above common flow diagram pathway.

MATERIALS AND METHODS

The precursor materials were obtained, reduced to size of approx. 0.5 cm x 0.5 cm. All the samples were first weighed using an electronic balance and repeatedly washed with distilled water in order to remove dust and other inorganic impurities, dried in sunlight and hot air oven subsequently and prepared for impregnation step as per given in the Table-1.

Experimental

Three samples of 200 gm of each dried precursor were then added to 150 ml solution 50% KOH (85% purity) in a ratio of 1 g of precursor per 0.75 mL of solution [8] and kept for 24 hrs for soaking. The excess KOH solution from all nine samples were decanted off and air-dried the materials and placed one sample of each precursor in self prepared vacuumed heating vessel for carbonization at 350 °C, 45 min. The second sample of each precursor in self prepared vacuumed heating vessel for carbonization at 400 °C, 60 min and third sample of each precursor in self prepared vacuumed heating vessel for carbonization at 450 °C, 75 min. The dried materials were powdered separately and activated in vacuumed heating vessel kept at 500 °C for a period of 20 minutes. This step gave activated charcoals which were then washed sufficiently with 5 N hydrochloric acid solution to remove the cations subsequently washed with plenty of water to remove excess hydrochloric acid, filtered out and dry materials were obtained. These final materials were dried, lumps were grinded and sieved into sizes of 20 x 30 mesh, heated in vacuumed vessel in absent of air at 110°C, cooled in vacuum desiccators and final weights were noted see the table-2.

RESULTS AND DISCUSSION

Yield of Activated Carbon

The % yields of all nine samples are given in the Table-2. The results shows that the % yields of ACs obtained from Kapok tree are in the range of 55.50–61.50 %, for Coconut Shell it is 55.00–58.50 % and for Peanut Shell it is found





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47.00–50.50 %, indicates lower yield in the case of Peanut Shell. It also shows that increasing the temperature and time or carbonization decreases the % yield of ACs. This is attributable to the fact that activation time and temperature have a major impact on the yield(Fig 3).

Methylene Blue Adsorption Test

Standard MB (methylene blue) Number tests were carried out using 0.05 g of ACs of each sample. Each of it was added in 150 mL of MB solutions with concentration of 100 mg/L for 120 min. After filtration of these solutions filtrates were used to find out the remaining concentration of MB was determined by measuring the absorbance UV/ Visible spectrophotometer. The results are shown in the Table-3. The MB number increases with carbonization time and temperature too. Moreover, MB number for coconut shell is some higher than kapok tree sample and for peanut shell it is quite higher than both of other respective samples with similar time and temperatures of carbonization (Fig 4).

Proximate analysis

The proximate analysis of prepared ACs was carried out according to ASTM standard D1762 – 84. Results of these tests are given in the table-3. Results shows that Average moisture content is found higher in the case of kapok tree than coconut shell and peanut shell. It is clearly observed that increasing the temperature of carbonization process decreases moisture content in all three precursors (Fig.5). As per the expectation the bulk density of the peanut shell AC is found lesser than both of the other samples. Increasing the temperature gives decrement in it (Fig. 6) similar results are also observed for ash content (Fig. 7) and volatile matter (Fig. 8). Amount of fixed carbon was found increased with temperature in all three sample precursors. Here one notable result is observed that fixed carbon % is found higher in peanut shell AC than both the other sample precursors (Fig. 9).

CONCLUSION

From the results of present experimental investigation, it can be concluded that:

- i) These three biomass waste precursors can be used for preparation of ACs conveniently and economically and environment-friendly.
- ii) The activation process employed here can be used for preparation of ACs from biomass with simplicity of the process.
- iii) High pyrolysis temperature requires high energy but optimum temperature can also give good quality ACs.
- iv) With compared to kapok tree and coconut shell ACs peanut shell AC has more absorption capacity and fixed carbon % too.

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Table.1: Preparation of dried precursor materials

Precursor material	Obtained from	Initial weight taken (gm)	Weight after drying in sunlight for a day (gm)	Weight after drying at 120° C for 6 hrs
Kapok tree	Campus Plant waste	800	704	635
Coconut Shell	Nearby temple waste	800	754	617
Peanut Shell	House hold waste	800	741	630

Table.2: % yield of activated carbons from dried precursor materials

Dwaguraar	C 1 -	Carbonization			Final Weight	Weight loss	
Precursor material	Sample code	Initial Weight in	Time	Temp.	in	in	% Yield
materiai	code	(X g)	(Min.)	(°C)	(Y g)	(Z g)	
	K1	200	45	350	123	77	61.50
Kapok tree	K2	200	60	400	119	81	59.50
	K3	200	75	450	111	89	55.50
Coconut	C1	200	45	350	117	83	58.50
Shell	C2	200	60	400	113	87	56.50
Sileii	C3	200	75	450	110	90	55.00
Peanut Shell	P1	200	45	350	101	99	50.50
	P2	200	60	400	97	103	48.50
	P3	200	75	450	94	106	47.00





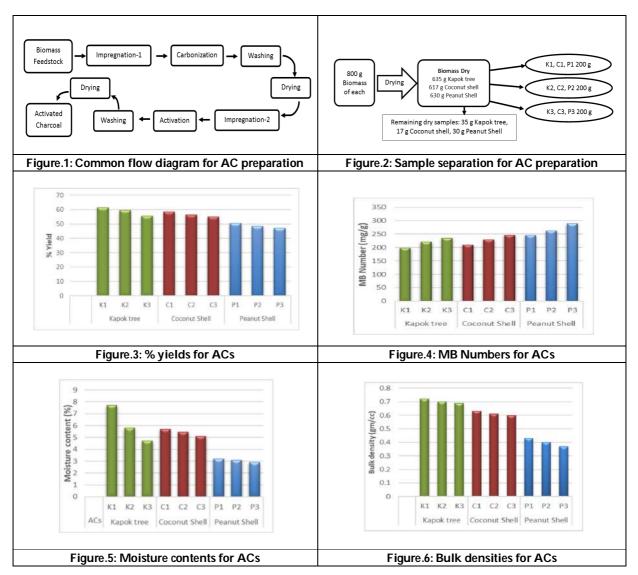
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Table.3: Characterization data of ACs

Precursor	Sample	Time	Temp.	MB	Bulk	Moisture	Volatile	Ash	Fixed
material	code	(Min.)	remp. (°C)	Number	density	content	matter	content	carbon
materiai	code	(IVIIII.)	(°C)	(mg/g)	(gm/cc)	(%)	(%)	(%)	(%)
Kanak	K1	45	350	198	0.72	7.70	8.80	7.85	75.65
Kapok tree	K2	60	400	221	0.70	5.80	8.20	7.80	78.20
tree	K3	75	450	235	0.69	4.70	7.35	7.65	80.30
Coconut	C1	45	350	210	0.63	5.70	5.50	8.90	79.90
Coconut Shell	C2	60	400	229	0.61	5.45	4.60	9.10	80.85
Sileii	C3	75	450	246	0.60	5.10	4.05	9.00	81.85
Peanut Shell	P1	45	350	245	0.43	3.20	4.15	5.50	87.15
	P2	60	400	262	0.40	3.10	4.00	5.70	87.20
Sileli	P3	75	450	289	0.37	2.90	3.80	5.30	88.00



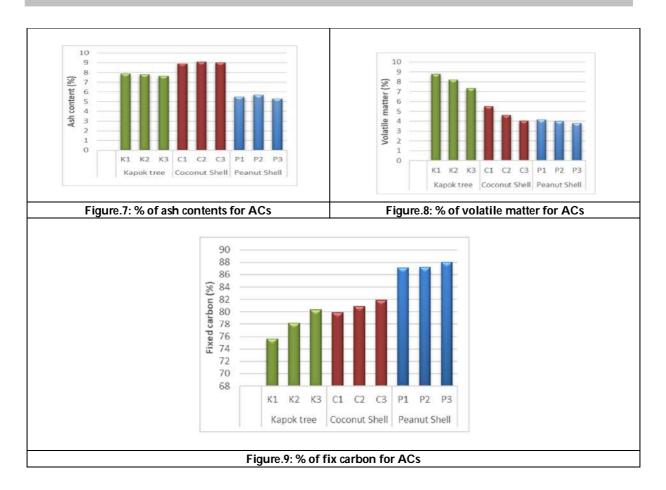




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RESEARCH ARTICLE

Phytotherapy used by the Common People of Assam (India) for the Treatment of Gynaecological Problems

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ABSTRACT

Phytomedicine or herbal medicine is the ancient Indian medical practice that depends on herbs and plants for maintaining good health. Assam is the land of large number of rural population with differences in vernacular, health practices, unique customs, values and traditions. Assam is also well known for its flora and fauna. Because of the climatic condition and diverse topology, Assam holds up the platform for producing variety of medicinal plants. These plants are used in the treatment of different diseases including gynaecological problems of women. This traditional knowledge is passed on to next generation which were somewhere published in different title.

Keywords: Medicinal plants, Female health, Assam

INTRODUCTION

Assam, one of the biodiversity hotspots, holds a special place in North eastern India located between 24º44' N to 27º 45'N latitude and 89° 41'E to 96° 02'E longitude, covering 2.4% of the geographical area of the country, i.e. 78,438 sq. km. The annual rainfall scale is in between 305 cm. max. to 178 cm. min. with an average of 211.76 cm. The temperature recorded in summer is 37 °C max. and 18 °C min. and 26 °C max. and 7 °C min. in winter, with an average humidity of 83.00%. These types of climatic conditions of Assam come up with a compatible environment for growing diverse medicinal plants [1]. North East India is well known for its affluent biodiversity of flora and fauna. The high rainfall prevailing in this region favours the growth and development of various types of vegetation. The climatic condition and diverse topology of Assam stand up for providing the platform for making it a hub for medicinal plant. Assam with its different agro climatic region, habitats and forest types has potentiality of growing





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plants of all types suitable to different habitats ranging from tropical plants to alpine forests [2]. Medicinal plants used by the local people and different tribes of the state of Assam are primarily primeval and most of them are not known to the huge world of phytochemical science and research. The functioning constituents and dominant phytochemicals with favourable pharmacological properties present in those plants are yet to be explored. Possibly some vitalizing drug formulation can be discovered from some of those indigenous plants of Assam which are being used with trust and confidence by the localites, the tribal and the villagers residing in Assam and can be a milestone in the world of pharmaceutical sciences and a blessing to mankind all over the globe [3]. Assam is residence of large number of rural population. These rural people have their own unique customs, traditions, beliefs and practices. Even within particular community, differences in vernacular, health practices, unique customs, values and traditions are noticeable. The rural life of Assam is full of problems. Other than extreme poverty and minimum living conditions, they have poor health conditions together with different diseases. Some health problems are more familiar and can be treated simply but when the problems are related to female gynaecological health, the circumstance is very bad. The rural women hide their gynaecological problems, and that is the reason their reproductive health is extremely poor and they suffer in silence [4]. The female health or gynaecological problems of women are crucial complications in the villages of Assam and such diseases are treated by using of folklore medicinal plants. This conventional knowledge is transmitted from one generation to another which were somewhere published in different headings [5]. This review focuses on different medicinal plants used by people of Assam for the treatment of different gynaecological problems and for the enhancement of female health.

Plants From Assam With Medicinal Property Used For Female Health Care

There are many plants and herbs grown in different parts of Assam. Some are found in forest area and some are grown by the people as domestic plants. These plants have magnificent medicinal uses since long time. Some of them are taken in various forms like spice, herb, vegetable etc and are known for their helpful effects in maintaining good health. Some of the medicinal plants with their local name and medicinal application are given below.

SI No	Local Name	Scientific Name	Family	Application
1	Dudhkosu	Colocasiaantiquorum Schott.	Araceae	Tuber of this plant is taken with <i>Piper</i> nigrumL by the Ahom community of Dibrugarh as Lactagogue[5].
2	Tubuki lota	Cissampelos pareira L.	Menispermaceae	Leaf is used in labour pain [5].
3	Tengesi	Oxalis corniculata L.	Oxalidaceae	Paste of leaves is taken orally to treat burning menstruation [5].
4	Aparajita	Clitoria ternateaL.	Papilionaceae	Juice of the flower is mixed with goat milk and given orally for 3 days to prevent miscarriage [5].
5	Ada	Zingiber officinale Rosc	Zingiberaceae	Rhizome id taken orally in labour pain [5].
6	Bhete	Clerodendrum viscosum Vent	Verbenaceae	Juice of young branches is taken orally in gynaecological disorder[5].
7	anaros	Ananas comosus L	Bromeliaceae	Unripe fruit juice is taken for regular menstruation and abortion [6].
8	piyaaz	Allium cepa	Liliaceae	Bulb juice is warmed and taken twice a day for treating menstrual trouble [6].
9	Makhioti	Flemingia strobiliferaL.	Fabaceae	Root decoction is given in menstrual irregularities [7].
10	Jaluk	Piper nigrumL.	Piperaceae	The fruits are consumed inthe post labour ailment [7].





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11	Raktachandan	Adenanthera pavoninaL.	Fabaceae	Seed of this plant is taken orally to treat menstrual problem [8].
12	Narasingha	<i>Murraya koenigii</i> L. Spreng.	Rutaceae	Leaves are taken orally to treat menstruation problem [8].
13	Teteli	Tamarindus indicaL	Fabaceae	Fruit and flower of this plant is soaked overnight and the water is taken to treat menstrual irregularity [8].
14	aamoi Iota/Shaguni- Iata	Tinospora cordifolia (Willd.) Miers.	Menispermaceae	
15	Durun	Leucas aspera (Willd.)	Lamiaceae	Leaves of aamoi lota, durum and chobchini are grind to make tablets and
16	Chobchini	Smilax glabra Roxb.	Smilacaceae	taken with powder of ashwagandha and with juice of Ranga Joba during
17	Ashwagandha	Withania somnifera (L.)	Solanaceae	menstruation [8].
18	Ronga Joba	Hibiscus rosa-sinensis L	Malvaceae	
19	Kola -Dhatura	Datura metel	Solanaceae	Leaves and seeds are used in Dysmenorrhea [9].
20	Kulthimah	Dolichos biflorus	Fabaceae	Seed of this plant is used to treat dysmenorrhea [9].
21	Podum	Nelumbo nucifera	Nymphaceae	Flower of this plant is used to prevents Miscarriage [9].
22	Kadam	Anthoce phalus cadamba	Rubiaceae	Leaves and flower are used in labour pain [9].
23	Maha- bhringaraj	Wedelia chinensis	Asteraceae	Leaves are used in Menorrhagia [9]. Leaf juice is applied as hair tonic; juice is taken orally for 2-3 days to delay the menstruation [10].
24	Jetuka	Lawsonia alba Lamb	Lythraceae	Leaves are kept in water overnight and in the morning water is taken in empty stomach in the treatment of dysmenorrhoea [10].
25	Nilkantha	Ecbolium linneanum Kurz.	Acanthaceae	Root juice is taken orally in the treatment of premenstrual colic [10].
26	Kopalphuta	Physalis minima L.	Solanaceae	Leaves wrapped with banana leaf are roasted; juice is given to lactating mother to reducelactation [10].
27	Sotiyana	Alstonia scholaris	Apocyanaceae	The latex of leaves is applied on breast nipples to induce lactation in mothers after child birth [11].
28	Matikanduri	Alternanthera sessilis	Amaranthaceae	Leaf and stem used as food to increase lactation [12].
29	Boga-bahok	Justiicia adhatoda L.	Acanthaceae	Warmed juice is used as message on lower abdomen after childbirth for uterus contraction [12].





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30	Belipoka	Coccinia indicaW & A	Cucurbitaceae	Root juice is taken orally to stop bleeding during pregnancy [12].
31	Gakhiyoti bon	<i>Lindernia pusilla</i> (Willd.) Bold	Scrophulariaceae	Decoction is given to women after childbirth to promote milk [12].
32	Era	Ricinus communis	Euphorbiaceae	Juice of leaves and root taken orally to increase lactation in mother [12].
33	Ashok phul	Saracaindica	Fabaceae	Dried bark powder is mixed with milk to treat irregular menstruation [12].
34	Methiguti.	Trigonella foenumgraecum Linn	Fabaceae	Grinded seed taken with milk to fight with uterus infection [12].
35	Hatikhutora	Amaranthus spinosus Linn.	Amaranthaceae	Roots or stems are used in menorrhagia and to increase milk flow [13]. It is also used by boro tribes to Enhance fertility and increase lactation in nursing mother. Leaf and stem taken as vegetables. Paste of stem is made along with clove and is taken orally during Menstrual Cycle up to 3 days, once daily [10].
36	Titakerela	<i>Momordica charantia</i> Linn.	Cucurbitaceae	Leaves, fruits and seeds are used in dysmenorrhea [13].
37	Takoriaalu	Argyreia speciosa Sweet.	Convolvulaceae	The tuber is used as a Contraceptive[13].
38	Bormanimuni	<i>Centella</i> asiatica(Linn.) Urban.	Apiaceae	Leaves are used as blood purifier, given to women after childbirth [13]. This is also used for treating jaundicein pregnantwomen andin mothersand theirbabies [14].
39	Pat sadha	Nicotiana tabacum L.	Solanaceae	Leaf juice is taken during pregnancy by boro tribes as Anti- implantation/Abortifacient [14].
40	Bhedailota	Paederiafoetida	Rubiaceae	Leaf juice is taken to cure Menorrhagia[14]. Leaves are taken as paste to relieve pain after child birth[12].
41	Taruakadam	Acacia farnesiana (L.)Willd	Fabaceae	Paste made from bark along with Piper nigrumL. and taken orally duringMenstrual Cycle upto 3 days, once daily to treat Dysmenorrhea [15].
42	Amita	Carica papaya L	Caricaceae	Fresh fruit is tekenorally during pregnancy as Anti implantation/Abortifacient [15].
43	Dubari bon	Cynodonda ctylon	Poaceae	Crushed leaf juice is taken along with honey to cure Menorrhagia. Mixture of raw leaf along with rice and sugar taken orally to cure Leucorrhoea[15].





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44	Bonorialata	Mikania scandens	Asteraceae	Cleaned Stem is directly used in the uterus and is used in Anti-implantation and abortifacient [15].
45	Lajuki bon	Mimosa pudica	Febaceae	Flower is taken orally during Menstrual Cycle for 3 days to Enhance fertility [15].
46	Dhekialata	Lygodium flexuosum(L.) Sw.	Lygodiaceae	Paste of leaves is made from leaf along with <i>Piper nigrum</i> L. and is taken orally duringmenstrual cycle to treat menorrhagia [15].
47	Ashok Kamal	Polygonum hydropiper	Polygonaceae	Paste of leaves is taken orally during menstrual cycle to increase fertility [15].
48	Xilikha	Terminalia chebula Retz.	Combretaceae	Paste of fresh or dried fruit is taken orally and is used in abortion [15].
49	Athiakal	Musa balbisianaColla.	Musaceae	Cleaned root is directly used in the uterus for Anti-implantation or abortion [15].
50	Bhatotita	Phlogocanthus thyrsiflorus Nees	Acanthaceae	Paste made from leaf is taken orally up to 3 days during menstrual cycle to cure dysmenorrhea [15].
51	khejur	Phoenix dactylifera L	Aracaceae	Dried Fruit is taken orally after menstrual cycle for 7 days to enhance fertility [15].
52	xookloti	Pogostemon benghalensis (Burm.f.)Kuntze	Lamiaceae	Can be taken as curry along with <i>Piper nigrum</i> L. to treat Menstrual Cycle irregularity. It also helps to fight uterus infection after delivery, and is good for lactating mother [15].
53	Era gach	Ricinus communis L.	Euphorbiaceae	Cleaned root is directly used in the uterus as anti- implantation /abortifacient [15].
54	Bhaluka bah	Bambusa balcooaRoxb.	Bambosaceae	Tender shoot is cut in to small pieces and taken with jaggery during Menstrual Cycle for 3 days to treat Dysmenorrhea. [15]. Decoction of leaves mixed with cow's milk is given in menorrhagia, about 25ml in a cup of milk, thrice daily in periods [16].
55	Kona simolu	Commelina benghalensis∟.	Commelinaceae	Curry made of tender shoots is given in Menorrhagia and irregular menstruation, once daily for a week [16].
56	Halodhi	Curcuma longa L.	Zingiberaceae	Rhizome juice with milk is given in empty stomach for curing anaemia & in menstrual problems [16].
57	Antamul	Tylophora asthamatica	Asclepiadaceae	Leaves and roots are boiled and taken inempty stomach to stop excessive vaginal discharge [17].





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58	Bionisapota	Desmodium Iaxiforum DC.	Leguminosae	Leaves and stem are boiled with water and taken to treat menstrual cycle irregularity and uterus Infection [17].
59	Boga agechita	Plumbago zeylanica Linn.	Plumbaginaceae	Leaf and roots are crushed and boiled and taken to treat excessive bleeding during menstrual cycle. Also helps in terminating/Abortion [17].
60	Kaurimoni	<i>Coixlachryma-jobi</i> Linn.	Gramineae	Root juice is taken along with milk and honey for the treatment of all sort of ovary problems, and irregular menstrual.[17]
61	Bosh gos	AcorascalamusLinn.	Anaceae	The rhizome is crushed and the juice is taken to treat irregularity in menstrual cycle. It cures excessive uterine bleeding [17].
62	Keheraj	Eclipta alba Hassk.	Family-Asteaceae	Leaf and Stem are used in irregularity in menstrual cycle and labour pain [17].
63	Modar	ErythrinaindicaLim.	Leguminosae	Root is grinded and taken along with milk. It helps in conceiving, provides stamina [17].
64	Sewa-tamol	CaryotaurensL.	Arecaceae	Decoction of root is as galactogue to nursing mothers [18].
65	Sukuta	CeltistetrandraRoxb	Ulmaceae	Tender leaf is taken as vegetable and is said to be useful to relieve pain after childbirth [18].
66	Kawri-moni	Coixlachrya-jobiL.	Poacceae	Root juice is good for menstrual trouble [18].
67	Borahu	Curcuma zedoaria Rosc.	Zingiberaceae	Juice of the rhizome is given to women after child birth to remove weakness and is said to act as blood purifier [18].
68	Gakhiroti bon	Euphorbia hirtaL.	Euphorbiaceae	Tender shoot is taken as vegetable by nursing mother to help in the lactation[18].
69	Sthalapadma	Hibiscus mutabilisL.	Malvaceae	Paste of flower is given in menorrhagia [18].
70	Panikolmow	Ipomoea aquatica Forsk.	Convolvulaceae	Tender shoot is taken as a vegetable and said to be useful as galactagogue to nursing mothers [18].
71	Setkopora	Mollugo pentaphylla∟.	Aizoaceaea	Leaf juice is prescribed to women after childbirth [18].
72	Nephaphu	Clerodendrum colebrookianumWalp	Verbenaceae	Decoction of leaves is taken orally in menstrual pain [19].
73	Kola-halodhi	Curcuma caesia Roxb.	Zingiberaceae	Rhizome is taken orally to treat menstrual pain [19].
74	Bihlogoni	Dryopteris filix-mas (L.) Schott	Dryopteridaceae	Decoction of leaves is taken orally in recovery after giving birth [19].





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75	Maudimoru	Ficus racemosa L.	Moraceae	Decoction of leaves is taken orally in recovery after giving birth [19].
76	Sorumanimun i	Hydrocotyle sibthorpioidesLam.	Araliaceae	Paste of whole plant is used to treat leucorrhoea [19].
77	Tora	Alpinia nigra (Gaertn).B.L.Burtt	Zingiberaceae	Paste of rhizome is used in leucorrhea [19].
78	Dudhkorilota	Ichnocarpusfrutescens R.Br.	Apocynaceae	Whole plant is used as Galactogogue [19].
79	Damdeuka	Impatiens tripetalaL.	Balsaminaceae	Root, stem and leaves are used in Menstruation problem and leucorrhea [19].
80	Roghumola	Macrosolencochinchin ensis	Loranthaceae	Juice of leaves are taken orally in menstruation pain [19].
81	Himolualu	<i>Manihatesculenta</i> cra ntz.	Euphorbiaceae	Paste of bark is used to treat leucorrhea [19].
82	Nuni	Morus alba L	Moraceae	Raw fruit is Used in menstruation pain [19].
83	Gorokhia korai	Abromaaugusta L.	Sterculiaceae	Root paste is used in breast cancer [19] and juice is considered as uterine tonic [12].
84	Bonjaluk	Stenoclaenapalustris(Burm.f.) Bedd	Blechnaceae	Decoction is taken orally during Menstruation pain [19].
85	Bhuiamlakhi	Phyllanthus fraternusG.L.Webste r;	Phyllanthaceae	Juice of the fruit is taken oraly in menstration pain [19].
86	Pokmo	Physalis minima L.	Solanaceae	Paste of leaves are taken orally in menstrual pain [19].
87	Bon Jora/ Mirikatenga	Elaeagnus caudata	Elaeagnaceae	About 5ml of the fresh root extract is diluted in ~100 ml of water and taken once a week during pregnancy to prevent miscarriage [20].
88	Bhuterchira	Desmodium laxiforum	Fabaceae	Leaves and stem are used to treat amenorrhoea and uterine infection [3].
89	Bihlongoni	Macrothelypteris 2quati (Wall. Ex Bedd.) Ching	Thelypteridaceae	Tender leaves are cooked with chichen to feed mother to increase the milk production for new born baby[21].
90	Ronga- Kanchan	Bauhinia purpurea L	Caesalpiniaceae	Barkjuice is also useful in menstruation trouble [22].
91	Bon dhonia	Scoparia dulcis L	Scrophulariaceae	Plant juice is useful againstirregular menstruation trouble [22].
92	Ubhot - kata	Achyranthes aspera L	Amaranthaceae	Leaf juice is also usedin menstruation trouble [22]. Leaf juice is taken orally at a time to





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				induce labour [10].
93	BhringarajHuh uniSak	Spilanthes paniculata L	Asteraceae	Mishing womenboil the leaves with black-pepper (jaluk) and fed tomother after childbirth to increase the milk [23].
94	Neem	Azadirachta indica A Juss	Meliaceae	Grounded root juice is taken orally by the karbi women as antifertility agent [24].
95	Barun	Crataeva nurvala Buch. Ham.	Capparaceae	Tender shoots are taken as a tonic for general weakness and is given to women after child birth [24].
96	MahudiMakh udi	Croton joufra Roxb	Euphorbiaceae	Leaves are warm gently and massage over the abdomen to relieve from acute stomach pain soon after delivery. It is repeated many times daily till the pain subsides [24].
97	Chulumpui	<i>Inula cappa (</i> D.Don) DC	Asteraceae	Crushed leaf juice is applied with a quill on vaginal wound caused due to delivery. This is reported to give effective result by drying the wound within a short period [24].
98	Kath- alu	Dioscorea alata L	Dioscoreaceae	Boiled tubers are given to mother for general weakness after delivery [24].
99	Masundari	Houttuynia cordata Thunb	Saururaceae	Leaves are boiled and mixed with boiled tubers of Solanumtuberosum and taken orally in general weakness after delivery; it is taken 2-3 times daily till the mother regains strength [24].
100	Hila guti	Micromelum integerrimum (Roxb.	Rutaceae	Water extract of the stem is given to women to hasten the process of delivery. Or tender shoots are cooked and given at least one day before expected date of delivery for initiation of delivery [24].
101	Bapbongnaiari kang	<i>Mikania scandens</i> (L.) Willd	Asteraceae	Tender shoots of the twinning herbs are baked and applied thrice daily on the vaginal wound after delivery. It is repeated till the wounds are healed [24].
102	Dhaan	<i>Oryza sativa</i> L	Poaceae	Water extract of grains are taken during labour to relieve pain [24].
103	AgechitAgyiac hitRonga	Plumbago zeylanica L.	Plumbaginaceae	It is an important abortifacient known among Karbis folk of Assam. Stem is tied around the thigh region with a long thread and then inserted in the vagina to initiate abortion [24].
104	Ahot	Ficusreligiosa	Moraceae	About 15 ml bark decoction mixed with a pinch of rock salt is given to women after child birth to relieve pain twice daily for three days [25].





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	1	ı		T =
405			•	Boiled rhizome is prescribed for irregular
105	Chengmora	Lasiaspinosa(L.) Thw	Araceae	menstruation and juice of the same is
				given in leucorrhoea [26].
				Leaves are crushed and the juice is mixed
106	Dhania	Coriandrum sativum	Apiaceae	with equal volume of water. It is taken
		Linn.		orally (10 ml) three times daily to stop
				vomiting in pregnancy[27].
				Shoots are crushed and juice extracted,
		Alternanthera		half cup of juice mixed with one cup of
107	Bishalyakarani	brasiliana (L.)	Amaranthaceae	milk; taken internally; whole preparation
		Kuntze		once in empty stomach for 3 days to treat
				excessive bleeding during menstruation
				[28].
108	kopah	Gossypium herbacium	Malvaceae	Root extract is taken orally to treat
100	Корин	L.	Marvaccac	menstruation problem [29].
		Sagittaria		Plant juice (30-35 ml) mixed with half the
		guayanensis lappula		amount of honey is used regularly 3-5
109	Panikochu	(D.Don) Bogin	Alismataceae	times in a day to induce flow of lochia
				after childbirth [30].
				Two to three teaspoonful fresh leaves
				juice mixed with keheraj
110	Suhani-Bon	Spilanthes acmella	Asteraceae	(Ecliptrlaprostrata L.) and little amount
110	Sullatii-buii	Murr.	Asieraceae	of jaluk (Piper nigrumL.) seed powder is
				given once aday in empty stomach in
				leucorrhoea [31].
111	Coverdinate	Nyctanthes	Oleanes	Rootinfusion is given orally once a day in
111	Sewaliphool	arbor-tristis L.	Oleaceae	menstrualirregularities [31].
		Meyna		
112	Kotkora	laxiflora Robyns.	Rubiaceae	Pulpdecoction is given for abortion [31].
		Averrhoea carambola		
113	Kordoi	L.	Oxalidaceae	Fruit infusion of is prescribed once a day
113	Kuruur	L.	Oxanuaceae	for7 days in leucorrhoea [31].
		Adhatada yasisa Mill		Loof deposition is taken in othering
114	Titabahak	Adhatoda vesica Mill.	Acanthaceae	Leaf decoction is taken in uterine
-				problems [32].
115	Nayantora	Catharan thus roseus	Apocynaceae	Decoction of root is prescribed to
113				enhance reproductive health [33].
11/	Titolale el cons	Solanum indicum	Colomi	Root paste is given orally thrice a day for
116	Titabhekuri	Linn	Solanaceae	5-6 days to treat menstrual disorder [34].
	1			1 ,

DISCUSSIONS

Because of the moist tropical weather different types of plants are available in Asam. Though medical science has reached an illustrious position in recent times, use of herbal medicine still has massive popularity because of its immeasurable benefits and lesser known side effects. There is a considerable demandin extracting new medicines from identified parts of known plants.





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RESEARCH ARTICLE

Impact of Social Media on Consumer Behavior - A Study W.R.T. Bengaluru Urban

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ABSTRACT

Globalization has altered people's lifestyles, and as a result, they have become increasingly reliant on social media. Customers are traveling all over the world due to shifting demographics and the influence of western culture, and these consumers are forming tight networks and living more similar lives. Multiple social media platforms impact consumers and create new channels for online interaction. Users create material for online communities and connect with others via social media (Nick Hajli, 2013). Various possibilities exist in social media to pinpoint the advantages that corporations may gain. It takes work to interpret customer habits. As a result of their research into consumer habits, marketers have come to some beneficial conclusions about what their target demographic wants and needs. As such, the marketer surveys customer behavior to get insight into the target audience's social, psychological, and emotional motivations (Suja R. Nair, 2013). The new kind of customer spends much time on social media to create and spread cutting-edge information on specific goods and services. The research shows that there are various reasons why people utilize social media and that these characteristics affect consumers' behavior.

Keywords: Social media, consumer behavior, buying style, factors, reasons, brand popularity.

INTRODUCTION

The impact of social media on customers' perspectives and actions has been revolutionary. Because of the widespread availability of the internet, people can communicate with one another through online platforms like email, Twitter, and Facebook without having to meet (Grudz et al. 2011) physically. The proliferation of social media





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has increased the number of people who shop and learn about products online (Chen et al., 2011). The use of social media to increase a company's profile among customers is a powerful tool (de Vries et al., 2012). The many marketers who actively participate by tweeting, liking, and commenting are facilitating openness in social media. When social media grows into a powerful media platform and an intrinsic part of the purchasing and selling process, it will be impossible for them to deny the influence of social proof. As billions of people are actively using social media platforms, this kind of communication is highly potent. Marketer can find their ideal clientele through social media and learn more about their tastes and preferences. Influence, inspiration, and altered consumer behavior can all be attributed to renowned people and celebrities. Brands may benefit significantly from the efforts of influencers who use their considerable influence to increase product awareness and sales. Today's marketers must consider not just one but all three of the personal, psychological, and societal variables that shape customer choices. Individual preferences and perspectives are influenced by their background and upbringing, which are considered personal variables. Finally, social aspects include one's peer group, socioeconomic status, and the impact of social media, while psychological ones deal with one's views and attitudes.

Significance of the study

As the world becomes more interconnected, several social media platforms have emerged as a means by which product and service consumers may exchange and produce information. Multiple social media platforms provide, display, and deliver data-rich mate. People are drawn to online media to interact socially and share knowledge (Ridings & Gefen, 2004). There is value for both the consumer and the producer in the reviews that customers have willingly provided for various products and services (Nambison, 2002). Companies in today's highly competitive market aggressively solicit customer feedback through ratings and reviews (Bronner et al., 2010). Consumers have benefited greatly from the widespread adoption of new marketing strategies made possible by the proliferation of social media (Hanna et al., 2011). What consumers do before, during, and after a purchase is all influenced by social media (Mangold et al., 2009). The proliferation of online consumer reviews has been documented (Chris et al., 2008). Consumers care more about what their peers have to say about a product than they do about what the manufacturer or retailer has to say, according to studies of social (Ridings and Gefen, 2004). As a result of their ability to rally an online community behind a cause, social media have grown in influence. Trust in online transactions is more likely to be imposed by social interactions reinforced by social media for both vendors and buyers.

Review of literature

According to Sin et al. (2012), "social networks" are online communities that bring together millions of people from all over the world who share common goals, perspectives, and pastimes. The writers said that all consumers are avid social media users, including blogs, YouTube, MySpace, and Facebook. According to Kozinets (2002), users employ a variety of communication formats to discuss a product, service, or brand with one another and reach out to other customers, who are trusted as more impartial information resources. According to Margold and Paulus (2009), the unprecedented success of social networking sites has radically altered traditional forms of advertising and public relations. According to Mersey et al. (2010), companies may engage and communicate with potential clients on social media platforms, leading to closer ties between the two parties. According to Chitranshi Verma (2018), using social media for customer service and assistance has become increasingly common. Experts in this field have recommended that businesses have a digital presence to provide this aid.

Research Methodology

The focus of this analysis is only on Bengaluru. The research was undertaken in urban Bengaluru using a questionnaire to learn how social media influences customer behavior. Ninety percent of the respondents are from inside Urban Bengaluru, while the survey did receive responses from a few international participants. The survey used in the study used a Likert scale with three levels of agreement: strongly agree, agree, and disagree. The table's claims were culled from existing studies to bolster the reliability of the current research.





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Data collection and sources of data

This study draws from a variety of sources, both primary and secondary. In this case, a well-designed questionnaire served as the primary source of information. Through persuasion, request, and appeal, we gathered 240 surveys. A total of 240 surveys were administered, and 200 of these were deemed valid for an efficiency rate of 83% in monitoring and administration. Books, e-journals, and other types of published and unpublished materials are all examples of secondary resources.

Statistical tools performed

Kendall's coefficient of concordance (W) is performed to measure the factors impacting consumer behavior and reasons behind the use of social media. Further, Garrett's ranking technique is performed to rank why companies are keen on social media.

Objectives

- 1) To analyze and measure the factors impacting consumer behavior.
- 2) To analyze and measure the reasons behind using social media.
- 3) To analyze the factors behind companies keen on using social media.

Hypotheses

- 1) There are no factors impacting consumer behavior.
- 2) There are no reasons behind the use of social media.
- 3) There are no factors for keen on using social media by companies.

Limitations

- 1) The study is confined only to Bangalore Urban.
- 2) Any generalization requires further in-depth study.
- 3) The problem of transportation and social distance felt but successfully managed to collect the data.

Survey findings

Table - 2 reveals data about factors influencing consumer behavior. 120 respondents out of 200 forming 60% said strongly agree with the statements, followed by 50(25%) blend and 30(15%) somewhat agree. Kendall's coefficient of concordance is a crucial non-parametric measure of the relationship between two variables being higher than the TV and thus fails to accept H0 and accepts H1. Therefore, a significant relationship exists between factors and consumer behavior. Table - 3 highlights data about factors impacting social media. One hundred twenty-three respondents strongly agree with the statements, followed by 46 approve, and 31 disagree. Kendall's metric, being higher than TV, fails to accept null hypotheses and takes alternatives. Therefore, it is concluded here that there exists a significant relationship between the variables. Tables - 4 and 5 present data through the weighted average technique about the reasons behind companies keen on social media. Table - 5 reveals that a 61-score assigned to social interaction is rising. 60 score is given to customers who prefer to share, review, and recommend online & 59 score is given to changes in demographics, enhancing brand value, trust building, and reduction in perceived risk, facing competition, and recommendations are used by consumers online. The remaining statements are assigned a score depending on the strength of the total and WA.

CONCLUSION

Consumers are now more connected than ever because of social media's rise. Bengaluru is known as the "Silicon Valley" due to its prominence as a center for the IT, textile, and apparel sectors. Consumers in Bengaluru, India, are adopting a new method of online shopping known as "social commerce" because they are comfortable with the concept of "virtual word of mouth" and sharing personal experiences through such channels. Because of their busy schedules, people in Bangalore depend more on internet marketing and social media. Customers in Bengaluru expect to be able to reach a company for help through social media. Therefore, local businesses have a strong online presence. The trend of making digital media more visible is beneficial to the growth and development of brands.





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Table 1: Sample and sampling technique

S.No	Area	Population universe Sample	
1	Malleshwaram	36321	67
2	Rajajinagar	33231	62
3	Jayanagar	38151	71
	Total	107703	200

Sources: Population census - 2020 estimations.





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Table - 2: Factors impacting consumer behavior

Drivers of consumer behavior	SA	Α	SWA	RT	RT ²
Online campaign	18	7	4	29	841
Perception & attributes (Psychology)	12	5	3	20	400
Personality and lifestyle	8	5	4	17	289
Socio-economic characteristics	19	8	3	30	900
Trustworthiness	10	3	4	17	289
Attraction	9	3	3	15	225
Earned expert knowledge	8	4	2	14	196
Online influence	16	6	2	24	576
Motivation by influencers & social media	20	9	5	34	1156
Total	120	50	30	200	4872

Source: Field Survey

Note: RT - Row Total, w = Kendall's coefficient of concordance

SSR = Sigma RT^2 - [(Sigma RT) 2 / N]

 $= 4872 - [(200)^2 / 9] = 4872 - 40000 / 9 = 4872 - 4444.4444 = 427.56$

 $W = 12 SSR / [k^2n (n^2 - 1)]$ = 12 x 427.56 / 9 x 9 (81 - 1) = 5130.72 / 81 x 80

= 5130.72 / 6480 = 0.7918

Test for the significance of w using the Chi-square statistic

 $x^2 = k(n-1)w$ with n-1 df = 3(9-1) 0.7918 = 3 x 8 x 0.7918 = 19.0032

Decision: At eight df @ 5% significance level, TV = 15.507. The calculated value being 19.0032 higher than the TV, rejects the H0 and accepts the alternative; hence, it is concluded that there is a relationship between factors driving and consumer behavior.

Table - 3: Reasons behind the use of social media

Reasons behind used of social media	A	N	DA	RT	RT ²
Entertainment	15	8	6	29	841
Spending leisure time	16	7	3	26	676
Information seeking	25	8	6	39	1521
Social interaction	6	3	2	11	144
Online shopping	16	6	3	25	625
Comparing with other information sources	11	3	2	16	256
Easy access	10	3	2	15	225
Social support	14	5	4	23	529
Reduction in perceived risk	10	2	3	15	225
Total	123	46	31	200	5042

Source: Field Survey

Note: RT - Row Total, W - Kendall's coefficient of concordance.

SA - Strongly Agree, A - Agree, SWA - Some What Agree

SSR = Sigma RT^2 - [(Sigma RT) 2 / N]

 $= 5042 - [(200)^2 / 9] = 5042 - 4444.44 = 597.56$

 $W = 12 SSR / [k^2 n (n^2 - 1)]$

= 12 x 597.56 / 9 x 9(81 - 1) = 7170.72 / 6480 = 1.1065





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 x^2 = Test for the significance of w using the x^2 statistic

Decision: At eight df @ 5% significance level, TV = 15.507. The calculated value being 26.550 higher than the TV, rejects the H0 and accepts H1. Therefore, it is concluded here that a relationship exists between reasons and social media use.

Table 4: Factors impacting companies' use of social media

Areas impacting keenness	SA	Α	N	DA	SDA	Т
Brand Enhancement value	110	65	25	-	-	200
Enhanced sales & customers attraction	100	55	20	15	10	200
Increase in management reputation	90	50	30	20	10	200
Customer service	115	55	20	10	-	200
Changes in demographics	125	45	15	15	-	200
Social interaction is on raise	140	43	8	7	2	200
Word of mouth through electronic media	80	55	30	30	5	200
Trust building & reduction in perceived risk	123	54	12	9	2	200
Facing competition	130	40	12	15	3	200
Customers prefer to share reviews& recommend online	135	45	8	7	5	200
Recommendations are used by customers online	130	41	15	8	6	200

Source: Field Survey

Note: SA - Strongly Agree, A - Agree, DA- Disagree, SDA - Strongly Disagree

Table 5: Weighted average analysis

Areas impacting keenness	SA	Α	N	DA	SDA	T	WA
Brand Enhancement value	550	260	75	-	-	88559	59
Enhanced sales & customers attraction	500	220	60	30	10	820	55
Increase in management reputation	450	200	90	40	10	790	53
Customer service	575	220	60	20	-	875	58
Changes in demographics	625	180	45	30	-	880	59
Social interaction is on raise	700	172	24	14	2	912	61
Word of mouth through electronic media	400	220	90	60	5	775	52
Trust building & reduction in perceived risk	615	216	36	18	2	887	59
Facing competition	650	160	36	60	3	879	59
Customers prefer to share reviews& recommend online	675	180	24	14	5	898	60
Recommendations are used by customers online	650	164	45	16	6	881	59

Source: Authors Compilation

Note: (1) The researcher has used a scale such as above, and weights are assigned in the order 5, 4, 3, 2, 1.



 $x^2 = k(n-1)w$ with n-1 df = 3(9-1) 1.1065 = 3 x 8 x 1.1065 = 26.556



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RESEARCH ARTICLE

Total Global Weight Domination Number of S-Valued Graphs

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ABSTRACT

In [5] the authors introduced the notion of semiring-valued graphs. They also studied weight dominating vertex set and weight domination number on S-valued graphs. In this paper we investigate total global weight dominating vertex set on S- valued graphs and the total global weight domination number $\gamma_{TG_D}(G^S)$ Also bounds are obtained for $\gamma_{TG_D}(G^S)$ and we discuss about the total global domination number for S-valued paths, cycles and bipartite graphs.

2010 AMS Classification: 16Y60, 05C25, 05C76

Keywords: S - Valued graphs, Weight dominating vertex set, Total weight domination vertex set in Svalued graphs, Total global weight domination on S-valued graphs.

INTRODUCTION

All graphs G^{S} (S-valued graphs) [3] considered here have order p and size q (i.e., p vertices and q edges) and both G^{S} and their complements $\overline{G^S}$ have no isolates. The concepts of total dominating sets and total domination number were introduced in [2]. In [3] the authors studied about the weight dominating vertex sets, weight domination number, global weight dominating vertex sets and global weight domination number on S-valued graphs. In [6] the authors gave extension to total weight dominating vertex sets on S-valued graphs. A total weight dominating vertex set $T_D \subseteq V$ of G^S is a total global weight dominating vertex set of a S-valued graph if $T_D \subseteq V$ is also a total weight





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dominating vertex set of $\overline{G^S}$ It is denoted by TG_D . A subset $TG_D \subseteq V$ is said to be a minimal total global weight dominating vertex set of G^S and no proper subset of TG_D is a total global weight dominating vertex set of G^S . In the present work, we derive some results for the total global dominating vertex sets of a S-valued graph and find the total global weight domination number of certain graphs.

Preliminaries

Definition 2.1 [4]

A Semiring (S, +, .) is an algebraic system with a non-empty set S together with + and \bullet such that 1.(S, +, 0) is a monoid. $2.(S, \bullet)$ is a semigroup. $3.\text{For all a,b,c} \in S, a \bullet (b+c) = (a \bullet b) + (a \bullet c) \text{ and } (a+b) \bullet c = (a \bullet c) + (b \bullet c)$ $4.0 \bullet x = x \bullet 0 = 0 \forall x \in S$

Definition 2.2 [3]

Let $(S, +, \bullet)$ be a semiring. A relation \leq is said to be a canonical pre-order if for $a,b \in S$, $a \leq b$ if and only if there exists $c \in S$ such that a+c=b

Definition 2.3 [3]

Let $G = (V, E \subset VXV)$ be the underlying graph with both $V, E \neq \emptyset$. For any semiring $(S, +, \bullet)$ a semiring valued graph (or an S-valued graph) G^S is defined to be the graph $G^S = (V, E, \sigma, \psi)$ where $\sigma: V \to S$ and $\psi: E \to S$ is defined to be $\psi(x, y) = \begin{cases} \min(\sigma(x), \sigma(y)), & \text{if } \sigma(x) \leq \sigma(y) \text{ or } \sigma(y) \leq \sigma(x) \\ 0 & \text{otherwise} \end{cases}$ For every unordered pair (x, y) of $E \subset VXV$ we call σ a S-vertex set and ψ an S-edge set of S-valued graph G^S

Definition 2.4 [3]

Consider the S-valued graph G^S = (V, E, σ , ψ). For $v_i \in V$, the open neighborhood of v_i in G^S is defined as a subset of V x S such that that

 $N_S(v_i) = \{(v_j, \sigma(v_j))/(v_i, v_j)\} \in \mathbb{E} \cdot \psi(v_i, v_j) \in \mathbb{S}\}$. For $v_i \in V$ a closed neighborhood of v_i in G^S is defined to be the subset of VXS such that $N_S[v_i] = N_S(v_i) \cup \{(v_i, \sigma(v_i))\}$

Definition 2.5 [3]

The degree of the vertex v_i of the S-valued graph G^S is defined as $deg_S(v_i) = (\sum_{(v_i,v_j) \in E} \psi(v_i,v_j)), l$) where l is the number of edges incident with v_i

Definition 2.6 [3]

In the S-valued graph $G^S = (V, E, \sigma, \psi)$, to compare the degree of two vertices $v, w \in G^S$, we define the \leq as follows:

- $(\sigma(v), \deg(v)) \leq (\sigma(w), \deg(w)) \Leftrightarrow (\sigma(v) \leq \sigma(w))$ and $\deg(v) \leq \deg(w)$
- If $(\sigma(v) \le \sigma(w))$ and $\deg(v) \ge \deg(w)$, the comparison is with respect to the S-values

Definition 2.7 [3]

Let $G^S = (V, E, \sigma, \psi)$ be a given S-valued graph. A vertex v in G^S is said to be a weight dominating vertex if $\sigma(u) \le \sigma(v) \forall u \in N_S[v]$

Definition 2.8 [3]

A subset $D \subseteq V$ is called a weight dominating vertex set of G^S if for each $v \in D$, $\sigma(u) \leqslant \sigma(v)$, $\forall u \in N_S[v]$. The minimum cardinality of a weight dominating set of G^S is called a weight domination number of G^S which is denoted by $\gamma^S(G^S)$ and the corresponding weight dominating set is called a γ^S – set of G^S .





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Definition 2.9. [3]

Let $G^S = (V, E, \sigma, \psi)$ be a given S-valued graph. The cardinality of the minimal weight dominating vertex set $D \subseteq V$ is called the weight dominating vertex number of G^S which is denoted by $\gamma^S(G^S)$ That is $\gamma^S(G^S) = \min\{(|D|s, |D|)/D \text{ is a weight dominating set vertex set of } G^S\}$

Here |D| sdenotes the scalar cardinality of D and |D| denotes the number of vertices in D

Definition 2.10 [3]

Let $G^S = (V, E, \sigma, \psi)$ be a given S-valued graph. If D is a weight dominating vertex set of G^S then the scalar cardinality of D denoted by |D|s is defined by $|D|s = \sum_{v \in D} \sigma(v)$.

Definition 2.11 [1]

The complement \bar{G} of a simple graph G is the simple graph with vertex set V, two vertices being adjacent in \bar{G} iff they are not adjacent in G.

Definition 2.12 [1]

A Dominating set $D\subseteq V$ of a graph G is said to be a global dominating set if D is also a dominating set in the complement of G.

Definition 2.13 [6]

Consider the S-valued graph G^S = (V, E, σ , ψ). A total weight dominating vertex set $T_D \subseteq V$ of G^S is a total global weight dominating vertex set of a S-valued graph if $T_D \subseteq V$ is also a total weight dominating vertex set of $\overline{G^S}$ It is denoted by TG_D

Definition 2.14 [6]

A subset $TG_D \subseteq V$ is said to be a minimal total global weight dominating vertex set of G^S if TG_D is a total global weight dominating vertex set of G^S and no proper subset of TG_D is a total global weight dominating vertex set of G^S

Definition 2.15 [6]

The cardinality of the minimal total global weight dominating vertex set $TG_D \subseteq V$ is called the total global weight domination vertex number of G^S and it is denoted by $\gamma_{TG_D}(G^S)$ That is $\gamma_{TG_D}(G^S) = \min\{|TG_D|s, TG_D\}$ where TG_D is a total global weight dominating vertex set of G^S

Definition 2.16 [6]

Let $G^S = (V, E, \sigma, \psi)$ be a given S-valued graph. V is said to be a total weight dominating vertex set of G^S if the following conditions are satisfied

- (i) For any $v \in T_D \sigma(u) \leq \sigma(v)$, $\forall u \in N_S[v]$.
- (ii) For each $e \in E$, $\psi(e) \le \sigma(v)$ and e is incident with some $v \in T_D$.
- (iii) $N_S[T_D] = V_S$.

ILLUSTRATION

Example 3.1

Consider the semiring $S=(\{0,a,b,c\},+,\cdot,\leq)$ with the following Cayley tables.

+	0	a	b	С
0	0	a	b	С
a	a	b	b	С
b	b	С	С	С
С	С	С	С	С

	0	a	b	С
0	0	0	0	0
a	0	a	b	с
b	0	b	b	С
c	0	С	С	С

\preceq	Elements of S
0	0, a, b, c
a	a, b, c
b	b, c
С	c,



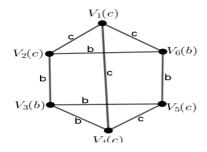


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Consider the S-valued graph $G^S = (V, E, \sigma, \Psi)$ where $\sigma: V \rightarrow S$ and $\psi: E \rightarrow S$ are defined to be $\sigma(v_1) = \sigma(v_2) = \sigma(v_2) = \sigma(v_3) = \sigma$

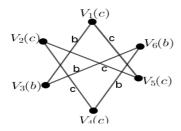


Consider $\overline{G^S}$ D = $\{v_1, v_4\}$. $N_S[v_1] = \{(v_1, c), (v_2, c), (v_4, c), (v_6, b)\}$

 $\sigma(v_2) = c \leq c = \sigma(v_1), \ \sigma(v_4) = c \leq c = \sigma(v_1) \ \text{and} \ \sigma(v_6) = b \leq c = \sigma(v_1)$

 $N_S[v_4] = \{(v_3,b), (v_4,c), (v_5,c), (v_1,c)\}, \ \sigma(v_3) = b \le c = \sigma(v_4), \ \sigma(v_5) = c \le c = \sigma(v_4) \ \text{and} \ \sigma(v_1) = c \le c = \sigma(v_4) \ \text{clearly}$ D is a weight dominating vertex set of G^S

Consider D= $\{v_1, v_4\}$



 $N_S[v_1] = \{(v_1, c), (v_3, b), (v_5, c)\}, \sigma(v_1) = c \le c = \sigma(v_1) \text{ and } \sigma(v_3) = b \le c = \sigma(v_1) \text{ and } \sigma(v_2) = b \le c = \sigma(v_1) \text{ and } \sigma(v_2) = b \le c = \sigma(v_1) \text{ and } \sigma(v_2) = b \le c = \sigma(v_1) \text{ and } \sigma(v_2) = b \le c = \sigma(v_1) \text{ and } \sigma(v_2) = b \le c = \sigma(v_1) \text{ and } \sigma(v_2) = b \le c = \sigma(v_1) \text{ and } \sigma(v_2) = b \le c = \sigma(v_1) \text{ and } \sigma(v_2) = b \le c = \sigma(v_1) \text{ and } \sigma(v_2) = b \le c = \sigma(v_1) \text{ and } \sigma(v_2) = b \le c = \sigma(v_1) \text{ and } \sigma(v_2) = b \le c = \sigma(v_1) \text{ and } \sigma(v_2) = b \le c = \sigma(v_1) \text{ and } \sigma(v_2) = b \le c = \sigma(v_1) \text{ and } \sigma(v_2) = b \le c = \sigma(v_1) \text{ and } \sigma(v_2) = b \le c = \sigma(v_1) \text{ and } \sigma(v_2) = b \le c = \sigma(v_1) \text{ and } \sigma(v_2) = b \le c = \sigma(v_1) \text{ and } \sigma(v_2) = b \le c = \sigma(v_$

 $\sigma(v_5) = c \leq c = \sigma(v_1)$

 $N_{S}[v_{4}] = \{(v_{4}, c), (v_{2}, c), (v_{6}, b)\}, \sigma(v_{4}) = c \le c = \sigma(v_{4}) \text{ and } \sigma(v_{2}) = c \le c = \sigma(v_{4}) \text{ and } \sigma(v_{6}) = c \le c = \sigma(v_{4})$

clearly D is a weight dominating vertex set of $\overline{G^S}$. Hence D is a global weight dominating vertex set of G^S . Also, D is a minimal global weight dominating vertex set of G^S and hence $\gamma_{G_D}(G^S)=(c,2)$.

In the above example,

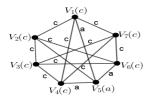
- (i) Clearly for any $v \in T_D = \{v_1, v_4\} \sigma(u) \le \sigma(v) \forall u \in N_S[v]$.
- (ii) For each $e \in E$, $\psi(e) \le \sigma(v)$ but e is not incident with some $v \in T_D$.

Therefore, D is not a total weight dominating vertex set of G^{S} .

Consider $T_D = \{v_1, v_2, v_3, v_4, v_5\}$. Here T_D is a total weight dominating vertex set which is minimal and the total weight domination number is $\gamma_{T_D}(G^S) = (c,5)$ and it is also a minimal total global weight dominating vertex set, therefore $\gamma_{TG_D}(G^S) = (c,5)$

Example 3.2

Consider the S-valued graph $G^S = (V, E, \sigma, \Psi)$ where $\sigma: V \rightarrow S$ and $\psi: E \rightarrow S$ are defined to be $\sigma(v_1) = \sigma(v_2) = \sigma(v_3) = \sigma(v_4) = \sigma(v_6) = \sigma(v_7) = c$ and $\sigma(v_5) = a$







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 $T_D = \{v_1, v_2, v_3, v_4, v_6, v_7\}$

Clearly

(i) For any $v \in T_D \sigma(u) \leq \sigma(v) \forall u \in N_S[v]$

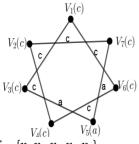
(ii) For each e \in E, ψ (e) $\leq \sigma(v)$ and e is incident with some $v \in$ TD

(iii) $N_S[T_D] = V_S$

So, T_D is a total weight dominating vertex set of G^S , it is a minimal total weight dominating vertex set of G^S and therefore

(iv) $\gamma_{T_D}(G^S) = (c,6)$

Consider $\overline{G^S}$



 $T_D = \{v_1, v_2, v_4, v_6, v_7\}$

Here also

(i) For any $v \in T_D \sigma(u) \leq \sigma(v) \forall u \in N_S[v]$

(ii) For each $e \in E$, $\psi(e) \leq \sigma(v)$ and e is incident with some $v \in T_D$

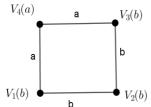
 $(iii)N_S[T_D] = V_S$

So, T_D is a total weight dominating vertex set of $\overline{G^S}$,it is a minimal total weight dominating vertex set of $\overline{G^S}$ and therefore, $\gamma_{T_D}(\overline{G^S}(c,6))$. It is a total global weight dominating vertex set of G^S . Also, T_D is a minimal total global weight dominating vertex set of G^S and therefore $\gamma_{T_{G_D}}(G^S)$ = (c,6)

Example 3.3

Consider the following S-valued graph G^S = (V, E, σ , Ψ) where σ : V \rightarrow S and ψ :E \rightarrow S are defined to be $\sigma(v_1) = \sigma(v_2) = \sigma(v_3) = b$ and $\sigma(v_4) = a$.

Here \leqslant is a canonical pre-order in S given by $0\leqslant 0$, $0\leqslant a$, $0\leqslant b$, $0\leqslant c$, $a\leqslant a$, $a\leqslant b$, $a\leqslant c$, $b\leqslant b$, $c\leqslant b$, $c\leqslant c$ $\Psi(v_1,v_2)=\Psi(v_2,v_3)=b$ and $\Psi(v_3,v_4)=\Psi(v_4,v_1)=a$



 $T_D = \{v_1, v_3\}$ is a total weight dominating vertex set of G^S since it is minimal $\gamma_{T_D}(G^S) = (b, 2)$. Here $\overline{G^S}$ is

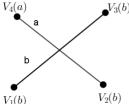




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 $TG_D = \{v_1, v_2, v_3\}$ is a total global weight dominating vertex set of G^S since it is minimal $\gamma_{TG_D}(G^S) = (b, 3)$ Therefore $\gamma_{T_D}(G^S) \leq \gamma_{TG_D}(G^S)$

RESULTS

Theorem 4.1

A total weight dominating vertex set T_D of G^S is a total global weight dominating vertex set of G^S if and only if for each vertex $(v, \sigma(v))$ εV there exists a vertex $(u, \sigma(u))$ εT_D such that $(v, \sigma(v))$ is not adjacent to $(u, \sigma(u))$

Proof

Let TG_D be the total global weight dominating vertex set of G^S .

Let $(v, \sigma(v))$ be any vertex in $V(G^S)$.

Suppose that this vertex v is adjacent to all the vertices in T_D then in $\overline{G^S}$, this vertex v is adjacent to all the vertices in TG_D . Then in $\overline{G^S}$, some edges connecting to this vertex v not incident with some $(u, \sigma(u)) \varepsilon T_D$.

This is a contradiction to our assumption that TG_D is the total global weight dominating vertex set of G^S .

Therefore, there exists a vertex (u, σ (u)) εT_D such that v is not adjacent to u

Converse is obvious from the definition.

Theorem 4.2

Let G^S be a S- valued graph such that neither G^S nor $\overline{G^S}$ have an isolated vertex then

 $\gamma_{TG_D}(G^S) = \gamma_{TG_D}(\overline{G^S})$

 $\gamma_{T_D}(G^S) \leq \gamma_{TG_D}(G^S)$

 $\gamma_g(G^S) \leq \gamma_{TG_D}(G^S)$

 $[\gamma_{T_D}(G^S) + \gamma_{T_D}(G^S)]/2 \leq \gamma_{TG_D}(G^S) \leq [\gamma_{T_D}(G^S) + \gamma_{T_D}(G^S)]$

Theorem 4.3

Let G^S be a S-valued graph such that neither G^S nor $\overline{G^S}$ have an isolated vertex then $\gamma_{TG_D}(G^S) = (\sum_{v \in V} \sigma(v), p)$ if and only if $G^S = P_A^S$ or mK_2^S or mK_2^S where $m \ge 2$

Proof

Suppose that $\gamma_{TG_D}(G^S) = (\sum_{v \in V} \sigma(v), p)$ then we consider the following cases

On the contrary let us assume that $G \neq P_4^S$, mK_2^S , $m\overline{K_2^S}$ where $m \ge 2$

Case (i)

If $\Delta_S(G^S)$ and $\Delta_S(\overline{G^S}) \leq (\sum \psi(uv), p-3)$

where $\Delta_S(G^S)$ is the maximum degree of G^S then both G^S and $\overline{G^S}$ have no vertices of degree 1 and hence for any vertex $(V, \sigma(v)) \in V$, $V \setminus \{(V, \sigma(v))\}$ is a total global weight dominating vertex of G^S a contradiction

Case(ii)

If either $\Delta_S(G^S)$ or $\Delta_S(\overline{G^S})$ = $(\sum \psi(uv), p-2)$

Say $\Delta_S(G^S) = (\sum \psi(uv), p-2)$

Then $(u, \sigma(u))$ is a vertex of degree $(\sum_{v \in N_s(u)} \psi(uv), p-2)$





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Then there exists exactly one vertex w such that w is not adjacent to u

If $(w,\sigma(w))$ is of degree $(\sum \psi(uv), p-2)$ then as $G^S=m\overline{K_2^S}$ m ≥ 2 then there exists a vertex x such that x is not adjacent to at least two vertices. If some non-neighbor of y of x has degree p-2 then $V\setminus (y,\sigma(y))$ is a total global weight dominating vertex set otherwise each non neighbor of $(x,\sigma(x))$ has at least two non neighbours and $V\setminus (x,\sigma(x))$ is a total global weight dominating vertex set. If degree of the vertex $(w,\sigma(w)) \leq (\sum \psi(uv), p-2)$ and if u has no neighbor of degree1then $V\setminus u$ is a total global weight dominating vertex set of G^S otherwise let u be adjacent to x, $deg_{G^S}(x)$ =1. Let y be adjacent to y0 (and necessarily adjacent to y2 and non-adjacent to y3). Then y4, y5 is a total global weight dominating vertex set and y5 end y6. This proves the necessity and sufficiency is obvious.

Theorem 4.4

Let G^S be a S-valued graph such that neither G^S nor $\overline{G^S}$ have an isolated vertex and T_D be a γ_{T_D} set of G^S with each X in T_D has non neighbor in T_D . If there exists a vertex $(\mathsf{u},\sigma(u)) \in V \setminus T_D$ which is adjacent only to vertices in T_D then $\gamma_{T_D}(G^S) \leq \gamma_{T_D}(G^S) + (\sum_{v \in V} \sigma(v), 2)$

Proof:

Case(i)

If $V \setminus T_D = \{(u, \sigma(u))\}\$ then there exists a vertex $(v, \sigma(v)) \in T_D$ such that v is not adjacent to v and hence v is a total global weight dominating vertex set therefore v is a v in

Case (ii)

If $V \setminus T_D \neq \{(u, \sigma(u))\}$ then there exists a vertex $(v, \sigma(v)) \in V \setminus T_D$ and hence $T_D \cup \{u, v\}$ is a total global weight dominating vertex set therefore $\gamma_{TG_D}(G^S) \leq \gamma_{T_D}(G^S) + (\sum_{v \in V} \sigma(v), 2)$

CONCLUSION

In S-valued graphs, we derived some results of total global weight dominating sets. Similar results can be obtained for different types of S-valued graphs.

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RESEARCH ARTICLE

A Comparative Study of Cawthorne Cooksey Exercises Versus Conventional Physiotherapy Exercises for the Patients Suffering from Migraine without Aura

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ABSTRACT

Migraine is an incurable, episodic predominant headache. Migraine is assumed to be a neurovascular ache affliction with changed central neuronal fining. Symptoms commonly lasts for several hours i.e., four to seventy-two hours and may be unpleasant. An aura means fortification spectrum. Cawthrone Cooksey exercise does the practice of eyes and head movement for the patients having dizziness. This exercise provokes the symptoms of the patients. Vestibular exercises make system fatigue the vestibular reaction which is the main aim of this exercise. This reaction is enrolled in CNS and makes the system to adapt the symptoms. Every type of uninterrupted aerobic exercise like cycling, jogging, Nordic walking or swimming can also the relief pain of migraine. 30 subjects were taken and divided into 2 groups where 15 subjects were taken in each group. The treatment was given for 5 days/week for 6 weeks. Independent sampling test was done and results were significant for both the groups i.e., 1.200 and -1.133. The here summarized that both the protocols here i.e., Cawthrone Cooksey exercises and Conventional physiotherapy are equally effective. There is only a little significant is seen in both the groups.

Keywords: Cawthrone Cooksey Exercises, Migraine without aura, Conventional Physiotherapy.

INTRODUCTION

Migraine is an incurable, episodic predominant headache[1]. It is assumed to be a neurovascular pain syndrome with changed central neuronal processing (starting of brainstem nuclei, cortical hyper excitability, and unfurling cortical depression) and complicity of the trigeminovascular system (triggering neuropeptide release, which brings





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out painful inflammation in cranial vessels and the dura matter)[2]. Symptoms commonly last 4 to 72 hours and may be nasty[2]. An aura means Fortification spectra and other transient focal neurologic deficits occur in a few patients which occurs commonly just before the headache[2]. Migraine without aura is the subtype of migraine. It is the commonest subtype It has a high-rise average barrage frequency. It is normally more debilitate i.e., disabling than Migraine with aura[3]. Migraine without aura may or may not present with photophobia or phonophobia. This type of migraine is most commonly seen in clinical practice which is commonly bilateral and periorbital [4]. Vomiting may sometime end the headache[4]. There are time and again various combinations of fatigue, difficulty in concentrating, neck stiffness, blurred vision, yawning, and pallor. Pain resembles the d increased neuronal activity in the trigeminal nucleus caudles and dorsal horn include upper cervical pain. Thus, cervical pain is commonly on one side only[4]. Migraine is centrally mediated pain from central nervous system. Migraine arises due to alteration in Central nervous system and association of trigeminal nerve complex[2].Migraine consists of four stages i.e. prodrome, aura, attack and post-drome. Not everyone who has migraines goes through all stages of the Migraine. Prodrome is a stage before migraine attack occurs, one or two days. The patient possibly notices small changes like indigestion, mood swings, from depression to sudden excitement, food cravings, stiffness of neck muscles, increase of urination, retention of fluid and yawning frequently. Aura might occur before or during migraines. Auras are reversible symptoms. These symptoms are usually visual but also can include other disturbances. Visual disturbances like seeing various shapes, bright patches or flashes of sun light, loss of vision, pricking sensations in hands and legs. Attack: A migraine attack usually lasts for several hours.

The suggested hours by the researchers are 4 to 72 hours. Pain usually presents on unilateral of your head, but often bilateral, Pain that throbs or pulses, Sensitivity to light, sound sometimes smell and touch, Nausea and vomiting. Post-drome: After a migraine attack the person might perceive a feeling of evacuation, confusion and washed out for up to a day. Some people report feeling over excited. Sudden movement of head might bring on the pain again briefly[5]. Migraine becomes more prevalent in women than in men and remains as such for the majority of the lifespan. Migraine reaches peak prevalence at 30-39 years of age[6]. All forms of continuous aerobic exercise (e.g., cycling, jogging, Nordic walking or swimming) were accepted. There are some studies that have showed physiotherapy is most effective for the treatment of migraine. When physiotherapy is combined with other treatments such as thermal biofeedback, relaxation training, and exercise it is more beneficial[7]. The vestibular exercise does the training of eyes to move free from the head, and to practice balance and head movements that cause dizziness. The goal of these exercises is to fatigue the vestibular response which there by force the central nervous system to compensate by dependence to the stimulus[8]. The MIDAS or Migraine Disability Assessment Test is used to determine how severely migraine affect a patient's life. The patients are asked questions about the frequency and duration of their headaches and also these headaches limited their ability to participate in activities at work, at school, or at home. The test was estimated by the professional journal Neurology in 2001; the reliability and validity of MIDAS is 83%[9]. VAS is often used in clinical research to measure the intensity or frequency of various symptoms. The amount of pain that a patient feels. It ranges from a none to an extreme amount of pain[10]. The reliability and validity of VAS is 70%-84%[11].

MATERIALS AND METHOD

The study comprised of (n=30) participants where Group A (n=15) Cawthrone Cooksey exercises were given and Group B (n=15) Conventional Physiotherapy exercises were given. The data was collected from Bhagwati General Hospital of Vastral, Ahmedabad. The study was done in 6 weeks. The participants suffering from Migraine without aura were included in the study with no history of any other neurological disorders, head injury, brain surgery or any cardiovascular disorders expect hypertension. The study design was Comparative study. The participants were divided into groups A and B with random sampling technique. The study included both the genders 21 females and 9 males with age group between 18 to 85 years of age with history of headache for 3 months or more than that. The participants were signed written consent form regrading study. The participants were assessed before and after the treatment. The outcome measures used in the study was





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MIDAS (Migraine Disability Assessment Scale) and VAS (Visual Analog Scale). The MIDAS is taken here to know how severely the Migraine does affect on patient's life. MIDAS measures the frequency duration and activities for participation in work, school and home. The VAS is taken for the pain during migraine attack whether the participant felt no pain or unbearable pain. Both groups were given exercises for 5 days/ week for 6 weeks. Group A was given Cawthrone Cooksey Exercises that includes:

- (1) Do the eye movement slowly at initial stage and quickly later on.
- (a) roll your eyes upward
- (b)roll your eyes side to side
- (c)Focus on index finger (3ft-1ft away from the eyes)
- (2) Movement of head slowly at first and then quickly afterwards eyes should be closed later in this exercise.
- (a)Bend your head forward.
- (b)turn your head from side to side.
- (3) Shrug and circle your shoulder.
- (4) You have to bend forward and pick up the objects.

Group B were given conventional physiotherapy exercises: (1) Do warm-up stretching (5 minutes).

- (a)Do breathing exercises i.e., deep breathing, diaphragmatic breathing exercise, Pursed lip breathing, (2 minutes)
- (b)Do slow walking(3 minutes)
- (2) Do brisk walking continues (10 minutes)
- (3) Do Marching (10 times)
- (4) Do Stepping (2 minutes)
- (5) Do Cool down stretching (5 minutes).
- (a)Do breathing exercise i.e., deep breathing, diaphragmatic breathing, pursed lip breathing, pranayama (2 minutes). (b)Do slow walking (3 minutes).
- (c) Perform mental imaginary techniques (1 minute).

The exercises were done for 20 times /day.

RESULT

30 patients suffering from Migraine without aura are taken for this study. Fifteen patients were in Group A (Cawthrone Cooksey Exercises) and fifteen were in Group B (Conventional Physiotherapy). The data were entered and analysed by using SPSS (statical package for social science) software version 20. After applying paired t-test the results were Group B shows significance difference compared to group B i.e., 0.781 and 2.812. of post MIDAS and VAS. The result was incomplete if independent sampling test was not done so independent sampling Levenes test for equality in the variance. The final result shows that both the protocols that were undertaken were equally significant as both shows almost similar results and similar output. The participants suffering from migraine without aura can be given both the protocols as both the exercises are effective.

DISCUSSION

This study signifies that both the protocol is equally significant no group shown more significance than other one. The result found here was after applying paired t-test on both the sample sizes of group A and group B that group B is more significant protocol for the patients suffering from migraine. The score here for group A MIDAS pre and post 1.082 and VAS pre and post 0.828. The score for Group B MIDAS pre and post 0.915 and VAS pre post is 3.167. The independent sampling test shows both the protocols are equally significant. Here the physical therapy protocol shows more significance than Cawthrone Cooksey exercises before applying independent sampling test. The results here in the study by L Pinar et.al., founded those exercises at sub maximal level reduces that disability and pain of





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migraineurs[12]. Cawthrone Cooksey exercises are mostly built on mechanism related to neural plasticity. CNS assisting optical stabilization ongoing the movements of head which improves vestibular visual interactions during head movements, enlarge static and dynamic postural stability. In this stability the conditions that produce contrary to sensorial information. This makes the patient sensitivity to head movements[13]. One of the studies suggest that if a person is suffering from migraine will also experience anxiety and the anxiety can lead to dizziness[14]. The patients received Cawthrone Cooksey exercises showed very little significance as the time taken to complete the study may be too fast. The study may have shown better results if the protocol should have included more than 6 weeks. Here in this study after applying independent sample test to both the group that is t-test was applied but independently to both the groups. As discussed above the result we found was insignificant between both the groups. The protocols show same effect no protocol given here is more significant both the protocols performed shows significance at same levels. The numbers show pre and post MIDAS 1.200 and for VAS pre and post -1.333. In this study the population mostly is young adults. The effect of migraine is felt during the attack as well as after the attack as it makes person emotionally distress and unstable also physically unwell[21]. The study comprises of only young adults and not older adults which is one of the limitations of this study. So, the conventional physiotherapy approach and Cawthrone Cooksey exercises are equally significant in this study as per the statics of the result none is more significant than the other one and therefore discussion is more important for this topic. Some study stated that migraine patients can lead to have depression more often than other general population[17,18,19,20]. If this study may have included the older adults, we may get an idea that these protocols work on older adults same as the younger adults. This is one of the most the biggest limitation of this study.

The aerobic exercises here given to the participants of group B suggest that this protocol influence pain, intensity of attack, disability and duration of the attack. The result shows that it significant to as equal as Cawthrone Cooksey protocol. The exercises in Cawthrone Cooksey protocol shows eye movement which are used to activate interconnected cortical and subcortical network such as visuo-motor, basal ganglia, superior colliculus, substantia nigra pars reticulata and many others like oculomotor in brainstem [15,16]. The network discussed here works on the principal of pain processing of exhibitory and inhibitory role [22,23]. These conjugated eye movement transmits the positive and negative charge in a sequential manner on the retina[24]. The international Headache Society criteria includes that migraine without aura are mild in nature and generalized. The symptoms for the Migraine without aura are not severe and also it may or may not be unilateral. The patients suffering from migraine without aura cannot distract themselves even performing aerobic exercises. In comparison to migraine without aura and tension type headache one may get better results of exercises in tension type headache[25]. Migraine is commonly seen more in females than males. It is corelated with puberty. 10% of the females experience migraine triggers during menstruation period[25]. The researchers suggested that the aerobic exercises improve the fitness of cardiovascular system, increase in the tone of cardiovascular vessels and also in decreases the frequency of the attacks of migraine[27,28,29]. A few study research on the nitric oxide theory and they stated that too much intensity exercises may lead to acute rise in increase in blood nitic oxide level which may induce migraine[29]. The insufficient warm-up may lead to trigger the migraine attack. The participants should be given proper warm-up time to prevent the migraine attack [30]. All there participants here in study is younger adults and that may limit this study. The same study can be done by taking older adults as sample size and difference in the result and conclusion can be noted. Further studies can be done including different outcome measures and different sample size which may conclude more significant result.

CONCLUSION

Here summarized that both the protocols here i.e., Cawthrone Cooksey exercises and Conventional physiotherapy are equally effect. There is only a little significant is seen in both the groups. The post results of MIDAS and VAS outcome measures shows only little decrease in pain and disability. The patients suffering from migraine without aura in this study are only younger adults and more researches can be done on this by involving older adults. The Cawthrone Cooksey exercises helped in improving pain intensity not much but yes if the protocol would have been





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followed up for more than 4 weeks it may show a significant effect. As both group shows equally significance Conventional exercises also helped the patient in improving their cardiovascular system fitness and as suggested earlier the protocol should have lasted longer than 6 weeks for more interesting and significant result.

Clinical Implication:

This study can be implemented in the daily practice of the clinic as they show a little significant in pain. The Conventional protocol may be given to the patients suffering from migraine without aura as their main complain and symptom was pain in head region unilateral or bilateral. The Cawthrone is beneficial for vestibular migraine and vestibular disorders but it showed a little improvement in pain for migraine without aura. This study is done including more females so one can implement this study on females. The implication can be done on younger adult age group. Warm-up – exercises – cool down is aerobic exercises which can be performed by any individual of age group of young adults so one can implement on the patients in clinical practice.

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Table 1: Standard Deviation and Mean Statics of both the groups

Group		Mean	Std. Deviation
MIDAS POST	Group A	10.20	3.726
IVIIDAS_POST	Group B	9.00	3.024
VAC DOCT	Group A	22.80	9.291
VAS_POST	Group B	23.93	10.892





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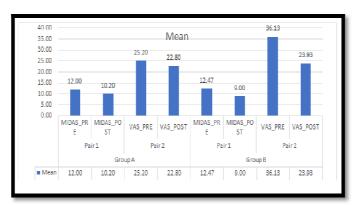
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Table 2: shows paired t-test between both the groups where group B shows significance difference

	Group			Mean	N	Std.
	Огоар			ivicari	14	Deviation
Croup	MIDAS_PRE	12.00)	15	3.645	0.941
Group A	MIDAS_POST	10.20)	15	3.726	0.962
	VAS_PRE	25.20		15	9.096	2.349
	VAS_POST	22.80		15	9.291	2.399
	MIDAS_PRE	12.47	'	15	3.248	0.839
Group	MIDAS_POST	9.00		15	3.024	0.781
В	VAS_PRE	36.13	}	15	11.813	3.050
	VAS_POST	23.93	3	15	10.892	2.812

Table 3: Levenes test for equally in variance which shows both the groups A and B are equally significant.

		Leven's Test for Equality of Variances									
		F	Sig.	t	df	Sig. (2- tailed)	Mean Difference	Std. Error Difference			
MIDAC DOCT		0.559	0.461	0.968	28	0.341	1.200	1.239			
MIDAS_POST				0.968	26.861	0.341	1.200	1.239			
VAC DOCT		0.555	0.463	-0.307	28	0.761	-1.133	3.696			
VAS_POST				-0.307	27.320	0.761	-1.133	3.696			



Graph 1: Shows that both groups show significance difference in group B the outcome measures with MIDAS and VAS.





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RESEARCH ARTICLE

Production of Degradable Bio Plastics from Invasive Alien Plants in Anamalais, the Western Ghats

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ABSTRACT

The work of the invention embraces to create ecofriendly bio plastics from the exotic plants species such as Cylindropuntia ramosissima (Engelm.) F.M. Knuth, Parthenium hysterophorus L., Lantana camara L. and Leucaena leucocephala (Lam.) de Wit. These plants are frequently available biomass and nonnative species, also most threat to our native plants and environment. The present work has entirely new report to produce bioplastics from exotic plants extract combinations; no work has been published for the selected study plants up to the product level. These plant samples were extracted by boiling method. The extracted samples were produce the formulations A, B and C. With the combination of these plants materials at different ratio with suitable non-hazardous chemical substances resulted in quality bioplastics production. Since the combination of various exotics yielded good quality bioplastics when compared with the individual plant. These bioplastics were tried to make different commercial products like sheets, pen stand, cup, plate, food packaging container, photo frame, pencil, glassware's packing materials, and mulching sheets for agriculture etc.

Keywords: bio plastics, environment, exotic plants, formulations, commercial products





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INTRODUCTION

Plastics are one of the worst full materials in the world, its everywhere, every part of our society. The problem is the plastics are not natural substance, it containing harmful chemicals like polyethylene terephthalate (PET). Plastics are made from synthetic polymers such as polypropylene and polyethylene are known to cause environmental concerns due to their non-biodegradable nature, because of long chain carbon molecules that are probably made from polymers of petroleum product [1]. It will create pollution and health issues. The enormous use of petroleum-based plastic compounds emphasized a need for sustainable alternatives derived from renewable resources [2]. One strategy to overcome this is the use of biobased polymers, which are derived from renewable materials, leading to the development of 'green or eco-composite materials' that can be easily degraded or bio assimilated [3]. These materials are expected to be one of the important materials to realize and maintain a sustainable productive society [4]. Therefore, this is a time to replace petroleum-based plastics as eco-friendly and eco-tolerable alternatives. Main goal of this project is to make the plastics from biodegradable plant materials. Plant materials are generally containing rich amount of starch, cellulose and lignin. It will replace the usage of the petroleum-based synthetic polymers. These degradable bio plastics are totally ecofriendly, safe and do not have any harmful chemicals and toxic substances. The main advantage of this bio plastic is that they degrade guickly in the environment without producing any pollution. Plastic from plant materials can be used for making bio plastic plates, bags etc. due to its flexibility and durability. Beyond deadly plastics, one more threat to our environment is the invasive alien or exotic plant species which destroys the indigenous plants and change the ecosystem. The eradication of these exotic species is unavoidable. Instead, the exotic plant species can be converted to biodegradable plastics without harming the environment. The exotic plant is available abundantly and throughout the year at all seasons in Tamil Nadu. Hence the present work has been designed to create ecofriendly bio plastics from the exotic plants species such as Cylindropuntia ramosissima (Engelm.) F.M. Knuth (Cactaceae), Parthenium hysterophorus L. (Compositae), Lantana camara L. (Verbenaceae) and Leucaena leucocephala (Lam.) de Wit. (Leguminosae).

MATERIALS AND METHODS

Collection and preparation of Plant Materials

The plant materials of the selected exotic species were collected from various regions of Anamalai, Western Ghats and plains of Pollachi, Tamil Nadu. The collected plant materials was washed and cleaned to remove the dust particles and converted into small pieces. The weighed 200 g of plant samples were grounded using distilled water 1000 ml and boiled for 30 minutes. These solutions was filtered and kept for further use.

Production of degradable bio plastics [5, 6]

The extracted solutions were mixed at different ratios and makeup into 100 ml. With this solution 1 M sodium bicarbonate were added as disinfectant. Further 6 ml of (0.25 N) HCl, 4 ml acetic acid, 3 gram starch and 2 ml Glycerol was added to this mixture, 0.5 N NaOH is added according to pH desired. Followed by the small amount of natural colours will be added for producing different colours of bioplastics. The solutions were boiled in distilled water for about 30 minutes. Further the samples are dried, placed in a beaker and using a hand blender, the samples are pureed until a uniform paste is formed. The paste was poured into a metal tray and put in the oven at 130°C about one hour. The metal tray was allowed to cool and the film is scraped off the surface. This process was continued with triplicates for rectifying complications.

Observations

Tensile strength

The strength of the plastics was determined by applying a pulling force on the plastic from the opposite sides and determines whether or not the plastic breaks. For the tensile strength calculations, the following formula was used:





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Force (N)

Tensile strength = -----
Cross sectional area (mm²)

Where, Force (N) = Weight of the sample (gm) X 10-3 X 9.81

The cross sectional area was measured by using Vernier caliper. The thickness and length of the bioplastic sample are converted to cross sectional area using the following equation [7]. Cross sectional area (mm^2) = thickness x length

Shelf-life Test:

The shelf life of the bio plastics was assessed by visual inspection on a daily basis (The darkening of the plastic suggested decay).

Biodegradation tests by composting [6, 9]

Known weight (1g) of the samples of the produced bio plastics was buried into the municipal solid waste mixture. The mixture consisted of leaves, paper waste, cow manure, food waste, composting seeds, urea, wood waste and water [8]. The mixture was kept in an oven at 55°C, at which the maximum growth of thermophilic microorganisms occurred. Then, they were examined for possible biodegradation. The samples were weighed every three days in order to determine the percentage of weight loss.

Effect of acids

The produced bio plastic samples were put into sulfuric acid solution and acidic soil with the concentrations of 10%, 20% and 30%. The samples were dried and weighed periodically for in order to determine the weight changes after each time period. The test was compared between the produced bio plastics and the other known types of plastics.

Effect of alkalis

Samples of the bio plastics were put into alkali solution and alkali soil (sodium hydroxide) with different concentrations (10%, 20%, 30% and 40%). The percentage of weight loss was calculated daily.

Effect of salts

Bio plastic samples were mixed with solid salts like ferrous sulfate, sodium chloride, tri-sodium orthophosphate and lead acetate and observed for its resistance to the action of salts in five days once.

Production of bio plastic by different molding method

The products of bio plastics were tried to make based on strength in different forms by molding method. The bio plastics products like photo frames, pen stands, plates, cups etc.

RESULTS AND DISCUSSION

Bio plastic production

The present study for the production of bioplastics the following exotic plants were selected *viz., Cylindropuntia ramosissima* (Engelm.) F.M. Knuth, *Parthenium hysterophorus* L., *Lantana camara* L. and *Leucaena leucocephala* (Lam.) de Wit. (Fig. 1). These plant materials were extracted by boiling method. The extracted samples were mixed together with different ratios to produce the formulations A, B and C. The weight of the final paste obtained, pH and weight of the bioplastic film produced were recorded and tabulated. (Table 1, 2, 3 & 4 and Fig. 2). The positive results are exhibited for all formulated (A, B and C) exotic plant sample extracts. The produced bioplastic films has good strength, stability and flexibility may better for alternative to chemical based synthetic plastics. Therefore, replacement of synthetic polymers with plant based polymers make the basis for a sustainable and ecofriendly plastic production [10]. Likewise so many authors have investigated widely for biopolymers from plants such as field grown crops of wheat, corn, potato, soy, and starch; sweet potato starch, cassava starch, cocoa pod husk and





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jackfruit seed starch, banana peels [11, 12, 13, 14, 15]. Generally plant materials contain starch, cellulose, lignin, oils and proteins. In bioplastics these compounds are constructed of linked molecules that form long polymer chains (biopolymers) [16].

Tensile strength

The significant tensile strength for the bioplastic samples were observed for A, B and C formulations. The results of tensile strength reaches a maximum of 0.100534 N/mm² and 0.091343 N/mm² for sample B and A respectively. Followed by the minimum tensile strength exhibits at 0.086814 N/mm² for C formulations. (Table 5 and Fig. 3). These results are agreed with [7]; they were conducted the bioplastics film production from waste banana peels in different phases and parameters. It is revealed that the maximum tensile strength ranged at 0.343511, 0.274844, 0.264248 N/mm² and the minimum tensile strength were reached at 0.118252 and 0.094858 N/mm². From the above results the moderate tensile strength was observed all compositions of samples and it is confirmed as biopolymers due to the components like starch, cellulose, lignin present in the bioplastic film. Additionally the author [17] was clearly pointed out the good result of tensile strength could be comes by adding glycerol to improve mechanical strength in the film production.

Biodegradation tests by composting

The biodegradation tests by composting were noted that the weight loss of bioplastic films at three days ones for twenty one days after soil burial (Table 6 and Fig. 4). The remarkable degradation is observed and it is confirmed accordingly the weight chances of known weighed samples placed in soil. But there is no weight changes were observed for known types of synthetic plastics. Bio plastics have higher percentage of biodegradability than all other synthetic plastic [18, 19, 20]. A synthetic polythene plastic will require a couple of hundred years to degrade under normal environmental condition resulting in high volume of accumulation [21, 22]. Here it may conclude the produced bioplastic materials to be transformed in to compost that can be used as fertilizer for plants. According to the American Society for Testing and Materials (ASTM), biodegradable plastics by definition undergo degradation by naturally occurring soil microorganisms, for which the plastic polymers serve as an energy source [23, 24, 25].

Effect of acids, alkalis and salts

The degradability of bioplastic samples were tested on acidified soil and solutions (Table 7 & 8 and Fig. 8 to 14). The results of acidified soil treated bioplastic samples were measured their weight changes at different interval days. It is revealed that the bioplastics are degraded and decayed gradually at all the concentrations of acids. The initial weight of one gram of bioplastics samples for A, B and C are changed their weight at all the concentrations of sulphuric acid with soil. After the twenty fourth day the maximum weight loss were observed at 0.40g at 10% concentration, 0.44g at 20% concentration and 0.54g at 10% concentration for sample A, B and C respectively (Table 7 and Fig. 5). The degradability of samples in acidic solutions, the bioplastic films was treated in four days for all the concentrations. The result indicates the degradation rate of bioplastics is faster in acid solution than acidic soil (Table 8). Because the degradation ability in acid solution the bioplastic film was directly affect by hydrolysis process which leads to faster degradation. But in soil burial with acid solution the bioplastic sample degradation is depending on the availability of soil moisture, oxygen and concentration of the acidic nature. The bioplastic degradability was also tested with alkali condition for samples A, B and C. The bioplastic films were placed in salts like ferrous sulfate, sodium chloride, tri-sodium orthophosphate and lead acetate, alkali solutions and alkali soil at different concentrations and observed at different time intervals. The changes of weight for bioplastic films were recorded and tabulated (Table 9 to 14). The result showed that the weight of the film was interestingly increased subsequent days. The reason for weight increasing of bioplastic films after subsequent days is due to salt accumulation. Later it was slightly decreased; this result indicates the degradability of bioplastics is take place due to osmostic imbalance in alkali environment. This condition leads to water loss of the bioplastic films resulted by weight loss. This process was continued until the degradation end. The observed results were shown in the table 9 to 14.





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Production of bio plastic products

Successfully produced bioplastics were tried to make different products such as multipurpose bioplastic sheets, pen stand, cup, plate, food packaging container, photo frame, pencil, glassware's packing materials, and mulching sheet are shown in figures 6 and 7. The highlight of this project is the produced bioplastic sheets can be suggested to use for mulching sheet in modern agricultural technology for covering the surface of the soil in order to increase the temperature, to retain moisture, to suppress the weeds, and to promote the germination of seeds. It can be ploughed into the ground at the end of the growth cycle, providing soil nutrition for future seasons. [26] Katherine and Douglas has pointed out the U.S. National Organic Program (NOP) maintains standards for the use of Biodegradable plastic mulches (BDMs) in certified organic agriculture. The Biodegradable Plastics Institute first petitioned for the use of BDMs to the National Organic Standards Board (NOSB) in 2012. In 2014, the petition was approved and BDMs were added to the National List (Organic Materials Review Institute) [27]. In forth coming years bio based mulching film technology can contribute towards purely organic and sustainable agriculture in Tamil nadu. It may enhance environment, productivity, economic sustainability and affordability.

CONCLUSION

In 2050 there will be more than nine billion people but only one earth we need more sustainable solution to conserve our planet from the harmful effects of synthetic plastics. To produce sustainable ecofriendly bioplastics is an only way to increase healthy environment. So this project may helpful to develop bio based polymers to replace the petroleum-based synthetic plastics. The results of our bio plastics are degraded quickly but the problem is the materials do not resist to over moisture level. So in future the upgraded bioplastics can be developed in to a complete bio polymers product.

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Table 1. Preparation of the exotic plants raw materials for bio plastic production

Sample name	Sample Weight (grams)	Weight of the final paste (grams)	Volume of the final paste obtained (ml)		
Cylindropuntia ramosissima	200	110	135		
Parthenium hysterophorus	200	220	250		
Lantana camara	200	400	420		
Leucaena leucocephala	200	220	250		

Table 2. Preparation of Formulations A

Sample name	Proportions of the final paste obtained (ml)	рН	Weight of the bio plastic film (grams)	Color of the bio plastic film
Cylindropuntia ramosissima	50			
Parthenium hysterophorus	30			
Lantana camara	Lantana camara 10		12.944	Dark Brown
Leucaena leucocephala	10			

Table 3. Preparation of Formulations B

Sample name	Proportions of the final paste obtained (ml)	рН	Weight of the bio plastic film (grams)	Color of the bio plastic film	
Cylindropuntia ramosissima	30				
Parthenium hysterophorus	20				
Lantana camara	20	Acidic	10.866	Dark Brown	
Leucaena leucocephala	30				

Table 4. Preparation of Formulations C

Sample name	Proportions of the final paste obtained (ml)	рН	Weight of the bio plastic film (grams)	Color of the bio plastic film
Cylindropuntia ramosissima	50			
Parthenium hysterophorus	50			
Lantana camara	50	Acidic	10.708	Dark Brown
Leucaena leucocephala	50			

Table 5 Tensile strength for the produced bioplastic flims

Bioplastic sample	Weight of the bioplastic sample (gm)	Force (n) (Weight of the sample X 10-3 X 9.81)	Thickness of the sample (mm)	Length of the sample (cm)	Cross sectional area (mm²)	Tensile strength (N/ mm²)
Α	12.944	0.126981	0.0562	24.8	1.39	0.091343
В	10.866	0.106566	0.0500	21.2	1.06	0.100534
С	10.708	0.105045	0.0495	24.5	1.21	0.086814





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Table 6. Biodegradation tests by composting

Bioplas		Weight of the samples in grams									
tic sample	Bioplastic film before soil burial	Synthetic plastics	3 rd day	6 th day	9 th day	12 th day	15 th day	18 th day	21st day	Synthetic plastics in 21st day	
Α	1	1	3.03	1.04	0.51	0.46	0.40	0.40	0.39	1	
В	1	1	1.89	1.05	0.85	0.79	0.46	0.40	0.34	1	
С	1	1	3.20	1.43	1.04	0.90	0.32	0.43	0.41	1	

Table 7. Effect of acids solution with soil

	sulphuric		Weight of the samples in grams										
Bioplastic sample	acid concentrati ons (%)	Bioplasti c film before treatment	Synthetic plastics	After 1 day	4 th day	8 th day	12 th day	16 th day	20 th day	24 th day	Synthetic plastics in 24th day		
Λ	10	1	1	1.94	1.26	1.46	0.88	0.85	0.41	0.40	1		
А	20	1	1	1.17	1.09	1.44	0.97	0.60	0.76	0.73	1		
	30	1	1	1.39	1.95	1.52	1.03	0.91	0.85	0.71	1		
В	10	1	1	1.69	1.20	1.01	0.96	0.67	0.60	0.60	1		
В	20	1	1	1.14	1.18	0.83	0.78	0.68	0.46	0.44	1		
	30	1	1	1.29	1.58	2.02	1.14	0.89	0.91	0.89	1		
	10	1	1	1.74	1.05	1.14	0.75	0.67	0.77	0.54	1		
С	20	1	1	1.82	2.09	1.77	1.71	1.43	1.33	1.29	1		
	30	1	1	1.53	1.91	1.11	1.10	1.00	0.98	0.86	1		

Table 8. Effect of acids solution without soil

		Weight of the samples in grams									
Bioplastic sample	Sulfuric acid concentration (%)	Bioplastic film before treatment	Synthetic plastics	After 1 day	2 nd day	3 rd day	4 th day	Synthetic plastics in 4th day			
	10	1	1	1.23	2.42	0.59	0.29	1			
Α	20	1	1	2.23	2.43	0.53	0.11	1			
	30	1	1	1.99	2.01	0.49	0.21	0.98			
D	10	1	1	1.93	1.32	0.93	0.40	1			
В	20	1	1	1.31	2.26	0.81	0.17	1			
	30	1	1	1.83	2.44	0.94	0.17	0.98			
	10	1	1	2.27	2.12	0.83	0.33	1			
С	20	1	1	2.08	2.23	0.84	0.19	1			
	30	1	1	2.52	1.83	0.93	0.14	0.98			





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Table 9. Effect of alkalis solution with soil

Bioplastic	Sodium			Weig	ght of t	he sam	ples in	grams			
sample	hydroxide concentrati -ons (%)	Bioplastic film before treatment	Synthe-tic plastics	After 1 day	4 th day	8 th day	12 th day	16 th day	21 st day	24 th day	Synthetic plastics in 24th day
	10	1	1	1.84	1.44	1.08	0.98	0.96	0.95	0.94	1
А	20	1	1	2.14	2.25	2.08	1.88	1.82	1.58	1.42	1
A	30	1	1	1.33	1.40	1.33	1.28	1.30	1.32	1.27	1
	40	1	1	2.44	2.62	2.46	2.37	2.53	1.20	1.84	1
	10	1	1	1.59	1.35	1.02	0.98	0.98	0.94	0.94	1
В	20	1	1	1.23	1.28	1.17	1.09	1.08	1.05	1.04	1
D	30	1	1	1.14	1.23	1.16	1.12	1.14	1.13	1.11	1
	40	1	1	1.32	1.40	1.39	1.32	1.53	1.66	1.53	1
	10	1	1	1.89	1.66	1.24	1.12	1.10	1.08	1.02	1
С	20	1	1	1.44	1.46	1.27	1.15	1.15	1.10	1.07	1
	30	1	1	1.21	1.30	1.22	1.15	1.15	1.30	1.10	1
	40	1	1	1.56	1.69	1.67	1.65	1.75	1.84	1.65	1

Table 10. Effect of alkalis solution without soil

Bioplastic	Sodium	ium Weight of the samples in grams									
sample	hydroxide concent- rations (%)	Bioplastic film before treatment	Synthetic plastics	After 1 day	2 nd day	3 rd day	4 th day	Synthetic plastics in 4 th day			
	10	1	1	1.93	1.51	0.53	0.13	1			
Α	20	1	1	1.31	1.89	0.84	0.23	1			
А	30	1	1	1.83	1.02	0.31	0.11	1			
	40	1	1	2.27	1.45	0.71	0.31	1			
	10	1	1	2.31	1.67	0.54	0.17	1			
В	20	1	1	2.29	1.72	0.74	0.26	1			
	30	1	1	1.81	2.47	0.27	0.13	1			
	40	1	1	1.83	2.47	0.89	0.34	1			
•	10	1	1	1.62	1.41	0.58	0.19	1			
С	20	1	1	2.37	1.64	0.33	0.13	1			
C	30	1	1	2.71	2.49	0.12	0.02	1			
	40	1	1	3.07	2.96	0.93	0.39	1			

Table 11. Effect of solid salt ferrous sulfate

Bioplastic sample	Weight of the Bioplastic film before treatment (grams)	Weight of the Synthetic plastics (grams)	Weight of the samples in grams for one month 5th 10th 15th 20th 25th 30th day day day day						Weight of the Synthetic plastics after 1 month
Α	1	1	1.04	0.99	0.99	0.99	1.00	1.01	1
В	1	1	1.06	1.02	1.01	1.01	1.05	1.03	1
С	1	1	1.02	0.98	0.99	0.99	1.00	0.99	1





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Table 12. Effect of solid salt sodium chloride

	Weight of the the				Weight of the samples in grams for one month					
Bioplastic sample	Bioplastic film before treatment (grams)	Synthetic plastics (grams)	5 th day	10 th day	15 th day	20 th day	25 th day	30 th day	Synthetic plastics after one month	
А	1	1	1.11	1.05	1.024	1.10	1.08	1.01	1	
В	1	1	1.03	1.04	1.34	1.10	1.11	1.12	1	
С	1	1	1.13	1.08	1.06	1.11	1.14	1.14	1	

Table 13. Effect of solid salt tri-sodium orthophosphate

Ri	Weight of the Bioplastic	Weight of the	Weig	Weight of the					
Bioplastic sample	film before treatment (grams)	Synthetic	5 th day	10 th day	15 th day	20 th day	25 th day	30 th day	Synthetic plastics after one month
Α	1	1	1.04	1.03	1.04	1.07	1.08	1.11	1
В	1	1	1.03	1.06	1.04	1.08	1.05	1.12	1
С	1	1	1.06	1.07	1.08	1.10	1.14	1.14	1

Table 14. Effect of solid salt lead acetate

Weight of the	Weight of	Wei	Weight of						
Bioplastic sample	Bioplastic film before treatment (grams)	the Synthetic plastics (grams)	1 st day	2 nd day	3 rd day	4 th day	5 th day	6 th day	the Synthetic plastics after one month
Α	1	1	1.05	1.01	0.96	0.96	1.02	1.00	1
В	1	1	1.02	0.99	0.96	1.01	0.99	0.90	1
С	1	1	1.16	1.11	1.04	1.08	1.16	1.10	1

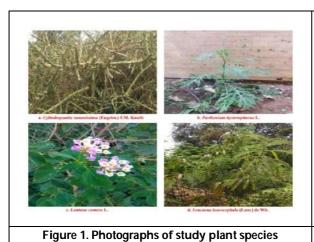




Figure 2. Showing the steps in bioplastic production



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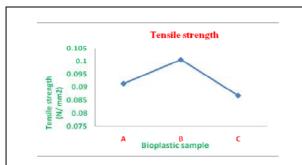




Figure 3 Tensile strength for the produced bioplastic flims

Figure 4. Biodegradation test by composting

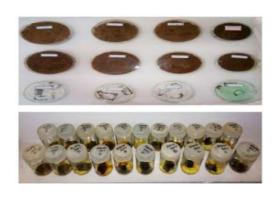




Figure 5. Effect of acids, alkalis and salts on bioplastic degradability

Figure 6. Multipurpose bioplastic film sheets produced in different colours and bioplastic products





Figure 7. Bioplastic films used as glassware's packing materials and used as mulching sheet





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REVIEW ARTICLE

A Review: Development and Advances in Hyphenated Techniques and its Applications

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ABSTRACT

The hyphenated technique was made by combining a separation detection technology with another technology. Since the 1990s, hyphenated analytical methods have gotten a lot better, which has made them a lot, more useful for analysing biomolecules contains especially natural products. This article discusses the benefits of employing different hyphenated techniques used in natural products, chemotaxonomic research, chemical fingerprinting, quality control of herbal goods, derivation of herbal goods, and metabolic research. These approaches have undergone notable advances, which are anticipated to improve combined selectivity and increase the amount of real information gathered. However, there will be synergies and drawbacks when the hyphenated analytical procedures are coupled. This review focuses on few hyphenated techniques and their applications in detail.

Keywords: LC-MS/MS, Hyphenated, Applications, Chromatography, LC-NMR

INTRODUCTION

A hyphenated approach is the combining of two separate analytical methods via a suitable interface. In conjunction with spectroscopic techniques, chromatographic methods are commonly utilised. Chromatography extracted pure or almost pure chemical components from a mixture, while spectroscopy produced selected information for





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identification using standards or library spectra [1]. A hyphenated technique will be created by combining separation methods with an online spectroscopic detecting technology[2]. The phrase hyphenated methods refer to a technique that combines two or more approaches[3]. In 1980, Hirsch Feld created the term "hyphenation" to characterize the ability to perform many instrumental analysis techniques in a single run (Hirsch Feld, 1980). The objective of coupling, compared to a single analytical approach, is to offer a detection that is rich in information for both identification and qualification[2].

Advantages

- To riddle out complex analytical problem
- Increased automation
- Increased sampling throughput
- Closed-loop system protects against contamination.
- Better reproducibility

Various hyphenated methods

- 1. Double hyphenated methods
- 2. Triple hyphenated methods

1. Double hyphenated Methods

- Liquid Chromatography with tandem mass spectrometry (LC-MS)
- Liquid Chromatography with Nuclear Magnetic Resonance Spectroscopy (LC-NMR)
- Liquid chromatography infrared spectroscopy (LC-IR)
- Capillary electrophoresis mass spectrometry (CE-MS)
- Gas chromatography infrared spectroscopy (GC-IR)
- Gas Chromatography-Mass Spectrometry (GC-MS)
- High-Performance Liquid Chromatography with Diode-Array Detection (HPLC-DAD)
- Gas Chromatography—Fourier Transform Infrared Spectroscopy (GC-FTIR)

2. Triple hyphenated Methods

- Liquid chromatography atmospheric pressure ionization mass spectrometry (LC-API-MS)
- Atmospheric pressure chemical ionization-MS-MS (APCI-MS-MS)
- Electrospray ionization-MS-MS (ESI-MS-MS)
- Large-Volume Injection Gas Chromatography Mass Spectrometry (LVI-GC-MS)
- Liquid Chromatography-Electrospray Ionization-Mass Spectrometry (LC-ESI-MS)
- Liquid chromatography with parallel NMR and mass spectrometry (LC-NMR-MS)
- Liquid chromatography-photodiode-array-mass spectrometry (LC-PDA-MS)
- Solid-phase extraction-liquid chromatography mass spectrometry (SPE-LC-MS)
- LC-UV-NMR-MS-ESI
- LC-MS-TSPLC-UV-NMR-MS
- LC-DAD-API-MS
- LC-PDA-NMR-MS

Available Hyphenated Method

Gas Chromatography-Mass Spectrometry (GC-MS)

It's a technique for separating substances that can be volatilized in a gas stream. Typically, this is performed by injecting 0.1-1 mL of sample into an injection port heated to 250 °C. Because the sample is in the vapour phase, both methods are very compatible with one another.





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Instrumentation

In GC-MS, a sample is first vaporized, then separated in the GC column, then evaluated by the MS detector, and finally recorded. All of these steps take place at the injection port of the GC instrument. Time between injection and elution is referred to as "retention time." In general, GC-MS equipment includes of,

- 1. A metal column with an injection port at one end (To promote maximum separation, they are frequently packed with a sand-like material).
- 2. In another end of the column, a detector (MS) is present.
- 3. A carrier gas like helium, argon, hydrogen, nitrogen, pushes the sample down to the column. The components of mixtures are separated in GC at the retention time of the sample, while the MS detector gives information about the identification of the structure.

There are two types of column in GC-MS

Capillary columns and macrobore and packed columns. The interface efficiently carries effluent from the GC to the MS [5]. The analyze must not condense and not disintegrate at the interface, and the volume of gas not exceed the MS's pumping capability which is entering into the ion source.

Applications Of GC-MS

- 1. It's good for research and development.
- 2. Molecular ion identification.
- 3.At high temperatures, volatile, tiny and stable compounds are determined using GC-MS.
- 4.By measuring and determining the precise mass of the components, it is possible to verify their purity and identity [5].

Advantages Of GC-MS

- The high-resolution capability of GC allows it to compare different approaches.
- When combined with thermal detectors, this approach has a high sensitivity.
- This technology, which allows for the separation and analysis of samples in a short amount of time, is also used for low-quality samples.
- This approach has a high level of precision and accuracy.

Disadvantages OF GC-MS

- Only volatile samples (or ones that may be turned volatile) are separated using this approach in GC.
- It is necessary to pay close attention during the injection of the gaseous sample [4].

Liquid Chromatography with tandem mass spectrometry (LC-MS)

Liquid chromatography mass spectrometry is a technique used in analytical chemistry that combines the physical separation skills of liquid chromatography (LC) with the mass analysis capabilities of mass spectrometry (MS) [7].

Instrumentation

In order to combine the two approaches effectively, a switching valve can help. Typically, an automated LC-MS system contains two three-way diverters in line with an:

- 1. Auto sampler
- 2. LC system
- 3. Mass spectrometer.

The diverter functions as an automated switching valve before the sample reaches the MS, transferring any excess eluate from the LC system to the trash. In LCMS, soft ionization techniques are frequently used, and they mainly disclose molecular ion species with a small number of fragment ions. As a result, a single LC-MS run provides a somewhat inadequate representation of the chemical's structure. In tandem mass spectrometry, fragments are generated by collision-induced dissociation, and this problem has been handled. Due to this, LC-MS-MS is used rapidly [5].





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Application

- 1. Detect and confirm molecular identification selectively.
- 2. Reveals details about the molecular weight and fragmentation pattern of the analyze.
- 3. Assist the process of determining the identity of analyze molecules.
- 4. This qualitative investigation enables the reconstruction of an unknown chemical using MS data.
- 5. During pharmaceutical development and scaling up, it tracks impurity profiles, as well as to assess the safety of batches utilised in clinical trials[5].

Liquid Chromatography with Nuclear Magnetic Resonance Spectroscopy (LC-NMR)

In 1978, it was revealed that liquid chromatography may be directly coupled to NMR utilising the stop flow approach. This approach may be used to analyse natural materials, organic compounds, biomolecules, drug contaminants, reaction mixtures, drug degradation products and by-products, among other things [9].

Instrumentation

Generally, in LC-NMR system, the LC unit was made up of

- 1.Auto sampler
- 2. LC pump
- 3. Column
- 4.Non-NMR detector.

The detector transmits the flow into the LC-NMR interface and can include extra loops for short-term storage of particular LC peaks. Following that, the flow from the LC-NMR interface is either directed toward the flow-cell NMR probe-head or the garbage. The flow is directed to a fraction collector after passing through the probe-head for recovery and further analysis of the distinct fractions evaluated by Nuclear Magnetic Resonance Spectroscopy. Reversed-phase columns with a binary or tertiary solvent combination and gradient isocratic / elution are employed in the majority of LC-NMR procedures. The protons in the mobile phase solvents make getting an acceptable NMR spectrum extremely difficult. The receiver of the NMR spectrometer has a hard time processing both the powerful signals from the solvent and the delicate signals from the material at the same time. Solvent signal suppression is a solution that may be done by one of the following three primary approaches in order to resolve this issue: prestarvation, water suppression enhancement or soft-pulse repeated irradiation through T1 effects can all lead to an increase in enhancement (WET) Utilizing a z-gradient of 10, presaturation was performed [5].

Advantages of LC-NMR

- 1. Because the information between the two (three) procedures is so different, HPLC methods separate out the "complexity of a mixture."
- 2. In NMR, impure peak present in LC can be determined.
- 3. There is no need to separate the mixture completely to collect the NMR data.
- 4. A non-destructive method is employed.
- 5. The sample might be kept for later use with a different method of analysis.

Disadvantages of LC-NMR

- 1. Extremely high in cost.
- 2. Long experiment times, high equipment expenses, and limited usage of 2 H solvents.
- 3. Skilled technician is required.
- 4. A special set of skills are needed to perform LC-NMR/MS.
- 5. Choosing the appropriate solvent is difficult.

Liquid chromatography infrared spectroscopy (LC-IR)

LC-IR or HPLC-IR is the name of the hyphenated method that results from the combination of an LC with the detection technique of infrared spectrometry (IR) or FTIR. The identification of organic substances can be





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accomplished with the help of infrared (IR) or Fourier transform infrared (FTIR) spectroscopy. This is due to the fact that multiple absorption bands characteristic of different functionalities can be seen in the structures of organic molecules in the mid-infrared range. One of the most potent separation methods now in use is high-performance liquid chromatography (HPLC). Example: -OH, -COOH, etc.....[5][6]. For the method known as solvent elimination, there are two different kinds of interfaces: - The diffuse-reflection infrared Fourier transform (DRIFT) methodology — A method utilizing buffer memory. Therefore, the LC-IR hyphenation procedure needs to take place in order to create a practical apparatus that generates whole mid-infrared spectra.

- 1. Take away the solvent without causing any heat damage to the analysis or filling up the vacuum system with an excessive amount of diluent gases.
- $2. \ Ensure \ that \ analyses \ are \ transmitted \ to \ the \ spectrometer \ in \ an \ effective \ manner.$
- 3. Put the analyses on a thick deposit and present them to the FT-IR.
- 4. Chromatographic resolution to be maintained[4].

Application

The determination of the identities of organic substances.

Capillary Electrophoresis Mass spectrometry (CE-MS)

CE analysis, Fig.5 which is carried out in tiny tubes and can quickly separate hundreds of different compounds, is powered by an electric field. Because of CE's adaptability and the myriad of applications that it may serve, virtually any molecule can be segmented with the assistance of this potent technique. It accomplishes this by applying voltage across capillaries that are filled with buffer, and it is most commonly employed for separating ions that, when the voltage is applied, travel at varying speeds based on their size and charge. As solutes pass the detector, they appear as peaks whose areas are proportionate to their concentrations, allowing quantitative measurements. The analysis consists of determining the purity of the substance, performing tests, and determining trace levels. The combination that results from the linking of an Mass Spectroscopy detector to a Capillary Electrophoresis system for the purpose of gathering on-line MS data of the substance that has been separated is referred to as CE-MS [11]. Instrumentation: It consists of;

- 1. High-Voltage Supply;
- 2. Capillary
- 3. UV-Vis or PDA detector
- 4. MS detector:
- 5. Buffer solution
- 6. PC control

Application

Separation of several hundreds of unique chemicals in a very short amount of time

Gas chromatography infrared spectroscopy (GC-IR)

Due to the fact that IR is a non-destructive approach, sample recovery is also feasible, despite the fact that this method is exceedingly sensitive and costly. In this method, the GC is responsible for the part that involves separation, while the IR is in charge of the part that involves identification. The GC effluent is delivered to the IR at atmospheric pressure[9].

Application Of Hyphenated Techniques

Many novel natural products derived from plant and marine sources have been found and their properties have been characterized with the use of hyphenated procedures. There are a variety of combination devices that make it possible to find novel natural products, to elucidate the structure in its whole and definitively, and to configure them in a way that is related to the time-consuming and expensive process of isolation and purification. This article will explore how hyphenated approaches may be used in the investigation of natural goods, providing a few examples along the way.





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Isolation and analysis of Crude Natural Products

The hyphenated approaches are able to be used for the analysis of crude natural products. LC-PDA and LC-MS are the two major approaches that are utilized widely in the analysis of crude natural products, although there are many more techniques as well. The LC-MS approach, which is presently the only system of the hyphenated techniques that is commercially accessible, may be used to conduct an analysis of certain tiny non-polar components that are found in natural goods. These constituents include oligosaccharides, proteins, and tannins [6,12].

Alkaloids

In order to study the alkaloids, scientists employ a wide variety of methodological combinations. The GC-MS system is the most common one, and it is used to examine compounds such as quinolizidine and pyrrolizidine, as well as numerous groups of nitrogen-containing secondary metabolites of, microbial, plant or animal origin. GC-MS analysis has recently been performed on the primary class of alkaloids that may be found in the *Leguminosae* family of plants [13]. The majority of these alkaloids are able to undergo GC analysis without any chemical modification since they are sufficiently volatile and thermostable under the circumstances [14].

Coumarins

The analysis of coumarin, which is the most common member of the 1-benzopyran family and is only present in higher plants, is the primary application for HPLC-PDA. These methods are also helpful in locating phenolic chemicals, such as coumarins, among other things. This apparatus identifies the coumarins and gives PDA with the helpful information necessary to establish the molecule's composition. In any crude extract, the identification of specific coumarins can also be assisted by the use of another technique known as MS to LC-PDA. Information on molecular weight and fragment ions was obtained through the recording of MS spectra at various voltages, which in turn made it possible to identify the primary components included in the extracts[6,15].

Carotenoids

LC-TLS technique is used to separate diatoxanthin, diadinoxanthin and other carotenoids by isocratic method. The approach was successful in determining the carotenoids present in all four species of marine phytoplankton. compared to UV detection, the sensitivity and selectivity of HPLC elution is significantly higher. Because of this approach, we have been able to monitor the interconversion of diadinoxanthin to diatoxanthin, as well as the changes that occur in other carotenoids when exposed to varying intensities of light [6,12,13].

Essential oil and volatile components

For the investigation of volatile and non polar natural components like mono and sesquiterpenes, GC-MS is a beneficial analytical technique. Because these compounds are difficult to separate by other methods. GC-MS analysis has pinpointed the volatile component at about 120-130 as being present in a number of Chinese herbal remedies. Chinese crude drugs like Jilin Ginseng, Radix Aucklander, and Citrus tangerine peels are separated and structure has been determined by using the combination method called GC-MS with EI. This method was used to study Jilin Ginseng, Radix Aucklander, and Citrus tangerine peels.

Saponins

GC-MS is mostly limited to the examination of aglycones like sapogenins or saponins since these compounds are strongly polarised and challenging to volatilize. Saponins are either steroidal or triterpenoid glycosides, and they are found in a wide variety of plant species spanning close to one hundred families. Saponins, with the exception of a very small number of them, do not have the chromophores that are necessary for detecting UV light. When searching for the presence of saponins in crude extracts or fractions, the hyphenated methods of, CE-MS,LC-NMR and LC-MS might be helpful for the speedy first screening process. The LC-NMR-MS technique proved suitable for analysing saponins with molecular masses in the range of 1200–1400 amu and gave structural information in a single chromatographic run [6].





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Dereplication

Combining the use of spectroscopic detection methods, online database searching, and the separation of science are the three components that comprise dereplication tactics. Combining HPLC with structurally informative spectroscopic detection techniques, such as PDA, MS, and NMR, may make it possible to screen crude extracts or fractions for biological activity and structural classes. HPLC with UV photodiode array detection (LC-DAD-UV) and mass spectrometry (LC-MS or LC-MS-MS) offer on-line analytical data of extract ingredients before isolation. These data can then be used to isolate the desired component. The use of HPLC in conjunction with nuclear magnetic resonance (NMR) in the form of LC-NMR is a potent addition to LC - UV-MS screening. With the use of these techniques, which combine mass spectrometry and liquid chromatography as well as mass spectrometry and liquid chromatography, it is now possible to identify known substances quickly and with the use of only a small amount of the original substance. LC-MS-MS spectra are typically repeatable. Additionally, Q-DIS/MARLINTM is ideal for autonomously validating suggested chemical structures derived from combinatorial chemistry studies using LC-MS data. The majority of the dereplication procedures that are now available for the examination of natural products primarily make use of LC-PDA-MS. Despite having the ability to offer more useful structural information, LC-NMR has only achieved modest success due to its weak sensitivity, the challenge of ensuring universal access to high-field NMR equipment, and the cost of employing deuterated solvents[6].

Chemical fingerprinting and quality control of herbal drugs

The following methods are utilised in the process of fingerprinting: LC-MS, CE-MS, LC-NMR-MS and LC-NMR. For drug analysis, the fingerprinting method is often used to highlight the sample matrix's profiles. This is typically adequate to offer indicators regarding the source as well as the manner of manufacture. When it comes to herbal remedies, the profile is dependent not only on the techniques that are used to prepare the herb, but also on the quality of the raw herb that is used. As a result, it is possible to interpret the uniformity and consistency of the chemical profiles as an indication of the calibre of the raw herbs. Fingerprinting analysis is performed to evaluate the quality of herbal material's helpful in good agriculture practise (GAP) and also in good manufacturing practise (GMP). GAP stands for Good agricultural practice, Good manufacturing practice. The main objective of this approach is to establish relationships between chromatographic or spectroscopic profiles based on marker compounds and the efficacy of herbal products. For decades, thin-layer chromatography (TLC) has dominated the field of traditional fingerprinting analysis in Chinese medicine. It is possible to identify and verify the identification of these trace marker chemicals by using either GC-MS or LC-MS. The HPLC - MS based comprehensive chemical fingerprinting of sangi, danshena and ginkgo included the application of the ESI method. When doing a fingerprint analysis, it is absolutely necessary to perfect both the laboratory apparatus and the procedure in order to exclude any arte facts from the findings. A high-throughput analytical programme that has seen the development of a number of analytical protocols based on LC-MS fingerprinting and their subsequent incorporation into the programme has included standard methods, the determination of template structures, and structural libraries. For instance, a taxane database was created and mixtures of taxanes that were produced from Taxus brevifolia extracts were described using LC-MS. Traditional medical practises like traditional Chinese medicine and Ayurveda attribute herbs' therapeutic properties to a variety of physiologically active substances. One example of such a system is traditional Chinese medicine. Any modification to the chemical profile of the plant, whether qualitative or quantitative, has the potential to completely eliminate any medicinal benefits, decrease potency, or even increase toxicity. By utilising a method that allows online identification of molecules in the herbal extract, quality control can confirm the presence of specific molecules and the amount of each active principle. This is because it is crucial to the product's efficacy and safety. The mass spectra of the numerous components that may be found in the extracts of Chinese medicine have been collected on-line from the LC-MS run, and they have been compared with established standards in order to validate the structures of the components[16].

Chemotaxonomy

Chemical taxonomy, often called chemotaxonomy, is based on the idea that the activity of particular enzymes involved in secondary metabolite formation determines their presence, our body's genetic makeup must be unique to have these enzymes. Secondary metabolite chemical profiles, obtained through either exhaustive isolation and





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identification or separation and online identification using cutting-edge hyphenated techniques, may shed light on the phylogenetic or taxonomic relationships between different species. Based on the profiles of quinolizidine alkaloids, it was looked into how useful GC-MS for studying the chemical classification of legumes using chemotaxonomy. To learn about the quinolizidine alkaloids of rare plants, researchers employed extracts prepared from minute fragments of herbarium specimens. This provided for a well-founded information base on the distribution of such compounds in methanolic extracts of the leaves or seeds of many legume species. Residue was dissolved in 100 mL of acetone before being injected into an HP1090M HPLC-PDA-MS system. These results lead to a dry filtered product after the solvent was removed and a solid understanding of the distribution of these chemicals in legumes. The residue was dissolved in 100 mL of acetone to prepare it for injection into an HP1090M HPLC-PDA-MS system [6,13].

CONCLUSION

Therefore, it is possible to arrive at the conclusion that hyphenated approaches are significantly more beneficial and effective than regular single techniques.

- Hyphenated techniques involves both separation and identification, which simplifies the process of doing an analysis on a sample.
- The hyphenated method is utilised far more frequently than traditional spectroscopic or chromatographic methods.
- This development assists biological research, food, and drug analyses. This innovation also impacted natural product analyses and elemental specifications.
- These hyphenated approaches reduce analytical times, increase automation, sample throughput, reproducibility, and contamination.
- Hyphenated procedures are a boon in the modern world since separation and quantification are done quickly and efficiently.

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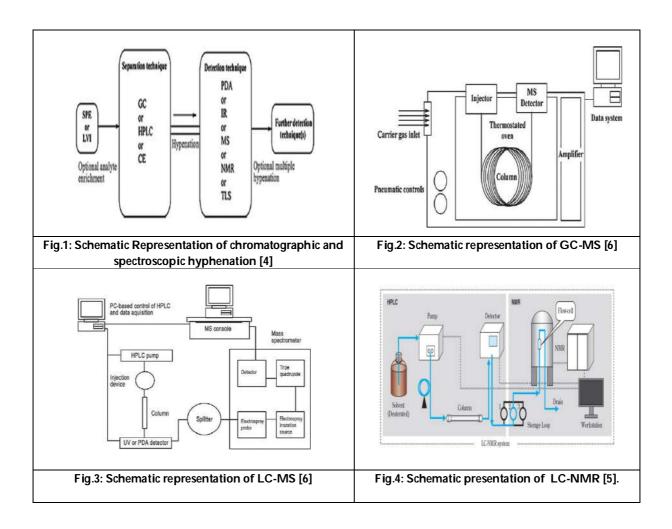


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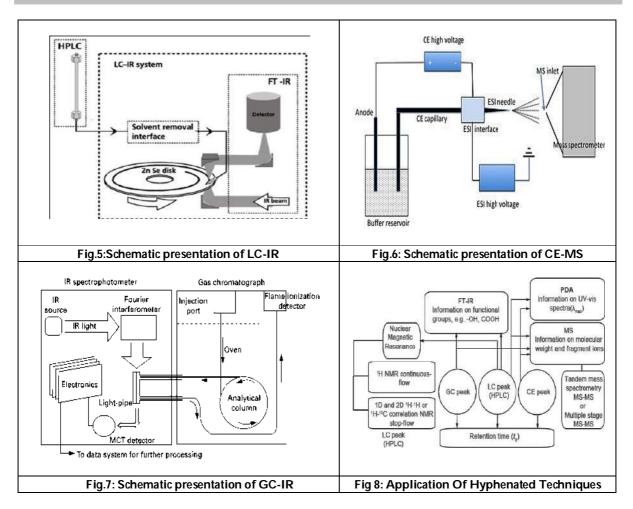




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RESEARCH ARTICLE

Effectiveness of Theraband Exercises and Bosu Ball Exercises on Core Stability and Dynamic Balance in Basketball Players: A Comparative Study.

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ABSTRACT

To see the effectiveness of Theraband exercises and Bosu ball exercises on core stability and dynamic balance in basketball players: A comparative study. Basketball is one of the most popular sports in the world and one of the most widely viewed. It is a fast moving game that involves a lot of variety, including shooting, dribbling, passing, rebounding, defence and much more. In basketball, to achieve better performance both static and dynamic balance plays a crucial role. Nowadays, core training has been very popular and is also an important role in training program. There are lack of evidence comparing the effect of theraband and bosu ball exercises among basketball players. In this study 60 players were taken. Players were randomly divided into 2 groups, each group has 30 players. Outcome measures such as Y-balance test and plank test were taken pre and post treatment. Group-A received Bosu ball exercises and group-B received Theraband exercises for 3 days per week up to 6 weeks. There was significant improvement in Y-balance test and plank test in group-A(Bosu ball exercises) and group-B(Theraband exercises). In unpaired t test there was a significant improvement in Y-balance test and plank test in group A(p - <0.005) then group B. This study concluded that Bosu ball exercises are more effective than Theraband exercises to improve core stability and dynamic balance in basketball players.

Keywords: Bosu ball, Theraband, Plank test, Y- balance test, basketball players, core stability, dynamic balance





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INTRODUCTION

Basketball is a fast-moving sport which includes a lot of mixture of movements such as shooting, dribbling, passing, rebounding, and defense. Basketball is one of the highest physically exhausting sports, which depends on intense lateral, sprinting and jumping movements [1]. Furthermore, in this game, better equilibrium leads to better control of players on their body which decreases the rate of fall while changing direction and also put their skills in postural control is the main component for conservation and control of balance [2]. In this history of 10 years was taken and according to them, the whole team has the history of injury. They concluded that lower limb injury is most commonly seen which is approx(63.7%), which is followed by ankle injuries (21,9%) and knee injuries (17.8%)[3]. In addition, an earlier study noted a high prevalence of neck pain (38.8%) and low back pain (42.0%) among Iranian basketball players [4]. One more study described that the most frequent site of injuries seen in basketball players was lower extremities which are 47%[5]. In contrast, several studies have focused on one joint like ankle and shoulder injuries[6] while certain studies have compared injury rates based on gender[7,8]. In basketball, Maintaining balance requires moving, passing, shooting, dribbling. Furthermore, during the game, fine stability allow players to curbs their bodies, decrease errors, defend themselves against falling while switching direction and move speedily within the pitch to implement technical skills productively [9]. In basketball, to achieve better performance both static and dynamic balance plays a crucial role[10]. Nowadays, core training has been very popular and is also an important role in training programs [11]. Core strengthening is defined as the provision of muscular control around the lumbar spine to maintain functional stability [12]. Higher the strength of core region leads to more accurate movement with less energy expenditure in athletes as it grants player to take more load [13].

In healthy individual core strengthening exercises are used for fitness training as in athletes it decrease the rate of injury and thus improves overall performance [14,15]. Bosu ball is a device which was customised by David weck in 1999. Which is especially for balance training and competitive activities in athletes. Bosu ball has a solid plastic base which is united with inflatable rubber bladder tools same as half swissball [16]. Generally, It is used for athletes by placing them in a vertical and horizontal position to improve stability and maintain correct posture [17]. Also used for cardio exercises on rotunda surface. The unstable surface of the Bosu ball is mostly used to improve strength, balance and coordination [18]. The main characteristic of the Bosu ball is furnishing a rocky surface on stable ground. Elastic resistance band is used for resistance training[19]. Which in-turn increases flexibility, mobility, daily activities, metabolic parameters etc [20]. It also play an important role in sports rehab by increasing muscular strength and endurance [21]. To increase the range of motion of the joint the resistance controlled by the theraband increases the adaptation of the muscle to increase its strength [22]. Using a resistance-band which is very light will make exercise less demanding and perhaps pointless. While Using one that is very heavy will make exercise more difficult or lead to injury. TheraBands provides a simple, easy to use exercise tool so that you can get your home exercise program done quickly and easily [23]. The Y-balance test used to asses players risk for injury. To assess the dynamic balance this test is applied. The purpose of this test is to assess the athletes strength, stability and balance in different directions. Y-balance test has proven to have very good interrater test-retest reliability (ICC = 0.80-0.85)[24]. The plank test, also called as prone bridge test. It is fitness test to improve core muscle strength and it can also be used as a fitness exercise for improving core strength. This test is valid reliable and practical both the methods for assessing core muscle endurance in athletes. Which is ICC:0.99 (95% CI = 9.4-0.99) [25].

MATERIALS AND METHODS

Source of data: Various basketball Academy in Ahmedabad

Study design: An experimental

study Sample size: 60

Sample design: A simple random sampling Duration of study: 3 days/week, up to 6 weeks.





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Inclusion Criteria

Willing to participants in this study Age group is 18 to 24 years Both male and female Minimum 6 months of training Participants able to hold plank position up to 2 minutes person who can able to read and write English

Exclusion criteria

Any history of lower limb injury. Lack of cooperation Any other illness that could compromise daily training Any neurological disorders Any orthopedic conditions like fracture, surgery, etc.

Procedure

Total 70 players were assess for the study but 10 players were drop out due to injuries of the lower limb during intervention and lake of cooperation. So, finally 60 players were selected on the basis of inclusion and exclusion criteria. Prior consent form were taken from basketball players. Explanation about the training programme which was given to the players. Players were randomly assigned in to two groups by simple random sampling method. Group 1 was treated with Bosu ball (stable surface). And group 2 was treated with Theraband (Green colour 5.0 lb and blue colour 7.5 lb). Total treatment session were 3 days/week up to 6 weeks. Each session were consist of 3 sets of 15 repetition in 1st week; 4 sets of 15 repetition in 2nd week; 4 sets of 20 repetition in 3rd and 4th weeks and 4 sets of 25 repetition in 5th and 6th weeks. Pre and post assessment were taken in the form of Plank test and y - balance test. Both the groups were treated with same protocol; Curl-up, Bridging, Side bridge, quadruped stabilization, push-ups, Lower abdomen crunches.

RESULTS

In this study 60 basketball players were included out of which 22 were male and 8 were female. In this study the players were taken who had fulfilled the inclusion criteria and randomly allocated in group A Bosu ball exercises and group B theraband exercises. Y-balance test on left leg pre intervention mean score in which anterior = 76.77, PL = 74.00 and PM = 71.33 and post intervention mean score anterior = 83.27, PL = 79.50 and PM = 77.40 of group A. Y-balance test on right leg pre intervention mean score in which anterior = 76.07, PL = 74.13 and PM = 70.83 and post intervention mean score anterior = 81.90, PL = 80.03 and PM = 77.17 of group A. President test on left leg pre intervention mean score plank test = 67.63 and post intervention mean score plank test = 163.07 of group A. Y-balance test on left leg pre intervention mean score in which anterior = 70.83, PL = 68.87 and PM = 67.47 and post intervention mean score anterior = 75.93, PL = 72.83 and PM = 71.33 of group B. Y-balance test on right leg pre intervention mean score in which anterior = 69.00, PL = 66.17 and PM = 70.40 and post intervention mean score anterior = 74.27, PL = 72.77 and PM = 70.49 of group B. pre intervention mean score plank test = 73.37 and post intervention mean score plank test = 144.97 of group B.

DISCUSSION

In this study bosu ball exercises and theraband exercises both are individually and statistically significant in improving core stability and dynamic balance in basketball players; but bosu ball (group A) showed more significant than theraband exercises (group B). The Bosu Ball forms a major part of numerous exercises due to its special design, which specially targets the core strength and balance, these are essential skills for any players to master upon. Standing on its rubber half-dome increases the difficulty and effectiveness of any exercise by forcing to activate the core muscle groups. However Bosu can also be flipped upside down standing on its rigid platform creates even more unstable surface for training static and dynamic balance and stability. This will help improve your footwork once you're back on the basketball court, which is used to enhance and coordinate, while making your shots more controlled and effective [59]. Mutlu Cugstu died Comparative Effects of Different Balance Training Progression Styles on Postural Control and Ankle Force Production. After balance training protocols which helps to gain in force production. Balance training, specially performed on unstable surfaces, increment muscle activation and helps enhance synergist coordination, agonist-antagonist timing, stabilizer function, and motor unit recruitment. These neuromuscular alterations are thought to better sustain the centre of mass with a steady or changing base of support





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or while transitioning from an unstable to a static state [60]. These neuromuscular adaptations assist to maintain postural control and could also enhance muscular force during isometric testing. The results of this study support the current study[61]. Mahmoud (2011) pointed to the importance of balance in implementing technical skills, such as passing, dribbling and shooting while changing direction, stopping, jumping and pretending. The same researcher mentioned that effective execution of the above skills on a small balance base was an indication of a high fitness level and creativity on the player's part [63]. One study involving 8 weeks of core programme training involving plank, bridge, birddog, diagonal crunch, crunch, reverse hyperextension, jack-knife, Russian twist, lateral roll, hip crossover, reverse crunch movements in both the group performing both static dynamic exercises, the core endurance test which are plank, double leg lift, back extension scores increases [64]. The effects of strength and endurance skills of core muscles, which have expanded with training, improvements in the measurements made in tests with similar motion properties similar to the exercises applied are more pronounced [66]. The configuration and quantity of contraction of the overall and local muscles of the lumbar-pelvic-hip complex, acting statically and dynamically in the crunch, quadruped, lateral bridge and hip flexion movements enforced with Theraband and Swiss Ball are suitable with the prone bridge, lateral bridge, trunk flexion, and back extension tests. In this view, both the groups have demonstrated a significant improvement[67].

CONCLUSION

This study showed both the techniques theraband exercises and bosu ball exercises are statistically significant to improve core stability and dynamic balance. But bosu ball exercises are more effective than theraband exercises to improve core stability and dynamic balance in basketball players.

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Table.1. Shows Between Groups Comparison of Y-Balance Test Of Right Leg Group A(Bosu ball) And Group B(Theraband):

Out come	Group	MEAN±SD	Un Paired -T Test	p value
Anterior Direction	Group A	81.90±13.168	2.48	<0.05
Anterior Direction	Group B	74.27±10.531	2.40	<0.05
Postero lateral	Group A	80.03±13.200	2.397	<0.05
Direction	Group B	72.77±10.07	2.397	<0.05
Postero medial	Group A	77.17±13.10	2.131	<0.05
Direction	Group B	70.40±11.43	2.131	<0.05





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Table. 2. Shows Between Groups Comparison of Y-Balance Test of Left Leg Group A(Bosu ball) And Group B (Theraband):

Out come	Group	MEAN ± SD	Un Paired -t test	P value	
Amtorior Direction	Group A	83.27±10.37	2.62	<0.05	
Anterior Direction	Group B	75.93±11.28	2.02	<0.05	
Postero lateral	Group A	79.50±11.79	2.233	<0.05	
Direction	Group B	72.83±11.32	2.233	<0.05	
Postero medial	Group A	77.40±13.63	1.920	<0.05	
Direction	Group B	71.33±10.66	1.920	<0.05	

Table 3. Shows Between Groups Comparison of Plank Test of Group A (Bosu Ball)And Group B(Theraband):

Out come	Group	MEAN±SD	un paired -t test	p value
Plank test	Group A	163.07±36.83	1.042	0.05
	Group B	144.97±34.55	1.963	<0.05





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RESEARCH ARTICLE

Protagonist Role of Chrysin against Mk-801 and Valproic Acid Induced Neurotoxicity in Neuro 2a Cell Lines - A NMDA Receptor Perspective Via Network Pharmacology

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ABSTRACT

Chrysin, a bioactive compound distributed in plants has emerging significance in various neurological disorders. The present investigation was undertaken to assess the efficacy of chrysin against neurotoxicity induced by MK-801 and valproic acid (VPA) in Neuro2A cell lines. The potential molecular interactions between chrysin and NMDA were explored via network pharmacology.Mk-801 revealed to have a higher probability of triggering neurotoxicity than valproic acid. The observations made from the perspective of cell viability indicated that pre-treatment with 1µM chrysin (preceding Mk-801 exposure) was more effective. Morphological abnormalities and existence of apoptosis (Acridine orange staining) were detected in MK-801-treated cells which eventually declined with chrysin pretreatment. The network pharmacology analysis demonstrated 41 hub genes linking chrysin and NMDA. GSK 3, COX 2, CaM, CK2, and Tau were identified as key genes associated to neurodegeneration by the Protein-Protein Interactions. Further, in vivo experiments needs to be conducted to validate the molecular mechanisms underlying the identified genes targets.

Keywords: Chrysin, Neuro2A, MTT, Network pharmacology, Gene Ontology, KEGG, NMDA, Mk-801, Valproic acid





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INTRODUCTION

Valproic acid is a branched, short chain fatty acid is used as an anti-seizure drug[1] whose anticonvulsant effects are regulated by the N-methyl-D-aspartate (NMDA). It is beneficial as it can change the ratio of excitation to inhibition through many pathways, which likely adds to its wide range of clinical benefits[2].NMDA-induced convulsions, and other components of brain glutamatergic activity have been found to be blocked by VPA both in vivo and in vitro by examining the evidence of altered glutamatergic neurotransmission in the brain [3]. MK-801 a non-competitive blocker of the open ion channel of the NMDA receptoran anaesthetic and anticonvulsant whose action is exerted by inhibiting receptors in a voltage-dependent way [4]. It is a well-known neuro-protectant that balances out NMDA-R agonists' excitotoxicity effects. NMDA-R antagonists can prevent GABA, a neuroprotective substance, from being released into the body, which leads to excessive glutamate levels and neurotoxicity[5]. The NMDAR antagonist Mk-801 has been used to mimic the electrophysiological alterations seen in both autism and schizophrenia in behavioural animal models [6]. Chrysin (5,7-dihydroxyflavone) a natural polyphenol found in honey, propolis, and a plethora of medicinal plants and fruits which helps in memory processes [7] and regulates neurogenesis in aging-related memory loss [8]. According to research, chrysin exerts neuroprotective effects via a variety of pathways, including anti-oxidant, anti-inflammatory, and anti-apoptotic properties, MAO inhibition, and GABA mimic capabilities [9]. In this present study, an in vitro strategy using Neuro2a cell lines was adopted to comprehend the potential of chrysin to alleviate MK-801 and Valproic acid independently-induced neurotoxicity. In addition, we employed a network pharmacology method to uncover prospective associations between chrysin and NMDA-related genes. This research would provide a key framework for prosperous exploration of chrysin's pharmacological mechanism.

MATERIAL AND METHODS

Cell culture

In a humidified atmosphere of 5% (v/v) CO_2 and 95% (v/v) air at 37 \square , the mouse neuroblastoma Neuro-2A cell line was maintained in minimal essential medium enriched with 10% foetal bovine serum, 100U/mL penicillin, and 100U/mL streptomycin.

Drug treatment

MK-801, valproic acid, and chrysin were administered at concentrations of 1, 5, 10, 25, 50, and 100µM, respectively. For 1 hour, the cells were pre-treated with chrysin followed by another 24 hours exposure with MK-801 and Valproic acid individually. The same medium without chrysin, MK-801, and Valproic acid was considered as control cells.

Determination of cell viability

Neuro-2A cells were seeded on 96-well plates which were exposed to MK-801 and valproic acid discretely followed by $20\mu I$ of MTT. The cells were maintained at $37\Box$ for 4 hours to disintegrate the dark blue formazan crystals. The supernatants were carefully aspirated and $150\mu I$ of DMSO was injected to each well. A microplate reader at 555nm was employed to measure the absorbance. Cell viability was expressed as percentage of MTT decline with untreated cells exhibiting a 100% absorbance [10].

Investigation of Morphological characteristics in Neuro2A cells

The cells were seeded at a density of 1×10^5 on 6-well plates and treated for 24 hours with chrysin, MK-801, and Valproic acid at doses of 50 μ M and 100 μ M(depending on the % cell viability), respectively. Using a microscope, the cellular morphology was studied and photographs were captured [11].

Fluorescence microscopy investigations to determine apoptosis and necrosis using acridine orange/ethidium bromide double labelling

AO and EB are fluorescent dyes attach to DNA. The unique incorporation of these dyes by cells permits healthy cells to be distinguished from apoptotic and necrotic cells. The following cell fractions were detected: Viable cells have a



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green nucleus that is morphologically normal; early apoptotic cells have a green nucleus with condensed or fragmented chromatin; late apoptotic cells have condensed or fragmented orange/red chromatin; and necrotic cells have a morphologically normal orange/red nucleus [12]. The cells were implanted in 96-well plates subsequently centrifuged for 5 minutes at 1,000 RPM. Each well received 8µl of dye mix, and cells were examined under the microscope [13].

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Chrysin target prediction

The Swiss target prediction database (http://www.swisstargetprediction.ch/) is a web-based tool that may predict bioactive small molecule targets based on ligands [14]. The "SMILES" format of chrysin was retrieved and deposited into the Swiss target prediction database using Pub Chem. Only human targets that have been annotated and examined were chosen as targets.

Predicted targets of NMDA

The GeneCards database (https://www.genecards.org/) was used to identify NMDA-related targets. Using Venny 2.1.0- BioinfoGP, a Venn diagram is constructed illustrating the intersection of genes dictating the predicted chrysin targets pertinent to NMDA [15].

Dataset on Protein-Protein Interactions (PPI)

STRING database was used to obtain the PPI data, the human species "H. sapiens" and a confidence score more than 0.4 were employed to find proteins for predicting protein–protein interactions that interacted directly or indirectly with the identified chrysin and NMDA targets [16].

Pathway Analysis and Gene Ontology

To investigate the biological process, cellular component, and associate pathways for all proposed targets were investigated using the KEGG (Kyoto Encyclopedia of Genes) enrichment analyses [17] and Gene Ontology (ShinyGO) database [18].

Statistical analysis

The experimental data were articulated as mean \pm SD. The graphical depiction of data was carried out using Graph Pad Prism software. All the groups were examined to scrutinize the statistical significance of the differences between the groups using Student's t tests.

RESULTS AND DISCUSSION

Cell viability

The cellular viability of Valproic acid and MK-801 were tested for 24 hours after administration to determine lethal doses at a ranges of 1,5,10,25,50, and 100 μ M, respectively. The potential neuroprotective benefits of chrysin were investigated in Neuro2A cells versus Valproic acid and MK-801 triggered neurotoxicity individually. The cell viability was drastically diminished by MK801 at 1-100 μ M, as shown in the Figure 1 on comparison with control cells, no viable cells were found to be present (25-100 μ M Mk-801). Non-viable cells were found to be present only in trace amounts (1-10 μ M) showing 100% viability and 25 μ M, 50 μ M, and 100 μ M showing 98.65 %, 94.124 %, and 88.457 % viability, respectively (Figure 1). Mk-801 has shown maximum probability of causing neurotoxicity in Neuo2A cell lines when compared to valproic acid. As an outcome, in the following experiments, Mk-801(100 μ M) treatment was used to induce neurotoxicity in Neuro-2acells.

According to previous investigations, the chrysin dosage and administration were described by Tripathi & Mazumder, (2021) [19]. The cells were pre-treated for one hour with varying concentrations of chrysin (1, 5, 10, 25, 50, and 100µM), after which they were incubated for another 24 hours with 100µM Mk-801. Mk-801-induced neurotoxicity was mitigated by pretreatment with chrysin, which restored cell viability (Figure 2). The lowest concentration of chrysin, (1µM), resulted in 90 percent cell viability, whereas the highest concentration (100µM)





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resulted in 59.12 percent cell vitality. Neuro-2A cells treated only with chrysin continued to grow in new media which clearly indicated no significant change in cell viability. Similarly, the DMSO solvent did not to trigger any cytotoxicity. Figure 3 represents the significant difference between the groups analysed by Students t test. The statistical analysis carried out for the experimental data exhibited the subsequent outcomes: **** - Mk-801 vs control representing P value <0.0001, *** - Chrysin pre-treatment and Mk-801 vs control representing P value 0.0006, ** - Mk-801 vs chrysin pre-treatment and Mk-801 representing P value 0.002. Using SH-SY5Y cells, a related research was carried out by Unal & Erdogan, 2020[20].

Morphological changes in Neuro2A cells:

A morphological investigation using phase contrast microscope validated the beneficial property of chrysin. N2a cells were treated with 100 μ M Valproic acid and MK-801, inducing cytotoxicity and morphological changes. Pretreatment of N2a cells with chrysin led to healthy neuritis formation and neuronal outgrowth. Healthy morphology was observed after pre-treatment with 1 μ M chrysin cells. A similar trend was identified in Neuro 2a cell lines which show identical morphology[11]. The cells exhibited significant difference between Mk-801 exposed cells and chrysin pre-treated cells which imply that chrysin has a positive impact to act against Mk-801 induced neurotoxicity (Figure 4-7).

Acridine orange/ethidium bromide staining:

A fluorescence microscope has been used to investigate the dual staining. In the negative control group, no substantial apoptosis was detected. In the MK-801-treated group, early-stage apoptotic cells were distinguished by crescent-shaped or granular yellow-green AO nuclear staining. The number of early-stage apoptotic cells were found to be decreased (1μ M and 5μ M) after chrysin pre-treatment. Only a few remnants of late-stage apoptotic cells were detected (Figure 8). Patnaik & Padhy, (2018) [21]performed a similar comprehensive evaluation to comprehend the toxicity of substances.

Pharmacological Network of Protein-protein interactions and fetched targets of chrysin and NMDA:

There were 100 targets of chrysin acquired from Swiss Target Prediction and 1264 targets of NMDA obtained from Genecards. Following that, 41 recurrent targets were sorted out as prospective chrysin targets in the therapy of NMDA Figure 9. The online complex protein database STRING was used to investigate the interactions among the resulting target proteins. The PPI network was established with 91 nodes and 942 edges, with an average node degree of 20.7 and an average clustering coefficient of 0.593, and was calibrated at low confidence (0.150). P <1.0 x 10-16 value was used to scrutinize whether the PPI enrichment was significant. Three clusters were displayed in the PPI network (Figure 10). Yuan et al., (2021)[22] executed an identical study to comprehend the basis of scopoletin's antilung cancer action.

Gene enhrichment and KEGG pathway Network:

KEGG is a repository that permits investigators to interpret genomic sequences and other high-throughput data biologically. The KEGG Orthology (KO) database records gene and protein molecular events pertaining with ortholog categories. Networks of KO nodes that represent the cell's and organism's high-level functions are generated via the KEGG pathway maps, BRITE hierarchies, and KEGG modules [23]. Utilizing the KEGG and Shiny GO databases, a network was generated to all the relevant genes targeted by chrysin interacting with NMDA in order to establish target gene pathway networking. The KEGG diagram's pathway analysis emphasizes on NMDA-related signalling pathways. GSK 3 β , COX 2, CaM, CK2, and Tau are the key genes responsible for neurodegeneration, as shown in KEGG-labeled diagram Figure 11.

GO concepts are hierarchical ontologies that incorporate biological data in three domains (Biological Process, Molecular Function, and Cellular Component). The top pathways in the biological process category postulated by GO terms associated to NMDA are Regulation of cell population proliferation, Ion transport, homeostatic process, Regulation of response to stress followed by regulation of programmed cell death, positive regulation of signal transduction, regulation of cell death, lipid metabolic process and regulation of apoptotic process (Figure 12). The major pathways involved in the cellular compound category are Organelle sub-compartment, nuclear outer





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membrane- endoplasmic reticulum membrane network, endoplasmic reticulum membrane, mitochondrial membrane, perinuclear region of cytoplasm and dendrite (Figure 13). In order to determine the underlying mechanism of the Yinchensini decoction, Chen et al., (2018) [24] performed GO analysis. Studies of Kang et al., (2021) [25] corroborates our present results. To conclude, chrysin exhibited a neuroprotective effect against MK-801 and Valproicacid induced neurotoxicity. The network pharmacology analysis revealed the molecular mechanism that might interfere in the therapeutic action of chrysin against NMDA. Our present study shall pavethe way to further analyze the efficacy of chrysin in neurodegenerative diseases.

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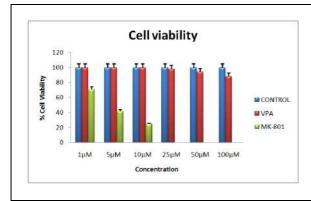


Figure 1: The effects of Mk-801, Valproicacid on Neuro 2a cell lines.

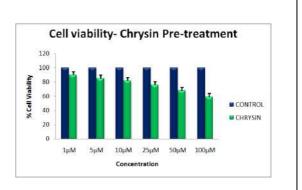


Figure 2: The effects of pre-treatment of chrysin and Mk-801exposure on Neuro 2a cell lines.



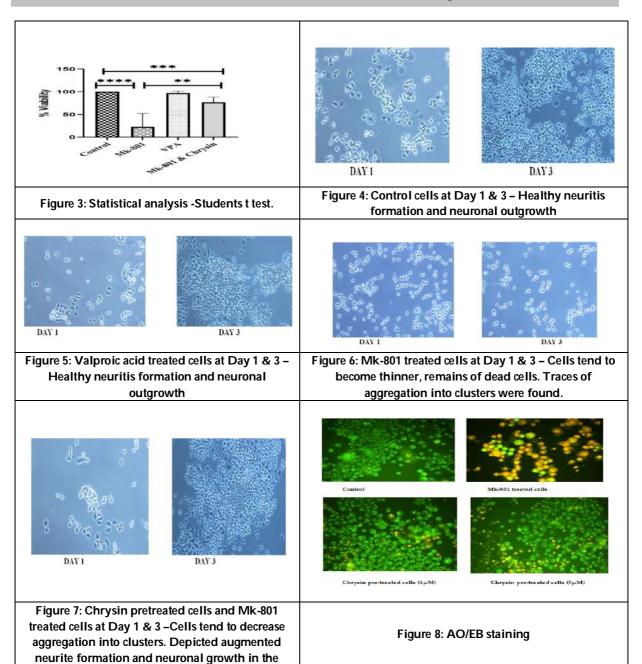


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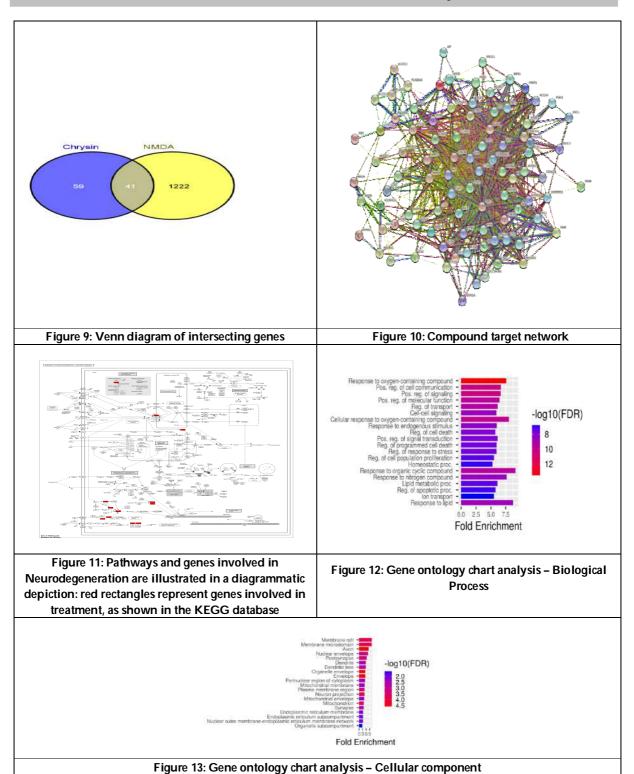




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RESEARCH ARTICLE

Entrepreneurial Attitude and Entrepreneurial Intentions: Mediation of Passion and Mediated Moderation of Creativity

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ABSTRACT

Entrepreneurship plays a vital role in solving social and economic problems such as unemployment, and regional disparities. Recognising the importance of entrepreneurship, policy makers and academia make numerous efforts to develop entrepreneurs. Many factors contribute a person to become an entrepreneur. This study aims at analysing role of attitude on entrepreneurial intentions keeping passion as a mediator and creativity as a mediated moderator. This is a descriptive study and employs a survey method. This study found that entrepreneurial attitude affects entrepreneurial intentions of the engineering students. Further, it is revealed that passion significantly mediates the relationship between entrepreneurial attitude and entrepreneurial intentions.

Keywords: Entrepreneurship, engineering, economic, students

INTRODUCTION

Entrepreneurship has gained importance in the developed and developing countries because it contributes to economic prosperity of the country (Jiatong et al., 2021). Entrepreneurship contributes to employment, and economic growth of the country (Anjum et al., 2022; Bignetti et al., 2021; IwanPrasodjo, Rita Amelinda, 2019). Entrepreneurial intention is a state of mind that motivates to start new business (Biraglia & Kadile, 2017a). Entrepreneurial intentions depend on various internal, external, and personal characteristics of the individuals (Anjum et al., 2022). Many studies established impact of entrepreneurial education on entrepreneurial intentions (Jiatong et al., 2021; Setia & Nuringsih, 2022). Apart from education and training, many internal factors such as motivation, self-efficacy, passion,





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mindset, attitude, passion, altruism, alertness, and creativity have a significant contribution to the entrepreneurial intentions of the individuals. Many external factors such as support of family, support of institution, and government support have also contributed to the entrepreneurial intentions of the study. Theory of Planned Behaviour emphasises that attitude leads to intention and intention, in turn leads to behaviour (Ajzen, 1991). So, attitude of a person towards entrepreneurship plays a significant role on his entrepreneurial intentions. Individuals who are more passionate towards entrepreneurship have more chance of becoming entrepreneurs (Murad *et al.*, 2021). Thus, entrepreneurial passion may contribute more towards entrepreneurial intentions of a person. Creativity indicates the ability of an individual to provide feasible and appropriate solution to the existing problem and in entrepreneurship, creativity refers to creation of new products and services (Bignetti *et al.*, 2021). "Creativity is associated with new ideas and innovation for starting a new business" (Murad *et al.*, 2021). Persons having more entrepreneurial passion are more creative to achieve his/her goal (Murad *et al.*, 2021). This study aims at measuring role of attitude on entrepreneurial intentions keeping passion as a mediator and creativity as a mediated moderator.

Review of the existing research works

Entrepreneurship is a career choice of individuals(Nguyen et al., 2022). Entrepreneurship is not everyone's career path. Being entrepreneur requires certain salient qualities of internal, external, contextual, and personal factors. Entrepreneurial education positively affects entrepreneurial intentions of the individuals (Al Ghafri & Malik, 2021; Boahemaah et al., 2020; Fadli et al., 2020; Hattab, 2014). Entrepreneurial intention is an individual's cognitive image of his entrepreneurial goal that is to be achieved (Fadli et al., 2020). Entrepreneurial intention plays a vital role on entrepreneurial actions (Al Ghafri & Malik, 2021). Intentions are shaped by Individuals' attitude (Ajzen, 1991). Entrepreneurial attitude means how the individuals think and feel about the entrepreneurship (Amofah & Saladrigues, 2022). Entrepreneurial passion is a distinct quality that every entrepreneur possesses (Biraglia & Kadile, 2017a). Entrepreneurial passion indicates intense and positive feeling towards entrepreneurial activities (Cardon & Kirk, 2015). Entrepreneurial passion is a motivational factor towards entrepreneurial activities (Murad et al., 2021). Entrepreneurial passion matters more when the entrepreneurial environment and situation are not favourable (Cardon et al., 2009). Entrepreneurial passion positively impacts entrepreneurial intentions (Moses et al., 2016). Creativity refers to generation of unique and feasible ideas (Biraglia & Kadile, 2017a). Creativity is an essential to entrepreneurial intent(Biraglia & Kadile, 2017a). Entrepreneurial passion enhances creativity (Murad et al., 2021). The existing research works depict that entrepreneurial intentions, attitude, passion, and creativity are interlinked. A lot of research works have been undertaken considering these variables in the entrepreneurial context. However, there are limited studies have been done especially in Indian context about role of attitude, passion, and creativity on entrepreneurial intentions of the individuals. This study tries to bridge the research gap. This study aims at analysing role of attitude on entrepreneurial intentions of the engineering students in India keeping passion as a mediator and creativity as a mediated moderator. Based on the objectives of the study, the following hypotheses have been formulated.

Hypothesis 1: Entrepreneurial Attitude significantly and positively affects entrepreneurial intentions of the engineering students.

Hypothesis 2: Passion significantly and positively mediates entrepreneurial attitude to entrepreneurial intentions relation.

Hypothesis 3: Creativity significantly and positively moderates the mediation effect on passion in entrepreneurial attitude to entrepreneurial intentions relation.

The proposed research model developed is given in figure – 1.

RESEARCH METHODS AND MATERIALS

Research framework

This study is based on descriptive research design to study about entrepreneurial attitude, passion, creativity, and entrepreneurial intentions. Primary data are collected from the engineering students who are the elements of the





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study administering a structured questionnaire. The questionnaire has 2 sections. Section A deals with personal characteristics of the respondents and Section B measures the core variables such entrepreneurial attitude, passion, creativity, and entrepreneurial intentions employing the scales that are validated. This study is a cross-sectional one.

Sample design

Population of this study is the engineering students in India. However, target population for the study is the engineering students in Bangalore because of feasibility and affordability. Bangalore has been chosen as it is a start-up hub and houses many higher educational institutions. More than 5 lakh engineering students pursue their engineering programmes in Bangalore. When the population of the study is more than 75000, but less than 10,00,000, the sample size is 382 at a 95% level of significance(Krejcie & Morgon, 1970). So, the sample size considered for the study is 382. The judgment sampling technique is applied.

Measurement of the variables

Constructs of the study such as entrepreneurial attitude, passion, creativity, and entrepreneurial intentions of engineering students are quantified using appropriate scales available. Measurement scales of the variables are finalised and presented in Table – 1.

Pilot study

Preliminary study is conducted to check the reliability. 63 responses are taken from the engineering students across Bangalore. α scores for the variables such as entrepreneurial attitude, passion, creativity, and entrepreneurial intentions are 0.713, 0.900, 0.754, and 0.712 respectively. As α scores are satisfactory, the main study is carried out.

RESULTS AND ANALYSIS

Normality tests are applied to check the normality of the variables such as entrepreneurial attitude, passion, creativity, and entrepreneurial intentions and test results show that the variables are not normally distributed. Most of the students are male students (64.9%). More than half of the students are in the age group of 17 years to 20 years (56%). 94.2% of the sample engineering institutions are private institutions and they are in urban area. Moreover, differences in perceptions on entrepreneurial attitude, passion, creativity, and entrepreneurial intentions are measured and presented here. Non-parametric mean difference analysis results convey that entrepreneurial attitude, passion, and creativity of the sample engineering students remain unchanged irrespective of personal characteristics of gender, age, branch of the engineering study, and nature of engineering institutions. Similarly, entrepreneurial intentions of the students do not significantly vary based on age, branch of the engineering study, and nature of engineering institutions. But entrepreneurial intentions of the students significantly differ based on gender of the students. Mediating role of passion in entrepreneurial attitude to entrepreneurial intentions is measured and estimated using PROCESS model 4 (Hayes, 2012). Tables 2 and 3 reveal that model is significant (Table – 2). Entrepreneurial attitude significantly impacts passion of the sample students (Table – 3).

The mediating effect of passion of the students are presented Tables 4 and 5. The mediation model is significant (Table – 4). 59.73% variance is explained by entrepreneurial attitude and passion The impact of entrepreneurial attitude and passion on entrepreneurial intentions of the sample students are shown in Table - 5. Both entrepreneurial attitude and passion impact entrepreneurial intentions significantly. So, indirect effects from entrepreneurial attitude to passion (Table – 3) and passion to entrepreneurial intentions (Table – 4) are significant. Entrepreneurial attitude significantly affects entrepreneurial intentions of the students by 78.95% (Table – 6). So, hypothesis 1 is accepted. Total indirect effect of passion in entrepreneurial attitude and entrepreneurial intentions relation is 28.1% and it is significant (Table – 7). Hypothesis 2 is also accepted. Tables 2 to 7 indicate that entrepreneurial attitude affects entrepreneurial intentions significantly and further, passion significantly mediates the relationship between entrepreneurial attitude and entrepreneurial intentions of the sample students. In this study, moderation role of creativity on indirect effect of passion in entrepreneurial attitude and entrepreneurial





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intentions is analysed using model 7 of Process macro. Tables 8 to 10 present mediated moderation analysis. Table – 8 indicates that mediated moderation analysis model is significant. Entrepreneurial attitude affects passion significantly. But creativity and interaction of entrepreneurial attitude and creativity insignificantly affect passion of the students (Table – 9). Since interaction of entrepreneurial attitude and creativity is insignificant, moderation role of creativity on mediation is also insignificant. Unconditional interaction results show that change in impact (r² change) is very nominal, and this impact is not significant. So, it can be said that creativity does not have mediated moderation effect on indirect effect of passion. Hypothesis 3 is rejected.

DISCUSSIONS

Entrepreneurship is a function of many factors. Entrepreneurial intentions are influenced by attitude, passion, alertness, mindset, self-efficacy, and creativity. This study aims at measuring and analyzing the role of entrepreneurial attitude of the students on their entrepreneurial intentions keeping passion as a mediator and creativity as a mediated moderation. Research results indicate that entrepreneurial attitude, passion, and creativity of the students are not based on personal characteristics of the sample students such as gender, age, branch of the study, nature of educational institution, and location of the institution. Similarly, entrepreneurial intentions of the students do not significantly vary based on age, branch of the engineering study, and nature of engineering institutions. But entrepreneurial intentions of the students significantly differ based on gender of the students. Entrepreneurial attitude significantly and positively affects entrepreneurial intentions. Passion significantly and positively mediates the self-efficacy and entrepreneurial intentions relation. Thus, passion encourages the entrepreneurial intentions of the students along with their attitude. However, creativity does not contribute to entrepreneurial intentions along with entrepreneurial attitude and passion of the students.

CONCLUSIONS

This research study aims at measuring and analysing impact of entrepreneurial attitude of the engineering students in India on their entrepreneurial intentions keeping passion as a mediator and creativity as a mediated moderator. Research results reveal that entrepreneurial attitude, entrepreneurial passion, and creativity are not based on gender, age, branch of the study, nature of educational institution, and location of the institution. However, entrepreneurial intentions of the students vary based on gender. Entrepreneurial attitude significantly affects entrepreneurial intentions of the students. Passion significantly and positively mediates the entrepreneurial attitude and entrepreneurial intentions relation. However, creativity does not contribute to entrepreneurial intentions along with entrepreneurial attitude and passion of the students.

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Table.1: Measurement Scales

Variable	Scale Name	Author (s) and Year	No of items
Entrepreneurial Attitude	Entrepreneurial Attitude scale	(Fayolle & Gailly, 2015)	5
Entrepreneurial passion	Entrepreneurial passion scale	(Cardon et al., 2013)	13
Creativity	Creativity scale	(Biraglia & Kadile, 2017b)	6
Entrepreneurial intentions	Entrepreneurial intentions scale	(Liñán <i>et al.,</i> 2011)	6

Table.2: Summary of Model

Table E all may of Model							
r	r²	MSE	F	P			
0.476	0.3821	0.4097	6.7879	0.004			

Outcome variable: Passion

Table.3: Model

Particulars	Coefficients	SE	t	Р
Constant	3.3389	0.2169	15.6208	0.000
Entrepreneurial attitude	0.1373	0.0527	2.6054	0.004

Outcome variable: Passion

Table.4: Summary of Model

r	r²	MSE	F	Р
0.7729	0.5973	0.1646	281.0851	0.000

Outcome variable: Entrepreneurial Intentions

Table.5: Model

Particulars	Coefficients	SE	t	Р
Constant	0.6020	0.1762	3.4163	0.000
Entrepreneurial Attitude	0.7895	0.0337	23.4196	0.000
Passion	0.7184	0.0325	13.5653	0.000

Outcome variable: Entrepreneurial Intentions

Table.6: Direct Effect

Effect	SE	t	P
0.7895	0.0337	23.4196	0.000

Table.7: Indirect Effect

Mediator	Effect	Boot SE	Boot LLCI	Boot ULCI
Passion	0.2810	0.0495	0.1325	0.2152

Table.8: Summary of Model

r	r²	MSE	F	P
0.1996	0.0398	0.4025	5.2282	0.001

Outcome variable: Passion





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Table.9: Model

Particulars	Coefficients	SE	t	Р
Constant	2.9580	1.2043	2.4562	0.014
Entrepreneurial attitude	0.0403	0.3014	0.1338	0.893
Creativity	0.2640	0.3293	0.8017	0.423
Interaction term	0135	0.0783	- 1726	0.863

Outcome variable: Passion

Table. 10: Test of Unconditional Interaction

Particulars	r ² Change	F	Р
Entrepreneurial attitude * creativity	0.0001	.0298	0.863

Outcome variable: Passion

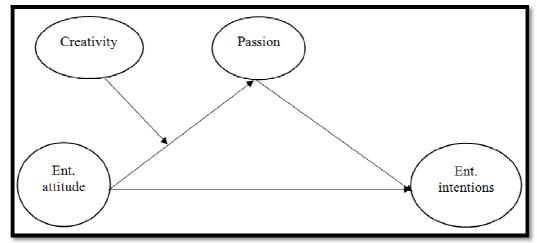


Figure.1: Proposed Research Model





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RESEARCH ARTICLE

Consensus on the Effectiveness of Various Factors Affecting Pulmonary **Function Test**

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ABSTRACT

There are various factors which affect the pulmonary function test of an individual and also is affected by various diseases. The aim of this study is togather suggestive consensus regarding factors affecting pulmonary function in individual from various expert team. The study was conducted as a literature review for factors affecting the pulmonary function from various sources. After gathering the factors, they were entered into the questionnaire. Using the google form, links were generated and were sent to different persons who were present in the panel of experts. Their responses were recorded under the category of Delphi method and the factors which were more affecting according to them were concluded as more effective. The study hereby concluded that lung function testing is related to every factor which is responsible for the medical field or health sector either in the positive way or negative way. Therefore the conclusion of the study was that the various factors have effect on pulmonary function of the lungs

Keywords: pulmonary function, consensus, various factors, pulmonary condition

INTRODUCTION

Pulmonary function test or lung function test are useful in estimating or finding out the functional status of the respiratory system both in physiological and pathological conditions. It is a process of having the patient perform specific inspiratory and expiratory maneuvers. PFT are valuable investigation in the management of patients with suspected or previously diagnosed respiratory disease. They help diagnosis and monitor response to the treatment and can guide decisions regarding further treatment and intervention. The interpretation of PFT requires knowledge of respiratory physiology. In general, this is design to measure lung volumes, bronchial obstruction, gas exchange, lung compliance and ventilator capacity. Therefore, we can suggest that using pulmonary function testing by the





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individual with respiratory conditions will benefits them with required values of their capacities and volumes. It will deal with different conditions and pathologies in the beneficial way and also give appropriate results.

Types of lung function test

- 1. static lung function test
- 2. dynamic lung function test

Static lung function test

It is defined as the volume of air that flows in and out of lung. These tests does not depend upon the rate of the air flow inside the lung rather this test includes static lung volume and capacities.

Lung volumes

Static lung volumes are the volumes of air breathed by an individual. Each of these volumes describes the volume of air present in the lung under specified condition. Tidal volume, Inspiratory reserve volumes, Expiratory reserve volumes and Residual volumes.

Lung capacities

static lung capacities are the combination of two or more lung volumes. Static lung capacities are of four types: Inspiratory capacity, Vital capacity, Functional residual capacity and Total lung capacity.

Dynamic lung function test

It is depend son time, i.e. the rate of air flows in to or out of lungs. These tests include FVC, FEV, MVV and PEF. These tests are useful in determining the severity of obstructive and restrictive lung diseases[1]. Spirometry is the method to measure lung volumes and capacities. Simple instrument used for this purpose is called spirometer. Modified spirometer is known as respirometer. Nowadays plethysmograph is also used to measure lung volumes and capacities. Spirometer is made up of metal and it contains two chambers namely outer chamber and inner chamber. Outer chamber is called the water chamber because it is filled with water. A floating drum is immersed in the water in an inverted position. Drum is counter balanced by a weight. Weight is attached to the top of the inverted drum by means of string or chain. A pen with ink is attached to the counter weight. Pen is made to write on a calibrated paper, which is fixed to a recording device.[2] Inner chamber is inverted and has a small hole at the top. Along metal tube passes through the inner chamber from the bottom towards the top. Upper end of this tube reaches the top portion of the inner chamber. Then the tube passes through a hole at the top of inner chamber and penetrates into outer water chamber above the level of water. A rubber tube is connected to the outer end of the metal tube. At the other end of this rubber tube, a mouth piece is attached. Subject respires through this mouthpiece by closing the nose with a nose clip. When the subject breathes with spirometer, during expiration, drum moves up and the counter weight comes down. Reverse of this occurs when the subject breathes the air from the spirometer, i.e. during inspiration. Upward and downward movements of the counter weight are recorded in the form of a graph. Upward deflection of the curve in the graph shows inspiration and the downward deflection denotes expiration.

Spirometer is used only for a single breath. Repeated cycles of respiration cannot be recorded by using this instrument because carbon dioxide accumulates in the spirometer and oxygen or fresh air cannot be provided to the subject. Computerized spirometer is the solid-state electronic equipment. It does not contain a drum or water chamber. Subject has to respire into a sophisticated transducer, which is connected to the instrument by means of a cable. Spirogram is the graphical record of lung volumes and capacities using spirometer. Upward deflection of the spirogram denotes inspiration and the downward curve indicates expiration. In order to determine the lung volumes and capacities, following four levels are to be noted in spirogram Normal end expiratory level, Normal end inspiratory level, Maximum expiratory level and Maximum in spiratory level[3]. Obesity is becoming more and more dangerous and prevalence rate for obesity is becoming greater worldwide and can be defined by the presence of body mass index (BMI) >30 kg/m2. Regarding changes in function of lung it is related to morbid obesity and been described a consequent decrease in functional residual capacity (FRC) and expiratory reserve volume (ERV)[4]. Diabetes Mellitus-the poorly controlled diabetes is associated with restrictive pattern of pulmonary abnormalities.





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There is disturbed function of lung with FVC,FEV1,DLCObeing significantly lower in poorly controlled comparative with the well- controlled group[5]. Massive weight loss-results point out that the severe morbid obesity (BMIX60 kg/m2) may lead to pulmonary function impairment and presents more prominent gain after massive weight reduction[6]. Pulmonary hypertension - Patients with PPH commonly have abnormalities in lung mechanics and DLCO levels that correlate significantly with disease severity[7]. Body weight and weight gain- might increase the risk of cardiovascular disease hypertension, diabetes, and cancer, as well as other diseases including arthritis, gout, kidney stones, and gallbladder disease. Weight gain is significantly related to lung dysfunction[8]. Dietary factors -The magnitude of the effect of individual dietary factors on pulmonary function observed so far is small. A small number of mis- classification of dietary intake may therefore have a substantial effect on the studied associations. Furthermore, one dietary factor with antioxidant capacity may not be a good indicator of the antioxidant status in the lungs related to diet. It may therefore be better to study the joint Vector of several antioxidant factors on pulmonary function[9]. Sicklecell anemia- adults with Hb-SS were characterized by decreased total lung capacities (70.2 14.7% predicted) and DLCO (64.5 19.9%). The most common PFT patterns were restrictive physiology (74%) and isolated low DLCO (13%). Decreased DLCO was associated with thrombocytosis (p 0.05), with hepatic dysfunction (elevated alanine amino transferase;p0.07), and atrend toward renaldys function (elevated blood urea nitrogen and creatinine; p 0.05 and 0.07, respectively). Pulmonary function is abnormal in 90% of adult patients with Hb-SS. Common abnormalities include restrictive physiology and decreased DLCO. Decreased DLCO may indicate more severe sickle vasculopathy characterized by impaired hepatic and renal function.(10) Effect of body position-Body position influences the results of PFTs, but the optimal position and magnitude of the benefit varies between study populations. One of the main goals of positioning, and specifically the use of upright positions, is to improve lung function in patients with respiratory disorders, heart failure, neuromuscular disease, spinalcord injury(SCI), and obesity, and in the past 20 years, various studies regarding the influence of body position on respiratory mechanics and/or function have been published.(11)Osteoporosis -We conducted a systematic review to examine the relationship between osteoporotic vertebral fractures, kyphosis and pulmonary function. Findings suggest modest but predictable declines in vital capacity related to the degree of kyphosis[12]. Abnormal PFT in NSCL-frequent complication of concurrent chemo radiotherapy(CCRT)and is associated with severe symptoms that decrease quality of life and might result in pulmonary fibrosis or death.

Exposure to radiation frequently induces pulmonary toxicity, which affecting quality of life(QoL) and oxygen dependence and leading to death in up to 50% of cases(13)Pollutinal haze: basic components of pollutional haze are gases (e.g., ozone, sulfur dioxide, nitric oxide, nitrogen dioxide, carbon monoxide, carbon dioxide), volatile organic compounds (e.g., Benzene), and PM(e.g., metals, nitrates, sulfates, organic carbon, microbial components, pollen). these air pollutants undermine lung function principally by way of changing FVC or FEV1[14]. Effect of passive smoking - The father's cigarette smoking status during child's lifetime was linearly related to a decrease in the percent predicted value so FEVI, MMEF and FEF. The Children of who have smoking parents have been observed to have reduced pulmonary function[15]. Effect of pseudomonas aeruginosa - Bronchiectasis patients is susceptible to infection with Pseudomonas aeruginosa. Isolation is associated with increased severity of disease, greater airflow obstruction and poorer quality of life. Infection by Pseudomonas aeruginosa occurs in bronchiectasis patients with more severe impairment of pulmonary function but does not influence rate of decline in pulmonary function either before or after adjustment for baseline diseasese verity[16]. Age and sex -that there are sexual and age differences in respiratory muscle strength and pulmonary function and that smoking or physical activity may affect respiratory muscle function[17]. Genetic and environmental factors-Genetic and environmental factors contribute equally to lung function variation in cystic fibrosis. The gene responsible for CF, the cystic fibrous is trans membrane conductance regulator gene (CFTR). environmental factors have been demonstrated to affect cystic fibrosis lung disease, including second-hand smoke exposure, socio- economic status, healthcare access, and air pollution[18]. Central arterial stiffness in men-that reduced lung function predicts the development of carotid atherosclerotic plaques. Aortic stiffness was estimated from the carotid-femoral pulse- wave velocity (PWV), which increases proportionally with an increase in aortic stiffness[19]. Scoliosis- scoliosis patients is a lack of cardiovascular ability or exercise capacity due to the progression of restrictive lung disease and respiratory insufficiency[20]. Weight gain- after smoking cessation-weight gain that occurs with smoking cessation in persons with early chronic





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obstructive pulmonary disease leads to a relative reduction in FVC and FEV1, with a greater effect on men than on women[21]. Industry-Public health statuses were affected by four main factors, namely environmental factors, community behavior, health services, and heredity. Environmental factors include the physical, chemical, biological, social, and economic environment. Environmental risk factors include total dust levels, inhaled dust levels, exposure time, room ventilation, room temperature, and humidity. Individual risk factors that can affect pulmonary function disorders based on nutritional status, age, working period, use of personal protective equipment, length of exposure, history of the disease, smoking habits, and years of work[22], and in cement industry The aerodynamic diameter of cement particles range from 0.05 to 5.0 µm. These particles are respirable in size, hence Portland cement is important as a potential cause of occupational lung disease. A high prevalence of respiratory symptoms and varying degrees of airway obstruction in relation to Portland cement exposure. The effect of postoperative change in bronchial angle on postoperative pulmonary function after upper lobectomy in lung cancer patients-the change in the angle of the bronchi following upper lobectomy is a physiological change, in some patients with increased angulation, this can lead to a significant decline in pulmonary function. There was an association between the extent of increase in the angle and the decline in pulmonary function[23]. Hence, reviewing the various articles we observed that there are various changes that occur for the pulmonary function testing by the factors so the following study is forgathering the various details for them. Thus there is need to evaluate the more factors in various field of the heath care to be associated whether in the either in a good or bawdy.

Aims And Objectives

AIMS

Aim is to review the factors which affect the PFT values in different field of health by taking consensus.

Objectives:

- To review the factors in various health sectors.
- To conclude the factors by using the consensus method.

Review of Literature

1.AtiNurhayati *et al* published an article on September 2020 "Analysis of Risk Factors that Affect Pulmonary Function Disorder in the Manufacturing Industry" for this study has been conducted on 150 workers in the production section of the manufacturing industry. The research was conducted by measuring workers 'pulmonary function, total dust level, inhaled dust level, room temperature, room humidity, room ventilation, workers' height, and weight. The production unit of the manufacturing industry consisted of 9 parts, namely 1) painting, 2) welding, 3) warehouse,4) assembling, 5) frame, 6) chrome plating & polishing 7) woodworking 8)nailing and9)pipe bending. There was an effect of dust exposure, ambient temperature, nutritional status, and use of personal protective equipment or masks on pulmonary function disorders. Dust exposure has the highest effect on workers' pulmonary function disorders[24].

2.Katz *et al* published an article on 2018"Effect of body position on pulmonary function: a systemic review" for measurement device and patient comfort PFT is usually performed in upright position. the study populations included 29 healthy subjects, 9 with lung disease, 4 with heart disease, 7 with spinal cord injury, 3 with neuromuscular disease and obesity, in which FEV1,FVC,FRC,PEmax,PImax or PEF values were higher in more erect positions. For tetraplegic spinal cord injury FVC and FEV1 Were higher in supine as compared to sitting. In healthy individual DLCO was higher in supine as compared to sitting and in sitting vs side line positions body position influences the result of PFTs but the optimal position and magnitude of the benefit varies between study populations[11].

3.Charles Gerald T. *et al* published an article on 20 June 2016"Pulmonary Function Tests Correlated with Thoracic Volumes in Adolescent Idiopathic Scoliosis" aims to correlate pulmonary function and thoracic volume before and after scoliosis correction. Scoliosis deformity has been linked with deleterious changes in the thoracic cavity that affect pulmonary function.10 patients with the best and 10 patients with the worst TLC values were included.





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Modeled thoracic volume and TLC values were compared before and 2 years after surgery. Scoliosis correction resulted in an increase in the thoracic volume for patients with the worst initial TLCs (11.7%) and those with the best initial TLCs and correction in adolescents was found to increase thoracic volume and is strongly correlated with improved TLC in cases with severe restrictive pulmonary function, but no correlation was found incases with pulmonary function being normal [25].

4."A study on respiratory problems and pulmonary function indexes among cement industry workers in Mashhad, Iran" (2015)This study was conducted to determine the effects of occupational exposure to cement dust on the respiratory system more thoroughly. pulmonary function tests were carried out on 100 exposed and 120 non-exposed workers at the cement factory in Mashhad, Iran. Among the exposed group, respiratory symptoms as cough and sputum weresignificantlymoreprevalentandForcedexpiratoryflowwas25–75%(FEF25–75%)which is significantly lower in the exposed workers compared with non-exposed ones[22].

5.Shao-Kun Liu et al published an article on Nov 20, 2015. "The effect of pollutional haze on pulmonary function" conducted for how much Exposure to pollutional haze, the carrier of air pollutants such as PM and nitrogen dioxide (NO2) has been linked to lung and cardiovascular disease, resulting increases in both hospital admissions and mortality. risk for undermining lung function due to pollutional haze could be related to several factors, such as dose, acquired predisposing diseases, age and genetic susceptibility, etc. according to available information and investigation that higher dose or long-term of exposure to pollutants including PM, sulfur dioxides, nitrogen dioxides, ozone, nitrates, and sulfates induce more observable symptoms and PM2.5 is the most common particle able to initiate detrimental health effects compared to other inhalable particles. The haze particles, Sulfur dioxides, nitrogen dioxides and ozone are the most harmful pollutants and these air pollutants undermine lung function principally by way of changing FVC orFEV1[14].

6.Eitan Kerem *et al* "Factors associated with FEV1 decline in cystic fibrosis: analysis of the European Cystic fibrosis surgery Patient Registry" (2014)Pulmonary insufficiency is the main cause of death in cystic fibrosis (CF). We analysed forced expiratory volume in 1 s (FEV1) data of 14 732 patients. We used linear and logistic regressions to investigate associations between FEV1 % predicted and clinical outcomes. Body mass index (BMI), chronic infection by *Pseudomonas aeruginosa*, pancreatic status and CF-related showed a statistically significant and clinically relevant effect on FEV1 % pred after adjusting for age. Patients with a lower BMI experience a six-fold increased odds ratio of having severe lung disease (FEV1,40% pred) compared to patients with normal BMI. Being chronically infected with *P. aeruginosa* increases the odds ratio of severe lung disease by2.4 and patients with pancreatic in sufficiency experience a 2.0-fold increased odds ratio of severe lung disease compared to pancreatic sufficient patients. Patients with CFRD have a1.8-fold increased odds ratio compared to patients not affected. These potential risk factors for pulmonary disease in patients with CF are to some degree preventable or treatable[26].

7.YangkiSeoka *et al* published an article on 14 November 2013"the effect of postoperative change in bronchial angle on post operative pulmonary function after upper lobectomy in lung cancer patients" Vascular and Thoracic Surgery patients were enrolled in this study. The number of patients involve 99 Among which, 50 underwent left upper lobectomy (LUL) and 4 underwent right upper lobectomy (RUL). Nine patients who underwent LUL showed worsening symptoms, and among them,8 presented an increase in the angle. However, among the 9 patients with worsening symptoms after RUL, only 4 presented an increase in the angle. Decreased FEV1after surgery was observed in 16 patients in the LU L group and 14 in the RUL group. The degree of angle change was also associated with the extent of FEV1reduction[23].

8.J.MichaelCollaco, M.D. *et al* published an article on 2010 November" Quantification of the Relative Contribution of Environmental and Genetic Factors to Variation in Cystic Fibrosis Lung Function" A study of 134 monozygous twins and 272 dizygous twins and siblings in which Lung function among monozygous twins was more similar than among dizygous twin and sibling pairs, regardless of living environment, affirming the role of genetic modifiers in





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cystic fibrosis lung function and concluded Genetic and environmental factors contribute equally to lung function variation in cystic fibrosis[18].

9.Alfredo N.C. Santanaa(2006) *et al* "The effect of massive weight loss on pulmonary function of morbid obese patients" this study especially was take non subjects with BMI>60kg/m2. Thirty- nine morbid obese subjects was taken before and after massive weight loss. patients were divided in groups A with BMI was 40–59.9 kg/m2 and B with BMI>60 kg/m2. Initially, group A had normal PFT, however group B was presented with FVC and FEV1 measurements in the lowest limit of normality with normal FEV1/FVC, significantly different from group A. After massive weightloss,thegroupBcomparedtoAhadasignificantimprovementinFVCandFEV1.These results pointed out that the severe morbid obesity (BMI>60 kg/m2) may lead to pulmonary function impairment and presents more prominent pulmonary function gain after massive weight reduction[6].

10.Eur Respir j et al published an article on 2006 "Effect of Pseudomonas aeruginosa in pulmonary function in patient with bronchiactasis" this study was conducted between group for consecutive non cystic fibrosis adult bronchiectasis outpatient n163 with multiple sputum cultures and follow up pulmonary function tests were designated, according to isolation of P. aeruginosa, as never infected group 1, n67 intermittently isolated group 2 n82 and chronically infected group 3 n14. infection by Pseudomonas aeruginosa occurs in bronchiectasis patients with more severe impairment of pulmonary function but does not influence rate of decline in pulmonary function either before or after adjustment for baseline disease severity, this study shows a significant reduction in FEV1 and FVC in those chronically infected with P. aeruginosa compared with those who had never isolated P. aeruginosa. An accelerated decline in FEV1, FVC was also observed in patient with chronic P. aeruginosa infection but possibility of deterioration prior to P.aeruginosa isolation could not be excluded [16].

- 11.Robyn A Harrison *et al* published an article on December 18 2006"Osteoporosis-Related Kyphosis and Impairments in Pulmonary Function: A Systematic Review" conducted a systematic review to examine to which osteoporosis-related vertebral fractures and kyphosis affect pulmonary function. All studies reported reductions in vital capacity (VC), with values ranging from 68% to 94% of predicted values and The degree of kyphosis clinicallyon one study or radio graphically on three studies correlated with declines in vital capacity; impairments were mostnotableatkyphoticangles>55°.Onthebasisofavailablestudies,declinesinvitalcapacity secondary to kyphosis seem modest and directly related to the number of vertebral fractures or degree of kyphosis[12].
- 12. Elizabeth S. Klings *et al* published an article on March 23, 2006" Abnormal Pulmonary Function in Adults with Sickle Cell Anemia" pulmonary function tests conducted on 310 adults with Hb-SS were analyzed to determine the pattern of pulmonary dysfunction and their association with other systemic complications of sickle cell disease. Overall, adults with Hb-SS were characterized by decreased total lung capacities and DLCO and concluded Pulmonary function is abnormal in 90% of adult patients with Hb-SS[10].
- 13.Xingmgguosun (2003) *et al* " pulmonary function in primary pulmonary hypertension" This study was conducted to know the degree to which abnormalities in resting lung function correlated with the disease severity of patients with primary pulmonary hypertension (PPH).79 patients were assessed for Resting lung mechanics and diffusing capacity for carbon monoxide DLco. whose findings conformed to the classical diagnostic criteria of PPH and who had no evidence of secondary causes of pulmonary hypertension and These findings were correlated with severity of disease as assessed by cardiac catheterization, New York Heart Association(NYHA) class, and cardiopulmonary exercise testing. This study confirmed that Patients with PPH commonly have abnormalities in lung mechanics and DLCO levels that correlated significantly with disease severity[7].
- 14. A M Li, et al published an article on 10 October 2002 "The effects of obesity on pulmonary function" conducted for To determine the predominant pulmonary function abnormality in population of obese children; and to assess the correlation between the severity of lung function impairment and the degree of obesity as assessed by dual energy xray absorptiometry. Sixty four obese patients underwent physical examination, standardized pulmonary





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function test and DEXA scan measurements. This study confirmed that Reduction in FRC and diffusion impairment were the commonest abnormalities found in our cohort of obese patients. Reduction in static lung volume was correlated with the degree of obesity[4].

15.Coratabak(1999)et al" dietary factors and pulmonary function: Across sectional study in middle aged men from three European countries" to evaluated this the data were collected from middle aged men in three European countries - Finland , Italy , Netherlands . In these European countries a high in take of fruit and vegetables was positively associated with pulmonary function before, but not after[9].

16.Robert A. wise (1999) et al "Effect of Weight Gain on Pulmonary Function after Smoking Cessation in the Lung Health Study" The objective of this study was to determine if the weight gain that accompanies smoking cessation is independently associated with reductions in FEV1 and FVC. The analysis included the 5,346 participants who completed PFT at both the baseline and the fifth annual visits. Male and female participants were categorised by assignment group, subjects were categorized by smoking status as either "sustained quitters," "intermittent smokers," or continuing smokers". The major finding of this study was that the weight gain that occurs with smoking cessation, as well as normal aging, is associated with a reduction in both FEV1andFVC. The magnitude of the changes are significant, but these changes do not out weight the benefit of stopping smoking. Weight gain caused greater impairment in FVC than in FEV1, whereas continuing to smoke caused greater decline in FEV1 than in FVC. The effect of weight gain after smoking cessation on FVC was greater in men than in women[21].

17.Robert J. cerfolio *et al* published an article on 1996 "Lung Resection in Patients With Compromise pulmonary function" for this study we reviewed 85 consecutive patients out of which 53 were men and 32 were women whose median age was 70 years with a preoperative forced expiratory volume in 1second of less than1.2L.We concluded that some patients with — lung cancer and compromised pulmonary function can safely undergo pulmonary resection if selected appropriately. We were unable to identify any specific preoperative pulmonary function test as a predictor of postoperative morbidity the study populations included healthy subjects diseases in which FEV1,FVC, FRC, PEmax, PImax or PEF values were higher in more erect positions and DLCO was higher in supine vs sitting and in sitting vs sideling[27].

18.Y Chen *et al* published an article on 1stapril1993 " Body weight and weight gain related to pulmonary function decline in adults: a six year follow up study" The analysis was based on data of the Humboldt cohort study which was conducted in the town of Humboldt, Saskatchewan, Canada. The base line survey in1977 included 1202 adults, comprising94% of all residents aged25-59 years. Of these, 709 (59%) were followed up in 1983. Pulmonary function (FVC), (FEV1s)and maximal mid expiratory flow rate (MMFR)) and weight were measured in both surveys. A residual analysis was used to examine the relationship between body mass index (BMI) at baseline, weight gain, and pulmonary function decline. The results showed that both mean residual FVC and FEV, were highest in the group that gained <10 kg, lowest in the group thatgained>4.0kg,andintermediateinthegroupthatgained1-03-9kginbothmenandwomen after taking age, BMI at baseline, and smoking into account. The effect of weight gain on pulmonary function was greater in men than in women. Weight gain is significantly related to lung dysfunction. The effect of weight gain on pulmonary function is greater then in men and women[15].

19.J.Appl. Physiol. *et al* published an article on 1989" Relationship between respiratory muscle function and age , sex and other factors" for this study 160 healthy volunteers were taken 80 men and 80 women each were divided into four groups according to age, in which 28 male were smokers. Group 1 had the age group from 16 – 30 year , group 2 had the age group from 31-45 year, group 3 had the age group from 46-60 year, group 4 had the age group from 61-75 year and in each group consisted of 20 males and 20 females. After they were familiarized with experimental procedure respiratory muscle strength , inspiratorys muscle endurance and spirometric functions such as FVC, FEV1, FEV1/FVC,TV ,breathing rate and duty cycle were measured . By the end of the study it was concluded that male subjects were more active physically than female[17].





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20.AJPH *et al* published an article on 1986"the Effect of passive smoking on children's pulmonary function in shanghai" the aim of findings of relationship between passive smoking and pulmonary function of children in Shanghai. For this study total of 571childrens were taken which included 303 boys and 268 girls, ranging inagefrom8 to 16 years, from a primary school and a secondary school at Xu-Hui District .Lung function tests were performed at the schools, and questionnaires were completed by parents. Our results showed an obstructive pattern and suggested that exposure to cigarette smoking may have more obstructive than restrictive effect on the lungs. the data showed that the effect of passive smoking is greater in school girls than in boys. School boys may spend more time outdoors than schoolgirls, and hence be less exposed to indoor smoking[15].

MATERIALS AND METHOD

- 1. Research Design- Consenses- (mixed method study)
- 2. Population- any population having any symptoms and disease and who have gone through PFT
- 3. Inclusion and exclusion criteria.

Inclusion criteria

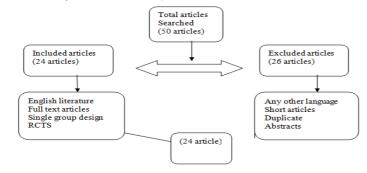
- · Study cross-sectional study, correlational study, cohort study, survey method, regression
- Design- single group design, two group design, randomized control trial, experimental study design, retrospective design.

Exclusive criteria

- We took every factors which was available for the PFT and we did not excluded any study.
- 4. Data collection technique- 1) any articles which were in inclusion criteria where taken as data. 2) Questionnaire through google form was send creating link to the experts.
- 5. Search database-Pubmed
- SciEL O
- Elsevier
- · Research gate
- Google scholor
- Scope Med
- Google

6. Procedure

In our study we followed the procedure described as flow chart-







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RESULTS

According to the review of literature from various sources, there were various factors which were Age, Height, Weight, BMI, Diet, Smoking, Occupation, Hypertension, Obesity, Anemia, Environment, Chest deformities, Altitude, Pollution, Post operative, Osteoporosis. After that they were put in the questionnaire format under various headings and subheadings and then consensus was taken from the panel of experts. The panel of experts included were MD medicine with 7-8 years of experience, masters of physiotherapy in various fields like cardiology, rehabilitation (5-6 years of experience), clinical therapist working with cardiac hospital, pulmonary OPDs and having with 7-8 years of experience. Total of 15-20 experts were selected for the collection of consensuses of the factors but only 10 experts responded well to the questionnaire which was sent to them through link. Thus, with the responses from the team the factors were again sorted and the following factors were finally listed which were Age, Weight, BMI, Smoking, Surgery, Chest deformities and Pollution. The responses were noted and the maximum amount of agreement from the experts were taken as the positive factor. The factor with 90% agreement from the panel was selected to be affecting more in the pulmonary function of the individual. The responses are being explained by the graphs and also excel sheets.

1) DISCUSSION

In this study we reviewed the factors from different literatures and found importance of that factors and how it affects the normal pulmonary function. Factors were jotted down and their positive as well as negative effect were analyzed. So, the number of factors were found and they were presented to the team of experts in the form of google form by creating link. Therefore final conclusion were given after they decided their final result. The list of factors which were analyzed from the review of literature were Age, Height, Weight, BMI, Diet, Smoking, Occupation, Hypertension, Obesity, Anemia, Environment, Chest deformities, Altitude, Pollution, Post operative, Osteoporosis. Thus, with the responses from the team the factors were again sorted and the following factors were finally listed which are Age, Weight, BMI, Smoking, Surgery, Chest deformities and Pollution. The effect of aging can be related to the changes in PFT due to the association where it was proved that both respiratory muscle strength and inspiratory muscle endurance decreased with aging. Harrison RA, Siminoski K, et al (12) in their study showed the effects of gender and age on the pulmonary function and a similar pattern;i.e.1)men had greater spirometric results than women except for the values of FEV/FVC and 2) the pulmonary function decayed as the age increased. Also the previous reports indicated that men have difference in muscle strength than women and this probably is due to different body size between men and women. Although a positive correlation between physical activity and inspiratory muscle endurance is being found. Thus the conclusion by this article studied was, 1) men have greater respiratory muscle function and spirometric values than women, except the inspiratory muscle endurance and FE&/FVC. which are similar in both sexes, 2) the respiratory muscle function and pulmonary function deteriorate with aging, 3) smoking may affect breathing pattern, and 4) physical activity may enhance inspiratory muscle endurance[17]. Therefore, we can prove that aging can be related to respiratory mechanics function and can affect the lungs capacity and function.

Another reason for the same is by Aging-related changes in arterial functional properties that could be attributed to the fatiguing effects of cyclic stress, actingon the inert, nonliving elastic fibers of the arterial wall, with subsequent stretching of the wall and remodeling. Increased central arterial stiffness may also occur, in parallel with increased pulmonary vascular resistance and vessel stiffness. Given the highly vascular nature of the lung and the intimate anatomic coupling of vascular and parenchymal elements, the loss of elasticity of the pulmonary vascular tree would probably affect pulmonary function by not changing any of parenchyma. Another possible explanation for our results is that inflammatory mechanisms act as a contributing factor to both vascular stiffness and reduced lung function. On the one hand, poor lung function could result from increased airway responsiveness and allergy, both of which are prototypical of inflammatory diseases. On the other hand, immune complexes and abnormal inflammatory responses have been implicated in arterial injury, which could result in vascular changes and stiffness.





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The impact of weight gain and loss over pulmonary function is massive. This can be justifiable by reviewing the various article if considered the obesity as a factor. It was found in the study that when there is massive weight loss of the person with morbid obesity than there is change in FVC and in FEV1 despite the same degree of relative weight loss than morbid obese patients with lower BMI. Santana AN, Souza Ret al in their study considered Obesity that can be inducing restrictive disturbance of respiratory function, related to reduce compliance of chest wall and/or pulmonary parenchyma. The chest mechanics in morbid obese are altered due to the subcutaneous adipose tissue and the splinting of diaphragm by intra-abdominal fat, and the decreased lung compliance has been related to increased pulmonary blood volume. Considering PFT changes related to weight reduction, there was a significant correlation between absolute weight loss and absolute changes in FVC. These findings reinforce the data that respiratory function decline related to weight gain, is worse among subjects with higher baseline BMI. Also this should be taken into account that severe morbid obese patients should be encouraged to lose weight to improve their pulmonary function [4] . Another study which relate to this is by the major finding of this study is that the weight gain that occurs with smoking cessation, as well as normal aging, is associated with a reduction in both FEV1 and FVC.(Katz S, Arish N et al). They suggested that Weight gain caused greater impairment in FVCthaninFEV1, where as continuing to smoke caused greater decline in FEV1 than in FVC. Gaining weight has a similar effect on FEV1 and FVC whether subjects continued to smoke or stopped smoking. The association of low body weight with poorer lung function might be the result of either heavier smoking and a greater nicotine effect in this group, or the adverse effects of COPD on nutrition. They found that age had an important effect on the relationship of both FVC and FEV1 with body weight. increasing weight was associated with increases in FEV1 and FVC, presumably because of increased respiratory muscle strength. For any smoking classification (sustained quitter, intermittent smoker, or continuing smoker), men lost more lung function than did women when they gained weight.

This was more no table in FVC thaninFEV1, presumably because the benefits of smoking cessation had greater influence on air flow obstruction as reflected by the FEV1. we have found that the weight gain that occurs with smoking cessation in persons with early COPD leads to a relative reduction in FVC and FEV1, with a greater effect on men than on women[11]. Thus same effect will be seen with the BMI. Body mass index is also impacting the changes in the functioning of the lungs. The chest deformities will it occurs than it affect the patients suffering from them. Even if the deformity is present from the birth it will have effect. Reviewed studies (Harrison RA, Siminoski K)suggested that a restrictive pattern in pulmonary function predominates and that this is directly related to the number of vertebral fractures present and clinical measures of kyphosis. This is not necessarily unexpected, because there is a strong relationship between the number of vertebral fractures and kyphosis and height loss. Kyphosis angle was negatively correlated with percent predicted FEV1. Declines in maximal forced expiratory flows were also observed in the women with osteoporotic fractures, respiratory muscle strength and respiratory muscle endurance Both these factors are associated with impairments in pulmonary function. Thus it can be concluded that paying more attention to the pulmonary status of patients with, or at risk for, osteoporosis related fractures of the spine can improve and suggest the values for pulmonary function[12]. YangkiSeoka, Sukki Cho et al in their reviewed article suggested that in this study, the effect of the degree of bronchial deviation following upper lobectomy on postoperative pulmonary function. It was found that when there is post operative changes that impacts the pulmonary function of the lung. The bronchi are made of cartilage, so with long periods of strong bending force the deformity may be permanent and can lead to the loss of the mechanical force of the bronchi and to bronchomalacia. In order to measure the rate of the angle change, the degree of bronchi deviation was measured between the two points in time. Upward displacement of the remaining lung and the diaphragm following upper lobectomy can lead to deformity of the residual bronchus. In severe cases, this can lead to bronchial kinking or obstruction, causing postoperative total collapse of the remaining lung[23].

There was an effect of dust exposure, physical environment (ambient temperature), individual characteristics (nutritional status),and behavior(use of personal protective equipment /masks)on pulmonary function disorders. Respiratory system disorders can occur in workers, characterized by excessive mucus secretion which causes the main symptoms that of ten occur that are coughing, shortness of breath, and general fatigue. One of the effects of malnutrition is the decline in a person's immune function so that they are prone to infections such as coughs, colds,





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diarrhea, and a decreased ability of the body to detoxify strange objects that enter the body such as dust. Research has been conducted to determine the risk factors that affect pulmonary function disorders. These risk factors come from the environment and individual workers. Pulmonary function disorders are determined by conducting a spirometer test. The spirometer value in women is lower than in men, in women, the FEV1 and FVC values decline at an earlier age (20 years) compared to men who began to decline at the age of 25 years, this is because it is related to physiological and anatomical conditions no correlation between age and pulmonary function disorders. The research was conducted by measuring workers 'pulmonary function, total dust level, inhaled dust level, room temperature, room humidity, room ventilation, workers' height, and weighing. Interviews were conducted to determine age, exercise habits, smoking habits, medical history, the period of work, length of exposure to dust, while the use of protective equipment was accompanied by observation[13]. Thus by this we can say that dust exposure and pollution can increase and decrease the lung function. The smoking has impact on the pulmonary function testing. The active smokers and passive smokers both have effect. Whether adult or child all will have the effect. Supporting article for the same is gained by reviewing the article of passive smoking. It showed that there is significant decrease in the pulmonary function of the child which is indirect contact with the passive smoking. The smoking done by the parents whether mother or the father it will decrease the child's FEV1, MMEF and FEF. The authors felt that smoking information of the mother might be more accurate than that of the father and that the mother might spend more time with her child. The hazardous effect of cigarette smoking on the health of passive smokers depends not only on the number of cigarettes smoked in the families but also on ventilation and the volume of enclosed space.

There were no important effects on the lung function of children of the other environmental factors considered in their study. This is due to the presence of respiratory symptoms in children which is not only associated with parental smoking, but also with respiratory symptoms among parents. Thus it has been reported that tobacco smoke can be a significant source of atmospheric pollution in enclosed areas. Passive smoking may constitute a real threat to the health of many urban children. Passive smoking is not only associated with lower levels of pulmonary function but also with the occurrence of both acute respiratory illness and chronic respiratory symptoms in children.' Concern for the health of their children could be a strong incentive to encourage smoking parents to quit and nonsmokers not to start[14]. The air pollution and smoking are the factors which are responsible for the FEV1 in active smokers requires explanation. At least part of the nonsmokers' function "deficit" may be real, assuming that subjects with initially poorer lung function were less likely to take up smoking, and that most of those who did smoke had not done so long enough to cause appreciable function loss. Previous evidence concerning effects of active smoking by young people is mixed but shows some consistency with those assumptions. Gold and coworkers [12], examining 10to 18- yr-old subjects in the Six Cities Study, found higher FVC in smokers compared with nonsmokers, and similar or higher FEV1, but lower MMEF and FEV1/FVC ratios. Tager and colleagues [14] found generally lower FEV1 in children and adolescents who smoke but little difference between nonsmokers and smokers with low total consumption. Beck and coworkers [15], comparing young smokers and nonsmokers, found higher FEV1 in female smokers aged 7-14 and lower FEV1 in their male counterparts, but the sex differences reversed in the 15-24 age range. Clear negative effects of smoking were seen in college students by Peters and Ferris [27].

The list of factors which were analyzed from the review of literature were Age, Height, Weight, BMI, Diet, Smoking, Occupation, Hypertension, Obesity, Anemia, Environment, Chest deformities, Altitude, Pollution, Post operative, Osteoporosis. After that they were put in the questionnaire format under various headings and subheadings and then consensus was taken from the panel of experts. The panel of experts included were MD medicine with 7-8 years of experience, masters of physiotherapy in various fields like cardiology, rehabilitation (5-6 years of experience), clinical therapist working with cardiac hospital, pulmonary OPDs and having with 7-8 years of experience. Total of 15-20 experts were selected for the collection of consensuses of the factors but only 10 experts responded well to the questionnaire which was sent to them through link. Thus, with the responses from the team the factors were again sorted and the following factors were finally listed which are Age, Weight, BMI, Smoking, Surgery, Chest deformities and Pollution. The study hereby concluded that Pulmonary function testing is related to every factor which is responsible for the medical field or health sector either in the positive way or negative way. Mostly the factors which we have reviewed in this study have suggested the negative effect which have changed the pulmonary





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function values to the greater extent. Thus, we conclude that the various factors have effect on pulmonary function of the lungs

CONCLUSION

Thus, the following study concluded that the factors which were responsible for affecting the PFT were Age, Weight, BMI, Smoking, Surgery, Chest deformities and Pollution.

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2)

3) Table 1: summary table of reviewed articles.

0 - 41	0:	Research	Sample	Outcome	17 6'
Author	Aim	design	characteristics	measures	Key findings
1 Robert J. Cerfolio <i>et al</i>	To identify factor s that affect post operative morbidity and mortality	cohort study	The total sample of 85 consecutive patients 53 men and 32 women	DLCO,PFT	Unable to identify specific preoperative PFT as a predictor of postoperative morbidity
2 JOHN M. PETERS et al	To study the possible chronic respiratory effect s of air pollut ants	Quasi - experimental study	The total sample of 3,293 subjects	PFT	Losses in FVC,FEV1,P EFR,MMEF were associated with pollution level in female and decreased in FVC and FEV1 were associated with peak O3 exposure in male spending more time outdoors
3 J. Michael Collaco <i>et al</i>	To assess the relative contri butions of environmental and genetic factor s to variation in cystic fibros	questionn aires	The total sample of 134 monozygous twins and 272 dizygous twins	Cystic- fibrosis specific FEV1	Genetic and environmental factors contribute equally to lung function variation in CF. Environmental effects are dominated by unique and





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	in/OF) musting a second				ata ala atta affitti-
	is(CF)pulmonary disease				stochastic effects rather than common
	uisease				exposures.
4 EitanKerem et al	aim of this study was to review the association between FEV1 and factors previously described in the literature as major risk factor s of CF lung disease.	regression	The total sample of f 14 732 patients	PFT,BMI, CF-related diabetes (CFRD)	Lower BMI experience a increased odds ratio of having severe lung diseases compared to patients with normal BMI
5 ROBERT A. WISE et al	The aim of this study was to deter mine if the weigh t gain that accompanies smoking cessation is independently associated with reductions in FEV1 and FVC	questionnair e	The total sample of 5,346 participants with 35 to 60 year of age	Forced expiratory Spirometry , body height, BMI	Weight gain that occurs with smoking cessation is associated with reduction in both FEV1 and FVC and on FVC was greater in men than in women
6 Cora Tabak <i>et al</i>	oevaluate the cross sectional association of dietary factors with pulmonary function	Cross sectional study	The total sample of 16 population samples of men aged 40–59 years	PFT, Spirometry	A high intake of fruit and vegetables was positively associated with pulmonary function before but not after
7 YUE CHEN et al	the aim of findings of relationship between passive smoking and pulmonary function of children in Shanghai	Cross sectional study	The total sample of571 study subjects included 303 males and 268 females	PFT, MMEF, chest measurem ent	Results showed an obstructive pattern and suggested that exposure to cigarette smoking may have more obstructive than restrictive effect on the lungs. The data showed that the effect of passive smoking is greater in schoolgirls than in boys. School boys may spend more time outdoors than schoolgirls, and hence be less exposed





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					to indoor
					smoking
8 G.Davies	The purpose of the study was to assess whether P.aeruginosa infection in patients with bronchiectasis is associated with a greater rate of decline in pulmonary function	ANNOVA and linear regression	The total sample of 163 patients	PFT,DLCO ,FE V1,HRCT	Significant reduction in FEV1 and FVC in those chronically infected with <i>P. aeruginosa</i> compared with those who had never isolated <i>P. aeruginosa</i> .
9 AtiNurhayati et al	aimed to determine the risk factors that affected the pulmonary function disorder of workers in the manufacturing industry.	observationa I with cross- sectional design,	The total sample of 150 worker with age between 20-25	Dust, Risk Factors, Pulmonary Function Disorder, Manufactu ring	There was an effect of dust exposure, physical environment (ambient temperature), individual characteristics (nutritional status), and behavior (use of personal protective equipment/masks) on pulmonary function disorders
10 Ehsan Rafeemanes h <i>et al</i>	This study was conducted to determine the effects of occupational exposure to cement dust on the respiratory system more thoroughly	cross sectional study	100 exposed and 120 non- exposed work	occupation al exposure, pulmonary function test, occupation al diseases	Among the exposed group, respiratory symptoms as cough and sputum were significantly more prevalent and Forced expiratory flow was 25–75%(FEF25–75%) which is significantly lower in the exposed workers compared with non-exposed ones.
11 YangkiSeok et al	This Study investigated the effect of the degree	Correlation and comparative study	The total sample of 99 patients where 51	Bronchial angulation, Pulmonary function,	Who underwent LUL showed worsening symptom and





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				1	T
	of bronchial		were male and	Upper	increase in angle
	deviation		48 were	lobectomy	and worsening
	following upper		female		symptoms and
	lobectomy on				increase in angle
	postoperative				after RUL With
	pulmonary				decreased FEV1
	function				after surgery.
	To test if				Results pointed
	morbidobesity				out that the
	causes		The total		severe Morbid
40.416	pulmonary		sample of		obesity may lead
12 Alfredo	function changes	Comparative	39		to pulmonary
N.C.	and if massive	study	morbid	PFT,BMI	function impairment and
Santana et al	weight loss have		obese		presents more prominent
	effect on		subject.		pulmonary function gain
	pulmonary		3 dibjoot.		after massive weight
	function				reduction.
	The study				reduction.
	was done				
	to ascertain				
					Patients with
	the degree to			DET	primary pulmonary
	which			PFT,	hypertension commonly
13 Xing-	abnormalities in		The total	DLCO,	have abnormalities in
Guo Sun et	resting lung	Regression	sample of	New York	lung mechanics
al	function correlate	J	79 patients.	heart	and DLCO levels
	with the disease		'	association	that correlated
	severity of			(NYHA),	significantly with
	patients with				disease severity
	primary				
	pulmonary				
	hypertension				
	Aim to				
	determine the				Adults with Hb-SS were
	pattern				characterized by decreased
	of pulmonary				total
14 51:	dysfunction and	0	The total		lung capacities
14 Elizabeth	their association	Comparative	sample of 310	PFT,DLCO	and DLCO and
S. Klings et al	with other	study	adults		concluded pulmonary
	systemic				function is abnormal in
	complications of				90% of adult patients with
	sickle cell				Hb-SS.
	disease.				55.
	Aim to				FEV1, FVC, FRC, PEmax ,
	investigated the				Plmax or PEF values were
	influence of body	Quasi-	The total	PFT, FEV1,	higher in more erect
15 Shikma	position	experimental	sample of	DLCO,PEF	positions. For tetraplegic
Katz et al	on lung function	study	29	DLCO,FEP	spinal cord injury FVC and
	in healthy	study	subjects		FEV1 Were higher in
	_				o o
	persons and				supine as compared to





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	161 .1 .	1		I	
	specific patient groups.				sitting. In healthy individual DLCO was higher in supine as compared to sitting and in sitting vs side line positions .
16 Robyn A Harrison et al	Aim to systematically review the extent to which osteoporosis- related vertebral fractures and kyphosis affect ulmonary function.	Comparative study	The total sample of 109 patients	PFT, Spirometry , diffusion capacity	declines in vital capacity secondary to kyphosis seem modest and directly related to the number of vertebral fractures or degree of kyphosis
17 L. Torre- Bouscoulet et al	The aim of this study is to identify whether pulmonary function test (PFT) abnormalities may predict RP in non small cell lung cancer (NSCLC) patients	Multi institutional cohort study	The total sample of 52 patients	PFT, Spirometry , six minute walk test, DLCO	All PFT results decreased after complication of concurrent chemoradio therapy (CCRT) and did not return to basal values at follow-up.
18 A M Li et al	To determine the predominant pulmonary function abnormality in our population of obese children; and assess the correlation between the severity of lung function impairment and the degree of obesity as assessed by DEXA	Correlation and Cohort study	The total sample of 64 children includes 16 girls and 48 boys.	DLCO ,Spirometr y, PFT,DEXA	the severe morbid obesity (BMI>60 kg/m2) may lead to pulmonary function impairment and presents more prominent pulmonary function gain after massive weight reduction
19 Shao- Kun Liu et al	conducted for how much Exposure to	Correlation study		PFT	long-term of exposure to pollutants induce





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	pollutional haze, the carrier of air pollutants such as PM and nitrogen dioxide (NO2) has been linked to lung and cardio vascular disease, resulting increases in both hospital admission s and mortality				more observable symptoms and PM2.5 is the most common particle able to initiate detrimental health effects compared to other inhalable particles. The hazeparticles, Sulfur dioxides, nitrogen dioxides and ozone are the most harmful pollutants and these air pollutants undermine lung function principally by way of
20 Chen et al	investigate the effects of gender and age on respiratory muscle function	Experimenta I and comparison study	The total sample of 160 subjects were taken 80 men and 80 women	Respirator y Muscle Function Test, Inspiratory muscle endurance, PFT	changing FVC or FEV1 it was concluded that male subjects were more active physically than female
21 Mahmou D zureik <i>et al</i>	associations of aortic stiffness with lung function measurements.	Cross sectional study	The total sample of 194 men aged 30- 70 year	PFT, blood pressure, pulse wave velocity	reduced pulmonary functionis independently associated with aortic stiffness in men. The interrelations between pulmonary and vascular alterations should be thoroughly investigated.



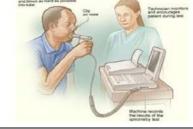


Fig 1- diagram of the huchingston's spirometer

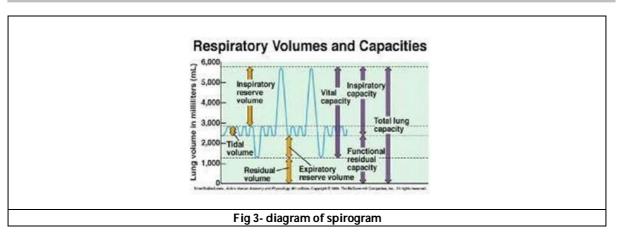
Fig-2 diagram of computerized spirometer





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Graph 1- Personal factors affecting the pulmonary function test

Graph 3- Medical factors affecting the pulmonary function test

Graph 5- Pie chart representing the pulmonary function test

Graph 5- Pie chart representing the chest deformities if affects pulmonary function test than various chest deformities if affects pulmonary function test than various chest deformities affecting the chest deformities if affects pulmonary function test than various chest deformities affecting the pulmonary function test than various chest deformities affecting it.

