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RESEARCH ARTICLE

Solving System of Linear Equations with Fuzzy Parameters

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ABSTRACT

The main objective of this paper is to find the positive fuzzy solution of the fully fuzzy linear system and the dual fully fuzzy linear system whose parameters are positive triangular fuzzy numbers, using the method of least squares. Numerical examples to illustrate the use of this method are shown.

Keywords: Fully fuzzy linear system, Dual fully fuzzy linear system, Triangular fuzzy numbers. Method of Least squares.

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1. Introduction

The system of linear equations plays a vital role in various fields, including mathematics, operations research, statistics, physics, social sciences, and engineering. Many applications of linear systems involve parameters and measurements represented by fuzzy numbers rather than crisp numbers. Therefore, it is essential to develop a mathematical model and numerical procedures to solve linear systems with fuzzy parameters. When all the parameters in a linear system are fuzzy numbers, it is referred to as a fully fuzzy linear system. In this context, the coefficient matrix, the unknown vector, and the right-hand side column vector are all fuzzy numbers. A linear system of this form $\widetilde{D}_1 \otimes \tilde{x} = \widetilde{D}_2 \otimes \tilde{x} \oplus \tilde{e}$ and $\widetilde{D}_1 \otimes \tilde{x} \oplus e_1 = \widetilde{D}_2 \otimes \tilde{x} \oplus \tilde{e}_2$ is known as a dual fully fuzzy linear system.

The concept of fuzzy numbers and fuzzy arithmetic operations was first introduced by Zadeh [5, 6]. Dehghan et al. [1] have solved the $n \times n$ fully fuzzy linear system with triangular fuzzy numbers using Cramer's rule, the Gauss elimination method, the LU decomposition method, and the linear programming approach. Nasseri et al. [2] proposed an LU decomposition method for a fully fuzzy linear system with triangular fuzzy numbers. Radhakrishnan et al. [3, 4] solved the $m \times n$, $(m \geq n)$ fully fuzzy linear system with trapezoidal fuzzy numbers using the QR decomposition method and simplex method.

In this paper, the fully fuzzy linear system of the form $\widetilde{D} \otimes \tilde{x} = \tilde{e}$, the dual fully fuzzy linear systems of the form $\widetilde{D}_1 \otimes \tilde{x} = \widetilde{D}_2 \otimes \tilde{x} \oplus \tilde{e}$ and $\widetilde{D}_1 \otimes \tilde{x} \oplus e_1 = \widetilde{D}_2 \otimes \tilde{x} \oplus \tilde{e}_2$ are considered and the positive solution is obtained by using the method of least squares, where $\widetilde{D}, \widetilde{D}_1, \widetilde{D}_2$ are $m \times n$, $(m \geq n)$ fuzzy matrices consisting of positive triangular fuzzy numbers, the unknown vector $\tilde{x}$ is a fuzzy vector consisting of n positive triangular fuzzy numbers, and the constants $\tilde{e}, \tilde{e}_1, \tilde{e}_2$ are fuzzy vectors consisting of m positive triangular fuzzy numbers.

2. Preliminaries

2.1. Definition

Let X be a universal set. Then, we defined the fuzzy subset $\widetilde{D}$ of X by its membership function $\mu_{\widetilde{D}} : X \to [0, 1]$ which assigns to each element $x \in X$ a real number $\mu_{\widetilde{A}}(x)$ in the interval $[0, 1]$, where the function value of $\mu_{\widetilde{D}}(x)$ represents the grade of membership of x in $\widetilde{D}$. A fuzzy set $\widetilde{D}$ is written as $\widetilde{D} = \{(x, \mu_{\widetilde{D}}(x)), x \in X, \mu_{\widetilde{D}}(x) \in [0, 1]\}$.

2.2. Definition

A fuzzy number $\widetilde{D} = (a, b, c)$ is said to be a triangular fuzzy number, if its membership function is given by

$$\boldsymbol{\mu_{\widetilde{D}}(x)} = \begin{cases} \mathbf{1} - \frac{\boldsymbol{a}-\boldsymbol{x}}{\boldsymbol{b}}, & \boldsymbol{a} - \boldsymbol{b} \leq \boldsymbol{x} \leq \boldsymbol{a}, \boldsymbol{b} > 0 \\ \mathbf{1} - \frac{\boldsymbol{x}-\boldsymbol{a}}{\boldsymbol{c}}, & \boldsymbol{a} \leq \boldsymbol{x} \leq \boldsymbol{a} + \boldsymbol{c}, \boldsymbol{c} > 0 \\ \mathbf{0}, & \boldsymbol{otherwise} \end{cases}$$

2.3 Definition

A triangular fuzzy number $\widetilde{D} = (a, b, c)$ is said to be positive (negative) if and only if $a - b \geq 0$ $(a + c \leq 0)$.

2.4. Definition

Two triangular fuzzy numbers $\widetilde{D} = (a, b, c), \widetilde{E} = (d, e, f)$ are equal if and only if $a = d, b = e, c = f$

2.5. Definition

Let $\widetilde{D} = (a, b, c), \widetilde{E} = (d, e, f)$ are two triangular fuzzy numbers then

$\widetilde{D} \oplus \widetilde{E} = (a + d, b + e, c + f)$

2.6. Definition

Let $\widetilde{D} = (a, b, c), \widetilde{E} = (d, e, f)$ are two triangular fuzzy numbers then

$(i)\ \widetilde{D} \ominus \widetilde{E} = (a - d, b + f, c + e)$

$(ii)\ \widetilde{D} \ominus \widetilde{D} = (a - a, b + c, c + b) = (0, b + c, b + c)$
$\neq (0,0,0) = \tilde{0}$

$(iii)\ \widetilde{E} \ominus \widetilde{E} = (d - d, e + f, e + f) = (0, e + f, e + f)$
$\neq (0,0,0) = \tilde{0}$

2.7. Definition

Let $\widetilde{D} = (a, b, c) > 0, \widetilde{E} = (d, e, f) > 0$ are two triangular fuzzy numbers then$\widetilde{D} \otimes \widetilde{E} = (ad, ae + bd, af + cd)$

2.8. Definition

A matrix $\widetilde{D} = (\widetilde{d}_{ij})$ is called a fuzzy number matrix or fuzzy matrix, if each element of $\widetilde{D}$ is a fuzzy number. A matrix $\widetilde{D}$ is a positive fuzzy matrix ($\widetilde{D} \geq 0$), if each element of $\widetilde{A}$ is positive.

2.9. Definition

Let $\widetilde{D} = (\widetilde{d}_{ij})\ and\ \widetilde{E} = (\tilde{e}_{ij})$ be two $m \times p$ and $p \times n$ fuzzy matrices. We define $\widetilde{D} \otimes \widetilde{E} = \widetilde{F} = (\tilde{f}_{ij})$ which is the $m \times n$ matrix where $\tilde{f}_{ij} = \sum_{k=1,2....n}^{\oplus} \widetilde{d}_{ik} \otimes \tilde{e}_{kj}$

2.10. Definition

A vector $\widetilde{X} = (\tilde{x}_1, \tilde{x}_2, ..., \tilde{x}_n)^T$ is called a fuzzy vector, if element of $\widetilde{X}$ are a fuzzy numbers.

2.11. Definition

The $m \times n$ fuzzy linear system

$(\widetilde{d}_{11} \otimes \tilde{x}_1) \oplus (\widetilde{d}_{12} \otimes \tilde{x}_2) \oplus ... \oplus (\widetilde{d}_{1n} \otimes \tilde{x}_n) = \tilde{e}_1$
$(\widetilde{d}_{21} \otimes \tilde{x}_1) \oplus (\widetilde{d}_{22} \otimes \tilde{x}_2) \oplus ... \oplus (\widetilde{d}_{2n} \otimes \tilde{x}_n) = \tilde{e}_2$
$\vdots$
$(\widetilde{d}_{m1} \otimes \tilde{x}_1) \oplus (\widetilde{d}_{m2} \otimes \tilde{x}_2) \oplus ... \oplus (\widetilde{d}_{mn} \otimes \tilde{x}_n) = \tilde{e}_m$

This can be written as $\widetilde{P} \otimes \tilde{x} = \tilde{a}$. Where the coefficient matrix $\widetilde{D} = (\widetilde{d}_{ij}),\ i = 1\ to\ m,\ j = 1\ to\ n,$ is a triangular fuzzy matrix and $\widetilde{d} = (\widetilde{d}_1, \widetilde{d}_2, ..., \widetilde{d}_m)^T$ is a triangular fuzzy number vector and the $\tilde{x} = (\tilde{x}_1, \tilde{x}_2, ..., \tilde{x}_n)^T$ is the unknown triangular fuzzy number vector. It is called a fully fuzzy linear system.

2.12. Definition

We may represent $m \times n$ fuzzy matrix $\widetilde{D} = (\widetilde{d}_{ij})_{m \times n}$, such that $\widetilde{D}_{ij} = (a_{ij}, b_{ij}, c_{ij};\ )$ with the new notation $\widetilde{D} = (A, B, C)$, where $A = (\boldsymbol{a_{ij}}), \boldsymbol{B} = (\boldsymbol{b_{ij}}),\ \boldsymbol{C} = (\boldsymbol{c_{ij}})$, are three $m \times n$ crisp matrices.

3. The method of Least Squares

3.1. Least-Squares Solutions [7]

Suppose that $Ax = b$ does not have a solution.

The best approximate solution of $Ax = b$ is called the least-squares solution.

That is, "best approximate solution" to an inconsistent matrix equation $Ax = b$.

3.2. Definition [7]

Let A be an $m \times n$ matrix and let b be a vector in R^m. A least-squares solution of the matrix equation $Ax = b$ is a vector $\hat{x}$ in R^n such that $dist(b, A\hat{x}) \leq dist(b, Ax)$ for all other vectors x in R^n.

3.3. Theorem [7]

Let A be an $m \times n$ matrix and let b be a vecotr in R^m. The least squares solutions of $Ax = b$ are the solution of the matrix equation $A^T Ax = A^T b$.

4. Method of finding the positive solution of the fully fuzzy linear system

4.1. Consider the fully fuzzy linear system $\widetilde{D} \otimes \tilde{x} = \tilde{e}$ (Here all the parameter are triangular fuzzy numbers)

Where $\widetilde{D} = (A, B, C) \geq 0, \tilde{x} = (x, y, z) \geq 0;\ \tilde{e} = (f, g, h) \geq 0$

$(A, B, C) \otimes (x, y, z) = (f, g, h)$

$(Ax, Ay + Bx, Az + Cx) =$
$(f, g, h)\quad using\ definition\ 2.7.$

$(A^T Ax, A^T(Ay + Bx), A^T(\ Az + Cx))$
$= (A^T f, A^T\ g, A^T h)$

Using definition 2.4., we have

$A^T Ax = A^T f$

$A^T(Ay + Bx) = A^T\ g$

$A^T(\ Az + Cx) = A^T h$

The above equation can be written as

$A^T Ax = A^T f$
$A^T Ay = A^T(g - Bx)$

$A^T Az = A^T(h - Cx)$

Therefore,

$x = (A^T A)^{-1}\ (A^T f)$

$y = (A^T A)^{-1}[A^T(g - Bx)]$

$z = (A^T A)^{-1}[A^T(h - Cx)]$

Where $(A^T A)^{-1} \neq 0$

4.2. Consider the dual fully fuzzy linear system $\widetilde{D}_1 \otimes \tilde{x} = \widetilde{D}_2 \otimes \tilde{x} \oplus \tilde{e}$ (Here all the parameter are triangular fuzzy numbers)

Where $\widetilde{D}_1 = (A_1, B_1, C_1) \geq 0, \widetilde{D}_2 = (A_2, B_2, C_2) \geq 0, \tilde{x} = (x, y, z) \geq 0;\ \tilde{e} = (f, g, h) \geq 0$

$(A_1, B_1, C_1) \otimes (x, y, z) = (A_2, B_2, C_2) \otimes (x, y, z) \oplus (f, g, h)$

$(A_1x, A\ _1y + B_1x, A_1z + C_1x) = (A_2x, A\ _2y + B_2x, A_2z + C_2x) \oplus (f, g, h)$ using definition 2.7.

$(A_1x, A\ _1y + B_1x, A_1z + C_1x) = (A_2x + f, A\ _2y + B_2x + g, A_2z + C_2x + h)$ using definition 2.5.

Using definition 2.4., we have

$A_1x = A_2x + f$

$A\ _1y + B_1x = A\ _2y + B_2x + g$

$A_1z + C_1x = A_2z + C_2x + h$

The above equation can be written as

$(A_1 - A_2)x = f$

$(A_1 - A_2)y + (B_1 - B_2)x = g$

$(A_1 - A_2)z + (C_1 - C_2)x = h$

Let us take $A_1 - A_2 = A, B_1 - B_2 = B, C_1 - C_2 = C$, then

$Ax = f$

$Ay = g - Bx$

$Az = h - Cx$

Using the method for computing a least-squares solutions of $Ax = f$ are the solutions of the matrix equation $A^TAx = A^Tf$

Therefore, $x = (A^TA)^{-1}\ (A^Tf)$

Similarly we have

$y = (A^TA)^{-1}[A^T(g - Bx)]$

$z = (A^TA)^{-1}[A^T(h - Cx)]$

Where $(A^TA)^{-1} \neq 0$

4.3. Consider the dual fully fuzzy linear system $\widetilde{D}_1 \otimes \tilde{x} \oplus \tilde{e}_1 = \widetilde{D}_2 \otimes \tilde{x} \oplus \tilde{e}_2$ (Here all the parameter are triangular fuzzy numbers)

Where $\widetilde{D}_1 = (A_1, B_1, C_1) \geq 0, \widetilde{D}_2 = (A_2, B_2, C_2) \geq 0, \tilde{x} = (x, y, z) \geq 0,$

$\tilde{e}_1 = (f_1, g_1, h_1) \geq 0, \tilde{e}_2 = (f_2, g_2, h_2) \geq 0$

$(A_1, B_1, C_1) \otimes (x, y, z) \oplus (f_1, g_1, h_1) = (A_2, B_2, C_2) \otimes (x, y, z) \oplus (f_2, g_2, h_2)$

$(A_1x, A\ _1y + B_1x, A_1z + C_1x) \oplus (f_1, g_1, h_1) = (A_2x, A\ _2y + B_2x, A_2z + C_2x) \oplus (f_2, g_2, h_2)$ using definition 2.7.

$(A_1x + f_1, A\ _1y + B_1x + g_1, A_1z + C_1x + h_1) = (A_2x + f_2, A\ _2y + B_2x + g_2, A_2z + C_2x + h_2)$ using definition 2.5.

Using definition 2.4., we have

$A_1x + f_1 = A_2x + f_2$

$A\ _1y + B_1x + g_1 = A\ _2y + B_2x + g_2$

$A_1z + C_1x + h_1 = A_2z + C_2x + h_2$

The above equation can be written as

$(A_1 - A_2)x = f_2 - f_1$

$(A_1 - A_2)y + (B_1 - B_2)x = g_2 - g_1$

$(A_1 - A_2z + (C_1 - C_2)x = h_2 - h_1$

Let us take $A_1 - A_2 = A, B_1 - B_2 = B, C_1 - C_2 = C$, $f_2 - f_1 = f, g_2 - g_1 = g,$ $h_2 - h_1 = h,$ then

$Ax = f$

$Ay = g - Bx$

$Az = h - Cx$

Using the method for computing a least-squares solutions of $Ax = f$ are the solutions of the matrix equation $P^TPx = p^Ta$

Therefore, $x = (A^TA)^{-1}\ (A^Tf)$

Similarly we have

$y = (A^TA)^{-1}[A^T(g - Bx)]$

$z = (A^TA)^{-1}[A^T(h - Cx)]$

Where $(A^TA)^{-1} \neq 0$

5. Numerical examples

5.1. consider the following fully fuzzy linear system

$(5,3,3) \otimes \tilde{x} \oplus (6,2,2) \otimes \tilde{y} = (39,28,28)$

$(7,1,1) \otimes \tilde{x} \oplus (6,3,3) \otimes \tilde{y} = (45,28,28)$

$(7,2,2) \otimes \tilde{x} \oplus (4,2,2) \otimes \tilde{y} = (37,25,25)$

Where $\tilde{x}, \tilde{y} \geq 0$

Solution:

$$\begin{bmatrix} (5,3,3) & (6,2,2) \\ (7,1,1) & (6,3,3) \\ (7,2,2) & (4,2,2) \end{bmatrix} \begin{bmatrix} \tilde{x} \\ \tilde{y} \end{bmatrix} = \begin{bmatrix} (39,28,28) \\ (45,28,28) \\ (37,25,25) \end{bmatrix}$$

$A = \begin{pmatrix} 5 & 6 \\ 7 & 6 \\ 7 & 4 \end{pmatrix}, B = \begin{pmatrix} 3 & 2 \\ 1 & 3 \\ 2 & 2 \end{pmatrix}, C = \begin{pmatrix} 3 & 2 \\ 1 & 3 \\ 2 & 2 \end{pmatrix}, f = \begin{pmatrix} 39 \\ 45 \\ 37 \end{pmatrix}, g = \begin{pmatrix} 28 \\ 28 \\ 25 \end{pmatrix}, h = \begin{pmatrix} 28 \\ 28 \\ 25 \end{pmatrix}$

$x = (A^T A)^{-1} (A^T f)$

$y = (A^T A)^{-1}[A^T (g - Bx)]$

$z = (A^T A)^{-1}[A^T (h - Cx)]$

Solving the above system of equations we have

$\tilde{x} = (3,1,1);\ \tilde{y} = (4,1,1)$

5.2. consider the following fully fuzzy linear system

$(10,3,3) \otimes \tilde{x} \oplus (5,2,2) \otimes \tilde{y} = (5,3,3) \otimes \tilde{x} \oplus (6,2,2) \otimes \tilde{y} \oplus (11,4,4)$
$(12,1,1) \otimes \tilde{x} \oplus (6,3,3) \otimes \tilde{y} = (7,1,1) \otimes \tilde{x} \oplus (6,3,3) \otimes \tilde{y} \oplus (15,5,5)$
$(16,2,2) \otimes \tilde{x} \oplus (8,2,2) \otimes \tilde{y} = (7,2,2) \otimes \tilde{x} \oplus (4,2,2) \otimes \tilde{y} \oplus (43,13,13)$
Where $\tilde{x}, \tilde{y} \geq 0$

Solution:

$A_1 = \begin{pmatrix} 10 & 5 \\ 12 & 6 \\ 16 & 8 \end{pmatrix}, B_1 = \begin{pmatrix} 3 & 2 \\ 1 & 3 \\ 2 & 2 \end{pmatrix}, C_1 = \begin{pmatrix} 3 & 2 \\ 1 & 3 \\ 2 & 2 \end{pmatrix}$
$A_2 = \begin{pmatrix} 5 & 6 \\ 7 & 6 \\ 7 & 4 \end{pmatrix}, B_2 = \begin{pmatrix} 3 & 2 \\ 1 & 3 \\ 2 & 2 \end{pmatrix}, C_2 = \begin{pmatrix} 3 & 2 \\ 1 & 3 \\ 2 & 2 \end{pmatrix}, f = \begin{pmatrix} 11 \\ 15 \\ 43 \end{pmatrix}, g = \begin{pmatrix} 4 \\ 5 \\ 13 \end{pmatrix}, h = \begin{pmatrix} 4 \\ 5 \\ 13 \end{pmatrix}$

$A = A_1 - A_2 = \begin{pmatrix} 5 & -1 \\ 5 & 0 \\ 9 & 4 \end{pmatrix}, B = B_1 - B_2 = \begin{pmatrix} 0 & 0 \\ 0 & 0 \\ 0 & 0 \end{pmatrix}, C = C_1 - C_2 = \begin{pmatrix} 0 & 0 \\ 0 & 0 \\ 0 & 0 \end{pmatrix}$

$x = (A^T A)^{-1} (A^T f)$

$y = (A^T A)^{-1}[A^T (g - Bx)]$

$z = (A^T A)^{-1}[A^T (h - Cx)]$

Solving the above system of equations we have
$\tilde{x} = (3,1,1);\ \tilde{y} = (4,1,1)$

5.3. consider the following fully fuzzy linear system

$(10,3,3) \otimes \tilde{x} \oplus (5,2,2) \otimes \tilde{y} \oplus (12,10,14) = (5,3,3) \otimes \tilde{x} \oplus (6,2,2) \otimes \tilde{y} \oplus (23,14,18)$
$(12,1,1) \otimes \tilde{x} \oplus (6,3,3) \otimes \tilde{y} \oplus (14,8,12) = (7,1,1) \otimes \tilde{x} \oplus (6,3,3) \otimes \tilde{y} \oplus (29,13,17)$
$(16,2,2) \otimes \tilde{x} \oplus (8,2,2) \otimes \tilde{y} \oplus (16,6,10) = (7,2,2) \otimes \tilde{x} \oplus (4,2,2) \otimes \tilde{y} \oplus (59,19,23)$
Where $\tilde{x}, \tilde{y} \geq 0$

Solution:

$A_1 = \begin{pmatrix} 10 & 5 \\ 12 & 6 \\ 16 & 8 \end{pmatrix}, B_1 = \begin{pmatrix} 3 & 2 \\ 1 & 3 \\ 2 & 2 \end{pmatrix}, C_1 = \begin{pmatrix} 3 & 2 \\ 1 & 3 \\ 2 & 2 \end{pmatrix}, f_1 = \begin{pmatrix} 12 \\ 14 \\ 16 \end{pmatrix}, g_1 = \begin{pmatrix} 10 \\ 8 \\ 6 \end{pmatrix}, h_1 = \begin{pmatrix} 14 \\ 12 \\ 10 \end{pmatrix}$
$A_2 = \begin{pmatrix} 5 & 6 \\ 7 & 6 \\ 7 & 4 \end{pmatrix}, B_2 = \begin{pmatrix} 3 & 2 \\ 1 & 3 \\ 2 & 2 \end{pmatrix}, C_2 = \begin{pmatrix} 3 & 2 \\ 1 & 3 \\ 2 & 2 \end{pmatrix}, f_2 = \begin{pmatrix} 23 \\ 29 \\ 59 \end{pmatrix}, g_2 = \begin{pmatrix} 14 \\ 13 \\ 19 \end{pmatrix}, h_2 = \begin{pmatrix} 18 \\ 17 \\ 23 \end{pmatrix}$

$A = A_1 - A_2 = \begin{pmatrix} 5 & -1 \\ 5 & 0 \\ 9 & 4 \end{pmatrix}, \quad B = B_1 - B_2 = \begin{pmatrix} 0 & 0 \\ 0 & 0 \\ 0 & 0 \end{pmatrix}, C = C_1 - C_2 = \begin{pmatrix} 0 & 0 \\ 0 & 0 \\ 0 & 0 \end{pmatrix}$

$f = f_2 - f_1 = \begin{pmatrix} 11 \\ 15 \\ 43 \end{pmatrix}, g = g_2 - g_1 = \begin{pmatrix} 4 \\ 5 \\ 13 \end{pmatrix}, h = h_2 - h_1 = \begin{pmatrix} 4 \\ 5 \\ 13 \end{pmatrix}$

$x = (A^T A)^{-1} (A^T f)$
$y = (A^T A)^{-1}[A^T (g - Bx)]$
$z = (A^T A)^{-1}[A^T (h - Cx)]$

Solving the above system of equations we have

$\tilde{x} = (3,1,1);\ \tilde{y} = (4,1,1)$

6. Conclusion

The $m \times n$, $(m \geq n)$ fully fuzzy linear system is converted into three different $m \times n$ crisp linear systems, and then the positive solution is obtained by using the method of least squares. Also given the procedure to solve the dual fully fuzzy linear systems of the form $\tilde{D}_1 \otimes \tilde{x} = \tilde{D}_2 \otimes \tilde{x} \oplus \tilde{e}$ and $\tilde{D}_1 \otimes \tilde{x} \oplus e_1 = \tilde{D}_2 \otimes \tilde{x} \oplus \tilde{e}_2$. Radhakrishnan et al. [3, 4] solved the $m \times n$, $(m \geq n)$ fully fuzzy linear system with trapezoidal fuzzy numbers using the QR decomposition method and simplex method, but these procedures are very lengthy, and the time consumption is very high when compared to method of least squares.

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RESEARCH ARTICLE

Quantitative Estimation and Comparative Analysis of Polysaccharides and Proteins in Common Dietary Pulses

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ABSTRACT

Pulses are edible seeds from legume plants and are a rich source of polysaccharides and proteins. Estimating the polysaccharide and protein content in pulses provides valuable insights into their nutritional composition. Polysaccharides, primarily in the form of starch and dietary fiber, contribute to the energy content and functional properties of pulses. Proteins, as a major macronutrient in pulses, play a crucial role in human nutrition, particularly as a plant-based protein source.

In the present study, there are four main pulses has been selected for the estimation of polysaccharides and proteins such as *Vigna mungo* (Black gram), *Pisum sativum* (Green peas), *Trigonella foenum graecum* (Fenugreek), and *Cicer arietinum* (Bengal gram) using standardized methods. The dried pulse seeds were powdered and extracted with water to obtain gum.

Total polysaccharides were significantly higher in *Trigonella foenum-graecum* and *Vigna mungo*, while total protein content was highest in *Trigonella foenum-graecum*, followed by *Cicer arietinum*. In both analyses, *Trigonella foenum-graecum* showed the highest levels of estimated phytochemicals, indicating that it contains the highest amounts of bioactive primary metabolites (polysaccharides and proteins). This research report suggested that mung dal and chickpea may also offer significant health benefits. The study recommends including *Trigonella foenum-graecum*, followed by *Vigna mungo* and *Cicer arietinum*, in regular diet to obtain both medicinal and nutritional health benefits.

Keywords: Pulses, Polysaccharides, Proteins, Fenugreek, Bengal gram, Black gram, Green pea

1. Introduction

Plants synthesize a wide array of primary metabolites that serve as essential nutritional resources for humans and herbivorous animals. These compounds not only provide the energy necessary for survival but also contribute significantly to human health by supporting physiological processes and offering various medicinal benefits [1]. Primary metabolites are also involved in the synthesis of essential nutrients and signaling molecules that regulate plant growth and development.

An important application of primary metabolism in food systems is fermentation, a process widely utilized in the production of foods such as bread, yogurt, and alcoholic beverages. During fermentation, microorganisms—particularly yeasts and bacteria—convert carbohydrates into metabolic products like alcohol, carbon dioxide, and organic acids. These by-products influence the texture, flavor, and preservation of food, making fermentation a cornerstone in food processing and preservation [2]. Central metabolic pathways, such as glycolysis and the citric acid cycle, are vital in converting nutrients into ATP, which fuels essential cellular functions across organisms.

Among the primary metabolites, polysaccharides play diverse and critical roles. Biologically, they are involved in cell signaling, adhesion, immune responses, and structural integrity. Industrially, polysaccharides are widely applied in food, pharmaceutical, and biotechnology sectors [3]. Their estimation in food grains is of significant scientific, nutritional, and economic importance. For instance, polysaccharides such as chitosan and alginate are valued for their biocompatibility and are utilized in drug delivery systems, wound healing, and as excipients in pharmaceutical formulations [4]. Hyaluronic acid and heparin serve in tissue engineering, medical implants, and as anticoagulants due to their biological functionality and safety [2].

In the food industry, polysaccharides such as starch, pectin, and carrageenan are employed as thickening agents, stabilizers, and emulsifiers, contributing to improved texture, water retention, and shelf life of food products [5]. Pectin, found abundantly in fruit cell walls, functions as a gelling agent and plays a role in cellular adhesion [6]. Hemicelluloses, including xylans and mannans, interact with cellulose and lignin to confer structural integrity to plant cell walls [7].

Proteins are another fundamental nutrient class, crucial for tissue repair, enzyme and hormone production, and immune function. Adequate intake from diverse sources—including

legumes, fish, eggs, and plant-based proteins—supports overall health and immune resilience. Notably, legumes like beans contain not only high-quality protein but also antioxidants and resistant starches that enhance gut health and modulate inflammation [8].

Rural and traditional diets often emphasize plant-based and legume-rich foods, which are naturally high in fiber, vitamins, and minerals. Such diets are associated with a diverse gut microbiome, reduced systemic inflammation, and improved gastrointestinal health, reflecting benefits similar to those observed in Mediterranean and other plant-based dietary patterns [9].

Among legumes, pulses—the dry edible seeds of the Fabaceae family—have been a cornerstone of global diets for millennia. Rich in plant protein, polysaccharides, dietary fiber, micronutrients, antioxidants, and bioactive compounds, pulses offer substantial nutritional and functional benefits. Numerous studies have highlighted their role in cardiovascular health, glycemic control, and prevention of chronic diseases, underscoring their potential in promoting sustainable and health-conscious diets [10].

Quantifying polysaccharides and protein contents in plants are essential for understanding their nutritional value, monitoring physiological responses, and conducting various biochemical analyses. Accurate estimation of these macromolecules aids in enhancing the utilization of pulses in food and feed industries. Hence, commonly consumed pulses such as *Vigna mungo*, *Pisum sativum*, *Cicer arietinum*, and *Trigonella foenum-graecum* were selected for this study. The research focuses on estimating the polysaccharide and protein contents of these pulses to evaluate their nutritional potential.

2. Materials and Methods

2.1 Collection of Pulses and Isolation of gum

The pulses such as *Vigna mungo, Pisum sativum, Trigonella foenum graecum,* and *Cicer arietinum* are purchased from market. These pulses are belonging to the family Fabaceae. The selected dried pulses of 50g were subjected to dry milling in a mixer and obtained as powder. The obtained powder is soaked in distilled water and shaken frequently for 4-5 hrs. The viscous solution obtained was passed through muslin cloth. The mucilage was precipitated out by adding of 95% ethanol in the ratio of 1:1 by continuous stirring. This solution is dried in oven at moderate temperature and powdered and stored.

2.2 Quantitative Estimation of total Polysaccharides

About 10 mg of gum was dissolved in 100 ml of distilled water. From this 1 ml is used for polysaccharides analysis. To estimate the polysaccharides content in pulses, 1ml of 5% phenol was added to the 1 ml gum solution, followed by 5 ml concentrated H_2SO_4. The absorbance was measured after 10 minutes at 488nm against blank. Aliquots (60-90 µg/ml) of glucose was used as standard [11].

2.3 Quantitative Estimation of total Protein (Lowry's method) [12]

500 mg of gum powder is taken in a mortar and pestle which was added with 10 ml of 0.2M phosphate buffer and ground well, and then it was centrifuged at 2000 rpm for 10 minutes. A clear supernatant is obtained and 1 ml of supernatant is taken and dissolved in 9 ml distilled water. Took 0.5 ml of the unknown protein sample in separate test tubes as triplicates. Added 5 ml of reagent C (Reagent C was obtained from reagent A -2% sodium carbonate in 0.1 N Sodium hydroxide solution and reagent B- 0.5 % of copper sulphate in 1% Potassium sodium tartrate as 50 ml from A is dissolved in 1 ml of B) to each test tubes (aliquot standard-0.2, 0.4, 0.6, 0.8, 1.0ml & samples). Mixed well and incubated at room temperature for 10 minutes. Added 0.5 ml of 50% Folin-ciocalteu reagent to each test tubes. Mixed thoroughly and incubated for 30 minutes at room temperature in the dark. Measured the absorbance at 660 nm using spectrophotometer. Distilled water of 1ml along with all the reagents used as blank.

2.4 Statistical Analysis

The experiments were made as triplicates and calculate Mean ±Standard deviation for each sample. Results were expressed as equivalents of standard per sample.

3. Results and Discussion

3.1 Isolation of gum

The selected pulses such as *Vigna mungo, Pisum sativum, Trigonella foenum graecum,* and *Cicer arietinum* (Figure 1) were powdered. The mucilaginous gum was extracted from each pulse has been oven dried and powdered for the further experiments as an initial step in this study (Figure 2). In a previous study, Camelina (*Camelina sativa* L. Crantz) seeds, especially their bran, contain a significant quantity of monosaccharides and polysaccharides (gums). A decortication procedure was used for improving gum isolation as well as increasing the efficiency of camelina protein isolation and protein quality [13]. Similarly, a new gum was isolated from the roots of *Acanthophyllum bracteatum* (ABG) by warm-water extraction [14].

3.2 Estimation of total Polysaccharides

The extracted gum powder was subjected to polysaccharides estimation (Figure 2 & Table 1). The fenugreek botanically *Trigonella foenum graccum* shows highest polysaccharides as 66.17 µg equivalence Glucose/100 µg followed by Mung dhal (*Vigna mungo*) reported as 61.73 µg equivalence Glucose/100 µg.

Pectic polysaccharides were isolated from the husks of field bean (*Dolichos lab lab*), cowpea (*Vigna sinensis*) and pea (*Pisum sativum*), using HCl (pH 2.0) and 0.5% EDTA at extractants at 70°C, in yields varying from 1.43 to 5.37% [15]. *Kalanchoe pinntum* and *Kalanchoe crenata* (crassulaceae family) leaves and stems of these plants were collected for the gum preparation. According to study, *K. pinnatum* leaves have high content of polysaccharides followed by *K. crenata* leaves as 2.21 and 2.04 (%w/w) respectively [16,17]. In our present study, by comparing the results concluded that *Trigonella foenum graccum*, and *Vigna mungo* have high polysaccharide content than *Cicer arietinum* and *Pisum sativum.*

Table 1: Estimation of total Polysaccharides and total Protein

S.No.	Name of the Pulses	Total Polysaccharides (µg equivalence Glucose/100 µg)	Total Protein (µg BSA equivalence /100 µg)
1.	*Cicer arietinum*	42.06±8.81	**52.61±8.2**
2.	*Pisum sativum*	38.73±6.66	46.81±5.78
3.	*Trigonella foenum graccum*	**66.17±13.56**	**59.11±11.87**
4.	*Vigna mungo*	**61.73±11.46**	24.49±2.44

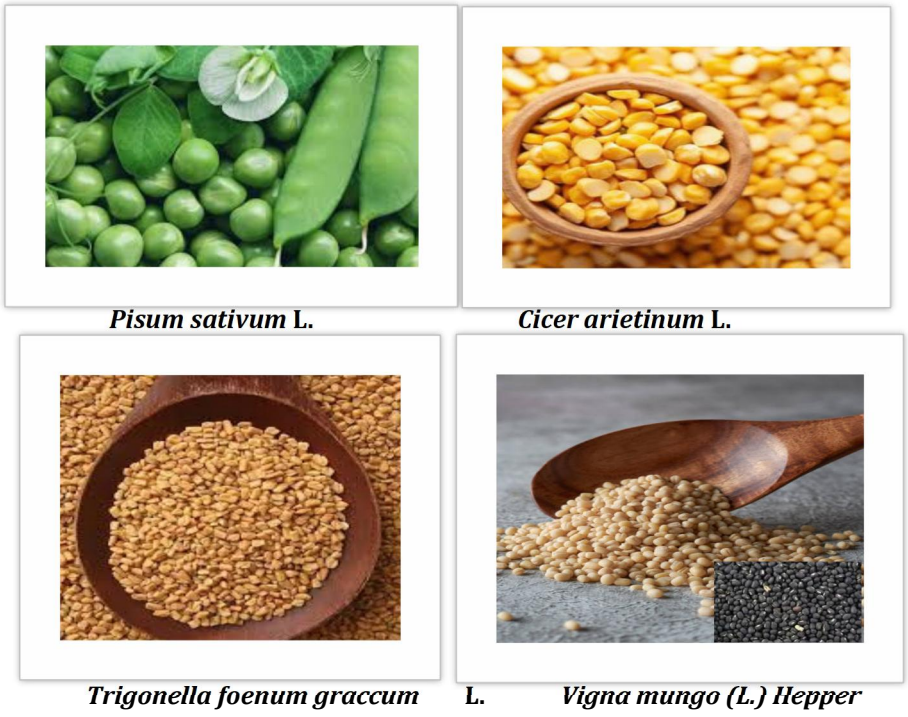
Values are Mean ±Standard Deviation estimated for pulses gum

3.3 Estimation of total Protein

The extracted gum powders were subjected to protein estimation where the fenugreek (*Trigonella foenum graccum*) reported highest protein content followed by chickpea (*Cicer arietinum*) (59.11 and 52.61 µg BSA equivalence /100 µg respectively) (Figure 2 & Table 1). In a previous study, the protein content was estimated for Mung bean which shown the range of 25.8–27.5 % is the highest in the pulse protein after Lupin (32–55.3 %) [18].

From the results, it has been found that under tree category, fruits of *Psidium guajava* shows the highest (98.51 mg BSA Equivalent/ g of Fresh Weight) and *Dillenia indica* shows the lowest (13.73 mg BSAE/ g of FW) amount of protein content. In case of shrubs, *Justicia adhatoda* showed the maximum (86.37 mg BSAE/ g of FW) and *Ocimum canum* shows the minimum (10.59 mg BSAE/ g of FW) amount of protein content. Among the herbs, red *Amaranthus viridis* contains highest (97.43 BSAE/ g of FW) and *Marsilea quadrifolia* contains the lowest (15.04 mg BSAE/ g of FW) content of protein. The study findings conclude that the protein content obtained from the leaves of different plant categories varies in their quantity [19]. In present study, the *Trigonella foenum graccum* (59.11 µg BSA equivalence /100 µg**)** reported as highest among selected pulses which may vary when estimate directly from powder sample of pulses.

Figure 1: Selected Pulses from Legume family



***Pisum sativum* L.** ***Cicer arietinum* L.**

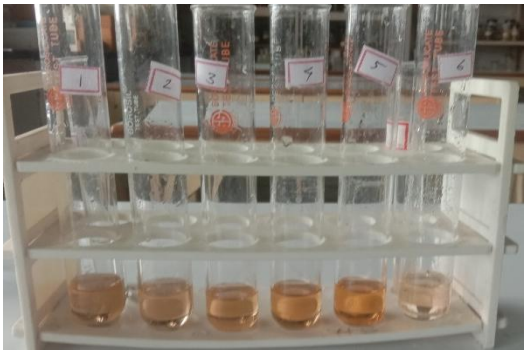
Trigonella foenum graccum* L.** ***Vigna mungo (L.) Hepper

Figure 2: Extraction of gum and Quantitative Estimation for selected Pulses

Pulses powder

Powder mixed with distilled water

Gum Powder of selected Pulses



Estimation of Total Polysaccharides

Estimation of Total Protein

4. Conclusion

Pulses are edible seeds from legume plants and are a rich source of polysaccharides and proteins. Estimating the polysaccharide and protein content in pulses provides valuable insights into their nutritional composition. Polysaccharides, primarily in the form of starch and dietary fiber, contribute to the energy content and functional properties of pulses. Proteins, as a major macronutrient in pulses, play a crucial role in human nutrition, particularly as a plant-based protein source. In the present study, total polysaccharides and proteins were significantly higher in *Trigonella foenum-graecum*. *Vigna mungo* and *Cicer arietinum* contain more polysaccharides and protein, respectively. Regular consumption of these legumes can contribute to balanced nutrition, particularly in plant-based and rural diets, and may play a role in the prevention of chronic diseases such as diabetes, cardiovascular conditions, and obesity. Therefore, promoting the dietary inclusion of these pulses can support both public health and sustainable food systems.

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Conflict of interest

The author declares no conflict of interest.

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RESEARCH ARTICLE

Phytochemical Screening and FTIR Analysis of two Important Medicinal Plant Species of Madurai District

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ABSTRACT

The present study deals with the phytochemical screening and FTIR analysis of available parts (mainly leaves and seeds) of two traditional medicinal plants of two different families found in selected region of Madurai District. Test plants were extracted with methanolic solvent for the presence of flavonoids, glycosides, saponins, tannins, steroids, terpenoids, resins, phenolic compounds, proteins and aminoacids and acidic compounds. We found that the selected plants are good source of various phytochemicals. This study revealed the presence of various biologically active secondary metabolites which could be helpful in the prevention of chronic diseases.

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Keywords: Screening, Alcoholic extracts, secondary metabolites, FTIR analysis

1. Introduction

Plants produce various bioactive phytochemicals which can be grouped under two catagories; primary and secondary metabolites. Primary metabolites include proteins, carbohydrates, amino acids and chlorophyll while polyphenols, alkaloids, terpenoids are some examples of secondary metabolites. Secondary metabolites are the chemicals that are not required for the immediate survival of the plant but synthesized to increase the survival of the plant by allowing it to interact with pathogens, herbivores insects and environment. The plant kingdom is a treasure house of potential drugs and in the recent years there has been an increasing awareness about the importance of medicinal plants. Plants are the richest resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (1). The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. The earliest mention of medicinal use of plants is founds in "Rigveda", which is said to have been written between 4500 - 1600 B.C. and is supposed to be the oldest repository of human knowledge.

Ayurveda is the foundation of medicinal science, in its eight division deals with specific properties of drugs and various aspects of science of life and the art of healing (2). The world health Organization (WHO) estimated that 80% of the population of developing countries still relies on traditional medicines, mostly plant drugs, for their primary health care needs (3). Medicinal plants are a source of great economic value all over the world. Plant products have been part of phytomedicines since time immemorial. Knowledge of the chemical constituents of plants is desirable because such information will be valuable for synthesis of complex chemical substances. There is widespread interest in evaluating drugs derived from plant sources. This interest mainly arises from the belief that green medicine is safe and dependable, compared to costly synthetic drugs which are invariably associated with adverse effects (4). The adverse effects of the drugs available today, necessitate the discovery of new harmless pharmacotherapeutic agents from medicinal plants (5, 6). A knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of such economic materials such as flavoniods, tannins, essential oils, gums, precursors for the synthesis of complex chemical substances, etc. The present work is aimed to screen different phytoconstituents found in two important traditional medicinal plants of Madurai District.

2. MATERIALS AND METHODS

2.1. Collection and Identification of Plant Material

Fresh plant/plant parts were collected from Goripalayam in and around region of Madurai District, Tamilnadu. The plants and the parts were screened, together with their family and vernacular names. The taxonomic identities of these plants were confirmed by Dr. D. Stephen, The American College, Madurai, Tamilnadu, and

the voucher specimen numbers of the plants were preserved. Fresh plant material was washed under running tap water, air dried and then homogenized to fine powder and stored in tight air bottles.

2.2. Preparation of plant extracts

To know the presence of major phytochemicals, the healthy leaf and seed samples were collected and dried in the shade for 2-3 weeks. Then the shade dried leaf and seed samples of *Ziziphus jujuba* and *Myristica fragrans* respectively were made into a fine powder. Following that, 30 g of the powder was filled in the filter paper and successively extracted using 250 ml solvent *viz.*, Methanol using the soxhlet extractor for 8 – 10 hours (7). The extract was filtered through Whatman No.1 filter paper to remove all undissolved matter, including cellular materials and other constitutions that are insoluble in the extraction solvents.

2.3. Preliminary phytochemical studies

The extract was tested for the presence of bioactivecompounds by using following standard methods (8, 9 & 10). The extracts were subjected to preliminary phytochemical tests to determine the groups of secondary metabolites present in the plant material as follows:

2.3.1. Test for flavonoids

The stock solution (1 mL) was taken in a test tube and added few drop of dilute NaOH solution. An intense yellow colour was appeared in the test tube. It became colourless when on addition of a few drop of dilute acid that indicated the presence of flavonoids.

2.3.2. Test for glycosides

Salkowski's test: To the 2 ml of extract, add 2 ml of concentrated sulphuric acid. The appearance of reddish brown colour indicates the presence of glycosides.

2.3.3. Test for saponins

One ml of extract was taken in a test tube and 5 ml of distilled water was added and vigorously shaken. A persistent froth that lasted for at least 15 minutes indicated the presence of saponins.

2.3.4. Test for tannins

Two ml of the extracts were diluted with distilled water in separate test tubes and 2-3 drops of 5 % ferric chloride ($FeCl_3$) solution was added. A green-black or blue-black colouration indicated the presence of tannins.

2.3.5. Test for steroids

2 ml of chloroform and 1ml of concentrated sulphuric acid were added with the 5 ml aqueous plant extract. In the lower, if chloroform layer shows red color appearance that indicates the presence of steroids.

2.3.6. Test for terpenoids

5ml of extract were mixed with 2ml of chloroform and 1ml of concentrated sulphuric acid to form a layer. A reddishbrown coloration of the interface shows the presence of terpenoids.

2.3.7. Test for Resins

1ml of extract was dissolved in acetone and then 1 ml of distilled water is added. Turbidity indicates the presence of resin.

2.3.8. Test for phenolic compounds

Tothe 3 ml of extract, 2 ml of lead acetate solution is added and observed for formation of precipitate.

2.3.9. Test for Proteins and Amino acids

To 2 ml of extract, few drops of nitric acid is added by the sides of the test tube and observed for formation of yellow colour.

2.3.10. Test for Acidic compounds

To 2 ml of the extract, 3 ml of sodium bicarbonate solution is added and observed for the production of effervescences.

2.4. FTIR analysis

The FTIR analysis was conducted on non-extracted leaf and seed powder of *Ziziphus jujuba* and *Myristica fragrans* sample respectively. The 50ºC oven-dried leaves (*Ziziphus jujuba)* and seeds (*Myristica fragrans*) were blended into a fine powder. As much as 1 mg of the sample was mixed with 50 mg KBr (FTIR-grade); then, some of the mixture was placed into the sample holder. All investigations were performed with an IRPrestige-21 (Shimadzu). The scanning absorption range was 400 to 4000 cm^{-1}.

3. RESULTS AND DISCUSSION

The present study subjected to screen the phytochemical constituents and identification of functional group of two medicinal plants namely *Ziziphus jujuba* (Rhamnaceae) and *Myristica fragrans* (Myristicaceae) generally inhabiting at Goripalayam and in and around region of Madurai district, Tamil Nadu, India were selected. The qualitative phytochemical analysis of *Ziziphus jujuba* and *Myristica fragrans* leaf and seed samples respectively with alcoholic solvent, viz., methanol extracts were presented in Tables 1 and 2.

Preliminary phytochemical screening of methanolic leaf extract of *Ziziphus jujuba* was carried out using different methods in order to identify either the presence or absence of secondary metabolites such as tannins, saponins, flavonoids, glycosides, resins, terpenoids, steroids, protein, phenolic compounds and acetic compounds are presented in Table 1.The leaf extract showed positive result for saponins, flavanoids, glycosides andsteroids and the

phytochemicals like tannins, resins, terpenoids, protein, phenolic compounds and acetic compounds were absent.

Another plant species *Myristica fragrans*, study also revealed that the methanolic seed extract was carried out using different methods in order to identify either presence or absence of bioactive compounds such as tannins, saponins, flavonoids, glycosides, resins, terpenoids, steroids, protein, phenolic compounds and acetic compounds are presented in Table 2. The seed extract showed positive result for tannins, saponins, flavanoids, resins, terpenoids, steroids and phenolic compounds and the phytochemicals like glycosides, protein and acetic compounds were absent.

FTIR analysis

The chemical bonds or functional groups present in the dried leaf and seed powder of *Ziziphus jujuba* and *Myristica fragrans* respectively were predicted using FTIR (KBr method). The bonds were determined by interpreting the infrared absorption spectra. Figure I shows the FTIR spectrum of the dried leaf powder, while Table 3 shows the interpretation of the chemical bonds in the non-extracted leaf powder of *Z. jujuba*. Seven major peaks are 3420.14, 2919.7, 1637.27, 1542.77, 1455.08, 1245.79 and 1056.8cm-1in the region between 400-4000 cm-1 (Figure I). Functional groups like amine group, carboxylic acid, conjugation of allergies with two aromatic ring, amide, aromatic ring, aliphatic nitro, amine group, alcohol, ether, carboxylic acid, alcohol and ether were identified. The corresponding functional groups are amine group (3420.14), carboxylic acid (2919.7), conjugation of allergies with two aromatic ring, amide (1637.27), aromatic ring, aliphatic nitro (1542.77), amine group (1455.08), alcohol, ether, carboxylic acid (1245.79), and alcohol and ether (1056.8) respectively (Table 3).

Whereas, Table 4 shows that the interpretation of the chemical bonds in the non-extracted seed powder of *M. fragrans*. Thirteen major peaks are 3391.21, 2922.59, 2852.2, 1707.6, 1635.34, 1514.81, 1460.81, 1375, 1239.04, 1128.15, 1047.16, 862.989 and 720cm-1in the region between 400-4000 cm-1 (Figure II). Functional groups like alcohol, carboxylic acid, carboxylic acid, aldehyde hydrogen, carboxylic acid, carboxylic acid, conjugation of aldehyde with two aromatic ring, amide, aromatic nitro, aromatic ring, amino related, aliphatic nitro, ether, alcohol, carboxylic acid, alcohol, ether, alcohol, ether, aromatic ring and alcohol, ether, aromatic ring were identified. The corresponding functional groups are alcohol, carboxylic acid (3391.21), carboxylic acid (2922.59), aldehyde hydrogen, carboxylic acid (2852.2), carboxylic acid (1707.6), conjugation of aldehyde with two aromatic ring, amide(1635.34), aromatic nitro, aromatic ring (1514.81), amino related (1460.81), aliphatic nitro (1375), ether, alcohol, carboxylic acid (1239.04), alcohol ether (1128.15), alcohol, ether (1047.16), aromatic ring (862.989) and alcohol, ether, aromatic ring (720) respectively (Table 4).

Qualitative phytochemical analysis of *Ziziphus jujuba* and *Myristica fragrans* leaf and seed extracts showed possible presence of chemical principles respectively. The methanolic solvent used to leaf extract from *Ziziphus jujuba* and seed extract from *Myristica fragrans* determined the bioactive compounds. Among the two extract methanolic solvents, the seed methanolic extracts of plant species showed the positive results for seven bioactive compounds like tannins, saponins, flavanoids, resins, terpenoids, steroids and phenolic compounds. Whereas, the methanol leaf extracts showed the positive results for only four bioactive compounds like saponins, flavanoids, glycosides and steroids.

Based on the results, the methanolic seed extracts of the *M. fragrans* plants showed positive results for many bioactive compounds and followed by methanol leaf extracts showed moderate positive results of active compounds. It is explained that the polarity level and species nature are playing major role in extracting the secondary metabolites. Many researchers also informed that the components arranged plants are largely polar. There are different factors that will affect the quantity and composition of the phytocompounds present in an extract. Among these are the types of extraction, time of extraction, temperature, nature of the solvent, solvent concentration and lastly polarity of the solvent (11). These differences may be attributed to the microclimate, processing method as well as the type of solvent employed (12, 13) and genetic variation (14). It also reported that the plants components are more soluble in high polar solvents. It can therefore, be deduced that the amount of extracts recovery is polarity dependent (15). Aqueous could dissolve alkaloid and glycoside compounds, but ethanol was effective to extract sterol, flavonoid, phenolic, and alkaloid (16). FTIR spectrum is generally used tool in plant biological studies (17).

Various chemicals have been used to extract bioactive compounds from plants. In this extraction, the high polar solvent like methanol showed differential extraction i.e., more compounds from seed and some of the compounds from leaf organs of the two studies plant species. The differential extractions may be due to degrading enzymes that may be active or denatured in the alcoholic extractants (18). These secondary metabolites are reported to have many biological and therapeutic properties (19 & 20). The presence of phenolic compounds in the plants indicates that these plants may be an antimicrobial agent (21). Tannins are known to be inhibiting pathogenic fungi (22). Saponin has the property of

precipitating and coagulating red blood cells (23 & 24). Glycosides also have vast therapeutic efficacy as they are found in almost every medicinal plant. Proteins are the building blocks of life. The body needs protein to repair and maintain itself (25).

Therefore, the data obtained from the experiments have provided the chemical basis for the wide use of these plants as therapeutic agents for treating different ailments. The traditional medicine practice is recommended strongly for these plants as well as it is suggested that further work should be carried out to isolate, purify, and characterize the active constituents responsible for the activity of these plants. Also additional work is encouraged to elucidate the possible mechanism of action of these extracts.

4. CONCLUSION

In conclusion, the study findings support these plants can also be used to discover bioactive natural products that may serve as leads for the development of new pharmaceuticals. Hence, the above plants extract could be explored for its highest therapeutic efficacy due to the presence of bioactive components by pharmaceutical companies in order to develop safe drugs for different ailments in future.

Table – 1 Phytochemical screening of Methanol leaf extract of *Ziziphus jujuba*

S.No	Tests	Leaves of *Ocimum tenuiflorum*
1.	Tannins	-ve
2.	Saponins	+ve
3.	Flavanoids	+ve
4.	Glycosides	+ve
5.	Resins	-ve
6.	Terpenoids	-ve
7.	Steroids	+ve
8.	Protein	-ve
9.	Phenolic compounds	-ve
10.	Acetic compounds	-ve

Table – 2 Phytochemical screening of Methanol seed extract of *Myristica fragrans*

S.No	Tests	Leaves of *Ocimum tenuiflorum*
1.	Tannins	+ve
2.	Saponins	+ve
3.	Flavanoids	+ve
4.	Glycosides	-ve
5.	Resins	+ve
6.	Terpenoids	+ve
7.	Steroids	+ve
8.	Protein	-ve
9.	Phenolic compounds	+ve
10.	Acetic compounds	-ve

Table – 3: FTIR spectral peak values and functional groups of dried leaf powder of *Ziziphus jujuba*

Spectrum No.	Wave Number cm-1	Functional group	Predicted compound
1	3420.14	N-H :stretching mode	Amine group
2	2919.7	O-H: Stretching mode	Carboxylic acid
3	1637.27	C=C,C=O :stretching mode	Conjugation of allergies with two aromatic ring, amide

4	1542.77	C=C: stretching mode, N-H:Bending in secondary amine, -NO2:Asymetric stretching mode	Aromatic ring, Aliphatic nitro
5	1455.08	N-H : Bending in secondary amine	Amine group
6	1245.79	C-O, C-F Stretching mode, C=O, C-O-H:Bending mode	Alcohol, Ether, Carboxylic acid
7	1056.8	C-O : Stretching mode C-O : Stretching mode	Alcohol Ether

Table – 4:FTIR spectral peak values and functional groups of dried seed powder of *Myristica fragrans*

Spectrum No.	Wave Number cm-1	Functional group	Predicted compound
1	3391.21	Trible Bound C-H, N-H, O-H:stretchingmode,Hydrogen bonded O-H band	Alcohol, Carboxylic acid
2	2922.59	O-H :stretching mode	Carboxylic acid
3	2852.2	C-H, O-H: stretching mode	Aldehyde hydrogen, Carboxylic acid
4	1707.6	C=O: stretching mode	Carboxylic acid
5	1635.34	C=C,C=O:stretching mode, N-H Bending mode	Conjugation of Aldehyde with two aromatic ring,Amide
6	1514.81	C=C:Stretching mode, N-H: Bending in secondary amine, -NO2:Asymmetric stretching mode	Aromatic nitro, aromatic ring
7	1460.81	N-H: Bending in secondary amine,	Amino related
8	1375	C-O-H bending mode, -NO2:symmetric stretching mode	Aliphatic nitro
9	1239.04	C-O-H, C=O bending mode, C-O, C-F: stretching mode	Ether, Alcohol, Carboxylic acid
10	1128.15	C-O : Stretching mode C-O : Stretching mode	Alcohol Ether
11	1047.16	C-O : Stretching mode C-O : Stretching mode	Alcohol Ether
12	862.989	=C –H : out of- plane bending mode	Aromatic ring
13	720	=C-H: out of plane bending mode, C-CI: stretching mode	Alcohol, Ether, Aromatic ring

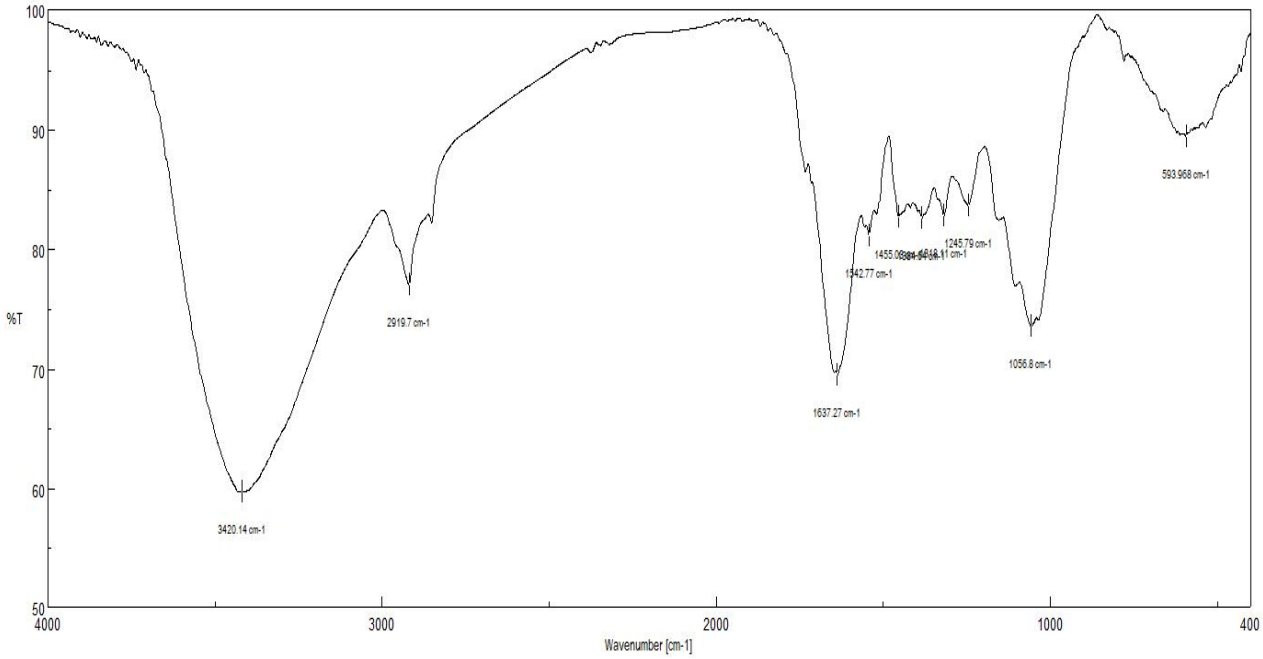

Fig.I: FTIR spectrum of dried leaf powder of *Ziziphus jujube*

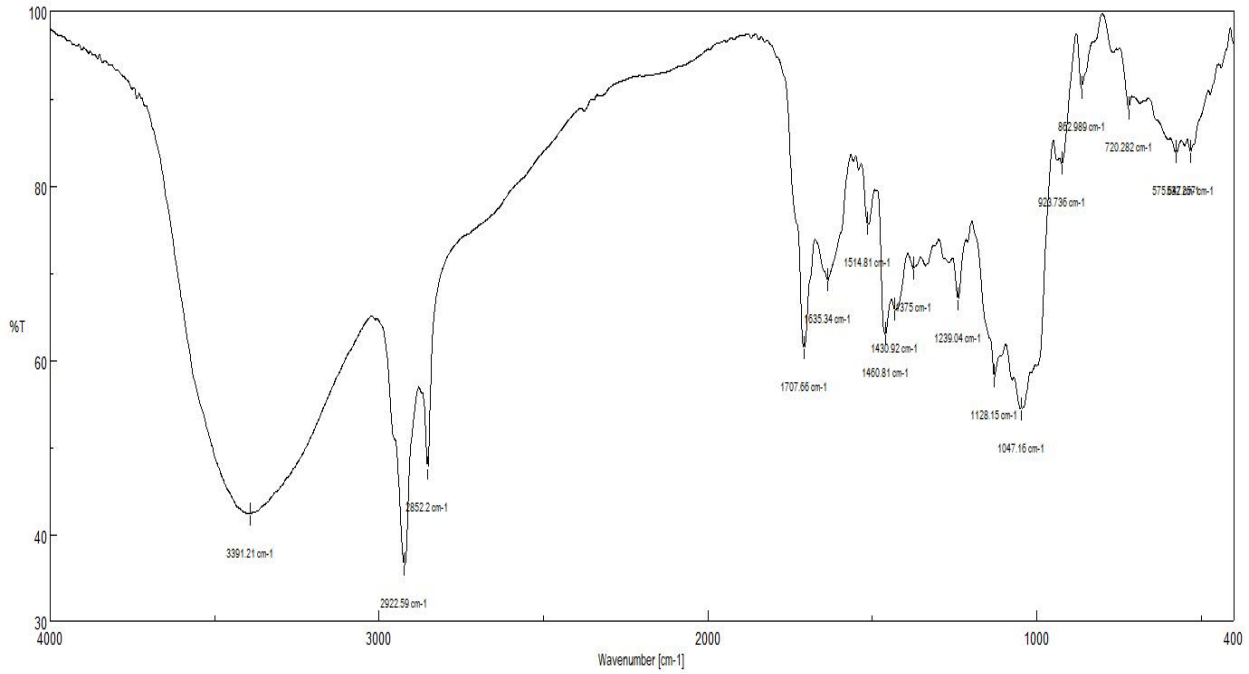

Fig.II: FTIR spectrum of dried seed powder of *Myristica fragrans*

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RESEARCH ARTICLE

NANO g* α- NORMAL SPACES IN NANO TOPOLOGICAL SPACES

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ABSTRACT

The aim of this paper, we introduce a new class of nano normal spaces in a nano topological space. We obtain the relationships of such normal spaces and present some properties and establish various preservation theorems.

Keywords: Nano open, nano α-open, nano g*α -normal space, nano g*α-continuous

1.Introduction

In the year 1971 Viglino[10] who defined semi normal space and the concept of almost normal spaces was introduced by Signal and Arya [9] and they demonstrated that a space is normal only if it is also both a semi-normal and an almost normal space. In 2013 L.Thivagar [5,6] introduced the notion of nano topological spaces for a subset X of a universe that is defined in terms of lower and upper approximations of X. The elements of a nano topological space are called the nano-open sets. He has also studied nano closure and nano interior of a set. Later he was introduced and studied the certain weak forms of nano-open sets namely nano α-open sets, nano pre-open sets etc. The characterizations of mildly nano gb-normal spaces were studied by Arul Mary and Arockiarani I [1].

2. PRELIMINARIES

DEFINITION 2.1[8] :

Let U be a non-empty finite set of objects called the universe and R be an equivalence relation on U named as the indiscernibility relation. Elements belonging to the same equivalence class are said to be indiscernible with one another. The pair (U, R) is said to be approximation space. Let X $\subseteq$ U.

(i) The lower approximation of X with respect to R is the set of all objects, which can be for certain classified as X with respect to R and its is denoted by $L_R(X)$. That is

$L_R(X) = \bigcup_{x \in u} \{R(x): R(x) \subseteq X\}$, where R(x) denotes the equivalence class determined by x.

(ii) The upper approximation of X with respect to R is the set of all objects, which can be possibly classified as X with respect to R and its is denoted by $U_R(X)$. That is $U_R(X) = \bigcup_{x \in u} \{R(x): R(x) \cap X \neq \emptyset\}$, where R(x) denotes the equivalence class determined by x

(iii) The boundary region of X with respect to R is the set of all subjects, which can be classified neither as X nor as not X with respect to R and it is denoted by $B_R(X)$. That is $B_R(X) = U_R(X) - L_R(X)$.

DEFINITION 2.2 [5]:

Let U be the universe, R be an equivalence relation on U and $\tau_R(X) = \{U, \emptyset, L_R(X), U_R(X), B_R(X)\}$ Where X $\subseteq$ U. Then, $\tau_R(X)$ satisfies the following axioms:

(i) U and $\emptyset \in \tau_R(X)$,

(ii) The union of the elements of any subcollection of $\tau_R(X)$ is in $\tau_R(X)$.

(iii) The intersection of the elements of any finite subcollection of $\tau_R(X)$ is in $\tau_R(X)$.

That is , $\tau_R(X)$ is a topology on U called the nanotopology on U with respect to X. We call $(U,\tau_R(X))$ as the nanotopological space. The elements $\tau_R(X)$ are called as nano-open sets.

REMARK 2.3 [5]:

If $\tau_R(X)$ is the nano topology on U with respect to X, then the set $B = \{U, L_R(X), B_R(X)\}$ is the basis for $\tau_R(X)$.

DEFINITION 2.4 [5]:

If $(U, \tau_R(X))$ is a nano topological space with respect to X where X $\subseteq$ U and if A $\subseteq$ U, then the nano interior of A is defined as the union of all nano -open subsets of A and it is denoted by NInt(A).

DEFINITION 2.5 [45]:

If $(U, \tau_R(X))$ is a nano topological space with respect to X where X $\subseteq$ U and if A $\subseteq$ U, then the

nano closure of A is defined as the intersection of all nano -closed sets containing A and it is denoted by NCl(A).

DEFINITION 2.6 [6]:

If $(U, \tau_R(X))$ is a nano topological space and $A \subseteq U$. Then A is said to be (i) Nano semi -open if $A \subseteq NCl(NInt(A))$ (ii) Nano pre- open (briefly nano p-open) if $A \subseteq NInt(NCl(A))$ (iii) Nano α - open if $A \subseteq NInt(NInt(NCl(A)))$ NSO(U, X), NSPO(U,X) and NαO(U,X) respectively we denote the families of all nano semi-open, nano pre-open and nano α-open subsets of $(U, \tau_R(X))$. Let $(U, \tau_R(X))$ be a nano topological space and $A \subseteq U$. A is said to be nano semi-closed, nano pre-closed and nano α-closed if its complement is respectively nano semi-open, nano pre-open and nano α-open.

DEFINITION 2.7 [2] :

A subset A of $(U, \tau_R(X))$ is called nano generalized closed set (briefly Ng-closed) if $Ncl(A) \subseteq V$ whenever $A \subseteq V$ and V is nano open in $(U, \tau_R(X))$.

DEFINITION 2.8 [3] :

A subset A of $(U, \tau_R(X))$ is called nano generalized α closed set (briefly Ngα -closed) if $N\alpha cl(A) \subseteq V$ whenever $A \subseteq V$ and V is nano open in $(U, \tau_R(X))$.

DEFINITION 2.9 :

A subset A of $(U, \tau_R(X))$ is said to be a α -neighborhood of u, if there exists a nano-open set V such that $u \in V \subseteq A$

DEFINITION 2.10 :

A function $f: (U, \tau_R(X)) \to (V, \tau_{R'}(Y))$ is said to be (1) nano continuous [6] if the inverse image every nano open set V is nano open in U. (2) almost nano α-irresolute if for each u in U and each nano α-neigjbourhood N of f(u) in V, $N\alpha Cl(f^{-1}(V))$ is a nano α-neighbourhood of u in U. (3) nano Mα-closed (nano Mα-open), if f(A) is nano α-closed(resp. nano α-open) set in V for each nano α-closed (resp. nano α-open) set A in U. (4) nano gα closed if f(F) is nano gα-closed set in V for every nano closed subset F of U.

LEMMA 2.11:

A function $f: (U, \tau_R(X)) \to (V, \tau_{R'}(Y))$ is weakly nano open nano continuous function, then f is nano Mα -open and nano R-map.

2. NANO g*α- NORMAL SPACES

DEFINITION 3.1:

Let $(U,\tau R(X))$ be a Nano topological spaces. A subset A of $(U,\tau R(X))$ is called Nano g*α-closed set if $Ncl(A) \subseteq V$ Where $A \subseteq V$ and V is Nano g-open.

DEFINITION 3.1 :

A nano topological space $(U, \tau_R(X))$ is said to be nano g*α- normal spaces if for any pair of disjoint nano closed sets A and B of U there exist nano g*α- pen sets V and W of U such that A M and B N.

EXAMPLE 3.2:

Let U = {a,b,c}with U/R = {{a}, {b, c}}. Then the nano topology, $\tau_R(X)$= {U, , {a}, {b,c}}. Hence the only pair of disjoint closed subsets of $(U, \tau_R(X))$ is {b, c}, {a}. Also {a},{b,c} are nano g*α-open sets such {a} {a} and {b,c} {b,c}.

We have the following characterization of nano g*α - normal spaces.

THEOREM 3.4:

For a nano topological spaces $(U, \tau_R(X))$ and $(V, \tau_{R'}(X))$ the following are equivalent.

(i) U is nano g*α- normal space.

(ii) for every pair of nano open sets M and N whose union is U, there exist nano g*α-closed sets A and B such that A M and B N and A B=U.

(iii) for every nano closed set H and every nano open set B containing A, there exists a nano g*α- open set M such that H M N g*α Cl(M) K.

PROOF:

(i) $\Rightarrow$ (ii): Let M and N be a pair of nano open sets in a nano g*α- normal space U such that $M \cup N = U$. Then, U\M and U \N are disjoint nano closed sets. Since U is nano g*α- normal, there exist disjoint nano g*α -open sets M_1 and N_1 such that $U\backslash M \subseteq M_1$ and $U \backslash N \subseteq N_1$. Let $A = U \backslash M_1$ and $B = U \backslash N_1$. Then A and B are nano g*α-closed sets such that $A \subseteq M$ and $B \subseteq N$ and $A \cup B = U$.

(ii) $\Rightarrow$ (iii): Let H be a nano closed set and K be a nano open set containing H. such that $H \subseteq K$. Then, U\H and K are nano open sets whose union is U. Then by (ii), there exist nano g*α -closed sets P_1 and P_2 such that $P_1 \subseteq U\backslash H$ and $P_2 \subseteq K$ and $P_1 \cup P_2 = U$. Then $H \subseteq U\backslash P_1$ and $U\backslash K \subseteq U\backslash P_2$ and $(U\backslash P_1) \cap (U\backslash P_2) = \phi$. Let $M = U\backslash P_1$ and $N = U\backslash P_2$. Then M and N are disjoint nano g*α -open sets such that $H \subseteq M \subseteq U\backslash N \subseteq K$. Since U\N is nano g*α -closed, then we have $N\ g^*\alpha Cl(M) \subseteq U\backslash N$ and $H \subseteq M \subseteq N\ g^*\alpha Cl(M) \subseteq K$.

(iii) $\Rightarrow$ (i): Let H_1 and H_2 are any two disjoint nano closed sets of U. Put $K = U\backslash H_2$, then $H_2 \cap K = \phi$, $H_1 \subseteq K$, where K is an nano open set. Then, by (iii), there exists a nano g*α -open set M of U such that $H_1 \subseteq M \subseteq N\ g^*\alpha Cl(M) \subseteq K$. It follows that $H_2 = U\backslash N$

g*αCl(M) = N, say, Then N is nano g*α -open and M ∩ N= ϕ . Hence, H_1 and H_2 are separated by nano g*α-open sets M and N. Therefore, U is nano g*α -normal.

Preservation theorems of nano g* α-normal spaces.

DEFINITION 3.5:

A function f: (U, τ_R (X)) → (V, $\tau_{R'}$ (Y)) is called strongly nano g*α-open if f(M) ∈ Ng*αO(V) for each M ∈ Ng*αO(U).

DEFINITION 3.6:

A function f: (U, τ_R (X)) → (V, $\tau_{R'}$ (Y)) is called strongly nano g*α-closed if f(M) ∈ Ng*αC(V) for each M ∈ Ng*αC(U)

THEOREM 3.7:

A function f : (U, τR(X)) → (V, τR′(Y)) is called strongly nano g*α -closed if and only if for each subset B in V and for each nano g*α -open set M in U containing f^{-1}(B), there exist a nano g*α -open set N containing B such that f^{-1}(N) ⊂ M

PROOF:

Suppose that f is strongly nano g*α-closed. Let B be a subset of V and M ∈ N g*αO(U) containing f^{-1} (B). Put N = V − f (U − M), then N is a nano g*α-open set of V such that B ⊂ N and f^{-1} (N) ⊂ M. Conversely let K be any nano g*α-closed set of U. Then f^{-1} (V −f (K)) ⊂ U −K and U − K ∈ N g*αO (U). There exists a nano g*α-open set N of V such that V − f (K) ⊂ N and f^{-1} (N)⊂ U − K. Therefore, we have f (K) ⊃ V − N and K ⊂ f^{-1} (V − N). Hence, we obtain f (K) = V − N and f(K) is nano g*α-closed in V. This shows that f is strongly nano g*α-closed.

THEOREM 3.8:

If f :(U, τR(X)) → (V, τR′(Y)) is a strongly nano g*α-closed continuous function from a nano g*α-normal space U on to a space V, then V is nano g*α-normal.

PROOF:

Let K_1 and K_2 are disjoint nano closed sets in V. Then f^{-1} (K_1) and f^{-1} (K_2) are nano closed sets in V. Since U is nano g*α-normal then there exists disjoint nano g*α-open sets M and N such that f^{-1} (K_1) ⊂ M and f^{-1} (K_2) ⊂ N. Then there exists nano g*α-open sets A and B such that K_1 ⊂ A, K_2 ⊂ B, f^{-1} (A) ⊂ M and f^{-1} (B) ⊂ N. Also, A and B are disjoint. Thus, V is nano g*α-normal.

DEFINITION 3.9:

A function f: (U, τR(X)) → (V, τR′(Y)) is called almost nano g*α- irresolute if for each u in U and each nano g*α-neighbourhood N of f (u), Ng*α -cl (f^{-1}(N)) is a nano g*α-neighbourhood of u.

LEMMA 3.10:

For a function f: (U, τR(X)) → (V,τR′(Y)) the following statements are equivalent.

(i) f is almost nano g*α-irresolute.

(ii) f^{-1} (N) ⊂ N g*α-int(N g*α-cl(f^{-1} (N))) for every N∈ Ng*αO(V).

THEOREM 3.11:

A function f :(U, τR(X)) → (V, τR′(Y)) almost nano g*α-irresolute if and only if f (N g*α -cl(M)) ⊂ Ng*α -cl(f(M)) for every M ∈ N g*αO(U).

PROOF:

Let M ∈ N g*αO(U). Suppose V ∈/ N g*α -cl(f(M). Then there exists N ∈ N g*αO(V, v)) such that N ∩ f (M) = ϕ. Hence, f^{-1}(N) ∩ M = ϕ .Since M ∈ N g*αO(U). We have N g*α -int(N g*α -cl(f^{-1}(N))) ∩ N g*α - cl(M) = ϕ. Then by lemma [3.10], f^{-1}(N) ∩ N g*α -cl(M) = ϕ and hence N ∩ f (Ng*α-cl(M)) = ϕ. This implies that V∉f (N g*α cl(M)). Conversely if N ∈ Ng*αO(V) then P = U/N g*α -cl(f^{-1}(N)) ∈ Ng*αO(U). By hypothesis, f (Ng*α -cl(P)) ⊂ Ng*α –-cl(f (P)) and hence, U/N g*α −int(N g*α − cl(f $^{-1}$(N))) = Ng*α -cl(P)⊂ f^{-1}(N g*α −cl(f (P))) ⊂ f^{-1}N g*α − cl(f (U)/ f^{-1}(N))⊂ f^{-1}Ng*αcl(V/N)⊂ f^{-1}(V/N) = U/f^{-1}(N). Therefore f^{-1}(N) ⊂ Ng*α -int(Ng*α -cl(f $^{-1}$(N)). By Lemma [3.10], f is almost nano g*α-irresolute.

THEOREM 3.12:

If f: (U, τR(X)) → (V, τR′ (Y)) is a strongly nano g*α-open continuous almost nano g*α-irresolute function from a nano g*α-normal space U onto a space V. Then V is nano g*α-normal space.

PROOF:

Let A be a nano closed set of K and B be a nano open set containing A. Then by continuity of f, f^{-1}(A) is nano closed and f^{-1}(B) is nano open set of U such that f^{-1}(A) ⊂ f^{-1}(B). As U is nano g*α-normal, there exists a nano g*α-open set M in U such that f^{-1}(A) ⊂ M ⊂ Ng*α-cl(M) ⊂ f^{-1}(B) by Theorem 2.4. Then f (f^{-1}(A)) ⊂ f (M) ⊂ f (N g*αcl(M)) ⊂ f (f $^{-1}$(B)). Since f is strongly nano g*α-open almost nano g*α-irresolute surjection, we obtain A ⊂ f (M) ⊂ N g*α -cl(f (M) ⊂ B. Then again Theorem 3.4 the space V is a nano g*α- normal.

4. ALMOST NANO g* α– NORMAL SPACES

DEFINITION 4.1:

A nano topological spaces (U, τR(X)) is said to be almost nano g*α-normal if for each nano g*α -closed set A and nano regular closed set B such that A ∩ B= ϕ, there exist disjoint nano g*α -open sets M and N such that A ⊂ M and B ⊂N.

DEFINITION 4.2:

A nano topological spaces (U, τR(X)) is said to be quasi nano g*α -closed if f (A) is nano g*α -closed in V for each A ∈ Ng*α C(U).

THEOREM 4.3:

For a nano topological spaces (U, τR(X)) the following statements are equivalent:

(i) U is almost nano g*α -normal.

(ii) For every pair of nano sets A and B, one of which is nano g*α-open and the other is nano regular open whose union is U, there exist nano nano g*α-closed sets H and K such that H ⊂ A and K ⊂ B and H ∪ K =U.

(iii) For every nano g*α-closed set H and nano regular open set K containing H, there exists a nano g*α -open set N such that H ⊂ B ⊂ Ng*α cl(B) ⊂ K.

PROOF:

(i) ⇒ (ii) Let A and B be a pair of nano open sets in a nano g*α -normal space U such that U = A ∪ B. Then U − A and U − B are two disjoint nano g*α -closed sets. Since U is nano g*α-normal there exist nano g*α-open sets A_1 and such B_1, such that U − A ⊂ A_1 and U− B ⊂ B_1. Let H = U − A_1, K = U − B_1. Then H and K are nano g*α -closed sets such that H ⊂ A and K ⊂ B and H ∪ K = U .

(ii)⇒ (iii) Let A be a nano g*α -closed set and B be a nano g*α -open set containing A. The U – A and B are nano g*α -open sets whose union is U. Then by (ii), there exist nano g*α -closed sets W_1 and W_2 such that W_1 ⊂ U − A and W_2 ⊂ B and W_1 ∪ W_2 = U. Then A ⊂ U − W_1 and U − B ⊂ W_2 and (U − W_1) ∩ (U − W2) = ϕ. Let X = U − W_1 and Y = U − W_2. Then A and B are disjoint nano g*α -open sets such that A ⊂ U − Y ⊂ B. As U − Y is nano g*α-closed set, we have N g*α cl(X) ⊂ U − Y and A ⊂ X ⊂ N g*αcl(X) ⊂ B.

(iii)⇒ (i): Let A_1 and A_2 be any two disjoint nano g*α -closed sets of U. Put B = U − A_2, then A_2 ∩ B = ϕ. A_1 ⊂ B where B is a nano g*α -open sets. Then by (iii), there exists a nano g*α -open set X of U such that A_1 ⊂ U − N g*αcl(X) = Y, then Y is nano g*α-open and X ∩ Y = ϕ . Hence A_1 and A_2 are separated by nano g*αopen sets X and Y. Therefore U is nano g*α-normal.

THEOREM 4.4:

If f : (U, τR(X)) → (V, τR′(Y)) is a nano g*α -continuous, quasi nano g*α-closed surjection and U is nano g*α-normal, then V is normal.

PROOF:

Let W_1 and W_2 be any disjoint nano g*α -closed sets of V. Since f is nano g*α-continuous, $f^{-1}(W_1)$ and $f^{-1}(W_2)$ are disjoint nano g*α-closed sets of U. Since U is nano g*α-normal, there exist disjoint N_1 and N_2 ∈ N g*αO(V), such that $f^{-1}(W_1)$ ⊂ Ni for i = 1, 2. Put Qi = V − f (U − Ni then Qi is nano g*α -open in V, W_2 ⊂ Qi and f^{-1}(Qi) ⊂ Ni for i = 1, 2. Since N_1 ∩ N_2 = ϕ and f is surjective. We have Q_1 ∩ Q_2 = ϕ. This shows that V is nano g*α-normal

5. MIDLY NANO g* α – NORMAL SPACES

DEFINITION 5.1 :

A nano topological (U, τ_R (X)) is said to mildly nano g*α -normal if for every pair of disjoint nano regular closed set A and B of U, there exist disjoint nano g*α -open sets M and N such that A ⊂ M and B ⊂ N.

THEOREM 5.2:

For a nano topological space (U, τ_R (X)) the following are equivalent.

(i) U is mildly nano g*α - normal space.

(ii) for every pair of nano regular open sets M and N, whose union is U, there exists nano g*α-closed sets A and B such that A ⊂ M and B ⊂ N and A ∪ B=U.

(iii) for any nano regular closed set A and every nano regular open set B containing A, there exists a nano g*α -open set M such that A ⊂ M ⊂ N g*α-cl(M) ⊂ B.

(iv) for every pair of disjoint nano regular closed sets A and B, there exists a nano g*α -open sets M and N such that A ⊂ M and B ⊂ N, N g*α-cl(M) ∩ N g*α-cl(N)=ϕ.

PROOF:

(i) ⇒ (ii): Let M and N be a pair of nano regular open sets in a mildly nano g*α- normal space U such that M ∪ N = U. Then, U\M and U \N are disjoint nano regular closed sets. Since U is mildly nano g*α-normal, there exist disjoint nano g*α-open sets M_1 and N_1 such that U\M ⊂ M_1 and U \N ⊂ N_1. Let A = U\ M_1 and B= U \N_1. Then A and B are nano g*α-closed sets such that A ⊂ M and B ⊂ N and A ∪ B=U.

(ii) ⇒ (iii): Let A be a nano regular closed set and B be a nano regular open set containing A such that A ⊂ B. Then, U\A and B are nano regular open sets whose union is U. Then by (ii), there exist nano g*α-closed sets P_1 and P_2 such that P_1 ⊂ U\A and P_2 ⊂ B and P_1 ∪ P_2 = U. Then A ⊂ U\ P_1 and U\B ⊂ U\ P_2 and (U\ P_1) ∩ (U\ P_2)= ϕ . Let M= U\ P_1 and N= U\ P_2. Then M and N are disjoint nano g*α-open sets such that A ⊂ M ⊂ U\N ⊂ B. Since U\N is nano g*α-closed, then we have N g*α-cl(M) ⊂ U\N and A ⊂ M ⊂ N g*α-cl(M) ⊂ B.

(iii) ⇒ (iv): Let A and B be two nano regular closed sets such that A ∩ B =ϕ. Then, A ⊂ U\B which is nano regular open. Therefore, there exists a g*α-open set M such that A ⊂ M ⊂ N g*α-cl(M) ⊂ U\B. Again, N is a g*α-open set containing the nano regular closed set A. Therefore, there is a g*α-open set N such that A ⊂ N ⊂ N g*α-cl(N) ⊂ M. Let U\ N g*α-cl (M) = V. Then, A ⊂ M and B ⊂ N, N g*α-cl(M) ∩ N g*α -cl(V)=ϕ.

(iv) ⇒ (i): Obvious.

THEOREM 5.3:

If f: $(U, \tau_R(X)) \to (V, \tau_{R'}(Y))$ is strongly nano g*α -open nano R-map and almost nano g*α -irresolute function from a mildly nano g*α -normal space U onto a space V, then V is mildly nano g*α -normal.

PROOF:

Let A be nano regular closed set and B be a nano regular open set containing A. Then by nano R-map of f, $f^{-1}(A)$ is a nano regular closed set contained in the nano regular open set $f^{-1}(B)$. Since U is mildly nano g*α -normal, there exists a nano g*α -open set N such that $f^{-1}(A) \subset N \subset N$ g*α -cl(N) $f^{-1}(B)$ by Theorem 4.3. As f is strongly nano g*α -open and an almost nano g*α -irresolute surjection, it follows that f(N) N g*α O(V) and $A \subset f(N) \subset N$ g*α-cl(f(N)) $\subset$ B. Hence V is mildly nano g*α -normal.

THEOREM 5.4:

If f: $(U, \tau_R(X)) \to (V, \tau_{R'}(Y))$ is nano R-map, strongly nano g*α -closed function from a mildly nano g*α -normal space U onto a space V, then V is mildly nano g*α -normal.

PROOF:

Let K_1 and K_2 be disjoint nano closed sets in V. Then by nano R-map of f, $f^{-1}(K_1)$ and $f^{-1}(K_2)$ are disjoint nano closed sets of U. Since U is nano g*α -normal, there exists a nano g*α -open sets M and N such that $f^{-1}(A) \subset M$ and $f^{-1}(B) \subset N$. By Theorem 4.3, there exists nano g*α -open sets A and B such that $K_1 \subset A$, $K_2 \subset B$, $f^{-1}(A) \subset M$ and , $f^{-1}(B) \subset N$. Also, A and B are disjoint. Thus, V is nano g*α-normal.

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RESEARCH ARTICLE

INDIAN KNOWLEDGE SYSTEM OF USING ILLICIUM VERUM.L WOUND HEALING IN DIABETIC FOOT ULCER – A TRADITIONAL APPROACH

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ABSTRACT

Since its beginning, Indian Traditional Medicine, the cornerstone of the world's ancient medical practice, has been vital to human health and well-being. Traditional medicine in India refers to the use of medicines that are believed to have Indian origins or that were imported to India and assimilated into Indian culture. Ayurveda, Siddha, Unani, Yoga, Naturopathy, and Homoeopathy are just a few examples of the country's recognized traditional medicine. The need for natural products and plant-based medications is rising globally these days. Herbal preparations can be used for extended periods of time due to their non-toxic nature, which also makes them potentially more effective than conventional medications. This study investigated the phytochemical, antioxidant, and antimicrobial effects of *Illicium verum*.L. Phytochemical analysis, total antioxidant capacity, DPPH radical scavenging effect, Nitric oxide scavenging, Hydrogen peroxide radical scavenging were studied by established methods. Antibacterial, antifungal effects were screened by disk diffusion respectively Significant ($P < 0.05$) IC50 values compared to respective standards were recorded in DPPH radical scavenging (54.141µg/ml), Nitric oxide scavenging (93.542 µg/ml), Hydrogen peroxide radical scavenging (93.542 µg/ml) methods. In antibacterial screening, the extract showed significant ($P < 0.05$) zone of inhibitions compared to positive controls Chloramphenicol against Gram Positive *Enterococcus faecalis* and *Staphylococcus aureus* and Gram negative Escherichia coli and *Klebsiella pneumoniae*. In antifungal assay, the greater zone of inhibition was obtained for growth of *Candida albicans*.

Keywords: *Illicium verum.L*, Phytochemical, Bioactive compounds, Antioxidant, Antibacterial.

1. Introduction

Plants are an important part of the traditional medical system, used to cure a variety of infectious and non-infectious diseases all over the world. They are a valuable source of drugs due to their abundance of bioactive chemicals such as phenols, terpenoids, and alkaloids. In general, the usage of herbal remedies for treating various disease conditions is more widespread in rural areas where there is limited access to food and medical services. People typically consume plants in a variety of ways, including infusions, spices, and medicinal smoke.

Chronic inflammation, poor vascularization and tissue regeneration, decreased growth factor synthesis, high protease activity, and oxidative stress are all brought on by the hyperglycemic state at the wound site. Controlling bacterial infections is therefore crucial to the usual therapy of diabetic wounds, which also includes wound debridement, revascularization, and accelerating the healing process. The risk of progressive infection cannot always be decreased by topical antimicrobial treatments (such as silver nitrate or povidone-iodine) or systemic antibiotic therapy (such as silver sulfadiazine, mafenide, mupirocin, or bacitracin), particularly if the bacteria resistant to antibiotics. In particular, reducing the duration of wound healing is essential for diabetics in order to minimize the risk of infection and reduce complications and expenses. Herbal products and the active ingredients in them have the potential to stop the growth of germs and to be very useful in treating resistant microbial strains. Also, some herbal products affect wound healing activities through anti-inflammatory and antioxidant activities, cell proliferation, and angiogenesis. The purpose of this study is to provide on the current knowledge acquired in herbal products (formulations and dressings) with diabetic wound healing activity. Moreover, herbal products and their active constituents used for microbial diabetic wound infections, and the various cellular and molecular mechanisms of their actions will also be described.

Antioxidant defense mechanisms protect all aerobic organisms from the damaging effects of free radicals. Antioxidants must be provided from outside sources if the antioxidant defense mechanism fails. Antioxidants are the substances that may protect the cells from the oxidative damage caused by free radicals. Natural goods may be good for human health and have significant antioxidant activity. Numerous plant species and their active components have been studied to find naturally occurring antioxidants with pharmacological qualities. Over the past few decades, there has been a growing interest in the study of the therapeutic qualities of many plants because of their strong pharmacological activities, ease of use, feasibility from an economic standpoint, and low toxicity. Although several studies on *illicium verum*.L are currently ongoing, the extract has not yet been employed as an antioxidant compound for skin care. Thus, the current work focused on developing a formulation that can scavenge free radicals and protect against oxidative damage.

Star anise also known as Chinese SA, belongs to the Magnoliaceae family and is an aromatic plant. *Illicium verum*.L has also been researched for its antimicrobial, anti-inflammatory, anthelmintic, and gastro protective properties. In this work, we reported on the next steps, in which the ethanol extract of *Illicium verum*.L was analysed for phytochemical status, total antioxidant capacity, DPPH radical scavenging impact, Nitric oxide scavenging, and hydrogen peroxide radical scavenging effect. This study also reported on the antibacterial and antifungal properties of the leaf extract using reference standards in each case. Thus, the result shows that *Illicium verum.L* has good biological activity, and further it can be carried out in pharmaceutical industries. From these results, it could be concluded that the prominent bioactive compounds in tested this spice have the potential to treat a wide range of serious diseases. These findings suggest that *Illicium verum.L* holds promise as a source of natural emollient.

Materials and Methods

Sample Collection and Preparation

The plant *Illicium verum*.L was collected during the month of January 2024 from Coimbatore,Tamil Nadu,India. The plant was identified and authenticated by Department of Botany Kongunadu Arts and Science College Coimbatore. The collected *Illicium verum.*L was washed thoroughly with distilled water and dried for 7 days in room temperature. The dried sample was ground to coarse powder with a mechanical grinder and powered sample were kept in clean closed container. The powered sample was subjected to ethanol extraction.25g of powered sample was mixed with 250ml of ethanol in Soxhlet and the extract was used for phytochemical.

Chemicals

Totally Four bacterial strains and one fungal strain were used throughout investigation. All the bacterial and fungal cultures were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, and Chandigarh, India. The bacteria used were *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae*. The fungal strains used were *Candida albicans.*

Qualitative analysis

Qualitative phytochemical analysis of each of *Illicium verum.*L was carried out by using Ethanol solventto identify the major natural chemical groups such as carbohydrates, tannins, saponins, flavonoids, alkaloids, glycosides, terpenoids, phenols, steroids and as per the procedures.

Preparation of Cream

Take the grated beeswax (3g) in a beaker and double boil it. After the beeswax is melted add 5ml white liquid paraffin and stir the solution for 5 minutes. Now add 5ml of *Illicium verum.*L extract. Stir it well and add 0.5 g of zincoxide to the solution. Cool the beaker for few minutes. After cooling keep the cream in a air tight container.

In-Vitro Characterization of Cream Formulation

Cream pH was recorded with a digital pH meter (Mettler & Toledo et al 2014) by inserting probe the cream formulation and allowing it to equilibrate for 1minute. Viscosity were conducted using a Model RVTDVII Brook field viscometer (Stougton, MA) AC-5Ospindle was employed with a rotation rate of 220 rpm. The gap value was set to 03 Temperature was set at 25°C ± 2 and these experiments were conducted in triplicate to statistically significant data.

The spreadability of the cream was determined by the wooden block and glass slide previously detailed somewhere else. Essentially, a 5ml. volume (100 mg) of cream was added to a dedicated pan and the time taken for a movable upper slide to separate completely the fived slides was noted

All the formulated cream were subjected to a 6 month-long protocol of accelerated stability conducted at a temperature of 40 ± 2 °C, 75% relative humidity. The accelerated stability was performed in accordance to the ICH guidelines. At 12h, 1day, 7days, 1month, 3 and 6 months, each formulation was examined for changes in appearance, pH, viscosity drug content.

The formulations were evaluated with different evaluation parameters like colour, odour state, consistency, pH, spreadability, washability, non-irritancy test phase separation test, After feel, In vitro permeation studies, Patch test. The objective of this review is to compile the information of herbal formulations of cream and its evaluation Herbal cream formulations studied by many researchers and this information can be researchers for novel herbal cosmetic formulations with new herbs.

***In Vitro* Antioxidant Activity**

DPPH Radical Scavenging Assay

The antioxidant potential of the plant extract was determined by DPPH method. The antioxidant activity of the extract was assessed on the basis of the radical scavenging effect of the stable DPPH free radical. Then a volume of 1.9ml of DPPH solution was added into a test tube and 100ul of the plant extract was added to it (At). The mixture was kept in dark for 30 minutes at room temperature. A solution containing 1.9ml methanol and 100ul of plant extract was taken as the test blank. A volume of 2.0ml of DPPH solution was taken as the control (Ac) and a volume of 2.0ml of methanol was used as the control blank. All of the sample were incubated in dark for 30minutes at room temperature and the absorbance values of these samples were measured at 517am. Then percentage DPPH radical scavenging concentration. All experiments were carried out in triplicate. Percentage inhibition (%) =[(Ac)-(AT)/Ae]x100%Ac Absorbance of the control sample at 540nm, AT Absorbance of the test sample at 540nm.

NO Radical Scavenging Assay

The procedure is based on the method, where sodium nitroprusside in aqueous solution at physiological pH, spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitrite ions. Take 100 µl of plant extract and standards (BHT and rutin) in triplicates. Add 3 mL of sodium nitroprusside (10 mM) to the extracts in test tubes. Incubate all the test tubes at room temperature for 150 min. Add 3mL of Griessreagent (1%sulphanilamide, 2%H3PO4 and 0.1%N-(1-naphthyl) ethylene diaminedihydrochloride) to all the test tubes. The same reaction mixture without the sample is the negative control. A test tube with phosphate- buffered saline alone will act as blank. Read the absorbance of the chromophore formed at 546 nm against the blank.

Hydrogen PeroxideRadical Scavenging Assay

Hydrogen peroxide radical scavenging activity of the test sample was estimated by the method of Ruch et al. (2018). A solution of hydrogen peroxide was prepared in phosphate buffer (pH 7.4) 200.0 µl of sample containing different concentration was mixed with 0.6 ml of H2O2 solution. Absorbance of H2O2 was determined 10 minutes later against a blank solution containing phosphate buffer without H2O2. A test tube containing 200µl of phosphate buffer and processed as described above served as the control tube. Different concentration of ascorbic acid was used as reference compound.

In Vitro Anti-Microbial Activity

The well diffusion method was used to screen the antimicrobial activity. *In vitro* anti microbial activity was screened by using Muller HintonAgar(MHA) obtained from Hi-media(Mumbai). The MHA plates were prepared by pouring 15 ml of molten media into sterile petri plates. The plates could solidify for 5 minutes and 0.1% inoculums suspension was swabbed uniformly, and the inoculums could dry for 5 minutes. Wells were cut and 20 µl of the different concentration of test drug were added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993). Chloramphenicol disc was used as a positive control.

Anti - Fungal Activity

Antifungal activity was measured using methods of well diffusion plates on agar.Totest the antifungal activity, the fractions of different concentration of plant extract were dissolved in 70% ethanol. 20 mL of Sabouraud Dextrose Agar was poured into each 15 cm Petri dish. *C. albicans* were grown in sabouraud dextrose broth at 27˚ C for 48 h. Growth was adjusted to OD (600 nm) of 0.1 by dilution with sabouraud dextrose broth. Then, Wells were cut and 20 µl of the different concentration of test drug were placed on agar to load 10 and 15 µL of each spice sample (1 mg/mL). 100 units of Fluconazole, obtained from a local pharmacy, were used as a positive control. Inhibition zones were determined after incubation at 27˚ C for 48 hrs.

Results and Discussion

Photochemical are the derivatives present in the plants are promising options to improve treatment efficiency in Diabetic foot ulcer patients and decrease adverse reactions. A number of these photochemical are naturally occurring biologically active compounds with significant ant diabetic potential. (Amit choudari *et al*., 2020)

Therapeutic efficacy of any medicinal plant depends upon the quality and quantity of the active phytoconstituents, which vary with latitude, altitude, climate and season. Different parts of these plants may possess different level of pharmacological activity. Additive or synergistic effects of bioactive phytoconstituents may be responsible for the concerned pharmacological function rather than the purified one. (Singh Sukhdev et al., 2016) Scientific evidence indicate that photochemical have significant anti diabetic potential.(Newman and Cragg et al., 2016).

Alkaloidsare important chemical compounds that serve as a rich reservoir for drug discovery. Several alkaloids isolated from natural herbs exists anti proliferation and anti metastasis effect on various types of diabetes both in-vitro and in-vivo.

Terpenoids found in a variety of plants. Fruits, vegetables, spices, plant derived beverages such as green tea, wine and cocoa-based products are the main dietary sources of flavonoids. Terpenoids have been shown to possess a wide variety of anti-diabetic effects. The value of medicinal plants lies in some chemical substances that produce a definite physiological action on the human body and the most important phytochemicals are alkaloids, flavonoids, tannins and phenolic compounds. The Medicinal plants have potent phytochemical components which are important source of antibiotic compounds and are responsible for the therapeutic properties (Florence et al., 2020**).**

Table 1: Phytochemical Analysis of ethanolic extract of *IlliciumVerum*.L

Phytochemical	**Tests**	***IlliciumVerum*.L (Ethanol Extract)**
Carbohydrate	Molisch'sTest	**+**
Protein	NinhydrinTest	**-**
Alkaloids	Hager'sTest	**+**
Saponins	Lieberman-Burchard	**+**
Steroids	ForthTest	**-**
Tanin	FerricChlorideTest	**+**
Flavanoids	AlkalineReagent Test	**-**
Glycoside	CardiacGlycosides Test	**+**
Phenols	LeadAcetate Test	**+**
Terpenoids	Salkowski Test	**+**

Preparation of Cream

To formulate and evaluate herbal body cream using beeswax, white liquid paraffin, Zinc Oxide, *Illicium verum.*L to give multipurpose effect. Creams maintain skin's hydration levels by locking in the moisture, keeping the skin healthy, soft, and supple and also heals the wounds. Unlike a lotion, the creams are less greasy and have more water content.

Beeswax exfoliates, conditions, soothes, and calms the skin, white liquid paraffin eradicates skin bacteria and remove dead skin cells and act as a emollient*, Illicium verum*.L extract fight skin infections, promote wound healing, zinc oxide acts as a good moisturizer.

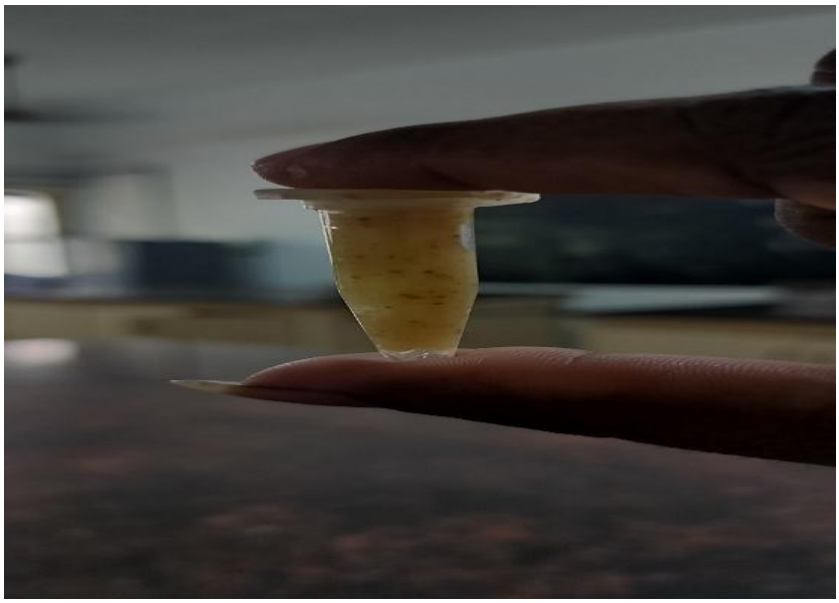


Fig 1: Preparation of *Illicium verum*.L

***In - Vitro* Studies of *Illicium Verum*.L Cream**

To formulate and evaluate herbal body cream using beeswax, white liquid paraffin, Zinc Oxide, *Illicium verum.*L to give multipurpose effect. Creams maintain skin's hydration levels by locking in the moisture, keeping the skin healthy, soft, and supple and also heals the wounds. Unlike a lotion, the creams are less greasy and have more water content.

Beeswax exfoliates, conditions, soothes, and calms the skin, white liquid paraffin eradicates skin bacteria and remove dead skin cells and act as a emollient, *Illicium verum.*Lextract fight skin infections, promote wound healing, zinc oxide acts as a good moisturizer.

The colour and odour of the cream was found to be attractive for use. The pH 6.9 make the cream suitable for all skin type as it in basic range. The absorbing capacity of the cream by the skin is high due to its semi solid state. The viscosity value of the product is measured as 6.4g.cm/cm, which exhibits a good spreadability score. The other parameters like washability, non irritancy and phase separation where found to be negative. The cream provided a soothing feel after its application, which is indicated as emollient.

Table 2*: In vitro* study and evaluation of *Illicium verum* cream

S. NO	PARAMETERS	RESULT
1	**Color**	White brown
2	**Odor**	Anise like odor
3	**State**	Semi-solid
4	**Consistency**	Smooth
5	**PH**	6.9
6	**Spreadability Test**	6.4g.cm/cm
7	**Washability Test**	Not easily washable
8	**Non-Irritancy Test**	Non irritant
9	**Phase Separation Test**	No phase separation
10	**After Feel**	Emollient

Antioxidant and Free Radical Scavenging analysis of *Illicium verum*.L Cream By Radical Scavenging Assay

Several concentrations ranging from 10-250 µg/ml *Illicium verum.*L were tested for their antioxidant activity in different in vitro models. The percentage of inhibition was observed and found that free radicals were scavenged by the test compounds in a concentration dependent manner up to the given concentration in all the models.

Natural antioxidants are widely utilized in the food and medicine industries as they counteract the cellular free radicals. Antioxidant capacity is important marker for assessing medicinal bioactive components. A variety of methods are used to study antioxidant potential of medicinal plants. Owing to the different chemical nature and complexity of antioxidant compounds present in extract of medicinal plants, there is variation in their mode of actions, so more than one assay are advised for evaluation of antioxidant activity. In this study three radical scavenging (DPPH, Hydrogen Peroxide, Nitric oxide) and reducing power assays were performed to determine the antioxidant potential. DPPH radical Scavenging Assay

The important property of an antioxidant is its ability to scavenge free radicals. DPPH radical scavenging is one of the most commonly used method for assessment of antimicrobial activity of medicinal plants. The DPPH method is simple and time saving method. DPPH contains an odd electron which gives absorption maximum at 517 nm and is

purple in colour. When freeradical scavenging antioxidants (phenolics) donates the hydrogen to freeradical, it becomes paired with hydrogen and formed reduced form of DPPH (Gulcin et al. 2019, Bahramikia et al., 2018). After reduction, the colour of DPPH is changed from purple to yellow This discoloration is stoichiometric with respect to radical scavenging activity.The DPPH radical scavenging activity was detected and compared with Ascorbic Acid. The activity of DPPH radical scavenging of *Illicium verum.*L and ascorbic acid was presented. The percentage of inhibition in DPPH in different concentration like10, 50, 100, 150 , 200 and 250 µg/ml were observed in 32.32, 42.86, 49.29, 52.28 and 54.93 respectively whereas the percentage inhibition of ascorbic acid concentrationlike50, 100, 150, 200 and 250 µg/ml were found to be 32.6 , 43.53 , 59.96 , 69.23 , 79.56 respectively. The IC50 values for DPPH scavenging activity for *Illicium verum*.L and ascorbic acid were 54.141µg/ml and93.542 µg/ml. The higher inhibition activity was recorded in *Illicium verum.*Lin dose dependent manner Values are the average of triplicate experiments and represented as mean standard deviation.

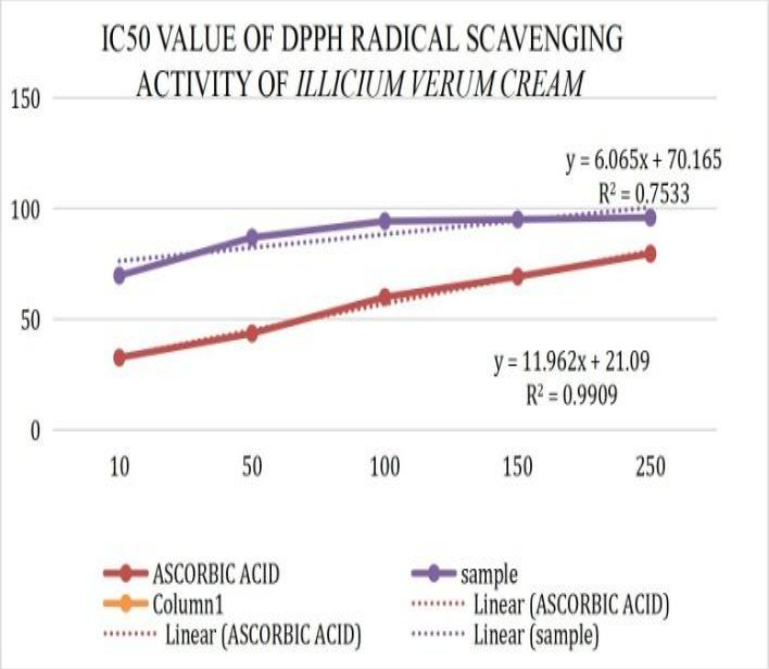


Fig 2: Graphical representation of DPPH Radical Scavenging activity of *Illicium verum cream*

H_2O_2 Scavenging Assay

He H_2O_2Scavenging activity was detected and compared with Ascorbic acid the activity of H_2O_2 scavenging of *Illicium verum*.L and Ascorbic acid was presented in Figure. The percentage of inhibition in H_2O_2 in different concentration like like10, 50, 100, 150 and 250 µ/ml were observed in 32.6,43.53,59.96,69.23 and 79.56 respectively whereas the percentage inhibition of Ascorbic acid in concentration like like10, 50, 100, 150 and 250 µ/ml were found to be 15.21 , 31.50 , 33.05 , 54.53 , and 55.20 respectively. The IC50 values for H_2O_2 scavenging activity for *Illicium verum*.L and ascorbic acid was 93.542 µg/ml and 65.350µg/ml. The higher inhibition activity was recorded in *Illicium verum.*Lin dose dependent manner. Values are average of triplicate experiments and represented as mean standard deviation (Lawenda et al 2018).

Free radical generation is a normal physiological process with a variety of effects. But increased production of these free radicals will render the lipids susceptible to lipid peroxidation. A common reliable marker of lipid peroxidation is malondialdehyde (MDA) which is measured Thiobarbiturate assay Evolution has also provided the cells with a number of counter acting antioxidant defense. These antioxidant defense mechanisms can be categorized in to two types- free radical scavenging and chain breaking antioxidants (Rosey Lekhru et al., 2017).

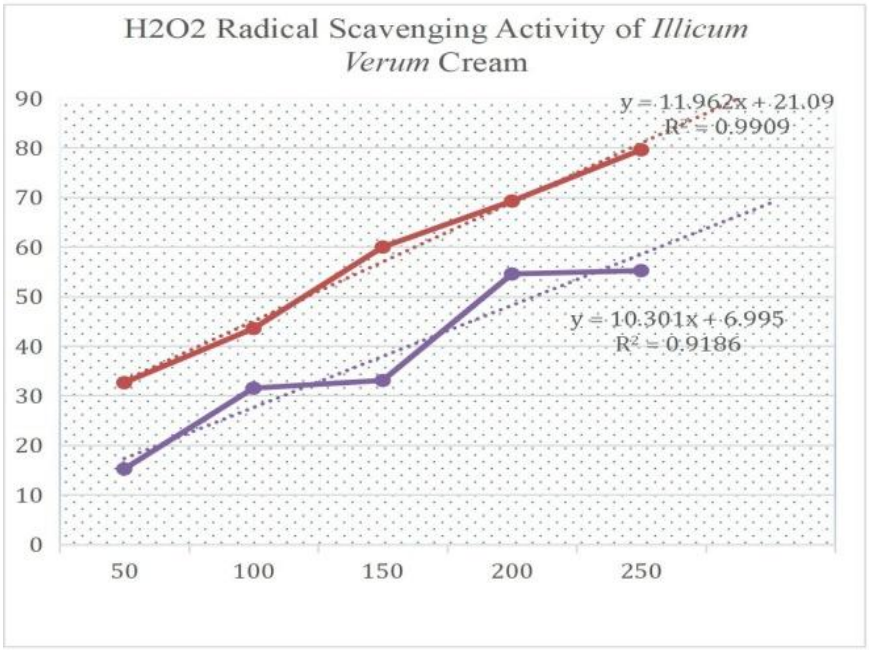


Fig 3: Graphical representation of H2O2 Scavenging Activity *lliciumverum cream*

NO Radical Scavenging Assay

Nitric oxide is a potent pleiotropic mediator of physiological processes such as smooth muscle relaxation, neuronal signalling, inhibition of platelet aggregation and regulation of cell mediated toxicity. This extract inhibits nitric oxide in a dose dependent manner (Rana et al.,2010) . The nitric oxide radical scavenging activity of 80% ethanol extract of *Illicium verum.*L. The concentration was taken with 50, 100, 150, 200 and 250 µg/ml, produce a dose dependent scavenging of nitric oxide radicals. The effect was compared with standard ascorbic acid, the maximum scavenging effects of nitric oxide radical was obtained at 77.73 and 79.56% of inhibition in 250 µg/ml and IC50 values were found to be 93.542 and 86.120 µg/ml

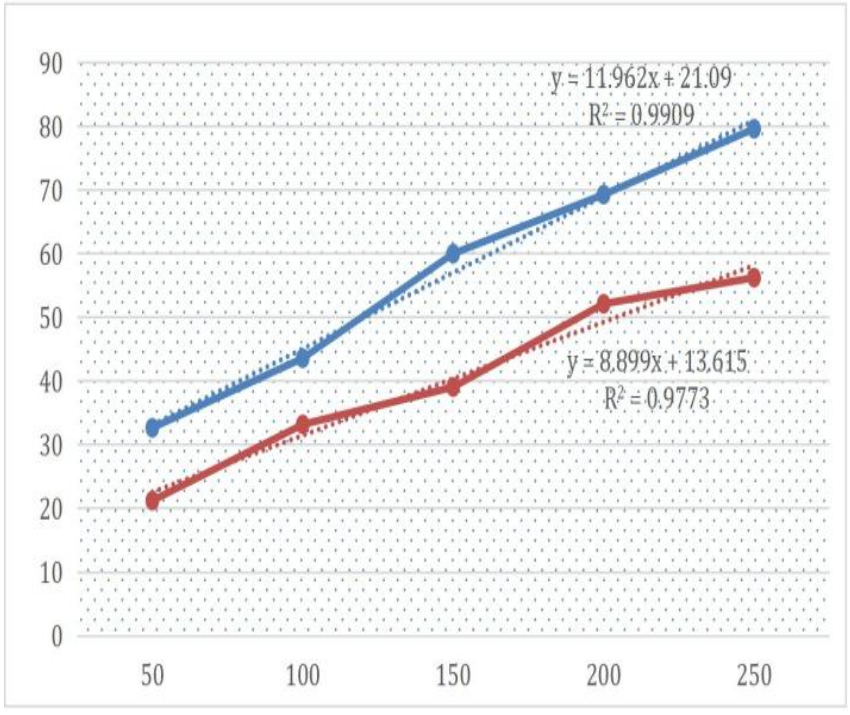
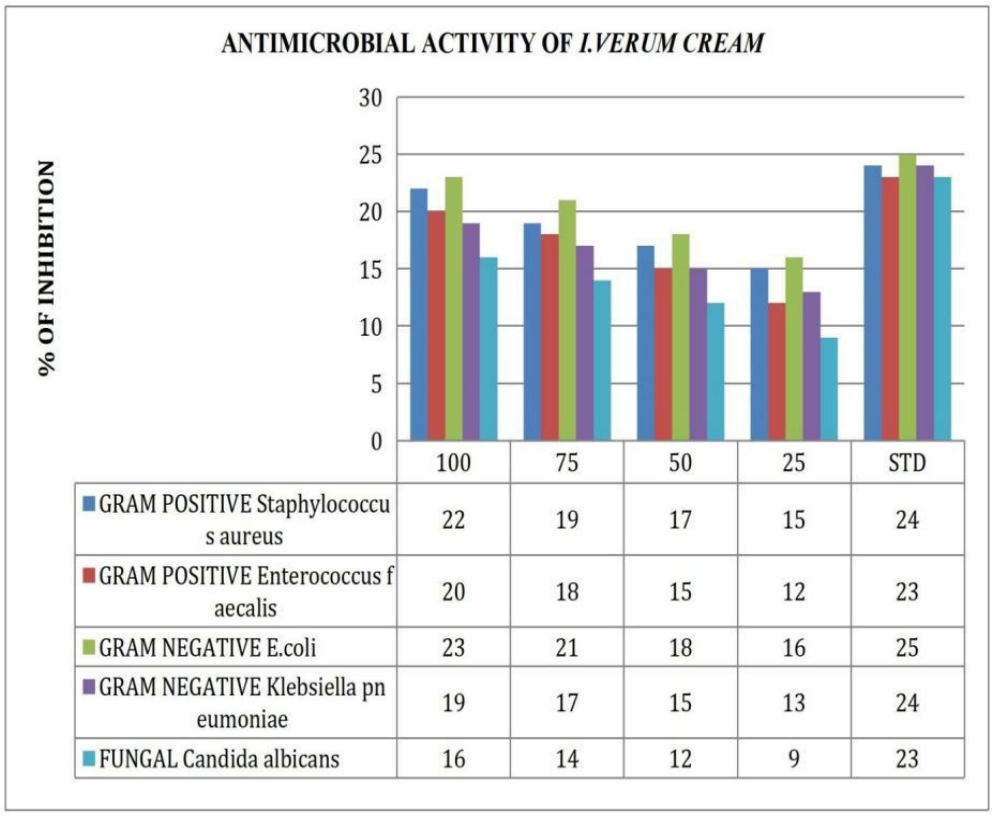


Fig 4:Graphical representation of Nitric Oxide radical Scavenging of *llicium verum* cream

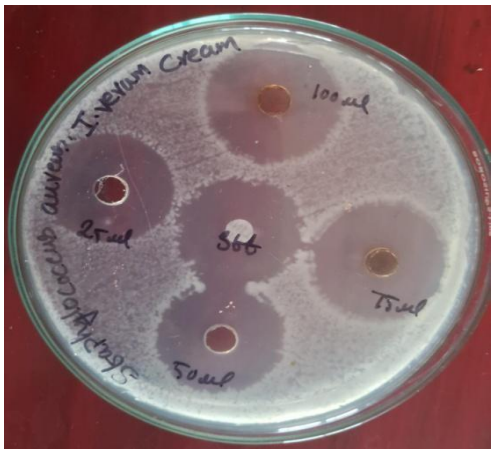
Anti -Microbial activity of *Illicium Verum*.L Cream

Results of antifungal activity showed almost similar trends as antibacterial activity. The results of this study revealed that diameter of the zone of inhibition for fungal strains was less than the diameter measured for bacterial strains. *Illicium verum*.L showed greater inhibitory effect on bacterial strains as compared to fungal strains. This distinction is due to difference in cell wall structure and protein synthesis of fungal and bacterial strains. These findings are in agreement with the observations of many other researchers (Papadopoulou *et al.*, 2005). The greater zone of inhibition was obtained for *Staphylococcus aureus* with 22mm.

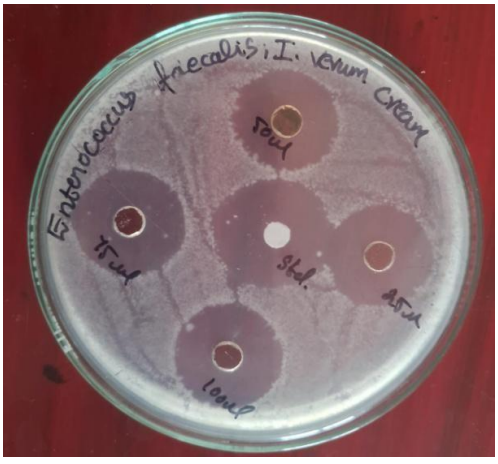


	100	75	50	25	STD
GRAM POSITIVE Staphylococcus aureus	22	19	17	15	24
GRAM POSITIVE Enterococcus faecalis	20	18	15	12	23
GRAM NEGATIVE E.coli	23	21	18	16	25
GRAM NEGATIVE Klebsiella pneumoniae	19	17	15	13	24
FUNGAL Candida albicans	16	14	12	9	23

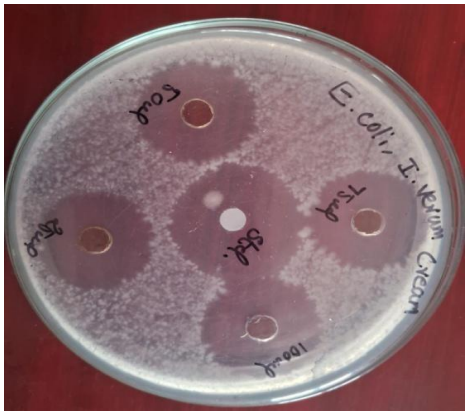
Fig 5: Graphical representation of antimicrobial activity o f *I.Verum Cream*



Staphylococcus aureus



Enterococcusfaecalis



Ecoli *Klebsiellapneumonia*

Cabdida albicans

Fig 6: Diagrammatic representation of antimicrobial activity of *illiciumverum* cream.

Conclusion

Herbal products and their dynamic ingredients through different mechanisms of action, including antimicrobial, anti-inflammatory, and antioxidant activities. The present study was formulated cream to understand the antimicrobial, antioxidant and phytochemical properties of the cream *Illicium verum.*L which is identified alkaloid, saponin, flavanoids and glycoside type compounds, Based on the results obtained in the present study, it might be *Illicium verum.*L cream exhibits high anti - fungal and kills by preventing the growth of microorganism and exhibits high anti-oxidant activity and scavenging of free radicals. It is may be considered as an important support during conventional therapy or even as a substitute for synthetic drugs used for diabetic wounds treatment. Better quality control techniques for identification, screening and quantification herbal components along with well-designed pre-clinical and clinical studies will open new research gateways in wound management.

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RESEARCH ARTICLE

Fortifying the Cloud for Comprehensive Survey of Data Protection Strategies and Emerging Challenges

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ABSTRACT

This paper presents a survey on the adoption of cloud use has increased among researchers, academia, government, and enterprises. Cloud advantages including initial investment, ideal scalability, and a variety of services are driving this trend. The cloud has many benefits, but data protection is a major problem in cloud computing and information security. Numerous strategies have been developed to address information security. These remedies exist, but the literature lacks a detailed study of their efficacy. This gap highlights the necessity to identify and assess the current body of work to see whether these solutions fit certain needs. This article fills this gap by meticulously examining cutting-edge cloud data exchange and protection approaches. Each approach is examined for its information security functions, potential breakthrough solutions, workflow, successes, scope, gaps, future directions, and more. A detailed assessment that compares methodologies is vital to the article. This review reveals method strengths and flaws. Then, these strategies' suitability for certain needs is addressed. The article finishes with a discussion of research gaps and future perspectives, inspiring prospective researchers to study and contribute to safe cloud data management.

Keywords: Cloud Computing, Data Protection, Information Security, Cloud Security Strategies, Future Challenges in Cloud Adoption.

1.Introduction

Information is widely regarded as the most vital asset for any organization, defining the uniqueness of each enterprise. Data forms the essential foundation for generating information, building knowledge, and making informed decisions, impacting everything from disease treatment [1] to revenue growth and performance enhancement. To optimize their operations, organizations rely heavily on data analysis, sharing, and storage. However, as information continues to expand rapidly, organizations face significant challenges in managing large volumes of data locally, especially [2] when limited resources hinder data exploration. Cloud services offer a solution, providing accessible, flexible, scalable, and reliable support for data storage and sharing.

Figure 1 illustrates various functionalities and methodologies used in Secure Data Storage and Sharing Techniques for data protection.

Despite the numerous advantages of cloud computing, it faces challenges that could hinder its growth if left unaddressed. For example, when a company enables its employees or departments to use the cloud for data storage and sharing [6], it reduces the load of local data management but also introduces security risks, a major concern for cloud users. By outsourcing data to cloud servers, organizations lose a degree of control, which can be unsettling, particularly when sensitive information is involved. Furthermore, data sharing in open environments exposes cloud servers to potential attacks, increasing the risk of unauthorized access and the possibility of user data being misused for illegal purposes. Additionally, the need to share data with various stakeholders, both inside and outside the organization, introduces [8] potential risks. There is concern that the receiving party may misuse or intentionally disclose shared data to unauthorized third parties, jeopardizing data integrity and security. Effectively addressing these security challenges is essential for fostering the continued growth and widespread adoption of cloud computing.

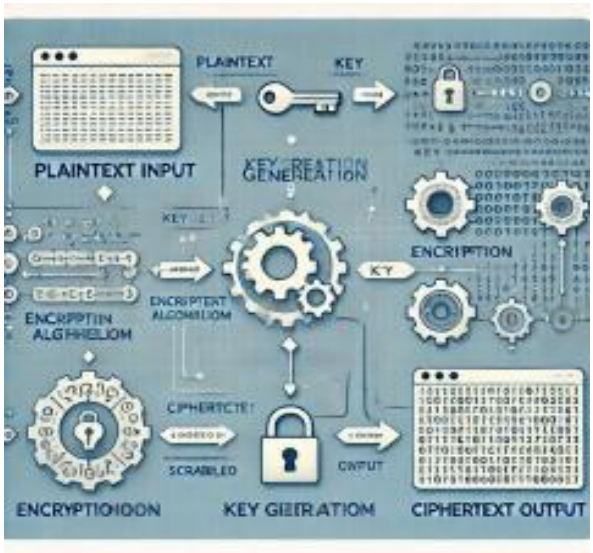


Figure 2: Overview of Secure Data Storage and Sharing Techniques for Data Protection

Figure 2 illustrates the detailed process of encryption. In our survey paper, we reference various studies, each focusing on specific components of this system. The encryption process is composed of well-defined phases, including key expansion, key mixing, and the substitution-permutation network (SPN) [8] transformation ages collectively strengthen the security of the algorithm by adding complexity and obscuring the relationship between plaintext and ciphertext. The substitution phase introduces a non-linear layer, using a fixed substitution table (S-box) to replace bytes, which is crucial for defending against cryptanalysis attempts.

I. RELATED WORK

Kao et al. introduced a user-centric key management system for cloud security that leverages RSA encryption to secure data by indirectly using users' public keys, with private keys stored only on users' mobile devices rather than on servers or personal computers. In this system, the private key can be represented as a two-dimensional (2D) barcode image for decrypting sensitive information. Al-Haj et al. proposed two cryptographic methods that ensure data security, privacy, and verifiability. They devised an approach that combines hash codes and symmetric keys, with digital signatures based on the elliptic curve method to reinforce data integrity and authenticity. To further enhance security and confidentiality [9], their approach integrates the Whirlpool hash function with the advanced encryption standard in Galois counter mode. Liang et al. introduced a proxy re-encryption technique using ciphertext rules to enable secure data transmission in the cloud, focusing on reducing the computational and communication resources required for re-encryption [10]. This method allows data owners to selectively grant access to encrypted data in the cloud. Wang et al. proposed an encryption technique utilizing file hierarchy features to secure cloud-stored data through filter hierarchy-ciphertext policy-attribute based encryption (FH-CP-ABE). Proven secure against selected plaintext attacks (CPA) via Decisional Bilinear Diffie-Hellman (DBDH), this scheme uses a layered access control method to streamline the management of hierarchical files. However, it presents a challenge in dynamically increasing computation costs when integrating features and generating unified ciphertext.

Liu et al. introduced an equitable key rebuilding mechanism to prevent unauthorized data access in cloud storage [11]. Their approach generates numerous decoy keys to obscure the decryption key, ensuring each user's contribution remains integral to accessing shared data. Although the authentication process could be more efficient, the approach reduced computation time and communication costs.

Additionally, Liu et al. developed a CP-ABE approach to address the escalating computational demands placed on users by complex access policies. This solution supports outsourced decryption, user attribute revocation [12], and rule modification. While effective in managing performance metrics related to processing and storage, it does have limitations in terms of privacy protection.

II. PROPOSED SYSTEM

The current focus on this topic highlights information security and cloud computing. A major concern is the absence of a thorough evaluation of existing methods addressing this issue. This informational gap necessitates the study [13], analysis, and assessment of significant previous research to determine if these solutions fulfill specific requirements. There are several issues with the current approach.:

The lengthy processing times may make it unsuitable for applications requiring immediate or real-time responses.

Insufficient security: Existing systems do not provide adequate protection, potentially jeopardizing data integrity and confidentiality.

The method does not guarantee the privacy of sensitive information.

The proposed technique can enhance the security of cloud storage and sharing. We apply encryption to all actions using access control and cryptographic techniques, including SHA-256 hashing and [14] encryption methods. This approach ensures that textual information remains accurate and confidential. Data protection is achieved through hashing, with SHA-256 providing robust cryptography [15]. Various techniques are employed to develop a secure and advanced cloud storage and transmission system for sensitive information. Researchers have developed and refined data security solutions for various cloud applications. Common data protection strategies focus on preventing data leakage and identifying unauthorized [16] disclosures. This article addresses methods for preventing data breaches and identifying the responsible parties. Most data leakage prevention strategies involve customized encryption and access control measures.

III. MODULE DESCRIPTION

A proposed approach for secure data storage and exchange involves implementing a modular structure that clearly outlines the roles and responsibilities [17] of the cloud service provider, data owner, and data consumer.

Cloud Service Providers (CSPs):

The CSP establishes a strong framework for data security by enforcing strict access controls and encryption protocols within the cloud environment. Security Compliance: The CSP ensures compliance with regulations and legislation designed to protect data. Regular audits and assessments aim to identify and rectify any security vulnerabilities. The CSP must implement and routinely update a reliable backup and recovery system to guarantee [18] that, in the event of data loss or a security incident, data can be restored quickly and effectively without damage. The cloud environment is continuously monitored by incident response systems to detect any suspicious activities. Additionally, the CSP develops and evaluates an incident response plan to ensure timely detection and resolution of security issues.

Data Owners:

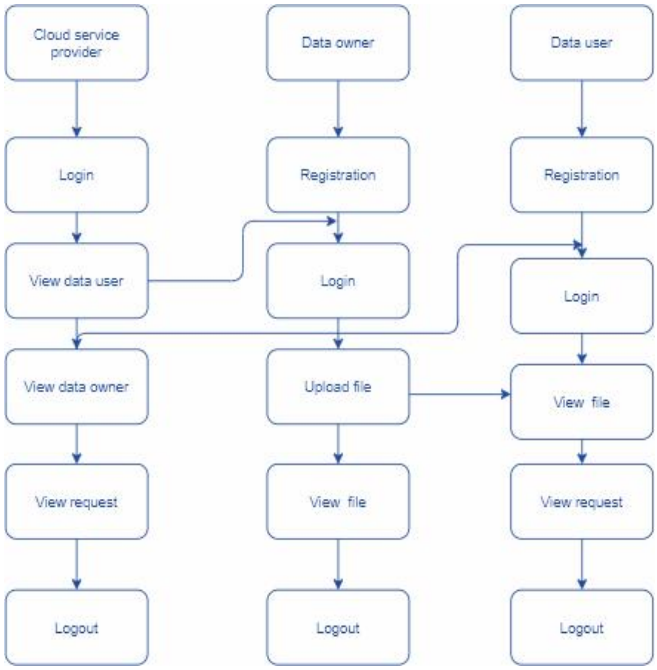
The data owner categorizes data based on its importance and sensitivity, subsequently applying encryption to protect it. This encryption safeguards data during transmission and storage, ensuring that only authorized users can access it. The data owner controls access permissions using security mechanisms such as multi-factor authentication and role-based access control (RBAC) [19], ensuring that only individuals with proper authorization can manage confidential information. The data owner defines and enforces sharing rules, specifying who has permission to access certain data and how it can be accessed. Secure methods of sharing are utilized to oversee and monitor data dissemination. Data owners are also responsible for ensuring compliance with data protection regulations, such as GDPR and HIPAA, and they educate users on enhancing their internet security.

Data Users:

Users must securely authenticate themselves before accessing data, with their access determined by the permissions set by the data owner. Multi-factor authentication enhances security during this process. Data Security: Users are educated on how to securely store and transmit data, emphasizing the importance of protecting sensitive information. By adhering to sharing restrictions, data users help prevent unauthorized transmissions. Creating a Security Incident Report: Data users are responsible for promptly notifying the Cloud Service Provider (CSP) [20] and the data owner of any suspicious security activities. This enables a swift response according to the incident response strategy. This

approach leverages the critical roles of each component to safeguard information and establish a robust data security framework, ensuring a collaborative and comprehensive strategy for secure data storage and sharing.

Figure.3. Workflow of Module Description



IV. RESULT AND DISCUSSION

The system's functionality relies on secure methods for data storage and transfer. It is essential for the system to generate data user keys. The effectiveness of data protection measures is assessed by comparing the expected results with actual outcomes. During inactive periods, no data should be in use, ensuring its protection remains confidential. It is recommended to employ AES-256 encryption before storing sensitive data in the cloud. Access to the contents of the storage system is restricted to those with the necessary decryption keys, preventing unauthorized physical access. Data transmitted between the client and the cloud is secured using SSL and TLS, allowing for communication without interception. For comprehensive functionality testing, all features and capabilities specified in user manuals, system documentation, and business and technical requirements must be addressed. Full functional testing safeguards all system documentation.

Processing illegal input is unacceptable, and it is impossible to ignore certain types of erroneous data. All responsibilities must be upheld, and the application's output should be checked for accuracy. The outcome of key generation involves unique test cases, critical functionalities, and requirements as part of functional testing. Every step, data field, method, and action within a company's process should undergo rigorous testing. Before concluding functional testing, the relevance of previous tests and the necessity for new tests must be evaluated.

V. CONCLUSION

There have been several attempts to address and alleviate the worries around the enormous problem of guaranteeing data protection in the context of cloud computing and information security. The literature is noticeably lacking in a complete examination of the available solutions, despite the volume of effort committed to solving this topic. To fill this need, this article presents an in-depth evaluation of the most popular methods for safe data sharing in the cloud, with the goal of bolstering data security there. The report dives intothe practicality and relevant solutions linked to each method, going beyond a cursory review. The main components of each approach are shown, together with study gaps and potential avenues for further investigation, thanks to the inclusion of critical and adequate facts. In addition, in order to find out what works and what doesn't, the study compares and analyses all of the above methods extensively. An in-depth analysis of each method's usefulness in the complicated cloud data security environment is provided by analyzing it within the given context.

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RESEARCH ARTICLE

EFFECT OF INTEGRATED NUTRIENT MANAGEMENT ON PRODUCTIVITY OF GREEN GRAM (*Vigna radiata*)

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ABSTRACT

The present study is to evaluate the practice of Integrated Nutrient Management (INM) effect on growth and yield response of green gram and their effect on soil fertility. Field experiment was conducted using various INM treatments of combinations of organic and inorganic fertilizers and biofertilizers and recorded the observations on plant height, number of branches, leaf count, secondary root, leaf area and yield. The integrated treatments were satisfactory and provided positive response with respect to plant growth and productivity with the integrated application of partially reduced use of chemical fertilizers, vermicompost and biofertilizers. The study concluded that INM could be an efficacious practice for achieving promising yield of green gram.

Keywords: Crop Yield, Soil Fertility, Biofertilizer, Vermicompost, FYM, Integrated Nutrient Management, green gram, etc.

1. Introduction

Green gram (*Vigna radiata*) commonly called as Mung bean is one of the predominantly consuming legumes and being cultivated in the tropical and subtropical regions. It has high value for protein, digestibility, and lesser incidence of flatulence and, therefore, suitable for infants and convalescents [1]. The crop is also rich in lysine and consumed in various forms like whole beans, dhals, sprouts, and flour. A demand of green gram is gained importance to meet regular supply. Whereas the productivity faces not expected yield and productivity to quench the requirement. In this scenario, the approach called integrated nutrient management (INM) is a global agriculture strategy through which organic, inorganic, and biological inputs are combined to improve soil fertility for optimal productivity of crops under diverse environments[7],[16]. INM emerges as a sustainable strategy with balanced input from different sources, increased nutrient-use efficiency, and long-term maintenance of soil productivity [6]. The evil of chemical fertilizers is manifest in agriculture all over the world.

Integrated Nutrient Management (INM) has been recognized for enhancing the productivity and sustainability of green gram cultivation[20]. Application of Farmyard Manure (FYM) has been shown to improve soil fertility by its positive influence on physical, chemical, and biological properties [5][7][12]. Rhizobium fixes the atmospheric nitrogen by symbiotic means and increased root nodules. *Azospirillum* increased root elongation and fixed atmospheric nitrogen non-symbiotically[3]. Phosphorus Solubilizing Bacteria (PSB) made available fixed phosphorus and thus flowered [13]. Zinc, one of the essential micronutrients, is very much important for nodulation and nitrogen fixation of legumes [10]. Observatios were made the authors of [11][10][8], the application of zinc did not have a significant effect on the dry weight of root nodules, but it greatly enhanced their number and, thus, efficient nitrogen fixation.

2. MATERIALS AND METHODS

2.1. Experimental Site and Design

There were eight number of treatments planned with each treatment had three replications to ensure statistical reliability. Involves location-uniform soil conditions and the study was done under standard cultivation practices[2]. The soil was loamy and reasonable pH, with moderately available organic matter. The treatment details are: T1: Inorganic fertilizers - IOF (100%); T2: Organic Manure - OM (100%), T3: Biofertilizers - BOF (100%), T4: Inorganic fertilizers(IOF) + Organic Manure (OM)(50:50), T5: Organic Manure(OM) + Biofertilizers(BOF) (50:50)[19], T6: Inorganic fertilizers(IOF) + Biofertilizers (BOF)(50:50), T7: Inorganic Fertilizers(IOF) + Organic Manure (OM)+ Bio fertilizers(BOF) (33:33:33)[24] and T8: Untreated Control.

2.2. Observation of biometric parameters

After implement of treatments, the plants were uprooted and biometric and biochemical contents were analysed and observation taken on two months old plants. The plants were chosen for observation based on its healthy appearance and unbiased approach. The biometric parameters such as plant fresh weight (g), plant height (cm), number of branches and leaves per plant, yield per plot (kg), leaf area, and number of secondary roots.

2.3. Observation of chlorophyll & protein estimation

Chlorophyll content actually reflects photosynthetic activity under different nutrient-rich soil conditions since it can be enhanced through such characteristics. Similar effects on chlorophyll levels through improved nitrogen assimilation and availability of micronutrients occur due to integrated nutrient management. Freshly collected green leaves were taken for both chlorophyll and protein analysis from the pooled sample randomly collected from replicated of each treatment.

3. RESULTS AND DISCUSSION

3.1 Biometric parameters observation

INM treatments were given as per the treatments to green gram and the data were observed at 60 days after planting. Among the various INM treatments, organic and biofertilzers along with reduced level of chemical fertilizers registered the satisfied biometric results. Results showed that the highest plant height was recorded in T7 (34 cm) and lowest in T8 (23.5cm). Number of branches/plant peaked at 12.4 in T6. Leaf count reached a maximum of 07 in T1,T4 and T6 (Table 1), showing enhanced photosynthetic area. Number of secondary roots was observed more in T1. Shoot and root length were also enhanced (T1: 34 cm shoot, T7: 5.7 cm root). Dry matter weight was significantly higher under INM. Shoot height was noted in 100% chemical treatment followed by 33% each IOF, OM and BF treated green gram plants (Fig 1). Same trend of observation also was reflected in shoot and root fresh and dry weight biomass weight (Fig 2 & 3)

3.2 Estimation of chlorophyll content

Leaf chlorophyll content was noted in 100% chemical treatment followed by 33% each IOF, OM and BF treated green gram plants (Table 2). Increased chlorophyll content was recorded under the INM treatment of 33:33:33 (0.638 mg/g), indicating an increase in photosynthesis due to greater nitrogen and micro-nutrient uptake; this was followed by the treatment with inorganic fertilizer only (0.615 mg/g), while the lowest level was in the untreated control. With the Bradford assay, protein content also measured highest under the 33:33:33 treatment (0.993 mg/ml), reinforcing the fact of better nitrogen assimilation and metabolic efficiency over other treatments. They [17] reported that combining 75% RDF with *Azotobacter* and PSB treatments led to a significant increase in chlorophyll content due to improved uptake of magnesium and iron.

A study conducted by [21] also found that INM treatments produced maximum leaf area and chlorophyll levels relative to treatments with chemical fertilizers, which supports INM's efficacy in improving photosynthetic efficiency. In the field trials, INM treatments have shown superior performance. The increase in plant height, branching, leaf area, and dry matter with the treatment of 75 percent Recommended Dose of Fertilizers (RDF) with *Rhizobium*, PSB, and vermicompost has been reported by [22]. Similarly, they also [4] reported that dual inoculation of *Azospirillum* and PSB enhanced root and shoot growth over single treatments or control. They [6] reported that integration of 100% NPKS with biofertilizers and FYM significantly increased plant height, pod number, and dry matter accumulation. In the rice-green gram systems,[8] reported that combining chemical fertilizers with organic sources improved nitrogen uptake and contributed positively to soil nitrogen balance.

Foliar spraying with zinc has been effective. Foliar application of 1% $ZnSO_4$ at 25 days after sowing improved nodulation and grain yield, according to [18]. Co-inoculation of biofertilizers, like Rhizobium and PSB, along with mycorrhizae, further enhance phosphorus availability and nitrogen fixation [9]. They [23] documented a positive impact of vegetative mulching and application of biofertilizers on soil moisture conservation and water-use efficiency. Other studies have confirmed that phosphorus levels up to 40 kg/ha enhance nodulation, shoot height, and dry matter accumulation in green gram[19].

3.3 Estimation of Protein content

Similar trend like chlorophyll contents of observation also was reflected in protein content of the leaves (Table 3, Fig 4 & 5). Protein amount in green gram is closely related to nitrogen, and it has been observed that INM practices enhance protein concentration. The combined application of chemical fertilizers, organic manures, and biofertilizers resulted in higher nitrogen fixation and metabolic activity, thus a higher protein content [18];[15]. In accordance with this view, similar findings were recorded by [14], which confirmed that multi-strain microbial inoculation enhanced the uptake of nutrients and protein levels in legumes, proving that INM has the nutritional benefits.

4. CONCLUSION

An integrated application of fertilizers served as a great supplement for plant growth, yield, and soil fertility enhancement. Among the treatments, organic and biofertilzers along with reduced level of chemical fertilizers registered the

satisfied biometric results. The INM could make possibility to stabilize the soil pH, increases microbial biomass, and serves as an economically viable alternative to chemical fertilizers in terms of sustainability. Future studies may look into multi-season studies, crop-specific INM protocols, and training of farmers to adopt these practices.

Table 1.Influence of INM on biometric parameters of green gram

TREATMENTS	SHOOT LENGTH (cm)	ROOT LENGTH (cm)	No. OF LEAVES	SHOOT FRESH WEIGHT (g)	ROOT FRESH WEIGHT (g)	SHOOT DRY WEIGHT (g)	ROOT DRY WEIGHT (g)	No. OF SECONDARY ROOTS	Leaf area (sq mm)
INORGANIC FERTILIZER (IOF)	33.2	5.5	7	0.413	0.043	0.081	0.013	9	721
ORGANIC MANURE (OM)	27.6	2.8	4	0.275	0.028	0.048	0.003	5	503
BIOFERTILIZERS (BOF)	26.5	3.3	5	0.283	0.035	0.051	0.005	5	398
IOF + OM	29.8	4.3	7	0.378	0.04	0.074	0.007	8	515
OM + BOF	28.4	3.6	6	0.348	0.038	0.058	0.005	6	571
IOF + BOF	28.6	4	7	0.374	0.039	0.069	0.011	8	539
IOF + OM + BOF	34.0	5.7	8	0.508	0.048	0.119	0.013	11	856
UNTREATED CONTROL	23.5	2.4	2	0.196	0.011	0.022	0.003	4	90

Values are mean of three replicates

Table 2. Effect of INM practice on chlorophyll content

Treatment	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total chlorophyll (mg/g)
Chemical fertilizers	0.496	0.727	0.615
Organic Manure	0.253	0.399	0.297
Bio fertilizers	0.247	0.395	0.289
Chemical fertilizers + Organic Manure	0.267	0.463	0.314
Organic Manure + Bio fertilizers	0.279	0.465	0.331
Chemical fertilizers + Bio fertilizers	0.268	0.448	0.301
Chemical Fertilizers + Organic Manure + Bio fertilizers	0.585	0.882	0.638
Untreated Control	0.105	0.113	0.111

Values are mean of three replicates

Table 3. Impact of INM Treatment on Protein content

Treatment	Protein Concentration (mg/g.fr wt)
Inorganic fertilizers (IOF)	0.957
Organic Manure (OM)	0.363
Biofertilizers (BOF)	0.578
IOF + OM	0.722
OM + BOF	0.899
IOF + BOF	0.863
IOF + OM + BOF	0.993
Untreated control	0.146

Values are mean of three replicates

Fig 1. Influence of INM of shoot length of two months old green gram

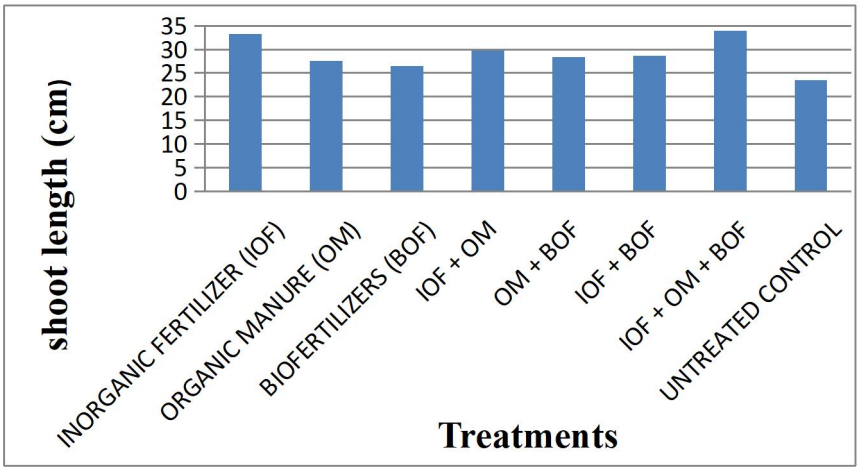


Fig 2. Influence of shoot fresh and dry weight of two months old green gram

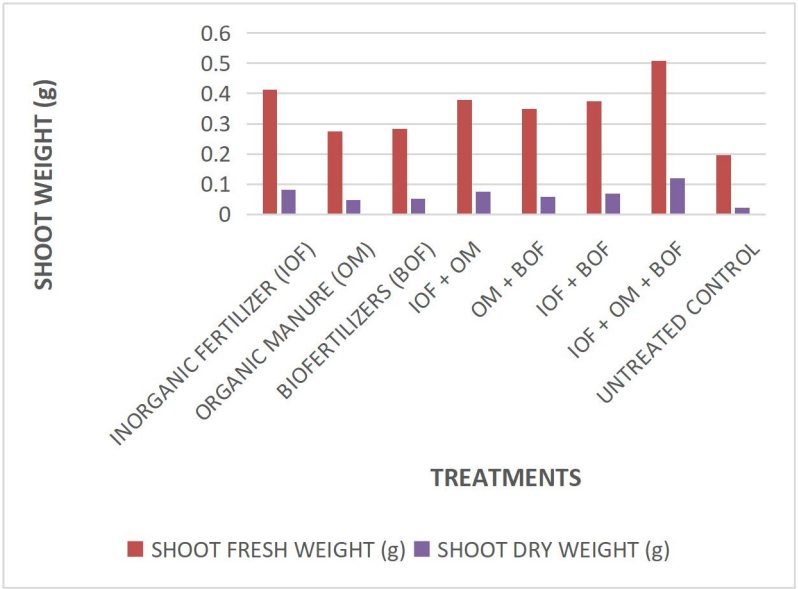


Fig 3. Influence of root fresh and dry weight of two months old green gram

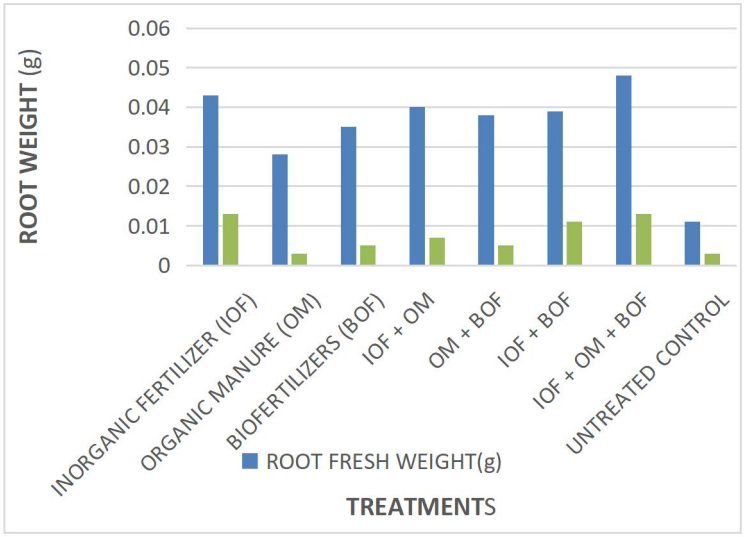


Fig 4.Effect of INM on Total chlorophyll content of green gram

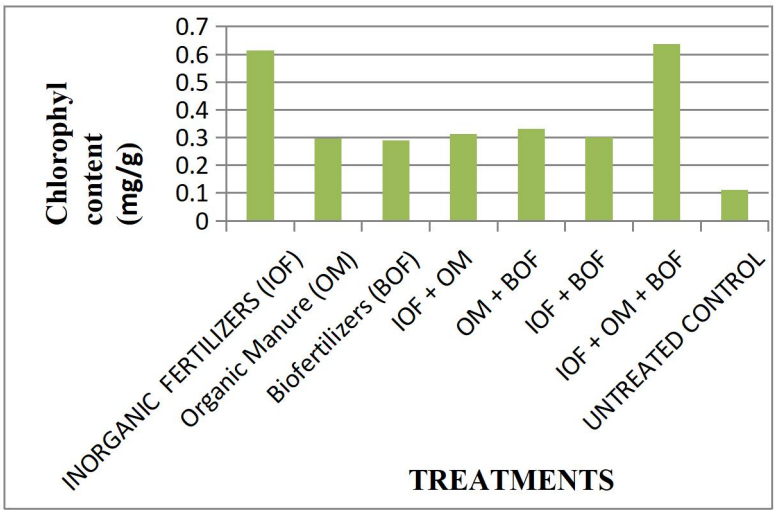
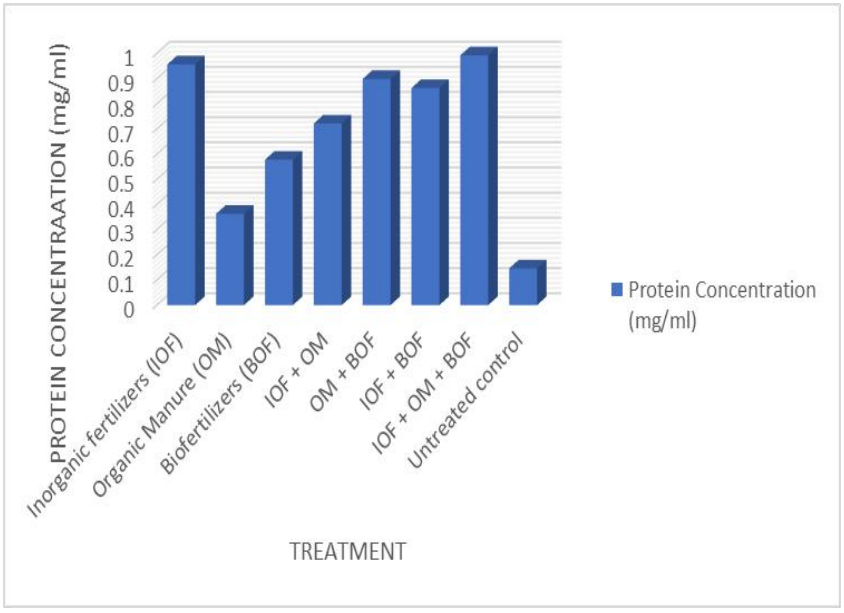


Fig 5. Effect of INM on protein



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RESEARCH ARTICLE

ASSESSMENT OF NUTRITIONAL STATUS AND BEHAVIORAL CHARACTERISTICS OF ADHD CHILDREN (4-10 YEARS) IN SPECIAL SCHOOLS OF PUDUCHERRY

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ABSTRACT

ADHD is one of the most common childhood brain disorders and can continue through adolescence and adulthood. The objective is to study the causes, nutritional status and behavioural characteristics of attention deficit hyperactivity disorder children aged 4-10 years in special need schools of Puducherry. The special need schools and the subjects was selected using purposive sampling technique to collect details on pre diagnosed attention deficit hyperactivity disorder children and other learning-disabled children, as the study was exclusive. The structured questionnaire was formulated to collect the details pertaining to the study. Further by using Statistical Package of Social Science, the mean results were calculated. The results of the study reveal that were majority of 63 percent of children with ADHD belong to first ordinal position, a higher value of 61.9 percent of male children were pre diagnosed with ADHD compare to female children of 58.3 percent. The major number of children was identified with combined type of ADHD. The maternal life style of ADHD children's mother was found to be 70.4 percent were leading sedentary life style. The consanguineous marriage was highly prevalent in parents of ADHD children. The energy and calcium intake is found to be deficit and fat content was excess in all three-age group. Eighty-three-point three percent of children's dietary pattern was non vegetarian. The intake of fruits and vegetables was identified with less frequency of consumption which mainly contains all nutrients for brain development. The processed and miscellaneous food consumption frequency was found to be higher 79.6 and 59.3 percent respectively. The awareness of sugar free, casein free, Feingold diet and gluten free diet along with food exchange list were provided for parents through awareness program. Which indicates to avoid artificial or processed foods and additives and besides gluten free diet also plays major role of ADHD children behaviour. Thus, the change in diet pattern can help children with ADHD. Future research in supplementation of foods can majorly help the community to manage ADHD children.

Keywords: ADHD, Nutritional status, behavioural, sugar free, gluten free, brain development, supplementation.

1. Introduction

Attention Deficit Hyperactivity Disorder (ADHD) is one of the most common childhood brain disorders and can continue through adolescence and adulthood (NIMH) U.S. (Stephen v farone *et al.,* 2003). Attention-deficit/hyperactivity disorder (ADHD) is affecting up to 1 in 20 children in the USA and many research states that prevalence of attention deficit hyperactivity disorder is higher in boys than girls. Australian guide line on ADHD states that children with attention deficit hyperactivity disorder are usually initiated with the symptoms from early child hood, they creates the problems when the child going to school and they become as the troublesome issue in their life span. They will find difficulty in paying attention, being focused, and controlling behaviour and over activeness will be more. These difficulties will further lead the children to lack in connected with educational, social and emotional function.

There are three subtypes of Attention deficit hyperactivity. They are predominantly inattentive type, predominantly hyperactive-impulsive type, Combined type (inattentive, hyperactive – impulsivity). All children will not have same type of

ADHD problem. It will differ from individual to individual. Some children may have inattentive behaviour alone or hyperactive behaviour alone but children also have combination of inattention, hyperactive and impulsivity together. Scientist has discovered a genetic basis for part of the dopamine problem that exists in some individual with ADHD. When the neurotransmitter like dopamine is not working properly will cause ADHD with all subtypes (Castellanos & swanson, 2002).

Attention deficit hyperactivity disorder can occur due to genetical reasons, prenatal exposure to nicotine and alcohol, prenatal exposure to maternal stress, exposure to toxins like lead, mercury and so on. Birth complications like premature birth, type of deliver, injury during pregnancy, acquired brain injury; thyroid function and improper diet can also lead to Attention deficit hyperactivity problem. (Konikowska K *et al.,* 2012).

Treatments can help to relive from ADHD symptoms but cannot cure completely. Researchers are making and finding more effective treatments and interventions, and using new tools as like brain imaging.

To analyse and gather information on nutritional status of ADHD, the health care people make use of questionnaire as an instrument and the assessment methods like anthropometry, clinical symptoms (based on their behaviour) and diet survey (3 day food recall and food frequency).

The aim is to study on causes, nutritional status and behavioural characteristics of attention deficit hyperactivity disorder children aged 4-10 years in special schools of Puducherry.

2. Materials and Methods:

Selection of Schools from urban area of Puducherry

Special need schools were selected for the study to collect details on pre diagnosed attention deficit hyperactivity disorder children and other learning-disabled children, as the study was exclusive. Special need schools are the place which improves (Bradley.K., *et al.,* 2009) their educational progress and helps to provide improvement out of their disabilities. The study was carried out by obtaining the prior permission from the higher authority of special need schools. The lists of schools are as follows below.

Table 1. List of special need schools selected for the study

S.No	Name of the schools	Area
1.	Bridges Learning Vidhyalaya	Reddiarpalayam
2.	Sathya Special school	Shivaji Nagar
3.	Mother special school for Mentally Retarded	Boomiapettai
4.	Certh India	Uppalam
5.	Carunnai society for education research and rehabilitation for mentally challenged	Reddiarpalayam
6.	Aseerva Special school	Reddiarpalayam

Phase II

Selection of subjects (4-10yrs) in special needs schools of puducherry

In order to find out the early causes and intervention, the children in the age group of 4-10 years were taken as subjects in this study. The subjects were selected based on the method of purposive sampling technique which refers a particular participant selected as subject for the study as they illustrate some specific characteristics which required for the study. With reference to the medical report from the special need school the children with ADHD and other learning disorder at age of 4-10 were selected. Table 2 represents number of children selected for the study.

Table 2. Number of children selected for the study

S.No	Name of the schools	Number of subjects
1.	Bridges Learning Vidhyalaya	14
2.	Sathya Special school	8
3.	Mother special school for Mentally Retarded	2
4.	Certh India	4
5.	Carunnai society for education research and rehabilitation for mentally challenged	6
6.	Aseerva Special school	20
	Total	54

Phase III

Formulation of questionnaire

A questionnaire is a dignified set of questions for obtaining information from respondents. The prime objective is to translate the researcher's information in form of specific questions that respondents can able to answer. It is the best tool and common method for data collection as it will be easy to collect information in large scale and to interpret it. A questionnaire is the main part of collecting quantitative primary data. The questionnaire enables quantitative data to be collected in a standardized way so that the data are internally consistent for analysis.

With this as a background the community-based survey was carried out to study the nutritional status and behavioral characteristics of attention deficit hyperactivity disorder children aged (4-10years) in special schools of Puducherry. A structured questionnaire was formulated and it was pre tested by the small crew of parents and with support of psychiatrics. They put forth their valuable suggestion regarding the questionnaire accordingly few changes were made and eventually the questionnaire was tested and it was finalized for further study.

3. Nutritional assessment of attention deficit hyperactivity disorder

3.1 Anthropometric measurements

Anthropometries which focus on obtaining physical measurement of an individual and matching them to standards that reflects on the growth and development of the individual. The height that is the length of child and weight that is mass of body where for height and weight it should be balanced so the BMI i.e. body mass index is calculated They were calculated by using formula:

Body Mass Index:

$$\frac{\text{Weight (kg)}}{\text{Height (m)}2}$$

Clinical Assessment

Any abnormality in hair, face, mouth, eyes, tongue, teeth, gums, skin, nails, lips, subcutaneous tissue, muscular and skeletal system were clinically assessed with help of physician.

Dietary assessment

A dietary history is the best means of getting dietary intake information, and refers to a review of an individual's usual patterns of food intake and the food selection variables that dictate the food intake. Food intake data was assessed by collecting using 3 day food recall method and the food frequency questionnaire.

Conduct of the study

Accordance with the prior permission the study was conducted in the respective institute. Among selected six special needs school 54 subjects were drawn. As the study was exclusive the children with ADHD and with other learning and behavioural problem were included. Based on purposive sampling technique by using medical reports of children as tool the subjects were selected for the study. The institute helped to assemble parents for collecting the data's pertaining to the children with attention deficit hyperactivity disorder and with other brain related problem. A pretested structured questionnaire was used as main instrument which was parent targeted questionnaire enclosed with following details like basic demographic details, mother health profile, father health profile, child's health profile with dietary pattern taken by Children and nutrients excess and deficit in them was calculated. The questionnaire was also enclosed with Diagnostic statistical manual for mental disorder edition IV check list to assess the children under which sub type of ADHD. The details in questionnaire were filled by interviewing parents individually. They responded patiently in the interview. All the collected data's were entered in excel sheet for statistical interpretation of data. Based on the response of parents to the study the awareness program was conducted on the topic of "FOOD and ADHD" which was taken place in the respective institutes where parents were assembled for the program.

4. Awareness program for parents of children with attention deficit hyperactivity disorder

After the collection of data, the awareness program was conducted in the schools to endow with knowledge on "FOOD and ADHD." Based on the research studies the details regarding what is ADHD and their types, causes, characteristics behaviour, modified food for ADHD children, food exchange for allergic foods and a sample of menu plan were provided and explained to them in the known language tamil. Power point presentation and videos were the mode of providing knowledge to parents. (Konikowska K *et al.,* 2012) states that the diet of pregnant and lactating woman and child may have significant role on the development and deepening of the hyperkinetic syndrome. There is much proof to show that it is connected to nutritional factors.

Chronic deficiencies of certain minerals such as zinc, iron, magnesium and iodine and insufficient dietary intake of long-chain polyunsaturated fatty

acids may have an important role on the development and deepening of the symptoms of ADHD in children. Polyunsaturated omega-3 fatty acids, mainly DHA, which are must for proper development and function of brain. Chronic deficiency of these minerals may lead to increased risk of ADHD in children. Thus, the importance of diet during pregnancy and infancy were explained to the parents with ADHD children. Many researchers were found a positive effect by eliminating food products containing synthetic food additives, like artificial food dyes and preservatives on the behavior of children with ADHD. This information was explained to the parents. Foods to be included and avoided and in which form they can be included for children was also explained in the awareness program. The significance of types of diet like casein free, sugar free, gluten free and Feingold diet for ADHD children was provided.

Further the importance of taking children to psychiatrist, psychologist or child paediatrician to manage children with ADHD were provided to the parents. As a take home message, the pamphlet was distributed to the parents which enclosed with explained details regarding ADHD, it types, causes, foods for them, food replacement or exchange list and a sample menu plan and how to manage children with proper positive behaviour were given in that which would be beneficial to them.

Data Analysis

The mean of results was calculated using SPSS software i.e. Statistical Package for Social Science. The mean value results were also interpreted with the graphical representation using bar diagrams and pie charts with detailed discussion.

Results and Discussion

From the selected 54 children the majority (51.9) percent were assessed with sub type of combined type, (37) percent children were comes under predominantly inattentive type and remaining (11.1) percent were predominantly hyperactive impulsive type. The development, clinical health and dietary aspects of the selected children were discussed.

5. Milestone development in children with ADHD at age of 24 months

Milestone development in children with ADHD at age of 24 months was shown in table 3.

Table 3 Milestone development in children with ADHD at age of 24 months

S.NO	Categories	Duration	Number %
1.	Social smile	Before 2 month	27(50)
		After 2 month	27(50)
2.	Head holding	Before 4 months	17(31.5)
		After 4 months	37(68.5)
3.	Sitting alone without support	Before 8 months	21(38.9)
		After 8 months	33(61.1)
4.	Standing without support	Before 1 year	17(31.5)
		After 1 year	37(68.5)
5.	Walking	Before 18 year	20(37)
		After 18 year	34(63)
6.	Speech	Before 2 years	12(22.2)
		After 2 years	42(77.8)

Number in parenthesis indicates percentage

The present study reveals that there is milestone developmental delay in children with attention deficit hyperactivity disorder in their 24 month of age. Among 54 children with ADHD equivalent of fifty percent of children were obtained with delayed social smile. A major of (77.8) percent children were identified to be with delayed speech in 24 month of milestone developmental stage. The head holding mile stone was delayed for (68.5) percent of children, (61.1) percent of children were undergone delay in sitting alone without support at 24 month of age, sixty eight point five percent of children were found to be with mile stone delay in standing without support and subsequently sixty three percent of children were assessed with delayed walking. This present study's result was

correspondence with Robert perna *et al.,* 2013 states that early delays in speech and motor milestones remains unclear in children with ADHD.

Morbidity pattern of children with ADHD

Thirty eight point nine percent of children with attention deficit hyperactivity disorder were affected with fever and cold frequently and (55.6) percent affected rarely. Meager five point six percent of children not affected to fever and cold.

Morbidity pattern of children with ADHD discussed in table 4

Table 4. Morbidity pattern of children with ADHD

S.NO	Morbidity pattern	Frequently	Rarely	Not at all
1.	Fever and cold	21(38.9)	30(55.6)	3(5.6)
2.	Intestinal infestation	0	21(38.9)	33(61.1)
3.	Abdominal pain	2(3.7)	30(55.6)	22(40.7)
4.	Common ear, nose and throat problems	1(1.9)	18(33.3)	35(64.8)
5.	Common eye problems	2(3.7)	8(14.8)	44(81.5)
6.	Common skin disorders	0	6(11.1)	48(88.9)
7.	Respiratory diseases	1(1.9)	3(5.6)	50(92.6)
8.	Heart diseases	0	1(1.9)	53(98.1)
9.	Genito urinary problems	6(11.1)	5(9.3)	43(79.6)
10.	CNS disorders	1(1.9)	6(11.1)	47(87)

Number in parenthesis indicates percentage

Subsequently eleven-point one percent of children were found with genitor urinary problems and the remaining children with other disorders like abdominal pain, ear, nose, throat problems, skin problem, respiratory problem, heart problem, central nervous system problem found to be very few. Therefore, the present study reveals that children with ADHD identified mostly with fever & cold eventually the prevalence rate of other disorder was very low.

Nutrient excess and deficit of children with ADHD

According to the study regarding role of nutrients in brain development (Michael georgieff K 2007) states that Nutrients and growth factors control brain development during fetal and early postnatal life. These include protein, energy, certain fats, iron, zinc, copper, iodine, selenium, vitamin A and so on. Based on this the current study focused on nutrients like carbohydrate, protein, fat, calcium, iron, magnesium, zinc and energy were assessed for the children with attention deficit hyperactivity disorder with a segmented age groups of 4-6, 7-9 and 10-12 years. Table 5 shows the nutrient excess and deficit of children with ADHD.

Table 5 Nutrient excess and deficit of children with ADHD

Nutrients	Age group 4-6	Excess deficit of age group 4-6	Age group 7-9	Excess deficit of age group 7-9
	Mean ± standard deviation		Mean ± standard deviation	
Energy (kcal)	1258.91± 1350.00	-91.09	1387.56±1690.00	-302.44
Protein (g)	31.56± 20.10	11.46	37.61±29.50	8.11
Fat (g)	44.50± 25.00	19.50	49.85±30.00	19.85
Carotene (ug)	849.11±3200.00	-2350.82	991.38±4800	-3808.62
Calcium (mg)	571.80±600	-28.20	621.05±600.00	21.05
Iron (mg)	13.78±13.00	0.78	15.84±16.00	-0.16
Magnesium (mg)	101.37±70.00	31.37	118.65±100.00	18.65
Zinc (mg)	5.27±7.00	-1.73	5.81±8.00	-2.19

Number in parenthesis indicates percentage

Nutrients	Age group 10-12 Mean ± standard deviation	Excess deficit of age group 10-12
Energy (kcal)	1936.74±2190.00	-253.26
Protein (g)	56.58±39.90	16.68
Fat (g)	66.46±35.00	31.46
Carotene (ug)	2326.44±4800	-2473.56
Calcium (mg)	752.31±800	-47.69
Iron (mg)	21.40±21.00	0.40
Magnesium (mg)	170.48±120.00	50.48
Zinc (mg)	8.01±9.00	-0.99

Age group of children with 4-6 years was assessed with the nutrients like carbohydrate, protein, fat, carotene, calcium, iron, magnesium, zinc and energy based on the three day food recall method and then nutrients amount were calculated. The mean protein content was 31.56(g) in children with ADHD when compared with the standard of 20.10 (g) it was 11.46(g) excess. The comparison of fat content's standard value 25 (g) with mean value 44.50 (g) shows that children were excess 19.50 (g) in fat consumption. Children majorly showing deficit in carotene, calcium, zinc and energy content as 2350.89 (ug), 28.20 (mg), 1.73(mg) and 91.09 (kcal) respectively. Eventually 0.78 (mg) iron and 31.37 (mg) magnesium were excess in children of 4-6 years with ADHD. Therefore from this study it was found that children with attention deficit hyperactivity disorder in the age group of 4-6 years were not meeting their daily requirements for the major nutrients like energy, calcium, carotene and zinc further the consumption of fat, protein and magnesium was higher than the recommended amount.

The nutrients for the children in the age group 7-9 years were assessed. The mean value of the nutrients was compared with the standard values in order to find out excess and deficit. Children with ADHD in this age group were showing deficit value in energy, carotene, iron and zinc as 302.44 (kcal), 3808.62 (ug), 0.16 (mg) and 2.19 (mg) out of 1690 (kcal), 4800 (ug), 16 (mg) and 8 (mg) respectively. Subsequently the major nutrients of protein were seems to be higher of 8.11 (g) in children with ADHD when compared with standard of 29.50(g), the fat intake was quite significant 19.85 (g) than compared to standard of 30 (g), the mean value of calcium is excess of 21.05 (mg) with standard of 600 (mg) and further magnesium was assessed with excess level in children with ADHD for 18.65 (mg) with standard of 100 (mg). Hence this currents study shows children with ADHD in the age group 7-9 were majorly deficit in energy intake and carotene and consumption of fat was significantly higher in children diet pattern.

The present study reflects that the children in the age group of 10-12 years were found to significantly deficit in energy mean value of 1936.74 (kcal) out of the standard 2190 (k cal) it was 253.26 (kcal) deficit, the calcium content of children was found to be deficit of 47.69 (mg) out of standard 800 (mg), 2473.56 (ug) of carotene seems to be deficit with the standard value of 4800 (ug) and meager deficit of 0.99 (mg) in zinc level out of standard 9 (mg). Further the content of fat was obtained to be higher 32.46 (g) in standard value of 35 (g), secondly 50.48 (mg) of magnesium is excess in children with attention deficit hyperactivity disorder, the protein content was found to be excess of 16.68 (g) in children and meager excess of 0.40 (g) of zinc in children with attention deficit hyperactivity disorder. Therefore, the current study reveals that children in the age group of 10-12 years were significantly deficit in energy intake, calcium intake level, meager deficit in milk whereas excess in amount of fat consumption.

Skipping of meals in children with ADHD

Skipping of meals in children with ADHD was shown in table 6.

Table 6. Skipping of meals in children with ADHD

S.NO	Categories	Number (%)
1.	Skipping milk	12(22.2)
2.	Skipping breakfast	4(7.4)
3.	Skipping lunch	1(1.9)
4.	Skipping dinner	3(5.6)
5.	Skipping milk & break fast	4(7.4)
6.	Not skipping any of the above	30(55.6)

Number in parenthesis indicates percentage

Children with attention deficit hyperactivity disorder were mostly twenty-two-point two percent were found to be skipping milk every day, secondly seven point four children were identified of skipping breakfast alone and skipping milk along with breakfast, besides five point six children were not taking dinner. Finally, fifty-five point six children were obtained without skipping any meals of the day. Therefore, in this study the prevalence of children with ADHD skipping meals was found to be significantly lower.

Awareness of parents on type diet for children with ADHD

Awareness of parents on types of diet for children with ADHD represented in table 7

Table 7. Awareness of parents on types diet for children with ADHD

S.no	Types of Diet	Number (%)
1.	Sugar free diet	11(20.4)
2.	Sugar & casein free diet	1(1.9)
3.	Sugar & gluten free diet	3(5.6)
4.	Casein & Gluten free diet	1(1.9)
5.	All four diet known	1(1.9)
6.	Gluten free	1(1.9)
7.	No awareness of any of above	36(66.7)

Number in parenthesis indicates percentage

Parents of children with attention deficit hyperactivity disorder of 54 were assessed for their awareness on types of diet that helps to manage ADHD children. From the study it was found that a majority of (66.7) percent of parents were not found to have the awareness about any of the diet. Whereas mainly twenty-point four percent were identified with awareness on sugar free diet, meager of one point nine percent parents was found with awareness on casein free diet, gluten & casein free diet, gluten free diet alone and all four diet of sugar free, casein free, gluten free, Feingold diet. Hence this study reveals that significant of parents of this population were not having awareness about diet for attention deficit hyperactivity disorder.

Food frequency of children with ADHD

Table 8 reveals the food frequency of children with ADHD.

Table 8. Food frequency of children with ADHD (Number%)

Food items	Daily (n	Weekly	Biweekly	Monthly	Rarely
Cereals like Rice, Wheat, Bread, Ragi, Corn	49(90.7)	4(7.4)	1(1.9)	0	0
Pulses like Red gram, Bengal gram	40(74.1)	11(20.4)	1(1.9)	0	2(3.7)

Vegetables Beans, Lady's finger, Drumstick	32(59.3)	12(22.2)	6(11.1)	2(3.7)	2(3.7)
Green leafy vegetables	3(5.6)	25(46.3)	9(16.7)	5(9.3)	12(22.2)
Roots and tubers like Onion, Potato, Carrot	39(72.2)	14(25.9)	1(1.9)	0	0
Fruits like Apple, Guava, Papaya	12(22.2)	18(33.3)	7(13)	5(9.3)	12(22.2)
Non vegetarian foods like Egg, Chicken, Fish	15(27.8)	30(55.6)	3(5.6)	0	4(7.4)
Milk & milk products like Buttermilk, Curd, Butter	44(81.5)	5(9.3)	0	1(1.9)	4(7.4)
Sugar &Jaggery	36(66.7)	6(11.1)	0	2(3.7)	10(18.5)
Oils like Palm oil, Ghee	39(72.2)	3(5.6)	1(1.9)	2(3.7)	9(16.7)
Nuts like Groundnuts, Almonds, Coconuts	35(64.8)	13(24.1)	3(5.6)	2(3.7)	1(1.9)

Number in parenthesis indicates percentage

The exploration of consumption pattern of diet in children with attention deficit hyperactivity disorder reveals that the cereals were majorly (90.7) percent consumed daily, secondly seven-point four percent of children were found to be taking cereals weekly and remaining one point nine of them consuming weekly twice. Mainly of fifty-nine-point three percent of children were obtained with consumption of pulses daily, twenty-two-point two percent were consuming pulses included food weekly, meager children were found to be taking pulses biweekly, monthly, and rarely of (11.1), (3.7) & (3.7) respectively. The majority of children were given with dosa, idly regularly and only in this form of cereal and pulses were combined and given to children where there are also other cereals, legumes and millets which have higher nutrient value for the brain development were not included in their diet. A major of (59.3) percent were found to be consuming vegetables every day where twenty-two-point two percent children consuming weekly. A few of (5.6) percent of children alone found to be taking green leafy vegetables daily and remaining (46.3) percent were given green leafy vegetables weekly, further (22.2), (16.7) and (9.3) were found to be given green leafy vegetables rarely, biweekly and monthly respectively.

From this the study identify that many important mineral consumptions through green leafy vegetables are not properly taken by children and only one type of green leafy like mulla keerai and agathi are included but many other green leafy like spinach, brahmi which containing high minerals required for brain development is missed out of consumption of children with ADHD. The children are not much interested in taking green leafy vegetables. A major of (72.2) percent of children with ADHD were assessed with the dietary pattern of consuming roots and tubers daily. Mostly potato, onion, carrot was taken significantly where raddish, yam, beetroot these were found to included very rare in their diet. Thirty three point three percent of children with ADHD were found with weekly consumption of fruits which are mainly coming under children's dislike section that mainly gives more vitamins, minerals, fibre and antioxidant content. The intake of non-vegetarian was more (55.6) percent children taking weekly and (27.8) percent of children are not eating without non vegetarian food.

As far as the consumption of non-vegetarian foods many of them are given in the form of fried cooking method like fish fry, chicken rice with chicken fry and even egg given in form of egg omelette but not in form of boiled egg. Thus, the nutrient content of these food gets destroyed in heat and frying. Milk which is one of the major sources of protein was identified to be consumed regularly by major of (81.5) percent of children but mostly they are consumed with the commercial products which due to the added preservative might cause hyperactive behaviour or they consumed in form of tea and coffee. The currents study recognized that among 54 children with ADHD a major part of (68.5) percent of them found with consumption of tea & coffee daily.

Subsequently the intake level of sugar was found to significantly higher in children with ADHD of (66.7) percent were consuming daily. This study states that prevalence in consumption of sugar was higher in children which mainly might cause increase in blood glucose level and leads to ADHD. A majority of seventy two point two percent children were found with oil in their diet pattern daily. Further sixty four point eight percent of them were recognized with consuming nuts daily.

Miscellaneous foods consumption of children with ADHD

Miscellaneous foods consumption of children with ADHD was shown in table 30

Table 9. Miscellaneous foods consumption of children with ADHD(Number%)

Miscellaneous foods	Daily	Weekly	Biweekly	Monthly	Rarely
Mixture, murukkuetc	32(59.3)	13(24.1)	0	2(3.7)	7(13)
Fried snacks (Bhajji, Samosa)	24(44.4)	20(37)	2(3.7)	3(5.6)	5(9.3)
Sweets	19(35.2)	14(25.9)	5(9.3)	2(3.7)	14(25.9)
Eating out	6(11.1)	9(16.7)	2(3.7)	11(20.4)	26(48.1)
Tea / coffee	37(68.5)	6(11.1)	1(1.9)	0	10(18.5)

Number in parenthesis indicates percentage

As far as the children with consumption of miscellaneous food were found to be significant, the snacks like mixture or murukku were taken mainly (59.3) percent of children daily and (24.1) percent children were taking weekly. The other junk foods like bhaji, samosa was consumed by children majorly (44.4) percent regularly and secondly thirty seven percent of them consumed weekly. Junk foods which mainly to be reduced in their diet was quiet in high frequency of consumption in the population of current study. Sweets which mainly contains high amount of sugar taken largely of (35.2) percent of children which plays a major in blood sugar level with prevalence of ADHD. Children in consumption of tea and coffee were seems to be greater of (68.5) percent every day. Eventually the frequency of eating outside in children with ADHD were (11.1) percent only taking each day and a major of fourty eight point one percent children were taking it rarely.

Processed food consumption of children with ADHD

Processed foods consumption of children with ADHD discussed in table 10.

Table 10. Processed foods consumption of children with ADHD(Number%)

Processed foods:	Daily	Weekly	Biweekly	Monthly	Rarely
Biscuits	43(79.6)	6(11.1)	1(1.9)	1(1.9)	3(5.6)
Processed chips snacks	37(68.5)	8(14.8)	2(3.7)	1(1.9)	6(11.1)
Soft drinks, flavoured	13(24.1)	8(14.8)	7(13)	2(3.7)	24(44.4)
Ice items	19(35.2)	10(18.5)	3(5.6)	4(7.4)	18(33.3)
Icecreams	12(22.2)	16(29.6)	4(7.4)	7(13)	15(27.8)
Candies, lollypops	33(61.1)	7(13)	1(1.9)	1(1.9)	12(22.2)
Chocolates	38(70.4)	8(14.8)	0	0	8(14.8)
Artificially coloured / flavored foods	21(38.9)	10(18.5)	6(11.1)	3(5.6)	14(25.9)

Number in parenthesis indicates percentage

Processed foods which mainly contains high amount of artificial colors and additives, the frequency of consuming biscuits in children with ADHD was greater (79.6) percent of them taking it every day in their diet pattern. As far as the processed chips and snacks concerned in children with ADHD significant percent of (68.5) of them consuming daily, secondly (14.8) percent of them taking it weekly, soft drink or flavoured which are available in different flavors, color and taste but it mainly contains high amount of sugar and food additives, gas which provides large amount of calories twenty four point four percent of children drinking soft drinks daily where fourty four point four percent children taking it rarely. Different flavored ice creams and ice items like strawberry, pista and more which enclosed with many colors that may play role of causes of ADHD in children were taken by a major of (22.2) and (35.2) percent of children respectively. But ice cream that mainly come in the liking food of children. Besides the main processed food which was mostly liked and taken by major of seventy point four percent of children every day and candies and lolly pops which taken mainly (61.1) percent of children each day. Finally the frequency of consumption of artificially coloured foods like cotton candy, kesari were studied from which it found that (38.9) percent of children with ADHD taking it regularly.

Behaviour of children with ADHD in hungry time

Table 11. Behaviour of children with ADHD in hungry time

S.No	Behaviours	Number (%)
1.	Having head aches	1 (1.9)
2.	Dizzy	1(1.9)
3.	Moody	2(3.7)
4.	Tired	9(16.7)
5.	Shaky	0
6.	Irritable	14(25.9)
7.	Hyperactive	5(9.3)
8.	Normal	14(25.9)
9.	Tired with irritable	3(5.6)
10.	Tired with hyperactive	1(1.9)
11.	Irritable with hyperactive	4(7.4)

Number in parenthesis indicates percentage

Irritable behaviour was majorly (25.9) percent found to be in children with ADHD, Sixteen point nine percent of children were with behaviour of getting tired in their hungry time, nine point three percent of children were found to be hyperactive, irritability along with the behaviour of hyperactive was assessed in seven point four percent of children with ADHD, five point six percent of children were experiencing tiredness with irritable behaviour during hungry, response behaviour of moodiness was seen in three point seven percent of children, no behaviour of shakiness was found in children, besides twenty five point nine percent of children with ADHD was identified with normal behaviour during hungry time.

Conclusion

From the current study the importance of nutrition is emphasized that children with age group of 4-12 were found to be deficit in major nutrients like energy, calcium, protein and significantly excess in fat content which means that the consumption level of fat rich foods in form of fried foods like bhaji, samosa and further many artificially colored foods and the sugar consumption of children with ADHD are which contribute aggressiveness. The combined type of ADHD in children was highly prevailed. The behavior of irritability also found in children. Hence the diet of sugar free, casein free in which casein protein cause hyperactivity, Feingold diet which indicates to avoid artificial or processed foods and additives and besides gluten free diet also plays major role of ADHD children behavior. Thus, the change in diet pattern can help children with ADHD. Future research in supplementation of foods can majorly help the community to manage ADHD children.

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RESEARCH ARTICLE

PREPARATION AND CHARACTERIZATION OF CHITOSAN BASED SILVER-OXIDE NANOPARTICLES

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ABSTRACT

Silver oxide nanoparticles (AgO NPs) have attracted significant interest due to their remarkable physicochemical properties, antimicrobial activity, and biocompatibility, making them ideal candidates for a variety of industrial, environmental, and biomedical applications. However, their practical utilization requires stabilization and biocompatibility, which can be achieved by integrating them with natural biopolymers like chitosan. Chitosan, a biodegradable and non-toxic polysaccharide, serves as an effective stabilizer and functionalizing agent, preventing nanoparticle aggregation and enhancing their biological activity. The nanocomposites were synthesized through an eco-friendly approach, with chitosan acting as both a reducing and capping agent. Characterization techniques, including UV, XRD, SEM with EDX, were employed to confirm the formation, size, and dispersion of the silver oxide nanoparticles. In conclusion, chitosan-based silver oxide nanoparticles represent versatile, eco-friendly materials with broad potential for applications. Further research is needed to optimize synthesis methods and enhance nanoparticle stability.

Keywords: Silver-oxide**,** Chitosan, Aggregation, Nanocomposites, Biocompatibility.

Introduction

Silver oxide nanoparticles (AgO NPs) have gained significant attention due to their remarkable catalytic, physicochemical properties, biocompatibility, and antimicrobial activity, making them valuable in various industrial, environmental, and biomedical applications. These nanoparticles exhibit unique physicochemical characteristics, such as high surface-to-volume ratio and exceptional reactivity, which contribute to their effectiveness in inhibiting microbial growth and promoting wound healing. However, the successful deployment of AgO nanoparticles in such domains requires stabilization and biocompatibility, which can be achieved by combining them with biopolymeric materials such as chitosan [1].

Chitin is a linear copolymer consisting of β-(1-4)-linked units of 2-amino-2-deoxy-D-glucan and 2-acetamido-2-deoxy-D-glucan. Structurally, it is composed of β-(1-4)-linked D-glucosamine units. Unlike cellulose, where the hydroxyl group is present, chitin has an N-acetyl group ($–NHCOCH_3$) substituting it. Chitin primarily exists in three polymorphic forms: α, β, and γ.Among these, α-chitin is the most abundant form [2]. The major source of α-form of chitin is generally shrimps, insect cuticle, crab, krill, lobster, cell wall of yeast and Zygomycetes. The abundancy of -chitin favors the significant quality of chitin as high crystallinity and purity due to the absence of calcium carbonate, proteins, and pigments. Instead β-chitin is found in connotation with proteins in squid pens while γ-chitin is found in cuttlefish stomach lining [3]. X-Ray diffraction revealed that the inner ring present in α-form of chitin is unaffected from hydration while the inner ring of β-chitin is sensitive to hydration. Moreover crystallographically, α-chitin exhibits two antiparallel molecules per unit cell, whereas β-chitin exhibits one parallel arrangement. As far as similarity is concerned, both the allomorphs have same moiety of N-acetylglycosamine [4].

METHODOLOGY

2.1 MATERIALS:

The materials used in this experiment include chitosan, a natural biopolymer known for its biocompatibility and biodegradability, which was dissolved in 10% acetic acid to form a chitosan solution. Silver Nitrate ($AgNO_3$) were used as metal precursors for the synthesis of metal oxide nanoparticles, Sodium hydroxide (NaOH) was employed to adjust the pH and precipitate metal hydroxides and purchased from Global chemicals, Hosur, Tamil Nadu. The reaction mixtures were prepared using standard laboratory glassware, including a beaker and standard measuring flask, to ensure accurate measurement of all solutions. A magnetic stirrer machine equipped with a stirrer

bar was used continuously to stir the solutions, ensuring uniform mixing throughout the synthesis process.

2.2 METHODS:

2.2.1 Preparation of chitosan:

For chitosan to be extracted from dried mushroom it is necessary to convert it first to chitin. Generally, extraction of chitin from dried mushroom consists of three steps including demineralization for removal of calcium carbonate/phosphate, deproteinisation for removal of protein and then, chitin can be converted into chitosan by N-deacetylation which partially removes the acetyl group from the polymers chain composition. Mushroom was washed to remove the residue attached to it and then dried in intense sunlight and left for autolysis for 24 hours at room temperature which improve the quality of chitosan.[5]

2.2.2 Preparation of Chitosan / Silver Nitrate Oxide Nanoparticles:

Chitosan-based silver oxide (AgO) nanoparticles were prepared using a chemical co-precipitation method involving silver nitrate (AgNO3) and sodium hydroxide (NaOH). Initially, a 1% (w/v) chitosan solution was prepared by dissolving chitosan in 1% (w/v) acetic acid under constant stirring at room temperature until a clear solution was obtained. Separately, an aqueous solution of AgNO3 was prepared, which served as the silver precursor for AgO formation. This silver nitrate solution was slowly added to the chitosan solution under vigorous stirring to ensure uniform dispersion of copper ions within the chitosan matrix. To initiate the formation of AgO nanoparticles, NaOH solution was added dropwise to the $AgNO_3$-chitosan mixture, leading to the precipitation of silver hydroxide $Ag(OH)_2$. The reaction was allowed to proceed under continuous stirring with 600-700 rpm for about 18 hrs, Until the pH of the mixture reached around 9-10, at which point $Ag(OH)_2$ the blue color precipitated out. The mixture was then heated to convert $Ag(OH)_2$ into AgO nanoparticles. The resulting chitosan-AgO nanoparticle suspension was centrifuged to separate the nanoparticles, which were then washed with distilled water to remove any residual reactants.

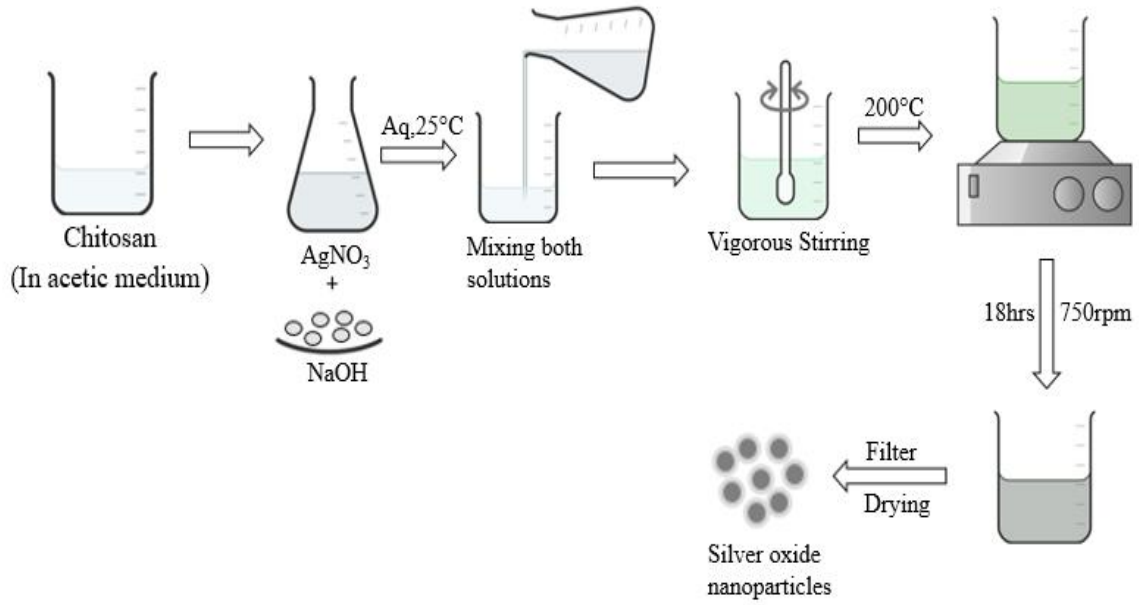


Figure.1 Preparation of chitosan based silver oxide nanoparticles

Reaction:

$$AgNO_3 + 2NaOH \xrightarrow{(i)} Ag(OH)_2 + Na_2NO_3$$

$$Ag(OH)_2 \xrightarrow{(ii)} AgO + H_2O$$

*(*i) Aqueous medium,25°C (ii) In air, 200°C - 300°C

RESULTS AND DISCUSSIONS:

3.1 Ultraviolet – Visible Spectroscopy (UV) Analysis:

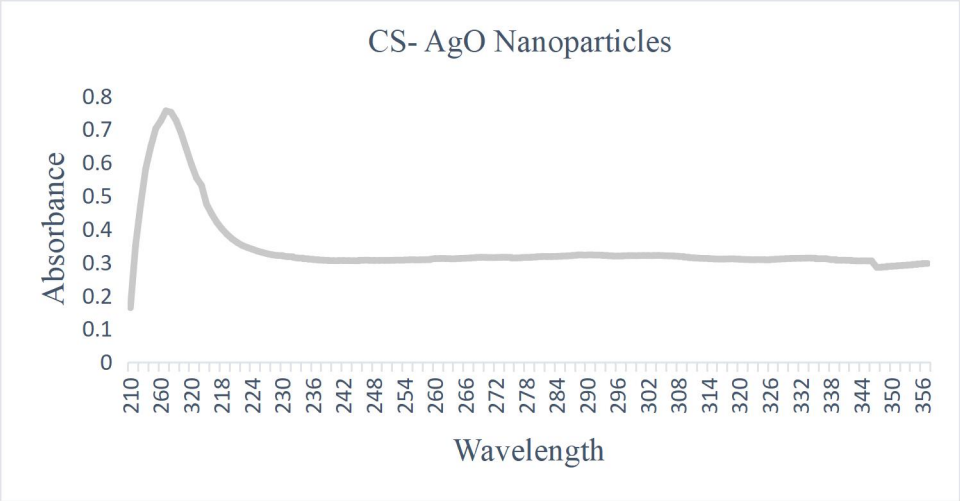


Figure.2 UV Spectra of Chitosan/AgO nanoparticles

Silver oxide nanoparticles exhibit a characteristic SPR absorption peak typically in the 300 nm range, which is primarily attributed to the excitation of surface electrons in the silver (Ag) component of the nanoparticles. The presence of chitosan often leads to narrowing of the SPR peak compared to unmodified AgO nanoparticles, indicating better stabilization and less aggregation. Impact of chitosan: The band gap of AgO nanoparticles may be slightly reduced in the presence of chitosan due to changes in the electron density and energy states at the nanoparticle surface, which enhances photocatalytic and antimicrobial activity.

3.2 Fourier Transform Infra-Red (IR) Analysis:

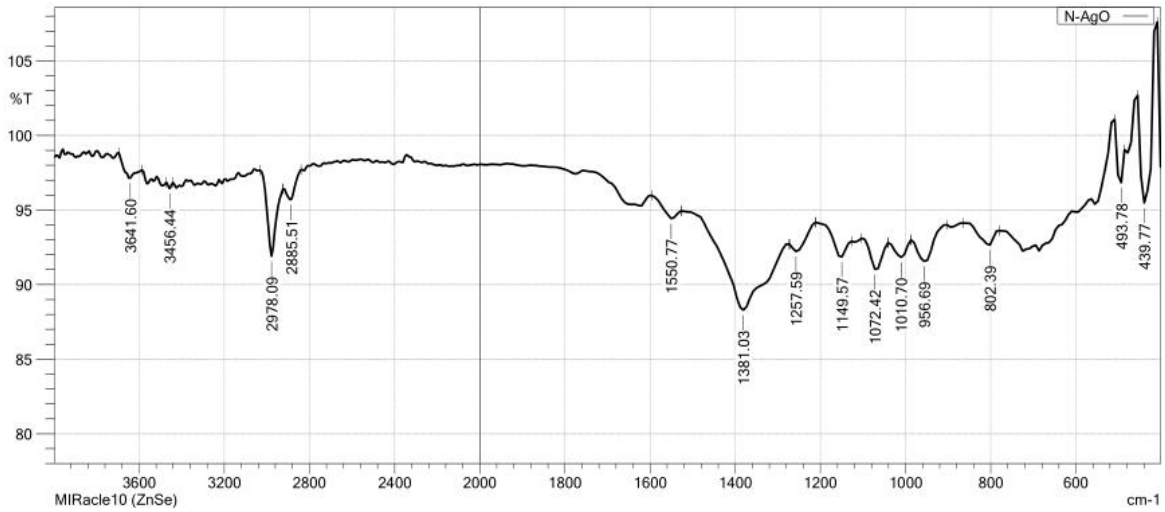


Figure.3 FTIR analysis of Chitosan/AgO

A broad absorption band 3456.44 cm^{-1} corresponds to the O-H and N-H stretching vibrations, indicating the presence of hydroxyl and amine groups in chitosan. The peak at 2885.51 cm^{-1} in the IR spectrum of chitosan typically corresponds to C-H stretching vibrations of the aliphatic -CH_2 and -CH_3 groups present in the chitosan backbone. This is associated with the saccharide structure of chitosan. The peak at 1550 cm^{-1} represents the amide II band (N-H bending) which is also C=O axial deformation band. The broad peak at 1381 cm^{-1} corresponds to CH_3 symmetrical deformation, confirming the structure of chitosan.

For AgO Nanoparticles, A band 439.77 cm^{-1} confirms the presence of Ag–O bonds. The interaction of AgO with chitosan may enhance or shift the absorption bands related to the amine or hydroxyl groups.

- The FTIR results confirms that the chitosan structure remains intact during the nanoparticle synthesis process, as the characteristic peaks of chitosan were preserved.
- The successful incorporation of ZnO, CuO, and AgO nanoparticles is evident from the appearance of characteristic metal-oxide peaks in the spectrum.
- The interactions between chitosan and metal oxides (ZnO, CuO, AgO) can be attributed to the formation of hydrogen bonds or electrostatic interactions between the amine, hydroxyl groups of chitosan, and the surface of the nanoparticles.

These interactions enhance the stability of the composite nanoparticles and potentially improve their antimicrobial, catalytic, or other functional properties.

3.3 X- RAY DIFFRACTION (XRD) ANALYSIS:

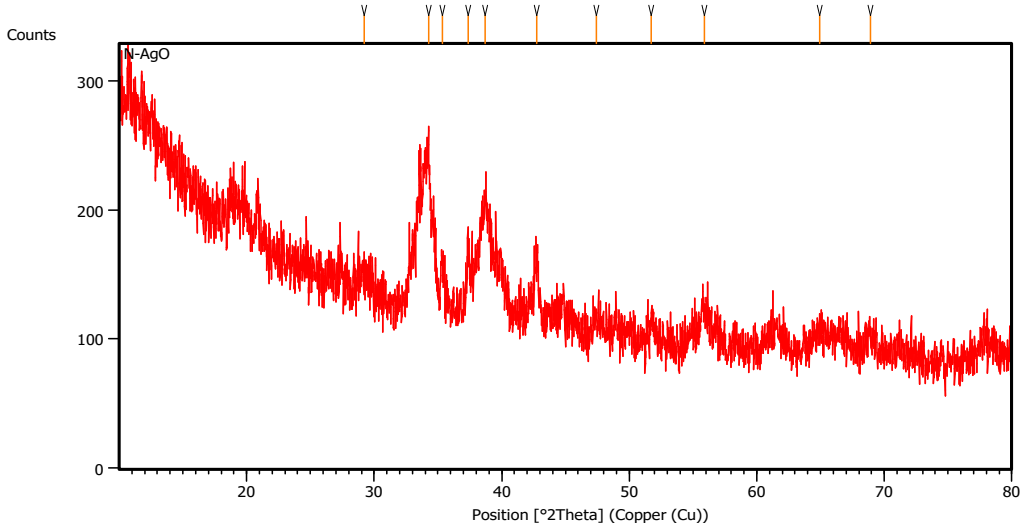


Figure.4 XRD Main graphics, Analyze view for AgO

2θ	FWHM	d-spacing [Å]	D	delta	Micro strain	Lattice parameters, a	Relative intensity
29.2301	0.8029	3.05535	8.63	13.41	6.260	157.74	12.31
34.263	0.2007	2.61719	32.71	0.93	1.285	136.822	100.00
35.3434	0.2007	2.53963	32.28	0.95	1.234	133.16	27.17
37.3549	0.2007	2.40737	31.46	1.01	1.147	126.95	37.38
38.6828	0.5353	2.32774	11.58	7.45	2.917	123.24	60.17
42.7214	0.3346	2.11658	17.44	3.28	1.580	113.54	38.41
47.4113	0.6691	1.91757	8.03	15.48	2.683	104.62	9.92
51.7458	0.4015	1.76668	12.25	6.66	1.381	98.09	16.11
55.87	0.5353	1.64565	8.32	14.42	1.5837	93.05	24.73
64.9486	0.8029	1.43585	4.18	56.97	1.637	85.02	12.34
68.9212	0.8029	1.36247	3.55	78.96	1.350	82.55	10.14

Table 4.3 Calculation of Crystallite Size, Microstrain, Lattice parameters of AgO

For AgO- Chitosan Nanoparticles,

(i) Peak Positions (2θ):

- The characteristic peaks of AgO are observed at: 29.2°,*34.2°*,*35.3°*,*37.3°*,38.6°
- For AgO at 2θ=34.2° which is corresponding to the (100) plane,
- The inter planar spacing d is approximately 0.261 nm.
- The X-ray wavelength λ is approximately 0.1563 nm (or 1.563 Å).

(ii) Crystallite Size:

The crystallite size of AgO nanoparticles in chitosan composites ranges of 32 nm.

(iii) Chitosan Interaction:

The incorporation of chitosan often leads to peak broadening and reduced intensity, indicating effective dispersion and reduced crystallinity of AgO.

(iv) Structure:

Silver oxide (AgO) has a monoclinic crystal structure, belonging to the $P2_1/c$ space group AgO in nanoparticle form shows peak broadening in XRD due to reduced particle size and Lattice strain, allowing estimation of crystallite size via the Debye-Scherer equation.

This structure makes AgO an interesting material for applications like catalysis, batteries, and sensors.

3.4 SEM WITH EDAX:

3.4.1 Sem Analysis

(i) Morphology: SEM images of chitosan-AgO nanoparticles predominantly spherical or quasi-spherical shapes. Silver oxide (AgO) nanoparticles often exhibit a more uniform and smaller size due to the strong stabilizing effect of chitosan.

(ii) Particle Size: The SEM images typically show sizes of 23.68nm, 25.01nm, 23.93nm, 19.40nm, 23.58nm and 31.44nm with well-dispersed particles, with a tendency to form more compact clusters. Chitosan prevents excessive agglomeration, keeping the particles well-separated.

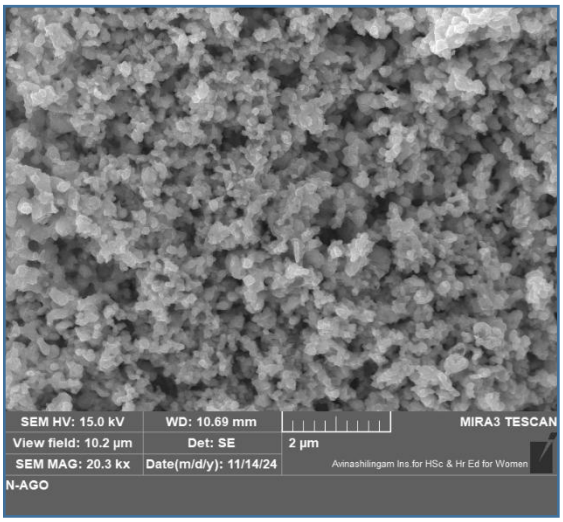
(iii) Dispersion: The chitosan matrix effectively prevents agglomeration of AgO particles. SEM images show a high degree of uniformity and well-separated nanoparticles.

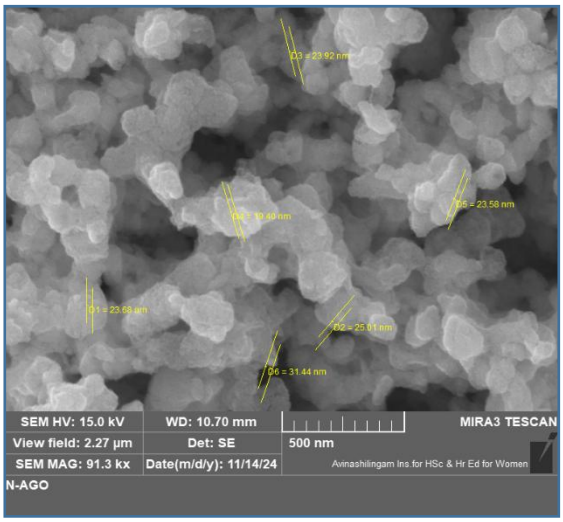
(iv) Surface Texture: The surfaces of AgO nanoparticles are typically smooth, but slight irregularities may be visible due to the AgO crystal structure. Chitosan-coated AgO nanoparticles show a thin, uniform layer of chitosan surrounding the metal oxide core.

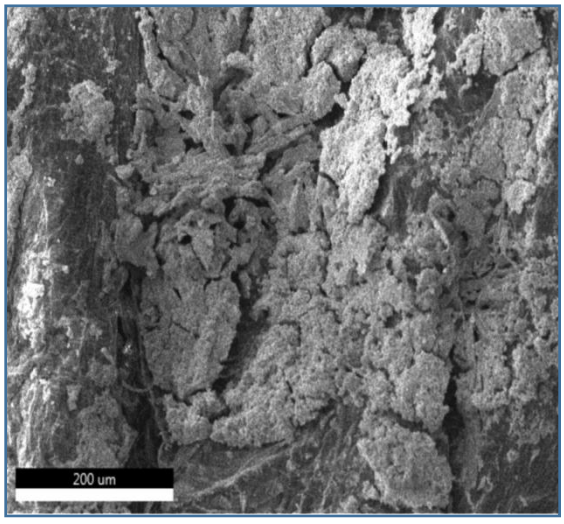
3.4.2 EDAX Analysis:

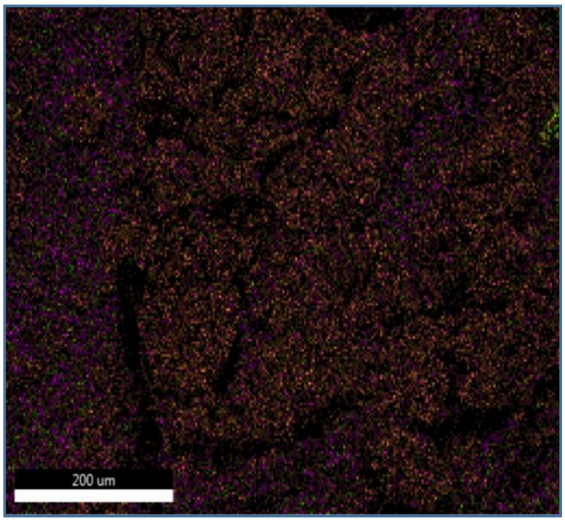
(i) Composition: The EDAX spectrum confirms the presence of silver (Ag) and oxygen (O), along with carbon (C) and nitrogen (N) from chitosan.

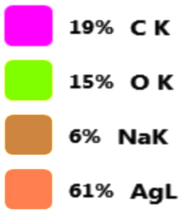
(ii) Elemental Ratios: The Ag-to-O ratio is consistent with the expected stoichiometry of AgO. The C and N peaks confirm the chitosan coating around the nanoparticles.











Element	Weight%	Atomic%
C K	9.02	26.28
O K	21.12	46.20
Na K	4.05	6.17
Ag L	65.81	21.35

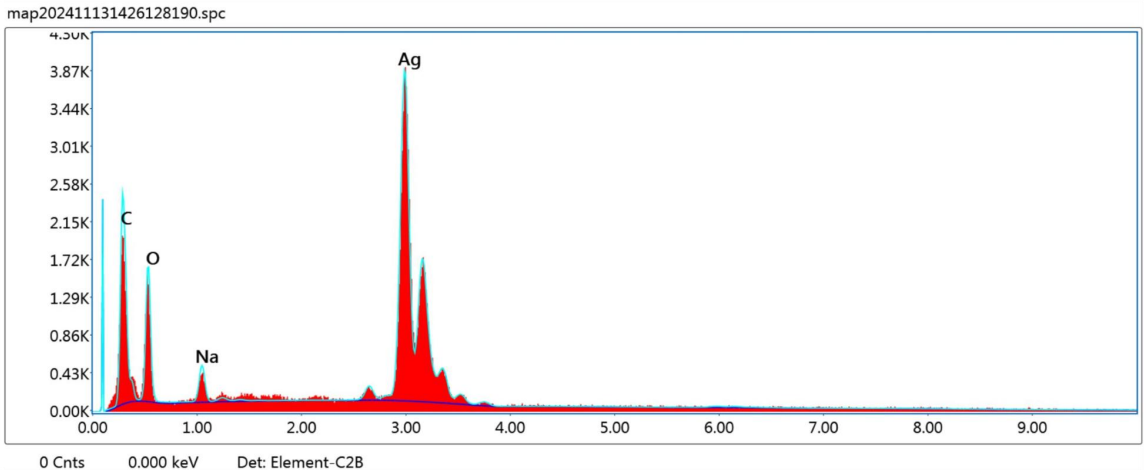


Figure.5 SEM and EDAX images with different magnifications for AgO nanoparticles

3.4.3 General Discussion for SEM-EDAX Results:

(i) Morphology and Particle Size:

- The morphology and particle size variation are influenced by synthesis parameters such as precursor concentration, pH, temperature, and chitosan concentration.
- SEM images confirm that ZnO, CuO, and AgO nanoparticles exhibit distinct morphologies—spherical (ZnO, AgO) and irregular or flake-like (CuO)—influenced by chitosan.
- ZnO and CuO nanoparticles show slightly larger particle sizes compared to AgO due to differences in crystal growth mechanisms and precursor reactivity.

(ii) Role of Chitosan:

- Chitosan stabilizes the nanoparticles, preventing aggregation , ensuring uniform size dispersion and reduced agglomeration.
- This is evident from the smooth coating seen in SEM and the carbon and nitrogen signals detected in EDX.
- The interaction of chitosan with metal ions (Zn^{2+}, Cu^{2+}, Ag^{+}) through hydroxyl and amine groups helps in nanoparticle stabilization.

(iii) Elemental Composition:

- EDAX analysis confirms the presence of Zn, Cu, and Ag along with oxygen, validating the synthesis of the respective nanoparticles.The C and N peaks in all spectra confirm the successful encapsulation of nanoparticles by the chitosan matrix.

(iv) Surface Characteristics

- The encapsulation of nanoparticles by chitosan results in smooth surfaces for ZnO and AgO, while CuO exhibits some roughness due to intrinsic material properties.
- SEM images show evidence of a thin chitosan layer, confirming its role as a biopolymer coating.

(v) Aggregation Behavior

- Aggregation is minimal due to the electrostatic stabilization provided by chitosan, as observed in the SEM images.
- Without chitosan, bare nanoparticles tend to cluster, reducing their effectiveness in applications.

CONCLUSION:

In this investigation, the synthesis, characterization of chitosan-based silver oxide nanoparticles (AgO NPs) have been systematically studied. The use of chitosan as a biocompatible and

eco-friendly stabilizer has proven to be effective in controlling the size, shape, and dispersion of silver oxide nanoparticles, which significantly enhances their stability and functional properties. The interaction between chitosan and silver ions leads to the formation of highly stable silver oxide nanoparticles with well-defined characteristics.In conclusion, the successful synthesis and application of chitosan-based silver oxide nanoparticles offer a pathway for developing highly functional, eco-friendly nanomaterials with a wide array of practical uses. Their unique properties, including antimicrobial activity, photocatalytic potential, and biocompatibility, position them as valuable candidates for future research and technological advancements.

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