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RESEARCH ARTICLE

FIBONACCI MEAN ANTI-MAGIC LABELING OF SOME GRAPHS

Ameenal Bibi, K. and T. Ranjani*
P.G. and Research Department of Mathematics, D.K.M College for Women (Autonomous), Vellore - 632 001, Tamil Nadu, India.

ABSTRACT

In this paper, we introduced Fibonacci mean anti-magic labeling in graphs. A graph G with p vertices and q edges is said to have Fibonacci mean anti-magic labeling if there is an injective function $f: E(G) \to F_j$, ie, it is possible to label the edges with the Fibonacci number F_j where (j= 0,1,1,2…n) in such a way that the edge uv is labeled with

$\frac{|f(u)+f(v)|}{2}$ if $|f(u)+f(v)|$ $is\ even$,

$\frac{|f(u)+f(v)|+1}{2}$ if $|f(u)+f(v)|$ $is\ odd$ and the resulting vertex labels admit mean anti-magic labeling. In this paper, we discussed the Fibonacci mean anti-magic labeling for some special classes of graphs.

Keywords: Fibonacci mean labeling, circulant graph, Bistar, Petersen graph, Fibonacci mean anti-magic labeling.

AMS Subject Classification (2010): 05c78.

1. INTRODUCTION

The concept of Fibonacci labeling was introduced by David W. Bange and Anthony E. Barkauskas in the form Fibonacci graceful (1). The concept of skolem difference mean labeling was introduced by Murugan and Subramanian (2). Somasundaram and Ponraj have introduced the notion of mean labeling of graphs. Hartsfield and Ringel introduced the concept of anti-magic labeling which is an assignment of distinct values to different vertices in a graph that in such a way that when taking the sums of the labels, all the sums will be having different constants.

For various graph theoretic notations and terminology, we followed Gross and Yellen (3). Sridevi *et al.* (4) proved the path and cycle graphs are Fibonacci divisor cordial graphs. A dynamic survey of graph labeling is updated by Gallian (5). Rokad and Ghodasara (6) proved that Fibonacci cordial labelingexists for some special graphs. In this paper, we have discussed different families of graphs which satisfy the conditions of Fibonacci mean anti-magic labeling.

Definition 1.1.

Fibonacci number can be defined by the linear recurrence relation $F_n = F_{n-1} + F_{n-2}, n \geq 2$ where $F_0 = 0, F_1 = 1$ .This generates the infinite sequence of integers in the form 0,1,1,2,3,5,8,13,21,34,55,89,144…

Definition 1.2.

A graph G with p vertices and q edges admits mean anti-magic labeling if there is an injective function $f$from the edges $E(G) \to \{0,1,1 \dots q\}$ such that when each uv is labeled with $\frac{|f(u)+f(v)|}{2}$ if $|f(u)+f(v)|$ $is\ even$ and $\frac{|f(u)+f(v)|+1}{2}$ if $|f(u)+f(v)|$ $is\ odd$ then the resulting vertices are distinctly labeled.

Note: A graph which admits mean anti –magic labeling is called mean anti-magic graph.

Definition 1.3.

A graph G is called anti-magic if the q edges of G can be distinctly labeled in such a way that when taking the sum of the edge labels incident to each vertex, they all will have different (distinct) constants.

2. RESULTS

Theorem 2.1.

The circulant graph $C_n (n \geq 6)$ admits Fibonacci mean anti-magic labeling with the generating set (1,2).

Proof:

Let $G = C_n(1,2)$ be the 4-regular graph with $(n \geq 6)$.

*Correspondence: T. Ranjani, P.G. and Research Department of Mathematics, D.K.M College for Women (Autonomous), Vellore - 632 001, Tamil Nadu, India. E.mail: ranjanithangavelu2016@gmail.com

We define the labeling function $f: E(G) \rightarrow F_j$ where (j=0,1,1...n)

Then apply mean labeling for the edges so that the sum of the labels of the vertices are all distinct.

Thus, the above labeling pattern gives rise to a Fibonacci mean anti-magic labeling on the given graph $G = C_n(1,2)$.

Example 2.2.

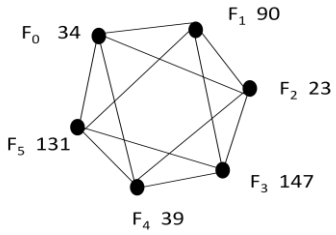


Fig. 1. Fibonacci mean anti-magic labeling of circulant graph C_6

Theorem 2.3.

Petersen graph admits Fibonacci mean anti-magic labeling.

Proof.

Petersen graph is a three regular graph with 10 vertices and 15 edges.

Let $u_0, u_1, u_2 \ldots . u_{14}$ be the edges and

Let $v_0, v_1, v_2 \ldots . v_9$ be the vertices of Petersen graph.

We define the labeling function $f: E(G) \rightarrow F_j$ where (j=0,1,1...n) such that each uv is labeled with $\frac{|f(u)+f(v)|}{2}\ if\ |f(u)+f(v)|\ is\ even$ and $\frac{|f(u)+f(v)|+1}{2}\ if\ |f(u)+f(v)|\ is\ odd$ then the resulting vertices are distinctly labeled.

By applying the above mean labeling to the edges, we obtained the sum of the vertex labels are all distinct (different constants).

Hence Petersen graph admits Fibonacci mean anti-magic labeling.

Example 2.4.

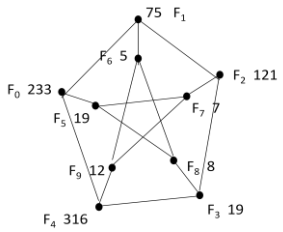


Fig. 2. Fibonacci mean anti-magic labeling of Petersen graph.

Theorem 2.5.

The Wheel graph W_n admits Fibonacci mean anti-magic labeling.

Proof:

Let $u_0, u_1, u_2 \ldots . u_{2n}$ be the edges of W_n and

Let $v_0, v_1, v_2 \ldots . v_n$ be the vertices of the Wheel graph W_n.

We defined the labeling function $f: E(G) \rightarrow F_j$ where (j=0,1,1...n) such that each uv is labeled with $\frac{|f(u)+f(v)|}{2}\ if\ |f(u)+f(v)|\ is\ even$ and $\frac{|f(u)+f(v)|+1}{2}\ if\ |f(u)+f(v)|\ is\ odd$ then the resulting vertices are distinctly labeled.

By applying mean labeling to the edges of W_n we obtained the sum of the vertex labels are all distinct (different constants).

Hence the Wheel graph W_n admits Fibonacci mean anti-magic labeling.

Example 2.6:

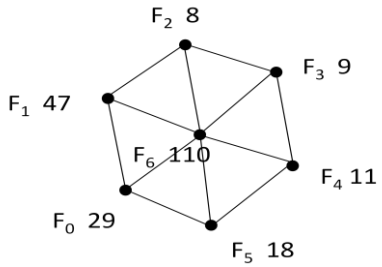


Fig. 3. Fibonacci mean anti-magic labeling of Wheel graph W_6.

Theorem 2.7.

Bistar $B_{n,n}$ admits Fibonacci mean anti-magic labeling.

Proof:

Let $v_{1,0}\ and\ v_{2,0}$ be the apex (central) vertices of $B_{n,n}$.

Let $v_{1,1} \ldots . v_{1,n}$ be the pendent vertices adjacent to the vertex $v_{1,0}$.

Let $v_{2,1} \ldots . v_{2,n}$ be the pendent vertices adjacent to the vertex $v_{2,0}$.

Let u_0 be the edge of the two apex vertices.

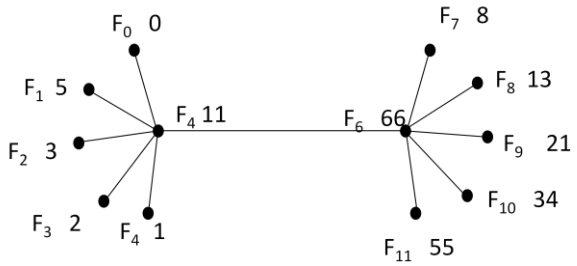
Let $u_1, u_2 \ldots$ be the edges of all the pendent vertices.

We defined the labeling function $f: E(G) \rightarrow F_j$ where (j=0,1,1...n) such that each uv is labeled with $\frac{|f(u)+f(v)|}{2}\ if\ |f(u)+f(v)|\ is\ even$ and

$\frac{|f(u)+f(v)|+1}{2}$ if $|f(u)+f(v)|$ $is\ odd$ then the resulting vertices are distinctly labeled.

Hence Bistar $B_{n,n}$ admits Fibonacci mean anti-magic labeling.

Example 2.8.



3. CONCLUSION

In this paper, we have obtained Fibonacci mean anti-magic labeling for the circulant graph, the Wheel graph, Petersen graph and the Bistar. Further study on some more special graphs is under progress.

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RESEARCH ARTICLE

DOMINATION AND TOTAL DOMINATION IN INTUITIONISTIC TRIPLE LAYERED SIMPLE FUZZY GRAPH

Jehruth Emelda Mary, L.[a*] and K. Ameenal Bibi[b]
[a]P.G. and Research Department of Mathematics, St. Joseph's College of Arts and Science College (Autonomous), Cuddalore - 607 001, Tamil Nadu, India.
[b]P.G. and Research Department of Mathematics, D.K.M College for Women (Autonomous), Vellore - 632 001,Tamil Nadu. India.

ABSTRACT

In this paper, we discussed domination and total domination in Intuitionistic fuzzy graph. We determined the domination number (γ) and total domination number (γ_t) in Intuitionistic Triple Layered Simple fuzzy graph (ITLFG) and also verified the existence of 2-domination in intuitionistic Triple Layered simple fuzzy graph.

Keywords: Intuitionistic Triple Layered Simple fuzzy graphs, Domination, Total domination, 2- Domination.

1. INTRODUCTION

Atanassov introduced the concept of intuitionistic fuzzy graphs (1). Parvathi and Karunambigai are also studied the concept of intuitionistic fuzzy graphs and its properties (2). Some of the properties of intuitionistic fuzzy graphs are introduced by Nagoorgani and Shajitha Begum (3).

Pathinathan and Jesintha Roseline defined the Triple Layered fuzzy graph and its properties (4,5). In this paper, Jethurth Emelda Mary and Ameenalbibi introduced the concept of domination (γ) and total domination (γ_t) in Intuitionistic Triple Layered Simple fuzzy graph (ITLFG) under certain conditions and illustrated with some examples.

2. PRELIMINARIES

In this section, we presented some of the basic definitions.

2.1. Fuzzy graph

A fuzzy graph G is a pair of functions G: (σ,μ) where σ is a fuzzy subset of a non-empty vertex set S and μ is a symmetric fuzzy relation on σ. The underlying crisp graph of G: (σ,μ) is denoted by G*: (σ*,μ*)

2.2. Complement of Fuzzy graph

The Complement of the fuzzy graph G: (σ,μ) is a fuzzy graph $\bar{G} = (\bar{\sigma}, \bar{\mu})$ where $\bar{\sigma} = \sigma$ and $\bar{\mu}(u,v) = 0 \; if$ μ(u, , v) > 0 and $\bar{\mu}(u,v) = \sigma(u) \wedge \sigma(v)$ otherwise.

2.3. Intuitionistic Triple Layered Fuzzy Graph (ITLFG)

Let $G: \langle (v_i, \mu_1, \nu_1), (e_{ij}, \mu_2, \nu_2) \rangle$ be an intuitionistic fuzzy graph with the underlying crisp graph $G^*: (\sigma^*, \mu^*)$. The pair $TL(G): \langle (v_i, \mu_{TL_1}, \nu_{TL_1}), (e_{ij}, \mu_{TL_2}, \nu_{TL_2}) \rangle$ is called the Intuitionistic Triple Layered Fuzzy graph and is defined as follows. The Vertex set of ITL (G) be $\langle \mu_{TL_1}, \nu_{TL_1} \rangle$. and The fuzzy subset $\langle \mu_{TL_1}, \nu_{TL_1} \rangle$ is defined as

$$\langle \mu_{TL_1}, \nu_{TL_1} \rangle = \begin{cases} \langle \mu_1(u), \nu_1(u) \rangle & if \; u \in \sigma^* \\ \langle \mu_2(uv), \nu_2(uv) \rangle & if \; uv \in \mu^* \end{cases}$$

Where, $0 \leq \mu_{TL_1} + \nu_{TL_1} \leq 1$.

The fuzzy relation $\langle \mu_{TL_2}, \nu_{TL_2} \rangle$ *on* $\sigma^* \cup \mu^* \cup \mu^*$ is defined as

$$\langle \mu_{TL_2}, \nu_{TL_2} \rangle = \begin{cases} \langle \mu_2(uv), \gamma_2(uv) \rangle & if \; u, v \in \sigma^* \\ \langle \mu_2(e_i) \wedge \mu_2(e_j), \nu_2(e_i) \vee \nu_2(e_j) \rangle & if \text{ the edgese}_i \text{and } e_j \text{ have a vertex in common between them} \\ \langle \mu_1(u_i) \wedge \mu_2(e_j), \nu_1(u_i) \vee \nu_2(e_i) \rangle & if \; u_i \in \sigma^* \text{ and } e_i \in \mu^* \text{ and each } e_i \text{ is incident with sigle } u_i \\ & \text{either clockwise or anticlockwise} \\ 0 & \text{otherwise} \end{cases}$$

By definition $0 \leq \mu_2(uv) + \nu_2(uv) \leq 1$ for all (u,v) in $\sigma^* \cup \mu^* \cup \mu^*$. Here $\langle \mu_{TL_2}, \nu_{TL_2} \rangle$ is a fuzzy relation on the fuzzy subset $\langle \mu_{TL_1}, \nu_{TL_1} \rangle$

2.4. Domination in Intuitionistic Fuzzy Graph

Let $G = (\sigma, \mu)$ be an intuitionistic fuzzy graph on the set V. Let $x, y \in V$, we say that x dominates y in G if $\mu(x,y) = \sigma(x) \wedge \sigma(y)$ and $\mu(x,y) = \sigma(x) \vee \sigma(y)$. A Subset D of V is called a dominating set of G if for every vertex $v \notin D$ there exist $u \in D$ such that u dominates v. The minimum cardinality of a dominating set in G is called the domination number of G and is denoted by $\gamma(G) or \; \gamma$.

2.5. Total Domination in Intuitionistic Fuzzy Graph

Let $G = (\sigma, \mu)$ be an intuitionistic fuzzy graph without isolated vertices. A Subset S of V is said to be total dominating set if every vertex in V is dominated by a vertex in D.

*Correspondence: Jehruth Emelda Mary, L. Research scholar, PG and Research Department of Mathematics, St. Joseph's College of Arts and Science College (Autonomous), Cuddalore - 607 001, Tamil Nadu, India. E.mail: jethruth15@gmail.com

2.6. 2-Domination in Intuitionistic Fuzzy graph

The 2-Domination number of a fuzzy graph G denoted by $\gamma_2(G)$, is the minimum cardinality of a 2-dominating set of G.

3. DOMINATION IN INTUITIONISTIC TRIPLE LAYERED FUZZY GRAPH

In this section, we introduced the Domination in Intuitionistic Triple Layered Fuzzy graph and illustrate with some examples.

3.1. Definition

Let $G: \langle (v_i, \mu_1, \nu_1), (e_{ij}, \mu_2, \nu_2) \rangle$ be an intuitionistic fuzzy graph with the underlying crisp graph $G^*: (\sigma^*, \mu^*)$. Let $ITL(G): \langle (v_i, \mu_{TL_1}, \nu_{TL_1}), (e_{ij}, \mu_{TL_2}, \nu_{TL_2}) \rangle$ be an Intuitionistic Triple Layered Fuzzy graph on the vertex set $(\sigma^* \cup \mu^* \cup \mu^*)$. Let $x, y \in (\sigma^* \cup \mu^* \cup \mu^*)$, we say that x dominates y in G if $\mu_{TL_1}(x,y) = \sigma_{TL}(x) \wedge \sigma_{TL}(y)$and $\nu_{TL_1}(x,y) = \sigma_{TL}(x) \vee \sigma_{TL}(y)$. A subset D of $(\sigma^* \cup \mu^* \cup \mu^*)$ is called adominating set in G if every vertex $v \notin D$ there exists $u \in D$ such that u dominates v.

The minimum cardinality of the minimal dominating set in G is called the domination number of G and is denoted by $\gamma(G) or\ \gamma$.

Example 3.1.

Consider the Intuitionistic fuzzy graph $G: (\sigma, \mu)$ with n=3 vertices whose crisp graph is a cycle.

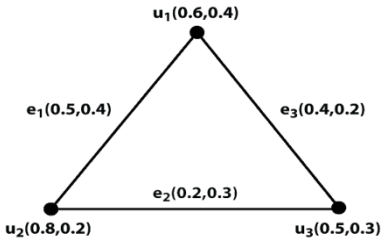

Fig. 1. Intuitionstic Fuzzy Graph$G$: (σ, μ)

Then the Intuitionistic Triple Layered Fuzzy graph is given by

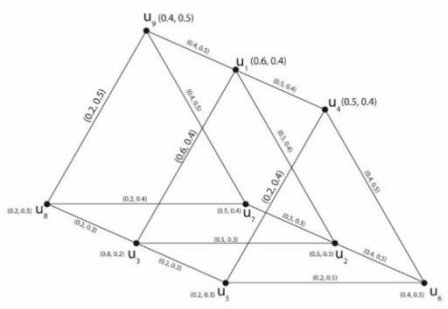

Fig. 2. ITLFG$TL(G)$: (σ_{TL}, μ_{TL})

The minimal cardinality of the minimal dominating set of the Intuitionistic Triple Layered fuzzy graph is $D = \{u_3, u_6, u_9\}$ is 3. ie) The domination number is $\gamma(G) = 3$

Theorem 3.1.

For any intuitionistic triple layered fuzzy graph ITL (G) $\gamma(G) + \gamma(\bar{G}) \leq 2p$, where p – number of vertices and $p \geq 3$.

Proof:

Let $G: \langle (v_i, \mu_1, \nu_1), (e_{ij}, \mu_2, \nu_2) \rangle$ be a cycle graph with p vertices and the vertex set of G be σ*.Let $ITL(G): \langle (v_i, \mu_{TL_1}, \nu_{TL_1}), (e_{ij}, \mu_{TL_2}, \nu_{TL_2}) \rangle$ be an intuitionistic triple layered fuzzy graph of G with 3p vertices and a vertex set of ITL(G) be $(\sigma^* \cup \mu^* \cup \mu^*)$.

Let D be the Dominating set of the Intuitionistic Triple Layered fuzzy graph. The minimuml cardinality of the minimal dominating set D is p.

Ie) $\gamma(G) = p$……(1)

Let ITL $(\bar{G})$ be the complement of the intuitionistic triple layered fuzzy graph $ITL(G)$ and a vertex set of ITL $(\bar{G})$ be $(\sigma^* \cup \mu^* \cup \mu^*)$.

Let $\overline{D}$ be the dominating set of the complement of the intuitionistic triple layered fuzzy graph. The minimum cardinality of the minimal dominating set D is$\leq p$.

$\gamma(\bar{G}) \leq p$……(2)

The sum of the dominating set $ITL(G)\ and$ ITL$(\bar{G})$ is,

$$\gamma + \bar{\gamma} \leq p + p$$

$$\leq 2p$$

$\gamma(G) + \gamma(\bar{G}) \leq 2p$. (by (1) and (2))

Example: 3.2.

Consider the Intuitionistic fuzzy graph $G: (\sigma, \mu)$ with p = 3 vertices whose crisp graph is a cycle.

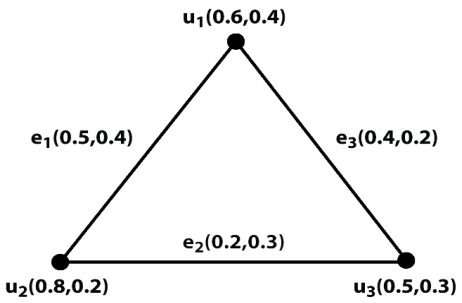

Fig. 3.1. Intuitionstic Fuzzy Graph$G$: (σ, μ)

The Intuitionistic Triple Layered Fuzzy graph of G with vertices u_1 = (0.6,0.4), u_2 = (0.8,0.2), u_3=(0.5,0.3); and edges u_1u_2 = (0.5,0.4), u_2u_3 = (0.2,0.3) and u_3u_1 = (0.4,0.3)

The Intuitionistic Triple Layered Fuzzy graph is given by

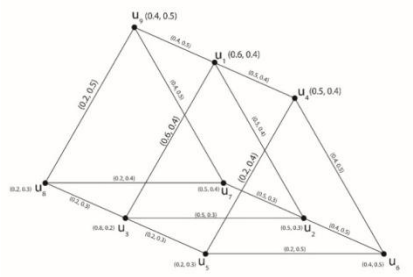

Fig. 3.2. ITL (G)

The minimal dominating set of an Intuitionistic Triple Layered fuzzy graph is

$$D = \{u_3, u_6, u_9\}$$

$$\gamma(G) = 3$$

The Complement of the Intuitionistic Triple Layered Fuzzy graph is given by

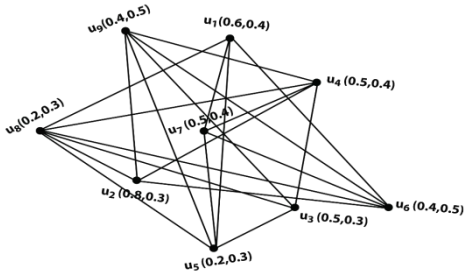

Fig. 3.3. ITL ($\bar{G}$)

The minimal dominating set of the complement of an Intuitionistic Triple Layered fuzzy graph is

$$\bar{D} = \{u_1, u_7\}$$

$$\gamma(\bar{G}) = 2$$

The sum of the dominating set $ITL(G)$ *and* ITL($\bar{G}$) is $\gamma(G) + \gamma(\bar{G}) \leq 2p$.

$$\gamma(G) + \gamma(\bar{G}) = 2 + 3$$

$$= 5$$

$$2p = 2(3)$$

$$= 6$$

$$5 \leq 6.$$

$$\gamma(G) + \gamma(\bar{G}) \leq 2p.$$

Remark: 3.1.

Since there does not exist any set which satisfying the condition of 2-domination and so 2-Domination is not applicable for the Intuitionistic Triple Layered simple fuzzy graph.

4. TOTAL DOMINATION IN INTUITIONISTIC TRIPLE LAYERED FUZZY GRAPH

In this section, we introduced the Total Domination in Intuitionistic Triple Layered Fuzzy graph and illustrated with some example.

Definition: 4.1.

Let $G: \langle (v_i, \mu_1, \nu_1), (e_{ij}, \mu_2, \nu_2) \rangle$ be an intuitionistic fuzzy graph without isolated vertices the underlying crisp graph $G^*: (\sigma^*, \mu^*)$. Let $ITL(G): \langle (v_i, \mu_{TL_1}, \nu_{TL_1}), (e_{ij}, \mu_{TL_2}, \nu_{TL_2}) \rangle$ be an Intuitionistic Triple Layered Fuzzy graph on the vertex set $(\sigma^* \cup \mu^* \cup \mu^*)$. A subset $D_t$of $(\sigma^* \cup \mu^* \cup \mu^*)$ is said to be a total dominating set if every vertex in $(\sigma^* \cup \mu^* \cup \mu^*)$is dominated by a vertex in D_t.

The minimum intuitionistic cardinality of the minimal total dominating set in G is called total domination number of G and is denoted by γ_t.

Theorem. 4.1.

For any Intuitionistic Triple Layered Simple Fuzzy graph ITL (G), the total domination number $\gamma_t(G) = p$ if and only if every vertex of ITL(G) has a unique neighbor.

Proof:

Let $G: \langle (v_i, \mu_1, \nu_1), (e_{ij}, \mu_2, \nu_2) \rangle$ be a cycle graph with p vertices. A vertex set of G be σ*.

Let $ITL(G): \langle (v_i, \mu_{TL_1}, \nu_{TL_1}), (e_{ij}, \mu_{TL_2}, \nu_{TL_2}) \rangle$ be an intuitionistic triple layered fuzzy graph of G with 3p vertices. A vertex set of ITL(G) be$(\sigma^* \cup \mu^* \cup \mu^*)$.

Let D_t be the total Dominating set of the Intuitionistic Triple Layered fuzzy graph which is the minimum cardinality of the minimal dominating set D_t *of* $ITL(G)$ is p.

$$\gamma_t(G) = p$$

Conversely,

Suppose $\gamma_t(G) = p$. If there exists a vertex v with two neighbors x and y then V – {x} is a total dominating set of G so that $\gamma_t(G) < p$ which is a contradiction.

Example:

Consider the Intuitionistic fuzzy graph $G: (\sigma, \mu)$ with p = 5 vertices whose crisp graph is a cycle.

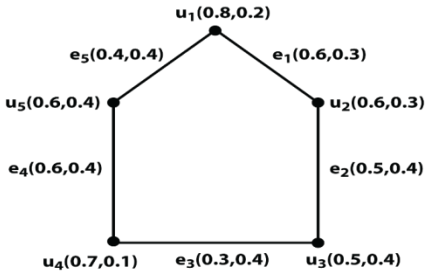

Fig. 4.1. Intuitionstic Fuzzy Graph G: (σ, μ)

The Intuitionistic Triple Layered Fuzzy graph is given by

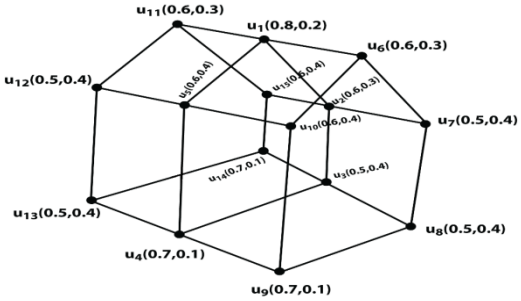

Fig. 4.2. ITL(G)

The Total dominating set is $D_t = \{u_1, u_2, u_3, u_4, u_5\}$

The minimum cardinality of the minimal total dominating set $\gamma_t(G) = 5$

5. CONCLUSION

In this paper, the Domination and the total domination in Intuitionistic Triple layered fuzzy graph is found under certain conditions and illustrated with some example. This work further can be extended to any simple Intuitionistic Triple Layered Fuzzy graph.

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RESEARCH ARTICLE

FUZZY ANTI-MAGIC LABELING ON SOME GRAPHS

Ameenal Bibi, K. and M. Devi *
PG and Research Department of Mathematics, D.K.M College for Women (Autonomous), Vellore - 632 001, Tamil Nadu, India.

ABSTRACT

In this Paper, we introduced the concept of fuzzy anti-magic labeling in graphs. We defined Fuzzy Anti-Magic Labeling (FAML) for Cycle, Star, Path and Antiprism graphs. A fuzzy graph $G:(\sigma,\mu)$ is known as fuzzy anti-magic graph if there exists two bijective functions $\sigma: V \to [0,1]$ and $\mu: V \times V \to [0,1]$ such that $\mu(u,v) < \sigma(u) \wedge \sigma(v)$ with the property that the sum of the edge labels incident to each vertex, the sums will all be different. We investigated and verified that fuzzy Cycle graphs, fuzzy Star graphs, fuzzy Path graphs and fuzzy antiprism graphs admits fuzzy anti-magic labeling.Further some properties related to fuzzy bridge and fuzzy cut vertex have been discussed.

Keywords: Fuzzy Anti-Magic labeling, FAM Cycle, FAM Star, FAM Path, FAM Antiprism.

AMS Mathematical Subject Classification: 03E72, 05C72, 05C78, 05C38.

1. INTRODUCTION

We begin with a finite, connected and undirected graph $G:(\sigma,\mu)$without loops and multiple edges. Throughout this paper $\sigma(G)$ and $\mu(G)$ denote the vertices and edges respectively. In recent years, graph theory has been actively implemented in the fields of Bio-chemistry, Electrical engineering, Computer science, Algebra, Topology and Operations Research. A Mathematical background to describe the phenomenon of uncertainty in real life situation has been suggested by Zadeh (1). The theory of fuzzy graphs was independently developed by Rosenfeld, Yeh and Bang. Fuzzy graph theory is finding extensive applications in modeling real time systems where the level of information congenital in the system varies with different levels of precision. Nagoorgani *et al.* (2) introduced the concept of fuzzy magic labeling and properties of fuzzy labeling. Akram *et al.* introduced interval valued fuzzy graphs, Strong intuitionistic fuzzy graphs, m-polar fuzzy graphs and novel properties of fuzzy labeling graphs (3).

Already we published two articles in fuzzy Bi-magic labeling and Interval valued fuzzy Bi-magic labeling (4,5). In this paper, we introduced the concept of Fuzzy anti-magic labeling on some standard graphs.

2. PRELIMINARIES AND OBSERVATIONS

Let U and V be two non-empty sets. Then ρ is said to be a fuzzy relation from U into V if ρ is a fuzzy set of UxV. A fuzzy graph $G:(\sigma,\mu)$ is a pair of functions $\sigma: V \to [0,1]$ and $\mu: V \times V \to [0,1]$where for all u,v $\in V$, we have $\mu(u,v) < \sigma(u) \wedge \sigma(v)$. A graph $G:(\sigma,\mu)$ admits fuzzy labeling and if the mapping $\sigma: V \to [0,1]$ and $\mu: V \times V \to [0,1]$ are bijection such that the membership values of edges and vertices are distinct and $\mu(u,v) < \sigma(u) \wedge \sigma(v)$ for all u,v $\in V$. Let $G:(\sigma,\mu)$ be a fuzzy graph. The strong degree of a vertex v is defined as the sum of membership values of all strong neighbours of v, then $d_s(v) = \sum_{u \in N_s(v)} \mu(u,v)$.

An edge uv is called a fuzzy bridge of G, if its removal reduces the strength of connectedness between some pair of vertices in G. Equivalently (u,v) is a fuzzy bridge iff there are nodes x,y such that (u,v) is a arc of every strongest x-y path.A vertex is a fuzzy cutvertex of $G:(\sigma,\mu)$ if removal of it reduces the strength of connectedness between some pair of vertices in G. A fuzzy graph admits anti-magic labeling,if the sum of the edge labels incident to each vertex, the sums all will be different and it is denoted by $A\widetilde{m_0(G)}$. A fuzzy graph which admits an anti-magic labeling is called Fuzzy anti-magic labeling graphs.

3. RESULTS

Definition 3.1.

A Cycle or Circulant graph is a graph that consists of a single cycle. The number of vertices in a cycle graph C_n equals the number of edges and every vertex has degree 2.

A Cycle graph which admits fuzzy labeling is called a fuzzy labeling Cycle graph and anti-magic

*Correspondence: M. Devi, PG and Research Department of Mathematics, D.K.M College for Women (Autonomous), Vellore - 632 001, Tamil Nadu, India. E.mail: devisivaji17@gmail.com

labeling exists then it is called a fuzzy anti-magic labeling cycle graph and it is denoted by $A\widetilde{m_0(C_n)}$.

Example 3.2.

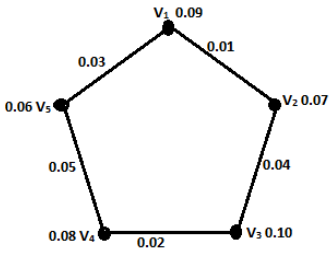

Fig. 1. $\boldsymbol{A\widetilde{m_0(C_5)}}$.

Theorem 3.3.

If n is odd, then the cycle C_n admits a fuzzy anti-magic labeling.

Proof:

Let G be a cycle with odd number of vertices and $v_1, v_2, v_3, \ldots v_n$ and $v_1v_2, v_2v_3, \ldots v_nv_1$ be the vertices and edges of C_n respectively. Let $z \to [0,1]$ such that one can choose $z = 0.01$ if $n \geq 3$.

The fuzzy labeling is defined as follows:

$$\sigma(v_{2i}) = (n+1+i)z \text{ for } 1 \leq i \leq \frac{n-1}{2}$$

$$\sigma(v_{2i-1}) = Max\left\{\sigma(v_{2i})/1 \leq i \leq \frac{n-1}{2}\right\} + i(z) \text{ for } 1 \leq i \leq \frac{n-1}{2}$$

$$\sigma(v_{2i-1}) = Min\left\{\sigma(v_{2i})/1 \leq i \leq \frac{n-1}{2}\right\} - i(z) \text{ for } i \leq \frac{n+1}{2}$$

$$\mu(v_{n-i}, v_{n-i+1}) = \frac{1}{2}Max\{\sigma(v_i)/1 \leq i \leq n\} \text{ for } i=1.$$

$$\mu(v_{n-i}, v_{n-i+1}) = \frac{1}{2}Max\{\sigma(v_i)/1 \leq i \leq n\} - \{i(z)/1 \leq i \leq n\} \text{ for } i = 3,5,\ldots.$$

$$\mu(v, v_n) = \frac{1}{2}Min\{\sigma(v_i)/1 \leq i \leq n\}$$

$$\mu(v_{n-i}, v_{n-i+1}) = \{\mu(v, v_n) - i(z)/1 \leq i \leq n\} \text{ for } i=2,4,\ldots..$$

Here, we investigated the results for fuzzy anti-magic cycle $\widetilde{Am}_0(C_7)$ for n=7.

Case (i): i is even

Then i=2x for any positive integer x.

For each edge v_i, v_{i+1}, the fuzzy anti-magic labelings are as follows:

Subcase (i):

i=2x for any positive integer $\left(x \leq \frac{n-5}{2}\right)$

$$\widetilde{Am}_0(C_7)\ \sigma(v_i) + \mu(v_i, v_{i+1}) + \sigma(v_{i+1})$$

$$\sigma(v_{2x}) + \mu(v_{2x}, v_{2x+1}) + \sigma(v_{2x+1})$$

$$= \sigma(v_2) + \mu(v_2, v_3) + \sigma(v_3)$$

$$\left\{(n+1+x)z/1 \leq i \leq \frac{n-1}{2}\right\} + \frac{1}{2}Min\{\sigma(v_i)/1 \leq i \leq n\} +$$

$$(n-4-2x)z + Max\left\{\sigma(v_{2i})/1 \leq i \leq \frac{n-1}{2}\right\} + (x+1)z$$

$$(2n-2)z + \frac{1}{2}Min\{\sigma(v_i)/1 \leq i \leq n\} +$$

$$Max\left\{\sigma(v_{2i})/1 \leq i \leq \frac{n-1}{2}\right\}$$

Subcase (ii):

i=2x for any positive integer $\left(x \leq \frac{n-3}{2}\right)$

$$\widetilde{Am}_0(C_7)\ \sigma(v_i) + \mu(v_i, v_{i+1}) + \sigma(v_{i+1})$$

$$= \sigma(v_4) + \mu(v_4, v_5) + \sigma(v_5)$$

$$\left\{(n+1+x)z/1 \leq i \leq \frac{n-1}{2}\right\} + \frac{1}{2}Min\{\sigma(v_i)/1 \leq i \leq n\} +$$

$$(n-1-2x)z + Max\left\{\sigma(v_{2i})/1 \leq i \leq \frac{n-1}{2}\right\} + (x+1)z$$

$$(2n+1)z + \frac{1}{2}Min\{\sigma(v_i)/1 \leq i \leq n\} +$$

$$Max\left\{\sigma(v_{2i})/1 \leq i \leq \frac{n-1}{2}\right\}$$

Subcase (iii):

i=2x for any positive integer $\left(x \le \frac{n-1}{2}\right)$

$$\tilde{A}m_0(C_7) = \sigma(v_6) + \mu(v_6, v_7) + \sigma(v_7)$$

$$= \left\{(n+1+x)z / 1 \le i \le \frac{n-1}{2}\right\} + \frac{1}{2} Min\{\sigma(v_i) / 1 \le i \le n\} +$$

$$(n-5-2x)z + Max\left\{\sigma(v_{2i}) / 1 \le i \le \frac{n-1}{2}\right\} + (x+1)z$$

$$= (2n-3)z + \frac{1}{2} Min\{\sigma(v_i) / 1 \le i \le n\} + Max\left\{\sigma(v_{2i}) / 1 \le i \le \frac{n-1}{2}\right\}$$

Case (i): i is odd

Then i=2x-1 for any positive integer x.

For each edge v_i, v_{i+1} , the fuzzy anti-magic labelings are as follows:

Subcase (i):

i=2x-1 for any positive integer $\left(x \le \frac{n-5}{2}\right)$

$$\tilde{A}m_0(C_7) = \sigma(v_i) + \mu(v_i, v_{i+1}) + \sigma(v_{i+1})$$

$$= \sigma(v_{2x-1}) + \mu(v_{2x-1}, v_{2x}) + \sigma(v_{2x})$$

$$= \sigma(v_1) + \mu(v_1, v_2) + \sigma(v_2)$$

$$= Max\left\{\sigma(v_{2i}) / 1 \le i \le \frac{n-1}{2}\right\} + (x+1)z + \frac{1}{2} Min\{\sigma(v_i) / 1 \le i \le n\} - (2x+1)z / 1 \le i \le n-1 + \{(n+x)z\}$$

$$= Max\left\{\sigma(v_{2i}) / 1 \le i \le \frac{n-1}{2}\right\} + \frac{1}{2} Min\{\sigma(v_i) / 1 \le i \le n\} + nz$$

Subcase (ii):

i=2x-1 for any positive integer $\left(x \le \frac{n-3}{2}\right)$

$$\tilde{A}m_0(C_7) = \sigma(v_i) + \mu(v_i, v_{i+1}) + \sigma(v_{i+1})$$

$$= \sigma(v_3) + \mu(v_3, v_4) + \sigma(v_4)$$

$$= Max\left\{\sigma(v_{2i}) / 1 \le i \le \frac{n-1}{2}\right\} + (x+1)z + \frac{1}{2} Min\{\sigma(v_i) / 1 \le i \le n\} - (2x-2)z / 1 \le i \le n-1 + \{(n+x)z\}$$

$$= Max\left\{\sigma(v_{2i}) / 1 \le i \le \frac{n-1}{2}\right\} + \frac{1}{2} Min\{\sigma(v_i) / 1 \le i \le n\} + (n+3)z$$

Subcase (iii):

i=2x-1 for any positive integer $\left(x \le \frac{n-1}{2}\right)$

$$\tilde{A}m_0(C_7) = \sigma(v_i) + \mu(v_i, v_{i+1}) + \sigma(v_{i+1})$$

$$= \sigma(v_{2x-1}) + \mu(v_{2x-1}, v_{2x}) + \sigma(v_{2x})$$

$$= \sigma(v_5) + \mu(v_5, v_6) + \sigma(v_6)$$

$$= Max\left\{\sigma(v_{2i}) / 1 \le i \le \frac{n-1}{2}\right\} + (x+1)z + \frac{1}{2} Min\{\sigma(v_i) / 1 \le i \le n\} - (2x-5)z / 1 \le i \le n-1 + \{(n+x)z\}$$

$$= Max\left\{\sigma(v_{2i}) / 1 \le i \le \frac{n-1}{2}\right\} + \frac{1}{2} Min\{\sigma(v_i) / 1 \le i \le n\} + (n+6)z$$

Subcase (iv):

i=2x-1 for any positive integer $\left(x \le \frac{n+1}{2}\right)$

$$\tilde{A}m_0(C_7) = \sigma(v_7) + \mu(v_7, v_1) + \sigma(v_1)$$

$$= Min\left\{\sigma(v_{2i}) / 1 \le i \le \frac{n-1}{2}\right\} + (x+1)z + \frac{1}{2} Min\{\sigma(v_i) / 1 \le i \le n\} - (2x-3)z / 1 \le i \le n-1 + \{(n+x)z\}$$

$$= \begin{matrix} Min\left\{\sigma(v_{2i})/1 \le i \le \frac{n-1}{2}\right\} + \\ \frac{1}{2}Min\{\sigma(v_i)/1 \le i \le n\} + (n+4)z \end{matrix}$$

Therefore, from the above cases, we verified that C_n is a fuzzy anti-magic graph if C_n has odd number of vertices.

Definition 3.4.

A fuzzy Star graph consists of two vertex sets V and U with $|V| = 1$ and $|U| > 1$ such that $\mu(v,u_i) > 0$ and $\mu(u_i,u_{i+1}) = 0$ for all $1 \le i \le n$.

In a fuzzy Star graph, if an anti-magic labeling exists then it is called a fuzzy anti-magic labeling Star graph and it is denoted by $\tilde{A}m_0(S_{1,n})$.

Example 3.5.

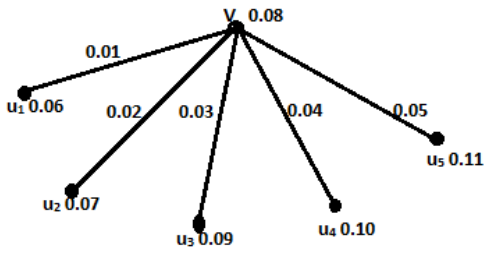

Fig. 2. $\widetilde{Am_0(S_{1,n})}$.

Theorem 3.6.

For n ≥ 3, the Star graph $S_{1,n}$ admits a fuzzy anti-magic labeling.

Proof:

Let $S_{1,n}$ be the Star graph with v,u_1, u_2,...u_n as vertices and vu_1, vu_2, ...vu_n as edges. Let $z \to [0,1]$ such that one can choose z=0.01 if n ≥ 3. The fuzzy labeling is defined as follows:

$$\sigma(u_i) = (n+i)z \text{ for i=1,2}$$

$$\sigma(u_i) = [(n+i)+1]z \text{ for } 3 \le i \le n$$

$$\sigma(v) = \sum \frac{\sigma(u_i)}{n} \text{ for } 1 \le i \le n$$

$$\mu(v,u_1) = Max\{\sigma(v),\sigma(u_1)\} - Min\{\sigma(v),\sigma(u_1)\} - z \text{ for i=1}$$

$$\mu(v,u_2) = Max\{\sigma(v),\sigma(u_2)\} - Min\{\sigma(v),\sigma(u_2)\} + z \text{ for i=2}$$

$$\mu(v,u_i) = Max\{\sigma(v),\sigma(u_i)\} - Min\{\sigma(v),\sigma(u_i)\} + 2z \text{ for } 3 \le i \le n$$

Here, we investigated the results for fuzzy anti-magic labelings of $\tilde{A}m_0(S_{1,n})$.

Case (i):

$$\tilde{A}m_0(S_{1,n}) = \sigma(v) + \mu(v,u_1) + \sigma(u_1)$$

$$= \begin{matrix} \left\{\sum \frac{\sigma(u_i)}{n}/1 \le i \le n\right\} + Max\{\sigma(v),\sigma(u_1)/i=1\} - \\ Min\{\sigma(v),\sigma(u_1)/i=1\} - z + (n+1)z. \end{matrix}$$

$$= \begin{matrix} \left\{\sum \frac{\sigma(u_i)}{n}/1 \le i \le n\right\} + Max\{\sigma(v),\sigma(u_1)/i=1\} - \\ Min\{\sigma(v),\sigma(u_1)/i=1\} + nz \end{matrix}$$

Case (ii):

$$\tilde{A}m_0(S_{1,n}) = \sigma(v) + \mu(v,u_2) + \sigma(u_2)$$

$$= \begin{matrix} \left\{\sum \frac{\sigma(u_i)}{n}/1 \le i \le n\right\} + Max\{\sigma(v),\sigma(u_1)/i=2\} - \\ Min\{\sigma(v),\sigma(u_1)/i=2\} + z + (n+2)z. \end{matrix}$$

$$= \begin{matrix} \left\{\sum \frac{\sigma(u_i)}{n}/1 \le i \le n\right\} + Max\{\sigma(v),\sigma(u_1)/i=2\} - \\ Min\{\sigma(v),\sigma(u_1)/i=2\} + (n+3)z \end{matrix}$$

Case (iii):

$$\tilde{A}m_0(S_{1,n}) = \sigma(v) + \mu(v,u_i) + \sigma(u_i)$$

$$= \begin{matrix} \left\{\sum \frac{\sigma(u_i)}{n}/1 \le i \le n\right\} + Max\{\sigma(v),\sigma(u_i)/3 \le i \le n\} - \\ Min\{\sigma(v),\sigma(u_i)/3 \le i \le n\} + 2z + \{(n+i)+1\}z. \end{matrix}$$

$$= \begin{matrix} \left\{\sum \frac{\sigma(u_i)}{n}/1 \le i \le n\right\} + Max\{\sigma(v),\sigma(u_i)/3 \le i \le n\} - \\ Min\{\sigma(v),\sigma(u_i)/3 \le i \le n\} + \{(n+3+i)z/3 \le i \le n\}. \end{matrix}$$

Definition 3.7.

A Path with atleast two vertices is connected and has two terminal vertices (vertices that have degree 1) while all others (if any) have degree 2.

In a graph which admits fuzzy labeling is called a fuzzy path graph and anti-magic labeling exists then it is called as a fuzzy anti-magic Path graph.

Example: 3.8

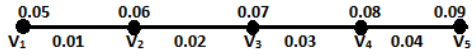

Fig. 3. $\widetilde{Am_0(P_5)}$.

Theorem: 3.9

For n ≥ 3, the Path graph P_n has (n-1) fuzzy anti-magic labeling.

Proof:

Let P be a Path with length n ≥ 1 and $v_1,v_2,v_3,\ldots v_n$ and v_1v_2, $v_2v_3,\ldots,v_{n-1}v_n$ are the vertices and edges of P.

Let $z \to [0,1]$ such that one can choose z=0.01 if n ≥ 3. If the length of the path P is Odd, then the fuzzy labeling is defined as follows:

$$\sigma(v_{2i-1}) = (n+2i-2)z \text{ for } 1 \le i \le \frac{n+1}{2}$$

$$\sigma(v_{2i}) = Min\left\{\sigma(v_{2i-1})/1 \le i \le \frac{n+1}{2}\right\} + (2i-1)z$$

for $1 \le i \le \frac{n+1}{2}$

$$\mu(v_i,v_{i+1}) = Max\{\sigma(v_i)/1 \le i \le n-1\} - M \text{ for } 1 \le i \le n$$
$$in\{\sigma(v_i)/1 \le i \le n-1\} - (n-i-1)z$$

Here, we investigated the results for fuzzy anti-magic labeling of Path graph for n=5.

Case (i): i is even

Then i=2x for any positive integer x

For each edge v_i,v_{i+1}

Subcase (i):

i=2x for any positive integer $\left(x \le \frac{n-3}{2}\right)$

$$\tilde{A}m_0(P_5) = \sigma(v_i) + \mu(v_i,v_{i+1}) + \sigma(v_{i+1})$$
$$= \sigma(v_2) + \mu(v_2,v_3) + \sigma(v_3)$$
=
$$Min\left\{\sigma(v_{2i-1})/1 \le i \le \frac{n+1}{2}\right\} + (2n-x)z + M$$
$$ax\{\sigma(v_i)/1 \le i \le n-1\} -$$
$$Min\{\sigma(v_i)/1 \le i \le n-1\} - (n+x+2)z + (n+2x-2)z$$
=
$$Min\left\{\sigma(v_{2i-1})/1 \le i \le \frac{n+1}{2}\right\} + Max\{\sigma(v_i)/1 \le i \le n-1\} -$$
$$Min\{\sigma(v_i)/1 \le i \le n-1\} + (2n-4)z$$

Subcase (ii):

i=2x for any positive integer $\left(x \le \frac{n-1}{2}\right)$

$$\tilde{A}m_0(P_5) = \sigma(v_4) + \mu(v_4,v_5) + \sigma(v_5)$$
=
$$Min\left\{\sigma(v_{2i-1})/1 \le i \le \frac{n+1}{2}\right\} + Max\{\sigma(v_i)/1 \le i \le n-1\} -$$
$$Min\{\sigma(v_i)/1 \le i \le n-1\} + (2n+2)z$$

Case (ii): i is odd

Then i=2x for any positive integer x

For each edge v_i,v_{i+1}

Subcase (i):

i=2x-1 for any positive integer $\left(x \le \frac{n-3}{2}\right)$

$$\tilde{A}m_0(P_5) = \sigma(v_i) + \mu(v_i,v_{i+1}) + \sigma(v_{i+1})$$
$$= \sigma(v_{2x-1}) + \mu(v_{2x-1},v_{2x}) + \sigma(v_{2x})$$
$$= \sigma(v_1) + \mu(v_1,v_2) + \sigma(v_2)$$
=
$$(n-2x)z + Max\{\sigma(v_i)/1 \le i \le n-1\} - M$$
$$in\{\sigma(v_i)/1 \le i \le n-1\} -$$
$$(n+x+2)z + Min\left\{\sigma(v_{2i-1})/1 \le i \le \frac{n+1}{2}\right\} + (n-x)z$$
=
$$(n-2)z + Max\{\sigma(v_i)/1 \le i \le n-1\} - M$$
$$in\{\sigma(v_i)/1 \le i \le n-1\} +$$
$$Min\left\{\sigma(v_{2i-1})/1 \le i \le \frac{n+1}{2}\right\}$$

Subcase (ii):

i=2x-1 for any positive integer $\left(x \le \frac{n-1}{2}\right)$

$$\tilde{A}m_0(P_5) = \sigma(v_3) + \mu(v_3,v_4) + \sigma(v_4)$$
=
$$(n-2x)z + Max\{\sigma(v_i)/1 \le i \le n-1\} -$$
$$Min\{\sigma(v_i)/1 \le i \le n-1\} -$$
$$(n+x+2)z + Min\left\{\sigma(v_{2i-1})/1 \le i \le \frac{n+1}{2}\right\} + (n-x)z$$

$(n+4)z + Max\{\sigma(v_i)/1 \le i \le n-1\} -$

$= Min\{\sigma(v_i)/1 \le i \le n-1\} +$ If the length

$Min\left\{\sigma(v_{2i-1})/1 \le i \le \frac{n+1}{2}\right\}$

of the path P is even then it has the following membership functions:

$\sigma(v_{2i-1}) = (n+2i-2)z$ for $1 \le i \le \frac{n}{2}$

$\sigma(v_{2i}) = Min\left\{\sigma(v_{2i-1})/1 \le i \le \frac{n}{2}\right\} + (2i-1)z$ for $1 \le i \le \frac{n}{2}$

$\mu(v_i, v_{i+1}) = Max\{\sigma(v_i)/1 \le i \le n-1\} -$ $Min\{\sigma(v_i)/1 \le i \le n-1\} - (n-i-1)z$ for $1 \le i \le n$

By using the above membership functions, we can prove that the Path with even length also admits fuzzy anti-magic labeling.

Definition 3.10.

An Antiprism graph is a graph corresponding to the skeleton of an Antiprism. Antiprism graphs are therefore polyhedral and planar graphs.

The n-antiprism graph has 2n-vertices and 4n-edges and is isomorphic to the circulant graph $C_{i2n}(1,2)$.

An Antiprism graph is a special case of Circulant graph. In a graph which admits fuzzy labeling is called a fuzzy antiprism graph and anti-magic labeling exists then it is called as a fuzzy anti-magic antiprism graph.

Example 3.11.

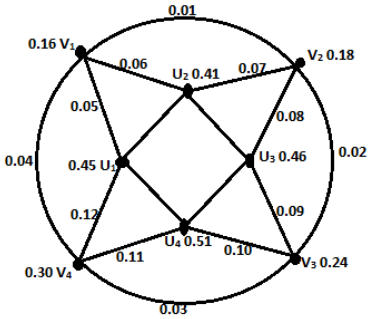

Fig. 4. $\widetilde{Am_0(AP_4)}$.

Theorem 3.12.

For n ≥ 2, the antiprism graph AP_nhas 4n fuzzy antimagic labelings.

Proof:

Let AP_n be any n-sided Antiprism graph with 2n vertices and 4n edges.

It consists of two vertex sets U and V with $|U| > 1$ and $|V| > 1$ such that $\mu(u_i, v_i) > 0$and $(u_{i+1}, v_i) > 0$, $\mu(v_i, v_{i+1}) > 0$ and $\mu(u_i, u_{i+1}) > 0$.

Let $z \to [0,1]$ such that one can choose z=0.01 if n ≥ 3. The fuzzy labeling is defined as follows:

$\mu(u_i, v_i)$= (n+2i-1)z for i=1,2,...

$\mu(u_{i+1}, v_i)$ = (n+2i)z for i=1,2,...

$\mu(v_i, v_{i+1})$ = iz for i=1,2,...

$\mu(u_i, u_{i+1})$=(3n+j)z for i=1,3,..., j=1,2,... for n ≥ 2

$\mu(u_i, u_{i+1})$=(3n+j+2)z for i=2,4,..., j=1,2,... for 2 ≤ n ≤ 4

$\mu(u_i, u_{i+1})$=(3n+j+3)z for i=2,4,..., j=1,2,... for 5 ≤ n ≤ 6

$\mu(u_i, u_{i+1})$=(3n+j+4)z for i=2,4,..., j=1,2,... for 7 ≤ n ≤ 8

$\mu(u_i, u_{i+1})$=(3n+j+5)z for i=2,4,..., j=1,2,... for 9 ≤ n ≤ 10

and so on.

For vertex labeling,

$\sigma(u_i) = \mu(u_i, u_{i+1}) + \mu(u_i, u_n) + \mu(u_i, v_i) + \mu(u_i, v_n)$for i=1

$\sigma(u_i) = \mu(u_i, u_{i-1}) + \mu(u_i, u_{i+1}) + \mu(u_i, v_i) + \mu(u_i, v_{i-1})$for 2 ≤ i ≤ n-1

$\sigma(u_i) = \mu(u_i, u_{i-1}) + \mu(u_n, u_{n-i+1}) + \mu(u_n, v_n) + \mu(u_n, v_{i-1})$for i = n

and

$\sigma(v_i) = \mu(v_i, v_{i+1}) + \mu(v_i, v_n) + \mu(v_i, u_i) + \mu(v_i, u_{i+2})$for i=1 $\sigma(v_i) = \mu(v_i, v_{i-1}) + \mu(v_i, v_{i+1}) + \mu(v_i, u_i) + \mu(v_i, u_{i+1})$ for 2 ≤ i ≤ n-1 $\sigma(v_i) = \mu(v_i, v_{i-1}) + \mu(v_n, v_{n-i+1}) + \mu(v_n, u_n) + \mu(v_n, u_{i-1})$ for i=n

In view of the above labeling pattern, the edges are distinctly labeled in such a way that when taking the sum of the edge labels incident to each vertex, the sums will be different.

Hence, the Antiprism graph admits fuzzy anti-magic labelings.

4. PROPERTIES OF FUZZY ANTI-MAGIC GRAPHS

Proposition 4.1.

For every fuzzy anti-magic graph G, there exists atleast one fuzzy bridge.

Proposition 4.2.

Removal of a fuzzy cut vertex from a fuzzy anti-magic Star graph G, the resulting graph G^* also admits a fuzzy anti-magic labeling if $n \geq 4$.

Proof:

Since G is a Star graph, there exists atleast one fuzzy cut vertex. Now if we remove that fuzzy cut vertex from G, then it becomes a smaller Star G^*. However, G^* remains to be admit fuzzy anti-magic labeling if $n \geq 4$.

Hence, we conclude that removal of a fuzzy cut vertex from a fuzzy anit-magic Star graph results in a fuzzy anti-magic Star graph if $n \geq 4$.

Proposition 4.3.

Removal of a fuzzy cut vertex from a fuzzy anti-magic graph G such that G* is a path is also a fuzzy anti-magic graph.

Observation 4.4.

1. Every fuzzy anti-magic graph is a fuzzy labelled graph, but the converse is not true.
2. If G is a fuzzy anti-magic graph then $d(u) \neq d(v)$ for any pair of vertices $u, v \in V(G)$.
3. For all fuzzy anti-magic cycle graph G, there exists a subgraph G^* which is a cycle with odd number of vertices and there exists atleast one pair of vertices u and v such that $d_s(u) = d_s(v)$

5. CONCLUSION

In this paper, the concept of fuzzy anti-magic labeling has been introduced. Fuzzy anti-magic labeling for cycle, Star, Path and Antiprism graphs have been discussed.Properties of fuzzy anti-magic graphs are investigated. We further extend this study on some more special classes of graphs.

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RESEARCH ARTICLE

TWO-OUT DEGREE EQUITABLE DOMINATION IN THE MIDDLE, CENTRAL AND THE LINE GRAPHS OF P_N, C_N AND $K_{1,N}$

Ameenal Bibi, K. and P. Rajakumari*
P.G. and Research Department of Mathematics, D.K.M.College for Women (Autonomous), Vellore - 632 001, Tamil Nadu, India.

ABSTRACT

Let G=(V,E) be a simple, finite, connected and undirected graph. A dominating set D of G is said to be two-out degree equitable dominating set if for any two vertices $u, v \in D$ such that $\left| od_D(u) - od_D(v) \right| \leq 2$, where $od_D(u) = \left| N(v) \cap (V - D) \right.$. The minimum cardinality of two -out degree equitable dominating set is called two- out degree equitable domination number and it is denoted by γ_{2oe}(G). In this paper, we introduced the two-out degree equitable domination numbers in the middle, central and the line graphs of the path P_n, cycle C_n and star $K_{1,n}$ graphs.

Keywords: Two-out degree equitable domination number, Middle graph, Central graph, Line graph, Path P_n, Cycle C_n and Star graph $K_{1,n}$.

AMS Subject Classification: 05C9, 05C69, 05C70.

1. INTRODUCTION

The concept of domination was first studied by Ore and Berge (1962). A non-empty set $D \subseteq V$ is said to be a dominating set of G if every vertex in V-D is adjacent to atleast one vertex in D. The minimum cardinality of the minimal dominating set D is = called the domination number and it is denoted by γ(G).

An equitable domination has interesting application in the context of social network. In a network, nodes with nearly equal capacity may interact with each other in a better way. In society, persons with nearly equal status, tend to be friendly. Ali Sahal and V.Mathad (Sahal,2013) introduced the concept of two out degree equitable domination in graphs. In this paper, we investigated the two out degree equitable domination number in the middle and the central graphs of P_n , C_n and $K_{1,n}$ graphs.

Definition 1.1 (8)

The middle graph of a connected graph G denoted by M(G) is the graph whose vertex set is V(G)UE(G) where two vertices are adjacent if

(i) They are adjacent edges of G (or)

(ii) One is a vertex of G and the other is an edge incident with it.

Definition 1.2 (8)

For a given graph G=(V,E) of order n, the central graph C(G) is obtained, by subdividing each edge in E exactly once and joining all the non adjacent vertices of G. The central graph C(G) of a graph G is an example of a split graph, where a split graph is a graph whose vertex set V can be partitioned into two sets, V1 and V2, where each pair of vertices in V1 are adjacent, and no two vertices in V2 are adjacent.

Definition 1.3 (7)

A dominating set D in a graph G is called a two-out degree equitable dominating set if for any two vertices $u, v \in D$ such that $\left| od_D(u) - od_D(v) \right| \leq 2$, where $od_D(u) = \left| N(V) \cap V - D \right|$. The minimum cardinality of a two-out degree equitable dominating set is called the two-out degree equitable domination number of G and is denoted by γ_{2oe}(G).

In the consequent section, we obtained the two-out degree equitable domination number γ_{2oe}(G) of the middle graph of P_n, C_n and $K_{1,n}$ graphs.

Definition 1.4 (9)

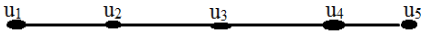
Line graph L(G) of a graph G is defined with the vertex set E(G), in which two vertices are adjacent if and only if the corresponding edges are adjacent in G.

2. Two-out degree Equitable domination in the Middle graphs of P_n , C_n and $K_{1,n}$.

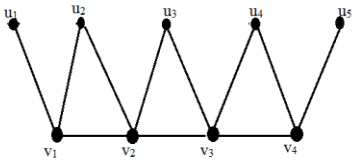
Example 2.1

Let G be the middle graph as in the figure. We obtained the two-out degree equitable domination number.

*Correspondence: P. Rajakumari, P.G. and Research Department of Mathematics, D.K.M.College for Women (Autonomous), Vellore-632001, Tamilnadu, India. E.mail: rajakumari0990@gmail.com



Path P_5



Middle graph of P_5 $M(P_5)$

Consider the set D= $\{v_1, v_3\}$.It is a dominating set and $V - D = \{v_2, v_4, u_1, u_2, u_3, u_4, u_5\}$

$od_D(v_1) = \left| N(v_1) \cap \{ v_2, v_4, u_1, u_2, u_3, u_4, u_5 \right.$

$= \left| \{u_1, u_2, v_2\} \cap \{ v_2, v_4, u_1, u_2, u_3, u_4, u_5\} \right|$

= 3

$od_D(v_3) = \left| N(v_3) \cap \{ V - D \right|$

$= \left| \{u_3, u_4, v_2, v_4\} \cap \{ v_2, v_4, u_1, u_2, u_3, u_4, u_5\} \right|$

= 4

From the above, any two vertices $u, v \in D$ such that $\left| od_D(u) - od_D(v) \right| \leq 2$.

Therefore $\{v_1, v_3\}$ is the two-out degree equitable dominating set with the minimum cardinality is 2. That is $\gamma_{2oe}[M(P_5)] = 2$.

Theorem2.2

For any Path P_n, $\gamma_{2oe}[M(P_n)] = \left\lceil \frac{n-1}{2} \right\rceil$ *where* $n \geq 5$.

Proof

Let $P_n: u_1, u_2, \ldots . u_{n+1}$ be the path of length n and $u_i u_{i+1} = v_i$, By the definition of middle graph, $M(P_n)$ has the vertex set $V(P_n) \cup E(P_n) = \{u_i / \ 1 \leq i \leq n+1\} \cup \{v_i / 1 \leq i \leq n\}$ in which each u_i is adjacent to v_i and v_i is adjacent to u_{i+1}. The vertices $u_1, v_1, v_2 \ldots . v_{2k}, v_{2k-1}$ of $M(P_n)$ induces a path of length 4k.

Let $D = \{(v_{i+1}, v_{i+3}/i = 1), (v_i, v_{i+2}/i = 2), (v_{i-1}, v_{i+1}, v_{i+3}/i = 3), \ldots .\}$ be a dominating set of $M(P_n)$ and

$V - D = \{(v_i, v_{i+2}/i = 1), (v_{i-1}, v_{i+1}, v_{i+3}/i = 2), \ldots \cup (u_i / 1 \leq i \leq n)\}$.

Now $v_{i+1} \in D$ then $od_D(v_{i+1}) = \left| N(v_{i+1}) \cap V - D \right|$ *for* $i = 1, od_D(v_2) = \left| N(v_2) \cap V - D \right|$

$= \left| \{(v_i, v_{i+2}, u_{i+1}, u_{i+2}/i = 1), (v_{i-1}, v_{i+1}, u_{i-1}, u_{i+1}/i = 2), \ldots\} \cap \{(v_i, v_{i+2}/i = 1), (v_{i-1}, v_{i+1}, v_{i+3}/i = 2), \ldots \cup (u_i / 1 \leq i \leq n)\} \right|$

$\left| \{(v_i, v_{i+2}, u_{i+1}, u_{i+2}), (v_{i-1}, v_{i+1}, u_{i-1}, u_{i+1}), \ldots . .\} \right|$

$= 4$

Then $\left| od_D(v_i) - od_D(v_{i+1}) \right| \leq 2$, for any $v_i v_{i+1} \in D$. Therefore D is the minimum two-out degree equitable dominating set, Hence, $\gamma_{2oe}[M(P_n)] = \left\lceil \frac{n-1}{2} \right\rceil$ *where* $n \geq 5$. .

Theorem 2.3

For any Cycle C_n, $\gamma_{2oe}[M(C_n)] = \left\lceil \frac{n}{3} \right\rceil + 1$ *where* $n \geq 4$.

Proof

Let $V(C_n) = \{u_1, u_2 \ldots . u_n\}$ and $E(C_n) = \{v_1, v_2 \ldots . v_n\}$ Where $v_i = u_i u_{i+1}$ $(1 \leq i \leq n-1)$, $v_n = u_n u_1$. By the definition of middle graph, $M(C_n)$ has the vertex set $V(C_n) \cup E(C_n)$ in which each v_i *is* adjacent to v_{i+1} $(i = 1,2 \ldots . n-1)$ and v_n is adjacent to u_1. In $M(C_n)$, $\{u_1, v_1, u_2, v_2 \ldots . v_{n-1}, u_1\}$ induces a cycle of length 2n. That is $\left| V(M(C_n)) \right| = 2n$ and $\left| E(M(C_n)) \right| = 3n$.

Let $D = \{(v_i, v_{i+2}/i = 1), (v_{i-1}, v_{i+1}, v_{i+3}/i = 2), \ldots\}$ be a dominating set of $M(C_n)$ and

$V - D = \{(v_{i+1}, v_{i+3}/i = 1), (v_i, v_{i+2}/i = 2), \ldots . \cup \{ui / 1 \leq i \leq n\}$.

Now $v_i \in D$ then $od_D(v_i) = \left| N(v_i) \cap V - D \right|$

for $i = 1$, $od_D(v_1) = \left| N(v_1) \cap V - D \right|$

$= \left| \{(v_{i+1}, v_{i+3}, u_i, u_{i+1}/i = 1), (v_i, v_{i+3}, u_{i-1}, u_i/i = 2), \ldots .\} \cap \{(v_{i+1}, v_{i+3}/i = 1), (v_i, v_{i+2}/i = 2), \ldots .\} \cup \{u_i / 1 \leq i \leq n\} \right|$

$= \left| \{(v_{i+1}, v_{i+3}, u_i, u_{i+1}/i = 1), , (v_i, u_{i-1}, u_i/i = 2), \ldots .\} \right|$

= 3 or 4.

Then $\left| od_D(v_i) - od_D(v_{i+1}) \right| \leq 2$, for any $v_i v_{i+1} \in D$. Therefore D is the minimum two- out degree equitable dominating set. Hence, $\gamma_{2oe}[M(C_n)] = \left\lceil \frac{n}{3} \right\rceil + 1$ *where* $n \geq 4$..

Theorem 2.4

For any Star graph $K_{1,n}$, $\gamma_{2oe}\left[M(K_{1,n})\right] = n$.

Proof

Let $V(K_{1,n}) = \{u, u_1, u_2 \ldots . u_n\}$ and $E(K_{1,n}) = \{v_1, v_2 \ldots . v_n\}$.By the definition of middle graph, we have $V\left(M(K_{1,n})\right) = \{u\} \cup \{v_i / \leq i \leq n\} \cup \{u_i / \leq i \leq n\}$ in which the vertices $v_1, v_2 \ldots . v_n, u$

induces clique of order $n+1$. Let $D=\{v_i\ /1\le i\le n\}$ be the dominating set of $M(K_{1,n})$ and $V-D=\{(u,u_i)/1\le i\le n\}$.

Now $v_i\in D$ then $od_D(v_i)=\left|N(v_i)\cap V-D\right|$ $for\ i=1, od_D(v_1)=\left|N(v_1)\cap V-D\right|$

$=\left|\{v_{i+1},\dots v_n\}\cup\left\{\frac{u_i}{\le i}\le n\right\}\cap\{u,u_i\}\right.$

$=\left|\{u,u_i\}\right|$ $since\ i=1$

=2

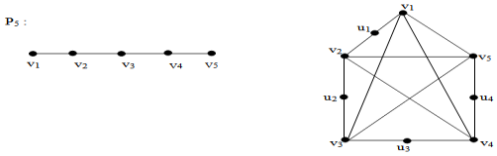
Then $\left|od_D(v_i)-od_D(v_j)\right|\le 2$, for any $v_iv_j\in D$. Therefore D is the minimum two - out degree equitable dominating set, Hence, $\gamma_{2oe}\left[M(K_{1,n})\right]=n$.

3. Two-out degree Equitable domination in the Central graphs of P_n , C_n and $K_{1,n}$.

In this section, we obtained the two-out degree equitable domination number $\gamma_{2oe}(G)$ of the central graphs of the path P_n, cycle C_n and the star graph $K_{1,n}$.

Example 3.1.

Let G be the central graph as in the figure. we obtained the two-out degree equitable domination number.



Central graph of P_5

Consider the set $D=\{v_1,u_2,u_3,u_4\}$ .It is a dominating set and $V-D=\{v_2,v_3,v_4,v_5,u_1\}$

$od_D(v_1)=\left|N(v_1)\cap V-D\right|$

$=\left|\{u_1,v_3,v_4,v_5\}\cap\{v_2,v_3,v_4,v_5,u_1\}\right|$

= 4

$od_D(u_2)=\left|N(u_2)\cap V-D\right|$

= 2

Similarly, $od_D(u_3)=od_D(u_4)=2$

From the above, any two vertices $u,v\in D$ are such that $\left|od_D(u)-od_D(v)\right|\le 2$.

Therefore $\{v_1,u_2,u_3,u_4\}$ is the minimum two-out degree equitable dominating set with the minimum cardinality 4. Hence, $\gamma_{2oe}[C(P_5)]=4$.

Theorem 3.2.

For any Path P_n, $\gamma_{2oe}[C(P_n)]=n-1\ for\ n\le 5$.

Proof

Let P_n be the path of length $(n-1)$ with vertices $v_1,v_2\dots.v_n$.By the definition of central graph, the non-adjacent vertices $v_i\ and\ v_j$ of P_n are adjacent in $C(P_n)$.

Therefore,$V(C(P_n)=\{v_i/1\le i\le n\}\cup\{u_i/1\le i\le n-1$and

$E(C(P_n)=\{e_i/1\le i\le n-1\}\cup\{e'_i/1\le i\le n-1\}\cup\{e_{ij}:1\le i\le n-2, i+2\le j\le n\}$.

Let $\{v_i\}\cup\{u_i/2\le i\le n-1\}$ will be the dominating set.

Let

$D=\{(v_1,u_{i+1}),(v_1,u_{i+1}),(v_1,u_{i-1},u_i,u_{i+1})\}, where\ 1\le i\le 3$ be the dominating set of $C(P_n)\ and$

$V-D$

$=\{(u_1,v_{i+1},v_{i+2}),(u_1,v_i,v_{i+1},v_{i+2}),(u_1,v_{i-1},v_i,v_{i+1},v_{i+2})\}$

$where\ 1\le i\le 3$

Now $v_1\in D$ then $od_D(v_1)=\left|N(v_1)\cap V-D\right|$

$=\left|\{(u_1,v_{i+2}),(u_1,v_{i+1},v_{i+2}),(u_1,v_i,v_{i+1},v_{i+2})\}\right.$
$\cap\ \{(u_1,v_{i+1},v_{i+2}),(u_1,v_i,v_{i+1},v_{i+2}),(u_1,v_{i-1},v_i,v_{i+1},v_{i+2})\}\Big|$

$=\left|\{(u_1,v_{i+2}),(u_1,v_{i+1},v_{i+2}),(u_1,v_i,v_{i+1},v_{i+2})\}\right|$

$=n-1$

Now $u_i\in D$ then $od_D(u_i)=\left|N(u_i)\cap V-D\right|$

=2

Then $\left|od_D(v_1)-od_D(u_i)\right|\le 2$, for any $v_1,u_i\in D$. Therefore D is the minimum two - out degree equitable dominating set. Hence, $\gamma_{2oe}[C(P_n)]=n-1\ for\ n\le 5$.

Theorem 3.3.

For any Cycle C_n, $\gamma_{2oe}[C(C_n)]=n-1\ for\ n\le 5$.

Proof

Let C_n be any cycle of length n and let $V(C_n)=\{v_1,v_2\dots v_n\}$ and $E(C_n)=\{e_1,e_2\dots e_n\}$.By the definition of central graph $C(C_n)$ has the vertex set $V(C_n)\cup\{u_i:1\le i\le n\}$ where u_i is a vertex of subdivision of the edge v_iv_{i+1} $(1\le i\le n-1)$ and u_n is a vertex of subdivision of the edge v_nv_1 .

Let

$D=\{(v_1,u_{i+1}),(v_1,u_i,u_{i+1}),(v_1,u_{i-1},u_i,u_{i+1})\}, where\ 1\le i\le 3$ be a dominating set of $C(C_n)$ and $V-D=\{(v_{i+1},v_{i+2},u_i,u_{i+2}),(v_i,v_{i+1},v_{i+2},u_{i-1},u_{i+2})(v_{i-1},v_i,v_{i+1},v_{i+2},u_{i-2},u_{i+2})\}$, $where\ 1\le i\le 3$.

Now $v_1 \in D$ then $od_D(v_1) = |N(v_1) \cap V - D|$
$= |\{(u_{i+2}), (u_{i-1}, u_{i+2}, v_{i+1}), (u_{i-2}, u_{i+2}, v_i, v_{i+1})\} \cap$
$\{(v_{i+1}, v_{i+2}, u_i, u_{i+2}), (v_i, v_{i+1}, v_{i+2}, u_{i-1}, u_{i+2})(v_{i-1}, v_i, v_{i+1}, v_{i+2}, u_{i-2}, u_{i+2})\}|$

$= |\{(u_{i+2}), (u_{i-1}, u_{i+2}, v_{i+1}), (u_{i-2}, u_{i+2}, v_i, v_{i+1})\}|$

$= n-2$ or $n-1$

Similarly, $od_D(u_i) = od_D(u_{i+1}) = 2$

Then $|od_D(v_1) - od_D(u_{i+1})| \leq 2$, for any $v_1 u_i \in D$. Therefore D is the minimum two - out degree equitable dominating set. Hence, $\gamma_{2oe}[C(C_n)] = n-1 \; for \; n \leq 5$.

Theorem 3.4.

For any Star graph $K_{1,n}$, $\gamma_{2oe}[C(K_{1,n})] = 2$.

Proof

Let $V(K_{1,n}) = \{v, v_1, v_2, \dots v_n\}$ *where* $\deg(v) = n$. By the definition of central graph of $K_{1,n}$ we denote the vertices of subdivision by $v_1', v_2', \dots v_n'$. That is vv_i is subdivided by $u_i (1 \leq i \leq n)$.

Let $D = \{v, v_1\}$ be the dominating set of $C(K_{1,n})$ and $V - D = \{(v_i', v_{i+1}, \dots v_n)\}, 1 \leq i \leq n$

Now $v \in D$ then $od_D(v) = |N(v) \cap V - D|$

$= |\{v_1', v_2', \dots v_n'\} \cap \{(v_i', v_{i+1}, \dots v_n)\}\}$

$= |\{v_1', v_2', \dots v_n'\}| \quad = n$

Similarly, $od_D(v_1) = n$

Then $|od_D(v) - od_D(v_1)| \leq 2$, for any $v, v_1 \in D$. Therefore D is the minimum two - out degree equitable dominating set. Hence, $\gamma_{2oe}[C(K_{1,n})] = 2$.

4. Two-out degree Equitable domination in the Line graphs of P_n , C_n, and $K_{1,n}$.

In this section, we obtained the two-out degree equitable domination number $\gamma_{2oe}(G)$ of the line graphs of the path P_n, cycle C_n and the star graph $K_{1,n}$.

Theorem 4.1.

For any Path P_n, $\gamma_{2oe}[L(P_n)] = n-3$.

Proof

Let P_n has n vertices n-1 edges. Let $V = \{v_1, v_2, \dots . v_{n-1}\}$ be the vertices of $L(P_n)$ and let $E = \{u_1, u_2, \dots . u_{n-2}\}$ be the edges of $L(P_n)$.

Since the degree of any vertex in $L(P_n)$ is 2 except the initial and terminal vertices.

Let us consider $D = \{v_1, v_2, \dots . v_{n-3}\}$ be a dominating set of $L(P_n)$ *and* $V - D = \{v_{n-2}, v_{n-1}\}$`

Now $v_i \in D$ then $od_D(v_i) = |N(v_i) \cap V - D|$

$od_D(v_{i+1}) = |N(v_{i+1}) \cap V - D| \quad = 0$

Similarly, $od_D(v_{n-3}) = 1$. Hence for every $v_i, v_j \in D$ then $|od_D(u) - od_D(v)| \leq 2$. So D is the minimum two-out degree equitable dominating set. Then $\gamma_{2oe}[L(P_n)] = n-3$.

Theorem 4.2.

For any Cycle C_n, $\gamma_{2oe}[L(C_n)] = n-2$.

Proof

Let $L(C_n)$ have n vertices and n edges in which each vertex is of degree 2. That is each vertex dominates two vertices. Let $V = \{u_1, u_2, \dots . u_n\}$ be the vertices of $L(C_n)$.

Let us consider $D = \{u_1, u_2 \dots . u_{i-1}, u_{i+1}, \dots u_n\}$ be the dominating set of $L(C_n)$ and V–D=$\{u_i, u_{i+1}\}$.

Now $od_D(u_j) = 0, j = 1,2 \dots i-2, i+3, \dots n$

$od_D(u_i) = 1$ *and* $od_D(u_{i+1}) = 1$

Then $|od_D(u_i) - od_D(u_j)| \leq 2$

Then D is the minimum two out degree equitable dominating set

So $\gamma_{2oe}[L(C_n)] = n-2$.

Note: The line graph of C_n, $L(C_n)$ *is* C_n itself.

Theorem 4.3.

For any Star graph $K_{1,n}$, $\gamma_{2oe}[L(K_{1,n})] = 1 \; for \; n \leq 3$.

Proof

Let $V = \{v, u_1, u_2, \dots u_n\}$ be the vertices of $K_{1,n}$. Let $V = \{v, u_1, u_2, \dots u_{n-1}\}$ be the vertices of $L(K_{1,n})$. Let us consider $D = \{v\}$ be a dominating set of $L(K_{1,n})$ *and* $V - D = \{u_1, u_2\}$.

Now $v \in D$ then $od_D(v) = |N(v) \cap V - D|$

$= |(u_1 u_2) \cap (u_1 u_2)| \quad = 2$

Then $|od_D(u) - od_D(v)| \leq 2$, for any $u, v \in D$. Therefore D is the minimum two - out degree equitable dominating set. Hence, $\gamma_{2oe}[L(K_{1,n})] = 1 \; for \; n \leq 3$.

5. CONCLUSION

In this paper, we introduced two-out degree equitable domination number in the middle, central and line graph of $P_n, C_n,$ *and* $K_{1,n}$ graphs. We extend this study on some more special classes of graphs.

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RESEARCH ARTICLE

PREPARATION OF POLY (METHYL METHACRYLATE) THIN FILMS BY SPIN COATING TECHNIQUE FOR OTFT AND WOUND HEALING APPLICATIONS

Chandar Shekar, B.[1*], R. Ranjit Kumar[2], K.P.B. Dinesh[3], C. Sulana Sundari[4] and K. Punithavathi[5]
[1]Department of Physics, Kongunadu Arts and Science College, Coimbatore. Tamil Nadu. India.
[2]Department of Biotechnology, Nehru Arts and Science College, Coimbatore. Tamil Nadu, India.
[3]Applied Biology, Higher College of Technology, Muscat, Oman.
[4]KSIR'S, Chinavedampatti, Coimbatore, Tamil Nadu, India.
[5]PSG Institute of Medical Sciences and Research, Coimbatore, Tamil Nadu, India.

ABSTRACT

Thin films of poly (methyl methacrylate) (PMMA) were prepared on cleaned glass slides by using spin coating technique. The prepared films were identified by using FTIR spectrum. Surface morphology of the coated films was studied by using SEM and AFM. Both as grown and annealed films showed smooth and amorphous structure. It also revealed the absence of pits, pin holes and dendritic features in the surface. Both as grown and annealed films showed very low RMS roughness value. The morphology analysis revealed that the prepared film could be used as dielectric layer in thin film transistors and as drug delivery system for wound healing.

Keywords: PMMA, Morphology, Roughness, Spin coating.

1. INTRODUCTION

Poly(methyl methacrylate) (PMMA) is one of the promising polymers and there are numerous proposals for its application as dielectric in organic thin film transistors (OTFTs)[1-2], as optical lenses in camera and optical fiber [3-6]. Due to its excellent bio- and hemocompatibility and ease of manipulation, it is used in many medical devices, including blood pumps and dialyzers. Its optical properties make it a candidate material for implantable ocular lenses and hard contact lenses. It is a non-metallic implant material in orthopaedics and it is also used in denture fabrication, *in situ* drug delivery system for antibiotics in cavities produced by osteomyelytis. It has also been proposed as a drug delivery system for anti-blastic drugs in the *in situ* therapy of tumours affecting the bone and a new application of PMMA comes from spinal surgery. With the technique of vertebroplasty, a crushed vertebral body can be restored to its original volume and its inner space can be filled with PMMA cement to assure mechanical strength. Though lot of work has been reported on the preparation and characterization of PMMA thin films, to the best of our knowledge, there is no report on the nanoscale thick PMMA films prepared by spin coating method. In the present work an attempt has been made to prepare nanoscale PMMA thin films by spin coating technique and to study their surface morphology using SEM and AFM in order to find out the feasibility of using these thin films as dielectric in OTFTs and in wound healing applications.

2. EXPERIMENTAL

Conventional PMMA obtained from Sigma-Aldrich was used without further purification to prepare PMMA thin films. Anisole was used as a solvent to dissolve PMMA. The solution was spun on cleaned glass slides at room temperature to prepare PMMA thin films. After spin coating process, the samples were dried in the vacuum chamber to evaporate the solvent remained in the film. The films were annealed in Ar ambient. The PMMA films coated were identified by using a FTIR spectrometer (NICOLET 6700 FT-IR). The surface morphologies of the as grown and annealed PMMA films were investigated by means of SEM (FEI company, XL-305) and TM-AFM (Digital Instrument, Nanoscope IIIa).

3. RESULTS AND DISCUSSION

The FTIR spectrum of as grown PMMA thin film of 300 nm is shown in the Fig. 1

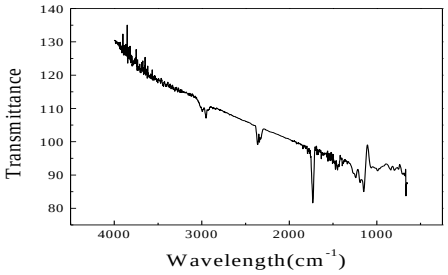
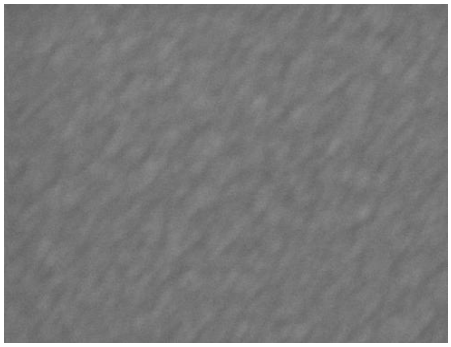


Fig.1 FTIR spectrum of PMMA thin film.

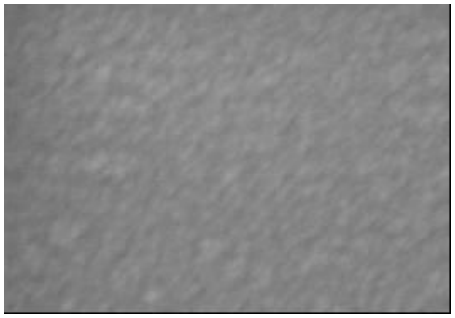
*Correspondence: Dr. B. Chandar Shekar, Assistant Professor, Department of Physics, Nanotechnology Research Lab, Kongunadu Arts and Science College, Coimabatore-641029, Tamil Nadu, India. E.mail: chandar.bellan@gmail.com

The peak observed at 1150 cm^{-1} is assigned to C-O stretching (ester) where as the peaks observed at 1450 cm^{-1} and at 1740 cm^{-1} are assigned to O–CH_3 bending and C=O stretching respectively. Films subjected to various annealing temperatures ranging from 50^0C to 200^0C showed no changes in the FTIR spectrum (Figs not included).

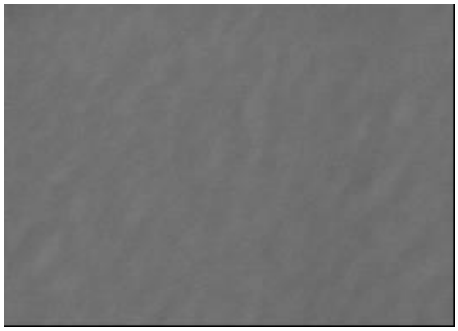
Surface morphology of dielectric layer is very important because it affects the property of the semiconductor layer coated over it. Fig. 4a- d shows the SEM image of the PMMA films annealed at different temperatures. The film surface of as grown and films annealed at 100^0C, 150^0C and 200^0C is compact. No pits, pin holes and dendritic features are found in the surface. Macroscopic granular chains appear at the surface in the stretching direction of PMMA film. The granular structures vary in size from few nanometers to hundreds of nanometers.



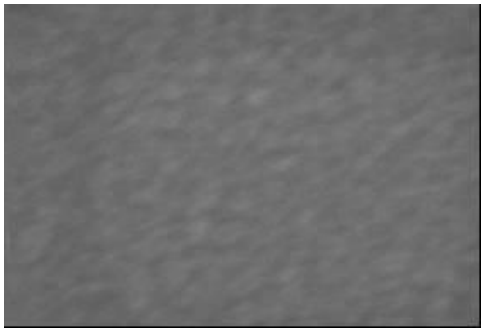
a) As deposited (x250000)



b) Annealed at 100°C



c) Annealed at 150°C



d) Annealed at 200°C

Fig. 4. SEM image of a) as grown, b) 100°C annealed, c) 150°C annealed and d) 200°C annealed samples.

The surface morphology of both as grown and annealed films is quite homogeneous and amorphous in nature.

Fig. 5 shows the atomic force micrographs of as grown and annealed PMMA films. Both as grown and annealed films exhibited random morphologies with smooth surface having micro-domains of less than 100 nm. The RMS roughness was found to be very low for as grown and annealed films.

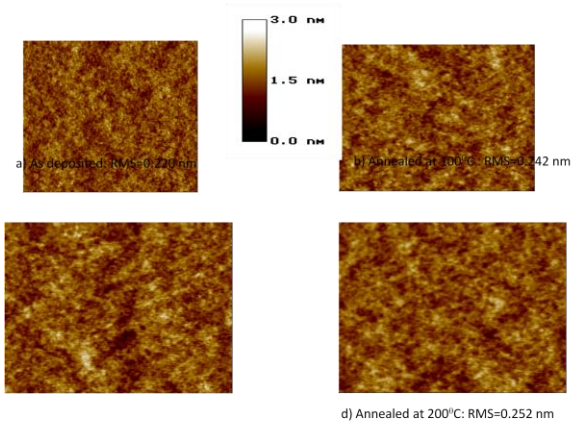


Fig. 5. AFM image of a) as grown, b) Annealed at 100°C, c) Annealed at 150°C and d) Annealed at 200°C.

The roughness increased a bit with annealing cycle. No pit, pin holes and dendritic feature are observed in the AFM topographical image of the samples studied. The only topographic features observed is the hillock of about 10 – 100 nm large and a peak to valley distance of about 0.5 – 1 nm.

Annealing is a process related with stress relief and local structural rearrangement of polymer chains. It is observed from the AFM and SEM analysis that films annealed above 100°C showed very smooth surface, which is one of the most

important requirement of a dielectric layer in thin film transistors. As the annealing temperature increases, intrinsic changes in the microstructure of PMMA as well as in interface are expected. The evidence for the formation of rougher surface with annealing cycles is observed in the AFM spectrum (Fig.5). It is observed that the RMS roughness value increased with the increase of annealing temperature. As grown film showed a RMS roughness of 0.220 nm where as the film subjected to 200°C annealing showed a RMS roughness of 0.252 nm.

4. CONCLUSIONS

Nano scale thick PMMA thin films were prepared by simple spin coating technique. Surface morphology of the PMMA thin films have been studied by AFM and SEM. No pits and pin holes were found in the surface. Both as grown and annealed films showed smooth surface. The RMS roughness was found to be very low for as grown (0.220nm) and it increases a little with increase of annealing temperature. The observed thermal stability, amorphous and smooth surface and lower roughness values implies that thin films of PMMA formed by spin coating could be used as an efficient dielectric layer in organic thin film transistors (OTFTs) and as drug delivery system for wound healing.

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RESEARCH ARTICLE

SYNTHESIS, CHARACTERISATION, THERMAL ANALYSIS AND DNA CLEAVAGE ACTIVITY OF COPPER PYRAZOLE SCHIFF BASE

Jayanthi, E.* and M. Anusuya
Department of Chemistry, Kongunadu Arts and Science College, Coimbatore - 641 029, Tamil Nadu, India.

ABSTRACT

Thiophene-2-carboxylic acid hydrazide and 1, 3-diphenyl-1*H*-pyrazole-4-carboxaldehyde reacted together in 1:1 mole ratio to form Schiff base ligand(**L**) which was subsequently, reacted with $CuCl_2.2H_2O$formed complex of the composition ($Cu(L)Cl_2$). The Schiff base ligand and its Cu(II) complex were characterized on the basis of elemental analysis,thermogravimetry, UV-Visible spectroscopy, FT-IR spectroscopy and NMR spectroscopy. IR spectrum of the copper complex shows that the ligand coordinated through imine nitrogen and amide oxygen atom forming a neutral bidentate chelate with the metal centre.The thermal behaviour of thecomplex shows a single step decomposition pattern leaving CuO residues.DNA cleavage activity of the complexshowed the potential of the complex to cleave supercoiled DNA.

Keywords: Characterisation, Thermal analysis , DNA Cleavage.

1. INTRODUCTION

Schiff bases, theimportant class of ligands can readily coordinate with different metal ions forming stable chelate complexes. They are of synthetic importance due to the various modes of coordination under different conditions and exhibiteddifferent chemical, physical, biological and catalytic properties (1-3). Schiff bases of pyrazole heterocycles found their place in different fields of chemistry because of their wide biological activity like antimicrobial (4), anti-inflammatory (5), antitubercular (6), antitumor (7), antiangiogensis (8), antiparasitic (9), antiviral (10) and also possessesanalgesic and anxiolytic activity (11). Many transition metal pyrazole Schiff base complexes have been synthesized and tested for their biological activity (12). Copper (II) complexes show distorted octahedral and tetrahedral symmetries due to d^9configuration (Jahn Teller effect). The distortion is usually seen as axial elongation consistent with the lability and geometric flexibility of the complex.The fundamental role of copper and the recognition of its complexes asbioactive compounds created interest in their synthesis and their potential application inpharmaceutical industry. Copper complexes are of particular interest with regard to DNA cleavage through oxidative pathways (13).

Some pyrazole Schiff base complexes showed better cytotoxic effect against the fast growing head and neck squamous carcinoma cells SQ20B and SCC-25 and were found to have higher clonogenic cytotoxic effect than cisplatin when tested on SQ20B cell line (14). Copper complexes have a significant place due to its presence in various enzymes and proteins. Copper pyrazole complexes were found to be one of the most effective apoptosis inducers and inhibited angiogenesis on Matrigel and HUVEC migration *in vitro* (15). Thus, in the present work, synthesis of copper complex of pyrazole heterocycle and its characterisation by elemental analysis, FT-IR, UV visible and NMR techniques.

2. MATERIALS AND METHODS

Reagent grade chemicals were procured commercially and used without subsequent purification. 1,3-diphenyl-1*H*-pyrazole-4-carboxaldehyde and thiophene carboxylic acid hydrazide were purchased from Sigma Aldrich. $CuCl_2.\ 2H_2O$ purchased from Lobachemie Pvt. Ltd. and Rankem. The commercial solvents were used.

2.1. Physical measurements

Melting points of the sample were determined using Raaga apparatus**.** FT-IR spectra of solid sample of ligand and the complex were recorded using KBr pellets on a Nicolet Avatar instrument in the frequency range 400-4000 cm^{-1}. Microanalyses (C, H & N) were performed on a Vario EL III CHNS analyser. Electronic absorption spectra of the samples were recorded using a Jasco V-630 spectrophotometer. ^1H NMR spectrum of the ligand was recorded on a Bruker Avance-3 spectrometer at 400 MHz.

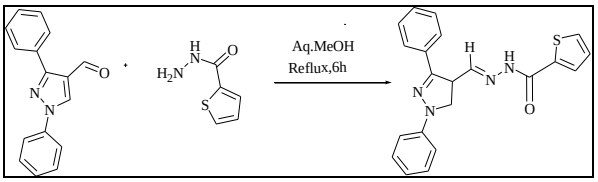
2.2. Synthesis of ligand (L)

The Schiff base ligand was prepared by reacting a mixture of thiophene carboxylic acid hydrazide (0.273g, 1 mM) and 1,3-diphenyl-1*H*-pyrazole-4-carboxaldehyde (0.173g , 1mM) in 50 mL of aqueous methanol. A few drops of glacial acetic acid were added to the reaction mixture. The

*Correspondence: Dr. E. Jayanthi, Assistant Professor, Department of Chemistry, Kongunadu Arts and Science College, Coimbatore - 641 029, Tamil Nadu, India. E.mail: jayakumar.jayanthi@gmail.com

resulting solution was refluxed for 6 h, cooled and the precipitate obtained was checked for purity. The analysis of the product by TLC revealed the formation of the ligand. Synthetic scheme for the preparation of the Schiff base is given in below.

Molecular formula: $C_{21}H_{16}N_4OS$, Yield: 85%, Colour:Pale yellow, Melting Point: 210°C, Elemental analysis $C_{21}H_{16}N_4OS$, Found (Calc.) in %: C: 67.43(67.72), H:4.21(4.33), N: 14.95 (15.04)

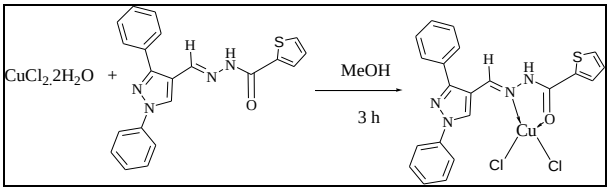


Scheme 1. Synthetic scheme for the preparation of ligand

2.3. Synthesis of complex

Methanolic solution of ($CuCl_2.2H_2O$) (0.162g; 0.5 mM) was refluxed with equimolar quantity of the ligand L (0.1627g; 0.5 mM) in 20 mL of methanol for 3 h (Scheme 2). After two hours, the reddish brown colour complex is precipitated which was filtered, washed several times with petroleum ether and water and then dried *in vacuum*. The purity of the complex was checked by TLC that showed single spot.

Molecular formula: $C_{21}H_{16}Cl_2CuN_4OS$, Yield: 44 %, Colour: reddish brown, Melting point: 230°C, Elemental analysis $C_{21}H_{16}Cl_2CuN_4OS$, Found (Calc.)in %: C: 49.68(49.76), H: 3.11(3.18), N: 10.67(11.05)



Scheme 2. Synthetic scheme of preparation of copper complex

2.4. Determination of oxidative plasmid DNA strand breakage

The potential of newly synthesized complex to cause oxidative plasmid DNA breakage was assessed by the plasmid DNA breakage assay followed by our previous protocol (16). The 10, 20and 30 µMconcentration of the test compounds were added to 500 ng of pBR322 supercoiled plasmid DNA along with the blank and incubated for 6h at ambient temperature under dark. Then, the sample is mixed with 6X orange loading dye (Fermentas, Mumbai) and loaded into 1% agarose gel containing Ethidium bromide. After 30 minutes of gel run, the extent of damage caused by the test compounds were visualized under UV light and documented using G-BOX (GE-health care, USA).

3. RESULTS AND DISCUSSION

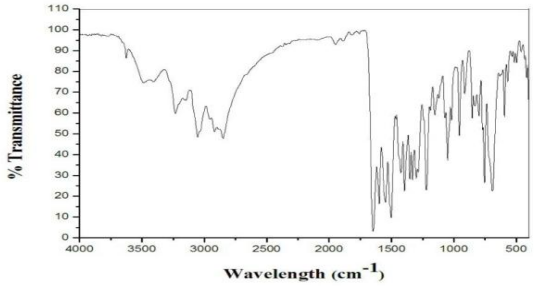
We synthesized the ligand by reacting equimolar quantities of thiophene carboxylic acid hydrazide and 1,3-diphenyl-1*H*-pyrazole-4-carboxaldehyde in methanol medium to yield pale yellow colour ligand thiophene 2-carboxylic acid (1,3 diphenyl 4,5- dihydro-1H- pyrazol-4-yl methylene)-hydrazide (L). The reactions of L with ($CuCl_2.2H_2O$) in methanol medium yielded complex of composition ($Cu(L)Cl_2$)(Scheme **2**). Analytical data of the Schiff base ligand and its copper complex are in well agreement with the proposed molecular formulae. The yellowish brown complex is non-hygroscopic solid and stable in air. It is sparingly soluble in common organic solvents, but soluble in DMF and DMSO. The ligand and the complex are characterized using IR, UV-visible, NMR spectroscopic techniques and elemental analysis. Thermal analysis of the complex was done to ascertain its formation as proposed.

3.1. FT-IR spectral data of the ligand and complex

FT-IR spectrum of the ligand showed a sharp band in the region 3232 cm^{-1} due to the presence of $\nu_{(N-H)}$ stretching vibrations. A very strong band found around 1647 and 1598 cm^{-1} was assigned as due to amide carbonyl symmetric and asymmetric stretching vibration. The other bands at 1547 and 1073 cm^{-1} were assigned to the $\nu_{(C=N)}$ and $\nu_{(N-N)}$ stretching frequencies of the ligand.

The bands due to the $\nu_{(N-H)}$ and $\nu_{(C=O)}$ vibrations remained intact in the IR spectrum of thecomplex but were present at lower frequencies, implied the coordination of amide carbonyl oxygen and imine nitrogen with the copper centre (17). Thus the ligand coordinated to the metal as neutral bidentate fashion. The strong band appeared in the IR spectrum of the copper complex at 1582 cm^{-1} is assigned to $\nu_{(C=N)}$ stretching frequency of the pyrazole ring.

The analytical data and IR characteristics are in good agreement with the proposed structure of copper complex.



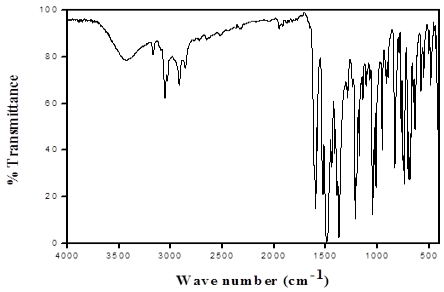


Fig. 1. IR spectrum of ligand and complex

3.2. Electronic spectrum

The electronic spectra of the ligand and complex were recorded in DMSO solution. The ligand spectrum exhibited one broad band in the range 240-360 nm were assigned to the n→π* and π→π* intra ligand transitions. The spectrum of complex exhibited two bands in the range 240-380 nm region. The higher energy bands below 300 nm are attributable to n→π* and π→π* intra ligand transitions (17). Other broad band that was observed in the 300-370 nm regions can be assigned to a ligand to metal charge transfer (LMCT) transitions of the imine group (14).

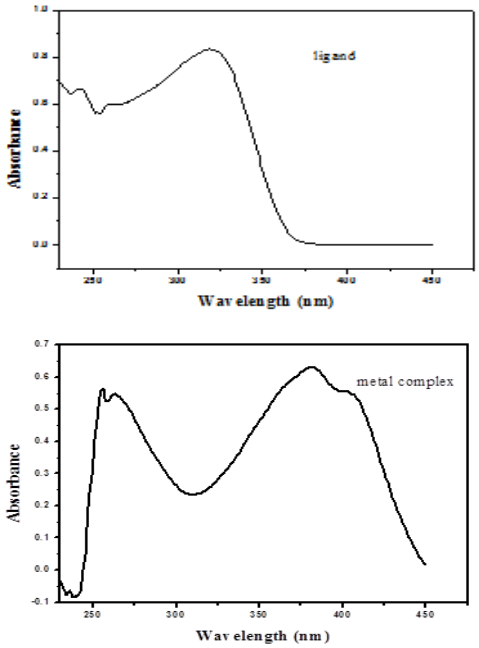


Fig. 2. Electronic spectrum of ligand and complex

3.3. Proton NMR spectrum of the ligand

^{1}H-NMR spectrum of the free hydrazone ligand recorded using $CDCl_3$ as solvent was assigned on the basis of observed chemical shift. The spectrum displayed a singlet due to an NH proton δ 9.0 ppm. The ligand showed a sharp singlet for azomethine (HC=N) at δ 8.87 ppm. Signals corresponding to the protons of benzene proton and thiophene proton of the ligand were observed as multiplets in the range δ 7.22-8.58 ppm. NMR spectrum of the ligand ascertained its formation as expected.

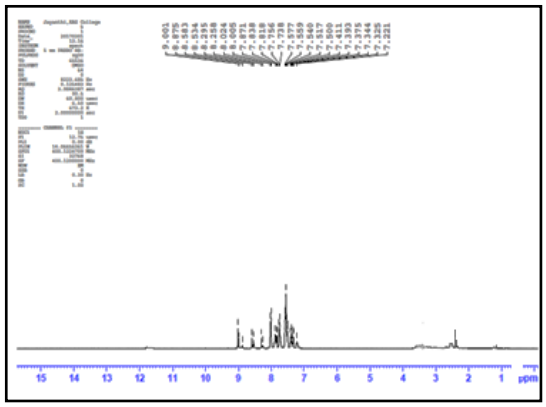


Fig. 3. ^{1}H NMR spectrum of the ligand

3.4. Thermal analysis of the complex

Thermo-gravimetric analysis of the copper complex showed a single step decomposition pattern. It decomposed at 401°C with the formation of CuO. The percentage weight loss for the decomposition of the ligand was found to be 86.28 %. 13.72% that matches with the formation of CuO. The calculated value for the same is 13.99%. Thus it confirmed the compositon of the complex.

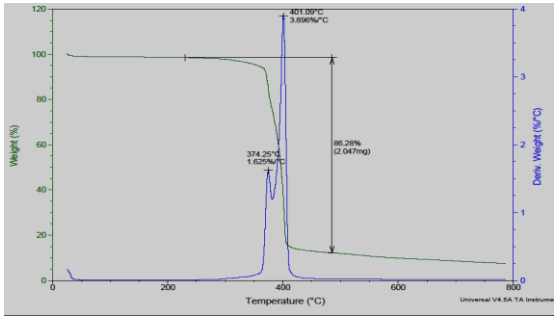


Fig. 4.TG-DSC spectrum of complex

Based on the above spectral and micro analysis data, a four coordinate square planar geometry has been proposed for the complex with 1:1 metal to ligand stoichiometry. The proposed structure of the complex is given below.

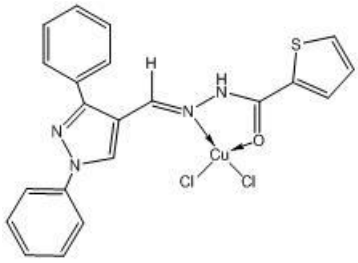


Fig. 5. Proposed structure of copper complex

3.5. DNA cleavage study

Generally DNA damage is indicated by the conversion of supercoiled form of plasmid DNA to circular form. To check the role of synthesized complex on DNA breakage, plasmid DNA damage assay was performedand the efficiency of the cleavage was monitored by agarose gel electrophoresis. The DNA cleavage efficiency of the complex was due to the difference in the binding affinity of the complex to DNA. Results of the experiment revealed that complex significantly damaged the plasmid DNA upon treatment for 30 min (Fig. 6). The efficiency of studied complex to cleave super-coiled DNA to linear form is the characteristic of anticancer drugs those could effectively bind to the nuclear DNA and impart damage to it and thus arrest the proliferation of cancerous cells.

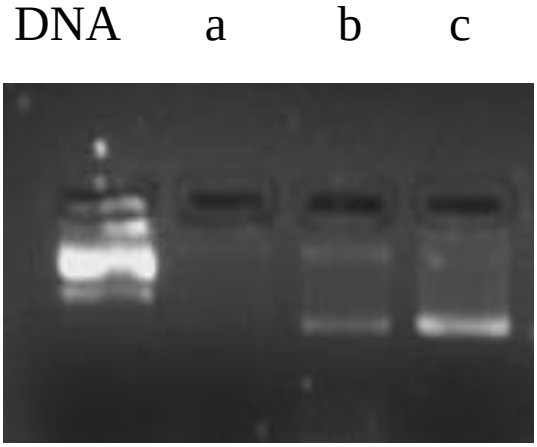


Fig. 6. pBR322 plasmid DNA cleavage using different concentration of complex (a-10 µM, b-20 µM and c-30 µM)

4. CONCLUSION

Interesting coordination modes of hydrazone and their biological perspective provoked us to synthesize new copper hydrazone complex by using the ligand prepared from 1,3 diphenyl pyrazole-1H 4 carbaldehyde andthiophene carboxylic hydrazide (L). The ligand was characterised by FT-IR, UV-visible and NMR spectral method. The elemental analysis data of the ligand and the complex are in good agreement with the proposed molecular formulae. The presence of NH stretching vibration and reduction in the C=N and C=O stretching frequencies suggest the neutral bidentate coordination of the ligand in copper complex. The DNA cleavage studies showed that the complex has the potential to cleave DNA. Thus cytotoxic potential of the complex and mechanism of inducing apoptosis by oxidative pathway can further be analysed.

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RESEARCH ARTICLE

INDOLE DERIVATIVES: DESIGN, SYNTHESIS, *IN-VITRO* BIOLOGICAL EVALUATION AND MOLECULAR DOCKING STUDY AS ANTICANCER AGENTS

Amuthavalli, A., B. Prakash and R. Velmurugan*
Department of Chemistry, Kongunadu Arts and Science College, Coimbatore-641029, Tamil Nadu, India.

ABSTRACT

New hetero annulated indoles were synthesized and structurally characterized by spectral means. In order to understand the nature of interactions of these molecules, we carried out molecular docking studies using the protein kinase CK2 inhibitors. The docking results provided some useful information for the future design of more potent inhibitors. The *in vitro* cytotoxicity was evaluated for all the new compounds by MTT assay against HeLa and compared with the standard drug ellipticine. All the compounds showed moderate to potent activity against the cell lines. The preliminary structure–activity relationships were carried out.

Keywords: Cyclopenta[*b*]indoles, Molecular docking, Anticancer.

1. INTRODUCTION

Cancer continues to be one of the major health problems worldwide and one of the leading causes of death despite the advances that have led to the development of new therapies. So, there is a continuing need for designing and developing new chemotherapeutic agents for cancer treatment.

The indole ring is the most ubiquitous heterocyclic substructure in nature. Owing to its great diversity in both structure and biological activity, it is not surprising that the indole ring is an important structural component in many pharmaceuticals (1-5). Particularly fused-polycyclic indole framework is potential candidates for drug discovery because this structural motif is present in a wide variety of biologically active alkaloids (6). It is known for its variety of pharmacological characteristics as e.g. anti-fungal, anti-bacterial, anti-tumor, anti-HIV and DNA interaction properties (7-12).

The development of an efficient synthetic method of cyclopenta[*b*]indole derivatives has attracted broad attention in medicinal chemistry and synthetic organic chemistry. Extensive efforts are therefore focused on this topic (13-15).

Prompted by the findings from the literature studies, we set out to explore and synthesise new pyrazolo- and isooxazolo- cyclopenta[*b*]indole derivatives. Furthermore, the compounds were evaluated for their active site with Human Kinase CK2 Protein by molecular docking study and cytotoxicity against HeLa human cervical cancer cell line also was carried out.

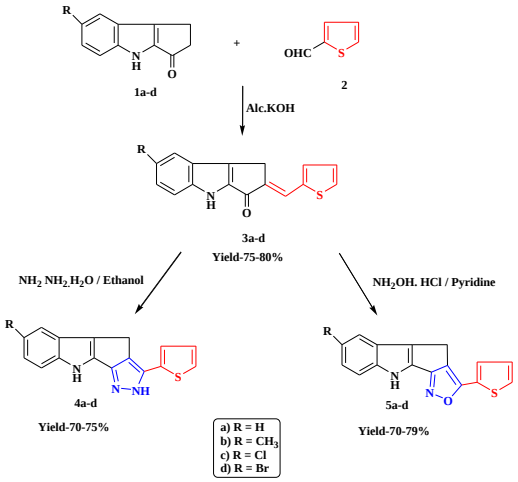
2. RESULTS AND DISCUSSION

2.1. Chemistry

In the attempt of synthesising bioactive isoxazolo- and pyrazoloindole derivatives, first step is the synthesis of thiophen-2-ylmethylene by mixed aldol condensation of 1,4-dihydro-2H-cyclopenta[*b*]indol-3-one 1a-d with thiophene-2-carbaldehyde 2. Further the thiophen-2-ylmethylene derivatives 3a-d was treated with hydroxylamine hydrochloride and hydrazine hydrate to give the corresponding cyclised hetero annulated isoxazolo 4a-d and pyrazoloindole 5a-d derivatives respectively. The synthetic routes were shown in the Scheme 1.

The formation of compound 3a confirmed by its IR spectrum which showed sharp and strong bands at 3153 and 1671 cm^{-1} assigned to NH and C=O group respectively. ^1H NMR spectrum lacked C_2-methylene proton signals and the displayed signals for C_1-methylene and thiophene-CH protons, suggested the structure of 3a to be a thiophen-2-ylmethylene compound. The proton NMR spectrum of 4a exhibited two broad singlets at 11.94 ppm and 9.01 ppm attributed to indole-NH and pyrazole-NH protons respectively. The aromatic protons appeared as multiplet in the region 6.76-7.87 ppm. IR spectrum of 5a registered absorption band at 1626 cm^{-1} assigned to cyano functional group. The proton NMR spectrum of 5a showed one broad singlet at 11.96 ppm attributed to indole-NH. The aromatic protons appeared as a multiplet between the regions δ 7.20-7.96 ppm. Analytical data are in accordance with the proposed structure for compound 5a. The identities of the other compounds were established in the same way with all spectroscopic data readily assignable.

*Correspondence: Dr. R. Velmurugan, Assistant Professor, Department of Chemistry, Kongunadu Arts and Science College, Coimbatore-641029, Tamil Nadu, India. E.mail: velmuruganchemist57@gmail.com



Scheme 1. Synthesis of pyrazolo and isoxazolo cyclopenta[*b*]indoles (4a-4d) and (5a-5d) derivatives.

3. BIOLOGICAL EVALUATION

3.1. Molecular docking studies

To understand the interaction of Human Kinase CK2 protein receptor with (3a–3d, 4a–4d and 5a-5d), the crystal structure of CK2 protein was downloaded from protein data bank and studied with the glide program. All the glide and E model scores and the details of docked compounds are presented in Table 1. The use of glide and E model scores for ranking the different derivatives within a series is always not dependable. The glide scores are mainly used to identify the active and inactive compounds. In addition, glide is primarily concerned with generating an accurate pose for each compound and enrichment (the separation of actives from inactive) Fig.1.

Table 1. Molecular docking studies of 14 analogues taken for study with Human Kinase CK2 protein (PDB: 3OWJ)

Compound	Glide score (kcal/mol)	E model score	Glide energy	Hydrophobic interaction
3a	-6.999	-36.014	-23.094	ALA 148, TYR 32, PHE 169, PHE 97, ILE 79, MET 167, LEU 70, ALA 165, VAL 35, ALA 50, LEV 151.
3b	-6.243	-48.931	-36.337	ALA 148, TRP 105, VAL 35, TYR 32, MET 167, LEU 70, PHE 97, ALA 165, PHE 169, ILE 79, ALA 50, LEU 151.
3c	-6.400	-46.739	-36.360	ALA 148, VAL 27, TYR 32, MET 167, PHE 169, LEU 70, ALA 165, PHE 97, ILE 79, ALA 50, VAL 35, LEU 151.
3d	-5.954	-45.286	-35.422	ALA 148, IEU 151, VAL 35, ALA 50, ILE 79, PHE 97, LEU 70, ALA 165, PHE 169, MET 167, TYR 32
4a	-6.859	-39.633	-36.460	VAL 27, VAL 35, ALA 100, LEU151, ALA 50, ILE 79, LEU 70, PHE 97, PHE 169, MET 167, ALA 165, TYR 32
4b	-6.491	-50.447	-36.007	VAL 27, TYR 99, VAL 35, ALA 50, LEU 151, PHE 97, ILE 79, LEU 70, ALA 165, PHE 169, MET 167, TYR 32, LEU 328.
4c	-7.097	-54.270	-35.105	VAL 27, VAL 35, ALA 50, LEU 151, PHE 97, ALA 165, ILE 79, LEU 70, MET 167, PHE 169, TYR 32
4d	-6.313	-56.113	-39.885	VAL 27, TYR 99, VAL 35, LEU 151, ALA 50, PHE 97, ILE 79, LEU 70, ALA 165, PHE 169, MET 167, TYR 32.
5a	-7.650	-47.866	-32.416	VAL 27, TYR 99, VAL 35, ALA 50, LEU 151, PHE 97, ILE 79, LEU 70, ALA 165, PHE 169, MET 167, TYR 32, LEU 328.
5b	-6.826	-52.164	-36.540	VAL 27, TYR 99, VAL 35, ALA 50, LEU 151, PHE 97, ILE 79, LEU 70, ALA 165, PHE 169, MET 167, TYR 32, LEU 328.
5c	-7.198	-50.162	-36.784	VAL 27, VAL 35, LEU 151, ALA 50, ILE 79, PHE 97, MET 167, LEU 70, PHE 169, ALA 165, TYR 32.
5d	-7.590	-53.858	-33.325	VAL 27, TYR 99, VAL 35, ALA 50, LEU 151, PHE 97, ILE 79, LEU 70, ALA 165, PHE 169, MET 167, TYR 32, LEU 328.

In the binding mode, compounds were attractively bound to CK2 via hydrophobic interaction, and Pi-Pi stacked interaction. The scoring functions of the docking program ranked that the binding interactions of the intermediate were less than those of the cyclised products. The compounds 5a-5d displayed better binding interaction compared to the intermediate; this might be due to the presence of the isoxazole moiety. The next best interactions were found among the compounds 4a-4d which hold the pyrazole moiety. Among the cyclised products compound 5c exhibited the best lowest binding energy and ligand efficiency; this might be due to the presence of the isoxazole moiety which was further reinforced by favourable electrostatic interaction of the chloro group at the 7th position of the indole moiety. In general it was found that the isoxazole moiety favours better binding interactions compared to pyrazole moieties and intermediates.

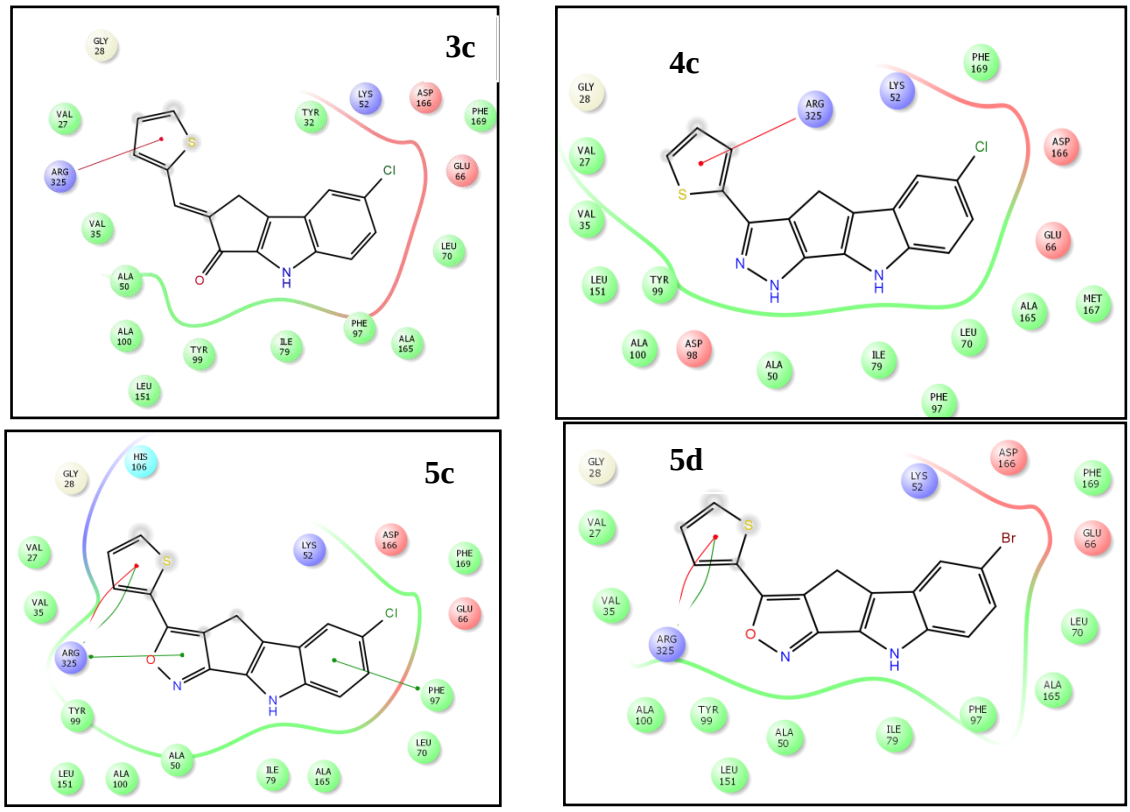


Fig. 1. Docking model structure of compound 3c, 4c, 5c, 5d into the Protein kinase (PDB ID: 3OWJ) binding pocket.

3.2. Cytotoxicity studies

The *in vitro* cytotoxicity assay proves that the compound 5c has potent anticancer effect on human cervical cancer cells. Morphological shift of HeLa cell line by the compound is shown in Fig.2, revealing that the morphological alteration occurs in all the concentrations. From Fig.3, it is evident that compound 5c showed a dose-dependent anticancer activity. The maximum anticancer activity was obtained at 100 µM. The percentage of cell growth inhibition is found to be 100 % for compound 5c. The estimated IC_{50} value is 15.24 µ. Therefore, it is evident that the compound 5c has cell growth inhibition value closer to the standard drug ellipticine (9.62µ). The results obtained from the MTT assays help us to understand that the compound 5c have higher efficiency due to the presence of chloro group in the exact position in benzene moiety to have a good interaction with receptor protein active site. This, again, may be reason for the notable difference in IC_{50} value.

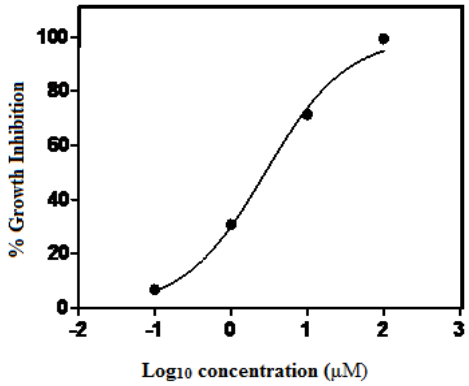
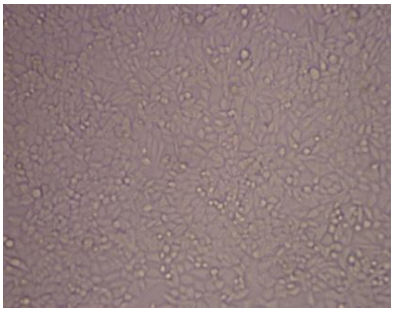
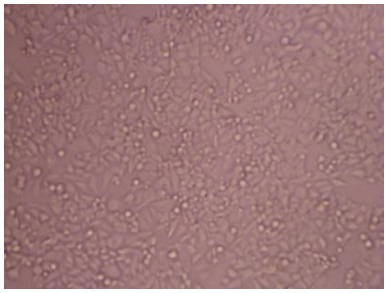


Fig. 2. Effect of % cell growth inhibition in different concentration (lM)



0.1µM



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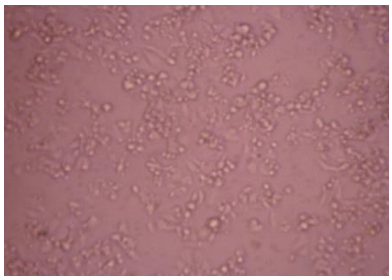
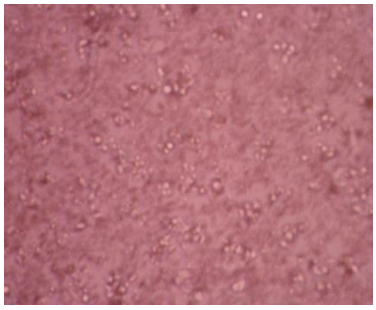
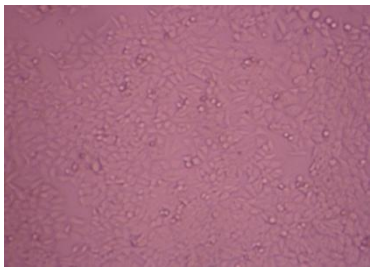


10 µM



100 µM



Control

Fig. 3. Images of cytotoxic activity of compound 5c in HeLa cells

4. STRUCTURE ACTIVITY RELATIONSHIP (SAR) STUDIES

For the structure activity relationship, heterocyclic indole motif derivatives (3a-5d) such as pyrazolo and isoxazolo groups with indole ring were synthesized and evaluated for anticancer activity. It was observed that compounds 5a-5d comprising the isoxazolo framework possessed excellent anticancer activity against the tested cell line, where the anticancer activity was found to be in the order (5c (15.24µM)> 5d (17.67 µM) > 5b (19.33 µM) > 5a (25.63 µM)). Such results suggested that the halogen substituents enhance the antitumor activity when compared to other substituent. Compounds 4a-4d comprising pyrazolo moiety displayed moderate anticancer activity. It was noted that the intermediates showed comparatively less cytotoxic activity than the cyclised derivatives Fig. 4.

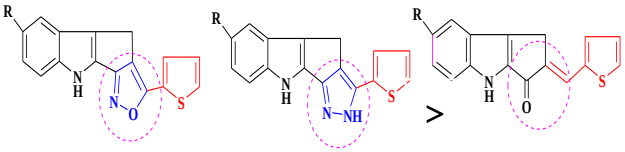


Fig. 4. The increasing order of efficiency of cytotoxic activities.

5. CONCLUSION

In conclusion, a new series of fluorine substituted pyrazolo- and isoxazolo- derivatives were chosen through target based drug discovery and synthesized, and characterized by IR, ^{1}H NMR and ^{13}C NMR spectroscopy. Molecular docking studies establish that these molecules exhibit significant molecular interactions with Protein kinase CK2 target protein. *In vitro* cytotoxicity study of the synthesized molecules against cervical cancer cells (HeLa), revealed that compound 5c exhibited IC_{50} value 15.24 µ. Compound 5c inhibited the growth of HeLa cells, showing a cell growth inhibition of 100%. The structure activity relationship of cytotoxic studies discovered that the chloro substituted isoxazolo-cyclopenta[*b*]indole display high activities than their counterparts.

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RESEARCH ARTICLE

DETERMINATION OF CHLOROPHYLL CONTENT OF SOME MANGROVES AND ASSOCIATED PLANT SPECIES OF PAYANGADI, KANNUR

Gayathri, P.V*. and C.R. Lalitha
Department of Botany, Sree Narayana College, Kannur, Kerala, India.

ABSTRACT

Mangroves are trees inhabiting the intertidal zones of tropical and subtropical coast. They are classified as true mangroves and mangrove associates. In the present study leaf samples of various age groups from five mangrove species and six mangrove associated species were selected for the estimation of chlorophyll content. True mangroves showed comparatively high amount of chlorophyll than mangrove associates and moreover in medium aged leaves chlorophyll contents were more than young and old leaves.

Keywords: Mangrove, mangrove associate, chlorophyll.

1. INTRODUCTION

Mangroves create a unique ecological environment, having a rich assemblage of various species. It has many peculiar features than other terrestrial plants. However, mangrove species are classified into true or exclusive mangroves or strict or obligate mangroves, and Mangrove associate or nonexclusive or semi mangroves (1). A true or exclusive Mangrove occurs only in mangrove environment and not extends into terrestrial habitat. In addition to this morphological specialization such as profuse lateral roots, exposed aerial roots, viviparous, water dispersed propagules and physiological mechanism for salt excretion or salt exclusion are also observed. Non exclusive species or mangrove associates are mainly distributed in terrestrial or aquatic habitat but also occur in the mangrove ecosystem (1,2). Of the 14 districts of Kerala, mangroves are spread over about in ten districts of which majority occur in the northern region. Kannur has highest area of mangroves (755Ha) followed by Kozhikode (293Ha) and Ernakulam (260Ha) (3).

In green plants, photosynthesis takes place in the chlorophyll containing thylakoid membrane of the chloroplast. Chlorophyll a is the primary photosynthetic pigment which captures light with narrow and specific visible range of sunlight. Chlorophyll b and carotenoids act as accessory supportive photosynthetic pigments and they accept light of wide range (4). For mangroves, the concentrations of leaf pigment can be associated with environmental factors such as ambient temperature/sunlight (5), water availability and salinity (6). Chlorophylls are the most important leaf pigment responsible for photosynthesis (7) and relative chlorophyll content has positive relationship with photosynthetic rate (8).

2. MATERIALS AND METHODS

2.1. Plant material

For the present study, young, medium and old leaves were collected randomly from about five mangroves and six mangrove associated species from Payangadi, Kannur, Kerala. Leaves of three developmental stages were selected considering the colour variation, leaf size and their position on the stem from apex to base direction, ie; leaves from the young tip/apex of the stem as juvenile/ young leaves and so on, for comparing the relative chlorophyll content. The selected mangrove plants are *Rhizophora mucronata* Poir, *Avicennia marina* (Forssk.) Vierh., *Avicennia officinalis* L., *Acanthus ilicifolius var. subinteger* Nees, and *Bruguiera cylindrica* (L) Blume. The selected mangrove associated plants are; *Ipomoea macrantha* Roem. & Schult., *Cayratia trifolia* (L.) Domin., *Derris trifoliata* Lour. *Premna serratifolia* L., *Clerodendrum inerme* (L.) Gaertn. and *Cyperus* sp. The leaves were collected in poly bags and were immediately brought into the laboratory for biochemical analysis.

2.2. Quantitative estimation of chlorophyll

100 mg. fresh leaf tissues were homogenized with 80% acetone. The extract was centrifuged for five minutes and the supernatant was collected. The residue was re extracted with 80% acetone and centrifuged. The process was repeated till the pellet become colorless. The final volume of the combined supernatant was noted. The absorbance of the extract was noted at 663nm and 645nm. using UV-Visible spectrophotometer. The total chlorophyll, chlorophyll a, chlorophyll b, chlorophyll a/ chlorophyll b ratio were calculated using the formula suggested by Arnon (9).

Chlorophyll expressed as mg/g fresh tissue.

*Correspondence: Gayathri P.V., Department of Botany, Sree Narayana College, Kannur, Kerala, India.
E.mail: gayu28041986@gmail.com

Chlorophyll a = 12.7(A_{663}) – 2.69(A_{645}) * V/1000*W.

Chlorophyll b = 22.9(A_{645}) - 4.68(A_{663}) * V/1000*W.

Total Chlorophyll = 20.2(A_{645}) + 8.02(A_{663}) * V/1000*W.

A = Absorbance at specific wave length

V = Final volume of chlorophyll extract in 80% acetone.

W = Fresh weight of tissue extracted.

3. RESULTS AND DISCUSSION

The present experiment on quantitative estimation chlorophyll a and b, total chlorophyll, the ratio of chl.a/b for selected mangroves and associated plant species, (Table 1) showed little differences between mangroves and associated plant species. In the study, it is estimated that the old leaves of *Avicennia marina* contained highest amount of chlorophyll a and chlorophyll b (1.37,1.2 mg/g fresh tissue respectively) where as young leaves of *Acanthus ilicifolius* showed the lowest amount (0.23mg/g fresh tissue-chlorophyll a and 0.13 mg/g fresh tissue-chlorophyll b). Considering the mangrove associated plants, young leaves of *Derris trifoliata* showed highest amount of chlorophyll a (1.07 mg/g fresh tissue) and intermediate or medium aged leaves have shown increased amount of chlorophyll b (1.44 mg/g fresh tissue). Medium aged and old aged leaves of *Cayratia trifolia* showed the lowest amount of chlorophyll a (0.39 mg/g fresh tissue) and chlorophyll b (0.18 mg/g fresh tissue). Leaf age and physiological state are important determinant of the chlorophyll content (10).

Table1. Estimation of chlorophyll for different mangroves and associated plant species.

Plant	Plant age	Chl. a	Chl. b	Tot. Chl.	Chl. a/b
	Y	0.57	0.27	0.84	2.11
Ao	**M**	0.82	0.7	1.52	1.17
	O	0.83	0.33	1.16	2.52
	Y	1.12	0.63	1.75	1.78
Am	**M**	1.23	0.71	1.94	1.73
	O	1.37	1.2	2.57	1.14
	Y	0.5	0.26	0.76	1.92
Rm	**M**	0.74	0.37	1.11	2.00
	O	0.87	0.3	1.17	2.90
	Y	0.23	0.13	0.36	1.77
Ai	**M**	0.32	0.18	0.5	1.78
	O	0.25	0.17	0.42	1.47
	Y	0.81	0.29	1.1	2.79
Bc	**M**	0.77	0.9	1.67	0.86
	O	0.88	0.5	1.38	1.76
	Y	0.42	0.61	1.03	0.69
Iv	**M**	0.45	0.72	1.17	0.63
	O	0.51	0.68	1.19	0.75
	Y	1.07	1.24	2.31	0.86
Dt	**M**	0.78	1.44	2.22	0.54
	O	0.88	0.67	1.55	1.31
	Y	0.61	0.3	0.91	2.03
Ps	**M**	0.71	0.33	1.04	2.15
	O	0.8	0.35	1.15	2.29
	Y	0.59	0.32	0.91	1.84
Ct	**M**	0.39	0.41	0.8	0.95
	O	0.44	0.18	0.62	2.44
	Y	0.47	0.18	0.65	2.61
Ci	**M**	0.57	0.25	0.82	2.28
	O	0.57	0.24	0.81	2.38
	Y	0.84	0.31	1.15	2.71
Cy.	**M**	0.81	0.34	1.15	2.38
	O	0.65	0.37	1.02	1.76

Ao- *Avicennia officinalis*,**Am**-*Avicennia marina*,**Rm**- *Rhizophora mucronata*, **Ai**-*Acanthus ilicifolius*, **Bc**-*Breguiera cylindrica*,**Iv**-*Ipomoea violacea*, **Dt**-*Derris trifoliate*,**Ps**-*Premna serratifolia*,**Ct**-*Cayratia trifolia*, **Ci**-*Clerodendrum inerme*, **Cy**. *Cyperus sp.* **Y**-young leaves, **M**-medium aged leaves, **O**- old aged leaves.

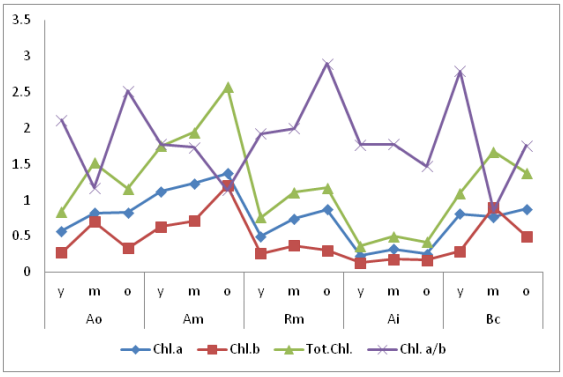

Fig. 1. Showing chlorophyll a, chlorophyll b, total chlorophyll and chlorophyll a/b ratio of mangroves.

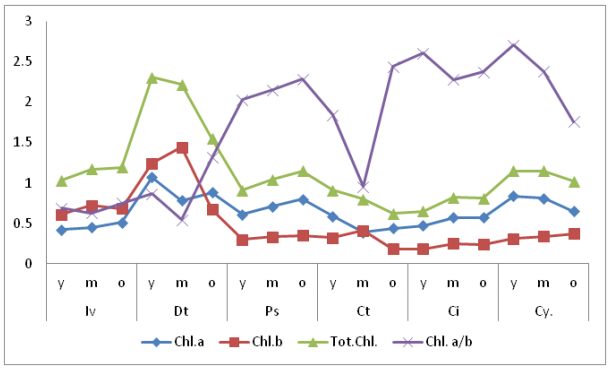

Fig. 2. Showing chlorophyll a, chlorophyll b, total chlorophyll and chlorophyll a/b ratio of mangrove associates.

The chlorophyll content in the leaves depend on the endogenous factors such as pigment synthesis and degradation and stage of leaf development. In addition, environmental factors like shade, light, temperature, drought, water logging, soil salinity etc affect the chlorophyll content. Generally chl.a/b ratio of mangroves and associated plant species was higher in p growing in non saline soil than saline soil (11). The reduction in the chl.a/b ratio which is an adaptation to unfavorable conditions may be due to the fact that chlorophyll b is more resistant for degradation than chlorophyll a (12). The total chlorophyll content in the mangrove plants *Bruguiera gymnorrhiza, Excoecaria agallocha* and *Heritiera fomes* grown in nonsaline condition was higher than that of in saline condition (13). Increased count is an indication of increasing rate of assimilation thus enhancement in the rate energy transfer and production. In the present study an increase in the total chlorophyll content is observed in old leaves of *Avicennia marina (*2.57 mg/g fresh tissue) and young leaves of *Derris trifoliata* (2.31 mg/g fresh tissue).

Seasonal change in the chlorophyll a content is observed (14), where during rainy season an increase in chlorophyll a content in mangroves than that of dry seasons. It is observed that during winter an increase in total chlorophyll and the highest amount of carotenoids in summer reflect an adaptation to enhance the photo protection properties of mangrove leaves (4). An increase in the chlorophyll a and b in the leaves under shaded, receiving diffused light is observed than the leaves under direct sun light (15). Simultaneously analysis of leaves of different age group shows that leaves with intermediate or medium age comparatively have the highest content of chlorophyll a and b. these results also well support present study which also indicates that intermediate leaves are more productive than the remaining aged leaves. In general, true mangroves show comparatively more chlorophyll content than associated plant species.

4. CONCLUSION

The result of the work adds to our understanding of the relationship of leaf age and its physiological state to the chlorophyll content. Low chlorophyll concentration may also indicates plant physiological stress. Since chlorophyll take part in the conversion of solar energy in to chemical energy, their level in the leaf tissue is one of the important features governing photosynthetic efficiency of plants. Thus it can be concluded that in relation to ecophysiological adaptation understanding of the nature of the pigments in mangroves and their associated plants is a parameter for their conservation and propagation under different salinity condition.

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RESEARCH ARTICLE

CLIMATE CHANGE AND ITS IMPACT ON BIO-DIVERSITY

Mary Kensa, V.*
P.G. Department of Botany and Research Centre, S.T. Hindu College, Nagercoil -629 002, Tamil Nadu, India.

ABSTRACT

The climate change is unprecedented scale and speaks of an emerging crisis throughout the world. It is an alarming issue beyond human control. The change is caused by so called development. This article is pen down the climate change and its impacts on various levels of biodiversity.

Keywords: Alarming, biodiversity, climate change, impacts and unprecedented.

1. INTRODUCTION

The climate change is resulted in greenhouse gases. Among the gases CO_2 plays a vital role to change it. This is emitted more from the combustion of fossil fuel. The concentration of CO_2 in the atmosphere increased from 280 ppm in 1760 to 379 ppm in 2000. If the trend continues, the end of the century it will increase to 560 ppm. The CH_4 and N_2O increased from 700 ppb to 1774 ppb and 270 ppb to 319 ppb respectively during the same period. The other green house gases are such as methane, black carbon and nitrous oxide which currently contribute about 25 percent of the total warming. Effects of the concentration of CO_2. Concentration of CO_2 makes the earth hotter that is global warming. This global warming leads to negative impact on physical structure of the earth. The effects are

2. ICE MELTING

As a result of global warming mountain glaciers are melting rapidly. Artic ocean ice sheet has thinned by 42 percent in 2001. Within 50 years, the Antarctic continent will shrink and Europes Alps and Himalaya, the glacial volume shrinks more than half since 1850.

3. SEA-LEVEL RISING

Sea level is rising due to global warming. The main causes include thermal expansion of oceans and melting of the glaciers and ice caps. From 1961 to 2003, the average rate of global mean sea level rise is estimated to be 1.8 ± 0.5 mm/year. The contribution from thermal expansion is estimated to be 0.42 ± 0.12 mm/year. The contribution from glaciers, ice caps and ice sheets is estimated to be 0.7 ± 0.5 mm/year. During 20th century the sea level rose by 10 – 20 cm (4.8 inches). For each millimeter rise in sea level, the seashore will retreat an average of 1.5 meters. The Inter Governmental Panel on Climate Change 2001 assessed that the sea level could rise by as much as one meter during 21st century when the present global warming continues. Thus one meter rise in sea level will retreat 1500 meters or nearly one mile. As a result two thirds of the Marshall Island and Kiribati 3600 square kilometers of the USA would be under sea water.

4. GLOBAL TEMPERATURE

There were not many changes from 1850 to 1915, besides the fluctuations associated with the natural variability. An increase in the warming occurred from 1919 to 1940s followed by a slight cooling due to aerosol reflectance and decline in the sun pot cycle period, followed by a rapid warming up to the end of 2006. Notable increases were reported from 1950 onwards. The global average surface temperature has increased and the 100 year end (1906-2005) indicates an increase of 0.74^0 C ± 0.18^0 C. Eleven of the last 12 years (1995 to 2006), with the exception of 1996, rank amongst the 12 warmest years on record. The high level concentration of CO_2 increases the global temperature. If the concentration of CO_2 reaches 560 ppm, the temperature is projected to increase 1.4 to 5.8 degree Celsius.

5. CHANGES IN THE HYDROLOGIC CYCLE

The increase in the global mean temperature result in increase in the evaporation rates resulting in an increase in humidity levels which have been observed to increase since 1976 over both the land and oceans. The upper troposphere water vapour has been increased in the last two decades. This troposphere water vapour absorbs radiation and amplifies the warming rates.

An interrelationship between the El Nino event and cloud cover exists. The radiation changes from 1980s to 1990s during the E1 Nino phenomenon appear to be associated with the reduction in the tropical upper level cloud cover. These changes bring changes in the energy balance. The number of hurricanes in the North Atlantic

*Correspondence: Mary Kensa, V. P.G. Department of Botany and Research Centre, S.T. Hindu College, Nagercoil -629 002, India.
E.mail: surejkensa@gmail.com

region has increased when compared with their occurrences over the last 25 years.

6. CHANGES IN THE CRYOSPHERE

The main components of the cryosphere are snow, river and lake ice, sea, ice glaciers, ice caps and frozen ground. Nearly 90 percent of the solar radiation gets reflected by the cryosphere, whereas only 10 percent gets reflected from oceans and forest lands. Increase in temperature causes an increase in the melting of snow and ice cover, reducing their rate of reflectively, thereby enhancing the absorption of radiation in the melted areas.

The mountain snowpack in western North America and the Swiss Alps have been declined. One hundred and fifty records on freezing and melting of river and lake ice delayed in the freezing dates and an early melting date. Ice sheets shelves lose mass by calving ice bergs and melting and melting at the base into the ocean. A warming of about 1^0 C in the oceans can increase the melting of the base of an ice shelf at a rate of 10m/year. The ice sheets have shrunk in response to warming and increased in volume in response to cooling. The volumes of the Greenland and Antarctic ice sheets are equivalent to approximately 7m and 57 m of the sea level rise, respectively. Thinning or loss of ice shelves in Greenland, the Antarctic Peninsula and West Antarctica has been associated with an accelerated flow of water in the form of ice streams.

7. HEAT WAVES

Climate change is indicated by the heat waves. As a result of global warming, the number of days per year is increasing continuously. Heat waves create spatial death and affect the health of the people. The recent heat waves killed about 70,000 people in Europe in 2003. IPCC report indicated that Romania will have 28.9 days in 2050. Majority of the countries in the world will have more than 10 days of heat waves in near future. The high level of temperature will lead to more mortality.

8. NATURAL DISASTER

Frequent natural disasters have been realized by the world. Flood, earthquake, cyclone, hurricane have been faced by many countries in recent years. The natural disasters affect the life of the people and a very huge economic loss. The mortality due to drought was 1728 since 1971-2008.

9. CLIMATE CHANGE AND BIO-DIVERSITY

Earth supports a web of a 5 million to 10milion species of plants and animals. Single species human destroys or disturbs the every functioning of that web. A few dozen species provide basic nutrition, 20 percent of human calorie intake comes from rice, 20 percent comes from wheat, a few species of cattle, and poultry supply 70 percent of animal protein. Only among the 20 percent of animal protein from fish and shell fish is a diversity of dietary species found.

Climate change has been accelerated many changes in the biosphere. Biosphere is the place where living organism is interacting with them. Biodiversity is the variety of all forms of life, including genes, populations, species and ecosystem. The number of species is often used as an indicator of the diversity of an area. Most of them are micro organisms and only about 1.75 million have been formally described. Two-thirds of the diversity is the tropics.

Global warming has significantly impact on the timing of the species life cycle. In future, the impacts are expected to be much more extreme. In many plant species, the timing of spring growth phases such as budding, flowering and fruiting is a response to accumulated temperature. In animals, the timing of migrations, breeding emergence and metamorphosis has changed significantly. These phenological events have been and will continue more than earlier periods. Delay in autumn events such as leaf colour, leaf fall and migration of animals and birds.

The characteristics of all individual animals and plants are determined by the interaction between their genes and environment. The physical environment experienced by each individual includes temperature, rainfall, lay length and geological subtract, while the biotic environment includes the presence of food, competitors and natural enemies.

When the environmental changes, phenotypes of organism may change in direct responses. Most of the insects grow faster and have shorter generation time at higher environmental temperature. Under warmer conditions in the Northern hemisphere, plants have flowered earlier, insects have emerged earlier in the season, amphibians have returned to their breeding ponds earlier, migrant birds have returned earlier in spring and non-migrant birds have nested earlier in spring. There can be longer breeding periods for both plants and animals. The largest phenological changes will be more at higher latitudes and altitudes because the warming of the globe is more intense in these areas.

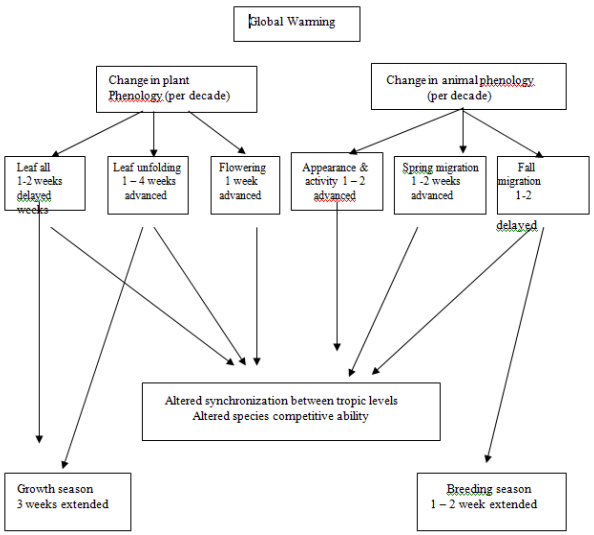
10. IMPACTS ON FRESHWATER ECO-SYSTEM

Fresh water ecosystem will naturally be sensitive to change in the hydrologic cycle. A warmer climate will result in evaporation from water surfaces a greater transpiration by plants, which will

result in a more vigorous water cycle. Future climate change will directly affect lake ecosystems through warmer temperatures and changes to the hydrologic cycle.

A strong case can be made that future climate warming will alter the extent of habitats available cold, cool and warm water organism depending upon region and result in range expansions and contractions. Rapid climate change has many negative implications for the biodiversity of rivers and streams. Streams are coolest in the headwaters and a warming will tend to push species upstream to find thermally optimal habitats. Climate change may cause extinction at several taxonomic levels.

Ecological consequences of climate warming for plants and animals phenology



Source: Lovejoy and Hannak (Ed.) 2006.

11. CLIMATE CHANGE AND MARINE ECOSYSTEM

At about 70 percent of the earth's surface is covered by sea water. Climate change is already changing the distribution of ecosystems. Marine plants and animals live within fairly narrow set of physical and chemical conditions. Rapid change of environmental conditions results in changes in the abundance of organisms.

The increase in greenhouse gases within the earth's atmosphere is set to change three fundamental variables associated with oceanic environments: the calcium carbonate saturation state, sea level, and temperature of the earth's oceans. These bring negative impact on marine bio-diversity. Total carbonate alkalinity of seawater will decrease as carbon dioxide increases within the earth's atmosphere. The doubling carbon dioxide concentrations in the atmosphere will decrease the aragonite saturation state in the tropics by 30 percent in 2050.

The level of the ocean has fluctuated by more than 100 over the past 100000 years as ice stored on land has changed in volume. During the last ice age, sea level was 120 m below where it is today. During the transition out of this period of glaciation, sea level changed at an average rate of 10 mm/per year. During the interglacial periods rates of sea level rise have been lower that is 0.1 – 0.2 mm/per year over the past 3000 years. Changes in sea level have had major impacts on the abundance and particularly the distribution of both marine and terrestrial diversity. Ocean temperature has increased 2.3 x 10^{23} J between the mid 1950s and mid 1990s. The mean warming is 0.60^{0} C. The changes in global temperature bring the changes in direction of ocean water movement. Coastal ecosystems are generally dominated by food webs that depend on attached plants such as algae or water borne microalgae. They are important for the flow of resources within the ocean and are the basis for more than 60 percent of the productivity of the ocean.

Tropical intertidal and sub tidal regions are dominated by ecosystem that are characterized by a framework of scleractinian corals, The biodiversity of coral reef is extra ordinary with an estimated million species of plants, animals and protists living in an estimated 400,000 km. of coral reef. These ecosystems form rich and complex food chains that support large populations of fish, birds, turtles and marine mammals. Coral reefs have already experienced major impacts from climate change. Tropical oceans are 0.5 – 1.0^{0} C warmer than 100 years ago. Major disturbances of mass coral bleaching have increased dramatically over the last 30 years. Changes in reef building coral communities create a huge impact on marine diversity. Fishes depend on corals for food, shelter or settlement cues may experience dramatic changes in abundance or go extint. Thousands of other organisms are also vulnerable changes in sea temperature are affecting plankton processes in polar and temperature regions. The mid 1970s fledgling survival has declined and penguin as well. The population size decreased by 70 percent since 1987. The emperor penguin numbers have declined by 50 percent. In the Indian Ocean some regions lost all their reef building coral communities and the over all average loss for the Indian Ocean was 46 percent. Between 1992 and 2000, the share of severely damaged reefs world wide expanded from10 percent to 27 percent.

The carbon dioxide – carbonate system is the most important chemical equilibrium in ocean. It influences nearly every aspects of marine science, including the ecology, and ultimately, the bio diversity of the ocean. It is largely controlling the PH

of sea water and thus affects directly much other chemical equilibrium as well. By the middle of the century atmospheric carbon dioxide is expected to reach double pre industrial level. CO_2will give rise via passive diffusion to a two fold increase in surface ocean CO2 concentrations and cause a drop in surface PH of about 0.4 units because of the CO_2 carbonate buffer system. The breeding centre for the fishes is disturbed that the fish population will decrease drastically. Finally, the poor people's food will be affected to them.

12. ECONOMIC LOSS

The components of biological diversity are important from ecological point of view. Elements of bio-diversity provide food, medicine and raw materials for industries and maintaining the ecological balance of nature. It protects and stabilizes soil, local climate, soil hydrology and efficiency of the nutrient cycle between soil and vegetation. The survival and well beings of society depend on a large number of antibiotics and anti cancer drugs developed from plants, animals and micro organisms. World attention was focused on natural drugs only in the late 20th century. Plants derived drugs represented a market value of $ 40 million all over the world. It has been estimated that 3 billion people depend on traditional medium. In the USA a quarter of all prescription dispensed by pharmacies are substance isolated from plants. More than 5000 species of plants are used in Chinese traditional medicines. If the climate change continues, the plants diversity will affect and consequently irrecoverable huge loss to the society.

Table 1. Climate change and agricultural production

	Change in temperature 0°C	Change in heat wave duration No. of days	Precipitation % change	Agricultural output %change	Agric. Yield
	2000-50	2000-50	2000-50	2000-50	2000-50
Australia	1.5	10.9	-1.4	- 26.6	- 16.4
Bangladesh	1.4	8.7	1.4	- 21.7	8.9
China	1.7	16.1	4.5	- 7.2	8.4
Finland	2.1	29.6	5.6	-	15.7
Russian Federation	2.2	29.5	8.8	- 7.7	11.0
Pakistan	1.8	19.8	- 3.0	- 30.4	- 32.9
United States	1.8	24.4	2.7	- 5.9	- 1.7
India	1.6	10.8	1.9	- 38.1	- 12.2

Source: World Development Report, World Bank, 2010.

Table 2. Climate change and natural disasters

	Mortality		People affected		Economic loss	
	No. of people		No. of people thousand		$ thousands	
	Drought	Flood & storms	Drought	Flood & storms	Drought	Flood & storms
	1971-2008	1971-2008	1971-2008	1971-2008	1971-2008	1971-2008
Bangladesh	0	5673	658	8751	0	44576
Australia	0	10	186	108	262447	390461
China	93	1304	9642	53460	522350	4791624
Ethiopia	10536	51	1361	59	2411	424
India	8	2409	25294	22314	61608	1055375
Iran	0	102	974	101	86842	202133
Italy	0	8	0	2	21053	597289
Philippines	0	743	172	2743	1696	164362
United States	0	272	0	672	187763	12104146
Vietnam	0	393	161	1749	17082	157603
Mozambique	2633	65	455	328	1316	22846

Source: World Development Report, World Bank, 2010.

13. BIO-DIVERSITY IN AGRICULTURE

Biological diversity is an important resource essential to sustainable development in agriculture. Loss of species and genetic diversity presents a serious threat to sustainable agriculture. Species and genetic diversity provide raw materials for plant breeding. Climate change is highly influenced the

agricultural production. The production is affected by rainfall, temperature and precipitation. There are crops respondent to carbon fertilization and it increases the production. The negative impact is increase the requirement of water and shrinking of crop land result in decrease production. The wetland will be changed into dry land as a result agricultural bio diversity will be declined.

The rising trend of sea water results in retreat nearby areas of coastal line. It creates alkalinity of agricultural lands and the ultimate result is that, the soil is not suitable for cultivation crops. The cropped area will be reduces and the agricultural production will be fallen. The desertification falling of water availability and intrusion of sea water affect the entire agriculture production of the world. Some parts of the world will not have enough water to always grow all of their food. Many countries already import a large share of their food from other countries.

Table 1 depicts that the change in temperature ranges from 1.4^0 C to 2.2^0 C in different regions. As a result of hotter temperature, the heat waves will be higher in all regions. All the developing countries will face the problem less yield and production. People will in secured food. This kind of problem will lead to huge economic problem to society as well as the nation. There were 70 countries projected the heat waves, agricultural output and agricultural yield. Almost all the countries change in temperature is more than 1^0 C. There are countries will face problem of reduction in output from agriculture. The developing countries will have more threat because of decline in agricultural output and agricultural yield. This kind of problem reduces the food security to the people of developing countries.

14. NATURAL DISASTERS AND ECONOMIC LOSS

Climate change is one the reasons for natural disasters. Climate change influences the hydrological cycle and as a result flood and drought experienced by the world. There were 70 countries estimated the human loss and economic loss. Both the developed and developing countries faced the problem of human loss and economic loss. The loss of China was 2.9 percent, the USA 1 percent, India 2.5 percent, Australia 3.2 percent, Belize 2002.2 percent, Grenadu 20.5 percent Samoa 248.4 percent of GDP. Table 2 indicates that many countries faced the problem of drought, flood and storms.

15. BIO-DIVERSITY IN AQUATIC ECOSYSTEM

Increasing sea and river water temperature is likely to affect fish breeding and migration and harvest. A rise in temperature 1^0 C could have rapid effects on the mortality of fish and geographical distribution. The marine ecosystem provides a very huge amount food to all section of the people especially protein nutrient provider to the entire mass of the world. Fish and selfish currently supply about 8 percent of the world animal protein consumed with the world population growing by about 78 million people every year, fish and shellfish production must grow by about 2.2 million metric tones every year to maintain current consumption. The climate change already affected marine ecosystem and there is a threat to the future. If the trend continues, the stock of fish and animals will fall. There are countries earning sizeable amount of income through fishing industry. This industry face loss and finally the major section of the population loss their nutrient food. The most vulnerable section of people is the poor section of the population. They are not missed the food but also millions of people lost their livelihood.

16. FOREST ECOSYSTEM

Forest eco-system is not only expanding species and micro organism but also the plants and trees are oxygen producers. The climate change makes the forest drier. The most threat to the forest is forest fire. The high level of temperature creates fire in the forest. If this situation continues, the forest ecosystem will highly impair. As a result forest based industries lost their inputs and loss of the oxygen. An average a tree inhales 12 kg of CO_2 and exhales enough O_2 to keep a family of four breathing per year. On an average one tree produces nearly 260 pounds of oxygen every year. More than 20 percent of the entire world's oxygen is produced in the Amazon region. These rain forests generate about 40 percent of the world's oxygen. The level of carbon dioxide concentration will increase in the atmosphere.

17. CONCLUSION

These kind of problems have been arising allover the world. A beautiful planet is facing threat by so called development. A man is not satisfied by a single wants. The man wants have been expanded and therefore exploit the natural resources for his comfort and luxury life. People enjoy the comfort and luxury life that they called development. This development brings more income. This development affects the people's life and brings economic loss to the entire world. Will the people limit their wants? Not at all. They will bring modification and try to adopt the existing nature. How long? No answer for it. Educate the people in the line of limiting wants then only this planet will be saved and limit the economic loss in near future.

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RESEARCH ARTICLE

GC-MS ANALYSIS OF BIO-ACTIVE COMPOUNDS FROM THE ETHANOLIC EXTRACT OF *BALIOSPERMUM MONTANUM* (WILD.) MUELL. ARG.

Reshma, K., T. Baluprakash, H. Abdul Kaffoor, A. Venkatachalapathi and K. Arumugasamy*
Department of Botany, Kongunadu Arts and Science College, Coimbatore - 641 029, Tamil Nadu, India.

ABSTRACT

Baliospermum montanum (Wild.) Muell. Arg*., (*Euphorbiaceae) is a well known medicinal plant which is used in treatment of various diseases. The present study was focussed on the separation and investigation of the phytochemical compounds from ethanolic extract by GC-MS technique. The mass spectra of the compounds found in the extract were matched with the National Institute of Standards and Technology (NIST) library. The ethanolic extract revealed the presence of 30 bioactive compounds. The major and minor phytochemical compounds are 2,15-Dithia[3](9,10)anthracenol[3](2,6)pyridinophane,1,2,3,4-tetrahydro showed the highest peak 9.32% followed by phytol-9.08%, Neophytadiene-7.38%, 8,11-Octadecadienoic acid, methyl ester (CAS)-7.06%, 4-ethyl-6-[2-(methoxycarb onyl)ethenyl]-7-[2-(methoxycarbonyl)ethyl]1,3,5,8-tetramethyl-2-vinylporphyrin-6.72%, 2-pentafluorophenylpropanal-6.43%, 4,4'-Isopropylidene-bis-(2-cyclo hexyl phenol)-5.91% etc.. Further pharmacological studies are needed to find out the medicinal aspect of these compounds.

Keywords: *Baliospermum montanum,* euphorbiaceae, ethanol extract, Phytocompounds.

1. INTRODUCTION

A plant has been an important source of medicine with qualities for thousands of years. Phytochemical compounds of medicinal plants is desirable not only for the discovery of therapeutic agents, but also because such information may be of great value in discovering new sources of economic phytocompounds for the synthesis of complex chemical constituents substances and for discovering the actual significance of folkloric medicine (1). *Baliospermum montanum* with synonym *B. axillare* , *B. polyandrum, Croton polyandrus* belongs to the family euphorbiaceae with common names as in, English name - Red physic nut, Wild castor, Wild croton, Hindi name - Danti, Hakum, Hakun, Malayalam name - Dantika, Katalavanakku, Nagadanti, Tamil name - Kattamaraku, Niradimutta, Euphorbiacea family includes 280 genera with 730 species with largest genus Euphorbia (2). The plant is monoecious,s tout under shrub with numerous branches. *B. montanum* is widely distributed throughout the sub Himalayan tracts from khasi hills to Kashmir. It is a shade loving plant grows well in humid climate. It is easily available in Bengal, Bihar, Madhya Pradesh and peninsular India. Phytochemical compounds of *B. montanum* have high medicinal importance in the treatment of diseases like hemorrhoids, calculi, abdominal pain, itching, leprosy, burning sensation, inflammation, abdominal disorders, bleeding disorders and worm infestation, tumors etc. It is also used as blood purifier. However this study is to find out the phytochemical and the bioactive compounds which are present in the whole plant body of *B.monatanum* by GC-MS technique.

2. MATERIALS AND METHODS

2.1. Plant collected and preparation extraction

The Fresh plant collected in *B. montanum* was washed 1 to 2 times with water followed by distilled water and shade dried. All the dried parts were pulverized by mechanical grinder to get the powder which passed through 100 mesh sieve and then stored in an air tight container. 50 g of plant powder extracted with 300 mL of ethanol using the soxhlet extractor for 10 to 12 hours. The ethanolic extract was concentrated under reduced pressures at low temperature (40-50°C) for crude residues. The concentrated residue was stored in the refrigerator at 4ºC and it was used for further studies.

2.2. GC-MS Analysis

GC-MS analysis of the extract was performed using a Thermo Gc - Trace Ultra VER: 5.0, Thermo MS DSQ II employing the following conditions : column Elite -DB 35 - MS Capillary standard non - polar column , Helium (He) was used as a carrier gas at a constant flow of 1.0 ml /min and an injection volume of 1micro litre was employed, the oven temperature was programmed from 70^0C raised to 260^0C at 6^0C/min. Total running time was 37.49 Min. GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the

*Correspondence: Dr. K. Arumugasamy, Associate Professor, Department of Botany, Kongunadu Arts and Science College, Coimbatore - 641 029. E-mail: punnagaiibala@gmail.com

spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

3. RESULTS

The Gas Chromatography-Mass Spectrometry indicates the presence of 30 phytochemicals compound which is represented in table, the active compounds with their retention time (RT), Molecular formula and Molecular weight (MW) in the ethanol extract of rhizomes of *B. montanum* are presented in (Figure 1) and Table 1. The major phytochemical constituents were 2,15-Dithia [3] (9,10) anthracenol [3] (2,6) pyridinophane,1,2,3,4-tetrahydro showed the highest peak of 9.32% followed by phytol-9.08%, Neophytadiene-7.38%, 8,11-Octadecadienoic acid, methyl ester(CAS)-7.06%, 4-ethyl-6-[2-(methoxycarb onyl)ethenyl]-7-[2-(methoxycarbonyl)ethyl]1,3,5,8-tetramethyl-2-vinylporphyrin-6.72%, 2-pentafluoro phenyl propanal- 6.43%, 4,4'-Isopropylidene - bis- (2-Cyclo Hexyl Phenol) -5.91% Hexadecanoic acid, methyl ester (CAS) -5.78%, bis (4-methoxy-1-naphthyl) sulphoxide - 4.44%, 2à,3à – Diacetoxy - 22, 23-i sopropylidenedioxy -24- methyl- 25-hydroxy-5. alpha,- cholestan- 6 -one- 3.46%, 1- [2,4,6-tris (trimethylsiloxy) phenyl] -3- [3-methoxy-4 (trimethylsiloxy) phenyl]-2-propen-1-one-2.58%, 3-Cyano-3-(3',4'-dimethoxyphenyl)-3-phenyl propionyl chloride-1.77% along with other minor constituents were also present in the ethanol extract of whole plant of *B. montanum.*

Table 1. Compounds present in the ethanolic extract of Baliospermum montanum (Wild.) Muell. Arg.

S. NO	RT	Compound Name	Probability	Molecular Formula	Molecular Weight	Area %
1.	6.75	2,2,7-trimethyl-11-methoxycarbonylethyl-3-oxa-7, 11-diazas piro[5.6]dodecane	12.41	$C_{16}H_{30}N_2O_3$	298	1.00
2.	10.55	6á-Methoxy-22,23-methano-3à5-cyclo-26, 27-dinor-5à-ergost -24(28)-ene	82.33	$C_{28}H_{44}O$	396	1.70
3.	15.36	3-Cyano-3-(3',4'-dimethoxyphenyl)-3-phenylpropionyl chloride	10.13	$C_{18}H_{16}C_lNO_3$	329	1.77
4.	18.61	Neophytadiene	53.40	$C_{20}H_{38}$	278	7.38
5.	19.24	3-Cyano-2-methyl-4-(4-chlorophenyl)-6-[2-bis(methylthio)ethenyl]pyridine	74.92	$C_{17}H_{15}C_lN_2S_2$	346	1.63
6.	19.91	bis(4-methoxy-1-naphthyl)sulphoxide	8.73	$C_{22}H_{18}O_3S$	362	4.44
7.	21.35	4,4'-ISOPROPYLIDENE-BIS-(2-CYCLOHEXYLPHENOL)	45.70	$C_{27}H_{36}O_2$	392	1.39
8.	21.66	Hexadecanoic acid, methyl ester (CAS)	72.54	$C_{17}H_{34}O_2$	270	5.78
9.	21.99	2-pentafluorophenylpropanal	33.76	$C_9H_5F_5O$	224	6.43
10.	22.79	Methyl 1,3-dihydro-2H-isobenzofuran-4-carboxylate	10.34	$C_{10}H_{10}O_3$	178	2.29
11.	23.52	(all E)-8-Bromo-2,7-dimethyl-2,4,6-octatrienal	32.43	$C_{10}H_{13}BrO$	228	1.06
12.	23.83	(4aR*,12cR*)-5-Ethoxycarbonyl-4a,5,6,7,12,12c-hexahydro-4H-5,7a-diazabenzo[5,6]cyclohepta[def]fluorene	31.17	$C_{21}H_{22}N_2O_2$	334	1.12
13.	25.15	Phytol	43.15	$C_{20}H_{40}O$	296	9.08
14.	25.46	8,11-Octadecadienoic acid, methyl ester (CAS)	8.71	$C_{19}H_{34}O_2$	294	7.06
15.	25.89	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	21.72	$C_{19}H_{32}O_2$	292	5.00
16.	26.64	Dimethyl 2,12-dibromodecacyclo[9.9.0.0(1,8).0(2,12).0(6,10).0(11,18) 0(13,17).0(16,20)] icosane-syn-4,syn-9-dicarboxylate	17.38	$C_{24}H_{26}Br_2O_4$	536	0.95
17.	28.05	2à,3á,4à,6á,11,19-hexahydroxy-9,11-secocholest-(22E,24S)-24-methylen-9-one (Euryspongiol A3)	14.58	$C_{28}H_{48}O_7$	496	1.56
18.	28.41	4-(2-Nitrophenyl)-6-methoxypyridino[3,2-	23.24	$C_{19}H_{11}N_3O_5$	361	0.96

		g]quinoline-5,10-dione				
19.	29.02	3',4'-Dihydro-Stephasubine	79.40	$C_{36}H_{36}N_2O_6$	592	1.08
20.	29.33	6,7-Isopropylidenedioxy-1,15-dihydroxy-8,15-seco-maoerystals A	12.63	$C_{23}H_{36}O_5$	392	1.02
21.	30.47	2,15-Dithia[3](9,10)anthraceno[3](2,6)pyridinophane, 1,2,3,4-tetrahydro	9.64	$C_{23}H_{23}NS_2$	377	9.32
22.	31.88	4-ethyl-6-[2-(methoxycarbonyl)ethenyl]-7-[2-(methoxycarbonyl)ethyl]1,3,5,8-tetramethyl-2-vinylporphyrin	28.85	$C_{36}H_{38}N_4O_4$	590	6.72
23.	33.10	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (CAS)	6.94	$C_{24}H_{38}O_4$	390	2.35
24.	33.93	{[Thorium-(pentamethylcyclopentadienyl)]-tris[(trimethylsilylamino)-1',2'-ethylideneamino]}	97.44	$C_{25}H_{54}N_4Si_3$ Th	726	2.12
25.	34.73	3-(4-Chlorobenzoyl)-6-methoxy-9-N-methyl-4-[2'-(4-chlorobenzoyl)ethyl]carbazole	25.57	$C_{30}H_{23}Cl_2N_3$	515	0.92
26.	36.11	(E)-1,6-Dibromo-3,4-bis[(triisopropylsilyl)ethynyl]hex-3-en-1,5-diyne	73.36	$C_{28}H_{42}Br_2Si_2$	592	1.39
27.	36.48	N-[3'-Cyano-6'-(3''-methyl-5''-oxo-1''-phenyl-2''-pyrazolin 4''-yl)-4'-phenylpyridin-2'-yl]benzamide	24.30	$C_{29}H_{21}N_5O_2$	471	2.54
28.	37.85	1-[2,4,6-tris(trimethylsiloxy)phenyl]-3-[3-methoxy-4-(trimethylsiloxy)phenyl]-2-propen-1-one	98.47	$C_{28}H_{46}O_6Si_4$	590	2.58
29.	39.09	2à,3à-Diacetoxy-22,23-isopropylidenedioxy-24-methyl-25-hydroxy-5.alpha,-cholestan-6-one	58.59	$C_{35}H_{56}O_8$	604	3.46
30	39.90	4,4'-ISOPROPYLIDENE-BIS-(2-CYCLOHEXYLPHENOL)	40.08	$C_{27}H_{36}O_2$	392	5.91

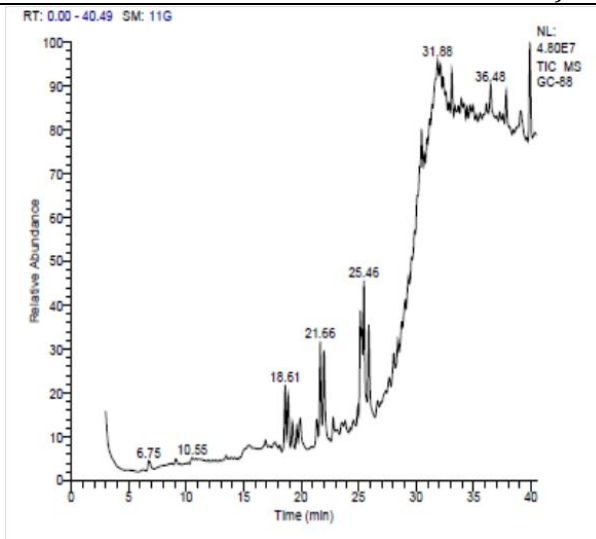

Fig. 1. GC-MS chromatogram of the ethanolic extract of *B. montanum*.

4. DISCUSSION

The phytochemical constituents refer to chemicals produced by medicinal plants which are important to health. The major phytocompounds isolated from *B.montanum* ethanol extract were 2,15-Dithia [3] (9,10) anthracenol [3] (2,6)pyridinophane,1,2,3,4-tetrahydro showed the highest peak 9.32% followed by phytol-9.08%, Neophytadiene-7.38%, 8,11-Octadecadienoic acid, methyl ester (CAS)-7.06%,etc.. The identified compounds possess many biological and pharmacological properties. The chromatogram showed in fig 1. Previous report on the phytochemical analysis of ethanolic extract of *B. montanum* also revealed the presence of alkaloids, flavonoids, tannins, phenols, saponins, steroids, terpenoids and resins (Starlin *et al*., 2012).The phytochemical constituent rich ethanolic extract of *B.montanum* was subjected to Gas Chromatography-Mass spectrometry (GC-MS) analysis. The result revealed the presence of 30 compounds. Earlier studies on phytochemical investigation of *B.montanum* ethanolic extract by GC-MS indicated the presence of thirty compounds (4). Similar work was reported for chemical composition analysis of essential oil of *Curcuma amada* by Vishnupriya *et al*., (5). The phytochemical characterization of the extracts, the isolation of responsible bioactive

compounds and their biological activity are necessary for future studies**.**

5. CONCLUSION

Present evaluations 30 bioactive phytochemical compounds have been identified from ethanolic extract of *B.montanum* by Gas Chromatogram-Mass spectrometry (GC-MS) analysis. Presence to the different bioactive compounds in *B.montanum* proved that the pharmaceutical drug importance. Further studies will require finding out its bioactivity, pharmacological activities.

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RESEARCH ARTICLE

A CHECK LIST OF MACROFUNGAL DIVERSITY IN PILLUR VALLEY, COIMBATORE DISTRICT TAMIL NADU, INDIA

Santhoshkumar, S.* and N. Nagarajan
Department of Botany, Kongunadu Arts and Science College (Autonomous), Coimbatore - 641 029, Tamil Nadu, India.

ABSTRACT

The present study carried out in exploring the macrofungal diversity in Pillur Valley, Coimbatore district, Western Ghats of Tamilnadu, India. The study was assessed from lower to higher altitude ranges in the different vegetation during the June, 2016 to August, 2017. The results of the survey revealed that the totally 20 species belonging to 11 families, 17 genera and 3 different orders were identified and in addition with 65 % Edible, 35 % Non edible mushrooms were identified. The documented mushroom species are *Agaricus campestris*, *Ganoderma lucidum*, *Coprinus comatus*, *Marasimus sp*, *Hygrophorous sp* and *Termitomycets microcarpus*. In conclusion, these wild species of mushrooms had rich amount nutritional properties and dietary fibres which is used as supplement for malnutrition deficiency especially Vitamin D and it is suggested for cultivation practices for large scale production and commercialization.

Keywords: Wild mushrooms, *Hygrophorous sp*, *Termitomycets* and Western Ghats.

1. INTRODUCTION

Mushrooms can be found in forests worldwide and have long been exploited as resources in developed economies because of their important agro-industrial, medicinal and commercial uses. The Indian sub-continent is blessed with favorable agro climatic conditions that are suitable to a varied range of fungal species. Fungi are among the most important organisms in the world, to the extent that their vital roles in ecosystem functions together with their influence on humans and human-related activities. macrofungi are fleshy fruiting body, which naturally grows all types of soil, grassy ground, rotten wood, leaf litter and decaying organic matter, they ability to grow in different seasons, yet all exhibit enhanced growth during the rainy season (1). It has represented by 41,000 species across the globe; however, only 2% have been reported from India, despite the fact that one-third of the total global fungal diversity exists in the tropical Indian region (2).

Wild Edible and medicinal mushrooms, whose history can be traced back to 4000 years, are treasured as a health food because of their unique edible and medicinal values (3). In recent years, increasing attention has been given to wild edible as well as medicinal purposes, because of their special nutritional and therapeutic attributes. The United Nations Food and Agriculture Organization proposed that a rational diet should be "meat, vegetable, and mushroom every meal".

It is represented by 41,000 species across the globe; however, only 2% have been reported from India, despite the fact that one-third of the total global fungal diversity exists in the tropical Indian region (2), Macrofungi are important economically due to their importance in food, medicine, bio-control, chemical, biological and other industries (4). Although the macrofungi are an integral part of a given ecosystem, their diversity and types are poorly studied, with a particular knowledge gap in the tropical regions including India (5).

2. MATERIALS AND METHODS

2.1. Study area

Fig. 1. Study area of Pillur valley.

The present study area, Pillur valley is confined to a major range in the Western Ghats of Nilgiri Biosphere Reserve, Coimbatore District, Tamil Nadu, India. The area of investigation approximately lies 110 - 18' latitude and 760 - 53" longitude. The altitude ranges from 1100 to 1428 m above mean sea level. The annual rain fall ranges from 1000 to 1400 mm. Pillur is continuous with Kerala forest in

*Correspondence: S. Santhoshkumar, Ph.D., Research Scholar, Department of Botany, Kongunadu Arts and Science College (Autonomous), Coimbatore- 641 029, Tamil Nadu, India. E-mail: santhosh.biology@gmail.com

the west and coonoor slops in the Nilgiri massif in the north. The forest area in the unique they exhibit a wide variety of floral diversity in different altitudinal and geographical zones. The major vegetation of Pillur valley is broadly classified in to scrub jungle, and deciduous forest (Fig.1).

2.2. Sample collection

During frequent field surveys from June, 2016 to August, 2017, mushroom species were collected. For collection of mushrooms various equipment, such as hunting knife, scissor, digging tools and zipped polythene packets for preserving the collected mushrooms were used. During survey the morphological & ecological characters of observed specimens were properly noted. Photographs of specimen from different angles were also taken for future studies. Collected specimens were then preserved in a mixture of liquid preservatives using rectified alcohol, formalin, and distilled water at a ratio of 25:5:70.

a) *Agaricus silvicola* b) *A. xanthodermus* c) *Amanita fulva* d) *Ganoderma lucidum*
e) *Schizophyllum commune* f) *Termitomycetes microcarpus*

Fig. 2. Collection of mushroom species in Pillur valley Western Ghats, Coimbatore district.

2.3. Identification

The identification of edible mushrooms were based on the morphological characters of the fruiting bodies following the guidelines mentioned in the websites, *viz.,* www.mushromexpert.com, www.rogersmushrooms.com, http://lifehacker.com, http://www.wisegeek.com, http://www.soppognyttevekster.no, http://www.mnn.com, Manual of Common Edible Mushrooms and scholarly article The collections of wild edible mushrooms have been identified by integrating their macroscopic and microscopic characterization by following method. Specimens were identified to their respective families, genera and species by consulting the available help of expert Valuable literature. Some of the mushroom samples were sent to Agharkar Research Institute Pune for the identification.

2.4. Preservation/drying of material

The specimens were preserved dry. The collections were dried in folding portable wooden driers, specifically designed for the purpose. The dried collections were wrapped in polythene bags and properly sealed. These bags were kept systematically in card board boxes which were stored in damp proof conditions. 1:4 P-Dichlorobenzene and Naphthalene balls were used as insect repellants. Specimens to be used for raising culture and systematic studies were usually sun dried or dried gradually at 25-30°C in the drier.

3. RESULTS AND DISCUSSION

Wild edible mushrooms are one of the higher valued non-timber forest products (6,7) China and most Asian countries. In the present result revealed that totally 20 wild mushroom species, belonging to 11 families, 17 genera were collected at Pillur valley, Western Ghats of Coimbatore district, Tamilnadu, India. The total mycotain this area are dominated by the family Agaricaceae (6 species), Polyporaceae (3 species), Lyophyllaceae (2 species) and other species belonged to the families Bolbitaceae, Ganodermataceae, Hygrophoraceae, Marasmiaceae, Mycenaceae, Plurotaceae, Russulaceae, Schizophyllaceae and Tricholomataceae each single species (Fig. 2 and Fig. 3). Similarly, Andrew *et al*. (1) reported that diversity and distribution of macrofungi in the Mount Cameroon totally 177 macrofungal species belonging to 83 genera and 38 families were recorded.

Table 1. Collection and identification of macrofungal species in Pillur valley Western Ghats of Coimbatore District.

S.No.	Name of the species	Order	Family	Habit	Edibility
1	*A. campestris*	Agaricales	Agaricaceae	Soil	Edible
2	*Agaricus silvicola*	Agaricales	Agaricaceae	Soil	Edible
3	*Agaricus xanthodermus*	Agaricales	Agaricaceae	Soil	Non-edible
4	*Coprinus* sp	Agaricales	Agaricaceae	Soil	Edible
5	*Collybia* sp	Agaricales	Tricholomataceae	Soil	Non-edible
6	*Ganoderma lucidum*	Polyporales	Ganodermataceae	Decaying wood	Edible
7	*Hygrophorous* sp	Agaricales	Hygrophoraceae	Soil	Edible
8	*Lentinus* sp	Polyporales	Polyporaceae	Decaying wood	Edible
9	*Lycoperdon perlatum*	Agaricales	Agaricaceae	Soil	Non-edible
10	*Macrolepiota procera*	Agaricales	Agaricaceae	Soil	Edible
11	*Mycena* sp	Agaricales	Mycenaceae	Soil	Non-edible
12	*Marasmeus* sp	Agaricales	Marasmiaceae	Soil	Edible
13	*Paneolus accuminatus*	Agaricales	Bolbitaceae	Soil	Non-edible
14	*Polyporus* sp	Polyporales	Polyporaceae	Decaying wood	Edible
15	*Pleurotus platypus*	Polyporales	Plurotaceae	Decaying wood	Edible
16	*Russula* sp	Russulales	Russulaceae	Symbiotic	Edible
17	*Schizophyllum commune*	Agaricales	Schizophyllaceae	Decaying wood	Edible
18	*Termitomycetes microcarpus*	Agaricales	Lyophyllaceae	Soil	Edible
19	*Termitomycetes* sp	Agaricales	Lyophyllaceae	Soil	Edible
20	*Trametes vesicolor*	Polyporales	Polyporaceae	Decaying wood	Edible

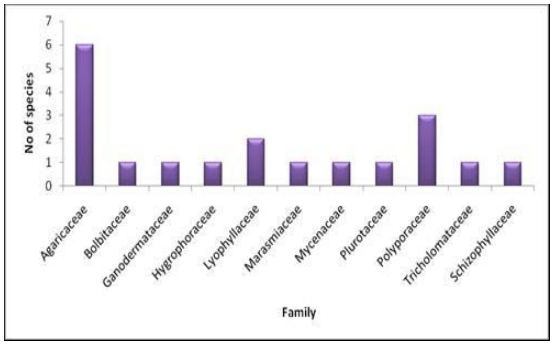

Fig. 3. Dominant families of mushroom species in pillur valley.

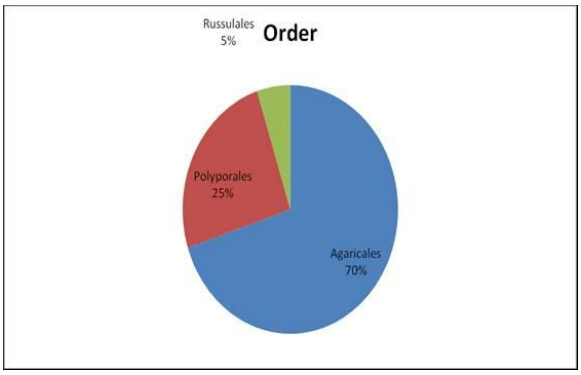

Fig. 4. Distribution of mushrooms in different order.

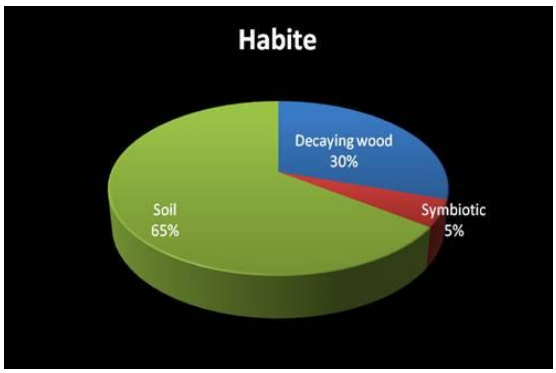

Fig. 5. Different habits of mushroom species in Pillur valley of western Ghats.

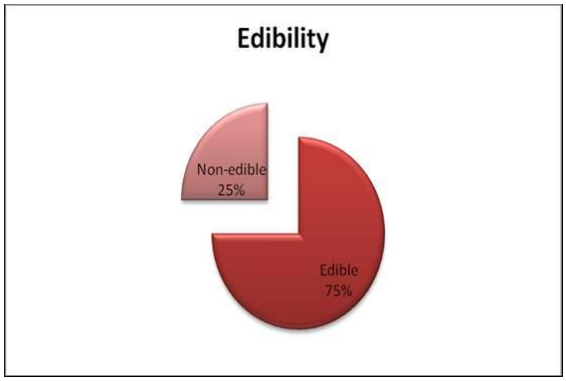

Fig. 6. Mushroom species and their edibility status.

Angelini *et al*. (8) also reported the diversity and ecological distribution of Macrofungi totally 305 species belonging to 61 families and 121 genera were identified and documented in Italy. The macro-fungi diversity in Patharia Forest of Sagar at Madhya Pradesh was reported by Vyas *et al*. (9). Earlier, Manjula (10) has been reported that the 300 agarics species belonging to 59 genera and 15 families of Agaricales were observed in the North West Himalayas. The diversity of agarics (Gilled mushrooms) was identified and documented in different region of Maharashtra, India (11). Singer (12) has been reported 1320 species belonging to 129 genera under Agaricales were documented. The order Agaricales (70%) was most dominant followed by Polyporales (25%) and Russulales (5%) in addition with soil was found as major habitat for 65 % of the macrofungi while 30% species Decaying wood and 5 % symbiotic associated in tree species are represented in (Fig. 4 and Fig. 5). Further the collected wild mushrooms 75 % are edible along with 25 % non-edible (Fig.6). The detailed information about the mushroom species different order, family habit and edibility status are presented in Table-1. The collected wild edible mushroom species were utilized as supplementary food by local tribal's especially in rainy seasons Greeshma *et al*. (13) observed that the leven species and ten genera of macrofungi in lateritic scrub jungles of Southern Western Ghats. In India, different kinds of tribes are lived in different region. They were utilized not only medicinal plants and also traditionally utilized in wild mushroom. More ethnomycological studies were conducted in different regions of India (14,15). In recently, Santhoshkumar *et al*. (16) has been studied sirumalai hills totally 38 macrofungi species, belonging to 20 families and 29 genera were recorded. The present finding clearly shows that the Pillur valley is rich store house of macrofungi.

4. CONCLUSION

From this result, it concluded that the wild habitat mushrooms are rich source of vitamins, minerals and amino acids for fulfill the nutrition deficiency in human food dietary. The development of the cultivation method and practices to improve the livelihood status of the rural peoples and also conservation of wild resources.

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RESEARCH ARTICLE

ETHNOBOTANICAL SURVEY OF MEDICINAL PLANTS IN AND AROUND MARANDAHALLI VILLAGE, DHARMAPURI DISTRICT, TAMIL NADU

Vijayakumar, K.K.* and M. Parimala
Department of Botany, Kandaswami Kandar's College, Namakkal - 638 182, Tamil Nadu, India.

ABSTRACT

The present ethnobotanical investigation was carried out from December, 2016 to February, 2017 to identify the medicinal plants used by local people residing in and around Marandahalli village, Dharmapuri District regularly. A total of 58 medicinal plants were identified on basis of available first hand information from local people inhabiting in the study area, from literature survey and internet. Out of 58 medicinal plants documented in the study area, only 27 plants have been used by them for the treatment of various diseases like fever, intermittent fever, cough, asthma, jaundice, gastric problems, urinary disorders, dry skin disease, psoriasis, skin allergies, leucoderma, burning skin, liver disorders, snake bite, memory power, ulcer, diabetes, stomach aches etc., The plants were also to be used in different forms such as juice, decoction, powder and past. Authentication is needed to validate the usage.

Keywords: Ethnobotany, Herbal medicine, Local people, Marandahalli, Dharmapuri.

1. INTRODUCTION

In India medicinal plants based traditional systems of medicines are playing major role in providing health care to large section of population in both rural and urban areas. Indian Systems of Medicine are well known among the global traditional systems of medicine which has been included Ayurveda, Unani, Siddha, Indigenous systems of medicine and Traditional systems of medicine. Traditional Systems of medicines always played important role in human welfare. These systems are continuing at present and also play major role in future. The system of medicines which are considered to be Indian in origin or the systems of medicine which have come to India from outside and got assimilated in to Indian culture are known as Indian Systems of Medicine (1).

Now India has only six recognized systems of medicine namely Ayurveda, Siddha, Unani and Yoga, Naturopathy and Homoeopathy. Even though Homoeopathy came to India in 18th Century, it is completely assimilated into the Indian culture and got enriched like any other traditional system hence it is considered as part of Indian Systems of Medicine (1). This system consists of both internal and external medicines which are today available in market, manufactured by various companies. However there are traditional practioners still practicing with their self prepared drugs.

The ancient Indian system of medicine reports diverse medicinal plants ranging from higher plants to lower forms from which more than 70% of medicinal drugs are derived which have been used to treat various diseases for 6000–7000 years (2). The indigenous knowledge of medicinal plants has been documented in different Indian system of medicines such as Ayurveda, Unani and Siddha (3)

Ethnobotanical investigations are a suitable source of information about medicinal plants for the treatment of various diseases. These studies give idea to enhance our traditional knowledge, skills and technology about cultivation and uses of medicinal plants for the welfare of local or tribal communities. The use of ethnobotanical information on medicinal plants has given considerable attention to research community (4). In Eastern Ghats and also Dharmapuri District, many ethnobotanical studies were conducted among the tribal communities and documented their indigenous knowledge on medicinal plants also. But the reports on indigenous knowledge of local people on medicinal plants are considerable less in number. Hence the present ethnobotanical study was aimed to conduct among the local communities in and around Marandahalli village, Dharmapuri District to document their indigenous ethnobotanical knowledge on the utilization of commonly available medicinal plants.

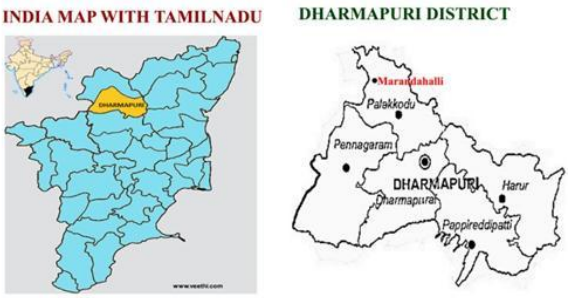
2. MATERIALS AND METHODS

2.1. *Study area*

The present ethnobotanical study was conducted in and around Marandahalli village, Dharmapuri District, Tamil Nadu (Fig. 1). The study area lies between 12.4°N and 78°E. It has an average elevation of 581meters above msl. Marandahalli is approximately 40 km away from Dharmapuri and 80 km away from Bengaluru.

*Correspondence: Dr. K.K. Vijayakumar, Assistant Professor, Department of Botany, Kandaswami Kandar's College, Namakkal - 638 182, Tamil Nadu, India. E-mail: kkvijay4@gmail.com m

Fig.1. Location map of the study village, Marandahalli.



2.2. *Data collection and identification of medicinal plants*

Periodic field survey for ethnobotanical exploration was conducted during December, 2016 to February, 2017 in Marandahalli village, Dharmapuri District. The local people and other traditional healers in and around the study area has been enquired and interviewed to collect the first hand information about vernacular name, medicinal uses, parts used and mode of administration of medicinal plants.

The data collection has been confirmed by contacting many people. The local people accompanied us to find out the right plant material from the study area and other nature habitat, the plants photographed in the field itself. The twigs of the medicinal plants are collected from study area and identification was done with the help of local and regional floras such as hand book of flora of presidency of Madras (5), the flora of the Tamilnadu Carnatic (6) and other flora of different areas (7,8). This identification was later confirmed by matching the plants with authentic specimen at Botanical Survey of India, Southern Circle, Coimbatore.

3. RESULTS AND DISCUSSION

The present study revealed that a total number of 58 medicinal plants were documented in and around the study area. The details of medicinal uses, parts used and mode of administration of medicinal plants are presented in Table 1. In this study, 58 plants species belonging to 54 genera under 33 families have been reported. Most dominant families in the study area were Solanaceae (6 species) followed by Euphorbiaceae, Asteraceae, Fabaceae, Malvaceae and Cucurbitaceae (4 species each), Lamiaceae (3 species), Amaranthaceae, Asclepiadaceae, Rutaceae, and Apocynaceae (2 species each) and other families with 1 species are Annonaceae, Clusiaceae, Convolvulaceae, Combretaceae, Moringaceae, Passifloraceae, Salvatoraceae, Nyctaginaceae, Caricaceae, Moraceae, Arecaceae, Piperaceae, Mimosaceae, Plumbaginaceae, Liliaceae, Lythraceae, Myrtaceae, Punicaceae, Verbenaceae, Menispermaceae and Rhamanaceae.

Table 1. List of medicinal plants present in the study area with their medicinal uses.

S. No.	Species	Family	Parts used	Medicinal uses	Mode of administration
1	**Acalypha indica* (Linn.)	Euphorbiaceae	Whole plant	Skin Disease, Ulcer, Bronchitis	Juice
2	**Aloe vera* (Linn.) Burm.f.	Liliaceae	Leaf	Sunburns, purgative, carminative	Juice
3	**Alternanthera sessilis* Linn.	Amaranthaceae	Whole plant	Leprosy, dyspepsia, Skin disease	Powder
4	*Amaranthus viridis* Linn.	Amaranthaceae	Whole plant	Diuretic, purgative	Powder
5	**Annona squamosa* Linn.	Annonaceae	Fruit	Constipation	Raw
6	*Asclepias curassavica* L.	Asclepiadaceae	Whole plant	Anodyne, antitumor	Juice
7	*Azima tetracantha* Lam.	Salvadoraceae	Leaf	Asthma, rheumatism	Juice
8	**Bidens pilosa* L.	Asteraceae	Leaf	Antitumor, antibacterial	Decoction
9	*Boerhaavia diffusa* Linn.	Nyctaginaceae	Whole plant	Diabetes, jaundice	Decoction
10	*Cajanus cajan* (Linn.) millsp.	Fabaceae	Seed	Tumours, oral ulcers, fever	Powder
11	*Calophyllum inophyllum* Linn.	Clusiaceae	Seed	Dermatitis, burning, sensation	Decoction
12	**Calotorpis gigantea* R.Br.	Asclepiadaceae	Leaf	Cough, asthma	Powder
13	**Capsicum annuum* Linn.	Solanaceae	Fruit	Malarial, intermittent fevers, indolent ulcers	Powder

14	**Carica papaya* Linn.	Caricaceae	Fruit	Skin diseases, dyspepsia, urinary, leprosy	Juice
15	**Catharanthus roseus* (Linn.) G.Don	Apocynaceae	Whole plant	Leocoderma, cancer, chemotherapy	Powder
16	**Citrus limon* (Linn.)	Rutaceae	Fruit	Diarrhea, cold, antibacterial	Juice
17	**Clitoria ternatea* Linn.	Fabaceae	Leaf	Asthma, ulcers, pulmonary	Tonic
18	**Coccinea indica* (Wight & Arn.) Naud.)	Cucurbitaceae	Leaf	Cough, asthma, diabetes	Powder
19	*Cucurbita maxima* Duchesne ex Lam.	Cucurbitaceae	Fruit	Inflammations, nervous	Tonic
20	*Dolichos lablab* L.	Fabaceae	Flower	Stomach, disorders, heart problems	Decoction
21	*Eupatorium odoratum* L.	Asteraceae	Leaf	Skin wounds, fevers	Decoction
22	*Euphorbia heterophylla* L.	Euphorbiaceae	Whole plant	Asthma, bronchitis	Paste
23	**Ficus religiosa* Linn.	Moraceae	Fruit	Dysentery, diarrhea	Powder
24	*Hibiscus callyphyllus* av.	Malvaceae	Flower	Bowel diseases	Juice
25	*Hibiscus cannabinus* Roxb.	Malvaceae	Leaf	Dyspepsia, ophthalmopathy	Powder
26	**Hibiscus rosa-sinensis* Linn.	Malvaceae	Flower	Venereal diseases, skin diseases	Tonic
27	**Hyptis suaveolens* (L) Roit	Lamiaceae	Seed	Uterus, stomach aches	Powder
28	**Ipomea staphylina* Roemer and schultes	Convolvulaceae	Leaf	Snake bites, stones	Paste
29	**Lawsonia inermis* Linn.	Lythraceae	Flower	Skin diseases, ulcers, dysentery	Tonic
30	*Lycopersicon lycopersicum* (Linn.) karsten)	Solanaceae	Fruit	Liver, kidney, stimulant, asthma	Decoction
31	*Mukia madraspatanas* (Linn.) Roem.	Cucurbitaceae	Whole plant	Neuralgia, nostalgia colic	Tonic
32	**Mentha arvensis* Linn.	Lamiaceae	Leaf	Ulcer, colic, peptic ulcer	Juice
33	**Momordica charantia* Linn.	Cucurbitaceae	Leaf	HIV, cancer	Decoction
34	**Moringa olefera* Lam.	Moringaceae	Leaf	Antioxidants, lower cholesterol	Decoction
35	**Murraya koenigii* (Linn.) Spreng.	Rutaceae	Leaf	Vomiting, leprosy, skin disease	Tonic
36	**Nerium oleander* Linn.	Apocynaceae	Leaf	Asthma, leprosy, ulcer	Juice
37	**Ocinum sanctum* Linn.	Lamiaceae	Leaf	Dyspepsia, gastric disease, vomiting	Decoction
38	*Parthenium hysterophorus* L.	Asteraceae	Whole plant	Skin inflammation, dysentery	Decoction
39	*Passiflora foetida* L.	Passifloraceae	Whole plant	Diarrhea, debility	Decoction
40	*Phoenix pussila* Gaertn.	Arecaceae	Fruit	Blood purifier, diabetes	Raw
41	**Phyllanthus amarus* Schum. & Thonn.	Euphorbiaceae	Whole plant	Jaundice, ulcer problems, urinary diseases	Paste
42	*Pithecolobium dulce* (Roxb.)	Mimosaceae	Whole plant	Constipation fever	Decoction
43	*Plumbago zeylanica* L.	Plumbaginaceae	Whole plant	Cancer, lung cancer, rheumatism	Powder
44	*Psidium guajava* Linn.	Myrtaceae	Whole plant	Vomiting, vitamin c, antimalarial	Tonic

45	*Punica granatum* (Linn.)	Punicaceae	Seed	Urinary infections, treat sore throats	Raw
46	**Ricinus communis* Linn.	Euphorbiaceae	Seed	Skin diseases, gulma, fever	Powder
47	*Sesbania grandiflora* (Linn.) Poir.	Fabaceae	Whole plant	Anaemia, gastralgia, diarrhoae, gulma	Juice
48	*Sida acuta* Burm.f	Malvaceae	Leaf	Diarrhea, snake bite	Powder
49	*Solanum melongena* (Linn.)	Solanaceae	Fruit	Ulcer, neuralgia, asthma, cholera	Powder
50	**Solanum nigrum* Linn.	Solanaceae	Leaf	Heal mouth, ulcer, diuretic	Juice
51	**Solanum trilobatum* (Linn.)	Solanaceae	Leaf	Cold and cough, chronic bronchitis, antibacterial, antifungal	Decoction
52	*Solanum turvum* Linn.	Solanaceae	Leaf	Diuretic cold, skin disease	Powder
53	*Stachytarpheta indica* (L.) vahl	Verbenaceae	Whole plant	Ulcers, allergy	Tonic
54	*Synadenium grantii* Hook.f.	Euphorbiaceae	Leaf	Backache, swelling	Powder
55	*Synedrella nodiflora* (L.) Gaerth.	Asteraceae	Leaf	Rheumatism, stomach pains	Powder
56	*Terminalia catappa* L.	Combretaceae	Seed	Liver diseases, ulcers	Powder
57	*Tinospora cordifolia* (Willd). Miers ex Hook. F. & Thoms.	Menispermaceae	Stem	Anaemia, asthma, skin disease	Tonic
58	*Ziziphus jujuba* Linn.	Rhamanaceae	Fruit	Anxiety, insomnia	Raw

' * ' mark in the columns indicates the species used by local people.

Fig. 2. Life form analysis of documented medicinal plants in the study area.

Fig. 3. Percentage of plant parts used for the preparation of medicine.

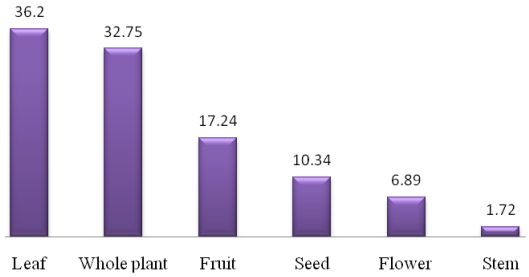
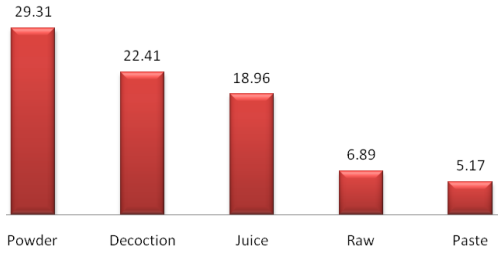


Fig. 4. Analysis of method of preparation and utilization of plant parts



Herbs were considered as a primary source of medicine (45%) followed by trees (26%), climbers (15%) and shrubs (14%) (Fig.2). It indicates that the study area contains more number herbs as compared to other life forms namely trees, shrubs and climbers. Among the reported plants, the leaves were mostly used for the preparation of medicine (36.20%) followed by whole plant (32.75%), fruit (17.24%), seed (10.34%), flower (6.89%) and stem (1.72%) (Fig.3). This may be due to the easy collection of leaves than that of other parts of plants such as underground parts, flowers, barks, flowers, fruits and seeds (9). Many local people throughout the world also use leaves for the preparation of herbal medicine. The mode of preparation and parts used were grouped into five categories (Fig.4). Of these, mostly used method of preparation was powder (29.31%) followed by decoction (22.41%), juice (18.96%), raw (6.89%) and paste (5.17%).

Out of 58 medicinal plants documented in the study area, only 27 plants have been used by local people inhabiting in around the study area for the treatment of various diseases like fever, intermittent fever, cough, asthma, jaundice, gastric problems, urinary disorders, dry skin disease, psoriasis, skin allergies, leucoderma, burning skin, liver disorders, snake bite, memory power, ulcer, diabetes, stomach aches etc., The plants were also to be used in different forms such as juice, decoction, powder and past (Table 1).

The list of following some medicinal plants documented from the study area viz., *Acalypha indica*, *Calotropis gigantea, Ocimum Sanctum, Lawsonia inermis, Solanum trilobatum* and *Clitoria ternatea* are commonly used by local people in the treatment of fever, cough and asthma. The following plant species such as *Acalypha indica, Aloe vera*, *Capsicum annuum*, *Carica papaya,Clitoria ternatea, Murraya koenigii and Ocimum sanctum* are also used for the treatment of dry skin disease, psoriasis, skin allergies, leucoderma, burning skin and other skin diseases.

4. CONCLUSION

The overall results of the present ethnobotanical investigation indicated that, the study area is rich in plants having ethno-medicinal properties that may be used to treat various diseases. But the results of present study also indicate that only less number (27) of medicinal plants is used by local people in the treatment of various ailments. Hence the people must be motivated to use more number of medicinal plants instead of using allopathic medicine and also suggested that priority must be given to conserve these medicinal plants for the welfare of humanity.

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RESEARCH ARTICLE

PRELIMINARY PHYTOCHEMICAL SCREENING AND HPTLC FINGER PRINTING ANALYSIS OF TRADITIONAL MEDICINAL PLANT *PUERARIA TUBEROSA* (ROXB. EX WILLD.)DC

Zereena Viji[1*] and S. Paulsamy[2]
[1]Department of Botany, NSS College, Nemmara, Palakkad - 678 508, Tamil Nadu, India.
[2]Department of Botany, Kongunadu Arts and Science College, Coimbatore - 641 029, Tamil Nadu, India.

ABSTRACT

The medicinal value of a plant lies in the phytoconstituents present in it. These phytochemical compounds form the base of modern drugs. The aim of the present study is to identify the phytochemical constituents present in the traditional medicinal plant *Pueraria tuberosa* and to develop HPTLC fingerprint profile of acetone extract. Preliminary phytochemical screening was done to identify the phytoconstituents and HPTLC studies were carried out. CAMAG make HPTLC system equipped with Linomat 5applicator, TLC scanner 3, Reprostar 3 and WINCATS-1.4.3 software were used. The present study revealed the presence of carbohydrates, proteins alkaloids, flavonoids, saponins, phenols and tannins in various extracts. The HPTLC fingerprint analysis of acetone extract of *Pueraria tuberosa* showed 10 peaks at 254nm. The components with Rf values 0.05, 0.21 and 0.72 were predominant with the percentage area of 34.52, 16.16 and 10.10 respectively. The preliminary phytochemical analysis revealed the presence of various phytochemicals, which were confirmed by the HPTLC fingerprint profile.

Keywords: *Pueraria tuberosa*, phytochemical analysis, HPTLC.

1. INTRODUCTION

Plants are endowed with various phytochemicals and secondary metabolites which include alkaloids, tannins, saponins, coumarins, glycosides etc. Products of primary metabolism like amino acids, carbohydrates and proteins are vital for the maintenance of life processes, while secondary metabolites like alkaloids, phenolics, steroids, and terpenoids are of toxicological, pharmacological and ecological importance (1). About three quarters of the world's population relies on plant products for good health care. People living in rural areas largely depend up on herbal remedies for the treatment of different types of diseases. *Pueraria tuberosa* is one such plant used in traditional medicine as fertility control agent, aphrodisiac, cardiotonic, diuretic, antihyperglycemic, anti hyperlipidemic etc. (2,3,4)

Pueraria tuberosa (Roxb.ex Willd.) DC belongs to Fabaceae family. It is an important plant used in Indian medicine, commonly called as Vidarikand or Indian Kudzu. The plant is described as rasayana and tonic in Ayurvedic Pharmacopoeia of India. The herb acts as rasayana and slows down the ageing process. It strengthens body and boosts its immunity. Kudzu is used for the treatment of dysuria, cough, rheumatism, erysipelas and malarial fever. The roots are used as a demulcent and refrigerant in fevers (5). The study by Nagendra Singh Chauhan *et al.* (6) provides evidence for the role of phytoestrogenic compounds from *Pueraria* function and testosterone production in male rats and thus adds to the evidence for its ethnopharmacological utilization as an Ayurvedic herb for improvement of sexual potency.

2. MATERIALS AND METHODS

For the present investigation the medicinal plant Vidari (*Pueraria tuberosa* (Roxb. ex Willd.) DC. (Fabaceae) was selected. The root tubers of *Pueraria tuberosa* were collected from Nelliampathy region of south Western Ghats during the month of April and identified. The collected samples were washed thoroughly in running tap water and shade dried under room temperature.

2.1. Preparation of extracts

The collected tubers were cut into small pieces, shade dried, powdered and extracted with organic solvents like petroleum ether, chloroform, acetone, methanol and hot water in the increasing order of polarity using a soxhlet apparatus for 8-10 hours. After each solvent extraction, the material was dried in hot air oven at 40°C. And all the extracts were dried under vacuum in a rotary evaporator at 40°C to pursue further analysis. The dried extracts were obtained and the percentage yield was expressed in terms of air dried weight of plant material. For further studies the evaporated extracts were dissolved in respective solvents at the concentration of 1mg/mL.

*Correspondence: Zereena Viji, Assistant Professor, Department of Botany, NSS College, Nemmara, Palakkad - 678 508, India.
E-mail: reenaravi1@rediffmail.com

2.2. Preliminary phytochemical analysis

The phytochemical test of these extracts was performed using the method adopted by Harborne (7) and Sofowora (8).

2.2.1. Test for Carbohydrates (Molisch's test)

To 2ml of plant extracts, 1ml of Molisch's reagent and a few drops of conc. sulphuric acid were added. Presence of purple or reddish colour indicates the presence of carbohydrates.

2.2.2. Test for Proteins (Ninhydrin test)

To the extract, 2 drops of freshly prepared 0.2% ninhydrin reagent was added and heated. The appearance of pink or purple colour indicates the presence of proteins

2.2.3. Test for Alkaloids (Mayer's test)

To 2ml of plant extract, 2ml of conc. hydrochloric acid was added. Then add few drops of Mayer's reagent presence of green colour or white precipitate indicates the presence of alkaloids.

2.2.4. Test for Flavonoids (Shinoda test)

To 2ml of plant extract, 1ml of 2N of sodium hydroxide was added. Presence of yellow colour indicates the presence of flavonoids.

2.2.5. Test for Saponins (Froth test)

To 2ml of plant extract, 2ml of distilled water was added and shaken in a graduated cylinder for 15 minutes length wise. Formation of a 1cm layer of foam indicates the presence of saponins.

2.2.6. Test for Tannins and Phenols (Ferric chloride test)

To 1ml of plant extract, 2ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

2.3. High Performance Thin Layer Chromatography - HPTLC Studies

HPTLC technique was carried out using the method of Harbone (9). HPTLC is an important tool in drug analysis. It has the ability to analyze several samples simultaneously with small quantity of mobile phase with precession and shorter time.

2.3.1. Sample preparation

The acetone tuber extract was dissolved in 1ml of acetone and centrifuged for about 5 minutes at 3000rpm, and this solution was utilized as test solution for HPTLC analysis

2.3.2. Developing solvent system

Mobile phase used was ethyl acetate - 80%, water-10%, acetic acid- 5% and formic acid- 5%.

2.3.3. Sample application

1µl of test solution was spotted on the form of band of 8mm length using Hamilton syringe on silica gel $60F_{254}$ (precoated on aluminium plate 10x10 cm) with the help of CAMAG LINOMAT 5 applicator which was programmed through WINCATS software.

2.3.4. Development of chromatogram

The chromatogram was developed in ascending order with CAMAG twin trough glass chamber (20x10cm) which was pre saturated with mobile phase for 15min.The length of each run is cm. The TLC run was performed under laboratory conditions of temperature 25±2 and humidity 60±5°C. The plates were air dried by hot air to evaporate solvents.

2.3.5. Photo documentation

The Reprostar 3 (CAMAG, Switzerland) was used for documenting and evaluating the planar chromatograms at UV 366 nm, UV 254 nm and in white light.

2.3.6. Scanning

After derivatization the plate was fixed in CAMAG TLC scanner 3 and scanning was done at UV254nm.The peak numbers with their height and area, peak display and peak densitogram and Rf values were programmed through WINCATS software 1.3.4 version.

3. RESULTS AND DISCUSSION

Phytochemicals are chemical compounds synthesized during various metabolic processes. These may possess a variety of pharmacological activities, some are found to have antimicrobial activity and serve as plant defense mechanisms against pathogenic organisms. The present study revealed the presence of carbohydrates, proteins alkaloids, flavonoids, saponins, phenols and tannins in various extracts (Table 1). In the root tubers of *Pueraria tuberosa* alkaloids were detected in all the fractions in *Pueraria tuberosa* root extracts carbohydrates, saponins and proteins were found only in methanol and hot water extracts. It was detected that methanol extract had higher number of secondary metabolites.

High performance Thin Layer chromatography (HPTLC) is most simple and reliable separation technique which gives better precision and accuracy at various steps. The acetone

root tuber extract of *Pueraria tuberosa* was subjected to high performance thin layer chromatography to analyse the fingerprint profile of secondary metabolites. The acetone extract of *Pueraria tuberosa* showed 10 peaks at 254nm in 2µl of the sample (Fig.1). Rf values ranged from 0.05 to 0.85. It was clear from Table 2 (Fig. 2 & 3), the components with Rf values 0.05, 0.21 and 0.72 were predominant as the percentage area is more with 34.52, 16.16 and 10.10 respectively. The photo documentation of acetone extract of *P. tuberosa*, observed at 254 nm and 366 nm is given (Fig. 2). The 3D densitogram of acetone extract at 254nm was given (Fig. 3).

Table 1. Phytochemical screening of various fractions of *Pueraria tuberosa* root tuber extracts

Fractions	C	P	A	F	S	T&P
Petroleum ether	-	-	+	-	-	+
Chloroform	-	-	+	+	-	+
Acetone	-	-	+	+	-	+
Methanol	+	+	+	+	+	+
Hot Water	+	+	-	+	+	+

C- Carbohydrates; P – Proteins; A – Alkaloids; F – Flavonoids; S-Saponins; T&P-Tannins & Phenols.
(+) – Presence (-) -Absence

Table 2. HPTLC –Flavonoid profile of acetone extract of root tuber of *Pueraria tuberosa*

Peak	Retention factor (Rf)	Height AU	Peak area AU	Area %
1	0.05	751.5	49956.4	34.52
2	0.14	283.2	10768.8	7.44
3	0.21	403.6	23388.5	16.16
4	0.37	109.0	3854	2.66
5	0.43	112.5	5039	3.48
6	0.51	229.5	7922.6	5.47
7	0.59	407.1	12381.3	8.56
8	0.66	364.1	9034.8	6.24
9	0.72	417.4	14615.8	10.10
10	0.85	226.2	7753.0	5.36

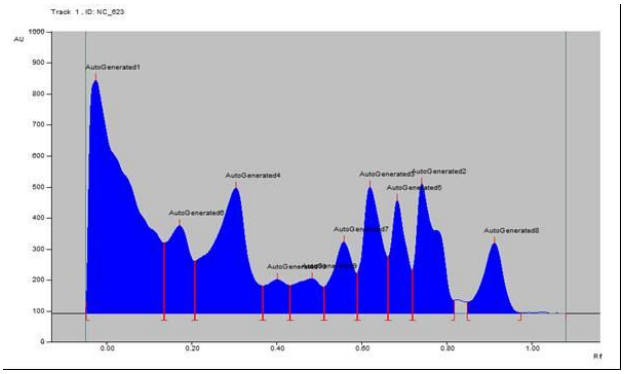

Fig. 1. Chromatogram for acetone solvent extract of *Pueraria tuberosa* at 254nm

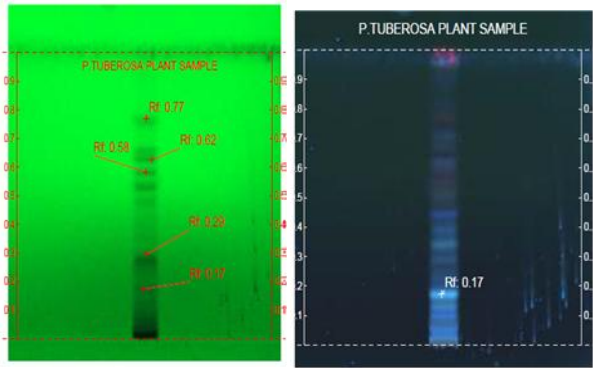

Fig. 3. 3D diagram of HPTLC densitogram for acetone solvent extract of *Pueraria tuberosa* at 254nm

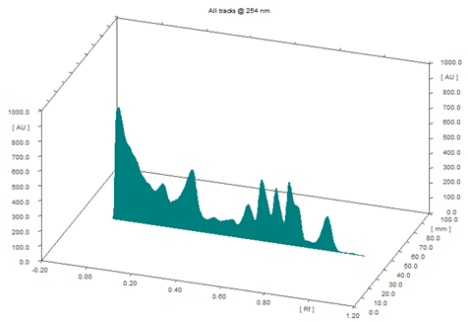

Fig. 3. 3D diagram of HPTLC densitogram for acetone solvent extract of *Pueraria tuberosa* at 254nm

Authentication of medicinal plants at chemical and genetic level is an imporant step for both research purposes and commercial preparations. A thorough understanding of the chemical composition is essential for conducting saftey assesment. HPTLC technique is an invaluable quality assesment tool for the analysis of broad number of compounds costeffectively and efficiently Prema *et al*. (10) has done the HPTLC fingerprint analysis of bark of *Stereospermum colais* which could be used as a diagnostic tool for the correct identification of the plant and also as a phytochemical marker and a good estimator of genetic variability in plant populations.

4. CONCLUSION

Herbal medicines are composed of many constituents and are therefore very capable of variation. Hence it is very important to obtain reliable chromatographic fingerprints that represent pharmacologically active and chemically characteristic components of the herbal medicine. HPTLC fingerprinting profile is very important parameter of herbal drug standardization for the proper identification of medicinal plants. It can serve as a tool for identification, authentication, and quality control of herbal drug. In the present study

preliminary phytochemical screening showed presence of alkaloid, flavonoids, tannin, and phenolic compounds. HPTLC chromatogram of acetone extract results showed that there are many compounds in *Pueraria tuberosa.* So it is established that the pharmacological activity shown by the study species is due to the cumulative effect of all the compounds in composite.

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RESEARCH ARTICLE

POLYAMINES: ROLE IN ATTENUATION OF HEAVY METAL TOXICITY

Barsha Majumder* **and Asok K. Biswas**
Plant Physiology and Biochemistry Laboratory, Centre of Advanced Studies, Department of Botany, University of Calcutta, Kolkata - 700 019, West Bengal, India.

ABSTRACT

Environmental changes resulted in a variety of stresses in plants of which heavy metal stress holds important position, affect the growth and development and trigger a series of morphological, physiological, biochemical and molecular changes in plants. When exposed heavy metal stress, the complex dynamic kinetics of polyamine biosynthesis was observed. Polyamines are small organic polycations present in all organisms and have a leading role in signaling, plant growth and development and deliver tolerance to a cultivar against stresses. High accumulation of polyamines (putrescine, spermidine and spermine) in plants during heavy metal stress has been well reported and is correlated with increased tolerance to different plants under stressed condition. Genetic engineering of polyamine biosynthetic genes in crop plants is the way to create resistance heavy metal toxicity.

Keywords: Heavy metal, Polyamine, GABA, Tolerance.

1. INTRODUCTION

Plants are sensitive towards a variety of environmental stresses throughout the life cycle which affect plant distribution, growth, development and productivity (1). Losses in the productivity of many of the agriculturally important crops are associated with the depletion in the economic returns of the country. Unprecedented bioaccumulation and biomagnification of heavy metals (HMs) in the environment have become a dilemma for all living organisms including plants. There are several natural ways of self-defense in the plants to cope with these stressful conditions: they can induce several functional or regulatory genes (2) or can undergo different physiological or biochemical changes. The accumulation of some functional substances is an important element of the physiological and biochemical response of plants to the stressful conditions (3,4).

Polyamines (PAs) are small, positively charged, low molecular weight, N-containing polycations found in all living organisms (5). They are known to be essential for growth and development in prokaryotes and eukaryotes (6). In plant cells, the diamine putrescine (Put), triamine spermidine (Spd) and tetramine spermine (Spm) constitute the major PAs. The total PA concentration and the ratios between individual PAs vary markedly. The characteristic feature of PAs structure is that they have methylene groups, participate in hydrophobic interactions, thereby influencing PAs activity. Polyamines are well known for their anti-senescence and anti-stress effects due to their acid neutralizing and antioxidant properties, as well as for their membrane and cell wall stabilizing abilities (7). Furthermore, it has been noted that genetic transformation with polyamine biosynthetic genes encoding arginine decarboxylase (ADC), ornithine decarboxylase (ODC), *S*-adenosylmethionine decarboxylase (SAMDC) or Spd synthase (SPDS) improved environmental stress tolerance in various plant species (3).

In this review, I tried to summarize the knowledge that has been gathered over the last couple of decades concerning the changes in polyamine metabolism (biosynthesis, catabolism and regulation) in plants under heavy metal toxicity.

2. POLYAMINE BIOSYNTHESIS

The PA biosynthetic pathway in plants has been thoroughly investigated (6,8). The biosynthesis of polyamines in plants has been well described (Fig. 1). Put is produced either directly from ornithine by ornithine decarboxylase (ODC, EC 4.1.1.17) or indirectly from arginine by arginine decarboxylase (ADC, EC 4.1.1.19) with two intermediates, agmatine and *N*-carbomoylputrescine, and two corresponding biosynthetic enzymes, agmatine iminohydrolase (EC 3.5.3.12) and *N*-carbamoylputrescine amidohydrolase (EC 3.5.1.53) (8,9). Put is converted into Spd via spermidine synthase (SPDS, EC 2.5.1.16) with the addition of an aminopropyl moiety provided by decarboxylated *S*-adenosylmethionine (dcSAM), which is catalyzed by *S* adenosylmethionine decarboxylase (SAMDC, EC 4.1.1.50) using *S*-adenosylmethionine (SAM) as the substrate. Similarly, Spm is produced from Spd via spermine synthase (SPMS, EC 2.5.1.22) with the same aminopropyl moiety rendered by dcSAM.

*Correspondence: Barsha Majumder, Plant Physiology and Biochemistry Laboratory, Centre of Advanced Studies, Department of Botany, University of Calcutta, Kolkata - 700 019, West Bengal, India. E-mail: barshamjmdr@gmail.com

Apart from biosynthesis, polyamine degradation plays an important role in the regulation of cellular polyamine titers, which is primarily ascribed to two amine oxidases, diamine oxidase (DAO, EC 1.4.3.6) and polyamine oxidase (PAO, EC 1.5.3.11). DAO catalyzesthe oxidation of Put to give pyrroline, which is further metabolized to gamma-aminobutyric acid (10) and PAO catalyzes the conversion of Spd and Spm to pyrroline and 1-(3-aminopropyl)-pyrroline, respectively, along with 1,3-diaminopropane in plants (8).

The biosynthesis of PAs and ethylene share a common precursor in S-adenosylmethionine (AdoMet). Although there is much evidence to suggest that increased biosynthesis of either PAs or ethylene can inhibit synthesis of the other, this is still a contentious issue. It is also important to note that AdoMet is a methyl donor for a variety of transmethylation reactions (5,11).

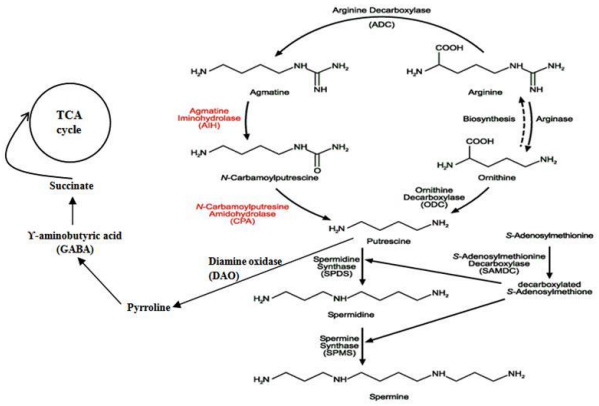

Fig. 1. Schematic representation of the pathway of polyamine-GABA biosynthesis

3. ROLE OF POLYAMINE IN PLANTS

In plant cells, the diamine putrescine (Put), triamine spermidine (Spd) and tetramine spermine (Spm) constitute the major PAs. Cadaverine is also present in legumes. These occur either in the free form or as conjugates bound to phenolic acids and other low molecular weight compounds or to macromolecules such as proteins and nucleic acids owing to their positive charge (12). Besides stimulating DNA replication, transcription and translation, they have contributed to various biological processes in plant growth, embryogenesis, organ development, leaf senescence, stress response (13). Plant polyamines also contribute towards several characteristics of agro-economical importance, such as phytonutrient content, fruiting and fruit quality, vine life, flowering and carnation of plants (14). Some of the observations suggest that PAs can act by stabilizing membranes, scavenging free radicals, affecting nucleic acids and protein synthesis, RNAse, protease and other enzyme activities, and interacting with hormones, phytochromes and ethylene biosynthesis (15,16). Because of these numerous biological interactions of PAs in plant systems, it has been difficult to determine their precise role in plant growth and development (12). PAs are involved in many plant developmental processes, including cell division, embryogenesis, reproductive organ development, root growth, floral initiation and development, fruit development and ripening as well as leaf senescence and in stress management (10,17-19) . It has been observed that cells undergoing division (apical shoots, meristems, flowers, etc) contain higher levels of PAs whereas cells undergoing expansion and elongation contain lower levels of PAs synthesized via ADC (12). Plants are exposed to continuous and rapid changing environmental factors (biotic and abiotic) such as light, temperature, water, nutrient availability and water. These have a major impact on plant growth and productivity. PAs play an important role in heavy metal stress tolerance as briefly discussed below:

4. ROLE OF POLYAMINES IN HEAVY METAL STRESS MANAGEMENT

Most recent reports on plant responses to As, Cu, Cd, Cr, Al, and other heavy metals have focused on the changes in the activities of antioxidant enzymes and more efforts are needed to identify the physiological and molecular significance of PAs in plant heavy metal tolerance (20). Wen *et al.* (21) have recently demonstrated Spd synthase overexpressing transgenic European pear showed tolerance to HMs by exerting antioxidant activities. Heavy metal toxicity leads to oxidative stress, resulted in production of reactive oxygen species such as superoxide free radicals ($O2^{\cdot-}$), hydroxyl free radicals ($OH^{\cdot-}$), or non-free radical species (molecular forms) such as singlet oxygen ($O2^{*}$) and hydrogen peroxide (H_2O_2) as well as cytotoxic compounds like methylglyoxal (MG). PAs are known to have a function in oxidative stresses. The antioxidative effect of PAs is probably due to a combination of their anionic and cationic-binding properties in radical scavenging, inhibiting properties of lipid peroxidation. Phenylpropanoid-PA conjugates can act as antioxidants against ROS and reactive nitrogen species in response to stress conditions (22). Inhibition of DNA oxidative degradation by OH^{-} in the presence of Spm in *Mesembryanthemum crystallinum* proved the efficiency of PAs to function as scavengers of free radicals. So, it may be concluded that plants not only accumulate free PAs to function as scavengers of free radicals, but also produce their conjugates which function as more efficient antioxidants also (23,24). ADC and ODC activity was increased in copper

stressed wheat (*Triticum aestivum*) plant led to an increment in put contents, suggesting that polyamines perform a pivotal function in heavy metal stress alleviation.

5. CONCLUSION AND FUTURE OUTLOOK

Heavy metal stresses including the global warming are negatively affecting the plant productivity worldwide. Soil and water contamination by HMs in changing environment poses a serious threat to public and food safety and is now emerging as a major health hazard to humans and plants. On the other hand the demand for food is expected to grow as a result of population growth and rising incomes. It is necessary to obtain stress-tolerant varieties to cope with this upcoming problem of food security. The involvement of PAs in regulation of various cellular processes including growth, development and stress tolerance in plants might have general implications. As much as it is apparent that plants with high PA contents (due to exogenous supply or endogenous production via genetic manipulation) can tolerate short term exposure to a multitude of stress factors, only a handful of studies on the survival and yield (fresh or dry biomass of usable product) in these plants under prolonged stress conditions or repeated exposure to the same stress, have been reported. Most importantly, no viable plant variety has yet been created or selected based upon genetic modification of PAs either via breeding or via transgene expression, which could be evaluated in comparison with other varieties showing similar characteristics. The knowledge gained so far about plant PAs has built a strong case for further studies towards careful analysis of the genes involved in abiotic stress tolerance.

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Abbreviations

ADC-Arginine decarboxylase; AdoMet-S-adenosylmethionine; DAO-Diamine oxidases; dcSAM-decarboxylated *S*-adenosylmethionine; HMs-Heavy metals; ODC-Ornithine decarboxylase; PAs-Polyamines; PAO-Polyamine oxidases; Put-Putrescine; ROS-Reactive oxygen species; SAM-S-Adenosylmethionine; SAMDC-SAM decarboxylase; Spd-Spermidine; Spm-Spermine; SPDS-Spermidine synthase; SPMS-Spermine synthase.

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REVIEW ARTICLE

SEED PRIMING: A MULTIFACETED AND COST-EFFECTIVE TECHNIQUE TO IMPROVE CROP PRODUCTION

Sabarni Biswas, Alivia Paul and Asok Kumar Biswas*
Plant Physiology and Biochemistry Laboratory, Centre for Advanced Study, Department of Botany, University of Calcutta, Kolkata - 700 019, India.

ABSTRACT

Seed priming is a cost-effective technique which involves prior seed exposure to an abiotic stress that makes the seed more resistant to future lethal exposure. Seed priming stimulates pre-germination metabolic processes and makes the seed ready for sprouting. It helps to up regulate the antioxidant enzyme activities and repairs membrane damage. These changes promote seed vigour and emergence under abiotic stress. This article aims to review the different priming processes as well as the physiological, biochemical and molecular changes induced by priming that lead to synchronized seed germination. Plants' responses to some priming agents under abiotic stress have been reported based on recent investigations.

Keywords: Seed priming techniques, seed germination, abiotic stress, crop productivity.

1. INTRODUCTION

Inspite of encountering multiple biotic and abiotic obstacles, plants being sessile, cannot escape such unfavourable conditions. This results in their retarded growth and productivity. Crop production is highly dependent on the use of high quality seeds. Rapid germination and sprouting of seeds help in determination of potential crop yield. Longevity of crops depends upon seed quality and vigour. Therefore, various seed priming or seed invigoration techniques can be adopted for improving crop productivity worldwide. Seed priming refers to pre-sowing treatments in water or in osmotic solution that allows seed to imbibe water or an osmotic solution to progress towards germination whereas seed invigoration not only helps to hasten germination and synchronize emergence but also enhances seed storability. Seed priming is an effective seed invigoration method adopted during the last twenty years to improve agronomic productivity worldwide. The controlled process of hydration that involves exposure of seeds to low water potentials which permits pre-germinative physiological and biochemical alterations resulting in better stand establishment and yield is called seed priming (1).

Several literatures reveal that seed priming advances germination and improves seed quality characters which lead to better establishment and increase crop yield in diverse environments. Seed priming is also known to alleviate ageing-induced deleterious events by improving seed performance (2). Seed priming has been employed for a wide range of crops expecting their increased crop stand and output. The beneficial effects have also been demonstrated for many food crops like sweet corn, wheat, mungbean, barley, lentil, cucumber, etc. (3). Priming resulted in better establishment, growth, earlier flowering, increased seed tolerance to adverse environment in maize (4). This review aims to focus on the different priming processes as well as the physiochemical and molecular changes induced by priming that lead to synchronized seed germination promoting better crop stand and yield.

2. PRIMING TECHNIQUES

Various priming techniques have been developed with an intention to boost crop production on a large scale which is necessary for feeding the increasing population worldwide. Windauer *et al.* (5) proposed different seed invigoration and seed priming techniques, viz., hardening, osmohardening, hydropriming, hormonal priming etc.

Hydropriming refers to the soaking of seeds in water prior to seed sowing. Hydropriming of wheat kernels has been reported to improve seed germination rate under saline conditions (6). Using hydropriming technique reported improved seed germination and seedling growth in wheat (*Triticum aestivum*) seeds under both saline and drought stressed conditions (7). Dastanpoor *et al.* (8) also reported that hydropriming improved germination percentage and mean germination time under varying temperature conditions in common sage (*Salvia officinalis*). Thus, this technique not only enhances seed germination and seedling emergence under saline condition but also has beneficial enzyme stimulatory activity that is essential for quick seed sprouting.

*Correspondence: Asok Kumar Biswas, Department of Botany, Department of Botany, University of Calcutta, Kolkata-700019, India. E-mail: asokkbiswas16@gmail.com

Halopriming refers to the treatment of seeds with salt in order to improve germination and decrease salt intolerance. Numerous studies have shown that halopriming of seeds resulted in improved seedling germination, growth, establishment and productivity. Seed treatment with $CaCl_2$ or KNO_3 resulted in improved accumulation of amino acids and proteins in pigeon pea (*Cajanus cajan*) (9), improved activity of amylases and proteases in germinating sorghum (*Sorghum bicolour*) seeds under salt stress. Priming of seeds with NaCl and KCl was effective in removal of deleterious effects of salts in wheat (*Triticum aestivum*) seedlings (10). NaCl primed hot pepper (*Capsicum annum*) seeds showed better performance than non-primed seeds at salinity levels 3, 6 and 9 dSm^{-1} by exhibiting improved seedling vigour and stand establishment under salt- stressed conditions (11). Preconditioning of mungbean (*Vigna radiata*), pigeon pea (*Cajanus cajan*) and blackgram (*Vigna mungo*) seeds with sublethal dose of NaCl (50mM) has also been reported to show improved seedling vigour and growth upon germination under lethal levels of NaCl (50mM, 100mM, 150mM) (12,13). Evidences of reduced DNA damage and improved respiratory cycle in NaCl pretreated mungbean seedlings have also been reported from our laboratory (14,15).

Hormonal priming is the pretreatment of seeds with different plant growth regulators i.e. salicylic acid, kinetin etc., which promotes growth and development of seedlings (16). Ashraf *et al.* (3) stated that gibberellic acid pretreated seeds showed better vegetative growth in wheat (*Triticum aestivum*), increased photosynthetic activity under salinity. Hussein *et al.* (17) speculated that salicylic acid pretreated maize (*Zea mays*) plants showed better growth performance under saline conditions.

Osmopriming technique involves seeds soaking of seeds in sugar solution, polyethylene glycol, glycerol, sorbitol or mannitol followed by air drying before sowing. Rehman *et al.* (18) reported that priming of seeds with low concentration boric acid that significantly increased seedling growth and parameters. Seedling growth in wheat was also found to improve by osmopriming (19).

3. PHYSIOCHEMICAL AND MOLECULAR ALERATIONS INDUCED BY SEED PRIMING

Seed priming triggers pre-germination metabolic processes and prepares seeds for radicle protrusion (20). It lowers resistance provided by the endosperm during water uptake, repairs membrane damage and eliminates germination inhibitors leading to the development of immature embryos (21,22,23). The imbibition phase and lag phase of water absorption are decreased when the primed seeds are allowed to germinate (11). Consequently, the seedlings develop faster, grow more vigorously, and perform better under adverse conditions (24) which is exhibited from their synchronized germination with certain physiological, biochemical, cellular and molecular changes (25,26,27). In addition, priming may generate moderate abiotic stress during procedural steps (28) which becomes beneficial for the seeds to withstand future abiotic stresses during seedling establishment (29). Improved stress tolerance of germinating seeds is achieved via two strategies. Firstly, seed priming triggers enhanced energy metabolism, early reserve mobilization, embryo expansion, and endosperm weakening (30,31,32) which hasten the transition of quiescent dry seeds into a germinating state. Secondly, priming generates an abiotic stress that represses radicle emergence, but stimulates cross-tolerance. These generate a 'memory response' in seeds, which can be recalled upon by the primed seeds later during stress exposure mediating greater stress tolerance in germinating primed seeds (33,34). Priming increases the activity of enzymes involved in metabolism of carbohydrates (α and β amylases), proteins (proteases) and lipids mobilization (isocitrate lyase) that are crucial for mobilization of stored reserves (25). Plants overcome salinity-induced osmotic effects through accumulation of inorganic ions or by synthesis of compatible solutes. Accumulation of these organic solutes is an essential mechanism of salt tolerance in plants that causes reduction of cell osmotic potential and allows an osmotic adjustment to stress (35). Seed priming ameliorates the adverse effects of salinity stress by promoting K^+ and Ca^{2+} accumulation and decreasing Na^+ and Cl^- accumulation in plants (10,36,37). This decreases the osmotic potential of plant and increases water uptake (38).

The antioxidant system is one of the major defense systems in seeds. Cells contain many enzymatic and non-enzymatic antioxidants that help in the scavenging of ROS and the protection of the seed (39,40). Priming treatments increase the activities of antioxidant enzymes, *viz.,* catalase, peroxidase and superoxide dismutase (41,42) and antioxidant compounds, *viz.,* ascorbic acid and reduced glutathione (43,44). These changes can optimize defense mechanisms during seed germination through a decrease in H_2O_2 production (41). In addition, multiple antioxidant enzymes are involved in the scavenging of ROS (45). Seed priming strategies decrease malondialdehyde accumulation under salt stress condition in *Allium fistulosum* seedlings (25,46).

Priming of seeds promote changes in cell division patterns, plasma membrane fluidity, induces stress-related proteins viz. heat-shock proteins and late embryogenesis abundant proteins which brings about changes in transcriptome as well as proteome, proton pump activity (47-49) and changes antioxidant enzyme activities (39). Enhanced progress towards germination in primed seeds is associated with an increased protein synthesis capacity, post-translational processing ability and targeted proteolysis (49). Earlier reports reveal that priming permits early DNA repair and replication, *de novo* synthesis of RNA and protein and reduces the leakage of metabolites. Many germination related genes are up-regulated during priming (42). Seed priming reprograms gene expression for antioxidant synthesis and defends the cells against oxidative damage and lipid peroxidation (49,50).

4. CONCLUSION

Seed priming is a commercially used technique developed mainly to hasten germination and minimize adverse environmental effects. It is a cost-effective technique that helps to solve germination related problems effectively especially under unfavourable conditions. Many priming techniques have been evolved now-a-days which are being utilized in many crops. Thus, seed priming is a process that improves seed performance under abiotically stressed conditions. Seed priming prior to germination helps to strengthen the defense mechanisms in seeds on exposure to lethal levels of salinity stress. Seeds develop a kind of 'memory response' that provokes greater stress tolerance of germinating primed seeds. Little work has been done adopting priming techniques in a limited variety of plants; further research needs to be carried out to standardize the concentration of priming agents that can successfully enhance seed germination and seedling growth of crops under abiotic stress. Future research should focus on understanding the basis of salt tolerance in primed seeds in relation to physiological, molecular, and metabolic pathway alterations.

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RESEARCH ARTICLE

INDIRECT ORGANOGENESIS OF *BOUCERSIA PROCUMBENS* (GAMBLE) PLOWES - A RARE SUCCULENT PLANT

Karuppusamy, S. *
Department of Botany, Centre for Botanical Research,
The Madura College (Autonomous), Madurai – 625 011, Tamil Nadu, India.

ABSTRACT

Efficient protocols of callus culture indirect organogenesis were established for mature internodal segments of *Boucerosia procumbens* (Asclepiadoideae). When MS medium was supplemented with different concentration of auxins, the texture of the callus varied according to the nature of auxin. Optimum callus was developed on MS medium supplemented with 3mg/l IAA. Best response (65%) of callus proliferation was obtained when MS medium fortified with 2iP 2mg/l + Zeatin 0.5 mg/l. IAA was most effective in producing the highest frequency of organogenic culture. Regeneration of callus into plantlets could not be achieved in the present study. The regenerated shoots were rooted on half strength MS medium fortified with 0.1 mg/l NAA. 57% of the rooted shootlets survived in the field.

Keywords: Indirect organogenesis, high frequency callus, *Boucerosia procumbens*.

1. INTRODUCTION

Boucerosia Plowes (Section *Caralluma*; Apocynaceae; Asclepiadoideae) is a genus of succulent herbs, which comprises about 200 genera and 2500 species world over. Out of 18 species reported from India, 5 species and 7 varieties are endemic to Peninsular India (1). *Boucerosia procumbens* is one such species which is rare and endemic to Southern Peninsular India. *Boucerosia procumbens* is growing wild on rocky areas in Maruthuvamalai hills of Kanyakuamri district, Tamil Nadu, India. No chemical investigation has been reported on this species, but plants belonging to this genus are rich in esterified polyhydroxy pregnane glycosides, some of which showed antitumor activity and others were postulated as precursors of cardenolides (2,3). Most of the species from this genus is also characterized by the presence of flavone glycosides (4,5). Young shoots of *Boucerosia* though very bitter, are commonly eaten raw in South India. But *Boucerosia procumbens* are particularly liked, for instead of being bitter they are pleasantly acid to the taste. Due to endemic nature of this plant, it has become imperative to establish a suitable protocol for its micropropagation. Present investigation therefore aims to develop a rapid and high frequency shoot regeneration system from shoot tip of *Boucerosia procumbens* for providing continuous supply of a better source of elite plants to be used as standard material.

2. MATERIALS AND METHODS

Fresh young and juvenile shoots of *Boucerosia procumbens* were collected from Maruthuvamalai hills, Kanyakumari District, Tamil Nadu. These shoots were washed thoroughly under running tap water to remove dust particles. The explants were then surface disinfected by agitating gently in 2% Tween-20 (v/v) for 15 minutes and washed in running tap water. Then the explants were taken inside the inoculation chamber for further sterilization. The materials were kept in 70% ethanol for 60 seconds, followed by repeated washings (3-4 times) in sterilized distilled water. These were then surface sterilized with 0.1% mercuric chloride for 4 minutes followed by 3-4 with sterile distill water.

Different types of media were used in the present study such as Murashige and Skoog's (MS) medium (6), B_5 medium (7) and Woody Plant medium (8). The culture medium consisted of MS salts supplemented with 2% (w/v) sucrose and various auxins (2,4-D, 2,4,5-T, NAA, IAA, IBA) and cytokinins(BAP, KN, 2iP,and Zeatin) at appropriate concentrations both individually and in combinations. All plant growth regulators were added to the medium before autoclaving. The pH of the medium was adjusted to 5.8 and autoclaved at 108KPa and 121^{o}C for 15 minutes. A quantity of 20 ml medium was dispensed in culture tubes closed with aluminium foil. All the cultures were maintained at a temperature of 25±2^{o}C under a light intensity of 2000lux provided by cool white fluorescent lamps. Subculturing was periodically carried out at 4 week interval. The nature and percentage of response were also recorded at an interval of 4 weeks. The regenerated shoots were rooted on half strength MS medium supplemented with different concentrations of auxins (IAA, IBA, and NAA).

*Correspondence: Dr. S. Karuppusamy, Assistant Professor, Department of Botany, Centre for Botanical Research, The Madura College (Autonomous), Madurai – 625 011, Tamil Nadu, India. E.mail: ksamytaxonomy@gmail.com

All the experiments were repeated thrice with 15 replicates each. The data was statistically analyzed using one way analysis of variance and means were compared using the Tukey test at the 0.05% level of significance.

3. RESULTS AND DISCUSSION

3.1. Medium Evaluation

Various types of media such as MS, B_5 and WPM fortified with optimum concentration of 2,4-D 3mg/l and 2% sucrose were tested for callus induction of mature internodal segments of *Boucerosia procumbens*. Among the 3 different media tested, MS was found to be the best basal medium with maximum fresh and dry weights of 614.00 ±81.10 mg and 33.50 ±1.22 mg respectively (Table 1). Internodes failed to induce callus on any media with out auxin but remained green and fresh. MS medium was found to be effective for *in vitro* induction of callus in several other Asclepiadaceae members such as *Decalepis hamiltonii* (9), *Leptadenia reticulata* (10), *Gymnema sylvestre* (11) and *Holostemma ada-kodien* (12).

3.2. Callus studies

The texture of the callus varied according to the nature of auxin. The internodal segments of *Boucerosia procumbens* cultured on MS medium at lower concentration (0.1mg/l) of 2,4-D, 2,4,5-T, NAA, IBA and IBA had failed to respond. When 2,4,5-T fortified individually with MS medium embryogenic callus produced. The texture of callus varied when the internodal segments cultured on MS medium fortified with 2,4,5-TP (0.1- 7mg/l) at 0.1 mg/l the texture of callus was white friable (Fig. 1a), at 2mg/l and 3mg/l green nodular callus was observed. At all concentrations of 2,4-D yellow compact callus was observed. Maximum percent of response (80%) was observed at 3 mg/l IAA and the nature of callus is nodular yellow (Fig.1 e). Callus developed on MS medium fortified with NAA was embryogenic and pale green in color. IBA gave the lowest and slowest callus production.

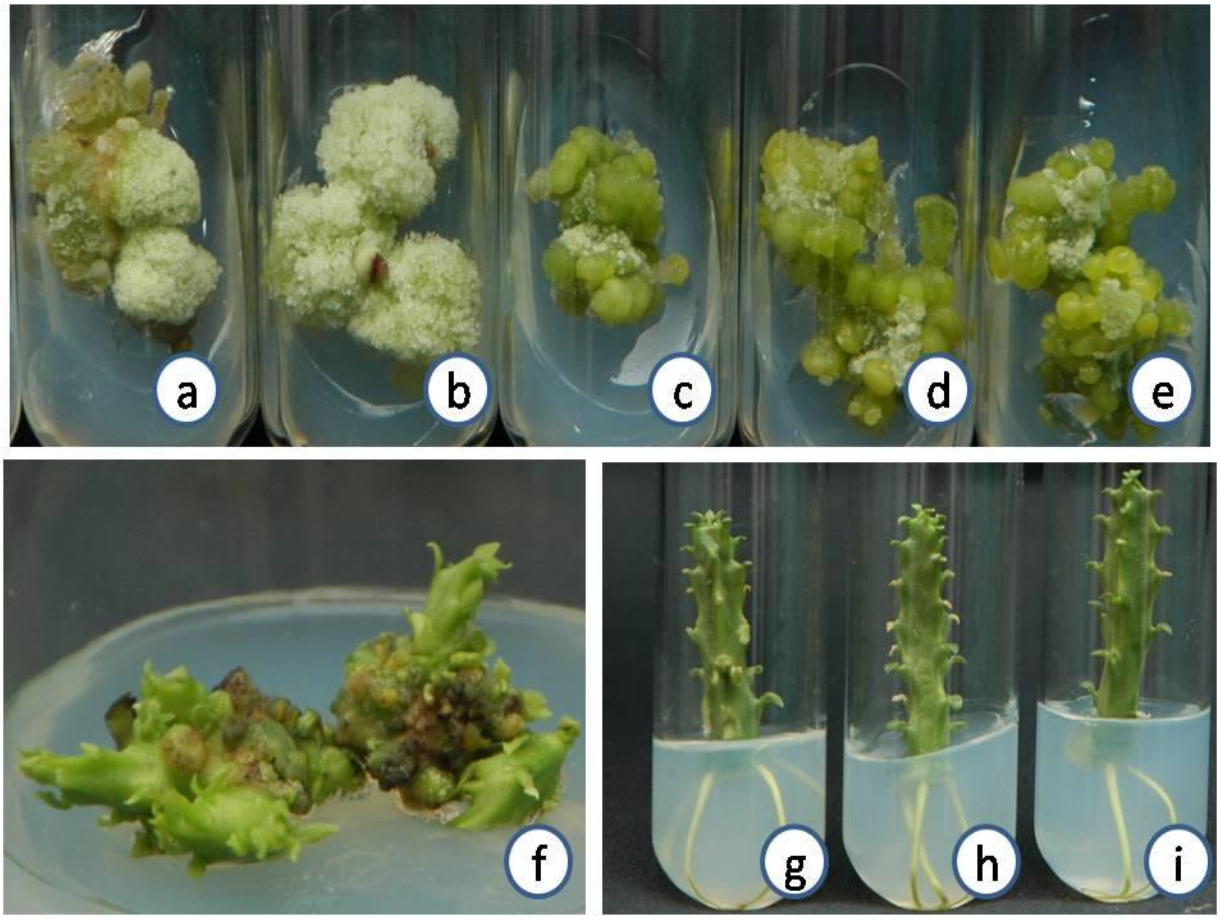

Fig. 1. Callus types of *Boucerosia procumbens* internode culture on MS medium containing various types of auxins. a & b. Friable callus; c-d. Nodular callus; f. Callus regeneration; g-i. *In vitro* rooting of regenerated shootlets on NAA containing medium.

Table 1. Effect of various types of media on callus induction from Internodal mature explants of *Boucerosia procumbens* cultured with 3mg/l 2,4-D.

Plant growth regulator (mg/l)	Type of medium	Internode		
		% of response	Fresh weight (mg) Mean ± SE	Dry weight (mg) Mean ± SE
2,4-D 3mg/l	MS	73	614.00 ± 81.10[a]	33.50 ± 1.22[a]
	B_5	62	420.00 ± 42.36[ab]	31.10 ± 0.84[ab]
	WPM	61	304.00 ± 45.25[b]	23.10 ± 0.87[b]

Means followed by the same letter not significantly different by the Tukey test at 0.05% probability level; NR-No Response

Table 2. Effect of various auxins on callus induction from mature internodal explants of *Boucerosia procumbens* cultured on MS medium.

Auxins	Conc. (mg/l)	Internode		
		% of response	Degree of callusing	Nature of response
2,4-D	0.1	0	-	NR
	1.0	30	+	YCC
	2.0	50	++	YCC
	3.0	75	+++	YCC
	5.0	60	++	YCC
	7.0	40	+	YCC
NAA	0.1	0	-	NR
	0.5	20	+	BCC
	1.0	40	++	PGEC
	2.0	55	+++	PGEC
	3.0	70	+++	PGEC
	5.0	60	+++	PGEC
IAA	0.1	0	-	NR
	0.5	10	+	BCC
	1.0	25	++	BCC
	2.0	40	++	WFC
	3.0	60	+++	WFC
	5.0	45	++	BFC
IBA	0.1	0	-	NR
	0.5	10	+	BCC
	1.0	35	++	WFC
	2.0	45	++	WFC
	3.0	55	+++	WFC
	5.0	40	++	BFC

YCC - Yellow compact callus; WEC - White embryogenic callus; BCC - Brown compact callus; WFC - White friable callus; NYC - Nodualr yellow callus; GNC - Green nodular callus; GCC - Green compact callus; PYEC - Pale yellow embryogenic callus; BEC - Brown embryogenic callus; PGNC - Pale green nodular callus; PGEC - Pale green embryogenic callus; BFC - Brown Friable callus; NR - No Response; + Scanty; ++ Less; +++ Moderate; ++++ Profuse; Experiments were repeated thrice with 15 replicated each.

3.3. Indirect organogenesis

The different growth regulators inducing the callus exhibited a significant influence on organogenesis. Callus obtained on MS medium fortified with IAA 3mg/l was selected for morphogenesis. The callus was subcultured on MS medium supplemented with different concentrations of cytokinins. Shoot buds are initiated from the surface of callus within 6 weeks of culture (Fig. 1g). Among the various concentrations of cytokinins tested, the highest shoot regeneration frequency (65%) and highest number of shoots (2.20 ± 0.20) were recorded at 2iP 2mg/l + Zeatin 0.5 mg/l (Table 3). Auxins and cytokinins are able to bring shoot or root formation from callus but the effective concentrations of these regulators may vary. Effectiveness of each treatment generally depends on the nature and origin of the explant, its endogenous hormone content and the conditions used for *in vitro* culture (13).

Table 3. Effect of various plant growth regulators on morphogenic response of callus induced from internodal mature explants of *Boucerosia procumbens* cultured on MS medium.

Plant growth regulator used for the callus induction	Media used for morphogenesis				Type of explant (leaf)	
	BAP	2iP	KN	Zeatin	% of response	No. of shoot buds / explant Mean + S.E
IAA 3 mg/l	0.1	-	-	-	0	NR
	0.5	-	-	-	15	0.60 ± 0.24[b]
	1.0	-	0.1	-	40	1.60 ± 0.25[ab]
	2.0	-	0.5	-	60	1.80 ± 0.20[ab]
	-	0.1	-	-	0	NR
	-	0.5	-	-	20	1.20 ± 0.20[b]
	-	1.0	-	0.1	50	1.60 ± 0.25[ab]
	-	2.0	-	0.5	65	2.20 ± 0.20[a]

Means followed by the same letter not significantly different by the Tukey test at 0.05% probability level; NR-No Response

3.4. Rooting and plantlet establishment

The shoots were regenerated from callus which are excised and transferred to half strength MS medium fortified with auxins for *in vitro* rooting. Half strength MS medium supplemented with auxins at different concentrations showed varied effect on *in vitro* rooting (Table 4). Maximum number of roots was observed on NAA0.1mg/l, 3.4 ± 0.13 roots per shoot with 2.08 ± 0.14 cm of root length (Fig. 1 h). Increase in concentration of NAA decreases the root number and length. With IBA treatments rooting was very slow and less effective. Lower concentration of IAA failed to induce rooting. In *Boucerosia procumbens* root formation is much better with NAA

than IAA and IBA. It was also proved in other Asclepiadaceae members such as *Decalepis hamiltonii* (9) and *Decalepis arayalpathra* (14).

Rooted plantlets were taken out carefully from culture tube and washed thoroughly to remove all the traces of agar. These plantlets were transferred to paper cups pots containing sterilized peatmoss and sand (3:1) and covered with polythene bags. Potted shootlets were first placed in culture room at 25±2°C, 16h photo period and 85% relative humidity. The potted plants were irrigated with MS basal salts solution (1/4 strength) devoid of sucrose every 5 days for 3 weeks. The hardened plants were transferred to earthen pots and kept under shade and finally acclimatized. Nearly 57% plants of *Boucerosia procumbens* were successfully acclimatized to field conditions.

Table 4. Effect of various auxins on rooting response from *in vitro* regenerated shoots of *Boucerosia procumbens* cultured on MS half strength medium after 30 days.

Auxins (mg/l)	% of response	No. of roots / shoots Mean ± SE	Length of roots (cm) Mean ± SE	Degree of callusing
IAA 0.1	-	NR	NR	-
IAA 0.5	20	1.06 ± 0.11^{c}	0.50 ± 0.05^{d}	-
IAA 1.0	40	1.86 ± 0.21^{bc}	1.70 ±0.08^{ab}	-
IBA 0.1	-	NR	NR	-
IBA 0.5	35	1.13 ± 0.16^{c}	1.02 ± 0.14^{cd}	-
IBA 1.0	20	0.73 ± 0.11^{c}	0.58 ± 0.05^{d}	-
NAA 0.1	75	2.06 ± 0.24^{a}	2.14 ± 0.14^{a}	-
NAA 0.5	60	1.13 ± 0.21^{b}	1.68 ± 0.08^{b}	-
NAA 1.0	50	1.20 ± 0.14^{c}	0.16 ± 0.09^{c}	+

Means followed by the same letter not significantly different by the Tukey test at 0.05% probability level; NR-No Response

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RESEARCH ARTICLE

DIVERSITY AND CONSERVATION OF PLANT RESOURECES OF TIRUMALAIAH GUTTA SACRED GROVE, WANAPARTHY, TELANGANA, INDIA

Sadasivaiah, B.[1]*, M. Sharath Goud[1], R. Devilal[1], M. Laxmikanth[1], A. Narasimha[1], M. Uday Kumar[1], A. Ramakrishna[1], L. Paramesh[1], P. Bharath Simha Yadav[1], B. Raghavendra[1], G. Janaki[1] and M. Sridhar Reddy[2]

[1]Department of Botany, Government Degree College (Men), Wanaparthy – 509 103, Telanagana
[2]Department of Environmental Science, Yogi Vemana University, Kadapa, Andhra Pradesh.

ABSTRACT

Tirumalaiah Gutta sacred grove is situated near Wanaparthy, Telanagana with dry deciduous and scrub forests and huge rock boulders. The study yields a total of 467 taxa belonging to 283 genera and 81 families. Of the 467 taxa, 332 are dicots, 129 are monocots and 6 are pteridophytes. Of the 81 families, Poaceae is the largest family with 77 taxa, followed by Fabaceae (51), Cyperaceae (25), Asteraceae (24), Rubiaceae (19) and Acanthaceae (18). A total number of 34 endemic taxa at different levels are recorded of which, *Alysicarpus mahabubnagarensis* is endemic to Mahabubnagar district, *Chryopogon velutinus* is endemic to Kadapa district of Andhra Pradesh, Rathnagiri hills of Maharashtra and Wanaparthy district of Telangana; *Euphorbia senguptea* and *Rostellularia vahlii* var. *rupicola* are endemic to Eastern Ghats. From the inventory it has been resulted in a total of 16 taxa which was identified and found as addition to the flora of Telangana state after a perusal of literature. *Ceropegia spiralis, Caralluma stalagmifera*, *Tripogon purpurascens, Chrysopogon velutinus* are some of the significant taxa of the study. Good number of insectivorous plants were also recorded from the study area. The "Sanjeevani" is mythical herb mentioned in the Ramayana as a wonderful medicinal plant was present in this area. A total number of 382 taxa can be considered as economically important.

Keywords: Tirumalaiah Gutta sacred grove, Telanagana, Conservation.

1. INTRODUCTION

Biodiversity is the totality of genes, species and ecosystem in a region. Biodiversity interacting with the physical environment form the foundation of sustainable development. The worldwide destruction of the natural environment by population explosion, urbanization, industrialization and habitat fragmentation has led to a tremendous loss of biological diversity over the past few decades. Population pressures and concomitant unscientific and unsustainable extraction of resources especially of timber, medicinal herbs, fuel wood and fodder from forests has alarming consequences on conservation of these resources. Overexploitation is likely to severely reduce the population sizes below the critical level and consequently the survival of the species *per sec*.

Flora refers to "the plants present in a particular geographic region or an area at a particular time, generally the naturally occurring or indigenous plants". Such flora will serve as documented inventory of plants and as a historic datasets for future monitoring of native plant species. According to conservation biologists, 25% of all species could become extinct during the next 20-30 years. The cause for the loss of species is numerous but the most important is the loss and fragmentation of natural habitats. The International Union for the Conservation of Nature (IUCN) is the world's main authority on the conservation status of species (1). The 2008 update of the IUCN Red List cover 44,838 species including 8,457 plant species classified under different threats. The existing information on Endemic, Rare, Endangered and Threatened (ERET) species is very thin and often provides inadequate data. There is a need to revive the red lists based on sound datasets as opined (2,3). In the context of unabated loss of biodiversity due to human interference, plant taxonomists throughout the world are documenting flora at different levels-national, regional, local, etc.

2. NEED OF STUDY

Sacred groves are the patches of native vegetation traditionally protected by local communities, and are unique, and significant, examples of in situ biodiversity conservation (4). The nature of religiousness associated with sacred groves suggests that the practice of sacred groves dates back to the nomadic hunter-gatherer age of human history (5). It is generally believed that, owing to their religious significance, sacred groves are better protected and managed, and hence harbor

*Correspondence: Sadasivaiah, B., Department of Botany, Government Degree College (Men), Wanaparthy – 509 103, Telanagana.
E. mail: sadasivaiahbyalla@gmail.com

richer plant diversity than other forests (6), though this has not been substantiated through systematic floristic and quantitative studies.

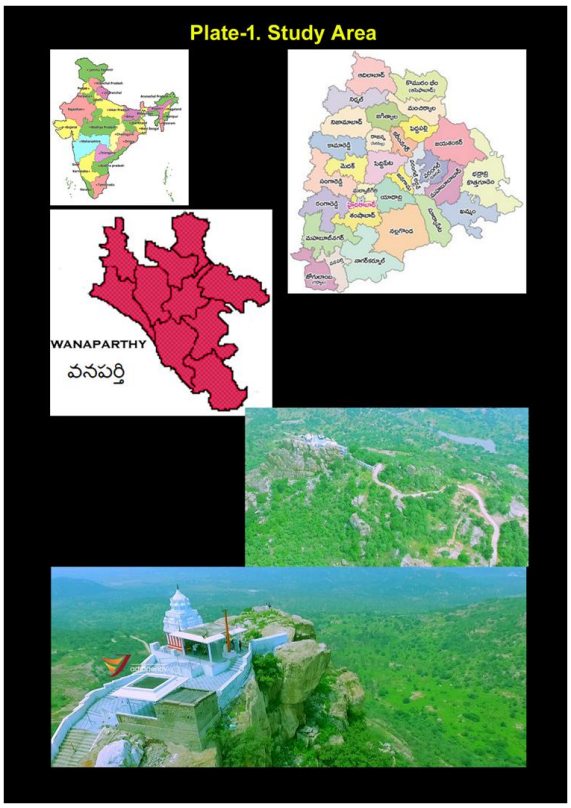
Tirumalaiah Gutta sacred grove is situated near Wanaparthy, Telanagana. No floristic and conservation works were done in this sacred grove. Hence the present work has undertaken.

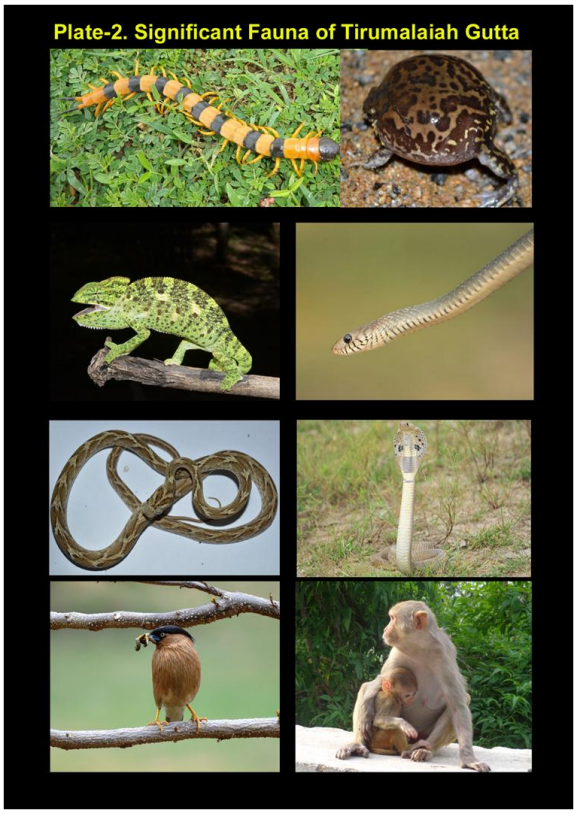
3. STUDY AREA

Wanaparthy is one of the districts of Telangana situated in southern part. The name of town itself indicates that, once upon a time it was with forests (Vanam-Forest; Parthy-Village). Tirumalaiah Gutta is one of the sacred groves of Telangana located 5 km away from Wanaparthy town. The lord Venkateswara is in the name of Tirumalanatha swamy present at the top of the hill worshipped by the local people for the past 300 years (Plate-1). According to the history of Wanaparthy Samsthan, the temple was built by the Raja Wanaparthy in 18th century. Sravanamasam (August-September) of every year nearly 2lakhs of people visited this temple.

The climate of the study area is that January, February and March months are pleasant with moderate winds from southeast with an average temperature varies from 24˚ to 28˚C. April and May are the hottest months of the year with the mean temperature of 35˚C-45˚C. The maximum temperature during this season ranges between 45˚C and 26˚C. During the succeeding four months, the wind blows from western side and brings fairly good rainfall. By the end of September, the wind is light and pleasant forecasting the onset of north-east monsoon. From November to February the temperature falls as low as 10˚C. The average rainfall of Tirumalaiah Gutta is about 100cm and is mostly due to south-west monsoon.

The forests of Tirumalaiah Gutta can be broadly categorized into three types: dry deciduous, scrub type and plantations. Dry deciduous forests are dominated by *Anogeissus latifolia, Chloroxylon switenia, Dalbergia lanceolaria, Deccania pubescens, Gyrocarpus americanus*. Scrub is usually confined to the base of hills and generally in the peripheries of much disturbed and degraded dry deciduous forests. On the western side of the sacred grove, scrub predominates and seen with species like *Acacia catechu, Dichrostachys cinerea, Diospyros chloroxylon, Maytenus emarginata,* etc. It is not only home for plants and also for various animals. The important animal species are represented in Plate-2.





4. METHODOLOGY

The present study aims at a first ever systematic attempt towards a fine scale assessment of the plant resources of Tirumalaiah Gutta Sacred Grove based on filed explorations.

Field explorations were conducted intensively for a period of 3 years, during 2013-2016 covering all the seasons. All the plant taxa encountered in the sampled quadrates were listed and representative specimens of every taxon were collected in quadruplicates. Specimens were then poisoned, dried and were made into herbarium according to methodology described by Santapau (7), Jain and Rao (8), Forman and Bridson (9). Identification of the specimens was done by the following Gamble and Fischer (10), Pullaiah (11) and further confirmed in certain cases, by comparing with the herbarium material housed at SKU; Botanical Survey of India Deccan Regional Circle, Hyderabad (BSID). A critical care should be taken in the confirmation of endemic, threatened taxa and new distributional records. Every attempt has been made in to study the habitat, soil, elevation, vegetation type, associates etc., which were recorded carefully in the field itself. With the help of local people and based on secondary literature, plants with medicinal importance were identified and the relevant information is documented.

All the endemic and threatened taxa recorded from the area were revisited with update population numbers. Apart from the recorded threatened taxa, significant taxa under different threat have also been analyzed following the latest version (3.1) of IUCN threat categories. The threatened and important endemic species were collected and grown in the Botanical Garden of Government Degree College, Wanaparthy and the sapling were distributed to the plant lovers.

Awareness is needed to conserve any biological resources at any point especially for local people who are residing in the forests or very near to forest areas. The importance of flora and fauna of Tirumalaiah Gutta and its conservation was explained by the research team headed by Dr. Sadasivaiah, to the local people through printed pamphlets, print and electronic media especially at the time of heavy pilgrimage. A team of 80 students along with Forest Department officials were checked and eradicated all the plastic covers and other plastic materials from the pilgrims and vendors at the time of Sravanamasam. All the collected plastic covers were deposited at Municipal Office, Wanaparthy.

5. RESULTS AND DISCUSSION

5.1. Floristic Analysis

In the present study, a total number of 467 wild and naturalized vascular plant taxa comprising 457 species and 10 intraspecific taxa were recorded in Tirumalaiah Gutta Sacred Grove. They are included in 283 genera and 81 families. Of the 467 taxa, 332 (71%) were dicots (208 genera), 129 (27.6%) were monocots (69 genera) and 6 (1.2%) were pteridophytes (06 genera). The enumerated species are presented in Table-1.

Table 1. Floristic Analysis of Tirumalaiah Gutta Sacred Grove

	Families	Genera	Species
Dicotyledons	66	208	383
Monocotyledons	09	69	129
Pteridophytes	06	06	21
Total	81	283	467

All the recorded 467 taxa are presented in Table-2 along with their botanical name, family and use value. All the taxa are arranged in alphabetical order by their families. The use value is abbreviated.

5.2. Analysis of Families and Genera

Analysis at family level revealed that Poaceae is the largest family with 77 taxa, followed by Fabaceae (51), Cyperaceae (25), Asteraceae (24), Rubiaceae (19) and Acanthaceae (18). Of the 81 families recorded in the present study, 34 are monotypic, viz., represented by only one species. Of these, 24 are dicot families, 3 are monocots and 6 are pteridophytes. A total of 09 families are represented with two species, 32 are represented by 3-15 species. The significant plant taxa are represented in Plate 4-7.

Table 2. List of recorded species in Tirumalaiah Gutta sacred grove.

S. No.	Name of the Taxon	Family	Habit	Use
1	*Andrographis paniculata* (Burm.f.) Nees	Acanthaceae	H	M, O
2	*Barleria cristata* L.	Acanthaceae	H	M, O
3	*Barleria prionitis* L.	Acanthaceae	S	M, O
4	*Blepharis maderaspatensis* (L.) B. Heyne ex Roth	Acanthaceae	H	M
5	*Blepharis integrifolia* (L.f.) E. Mey & Drege ex Schinz	Acanthaceae	H	M
6	*Dipteracanthus patulus* (Jacq.) Nees	Acanthaceae	H	O

7	*Dipteracanthus prostratus* (Poiret) Nees	Acanthaceae	H	M
8	*Hygrophila schulii* (Buch.-Ham.) M.R. Almeida & S.M. Almeida	Acanthaceae	H	M
9	*Indoneesiella echioides* (L.) Sreem.	Acanthaceae	H	M
10	*Indoneesiella longipedunculata* (Sreem.) Sreem.	Acanthaceae	H	M
11	*Justicia glauca* Rottl.	Acanthaceae	H	
12	*Lepidagathis cristata* Willd.	Acanthaceae	H	M
13	*Dicliptera paniculata* (Forssk.) I. Darbysh	Acanthaceae	H	M
14	*Rhinacanthus nasutus* (L.) Kurz.	Acanthaceae	H	M
15	*Rostellularia crinita* (Nees) Nees	Acanthaceae	H	
16	*Rostellularia simplex*Wight	Acanthaceae	H	
17	*Justicia vahlii* Roth var. *rupicola* Ellis	Acanthaceae	H	
18	*Justicia vahlii* Roth	Acanthaceae	H	
19	*Actiniopteris radiata* (Sw.) Link	Actiniopteridaceae	H	M
20	*Adiantum incisum* Forssk.	Adiantaceae	H	M
21	*Agave americana* L.	Agavaceae	S	Fibre
22	*Alangium salvifolium* (L.f.) Wangerin	Alangiaceae	T	M
23	*Achyranthes aspera* L.	Amaranthaceae	H	M
24	*Achyranthes aspera* L. var. *sicula* L.	Amaranthaceae	H	
25	*Aerva javanica* (Burm.f.) Juss. ex Schult.	Amaranthaceae	H	M
26	*Aerva lanata* (L.) Juss.	Amaranthaceae	H	M
27	*Allmania longepedunculata* (Trimen) Gamble	Amaranthaceae	H	E
28	*Allmania nodiflora* (L.) R. Br. ex Wight	Amaranthaceae	H	E
29	*Allmania nodiflora* (L.) R. Br. ex Wight. var. *roxburghii* Wight	Amaranthaceae	H	E
30	*Alternanthera sessilis* (L.) R. Br. ex DC.	Amaranthaceae	H	E
31	*Amaranthus viridis* L.	Amaranthaceae	H	E
32	*Celosia argentea* L.	Amaranthaceae	H	E
33	*Gomphrena serrata* L.	Amaranthaceae	H	
34	*Pupalia lappacea* (L.) Juss.	Amaranthaceae	H	M
35	*Trichurella monsoniae* (L.f.) Bennet	Amaranthaceae	H	
36	*Crinum asiaticum* L.	Amaryllidaceae	H	O
37	*Crinum defixum*Ker-Gawl.	Amaryllidaceae	H	O
38	*Pancratium longiflora* Roxb. ex Ker Gawl.	Amaryllidaceae	H	O
39	*Pancratium sp.*	Amaryllidaceae	H	O
40	*Pancratium sp.*	Amaryllidaceae	H	O
41	*Pancratium triflorum*Roxb.	Amaryllidaceae	H	O
42	*Annona squamosa* L.	Annonaceae	T	M
43	*Carissa carandas* L.	Apocynaceae	S	E
44	*Carissa spinarum* L.	Apocynaceae	S	E
45	*Catharanthus pusillus* (Murray) G. Don	Apocynaceae	H	M
46	*Wrightia tinctoria* (Roxb.) R. Br.	Apocynaceae	T	M
47	*Aponogeton natans* (L.) Engl.	Aponogetonaceae	H	O

48	*Amorphophallus sylvaticus* (Roxb.) Kunth	Araceae	H	M
49	*Theriophonum infaustum* N.E. Br.	Araceae	H	O
50	*Theriophonum minutum* (Willd.) Baill.	Araceae	H	O
51	*Borassus flabellifer* L.	Arecaceae	T	TADDY
52	*Phoenix sylvestris* (L.) Roxb.	Arecaceae	T	TADDY
53	*Aristolochia indica* L.	Aristolochiaceae	C	M
54	*Calotropis gigantea* (L.) Dryand	Asclepiadaceae	S	M
55	*Caralluma adscendens* (Roxb.) R.Br. var. *attenuata* (Wight) Grav. & Mayur.	Asclepiadaceae	H	M
56	*Caralluma stalagmifera* Fischer	Asclepiadaceae	H	M
57	*Ceropegia spiralis* Wight.	Asclepiadaceae	H	M
58	*Gymnema sylvestre* (Retz.) R.Br. ex Schultes	Asclepiadaceae	C	M
59	*Hemidesmus indicus* (L.) R.Br.	Asclepiadaceae	C	M
60	*Hemidesmus indicus* (L.) R.Br. var. *pubescens* (Wight & Arn.) Hook.f.	Asclepiadaceae	C	M
61	*Oxystelma esculentum* (L.f.) Sm.	Asclepiadaceae	C	M
62	*Pentatropis capensis* (L.f.) Bullock	Asclepiadaceae	C	M
63	*Pergularia daemia* (Forssk.) Chiov.	Asclepiadaceae	C	M
64	*Sarcostemma acidum*	Asclepiadaceae	C	M
65	*Tylophora fasciculata* Buch.-Ham.	Asclepiadaceae	H	M
66	*Tylophora indica* (Burm.f.) Merr.	Asclepiadaceae	C	M
67	*Wattakaka volubilis* (L.f.) Stapf	Asclepiadaceae	C	M
68	*Acanthospermum hispidum* DC.	Asteraceae	H	M
69	*Ageratum conyzoides* L.	Asteraceae	H	M
70	*Bidens bipinnata* L.	Asteraceae	H	O
71	*Blainvillea acmella* (L.) Philipson	Asteraceae	H	M
72	*Blumea mollis* (D.Don) Merr.	Asteraceae	H	
73	*Dicoma tomentosa* Cass.	Asteraceae	H	M
74	*Echinops echinatus* Roxb.	Asteraceae	H	
75	*Eclipta prostrata* (L.) L. Mant.	Asteraceae	H	M
76	*Emilia sonchifolia* (L.) DC.	Asteraceae	H	M
77	*Epaltes divaricata* (L.) Cass.	Asteraceae	H	
78	*Glossocardia bosvallea* (L.f.) DC.	Asteraceae	H	M
79	*Grangea maderaspatana* (L.) Poir.	Asteraceae	H	
80	*Lagascea mollis* Cav.	Asteraceae	H	F
81	*Oligochaeta ramosa* (Roxb.) Wagenitz	Asteraceae	H	
82	*Parthenium hysterophorus* L.	Asteraceae	H	M
83	*Sclerocarpus africanus* Jacq.	Asteraceae	H	O
84	*Senecio tenuifolius* Burm.f.	Asteraceae	H	O
85	*Sphaeranthus idicus* L.	Asteraceae	H	M
86	*Tagites erecta* L.	Asteraceae	H	Escape
87	*Tridax procumbens* L.	Asteraceae	H	M
88	*Vernonia albicans* DC.	Asteraceae	H	

89	*Vernonia cinerea* (L.) Less.	Asteraceae	H	M
90	*Vicoa indica* (L.) D.C.	Asteraceae	H	O
91	*Xanthium indicum* Koenig	Asteraceae	S	M
92	*Dolichandrane atrovirens* (Roth) K.Schum.	Bignoniaceae	T	T
93	*Dolichandrane falcata* (Wall. ex DC.) Seem.	Bignoniaceae	T	
94	*Stereospermum tetragonum* DC.	Bignoniaceae	T	T
95	*Heliotropium strigosum* Willd.	Boraginaceae	H	
96	*Trichodesma indicum* (L.) R. Br.	Boraginaceae	H	M
97	*Trichodesma sedgwickianum* S.P. Benerjee	Boraginaceae	H	
98	*Opuntia stricta* (Haw.) Haw.	Cactaceae	H	E
99	*Bauhinia racemosa* Lam.	Caesalpiniaceae	T	M
100	*Cassia fistula* L.	Caesalpiniaceae	T	M
101	*Chamaecrista absus* (L.) H.S. Irwin & Barneby	Caesalpiniaceae	H	M
102	*Chamaecrista mimosoides* (L.) Greene	Caesalpiniaceae	H	M
103	*Chamaecrista pumila* (Lam.) V. Singh	Caesalpiniaceae	H	M
104	*Hardwickia binata* Roxb.	Caesalpiniaceae	T	T
105	*Pterolobium hexapetalum* (Roth) Sant. & Wagh	Caesalpiniaceae	C	
106	*Senna auriculata* (L.) Roxb.	Caesalpiniaceae	S	M
107	*Senna sophora* (L.) Roxb.	Caesalpiniaceae	S	M
108	*Senna uniflora* (Mill.) H.S. Irwin & Barneby	Caesalpiniaceae	H	
109	*Cadaba fruticosa* (L.) Druce	Capparaceae	S	M
110	*Capparis divaricata* Lam.	Capparaceae	T	E, M
111	*Capparis roxburghii* DC.	Capparaceae	C	M
112	*Capparis sepiaria* L.	Capparaceae		
113	*Maerua oblongifolia* (Forssk.) A. Rich.	Capparaceae	C	M
114	*Polycarpaea corymbosa* (L.) Lam.	Caryophyllaceae	H	
115	*Cleome aspera* Koen. ex DC.	Cleomaceae	H	M
116	*Cleome monophylla* L.	Cleomaceae	H	M
117	*Cleome viscosa* L.	Cleomaceae	H	M
118	*Anogeissus latifolia* (Roxb.ex DC.) Wall. ex Bedd.	Combretaceae	T	T
119	*Commelina benghalensis* L.	Commelinaceae	H	M
120	*Commelina maculata* Edgew.	Commelinaceae	H	F
121	*Cyanotis fasciculata* (B. Heyne ex Roth) Schult. & Schult. f.	Commelinaceae	H	
122	*Cyanotis tuberosa* (Roxb.) Schultes & Schult. f.	Commelinaceae	H	M
123	*Murdannia edulis* (Stokes) Faden	Commelinaceae	H	F
124	*Murdannia nudiflora* (L.) Brenan	Commelinaceae	H	F
125	*Tonningia axillaris* (L.) Kuntze	Commelinaceae	H	F
126	*Argyreia sericea* Dalzell	Convolvulaceae	C	M
127	*Argyreia setosa* (Roxb.) Choisy	Convolvulaceae	C	M
128	*Evolvulus alsinoides* (L.) L.	Convolvulaceae	H	M
129	*Ipomoea barlerioides* (Chosy) Benth. ex C.B. Clarke	Convolvulaceae	C	
130	*Ipomoea carnea* Jacq. ssp. *fistulosa* (Choisy) D.Austin	Convolvulaceae	S	O
131	*Ipomoea coptica* (L.) Roth ex Roem. & Schult.	Convolvulaceae	C	

132	*Ipomoea wightii* (Wall.) Choisy	Convolvulaceae	C	
133	*Jacquenmontia paniculata* (Burm.f.) Hallier f.	Convolvulaceae	C	O
134	*Merremia tridentata* (L.) Hallier f.	Convolvulaceae	H	M
135	*Merremia tridentata* (L.) Hallier f. ssp. *hastata* (Desr.) Oostr.	Convolvulaceae	C	M
136	*Rivea hypocrateriformis* (Desr.) Choisy	Convolvulaceae	C	M, E
137	*Rivea ornata* Choisy	Convolvulaceae	C	M
138	*Coccinia grandis* (L.) Voigt.	Cucurbitaceae	C	M
139	*Ctenolepis garcinii* (L.) C.B. Clarke	Cucurbitaceae	C	M
140	*Cucumis pubescens* Willd.	Cucurbitaceae	H	E
141	*Diplocyclos palmatus* (L.) Jeffrey	Cucurbitaceae	C	M
142	*Mukia maderaspatana* (L.) Roemer	Cucurbitaceae	C	M
143	*Cuscuta reflexa* Roxb.	Cuscutaceae	C	
144	*Bulbostylis barbata* (Rottb.) Kunth ex C.B. Clarke	Cyperaceae	H	F
145	*Cyperus corymbosus* Rottb.	Cyperaceae	H	F
146	*Cyperus difformis* L.	Cyperaceae	H	F
147	*Cyperus distans* L.f.	Cyperaceae	H	F
148	*Cyperus haspan* L.	Cyperaceae	H	F
149	*Cyperus iria* L.	Cyperaceae	H	F
150	*Cyperus pulchellus* R.Br.	Cyperaceae	H	F
151	*Cyperus rotundus* L.	Cyperaceae	H	F, M
152	*Cyperus rubicundus* Vahl	Cyperaceae	H	F
153	*Cyperus teneriffae* Poir.	Cyperaceae	H	F
154	*Fimbristylis alboviridis* C.B. Clarke	Cyperaceae	H	F
155	*Fimbristylis argentea* (Rottb.) Vahl	Cyperaceae	H	F
156	*Fimbristylis bisumbellata* (Forssk.) Bubani	Cyperaceae	H	F
157	*Fimbristylis dichotoma* (L.) Vahl	Cyperaceae	H	F
158	*Fimbristylis quinquangularis* (Vahl) Kunth	Cyperaceae	H	F
159	*Fuirena capitata* (Burm. f.) T. Koyama	Cyperaceae	H	F
160	*Fuirena ciliaris* (L.) Roxb.	Cyperaceae	H	F
161	*Kyllinga bulbosa* P. Beauv.	Cyperaceae	H	F
162	*Kyllinga nemoralis* (Forst. & Forst.f.) Dandy ex Hutchins. & Dalziel	Cyperaceae	H	F
163	*Lipocarpha sphacelata* (Vahl) Kunth	Cyperaceae	H	F
164	*Mariscus clarkei* T. Koyama	Cyperaceae	H	F
165	*Cyperus paniceus* (Rottb.) Boeckeler	Cyperaceae	H	F
166	*Cyperus squarrosus* L.	Cyperaceae	H	F
167	*Lipocarpha squarrosa* (L.) Goetgh.	Cyperaceae	H	F
168	*Scleria lithosperma* (L.) Sw.	Cyperaceae	H	F
169	*Dioscorea pentaphylla* L.	Dioscoreaceae	C	M
170	*Drosera burmannii* Vahl	Droseraceae	H	Insectivorous
171	*Drosera indica* L.	Droseraceae	H	Insectivorous
172	*Diospyros chloroxylon* Roxb.	Ebenaceae	T	M
173	*Diospyros melanoxylon* Roxb.	Ebenaceae	T	M
174	*Eriocaulon quinquangulare* L.	Eriocaulaceae	H	

175	*Acalypha alnifolia* Klein ex Willd.	Euphorbiaceae	H	M
176	*Acalypha ciliata* Forssk.	Euphorbiaceae	H	M
177	*Acalypha indica* L.	Euphorbiaceae	H	M
178	*Croton bonplandianum* Baill.	Euphorbiaceae	H	M
179	*Euphorbia fusiformis* Buch.-Ham. ex D.Don	Euphorbiaceae	H	M
180	*Euphorbia hirta* L.	Euphorbiaceae	H	M
181	*Euphorbia indica* Lam.	Euphorbiaceae	H	M
182	*Euphorbia senguptae* N.P.Balakr. & Subr.	Euphorbiaceae	H	M
183	*Phyllanthus amarus* Schum. & Thonn.	Euphorbiaceae	H	M
184	*Phyllanthus kozhikodianus* Sivar. & Manilal	Euphorbiaceae	H	M
185	*Phyllanthus maderaspatensis* L.	Euphorbiaceae	H	M
186	*Phyllanthus reticulatus* Poir.	Euphorbiaceae	S	M
187	*Phyllanthus rheedei* Wight	Euphorbiaceae	H	M
188	*Phyllanthus virgatus* G. Forst.	Euphorbiaceae	H	M
189	*Sebastiniana chamaelea* (L.) Muell.-Arg.	Euphorbiaceae	H	M
190	*Abrus precatorius* L.	Fabaceae	C	M
191	*Aeschynomene indica* L.	Fabaceae	H	MISC.
192	*Alysicarpus bupleurifolius* (L.) DC.	Fabaceae	H	F
193	*Alysicarpus bupleurifolius* (L.) DC. var. *gracilis* (Edgew.) Baker	Fabaceae	H	F
194	*Alysicarpus hamosus* Edgew.	Fabaceae	H	F
195	*Alysicarpus mahabubnagarensis* Raghava Rao et al.	Fabaceae	H	F
196	*Alysicarpus monilifer* (L.) DC.	Fabaceae	H	F
197	*Alysicarpus pubescens* Law. ex Wight	Fabaceae	H	F
198	*Alysicarpus roxburghianus* Thoth. & A. Bramanik	Fabaceae	H	F
199	*Butea monosperma* (Lam.) Taub.	Fabaceae	T	M
200	*Cajanus cajan* (L.) Millsp.	Fabaceae	H	Escape
201	*Cajanus scarabaeoides* (L.) Thours	Fabaceae	C	WR
202	*Crotalaria hebecarpa* (DC.) Rudd.	Fabaceae	H	F
203	*Crotalaria hirsuta* Willd.	Fabaceae	H	O, F
204	*Crotalaria medicaginea* Lam.	Fabaceae	H	M
205	*Crotalaria pusilla* Heyne ex Roth	Fabaceae	H	
206	*Crotalaria ramosissima* Roxb.	Fabaceae	H	M
207	*Crotalaria willdinowiana* DC.	Fabaceae	H	
208	*Dalbergia lanceolaria* L. f.	Fabaceae	T	T
209	*Dalbergia latifolia* Roxb.	Fabaceae	T	T
210	*Dalbergia paniculata* Roxb.	Fabaceae	T	T
211	*Desmodium triflorum* (L.) DC.	Fabaceae	H	F
212	*Dysolobium pilosum* (Willd.) Marechal	Fabaceae	C	F
213	*Galactia tenuiflora* (Klein ex Willd.)Wight &Arn.	Fabaceae	C	
214	*Gliricidia sepium* (Jacq.) Kunth ex Walp.	Fabaceae	T	O
215	*Indigofera astragalina* DC.	Fabaceae	H	M
216	*Indigofera barberi* Gamble	Fabaceae	H	

217	*Indigofera caerulea* Roxb.	Fabaceae	H	
218	*Indigofera cordifolia* B. Heyne ex Roth	Fabaceae	H	O
219	*Indigofera hirsuta* L.	Fabaceae	H	
220	*Indigofera linifolia* (L. f.) Retz.	Fabaceae	H	F
221	*Indigofera linnaei* Ali	Fabaceae	H	M
222	*Indigofera trita* L. f.	Fabaceae	H	
223	*Pongamia pinnata* (L.) Pierre	Fabaceae	T	M
224	*Rhynchosia capitata* (B. Heyne ex Roth) DC.	Fabaceae	H	F
225	*Rhynchosia densiflora* (Roth) DC.	Fabaceae	C	F
226	*Rhynchosia minima* (L.) DC.	Fabaceae	H	M
227	*Rhynchosia rufescens* (Willd.) DC.	Fabaceae	C	F
228	*Rhynchosia suaveolens* (L.f.) DC.	Fabaceae	H	F
229	*Stylosanthes fruticosa* (Retz.) Alston	Fabaceae	H	F
230	*Stylosanthes scabra* Vog.	Fabaceae	S	F
231	*Tephrosia pumila* (Lam.) Pers.	Fabaceae	H	F
232	*Tephrosia purpurea* (L.) Pers.	Fabaceae	S	M
233	*Tephrosia strigosa* (Dalz.) Sant. & Mahesh.	Fabaceae	H	F
234	*Tephrosia villosa* (L.) Pers.	Fabaceae	H	M
235	*Teramnus labialis* (L. f.) Sprengel	Fabaceae	C	
236	*Teramnus mollis* Benth.	Fabaceae	C	
237	*Vigna aconitifolia* (Jacq.) Marechal	Fabaceae	H	F
238	*Vigna trilobata* (L.) Verdc.	Fabaceae	H	F
239	*Zornia diphylla* (L.) Pers.	Fabaceae	H	M
240	*Zornia gibbosa* Span.	Fabaceae	H	M
241	*Chloroxylon swietenia* DC.	Flindersiaceae	T	M
242	*Canscora alata* (Roth) Wall.	Gentianaceae	H	
243	*Enicostemma axillare* (Poir. ex Lam.) A. Raynal	Gentianaceae	H	M
244	*Gyrocarpus americanus* Jacq.	Hernandiaceae	T	M
245	*Isoites coromandeliana*	Isoitaceae	H	
246	*Anisochilus carnosus* (L.f.) Wall. ex Benth.	Lamiaceae	H	M
247	*Anisomeles indica* (L.) Kuntze	Lamiaceae	S	M
248	*Hyptis suaveolens* (L.) Poit.	Lamiaceae	H	M
249	*Leucas aspera* (Willd.) Link	Lamiaceae	H	M
250	*Leucas decemdenta* (Willd.) R. Br. ex Smith	Lamiaceae	H	M
251	*Ocimum americanum* L.	Lamiaceae	H	M
252	*Ocimum tenuiflorum* L.	Lamiaceae	H	M
253	*Orthosiphon rubicundus* (D.Don) Benth.	Lamiaceae	H	M
254	*Plectranthus barbatus* Andr.	Lamiaceae	H	M
255	*Utricularia aurea* Lour.	Lentibulariaceae	H	Insectivorous
256	*Utricularia caerulea* L.	Lentibulariaceae	H	Insectivorous
257	*Utricularia scandens* Benj.	Lentibulariaceae	H	Insectivorous
258	*Aloe vera* (L.) Burm. f.	Liliaceae	H	M, O
259	*Asparagus racemosus* Willd.	Liliaceae	C	M

260	*Chlorophytum laxum* R. Br.	Liliaceae	H	M
261	*Chlorophytum tuberosum* (Roxb.) Baker	Liliaceae	H	M
262	*Drimia indica* (Roxb.) Jessop	Liliaceae	H	M
263	*Gloriosa superba* L.	Liliaceae	C	M, O
264	*Iphigenia indica* (L.) A. Gray ex Kunth	Liliaceae	H	M
265	*Ledebouria revoluta* (L.f.) Jessop	Liliaceae	H	M
266	*Urgenia raogibikei* Hemadri	Liliaceae	H	
267	*Strychnos potatorum* L.f.	Loganiaceae	T	M
268	*Dendrophthoe falcata* (L.f.) Ettingsh.	Loranthaceae	S	M
269	*Ammannia baccifera* L.	Lythraceae	H	
270	*Ammannia multiflora* Roxb.	Lythraceae	H	
271	*Aspidopterys cordata* (Heyne ex Wall.) A. Juss.	Malphigiaceae	C	M
272	*Herissantia crispa* (L.) Brizicky	Malvaceae	H	M
273	*Abutilon indicum* (L.) Sweet	Malvaceae	S	M
274	*Hibiscus vitifolius* L.	Malvaceae	H	O
275	*Gossypium arborium* L.	Malvaceae	S	
276	*Hibiscus lobatus* (Murr.) Kuntze	Malvaceae	H	M
277	*Hibiscus ovalifolius* (Forssk.) Vahl	Malvaceae	S	M
278	*Malvastrum coromandelianum* (L.) Gracke	Malvaceae	H	
279	*Pavonia odorata* Willd.	Malvaceae	H	M
280	*Pavonia procumbens*	Malvaceae	H	M
281	*Pavonia zeylanica* (L.) Cav.	Malvaceae	H	M
282	*Sida acuta* Burm.f.	Malvaceae	S	M
283	*Sida cordata* (Burm.f.) Borssum	Malvaceae	H	M
284	*Sida cordifolia* L.	Malvaceae	S	M
285	*Sida ovata* Forssk.	Malvaceae	S	M
286	*Sida spinosa*	Malvaceae	H	M
287	*Marsilea minuta* L.	Marsileaceae	H	O
288	*Azadirachta indica* A. Juss.	Meliaceae	T	M
289	*Cissampelos pareira* L. var. *hirsuta* (Buch.-Ham.ex DC.) Forman	Menispermaceae	C	M
290	*Cocculus hirsutus* (L.) Diels	Menispermaceae	C	M
291	*Tinospora cordifolia* (Willd.) Meirs ex Hook. f. & Thomson	Menispermaceae	C	M
292	*Acacia auriculiformis* A. Cunn. ex. Benth.	Mimosaceae	T	O
293	*Acacia eburnea* (L.f.) Willd.	Mimosaceae	T	
294	*Acacia ferrugenia* DC.	Mimosaceae	T	
295	*Albizia amara* (Roxb.) Boivin	Mimosaceae	T	F
296	*Dichrostachys cinerea* (L.) Wight & Arn.	Mimosaceae	S	
297	*Gisekia pharnaceoides* L.	Molluginaceae	H	E
298	*Glinus lotoides* L.	Molluginaceae	H	
299	*Glinus oppositifolius* (L.) A. DC.	Molluginaceae	H	
300	*Mollugo nudicaulis* Lam.	Molluginaceae	H	

301	*Mollugo pentaphylla* L.	Molluginaceae	H	
302	*Ficus benghalensis* L.	Moraceae	T	M
303	*Ficus mollis* Vahl	Moraceae	T	M
304	*Ficus rumphii* Blume	Moraceae	T	M
305	*Eucalyptus globulus* Labill.	Myrtaceae	T	M
306	*Syzygium cumini* (L.) Skeels	Myrtaceae	T	E
307	*Boerhavia diffusa* L.	Nyctaginaceae	H	M, E
308	*Boerhavia erecta* L.	Nyctaginaceae	H	M, E
309	*Ximenia americana* L.	Olacaceae	T	M
310	*Jasminum auriculatum* Vahl	Oleaceae	C	O
311	*Jasminum arborescens* Roxb.	Oleaceae	C	O
312	*Nyctanthes arbor-tristis* L.	Oleaceae	T	M, O
313	*Ludwigia perennis* L.	Onagraceae	H	
314	*Oxalis corniculata* L.	Oxalidaceae	H	M, E
315	*Passiflora foetida* L.	Passifloraceae	C	E
316	*Martynia annua* L.	Pedaliaceae	H	
317	*Pedalium murex* L.	Pedaliaceae	H	M
318	*Sesamum radiatum* Schumach. & Thonn.	Pedaliaceae	H	WR
319	*Plumbago zeylanica* L.	Plumbaginaceae	H	M, O
320	*Alloteropsis cimicina* (L.) Stapf	Poaceae	H	F
321	*Andropogon pumilus* Roxb.	Poaceae	H	F
322	*Apluda mutica* L.	Poaceae	H	F
323	*Aristida adscensionis* L.	Poaceae	H	
324	*Aristida funiculata* Trin. & Rupr.	Poaceae	H	F
325	*Aristida hystrix* L.f.	Poaceae	H	F
326	*Aristida redacta* Stapf	Poaceae	H	
327	*Aristida setacea* Retz.	Poaceae	H	MISC.
328	*Arthraxon lanceolatus* (Roxb.) Hochst. var. *echinatus* (Nees) Hackel	Poaceae	H	F
329	*Arundinella nervosa* (Roxb.) Nees ex Hook. et Arn.	Poaceae	H	F
330	*Brachiaria distachya* (L.) Stapf	Poaceae	H	F
331	*Brachiaria ramosa* (L.) Stapf	Poaceae	H	F
332	*Brachiaria remota* (Retz.) Haines	Poaceae	H	F
333	*Brachiaria reptans* (L.) C. Gardner & C.E. Hubb.	Poaceae	H	F
334	*Chloris barbata* Sw.	Poaceae	H	F
335	*Chloris quinquesetica* Bhide	Poaceae	H	F
336	*Chloris virgata* Sw.	Poaceae	H	F
337	*Chrysopogon fulvus* (Spr.) Chiov.	Poaceae	H	F
338	*Chrysopogon velutinus* (Hook.f.) Bor	Poaceae	H	F
339	*Coelachyrum lagopoides* (Burm.f.) Senaratna	Poaceae	H	F
340	*Cynodon dactylon* (L.) Pers.	Poaceae	H	M
341	*Dactyloctenium aegyptium* (L.) P. Beauv.	Poaceae	H	F
342	*Dactyloctenium aristatum* Link Hort.	Poaceae	H	F
343	*Dendrocalamus strictus* (Roxb.) Nees	Poaceae	H	MISC.

344	*Dichanthium annulatum* (Forssk.) Stapf	Poaceae	H	F
345	*Dichanthium foveolatum* (Del.) Roberty	Poaceae	H	F
346	*Digitaria abludens* (Roemer & Schult.) Veldkamp	Poaceae	H	F
347	*Digitaria bicornis* (Lam.) Roemer & Schult.	Poaceae	H	F
348	*Digitaria ciliaris* (Retz.) Koel.	Poaceae	H	F
349	*Digitaria longiflora* (Retz.) Pers.	Poaceae	H	F
350	*Digitaria tomentosa* (Willd.) Henr.	Poaceae	H	F
351	*Dimeria orissae* Bor	Poaceae	H	F
352	*Echinochloa colona* (L.) Link	Poaceae	H	F
353	*Eragrostiella bifaria* (Vahl) Bor	Poaceae	H	F
354	*Eragrostiella walkeri* (Stapf) Bor	Poaceae	H	F
355	*Eragrostis ciliaris* (L.) R.Br.	Poaceae	H	F
356	*Eragrostis pilosa* (L.) Beauv.	Poaceae	H	F
357	*Eragrostis riparia* (Willd.) Nees	Poaceae	H	F
358	*Eragrostis tenella* (L.) P. Beauv. ex Roemer & Schult.	Poaceae	H	F
359	*Eragrostis tremula* Hochst.ex Steudel	Poaceae	H	F
360	*Eragrostis unioloides* (Retz.) Nees ex Steudel	Poaceae	H	F
361	*Eragrostis viscosa* (Retz.) Trin.	Poaceae	H	F
362	*Eriochloa procera* (Retz.) C.E. Hubb.	Poaceae	H	F
363	*Hackelochloa granularis* (L.) Kuntze	Poaceae	H	F
364	*Heteropogon contortus* (L.) Beauv. ex Roemer & Schultes	Poaceae	H	MISC.
365	*Heteropogon fischerianus* Bor	Poaceae	H	MISC.
366	*Ischaemum rugosum* Salisb.	Poaceae	H	F
367	*Iseilema anthephoroides* Hackel	Poaceae	H	F
368	*Iseilema laxum* Hackel	Poaceae	H	F
369	*Iseilema prostratum* (L.) Nees	Poaceae	H	F
370	*Lophopogon tridentatus* (Roxb.) Hackel	Poaceae	H	F
371	*Melanocenchris jacquemontii* Jaub.& Spach	Poaceae	H	F
372	*Microchloa indica* (L.f.) Beauv.	Poaceae	H	
373	*Oropetium thomaeum* (L.f.) Trin.	Poaceae	H	
374	*Oryza rufipogon* Griff.	Poaceae	H	WR
375	*Oryza sativa* L.	Poaceae	H	Escape
376	*Panicum trypheron* Schultes	Poaceae	H	F
377	*Paspalidium flavidum* (Retz.) A. Camus	Poaceae	H	F
378	*Paspalidium geminatum* (Forssk.) Stapf	Poaceae	H	F
379	*Paspalum scrobiculatum* L.	Poaceae	H	E
380	*Paspalum vaginatum* Sw.	Poaceae	H	WR
381	*Pennisetum pedicellatum* Trin.	Poaceae	H	WR
382	*Perotis indica* (L.) Kuntze	Poaceae	H	F
383	*Rhynchelytrum repens* (Willd.) C.E.Hubb.	Poaceae	H	F,O
384	*Sacciolepis indica* (L.) Chase	Poaceae	H	F
385	*Schizachyrium exile* (Hochst.) Pilg.	Poaceae	H	F

386	*Sehima nervosum* (Rottler) Stapf	Poaceae	H	F
387	*Setaria intermedia* Roemer & Schult.	Poaceae	H	WR
388	*Setaria pumila* (Poir.) Roemer & Schult.	Poaceae	H	WR
389	*Setaria verticillata* (L.) P. Beauv.	Poaceae	H	WR
390	*Sorobolus coromandelianus* (Retz.) Kunth	Poaceae	H	F
391	*Sporobolus indicus* (L.) R.Br. var. *diander* (Retz.) Jovet & Guedes	Poaceae	H	F
392	*Sporobolus indicus* (L.) R.Br. var. *fertilis* (Steud.) Jovet & Guedes	Poaceae	H	F
393	*Tragus roxburghii* Panigr.	Poaceae	H	F
394	*Tripogon bromoides* Roemer & Schultes	Poaceae	H	F
395	*Tripogon purpurescens* Duthie	Poaceae	H	F
396	*Urochloa panicoides* P. Beauv.	Poaceae	H	F
397	*Polygala chinensis* L.	Polygalaceae	H	
398	*Polygala elongata* Klein ex Willd.	Polygalaceae	H	
399	*Polygala erioptera* DC.	Polygalaceae	H	
400	*Polygala javana* DC.	Polygalaceae	H	
401	*Polygonum plebeium* R.Br.	Polygonaceae	H	
402	*Portulaca pilosa* L.	Portulacaceae	H	E
403	*Ventilago denticulata* Willd.	Rhamnaceae	C	M
404	*Ziziphus mauritiana* Lam. var. *fruticosa* (Haines) Sebastine & Balakr.	Rhamnaceae	S	E
405	*Ziziphus oenoplia* (L.) Mill.	Rhamnaceae	C	M
406	*Ziziphus xylopyra* (Retz.) Willd.	Rhamnaceae	T	M
407	*Psydrax dicoccos* Gaertn.	Rubiaceae	T	M
408	*Canthium coromandelicum* (Burm.f.) Alston	Rubiaceae	S	M
409	*Catunaregum spinosa* (Thunb.) Tirveng.	Rubiaceae	S	M
410	*Deccania pubescens* (Roth) Tirveng.	Rubiaceae	T	
411	*Gardenia latifolia* Aiton	Rubiaceae	T	E
412	*Haldinia cordifolia* (Roxb.) Ridsd.	Rubiaceae	T	M
413	*Hedyotis affinis* Roemer & Schultes	Rubiaceae	H	
414	*Hedyotis aspera* Heyne ex Roth	Rubiaceae	H	
415	*Hedyotis corymbosa* (L.) Lam.	Rubiaceae	H	
416	*Hedyotis herbacea* L.	Rubiaceae	H	
417	*Hedyotis puberula* (G.Don) Arn. & Pugill.	Rubiaceae	H	
418	*Ixora pavetta* Andrews.	Rubiaceae	T	M
419	*Morinda angustifolia* Roxb.	Rubiaceae	T	
420	*Morinda pubescens* J.E. Smith	Rubiaceae	T	M
421	*Pavetta indica* L. var. tomentosa (Roxb. ex Sm.) Hook.f.	Rubiaceae	T	M
422	*Spermacoce articularis* L.f.	Rubiaceae	H	
423	*Spermacoce hispida* L.	Rubiaceae	H	
424	*Spermacoce latifolia* Aubl.	Rubiaceae	H	
425	*Spermacoce pusilla* Wall.	Rubiaceae	H	
426	*Cardiospermum canescens* Wall.	Sapindaceae	C	M

427	*Cardiospermum halicacabum* L.	Sapindaceae	C	M
428	*Dodonaea angustifolia* L.f.	Sapindaceae	S	M
429	*Sapindus emarginatus* Vahl	Sapindaceae	T	M
430	*Bacopa monnieri* Wettst.	Scrophulariaceae	H	E, M
431	*Limnophila indica* (L.) Druce	Scrophulariaceae	H	
432	*Lindernia ciliata* (Colsm.) Pennell	Scrophulariaceae	H	
433	*Sopubia delphinifolia* (L.) G. Don	Scrophulariaceae	H	
434	*Striga asiatica* (L.) Kuntze	Scrophulariaceae	H	
435	*Selaginella bryopteris* (L.) Bak.	Selaginellaceae	H	M
436	*Ailanthus excelsa* Roxb.	Simaroubaceae	T	M
437	*Cheilanthus mysorensis* Wall. ex Beddome	Sinopteridaceae	H	
438	*Solanum melongena* L. *var. insanum* (L.) Prain	Solanaceae	S	WR
439	*Helicteres isora* L.	Sterculiaceae	S	M
440	*Melochia corchorifolia* L.	Sterculiaceae	H	
441	*Firmiana simplex* (L.) W. Wight	Sterculiaceae	T	Gum
442	*Waltheria indica* L.	Sterculiaceae	H	M
443	*Melhania incana* Heyne ex Wight & Arn.	Tiliaceae	H	
444	*Corchorus aestuans* L.	Tiliaceae	H	MISC.
445	*Corchorus olitorius* L.	Tiliaceae	H	MISC.
446	*Corchorus trilocularis* L.	Tiliaceae	H	MISC.
447	*Grewia damine* Gaertn.	Tiliaceae	T	E
448	*Grewia flavescens* Juss.	Tiliaceae	T	E
449	*Grewia hirsuta* Vahl	Tiliaceae	S	M
450	*Grewia rhamnifolia* Heyne ex Roth	Tiliaceae	C	M
451	*Grewia tenax* (Forssk.) Fiori	Tiliaceae	S	E
452	*Grewia villosa* Willd.	Tiliaceae	S	E
453	*Triumfetta pilosa* Roth	Tiliaceae	H	
454	*Triumfetta rhomboidea* Jacq.	Tiliaceae	S	
455	*Holoptelea integrifolia* (Roxb.) Planch.	Ulmaceae	T	M
456	*Pouzolzia auriculata* Wight	Urticaceae	H	
457	*Lantana camara* L.var. *aculeata* (L.) Mold.	Verbenaceae	S	O, E
458	*Phyla nodiflora* (L.) Greene	Verbenaceae	H	
459	*Premna mollissima* Roth	Verbenaceae	T	
460	*Priva cordifolia* (L.f.) Druce	Verbenaceae	H	
461	*Hybanthus enneaspermus* (L.) F.V. Muell.	Violaceae	H	M
462	*Hybanthus stellerioides* (Domin) P. I. Forst	Violaceae	H	M
463	*Cissus arnottiana* Shetty & P. Singh	Vitaceae	S	M
464	*Cissus quadrangularis* L.	Vitaceae	C	M
465	*Cissus repanda* Vahl	Vitaceae	C	M
466	*Cissus vitiginea* L.	Vitaceae	S	M
467	*Tribulus terrestris* L.	Zygophyllaceae	H	M

Habit: H- Herb; S- Shrub; C- Climber; T- Tree
Use: E- Edible; F- Fodder; M- Medicinal; O- Ornamental; WR- Wild Relative; Misc.- Miscillaneous

Seven herbaceous families are represented with more than 10 genera. Poaceae is the largest family with 42 genera followed by Asteraceae (23), Fabaceae (19), Cyperaceae (14), Acanthaceae (11), Asclepiadaceae (11) and Rubiaceae (10). The top 10dominant families are presented in Fig. 1.

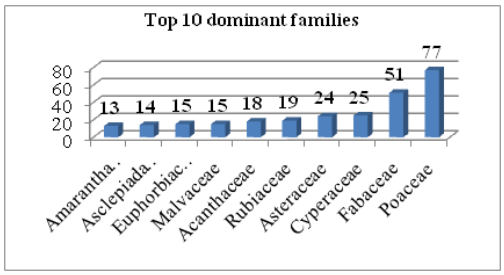


Fig. 1. Top 10 dominant families

5.3. Endemic taxa

A total of 34 endemic taxa at different levels (up to the level of Peninsular India) are recorded from study area. Endemic taxa up to Peninsular India level are presented in a tabular form, along with their earlier distribution (Table-3). Of the 34 taxa, *Alysicarpus mahabubnagarensis* is endemic to Mahabubnagar district of Telangana, *Chryopogon velutinus* is endemic to Kadapa district of Andhra Pradesh, Rathnagiri hills of Maharashtra and Wanaparthy district of Telangana; *Euphorbia senguptea* and *Rostellularia vahlii* var. *rupicola* is endemic to Eastern Ghats.

5.4. New distributional records

The inventory has resulted in a total of 16 taxa are identified and found as addition to the flora of Telangana state after a perusal of literature. The details are provided in Table-4 along with their earlier distribution in India. *Stylosanthes scabra* is reported as new distributional record for Eastern Ghats Eco region. The study has registered *Tripogon purpurascens* as second reports for the state of Telangana after Sadasivaiah (12).

Table 3. List of Endemics recorded in the study area

S. No.	Name of the Taxon	Family	Endemism
1	*Indoneesiella longipedunculata* (Sreem.) Sreem.	Acanthaceae	Peninsular India
2	*Rostellularia crinita* (Nees) Nees	Acanthaceae	Peninsular India
3	*Justicia vahlii* Roth var. *rupicola* Ellis	Acanthaceae	Eastern Ghats
4	*Theriophonum infaustum* N.E. Br.	Araceae	Peninsular India
5	*Caralluma adscendens* (Roxb.) R.Br. var. *attenuata* (Wight) Grav. & Mayur.	Asclepiadaceae	Peninsular India
6	*Caralluma stalagmifera* Fischer	Asclepiadaceae	Peninsular India
7	*Ceropegia spiralis* Wight.	Asclepiadaceae	Peninsular India
8	*Vernonia albicans* DC.	Asteraceae	Peninsular India
9	*Hardwickia binata* Roxb.	Caesalpiniaceae	Peninsular India
10	*Mariscus clarkei* T. Koyama	Cyperaceae	Peninsular India
11	*Euphorbia senguptae* N.P.Balakr. & Subr.	Euphorbiaceae	Eastern Ghats
12	*Phyllanthus kozhikodianus* Sivar. & Manilal	Euphorbiaceae	Peninsular India
13	*Alysicarpus mahabubnagarensis* Raghava Rao et al.	Fabaceae	Eastern Ghats
14	*Alysicarpus pubescens* Law. ex Wight	Fabaceae	Peninsular India
15	*Alysicarpus roxburghianus* Thoth. & A. Bramanik	Fabaceae	Peninsular India
16	*Crotalaria hirsuta* Willd.	Fabaceae	Peninsular India
17	*Crotalaria willdinowiana* DC.	Fabaceae	Peninsular India
18	*Indigofera barberi* Gamble	Fabaceae	Peninsular India
19	*Tephrosia strigosa* (Dalz.) Sant. & Mahesh.	Fabaceae	Peninsular India
20	*Acacia eburnea* (L.f.) Willd.	Mimosaceae	Peninsular India
21	*Andropogon pumilus* Roxb.	Poaceae	Peninsular India
22	*Aristida redacta* Stapf	Poaceae	Peninsular India
23	*Arthraxon lanceolatus* (Roxb.) Hochst. var. *echinatus* (Nees) Hackel	Poaceae	Peninsular India

24	*Arundinella nervosa* (Roxb.) Nees ex Hook. et Arn.	Poaceae	Peninsular India
25	*Chloris quinquesetica* Bhide	Poaceae	Peninsular India
26	*Chrysopogon velutinus* (Hook.f.) Bor	Poaceae	Peninsular India
27	*Digitaria tomentosa* (Willd.) Henr.	Poaceae	Peninsular India
28	*Dimeria orissae* Bor	Poaceae	Peninsular India
29	*Eragrostis riparia* (Willd.) Nees	Poaceae	Peninsular India
30	*Heteropogon fischerianus* Bor	Poaceae	Peninsular India
31	*Iseilema anthephoroides* Hackel	Poaceae	Peninsular India
32	*Lophopogon tridentatus* (Roxb.) Hackel	Poaceae	Peninsular India
33	*Tragus roxburghii* Panigr.	Poaceae	Peninsular India
34	*Tripogon bromoides* Roemer & Schultes	Poaceae	Peninsular India

Table 4. New distributional records

S. No.	Name of the Taxon	Family	Habit	New to
1	*Justicia vahlii* Roth var. *rupicola* Ellis	Acanthaceae	H	Telangana
2	*Caralluma stalagmifera* Fischer	Asclepiadaceae	H	Telangana
3	*Commelina maculata* Edgew.	Commelinaceae	H	Telangana
4	*Rivea ornata* Choisy	Convolvulaceae	C	Telangana
5	*Cyperus pulchellus* R.Br.	Cyperaceae	H	Telangana
6	*Phyllanthus kozhikodianus* Sivar. & Manilal	Euphorbiaceae	H	Telangana
7	*Alysicarpus pubescens* Law. ex Wight	Fabaceae	H	Telangana
8	*Stylosanthes scabra* Vog.	Fabaceae	S	Eastern Ghats
9	*Teramnus mollis* Benth.	Fabaceae	C	Telangana
10	*Arundinella nervosa* (Roxb.) Nees ex Hook. et Arn.	Poaceae	H	Telangana
11	*Chloris quinquesetica* Bhide	Poaceae	H	Telangana
12	*Chrysopogon velutinus* (Hook.f.) Bor	Poaceae	H	Telangana
13	*Heteropogon fischerianus* Bor	Poaceae	H	Telangana
14	*Paspalum vaginatum* Sw.	Poaceae	H	Telangana
15	*Polygala javana* DC.	Polygalaceae	H	Telangana
16	*Morinda angustifolia* Roxb.	Rubiaceae	T	Telangana

Records of significant herbaceous taxa

5.5. Ceropegia spiralis

This species is reported endemic plant of Peninsular India (Ahembedullah & Nayar, 1987), distributed in Andhra Pradesh, Karnataka, Kerala and Tamil Nadu. In Andhra Pradesh, the species is restricted to Kadapa hills. The present study revealed that it is found in Tirumalaiah Gutta sacred grove. This collection forms the second report of the taxon from different locality after Beddome's collection and extended its distribution from Kadapa to Wanaparthy.

5.6. Caralluma stalagmifera

Caralluma stalagmifera was first described by Fischer from Madras. The distribution of *Caralluma stalagmifera* is Eastern Peninsular India, from Visakhapatnam (Andhra Pradesh) to Ramanthapuram (Tamil Nadu). In the present investigation it is also recorded from the study area. Hence this forms new distributional record for Telangana State.

5.7. Grasses

Tripogon purpurascens was reported from Anantapur and Viziayanagaram districts of Andhra Pradesh, as new distributional record for Peninsular India (13). The present study resulted in its extended distribution from Northern Eastern Ghats to study area. Hence it can be considered as second report for the Peninsular India.

A total of 10 speccies of *Chrysopogon* is recorded from Telangana State (11), six of them are rare in distribution and collection is very poor including *Chrysopogon velutinus*. According to type specimens housed at Herbarium Royal Botanic gardens, Kew, it was collected by Robert Wight in early 19th century around 1819-1826 from Appayapalle in Kadapa district, which was in Mysore state (Presently in Andhra Pradesh) and by Meebold in September 1910 from Badami of Belgam district, which was in Bombay Presidency (Presently in

Karnataka state). Recently 18th November 2010 it was recollected from Badami plateau (14) but there is no subsequent collection of this from Andhra Pradesh, even though many workers sieved the area in their floristic works.

Recently, our team collected this from the study area. The present collection is the subsequent collection after Wight in Andhra Pradesh with gap of 150 years and far away from earlier locality.

5.8. Insectivorous Plants

A total of 9 insectivorous plants recorded from entire Telangna state by Pullaiah (11) of them *Drosera burmannii, D. indica*, *Utricularia aurea, U. caerulea, U. scandens* are reporting from the study area. The richness of insectivorous plants indicated that the study area is with less environmental pollution.

5.9. Resource potenitial taxa

A total of 382 taxa can be considered as economically important. They form 81% of the total recorded plants in the study. Of them, 189 (49.5%) are medicinal plants, 28 are edible plants (7.3%), 113 are under fodder value (29.5%), 9 genetic resource plants for crop plants (2.3%), 31 plants with ornamental properties (8%), 7 species are with timber value (1.8%) and 12 are with miscellaneous (3.1%) uses are utilized by the local people and also recognized based on secondary literature. All the details are tabulated in Table 2. Graphical representation for these taxa presented in Fig. 2.

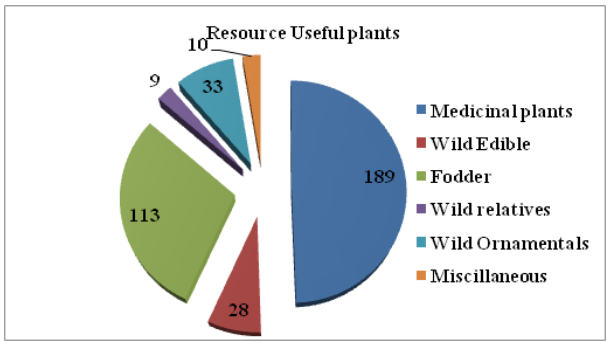


Fig. 2. Resource useful taxa from Tirumalaiah Gutta

5.10. Use Value

Of the recorded 382 economically important species, 365 are recorded with use value one. They included 21 edible plants, 110 fodder value species, 09 wild relatives of crop plants, 175 medicinal plants, 7 timber yielding plants, 20 plants with miscellaneous uses and 23 wild ornamentals. A total of 17 taxa are recorded with use value two.

5.11. Medicinal plants

A total of 189 taxa are having rich medicinal value, they belonging to 54 families. They are including, *Achyranthus apera, Acalypha indica, Aloe vera, Andrographis paniculata, Bacopa monnieri, Cyperus rotundus, Cynodon dactylon, Desmodium triflorum, Eclipta prostrata, Euphorbia hirta, Evolvulus alsinoides, Hybanthus ennaespermus, Hygrophyla auriculata, Leucas aspera, Ocimum tenuiflorum, Plectranthus barbatus, Pedalium murex, Phyllanthus amarus, Plumbago zeylanica, Selaginella bryopteris, Tylophora fasciculata, Vernonia cineria.* The tubers of *Ceropegia spiralis* are edible and used in local medicine for indigestion.

Euphorbiaceae and Asclepiadaceae are the top dominant families with 15 and 14 taxa respectively to medicinal plants, followed by Fabaceae, Acanthaceae, Malvaceae, Asteraceae with 12 species in each. *Adiantum incisum* and *Selaginella bryopteris* are the medicinal Pteridophytes recorded in the study area.

5.12. Wild Edible Plants

Of the 28 edible plant taxa, the species of *Allamania* and *Alternanthera sessilis, Amaranthus viridis, Boerhavia diffusa, Celosia argentea, Hygrophila auriculata,* tender leaves of *Tribulus terrestris* and *Zaleya decandra* are commonly used as leafy vegetables by local and people. The stems of *Caralluma adscendens* and *C. stalagmifera* are eaten as raw food and some time chutney prepared by local people. *Ceropegia spiralis* tubers are edible locally. The ripened fruits of *Opuntia stricta, Grewia flavescens, G. hirsuta, Canthium parviflorum* are edible. *Echinochloa colona, Oryza sativa* and *Paspalum scrobiculatum* grains are used as food grains. All these edible plants belonging to 18 families, of which Amaranthaceae is the dominant family comprising 6 taxa and Tiliaceae with 4 species. Khadar Basha *et al.* (13) reported 47 wild species that yield edible fruits from Southern Eastern Ghats, which are vital for livelihood of local communities.

5.13. Fodder

A good number of fodder species, 113 are recorded from the study. The majority of the fodder species are belonging to Poaceae (60), Cyperaceae (25) and Fabaceae (22). Some of the Fabaceae species like *Rhynchosia capitata, R. minima, R. suaveolens, Stylosanthes fruticosa, S. scabra, Vigna aconotifolia* and *V. trilobata* are the good fodder species. The important fodder grasses are, *Brachiaria ramosa*, *Chrysopogon fulvus, Cynodon dactylon, Dactyloctenium aegyptium, Dichanthium annulatum, Echinochloa colona, Hackelochloa*

granularis, Heteropogon contortus, Panicum trypheron, Pennisetum pedicillatum, Setaria intermedia and *Urochloa panicoides*. Grasses and their importance in NTFP are observed in Western Himalayan forests (15), they reported more than 16 species under fodder value, and some of them are recorded in the present investigation.

5.14. Wild Relatives of Crop plants

A total of nine Wild relatives to crop plants are reported from the study area. *Oryza rufipogon* is the very close relative to cultivated *Oryza sativa* and belongs to primary gene pool (16). *Pennisetum pedicillatum* is relative of Pearl millet distributed in Tirumalaiah Gutta sacred grove. *Panicum repens, P. trypheron* are the wild relative species for proso millet, *Panicum sumatrense.* Out of five wild relatives for Italian millet reported from Telangana, Three of them *Setaria intermedia, S. pumila* and *S. verticillata* are recorded from the present study. *Paspalum vaginatum* is the one wild relative of Kodo millet. The kodo millet, *Paspalum scrobilatum* is commonly found near marshy areas of the study area.

Vigna aconitifolia and *V. trilobata* are the genetic resource species for *Dolichos*. *Sesamum radiatum* is the important genetic resource species for *Sesamum orientale*. *Memordica dioica* is the genetic resource potential species for bitter guard. *Solanum melongena* var. *insanum* is the wild relative of Brinjal and it is used as vegetable by tribal people resided in Nallamalais (12). *Cajanus scarabaeioides* is wild relative of *Cajanus cajan*.

5.15. Wild ornamentals

In the present study a total of 33 wild plants with ornamental value are recorded. Some of them are already domesticated for the purpose of gardens and domestic uses. *Aloe vera, Barleria cristata, Crinum asiaticum, C. defixum* are commonly cultivated in gardens and in houses. The remaining species are having good ornamental properties. Some of them are potted in our college botanical garden. The members of Acanthaceae, *Barleria cristata, B. prionitis* are shows good reproductive capacity through stem cuttings.

The species of *Pancratium longiflora, P. triflorum* and other *Pancratium* spp. are potential ornamental plants with underground bulbs. The flowers of these species are of showy and large. Very less amount of water is needed for the cultivation of these species. There is an urgent need to domesticate these species and can improve the economy of the local people. Reddy *et al.* (17) reported 356 plants with ornamental value from the forests of Kadapa.

5.16. Miscellaneous use

Out of 382 recorded economically important plants, some of them are treated under miscellaneous uses. Of them, *Aeschynomene indica* (Bio fertilizer), *Aristida setacea, Heteropogon contortus, H. fischerianus* (Brooms), *Corchorus aestuans, C. olitorius and C. trilocularis* (Fibre) and *Dendrocalamus strictus* is used for many things.

5.17. Conservation aspects

A total of 123 species of 455 individuals are growing in the Botanical Garden of Government Degree College (Men), Wanaparthy and some of them are distributed to the plant lovers in the special occasions like Environmental Day, Biodiversity Day etc.

As a part of our research work nearly 800 kg of plastic covers and plastic materials were collected from pilgrims and venders at the time of Sravanamasam in the year 2012. The amount of plastic covers and plastic materials are slowly decreasing following years. In 2016 a total of 123 kg of plastic materials were collected and deposited in the Municipality office at Wanaparthy. The above result indicates the impact of awareness programmes conducted by the research team.

5.18. Conservation strategies

The field observations have strengthened that the herbs are habitat specific especially in the case of Insectivorous plants, some medicinal plants and lithophytes. Forests that are relatively undisturbed seem to possess these varied habitat conditions more. Human disturbance is high in western parts of the sacred grove than eastern part.

Ex-situ maintenance is one of the strategies to conserving the plants. This is mainly in gardens, germ-plasm banks. In the present investigation a total of 94 wild plants are conserving in the Botanical garden of Government Degree College (Men), Wanaparthy. The following key strategies are proposed for effective conservation of plant resources in Tirumalaiah Gutta sacred grove based on the present work sampling inventory.

1. State Forest department and GCC should ensure sustainable harvesting of medicinal plants presenting in the study area. Towards this, intensive training programmes to be organized for tribal and other communities by governmental and non-governmental agencies for promoting awareness.
2. Focus immediate attention on the threatened herbs identified as vulnerable and other categories by the forestry sector.

3. *Ex situ* conservation of identified threatened species of Tirumalaiah Sacred Grove in Tirumalanatha Swamy Eco Park (have been developing adjacent to Tirumalaiah Gutta) and other botanical gardens of the state.
4. Regular monitoring of plant resources of the study area is needed especially in the month Sravanamasam (August-September), where the pilgrim pressure is high.
5. A highly coordinated action-oriented multi-disciplinary approach on plant resources conservation integrating the forest department, Non-Governmental Organizations, scientific bodies at universities, Colleges and research institutions with the co-operation of local communities should be implemented.
6. The area with insectivorous plants needs to be prioritized and conserved by the forest department.

The information accumulated in the present work will be disseminated to the state forest department for further action. The information on threatened plant taxa will be intimated to Botanical Survey of India and IUCN, Plant Specialist Group of Indian Sub-Continent.

6. CONCLUSION

The present study on plant resources of Tirumalaiah Gutta, one of the sacred groves in Telangana has yielded significant results. A total of 467 plant taxa were recorded belonging to 283 genera and 81 families. A total of 16 species are additions to the flora of Telangana state indicated that rich diversity is present in the study area. Of the 81 families recorded in the study area, 34 are monotypic. The dominant family is Poaceae with 77 taxa indicating that the available resources are utilizing by them. A good number of endemic taxa recorded from the study area represent that there is an urgent need to conserve.

Of the 467 plant taxa recorded from the study area, 382 taxa are having on or other use value. Of these, 175 taxa (46%) used as medicinal, 21 (6.8%) are edible, 110 (28.7%) with fodder value, 09 (2.3%) are genetic resource for crop plants, 23 (6%) with ornamental value, 07 (1.8%) with timber value and 20 taxa (5.2%) of miscellaneous uses. A total of 20 taxa are considered threatened in the present study based on the field observations.

We have to protect and conserve the medicinal plants like *Selaginella bryopteris* (Sanjeevani), *Ceropegia spiralis* (Nimmatayi), *Chlorophytum tuberosum* (Safed Musli), *Gymnema sylvestre* (Podapatri), *Asparagus racemosus* (Sathavari). Even though many species of Poaceae, Cyperaceae and Fabaceae are used as fodder among them *Alysicarpus hamosus, Chrysopogon velutinus* are the important and are palatable in all stages of its life. There is a need to develop hybrids from these and to overcome the scarcity of fodder. *Chrysopogon velutinus* is a very rare grass relocated after 150 years from Eastern Ghats and it need to be reintroduce in the similar habitats of the study area and also Eastern Ghats due to its low population.

Instead of using the exotic species of *Pancratium* and *Crinum* as ornamentals, better to use our own species like *Pancratium logiflora*, *P. triflorum, Crinum asiaticum, C. defixum* and other species of *Pancratium* and *Urginea*, which are suitable to our environmental conditions.

If we conserve and utilize these medicinal, fodder and ornamentals the economy of local people will be increased. This area is rich in medicinal plants hence it should be recognized as Medicinal Plant Conservation Area (MPCA) by the Forest Department. The presence of Insectivorous plants, wild edibles, ornamentals, wild relatives to the crop plants indicate that this is the better area for biological tours at school and college level. Action should be taken to protect the plant resources at the time of Sravanamasam due to the heavy pressure from the pilgrims.

A highly coordinated action-oriented multi-disciplinary approach on potential plant resources conservation integrating the forest department, Non-Governmental Organizations, scientific bodies with the co-operation of local communities should be launched at the earliest.

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RESEARCH ARTICLE

STUDIES ON THE IMPACT OF A MALATHION INSECTICIDE ON CERTAIN BIOCHEMICAL CONSTITUENTS OF A FRESHWATER FISH, *LABEO ROHITA*

Anusiya Devi, K. and M. Lekeshmanaswamy*
Department of Zoology, Kongunadu Arts and Science College (Autonomous), Coimbatore – 641 029, Tamil Nadu, India.

ABSTRACT

Malathion is an insecticide which is commonly used for the agricultural and non-agricultural purposes in India. Malathion is found effective for controlling mosquitoes, flies, household insects, animal parasites (ectoparasites) and head & body lice. The effect of insecticide Malathion is found to be highly toxic even to the non-targeted aquatic organisms including fish. The aim of the study, was to determine the effect of insecticide malathion on some biochemical characteristics (protein, carbohydrate and cholesterol in gill, liver, muscle and kidney) of the fish, *Labeo rohita*. Toxicity evaluation tests were conducted to determine LC_{50} values. The $1/10^{th}$ of 96 hrs, LC_{50} value was selected as sublethal concentrations (0.5 ppm). All biochemical's parameters were found to be decreased in all tissues on comparison with control. The results indicated the toxic nature of the insecticide malathion.

Keywords: Malathion, *Labeo rohita*, biochemical and sublethal study.

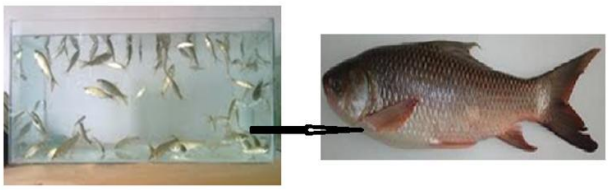
1. INTRODUCTION

Pesticides are most commonly used in agriculture to aid in the production of high quality food. However, some pesticides have the ability to cause serious health and environmental damage (1). The exposure to the sub - lethal doses of some pesticides repeatedly can cause physiological and behavioural changes in fish that bring down populations increaded susceptibility to increase and decreased efficiency to avoid predators (2). Malathion is one of the organophosphate insecticide. That was developed earlier (introduced in 1950). The over use of malathion on land may be washed along with the surface water which can adversely affect or kill the aquatic organisms and other higher organisms. The organisms of the aquatic habitat particularly fishes, are highly sensitive to this organo phosphate insecticide. The first indication of the stress is the biochemical changes occurring in the body of the organisms. A number of changes in biochemical parameters of aquatic organisms due to the pesticidal exposure have been reported by several investigators (3,4,5).

2. MATERIALS AND METHODS

2.1. Procurement and maintenance

A commercial formulation of malathion (Hi-Yield 55% EC 500gl-1) was purchased from a local market in Thudiyalur, Coimbatore district, Tamil Nadu and was used in this study. Bulk of sample of fishes (*Labeo rohita*) ranging in weight from 3-4gms measuring 3-5cm in length were procured from Aliyar reservoir. They were carried to the laboratory in suitable polythene bags containing oxygenated water. The fishes were acclimated to the laboratory temperature (26±1.5) in large glass aquarium. Another group was maintained as control. The fishes were acclimatized to the laboratory conditions for two weeks in glass aquaria. The period of acclimation lasted for 2 weeks. Batches of 10 healthy fishes were exposed to different concentrations of insecticide malathion to calculate the median lethal concentration LC_{50} value using probit analysis method (6).



Collection and Maintenance of fresh water fish, *Labeo rohita*

Morphological Characters of Experimental fish - *Labeo rohita*

Toxicant - Malathion

*Correspondence: Dr. M. Lekeshmanaswamy, Associate Professor, Department of Zoology, Kongunadu Arts and Science College (Autonomous), Coimbatore - 641 029, Tamil Nadu, India. E-mail: ml_swamy64@yahoo.co.in

2.3. Evaluation of median lethal concentration (LC_{50})

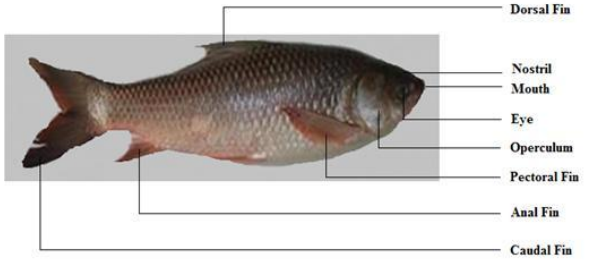
The concentration of the pollutant at which 50 percent of the test animals die during a specific test period of the concentration lethal to one half of the test population is referred to as median lethal concentration (LC_{50}) or median reference limit in aquatic toxicology the traditional LC_{50} test is often used to measure the potential risk of a chemicals (7). The fishes (Four groups) were exposed to the sublethal concentration (0.5 ppm) of malathion for 24, 48, 72 and 96hrs respectively.

2.4. Killing of Animals

The fish was caught very gently using a small dip net, one at a time with least disturbance. At the end of each exposure time, fishes were decapitated and tissues such as gill, liver, kidney and muscle were dissected and stored at 4°C until the analyses were performed. The tissues (10 mg) were homogenized in 80% methanol centrifuged at 3500 rpm for 15 min & the clear supernatant was used for the analysis of different parameters.

2.5. ESTIMATION OF BIOCHEMICAL PARAMETERS

2.5.1. Estimation of total protein



Total protein concentration was estimated by the method of Lowry *et al.* (1951) based on the following principle. In alkaline medium protein in the sample form a complex with copper ions. The amino acids containing aromatic groups, tyrosin and tryptophane present in copper protein complex react with Folin ciocalteu phenol reagent to give blue colour due to the reduction of phosphomolybdate. The intensity of the colour developed is proportional to the concentration of protein present in the sample. The value is expressed as mg/g of tissues.

2.5.2. Estimation of carbohydrate

Quantitative estimation of carbohydrate in the tissues was done following the method by Hedg's and Hofreiter (1962).

2.6. Principle

Carbohydrates are first hydrolysed into simple sugars using dilute hydrochloric acid. In hot acidic medium glucose is dehydrated to hydroxymethyl furfural. This compound forms with anthrone, a green coloured product with an absorption maximum at 630 nm. The value is expressed as mg/g of tissues.

2.7. *Estimation of lipid*

Estimation of Lipid was estimated by the method of Richmond, (1973) based on the following principle.

Cholesterol esterase hydrolyses cholesterol esters into free cholesterol and fatty acids. In the second reaction cholesterol oxidase converts cholesterol to cholest-4-en-3-one and hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide oxidatively occupies with 4-aminoantipyrine and phenol to produce red quinoeimine dye which has absorbance maximum at 510nm (500 - 530). The intensity of red colour is proportional to the amount of total cholesterol in the specimen. The value is expressed as mg/g of tissues.

$$\text{Cholesterol esters} \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol + fattyacids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} H_2O_2 + \text{Cholest + 4-en-3one}$$

$$2H_2O_2 + \text{4 - aminoantipyrine} + \text{Phenol} \xrightarrow{\text{Peroxidase}} \text{Redquinoeimine dye} + H_2O$$

2.6. Statistical analysis

Statistical analyses were performed using statistical package SPSS - 20, data are presented as mean ± SD.

3. RESULTS AND DISCUSSION

The percentages of dead fishes for different Malathion doses of 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 mg/L were determined for 24, 48, 72 and 96 hr (Table 1) was found in fingerling of *Labeo rohita*. Mortality of *Labeo rohita* increased with increasing concentrations of Malathion insecticide, while there was no mortality in the control. The various periods of Malathion exposure has revealed the depletion of the biochemical parameters like protein and glycogen in *Labeo rohita* (7). Protein plays a important role in almost every biological processes. Under stressed conditions, protein serve as the suppliers of energy for the metabolic pathways and biochemical reactions (8). The result of the present study showed that when the fish were exposed to malathion (0.5 ppm) the protein content were found to have decreased (Table 2). The present study revealed the reduction in protein levels in the tissues of *Labeo rohita* by following acute exposure of toxicant Malathion. Aruna *et al.* (9) observed that

the week of exposure to the sublethal concentrations of malathion showed a significant increase in total protein content in kidney of the fish, *Clarias batrachus* and the later periods of exposure showed a gradual decrease in the protein content. Similar results were obtained in *Channa punctatus* when exposed to technical grade malathion. Remia *et al.* (3) observed that the proteolysis and increased metabolism under toxicant stress caused the reduction of protein.

Table 1. Effect of Malathion on survival of *Labeo rohita* for 24, 48, 72 and 96 hr exposure time

S. No.	Concentration (ml)	No. of exposed	No. of dead	Percent of mortality
Control	**-**	**10**	**0**	**0**
1	1.0	10	0	0
2	2.0	10	1	10
3	3.0	10	3	30
4	4.0	10	3	30
5	5.0*	10	5	50
6	6.0	10	6	60
7	7.0	10	6	60
8	8.0	10	8	80
9	9.0	10	8	80
10	10.0	10	10	100

Table 2. Changes in the protein content in the tissues of *Labeo rohita* on short term exposure on short exposure

Sample (mg/g wet tissue)	Exposure periods				
	Control	**24hrs**	**48hrs**	**72hrs**	**96hrs**
Gill	2.46 ± 0.38	1.98 ± 0.43	1.64 ± 0.04	1.32 ± 0.04	1.21 ± 0.08
% change		- 0.19	- 0.33	- 0.46	- 0.50
Liver	1.76 ± 0.07	1.65 ± 0.07	1.18 ± 0.09	1.12 ± 0.11	0.98 ± 0.04
% change		- 0.06	- 0.32	- 0.36	- 0.44
Kidney	2.01 ± 0.07	1.98 ± 0.04	1.47 ± 0.21	1.32 ± 0.10	1.00 ± 0.12
% change		- 0.01	- 0.26	- 0.34	- 0.50
Muscle	3.60 ± 0.31	3.21 ± 0.16	3.04 ± 0.15	2.94 ± 0.06	2.71 ± 0.10
% change		- 0.10	- 0.15	- 0.18	- 0.24

Values were expressed as mean ± S.D of three replicates using SPSS statistical package

Table 3. Changes in the carbohydrate content in the tissues of *Labeo rohita* on short term exposure

Sample (mg/g wet tissue)	Exposure periods				
	Control	**24hrs**	**48hrs**	**72hrs**	**96hrs**
Gill	12.42 ± 0.09	9.58 ± 0.05	7.84 ± 0.41	6.74 ± 0.37	4.98 ± 0.28
% change		- 0.22	- 0.36	- 0.45	- 0.59
Liver	18.62 ± 0.08	15.12 ± 0.22	11.42 ± 0.31	11.20 ± 0.24	10.45 ± 0.33
% change		- 0.18	- 0.38	- 0.39	- 0.43
Kidney	31.00 ± 4.03	26.42 ± 0.27	20.00 ± 3.27	12.40 ± 0.29	10.82 ± 0.13
% change		- 0.17	- 0.35	- 0.60	- 0.65
Muscle	30.41 ± 0.79	24.58 ± 0.45	19.68 ± 0.39	15.54 ± 0.29	13.42 ± 0.25
% change		- 0.19	- 0.35	- 0.48	- 0.55

Values were expressed as mean ± S.D of three replicates using SPSS statistical package

Table 4. Changes in the cholesterol content in the tissues of *Labeo rohita* on

Sample (mg/g wet tissue)	Exposure periods				
	Control	**24hrs**	**48hrs**	**72hrs**	**96hrs**
Gill	21.54 ± 0.35	15.38 ± 0.38	10.25 ± 0.36	9.40 ± 0.21	7.54 ± 0.39
% change		- 0.28	- 0.52	- 0.56	-0.64
Liver	20.05 ± 2.13	16.45 ± 0.25	16.12 ± 0.17	14.00 ± 0.69	13.14 ± 0.22
% change		- 0.17	- 0.19	- 0.30	- 0.34
Kidney	37.12 ± 0.42	31.00 ± 3.45	26.24 ± 0.38	19.50 ± 0.37	11.00 ± 0.57
% change		- 0.16	- 0.29	- 0.47	- 0.70
Muscle	64.65 ± 0.20	56.75 ± 0.36	41.00 ± 4.53	34.10 ± 3.97	25.45 ± 0.37
% change		- 0.12	- 0.36	- 0.47	- 0.60

Values were expressed as mean ± S.D of three replicates using SPSS statistical package

Carbohydrates are stored in the form of glycogen in the tissues and organ like liver of fishes to supply energy needs during hypoxic condition and lack of food (10). The results of the present findings showed a significant decrease in carbohydrate content in all the tissues studied (Table 3). Arun Kumar and Jawahar Ali (11) reported that the sublethal concentrations of Malathin and glyphosate in the exposed shrimp *Streptocephalus dichotomus* showed a decrease in carbohydrate content. The decreased level of carbohydrates contents may affect the carbohydrate metabolism due to toxic effect (12).

Lipid play a crucial role in energy metabolism and providing energy to the metabolic processes (13). The results presented in Table 4 show a significant decrease in cholesterol content in the studied tissues of fish, *Labeo rohita*. Generally, the decrease in cholesterol contents in all tissues was found to be increased with the hours of exposure. The inhibition of cholesterol biosynthesis in the liver are the reduction in the absorption of dietary cholesterol might have resulted in the reduced cholesterol level (14). The reduction of lipids in various tissues have been studied by various authors. Mishra *et al.* (15) analysed the gradual depletion of lipid content of liver and muscle during the malathion exposure. Generally, the present results indicated the toxic nature of the insecticide malathion.

4. SUMMARY AND CONCLUSION

In the present study, we have observed the sub lethal exposure of the Malathion proved to be toxic to fish *L. rohita*, which effects on the protein, carbohydrate and lipid levels of vital organs like gill, liver, kidney and muscle. Depletion of Protein, Carbohydrate and Cholesterol occurs after pesticidal exposure shows greater tendency for accumulation of pesticide Malathion in the body of the fresh water fish, *Labeo rohita*. Reducing the use of pesticides and introduction of natural remedies for pest encroachment could minimize pesticide pollution.

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RESEARCH ARTICLE

SCIENTOMETRIC ANALYSIS OF ASIAN COUNTRIES MATHEMATICS PUBLICATIONS

Senthil Kumar, R*. and G. Ulaganathan
Department of Library and Information Science, Kongunadu Arts and Science College (Autonomous), Coimbatore - 641 029, Tamil Nadu, India.

ABSTRACT

This paper discusses about the Asian countries Mathematics publications during the period of 1996-2016 and its citation available in the Scimago Journal and Country Rank data base by the authors from top 15 countries (based on publications). The relevant data are collected from Scimago Journal and Country Rank data base and it was analyzed. It shows among the Asian countries Mathematics publications totally 833461 articles were published. Among the publications, maximum of 418170 (50.17%) articles published by China followed by Japan with 129674 (15.56%) publications and India is in 3rd place with 77303 (9.28%) publications during the study period.

Keywords: Asian Countries, Mathematics, Scimago Journal and Country Rank, Citations, Self Citations, Citable Documents, H- Index.

1. INTRODUCTION

The true measurement of assessing the quality and quantity of a journal is the Citation Index. While discussing citation, one needs to know the citation. Simply, when another refers other works in his/her article, we describe the article referred is cited. In other words the citation is called as the earlier work which is referred in the present work. The quality of a given work can precisely be deemed through the number of citations that it gets. Therefore, a firm piece of article or research paper is carrying more number of citations get more impact than the work carrying less citation. Therefore, we always refer to some indexing and abstracting databases like Web of Science, Scopus, or even Google Scholars to know the impact of a particular journal, a article or a particular author. Scimago Journal and Country Rank database developed by Scimago Lab and powered by Scopus

2. REVIEW OF LITERATURE

Senthilkumar *et al*. (1) this study analyzes the Astrophysics research output in India from 1989 to 2014. The study revealed that the highest number of publications is in the year 2013 with 913 records having a GCS of 4342 and LCS of 324. The major source of publication in Astrophysics research comes in the form of articles. Rajneesh *et al.* (2) have analyzed research output of Computer Science Literature, articles published in the "Journal of the ACM", for ten years in from 1999 to 2008. The study envisages that a total number of 336 papers comprise of 10799 citations. The highest average citations per article were 37.25 the overall average of the citations per article is 32.14. Journals and conference proceedings and both of them together have shared 77% of the total citations. 3926 (36.88%) citations authored by a single author, whereas 6719 citations (63.12%) were multiple authors. It is evident that Computer science is one of the emerging disciplines. Krishnan *et al.* (3) have studied the Current science Publications research output, for the period of 2000 to 2013. Among 2357 records, the most productive author was Aswal, V.K. with 108 papers and the highest number of records 334 published in 2011 and 322 records in the year 2010. Total 73.8% of the literature was published records were articles. India was the top produced country with 1363 publications (57.8%) followed by USA with 293 publications (12.4%). Most productive Institution was Bhabha Atomic Research Center (BARC), which topped with 143 publications. Seeman *et al.* (4) have analyzed the growth rate of environmental science literature output in nineteen Universities of South India the period of 2000 - 2012 were retrieved from Web of Science database Among total 6784 journal articles, the highest output was in the year 2012 that accounts for 13.97% and the 7694 journal articles occupy predominant position sharing 88.17% of total research output. A core set of 38 journals has covered about one third of the total publications made by the environmental science researchers in selected universities from South India. Khatun *et al.* (5) have examined the periodical articles on diarrheal disease research in Bangladesh. The articles were derived from PubMed, Web of Science and Scopus databases from the period of 1971 to 2009 (38 Years). The total number of retrieved records was 1.521 PubMed 488; WoS 419; and Scopus 614). The unique 711 records were retained for analysis. The literature growth increased with an average 18.23 articles published

*Correspondence: Dr. R. Senthil Kumar, Librarian (SG) and Head (Research), Department of Library and Information Science, Kongunadu Arts and Science College (Autonomous), Coimbatore - 641 029, Tamil Nadu, India. E.mail: kasclibrary@yahoo.com

per year. The majority of journals 99 (65.55%) were published in the USA and UK.

3. METHODOLOGY

This study aims to discuss about the Asian countries Mathematics publications and its citation available in the Scimago Journal and Country Rank data base by the top 15 countries (based on publications) (6). The relevant data are collected from Scimago Journal and Country Rank database. Based on the available sources, the following discussions are made.

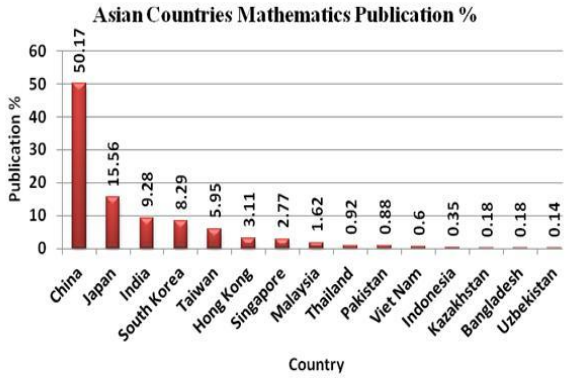
4. ANALYSIS AND INTERPRETATION

The distributions of the Asian countries Mathematics publications by the top 15 countries that is available in Scimago Journal and Country Rank data base which were analyzed in the Table 1.

4.1. Asian countries mathematics publications (Top 15 Countries)

Table 1. Asian Countries Mathematics Publications (Top 15 Countries)

S. No.	Country	Mathematics Publication	%
1	China	418170	50.17
2	Japan	129674	15.56
3	India	77303	9.28
4	South Korea	69104	8.29
5	Taiwan	49578	5.95
6	Hong Kong	25910	3.11
7	Singapore	23107	2.77
8	Malaysia	13528	1.62
9	Thailand	7649	0.92
10	Pakistan	7366	0.88
11	Viet Nam	4968	0.60
12	Indonesia	2890	0.35
13	Kazakhstan	1535	0.18
14	Bangladesh	1492	0.18
15	Uzbekistan	1187	0.14
	Total	833461	100



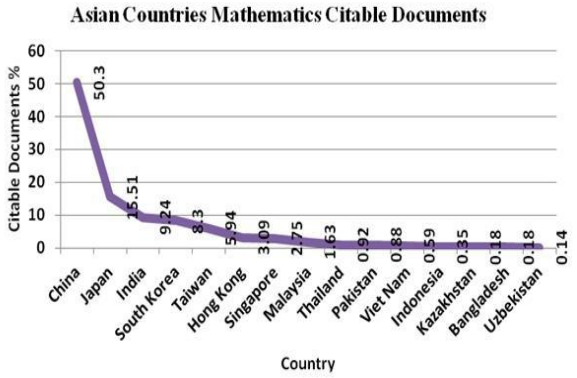
The above Table shows that the country-wise distribution of Asian Countries Mathematics Publications From 1996 to 2016, totally 833461 articles was published which are indexed in Scimago database. Among the publications, maximum of 418170 (50.17%) articles published by China and followed by Japan with 129674 (15.56%) publications and India is in 3rd place with 77303 (9.28%) publications.

4.2. Asian countries mathematics citable documents

Table 2. Asian Countries Mathematics Citable Documents

S. No.	Country	Mathematics Citable Documents	%
1	China	414307	50.30
2	Japan	127731	15.51
3	India	76183	9.24
4	South Korea	68341	8.30
5	Taiwan	48902	5.94
6	Hong Kong	25422	3.09
7	Singapore	22660	2.75
8	Malaysia	13391	1.63
9	Thailand	7564	0.92
10	Pakistan	7259	0.88
11	Viet Nam	4855	0.59
12	Indonesia	2862	0.35
13	Kazakhstan	1512	0.18
14	Bangladesh	1469	0.18
15	Uzbekistan	1175	0.14
	Total	823633	100

The above Table presents the country-wise distribution of Asian Countries Mathematics citable documents (includes articles, reviews and conferences papers), from top 15 countries from 1996 to 2016, 823633 citable documents were available which are indexed in Scimago database. Among the citable documents maximum of 414307 (50.30%) by China followed by Japan with 127731 (15.51%) and India contributed 76183 (9.24%) citable documents.

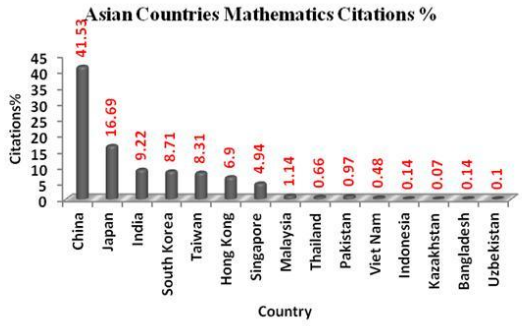


4.3. Asian countries mathematics citations

Table 3. Asian Countries Mathematics Citations.

S. No.	Country	Mathematics Citations	%
1	China	2126508	41.53
2	Japan	854615	16.69
3	India	471974	9.22
4	South Korea	446103	8.71
5	Taiwan	425341	8.31
6	Hong Kong	353458	6.90
7	Singapore	252969	4.94
8	Malaysia	58238	1.14
9	Thailand	33912	0.66
10	Pakistan	49702	0.97
11	Viet Nam	24797	0.48
12	Indonesia	7350	0.14
13	Kazakhstan	3823	0.07
14	Bangladesh	7086	0.14
15	Uzbekistan	4912	0.10
	Total	5120788	100

The above Table shows the distribution of Asian Countries Mathematics citations, from top 15 countries from 1996 to 2016. Among the citations maximum of 2126508 (41.53%) by China followed by Japan with 854615 (16.69%) and India contributed 471974 (9.22%) Citations.

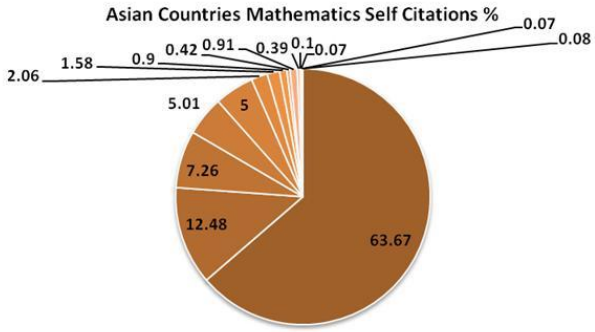


4.3. Asian countries mathematics self citations

Table 4. Asian Countries Mathematics Self Citations

S. No.	Country	Mathematics Self Citations	%
1	China	1376546	63.67
2	Japan	269878	12.48
3	India	156953	7.26
4	South Korea	108313	5.01
5	Taiwan	108068	5.00
6	Hong Kong	44632	2.06
7	Singapore	34073	1.58
8	Malaysia	19448	0.90
9	Thailand	8949	0.42
10	Pakistan	19619	0.91
11	Viet Nam	8515	0.39
12	Indonesia	2111	0.10
13	Kazakhstan	1617	0.07
14	Bangladesh	1430	0.07
15	Uzbekistan	1870	0.08
	Total	2162022	100

The above Table reveals the distribution of Asian Countries Mathematics self citations, from top 15 countries from 1996 to 2016. Among the Asian Countries Mathematics self citations maximum of 1376546 (63.67%) by China followed by Japan with 269878 (12.48%) and India's self citation is 156953 (7.26%)

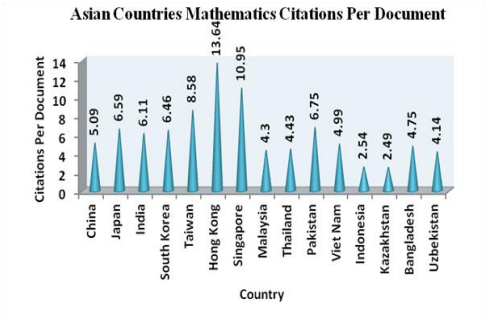


4.4. Ranking of asian countries mathematics citations per document

Table 5. Ranking of Asian Countries Mathematics Citations Per Document

S. No.	Country	Citations Per Document	Ranking
1	China	5.09	VIII
2	Japan	6.59	V
3	India	6.11	VII
4	South Korea	6.46	VI
5	Taiwan	8.58	III
6	Hong Kong	13.64	I
7	Singapore	10.95	II
8	Malaysia	4.3	XII
9	Thailand	4.43	XI
10	Pakistan	6.75	IV
11	Viet Nam	4.99	IX
12	Indonesia	2.54	XIV
13	Kazakhstan	2.49	XV
14	Bangladesh	4.75	X
15	Uzbekistan	4.14	XIII

The above Table depicts that the ranking of Asian Countries Mathematics Citations per Document (Average citations to documents published during 1996-2016), from top 15 countries. Among the Ranking of citations per document study Hong Kong is in first rank with 13.64 followed by Singapore with 10.95 in second rank and Taiwan is in third rank with 8.58 citations per document used.

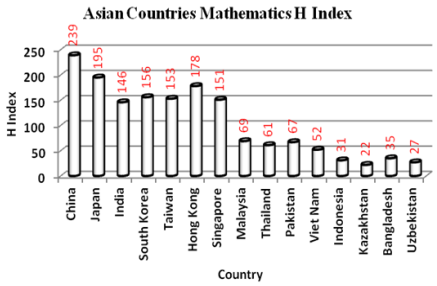


4.5. Ranking of asian countries mathematicsh index

Table 6. Ranking of Asian Countries Mathematics H Index

S. No.	Country	H Index	Ranking
1	China	239	I
2	Japan	195	II
3	India	146	VII
4	South Korea	156	IV
5	Taiwan	153	V
6	Hong Kong	178	III
7	Singapore	151	VI
8	Malaysia	69	VIII
9	Thailand	61	X
10	Pakistan	67	IX
11	Viet Nam	52	XI
12	Indonesia	31	XIII
13	Kazakhstan	22	XV
14	Bangladesh	35	XII
15	Uzbekistan	27	XIV

The data presented in the above table shows that the ranking of Asian Countries Mathematics distribution of H Index (country's number of articles (h) that have received at least h citations) the China is in the first rank with 239 H indexes followed by Japan with 195H indexes respectively and Hong Kong is in third rank with 178 H indexes. Also India is in Seventh rank with 146 H Indexes.



5. CONCLUSION

The superiority and magnitude of research are made obtainable through indexing journals with citations of various articles. There is wanting, for providing citations to other articles which authors cite. For reviewing the prior articles which are very much important for behind your article value added point for publishing. It is a good practice to give self citation for their previous works and it follows up of the previous one and improved one. During the study period from 1996 to 2016, among the publications, maximum of 418170 (50.17%) articles published by China and followed by Japan with 129674 (15.56%) publications and India is in 3rd place with 77303 (9.28%) publications. The above citable documents study shows that maximum of 414307 (50.30%) by China followed by Japan with 127731 (15.51%) and India contributed 76183 (9.24%) citable documents. The study envisages that maximum number of citations 2126508 (41.53%) by China followed by Japan with 854615 (16.69%) and India contributed 471974 (9.22%) citations. The above study reveals that maximum number of self citations 1376546 (63.67%) by China followed by Japan with 269878 (12.48%) and India's self citation is 156953 (7.26%). Among the Ranking of citations per document study Hong Kong is in first rank with 13.64 followed by Singapore with 10.95 in second rank and Taiwan is in third rank with 8.58 citations per document used. The H Index study shows that China is in the first rank with 239 H indexes followed by Japan with 195H indexes and Hong Kong is in third rank with 178 H indexes. India is in Seventh rank with 146 H Indexes. It is concluded that the maximum number of Asian Countries Mathematics publications, Citable documents, citations, self citations and H index are in the rank of China and Japan respectively.

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