# EXISTENCE OF SOLUTIONS FOR IMPULSIVE NEUTRAL FUNCTIONAL INTEGRO DIFFERENTIAL EQUATIONS WITH NONLOCAL CONDITIONS

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#### ABSTRACT

In this paper, by using fractional power of operators and Sadovskii's fixed point theorem, we study the existence of mild solution for a certain class of impulsive neutral functional integrodifferential equations with nonlocal conditions. The results we obtained are a generalization and continuation of the recent results on this issue.

Keywords: Sadovskii's fixed point theorem, integrodifferential equations.

#### **1. INTRODUCTION**

Impulsive differential equations, that are differential equations involving impulsive effect, appear as a natural description of several real world problems. Many evolution process that have a sudden change in their states such as mechanical systems with impact, biological systems such as heart beats, blood flows, population dynamics, theoretical physics, radiophysics, pharmacokinetics, mathematical economy, chemical technology, electric technology, metallurgy, ecology, industrial robotics, biotechnology process, chemistry, engineering, control theory, medicine and so on. Adequate mathematical models of such processes are systems of differential equations with impulses, see the monographs of Bainov and Simeonov (13), Bainov, Lakshmikantham and Simeonov (14), the papers (10,15) and the references therein.

The theory of integrodifferential equations can be used to describe a lot of natural phenomena arising from many fields such as electronics, fluid dynamics, biological models, and chemical kinetics. Most of these phenomena cannot be described through classical differential equations. That is Why in recent years they have attracted more and more attention of several mathematicians, physicists and engineers. Impulsive integrodifferential equations has undergone rapid development over the years and played very important role in modern applied mathematical models of real process. Recently, several authors (3,9,11) have investigated the impulsive integrodifferential equations in abstract spaces. We refer to the papers Wang and Wei (20) and Guo (21) and the references cited therein. Particularly, neutral (integro) differential equations arise in many areas of applied mathematics. For instance, the system of heat conduction with finite wave speeds, studied in (19) can be modeled in the form integrodifferential equation of neutral type. For more details on this theory and on its applications we refer to the monographs of Lakshmikantham *et al.* (14), and Samoilenko and Perestyuk (4) for the case of ordinary impulsive system and for partial differential and for partial functional differential equations with impulses.

The starting point of this paper is the work in papers (11, 12). Especially, authors in (12) investigated the existence of solutions for the system.

$$\frac{d}{dt} \Big[ x(t) + F\left(t, x(t), x(b_1(t)), \dots, x(b_m(t))\right) \Big] + A(t) x(t)$$

$$G\left(t, x(t), x(a_1(t)), \dots, x(a_n(t))\right), \quad 0 \le t \le a \quad (1)$$
$$x(0) + g(x) = x_0 \quad (2)$$

=

by using fractional powers of operators and Sadovskii's fixed point theorem. And in (11), authors studied the following impulsive functional integrodifferential equation with nonlocal conditions of the form

$$\begin{aligned} x'(t) &= A(t)x(t) \\ &+ F\left(t, x(\sigma_1(t)), \dots, x(\sigma_n(t)), \int_0^t h\left(t, s, x(\sigma_{n+1}(s))\right) ds\right) (3) \\ &\quad t \in J = [0, b], \ t \neq t_{k,} \ k = 1, \dots, m \\ &\quad x(0) + g(x) = x_0 \end{aligned}$$

$$\Delta x(t_k) = I_k(x(t_k)), \ k = 1, \dots, m$$
(5)

by using Schaefer's fixed point theorem.

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Motivated by above mentioned works (11,12), the main purpose of this paper is to prove the existence of mild solutions for the following impulsive neutral functional integrodifferential equations in a Banach space *X*.

$$\frac{d}{dt}\left[F\left(t,x(b_1(t)),\ldots,x(b_m(t)),\int_0^t h\left(t,s,x(b_{m+1}(s))\right)ds\right)\right]$$

$$= A(t)x(t) + G\left(t, x(a_1(t)), \dots, x(a_n(t)), \int_0^t e\left(t, s, x(a_{n+1}(s))\right) ds\right)$$
(6)

$$t \in J = [0, b], \ t \neq t_{k_{i}} \ k = 1, \dots, m$$
$$x(0) + g(x) = x_{0}$$
(7)

$$(t)U(t,s) \| \le \frac{C_{\alpha}}{(t-s)^{\alpha}} \qquad 0 \le t$$
$$\le b \qquad (9)$$

For our convenience let us take  $F(0, x(b_1(0)), \dots, x(b_m(0)), 0) = 0.$ 

Let  $M_0 = \| (-A)^{-\beta}(t) \|$  assume the following conditions

(*H*1)  $F: [0, b] \times X^{m+1} \to X$  is a continuous functions and there exists a  $\beta \in (0,1)$  and L > 0 such that the function  $(-A)^{\beta}F$  satisfies the condition:

$$\| (-A)^{\beta} F(s_{1}, x_{1}, x_{2}, \dots, x_{m}, y) - (-A)^{\beta} F(s_{1}, \overline{x_{1}}, \overline{x_{2}}, \dots, \overline{x_{m}}, \overline{y}) \|$$
  
$$\leq L \Big( \max_{i=1,\dots,m} \| x_{i} - \overline{x_{i}} \| + \| y - \overline{y} \| \Big)$$

 $\label{eq:sigma_state} \begin{array}{ll} \text{for any } 0 \leq s_1, s_2 \leq b, \ x_i, \overline{x_i}, y, \overline{y} \in X, \ i=1,2, \ldots, m. \end{array}$ 

Moreover, there exists constant N > 0 such that

$$\|\int_0^t [h(t,s,x) - h(t,s,y)] ds \| \le N \|x - y\| \text{ for } t, s \in 0, b, x, y \in X$$

(H2) The function  $G: [0, b] \times X^{n+1} \to X$  satisfies the following conditions:

each For  $t \in [0, b],$ function (i) the  $G(t,.): X^{n+1} \to X$  is continuous and for each  $(x_1,x_2,\ldots,x_n,y)\in X^{n+1}$ the function  $G(., x_1, x_2, \dots, x_n, y) : [0, b] \rightarrow X$ strongly is measurable.

(ii) For each positive number  $k \in \mathbb{N}$ , there is a positive function  $g_r \in L^1(J)$  such that

$$\sup_{\|x_1\|,\dots,\|x_n\| \le r} \|G(s_1, x_1, x_2, \dots, x_n, y)\| \le g_r(t) \text{ and }$$

$$\lim_{r \to \infty} \frac{1}{r} \int_0^b g_r(s) ds = \gamma < \infty$$
(H3)  $a_i, b_j \in C([0, b], [0, b]), i = 1, 2, ..., n + 1, j = 1, 2, ..., m + 1.$ 

(H4) There exist positive constants  $L_1$  and  $L_2$  such that

$$\|g(x)\| \le L_1 \|x\|_{\Omega} + L_2$$
 for all  $x \in \Omega$ 

and  $g : \Omega \rightarrow X$  is completely continuous.

(H5)  $I_k : X \to X$  is completely continuous and there exist continuous non decreasing functions  $L_k : R_+ \to R_+$  such that for each  $x \in X$ .

$$\|I_k(x)\| \leq L_k(\|x\|), \qquad \liminf_{r \to \infty} \frac{L_k(r)}{r} = \lambda_k < \infty$$

#### **Definition 2.1**

A family of linear operator  $\{U(t, s) : 0 \le s \le t \le b\}$  on *X* is called an evolution system if the following conditions hold:

(*i*)  $U(t, s) \in B(X)$  the space of bounded linear transformation on *X* whenever  $0 \le s \le t \le b$  and for each  $x \in X$  the mapping  $(t, s) \rightarrow U(t, s)$  is continuous;

(*ii*)  $U(t,s) U(s,\tau) = U(t,\tau)$  whenever  $0 \le \tau \le s \le t \le b$ .

#### Theorem 2.1 (Sadovskii)

Let *P* be a condensing operator on a Banach space, that is, *P* is continuous and takes bounded sets into bounded sets, and let  $\alpha(P(B)) \leq \alpha(B)$  for every bounded set *B* of *X* with  $\alpha(B) > 0$  of  $P(H) \subset H$  for a convex, closed and bounded set *H* of *X*, then *P* has fixed point in *H* (where  $\alpha(.)$  denotes Kuratowski's measure of noncompactness).

#### **3. EXISTENCE RESULTS**

In order to define the solution of the problem (6) - (8), we consider the following space

$$\Omega = \begin{cases} x : J \to X : x(t) \text{ is continuous at } t = t_k \text{ and left continuous at } \\ t = t_k \text{ and the right limit } x(t_k^+) \text{ exists for } k = 1, ..., m \end{cases}$$

which is Banach space with norm

$$||x||_{\Omega} = \sup_{t \in J} ||x(t)||.$$

Definition 3.1

A continuous function  $x(.) : [0, b] \to X$  is said to be a mild solution of the nonlocal Cauchy problem(6) - (8), if the function  $A(s)U(t,s)F(s,x(b_1(s)), ...x(b_m(s)), \int_0^s h(s,\tau,x(b_{m+1}(\tau))) d\tau),$  $s \in [0,b)$ 

is integrable on [0, b) and the integral equation

$$\begin{aligned} x(t) &= U(t,0)[x_0 - g(x)] \\ &- F\left(t, x(b_1(t)), \dots, x(b_m(t)), \int_0^t h\left(t, s, x(b_{m+1}(s))\right) ds\right) \\ &- \int_0^t A(s) U(t,s) F\left(s, x(b_1(s)), \dots, x(b_m(s)), \int_0^s h\left(s, \tau, x(b_{m+1}(\tau))\right) d\tau\right) ds \\ &+ \int_0^t U(t,s) G\left(s, x(a_1(s)), \dots, x(a_n(s)), \int_0^t e\left(s, \tau, x(a_{n+1}(\tau))\right) d\tau\right) ds \\ &+ \sum_{0 < t_k < t} U(t, t_k) I_k\left(x(t_k)\right) \end{aligned}$$
(10)

Theorem 3.1

If assumptions  $(H_1) - (H_5)$  are satisfied and  $x_0 \in X$ , then the nonlocal Cauchy problem (6) - (8) has a mild solution provided that

$$L_0 = L(N+1) \left[ M_0 + \frac{1}{\beta} C_{1-\beta} b^{\beta} \right] < 1$$
 (11)

and  

$$M\left[L_{2} + \gamma + \sum_{k=1}^{m} \lambda_{k}\right]$$

$$+ L(N+1)\left[M_{0} + \frac{1}{\beta} C_{1-\beta} b^{\beta}\right]$$

$$< 1 \qquad (12)$$

Proof:

Let us write,

$$\begin{pmatrix} t, x(b_1(t)), \dots, x(b_m(t)), \int_0^t h(t, s, x(b_{m+1}(s))) ds \end{pmatrix}$$
  
=  $(t, v(t))$  and

$$\begin{pmatrix} t, x(a_1(t)), \dots, x(a_n(t)), \int_0^t e(t, s, x(a_{n+1}(s))) ds \end{pmatrix}$$
  
=  $(t, u(t))$ 

Define the operator *P* on  $\Omega$  by the formula

$$(Px)(t) = U(t,0)[x_0 - g(x)] - F(t,v(t)) - \int_0^t A(s)U(t,s) F(s,v(s)) + \int_0^t U(t,s) G(s,u(s)) ds + \sum_{0 < t_k < t} U(t,t_k) I_k (x(t_k)) \quad 0 \le t \le b$$

For each positive integer r, let

 $B_r = \{ x \in \Omega \colon \| x(t) \| \le r, 0 \le t \le b \}.$ 

Then for each r,  $B_r$  is clearly a bounded closed convex set in  $\Omega$ . Since by (9) and (H1) the following relation holds:

$$\|A(t)U(t,s)F(s,v(s))\|$$
  
=  $\|(-A)^{1-\beta}(t)U(t,s)(-A)^{\beta}(t)F(s,v(s))\|$ 

$$\leq \frac{C_{1-\beta}}{(t-s)^{\beta}} [L(N+1)r + LC_1 + C_2]$$

Where,

$$\begin{array}{l} C_1 = b \ h(t,s,0), \quad C_2 = \\ \left\| (-A)^{\beta}(t) \right\| \, \left\| \, F(t,0,0,\ldots,0,0) \right\| \end{array}$$

then from Bocher's theorem (22) it follows that A(t)U(t,s)F(s,v(s)) is integrable on [0,b], so P is well defined on  $B_r$ . We claim that there exist a positive integer r such that  $PB_r \subseteq B_r$ . If it is not true, then for each positive integer r, there is a fuction  $x(.) \in B_r$ , but  $Px_r \notin B_r$ , that is  $||Px_r(t)|| > r$ for some  $t(r) \in [0, b]$ , where t(r) denotes t is dependent of r. However, on the other hand, we have

$$r < ||Px_{r}(t)|| = ||U(t,0)[x_{0} - g(x_{r})] - \int_{0}^{t} A(s)U(t,s)F(s,v_{r}(s))ds + \int_{0}^{t} U(t,s)G(s,u_{r}(s))ds + \sum_{0 < t_{k} < t} U(t,t_{k})I_{k}(x_{r}(t_{k}))||$$
  
$$\leq ||U(t,0)[x_{0} - g(x_{r})]|| + ||(-A)^{-\beta}(t)(-A)^{\beta}(t)F(t,v_{r}(t))||$$

$$+ \int_{0}^{t} \| (-A)^{1-\beta}(s)U(t,s)(-A)^{\beta}(s)F(s,v_{r}(s)) \| ds + \int_{0}^{t} \| U(t,s)G(s,u_{r}(s)) \| ds + \sum_{0 < t_{k} < t} \| U(t,t_{k}) I_{k}(x_{r}(t_{k})) \| \leq M[\| x_{0} \| + L_{1}r + L_{2}] + M_{0}[L(N + 1)^{2}]$$

$$+ \int_0^t \frac{C_{1-\beta}}{(t-s)^{\beta}} \left[ L(N+1)r + LC_1 + C_2 ds + M0tgr(s) ds \right]$$

$$+M \sum_{k=1}^{m} L_{k}(r)$$

$$\leq M[||x_{0}|| + L_{1}r + L_{2}] + M_{0}[L(N + L_{2})]$$

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$$+ \frac{\mathcal{C}_{1-\beta}}{\beta} \left[ \mathcal{L}(N+1)r + \mathcal{L}\mathcal{C}_1 + \mathcal{C}_2 \right] b^{\beta} + \mathcal{M} \int_0^t g_r(s) ds$$

 $+M\sum_{k=1}^{m}L_{k}(r)$ 

Dividing on both sides by r and taking the lower limit as  $r \to \infty$ , we get

$$\mathcal{M}_{2} + \mathcal{M}_{0}[\mathcal{L}(\mathcal{N}+1)] + \frac{\mathcal{C}_{1-\beta}}{\beta} [\mathcal{L}(\mathcal{N}+1)] b^{\beta} + \mathcal{M}_{\gamma}$$
$$+ \mathcal{M}_{k=1}^{2} \lambda_{k} \ge 1$$

$$M\left[L_2 + \gamma + \sum_{k=1}^{m} \lambda_k\right] + L(N+1)\left[M_0 + \frac{C_{1-\beta}}{\beta} b^{\beta}\right] \ge 1$$

This contradicts (12). Hence for some positive integer r,  $PB_r \subseteq B_r$ .

Next we will show that the operator *P* has a fixed point on  $B_r$ , which implies eq (6) – (8) has a mild solution. To this end, we decompose *P* as  $P = P_1 + P_2$ , where the operator  $P_1, P_2$  are defined on  $B_r$  respectively, by

$$(P_1x)(t) = -F(t, v(t)) - \int_0^t A(s)U(t, s) F(s, v(s)) and$$

$$(P_2 x)(t) = U(t, 0)[x_0 - g(x)] + \int_0^t U(t, s) G(s, u(s)) ds + \sum_{0 \le t_k \le t} U(t, t_k) I_k (x(t_k))$$

for  $0 \le t \le b$ , and we will verify that  $P_1$  is a contraction while  $P_2$  is a compact operator.

To prove that  $P_1$  is a contraction, we take  $x_1, x_2 \in B_r$ . Then for each  $t \in [0, b]$  and by condition (H1) and (11), we have

$$\| (P_1 x_1)(t) - (P_2 x_2)(t) \| \le \| F(t, v_1(t)) - F(t, v_2(t)) \|$$

$$+ \left\| \int_0^t A(t)U(t,s) \left[ F(s,v_1(s)) - F(s,v_2(s)) \right] ds \right\|$$

$$\leq \\ \| (-A)^{-\beta}(t) [ (-A)^{\beta}(t) F(t, v_{1}(t)) - \\ -A\beta(t) Ft, v2t \| \\ + \| \int_{0}^{t} (-A)^{1-\beta}(s) U(t, s) [ (-A)^{\beta}(s) F(s, v_{1}(s)) - \\ -A\beta(s) Fs, v2sds \| \\ \leq M_{0} L [max_{i=1,2,...,m} \| x_{1}(s) - x_{2}(s) \| + N \| x_{1}(s) - \\ x2s \| \\ + \int_{0}^{t} \frac{c_{1-\beta}}{(t-s)^{\beta}} L [max_{i=1,2,...,m} \| x_{1}(s) - x_{2}(s) \| + \\ \end{cases}$$

$$N \| x 1s - x 2s \| ds$$

$$\leq MOLsup0 \leq s \leq b \| x1s - x2s \| + Nsup0 \leq s \leq b \| x1s - x2s \|$$
$$+ \frac{1}{\beta}C_{1-\beta}b^{\beta} L[sup_{0 \leq s \leq b} \| x_{1}(s) - x_{2}(s) \| + Nsup0 \leq s \leq b \| x1s - x2s \|$$

$$\leq L(N+1) \left[ M_0 + \frac{1}{\beta} C_{1-\beta} b^{\beta} \right] \sup_{0 \leq s \leq b} \| x_1(s) - x2s \|$$

$$= L_0 \sup_{0 \le s \le b} \| x_1(s) - x_2(s) \|$$

Thus,

$$\|P_1x_1 - P_1x_2\|_{\Omega} \le L_0 \|x_1 - x_2\|_{\Omega}$$

So by assumption  $0 < L_0 < 1$ , we see that  $P_1$  is contraction.

To prove that  $P_2$  is compact, firstly we prove that  $P_2$  is continuous on  $B_r$ . Let  $\{x_n\}_{n=0}^{\infty} \subseteq B_r$  with  $x_n \to x$  in  $B_r$ , then by H2(i) and H5

$$\begin{split} (i) I_k, k &= 1, 2, \dots, m \text{ is continuous} \\ (ii) G\bigl(s, u_n(s)\bigr) &\to G\bigl(s, u(s)\bigr), \qquad n \to \infty \end{split}$$

Since

 $P_2 x_r$ 

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$$\left\|G\left(s,u_n(s)\right)-G\left(s,u(s)\right)\right\|\leq 2g_r(s)$$

By the dominated convergence theorem, we have

$$|| - P_2 x || = \sup_{0 \le t \le b} || U(t, 0)[g(x_n) - g(x)]$$
  
+  $\int_0^t U(t, s)[G(s, u_n(s)) - (s)]$ 

G(s,u(s))ds

$$+\sum_{0 < t_k < t} U(t, t_k) [I_k(x_n(t_k)) -$$

$$\leq M \| g(x_n) - g(x) \|$$
  
+  $M \int_0^t \| G(s, u_n(s)) - G(s, u(s)) \| ds$   
+  $M \sum_{0 < t_k < t} \| I_k(x_n(t_k)) - I_k(x(t_k)) \|$   
 $\rightarrow 0 \text{ as } n \rightarrow \infty$ 

ie, *P*<sub>2</sub> is continuous.

Next, we prove that  $\{P_2 x : x \in B_r\}$  is a family of equicontinuous functions. To see this we fix  $t_1 > 0$  and let  $t_2 > t_1$ , and  $\epsilon > 0$  be enough small. Then

$$\begin{split} \| (P_{2}x)(t_{2}) - (P_{2}x)(t_{1}) \| &\leq \| U(t_{2}, 0) - \\ U(t_{1}, 0) \| \| x_{0} - g(x) \| \\ &+ \int_{0}^{t_{1}-\epsilon} \| U(t_{2}, s) - \\ Ut_{1,s} \| \| G(s, u(s)) \| ds \\ &+ \int_{t_{1}-\epsilon}^{t_{1}} \| U(t_{2}, s) - \\ Ut_{1,s} \| \| G(s, u(s)) \| ds \\ &+ \\ \int_{t_{1}}^{t_{2}} \| U(t_{2}, s) \| \| G(s, u(s)) \| ds \\ &+ \sum_{0 < t_{k} < t_{1}} \| U(t_{2}, t_{k}) - \\ Ut_{1,tk} \| \| /kxtk \| \\ &+ \\ \end{split}$$

 $\sum_{t_1 < t_k < t_2} \left\| U(t_2, t_k) \right\| \left\| I_k \big( x(t_k) \big) \right\|$ 

Noting that  $||G(s, u(s))|| \le g_r(s)$  and  $g_r(s) \in L^1$ , we see that  $||(P_2x)(t_2) - (P_2x)(t_1)||$  tends to zero independently of  $x \in B_r$  as  $t_2 - t_1 \to 0$ , since the compactness of U(t,s) for t - s > 0, implies the continuity in the uniform operator topology. We can prove that the functions  $P_2x, x \in B_r$  are equicontinuous att = 0. Hence  $P_2$  maps  $B_r$  into a family of equicontinuous functions.

It remains to prove that  $V(t) = \{(P_2x)(t) : x \in B_r\}$  is relatively compact in *X*. V(0) is relatively compact in *X*. Let  $0 < t \le b$  be fixed and  $0 < \epsilon < t$ . For  $x \in B_r$ , we define

$$\begin{aligned} (P_{2,\epsilon}x)(t) &= U(t,0)[x_0 - g(x)] + \\ \int_0^{t-\epsilon} U(t,s) \, G(s,u(s)) ds \\ &+ \sum_{0 < t_k < t-\epsilon} U(t,t_k) \, I_k \left( x(t_k) \right) \\ &= U(t,0)[x_0 - g(x)] + U(t,t-\epsilon) \int_0^{t-\epsilon} U(t-\epsilon,s) Gs, u(s) ds \end{aligned}$$

+ 
$$U(t, t - \epsilon) \sum_{0 < t_k < t - \epsilon} U(t - \epsilon, t_k) I_k(x(t_k))$$

Then from the compactness of U(t, s) for t - s > 0, we obtain  $V_{\epsilon}(t) = \{(P_{2,\epsilon}x)(t) : x \in B_r\}$  is relatively compact in *X* for every  $\epsilon, 0 < \epsilon < t$ . Moreover, for every  $x \in B_r$ , we have

$$\|(P_2 x)(t) - (P_{2,\epsilon} x)(t)\| \le \int_{t-\epsilon}^t \|U(t,s) G(s,u(s))\| ds$$

$$\begin{aligned} + \sum_{t-\epsilon < t_k < t} \| U(t, t_k) \| \| \| I_k (x(t_k)) \| \\ &\leq M \int_{t-\epsilon}^t g_r(s) ds + \\ Mt - \epsilon < tk < tLk(r) \end{aligned}$$

Therefore, there are relatively compact sets arbitrarily close to the set V(t). Hence the set V(t) is also relatively compact in X.

Thus, by Arzela-Ascoli theorem,  $P_2$  is a compact operator. Those arguments enable us to conclude that  $P = P_1 + P_2$  is a condensing map on  $B_r$ , and by the fixed point theorem of Sadovskii there exists a fixed point x(.) for P on  $B_r$ . Therefore, the nonlocal Cauchy problem (6) – (8) has a mild solution and the proof is completed.

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# STRUCTURAL ANALYSIS OF ZINC OXIDE THIN FILMS PREPARED BY THERMAL EVAPORATION TECHNIQUE

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# ABSTRACT

Zinc oxide thin films of 800nm were successfully prepared by thermal evaporation technique. XRD analysis revealed polycrystalline nature of the as prepared ZnO films. The structural parameters such as crystallite size, dislocation density and micro strain were evaluated and discussed.

**Keywords:** Structural analysis, Zinc oxide thin film, thermal evaporation technique.

#### **1. INTRODUCTION**

Thin film technology is stretching its hands in all directions and the thin be defined as any solid or liquid object with one of the dimension is very much less than that of the other two (1). Faraday obtained first evaporated thin films using metal wires in an inert atmosphere in the year 1857(2). After 30 years Warhol in 1887 discovered thin metal film using a pure heating a platinum wire in presence of vacuum. Thin films can be considered to possess two dimensions. Thickness of thin film is usually discussed in terms of Angstrom (Å) and is of the same order of magnitude as the dimension of single atom depending on the properties to be investigated and technological application of the film thickness can be arranged from a few angstrom(Å) 1000 Å. Thin film properties are strongly to dependent on the method of deposition and the background pressure. Specific applications in modern technology demand film properties such a high optical reflection, non porosity, high inertness towards corrosive environment, stability with respect to temperature, stoichiometry and orientation in single film crystal films.

Deposition of thin films increases the contact area of the cell components, resulting in high fraction of reactants. Since its thickness is limited than the bulk material, thin films result in higher current density and cell efficiencies because the transport of ions is easier and faster. All films whether prepared by vacuum deposition or by other techniques are invariably associated with some growth defects or imperfections such as lattice defects , stacking faults , twining, disorders in atomic arrangement, dislocations, grain boundaries , foreign atom inclusion, etc. Thin films science and technology plays an important role in the high tech industries. Thin film technology has been developed primarily for the need of the integrated circuit

industry. The demand for development of smaller and smaller devises with higher speed especially in new generation of integrated circuits requires advanced materials and new processing techniques suitable for future Giga Scale Integration (GSI) technology. In this regard, physics and technology of thin film can play an important role to achieve this goal. The production of thin films for device purposes has been developed over the past 45 years. Thin film as a two dimensional system are of great importance to many real -world problems. Their material cost is very small as compared to the corresponding bulk material and they perform the same function when it comes to processes. Thus, knowledge surface and determination of the nature, function and new properties of thin films can be used for the development of new technologies for future applications (3-5).

Thin film technology is based on three foundations: fabrication, characterization and application. Some of the important applications of thin films are microelectronics, communication, optical electronics, and catalysis, coating of all kinds, and energy generation and conservation strategies. Transparent and highly conducting oxide thin films have attracted many research due to their wide range of applications in industry as well in research. Transparent and conducting layers of some metallic oxides such as CdO, SnO, In<sub>2</sub>O<sub>3</sub> has known for long time.

Zinc oxide is an inorganic compound with the formula ZnO. ZnO is a white powder that is insoluble in water, which is widely used as an additive in numerous materials. It occurs naturally as the mineral zincite but most zinc oxide is produced synthetically. In materials science, ZnO is a wide-bandgap semiconductor of the II-VI semiconductor group. This semiconductor has

7

several favorable properties, including good transparency, high electron mobility, wide bandgap, and strong room-temperature luminescence. Those properties are used in emerging applications for transparent electrodes in liquid crystal displays, in energy-saving or heat-protecting windows, and in electronics as thin-film transistors and light-emitting diodes.

Electrical properties of ZnO has a relatively large direct band gap of  $\sim 3.3 \text{ eV}$  at room temperature. Advantages associated with a large band gap include higher breakdown voltages, ability to sustain large electric fields, lower electronic noise, and high-temperature and high-power operation. The bandgap of ZnO can further be tuned to 3 - 4 eV by its alloying with magnesium oxide or cadmium oxide.

#### 2. MATERIALS AND METHODS

#### 2.1 Preparation of ZnO thin film

In this present work ZnO thin films were deposited using vacuum evaporation method Hind Hivac coating unit. The source material (ZnO (Aldrich 99.9%) is taken in the Mo boat of 200 Amps. Initially, the boat was heated using 10A till the material reach its melting point and a seed layer is allowed to form on the substrate which is placed on the substrate holder 17.5 cm above the source material. After that the amps get increased to get the thin film of the material with sufficient thickness. The thickness of the film can be monitored using digital thickness monitor, attach to the system. The rate of deposition of the film was kept constant at 0.2 Å/sec to get the uniform thickness of the film.

#### 2.2. Structural Characterization

The structural characterization is very important in explaining optical and electrical properties of ZnO thin films. The X-ray diffraction patterns were recorded from  $20^{\circ}$  to  $80^{\circ}$  for ZnO thin film of thickness 800nm prepared by thermal evaporation of ZnO powder at a pressure of  $3 \times 10^{-6}$  torr.

#### **3. RESULTS AND DISCUSSION**

The as deposited films are dark brown, rich in zinc and present a low transmittance; their microstructure was studied using XRD (Fig. 1).

The XRD results show that the prepared film is polycrystalline in nature at randomly oriented. All the peaks in the diffraction pattern correspond to the hexagonal structure of ZnO and are indexed on the basis of JCPDS data card no. 5-664. The prominent peaks around  $2\theta = 37.4$  and  $44^0$  are respectively corresponding to (101) and (203) crystal orientation. The preferential orientation was along (101) crystal plane. The strongest peak along (101) is observed and the other peaks are so weak; this suggests that the films will have the best structure along this orientation (6).

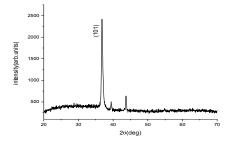


Fig. 1. X-ray diffraction pattern of ZnO thin film.

2.3. Determination of Structural Parameters:

From the XRD profiles, the crystalline size (D) was calculated using the Debye Scherer's formula from the full width at half maximum [FWHM],

$$D = \frac{k\lambda}{\beta\cos\theta} \qquad \dots \qquad (1)$$

Where the constant K is the shape factor = 0.94,  $\lambda$  is the wavelength of the X-rays [1.5406 Å for CuK<sub>α</sub>], θ is the Bragg's angle and β is the FWHM.

The dislocation density ( $\delta$ ) can be evaluated from the crystalline size (D) by the following relation,

$$\delta = \frac{1}{D^2} \qquad \dots \qquad (2)$$

The origin of the micro strain is related to the lattice misfit, which in turn depends upon the deposition conditions. The micro strain ( $\epsilon$ ) can be calculated from the following relation,

$$\mathcal{E} = \left[\frac{\beta \cos \theta}{4}\right] \qquad \dots \tag{3}$$

The structural parameters evaluated by using the above equations from the XRD pattern are given in the Table 1.

Table 1. Structural parameters of ZnO thin film.

Plane	D(Å)	FWH M (βº)	20	D(n m)	δ×10 <sup>-3</sup> (nm <sup>-2</sup> )	ε× 10 <sup>-</sup> 2
101	2.484 7	0.433 23	36. 77	18	3.08	0.1 7

### 4. CONCLUSION

Thin film of ZnO of 800 nm thickness was successfully prepared by thermal evaporation technique. The as prepared ZnO film is polycrystalline in nature. The crystallite size was evaluated to be 18nm. The structural parameters like dislocation density ( $\delta$ ) and micro strain ( $\epsilon$ ) are evaluated to be  $3.08 \times 10^{-3}$  nm<sup>-2</sup> and  $0.17 \times 10^{-2}$  respectively.

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#### SYNTHESIS AND CHARACTERIZATION OF LITHIUM TITANATE (LTO) NANOCOMPOSITES VIA SOLUTION GROWTH ROUTE FOR Li-ION BATTERIES

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# ABSTRACT

The novel bimetal oxide composite of  $Li_4Ti_5O_{12}$  was successfully synthesized by solution growth technique. The structural and microstructural properties of synthesized powders were characterized by powder X-ray diffraction (XRD), fourrier transform infrared spectroscopy (FT-IR), Raman spectroscopy, scanning electron microscopy (SEM) and energy dispersive X-ray-spectroscopy (EDX). The electrochemical performance of the  $Li_4Ti_5O_{12}$  anode was investigated using galvanostatic charge-discharge techniques. The electrochemical property of the Lithium titanate anode was investigated. The good electrochemical performance is ascribed to the stable lithium storage host structure, decreased electrochemical resistance and enhanced lithium-ion diffusion coefficient. Therefore,  $Li_4Ti_5O_{12}$  may be a promising alternative anode material for Li-ion batteries.

Keywords: Lithium titanate (LTO) nanocomposites, Li-Ion batteries.

# **1. INTRODUCTION**

There is a remarkable interest in developing alternative and sustainable energy storage systems to meet modern society needs due to fossil fuel depletion. Compared with other existing energy storage systems, batteries are promising and lots of efforts have been taken by researchers to develop efficient device with affordable price. The development of Lithium-ion batteries with enhanced safety and a long cycle life is vital for mainly energy storage devices used in specific some fields such as electric vehicles (EVs) or hybride electrical vehicles (HEVs), cameras, laptops and mobile phones. Recently, researchers are attempting to develop the advanced nanomaterials for energy storage devices especially for batteries. Among the Nanostructured materials such as lithium cobalt oxide  $(LiCoO_2)$  (1), lithium iron phosphate (LiFePO<sub>4</sub>) (2,3), lithium manganese oxide  $(LiMn_2O_4)$  (4) or oxides of vanadium  $(V_2O_5)$  (5-7), manganese  $(MnO_2)$  (8,9) have been used as the cathode in Lithium ion batteries. Similarly, anode (TiO<sub>2</sub> and graphite) materials have been developed for Lithium ion batteries (10-15). Ti-based materials have been intensively investigated and observed as good potential negative electrode materials for lithium-ion batteries owing to their safety, excellent rate capability and superior cyclic stability. These materials have shown several advantages for example easy and swift charging within ten minutes. Spinel lithium titanate has a high lithium intercalation voltage of 1.55 V against a lithium electrode with a theoretical capacity of 170 mAhg<sup>-1</sup> and an actual discharge capacity of over 160 mAhg<sup>-1</sup>. Now a day's new kind of anode materials being developed in order to reduce the cost as well as to make highly efficient devices. Among the new anode materials, Li<sub>4</sub>Ti<sub>5</sub>O<sub>12</sub> is one of the right choices for anode materials due to its superior performance nanomaterials have been (16-18). Li<sub>4</sub>Ti<sub>5</sub>O<sub>12</sub> prepared using several methods for instance hydrothermal methods (19), sol-gel process (20, 21), solid sate reaction (22), spray pyrolysis, hydrothermal- microwave synthesis, gel-emulsion, and gel combustion (23-25). In this work, the preparation and characterization of nanostructured novel bimetal oxide Li<sub>4</sub>Ti<sub>5</sub>O<sub>12</sub> by solution growth technique.

#### **2. EXPERIMENTAL**

#### 2.1. Materials preparation

Lithium titanate was prepared by solution growth technique. In a typical experimental procedure, titanium oxysulfate (TiOSO<sub>4</sub>) and lithium hydroxide (LiOH.H<sub>2</sub>O) were dissolved in double distilled water under strong stirring and consequently a precipitate was obtained. The precipitate was dried at 80°C in hot air oven for 10 hr. Finally, colorless powder was obtained which was then heat treated at 850°C in a muffle furnace for 3 h. The structural property of synthesized powder was studied using various advanced characterization techniques.

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# 2.2. Materials Characterization

The X-ray diffraction (XRD) patterns of all the samples were measured on a (XPERT-PRO) diffractometer with monochromatic CuK $\alpha$  – radiation ( $\lambda$  = 1.5406Å). FT-IR spectra of the samples were recorded on a (Thermo Nicolet 380, USA) spectrometer using a KBr pellet technique in the range of 4000–400 cm<sup>-1</sup>. The SEM - EDX were recorded on a (JEOL JSM-6360LV) using an accelerating voltage of 30.0 kV

#### 2.3. Fabrication of electrodes

1.3504 g of lithium titanate and 0.9079 g of carboxymethylcellulose (CMC) were taken in the porcelain dish and mixed well by adding few drops of distilled water. Then, 0.0966 g of binder was also added into the above mixer and then the mixture was homogeneously mixed manually with the aid of spatula. The paste is coated on the electrode using the Dr. Blade method. Then the electrode was dried in oven at 75°C for 1 hour. Lithium was used as counter electrode and polypropylene was used as the separator. 1 M LiPF<sub>6</sub> was dissolved in ethylene carbonate (EC) / dimethyl carbonate (DMC) /1, 2diethyl carbonate (DEC) and used as the electrolyte. The cell was assembled inside the glove box under the argon atmosphere. Galvanostatic charge/discharge cycle test was carried out for all the assembled cells at ambient temperature with constant current mode (0.1 mA) up to 1.5 V for charging and up to 2 V for discharging.

#### **3. RESULTS AND DISCUSSION**

# 3.1. X-ray Diffraction Analysis

Fig. 1 depicts the XRD pattern of synthesized powder. For Lithium titanate the crystalline peaks appear at 18.34, 35.60 and 43.28° and those peaks are assigned to (111), (311) and (400) crystalline planes of cubic phase (Li<sub>4</sub>Ti<sub>5</sub>O<sub>12</sub>). The appeared peaks clearly indicate the formation of Lithium titanate. It should be indicated that the appearance of three more peaks at 21.98, 22.21 and 22.63°. These peaks are assigned to the reduced form of titanium oxide. The formation of titanium oxide (JCPDS no. 76-1690) along with lithium titanate (JCPDS no. 49-0207) may be beneficial in terms of storage capacity when it is used as anode material in the lithium ion batteries. The average crystallite size of the prepared material was calculated from the scherrer equation:

#### $D = k\lambda / \beta \cos\theta$

Where D is the average crystallite size,  $\lambda$  is the X-ray wavelength,  $\beta$  is the full width at half maximum, K is a constant related to crystallite shape, and  $\beta$  in 2 $\theta$  axis of diffraction outline must be in

radians. The  $\theta$  can be in degrees, since the cos  $\theta$  corresponds to the equal number.

The crystallite size of 50 nm was achieved for synthesized materials.

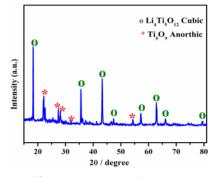
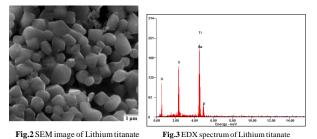


Fig.1 XRD patterns of Lithium titanate

#### 3.2. SEM and EDX Analysis

Fig. 2 shows the SEM image of lithium titanate. The SEM image clearly reveals that the formation of homogeneous cubic morphological features. EDX spectrum of mixture of lithium titanate and titanium oxide nanopowders was measured (Fig. 3). It was found from the EDX spectrum that the presence of appropriate percentage of Ti and O in the synthesized sample. It should be indicated that the Li does not present in EDX spectrum because the Li is light weight element.



#### 3.3. FT-IR Studies

Fig. 4 shows the FTIR p spectrum of the synthesized sample i.e a mixture of lithium titanate and titanium oxide nanopowder. The peaks were observed at 481 and 637 cm<sup>-1</sup> due to the asymmetric stretching vibration of Ti-O-Ti. The peaks positioned in the range of 400-900cm<sup>-1</sup> reveals the symmetric stretching vibration of octahedron groups (26, 27).

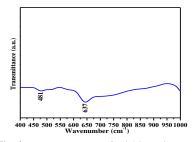


Fig.4 FT-IR spectrum of Lithium titanate

#### 3.4. Raman studies

Raman spectroscopy is an effective technique to characterize the functional groups present in the synthesized samples. Fig. 5 shows the Raman spectrum of the synthesized sample i.e a mixture of lithium titanate and titanium oxide nanopowder. Recently, Xue Li and Wei Liu *et al.* reported that the peaks observed at 671, 227 and 427 cm<sup>-1</sup> due to the  $A_{1g}$ ,  $A_{1g}$  and  $E_g$ , mode, respectively. (28-30). The peaks are observed at 670, 229 and 427 cm<sup>-1</sup> are due to the (2A<sub>1g</sub>) modes and  $E_g$  mode which are attributed to the vibrations of Ti-O bonds (TiO<sub>6</sub>), O-Ti-O and stretching vibrations of Li-O in LiO<sub>4</sub>. This is one of the evidence for the formation of mixture of lithium titanate and titanium oxide nanopowder.

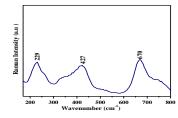
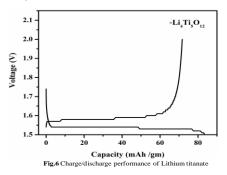


Fig.5 Raman spectrum of Lithium taitanate

#### 3.5. Electrochemical Analysis

The investigations on the electrochemical property of the prepared  $Li_4Ti_5O_{12}$  materials were also carried out. The charge/discharge curve of lithium titanate anode is shown in Fig. 6. The lithium titanate anode shows discharge capacity of 83mAhg<sup>-1</sup> at first cycle. The better lithium ion storage performance of the synthesized Lithium titanate anode may be due to the good electronic conductivity.



#### 4. CONCLUSIONS

Lithium titanate has been successfully synthesized by simple solution growth from aqueous precursor solution of titanium. The XRD studies reveal the formation of lithium titanate. SEM observation demonstrated the formation of homogeneous cubic morphology. The storage capacity of 83mAhg<sup>-1</sup> was achieved for mixture of lithium titanate and titanium oxide nanopowder. Further studies are underway to improve the storage capacity of the synthesized material.

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# *IN VITRO* ANTIOXIDANT ACTIVITY APPLYING AN ABTS<sup>++</sup> RADICAL SCAVENGING ABILITIES OF DIFFERENT EXTRACTS OF *EUPHORBIA ROUTHIANA* SPRENG. PLANT PARTS (EUPHORBIACEAE)

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# ABSTRACT

The aim of this work was to determine antioxidant activity in various parts of different extracts of *Euphorbia routhiana* Spreng. In Indian system of medicine the plant is used as an antifungal. Antioxidant activities were determined by ABTS<sup>++</sup> assay in leaf, stem and root extract of *Euphorbia routhiana*. ABTS<sup>++</sup> method, all the sample extracts of were able to quench ABTS<sup>++</sup> radical more efficiently with their TEAC values ranging between 187.2 to 6089.2 µmol Trolox equivalent/g extract. In this context, ethanol of *E. rothiana* root were found to be fast and potent scavengers of ABTS<sup>++</sup> radicals, as they were able to quench ABTS<sup>++</sup> radicals more readily than the other solvent extracts. Therefore the present study reveals that the study species has a reliable source of bioactive compounds which were highly correlated to their therapeutic properties and thus confirm the traditional medicinal usage of the plant practiced by the traditional healers.

Keywords: Euphorbia routhiana, in vitro antioxidant, ABTS++ radicals scavenging abilities.

### **1. INTRODUCTION**

Natural antioxidants have great interest among scientist because of their anticarcinogenic and health promoting properties (1). Plants are good source of phytochemicals such as vitamin E, vitamin C, carotenoid, flavonoids, glutathione, ascorbic acid etc. which having antioxidant properties. Natural antioxidants have great interest among scientist because of their anticarcinogenic and health promoting properties (2). Overall, free radicals have been implicated in the pathogenesis of at least 50 diseases (3, 4). ABTS is also frequently used by the food industry and agricultural researchers to measure the antioxidant capacities of foods (5).

*Euphorbia routhiana* (family Euphorbiaceae) is an annual erect, glabrous, profusely branched sub shrub of one-meter height and distributed in India (Maharastra and Tamil Nadu). In Indian system of medicine the plant is used as an antifungal (6), hypotensive agent and anthelmetic. Acne vulgaris is a disease which affects more than 90% of young people, which leads to permanent marking on the skin, disfiguring of the face. In view of the above, we designed the study to evaluate the ABTS<sup>++</sup> antioxidant potential content in *Euphorbia routhiana* different solvent extracts of various parts.

#### 2. MATERIALS AND METHODS

#### 2.1. Description of the selected plant

*Euphorbia laeta* Heyne ex Roth. syn. *Euphorbia rothiana* Spreng. Belonging to the family Euphorbiaceae, comprises of 300 genera with more than 6500 species. It is an annual or perennial erect herb with copiously branched stem, Leaves alternate, linear-lanceolate or oblanceolate. In India they are wide spread in an open sunny grasslands and evergreen forests at an attitude of 1000-2500m. They are commonly distributed in the regions like Madhya Pradesh, Gujarat, Maharashtra, Karnataka, Kerala and Tamil Nadu.

### 2.2. Collection of Plant material

Fresh leaves, stem and root parts of *Euphorbia rothiana* were collected from Dhottabetta hills, Niligri District. The authenticity of the selected plant material was confirmed by comparing with the reference specimen preserved at Botanical Survey of India, Southern Circle, Coimbatore. The leaf, stem and root parts were dried in shade at room temperature, chopped and ground to a fine powder in a mechanical blender. For extraction (50 g) of coarsely powdered plant samples were subjected to successive solvent extraction with petroleum ether, chloroform, ethyl acetate and ethanol using Soxhlet apparatus. The extracts were concentrated to dryness under reduced pressure using rotary vacuum evaporator, lyophilized and stored at -20°C for further phytochemical and in vitro antioxidant studies.

#### 2.3. Preparation of crude plant extracts

50 g of coarsely powdered plant samples were subjected to successive solvent extraction with petroleum ether, chloroform, ethyl acetate and ethanol. The extracts were concentrated to dryness under reduced pressure using rotary vacuum evaporator (Supervac R-185, India), lyophilized to remove traces of water molecules and the lyophilized powders were stored at -20°C for further studies.

#### 2.4. Determination of in vitro antioxidant activity

### 2.4.1. ABTS++ radical scavenging activity

Antioxidant activity was performed using an improved ABTS'+ method proposed by Siddhuraju and Manian (7). The ABTS radical cation (ABTS<sup>+</sup>) was generated by a reaction of 7 mmol/ L ABTS and 2.45 mmol/ L potassium persulfate after incubation for 16 h at laboratory temperature in dark. Blue green ABTS<sup>+</sup> was formed at the end of this period. Prior to assay, the solution was diluted in ethanol (about 1:89 v/v) and equilibrated at 30°C to obtain an absorbance of  $0.700 \pm 0.02$  at 734 nm, the wavelength of maximum absorbance in the visible region. The stock solution of the sample extracts in ethanol were diluted such that, after introduction of 10 µL aliquot of each dilution into the assay, they produced between 20- 80% inhibition of the blank absorbance. After the addition of 1.0 mL of diluted ABTS<sup>+</sup> solution to 10 µL of sample extracts or Trolox standards (final concentration 0-15 µM) in ethanol, absorbance was recorded at 30°C, exactly 30 min after the initial mixing. Appropriate solvent blanks were also run in each assay. Triplicate determinations were made at each dilution of the standard, and the percentage inhibition of the blank absorbance at 734 nm was plotted as a function of Trolox concentration. The unit of total antioxidant activity (TAA) was defined as the concentration of Trolox having the equivalent antioxidant activity expressed as  $\mu$ mol/g sample extracts on dry weight basis.

# 2.5. Statistical analysis

For *in vitro* antioxidant activity of the extracts, the result were recorded as mean  $\pm$  standard deviation (SD) (n=3) and were subjected to one-way analysis of variance (ANOVA) followed by post-hoc Duncan's multiple range test using SPSS (Version 9,SPSS Inc Chicago, USA). P<0.05 was chosen as the criterion for statistical significance.

# **3. RESULTS**

# 3.1. ABTS<sup>•+</sup> radical scavenging activity

Trolox equivalent antioxidant capacity (TEAC) was measured using the improved ABTS<sup>++</sup> radical decolorization assay. The decolorization of ABTS<sup>++</sup> cation radical is an unambiguous way to measure the antioxidant capacity of test drugs or plant samples. Since, TEAC is a measurement of the effective antioxidant activity of the extract; a higher

TEAC value would imply greater antioxidant activity of the sample. This assay was calibrated with the water-soluble  $\alpha$ -tocopherol analogue, Trolox. In the evaluation of total antioxidant capacity by ABTS<sup>++</sup> method, all the sample extracts of both the species were able to quench ABTS<sup>++</sup> radical more efficiently with their TEAC values ranging between 187.2 to 6089.2 µmol Trolox equivalent/ g extract. In this context, ethanol of *E. rothiana* root (Table 1) were found to be fast and potent scavengers of ABTS<sup>++</sup> radicals, as they were able to quench ABTS<sup>++</sup> radicals more readily than the other solvent extracts (Table 1).

Table.1. ABTS<sup>++</sup> radical scavenging activities of *E. rothiana* plant in different part extracts<sup>\*</sup>

Parts Extracts		ABTS•+ scavenging activity
	Petroleum ether	1387.1±30.9 <sup>j</sup>
	Chloroform	4572.5±91.9 <sup>f</sup>
Leaf	Ethyl acetate	$5088.4\pm56.4^{d}$
	Ethanol	$6082.5 \pm 46.4^{b}$
	Petroleum ether	$187.2\pm61.9^{1}$
	Chloroform	$1443.7\pm65.9^{i}$
Stem	Ethyl acetate	$3084.3\pm30.4^{h}$
	Ethanol	5586.8±74.6 <sup>c</sup>
	Petroleum ether	288.4±76.1 <sup>k</sup>
	Chloroform	3262.6±77.3 <sup>g</sup>
Root	Ethyl acetate	4675.0±86.1 <sup>e</sup>
	Ethanol	6089.2±71.8ª
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\*Values are mean ± standard deviation (SD) of three independent experiments.

Values not sharing a common letter in a column are significantly (P<0.05).

# Values are expressed as TEAC (Trolox equivalent antioxidant capacity/in  $\mu mol/g$  extract.

#### 4. DISCUSSION

ABTS radical scavenging activity is relatively a recent one, which involves a more drastic radical chemically, produced and is often used for screening complex antioxidant mixture such as plant extract, beverage and biological fluids (8). In the presented investigation, the ethanolic extract of *E.rothiana* root (6089 µmol Trolox equivalent/gm extract) present appreciable antioxidant activity (Table 1). Therefore, the high radical scavenge activities of the extract reported in the study might be due to its ability to scavenge free radical, thereby preventing lipid oxidation via a chain breaking reaction (9).

# **5. CONCLUSION**

Plants with medicinal properties have been known for thousands of years and have been used as traditional medicine for the people to treat diseases. The antioxidant potential of the extracts were assessed by employing similarly in ABTS<sup>++</sup>, radical scavenging assay, the ethanol extract of stem were found to be fast and potent scavenger of ABTS<sup>++</sup>.In reducing power assay, through all the extract exhibited significant activity, ethyl acetate of *E.rothiana* stem exerted stronger reductive abilities. Their therapeutic properties and thus confirm the traditional medicinal usage of the plant practiced by the traditional healers of southern district of Tamil Nadu, India.

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# PHYTOCHEMICAL ANALYSIS OF LEAF, STEM AND ROOT IN *EUPHORBIA ROTHIANA* SPRENG. (EUPHORBIACEAE), THE NILGIRIS WESTERN GHATS, TAMIL NADU

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# ABSTRACT

*Euphorbia rothiana* Spreng. is an important medicinal plant. It used in hypertensive agent in traditional medicine. The present study deals with the analysis of Phytochemical constituents by qualitative analysis of leaves, stem and root were done using Petroleum ether, chloroform, ethyl acetate and ethanol extracts. Alkaloids, flavonoids, phenols, terpenoids, triterpinoids, steroids, cardio glycosides and carbohydrates were analysed. Alkaloids, flavonoids, and phenols were highly present various extracts of leaves stem and root. Cardio glycosides triterpinoids and carbohydrates were minimum present in the various extracts.

Key words: *Euphorbia rothiana*, Medicinal plant, qualitative analysis.

#### **1. INTRODUCTION**

The evaluation of all the drugs is based on phytochemical and pharmacological approaches which leads to the drug discovery referred as natural product screening (1). Any part of the plant may contain active components such as bark, leaves, flowers, roots, fruits and seeds (2). The Euphorbiaceae, in common English sometimes called euphorbia's, which is also the name of a genus in the family, is a large family, the spurge family, of flowering plants with about 300genera and 6,500 species. Euphorbia laeta Heyne ex Roth. syn. Euphorbia rothiana Spreng. Belonging to the family Euphorbiaceae. It is an annual or perennial erect herb with copiously branched stem, Leaves alternate, linear-lanceolate or oblanceolate. The seeds are used by the tribes of Madhya Pradesh to remove warts (3). They are used as an hypertensive agent in traditional medicine.The present investigation to find out the qualitative analysis from the leaves, stem and root.

# 2. MATERIALS AND METHODS

#### 2.1. Collection of Plant material

Fresh leaves, stem and root parts of *Euphorbia rothiana* (Fig. 1 & 2) were collected from Dhottabetta hills, Niligri District. The authenticity of the selected plant material was confirmed by comparing with the reference specimen preserved at Botanical Survey of India, Southern Circle, Coimbatore. The leaf, stem and root parts were dried in shade at room temperature, chopped and ground to a fine powder in a mechanical blender. For extraction (50 g) of coarsely powdered plant samples were subjected to successive solvent

extraction with petroleum ether, chloroform, ethyl acetate and ethanol using Soxhlet apparatus. The extracts were concentrated to dryness under reduced pressure using rotary vacuum evaporator, lyophilized and stored at -20°C for further phytochemical and *in vitro* antioxidant studies.

#### 2.2. Preparation of crude plant extracts

50 g of coarsely powdered plant samples were subjected to successive solvent extraction with petroleum ether, chloroform, ethyl acetate and ethanol. The extracts were concentrated to dryness under reduced pressure using rotary vacuum evaporator (Supervac R-185, India), lyophilized to remove traces of water molecules and the lyophilized powders were stored at -20°C for further studies.

# 2.3. Qualitative phytochemical analysis

The concentrated extracts were subjected to qualitative tests for the identification of various phytochemical constituents according to the method set forth by Trease and Evans (4) and Sofowora (5).

#### **3. RESULTS AND DISCUSSION**

#### 3.1. Preliminary qualitative phytochemical analysis

In preliminary phytochemical analysis, the presence of major secondary metabolites such as alkaloids, flavonoids, phenols, saponins, terpenoids, steroids, cardiac glycosides tannins and carbohydrate were attempted and depicted. Among the various solvent types examined ethyl acetate and ethanol extracts indicated the presence of all the phytochemical constituents tested (Fig. 3). Preliminary qualitative phytochemical analysis made

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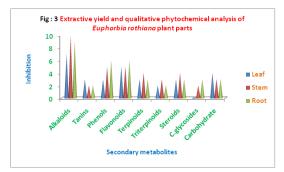
for the leaf, stem and root parts of *E.rothiana* revealed the presence of alkaloids, flavonoids, phenols, tannins, terpenoids and triterpenoids. These secondary metabolites are reported to have many biological and therapeutic properties (6-9).



Fig. 1. Study area "The Nilgiri"



Fig. 2. Euphorbia rothiana Spreng.



# **4. CONCLUSION**

The phytochemical constituents are mainly responsible for these medicinal properties of the plants. Now a day, medicinal plant based drug industries and enterprises were increasing day by day in this juncture, scientific validation of traditional medicinal plants is required to confirm their therapeutic properties and hence the commercial production. The qualitative phytochemical profile revealed the presence of important secondary metabolites in the leaf, stem and root parts of the plant. However, the ethanolic extract presented higher amount of secondary metabolites than the other solvent studied.

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# DIVERSITY STATUS AND MEDICINAL PLANT SPECIES PRESENT IN THE NATURAL VEGETATION OF KONGUNADU ARTS AND SCIENCE COLLEGE CAMPUS, COIMBATORE

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#### ABSTRACT

The present study is aimed to identify the diversity status and medicinal plant species present in natural vegetation of Kongunadu Arts and Science College Campus, Coimbatore. The study was conducted during the period between October, 2015 and February, 2016 through exploration was made periodically at weekly intervals in all vegetation areas of Kongunadu Arts and Science College, Coimbatore to enlist the species. A total of 50 plant species belongs to 47 genera which are included in 29 families are present in the campus. The total number of species in herbs is higher (27) followed by the trees and climbers with 8 species, shrubs with 4 species in the college campus. The documentation of this floristic list along with the economic uses of plants may be considered as a baseline data for future management and perspective of plant species diversity.

Keywords: Diversity status, Medicinal plants, Kongunadu Arts and Science College.

# **1. INTRODUCTION**

Institutional premises in the past few decades are becoming most conductive habitats for rich variety of wiled plant species as the management authorities are giving considerable attention to plant conservation. Despite the severe exploitation on wild bioresources in natural ecosystem, the premises of educational institutions generally have considerable green cover contributed by many number of plant species of different lifeforms due to the habitat protection offered by the authorities. The communities being maintained in the educational institutions are economically efficient, ecologically sound and biologically sustainable systems. Campus plant communities have attained characteristics which can be useful for making interesting models for research and design Some sustainable ecosystems. of of the characteristics include efficient nutrient cycling, high biodiversity, low use of external inputs and soil conservation potential (1). The nature and organization of plant communities and ecological features of constituent species are generally vary from place to place according to the local physical environment (2). In biodiversity point of view, the first step for effective conservation of species is the documentation of all available species followed by preparing databases for every possible local areas, which can enable to prepare regional and national biodiversity map. Categorization of documented species into various groups according to their economic uses is another important requisite to offer species specific conservation strategy.

Kongunadu Arts and Science College, Coimbatore is a most popular educational institution in Tamil Nadu, India that attained top rank in NACC reaccreditation. College management authorities and staff and students forums give more attention for establishing indoor gardens and also maintaining the natural plant communities in a well manner inside the campus. Around seven hectares of habitat with natural plant communities encompassed by different plant species are available in Kongunadu Arts and Science College, Coimbatore. However, documentation of flora for that habitat with economic uses is not completed so far. Therefore, the present study was aimed at to prepare a floristic list along with the medicinal and other economical uses of plants in Kongunadu Arts and Science College with particular reference to wild species. The data obtained can be useful to know the changes in species composition, community dynamics and level of conservation as influenced by the habitat protection in future.

# 2. MATERIAL AND METHODS

During the period between October, 2015 and February, 2016 through exploration was made periodically at weekly intervals in all vegetation areas of Kongunadu Arts and Science College, Coimbatore to enlist the species. Identification of plant species was made on the basis of guidelines and keys provided by Gamble (3). Herbarium specimens were collected and deposited in the Department of Botany, Kongunadu Arts and Science College, Coimbatore. Medicinal and other economic uses of the plant species were known through literature survey and information from local traditional healers by adequate interagations. The level of anthropologenic disturbances caused to the communities of the college campus was mentioned regularly.

# **3. RESULTS AND DISCUSSION**

The vegetation of college campus is heterogenous composed by various types of lifeforms such as trees, shrubs, herbs, climbers, creepers, parasites, etc. As the climatic condition is semi-arid, the plants are mostly mesophytic in the college campus. Generally, the herbs are distributed in patches and also all along the length of the hedges. The tree species such as *Delonix regia*, *Albizia amara*, *Azadirachta indica*, *Morinda tinctoria*, *Peltophorum ferrugineum*, *Pongamia pinnata*, *Samanea saman* and *Spathodea campanulata* are raised by college management and they are nearly 35 years old in age.

A total of 50 plant species belongs to 47 genera which are included in 29 families are present in the campus (Table 1). Euphorbiaceae is the dominant family in terms of species richness consists six species followed by Asteraceae with four species and Asclepiadaceae, Mimosaceae, Poaceae and Solanaceae with three species each in the community of college campus. Twenty three families such as Acanthaceae. Aizoaceae. Amaranthaceae. Apocynaceae, Bignoniaceae, Combretaceae, Commelinaceae, Convolvulaceae, Cucurbitaceae, Cyperaceae, Fabaceae, Malvaceae, Meliaceae, Menispermaceae, Myrtaceae, Nyctaginaceae, Passifloraceae, Sapindaceae, Rubiaceae, Sterculiaceae, Tiliaceae, Verbenaceae and Zygophyllaceae contributed one species each to the community is also varying widely (Fig. 1). The total number of species in herbs is higher (27) followed by the trees and climbers with 8 species, shrubs with four species in the college campus.

In the floristic list of college campus, interestingly all the 50 species (100%) recognized as medicinally important (Table 1). This may be explained due to the existence of semi-arid climatic condition inside the campus, a favourable environment for many constituent plant species (4,5). It has been observed further that the medicinal uses of the plants in the campus are diverse and multifaceted (Table 1). A higher number of 18 plants which include the species like Calotropis gigantea, Calotropis procera, Chloris barbata, Clitoria ternatea, Cynodon dactylon, Cyperus rotundus, Datura metel, Hibiscus micranthus, Justicia tranquebariensis, Lantana camara, Oldenlandia umbellata, Pergularia daemia, Physalis minima, Quisqualis indica, Sida acuta, Tinospora cordifolia, Vernonia cinerea and Waltheria indica are known to have curing the fever. This may be attributed to high variety of flavonoids, the active principle compounds naturally present in high amount in the species of semi-arid climatic condition (6,7). Next to curing fever, a sizable number of 13 species such as Albizia amara, Brachiaria ramose, Chloris barbata, Euphorbia hirta, E. microphylla, Eucalyptus tereticornis, Justicia tranauebariensis. Phyllanthus maderaspatensis. Pergularia daemia, Quisqualis indica, Samanea saman, Vernonia cinerea and Waltheria indica are used for the treatment of diarrhea. Twelve species such as Acalypha indica, Azadirachta indica, Corchorus tridens, Datura metel, Euphorbia heterophylla, Lantana camara, Peristrophe bicalyculata, Prosopis cineraria, Tabernaemontana divaricata, Tinospora cordifolia, Vernonia cinerea and Waltheria indica are reported to have property of curing skin diseases. Seven species such as Alternanthera pungens, Corchorus tridens, Hibiscus micranthus, Justicia tranquebariensis, Spathodea campanulata, Tabernaemontana divaricata and Tridax procumbens are reported to have antiinflammatory property. Seven species such as Acalypha indica, Brachiaria ramose, Euphorbia hirta, E. microphylla, Pongamia pinnata, Solanum nigrum and Tridax procumbens are used for treating ulcer. Seven species such as *Calotropis gigantea*, *Calotropis* procera, Datura metel, Millingtonia hortensis, Oldenlandia umbellata, Passiflora foetida and Solanum nigrum are generally used for curing asthma. The 7 species such as Cardiospermum halicacabum, Cyperus rotundus, Euphorbia hirta, Euphorbia heterophylla, Mukia maderaspatana, Phyllanthus amarus and Vernonia cinerea are prescribed abdominal/stomach disorders treatment.

A 5 species such as *Acalypha indica*, *Chloris* barbata, Phyllanthus maderaspatensis, Spathodea *campanulata* and *Quisqualis indica* are used for their anti-rheumatism. Five species such as *Alternanthera* pungens, Phyllanthus amarus, Physalis minima, Tinospora cordifolia and Tribulus terrestris are used for the treatment of kidney disorders. Five species such as Calotropis gigantea, Lantana camara, Mollugo nudicaulis, Mukia maderaspatana and Waltheria indica are used to reduce cough. Five species such as Chloris barbata, Gomphrena Hibiscus micranthus, decumbens, Iusticia tranguebariensis and Tinospora cordifolia are reported to have anti-diabetic property. Five species such as Justicia tranquebariensis, Millingtonia hortensis, Mollugo nudicaulis, Phyllanthus amarus and Solanum nigrum are used to treat liver and spleen disorders. Four species such as Euphorbia microphylla, Eucalyptus tereticornis, Lantana camara and Parthenium hysterophorus are used for the control of dysentery.

S. No.	<b>Bionomial Name</b>	Common Name	Family	Habit	Part Used	Medicinal Uses
1	Acalypha indica L.	Kuppaimeni	Euphorbiaceae	Herb	Leaf, root and flowers	Ulcers, snake bite, skin diseases and rheumatism.
2	Albizia amara (Roxb.) B.Boivin.	Arapu	Mimosaceae	Tree	Flower and seeds	Piles, diarrhea, gonorrhea, leprosy, leucoderma, erysipelas and abscesses.
3	Alternanthera pungens Kunth.	Ponnaganni	Amaranthaceae	Herb	Leaf	Blood Purification, anti-inflammatory and kidney disorders.
4	Azadirachta indica A. Juss.	Veppai	Meliaceae	Tree	Whole plant	Leprosy, intestinal helminthiasis and skin infections.
5	<i>Blumea obliqua</i> (Linn) Druce.	Kakronda	Asteraceae	Herb	Leaf	Insect repellent.
6	Boerhavia diffusa L.	Mukaratte kirai	Nyctaginaceae	Herb	Leaf, root and seed	Cooling, bowels complaint and blood purification.
7	Brachiaria ramosa (L.) Stapf.	Chamapothaval	Poaceae	Herb	Leaf	Ulcers and diarrhea.
8	Calotropis gigantea L.	Eruku	Asclepiadaceae	Shrub	Leaf, root, bark, seed and flower	Fever, rheumatism, cough, cold and asthma.
9	Calotropis procera (Aiton) W.T.Aiton.	Eruku	Asclepiadaceae	Shrub	Leaf, root, bark, seed and flower	Fevers, asthma, vomiting, nausea and indigestion.
10	Cardiospermum halicacabum L.	Mudakattan kirai	sapindaceae	Climber	Whole plant	Arthritis, constipation and abdominal problems.
11	Chloris barbata SW.	Mayil kondai pul	Poaceae	Herb	Leaf	Skin disease, fever, diarrhea and diabetes.
12	Clitoria ternatea L.	Thuthi	Fabaceae	Climber	Whole plant	Antimicrobial, antipyretic, analgesic and diuretic.
13	Commelina benghalensis L.	Kanangkozai	Commelinaceae	Herb	Whole plant	Mouth ulcer, psychosis, epilepsy, nose blockage in child.
14	Corchorus tridens L.	Yennai chedi	Tiliaceae	Herb	Leaf	Anti-inflammatory, gonorrhea and headache.
15	Cynodon dactylon Dress.	Arugampull	Poaceae	Herb	leaf and stem	Eye disorder and antipyretic.
16	Cyperus rotundus L.	Korai	Cyperaceae	Herb	Leaf and tuber	Fever, digestive system disorders and dysmenorrheal.
17	Datura metel L.	Umathai	Solanaceae	Herb	Leaf, seeds and flowers	Asthma, skin diseases and fever.
18	Eucalyptus tereticornis SM.	Thaila maram	Myrtaceae	Tree	Leaf	Diarrhea and dysentery.
19	Euphorbia heterophylla L.	Pall peruki	Euphorbiaceae	Herb	Leaf	Stomachache.
20	E. hirta L.	Amman pacharisi	Euphorbiaceae	Herb	Leaf	Diarrhea, peptic ulcers and stomach disorders.
21	<i>E. microphylla</i> B.Heyne ex Roth.	Pall peruki	Euphorbiaceae	Herb	Leaf	Jaundice, diarrhea, dysentery and ulcer.
22	Evolvulus alsinoides L.	Visnu kanthi	Convolvulaceae	Herb	Whole plant	Nerves tonic and memory loss.
23	Gomphrena decumbens Jacq.	Chengkruk	Amaranthaceae	Herb	Whole plant	Diabetes.

# Table 1. List of plant species in Kongunadu Arts and Science College campus, Coimbatore with their medicinal uses.

24	Hibiscus micranthus L.	Sitramutti	Malvaceae	Herb	Leaf, fruit and flowers	Hypoglycemia agent, anti pyretic, anti inflammatory and tumor.
25	Justicia tranquebariensis L.	Thavasi murungai	Acanthaceae	Herb	Leaf	Fever, inflammation, diabetes, diarrhea and liver disease.
26	Lantana camara L.	Unni chedi	Verbenaceae	Shrub	Leaf, bark, root and flower.	Itching, cold, cough, fever, dysentery and jaundice.
27	Millingtonia hortensis L.	Maramalli	Bignoniaceae	Tree	Whole plant	Asthma, sinusitis, cholagogue and tonic.
28	<i>Mollugo nudicaulis</i> Lam.	Parpadakapullu	Aizoaceae	Herb	Leaf	Whooping cough, wound healing and liver disorders.
29	<i>Mukia maderaspatana</i> (Linn.) M. Roemer.	Mosumouskai	Cucurbitaceae	Climber	Stem, bark and root	Cough, gas trouble and tooth ache.
30	Oldenlandia umbellata L.	Chaaya chedi	Rubiaceae	Herb	Whole plant	Asthma and febrifuge.
31	Parthenium hysterophorus L.	Parthenium	Asteraceae	Herb	Root	Dysentery and anti tumor.
32	Passiflora foetida L.	Mossukkattan	Passifloraceae	Climber	Whole plant	Asthma.
33	<i>Pergularia daemia</i> (Forssk.) Chiov.	Veliparuthi	Asclepiadaceae	Climber	Leaf and root	Laxative, antipyretic, diarrhea and malaria.
34	Peristrophe bicalyculata (Retz.)	Chebisa	Acanthaceae	Herb	Whole plant	Skin diseases.
35	<i>Phyllanthus amarus</i> Schum. & Thonn.	Sirunelli	Euphorbiaceae	Herb	Whole plant	Stomachache, liver, kidney and spleen disorders.
36	P. maderaspatensis L.	Arunelli	Euphorbiaceae	Herb	Whole plant	Diarrhea, menstrual problems and rheumatism.
37	Physalis minima L.	Kupanti	Solanaceae	Herb	Leaf, stem, root, fruit	Diuretic, laxative, head ache, fever and abscesses.
38	Pongamia pinnata (L). Pierre.	Pungai	Fabaceae	Tree	Whole plant	Bleeding hemorrhoids and ulcer.
39	Prosopis cineraria L.	Vanni	Mimosaceae	Tree	Leaf , bark, pad and flower	Scorpion bites and eye troubles.
40	Quisqualis indica L.	Irangun Malli	Combretaceae	Climber	Leaf, root and fruit	Fever, rheumatism and diarrhea.
41	Samanea saman (Jacq) Merr.	Thungumonji maram	Mimosaceae	Tree	Whole plant	Stomach cancer, colds, diarrhea, head ache and intestinal ailments.
42	<i>Sida acuta</i> Burm.	Palambasi	Malvaceae	Herb	Leaf and root	Fever.
43	Solanum nigrum L	Manathakkali	Solanaceae	Herb	Whole plant	Liver diseases, mouth ulcer and asthma.
44	<i>Spathodea campanulata</i> P. Beauv.	Neerkaai	Bignoniaceae	Tree	Leaf, root, bark, stem and fruit	Antiinflammatory, malaria and HIV.
45	Tabernaemontana divaricata (L.)	Nandia vattai	Apocynaceae	Shrub	Leaf, fruit, flower	Anti inflammatory, eye disease and skin disease.
46	<i>Tinospora cordifolia</i> (Thunb.)	Senthil kodi	Menispermaceae	Climber	Stem, bark and root	Fevers, diabetes, dyspepsia, jaundice, urinary problems and skin disease.
47	Tribulus terrestris L.	Nerunji	Zygophyllaceae	Herb	Leaf and root	Gonorrhea and urinary disorders.
48	Tridax procumbens L.	Vetukaya poondu	Asteraceae	Herb	Whole plant	Inflammation, wound, ulcers and hemorrhoids.
49	Vernonia cinerea Less.	Sahadevi	Asteraceae	Herb	Whole plant	Stomach pain, diarrhea, fever, eczema, ring worm and elephantiasis diseases.
50	Waltheria indica L.	Shengalipoondu	Sterculiaceae	Shrub	Leaf and root	Diarrhea, infertility, fever and cough.

Four species such as Calotropis gigantea, Lantana camara, Commelina benghalensis and Samanea saman are prescribed for the treatment of cold. Three species such as Albizia amara, Pongamia pinnata and Tridax procumbens are recommended to treat the problems of piles/bleeding hemorrhoids. Three species such as Albizia amara, Corchorus tridens and Tribulus terrestris are reported to have anti-gonorrhea property. Three species such as Corchorus tridens, Physalis minima and Samanea saman are prescribed for treating headache. Three species such as Cynodon dactylon, Prosopis cineraria and *Tabernaemontana divaricata* are used for curing eve disorders. Three species such as Cyperus rotundus, Phyllanthus maderaspatensis and Tribulus terrestris are used to ameliorate menstrual problem/dysmenorrhea. Three species such as Euphorbia microphylla, Lantana camara and Tinospora cordifolia are reported to cure jaundice. Three species such as Hibiscus micranthus, Parthenium hysterophorus and Samanea saman are reported to have anti-tumor/anti-cancer property. Two species such as Albizia amara and Azadirachta *indica* are prescribed for curing leprosy. Two species such as Albizia amara and Physalis minima are used for reduce obesity and abscesses. Two species such as Alternanthera pungens and Boerhavia diffusa are good blood purifiers. Two species such as Azadirachta indica and Samanea saman are used to treat intestinal helminthiasis/ailments. Two species such as *Pergularia daemia* and *Spathodea campanulata* are having anti-malarial property. Two species such as Calotropis procera and Tinospora cordifolia are used to control vomiting, indigestion and nausea. Two species such as Commelina benghalensis and Solanum nigrum possesses the property of control mouth ulcer. Two species such as Commelina benghalensis and Evolvulus alsinoides are used for improving memory disorders. Two species such as Mollugo nudicaulis and Tridax procumbens are reported to have wound healing property and the other two species such as Pergularia daemia and Physalis minima are reported to have laxative property.

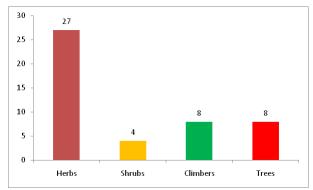


Fig. 1. The different life-form of KASC campus.

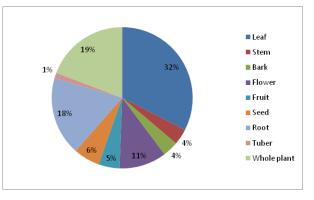


Fig. 2. The different plant parts used to cure various diseases.

The following species such as Acalypha indica used to treat snake bite, Albizia amara used to treat leucoderma and ervsipelas, Blumea oblique used as insect repellent, Boerhavia diffusa used for body cooling and to treat bowels complaint, *Cardiospermum halicacabum* is used to treat arthritis and constipation, Clitoria ternatea used for antimicrobial and anti-analgesic, Commelina benghalensis used to treat psychosis and epilepsy, *Evolvulus alsinoides* used to treat nervous problem. Millingtonia hortensis used to cure sinusitis and as tonic, Mukia maderaspatana used to treat toothache, Prosopis cineraria is used to treat scorpion bite, Vernonia cinerea used to cure eczema, ring worm and elephantiasis diseases and Waltheria indica used to treat infertility.

The plant parts used for treating the ailments also varying according to the types of ailments (Fig. 2). Among them leaf part for the higher number of (32%) plant species followed by whole plants (19%), root (18%), flower (11%), seed (6%), fruit (5%), stem and bark (4% each) and tuber (1%). It is explained that leaf is the primary site of photosynthesis and produce many secondary metabolites, in addition to reserves like carbohydrates which attributes the higher number of species for leaf as medicinal part (8-10).

Many species present in the community of college campus are multifaceted in medicinal and other economic uses. Such species are Acalypha indica, Albizia amara, Alternanthera pungens. Azadirachta indica, Boerhavia diffusa, Brachiaria ramose, Calotropis gigantea, С. procera, Cardiospermum halicacabum, Chloris barbata. Clitoria ternatea, Commelina benghalensis, Corchorus tridens, Cynodon dactylon, Cyperus rotundus, Datura metel, Euphorbia hirta, E. microphylla, Eucalyptus tereticornis, Evolvulus alsinoides, Hibiscus micranthus, Iusticia tranquebariensis, Lantana camara. Millingtonia hortensis, Mollugo nudicaulis, Mukia maderaspatana, Oldenlandia umbellata, Phyllanthus

maderaspatensis, Parthenium amarus, Р. hysterophorus, Pergularia daemia, Physalis minima, Pongamia pinnata, Prosopis cineraria, Quisqualis indica, Samanea saman, Solanum nigrum, Spathodea Tabernaemontana campanulata, divaricata, Tinospora cordifolia, Tribulus terrestris, Tridax procumbens, Vernonia cinerea and Waltheria indica which are used in the treatment of various ailments. This may be explained due to the various phytochemicals and nutraceuticals in these species (11-14). The rich diversity of plant species in the college campus may be due to the presence of different microclimatic sites like open habitats, shaded habitats by broad tree canopy coverage, slightly ever wet places, hedges with the habitat of more soil organic matter etc in the common macroclimate of semi-arid condition. In addition, very little or no disturbance by biotic factor including man is being caused to the vegetation may also be a possible factor for this high species richness in the college campus.

# 4. CONCLUSION

It is concluded from the observation that the campus of Kongunadu Arts and Science College, Coimbatore is a habitat for various plant species of different taxonomic categories. Furthermore, it is a place of vegetation that contains many species with different medicinal uses. Hence, the campus may be considered as a potential site for many medicinal species sue to its divers microclimatic conditions. In addition to the establishment of many indoor plants, the perpetuation of natural vegetation with high species richness adds still more significance to the biodiversity conservation. Documentation of this floristic list along with the economic uses of plants may be considered as a baseline data for future management and perspective of plant species diversity.

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# STUDIES ON THE AM FUNGAL DIVERSITY OF SOME SIGNIFICANT ETHANO-MEDICINAL PLANTS OF KARULAI HILLS, MALAPPURAM DISTRICT, KERALA

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#### ABSTRACT

The study was carried out to observe the AM fungal diversity in some important medicinal plant species of Karulai hills, Malappuram district, Kerala. The root samples of all the collected plant species showed mycorrhizal infection. The percentage of AM fungal colonization ranged from 17 to 87. The highest AM fungal infection was exhibited in *Desmodium triflorum* (87%) and lowest in *phyllanthes amarus* (17%). The maximum spore population was observed in *Desmodium gangeticum* (874/100g of soil) and minimum in *Piper longum* (171/100g of soil). Totally 13 genera of AM fungal were found to be associated with the rhizosphere soil samples. Among them AM fungal species isolated, the dominant species is *Rhizophagus fasciculatus*. Ethanobotanical study reveals that the Cholanaykans tribes of Karullai hills posses great knowledge about the use of various herbal medicines to cure different ailments and are also conscious about the loss of their traditional medicinal practices. They know about number of medicinal plants and their applications.

Keywords: AM fungal, diversity, Ethanobotany, Cholanaykans, Karullai hills.

# **1. INRODUCTION**

Limited availability of soil nitrogen and phosphorus is frequently a major factor limiting sustainable productivity of tropical tree plantations. The situation in developing countries like India, fertilizer could be applied only for a few cash crops and stable food crops such as rice and wheat and not for afforestation of waste lands. Hence, microbial technologies hold great promise in the operation of scientific forest nursery managements by inoculating containers with biofertilizers viz., nitrogen fixing organisms, phosphate solubilising organisms and mycorrhizae. Of these, inoculation of forest trees with mycorrhizal fungi could help the plants to scavenge sparingly available nutrients in soil including phosphorus and also provide protection against plant pathogens and drought Baltruschat and Schonbeck (1).

(AM) Arbuscular mycorrhizal fungal symbiosis facilitates the survival, growth and establishment of plants in extreme habitats Asmelash (2). Many factors stimulate differential spore production by AM fungi in the rhizosphere, leads to seasonal fluctuation in AM fungal colonization and spore densities Koske (3), Gemma and Koske, (4). The most wide spread symbiosis amongst plants is mycorrhizal association which involves various root inhabiting fungi and feeder roots. Among the different type of mycorrhizal fungi, the AM fungi are widely distributed in most ecosystems and associated with many plant species.

The beneficial effect of AM fungi on plant growth has been highlighted by Rafiq (5) and by several researchers. It has been found that AM fungi contributed to increased rate of nutrient absorption especially phosphorus from soil, longevity of feeder roots, increased tolerance to drought, heavy metals, soil toxins, extremes of soil pH and high temperature. Many commercially important tree species like *Acacia, Eucalyptus,* Teak etc. are naturally colonized by AM fungi. It is well known that AM fungi protect plants against soil and rootborne pathogens Bagyaraj (6) thereby improving plant growth and vigor.

Microorganisms are present in great number on and near the feeder roots and they play vital roles in numerous physiological processes. These dynamic processes are medicated by association of microorganisms participating in saprophytic, pathogenic and symbiotic root activities. The major symbiotic associations on tree species are mycorrhizal fungi. AM fungi, play an important role in plant survival and in the community stability of vegetation in natural ecosystems. Mycorrhizal symbiosis plays a critical role in mineral nutrition of terrestrial plants. The mycorrhizal fungi are an important part of the soil microbial system because the prevalence of these associations on plants is so common under natural soil conditions.

Plants also find innumerable uses in the human civilization since its conception. The plants

also find their use as medicine in human healthcare. Several traditional systems have evolved in the world, which use plants to cater to needs of healthcare and are still in practice around the world. The use of plants and natural products received a fillip when World Health Organization recognized plant and natural products based medicinal systems as alternative and complimentary. The use of medicinal plants for human healthcare is well documented in India, China, Egypt and Arab world Lalrinzuali (7).

The traditional systems of medicine prescribe drug as single plant products or a mixture of several plants depending on the disease, which are mainly administrated orally. The ethanobotanical and ethanomedicinal studies have great significance in the collection traditional knowledge, preparation of recorded data and in conservation of endangered medicinal plant species Prakash (8). The present work aims, to documentation of ethanobotanical importance of medicinal plants practiced by Cholanaikkan tribes and Enumeration of the arbuscular mycorrhizal fungal species in the rhizosphere soil samples of these plant species in Karulai, Malapuram district, Kerala.

# 2. MATERIALS AND METHODS

#### 2.1. Study area

Karulai village is located in Nilambur, Malappuram district, Kerala, India. It is situated 10 km away from the sub-district headquarters Nilambur and 48 km away from district headquarters Malappuram. Total extent of Karulai range is 26560.76 hecters which is notified under two reserve notifications viz., Amarambalam Reserve and Karimpuzha reserve. Karulai is the "Gods' own village" in Kerala state with green forest (Fig. 1). The average annual temperature in Karulai is 27.7°C in a year and the average rainfall is 2500 mm (Table- 1). Karimpuzha is the largest tributary of Chaliyar River, Kerala, India. It is very near to Nilambur. Karimpuzha originates from Western slopes between Mukuthi peak and Avanlanche Dam in Nilgiri district of Tamil Nadu.

# 2.2. Sample collection

Totally 45 plant species belonging to the 31 families were collected from Karulai during the September, 2016 – March 2017. Root samples and rhizosphere soil samples of plant species growing in and around areas of Karulai were collected. The root and soil samples were transported to the laboratory immediately after collection.

#### 2.3. Root samples

Root samples, 5-15 cm long, were collected from the plant species during all three seasons of 2016 to 2017. During collection, care was taken to ascertain individual plants for which roots could positively identified as belonging to a particular plant species. For identification and nomenclature of the plant species the following manual was used Gamble(9) Nair and Henry (10).

#### 2.4. Soil samples

The rhizosphere soils, dug up to a depth of 10 cm, were collected from each plant species after removing the surface of the soil and litter covering. These samples were kept in sterilized bags and were transported to the laboratory immediately after collection for the examination of arbuscular mycorrhizal fungal spore isolation.

#### 2.5. Soil pH

The pH of soil samples was determined (soil-water suspensions 1:5) with the help of pH meter (Elico).

#### 2.6. Sample preservation

In the laboratory, the roots were separated from the soil by wet sieving. The roots were washed with water and processed a fresh whenever possible. Otherwise the washed roots were fixed in formaldehyde-acetic acid-ethanol (FAA) solution (90:5:5 V/N) modified method of Phillips and Hayman (11). The soil sample was air dried and stored at 4°C until processed. Each soil samples was used for chemical analysis, spore counts and classification in to various types and multiplication, concentration and separation of AM fungal spore for identification.

# 2.7. Evaluation of AM infection

The root samples were cleared and stained in tryphan blue with a modified version of the Phillips and Hayman's (12) method. Roots were cut in to 1-2 pieces, heated at 90°C in 10% KOH for about 1 hour. For thicker and older roots, the duration was increased. The root segments were rinsed in water and acidified with dilute HCl. The root pieces were stained 0.05% tryphan blue in lacto phenol for 5 minutes and the excess stain was removed with clear lacto phenol.

The pigmented roots were heated at 90°C in 10% KOH for 2 hours, washed with fresh 10% KOH and immersed in an alkaline solution of  $H_2O_2$  for 30 minutes at 25°C until bleached. They were rinsed thoroughly with water to remove the  $H_2O_2$ , acidified in dilute HCl and stained as described earlier. In some cases the modified method of Merryweather

and Fitter (13) was followed where autoclaving and bleaching with  $H_2O_2$ , were omitted. In a few cases, direct observation of unstained, fresh and intact roots Arias(11) was made.

Arbuscular mycorrhizal infection in the roots was assessed following the grid line-intersect method of Giovannetti and Mosse (14). The stained root pieces were spread out evenly on a square plastic Petridish (10.2 x 10 cm). A grid of lines was marked on the bottom of the dish to form 1 cm inch squares. Vertical and horizontal gridlines were scanned under a dissecting microscope and the presence of infection was recorded at each point where the roots intersected a line. Four sets of observation were made, recording 100, 200, 300 and all the root gridline intersects. Each of the three replicates records was made on a fresh rearrangement of the same root sample.

The percentage of AM infection was calculated using the formula:

$$Percentage of infection = \frac{No.of root segments infected}{Total No.of root segments observed} \times 100$$

When sufficient root pieces are not available, the slide method Giovannetti and Mosse was followed. Root pieces, 1 cm long, were selected at random from a stained sample and mounted on microscope slide groups of 10. Presence of infection was recorded in each of the 10 pieces and present infection was calculated. To observe hyphae, vesicles and arbuscles under light microscope, the root pieces were mounted on glass slides either temporarily in lacto phenol. The cover slip was pressed gently to make the roots flattened and sealed with DPX medium.

# 2.8. Isolation of arbuscular mycorrhizal spores from the soil samples

Spores were recovered from the soil samples by the wet-sieving and decanting method Gerdemann and Nicolson (15). From each soil sample, 100 g of soil was taken and mixed with 1:1 of warm water in a large beaker until all the aggregates dispersed to leave a uniform suspension. Heavier particles were allowed to settle down. To remove organic matter and roots, the suspension was decanted through a 710  $\mu$ m sieve. The suspension that passed through 710  $\mu$ m was decanted 425  $\mu$ m, 250  $\mu$ m, 150  $\mu$ m, 75  $\mu$ m and 45  $\mu$ m sieves consecutively. The residues in the respective sieve were collected in petridishes with about 10-20 mL water observed under a dissecting microscope for AM fungal spores.

The total spore count was calculated by counting the spores. Then the spores were separated using a glass pipette and segregated. The spore were mounted on clear glass slides using lacto phenol or polyvinyl alcohol lacto phenol (PVL), covered with cover slips and sealed with DPX medium.

# 2.9. Identification of AM fungi

Based upon microscopic characters, the AM fungal spores were identified. For identification and nomenclature, keys of the following manual authors were used: Raman and Mohankumar (16) and Redecker (17). Classification on based on color, size, shape, surface, structure, general nature of the spore contents and hyphal attachment. Photomicrographs were taken with the help of a Magnus Olympus Microscope.

# 2.10. Ethnobotanical study

Frequent field trips were conducted in the tribal villages located at Karulai hills, during the study period (2016-2017). Initial field trips were utilized to know about the land and people. As the tribal's are mostly illiterate. no structural questionnaire approach was used. Ethno medicinal data were collected through conversation with beneficiaries, traditional healers and elder people. During the interviews, local names, useful plant parts, method of preparation and dosage were recorded. Subsequent field trips were conducted in different season in the same localities for confirming the data collected and also for gathering, additional medicinal information. The medicinal plant species were collected from wild and also from the tribal peoples homestead gardens for herbarium preparation. The method of gathering information was the same as suggested by Jain (18).

# 2.10.1. Cholanaikkan tribes

The Cholanaikkans are an ethnic group and primarily inhabit the southern Kerala state, especially silent valley national park. The Cholanaikkan traditionally reside Karulai and Chunkathara forest ranges near Nilambur. Malappuram district. Until the 1960s, they were leading a secluded life with very limited contact with mainstream urban society. Since then, the Cholanaikkans traditional lifestyle has been altered. They currently have a 16% literacy rate. The Cholanaikkan call themselves as 'Malanaikan' or 'Sholanaikan'. They are called Cholanaikan because they inhabit the interior forests. 'Chola' or 'sholas' means deep ever green forests. And 'naikan' means king. The Cholanaikkan numbered 360 indivduals in the 1991 but only 191 members today. They are found widely scattered in the forest ranges. They subsist on food-gathering, hunting and minor forest produce collection. Their language is a mixture of Kannada, Tamil and Malayalam. They use rice as

their staple food, also use wild tubers, roots, seeds, fruits, and meat.

Voor	Month	Tempera	ture(0°C)	Rainfall	Humidity
Year	Month	Maximum	Minimum	(mm)	(%)
	September	29.5	24.0	253.2	84
2016	October	30.6	24.0	280.8	81
2010	November	31.3	23.6	68.6	77
	December	31.6	22.7	82.7	74
	January	31.9	22.9	19.4	67
2017	February	32.2	23.3	7.8	71
	March	33.1	24.9	1.5	74

Table 1. Temperature and rain fall data of Malappuram, District, during the September 2016 to March

2017

Table 2. AM Fungal spore population and root colonization of plants species in Karulai, Malappuram district, Kerala.

S. Diatas		<b>F</b> !	H-h-b	Soil		ype o		% of	Spore
No	Plant name	Family	Habit	рН	<u> </u>	nizat V	ion A	Root Infection	Population /100g of soil
1	Abrus precatorius L.	Leguminosae	Climber	5	+	+	+	58	693
2	Andrographis paniculata (Burm.f.) Nees	Acanthaceae	Herb	5.8	+	-	-	27	372
3	Asparagus racemosus Willd	Asparagaceae	Armed vine	5.1	+	-	-	22	329
4	Biophytum sensitivum (L.) DC.	Oxalidaceae	Herb	5	+	+	-	27	268
5	Calotropis gigantea (L.) R.Br	Asclepiadaceae	Shrub	4.8	+	-	-	18	325
6	<i>Canavalia gladiate</i> (Jacq.) DC.	Leguminosae	Twinig herb	4.6	+	+	+	75	693
7	Cassia auriculata L.	Caesalpiniaceae	Shrub	5.6	+	+	-	58	683
8	<i>Catharanthus roseus</i> (L.) G.Don.	Apocynaceae	Shrub	5.9	+	-	-	19	427
9	<i>Centella asiatica</i> (L.) Urb.	Apiaceae	Herb	4.2	+	-	-	27	276
10	<i>Cheilocostus speciosus</i> (J.Koenig) C.D.Specht	Costaceae	Herb	5.5	-	-	-	-	197
11	<i>Clitoria ternatea</i> L.	Leguminosae	Climber	6	+	+	-	57	572
12	<i>Costus pictus</i> D.Don	Costaceae	Herb	5.1	-	-	-	-	213
13	<i>Crotalaria pallida</i> Aiton.	Leguminosaea	Shrub	5.2	+	+	+	73	842
14	<i>Curculigo orchioides</i> Gaertn	Hypoxidaceae	Herb	5.3	-	-	-	-	174
15	<i>Curcuma aromatic</i> Salisb	Zingiberaceae	Herb	4.8	-	-	-	-	266
16	<i>Cyclea peltata</i> (Lam.) Hook.f.&Thomson	Menispermaceae	Climber	5.7	+	+	-	26	271
17	<i>Cymbopogon</i> <i>flexuosus</i> (Nees ex steud) W.Watson	Poaceae	Herb	5.4	+	+	-	38	372
18	Datura metal L.	Solanaceae	Subshr ub	5.9	+	+	+	72	624
19	Desmodium	Leguminosae	Herb	5.6	+	+	+	64	874

	gangeticum (L.) DC.								
20	Desmodium triflorum (L.) DC	Leguminosae	Herb	5.4	+	+	+	87	862
21	Elephantopus scaber L.	Compositae	Herb	5.1	+	+	-	63	758
22	Emilia sonchifolia (L.) DC.ex DC.	Compositae	Herb	5.6	+	+	-	69	649
23	<i>Ensete superba</i> (Roxb.) Cheesman	Musaceae	Shrub	5.5	+	+	-	47	483
24	Euphorbia hirta L.	Euphorbiaceae	Herb	5.4	-	-	-	-	287
25	<i>Gliricidia sepium</i> (Jacq.) Walp.	Leguminosae	Shrub	5.1	+	+	-	48	472
26	Gloriosa superb L.	Liliaceae	Climber	4.7	+	-	-	21	273
27	Helicteres isora L.	Malvaceae	Shrub	5	+	+	-	58	792
28	<i>Hemidesmus indicus</i> (L.) R.Br.ex Schult	Apocynaceae	Climber	5.6	+	-	-	32	341
29	<i>Hydnocarpus pentandra</i> (Buch- Ham)	Flacourtiaceae	Tree	5.1	+	-	-	25	432
30	Justicia adhatoda L	Acanthaceae	Shrub	5.7	+	-	-	22	276
31	Justicia gendarussa Burm.f	Acanthaceae	Shrub	5.8	+	-	-	28	372
32	<i>Leucas aspera</i> (Willd.) Link	Lamiaceae	Herb	5.8	+	+	-	44	537
33	Maranda arundinaceae L.	Marandaceae	Shrub	5.6	+	-	-	27	281
34	Microsorum diversifolium G.Forst	Polypodiaceae	Shrub	5.3	+	-	-	31	562
35	Mimosa pudica L.	Mimosaseae	Sub shrub	4.7	+	-	+	38	541
36	Oscimum sanctum L.	Lamiaceae	Herb	5.3	+	+	-	47	483
37	Pandanus odoratissimus L.F	Pandanaceae	Shrub	5.3	-	-	-	-	168
38	<i>Phyllanthus amarus</i> Schumach & Thonn.	Euphorbiaceae	Erect herb	5.3	+	-	-	17	228
39	Phyllanthus emblica L.	Euphorbiaceae	Tree	5.4	-	-	-	-	256
40	Piper longum L.	Piperaceae	Scadent shrub	5.3	+	-	-	18	171
41	Plumbago zeylanica L.	Plumbaginaceae	Subshr ub	5.6	-	-	-	-	224
42	Pseudarthria viscida (L.) Wight & Arn.	Fabaceae	Subshr ub	5.3	+	+	+	58	642
43	Psidium guajava L.	Myrtaceae	Shrub	5.8	+	-	-	26	331
44	<i>Rotala aquatica</i> Lour	Lythraceae	Shrub	5.3	+	-	-	27	462
45	Scoparia dulcis L.	Scrophulariaceae	Herb	5.6	+	-	_	19	254
H- Hv	phae, A- Arbuscules, V- Vesci	cle. + - Present Absen	t						

H- Hyphae, A- Arbuscules, V- Vescicle, + - Present, - - Absent

# Table 3. Identified AM fungal spore species list from Karulai, Malappuram district, Kerala.

S. No.	Genera	Species
1	Acaulospora	Aca. alpine, Aca. foveat, Aca. tuberculata, Aca. undulate
2	Ambispora	Ambispora callosa
3	Archaeospora	Archaeospora trappei
4	Claroideoglomus	Claroideoglomus claroideum

5	Dentiscutata	Dentiscutata erythropus
6	Diversispora	Diversispora arenaria, Div. celata
7	Entrophospora	Entrophospora infrequens
8	Funneliformis	Funneliformis coronatum
9	Gigaspora	Gigaspora albida, Gi. decipiens, Gi. ramisporophora
10	Glomus	Gl. albidum, Gl. ambisporum, Gl. arborense, Gl. canadense, Gl. globiferum, Gl.
		multicaule
11	Pacispora	Pacispora scintillans
12	Rhizophagus	Rhizophagus fasciculatus
13	Scutellispora	Scutellispora spp, Scu. savannicola, Scu. striata
-		

Table 4. Distribution of AM fungal spores different plant species from Karulai, Malappuram district,
kerala.

kera S.			
No.	Plant name	Family	Spores name
1	Abrus precatorius L.	Leguminosae	Acaulospora alpine, Gigaspora albida, Glomus arborense, Rhizophagus fasciculatus
2	Andrographis paniculata (Burm.f.) Nees	Acanthaceae	Ambispora callosa, Diversispora arenaria, Pacispora scintillans
3	Asparagus racemosus Willd	Asparagaceae	Acaulospora foveat, Gigaspora ramisporophora, Glomus multicaule, Rhizophagus fasciculatus
4	Biophytum sensitivum (L.) DC.	Oxalidaceae	Acaulospora undulate, Dentiscutata erythropus, Pacispora scintillans, Rhizophagus fasciculatus
5	Calotropis gigantean (L.) R.Br	Asclepiadaceae	Ambispora callosa, Funneliformis coronatum, Rhizophagus fasciculatus
6	<i>Canavalia gladiate</i> (Jacq.) DC.	Leguminosae	Acaulospora alpine, Glomus albidum, Glomus multicaule, Scutellospora striata
7	Cassia auriculata L.	Caesalpiniaceae	Claroideoglomus claroideum, Glomus ambisporum, Rhizophagus fasciculatus
8	<i>Catharanthus roseus</i> (L.) G.Don.	Apocynaceae	Acaulospora foveat, Diversispora celata, Glomus arborense
9	Centella asiatica (L.) Urb.	Apiaceae	Acaulospora undulate, Gigaspora albida, Pacispora scintillans
10	<i>Cheilocostus speciosus</i> (J.Koenig) C.D.Specht	Costaceae	Acaulospora undulate, Glomus albidum, Glomus multicaule
11	Clitoria ternatea L.	Leguminosae	Acaulospora undulate, Glomus albidum, Glomus multicaule, Rhizophagus fasciculatus
12	<i>Costus pictus</i> D.Don	Costaceae	Acaulospora alpine, Diversispora celata, Glomus arborense, Rhizophagus fasciculatus
13	Crotalaria pallida Aiton.	Leguminosaea	Claroideoglomus claroideum, Glomus ambisporum Rhizophagus fasciculatus
14	Curculigo orchioides Gaertn	Hypoxidaceae	Acaulospora tuberculata, Funneliformis coronatun Rhizophagus fasciculatus
15	Curcuma aromatic Salisb.	Zingiberaceae	Claroideoglomus claroideum, Gigaspora ramisporophora
16	<i>Cyclea peltata</i> (Lam.) Hook.f.&Thomson	Menispermaceae	Dentiscutata erythropus, Glomus albidum, Glomus arborense
17	<i>Cymbopogon flexuosus</i> (Nees ex steud) W.Watson	Poaceae	Acaulospora foveat, Entrophospora infrequens, Glomus globiferum, Rhizophagus fasciculatus
18	Datura mental	solanaceae	Acaulospora undulate, Glomus albidum
19	Desmodium gangeticum (L.) DC.	Leguminosae	Claroideoglomus claroideum, Entrophospora infrequens, Rhizophagus fasciculatus
20	Desmodium triflorum (L.) DC	Leguminosae	Diversispora arenaria, Funneliformis coronatum, Rhizophagus fasciculatus
21	Elephantopus scaber L.	Compositae	Archaeospora trappei, Gigaspora albida, Glomus canadense
22	Emilia sonchifolia (L.) DC.ex	Compositae	Acaulospora foveat, Gigaspora ramisporophora,

	DC.		Glomus globiferum, Rhizophagus fasciculatus
23	<i>Ensete superbum</i> (Roxb.) Cheesman	Musaceae	Diversispora arenaria, Glomus albidum, Glomus arborense
24	Euphorbia hirta L.	Euphorbiaceae	Claroideoglomus claroideum, Funneliformis coronatum, Rhizophagus fasciculatus
25	<i>Gliricidia sepium</i> (Jacq.) Walp.	Leguminosae	Acaulospora foveat, Gigaspora albida, Glomus canadense, Scutellospora striata
26	Gloriosa superb L.	Liliaceae	Claroideoglomus claroideum, Glomus ambisporum, Glomus globiferum
27	Helicteres isora L.	Malvaceae	Entrophospora infrequens, Funneliformis coronatum, Glomus canadense, Rhizophagus fasciculatus
28	<i>Hemidesmus indicus</i> (L.) R.Br.ex Schult	Apocynaceae	Acaulospora alpine, Gigaspora ramisporophora, Glomus canadense, Rhizophagus fasciculatus
29	Hydnocarpus pentandra (Buch-Ham)	Flacourtiaceae	Archaeospora trappei, Gigaspora decipiens, Rhizophagus fasciculatus
30	Justicia adhatoda L	Acanthaceae	Archaeospora trappei, Glomus ambisporum, Glomus arborense
31	<i>Justicia gendarussa</i> Burm.f	Acanthaceae	Claroideoglomus claroideum, Gigaspora albida, Gigaspora decipiens, Rhizophagus fasciculatus
32	Leucas aspera (Willd.) Link	Lamiaceae	Archaeospora trappei, Glomus ambisporum, Glomus globiferum
33	Maranda arundinaceae L.	Marandaceae	Ambispora callosa, Gigaspora decipiens, Glomus canadense
34	<i>Microsorum diversifolium</i> G.Forst	Polypodiaceae	Acaulospora alpine, Glomus ambisporum, Scutellospora savannicola
35	Mimosa pudica L.	Mimosaceae	Dentiscutata erythropus, Entrophospora infrequens, Rhizophagus fasciculatus
36	Oscimum sanctum L.	Lamiaceae	Ambispora callosa, Glomus ambisporum, Glomus arborense
37	Pandanus odoratissimus L.F	Arecaceae	Acaulospora tuberculata, Gigaspora ramisporophora, Rhizophagus fasciculatus
38	<i>Phyllanthus amarus</i> Schumach & Thonn.	Euphorbiaceae	Dentiscutata erythropus, Gigaspora decipiens, Glomus canadense
39	Phyllanthus emblica L.	Euphorbiaceae	Diversispora celata, Entrophospora infrequens, Glomus arborense, Scutellispora spp
40	Piper longum L.	Piperaceae	Dentiscutata erythropus, Gigaspora decipiens, Rhizophagus fasciculatus
41	Plumbago zeylanica L.	Plumbaginaceae	Acaulospora tuberculata, Glomus arborense, Glomus globiferum, Rhizophagus fasciculatus
42	<i>Pseudarthria viscida</i> (L.) Wight & Arn.	Leguminosae	Claroideoglomus claroideum, Glomus ambisporum, Scutellospora savannicola Claroideoglomus claroideum, Gigaspora decipiens,
43	Psidium guajava L	Myrtaceae	Glomus canadense, Glomus globiferum, Scutellispora spp
44	<i>Rotala aquatica</i> Lour	Euphorbiaceae	Acaulospora foveat, Gigaspora decipiens, Glomus globiferum
45	Scoparia dulcis L.	Scrophularaceae	Acaulospora alpine, Gigaspora ramisporophora, Rhizophagus fasciculatus

# Table 5. Details of enumerated plants used by the Cholanaikkan tribes from Karulai.

S. No.	<b>Botanical Name</b>	Family	Local Name	Habit	Part used
1.	Abrus precatorius L.	Leguminosae	Kunnikkuru	Climber	Leaves, Seed
2.	Andrographis paniculata (Burm.f.) Nees	Acanthaceae	Kiriyattu,Kiriyathu	Herb	Leaves, stem.

3.	Asparagus racemosus Willd.	Asparagaceae	Sathavari.	Armed vine	Tuberous root.
4.	Biophytum sensitivum (L.) DC.	Oxalidaceae	Mukkuttihi	Herb	Aerial part
5.	Calotropis gigantea (L.) R.Br	Asclepiadaceae	Erikku	Shrub	Leaves
6.	<i>Canavalia gladiata</i> (Jacq.) DC.	Leguminosae	Valpayar	Twinig herb	Seed
7.	Cassia auriculata L.	Caesalpiniaceae	Avara	Shrub	Whole plant
8.	<i>Catharanthus roseus</i> (L.) G.Don	Apocynaceae	Shavam Naari	Shrub	Whole plant
9.	<i>Centella asiatica</i> (L.) Urb.	Apiaceae	Kudangal,Mutthil.	Herb	Whole plant
10.	Cheilocostus speciosus (J.Koenig) C.D.Specht	Costaceae	Anakuva	Herb	Rhizome
11.	Clitoria ternatea L.	Leguminosae	Sangu pushpam	Climber	Leaves.
12.	<i>Costus pictus</i> D.Don.	Costaceae	Insulin chedi	Herb	Leaves
13.	<i>Crotalaria pallida</i> Aiton.	Leguminosaea	Kilukkachedi	Shrub	Roots
14.	Curculigo orchioides Gaertn	Hypoxidaceae	Nelappana	Herb	Rhizome
15.	<i>Curcuma aromatica</i> Salisb.	Zingiberaceae	Kasthurimanjal	Herb	Rhizome, oil
16.	<i>Cyclea peltata</i> (Lam.) Hook.f.&Thomson	Menispermaceae	Padathali, Pattichevian	Climber	Leaves,Roots
17.	<i>Cymbopogon flexuosus</i> (Nees ex steud) W.Watson	Poaceae	Inchipullu, Thilappullu	Herb	Leaves
18.	Datura metal L.	Solanaceae	Ummathu	Subshrub	Fruit
19.	<i>Desmodium gangeticum</i> (L.) DC.	Fabaceae	Orila	Herb	Leaves
20.	Desmodium triflorum (L.) DC	Leguminosae	Nilamparanda	Herb	Leaves
21.	Elephantopus scaber L.	Compositae	Anachuvadi	Herb	Leaves, Root
22.	<i>Emilia sonchifolia</i> (L.) DC.ex DC.	Compositae	Muyalchevian	Diffuse herb	Whole plant.
23.	<i>Ensete superba</i> (Roxb.) Cheesman	Musaceae	Kalluvazha, Malavazha	Erect shrub	Rhizome
24.	Euphorbia hirta L.	Euphorbiaceae	Nilappala	Herb	Root, Leaf
25.	<i>Gliricidia sepium</i> (Jacq.) Walp.	Leguminosae	Simakkonna	Short tree	Leaves
26.	Gloriosa superb L	Liliaceae	Menthonni	Climber	Leaves
27.	Helicteres isora L.	Malvaceae	Edampiri-valampiri	Large shrub	Fruit
28.	Hemidesmus indicus (L.) R.Br.ex Schult	Asclepiadiaceae	Nannari	Climber	Root, Leaves
29.	Hydnocarpus pentandra (Buch-Ham)	Flacourtiaceae	Marrotti	Tree	Seed
30.	Justicia adhatoda L	Acanthaceae	Aadalodakam	Shrub	Leaves
31.	Justicia gendarussa Burm.f	Acanthaceae	Vathakkodi	Shrub	Leaves
32.	Leucas aspera (Willd.) Link	Lamiaceae	Thumba	Herb	whole plant
33.	Maranda arundinaceae L	Marandaceae	Kuvva	Shrub	Rhizome
34.	Microsorum diversifolium G.Forst	Polypodiaceae	Panal chedi	Shrub	Tuber
35.	Mimosa pudica L.	Mimosaceae	Thottavadi	Sub shrub	Whole plant
36.	Ocimum sanctum L.	Lamiaceae	Tulsi	Herb	Leaves
37.	Pandanus odoratissimus L.F	Pandanaceae	Kaitha	shrub	Inflorescenc
38.	<i>Phyllanthus amarus</i> Schumach & Thonn.	Euphorbiaceae	Keezharnelli	Erect herb	Whole plant
39.	Phyllanthus emblica L.	Euphorbiaceae	Nelli	Tree	Fruit
40.	Piper longum L.	Piperaceae	Thippali	Scadent	Fruit

41.	Plumbago zeylanica L.	Plumbaginaceae	Vellakoduveli	shrub Subshrub	Root
42.	<i>Pseudarthria viscida</i> (L.) Wight & Arn.	Fabaceae	Moovila	Sub shrub	Leaves
43.	Psidium guajava L.	Myrtaceae	Perakka	Shrub	Leaves
44.	<i>Rotala aquatica</i> Lour	Lythraceae	Kallurvanchi	Shrub	Root
45.	Scoparia dulcis L.	Scrophulariaceae	Kallurukki	Herb	Whole plant

# Table 6. Mode of administration for the ailments of the medicinal plants used by the Cholanaikkantribes from Karulai.

tribe	ribes from Karulai.				
S. No.	Botanical Name	Ailments	Mode of administration		
1.	Abrus precatorius L.	Swelling	The leaves and seed powder is made paste with water, applied externally to relieve, Joint pains, swelling.		
2.	<i>Andrographis paniculata</i> (Burm.f.) Nees	Diarrhea, Bronchitis, Chicken Pox and Coughs	Leaves and root decoction used for diarrhea, bronchitis, chicken pox, coughs, headaches and ear infection		
3.	<i>Asparagus racemosus</i> Willd.	Stomach pain	Cooked tubers are eaten for stomach pain.		
4.	Biophytum sensitivum (L.) DC.	Eye diseases	Juice taken from crushed plant parts is applied for eye itching and other eye problems		
5.	Calotropis gigantea (L.) R.Br	Earache	The juice from the heated leaves of the plant is applied in to ear for earache.		
6.	Canavalia gladiata (Jacq.) DC.	As a vegetable	The ripe seed can be eaten after cooking		
7.	Cassia auriculata L.	Diabetes	Grind the dried bark, flowers, leaves and fruits in qualities boil with water. It is used to treat diabetes.		
8.	<i>Catharanthus roseus</i> (L.) G.Don	Eye diseases	The extract of the plant is useful for eye infection and irritation.		
9.	<i>Centella asiatica</i> (L.) Urb.	Memory power	Consumption of whole plant juice can improves memory power		
10.	<i>Cheilocostus speciosus</i> (J.Koenig) C.D.Specht	Intestinal worms	Rhizome has been used to treat fever, rash, and intestina worms.		
11.	Clitoria ternatea L.	Head ache, Inflammation	Leaf juice is used as a nasal drops in headache. The leaf can be grind in to fine paste and applied any kind of inflammation.		
12.	<i>Costus pictus</i> D.Don.	Diabetes	Juice prepared from the leaves is used to treat diabetes.		
13.	<i>Crotalaria pallida</i> Aiton.	Swelling	The poultice made of the root applied in painful swelling of joint		
14.	<i>Curculigo orchioides</i> Gaertn	Blood purifier	Crushed tubers are mixed with milk is used as blood purifier.		
15.	<i>Curcuma aromatica</i> Salisb.	Skin diseases	The oil is used to reduce pain and inflammation associated with snake bite.		
16.	<i>Cyclea peltata</i> (Lam.) Hook.f.&Thomson	Hair cleaner, Stomach pain	Leaves crushed with water and it is applied over the hair as hair cleaner. Powder obtained from dried tubers are mixed with hot water used for stomach pain		
17.	<i>Cymbopogon flexuosus</i> (Nees ex steud) W.Watson	Headache, Stomachache	The oil extracted from the leaves is used directly to the skin for headache, stomachache, muscle pain		
18.	Datura metal L.	Snake poison	Fruit paste applied for snake poison		
19.	Desmodium gangeticum (L.) DC.	Kidney stones, Fever	A decoction of the leaves is used against kidney stones. The decoction of the root is employed to treat fever.		
20.	<i>Desmodium triflorum</i> (L.) DC	Skin problems, Digestion.	The crushed leaves are applied externally on wounds and skin problems. The whole plant is used to promoting		

			digestion
21.	Elephantopus scaber L.	Vomiting, Kill round worms	Fresh roots are used to prepare a blend which is best for combating vomiting. The decoction prepared from its roots or leaves is used to kill roundworms.
22.	Emilia sonchifolia (L.) DC.ex DC.	Diarrhoea, Protect teeth	The juice of the root is used to treat diarrhoea. The flower heads are chewed and kept in the mouth for abou 10 minutes to protectteeth from decay.
23.	<i>Ensete superba</i> (Roxb.) Cheesman	Kidney stones	Raw fruits are eaten for kidney stone, diabetes and stomach ache. Flowers and pseudostem used as a vegetable.
24.	Euphorbia hirta L.	Impprove lactation, Remove warts on the face.	A decoction made from the root increases lactation in women. Leaf paste is used to treat swelling. Latex is used to treat warts on face.
25.	<i>Gliricidia sepium</i> (Jacq.) Walp.	Insect repellent	The leaves paste is used as a sedative and insecticides. It is used for the treatment of fracturesand wounds.
26.	Gloriosa superba L	Insect bites , Scorpion bites, Hair cleaner.	The crushed leaves are applied on treatment of snake bites, scorpion stings and sexually transmitted diseases. The leaf juice used against head lice.
27.	Helicteres isora L.	Ear drops	Crushed pods heated with castor oil used as an ear drop.
28.	<i>Hemidesmus indicus</i> (L.) R.Br.ex Schult	Snake bites& Scorpion bites	It is used for the treatment of snake bite, scorpion bite and other poisonous insect bite cases.
29.	<i>Hydnocarpus pentandra</i> (Buch- Ham)	Body pain	Oil extracted from the seeds are externally used for body pain
30.	Justicia adhatoda L	Cough & cold	Oral administration of leaf juice is used for cough and cold.
31.	<i>Justicia gendarussa</i> Burm.f	Chronic rheumatism	The decoction of leaves and tender young shoots are used in the treatment of chronic rheumatism and used for bathing during child birth.
32.	<i>Leucas aspera</i> (Willd.) Link	Cold and cough.	Crushed leaves juice is directly applied to the nose to get relief from cold and cough.
33.	Maranda arundinaceae L	Intestinal disorders.	Boiled starch is used to treat several stomach disorders like digestion and ulcers.
34.	<i>Microsorum diversifolium</i> G.Forst	Stomachache	Tuber used in the treatment of stomach ache.
35.	Mimosa pudica L.	Cuts and wounds	Crushed leaf Juice is applied over cuts and wounds.
36.	Ocimum sanctum L.	Headache& Skin diseases	Leaves paste is used for curing stomachache, headache, skin diseases, insect bites and itching
37.	Pandanus odoratissimus L.F	Mosquito repellent	Crushed inflorescence is mixed with water and sprayed over mosquito affected areas.
38.	<i>Phyllanthus amarus</i> Schumach & Thonn.	Jaundice	The root juice along with milk consumed in the morning is good to cure jaundice.
39.	Phyllanthus emblica L.	Eye diseases & Diabetes	Amla juice used to treat eye disease, diabetes, common cold and cough
40.	Piper longum L.	Tooth ache	Chewing of crushed fruits can reduce tooth ache.
41.	Plumbago zeylanica L.	Swelling.	Root paste applied externally for inflammatory swellings
42.	<i>Pseudarthria viscida</i> (L.) Wight & Arn.	Internal bleeding	Oral administration of leaf paste is used for internal bleeding
43.	Psidium guajava L.	Stomach problems	Leaves paste is used to the treatment of diarrhea and stomachache.
44.	<i>Rotala aquatica</i> Lour	Stomach ache	Consumption of root decoction is used for stomach ulcer
45.	Scoparia dulcis L.	Kidney stones	Consumption of whole plant juice along with milk is remedy for kidney stone.

Their livelihood is totally dependent on the forest. The collection and selling of minor forest produce is the major source of income. The tribes, unlike any other tribes, under the leadership of the Mooppan (Elder) are willing to come out of the deep forest (Fig. 2).

# **3. RESULTS**

AM fungal infection and spore population 45 plant species belongs to the 31 families and pH of the rhizosphere soil samples present in the Table 2 to 4. Totally 13 genera of AM fungi belonging to Acaulospora, Ambispora, Archaeospora, Claroideoglomus, Dentiscutata. Diversispora, Entrophospora, Funneliformis, Gigaspora, Glomus, Pacispora, Rhizophagus and Scutellispora were found to be associated with the rhizosphere soil samples (Fig. 3). Among them AM fungal species isolated, the Glomus is dominant genera and Rhizophagus fasciculatus is dominant species.



Fig. 1. The map showing the study area Karulai, Mallapuram, Kerala



Fig. 2. The Cholanaikkan tribes Karulai and Chunkathara forest ranges near Nilambur, Malappuram.

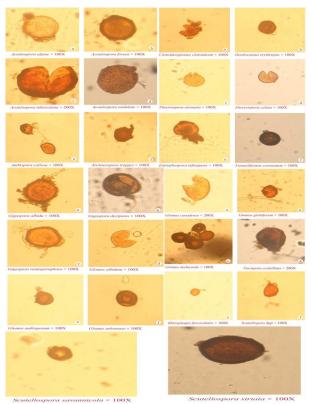


Fig. 3. Isolated AM fungal spore species from Karulai , Mallapuram, Kerala.



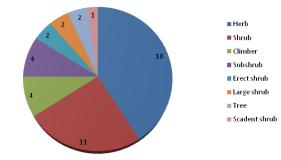


Fig. 4. Percentage of medicinal plants in different life-forms used by local healers of Karulai.



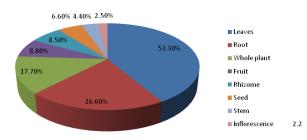


Fig. 5. Percentage of parts prescribed for the treatment of diseased by local healers of Karulai.

Totally 45 plant species belongs to 31 families were analyzed for AM fungal infection and spore population. Of these, the maximum spore population was observed in Leguminosae member of *Desmodium gangeticum* (874/100 g of soil) and minimum spore population was noticed in *Piper longum* (171/100 g of soil) belongs to the family Piperaceae.

The highest AM fungal infection was found Desmodium triflorum (87%) belongs to in Leguminosae and the least infection was recorded in Euphorbiaceae member of *Phyllanthus amarus* (17%). The plant species like Andrographis (27%), paniculata Acanthaceae, Asparagus racemosus (22%). Asparagaceae, Biophytum sensitivum (27%), Oxalidaceae, Calotrapis gigantea (18%), Asclepiadaceae, Catharanthus roseus (19%), Apocyanaceae, Centella asiatica (27%), Apiceae, Cyclea peltata (26%), Menispermaceae, Gloriosa superba (21%), Lilliaceae, Justicia adhatoda (22%), J. gendarussa (28%) both species belogs to Acanthaceae member, Maranda arundinaceae (27%), Phyllanthus amarus (17%), Euphorbiaceae, Scoparia Scrophulariaceae, dulcis (19%), *Hydnocarpus* pentandra (25%), Flacourtiaceae, Piper longum Piperaceae, Rotula aquatic (27%), (18%), Boraginaceae, Psidium guajava (26%), Myrtaceae showed 10 to less than 30% of AM fungal infection.

The other species like Abrus precatorius (58%), Leguminosae, *Cassia auriculata* (58%), Caesalpiniaceae, Clitoria ternatea (57%). Leguminosae, Cymbopogon flexuosus (38%), Poaceae, Ensete superbum (47%), Musaceae, Gliriciadia sepium (48%), Leguminosae, Helicteruse isora (58%), Malvaceae, Hemidesmus indicus (32%), Apocynaceae, Leucas aspera (4%), Laminaceae, Mimosa pudica (38%), Leguminosae, Oscimum sanctum (47%), Lamiaceae. Microsorum diversifolium (31%), Polypodiaceae, showed 30 to less than60% of AM fungal infection.

The rest of the species like Canavalia *aladiate* (75%), *Crotalaria pellida* (73%), *Desmodium* triflorum (87%), Desmodium gangeticum (64%) all the four species belongs to Leguminosae, the Compositae membersof Elephantobus scaber, Emilia sonchifolia infected 63 and 69% respectively, and one species Pseudarthria viscida (58%) the member of Fabaceae showed 60 to less than 90% of AM fungal infection was found in the Costaceae members of Costus pictus and Cheilocostus speciosus. The species Curculiao orchioides belongs to Hypoxidaceae, *Cureuma aromatic* belongs to Zingiberaceae, the Arecaceae member Pandanus odoratissimus, the Euphorbiaceae member Phyllanthus emblica and Plumbago zeylanica the member of Plumbaginaceae, also there is no hyphae, vesicles and arbuscular infection surprisingly these all the plant species rhizosphere soil simply showed the spore population.

In ethanobotanical study, 45 medicinal plant species belonging to 31 families used traditionally as herbal medicines for curing various diseases (Table5). The study as carried out related to Cholanaykan tribes. Medicinal plants used in folk herbal remedies are prepared and administered in various forms in the Karulai hills.

Among these medicinal plants, herbs (40%) were found to be most used plants folloed by shrub (24.4%), climber (8.8%), sub shrub (8.8%), erect shrub (4.4%), large shrub (4.4%), tree (4.4%) and scadent shrub (2.2%) (Fig. 4). Similar pattern of life form was reported by Giday *et al.*, (2014). The most frequently utilized medicinal plant parts were leaves (53.3%) used for the preparation of medicines solely or mixed with other plant parts. It was followed by roots (26.6%), whole plant (17.7%), fruit (8.8%), rhizome (8.8%), seed (6.6%), stem (4.4%), and inflorescence (2.2%) (Fig. 5).

Medicinal plants used in folk herbal remedies are prepared and administered in various forms in the Karulai hills. Majority of the plant remedies were prepared by decoction and juice. The paste was prepared by grinding the fresh or dried plant parts with oil or water. Powder was prepared by the grinding of shade dried parts. The most frequently used mode of remedy administration is oral ingestion, followed by tropical uses, nasal drops, face crams, hair cleaners, and bath. The most treated illness of the Karulai hills using a number of medicinal plants are grouped in to several disorders. We found the highest number of plant species are used against cold, followed by cough, diabetes, kidney stones, stomachache, swelling, headache, eve diseases, ageist intestinal worms, toothache, snake and scorpion bites, mosquito repellent, vomiting, jaundice and rheumatism (Table 6). The present study noticed that, single disease can be cured with infusions of more than one plant. Similarly, the single plant can be utilized to cure more than one disease.

# 4. DISCUSSION

The arbuscular mycorrhizae are reported to be ubiquitous both geographically and ecologically Mosse(14). Seasonal fluctuations in moisture, temperature,  $p^{H}$  and soil nutrient status show high and dramatic effects on arbuscular mycorrhizal spore population and percentage of root colonization. Soil physiological characters played an important role in distribution and density of mycorrhizal fungi. All the plant species 45 belongs to 31 families of rhizosphere soil samples observed the AM fungal spores. Among the AM fungal species 36 *Glomus* is most common. All the plant species colonized by AM fungi. The plant species infected by hyphae, vesicles and arbuscules. Grasses they have evolved the fibrous root system or an alternative phosphate acquisition strategy which enables them to do without mycorrhiza.

In the present finding the Poaceae member *Cymbopogon flexuosus* infected by arbuscular mycorrhizae. The infection in the plant species has 38%. Mycorrizal association occurred naturally with many important forest trees. Ectomycorrhizae mostly occur in temperate forest whereas in tropics endomycorrizae are more common. The present finding is in agreement with the results obtained by seasonal workers.

Brundrett and Abbott (20) analyzed the most of the plant species in tropical rain forests and the members of Leguminosae and the subfamilies of Papilonaceae and Mimosaceae. The same results was obtained in the present investigation that the Leguminosae members of Abrus precatorius, clitoria pictus, crotalaria pallida infected by AM fungal infection. Arbuscular micorrhiza is most common in Angiosperms, Gymnosperms, Pteridophytes and Bryophytes. The association of AM fungi with all the plants studies confirms the ubiquitous nature of AM Hayman(11) although the extent of root infection and number of AM spores found in the rhizosphere were different.

In this investigation, the mycorrhizal colonization was vary this may be the host specificity. The major ecosystem function of mycorrhizae is to assist host plants in the acquisition of resources from soil. This study displays the different degrees of AM fungi in plant host specificity. Such as mycorrhizal symbioses play fundamental roles in shaping plant communities, terrestrial ecosystems and high value for sustainability of this ecosystem.

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## **RESEARCH ARTICLE**

## SURVEY OF TREES AND SHRUBS IN MARUNGOOR, KANYAKUMARI DISTRICT, TAMIL NADU, SOUTHERN INDIA

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#### ABSTRACT

India is one of the twelve mega-biodiversity countries in the world, which has very rich floral vegetation with variety of plants of high economic value including plants of medicinal importance. Present survey was conducted in the plant species (Trees and Shrubs) growing in their natural habitats like grounds, roadsides, open land, home gardens. Plant specimens were collected (depending upon their availability) from the area under investigation. These specimens were identified and photographed. Maximum plants have been photographed in their natural habitat whereas others in the laboratory conditions. The present study site had a high species diversity for both tree and shrub species. Probably, the high species diversity for trees and shrubs could be attributed to the many tributaries and streams that empty rich organic content and mineral resources utilized by the species for growth and production. It is therefore recommended that measures to foster partnership between the community and other stakeholders in natural resources conservation in the areas should be encouraged to ensure sustainable natural resources management in the areas.

Keywords: Survey, Marungoor, Kanyakumari.

#### **1. INTRODUCTION**

Nature has blessed India with a wealth of medicinal plants, thus being designated as "Medicinal Garden of the World" (1). Since ancient times human health was taken care through traditional plant medicines (2, 3). Indian floral diversity may be due to variety of habitats and variable environmental and geographical conditions (4). Studies of forest flora provide useful information on several aspects related to species diversity like dominant families, life-form status etc. The most dominant life form was trees (36.9%), followed by shrubs (22.7%), grasses (17.1%), herbs (13.6%) climbers (8.5%) and sedges (1.1%) (5). Vegetative survey of Kunckles valley recorded a total of 204 flowering plant species in 70 families. Eighty-nine (44%) species are endemic to Sri Lanka, while 39 (20%) are nationally threatened. Among them 148 trees, shrub species identified are 74 (50%) have not been recorded during previous floral surveys of the Kunckles forest reserve, while 115 (78%) are common to the lowland rain forests of southwestern Sri Lanka (6).

### 2. MATERIALS AND METHODS

### 2.1. Description of the study area

The present study was carried out in Marungoor Panchayat and Agastheeswnram Taluk of Kanyakurnari District. Marungoor, is a panchayat town near Suchindrum in Kanniyakumari district in the state of Tamil Nadu. The place sprawls over an area of about 10 km<sup>2</sup>. Suchindram is about five km south-west of Marungoor. As of 2001 India census, Marungur had a population of 10,096 and most of them are farmers Males constitute 49% of the population and females 51%. Marungur has an average literacy rate of 82%, higher than the national average of 59.5%: male literacy is 85%, and female literacy is 80%. The annual rainfall of this area is low when compared to other areas of the Kanyakumari District. Its latitude and longitude are 8.23738 and 77.33989 respectively.

### 2.2. Floristic survey

Present survey was conducted in the plant species (Trees and Shrubs) growing in their natural habitats like grounds, roadsides, open land, home gardens. Plant specimens were collected (depending upon their availability) from the area under investigation. These specimens were identified and photographed. Maximum plants have been photographed in their natural habitat whereas others in the laboratory conditions. All species have been designated to their corresponding families. Plant species were also differentiated on the basis of their habit. Herbarium sheets were prepared and documented. Identification was done with the help of different floras Gamble and Fischer (7), Mathew (8), Nair and Henry (9).

#### **3. RESULTS AND DISCUSSION**

Total 78 plant species belonging to 43 families and 70 genera were recorded from the study site (Table 1). The most dominant life form was

shrub (57.5%) followed by trees, (30.8%), and climber (8.97%), herb (2.6%) (Table 2). Out of 78 plants, 75 were angiosperms and three gymnosperms. The contribution of dicotyledons was 89.74% and monocotyledons 10.25% (Table 3). Apocynaceae was the most dominant family with 6 species and 5 genera and other main contributing families were Euphorbiaceae (4 genera and 5 species), Annonaceae (1 genera and 3 species) and Bignoniaceae (3 genera 3 species), Caesalpiniaceae (2 genera and 4 species), Rubiaceae (4 genera and 4 species), Verbenaceae (3 genera and 3 species) (Table 6). Families Araceae, Moraceae, Ulmaceae, Santalaceae, Rosaceae, Punicaceae, Moringaceae, Muntingiaceae, Oxalidaceae, Plantaginaceae, Ranumculaceae, Rhizophoraceae, Sterculiaceae etc., had only one species each (Table 7). In the study area, most dominant life form was shrub generally; the high diversity of shrub is associated with undisturbed tropical areas.

Table 1. List of plant species recorded from the study area.

S. No.	Botanical Name	Family	Habit	Wild / Ornamental /Cultivated
1.	AcalyphahispidaBurm .f	Euphorbiaceae	Shrub	Ornamental
2.	Acantholippiaseriphioides (A. Gray)	Verbenaceae	Shrub	Ornamental
3.	Achrussapota L.	Sapotaceae	Tree	Cultivated
4.	Adeniumobesum (forssk). Roem. &Schult	Apocynaceae	Shrub	Ornamental
5.	AdhathodavasicaNees.	Acanthaceae	Shrub	Wild
6.	Allamandacathartica L.	Apocynaceae	Climber	Ornamental
7.	Anacardiumoccidentale L.	Anacardiaceae	Tree	Cultivated
8.	Annonamuricata L.	Annonaceae	Shrub	Cultivated
9.	Annonareticulata L.	Annonaceae	Shrub	Cultivated
10.	Annonasquamosa L.	Annonaceae	Shrub	Cultivated
11.	Aracauriasps.	Aracauriaceae	Shrub	Ornamental
12.	Argyreia nervosa (Burm .f.) Bojer	Convolvulaceae	Climber	Wild
13.	Averrhoablimbi L.	Oxalidaceae	Tree	Cultivated
14.	Bauhinia vahliiwt&Aron	Caesalpiniaceae	Shrub	Wild
15.	Borassusflabellifer .L	Arecaceae	Tree	Wild
16.	Calotropisgigantea (Ait.) R. Br	Asclepiadaceae	Shrub	Wild
17.	Caralliabracheata (Louro) merr.	Rhizophoraceae	Shrub	Wild
18.	Carica papaya L.	Caricaceae	Tree	Cultivated
19.	Cassia acacia L.	Caesalpiniaceae	Shrub	Wild
20.	Cassia alata L.	Caesalpiniaceae	Shrub	Wild
21.	Cassia auriculata Linn.	Caesalpiniaceae	Shrub	Wild
22.	Citrus medica L.	Rutaceae	Shrub	Cultivated
23.	Clematis recta L.	Ranunculaceae	Climber	Ornamental
24.	CoccusnuciferaL.	Arecaceae	Tree	Cultivated
25.	Colocasiasps	Araceae	Shrub	Wild
26.	Crataevamagna(Lour.) Dc.	Capparidaceae	Tree	Wild
27.	Cryptostegiagrandiflora R.Br.	Apocynaceae	Shrub	Ornamental
28.	Cycas revolute Thunb.	Cycadaceae	Tree	Ornamental
29.	Dichrostachyscinereawight et Arn.	Mimosaceae	Shrub	Wild
30.	Dodonaea viscosa Jacq	Rutaceae	Shrub	Wild
31.	Duranta erecta L.	Verbenaceae	Shrub	Ornamental
32.	FicuscaricaL.	Moraceae	Tree	Cultivated
33.	Flacourtiajangomas (Lour.) Rarusch	Flacourtiaceae	Tree	Cultivated
34.	GalphimiagracilisBartl.	Malphigiaceae	Climber	Ornamental
35.	Gardenia gummifera L.F.	Rubiaceae	Shrub	Ornamental
36.	Gliricidiasepium (Jacq.) Kunth ex walp	Fabaceae	Shrub	Wild
37.	Hibiscus mutabilis L.	Malvaceae	Tree	Ornamental
38.	Hibiscus rosasinensis L.	Malvaceae	Shrub	Ornamental
39.	Ixoracocinea L.	Rubiaceae	Shrub	Ornamental
40.	Jatrophagossipifolia L.	Euphorbiaceae	Shrub	Wild
41.	JatrophaintegrimmaJacq.	Euphorbiaceae	Shrub	Ornamental
42.	Klienhofia hospitaL.	Sterculiaceae	Tree	Wild
43.	Kopsiafruticosa A.D.C	Apocynaceae	Shrub	Ornamental

RicinuscommunisL. Rosa sps Santalum album L. Syzygium samarangens (Blume) Merr. & Perry Syzygium jambolanum L. Tecomastans L. Terminaliacatasppa L. Thujaoccidentalis L. ThunbergiagrandifloraRoxb ToreniafalconeriiL. Tremaorientalis (L.) Blume	Euphorbiaceae Rosaceae Santalaceae Myrtaceae Bignoniaceae Combretaceae Cupressaceae Acanthaceae Plantaginaceae Ulmaceae	Shrub Shrub Tree Shrub Tree Tree Tree Shrub Shrub Shrub	Wild Ornamental Cultivated Wild Wild Ornamental Cultivated Ornamental Ornamental Ornamental Wild
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RicinuscommunisL. Rosa sps Santalum album L. Syzygium samarangens (Blume) Merr. & Perry	Euphorbiaceae Rosaceae Santalaceae Myrtaceae	Shrub Tree Shrub	Ornamental Cultivated Wild
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RicinuscommunisL. Rosa sps	Euphorbiaceae Rosaceae	Shrub	Ornamental
RicinuscommunisL.	Euphorbiaceae		
		<b>a</b> ) )	
RhondeletiacalophyllaStandl.	Rubiaceae	Shrub	Wild
			Ornamental
			Ornamental
			Cultivated
		Tree	Cultivated
			Cultivated
Podranearicasoliana (Tanf.)	Bignoniaceae	Tree	Ornamental
	Apocynaceae	Shrub	Ornamental
	Apocynaceae		Ornamental
	Nyctaginaceae		Ornamental
Phyllanthusemblica L.	Euphorbiaceae	Tree	Wild
Oxystelmasecamone L.	Asclepidaceae	Climber	Wild
Nyctanthusarboretristis L.	Nyctaginaceae	Shrub	Wild
MyristicafragransHoult	Myrtaceae	Tree	Wild
Mussanda erythrophylla (Schumdch)	Rubiaceae	Shrub	Ornamental
Musa paradisiaca L.	Musaceae	Shrub	Cultivated
Muntingiacalabura L.	Muntinginaceae	Tree	Wild
Moullava spicata (Dalzell) Nicolson	Fabaceae	Climber	Wild
Moringaoleifera Lam.		Tree	Cultivated
Millingtonia hortensis L.	Bignoniaceae	Tree	Ornamental
Melastomamalabathricum (L.) smith	Melastomaceae	Shrub	Ornamental
Mangiferaindica L.	Anacardiaceae	Tree	Cultivated
Lantana camara Linn.	Verbenaceae	Shrub	Ornamental Ornamental
	Mangiferaindica L. Melastomamalabathricum (L.) smith Millingtonia hortensis L. Moringaoleifera Lam. Moullava spicata (Dalzell) Nicolson Muntingiacalabura L. Musa paradisiaca L. Mussanda erythrophylla (Schumdch) MyristicafragransHoult Nyctanthusarboretristis L. Oxystelmasecamone L. Phyllanthusemblica L. Pisonia alba span. PlumeriapudicaJacq Plumeriarubra L. Podranearicasoliana (Tanf.) Pouteria campechiana(kunth) Baehni Psidiumguajava L. Punicagranatum L. Quisqualisindica L. RavanalamadacascariensisSonn.	Lantana camara Linn.VerbenaceaeMangiferaindica L.AnacardiaceaeMelastomamalabathricum (L.) smithMelastomaceaeMillingtonia hortensis L.BignoniaceaeMoringaoleifera Lam.MoringaceaeMoullava spicata (Dalzell) NicolsonFabaceaeMuntingiacalabura L.MuntinginaceaeMusa paradisiaca L.MusaceaeMussanda erythrophylla (Schumdch)RubiaceaeMyristicafragransHoultMyrtaceaeNyctanthusarboretristis L.NyctaginaceaeOxystelmasecamone L.EuphorbiaceaePhyllanthusemblica L.EuphorbiaceaePisonia alba span.NyctaginaceaePlumeriapudicaJacqApocynaceaePlumeriarubra L.ApocynaceaePodranearicasoliana (Tanf.)BignoniaceaePoidranearicasoliana L.MyrtaceaePunicagranatum L.PunicaceaeQuisqualisindica L.CombretaceaeRavanalamadacascariensisSonn.Musaceae	Lantana camara Linn.VerbenaceaeShrubMangiferaindica L.AnacardiaceaeTreeMelastomamalabathricum (L.) smithMelastomaceaeShrubMillingtonia hortensis L.BignoniaceaeTreeMoringaoleifera Lam.MoringaceaeTreeMoullava spicata (Dalzell) NicolsonFabaceaeClimberMuntingiacalabura L.MuntinginaceaeShrubMussanda erythrophylla (Schumdch)RubiaceaeShrubMyristicafragransHoultMyrtaceaeTreeNyctanthusarboretristis L.NyctaginaceaeShrubOxystelmasecamone L.AsclepidaceaeClimberPhyllanthusemblica L.EuphorbiaceaeShrubPlumeriarubra L.ApocynaceaeShrubPlumeriarubra L.ApocynaceaeShrubPlumeriarubra L.ApocynaceaeShrubPlumeriarubra L.ApocynaceaeShrubPodranearicasoliana (Tanf.)BignoniaceaeTreePouteria campechiana(kunth) BaehniSapotaceaeTreePunicagranatum L.PunicaceaeShrubQuisqualisindica L.CombretaceaeClimberRavanalamadacascariensisSonn.MusaceaeTree

Table 2. Ha	abit wise distribution	on of plant species	Table J	LCOHOIN	ie uses of pl	ants	
	in the study area.			Fruit	Timber	Oil	Medicinal
Habits	No. of species	No. of species		Yield	Yield	Yield	Used
Climber	7	8.97%	5	4	4	3	7
Shrub	44	56.41%					

Tree	es 27		34.61%
Table	3. Cotyledon wise d	listribution	
S.	Presence of	No. of	Percentage

No.	cotyledonous	Plants	Percentage
1.	Dicot	70	89.74%
2.	Monocot	8	10.25%
2.	Monocot	8	10.25

# Table4. Percentageofplantspeciesunderwild/cultivatedandornamentalcategories.

Nature of plants	No. of species	Percentage
Wild	27	35.52%
Cultivated	19	24.35%
Ornamental	32	42.10%

## Table 6. Dominant families observed during the<br/>study period

S. No	Families	No. of plants
1	Apocynaceae	5
2	Euphorbiaceae	4
3	Rubiaceae	4
4	Bignoniaceae	3
5	Verbenaceae	3
6	Annonaceae	3
7	Acanthaceae	2
8	Anacardiaceae	2
9	Arecaceae	2
10	Asclepidaceae	2
11	Caesalpinaceae	2
12	Combretaceae	2
13	Fabaceae	2

14	Musaceae	2	
15	Nyctaginaceae	2	
16	Rutaceae	2	
17	Sapotaceae	2	

Table 7. Family wise distribution of plant speciesin the study area

Sl. No.	Family	Genus	Species
1.	Acanthaceae	2	2
2.	Anacardiaceae	2	2
3.	Annonaceae	1	3
4.	Apocynaceae	5	6
5.	Aracariaceae	1	1
6.	Araceae	1	1
7.	Arecaceae	2	2
8.	Asclepidaceae	2	2
9.	Bignoniaceae	3	3
10.	Caricaceae	1	1
11.	Caesalpiniaceae	2	4
12.	Capparidaceae	1	1
13.	Combretaceae	2	2
14.	Convolvulaceae	1	1
15.	Cupressaceae	1	1
16.	Cycadaceae	1	1
17.	Euphorbiaceae	4	5
18.	Fabaceae	2	2
19.	Flacourtiaceae	1	1
20.	Lythraceae	1	1
21.	Malvaceae	1	2
22.	Malphigiaceae	1	1
23.	Melastomaceae	1	1
24.	Mimosaceae	1	1
25.	Moraceae	1	1
26.	Moringaceae	1	1
27.	Muntingiaceae	1	1
28.	Musaceae	2	2
29.	Myrtaceae	2	2
30.	Nyctaginaceae	2	2
31.	Oxalidaceae	1	1
32.	Punicaceae	1	1
33.	Plantaginaceae	1	1
34.	Ranunculaceae	1	1
35.	Rosaceae	1	1
36.	Rhizophoraceae	1	1
37.	Rubiaceae	4	4
38.	Rutaceae	2	2
39.	Santalaceae	1	1
40.	Sapotaceae	2	2
41.	Sterculiaceae	1	1
42.	Ulmaceae	1	1
43.	Verbenaceae	3	3

Plants like Anacardium occidentale, Mangifera indica, Adhathoda vasica, Calotropis procera, Millingtonia hortensis, Tecoma stans, Cassia auriculata, Quisqualis indica, Phyllanthus emblica, Ricinus communis, Hibiscus rosasinensis, Ficus carica, Moringa oleifera, Musa paradisiaca, Rosa sps, Ixora cocinea, Lantana camara are abundantly found in the study area. Dominance of Apocynaceae shows that these areas are nutrient deficient especially nitrogen. Among the plant species, 27 were wild / naturalized, 19 are cultivated and 32 are ornamental (Table 4). The most diverse families in the study area include Apocynaceae, Euphorbiaceae, Rubiaceae, Bignoniaceae. Some number of exotic floras was reported from the study area which includes Annona squamosa, Psidium guajava, Punica granatum, Lantana camara.

Most plant species in the study area are considerable ecological and economic importance and useful as bioresources to wild fauna and human beings. Of the total 27 wild / naturalized plant species, most are useful as edible fruits, timbers, fuel wood etc (Table 5). Ecologically, the non woody species provide fleshy fruit resources to faunas indicating the extent of the faunal dependence of plants for various ecological processes. Some of the wild / naturalized edible fruits trees are Annonasquamosa, Annona muricata, Annona reticulata, Anacardium occidentale, Mangifera indica, Ficus carica, Cocos nucifera, Musa paradisiaca, Carica papaya, Pouteria campechiana, Averrhoa blimbi, Borassus flabellifer, Terminalia catappa, Achras sapota. Growing medicinal plants is a great way to ensure good health. These plants are recommended for their wide range of health benefits and basic healthing properties. The medicinally important species are Adhathoda vasica, Annona muricata, Annona reticulata, Annona squamosa, Carica papaya, ficus carica, Myristica fragrans. Timber yielding plants like Borassus flabellifer, Coccus nucifera, Manaifera indica, Santalum album and oil vielding plants are Coccus nucifera, Riccinus communis, Borassus flabellifer. There are 14 fruit yielding trees, 7 medicinal plants, 5 edible trees, 4 timber yielding trees and 3 oil yielding trees.

The Apocynaceae were observed to be the most prevalent family. This may be due to their fast germination ability, associated with symbiotic properties which have enabled species to easily establish within habitat types. This finding was in line with the works of Deka et al. (10), on vegetative assessment of tree species and shrubs indicating that legumes were the prominent species recorded in the study area. Moraceae, Meliaceae and Papilionaceae also their ability to produce numerous seeds which was eventually establish at suitable sites. This result was confirmed by Khan *et al.* (11) while working on regeneration and survival of tree seedlings in tropical forests. The reasons for the low number of species observed in some families could be attributed to diseases and browsing by

herbivores which resulted in poor growth and establishment and perhaps seeds need scarification treatment before germination. Similar results were reported by Coley and Barone (12) on herbivory and plant defences on herbivores. The low number of species could also be attributed to anthropogenic activities which affected species growth and production. Similar findings have been reported by on plant communities Sumina (13)on anthropogenically disturbed sites in Chukotka Peninsula.

The present study site had a high species diversity for both tree and shrub species. Probably, the high species diversity for trees and shrubs could be attributed to the many tributaries and streams that empty rich organic content and mineral resources utilized by the species for growth and production. Giliba *et al.* (14) reported similar findings on woodland of Bereku Forest Reserve in Tanzania. Some of the rare trees and shrubs species in the area observed during survey, Such as Crataeva magna, Averrhoa blimbi, Borassus flabellifer, Clematis recta, Hibiscis mutabilis, Klienhofia hospita, Moullava speicata, Oxystelma secamone, Pouteria compechiana etc.,

The dominance of this family could be as a result of habitat adaptation and favourable environmental conditions which encourage pollination, dispersal and eventual establishment of species. Similar situations were reported by Pausas and Austin (15) on species richness in relation to environment. Austin et al (16) found that edaphic parameter (soil nutrients) played a major role in species richness and establish-ment in an ecosystem. The reasons for the poor establishment of some families which showed lowest species may be attributed to competition for nutrients, limited light by canopy trees and destruction of undergrowth during tree snapped and logged on the forest floor. Egbe et al. (17) mentioned similar reports in a disturbed and natural regeneration forest in Korup. National Park and Coley and Barone (12) also recorded anthropogenic activities affecting growth and distribution of species.

## 4. CONCLUSION

Human activities including unsustainable resources exploitation in communities has greatly depleted the resources base of the community forest. However, tree species had the highest population density in the study area followed by the shrubs species. It is therefore recommended that measures to foster partnership between the community and other stakeholders in natural resources conservation in the areas should be encouraged to ensure sustainable natural resources management in the areas. Furthermore, public enlightenment on the need for sustainable natural resources exploitation should be intensified in the area to raise the level of awareness of the locals; also there is need for the provision of alternative means of livelihood for the local populace to reduce their rate of dependence on the available resources of the forest. Finally, afforestation and re-afforestation programs should be timely carried out to rehabilitate the degraded ecosystem.

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## **RESEARCH ARTICLE**

## REACTIVE OXYGEN OR NITROGEN SPECIES (ROS/ RNS) AND RESPONSE OF ANTIOXIDANTS AS SCAVENGERS DURING BIOTIC STRESS IN PLANTS: AN OVERVIEW

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### ABSTRACT

Phytopathogens have evolved diverse independent and complex modes of penetrating and accessing plant cellular contents. The synthesis of reactive oxygen or nitrogen species (ROS/RNS) by the utilization of molecular oxygen during plant-pathogen interactions results in to oxidative burst, a signaling cascade to defense. ROS array includes singlet oxygen, the hydroxyperoxyl radical, the superoxide anion, hydrogen peroxide, the hydroxyl radical and the closely related reactive nitrogen species, nitric oxide. ROS acts synergistically directs to signal amplification to drive the hypersensitive reaction (HR) and initiates systemic defenses. The role of ROS in successful pathogenesis, it is ideal to inhibit the cell death machinery selectively and simultaneously to monitor other defense and pathogenesis-related processes. With the understanding of the interplay underlying the localized activation of the oxidative burst following perception of pathogen avirulence signals and key downstream responses including gene activation, cell death, and long-distance signaling, novel strategies will be developed for engineering enhanced protection against pathogens by manipulation of the oxidative burst and oxidant-mediated signal pathways. In this over review, it is reported the different roles of ROS/RNS in host-pathogen interactions with example on *Alternaria- Sesamum* interaction.

Keywords: Antioxidants, ROS, RNS, plant, pathogen, defense, oxidative burst.

## **1. INTRODUCTION**

Plant diseases caused by biotic stresses such as bacteria, fungi, viruses, or nematodes forms the major concern in agriculture leads to economic loss of crop productivity. The increasing human population needs an increase in agricultural production. This challenge is made difficult by the fact that changes in the environmental conditions under which the crops grown have resulted in the appearance of diseases. Similarly, drastic genetic changes within the pathogen have resulted in the emergence of new plant diseases. To meet this challenge, study of plant-pathogen interactions in terms of physiology, biochemistry and molecular level is a pre-requisite to uncover the mechanisms by which disease resistance is achieved. Plant pathogens include a wide array of organisms such as fungi, bacteria, oomycetes and viruses. Pathogens have evolved different strategies to invade a host, as well as to feed on and multiply in the plant. Biotrophic pathogens need living tissue for growth and multiplication, in course of time, the tissue will die so that the pathogen becomes hemi-biotrophic. On the other hand, necrotrophic pathogens kill the host tissue at the initial stage of the infection and utilize the dead tissue. Bacteria and fungi show both bio-trophic as well as necrotrophic strategies. The jasmonate/ethylene pathway is more important in defending necrotrophic pathogens while salicylic acid dependent responses are more effective against biotrophic pathogens (1).

Exploring the interplay between plants and pathogens can lead to develop strategies to control or minimize the impact of pathogenicity on the host. Pathogen infection leads to alter the primary and secondary metabolism which in turn tunes defense strategies as well as growth and development of the host plant. The regulation of defense responses has been intensively studied for decades but less is known about the interplay between the pathogen infection and reactive oxygen and nitrogen cycles. Production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) are linked to signaling in both developmental and stress responses. Currently, interest in this research area has been growing in terms of biochemistry of antioxidant machinery and source-sink regulation in different types of plant-pathogen interactions. Further, the plant pathogen studies started analyzing the physiological status of the infected tissues to elucidate the infection mechanisms.

Plant-pathogen interaction is a multifaceted event, regulates pathogen ingress and establishment of disease. Studies revealed that plants and

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pathogens communicate with each other in a conversation through reactive oxygen species (ROS) and reactive nitrogen species (RNS) signalling network. Induction of ROS and RNS are the earliest observable manifestations of a plant defence strategy orchestrated by ROS and RNS gene network. The interplay between ROS and RNS production and their scavenging during plant pathogen interaction appears to be more and more complex. In this scenario, the ROS and RNS cycle seems to have an increasing importance. Despite this significance, there is still a lacuna to dissect the identities, activities and relative importance of the ROS and RNS generating system in host pathogen interaction.

The level of ROS and RNS are controlled by both production and removal through various scavengers including ascorbic acid and glutathione. Ascorbic acid and glutathione are central components in regulating the redox balance of the plant cell. It has become increasingly clear that signalling pathways in plants are not organized into linear pathways; instead, as a web of interactions. Not even individual ROS and RNS give uniform responses; instead, separate molecules (hydrogen peroxide, superoxide, singlet oxygen, and nitric oxide), acting at different subcellular locations give rise to unique changes in gene expression (2). ROS and RNS production and their scavenging are intimately linked, and the balance between them will determine defence signalling output as well as damage and cell-death responses. An active phase of enzymatic ROS and RNS scavengers (including catalase, superoxide dismutase, and ascorbate peroxidase) and low-molecular-weight non enzymatic scavengers (ascorbate, glutathione, and  $\alpha$ tocopherol) protect plants from excessive ROS and RNS production. Ascorbate and glutathione are connected through the ascorbic acid-glutathione cycle (3) and are essential for plants. The ROS and RNS -mediated plant response is variable and depends on the pathogen life style (biotrophy versus necrotrophy), the type of plant-pathogen interaction (compatibility versus incompatibility) and the stage of plant development. Thus, the tightly regulated balance between ROS and RNS production and its removal at the cellular and subcellular levels seems to be of primary importance for fulfilling the multiple functions of ROS and RNS controlling redox homeostasis.

Analysis of the host parasite relationships reveals the pattern of pathogenesis and the defence mechanisms exhibited by the plants challenged by the pathogen. The induction of defence mechanism may be either specific or non-specific. Perception of a pathogen by a plant triggers rapid defense responses via multiple signalling pathways that lead to the induced expression of genes encoding pathogenesis-related (PR) proteins and enzymes involved in the production of secondary metabolites and hormones (4). In many host-parasite interactions, substances such as phenolic compounds, phytoalexins, tannins and some fatty acid like compounds, which are potent inhibitors of many hydrolytic enzymes form the basis of resistance. Plant cells contain variable amounts of hydrolytic enzymes such as glucanases and chitinases that cause breakdown of cell wall components of the pathogen.

Biotic elicitors induced several dynamic defense mechanisms that act as physical and prevent which further chemical barriers, colonization or spread of the pathogen. The receptor-proteins located in cell membrane detect the pathogen or the factor translocated by pathogens. Plants have evolved sophisticated biochemical mechanisms to exert self-defense pathogen infections. The rapid and against transient production of ROS and RNS induce oxidative burst is one of the earliest plant cell responses following pathogen recognition and is involved in cell wall strengthening via crossof glycoproteins and induction of the linking hypersensitive response. The predominant types of ROS and RNS detected in plant pathogen interaction include  $O_2^{\bullet-}$ ,  $H_2O_2$ ,  $OH^-$  and NO (5). Enzymes such as NADPH oxidase and superoxide dismutase are responsible for the formation of reactive oxygen species. Increased synthesis and activity of phenylalanine ammonia lyase (PAL) has been reported in the plants against fungal and bacterial pathogens as active defense response. PAL plays key role in the synthesis of phenols, phytoalexins and lignin (6). The effectiveness of resistance depends on speed and amount of synthesized products and their movements to neighbouring healthy tissues to create defensive barriers. Due to the entry of the pathogen, a rapid and temporary increase in cellular activities capable metabolic of triggering hypersensitive cell death. The induced resistance offered by biochemical changes in host plants is the last line of host defence, which includes activation of lignin synthesis, enhanced activity of several antioxidant enzymes and suitable changes in plant metabolism.

## 2. Antioxidant systems vis-a-vis reactive oxygen species (ROS) and reactive nitrogen species (RNS) during plant - pathogen interaction

Plant resistance to pathogens requires the induction of complex metabolic pathways in the infected tissues, focused at recognizing pathogen and inhibiting its multiplication within the host plant tissues. In spite of compatible and incompatible

reactions induce alterations in plant metabolism, only incompatible reactions in the plant is able to efficiently inhibit pathogen invasion without substantial damage. The common incompatible responses is hypersensitive response (HR), in which cells surrounding the pathogen invasion switch on genes encoding for phenyl propanoid metabolism and other pathogenesis related proteins before activating programmed cell death (PCD) (3).

In plants, ROS continuously produced as byproducts of different metabolic pathways compartmentalized in different cellular organelles. Plants also produce ROS, by inducing various oxidases and peroxidases, which in turn produce ROS in response to environmental challenges. The major sites of ROS production in cell systems include chloroplast, mitochondrion, peroxisome, endoplasmic reticulum, plasma membrane and cell wall.

## 2.1. Singlet Oxygen $(^{1}O_{2})$

One of the most reactive forms of oxygen, the singlet oxygen generate by an input of energy that rearranges the electrons in the molecule. In singlet oxygen, the electron spins are opposed in a higher energy state and is many times more reactive than triplet oxygen. Singlet oxygen is the common name used for the two metastable states of molecular oxygen  $(0_2)$ , with higher energy than the ground state. In both forms of singlet oxygen, if the spin restriction removed then the oxidizing ability is greatly increased. Singlet oxygen can directly oxidize biomolecules like DNA and protein.

## 2.2. Superoxide radical or Superoxide radical anion $(0_2^{-})$

Supplying of single electron to  $O_2$ , it enters

one of the (  $\pi^*$ ) antibonding orbitals leading to the formation of superoxide radical. Membrane bound NAD(P)H oxidase (NOX) that generate the super oxide anions  $(O_2^{\bullet-})$  using NADPH as the electron donor.

 $O_2 + NADPH \rightarrow O_2^{\bullet-} + NADP^+ + H^+$ 

## 2.3. Hydrogen peroxide $(H_2O_2)$

Hydrogen peroxide is a relatively stable ROS being not very reactive and electrically neutral, is able to pass through cell membranes and reach cell locations remote from the site of its formation. Together with  $O_2^{\bullet-}$  it can be converted to hydroxyl radicals by the Haber-Weiss reaction. Superoxide dismutase (SOD) enzymes are responsible for  $H_2O_2$ production by dismutation reaction of O2.--.

Free radicals / ROSs have signalling function mediating defence gene activation and establishment of additional defences, by redox control of transcription factors or by interaction with other signalling components like phosphorylation cascades. ROS can generate lipid derivatives by nonenzymatic oxygenation that can produce membrane damage or function as signalling molecules like cyclic oxylipins of the jasmonate type. Similarly, these molecules can activate the generation of phytoalexins or other secondary metabolites that arrest pathogen growth and also in terms of lignification (7).

Moreover, H<sub>2</sub>O<sub>2</sub> also block pathogen invasion in plant cells because it contributes to wall strengthening by activating peroxidase reactions via intra- and inter-molecular cross-links between cell wall components and lignin polymerization. As H<sub>2</sub>O<sub>2</sub> is a diffusible molecule in biological membranes, it also acts as intracellular signal regulate ion flux across the membrane (Ca<sup>2+</sup> influx and K<sup>+</sup>, Cl<sup>-</sup> efflux) as well as changes in pH and plasma membrane depolarization.

## 2.4. Nitric oxide (NO)

Under biotic stress, plant cells exhibit a rapid synthesis and accumulation of reactive nitrogen species known as nitric oxide and these response trigger a programmed cell death (PCD) process leading to an intrinsic execution in plant cells. PCD is an integrated cellular process occurring in plant defense responses to facilitate normal growth and development and better survival against various stresses as a whole. Both NO and ROS play key roles in PCD. These redox active small molecules can trigger cell death either independently or synergistically. Nitric oxide and H<sub>2</sub>O<sub>2</sub> reciprocally enhanced the production of each other whereas NO and  $O_2^{-\bullet}$  showed reciprocal suppression on each other's production. It was the interaction between NO and  $O_2$ -but not between NO and  $H_2O_2$  that induced PCD, probably through peroxynitrite (ONOO-). Nitric oxide and reactive oxygen species are major players of biotic interactions in plants and are crucial components of plant immunity. Besides their role in plant defence response they have been demonstrated to be involved in symbiotic interactions between plants and microorganisms (8). In plants, nitric oxide could also function as a signal molecule in the transduction pathway leading to the induction of defence responses against pathogens and in damage leading to cell death.

## 2.5. Antioxidant (AOX) machinery and defence against phytopathogens

In plant cells, enzymes and redox metabolites act in synergy to detoxify ROSs. SOD dismutates  $0_2^{-}$ to  $H_2O_2$ , catalase (CAT) peroxidatively cleaves  $H_2O_2$  to oxygen and water, and ascorbate peroxidase (APX) reduces  $H_2O_2$  to water by utilizing ascorbate (ASC) as electron donor. These are the major enzymatic AOX systems for protecting cells against oxidative burst (Fig. 1). Most of the AOX enzymes are isoenzymatic and their expression is genetically regulated by developmental and environmental stimuli, accordingly ROS cycle will be regulated in the cells (7).

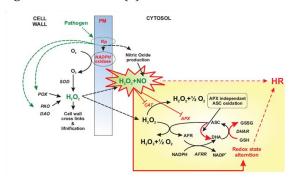


Fig. 1. ROS cycle induced during pathogen stress producing reactive oxygen and/or nitrogen species leading to oxidative burst and induction of antioxidant system

APX- ascorbate peroxidase, ASC- ascorbate, AFR- ascorbate free radical, AFRR- ascorbate free radical reductase, CAT- catalase, DAO- aiamine oxidase, DHA- dehydro ascorbic acid, DHARdehydro ascorbic acid reductase, GSH- glutathione, GSSG- glutathione disulfide, HR- hypersensitive response, NO- nitric oxide, PAO-polyamine oxidase, PM- plasma membrane, POX- peroxidase, Rpreceptor proteins, SOD- superoxide dismutase.

Glutathione (GSH) forms the ubiquitous antioxidants present in plant cells and play pivotal role in plant defence activation. Depletion of GSH or increase in its oxidised form, glutathione disulfide (GSSG), induces accumulation of phytoalexins (3). Up regulation of GSH content has been reported in leaves attacked by avirulent biotrophic pathogens. It is also reported that an increase in the expression of glutathione 5-transferase and glutathione peroxidase in the cells near to hypersensitive cell death region induced by an avirulent phytopathogen (9). H<sub>2</sub>O<sub>2</sub> accumulation and GSH oxidation occur in different sites and with different timing in leaves of resistant barley line attacked by powdery mildew (10). Decline in GSH content was noticed in tomato infected with the Botrytis cinerea (11) and in Avena sativa infected with virulent necrotrophic fungus (12). Both these cases, the decline in antioxidant defence could increase necrotic spots that facilitate the penetration of necrotrophic phytopathogens. Cotyledons of tomato carrying Avr genes and injected with race-specific elicitors of Cladosporium fulvum, showed an enhanced profile of GSH pool in the oxidised form (13). GSH responsive elements have been reported as promoters of phenylalanine ammonia lyase (PAL) and chalcone synthase (7). GSH acts in synergy with other signals in the induction of defence strategies has been reviewed on phytopathogen induced diseases in Arabidopsis mutants having GSH levels 70% lower than the wild type parental ecotype. The infection of the *Arabidopsis* mutant with either virulent or avirulent fungal and bacterial pathogens gives the same responses with wild type (14). A probable explanation of these findings could be GSH compensation with other antioxidant molecules and enzymes. Indeed, the ASC levels are higher in the mutant than in the wild type. Compensation between GSH and thioredoxin-glutaredoxin systems has also been reported in lower organisms also (15).

the ascorbate-glutathione system In suppression of APX is required in tissues undergoing HR. Similarly decrease in CAT activity has also been reported in cells undergoing HR. However, the mode of suppression of these scavenging enzymes is dissimilar (3). CAT is down-regulated at the transcription level, whereas APX regulation in HR involves changes both at the transcription and translation or post-translation levels. Tobacco mosaic virus (TMV) infection of tobacco enhance mRNA expression of APX probably as an antioxidant response triggered by increasing of H<sub>2</sub>O<sub>2</sub> within cells similar to that activated under other environmental constraints (16). In spite of the increase in its expression, the APX activity is strongly suppressed in the TMV-infected tissues by a mechanism that acts at the transcriptional or post-transcriptional level. transcriptional The / post-transcrip-tional regulation of APX is unique to HR-related incompatible response and indicates the necessity of accumulating ROS during this defence process. Meanwhile, it has been also reported that in a compatible reaction between barley and powdery mildew the cytosolic APX isoenzyme is up-regulated in epidermal and mesophyll cells. In spite of this, APXs are unable to block the pathogens, its increase limits the propagation of oxidative processes permitting cells to maintain their viability, a condition required for the penetration of biotrophic pathogens in to the plant tissues. This up-regulation of APX confirms previous results of enhancement in APX activity during successful infection of barley leaves by biotrophic compatible pathogens and in leaves of susceptible apricot infected by plum pox virus (17).

Ascorbate-glutathione redox enzymes and ASC/DHA levels play significant roles against plant pathogens but still the available data are

fragmentary. However, an increase in ascorbate free radical reductase, the enzyme responsible for the reduction of ascorbate oxidation, seems to occur in the compatible reactions, thus mimicking the behaviour of APX (17).

Similarly, the activities of ascorbate recycling enzymes such as dehydro ascorbate reductase, that reduces dehydroascorbate to ascorbate using GSH as reductant, and the enzyme GSSG reductase not clearly indicative of resistance or susceptibility in the host cells (3). This is probably due to multiple intrinsic factors such as plant species specific sensitivity, plant pathogen interactions, the suppression or strengthening of ROS detoxification that regulate ascorbate-glutathione interplay and the corresponding redox state (biosynthesis, oxidation pathways and recycling enzymes).

Further, increasing focus has also been given to reactive nitrogen species (RNS) such as nitric oxide (NO) as a signal molecule involved in plant pathogen interaction (18). Results obtained with cultured tobacco cells, in which hypersensitive programmed cell death (PCD) is induced by simultaneous treatments with NO and H<sub>2</sub>O<sub>2</sub> generators, indicate that suppression of APX and decrease in the ascorbate (ASC + DHA) and glutathione (GSH + GSSG) pools, the key events in PCD. Moreover, during the HR process, redox balances of ASC and GSH are strongly shifted towards the oxidized forms (Fig. 1). These changes in the cellular antioxidant systems are not only because of NO and  $H_2O_2$ , but also due to other intrinsic factors (19). Similarly, when programmed cell death was blocked by treatment with protein synthesis inhibitors in the tissues leads to the induction of HR with simultaneous generation of NO and  $H_2O_2$ . Parallely, the inhibition of APX and the decrease in ASC and GSH pools are also reversed (19). These results suggests that changes in the cellular redox balance are not as a consequence of disease impacts but part of the transduction signalling cascade that induces defense responses under the opportunate stimuli. Further, like GSH/GSSG pair, the pool and redox state of ascorbate play regulatory role in plant metabolism, both acting at the level of gene expression and also with enzymatic pathways (18). Thus, ROSs are secondary messenger cascades in stress responses and their level or redox status of the cell dictates the response types i.e., high level leads to cell death whereas low initiate defence genes and ends in adaptive responses. ROS sources and complex regulatory antioxidant systems provide the flexibility necessary to allow the dual role of ROS.

Free radicals like superoxide anions, reactive oxygen species (ROS) such as hydrogen peroxide ( $H_2O_2$ ), RNS like NO are potential reactive

molecule produced in the cell as by-products of normal cellular metabolism or under biotic stress. During plant-pathogen interactions ROS and RNS are generally involved in stimulating plant defense genes encoding pathogenesis-related (PR) proteins or regulating synthesis of secondary metabolites or/and genes encoding ROS and RNS scavenging enzymes.

ROS and RNS regulatory mechanisms at the biochemical level especially the communication and interplay across cellular organelles are still poorly documented. Due to the central role of ROS and RNS as signalling cascades, it is essential to obtain a comprehensive knowledge of the synthesis and regulation of ROS and RNS during plant-pathogen interaction. This adds additional information towards physiological, molecular and evolutionary research perspectives in phytopathology.

In this scenario, the present study aims to unravel the biochemical mechanism of ROS cycle in Sesamum orientale L. against Alternaria leaf spot disease in sesame. As an initial part conidium germination, inoculation, penetration and colonization of the pathogen on the plant surfaces were studied using scanning electron microscopy. Transmission electron microscopy studies showed structural changes in the chloroplast and mitochondria of diseased plants. Changes in different biochemical parameters in the diseased sesame plants were compared to control. Meanwhile the activity of different antioxidant enzymes such as catalase, peroxidase polyphenol oxidase and superoxide dismutase in diseased plants showed remarkable levels compared to control. Due to the infection, chlorophyll content, carbohydrates and total soluble protein decreased whereas free amino acid, proline, phenols and disease-related proteins increased in the host plants. Lubaina and Murugan, (20) reported the ultra-structural changes and oxidative stress markers appeared in S. orientale cultivar - Thilarani following Alternaria sesami infection. Subsequently Lubaina and Murugan (21) published the biochemical changes during oxidative burst in this particular plant pathogen interaction. To date no information is available on the involvement of the AsA-GSH cycle in Sesamum orientale L. against Alternaria sesami. The implication of ROS cycle and ascorbate-glutathione interplay in signaling and stress responses of the oxidative stress in sesame - Alternaria interactions was also well documented (22). The ROS such as H<sub>2</sub>O<sub>2</sub> formed can be detoxified via the ascorbateglutathione cycle. Increases in ascorbate peroxidase, and glutathione reductase activities concomitant with ascorbate (AsA) and glutathione interplay, as well as AsA regeneration ability, function to keep the

balance of cellular  $H_2O_2$  under pathogenicity. Dehydroascorbate reductase and monodehydroascorbate reductase are responsible for AsA regeneration. Oxidative damage in Thilarani is attributed by a lower induction of the ascorbate glutathione cycle as an antioxidant defense system and were not sufficient to protect the ultrastructural damage of chloroplasts and mitochondria. Overall, the availability of antioxidants and the induction of antioxidant enzyme activities for detoxifying reactive oxygen species (ROS) are not regulated effectively in sesame against A.sesami induced oxidative stress. The experiments using ROS scavengers demonstrate that the antioxidant defense system is modulated by  $O_2$  - or  $H_2O_2$  signals.

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## **RESEARCH ARTICLE**

## STUDIES ON THE ARBUSCULAR MYCORRHIZAL FUNGAL DIVERSITY OF SELECTED MEDICINAL PLANT SPECIES FROM KODIKUTHIMALA, MALAPPURAM, KERALA

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## ABSTRACT

The present investigation has brought out the AM fungal association in some plant species of Kodikuthimala, Malappuram district Kerala. Totally, 25 plant species belongs to 15 families were analyzed for arbuscular mycorrhizal association. The root samples of all collected plant species showed mycorrhizal infection. The percentage of colonization was varied with plant species and it ranges from 12 (*Commelina benghalensis*) to 79% (*Sida rhombifolia*). Maximum spore population was observed in *Gloriosa superba* (574/100g of soil) and minimum in *Euphorbia hirta* (143/10g of soil). Totally 26 AM fungal species belongs to 13 genera were found. Among this *Glomus* was most dominated. In most of the plants, spores of *Rhizophagus fasciculatus* are seen. Present study confirms the Arbuscular Mycorrhizal association in the collected plant species.

Keywords: AM fungal, Spore population, Colonization, Glomus, Rhizophagus fasciculatus.

## **1. INTRODUCTION**

Mycorrhizae, which are a key soil microbial component and known to play an important role in reclamation and revegetation of such, degraded ecosystems (1). They also detoxify certain soil toxins thereby enable seedlings to withstand extreme nutrient absorption capacity of plants (2, 3). Over 80% of terrestrial plants are able to associate symbiotically which mycorrhizal fungi and this usually results in positive plant growth response (4). Mutual nutrient transfer between the fungus and plant provides the plant with phosphate and micronutrients such as copper and zinc and the fungus with carbon- based compounds. The most common form of symbiosis involves arbuscular mycorrhizal (AM) fungi, which form two major structural classes of mycorrhizae with different host plants. In AM fungi, arbuscules are considered the major site of nutrient transfer to the plant (5, 6).

AM Fung can efficiency absorbs mineral nutrients from the soil and delivers them to their host plants in exchange for carbohydrates and it also enhance tolerance of or resistance to root pathogens. Vascular plants host a great variety of fungi. In additions to being susceptible to soil- borne pathogens, plant roots are also colonized by nonpathogenic or mutualistic fungi like arbuscular mycorrhizae (AM), ectomycorrhizae (EM) and dark septate endophytes (DSE). A vast majority of terrestrial plant species are mycorrhizal associations. The AM fungi are associated with most herbaceous plants and with various woody plant families, while the EM fungi are confined chiefly to a

limited number of woody plant families. It is now evident that the mycorrhizal fungi have many significant functions in ecosystems (7). Therefore, the present study aims to Enumeration of the arbuscular mycorrhizal fungal species in the rhizosphere soil samples of the plant species in Kodikuthimala, Malapuram district, Kerala.

## 2. MATERIALS AND METHODS

### 2.1. Study area

Kodikuthimala is located at 32 km from Malappuram at the Latitude: 10.9802 and Longitude: 76.2917. Kodikuthimala has a watch tower that is popular with tourists visiting this serene place because of the vantage point it offers. British hoisted their flag on this hilltop during survey, thus getting the name Kodikuthimala. This place is noted for its various kinds of medicinal plants and ever flowering springs (Fig. 1). This city has a tropical climate. During most months of the year, there is significant rainfall in Kodikuthimala. There is only a short dry season. The average annual temperature in Kodikuthimala is 27.7°c in a year and the average rainfall is 2500 mm (Table 1).

## 2.2. Sample collection

Totally 40 plant species belonging to the 28 families were collected from Kodikuthimala, Malappuram, Keralain the period of 2016. Root samples and rhizosphere soil samples of plant species growing in and around areas of Kodikuthimala were collected. The root and soil samples were transported to the laboratory immediately after collection.

## 2.3. Root samples

Root samples, 5-15 cm long, were collected from the plant species during 2016 to 2017. During collection, care was taken to ascertain individual plants for which roots could positively identified as belonging to a particular plant species. For identification and nomenclature of the plant species the following manual was used (8, 9).

## 2.4. Soil samples

The rhizosphere soils, dug up to a depth of 10 cm, were collected from each plant species after removing the surface of the soil and litter covering. These samples were kept in sterilized bags and were transported to the laboratory immediately after collection for the examination of arbuscular mycorrhizal fungal spore isolation.

## 2.5. Soil pH

The pH of soil samples was determined (soil-water suspensions 1:5) with the help of pH meter (Elico).

## 2.6. Estimation of arbuscular mycorrhizal colonization in roots

## 2.6.1. Sample preservation

In the laboratory, the roots were separated from the soil by wet sieving. The roots were washed with water and processed a fresh whenever possible. Otherwise the washed roots were fixed in formaldehyde-acetic acid-ethanol (FAA) solution (90:5:5 V/N) modified method of Phillips and Hayman (10). The soil sample was air dried and stored at 4°C until processed. Each soil samples was used for chemical analysis, spore counts and classification in to various types and multiplication, concentration and separation of AM fungal spore for identification.

## 2.6.2. Evaluation of AM infection

The root samples were cleared and stained in tryphan blue with a modified version of the Phillips and Hayman's (10) method. Roots were cut in to 1-2 pieces, heated at 90°C in 10% KOH for about 1 hour. For thicker and older roots, the duration was increased. The root segments were rinsed in water and acidified with dilute HCl. The root pieces were stained 0.05% tryphan blue in lacto phenol for 5 minutes and the excess stain was removed with clear lacto phenol.

The pigmented roots were heated at 90°C in 10% KOH for 2 hours, washed with fresh 10% KOH and immersed in an alkaline solution of  $H_2O_2$  for 30

minutes at 25°C until bleached. They were rinsed thoroughly with water to remove the  $H_2O_2$ , acidified in dilute HCl and stained as described earlier. In some cases the modified method of Merryweather and Fitter (11) was followed where autoclaving and bleaching with  $H_2O_2$ , were omitted. In a few cases, direct observation of unstained, fresh and intact roots (12) was made.

Arbuscular mycorrhizal infection in the roots was assessed following the grid line-intersect method of Giovannetti and Mosse (13). The stained root pieces were spread out evenly on a square plastic Petridish ( $10.2 \times 10 \text{ cm}$ ). A grid of lines was marked on the bottom of the dish to form 1 cm inch squares. Vertical and horizontal gridlines were scanned under a dissecting microscope and the presence of infection was recorded at each point where the roots intersected a line. Four sets of observation were made, recording 100, 200, 300 and all the root gridline intersects. Each of the three replicates records was made on a fresh rearrangement of the same root sample.

The percentage of AM infection was calculated using the formula:

Percentage of infection =  $\frac{\text{No. of root segments infected}}{\text{Total No. of root segments observed}} \times 100$ 

When sufficient root pieces are not available, the slide method Giovannetti and Mosse (1980) was followed. Root pieces, 1 cm long, were selected at random from a stained sample and mounted on microscope slide groups of 10. Presence of infection was recorded in each of the 10 pieces and present infection was calculated. To observe hyphae, vesicles and arbuscles under light microscope, the root pieces were mounted on glass slides either temporarily in lacto phenol. The cover slip was pressed gently to make the roots flattened and sealed with DPX medium.

## *2.6.3. Isolation of arbuscular mycorrhizal spores from the soil samples*

Spores were recovered from the soil samples by the wet-sieving and decanting method (14). From each soil sample, 100 g of soil was taken and mixed with 1:1 of warm water in a large beaker until all the aggregates dispersed to leave a uniform suspension. Heavier particles were allowed to settle down. To remove organic matter and roots, the suspension was decanted through a 710  $\mu$ m sieve. The suspension that passed through 710  $\mu$ m was decanted 425  $\mu$ m, 250  $\mu$ m, 150  $\mu$ m, 75  $\mu$ m and 45  $\mu$ m sieves consecutively. The residues in the respective sieve were collected in petridishes with about 10-20 mL water observed under a dissecting microscope

for AM fungal spores. The total spore count was calculated by counting the spores. Then the spores were separated using a glass pipette and segregated. The spore were mounted on clear glass slides using lacto phenol or polyvinyl alcohol lacto phenol (PVL), covered with cover slips and sealed with DPX medium.

## 2.6.4. Identification of AM fungi

Based upon microscopic characters, the AM fungal spores were identified. For identification and nomenclature, keys of the following manual authors were used: Raman and Mohankumar (15), Schenk and Perez (16) and Redecker *et al.*, (17). Classification on based on color, size, shape, surface, structure, general nature of the spore contents and

hyphal attachment. Photomicrographs were taken with the help of a Magnus Olympus Microscope.

## **3. RESULTS**

AM fungal infection and spore population of 40 plant species belongs to 28 families present in the Table 2 to 4 and pH of the rhizosphere soil samples present in the Table 3 was 4 to 5.8. In the present study, totally 14 AM fungal species belongs to 7 genre were identified. Where the *Glomus* (4) was dominate genus followed by *Gigaspora* (3),*Acaulospora* (2), *Ambispora* (2), *Claroideoglomus* (1), *Rhizophagus* (1) and *Scutellospora* (1). Moreover the *Rhizophagus fasciculatus* was the most frequently abundant species in the study area (Table 4).

Table 1. Temperature and rain fall data of Kodikuthimala, Malappuram, District, during the September 2016 to March 2017.

Voor	Month -	Temperat	ure(0°C)	Dainfall (mm)	Uumidity (0/)
Year	Monui	Maximum	Minimum	<ul> <li>Rainfall (mm)</li> </ul>	Humidity (%)
	September	29.5	24.0	253.2	84
2016	October	30.6	24.0	280.8	81
2016	November	31.3	23.6	68.6	77
	December	31.6	22.7	82.7	74
	January	31.9	22.9	19.4	67
2017	February	32.2	23.3	7.8	71
	March	33.1	24.9	1.5	74

S. No.	Plant name	Family	Habits	Soil pH	Type of coloni zation	% of root coloni zation	Spore populati on/ 100g of soil
1	Abrus precatorius L.	Leguminosae	Climber	4.5	HV	58	693
2	Abutilon indicum D.gon.	Malvaceae	Shrub	5.5	HA	44	279
3	Alysicarpus monilifer (L.) DC.	Fabaceae	Herb	4.2	ΗA	66	437
4	Anisomeles malabarica R.br.	Lamiaceae	Shrub	5.4	HV	36	435
5	Asparagus racemosus Willd	Asparagaceae	Climber	4.2	Н	27	332
6	Borreria hispida (L.) K.Schum.	Rubiaceae	Herb	4.9	HV	35	284
7	<i>Calotropis procera</i> (Aiton) W.T.Aiton	Asclepiadaceae	Tree	5.7	Н	29	332
8	Canavalia gladiataW&A.	Papilionaceae	Climber	4.3	HV	42	354
9	Cardiospermum halicacabum L.	Sapindaceae	Climber	5.6	HA	47	372
10	<i>Chrysopogon zizanioides</i> (L.) Roberty.	Poaceae	Herb	4.7	HA	56	467
11	Cissus vitiginea L.	Vitaceae	Climber	4.1	HV	44	366
12	Cleome aspera Koenig ex DC.	Capparidaceae	Herb	4.2	HV	27	241
13	Cleome monophylla L.	Capparidaceae	Herb	5.3	HV	22	385
14	Commelina benghalensis L.	Commelinaceae	Herb	4.7	Н	12	180
15	<i>Crotalaria pallida</i> Aiton.	Fabaceae	Shrub	4.1	Н	75	274
16	Euphorbia hirta L.	Euphorbiaceae	Herb	4.8	HV	13	147
	Evolvulus alsinoides L.	Convolvulaceae			Н	26	293

Table 2. AM Fungal spore population and root colonization of plants species in Kodikuthimala,

17			Herb	4.7			
18 19	Gloriosa superba L. Hemidesmus indicus (L.) R.Br.	Lilliaceae Asclepiadaceae	Climber Climber	5.6 4.8	HV H	48 25	574 329
20	<i>Hybanthus enneaspermus</i> (L.) F.Muell.	Violaceae	Herb	5.2	Н	24	381
21 22	Hyptis suaveolens (L.) Poit. Indigofera uniflora Roxb.	Lamiaceae Fabaceae	Shrub Herb	5.8 4.4	HV HA	47 55	472 382
23	Kyllinga alba Nees.	Cyperaceae	Herb	4.9	-	-	180
24	<i>Lindernia ciliata</i> (Colsm.) Pennell.	Scrophulariaceae	Herb	5.4	Н	14	173
25	<i>Lindernia parviflora</i> (Roxb.) Haines.	Scrophulariaceae	Herb	5.6	HV	15	285
26	Mimosa pudica L.	Mimosaceae	Herb	5.4	HV	33	247
27	<i>Mukia maderaspatana</i> (L.) Roem	Cucurbitaceae	Climber	4.8	HV	62	542
28	Ocimum gratissimum Linn.	Lamiaceae	Shrub	5.2	HV	45	473
29	Oldenlandia biflora L.	Rubiaceae	Herb	5.8	HV	34	312
30	Passiflora foetida L.	Passifloraceae	Climber	4.6	Н	27	189
31	Phyllanthus maderaspatensis L.	Euphorbiaceae	Herb	5.8	Н	21	473
32	Plectranthus barbatus Andrews.	Lamiaceae	Herb	4.7	HV	48	389
33	<i>Rauwolfia serpentina</i> (L.) Benth. ex Kurz	Apocynaceae	Herb	4.2	HV	31	431
34	Sida rhombifolia L.	Malvaceae	Shrub	5.5	HV	79	573
35	<i>Solanum xanthocarpum</i> Schrad. & H. Wendl.	Solanaceae	Herb	5.4	Н	32	412
36	Spillanthes calva DC.	Asteraceae	Herb	5.2	HV	32	352
37	<i>Stachytarpheta jamaicensis</i> (L.) Vahl.	Verbenaceae	Sub- shrub	5.4	Н	26	441
38	<i>Stachytarpheta urticifolia</i> (Salisb.) Sims.	Verbenaceae	Shrub	5.8	Н	18	249
39	Wattakaka volubilis (L. fil.) Stapf.	Asclepiadaceae	Climber	4.6	HV	34	285
40	<i>Ziziphus oenoplia</i> (L.) Miller. hyphae, A- Arbuscules, V- Vescicle, + - Pr	Rhamnaceae	Climber	5.1	HV	39	378

## Table 3. Distribution of AM fungal spores different plant species.

S. No.	Plant name	Family	AM Fugal species
1	Abrus precatorius L.	Leguminosae	Acaulospora alpine, Gigaspora albida, Glomus arborense, Rhizophagus fasciculatus
2	<i>Abutilon indicum</i> D.gon.	Malvaceae	Acaulospora tuberculata, Funneliformis coronatum, Glomus canadense, Rhizophagus fasciculatus
3	Alysicarpus monilifer (L.) DC.	Fabaceae	Acaulospora alpine, Claroideoglomus claroideum, Glomus albidum, Rhizophagus fasciculatus, Scutellospora striata
4	Anisomeles malabarica R.br.	Lamiaceae	Acaulospora foveat, Dentiscutata erythropus, Gigaspora ramisporophora, Scutellispora spp
5	Asparagus racemosus Willd	Asparagaceae	Archaeospora trappei, Dentiscutata erythropus, Glomus canadense, Rhizophagus fasciculatus
6	<i>Borreria hispida</i> (L.) K.Schum.	Rubiaceae	Acaulospora tuberculata, Claroideoglomus claroideum, Gigaspora albida, Scutellospora striata
7	Calotropis procera (Aiton) W.T.Aiton	Asclepiadaceae	Archaeospora trappei, Diversispora arenaria, Gigaspora decipiens, Glomus multicaule
8	Canavalia gladiataW&A.	Papilionaceae	Archaeospora trappei, Diversispora arenaria, Gigaspora decipiens, Rhizophagus fasciculatus

9	Cardiospermum halicacabum L.	Sapindaceae	Archaeospora trappei, Claroideoglomus claroideum, Glomus ambisporum
10	<i>Chrysopogon zizanioides</i> (L.) Roberty.	Poaceae	Claroideoglomus claroideum, Dentiscutata erythropus, Glomus 11canadense
11	Cissus vitiginea L.	Vitaceae	Acaulospora alpine, Dentiscutata erythropus, Glomus ambisporum, Rhizophagus fasciculatus, Scutellospora savannicola
12	<i>Cleome aspera</i> Koenig ex DC.	Cleomaceae	Acaulospora tuberculata, Diversispora arenaria, Glomus albidum, Glomus globiferum, Rhizophagus fasciculatus
13	Cleome monophylla L.	Cleomaceae	Acaulospora foveat, Diversispora arenaria, Gigaspora decipiens, Glomus multicaule, Scutellospora striata
14	Commelina benghalensis L.	Commelinaceae	Ambispora callosa, Diversispora celata, Glomus ambisporum, Rhizophagus fasciculatus, Scutellospora savannicola
15	<i>Crotalaria pallida</i> Aiton.	Fabaceae	Acaulospora undulate, Diversispora celata, Glomus albidum, Glomus globiferum
16	Euphorbia hirta L.	Euphorbiaceae	Acaulospora alpine, Entrophospora infrequens, Glomus ambisporum, Pacispora scintillans, Rhizophagus fasciculatus
17	Evolvulus alsinoides L.	Convolvulaceae	Acaulospora foveat, Diversispora arenaria, Gigaspora decipiens, Glomus multicaule, Rhizophagus fasciculatus
18	Gloriosa superba L.	Lilliaceae	Acaulospora foveat, Claroideoglomus claroideum, Glomus ambisporum, Rhizophagus fasciculatus
19	Hemidesmus indicus (L.) R.Br.	Asclepiadaceae	Acaulospora alpine, Dentiscutata erythropus, Glomus albidum, Glomus globiferum
20	Hybanthus enneaspermus (L.) F.Muell.	Violaceae	Ambispora callosa, Diversispora arenaria, Glomus albidum, Glomus arborense
21	Hyptis suaveolens (L.) Poit.	Lamiaceae	Acaulospora undulate, Diversispora celata, Gigaspora albida, Rhizophagus fasciculatus, Scutellospora savannicola
22	<i>Indigofera uniflora</i> Roxb.	Fabaceae	Ambispora callosa, Diversispora arenaria, Gigaspora albida, Glomus arborense
23	<i>Kyllinga alba</i> Nees.	Cyperaceae	Acaulospora tuberculata, Entrophospora infrequens, Glomus albidum, Rhizophagus fasciculatus
24	<i>Lindernia ciliata</i> (Colsm.) Pennell.	Linderniaceae	Ambispora callosa, Claroideoglomus claroideum, Gigaspora ramisporophora
25	<i>Lindernia parviflora</i> (Roxb.) Haines.	Linderniaceae	Acaulospora alpine, Diversispora celata, Glomus albidum, Glomus arborense
26	Mimosa pudica L.	Mimosaceae	Acaulospora tuberculata, Claroideoglomus claroideum, Glomus globiferum, Scutellispora spp
27	<i>Mukia maderaspatana</i> (L.) Roem	Cucurbitaceae	Claroideoglomus claroideum, Funneliformis coronatum, Glomus ambisporum
28	Ocimum gratissimum Linn.	Lamiaceae	Acaulospora alpine, Dentiscutata erythropus, Glomus albidum, Rhizophagus fasciculatus
29	Oldenlandia biflora L.	Rubiaceae	Acaulospora undulate, Diversispora arenaria, Gigaspora albida, Glomus ambisporum, Rhizophagus fasciculatu, Scutellospora savannicola
30	Passiflora foetida L.	Passifloraceae	Acaulospora undulate, Claroideoglomus claroideum, Glomus albidum, Rhizophagus fasciculatus, Scutellospora savannicola
31	Phyllanthus maderaspatensis L.	Euphorbiaceae	Ambispora callosa, Claroideoglomus claroideum, Glomus albidum, Pacispora scintillans, Rhizophagus fasciculatus
32	Plectranthus barbatus Andrews.	Lamiaceae	Archaeospora trappei, Gigaspora albida, Gigaspora decipiens, Glomus globiferum, Rhizophagus fasciculatus
33	Rauwolfia serpentina (L.)	Apocynaceae	Claroideoglomus claroideum, Diversispora arenaria, Glomus canadense, Glomus multicaule

	Benth. ex Kurz		
34	Sida rhombifolia L.	Malvaceae	Acaulospora foveat, Entrophospora infrequens, Glomus albidum, Glomus multicaule, Rhizophagus fasciculatus
35	<i>Solanum xanthocarpum</i> Schrad. & H. Wendl.	Solanaceae	Acaulospora undulate, Claroideoglomus claroideum, Glomus ambisporum, Rhizophagus fasciculatus
36	<i>Spillanthes calva</i> DC.	Asteraceae	Archaeospora trappei, Claroideoglomus claroideum, Glomus albidum
37	<i>Stachytarpheta jamaicensis</i> (L.) Vahl.	Verbenaceae	Acaulospora undulate, Funneliformis coronatum, Glomus arborense
38	<i>Stachytarpheta urticifolia</i> (Salisb.) Sims.	Verbenaceae	Claroideoglomus claroideum, Funneliformis coronatum, Gigaspora ramisporophora, Rhizophagus fasciculatus
39	<i>Wattakaka volubilis</i> (L. fil.) Stapf.	Asclepiadaceae	Archaeospora trappei, Entrophospora infrequens, Glomus arborense, Rhizophagus fasciculatus
40	Ziziphus oenoplia (L.) Miller.	Rhamnaceae	Ambispora callosa, Dentiscutata erythropus, Glomus arborense,

	Table 4. AM fungal s	spore species diver	rsity, Kodikuthimala	, Malappuram District.
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S. No.	Genus Name	Species Name
1	Acaulospora	alpine, foveat, tuberculata, undulate
2	Ambispora	callosa
3	Archaeospora	trappei
4	Claroideoglomus	claroideum
5	Dentiscutata	erythropus
6	Diversispora	arenaria, celata
7	Entrophospora	infrequens
8	Funneliformis	coronatum
9	Gigaspora	albida, decipiens, ramisporophora
10	Glomus	albidum, ambisporum, arborense, canadense, globiferum, multicaule,
11	Pacispora	scintillans
12	Rhizophagus	fasciculatus
13	Scutellispora	savannicola, striata, spp



Fig. 1. The map showing the study area.

The total number of 40 plant species belongs to 28 families were examined for AM fungal spore populations and colonization (Table 3 and 4). Of these, maximum spore population was recorded in the plant species of Gloriosa superba (574/100g of soil) belongs to the family Liliaceae and minimum spore population was noticed in the plant species of Euphorbia hirta belongs to Euphorbiaceae. The highest AM fungal infection was found in the roots of Sida rhombifloia (79%) belongs to Malvaceae and minimum infection was occurred in the plant species Commelina benghalensis (12%) belongs to Commelinaceae.

The plant species like *Cleome aspera* (27%) and Cleome monophylla belongs to the family Cleomaceae, Euphorbia hirta (13%), Euphorbiaceae, Evolvulus alsinoides (26%), Convolvulaceae, Hybanthus enneaspermus Violaceae, (24%), Lindernia *ciliate*(14%), L.parviflora (15%), Linderniaceae, Phylllanthus maderaspatensis (21%), Euphorbiaceae, Stachytarpheta jamaicensis (26%), S. urticifolia, Verbenaceae, Passiflora foetida (27%), Passifloraceae, Hemidesmus indicus (25%), Apocynaceae, Asparagus racemosus (27%), procera (29%), Calotropis Asparagaceae, *Stachytarpheta urticifolia* (18%), Verbenaceae, showed 10 to less than 30% of AM fungal infection.

The other plant species like Borreria hispida Rubiaceae, Oldenlandia (35%), biflora (34%), Rubiaceae, Wattakaka volubilis (34%), Asclepiadaceae, Zizypus oenoplia (39%), Rhambaceae, Mimosa pudica (33%), Mimosoideae, Rauwolfia serpentina (31%), Apocynaceae, Solanum xanthocarpum Solanaceae, Anisomeles (32%), malabarica (36%), Lamiaceae showed 30 to less than 40% of infection.

The other species *Hyptis suaveolens* (47%) Indigofera uniflora Lamiaceae, (55%), Fabaceae, Plectrantus barbatus (48%), Lamiaceae, Cardiospermum halicacabum (47%), Sapindaceae, vitiginea (44%), Vitaceae, Cissus **Ocimum** gratissimum (45%), Lamiaceae, Canavalia gladiate (42%). Papilionoideae. Cucurbitacae. Chrvsopogon zizanioides (56%), Poaceae, Abutilon indicum (44%), Malvaceae showed 40 to less than 60% of AM fungal infection. The rest of the species like Alysicarpus monilifer (66%), Crotalaria pallida (75%), both the species belongs to Fabaceae, Sida rhombifolia (79%), Malvaceae, Mukia madraspatana (62%). Cucurbitaceae, Abrus precatorius (66%), Fabaceae showed 60 to less than 80% of AM fungal infection.

In the present study, all the plants were examined form the study area have significantly influenced by AM fungal. Where, the plants were successfully surveyed by these fungal through their contribution in the plant community.

## 4. DISCUSSION

Vesicular-arbuscular mycorrhizal (VAM) association with plants is widely distributed and it is geographically ubiquitous. In the present investigation all tree species were found to have mycorrhizal association. Microscopic observation of root segments revealed the presence of AM fungal structures ramified by extra-matrical hyphae and intracellular infestation of angular thick-walled hyphae. AM fungi have a potential importance in the recovery of disturbed lands and can be used in wasteland or semi-arid land could be improved by incorporating AM fungi. The variation in the intensity of root colonization and sporulation due to varieties and AM fungi recorded in the present study must be on the basis of host-symbiont specificity. In the present investigation, there was a change in AM spore number and infection in all the plant species. Others have also reported similar changes in different constituents of microbial population (18, 19). Priya (20) showed that the activity of soil mycorrhizal population was greatly affected by soil pH, temperature and moisture.

In the present study the Cyperaceae family *Kyllinga alba* not infected by Arbuscular mycorrhizal infection. In contrast Cyperus conglomerathus, *Cyperus rotundus* both the species were found to be mycorrhizal (21). These findings are quite in line with the findings of Muthukumar and Udaiyan (22), Harikumar (23), Silva et al., (24). The probability of mycorrhizal colonization increases with the increase of soil pH because the availability of nutrients decreased with increasing pH (25). Chaudhry et al. (21) find out the AM fungal infection in the Poaceae members, particularly Cympopogon jwarancusa in an aromatic grass showed highest number of AMF species. The present study also revealed that the Poaceae member Chrysopogon zizanoides showed 56% of AM fungal infection. Most of the plant species in tropical rain forests and the members of Leguminosae sub families Papilonaceae and Mimosaceae form AM symbiosis (26). The same result was obtained in the present findings.

The present investigation of the AM fungal diversity in this study area, the tractability and ecological importance of mycorrhizal systems makes them ideal models to test and develop biodiversity in this study area. Consequently, recent studies have focused on the different functions of AM Fungal and their roles in ecosystem functioning. Hence, there is a new need of ecological concepts in AM Fungal community to increasing productivity and fitness of plants in ecosystems.

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#### **RESEARCH ARTICLE**

## AM FUNGAL DIVERSITY IN THE PLANT COMMUNITY OF VELLIANGIRI HILLS, WESTERN GHATS, COIMBATORE

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## ABSTRACT

The ecological mechanisms of AM fungal diversity ensure successful management for conservation and restoration of natural ecosystems. Here the study contacted to estimate the diversity of AM fungal and their function in Velliangiri hills, Western Ghats, Coimbatore. The community structure of AM fungi, as determined by number of spores present in 100g of soil, varied with sampling time in plant community. And all so the morphological identification was done by microscopic characters. Soil properties like pH, macro and micro nutrient and the climate data were collected for all tree years to know their impact on fungal community. The overall result conform the root colonization and spore population were higher in winter season and also lower in rainy. Totally 30 AM fungal species from 10 genera were identified the important genera were Acaulospora, Ambispora, Claroideoglomus, Funneliformis, Gigaspora, Glomus, Racocetra, Redeckera, Rhizophagus and Scutellospora. Among these five genera, Glomus occurred most frequently. In general, Rhizophagus fasciculatus was found to be most abundant species. Consequently, this result conform the rich diversity in the study area. This symbiotic relationship had important roles in establishment of plant community of this area.

Keywords: Arbuscular Mycorrhizal Fungi, Diversity, Velliangiri hills.

### **1. INTRODUTION**

There are many groups of fungi can establish associations with roots for facilitate plant growth and increase stress tolerance. Plants associated with mycobiota comprise taxonomically diverse, particularly mycorrhizal symbioses are extensively described due to the important role in improving plant nutrition and stress tolerance (1). AM fungi are integral components of most terrestrial ecosystems, with complex interactions between plants and production of glomalin (AM Fungal hyphal glycoprotein) may play a vital role in soil aggregation (2). The AM Fungal are essential for the function of ecosystems by the influence in plant diversity patterns in a variety of ecosystems. Where the mycelial network of AM fungi extends greatly increases the surface area for the uptake of immobile nutrients and they build up the macroporous structure in soil that allows penetration of water and air and thereby prevents erosion. They have great potential in the restoration of disturbed land and low fertility soil (3).

Mycorrhizal fungi usually enhance overall plant performance such as seed germination, early plant establishment, crucial steps in plant cycles and increased reproductive output (4). Moreover, the importance of mycorrhiza and the possibilities of its practical application strengthen the need for identification and cultivation of mycorrhizal fungi of natural plants (5). There is not enough focus on the mycorrhizal association with medicinal plants. Their species in different ecosystems are affected by edaphic factors, so it is necessary to investigate the spatial distribution and colonization of AM fungi in medicinal plants (6). Hence, in the present study contacted to estimate the diversity of AM fungal and their function in Velliangiri hills of Western Ghats.

## 2. MATERIALS AND METHODS

#### 2.1. Study area

The Velliangiri hills from a major hill range in Western Ghats and a part of Nilgriri Biosphere Reserve of southern Western Ghats of Coimbatore district at a distance of 40 km. The geographical position between the longitude  $6^{\circ}$ -40' and  $7^{\circ}$ -10' and E latitude 10°-55' and 11°N with the altitudinal range having the altitude 1840 ms above msl. The boundary of Velliangiri hills is Palghat district of Kerala at western side, Nilgiri mountains at northern side, Siruvani hills at the south and plains of Coimbatore district of eastern side (Fig. 1 & 2).

### 2.2. Sample collection

Root samples and rhizosphere soil samples of 25 plant species growing in area of Velliangiri hills were collected in all three different seasons in the period of January, 2013 to December, 2015. For identification and nomenclature of the plant species the following manual was used (7). The root and soil samples were transported to the laboratory immediately after collection. The roots were fixed in formaldehyde-acetic acidethanol (FAA) solution for further process (8). The soil sample was air dried and stored at 40 C until processed. Each soil samples was used for chemical analysis, spore counts and classification in to various types and multiplication, concentration and separation of AM fungal spore for identification.

## 2.3. Soil analysis and climate data

The soil factors, texture, macro and micro nutrients were estimated by the following methods such as soil pH, EC (9), OC (10), available N, available P (11) and available K and the micro nutrient (Zu, Cu, Fe and Mn) (12). The climate date of the study area was collected from the Tamil Nadu Agricultural University, Coimbatore, India.

## 2.4. Evaluation of AM infection

The root samples were cleared and stained in tryphan blue with a modified version of the Phillips and Hayman's (8) method, in some cases, the modified method of Merryweather and Fitter (13) and Arias *et al.* (14). Arbuscular mycorrhizal infection in the roots was assessed following the grid line-intersect and the slide methods of Giovannetti and Mosse (15).

## 2.5. Isolation of Arbuscular Mycorrhizal Spores from the soil samples

Spores were recovered from the soil samples by the wet sieving and decanting method (16). Identification of AM fungi based upon microscopic characters, the AM fungal spores were identified. For identification and nomenclature, keys of the following manual authors were used: Raman and Mohankumar (17), Schenk and Perez (18), Redecker *et al.* (19) and Schubler and Walker (20). Classification on based on color, size, shape, surface, structure, general nature of the spore contents and hyphal attachment. Photomicrographs were taken with the help of a Magnus Olympus Microscope.

## **3. RESULTS**

The study purpose was to isolate the diversity and function of AM Fungi associated with some medicinal plants located in Velliangiri hills. The infection and spread of AM fungal genera as influenced by as climatic and edaphic factors. The results relate to influence of soil properties and climatic variations on the AM fungal associations in medicinal plant. As well as the monthly rain fall, temperature and relative humidity of Velliangiri hills from January, 2013 to December, 2015 were presenting in Table 1. The soils were sandy loam,

non-calcareous and black in nature (Table 2). The soil physical factors such as soil pH, electric conductivity and organic carbon were reported in the Table 2. The soil pH was recorded 7.19 to 7.1 in the all seasons of three years, whereas electric conductivity was recorded in between 0.39 to 0.34 d sm-2. Likely, the organic carbon was noted in between 0.86 to 0.81 % in the vegetation zones in all seasons of tree years. Whereas, the detailed records of the macro and micro nutrients were given in the Table 3.

Totally in Velliangiri hills, 30 AM fungal species in the 10 genera were isolated and identified (Table 4). The important genera were identified as Ambispora, Acaulospora, Claroideoglomus, Funneliformis, Gigaspora, Glomus, Racocetra. Redeckera, Rhizophagus, and Scutellospora. Among these five genera, *Glomus* occurred most frequently. In general, Rhizophagus fasciculatus was found to be most abundant species. In winter season, Crotalaria barbata (47%), Plectranthus fruticosus (88%) and Crotalaria albida (66%) were noted higher percentage of root colonization and Begonia malabarica (12%), Abutilon hirtum (8%) Piper longum and Begonia malabarica (8%) were found as lower infected plant roots. In summer, the higher root infection were found in *Crotalaria albida* (63%), Plectranthus fruticosus (91%), Abutilon hirtum and Corchorus trilocularis (73%), and also the lower colonization were found in *Impatiens goughi* (6%) and Piper longum (7%). Where in rainy season, the high colonization were found in *Pogostemon mollis* (51%), Anaphalis aristata (11%) and Plectranthus fruticosus (57%) where the lower infection found in Biophytum polyphyllum and Begonia malabarica (6%), and Andrographis alata (5%). In the study period (2013 to 2015) some plant species were not have any infection or may have very poor percentage of infection such as Anaphalis aristata, Andrographis affinis, Cleome gynandra, Cleome monophylla, Begonia trichocarpa, Impatiens crenata, Impatiens goughii, Biophytum polyphyllum, Andrographis alata, Piper longum, Begonia malabarica, Cleome gynandra, Biophytum sensitivum, Begonia trichocarpa and Anaphalis aristata (Table 5-7).

In winter season, the highest spore populations were found in *Crotalaria barbata* (647), *Sida acuta* (784) and *Plectranthus fruticosus* (795), of the examined years 2013 to 2015, whereas also the lower spore population in *Impatiens goughii* (145), *Cleome gynandra* (189) and *Piper longum* (184). In summer, the higher population noted in *Crotalaria barbata* (758), *Pogostemon speciosus* (498) and *Impatiens goughii* (637) as well as the lowest population found in *Impatiens goughii* (128), *Anaphalis aristata* (120) and *Piper longum* (159).

	Rain fall (mm		ım)	a) Temperature °C						Relative humidity (%)			
Month	2013	2014	2015	20	2013		2014		15	2013	2014	2015	
	2013	2014	2013	MAX	MIN	MAX	MIN	MAX	MIN	<b>7.22 HOURS</b>	<b>7.22 HOURS</b>	<b>7.22 HOURS</b>	
JANUARY	0	14	0	31.6	19.0	29.4	17.9	30.1	19.5	86	61	86	
FEBRUARY	99.8	9.2	0	31.9	20.7	31.4	18.5	32.2	20	82	55	80	
MARCH	0	17.0	3.7	34.2	22.8	34.5	20.5	34.5	23.1	80	50	80	
APRIL	46.8	52.7	62.7	35.9	24.5	35.2	23.8	32.7	24	86	54	83	
MAY	14.8	66.5	195.8	33.4	24.5	34.0	23.2	32.3	23.5	82	55	91	
JUNE	54.5	42.8	46.9	30.6	23.3	31.6	22.9	32.3	23.7	80	56	82	
JULY	21.9	68.5	5.1	30.1	23.2	30.1	22.2	32.2	22.9	79	55	85	
AUGUST	27.3	30.1	28.1	31.3	22.6	30.1	22.2	32.3	23.2	86	62	86	
SEPTEMBER	46.5	68.0	66.2	31.2	22.6	31.6	21.8	33	23.8	85	63	83	
OCTOBER	140.12	146.0	65.2	31.5	21.7	30.6	21.4	31.6	23.3	88	72	87	
NOVEMBER	57.9	118.0	191.3	29.8	22.3	29.2	20.2	28.6	22	89	73	93	
DECEMBER	24.8	41.4	24.1	29.2	19.8	29.4	17.9	29.0	21.5	88	77	90	

Table 1. Climatic factor of Velliangiri hills, Western Ghats during 2013 to 2015.

 Table 2. Soil type, texture and Physical factor of Velliangiri hills, Western Ghats during 2013 to 2015.

Years	Seasons	PH	EC	<b>OC</b>	Soil type	laim
	Winter	7.14	0.38	0.85	Sandy loam	Non-calcareous, Black
2013	Summer	7.11	0.38	0.85	Sandy loam	Non-calcareous, Black
	Rainy	7.19	0.39	0.84	Sandy loam	Non-calcareous, Black
	Winter	7.13	0.38	0.86	Sandy loam	Non-calcareous, Black
2014	Summer	7.12	0.38	0.85	Sandy loam	Non-calcareous, Black
	Rainy	7.18	0.39	0.83	Sandy loam	Non-calcareous, Black
	Winter	7.12	0.36	0.82	Sandy loam	Non-calcareous, Black
2015	Summer	7.1	0.34	0.81	Sandy loam	Non-calcareous, Black
	Rainy	7.17	0.39	0.83	Sandy loam	Non-calcareous, Black

Table 3. Soil macro and micro nutrients of Velliangiri hills, Western Ghats during 2013 to 2015.

Years	Seasons	Available N (kg ha <sup>.1</sup> )	Available P (kg ha <sup>-1</sup> )	Available K (kg ha <sup>-1</sup> )	DTPA-Zn (pmm)	DTPA-Cu (pmm)	DTPA-Fe (pmm)	DTPA-Mn (pmm)
	Winter	224	16.1	555	0.98	1.26	7.58	13.37
2013	Summer	225	16.0	553	0.97	1.23	7.55	13.30
	Rainy	222	16.1	551	0.97	1.20	7.46	13.24
	Winter	234	16.2	544	0.92	1.39	7.91	12.48
2014	Summer	232	16.3	524	0.91	1.37	7.90	12.45
	Rainy	230	16.2	510	0.92	1.37	7.90	12.23
	Winter	226	16.2	536	1.1	1.40	7.52	14.96
2015	Summer	226	15.3	531	1.09	1.47	7.50	14.95
	Rainy	223	15.2	528	1.12	1.35	7.46	14.93

Table 4. AM fungal Species from Velliangiri hills Western Ghats with species code.

C N-	AM	Constant and a	
S. No.	New name	Synonym	— Species code
1.	Acaulospora denticulate	Acaulospora denticulate	ADTC
2.	Acaulospora foveata	Acaulospora foveata	AFVT
3.	Acaulospora mellea	Acaulospora mellea	AMLL
4.	Acaulospora nicolsonii	Acaulospora nicolsonii	ANCS
5.	Acaulospora sporocarpia	Acaulospora sporocarpa	ASPC
6.	Ambispora appendicula	Acaulospora appendicula	AAPD

7.	Claroideoglomus claroideum	Gloums claroides	LCRD
8.	Funneliformis caledonium	Glomus caledonium	LCLD
9.	Funneliformis constrictum	Glomus constrictum	LCST
10.	Gigaspora candida	Gigaspora candida	GCDD
11.	Gigaspora rosea	Gigaspora rosea	GRSA
12.	Glomus aggregatum	Glomus aggregatum	LAGR
13.	Glomus albidum	Glomus albidum	LABD
14.	Glomus ambisporum	Glomus ambisporum	LABS
15.	Glomus arborense	Glomus arborense	LABR
16.	Glomus austral	Glomus austral	LAST
17.	Glomus halonatum	Glomus halon	LHLN
18.	Glomus heterosporum	Glomus heterosporum	LHTS
19.	Glomus hoi	Glomus hoi	LHOI
20.	Glomus muticaule	Glomus muticaule	LMTC
21.	Glomus nanolumen	Glomus nanolumen	LNNL
22.	Glomus reticulatum	Glomus reticulatum	LRTC
23.	Racocetra coralloidea	Scutellospora coralloidea	CCRL
24.	Racocetra gregaria	Scutellospora gregaria	CGRG
25.	Racocetra verrucosa	Scutellospora verrucosa	CVRC
26.	Redeckera fulvum	Gloums fulum	LFLV
27.	Rhizophagus clarus	Glomus clarum	LCLR
28.	Rhizophagus fasciculatus	Glomus faciculatum	LFSC
29.	Scutellospora arenicola	Scutellospora arenicola	CARC
30.	Scutellospora savannicola	Scutellospora savannicola	CSVN

Table 5. AM fungal root colonization and spore population in the plant species of Velliangiri hi	ills
Vestern Ghats during 2013.	

S.				ype o			% Root			Spore population/100g		
No.	Plant name	Family	Colonization H V A		colonization W S R			of soil WSR				
1	Abutilon hirtum (Lam.) Sweet.	Malvaceae	+	• +	A -	47	62	23	376	<b>3</b> 12	<b>K</b> 189	
2	Anaphalis aristata (DC.) DC.	Compositae	+	+	+	12	36	11	271	189	123	
3	Andrographis alata (Vahl) Nees.	Acanthaceae	+	+	-	12	24	-	274	253	132	
4	Andrographis affinis Nees.	Acanthaceae	+	-	-	-	27	-	210	168	115	
5	Begonia malabarica Lam.	Begoniaceae	+	+	+	12	20	-	183	138	110	
6	Begonia trichocarpa Dalzell	Begoniaceae	+	-	-	-	10	-	234	172	121	
7	<i>Biophytum polyphyllum</i> Munro	Oxalidaceae	+	-	+	23	-	-	222	332	129	
8	Biophytum sensitivum (L.) DC.	Oxalidaceae	+	+	-	16	12	-	321	214	164	
9	Cleome gynandra L.	Cleomaceae	+	+	+	-	17	-	241	254	130	
10	Cleome monophylla L.	Cleomaceae	+	+	-	-	14	-	256	189	112	
11	Corchorus trilocularis L.	Malvaceae	+	-	+	36	53	25	473	481	243	
12	<i>Crotalaria albida</i> Roth	Leguminosae	+	+	-	42	63	32	564	742	184	
13	Crotalaria barbata Wight & Arn.	Leguminosae	+	-	-	53	57	28	647	758	213	
14	Hibiscus calyphyllus Cav.	Malvaceae	+	-	+	47	62	32	474	529	143	
15	<i>Hibiscus hispidissimus</i> Griff.	Malvaceae	+	+	-	38	46	29	546	435	179	
16	Impatiens crenata Bedd.	Balsaminaceae	+	+	-	-	10	-	243	156	124	

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17	Impatiens goughii Wight E	Balsaminaceae	+		+	-	6	-	145	128	112
18	Piper longum L.	Piperaceae	+	+	-	12	22	-	238	231	167
19	Plectranthus bishopianus Gamble	Lamiaceae	+	+	+	37	41	22	432	376	210
20	<i>Plectranthus fruticosus</i> L'Hér.	Lamiaceae	+	+	-	28	36	12	467	586	189
21	Pogostemon benghalensis Kuntze	Lamiaceae	+	+	-	47	56	32	546	487	129
22	Pogostemon mollis Benth.	Lamiaceae	+	+	+	32	44	51	466	378	121
23	Pogostemon speciosus Benth.	Lamiaceae	+	-	+	52	42	27	389	527	186
24	Pogostemon vestitus Benth.	Lamiaceae	+	+	+	39	40	18	425	253	130
25	<i>Sida acuta</i> Burm.f.	Malvaceae	+	-	+	44	56	23	274	738	180

H- Hyphal, V- Vesicles, A- Arbuscules; W-Winter; S – Summer; R – Rainy

Table 6. AM fungal root colonization and spore population in the plant species of Velliangiri hills Western Ghats during 2014.

S. No.	Plant name Family		Type of Colonization			% Root colonization			Spore population/100g of soil		
		-	Н	V	Α	W	S	R	W	S	R
1	Abutilon hirtum (Lam.) Sweet.	Malvaceae	+	+	+	32	46	18	265	483	192
2	Anaphalis aristata (DC.) DC.	Compositae	+	+	-	12	24	19	325	271	163
3	Andrographis alata (Vahl) Nees.	Acanthaceae	+	+	+	16	27	12	271	234	123
4	Andrographis affinis Nees.	Acanthaceae	+	-	-	18	22	20	292	189	136
5	<i>Begonia malabarica</i> Lam.	Begoniaceae	+	+	-	17	13	14	324	214	222
6	Begonia trichocarpa Dalzell	Begoniaceae	+	-	+	24	22	21	345	258	140
7	<i>Biophytum polyphyllum</i> Munro	Oxalidaceae	+	-	-	12	7	5	281	178	124
8	Biophytum sensitivum (L.) DC.	Oxalidaceae	+	+	-	8	9	-	299	189	142
9	Cleome gynandra L.	Cleomaceae	+	+	+	14	12	8	189	256	182
10	Cleome monophylla L.	Cleomaceae	+	+	-	12	7	-	201	173	122
11	Corchorus trilocularis L.	Malvaceae	+	-	+	45	84	22	372	478	123
12	<i>Crotalaria albida</i> Roth	Leguminosae	+	+	-	78	89	49	760	485	270
13	Crotalaria barbata Wight & Arn.	Leguminosae	+	-	-	88	91	57	768	389	213
14	Hibiscus calyphyllus Cav.	Malvaceae	+	-	+	56	62	39	580	482	294
15	Hibiscus hispidissimus Griff.	Malvaceae	+	+	+	63	41	29	440	341	289
16	Impatiens crenata Bedd.	Balsaminaceae	+	+	+	12	15	10	264	189	134
17	Impatiens goughii Wight	Balsaminaceae	+		+	11	8	5	284	149	111
18	Piper longum L.	Piperaceae	+	+	-	10	11	-	210	120	115
19	Plectranthus bishopianus Gamble	Lamiaceae	+	+	+	43	29	17	243	178	142
20	<i>Plectranthus fruticosus</i> L'Hér.	Lamiaceae	+	+	-	52	37	26	273	159	123
21	Pogostemon benghalensis Kuntze	Lamiaceae	+	+	-	67	48	29	658	493	184
22	Pogostemon mollis Benth.	Lamiaceae	+	-	+	58	39	22	597	498	281
23	Pogostemon speciosus	Lamiaceae	+	+	+	66	38	22	784	479	260

	Benth.										
24	Pogostemon vestitus Benth.	Lamiaceae	+	-	+	48	53	32	583	276	185
25	Sida acuta Burm.f.	Malvaceae	+	-	+	39	47	29	475	351	169

H- Hyphal, V- Vesicles, A- Arbuscules; W-Winter; S – Summer; R – Rainy

Table 7. AM fungal root colonization and spore population in the plant species of Velliangiri hills
Western Ghats during 2015.

S.	Plant name	Family		'ype o onizat		% Root colonization			Spore population/100g of soil		
No.		<sup>v</sup>	H	V	Α	W	S	R	W	S	R
1	<i>Abutilon hirtum</i> (Lam.) Sweet.	Malvaceae	+	+	+	64	73	39	584	396	259
2	Anaphalis aristata (DC.) DC.	Compositae	+	+	+	12	15	-	395	275	142
3	Andrographis alata (Vahl) Nees.	Acanthaceae	+	+	+	24	28	17	369	240	189
4	Andrographis affinis Nees.	Acanthaceae	+	-	-	32	25	15	463	273	168
5	Begonia malabarica Lam.	Begoniaceae	+	+	+	10	8	-	231	169	123
6	<i>Begonia trichocarpa</i> Dalzell	Begoniaceae	+	-	-	-	5	-	184	159	133
7	<i>Biophytum polyphyllum</i> Munro	Oxalidaceae	+	-	-	8	14	6	213	159	112
8	Biophytum sensitivum (L.) DC.	Oxalidaceae	+	+	+	12	14	8	243	260	127
9	Cleome gynandra L.	Cleomaceae	+	+	+	23	12	11	372	231	157
10	Cleome monophylla L.	Cleomaceae	+	+	-	21	22	13	543	274	189
11	Corchorus trilocularis L.	Malvaceae	+	-	+	47	52	25	473	243	163
12	<i>Crotalaria albida</i> Roth	Leguminosae	+	+	-	64	59	23	537	498	362
13	Crotalaria barbata Wight & Arn.	Leguminosae	+	-	-	87	91	46	758	637	341
14	Hibiscus calyphyllus Cav.	Malvaceae	+	+	+	53	42	28	576	453	236
15	<i>Hibiscus hispidissimus</i> Griff.	Malvaceae	+	-	-	66	43	35	564	376	132
16	Impatiens crenata Bedd.	Balsaminaceae	+	+	-	14	12	10	321	243	124
17	Impatiens goughii Wight	Balsaminaceae	+	-	+	11	9	-	246	194	153
18	Piper longum L.	Piperaceae	+	+	-	9	8	9	233	179	145
19	Plectranthus bishopianus Gamble	Lamiaceae	+	-	+	48	51	37	597	463	251
20	<i>Plectranthus fruticosus</i> L'Hér.	Lamiaceae	+	+	-	61	59	24	574	632	220
21	Pogostemon benghalensis Kuntze	Lamiaceae	+	-	-	41	73	28	689	473	197
22	Pogostemon mollis Benth.	Lamiaceae	+	+	+	52	63	31	489	372	186
23	Pogostemon speciosus Benth.	Lamiaceae	+	-	+	62	53	36	754	359	190
24	<i>Pogostemon vestitus</i> Benth.	Lamiaceae	+	+	+	42	49	21	479	352	189
25	<i>Sida acuta</i> Burm.f.	Malvaceae	+	-	+	57	62	70	795	473	255

H- Hyphal, V- Vesicles, A- Arbuscules; W-Winter; S – Summer; R – Rainy

Figure 1 Study area of Velliangiri hills, the Westrn ghats, Coimbatore district

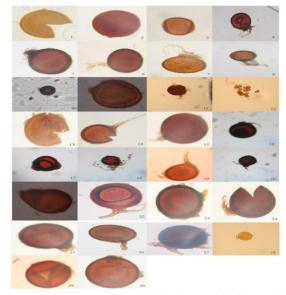


Figure 2 An overview and satellite view of study area



In rainy season, *Corchorus trilocularis* (243), *Crotalaria barbata* (294) and *Impatiens crenata* (362) were found higher spore population, at the same time *Begonia malabarica* (110), *Biophytum polyphyllum* (111) and *Begonia malabarica*, (112) were have minimum population in the study period. The overall aspirations the rainy season were influenced the spore population due to the lagging of rain water. Where, in the winter and summer seasons have more favor for the AM fungal. In Velliangiri hills, highest AM diversity was recorded which may be due to its location, which experiences optimum rainfall and temperature that are conducive for AM population (Table 5-7).

Figure 3 AM fungal Species from Velliangiri hills Western Ghats



 Acaulospora denticulate, 2. A. foveata, 3. A. mellea, 4. A. nicolsonii 5. A. sporocarpia, 6. Ambispora appendicula, 7. Claroideoglomus claroideum, 8. Funnelljornis caledonium, 9. F. constrictum, 10. Gigaspora candida, 11. G. rosea, 12. Glonus aggregatum, 13. Gl. albidum, 14. Gl. ambisporum, 15. Gl. Arborense, 16. Gl. austral, 17. Gl. halonatum, 18. Gl. heterosporum, 19. Gl. hoi, 20. Gl. muticaule, 21. Gl. nanolumen, 22. Gl. reticulatum, 23. Racocetra coralioldeu, 24. Ra. gregaria, 25. Ra. verrucoaa, 26.Redeckera fulvum, 27. Rhicophagus clarus, 28. Rb. fasciculatus, 29. Scutellospora arenicola, 30.52. savannicola.

Figure 4 The average spore population in the plant species of Velliangiri hills Western Ghats during 2013 to 2015

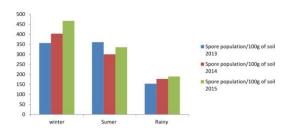
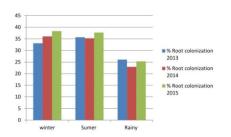


Figure 5 The average root infection in the plant species of Velliangiri hills Western Ghats during 2013 to 2015



## 4. DISCUSSION

The AM fungi are major components of soil biota that can determine the productivity of ecosystems (21). The rhizosphere of the mycorrhizal plant can be referred to as the mycorrhizosphere. Mycorrhizosphere comprises both the root and hyphae influence zones. Hence, the mycorrhizosphere provide a critical link between plants, other microorganisms and the soil (22). The number of mycorrhizal fungal individuals found in a given habitat is likely to depend on a range of factors that includes plant community composition and age, soil chemical, physical and biological properties, and climate, meaning that considerable variability can be expected this requires more effort to quantify intra specific diversity of mycorrhizal fungi (23). The present study contacts an experiment on mycorrhizal fungal community from the Velliangiri hills. In the study site 10 AM fungal genera were identified, among these Glomus has been the most dominant genus in this region, where also the Rhizophagus fasciculatus was the most dominant AM fungal species. Some other finding supported that the relatively higher frequency of Glomus species (24, 25). These species have good relation with edaphoclimatic factors of this area.

The present study clearly demonstrated for the first time that plant species from Velliangiri hills are revealed that both AM fungal spore population and percentage of root colonization, which may affected by edaphoclimatic factors such as effect of various climatic, physical and chemical properties of soils. The huge distinction takes place in the spore population within the plant species have in this study, this may be attributed to the variation in edaphic and climatic factors. Numerous biotic and abiotic factors influence into the structure of mycorrhizal fungal communities. . Similarly, Kulkarni (26) also proposed by the influence of edaphic factors and host compatibility, climate and soil microorganisms on mycorrhization. The soil study revealed that AM Fungal communities are influence by habitat and soil type. In addition, the soil properties are related to microbiological activity and triggering the distribution of AM Fungi. These results contribute to a better understanding of the ecological factors that can shape AM fungal communities, an important soil microbial group that affects multiple ecosystem functions. The pH of study area was very fine (7.19 to 7.1) and this got good relationship of AM population of the study area. The other factors like organic carbon, electric conductivity and micro and macro nutrients.

The present study have higher spore population in winter followed by summer, where rainy season got lower spore population, this may be a variation in moisture and temperature. There is an optimum soil and environmental conditions are required for the AM fungi development and infectiveness (27). Here, many species were recorded in lower colonization of the samplings in the test sites. This has been influenced on plant growth and community structure, due to the important relationship between biodiversity and their potential to control on plant diversity and productivity (28). Where also AM fungal colonization increase intra specific plant competition by different magnification among them. There the influence of mycorrhizal community appears to extant level of plant populations and communities. Fungal may profit from additional nutrient and water availability at relatively low energy cost compared to nonmycorrhizal. Mycorrhizal alien plant species may obtain a competitive advantage compared to nonmycorrhizal alien plant species (24).

## **5. CONCLUSION**

However, despite the importance of AMF to terrestrial ecosystems, little is known about the effects of environmental changes on AMF abundance, activity and the impact of these changes on the ecosystem services. Therefore, it is important to gain clearer understanding of the effects of а environmental changes on the AM fungal species to guide conservation and restoration efforts. The symbiosis has long been a focus for invasion biologists, we do not know of any study combining plant mycorrhizal status with other plant functional traits. Therefore, we encourage the consideration of mycorrhizal status and related mycorrhizal plant traits in future analyses of alien plant invasion success.

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## **RESEARCH ARTICLE**

#### PHYTOCHEMICAL INVESTIGATION BY USING TENDER LEAF PART OF GREWIA TILIIFOLIA VAHL.

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#### ABSTRACT

This study was aimed to evaluate the phytochemical potential of different extracts of tender leaf part of *Grewia tiliifolia* Vahl which are commonly used in Ayurveda drug preparations. Tender leaf part of *G.tiliifolia* subjected to analyze the phytochemical constituents by using qualitative and quantitative methods. The results of the present investigation revealed that the presence of flavonoid, phenol, tannin glycoside, resin, steroids, terpenoids and triterpenoids in different solvent extract like petroleum ether, chloroform, ethyl acetate, methanol and water. Tender leaves of the species which exhibited well marked potential activity and rich in secondary metabolite contents (flavonoids and phenols).

Keywords: Grewia tiliifolia Vahl, Tender leaf part, Phytochemical screening,

### **1. INTRODUCTION**

During last few decades there has been an increase in the study of medicinal plants and their traditional use in different parts of the world (1). A wide range of bioactive substances present in plants have traditional medicine that can be used for the treatment of infectious diseases (2). Recently, researchers all over the world focused on finding naturally occurring medicines from plants. The genus Grewia, (Family: Tiliaceae) is an important medicinal plant which comprises of shrubs and trees and is distributed in the warmer parts of the world. Nearly 40 species of this genus are found in India some of which are well known for their medicinal value (3, 4). Avurveda, the ancient Indian treatise on medicine, mentions the use of different plant parts of Grewia to cure inflammation, burning sensation, fever, blood disorders, wound healing, ulcerative colitis, heavy menstrual flow and diabetes etc. (5). *Grewia tiliifolia* Vahl is a medium sized tree up to 20 m in height, leaves simple, alternate, and ovate with oblique base, crenate-denate, acuminate, upper surface minutely stellately hairy. It is useful in vitiated conditions of kapha and pitta, burning sensation, hyperdipsia, rhinopathy, ulcers, skin diseases, haematemesis and general debility (6). Therefore, considering the traditional use of the plant, the present study has been designed to investigate the phytochemical constituent of different solvent extract of G. tiliifolia in tender leaf part.

### 2. MATERIALS AND METHODS

#### 2.1. Preparation of extract

Tender leaf part of *G tiliifolia* was collected from the Western Ghats region of Malappuram district, Kerala India. Plant materials (tender leaf part of plant) was collected and washed with distilled water and shade dried for a week. The dried sample were manually ground to fine powder using pulverizer and passed through 40 mesh sieve and stored in air tight containers. The coarsely powdered plant material was weighed to 50g and Soxhlet extracted with petroleum ether, chloroform, ethyl acetate and methanol separately for 12 hours. The filtrate was evaporated to dryness under reduced pressure using rotary vacuum evaporator and the solid mass obtained was stored at 4°C until further use. The stored filtrate was used for the various phytochemical and pharmacological studies.

## 2.2. Phytochemical Screening of tender leaf parts of G. tiliifolia.

### 2.2.1. Alkaloids test: (Mayer's test)

The plant extract was evaporated to dryness and the residue was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Mayer's reagent. Formation of turbidity or yellow precipitation showed the presence of alkaloid.

#### 2.2.2. Glycosides:

Glycosides are compounds which upon hydrolysis give rise to one or more sugars (glycone) and a compound which is not a sugar (glycone) to the solution of the extract, few drops of sodium hydroxide was added, and observed for yellow color which shows the presence of glycosides.

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#### 2.2.3. Terpenoids and steroids: (Salkowski's test)

1 ml of extract was taken in a boiling tube and 2 ml of concentrated sulphuric acid was added slowly and red violet color was observed for terpenoid and green bluish color for steroids.

## 2.2.4. Flavonoids: (Ferric chloride test)

In a test tube containing 1ml of extract, 5-6 drops of dilute hydrochloric acid was added and small pieces of magnesium were added. Red color was observed for flavonoids and orange color for flavones.

## 2.2.5. Saponins: (Foam test)

1gm of extracts was added to 5ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth which indicate the presence of saponin.

## 2.2.6. Phenols: (Ferric chloride test)

1ml of extract was taken in a test tube, to this few drops of neutral 5% ferric chloride solution are added. A dark green color indicates the presence of phenolic compounds.

## 2.2.7. Tannins: (Braemer's test)

1ml of extract solution 1-2 drops of lead acetate solution was added. Red precipitate was formed indicating the presence of tannins.

## 2.2.8. Cardiac glycosides: (Keller-killani's test)

5ml of extract was taken in a boiling tube to which 2ml of glacial acetic acid containing one drop of ferric chloride solution was added and 1ml of concentrated sulphuric acid was added slowly. Appearance of brown ring indicates the presence of cardiac glycosides.

#### 2.2.9. Resin: (sulphuric acid test)

5ml of extract was taken in a boiling tube to which 2-3ml of acetic anhydride was added, dissolved by gentle heating and 0.5ml of sulphuric acid was added. Bright purple color was produced it indicates the presence of resin.

#### 2.2.10. Triterpenoids

2ml of extract was added with 1 ml of acetic anhydride followed by the addition of 2ml concentrated sulphuric acid. Formation of reddish violet color indicates the presence of triterpenoids.

#### 2.2.11. Reducing sugar

The crude extract was shaken with 5 ml of distilled water and filtered. The filtrate was boiled with drops of Fehling's solution A and B for 2

minutes. An orange red precipitate indicates the presence of reducing sugar.

## **3. RESULTS AND DISCUSSION**

The present study investigates the phytochemical potential of tender leaf part of *G. tiliifolia*.

Table 1. Qualitative phytochemical screening in
different extracts of tender leaf plant part of G.
tiliifolia

Secondary Metabolites	Р	C	EA	М	A
Alkaloid	_	_	_	_	_
Flavonoid	+++	_	_	_	++
Phenol	+++	++	_	+++	_
Tannin	_	_	_	++	+
Glycoside	++	+	_	+++	++
Saponin	_	_	_	_	_
Resin	_	+++	_	+++	+
Steroids	_	+++	_	+++	++
Terpanoid	_	_	_	+++	++
Cardiac glycosides	_	_	_	_	_
Triterpenoids	_	+++	_	+++	++
Reducing sugar	_	_	_	_	

P- Petroleum Ether; C – Chloroform; EA – Ethyl Acetate; M – Methanol; A – Aquous.

Table 2. FT-IR Peak value and its functionalgroups in methanol extract of tender leaf Plantpart of G. tiliifolia

S.No.	FT-IR Peak Values	Functional groups
1.	995.27	C=C STRETCH (Benzene)
2.	1056.99	C-O STRETCH (Alcohol)
3.	1288.45	C-H STRETCH (Alcane)
4.	1435.04	C=C STRETCH (Benzene)
5.	1620.21	N-H STRETCH(Amine)
6.	1697.36	C=O STRETCH (Carbonyl)
7.	2476.60	N-H STRETCH(Amine)
8.	2762.06	N-H STRETCH(Amine)
9.	2862.36	O-H STRETCH(Carboxylic acid)
10.	3086.11	O-H STRETCH(Carboxylic acid)
11.	3950.22	N-H STRETCH(Amine)

## Table 3. Total Phenol content of tender leaf plant part of *G. tiliifolia* with different solvent Extracts.

Solvents	Sample (µl)	Total Phenol Content
Petroleum ether		2.4
Chloroform Ethyl acetate	20	0.88 0.21
Methanol		0.87

Table 4. Total flavanoid content of tender leaf plant part of *G. tiliifolia* with different Solvent extracts.

Solvents	Sample (µl)	Total Flavanoid Content
Petroleum ether		38.17
Chloroform	500	38.45
Ethyl acetate	500	48.1
Methanol		21.52



Fig. 1. FT-IR analysis of tender leaf plant part of *G. tiliaefolia* in methanol extract.



Fig. 2. Images of the tender leaf part of *G. tiliaefolia.* 

## 3.1. Phytochemical Analysis

*G. tiliifolia* (tender leaf powder 30g) were extracted with 4 solvents, *viz*; petroleum ether (1.0g), chloroform (1.2g), ethyl acetate (2.1 g), methanol (3.1g) and water (3.5g). The extractive values were useful to evaluate the chemical constituents present in the crude extract (drug).

The present study was undertaken to evaluate the presence of active principles in *Grewia tiliifolia* which is highly medicinal. The preliminary phytochemical qualitative test of extracts confirmed in the presence of flavonoid, phenol, tannin glycoside, resin, steroids, terpanoids and triterpanoids. The total phenolic content in the petroleum ether extract of tender leaf part of G. *tiliifolia* measured high (2.4 $\mu$ g). Ethyl acetate extract of tender leaf part *G. tiliifolia* measured very least value (0.21  $\mu$ g). Highest content of total flavonoids

(48.1  $\mu g)$  is found in ethyl acetate extract while methanol showed very low flavonoid (21.5  $\mu g$ ). The presence of the secondary metabolites in the investigated plants account that it can be used as medicinal plants.

## 3.2. Fourier Transform Infrared Spectrophotometer (FTIR)

FTIR spectroscopic analysis of the tender leaves of *G. tiliifolia* methanolic extract with infrared spectroscopy revealed the presence of C=C, C-O, C-H, C=C, N-H, C=O, N-H, O-H bonds stretching. The peaks revealed that the plant sample had the compounds like Benzene, Alcohol, Alcane, Amine, Carbonyl and Carboxylic acid.

## 4. CONCLUSION

*Grewia tiliifolia* is an important traditional folk medicine. Tender leaves of the species which exhibited well marked potential activity and rich in secondary metabolite contents. Thus the results obtained from this investigations indicate that tender leaf plant part extract of *G.tiliifolia*, rich in secondary metabolites and confirmed that the tender leaves have great importance as therapeutic agents in preventing diseases.

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### **RESEARCH ARTICLE**

## ASSESSMENT OF WATER QUALITY AND POLLUTION LOAD IN TEJASWINI RIVER NEAR THE MALAVETTUVAN TRIBAL SETTLEMENT, CHERUPUZHA PANCHAYATH, KERALA, INDIA

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#### ABSTRACT

Rivers are one of the primary sources of water for drinking, irrigation and other domestic purposes. The present study deals with the water quality assessment of Tejaswini river of Cherupuzha Panchayath, Kannur district, Kerala. The water samples collected from five different sites were analyzed for parameters such as temperature, color, pH, BOD, DO, calcium, magnesium and *Escherichia coli*. The analysis of the physico – chemical and microbiological characters of the river water indicated that the water quality study of site I was affected severely because the study site is very near to the tribal settlements and also the tribal settlement, the river water is not polluted as it is very far away from the tribal settlement and also nearer to forest canopy. Water from study site I which not suitable for drinking and other domestic use. Therefore, source protection is suggested for the site I water bodies for the benefit of mankind because it is not safe for human consumption.

Keywords: Water quality, Tejaswini River, Malavettuvan tribal settlement.

### **1. INTRODUCTION**

Water is essential to sustain life, and a satisfactory supply must be made available to consumers. Every effort should be made to achieve a drinking-water quality as high as practicable. Protection of water supplies from contamination is the first line of defence (1). Dirty water and poor sanitation cause more than 500,000 infant deaths a year in the Asia pacific region (2). The rapid industrialization, urbanization and modern civilization (increased population) have lead to the increasing demand for water in domestic, agricultural and industrial sectors (3).

Agricultural waste like Pesticides, fungicides and fertilizers as waste from agriculture human and animal faces, seepage from pit latrines and septic tanks, refuge dump, industrial, domestic and all municipal wastes released into water bodies are responsible for water contamination. often Contaminated water is responsible for health risks due to the spread of diseases such as dysentery, cholera, typhoid, diarrhea and so on (4). According to Grabow (5) the problem associated with polluted water supplies include camp bacteriosis, shigellosis, salmollosis, cholera and a varieties of other bacteria as well as fungi, viral and parasitic infection. The problem of water quality deterioration in the nation is mainly due to human activities such as disposal of dead bodies, discharge of industrial and sewage wastes and agricultural runoff which are major cause of ecological damage and pose serious health hazards (6).

The present study deals with the water quality assessment of Tejaswini river of Cherupuzha Panchayath, which is also known as Kariamkode River, is comparatively small among 44 rivers of Kerala. It originates from Brahmagiri hills of Coorg forest in Karnataka, enters Kerala near Pulingome and flows through Kannur and Kasargod district of Kerala finally drains at Arabian Sea. Which flows through the Malavettuvan tribal settlement between Odakally and Aratukadavu Tribals fully depend on the river water for their day to day activities.

#### 2. MATERIALS AND METHODS

#### 2.1. Study sites

Tejaswini River also known as Kariamkode River originates from Brahmagiri hills of Coorg forest in Karnataka, enters Kerala near Pulingome and flows through Kannur and Kasargod district of Kerala and finally drains to Arabian Sea. The study area which lies between latitudes 11°41' to 12°48' N and longitudes 74°52' to 76°07'E (Fig. 1). The elevation of the hills ranges between 500-900 meters from Mean Sea level (MSL). The study area which lies on the Western Ghats region of North Kerala, which is rich in Biodiversity and indigenous population (Fig. 1). Malavettuvan tribal settlement near Aratukadavu which is situated on the banks of river Tejaswini. Tribals usally depend directly on river for their day to day water requirements. The main objective of the study is to assess the water quality and pollution load of the river water which is used by Malavettuvan colony of Aratukadavu.

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#### 2.2. Methods

Five different study sites were selected in and around Malavettuvan tribal colony where each site is 200 meter apart lies in between Odakolly and Aratukadavu. Study site I is very near to the Malavettuvan tribal colony, from which people use water for their day to day domestic activities like bathing, washing etc. Study site II which is little bit away from the tribal settlement and study site III, IV and V which away from the tribal settlement and also nearer to forest canopy. The water samples were collected during June 2015 to July 2016. Samples were collected in a clean polythene container and brought to Botany Department laboratory, Kongunadu Arts and Science College, Coimbatore for further analysis. The samples collected were labelled properly and preserved in refrigerator at 4°C by r. The collection and analysis of samples had been done by Standard methods for examination of water and waste water (7). Water samples were analyzed for parameters such as temperature, color, pH, BOD, DO, calcium, magnesium and *E. coli*.

### **2.3. WATER QUALITY TEST PROCEDURE**

## 2.3.1. Physico - Chemical Analysis 2.3.1.1. Temperature

Temperature of the water was estimated using Thermometer.

#### 2.3.1.2. Color

Water is taken in a beaker and the color is estimated visually.

## 2.3.1.3. pH testing procedure

Electrometric and colorimetric methods were used for the determination of pH value (8).

#### 2.3.1.4. Biological oxygen demand (BOD)

The Biological oxygen demand was estimated using oxygen depletion method based on bio-assay procedure. (9)

## 2.3.1.5. Dissolved oxygen

Estimated using titrimetric and winkler's methods for determination of dissolved oxygen.

## 2.3.1.6. Calcium

Calcium is estimated by using EDTA as titrimetric method. (10)

### 2.3.1.7. Magnesium

Magnesium is estimated by using EDTA as volumetric method. (11).

## 2.3.2. Microbiological Analysis 2.3.2.1. Coliform analysis

Coliform bacteria and fecal coliform bacteria analysis was done by (Most Probable Number) MPN.

### **3. RESULTS AND DISSCUSIONS**

Variation in the physico – chemical and microbiological properties of Tejaswini River water with respect to different sampling sites were studied during June 2015 to July 2016. Water samples were analyzed for parameters such as temperature, color, pH, BOD, DO, calcium, magnesium and *E. coli*. (Table. 1).

Table 1. Water quality assessment of Tejaswini River Aratukadavu, of Cherupuzha Panchayath, Kannur.

Sample Collection Site	Temperature (ºC)	Colour	рН	BOD (mg/L)	DO (mg/L)	Calcium (mg/L)	Magnesium (mg/L)	Coliform bacteria (MPN)
Site I	34	Light brown	8.4	17.80	1.20	350	320	9.4
Site II	32	Dark green	7.6	15.64	2.44	345	300	5.6
Site III	30	Colourless	7.4	12.40	4.52	342	280	1.2
Site IV	29	Colourless	7.2	8.42	6.46	340	250	0.2
Site V	26	Colourless	7.2	7.82	6.82	338	245	0.2

3.1. Temperature

In the present investigation temperature showed a drastic difference at all the sites. Water temperature ranged between  $26^{\circ}$ C to  $34^{\circ}$ C at various study sites. In study site I, temperature recorded is  $34^{\circ}$ C, which is nearer to the tribal settlement, were the tribal use this sites mainly for their domestic activities. In study site II, the temperature recorded is  $32^{\circ}$ C which is little bit away from the tribal

settlement, so that the water is only little bit affected as compared to with site I. In the study sites III, IV and V there is a great variation in temperature compared to site I and II. The temperature recorded in site III, IV and V is 30°C, 29°C and 26°C respectively. These sites are away from tribal settlement and these areas are not affected as much when compared with study sites I and II. Temperature is an important physical parameter of water quality which has a direct effect on aquatic life because it reduces the dissolved oxygen (DO) concentration in the water, thus making oxygen less available for respiration (12). Temperature also affect chemical reactions and reaction rates within the water, thereby influencing its suitability for use (13).



Fig. 1. Map showing the study area

#### 3.2. Color

In the present study the color of the water near the study site I is brown in color as the study site is nearer to the tribal settlement, dark green in study site II and in study sites III, IV and V the water is colorless as the study sites are close to forest area.

#### 3.3. pH

pH is an important parameter for existence of most biological life forms. Aquatic organisms are affected by pH because most of their metabolic activities are pH dependent (14). The pH range of aquatic life is 6.5 to 9.5 (USEDA; 2002) and domestic use is 7.0 to 9.0 (ICMR; 1975). In the present study, pH values of water samples between "7.0 to 7.6". The study sites I have the pH values of 8.4 which is at moderate value of domestic use. In study site II pH value is 7.6 and in study site IV and V pH values is 7.2.

## 3.4. Biological oxygen demand

Biological Oxygen Demand (BOD) is an important parameter of surface water quality and which indicates the level of organic matter contamination in surface water (15).The value of BOD ranges between 7.82 mg/L to 17.80 mg/L from site I to site V respectively. Values reported in site II is 15.64 mg/L, site III is 12.40 mg/L and site IV is 8.42 mg/L. There is a great variation in the BOD values of site I and site V because site I is located near to the tribal settlements so that the level of pollution in water is high compared to other four sites eventually the BOD will be more in study site I. In study site V, which is located nearer to forest canopy and away from the tribal settlements then the chance of polluting water will be less when compared to other four sites so that the BOD will be less in study site V.

## 3.5. Dissolved oxygen

Dissolved oxygen (DO) plays an important role in water quality determination. Dissolved oxygen is one of the most important factors for aquatic life and most species become distressed when DO levels drop to 4-2 mg/L (16). There are two main sources of DO in water i.e., diffusion from air and photosynthetic activity within water. Oxygen demanding waste like organic wastes causes rapid deletion of dissolved oxygen from water. Maximum value of DO was found in site IV and site V 6.46 mg/L and 6.82 mg/L respectively and minimum value was in site I (1.20 mg/L) followed by site II(2.44 mg/L). The site study site I and II are very near to tribal settlement.

## 3.6. Calcium

Calcium determines the hardness of the water. In the present investigation the maximum value of calcium was reported in site I as 350mg/L during the study period and minimum value of calcium was reported in the study site V as 328mg/L. Values reported in site II is about 345mg/L, site III is 342 mg/L and site IV is about 340 mg/L. There is a great variation in the presence of Calcium in site I to site V because site I is situated near the tribal settlement and site V which is situated away from tribal settlement and near to forest area.

#### 3.7. Magnesium

In the present investigation, the maximum value of magnesium was reported in site I as 320mg/L and minimum value reported at site V is 245 mg/L, site II reported 300mg/, site III 280 mg/L and site IV 250 mg/L. High concentration of magnesium proves to be diuretic and laxative in action which reduces the utility of water for domestic uses while a concentration above 500mg/L imparts an unpleasant taste to water and renders it unfit for drinking (4).

## 3.8. Microbiological analysis

Higher sewage contamination would lead to increase in numbers of Coliforms in natural waste bodies. Normally faecal pellets contain several species of bacteria including human pathogens (12) .The settlement of Malavettuvan tribal colony is near the Tejaswini River, which flows through the forest in Aratukadavu of Pulingome. The tribals use the river water for drinking and other purposes. In the present investigation faecal Coliform bacteria reported in site I of the study area is 9.4 MPN/1L because study site I is near to the tribal settlement, where the tribal use the areas for their domestic purposes like washing the clothes, bathing etc and also the sanitary wastes are spills near the study area. So that the maximum number of Faecal coliforms are found in the areas near study site I. In study site II the presence of faecal coliforms bacteria is about 5.6 MPN/L as it is little bit away from the tribal settlements. The study site III, which have reported less when compared with study site II about 1.2 MPN/L. The study site IV and V reported very less number of faecal coliforms bacteria about 0.2 MPN/L and 0.2 MPN/L, which is away from the tribal settlement as there are only fewer disturbances to this area. So that the faecal coliforms are very less when comparing with other three study sites. The water near the tribal settlement is not fit for drinking and other household purposes as the number faecal coliform bacteria more. The number of Coliform bacteria is less in the study sites IV and V so that water in these study sites can be used for drinking and other house hold purposes.

# 4. CONCLUSION

By analysing the physico – chemical and microbiological characters of the river water indicate that the study site I affect severely because the study sites is very near to the tribal settlements also the tribals use this area mainly for spilling out the toilet waste . Study site II which is away from the tribal settlement and in study site III, IV and V the river water is not polluted as it is very far away from the tribal settlement and also nearer to forest canopy. So the study site I which not suitable drinking and other domestic use. Therefore, source protection is proposed for the site I bodies of water for the benefit of mankind because they were not safe for human consumption.

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# PHYTOSOCIOLOGICAL ATTRIBUTES – TOOLS FOR DETERMINING THE CURRENT STATUS OF PASTURE LAND IN JAKKANARI BEAT, SIRUMUGAI RANGE, WESTERN GHATS, COIMBATORE DISTRICT

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## ABSTRACT

The aim of this investigation was to study the species composition structure and phytosociological characters like frequency, density, dominance, abundance and their relative values and important values index (IVI) of Jakkanari beat located in Sirumugai Range, Coimbatore district. Phytosociological analyses were performed for 5 plots, each 150 m<sup>2</sup>. Subanalysis was performed for 5sq km. of the 141 species found in the study area the maximum ecological importance was attributed to the herb. The present floristic composition of the community types, which showed a high density of unpalatable species, suggests that intensive grazing has become a widespread problem across the entire study area. The result adds a new and important contest of understanding on the effect of plant diversity on ecosystem services and functioning in terrestrial ecosystem.

Keywords: Phytosociological, frequency, density, dominance, abundance, important values index.

## **1. INTRODUCTION**

The Indian subcontinent is remarkable for its exceptional level of biological diversity at broad habitat level and within these habitats at species level. About 75 million hectares of the land area in India is forest of various types from dry deciduous to evergreen forest and from alpine to tropical forests (1). It harbours about 45000 species of plants and 65000 species of animals. Owing to this fact of rich diversity of biotic resources, India is ranked one of the 17-megadiversity countries in the world (2). A large portion of this diversity is also found to be endemic to India.

The Western Ghats, an unbroken relief along the western coast in peninsular India, is one of the richest centres of biodiversity (3,4). Almost one third of all the flowering plant species in India are found in this region and among them, 40% are endemic also. Across the world, 25hot spots have identified on the basis of species endemism and degree of threats through habitat loss (4). Out of these, two are confined to India sub-continent that is, Western Ghats/Sri Lanka and Indo-Burma (5). The present study site falls within the Western Ghats. In the following account a case study of Jakkanari beat located in Sirumugai Range, Coimbatore district was taken. The study include species composition structure and phytosociological characters like frequency, density, dominance, abundance and their relative values and important values index (IVI).

## 2. MATERIALS AND METHODS

## 2.1. Site description

The present study area Jakkanari beat, Sirumugai Range are located about 55km North -East of Mettupalayam taluk of Coimbatore district.It occupy an area about 1358 hectors. The latitude and longitude of the study area was 11°,18 N and 76°59 E. The average rainfalls of the study are 800mm per annum. These forests are highly prone to fire. The grass and many herbs dry up by April and May and leaf fall starts. The forest floor is thickly covered with dry twigs and leaves. The north east portion of this Jakkanari beat of Sirumugai Range has a small patch of this type of forests covering about an area of 5 sq.km around foot hills of Kottagiri. The pasture land is dominated by grass Cynodon dactylon. The climate of study area is tropical and monsoon. The floristic composition include more than 145 species belongs to grasses, sedges, forbs and trees.

## 2.2. Vegetation analysis

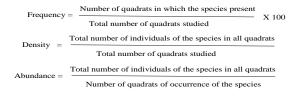
The herbaceous vegetation of the study area has been analysed over period of 6 months from September, 2012 to February, 2013. The sampling was done at the first week of every month for phytosociological studies.

## 2.3. Phytosociological studies

The minimum quadrate size of 1 x 1 m was fixed by the species-area curve method for phytosociological observations. Each time, 5 quadrates were laid by the randomised method. The minimum number of quadrates required (ie 5) was determined as described by Greig-Smith (6). For this mean number of individuals of the first two, four, six, eight and ten etc .Quadrates were calculated and plotted against the number of observations. It will be seen that the mean at first fluctuates, steadying as the required number of quadrates was reached.

The number and type of each species occurring in each quadrat were recorded. For grasses, each tiller counted as an individual because it is impossible to decide from aerial shoot whether it is separated or connect in the subterranean region, especially in perennial grasses. Different workers have used arbitrary units to represent an individual. Armstrong (7) and Stapleden (8) have counted the entire individuals as far as possible in the case of erect plants, but in creeping grasses each rooting unit has taken as an individual. Stove and Fryer (9) have considered an independent root system, as nearly as this could be determined without actually lifting the plant, to be a unit for counting. In the case of creeping plants, any portion of the plant, upto 5 cm in length and having functional root was counted as one plant. Only the plant beyond the seedling stage (ie., more than 2 cm height in case of monocots and beyond first leaf stage in dicots) were counted. The basal area at the point of emergence for the constituent species was measured. From the observation the quantitative characters such as frequency, density, abundance, relative frequency, relative density, relative dominance and importance value index were calculated.

Frequency, density and abundance were calculated using the following formulae



Basal area =  $\pi r^2$ 

Where,

 $\pi$  =3.14 and 'r' is the radius of the stem the point of emergence.

Relative frequency, relative density and relative dominance were calculated from the following formula:

Relative Frequency =	Number of occurence of the species	X 100
	Number of occurence of all species	
Relative density =	Number of individuals of the species	X 100
Tenative density =	Number of individuals of all species	X 100
Relative dominance =	Total basal area of the species	X 100
Kelauve dominance =	Total basal area of all species	X 100

Important Value Index (I.V.I) is the sum of quantities of relative frequency, relative density and relative dominance expressed per 300.

Dominance index was determined by the following formula as given by Simpson (10):  $C=\sum (ni/N)^2$ 

Where,

C = dominance index.

ni= number of individuals of a species over unit area.

N= corresponding total number of individuals of all species over the same unit area.

 $\Sigma$ = Summation.

The Shannon – Wiener's index of species diversity was worked out by the following formula as given by Margalef (1968):

 $H = -\sum \overline{Pi ln} Pi$ 

Where,

H= Shannon – Wiener's index of species diversity.

Pi= S/N

S = Number of individuals of one species.

N= Total number of all individuals in this sample.

ln= the logarithm to the base ' e'.

The evenness index has been calculated by using the following formula:

$$EI = \frac{-H}{Log S}$$

Where,

H = Species diversity index.

S = Number of species

## **3. RESULTS**

The present investigation was carried out in the pasture land of semi-deciduous forest, Sirumugai in Mettupalayam range of Coimbatore districts. The climatic data for the study period of the study area is given in Table 1. The maximum temperature was recorded ranged from 29.5°C September to 32.7°CMarch. The minimum temperature was varied between 23.2°C (October) and 21.8°C (August). The total rainfall recorded for the study period was 805 mm with the maximum of 356.6 mm during October and the minimum 3.7 mm during February. The rainy days were distributed in the early month of the study period (August). While the later months considerably decreasing rainy days. The relative humidity was generally above 68% and the February, experienced with lower humidity.

Basal Relative Relative Freque Abundance Density Relative S. Cover Density Name of the species ncy (individual (individuals/m Frequenc dominanc IVI (individuals/m (Sq.mm/5 No. (%) <sup>2</sup>) y (%) e (%)  $s/m^2$ ) <sup>2</sup>) quadrats) GRASS Chloris barbataL.Swartz 80 3.25 2.6 7.8 0.78 2.49 1 1.37 0.35 2 5.6 5.6 2.51 5.88 Cymbopogannardus (L.)Rendle 100 56 1.71 1.67 20.2 Cynodondactylon (L.)Pers. 3 100 56 56 42 1.71 16.70 1.88 8 Dactylocteniumaegyptium (L.) Willd. 100 12 12 33 1.71 3.58 6.76 4 1.48 5 Eragrostiellabifaria (Vahl) Bor 100 15 15 22.5 1.71 4.47 1.01 7.19 Eragrostistenella (L.)Roem.&Schult. 6 100 2 2 3.5 0.60 0.16 2.46 1.71 Heteropogoncontortus (L.) P.Beauv. 100 4 4 16 1.19 0.72 3.62 7 1.71 8 *Pennisetumciliare* (L.)Link 100 17.2 17.2 43 5.13 1.93 8.76 1.71 9 Setariaviridis (L.) Beauv 100 4 4 9 1.19 3.30 1.71 0.40 10 Digitariaciliaris (Retz.) Koeler 100 2 2 1 1.71 0.60 0.04 2.35 Eragrostisamabilis (L.) W.&A. 4 4 3 1.19 0.13 3.03 11 100 1.71 **SEDGES** 17.7 50 14.91 1.12 1 *Cyperusrotundus* L 100 50 25 1.71 3 Cyperusdistans L.f. 2 100 13 13 9.75 1.71 3.88 0.44 6.02 FORBS 5.96 6.02 1 Achyranthusaspera L. 100 20 20 100 1.71 4.48 Abrusprecatorius L. 2 2 2 100 15 1.71 0.60 0.67 1.71 12.1 3 AcalyphaindicaL. 100 4 4 20 1.71 1.19 0.90 5 2.5 2 Aervalanata (Linn.) Juss. 80 10 0.60 2.75 4 1.71 0.45 0 0 0 0 5 Aloe vera (Linn.) Burm.f. 0.00 0.00 0.00 0.00 6 Andrographispaniculata Nees. 100 3 3 2.4 0.89 0.11 2.71 1.71 2 2 7 Boerhaaviadiffusa L. 100 10 1.71 0.60 0.45 2.75 8 5 2 Cortalariaretusa L. 40 20 0.68 0.60 0.90 2.17 9 1 Datura metal L. 1.6 10 0.30 1.77 60 1.02 0.45 1.36 10 Dendrocalamusstrictus Nees. 20 2 0.4 20 0.34 0.12 0.90 11 Enicostemmaaxillare (Lam.) Raynal. 0 0 0 0 0.00 0.00 0.00 0.00 4.5 12 Evolvulusalisinodes L. 80 3.6 18 1.37 1.07 0.81 3.24

Table 1. The Frequency, abundance, density, relative frequency, relative density, relative dominance and IVI of constituent species in the community of study area

13	Gymnemasylvestre R.Br.	100	5	5	50	1.88	1.49	2.24	5.61
14	Ipomoea obscura (L.) Ker-Gawl.	60	3.3	2	15	1.02	0.60	0.67	2.29
15	Leucasaspera (Willd.) Link.	100	1	1	1.45	1.71	0.30	0.06	2.07
16	Mimosa pudica L.	60	1.3	0.8	8	1.02	0.24	0.36	1.62
17	<i>Mukiamadraspatana</i> (Linn.) M.Roemer.	100	2	2	20	1.71	0.60	0.90	3.20
18	Occimumbasilicum L.	100	2	2	20	1.71	0.60	0.90	3.20
19	Ocimumcanum Sims.	100	1	1	10	1.71	0.30	0.45	2.45
20	Ocimumgratissimum Linn.	0	0	0	0	0.00	0.00	0.00	0.00
21	Ocimum sanctum Linn.	60	1.6	1	5	1.02	0.30	0.22	1.55
22	Phyllanthusamarus Schum. & Thonn.	0	0	0	0	0.00	0.00	0.00	0.00
23	Solanum trilobatum L.	80	1	0.8	8	1.37	0.24	0.36	1.96
24	Solanum virginianum L.	0	0	0	0	0.00	0.00	0.00	0.00
25	Spermacoceocymoides (Brum.F.) DC.	100	10	10	100	1.71	2.98	4.48	9.17
26	Tribulus terrestis (L.)	100	1	1	10	1.71	0.30	0.45	2.45
27	Tridax ProcumbensL.	100	16	16	240	1.71	4.77	10.75	17.2 3
28	Abutilon hirtumSweet	100	2	2	20	1.71	0.60	0.90	3.2
29	Abutilon indicum (L.)Sweet	0	0	0	0	0.00	0.00	0.00	0.0
30	AzimatetracanthaLam.	40	1	0.4	6	0.68	0.12	0.27	1.0
31	Barleria prionitisL.	20	1	0.2	2	0.34	0.06	0.09	0.4
32	Calotropis gigantea (L.)W.T.Aiton	20	5	1	15	0.34	0.30	0.67	1.3
33	Canthium coromandelicum (Burm.f.) Alston	60	1.3	0.8	8	1.02	0.24	0.36	1.6
34	Capparisroxburghii DC.	100	3	3	45	1.71	0.89	2.02	4.6
35	CapparissepiariaLinn.	80	1	0.8	8	1.37	0.24	0.36	1.9
36	Carissa carandasLinn.	40	1.5	0.6	9	0.68	0.18	0.40	1.2
37	Carissa SpinarumL.	20	1	0.2	3	0.34	0.06	0.13	0.5
38	Carmona retusa (Vahl) Masam.	20	1	0.2	2	0.34	0.06	0.09	0.4
39	Cipadessa baccifera (Roth) Miq.	40	1	0.4	2	0.68	0.12	0.09	0.8
40	<i>Cissus quadrangularis</i> Linn.	100	1	1	20	1.71	0.30	0.90	2.9
41	Cissus vitiginea L.	100	1	1	15	1.71	0.30	0.67	2.6
42	Coleus aromaticus Benth.	100	1	1	15	1.71	0.30	0.67	2.6
13	Croton sparsiflorusMorong,Ann.	20	1	0.2	1	0.34	0.06	0.04	0.4
44	Euphorbia antiquorumL.	40	1	0.4	4	0.68	0.12	0.18	0.9
45	<i>Flacourtiaindica</i> (Brum.f.) Merr.	0	0	0	0	0.00	0.00	0.00	0.0
46	Flueggealeucopyrus Willd.	0	0	0	0	0.00	0.00	0.00	0.0

47	GmelinaasiaticasL.	20	1	0.2	5	0.34	0.06	0.22	0.62
48	GrewiaovalifoliaL.	20	1	0.2	2	0.34	0.06	0.09	0.49
49	JatropacurcasL.	100	2.4	2.4	48	1.71	0.72	2.15	4.57
50	JusticiatranquebariensisLinn.f.	60	4.6	2.8	14	1.02	0.83	0.63	2.49
51	Lantana camaraL.	100	4	4	40	1.71	1.19	1.79	4.69
52	Opuntiastricta(Haw.)Haw.	20	1	0.2	10	0.34	0.06	0.45	0.85
53	<i>Pachygoneovata</i> (Poir.)Meirs ex Hook.f.&Thomson	40	1.5	0.6	9	0.68	0.18	0.40	1.26
54	PartheniumhysterophorusL.	100	6	6	60	1.71	1.79	2.69	6.18
55	PavettaindicaLinn.	0	0	0	0	0.00	0.00	0.00	0.00
56	Pavoniazeylanica(L.) Car.	100	3.2	3.2	32	1.71	0.95	1.43	4.09
57	Phyllanthus reticulates L.	100	4.6	4.6	46	1.71	1.37	2.06	5.14
58	Pterolobiumhexapetalum(Roth) Sant.	60	1	0.6	9	1.02	0.18	0.40	1.61
59	Scutiamyrtina(Brum.f.) Kurz.	0	0	0	0	0.00	0.00	0.00	0.00
60	<i>Sidaaccuta,</i> Burm.	100	2.4	2.4	24	1.71	0.72	1.08	3.50
61	Solanum pubescensWilld.	100	4	4	40	1.71	1.19	1.79	4.69
62	Tephrosiapurpurea(L.) Pers.	100	6	6	150	1.71	1.79	6.72	10.2 1
63	<i>Toddaliaasiatica</i> (L.) Lam.	100	1.4	1.4	14	1.71	0.42	0.63	2.75
64	Vernoniacinerea(L.) Less.	100	5.6	5.6	112	1.71	1.67	5.02	8.39
65	<i>Zizyphusjujupa</i> Lam. <b>TREE</b>	20	1	0.2	2	0.34	0.06	0.09	0.49
1	Acacia chundra(Roxb.) DC.	20	1	0.2	15	0.34	0.06	0.67	1.07
2	Acacia concinna(Willd.) DC.	0	0	0	0	0.00	0.00	0.00	0.00
3	Acacia ferrugineaDC.	20	1	0.2	5	0.34	0.06	0.22	0.62
4	Acacia leucophloea(Roxb.) Willd.	20	1	0.2	8	0.34	0.06	0.36	0.76
5	Acacia melanoxylonR.Br.	0	0	0	0	0.00	0.00	0.00	0.00
6	Ailanthus excels Roxb.	0	0	0	0	0.00	0.00	0.00	0.00
7	Ailanthus malabaricaDC.	0	0	0	0	0.00	0.00	0.00	0.00
8	Albiziaamara(Roxb.) Boiv.	20	1	0.2	7	0.34	0.06	0.31	0.71
9	AlbizialebbeckBent.	20	1	0.2	21	0.34	0.06	0.94	1.34
10	Albiziaodoratissima (L.f.) Benth.	20	1	0.2	6	0.34	0.06	0.27	0.67
11	Annona squamosaL.	20	1	0.2	11	0.34	0.06	0.49	0.89
12	<i>Anogeissuslatifolia</i> (Roxb. Ex DC.) Wall. Ex Guill. &Perr.	0	0	0	0	0.00	0.00	0.00	0.00
13	ArtocarpusheterophyllusLamk.	20	1	0.2	7	0.34	0.06	0.31	0.71
14	Atalantiamonophylla(L.) Correa	0	0	0	0	0.00	0.00	0.00	0.00

15	AzadirachtaindicaA.Juss.	20	1	0.2	15	0.34	0.06	0.67	1.07
15 16	AzaairachtainaicaA.juss. BassialatifoliaRoxb.	20	1 0	0.2	0	0.34 0.00	0.06	0.67	1.07 0.00
10	Bauhinia racemosaLam.	÷	0	0.2	0	0.00	0.00	0.00	0.00
		20 20	-	0.2	3 20				
18 10	BischofiajavanicaBlume		1			0.34	0.06	0.90	1.30
19	Canthiumdiccoccum(Gaertn.) Merr.	0	0	0	0	0.00	0.00	0.00	0.00
20	CappariszeylanicaL.	20	1	0.2	8	0.34	0.06	0.36	0.76
21	Cassia auriculataL.	20	1	0.2	9	0.34	0.06	0.40	0.80
22	Cassia fistula L.	20	1	0.2	11	0.34	0.06	0.49	0.89
23	CassineglaucaRottb.Kuntze.	0	0	0	0	0.00	0.00	0.00	0.00
24	ChloroxylonswieteniaDC.	0	0	0	0	0.00	0.00	0.00	0.00
25	Cochlospermumreligiosum(L.) Alston	20	1	0.2	10	0.34	0.06	0.45	0.85
26	Cordia obliquaWilld.	0	0	0	0	0.00	0.00	0.00	0.00
27	DalbergiapaniculataRoxb.	0	0	0	0	0.00	0.00	0.00	0.00
28	DiospyrosmontanaRoxb.	20	1	0.2	3	0.34	0.06	0.13	0.54
29	DiospyrosmelanoxylonRoxb.	0	0	0	0	0.00	0.00	0.00	0.00
30	Eucalyptus tereticornisSm.	0	0	0	0	0.00	0.00	0.00	0.00
31	Feronia elephantumL.	0	0	0	0	0.00	0.00	0.00	0.00
32	FicusbenghalensisL.	0	0	0	0	0.00	0.00	0.00	0.00
33	FicusglomerataRoxb.	0	0	0	0	0.00	0.00	0.00	0.00
34	FicusmicrocarpaL.f.	0	0	0	0	0.00	0.00	0.00	0.00
35	<i>Ficustravancorica</i> King	20	1	0.2	6	0.34	0.06	0.27	0.67
36	FicusvirensL.	0	0	0	0	0.00	0.00	0.00	0.00
37	GrewiatiliifoliaVahl.	0	0	0	0	0.00	0.00	0.00	0.00
38	Haldiniacardifolia(Roxb.) Riddsdale	20	1	0.2	7	0.34	0.06	0.31	0.71
39	HardwickiabinataRoxb.	0	0	0	0	0.00	0.00	0.00	0.00
40	<i>Ixorapavetta</i> Andrews	20	1	0.2	9	0.34	0.06	0.40	0.80
41	Lagerstroemia lanceolataWall.	0	0	0	0	0.00	0.00	0.00	0.00
42	MachilusmacranthaNees.	0	0	0	0	0.00	0.00	0.00	0.00
43	MangiferaindicaL.	20	1	2	40	0.34	0.60	1.79	2.73
44	Melia azedarach L.	20	1	0.2	20	0.34	0.06	0.90	1.30
45	Melia dubiaCav.	0	0	0	0	0.00	0.00	0.00	0.00
46	MorindacoreiaiBunch.	20	1	0.2	6	0.34	0.06	0.27	0.67
47	MorindatinctoriaRoxb.	20	1	0.2	25	0.34	0.06	1.12	1.52
48	Palaquiumellipticum(Dalz.)Baill.	0	0	0	0	0.00	0.00	0.00	0.00
49	PhyllanthusemblicaL.	20	1	0.2	20	0.34	0.06	0.90	1.30
50	PterocarpussantalinusL.f.	20	1	0.2	15	0.37	0.06	0.67	1.10

51	Pongamiapinnata(L.) Pierre	20	1	0.2	30	0.34	0.06	1.34	1.74
52	Pterocarpus marsupium Roxburgh	0	0	0	0	0.00	0.00	0.00	0.00
53	Santalum album L.	20	1	0.2	40	0.34	0.06	1.79	2.19
54	Schleicheraoleosa(Lour.)Merr.	0	0	0	0	0.00	0.00	0.00	0.00
55	Strychnosnux-vomica L.	0	0	0	0	0.00	0.00	0.00	0.00
56	StrychnospotatorumL.	0	0	0	0	0.00	0.00	0.00	0.00
57	Swieteniamahagoni(L.)Jacq	0	0	0	0	0.00	0.00	0.00	0.00
58	TamarindusindicaL.	20	1	0.2	30	0.34	0.06	1.34	1.74
59	TectonagrandisL.f.	20	1	0.2	20	0.34	0.06	0.90	1.30
60	TermanaliachebulaRetz.	0	0	0	0	0.00	0.00	0.00	0.00
61	<i>Termanaliatomentosa</i> (Roxb.)Wight &Arn.	0	0	0	0	0.00	0.00	0.00	0.00
62	Wrightiatinctoria(Roxb.) R.Br.	20	1	0.2	32	0.34	0.06	1.43	1.83
63	ZiziphusmauritianaLam.	20	1	0.2	12	0.34	0.06	0.54	0.94
		∑f 5880	∑ab385.35	∑ d 335.6	∑ bc 2247.4				

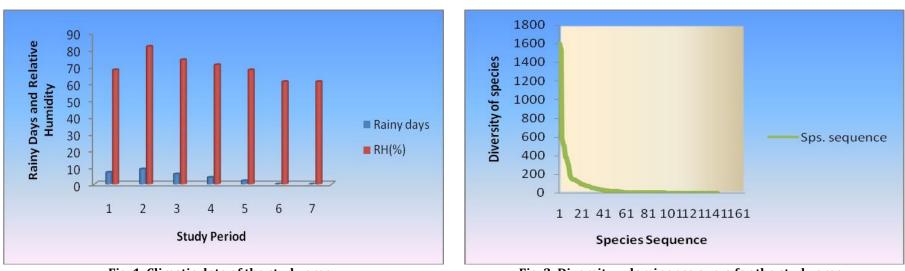


Fig. 1. Climatic data of the study area.

Fig. 2. Diversity - dominance curve for the study area.

# 3.1. Species diversity

During the study period September 2016 to March 2017 about 141 species of flowering plants belonging to 126 genera under 46 families were recorded from the study area. Regarding the habit of the plants, out of the 141 species, 63 were tree species, 65 belonged to forbs and shrub, 11 grasses and 2 sedges.

## 3.2. Family composition

Among the families, Poaceae is the largest one which constitutes 16species, followed by Fabaceae (15 species), Rubiaceae and Euphorbiaceae (9 species respectively), Lamiaceae (7 Species), Moraceae and Malvaceae(6 species respectively).

## 3.3. Status of grass, sedges, forbs and trees

During the study period 5quadrates are randomly laid down on the study area. Out of 141 individuals, the grasses belonging to 11 species were recorded from 5 quadrates covering 1358 hectors of Jakkanaribeat, Sirumugai. Based on Frequency values Cymbopogan nardus (100), Cynodon dactylon (100),Dactyloctenium aegyptium (100),Pennisetumciliare (100), Eragrostisbifaria (100), Digitariaciliaris (100), Eragrostisamabilis (100) etc., were dominant among the Grasses. Whereas Cypeus rotundus (100), Cyperusdistans (100) were the dominant components of Sedges. Achyranthus aspera (100),Acalypha indica (100),Andrographispaniculata (100), Gymnema sylvestre (100),Spermacoce ocymoides (100), Tridax procumbens (100), Abutilon hirtum (100), Capparis roxburghii (100) were the dominant components in Forbs layer. Based on Abundance values Cynodon dactylon (56), Cyperus rotundus (50), Achyranthus aspera (20), Tridax procumbens (16) etc., were the dominant components in the community. Based on the density values Cynodon dactylon (56), Cyperus rotundus (50), Achyranthus aspera (20), Tridax procumbens (14) etc., were the dominant component of the grasses and forbs layer. Based on the Basal cover values Tridax procumbens (240), Tephrosia purpurea (150), Achyranthus aspera (100), etc., were dominant components of shrubacious layers. Mangifera indica (40), Santalum album (40), Albizia leebeck (21), Tectona gradis (20), etc., were dominant among the tree species. Based on the IVI values Cynodon dactylon (20.28), Cyperus rotundus (17.73), Tridax procumbens (17.23), Acalypha indica (12.15), Eragros tiellabifaria (7.19), Dactyloctenium aegyptium (6.76), Achyranthus aspera (6.02) etc., were the dominant components of shrubacious layer (Table 1). The species composition, the number of individuals in each species and the number of sampling units in which each species were present are given in table. The total number of individuals and hence the density altogether in the studied period were positively related to the density of the species varied between 165.4 and 335.6 individuals/m<sup>2</sup>. Numerically the grass Cynodon *dactylon* contributed higher individuals than that of the other studied group. Apart from the grass, the sedge species, Cyperus rotundus shared maximum number of individuals. Among the grasses the species, Pennise tumciliare, Eragros teillabifaria, and Dactyloctenium aegyptium and sedges like Cyperus *distans* and forbs like *Achyranthus aspera*, and Tress like Acacia chundra. Manaifera indica and Pterocarpus santalinus showed their appearance at all times of sampling. The other species observed during the study period appeared only in limited time.

Frequency is usually expressed in terms of percentage occurrence of individual's species in an area. On global level, based on analysis on several thousands of quadrates. Raunkiaer (11) propounded the law of frequency. The frequency class 'A' include those species where the frequency values ranged between 1 and 20 percent, frequency class 'B' between 21 and 40 percent, frequency class 'C' between 41 and 60 percent, frequency class 'D' 61 and 80 percent and frequency class 'E' between 81 and 100 percent. Based on the relative proportion of different classes, Raunkiaer gave a law of frequency as

#### < A > B > C= D < E >

Relative frequency refers to relative dispersion of a species in respect to that of rest of the species. Density represents the number of individuals of a species in unit ground area in a community. Abundance however, refers, to the number of individuals per unit area on the basis of the number of quadrates of occurrence only. It is not like the density where the numbers of all the quadrates studied in the community are taken into Relative density is an expression for account. numerical strength of a species in relation to the total number of individuals of all the species of an area. The basal area is regarded as an index of dominance of a species and the term signifies the are occupied by the aboveground part of the plant at the region of emergence Relative dominance is a coverage value of species with respect to the coverage of the rest of the species. The Importance Value Index (IVI) of a species derived from the percentage values of the relative frequency, relative density and relative dominance is used to assess its ecological importance in the community. All the 82

sociological characters viz. frequency, density, basal area and their relative values abundance and importance values index of the study area the presented in table1. The study area was characterized by an even distribution of grasses in general. All other species have restricted distribution. The shrub, Spermacoce ocymoides was important in the study area in terms of its numerical strength. On the basis of increasing or decreasing values of basal cover and importance value index, species have classified as 'increasers' and 'decreasers' (12,13). Irena Simova (14) has regarded the frequency to be more artificial character than density and basal cover. The latter two are more reliable as they remain unaltered by varying quadrate size. It is known from the study are that the shrub Spermacoceo cymoides Occupied maximum basal cover area. In order to express the ecological success of any species with single values, the concept of importance value index has been developed. The quantitative values of relative dominance are added to get the IVI. Gupta and Das (15) used relative biomass in place of relative basal cover (relative dominance) to attain the IVI.

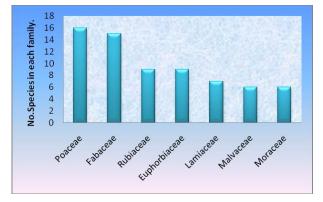


Fig. 3. Dominant families of the study area.

Of the 141 species found in the study area the maximum ecological importance was attributed to the herb, *Spermacoceocymoides* and followed by *Achyranthusaspera*, *Tridaxprocumbens*, and

Tephrosiapurpurea the all remaining 137 species altogether contributed very less ecological importance. Dominance - diversity curve for the study area have been drawn to interpret the community organizations in terms of resources sharing and niche space. It is confirmed that in the study area the single species Spermacoceocymoides the maximum share of the available community resources. The dominance index during the study period was varied from 0.067 to 0.081 and it was more pronounced during dry months. The diversity index was ranged from 1.31 to 1.36. It is observed that the species diversity and evenness were inversely proportional to dominance of the community. The result adds a new and important contest of understanding on the effect of plant diversity on ecosystem services and functioning in terrestrial ecosystem.

# 4. DISCUSSION

An analysis of the temperature data during the study period indicated that there is a well marked season in the study area, since the differences between seasonal temperatures ranged widely. The study conducted by Simon and Mohankumar (16) also reported that altitude and rain fall in Kerala are not correlated. On the other hand the study on rain fall in Palakkad Gap in Western Ghats by Raj and Azeez (17) show annual rain fall varying with altitude. The total annual rain fall of the Palakkad plains is lower than the total annual rain fall for the whole state Kerala (18,19). The ombrothermic graph shows that it is a humid region due to the wide spread moderate rainfall and higher humidity. Due to its specific geographical location, the climate of the Palakkad plain is highly influenced by the humid climate of Kerala as well as the more arid climate condition on the western side of the Western Ghats (17).

Forest degradation is usually accompanied by species extinction, reduction in biodiversity and decrease in primary productivity. Consequently, there is a growing interest in quantifying habitat characteristics like foret structure, floristic composition and species richness in Indian forest (20 - 24). Phytosociological analysis of plant community is the first and foremost basis of the study of any species of vegetation as it is a prerequisite for the understanding of community structure and organization. Species composition is one of the major characters of plant community. The dominance distribution pattern at the levels of species and family justifies mature, stable and complex nature of vegetation (25-27). It is evident from the data (Table 3) that the study area comprised a considerable number of individuals contributed by the shrubs were considerably higher that the grasses. This may be attributed to the presence of wide ecological amplitude in shrubs (27,28). There exists little authentic quantitative ecological information pertaining to vegetation aspects in relation to the soil nutrient status in this region.

Of the 141 species present in the community generally the shrubs showed even distribution. According to Misra (27) this may be due to their high reproductive capacity, quick dispersal of seeds and wind pollination to produce viable seeds. The herb, *Hyptissuaveolens* registered higher density and basal cover. Mc Naughton (29) opined that the presence of tolerance to poor conditions, adaptability and suitability to varies ecological niches for certain shrub species could be the possible reasons for the successful establishment in the grazing grasslands and the herbage yield as the dominant and important shrub. The relative frequency, relative density and relative dominance of the herb, *Hyptissuaveolens*was generally higher that the other species at all times of sampling. The quality and diversity of the species combination around individuals-so called neighbourhood diversity-is important from functional aspect as well (30-32)

The dominance-diversity curves prepared for the study are (Fig. 3) also confirmed the single species dominance in the community in terms of resource sharing. In addition, these curve fit for the geometric series confirming the niche pre-emption hypothesis of Whittaker (33) which stated that the single species (*Hyptissuaveolens*) occupied the maximum share of the available community resources. High species content and more even distributions of I.V.I among the species in dry deciduous forest depict high degree of stability and complexity of community (27).It has been argue that the ecosystem with high species diversity is more stable and resilient to environmental disturbance than those have low species diversity.

It is well known from the data that the species diversity of the study area was fluctuating according to seasons. Higher diversity was found in rainy seasons. During rainy months many species tend to grew equally. However in adversed conditions the species diversity decreased and farrowing only few drought tolerant species. The dominance index found to be decreased during rainy season in the study area. This is attributed to the favourable condition (26, 27).

# **5.** Conclusion

The phytosociological analysis for a pasture land community was carried out in the Jakkanari beat, Sirumugai Range of Coimbatore district, over a period of seven months from September, 2016 to March 2017. The habitat is humid and the climatic factors were determined to be favourable for the growth of the vegetation. Altogether it is estimated that 141 species were found in the study area. Among them, the herb, *Cyanodon dactylon* is found to be dominant as it received maximum impact of the environment. The distribution, numerical strength, basal cover, and their relative values and importance value index were higher for this species. The dominance-diversity curve obtained also indicates that this species shared the maximum resources of the habitat. However on basis of numerical strength no species are dominating in the study area. As the

diversity index is above 2 at most of the sampling times, it is known that the species diversity in the study area is well and adequate. It is desired that the community was a consociational unit due to the prevalence of single species dominance. There for it is expected to have still more productivity herbage which can be highly beneficial for many wild herbivores and hence the maintenance of ecological balance.

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# AN ETHNOZOOLOGICAL ASSESSMENT OF TRADITIONALLY USED ANIMAL-BASED THERAPIES IN ATTAPPADY OF PALAKKAD DISTRICT, KERALA, INDIA.

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# ABSTRACT

The present study is an attempt to collect and document the ethnozoological knowledge possessed by the traditional tribal people of Attappady hills of Western Ghats in Palakkad district. The study area consisted of three categories of tribes namely, Irula, Kurumba and Muduga. Irulas contribute the majority followed by Mudugas and Kurumbas respectively. The study involved collection of information regarding the topic from all the three categories of tribes. Informations were collected by direct personal interviews with traditional healers belonging to tribal community. The mentioned animals were identified by their local names and previous studies in ethnozoology and available biodiversity records of regions in and around Attappadv which involves Silent Valley National Park. The lack of biodiversity records posed difficulty in identifying the animal mentioned by the healers. The collected data was analyzed mathematically by calculating Informant Consensus Factor (ICF) to know the category of ailment for which more treatment is available, Fidelity level of animal species to identify the most preferred species for zootherapeutics, and Informant Agreement Index (IAR) to determine the agreement between informants for the use of a particular animal species for the treatment of a particular ailment category. ICF value obtained is highest for orthopedic ailments, FL is highest for Varanus bengalensis and Rusa unicolor and IAR is highest for seven species. The reduction in number of animals, lack of efficiency in implementing forest laws and their cultural taboos regarding the interaction with the outside world have resulted in the deterioration of traditional knowledge among the tribal population itself and also, due to legal issues, they have switched over almost completely to floral medicine. Therefore, they have a very little knowledge on the practice of zootherapeutics and ethnozoology.

Keywords: Ethnozoological assessment, animal based therapies, Attappady, Palakkad.

# **1. INTRODUCTION**

The tribal population is identified as the aboriginal inhabitants of our country. The traditional use of animals or their products for medicinal purposes has been documented throughout the history in ancient documents such as papyri, archives and several classical medicinal compendiums, even back to the practices of the ancient Mesopotamian, Assyrian and Babylonian civilizations (1). A major portion of the people dwelling in Western Ghats relies on traditional medical system, because their livelihood is based on their local environment. The people are trained in usage of natural systems for their living in the form of food and medicine and other uses (2). As other traditional communities, they also have the knowledge about the usage of biological diversity, through their traditional expertise and organization reflection.

The science of ethnozoology is a sub-field of anthropology concerned with how human beings perceive, manage, classify and use animal species. It also focuses on the ways in which animals influence the people they interact with and how man utilized animals for food, clothing, work, workshop and companionship.

Some of the best known medicinal compendiums containing animal samples are those from Hippocrates (Greece, V-IV century BC), Discorides (Greek physician born in Anatolia, first century AD), Avicenna (Persia, X-XI century AD) and Ibn al Baitar (Andalusia, XII-XIII century AD). About 10% of the medicinal samples included in the main classical works come from animals (3).

India is gifted with immense faunal and floral diversity. There are about 45,000species of plants and 81,000 species of animals. The tribals who depend on plants and animals for their day-today life and health problems are the real custodians of the knowledge of medicinally important plants and animals. Most of the knowledge accumulated by the tribes on medicinal plants and animals is unknown to the scientific community. Most of the biodiversity associated with tribes has either disappeared or is on the verge of extinction.

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Therefore, the immediate concern of the scientific community is to document the indigenous knowledge related to therapeutic use of plant and animal species and to devise strategies to preserve and tap this rich knowledge in a more sustainable way for the benefit of mankind.

The present study was aimed to explore and document the traditional uses of animals and to evaluate the importance of medicinal animals used in local healthcare system. It is believed that the present documentation will serve to record this vanishing knowledge before it is eroded completely from the hills and to the scientific community. It is also anticipated that the present documentation will be fundamental to protect traditional knowledge, for the conservation and sustainable use of rich biodiversity for future generations and to ensure the region and traditional people sovereign rights over its genetic resources for conservation and sustainable use by documenting them.

#### 2. MATERIALS AND METHODS

## 2.1. Study area

Attappady, the study area, is an extensive mountain valley at the head waters of Bhavani River. It is a part of the Nilgiri Hills of the Western Ghats. Attappadi comes under Mannarkkad Taluk in Palakkad District, Kerala State. The population of the valley is mostly Muduga (10%), Irula (84%) and Kurumba (6%) tribal people with a section of settlers from Tamil Nadu and other districts of Kerala. Thus, out of the whole, Irular inhabiting the plains and low elevations constitute the majority.

#### 2.2. Selection of traditional healers

The informants or traditional healers were selected based on their knowledge of medicinal animals in the study area. Totally, 10 informants were selected. Of them, 8 were men and only 2 were women. The healers selected were belonged to the age groups of around 40 years to 70 years to get the ethnozoological information through direct interviews or oral conversation.

## 2.3. Investigative method

Field investigations were conducted in all the three village panchayats of Attappady in 20 sites which includes Pattimalam, Ummathampadi, Nakkuppathi, Choriyannooru, Kulukkooru, Kadambara, Chavadivur, Nellippathi, Mulli, Malleeswaranmudi, Goolikkadavu, Pakkulam, Vengakkadavu, Veerannur, Mukkali, Mattathikkadu, Abbannooru, Kurukkuthikkallu, Kaniyoor and Dhonikkundu and obtained informations from 8 sites. The animals told by them are identified using the available biodiversity details and also by referring to reports of previous studies due to limitation of the duration of study and facilities for collecting specimen animals.

# 2.4. Quantitative analysis

## 2.4.1. Ailment categories

Based on the information obtained from the traditional healers in the study area, all the reported ailments were categorized into 13 categories viz. Respiratory ailments (RA), General health ailments (GHA), Dermal ailments (DA), Genito-urinal ailments (GUA), Hair care (HC), Neurological ailments (NA), Orthopedic ailments (OA), Gastrointestinal ailments (GIA), Veterinary ailments (VA), Fever ailments (FA), Oncology (O), Psychological ailments (PA) and Customary use (CU). Several diseases were placed in an ailment category based on the body systems treated and are mentioned in Table 1.

## Table 1. Ailment categories.

S. No.	Ailment category	Diseases
1	RA	Asthma, Cough,
2	GHA	Fatigue, Weight loss, Immunity,
3	DA	Wound, Albinism Rashes,Soriasis, Scabies
4	GUA	Delivery, Brest milk,
5	НС	Bald, Hair loss
6	NA	Epilepsy
7	OA	Muscle pain, Back pain, Joint pain, Rheumatism, Tetanus,
8	GIA	Appetite loss,Ulcer, Vomitting, Diarrhoea
9	VA	Rinder pest
10	FA	Fever, Plague, Typhoid
11	0	Cancer
12	PA	Anger
13	CU	Death ceremony, Food Ear Puncture, Black magic

## 2.4.2 . The fidelity level

To determine the most frequently used animal species for treating a particular ailment category by the informants of the study area, we calculate the fidelity level.

The FL was calculated using the following formula:

## $FL(\%) = (Np/N) \times 100$

Where Np is the number of informants that mentioned the specific animal species used to treat certain ailments, and N is the total number of the informants who utilized the animals as medicine for treating any given ailments.

## 2.4.3. Informant consensus factor

The informant consensus factor (ICF or FIC) is calculated by the following formula:

# FIC = Nur - Nt / (Nt - 1)

Where Nur is the number of use-reports of informants for a particular illness category and Nt refers to the number of species used for the illness category by all informants.

FIC values range from 0 to 1. A value close to one indicates a high intra cultural consensus (i.e. most informants use the same species for treatment of the same illness). A value close to zero indicates a high variation in the use of species (i.e. informants disagree over which species to use in the treatment within a category of illness)

# 2.4.4. Index of Agreement on remedies

To assess the importance of individual species in each illness category, Index of Agreement on Remedies was calculated. It is calculated by the following formula:

## Index of Agreement on Remedies (IAR) = nr-na / nr-1

Where nr is the total number of citations registered for species (s) and na is the number of use-categories that are treated with this species.

# **3. RESULTS**

# 3.1. Demographic characteristics of informants

Demographic characteristics of respondents were determined and recorded through face-to-face interviews. The number of practitioners between the age group 50-80 was high when compared to the other age groups. The percentage of practitioners with an age lower than 50 years was only 30%. Women's participation as a traditional medical practitioner was very low as indicated by high malefemale ratio and it remains almost a male-exclusive domain. The same fact was also documented in some previous works with traditional medical practitioners in India. 80% of the practitioners are uneducated but all have got certificates as traditional healers from Kerala Institute for Research Training and Development Studies of Scheduled Castes and Scheduled Tribes, Calicut (KIRTDS). Some of them were reluctant to reveal their traditional knowledge due to cultural taboos. They told that they believe that they have lost their tradition as a result of curse for they revealed it to the outsiders as against their value and thus took advantage over them.

In this study, a total of 29 species of animals categorized into 5 taxa were recorded that produced 33 usages. The 29 species consisted of 28 vertebrates and only one invertebrate.

Among them, mammals occupied 66% of the total animals followed by 17% of Aves, 10% of Reptiles, 4% of Amphibians and 3% of Insects of the whole respectively. Our survey indicated that only 8 informants were able to give some information about the use of animals in traditional medicine and no one is reported to use animals for their present medical treatments. It is also informed that instead of subscribing to pure animal-based preparations, it is advised to consume combinations of animal-based products with plant products. *Sus scrofa*, commonly known as pig has got more use citations i.e., for six diseases belonging to six ailment categories. Rusa unicolor which is commonly known as Sambar deer is mostly cited to be used for curing General health ailments by maximum number of healers . These results reveal that the animals belonging to mammalia and aves have been used mainly for medicinal purposes.

## 3.2. Data analysis

In this study, three quantitative indexes were used to study the medicinal animal species used by the local people.

# 3.2.1. Informant consensus factor (ICF)

The category with the highest degree of consensus from informants was orthopaedic ailments with ICF value of 0.83. The ranking is followed by General health ailments Respiratory ailments and other medical conditions. The lowest level of consensus was for Psychological, Oncological and veterinary ailments (ICF: 0) These results vary considerably from the case of Uganda (4), as gastro intestinal ailments ranked the highest; while in Waheed *et al.*, 2013, dermatological ailments, cardiovascular ailments, inflammation ailments, fever ailments and dental ailments ranked at the top. These differences are due to the geographical locations and local hygienic conditions of each nation (5).

No.	Ailments/Categories	Taxons	Use-reports	ICF
1	Respiratory ailments (RA)	8	21	0.65
2	Genito-urinary ailments (GUA)	5	6	0.2
3	General health ailments (GHA)	14	41	0.675
4	Cultural uses (CU)	4	5	0.25
5	Orthopedic ailments (OA)	3	13	0.83
6	Dermatological ailments (DA)	6	11	0.5
7	Hair care (HC)	2	4	0.66
8	Gastro-intestinal ailments (GIA)		5	0.25
9	Psychological ailments (PA)	1	1	0
10	Oncology (O)	1	1	0
11	Fever ailments(FA)	2	3	0.5
12	Neurological ailments (NA)	5	7	0.33
13	Veterinary ailments (VA)	1	1	0

# 3.2.2.Fidelity level (FL)

Fidelity value is useful for identifying the residents most preferred species in use for treating certain ailments.

FL values in this study varied from 10% to 80%. Generally, a FL of 100% for a specific animal indicates that all the informants use a particular animal for the treatment of a certain ailment category. The present study never revealed any species of animal with an FL value of 100%. The maximum FL value obtained for a species is 80% i.e., for *Varanus bengalensis* and *Rusa unicolor*. The results from this study indicate that 65.5% of the animal species are reported to cure more than one ailment. This trend is a common precise in Kerala (6) and other traditional medicines around the world (7).

# 3.2.3.Index of Agreement on Remedies (IAR)

The highest index of agreement on remedies values documented for & animal species in the current study implies that all informants agree upon he exclusive use of the medicinal animal species for a particular ailment condition. The highest IAR value of 1 is obtained for *Corvus splendens, Capra indicus, Manis craasicaudata, Loris sp., Macaca sp., Rusa unicolor* and *chamaeleo zelyanicus..* IAR value of ) 0.5 and above are reported for 11 species indicating moderate agreement within the informants and also no agreement among the informants exist for the use of 9 species indicating IAR value of 0.

# 4. DISCUSSION

Honey has had a valued place in traditional medicine for centuries

Mootoosamy and Mahmoodally (8) According to El-Soud (9), the usage of honey as a medicine has continued into present day traditional medicine. The ancient use of honey for coughs and sore throats has perpetuated into traditional medicine of modern times. Similarly, the present study also reported the use of honey against cough.

An ethnozoological study in Tamil Nadu by Solavan (10) reported the use of *P. cristatus* flesh for the treatment of paralysis. In this study also, citations were available for the use of feathers for the treatment of skeleton-muscular disorders. Charred feathers were reported to treat cough in other tribal communities.

This study indicated the importance of intensive studies not only considering the physiological but also ecological, anthropological, socioeconomic factors associated with the use of these animals for medicinal purposes.

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# PRELIMINARY PHYTOCHEMICAL QUALITATIVE AND QUANTITATIVE ANALYSIS OF *BRASSICA* OLERACEA LEAF EXTRACTS ON FOURTH INSTAR LARVAE OF AEDES AEGYPTI (CULICIDEA: DIPTERA)

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## ABSTRACT

*Aedes aegypti*, dengue fever mosquito, is primarily associated with the transmission of dengue in tropical and subtropical regions of the world. The present investigations was carried out to assess the larvicidal efficacy of aqueous and ethanol leaf extract of *Brassica oleracea var. botrytis L* against 4th instar larvae of *Ae.aegypti*. The concentration of extracts prepared from the leaf of plant *Brassica oleracea var. botrytis L*. were screened for their larvicidal activity against early fourth instars of dengue vector. The mortality rate of *Ae.aegypti* against aqueous and ethanol extracts of *B.oleracea* as follows 72% and 96%. Ethanol extract exposed to IV<sup>th</sup> larvae of Ae.aegypti is more efficiency than the aqueous extract. The present investigation suggest the possible use *B. oleracea L*. as an agent for the control of dengue vector, *A. aegypti*.

Keywords: Phytochemical analysis, Brassica oleracea, Aedes aegypti.

# **1. INTRODUCTION**

Mosquitoes can distributing a number of diseases than any other group of arthropods and affect more than 700 million people worldwide annually, including arboviruses responsible for yellow fever, dengue hemorrhagic fever, epidemic polyarthritis, several forms of encephalitis bancroftian filariasis (1) and pathogens which continue to have devastating effect on human beings (2). Personal protection from mosquito bites is currently the most important way to prevent transmission of this disease (3). To prevent these mosquito borne diseases and to improve quality of environment and public health, mosquito control is essential. Larvicide is successful way of reducing mosquito densities in their breeding places before they emerge into adults. Pesticides are indeed very effective in its use. However, the use of chemical insecticides are often toxic to both human and nontarget animals. The intensive use of chemical insecticides led to the development of resistant insect populations, resulting in reduced control, environmental pollution resulting in bioamplification in food chain and contamination (4).

Plants have the major advantage of still being the most effective and cheaper alternative green measure for the control of arthropods of public health importance (5,6). Natural products of plant origin are safe to use than the synthetic insecticides (7). *B. oleracea*, the sole species in the genus *Brassica* of the plant family *Brassicaceae* is widely cultivated. *Brassica* is a small herb-like plant, with a single stem growing from 3 to 5 feets short with spiral leaves. It is used as remedy against a variety of diseases (8,9). The leaf *Brassica oleracea* extracts have larvicidal and antioxidant activities (10). The main objective of the study was to test the larvicidal ability of aqueous and ethanolic leaf extracts of *Brassica oleracea var. botrytis* (Cauliflower) against *Aedes aegypti* mosquito larvae.

# 2. MATERIALS AND METHODS

# 2.1. Location of the study

This research was conducted at the Research Laboratory of P.G and Research Department of Zoology, Periyar E.V.R College, Tiruchirappalli.

# 2.2. Plant collection and Processing

The fresh leaves of plant *Brassica oleracea var. botrytis* were collected from weekly market Muthur, Tiruppur, district, Tamil Nadu, India. The selected plant parts were separated washed, dried, powdered and the extracts were filtered using whatman filter paper. Chemical test were carried out on the crude aqueous and ethanolic extracts using standard procedures to identify the phytochemical constituents like alkaloids, carbohydrates, proteins, coumarins, phenols, saponins, tannins, flavonoids, as described by Sofowora (11); Trease and Evans (12); Horborne (13).

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## 2.3. Mosquito species

Mosquito larvae of Aedes aegypti were collected from ICMR (Indian Council for Medical Research) Madurai, Tamil Nadu, India. *Ae. aegypti* was obtained an egg rafts on the filter paper and were reared in trays containing tap water and maintained at  $28 \pm 2C$ . When the eggs were hatched out into first instar larvae, they were fed with a mixture of yeast powder and dog biscuits in the ratio of (1:3). On the third day after hatching the first instar larvae moulted into second instar larvae on the fifth day, third instar larvae on the seventh day (14). The 4<sup>th</sup> instar of *Aedes aegypti* was experimented for the present study.

# 2.4. Larvicidal Assay

The larvicidal assay was conducted according to (15). In the Larvicidal assay on 4th instar larvae of Aedes aegypti were exposed to test concentration of 1% extract of crude aqueous and ethanol of B. oleracea L. separately. 1ml of solution was taken in separate bowl made up to 100 ml with distilled water and respective solvent separately for aqueous and ethanol extracts. crude Five concentrations of Aqueous and Ethanol leaf extracts were taken for experiments (2, 4, 6, 8 and 10%) and 20 numbers of 4th instars larvae were transferred gently to the test medium separately, simultaneously a control was maintained without extracts. The larval mortality in both treated and control were recorded after 6, 12, 24, 48, 72, and 96 hrs. Dead larvae were collected by tip of thin brush. This experiment was repeated five times. The percentage mortality was calculated by No of larvae Dead/Total No. of larvae×100.

# 2.4.1. Estimation of total Phenols

The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic components 15 mts. 5ml of the extract was pipette into a 50 ml flask, then 10 ml of distilled water was added. 2ml of ammonium hydroxide solution and 5ml o concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 mts for colour development. This was measured at 503nm.

## 2.4.2. Estimation of total Flavonoids

Ten grams of sample was repeatedly extracted with100ml of 80% aqueous and methanol at room temperature. The mixture was then filtered through a filter paper into a pre-weighed 250ml beaker. The percentage flavonoid was calculated by difference (16).

# 2.5. Statistical analysis

The concentration at which mortality observed (mg/ml) was corrected using Abbott's (17) formula. Statistical analysis of the experimental data was performed with MS Excel 2007 to find the Mean and Standard deviation values are tabulated.

# **3. RESULTS AND DISCUSSION**

The present study has been carried out to assess the mortality of the aqueous and ethanolic extracts of plant *B. oleracea* on mosquito larvae. Details of plant extracts used for the present study of the larval mortality and the phytochemical constituents noticed are in the Table 1, 2 and 3. The effects of plant extracts on mosquito larvae exposed to 96 hrs, for confirming mortality as per WHO standards are given in Table 3. Mortality larvae differences were observed in the toxicity of the aqueous and ethanolic leaf extracts of plants consisting of leaf against the fourth instar larvae of to be inducing 72 to 96% larvicidal property at 10ml of the extract at 96 hrs respectively on mortality against mosquito larvae at varying concentrations.

As per the preliminary phytochemical investigation, the constituents like flavanoids, tannins, alkaloids, saponins, phenolic compounds, coumarins, terpinoids, quinones and carbohydrates are equally present in leaf, of aqueous and ethanolic extracts as mentioned in Table 1. According to Bowers *et al.* (18) the biological activity of the plant extract is due to various compounds like Phenols and Flavonoids etc. These findings were in agreement of similar nature of study conducted by Okoye (19). Moreover, flavanoids are very important constituent of natural product and have got apart antioxidant activity (20).

# Table 1. Preliminary Qualitative analysis ofB.oleracea var. botrytis.

Compound	Aqueous	Ethanol
Carbohydrates	+	+
Tannins	+	+
Flavonoids	++	++
Alkaloids	-	++
Phenols	++	++
Terpinoids	+	+
Quinones	-	-
Coumarins	+	+
Saponins	+	+
Steroids	-	-

Table 2. Preliminary Quantitative analysis ofB.oleracea var.botrytis L.

Photochemical	Aqueous	Ethanol
TPC(CE/g)a	38.86±1.52	68.23±2.62
TFC(QE/g)b	26.76±0.95	42.33±1.92

a(mgCE/100g dry mass), C: Catechol, b(mg QE/100g of dry mass), Q: Quercetin, TPC: Total phenolic content, TFC: Total Flavonoid content.

Concen tration	29	%	4	%	6	%	8	%	10	%
Hrs	ALE	ELE								
12	3	10	4	12	5	13	6	17	10	18
24	4	12	5	12	7	15	10	17	12	19
48	4	14	6	14	10	16	12	18	15	19
72	6	14	9	16	10	18	13	18	17	20
96	7	16	10	18	12	19	15	20	17	20
Tot	24	66	34	72	44	81	56	90	72	96
Mortality%	24	66	34	72	44	81	56	90	72	96
М	4.8	13.2	6.8	14.4	8.8	16.2	11.2	18	14.4	19.2
SD	1.6431	2.2803	2.5884	2.6076	2.7748	2.3874	3.4205	1.2247	3.3615	0.8366

Table 3. Mortality of larvae of mosquito, *Aedes aegypti* exposed to aqueous and ethanolic leaf extract of *Brassica oleracea var. Botrytis* L.

Hrs= Hours., Tot= Total., % = Mortality percentage., M=Mean., SD=Standard Deviation.

ALE- Aqueous Leaf Extract., ELE- Ethanol Leaf Extract

Table 2 reveal the presence of total phenolic and flavanoid compounds in both aqueous and ethanol extracts of *B. oleracea*. High percentage of phenolic and flavanoids contents was reported in ethanol extract than aqueous extract. *B. oleracea* has been reported to possess potent mosquito larvicidal activity. The phytochemicals compounds present in the *B. oleracea* leaf extract show highest mortality of *Ae. egypti* larvae (Table 3). The aqueous and ethanolic leaf extracts show 72% and 96% mortality of 4<sup>th</sup> instar of *A. aeypti* when compared to the control. High insecticidal activity exhibited by ethanolic leaf extract may be due to presence of phenolic and flavanoids (Table 2).

A survey of literature on control of different phytochemicals obtained from various plants has been carried out by number of researchers in the field of vector control (21). There are many studies of toxicity carried out with other plants that reflect a similar behaviour against Aedes aegypti. Plant could be an alternate source of bioactive chemicals and generally free from harmful effects. Use of these botanical derivatives in mosquito control instead of synthetic insecticides could reduce the cost and environmental pollution. Many of the defensive components of plants are biodegradable with nonresidual effects on the biological environment. Hence an attempt has been made in the present investigation to identify the larvicidal potential of the locally available plant *B. oleracea*.

We can conclude from this study that in totality, the data collected show that B. oleracea indeed has larvicidal potential when treated to larvae in low concentrations, and can be used as substitute for commercial insecticides. Though the presence of phytochemicals in *B. oleracea* could be studied further in detail and its beneficial effect to control mosquitoes.

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# PREVALENCE OF MULTIPLE ANTIBIOTIC RESISTANT AND HAEMOLYSIN PRODUCING BACTERIA IN MARKETED FISH SAMPLES

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## ABSTRACT

Sea foods hold one of the greatest potentials to meet current and future demands of proteins to feed the world's ever-burgeoning population. Fresh seafood is an excellent source of proteins, a good source of minerals, and some vitamins, and it is low in fats, cholesterol, and sodium. Fishery products which are of great importance for human nutrition worldwide and provide clear health benefits. Food borne pathogens are the leading causes of illness and death in less developed countries killing approximately 1.8 million people annually. Bacteria are the most important cause of seafood spoilage. Percentage prevalence of bacterial population in fish samples collected from Ukkadam fish market, Coimbatore was significantly higher during the study period. About 7 fish intestinal samples were then enriched in nutrient broth and cultured. Biochemical test were performed to determine their phenotypic characteristics. The antibiotic resistance pattern and MAR index showed an increased antibiotic resistant bacterium in fish which may severe food borneillness in human. Thehemolytic activity and extracellular protein reveals the frequency of virulence and strong pathogenecity. On this basis, we came to the conclusion that all the isolates are highly pathogenic and cause various food poisoning in humans.

Keywords: MAR index, Hemolytic activity, Extracellular product.

## **1. INTRODUCTION**

Fish is one of the best supplies of proteins, vitamins and minerals and is an essential nutrient for fortifying both infant and adult diets. It is considered a healthy food and recommended by nutritionists and doctors as an ideal food to take care of almost all the nutritional requirements of man in all stages. Fish are of tremendous importance as food for people around the world, either collected from the wild or farmed in much the same way as cattle or chickens. It has high consumer preference due to its inherent nutritive values, taste and easy digestibility.

Fish is reservoir of large number of microorganisms. Bacteria are the main constituent of the gut microbiota in fish (1,2). The importance of intestinal bacteria in the nutrition and well-being of their hosts has been established for homeothermic species, such as birds and mammals (3). Fish receive bacteria in the digestive tract from the aquatic environment through water and food that are populated with bacteria. Being rich in nutrient, the digestive tract of fish confers a favourable culture environment for the microorganisms. Fish and fish products are eaten raw in many cultures, and these raw foods can be a route for direct transmission of pathogen.Many reports have demonstrated that Gram-negative, facultative anaerobic bacteria such Acinetobacter, Alteromonas. Aeromonas, as Bacteroides, Cytophaga, Flavobacterium, Micrococcus,

*Moraxella, Pseudomonas, Proteobacterium* d *Vibrio* spp. constitute the predominant endogenous microbiota of a variety of species of marine fish (4-9).

Foodborne illness usually arises from improper handling, preparation, or food storage. Good hygiene practices before, during, and after food preparation can reduce the chances of contracting an illness. *E.coli* was first recognized as food borne pathogen. Fish is highly perishable and should be handled with great care to preserve the natural attributes of fish and prevent microbial proliferation. With increasing demand for environment friendly aquaculture, the use of alternatives of antibiotic growth promoters in fish nutrition is now widely accepted.

## 2. MATERIALS AND METHODS

## 2.1. Collection of samples

Fish samples were collected from Ukkadam fish market, Coimbatore. Collected samples were packed in sterile plastic cover and transported to laboratory. The samples were stored in-20°C deep freezer for further use. The intestine was excised from the fish sample and was dropped into nutrient broth and overnight grown culture was taken and plated onto Nutrient agar medium and incubated at 37°C. The pure culture was grown in slants and were

\*Correspondence: R. Subashkumar, Associate Professor and Head PG and Research Department of Biotechnology, Kongunadu Arts and Science College, Coimbatore – 641 029, Tamil Nadu, India. E-mail.: rsubashkumar@gmail.com stored in 50% glycerol and stored at -20°C deep freezer.

# 2.2. Biochemical test

The isolates were subjected to different biochemical tests such as staining, Indole test, methyl red test, Voges Proskauer test, citrate utilization test, carbohydrate, catalase, oxidase, motility, protease, amylase, lipase test in order to find the biochemical characteristics of microorganisms.

# 2.3. Antibiotic sensitivity profiling

Antibiotic sensitivity of the isolates was determined on nutrient agar medium (Himedia). About 6 different antibiotic discs were used in this study and resistance profile of the isolates was obtained. Zone of inhibition was noted. Multiple antibiotic resistances (MAR) index was also calculated.

# 2.4. Haemolytic activity

Blood agar medium was prepared (5 mL of blood in100mL nutrient agar)and poured on to presterilized pertiplates under aseptic condition. The isolates were spot inoculated on the plates and incubated at 37°C overnight. Haemolysis was noted as zone of clearance around the isolate inoculated.

# 2.5. Extracellular protein precipitation

The isolates showing  $\beta$  haemolysis were subjected to TCA/Acetone precipitation method. About 1mL of culture supernatant was mixed with 8mL of ice cold ethanol and 1mL of Trichloro acetic acid and mixed gently. The tube was incubated at -20°C for one hour. The tube was centrifuged at 10,000rpm for 15 minutes at 4°C and the pellet

obtained was air dried and dissolved in  $250\mu L$  of PBS.

# 2.6. Agar well diffusion method

Agar well diffusion was performed on blood agar plates to confirm whether the precipitated proteins had the hemolytic property.  $50\mu$ L of the precipitated protein was added into the well and incubated at 37°C overnight. The zone of clearance was noted as hemolytic positive proteins.

# **3. RESULTS**

# 3.1. Sample collection

A total of 7 fishes were collected and their biological names were identified based on their local term. Upon culturing of the fish intestine, bacterial colonies were obtained on plates and 15 isolates were selected based on the morphological characteristics of the bacteria.

Table	1.	Fish	samples	collected	for	intestinal
bacter	ial	analy	sis			

S. No.	Common name	Scientific name
1	Mullan	Gazzaachlamys
	(Toothpony)	
2	Aylai (Mackerel)	Rastrelligerkanagurta
3	Oodalam (Flying	Potanichthysxingyiensis
	fish)	
4	Kilangan (White	Coregonusclupeaformis
	fish)	
5	Sangara (Red	Lutjanuscampechanus
	snapper)	
6	Parai (Malabar	Carangoidesmalabariws
	travelly)	
7	Ooli (Great	Sphyraneda barracuda
	barracuda)	

# Table 2. Biochemical characterization of the bacterial isolates

Fish	Cultur e name	Gram staining	Indole	Mr	Vp	Citrate utilisati on test	Carboh ytrate	Oxid ase	Catal ase	Motil ity	Amyl ase	Prote ase	Lipa se
	SA 01	+	-	-	-	+	-	+	+	+	+	+	-
Sankara	SA 02	+	-	+	+	+	+	+	-	+	+	+	-
Salikara	SA 03	+	-	+	+	+	+	+	-	-	+	+	-
	SA 04	+	+	+	+	+	+	+	+	+	+	-	+
Kelangan	KEL 01	-	+	+	-	+	+	+	+	+	+	-	+
	KEL 02	+	-	+	-	-	+	+	-	+	+	+	-
	PA 01	-	-	-	+	+	+	-	+	+	+	-	+
Parai	PA 02	-	+	+	-	+	+	+	-	+	+	+	+
	PA 03	+	+	+	+	+	+	+	-	+	-	+	+
0 - 1 - 1	00D 01	-	+	-	+	+	+	-	+	-	+	-	+
Oodalam	00D 02	-	+	+	+	+	+	+	-	-	+	+	+
Ooli	00L 01	-	+	+	+	-	+	+	+	-	+	-	+
	00L 02	+	+	+	+	+	+	+	-	-	+	+	+
Iylai	IY 01	+	-	+	-	-	-	+	+	+	+	+	-
Mullan	MUL 01	-	+	+	+	+	+	+	-	+	-	+	+

## 3.2. Biochemical characterization

All the strains were subjected to biochemical characterization and their phenotypic characteristics were noted. Both Gram negative and Gram positive isolates were distributed among the isolates.

## 3.3. Antibiotic sensitivity profiling

The antibiotic resistances of isolates were checked using six different antibiotics dics and the results were tabulated. About 80% of the isolates showed resistance towards Co-Trimoxazole ( $25\mu g$ ) and Tetracyclin ( $30\mu g$ ), 66.7% of the isolates showed resistance to Carbenicillin ( $100\mu g$ ), 33.3 % of isolates showed resistance to Vancomycin ( $30\mu g$ ), 53.3% of isolates showed resistance to Cefazolin ( $30\mu g$ ) and 60% of isolates showed resistance to Clindamycin ( $2\mu g$ ).

Table 3. Antibiogram assay for the bacterial isolates

Isolate	CO- TRI*	CAR*	TET*	VAN*	CEF*	CLI*
SA 01	+	+	+	+	+	+
SA 02	+	-	+	-	+	+
SA 03	+	+	+	-	-	-
SA 04	-	-	-	-	-	-
KEL 01	+	+	+	+	+	-
KEL 02	+	+	+	+	+	+
PA 01	+	+	+	-	+	+
PA 02	+	+	+	+	-	-
PA 03	-	-	-	-	-	-
00D 01	+	+	+	-	+	+
00D 02	+	+	+	-	+	-
00L 01	+	+	+	-	-	+
OOL 02	+	+	-	-	-	+
IY 01	+	-	+	-	+	+
MUL 01	-	-	+	+	-	+

\*CO-TRI – Co-Trimoxazole; CAR-Carbenicillin; TET-Tetracyclin; VAN-Vancomycin; CEF- Cefazolin; CLI-Clindamycin.

#### 3.4. MAR index value

The MAR (Multiple Antibiotic Resistance) index was calculated and the results were tabulated. The MAR index value clearly indicates the rate of resistance and sensitivity of the isolates which is very important to find out the pathogenicity.

#### Table 4. MAR index value

S. No.	Bacterial isolate	MAR index value
1.	SA 01	0.83
2.	SA 02	1.2
3.	SA 03	1.0
4.	SA 04	3.0
5.	KEL 01	1.2
6.	KEL 02	1.2
7.	PA 01	0.83
8.	PA 02	2.0
9.	PA 03	0.83
10.	OOD 01	0.67
11.	OOD 02	1.2
12.	00L 01	1.0
13.	OOL 02	1.2

14.	IY 01	1.0	
15.	MUL 01	1.0	

#### 3.5. Hemolytic activity

The hemolytic activity of the isolates confirmed the pathogenecity of the organism. Out of 15 strains, 5 strains showed  $\alpha$ - haemolysis, 4 strains showed  $\beta$ - haemolysis and 6 strains showed  $\gamma$ -haemolysis. Generally it is considered that the  $\beta$ -haemolytic strains are highly pathogenic.

Table 5. Haemolytic activity of the isolated strains

Culture Name	Growth	α	β	Ŷ
SA 01	+	-	-	+
SA 02	+	-	+	-
SA 03	+	-	-	+
SA 04	+	-	-	+
KEL 01	+	+	-	-
KEL 02	+	+	-	-
PA 01	+	-	-	+
PA 02	+	+	-	-
PA 03	+	-	+	-
00D 01	+	+	-	-
00D 02	+	-	-	+
OOL 01	+	-	+	-
OOL 02	+	-	+	-
IY 01	+	-	-	+
MUL 01	+	+	-	-

## 3.6. Extracellular protein precipitation

The supernatant of the strains OOL 01, SA 02, PA 03 and OOL 02 were taken. In order to make, confirmation where the haemolytic substance produced by the isolates were proteins, the precipitated proteins were loaded on to blood agar plate which shows haemolysis positive, confirming the haemolytic substance is proteinaceous in nature. **Table 6. Confirmation of \beta haemolysis caused by** 

extracellular proteins

CALIACCITAL	ai pi otein			
ISOLATES	10µL	20 µL	40 µL	50 µL
00L 01	+	+	+	+
SA 02	+	+	+	+
PA 03	+	+	+	+
OOL 02	-	+	+	+

## 4. DISCUSSION

Fish is considered as a healthy food and recommended by nutritionists and doctors as an ideal food to take care of almost all the nutritional requirements of man in all stages. Human infections due to many pathogenic bacteria are reported to have been transmitted through fin fish, shell fish and other sea food products. A high incidence of pathogen undoubtedly originates from the frequent consumption of Marine foods in these countries. In the present study, about 7 fish isolates collected and 15 isolates selected depend upon their phenotypical characteristics and morphology. The higher percentage of incidence were recorded which may be attributed to post-harvest contamination during handling, transportation, and selling through 96

fishermen and fish vendors (10,11). The antibiotic resistance and sensitivity of the isolates were checked using six different antibiotics and the isolates showed high resistance towards antibiotics. Multidrug resistance poses a serious clinical problem in the treatment of cancer and infectious diseases and is responsible for many tens of thousands of deaths each year (12,13). Increasing resistance of bacterial species to various antimicrobial agents and chlorine in potable water presents a significant threat to public health. Hemolysins are exotoxinprotein produced by bacteria which causes lysis of red blood cellsin vitro. Bacterial species is known to produce a variety of (14, 15).virulence factors Among them, haemolysin is the important one, also considered as the primary toxin, produced by most of the pathogenic bacteria (11,16,17). The present study showed high haemolytic activity and strong pathogenicity. The extracellular products from hemolysin also showed virulence towards fish and human. It emphasise that the respective protein of haemolysin is able to cause the diseases in human and animals (18). Based on the results obtained and the previous reports, it is concluded that the hemolysin and antibiotic resistance profile might be used to understand the existence of virulent bacterial isolates in diverse environments. It exhibits the presence of strain diversity among the strains and species. This study indicates that the presence of *A. hvdrophila* with virulence potential in fish samples collected from the study area, which may be a major threat to public health.

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## **BRITISH JOURNAL OF CANCER: A SCIENTOMETRIC STUDY**

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## ABSTRACT

The present study examined scientific publication research productivity in British journal of cancer for a period of selected 11 years between 2005 and 2015. Making use of various scientometric indicators like the annual growth rate, research document type, author productivity, Degree of collaboration, country wise Distribution, Institution wise distributionwas also used to analyze the data and interpretation. The study reveals that total 6818 records were published in the 264 issues of the journal.

Keywords: Authorship Pattern, Bibliometrics Analysis, Cancer, Publication Analysis, Scientometric analysis.

# **1. INTRODUCTION**

British journal of cancer (BJC) has been considered as a source journal for this study. BJC as a quarterly publication in 1947 to 1998, its popularity has led to a doubling in size and a move to fortnightly publication, the first issue of volume 1 and number 1 was published in March 1947 on behalf of Cancer Research UK by Nature Publishing Group, a division of Macmillan Publishers Ltd.Scientometrics analysis has been utilized by many research scholars to investigate conceptual network in different discipline in the most recent couple of decades. For this study, we have picked a couple of them and showed here.

Bharvi Dutta and Khaiser Nikam (1) the present study observed 10905 world publication output in solar cell research for five different years. Xiuwen Chen et al. (2) in this paper, a co-word method based on keywords from funded project is proposed to map the research trends. Dhawan *et al.* (3) the paper presents an analytical study of the research output in an e - publishing fieldin a series of scientometric indicators. As seen from Scopus database the total world output was 7010 publications published in 10 years during 2005-2014. Gnanasekaran and Balamurugan (4) this paper aim to identify the growth of literature on Intellectual Property Rights (IPRs) over a period of 10 years from 2005 to 2014. Ashok Kumar *et al.* (5) the present paper attempts to study the performance of India in RFID research using a series of bibliometric indicators. As seen from SCOPUS database, India's research output cumulated to 632 publications in 10 years during 2006-15. Kalmer Lauk (6) the present paper attempts to study the bibliometrical analysis of research published in oil shale. Palaniappan *et al.* (7) the study examined the

Bibliometric analysis of Indian Journal of Agricultural Research during 2010-2014. Marco Pautasso (8) the present paper attempts to study the Scientometrics of Forest Health and Tree Diseases. Prasad *et al.* (9) this study analyses, research output during 1989-2014 on the Himalayas. Sachithanantham and Raja (10) this study focuses the comparative analysis on the research publications in India and China on the rabies vaccine during 1980-2014. Sangam and Uma Arali (11) this study briefly explain Growth versus scientific collaboration in the field of genetics: A Senthilkumar scientometrics analysis. and Muthukrishnan (12) this study examined the Scientometric Analysis of Research Paper Published on Journal of Thoracic Oncology during 2006-2015.

## **2. OBJECTIVES**

Scientometric methods were used to analysis the research publications published in the British journal of cancer (BJC) during the selected eleven years between 2005 and 2015. The objectives of the present study are

- ✓ To analysis the year wise contribution of research publications during the selected period
- ✓ To determine the annual growth rate (AGR) of articles
- ✓ To examine the authorship pattern of the publications.
- ✓ To find out the author productivity of BJC
- ✓ To determine the Degree of collaboration (DC), and collaborative index (CI) of BJC
- ✓ To find out the country wise distribution of research papers during the selected period

✓ To analysis institution wise distribution of research publications.

# **3. METHODOLOGY**

All required data were collected from the "Thomson Reuters - Web of Science" database (WoS) and the search was completed on 10 November 2016 to download all the publications. The literature search was conducted via "Publication Name" search (SO) the term "British journal of cancer" selected in the search field and the timespan 2005-2015 was used as a restriction for the publication data. Finally 6818 publications were selected as the samples and these publications organized the database for further analysis.

The downloaded records were analyzed the standard Scientometric procedure to analysis various parameters like relative growth rate (RGR), doubling time (DT), Authorship Pattern (AP), Degree of collaboration (DI), Time Series Analysis (TA) etc.

4.	ANALYSIS	OF	THE	DATA	AND
INTE	RPRETATION	S			

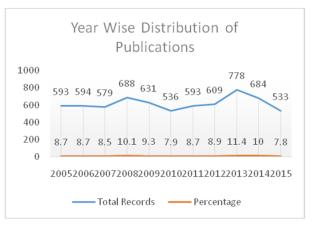
Year-Wise Distribution of Article Publications

**Table 1. Year Wise Distribution of Publications** 

S No	Year	Volume No.	Total Records	%
1	2005	92-93	593	8.7
2	2006	94-95	594	8.7
3	2007	96-97	579	8.5
4	2008	98-99	688	10.1
5	2009	100-101	631	9.3
6	2010	102-103	536	7.9
7	2011	104-105	593	8.7
8	2012	106-107	609	8.9
9	2013	108-109	778	11.4
10	2014	110-111	684	10
11	2015	112-113	533	7.8
		Total	6818	100

Analysis of the data indicates that the annual research output in BJC nearby around 09% of the total output during 2005-2015 are given in Table 1 and Fig. 1 above., the average number of article publication was 619.81 articles per year. According to the findings observed, it could be said that the numbers of research documents published from 2005 to 2015 are considerably closer to each other.It has been observed that the year 2013 has the highest number of publications (11.4%) followed by 2008 (10.1%), 2014 (10%) and 2009 (9.3%) respectively.

The year of 2007 has the lowest publication among the 11 years.



#### Fig. 1. Year Wise Distribution of Publications

## 4.1 Relative Growth Rate (Rgr)

The Relative Growth Rate (RGR) is the increase in the number of articles/ pages per unit of time. This definition is derived from the definition of relative growth rates in the study of growth analysis of individual plants and effectively applied in the field of Botany Hunt (1919), Blackman (1919) defined, which in turn had its origin from the study of the rate of interest in the financial investment. The mean Relative Growth rate (R) over the specific period of the interval can be calculated from the following equation.

R

1-2 = Loge 2 W - loge IW

Whereas, 1-2 R = mean relative growth rate over the specific period of interval.

Loge IW = logs of initial number of Articles.

Loge 2 W = logs of the final number of articles over a specific of the period of the interval.

2 T - 1 T = the unit difference between the initial time and final time.

The year can be taken here as the unit of time. The RGR for articles is hereby circulated. Therefore,

1-2 (aa-1 year-1) can represent the mean relative growth rate per unit of the year over a specific period of the interval.

#### 4.2 Doubling Time (Dt)

There exists a direct equivalence between the relative growth rate and the doubling time. If the numbers of articles/pages of subject double during a given period, then the difference the logarithms of numbers at the beginning and end of this period must be logarithms of number 2. If natural logarithm is used, this difference has a value of 0.693. Thus the corresponding doubling time for each specific period of interval and for both articles and pages can be calculated by the formula,

Doubling time (Dt) =0.693/R(p)

Therefore, Doubling time for articles D (t) = 0.693/1-2 R (aa-1 year-1)

Table 2. Relative growth rate (RGR) andDoublingTime (DT) of publications

S. No	Year	Total Reco	%	Cumulati ve	W1	W2	RG R	DT
		rds						
1	2005	593	8.7	593		6.38		
2	2006	594	8.7	1187	6.38	7.07	0.69	1.00
3	2007	579	8.5	1766	7.07	7.47	0.40	1.73
4	2008	688	10.1	2454	7.47	7.80	0.33	2.10
5	2009	631	9.3	3085	7.8	8.03	0.23	3.01
6	2010	536	7.9	3621	8.03	8.19	0.16	4.33
7	2011	593	8.7	4214	8.19	8.34	0.15	4.62
8	2012	609	8.9	4823	8.34	8.48	0.14	4.95
9	2013	778	11.4	5601	8.48	8.63	0.15	4.62
10	2014	684	10	6285	8.63	8.74	0.11	6.30
11	2015	533	7.8	6818	8.74	8.82	0.08	8.66
	Total	6818	100	40447				

Table 2 represents RGR and DT for publications for the period 2005-2015, that its relative growth rates has decreased from 2006 (0.69) to 2015 (0.08) in the 11 year period. The Doubling time increased from 1 in 2006 to 8.66 in 2015 and the doubling time is highest in the year 2015 with 8.66. It is clear that the relative growth rate and the doubling time are inversely correlative.

## 4.3 Authorship Patterns

Table 3 represents that the particulars about the authorship pattern of research articles published during the period of study. A total of 57553 authors has contributed the 6818 articles and the average number of authors per article observed to be 8.44. Among 6818 articles. 193 (2.83%) articles are written by a single author and 6625 (97.17%) articles are written by multiple authors. It could be identified that the Six authored articles involved highest percentage 659 (9.67%), seven authored articles 640 (9.39%) after eight authored articles 630 (9.24%) of the aggregate 6818 articles and nine to seventeen authored contributions are between 8 to 1 percent. The above seventeenauthored contributions are below one percent of the articles.In this way, indicating unmistakably the increased pattern towards multiple authorship is dominant as compared to single authorship.

Table 3. Presenting the Authorship pattern of BJC

S. No	No of Authors	No of Public ations	%	AP	%
1	Single	193	2.83	193	0.34
2	Two	347	5.09	694	1.21
3	Three	411	6.03	1233	2.14

4	Four	491	7.20	1964	3.41
5	Five	556	8.15	2780	4.83
6	Six	659	9.67	3954	6.87
7	Seven	640	9.39	4480	7.78
8	Eight	630	9.24	5040	8.76
9	Nine	599	8.79	5391	9.37
10	Ten	498	7.30	4980	8.65
11	Eleven	427	6.26	4697	8.16
12	Twelve	316	4.63	3792	6.59
13	Thirteen	259	3.80	3367	5.85
14	Fourteen	185	2.71	2590	4.50
15	Fifteen	132	1.94	1980	3.44
16	Sixteen	120	1.76	1920	3.34
17	Seventeen	75	1.10	1275	2.22
18	Eighteen	54	0.79	972	1.69
19	Nineteen	43	0.63	817	1.42
20	Twenty	44	0.65	880	1.53
21	Twenty+	139	2.04	4554	7.91
	Total	6818	100	57553	100

AP = Authorship pattern.



# Fig. 2. Presenting the authorship patternof BJC

## 4.4 Degree of author's collaboration

Table 4 Fig. 3 represents the degree of collaboration of BJC during the period of study between 2005 and 2015. It was statistically calculated using by the formula given by K Subramanyam,the mathematical deduction of the formula is

$$C = \frac{Nm}{Nm + Ns}$$

Where, C = Degree of collaboration in a discipline

Nm = Number of multi-authored papers in the discipline

Ns = Number of single-authored papers in the discipline

Table 4. Degree of Collaboration of BJC

S	Year	Single	%	Multi	%	D
1	2005-	193	2.	6625	97.	
Here	e, Nm = 662	5, Ns = 193,				

$$C = \frac{6625}{(625 + 102)}$$

$$= 0.97$$
, Thus, the degree of collaboration (C) is 0.97

The analysis of Table 4 shows that the degree of collaboration during the period of study between 2005 and 2015 is 0.97. The single authored Table 5. Year Wise Degree of Collaboration of BJC

articles are covered only 193 (2.83%) during the years. The multi authored articles 6625 (97.17%) are maximum throughout the years. which obviously shows its strength upon multi authored collaborative research. However, when we analysis the year-wise degree of collaboration for 11 years, the outcomes arise different and the mean value is 0.97.

S. No.	Year	SAP (Ns)	%	MAP (Nm)	%	Total (Nm+Ns)	%	DC
1	2005	25	12.95	568	8.57	593	8.70	0.96
2	2006	22	11.40	572	8.63	594	8.71	0.96
3	2007	15	7.77	564	8.51	579	8.49	0.97
4	2008	29	15.03	659	9.94	688	10.09	0.96
5	2009	17	8.81	614	9.27	631	9.25	0.97
6	2010	11	5.70	525	7.92	536	7.86	0.98
7	2011	24	12.44	569	8.59	593	8.70	0.96
8	2012	15	7.77	594	8.96	609	8.93	0.98
9	2013	19	9.84	759	11.45	778	11.41	0.98
10	2014	11	5.70	673	10.16	684	10.03	0.98
11	2015	5	2.59	528	8.00	533	7.82	0.99
	Total	193	100.00	6625	100.00	6818	100.00	Mean = 0.97

\*SAP = Single Authored Paper, \*MAP = Multi Authored Papers, \*DC = Degree of Collaboration

According to year wise analysis Table, 5 speaks to the year wise number of multi-authored articles and their degree of collaboration. In the study, the degree of collaboration was not a constant value, it reveals the variation of 0.96 to 0.99 and the mean value as 0.97. The analysis found that single author papers continuously reduced every year and the multi-authorship pattern is constantly stable above 7.82%.

MAP (Y)

# 4.5 Time series analysis

		-	-			
S. No.	Year	SAP (Y)	X	<b>X</b> <sup>2</sup>	XY	
1	2005	25	-5	25	-125	
2	2006	22	-4	16	-88	
2	2007	15	2	0	45	

# **Table 6. Time Series Analysisof BJC**

5	1041	5 (1)						<u> </u>	
1	2005	25	-5	25	-125	568	-2840	593	-2965
2	2006	22	-4	16	-88	572	-2288	594	-2376
3	2007	15	-3	9	-45	564	-1692	579	-1737
4	2008	29	-2	4	-58	659	-1318	688	-1376
5	2009	17	-1	1	-17	614	-614	631	-631
6	2010	11	0	0	0	525	0	536	0
7	2011	24	1	1	24	569	569	593	593
8	2012	15	2	4	30	594	1188	609	1218
9	2013	19	3	9	57	759	2277	778	2334
10	2014	11	4	16	44	673	2692	684	2736
11	2015	5	5	25	25	528	2640	533	2665
	Total	193	0	110	-153	6625	614	6818	461

\*SAP = Single Authored Paper, \*MAP = Multi Authored Papers, \*CP = Collaborative Papers

4.5.1 Single authored publications: time series analysis

The straight line equation is applied to arrive at projections for future growth under Time Series analysis. The Straight Line equation Yc = a+bX since  $\Sigma x = 0$ ,  $a = \Sigma Y/N$ ,  $\Sigma Y =$  (Total Number of Paper by Single Author), N = (Number of Years), a = 193/11, a = 17.54, b= $\Sigma XY / \Sigma$ ,  $\Sigma XY$  = (Total of XY Tables),  $\Sigma =$  (Total of X2 Table), b= -153/110, b=-1.39.

XY

CP (Y)

XY

Estimated literature in 2020 is, When X = 2020-2010(Mid-Year), X = 10, Apply Straight line equationYc = a+bX since  $\Sigma x = 0$ , Yc = 17.54+-1.39\*10, Yc = 17.54+13.9, Yc = 3.64. The time series analysis found that single author papers continuously reduced every year.

# 4.5.2 Multi authored publications: time series analysis

Straight Line equation Yc = a+bX since  $\Sigma x = 0$ , a= $\Sigma Y/N$ ,  $\Sigma Y =$  (Total Number of Paper by Multi Author), N = (Number of Years), a = 6625/11, a = 602.27, b= $\Sigma XY/\Sigma$ ,  $\Sigma XY =$  (Total of XY Tables),  $\Sigma =$  (Total of X2 Table), b= 614/110, b= 5.58.

Estimated literature in 2020 is, When X = 2020-2010(Mid-Year) X = 10,Apply Straight line equation, Yc = a+bX since  $\Sigma x = 0$ , Yc = 602.27+5.58\*10, Yc = 602.27 + 55.8, Yc = 658.07. The time series analysis also prove the multi-authorship pattern is constantly stable.

# 4.5.3 Collaborative publications: time series analysis

Straight Line equation Yc = a+bX since  $\Sigma x = 0$ , a= $\Sigma Y/N$ ,  $\Sigma Y =$  (Total Number of Paper by Multi Author),N = (Number of Years), a = 6818/11, a = 619.81, b= $\Sigma XY/\Sigma$ ,  $\Sigma XY =$  (Total of XY Tables),  $\Sigma =$  (Total of X2 Table), b= 461/110, b = 4.19.Estimated literature in 2020 is, When X = 2020-2010(Mid-Year), X = 10, Apply Straight line equationYc = a+bX since  $\Sigma x = 0$ , Yc = 619.81+4.19\*10, Yc = 619.81+41.9, Yc = 661.71

On the application of the formula of time series analysis for the expectation of BJC research output for the year 2020, it was found that the future trend and development in BJC research output may take an decreasing trend in single authored publications (Yc = 3.64) during the years to come and collaborative publications trends is constantly stable(Yc = 658.07).

# **5. CONCLUSION**

The findings of the study are summarized as follows.

- ✓ The numbers of research documents published from 2005 to 2015 are considerably closer to each other.
- ✓ It is clear that the relative growth rate and the doubling time are inversely correlative.
- ✓ It could be identified that the six authored articles involved highest percentages 659 (9.67%), in this way, indicating unmistakably the increased pattern towards multiple

authorship is dominant as compared to single authorship.

✓ It was found that the future trend and development in BJC research output may take a decreasing trend in single authored publications (Yc = 3.64)

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# MAPPING OF RESEARCH PRODUCTIVITY IN SRM UNIVERSITY: A SCIENTOMETRIC STUDY

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## ABSTRACT

This paper discusses about the published research articles and its citation available in the Indian Citation Index by the authors from SRM University. The relevant data are collected from Indian Citation Index and it was analyzed. It shows among the 510 articles, the maximum of 157(30.78%) articles published in 2015 and minimum of 1 (0.20%) articles published in 2005. Based on the citation during the period 153 citations were made. Among the 153 Citations, maximum of 32 (20.92%) citations in 2010 and minimum number of citation 1 (0.65%) in 2006 & 2007, was identified.

**Keywords:** Mapping, Research Productivity ICI, Year wise Distribution, Citation Analysis, SRM University, Scientometric.

## **1. INTRODUCTION**

The true barometer of assessing the quality and quantity of a journal is the Citation Index. While discussing citation, one needs to understand the citation. Simply, when another refers other works in his/her article, we call the article referred is cited. In other words the citation is called as the previous work which is referred in the present work. The quality of a given work can rightly be adjudged through the number of citations that it gets. Therefore, a certain 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 Scopus, Web of Science, or even Google Scholars to know the impact of a journal, a particular article or a particular author. Indian Citation Index which was initiated by Diva Enterprises is just an indexing and abstracting database.

## 2. REVIEW OF LITERATURE

Nicholas and Ritchie (1) view that, "study of bibliometrics concept provides information, knowledge and how it is communicated". Moreover, bibliometric studies are normally employed to evaluate the academic research output, the quality of the journal, impact and influence of articles, authors, and assorted parameters. Though there has been substantial growth of literature on bibliometric studies during the last decade, the authors focus on some of the pertinent literature that relate to the present study. Potter (2) defines bibliometric analysis as "the calculation and study of the research publication patterns of all types of written communication and their authorship nature". In a most interesting study Mooghali *et al.* (3) analyzed records of three premiere indexes known as, "SSCI", "SCI", and "AHCI", and it is projected in the field of "scientometrics" evolved between 1980 to 2009. The pattern of growth of literature in the field of Nanoscience during 1990 to 2009 was reported by Karpagam *et al.* (4). In the similar vein, Abramo (5) exercised bibliometric techniques on some national level research assessment. Lapon-Kandeishein and Prebor (6) bibliographical research on Hebrew printing also needs mention. In the similar light bibliometric studies by veterans like Krampen et al. (7), Kumar Suchetan (8) and others also presented findings on different directions. Dhanavandan and Tamizhchelvan (9) studied citations and research productivity of south Tamil Nadu universities from 2009 to 2013 based on Indian Citation Index (ICI).

## **3. METHODOLOGY**

This study aims to discuss about the analysis of the citation index of the research output by faculty members of SRM University. The relevant sources and data are collected from Indian Citation Index. Based on the available sources the following discussions are made.

## 4. ANALYSIS AND INTERPRETATION

The distributions of the research output by the authors from SRM University that are available in

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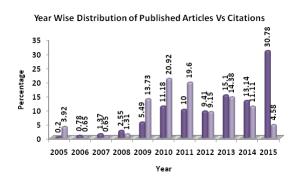
Indian Citation Index were analyzed in the table 1.

4.1. Year wise distribution of published articles vs citations

Table 1. Year Wise Distribution of Published Articles vs Citations.

S. No. Year	Article	s %	Citation	%	Citation Density	Article/Citation
1 2005	1	0.20	6	3.92	6.000	0
2 2006	4	0.78	1	0.65	0.250	4
3 2007	7	1.37	1	0.65	0.143	7
4 2008	13	2.55	2	1.31	0.154	7
5 2009	28	5.49	21	13.73	0.750	1
6 2010	57	11.18	32	20.92	0.561	2
7 2011	51	10.00	30	19.60	0.588	2
8 2012	48	9.41	14	9.15	0.292	3
9 2013	77	15.10	22	14.38	0.286	4
10 2014	67	13.14	17	11.11	0.254	4
11 2015	157	30.78	7	4.58	0.045	22
Total	510	100	153	100	9.323	56

The above Table shows that the year wise distribution of articles published by the various authors from SRM University. From 2005 to 2015, 510 articles were published which are indexed in Indian Citation Index. Among the 510 maximum of 157 (30.78%) articles published in 2015 and minimum of 1 (0.20%) articles published in 2005. Based on the citation study during the period 153 citations were made. Among the Citations, maximum of 32 (20.92%) citations in 2010 and minimum number of citation 1 (0.65%) in 2006 & 2007, was identified.



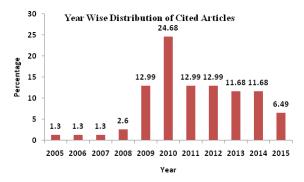
4.2. Year wise distribution of cited articles& cited density

Table 2. Year Wise Distribution of Cited Articles& Cited Density

S. No.	Year	Cited Articles	%	Cited Density	Article/Cited Articles
1	2005	1	1.30	1.000	1.000
2	2006	1	1.30	0.250	4.000

3	2007	1	1.30	0.143	7.000
4	2008	2	2.60	0.154	6.500
5	2009	10	12.99	0.357	2.800
6	2010	19	24.68	0.333	3.000
7	2011	10	12.99	0.196	5.100
8	2012	10	12.99	0.208	4.800
9	2013	9	11.68	0.117	8.556
10	2014	9	11.68	0.134	7.444
11	2015	5	6.49	0.032	31.400
	Total	77	100	2.924	81.600

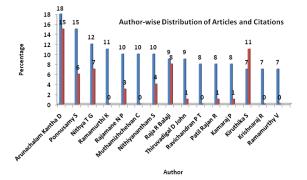
The above Table presents the year wise distribution of Cited articles, Cited density and Article/ Cited articles published by the various authors from SRM University. From 2005 to 2015, 77 cited articles were available which are indexed in Indian Citation Index. Among the 77 cited articles maximum of 19 (24.68%) in 2010 and minimum of 1 (1.30%) cited articles in the years of 2005, 2006 and 2007. Based on the cited density during the period maximum of 1 in 2005 and minimum number of 0.032 in 2015, was identified.



*4.3. Author wise distribution of articles and citations (Top 15):* 

S. No.	Author	Articles	Citation	Citation Density
1	Arunachalam Kantha D	18	15	0.833
2	Ponnusamy S	15	6	0.400
3	Nithya T G	12	7	0.583
4	Ramamurthi K	11	0	0.000
5	Rajamane N P	10	3	0.300
6	Muthamizhchelvan C	10	0	0.000
7	Nithiyanantham S	10	4	0.400
8	Raja R Balaji	9	8	0.889
9	Thiruvadigal D John	9	1	0.111
10	Ravichandran P T	8	0	0.000
11	Patil Rajan R	8	1	0.111
12	Kamaraj P	8	1	0.125
13	Kiruthika S	7	11	1.571
14	Krishnaraj R	7	0	0.000
15	Ramamurthy V	7	0	0.000
	Total	149	57	5.323

The above table reveals that the author wise distribution of the articles published and citations are available in the Indian Citation Index. Only we consider in the top fifteen authors. Among the 15, Arunachalam Kantha D occupied the first position with 18 articles and 15 citations followed 104 by the author Ponnusamy S in the second position with 15 articles and 6 citations and Nithya T G in the third position with 12 articles and 7 citations (Ranked by Articles only). In the case of highest citations again Arunachalam Kantha D is in the first position with 15 citations and Kiruthika S occupies the second position with 11 citations and Raja R Balaji occupies the third position with 8 citations.



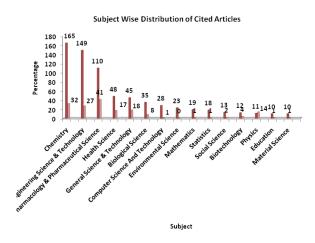
4.4. Subject wise distribution of cited articles (Top 15):

Table 4. Subject Wise Distribution of Cited
Articles (TOP 15)

				2
S. No.	SubjectCategory	Articles	Citation	3
1	Chemistry	165	32	4
2	Engineering Science & Technology	149	27	-
3	Pharmacology & Pharmaceutical Science	110	41	5 6
4	Health Science	48	17	_
5	General Science & Technology	45	18	7 8
6	Biological Science	35	8	9
7	Computer Science & Technology	28	1	10
8	Environmental Science	23	0	11
9	Mathematics	19	1	12
10	Statistics	18	1	
11	Social Science	13	2	13
12	Biotechnology	12	4	
13	Physics	11	14	14
14	Education	10	1	15
15	Material Science	10	1	. ———

The above Table presents the top 15 subjects it includes various articles published and cited from the SRM University that are available in the Indian Citation Index. As per the sources available in the Indian Citation Index. Among the articles 165 from Chemistry is in the first rank, 149 articles from Engineering Science & Technology with second rank and 110 articles from Pharmacology & Pharmaceutical Science subject in third rank were identified. It is revealed from the

table that Pharmacology & Pharmaceutical Science subject has the highest citations 41 when comparing toother subjects.

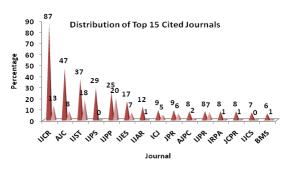


4.5. Distribution of top 15 cited journals

## Table 5. Distribution of Top 15 Cited Journals

S. No.	Publications	Articles	Citation
1	International Journal of Chemtech Research	87	13
2	Asian Journal of Chemistry	47	8
3	Indian Journal of Science & Technology	37	18
4	International Journal of Pharmaceutical Sciences: Review & Research	29	0
5	International Journal of Pharmacy &Pharmaceutical Sciences	25	20
6	International Journal of Engineering Science & Technology	17	7
7	International Journal of Advanced Research in Computer Science	12	1
8	Indian Concrete Journal (The)	9	5
9	Journal of Pharmacy Research	9	6
10	Asian Journal of Pharmaceutical &Clinical Research	8	2
11	International Journal of Pharmtech Research	8	7
12	International Review of Pure & Applied Mathematics	8	1
13	Journal of Chemical &Pharmaceutical Research	8	1
14	International Journal of Chemical Sciences	7	0
15	Bulletin of Materials Science	6	1
	Total	317	90

The data presented in the above table shows the top 15 journals articles published and cited by authors in SRM University. Among the top 15, International Journal of Chemtech Research occupies the first place with 87articles in the second place the Asian Journal of Chemistry with 47 articles and the third place in Indian Journal of Science & Technology with 37 based on the article publications. Based on the citation International Journal of Pharmacy &Pharmaceutical Sciences occupies the first position with 20 citations and Indian Journal of Science & Technology is in the second position with 18 citations.

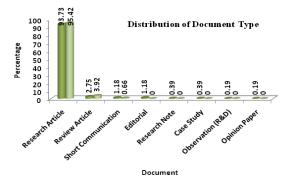


#### 4.6. Distribution of document type:

Table 6. Distribution of Document Type

S.	DocumentType	Articles	%	Citation	%
No.					
1	Research Article	478	93.73	146	95.42
2	<b>Review Article</b>	14	2.75	6	3.92
3	Short	6	1.18	1	0.66
	Communication				
4	Editorial	6	1.18	0	0
5	Research Note	2	0.39	0	0
6	Case Study	2	0.39	0	0
7	Observation	1	0.19	0	0
	(R&D)				
8	Opinion Paper	1	0.19	0	0
	Total	510	100	153	100

The above table expresses the distribution of document type based on the articles published from SRM University. It shows among the 510 articles, which includes 478(93.73%) Research Articles, 14(2.75%) Review Articles and 6(1.18%) Short communication and Editorial type. Among the 153 Citations, which include 146(95.42%) Research Articles type followed by 6(3.92%) Review Article and Short Communication type 1(0.66%). It is concluded that the highest articles and citations are from research article type of documents.



## **5. CONCLUSION**

Indian Citation Index to offer an easy-touse, reliable bibliographic and citation database to users. During the study period among the published articles 510 the maximum of 157(30.78%) articles published in 2015 and minimum of 1(0.20%) articles published in 2005. The research study shows that most of the years the number of article publications in gradually increasing. It shows the interest in publication of articles in SRM university. Based on the citation during the period 153 citations the maximum of 32(20.92%) citations in 2010 and minimum number of citation 1(0.65%) in 2006&2007, was identified. The cited articles study reveals that among the 77 cited articles maximum of 19(24.68%) in 2010 and minimum of 1(1.30%) cited articles in 2005-2007.Based on the cited density during the period maximum of 1 in 2005 and minimum number of 0.032 in 2015, was identified.. The Author Wise Distribution of Articles and Citations study tells that the author Among the 15, Arunachalam Kantha, D. occupied the first position with 18 articles and 15 citations followed by author Ponnusamy, S. in the second position with 15 articles and 6 citations and Nithya T G in the third position with 12 articles and 7 citations (Ranked by Articles only). In the case of highest citations again Arunachalam Kantha D is in the first position with 15 citations and Kiruthika, S. occupies the second position with 11 citations and Raja R Balaji occupies the third position with 8 citations. The Subject wise distribution of cited articles study reveals that the 165 articles from Chemistry is in the first rank, followed by Engineering Science & Technology with 149 articles and 110 articles from Pharmacology & Pharmaceutical Science subject in third rank were identified. It is revealed from the table that Pharmacology & Pharmaceutical Science subject has the highest citations 41 when comparing to other subjects. The Distribution of Top 15 Cited Journals depicts that, International Journal of Chemtech Research occupies the first place with 87 articles the second place in Asian Journal of Chemistry with 47 articles and the third place in Indian Journal of Science & Technology with 37 based on the article publications. Based on the citation International Journal of Pharmacy &Pharmaceutical Sciences occupies the first position with 20 citations and Indian Journal of Science & Technology is in the second position with 18 citations. The distribution of document type study shows that among the shows among the 510 published articles, it includes 478(93.73%) Research Articles, 14(2.75%) Review Article and 6(1.18%) Short communication and Editorial type. Among the 153 Citations, Research Articles are 146(95.42%) type followed by Review Articles with 6(3.92%) and Short Communication type 1 (0.66%). It is concluded that the highest articles

and citations are from research article type of documents.

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