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NONLOCAL CONTROLLABILITY OF IMPULSIVE FUNCTIONAL INTEGRO-DIFFERENTIAL EQUATIONS IN BANACH SPACES

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ABSTRACT

The paper is concerned with the controllability of impulsive functional integrodifferential equations with nonlocal conditions. Using the measure of noncompactness and Monch fixed point theorem, we establish some sufficient conditions for controllability and also our theorems extend some analogous results of (impulsive) control systems.

Keywords: Noncompactness, Integrodifferential equations.

1. INTRODUCTION

Impulsive differential equations are a class of important models which describes many evolution process that abruptly change their state at a certain moment,see the monographs of Bainov and Simonov (1993), Lakshmikantham *et al.* (1989) and have been studied extensively by many authors (Cuevas *et al.,* 2009; Fan and Li, 2010; Anguraj and Mallika Arjunan, 2009). On the other hand, the concept of controllability is of great importance in mathematical control theory. Many authors have been studied the control of nonlinear systems with and without impulses; see for instance (Guo *et al.,* 2004; Chen and Li, 2010; Ji *et al.,* 2011).

The starting point of this paper is the work in papers (Ji *et al.,* 2011; Jose *et al.,* 2013). Especially, authors in Jose *et al.* (2013) investigated the controllability results of mixed-type functional integro-differential evolution equations with nonlocal conditions

$$\begin{aligned} &x'(t) \\ &= A(t)x(t) \\ &+ f\left(t, x_t, \int_0^t h(t,s,x_s)\,ds, \int_0^b k(t,s,x_s)\,ds\right) \\ &+ Bu(t), \end{aligned} \tag{1.1}$$

$t\epsilon J = [0,b]\,, t \neq t_i, i = 1, \dots, s,$

$$\begin{aligned} &\Delta x|_{t=t_i} = I_i\left(x_{t_i}\right), i \\ &= 1, \dots, s, \end{aligned} \tag{1.2}$$

$$\begin{aligned} &x_0 \\ &= \emptyset + g(x)\,, t\epsilon[-r, 0], \end{aligned} \tag{1.3}$$

by using Monch fixed point theorem. And in (Ji *et al.,* 2011), authors studied the following controllability of impulsive differential systems with nonlocal conditions of the form

$$x'(t) = A(t)x(t) + f\big(t, x(t)\big) + (Bu)(t) \text{a.e} \quad \text{on } [0,b] \tag{1.4}$$

$$\begin{aligned} &\Delta x(t_i) = x(t_i^+) - x(t_i^-) = I_i\big(x(t_i)\big), i \\ &= 1, .., s. \end{aligned} \tag{1.5}$$

$$\begin{aligned} &x(0) + M(x) \\ &= x_0 \end{aligned} \tag{1.6}$$

Motivated by above mentioned works (Ji *et al.,* 2011; Jose *et al.,* 2013), the main work of this paper is to prove the controllability results of impulsive integro-differential systems with nonlocal conditions.

$$\begin{aligned} x'(t) &= A(t)x(t) + f\big(t, x(t)\big) \\ &+ \int_0^t h(t,s,x(s))\,ds \\ &+ (Bu)(t) \end{aligned} \tag{1.7}$$

$$\begin{aligned} &\Delta x(t_i) = x(t_i^+) - x(t_i^-) = I_i\big(x(t_i)\big), i \\ &= 1, .., s. \end{aligned} \tag{1.8}$$

$$\begin{aligned} &x(0) + M(x) \\ &= x_0 \end{aligned} \tag{1.9}$$

Where $A(t)$ is a family of linear operators which generates an evolution operator

$$\begin{aligned} U(t,s): \Delta = \{(t,s)\epsilon[0,b] \times [0,b]: 0 \leq s \leq t \leq b\} \\ \rightarrow L(X), \end{aligned}$$

here, X is a banach space, $L(X)$ is the space of all bounded linear operators in $X; f: [0,b] \times X \rightarrow X; G: [0,b] \times X \rightarrow X; O < t_1 < \dots\dots\dots < t_s < t_{s+1} = b; I_i = X \rightarrow X, i = 1, \dots, s$are impulsive functions; $M: PC([0,b]); X \rightarrow X$; B is a bounded linear

operators from a Banach space V to X and the control function$u(\cdot)$ is given in $L^2([0,b],V)$.

The paper is organized as follows: In section 2, we will recall some basic notations definition, hypothesis and necessary preliminaries. In section 3, we prove the controllability of impulsive integro-differential system with nonlocal system(1.7) –(1.9), usingMonch fixed point theorem.

2. PRELIMINARIES

In this section, we recall some basic definitions and lemmas which will be used to prove our main results of this paper.

Let (X,||. ||) be a real banach space .We denote by C([0,b];X) the space of X- valued continuous function on [0,b]with the norm $\|x\|$ = sup{ $\|x(t)\|$,t ε [0,b]} and by L^1([0,b];X) the space of X- valued Bochnerintegrable functions on[0,b] with the norm $\|f\|_{L^1} = \int_0^b \|f(t)\|$ dtS .

For the sake of simplicity, we put J=[0,b]; J_0 = [0,t_1]; $J_i = (t_i , t_{i+1}]$,i=1,……,s. In order to define the mild solution of problem (1.7)-(1.9), we introduce the set PC([0,b];X) = {u : [0,b]→X : u is]; continuous on J_i ,i = 0,1,….,s and the right limit u(t_i^+) exists, i = 1…..,s}. It is easy to verify that PC([0,b];X) is a banach space with the norm $\|u\|_{PC}$ = sup{ $\|u(t)\|$,t ε [0,b]} .

Definition 2.1: Let E^+ be the positive cone of an order Banach space (E,≤) . A function Φ defined on the set of all bounded subsets of the Banach space X with values in E^+ is called a measure of noncompactness (MNC) on X if Φ($\overline{co}\Omega$) = Φ(Ω) for all bounded subsets Ω ⊂ X,where $\overline{co}\Omega$ stands for the closed convex hull of Ω. The MNC Φ is said:

(1)Monotone if for all bounded subsets Ω_1, Ω_2 of X we have:

$(\Omega_1 \subseteq \Omega_2) \Rightarrow (\Phi(\Omega_1) \leq \Phi(\Omega_2))$;

(2)Nonsingular if Φ({a}∪ Ω) = Φ(Ω) for every a ϵX ,Ω ⊂ X;

(3)Regular if Φ(Ω) = 0 if only if Ω is relatively compact in x.

One of the most important examples of MNC is the noncompactness measure Of Hausdorff β defined on each bounded subset Ω of X by $\beta(\Omega)$= inf { ε> 0;Ω can be covered by a finite number of balls of radii smaller than ε}.for all bounded subset Ω, Ω_1, Ω_2 of X,

(1) $\beta(\Omega_1 + \Omega_2) \leq \beta(\Omega_1) + \beta(\Omega_2)$, where $\Omega_1 + \Omega_2 = \{x+y : x \epsilon \Omega_1, y \epsilon \Omega_2\}$

(2) $\beta(\Omega_1 \cup \Omega_2) \leq \max\{\beta(\Omega_1), \beta(\Omega_2)\}$;

(3) $\beta(\lambda\Omega) \leq |\lambda|\beta(\Omega)$ for any $\lambda \epsilon$ R;

(4) If the map Q: D(Q) ⊆ X →Z is Lipschitz continuous with constants k,then β_Z(QZ) ≤ k$\beta(\Omega)$ for any bounded subset $\Omega \subset D(Q)$, where Z is a Banach space.

Definition 2.2: A two parameter family of bounded linear operators U(t, s), 0 ≤ s ≤ t ≤ b on X is called an evolution system if the following two conditions are satisfied:

(i) U(s, s) = I, U (t, r)U(r, s) = U(t, s) for 0 ≤ s ≤ r ≤ t ≤ b;

(ii) U(t, s) is strongly continuous for 0 ≤ s ≤ t ≤ b

And there exists M_1> 0 such that U(t, s) ≤ M_1 for any (t ,s) ϵ T.

Definition 2.3: A function x (·) ϵ PC ([0,b];X) is a mild solution of(1.7)-(1.9) if

$$X(t) = U(t,0)[x_0 - M(x)] + \int_0^t U(t,s)\left[f\big(s,x(s)\big) + \int_0^s h(s,\tau,x(\tau))\,d\tau + Bu(s)\right]ds + \sum_{0<t_i<t} U(t,t_i)\,I_i(x(t_i)),$$

for all t ϵ [0,b], where $x(0) + M(x) = x_0$.

Definition 2.4: The system (1.7) –(1.9) is said to be controllable on the interval J if for every initial function $\varphi \epsilon$D and $x_1 \epsilon$ X, there exists a control u ϵL_2 (J, V) such that themild solution x(·) of (1.7) – (1.9) satisfies.$x(b) = x_1 + M(x)$.

Definition 2.5: A countableset $\{f_n\}_{n=1}^{+\infty} \subset L^1$([0,b];X) is said to be semicompact if:

(1) The sequence $\{f_n\}_{n=1}^{+\infty}$ is relatively compact in X for a.e. t ϵ [0,b]

(2) There is a function $\mu \epsilon L^1([0,b]; R^+)$ satisfying $sup_{n\geq 1}\|f_n(t)\| \leq \mu(t)$ for a.e.

tϵ [0,b].

Lemma 2.1: Let $\{f_n\}_{n=1}^{+\infty}$ be a sequence of function in L^1([0,b];R^+).Assume that there exist

$\mu, \eta \epsilon L^1([0,b]; R^+)$ satisfying $\overset{sup}{n\geq 1}\|f_n(t)\| \leq \mu(t)$ and $\beta(\{f_n(t)\}_{n=1}^{+\infty}) \leq \eta(t)$ a.e. t ϵ [0,b]. Then for all t ϵ [0,b], we have$\beta(\{\int_0^t U(t,s)f_n(s): n \geq 1\}) \leq 2M_1 \int_0^t \eta(s)\,ds$.

Lemma 2.2: Let (Gf) (t) = $\int_0^t U(t,s)\,f(s)ds$. If $\{f_n\}_{n=1}^{+\infty} \subset L^1$([0,b];X) is semicompact then the set $\{Gf_n\}_{n=1}^{+\infty}$ is relatively compact in C([0,b];X) and moreover , if $f_n \to f_0$, then for all t ϵ [0,b] ,

$(Gf_n)(t) \to (Gf_0(t)$as n$\to +\infty$.

Lemma 2.3: Let D be a closed convex subset of a Banach space X and 0ϵD. Assume that F: D$\to$X is a continuous map which satisfies Monch's condition, that is, M$\subseteq Discountable, M \subseteq \overline{co}(\{0\} \cup F(M)) \Rightarrow \overline{M}$ is compact. Then, there exists xϵD with x = F(x).

3. CONTROLLABILITY RESULTS

We first give the following hypothesis:

(H1) A(t) is a family of linear operators , A(t): D(A) $\to$ x, D(A) not depending on t and dense subset of X, generating an equicontinuous evolution system {U(t,s) : (t,s) $\in \Delta$}, i.e.,

(t,s) $\to \{U(t,s)x : x \in B\}$ is equicontinuous for t > 0 and for all bounded subsets B.

(H2) The function f: [0,b]$\times X \to X$ satisfies:

(i) For a.e. $t\epsilon[o,b]$,the function f(t,·):X $\to$X is continuous and for all xϵX,the function $f(\cdot, x)$:[0,b] $\to$X is measurable;

(ii)There exists a function m$\epsilon L^1([0,b];R^+)$ and a nondecreasing continuous function

$\Omega: R^+ \to R^+$such that$\|f(t,x)\| \leq m(t)\ \Omega(\|x\|)$, x $\epsilon X, t\epsilon[0,b]$and

$\lim_{n\to+\infty} inf \frac{\Omega(n)}{n} = 0.$

(iii) There exists h$\epsilon L^1([0,b];R^+)$ such that , for any bounded subset D$\subset X$,

$\beta\left(f\left(t,x(t)\right)\right) \leq h(t)\beta\left(x(t)\right)$fora.e. $t\epsilon[0,b]$, where β is the Hausdorff MNC

(H3) The function h:[0,b]$\times X \to X$ satisfies:

(i) For each t, $s\epsilon$[0,b],the function h(t,s,·):X $\to$X is continuous and for all xϵX,the

function h(·,·,x):[0,b] $\to$X is measurable;

(ii) There exists a function m$\epsilon L^1([0,b];R^+)$ such that

$\|h(t,s,x(s))\| \leq m(t,s)\|x(s)\|$, x$\epsilon X, t, s\epsilon[0,b]$ and $\lim_{n\to+\infty} inf \frac{x(n)}{n} = 0.$

(iii) There exists $\zeta\epsilon L^1([0,b];R^+)$ such that , for any bounded subset D $\subset X$,

$\beta\left(h\left(t,s,x(s)\right)\right) \leq \zeta(t,s)\beta\left(x(s)\right)$for a.e $t\epsilon J$,

For convenience let us take $L_0 = max \int_0^t m(t,s)ds$ and $\zeta^* = max \int_0^t \zeta(t,s)ds$

(H4) M: PC (J,X) $\to$ X is a continuous compact operator such that

$$\lim_{\|y\|_{PC}\to+\infty} \frac{\|M(y)\|}{\|y\|_{PC}} = 0;$$

(H5) The linear operator W:$L^2(J,V) \to X$ is defined byWu = $\int_0^b U(b,s)Bu(s)ds$ such that:

(i) W has an invertible operators W^{-1} which take values in $L^2(J,V)/kerW$ and there

exist positive constants M_2 , M_3 such that $\|B\| \leq M_2$ and $\|W^{-1}\| \leq M_3$;

(ii) there is $K_W \in L^1(J,R^+)$such that , for any bounded set Q$\subset X$

$$\beta\left((W^{-1}Q)(t)\right) \leq K_W(t)\beta(Q)$$

(H6) Let $I_i: X \to X$, $isi = 1, \dots, s$ be a continuous operator such that:

(i) There are non decreasing functions $I_i: R^+ \to R^+, i = 1, \dots, s$ such that

$\|I_i(x)\| \leq I_i(\|X\|)$ and $\lim_{n\to+\infty} inf \frac{I_i(n)}{n}$ = 0, i=1,....,s.

(ii) There exist constants $K_i \geq 0$, such that$\beta\left(I_i(x(t)\right) \leq K_i\beta(x(t))$. i=1,...,s.

(H7) The following estimation holds true:

L = $(M_1 + 2M_1^2M_2\|K_W\|_{L^1}) \sum_{i=1}^s K_i + (4M_1 + 8M_1^2M_2\|K_W\|_{L^1})(\|h\|_{L^1} + \zeta^* b) < 1$

Where $M_1 = sup\{\|U(t,s)\|, (t,s)\epsilon\Delta\}$

Theorem: Assume that (H1) – (H7) are satisfied, then the impulsive integro differential system

(1.7)-(1.9) is nonlocally controllable on J, provided that

$\frac{1}{n}[C_1 + C_2\|M(x_n)\| + C_3\Omega(n) + C_4\|x_n(\tau)\| + C_5 \sum_{i=1}^s I_i(n)] < 1.$

Proof : Using hypothesis (H5)(i),for every $x\epsilon PC(J,X)$,define the control

$$u_x(t) = W^{-1}\Bigg[x_1 - M(x_n) - U(b,0)[x_0 - M(x_n)] - \int_0^b U(b,s)\Big[f\left(s,x_n(s)\right) + \int_0^s h\left(s,\tau,x_n(\tau)\right)d\tau\Big]ds - \sum_{0<t_i<t} U(t,t_i)\, I_i\left(x_n(t_i)\right)\Bigg]$$

We shall show that, when using this control, the operator, defined by

$$(Gx)(t) = U(t,0)(x_0 - M(x)) + \int_0^t U(t,s)\left[f\big(s,x(s)\big) + \int_0^s h(s,\tau,x(\tau))\,d\tau + Bu_x(s)\right]ds + \sum_{0<t_i<t} U(t,t_i)\,I_i\big(x(t_i)\big) \quad (3.1)$$

has afixed point. This fixed point is then a solution of the system (1.7)-(1.9). Clearly

$x(b) = x_1 - M(x) = G(x)(b)$ which implies that the system (1.7)-(1.9) is controllable.

We define G=$G_1 + G_2$where

$(G_1x)(t) = U(t,0)(x_0 - M(x)) + \sum_{0<t_i<t} U(t,t_i)\,I_i(x(t_i))$

$(G_2x)(t) = \int_0^t U(t,s)\big[f\big(s,x(s)\big) + \int_0^s h(s,\tau,x(\tau))\,d\tau + Bu_x(s)\big]\,ds$

for all t$\epsilon[0,b]$.subsequently,we will prove that Ghas a fixed point by using lemma2.3. (Monch fixed point theorem).

Step1: There exist a positive integer $n_0 \geq 1$ such that $G(B_{n_0}) \subseteq B_{n_0}$,where$B_{n_0} = \{x\epsilon PC(J,X): \|x\| \leq n_0\}$.

Suppose the contrary. Then we can find $x_n\epsilon PC(J,X)$,$y_n = Gx_n\epsilon PC(J,X)$,such that $\|x_n\|_{PC} \leq n$ and$\|y_n\|_{PC} > n$ for every $n \geq 1$.

Now we have

$$y_n(t) = \mathrm{U(t,0)(\,x_0 - M}(x_n) + \int_0^t U(t,s)\left[f\big(s,x_n(s)\big) + \int_0^s h(s,\tau,x_n(\tau))\,d\tau + Bu_{x_n}(s)\right] + \sum_{0<t_i<t} U(t,t_i)\,I_i(x_n(t_i))$$

$$\|y_n\|_{PC} \leq M_1(\|x_0\| + \|M(x_n)\|) + M_1\Omega(\|x_n\|_{PC})\|\mathrm{m}\|_{\mathrm{L}^1} + M_1bL_0\|\mathrm{x_n}(\tau)\|_{PC} + M_1M_2b^{\frac{1}{2}}\big\|u_{x_n}\big\|_{L^2} + M_1\sum_{i=1}^{s} I_i\,(\|x_n\|_{PC}) \quad (3.2)$$

$$\big\|u_{x_n}\big\|_{L^2} \leq M_3[\|x_1\| + M_1\|x_0\| + (1+M_1)\|M(x_n)\| + M_1\Omega(\|x_n\|_{PC})\|m\|_{L^1} + M_1bL_0\|\mathrm{x_n}(\tau)\|_{PC} + M_1\sum_{i=1}^{s} I_i\,(\|x_n\|_{PC}) \quad (3.3)$$

Substituting (3.3) in (3.2) we get

$$< \frac{1}{n}\left[C_1 + C_2\|M(x_n)\| + C_3\Omega(\mathrm{n}) + \mathrm{C_4}\|\mathrm{x_n}(\tau)\| + \mathrm{C_5}\sum_{\mathrm{i}=1}^{\mathrm{s}} \mathrm{I_i(n)}\right] \quad (3.4)$$

where $C_1 = \left[M_1 + M_1^2M_2b^{\frac{1}{2}}M_3\right]\|x_0\| + M_1M_2b^{\frac{1}{2}}M_3\|x_1\|$

$C_2 = \left[M_1 + M_1M_2b^{\frac{1}{2}}M_3 + M_1^2M_2b^{\frac{1}{2}}M_3\right], C_3 = \left[M_1\|\mathrm{m}\|_{\mathrm{L}^1} + M_1M_2b^{\frac{1}{2}}M_3\|\mathrm{m}\|_{\mathrm{L}^1}\right]$

$\mathrm{C_4} = \left[M_1bL_0 + M_1^2M_2b^{\frac{3}{2}}M_3L_0\right]$, $\mathrm{C_5} = \left[M_1 + M_1^2M_2b^{\frac{1}{2}}M_3\right]$

by passing to the limit as $n \to +\infty$ in (3.4),we get$1 \leq 0$, which is a contradiction. Thus we deduce that there is $n_0 \geq 1$ such that $\mathrm{G}(B_{n_0}) \subseteq B_{n_0}$.

Step 2: The operators $G$is continuous on$PC[0,b];X$For thispurpose, we assume that

$x_n \to x$ in$PC[0,b];X$.Then by hypothesis (H4) and (H6), we have

$$\|G_1x_n \to G_1x\|_{PC} \leq M_1\|M(x_n) - M(x)\| + M_1\sum_{i=1}^{s}\big\|I_i\big(x_n(t_i)\big) - I_i\big(x(t_i)\big)\big\| \quad (3.5)\ \|G_2x_n \to G_2x\|_C \leq M_1\int_0^b \big\|f\big(s,x_n(s)\big) - f\big(s,x(s)\big)\big\|ds + M_1\int_0^b \left\|\int_0^s [h\big(s,\tau,x_n(\tau)\big) - h(s,\tau,x(\tau))]d\tau\right\| ds + M_1M_2b^{\frac{1}{2}}\big\|u_{x_n} - u_x\|_{L^2} \quad (3.6)$$

$$\big\|u_{x_n} - u_x\big\|_{L^2} \leq M_3[\|M(x_n) - M(x)\| + M_1\|M(x_n) - M(x)\| + M_1\int_0^b \big\|f\big(s,x_n(s)\big) - f\big(s,x(s)\big)\big\|ds$$

$$+M_1\int_0^b\left\|\int_0^s[h(s,\tau,x_n(\tau))-h(s,\tau,x(\tau))]d\tau\right\|ds+M_1\sum_{i=1}^{s}\|I_i(x_n(t_i))-I_i(x(t_i))\| \quad (3.7)$$

By domination convergence theorem, we have

$\|Gx_n \to Gx\|_{PC} \le \|G_1x_n \to G_1x\|_{PC} + \|G_2x_n \to G_2x\|_C \to 0$, as n→ +∞, ie., G is continuous.

Step 3: G(D) is equicontinuous on every J_i, i=1,...s.ie., D $\subseteq \overline{co}(\{0\}\cup G(D))$ is also equicontinuous on every J_i.To this end, let $y\epsilon G(D)$ and $t_1, t_2\epsilon J_i$, $t_1 \le t_2$. There is x ϵ D such that

$$\|y(t_2)-y(t_1)\| \le \|[U(t_2,0)-U(t_1,0)](x_0-M(x))\| + \int_0^{t_1}\|(U(t_2,s)-U(t_1,s))\left[f(s,x(s))+\int_0^s h(s,\tau,x(\tau))\,d\tau+Bu_x(s)\right]\|ds + \int_{t_1}^{t_2}\|U(t_2,0)\|\left\|\left[f(s,x(s))+\int_0^s h(s,\tau,x(\tau))\,d\tau+Bu_x(s)\right]\right\|ds \quad (3.8)$$

By the equicontinuity property of U(·, s) and the absolute continuity of the lebesgue integral, right hand side of the inequality equation (3.8) tends to zero independent of y as $t_2 \to t_1$.

Therefore G (D) is equicontinuous on every J_i

Step 4: Assume that D= $\{x_n\}_{n=1}^{+\infty}$. Since G maps D into an equicontinuous family, G (D) is equicontinuous on J_i.Hence D $\subseteq \overline{co}(\{0\}\cup G(D))$ is also equicontinuous on every J_i.

Now we shall show that (GD) (t) is relatively compact in X for each t∈ J.

From the compactness of M (·), we have

$$\beta(\{(G_1x_n)(t)\}_{n=1}^{\infty}) \le M_1\sum_{i=1}^{s}K_i\beta(x(t_i)) \quad (3.9)$$

for $t\epsilon[0,b]$ .by lemma(2.1),we have

$$\beta_V(\{u_{x_n}(s)\}_{n=1}^{\infty}) \le K_W(s)\left[2M_1\int_0^b h(s)\beta(x(s))ds+2M_1\zeta^* b\,\beta(x(s))+M_1\sum_{i=1}^{s}K_i\beta(x(t_i))\right] \quad (3.10)$$

Then this implies that

$$\beta(\{(G_2x_n)(t)\}_{n=1}^{\infty}) \le 2M_1\int_0^b h(s)\beta(x(s))ds + 4M_1^2M_2\left(\int_0^b K_W(s)ds\right)\left(\int_0^b h(s)\beta(x(s))ds\right) + 2M_1\zeta^* b\,\beta(x(s)) + 4M_1^2M_2\left(\int_0^b K_W(s)\,ds\right)\zeta^* b\beta(x(s)) + 2M_1^2M_2\left(\int_0^b K_W(\eta)\,d\eta\right)\sum_{i=1}^{s}K_i\beta(x(t_i)) \quad (3.11)$$

There fore

$$\beta((\mathrm{GD})(\mathrm{t})) \le M_1\sum_{i=1}^{s}K_i\beta(x(t_i)) + \left(2M_1 + 4M_1^2M_2\left(\int_0^b K_W(s)\,ds\right)\right)\int_0^b h(s)\beta(x(s))ds + \left(2M_1 + 4M_1^2M_2\left(\int_0^b K_W(s)\,ds\right)\right)\zeta^* b\beta(x(s)) + 2M_1^2M_2\left(\int_0^b K_W(\eta)\,d\eta\right)\sum_{i=1}^{s}K_i\beta(x(t_i)) \quad (3.12)$$

we have

$$\beta(GD) = [(M_1+2M_1^2M_2\|K_W\|_{L^1})\textstyle\sum_{i=1}^{s}K_i + (4M_1+8M_1^2M_2\|K_W\|_{L^1})(\|h\|_{L^1}+\zeta^* b)]\beta(x(s)) = L\beta(x(s))$$

Where L is defined in (H7). Thus, from the Monch's condition, we get

$$\beta(D) \le \beta(\overline{co}(\{0\}\cup G(D))) = \beta(G(D)) \le L\beta(D)$$

Which implies that β(D) = 0, since hypothesis (H7) holds. So we have that D is relatively compact. Finally, due to lemma, G has at least a fixed point and

thus the system (1.7)-(1.9) is non locally controllable on [0,b].

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A STUDY OF *MUSA ACUMINATA* (AAA GROUP) 'RED DACCA' FLOWER EXTRACT AS CORROSION INHIBITOR FOR MILD STEEL IN ACID MEDIA

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ABSTRACT

The inhibitive effect of *Musa acuminata* Red Dacca flower extract in 1N HCl / H_2SO_4 acid on mild steel was calculated using weight loss method and surface examination study by FTIR. The extract of the Red Dacca flower extract was subjected to preliminary phytochemical screening to identify the chemical constituents of the plant. The results revealed strong presence of tannins and steroids and moderate presence of, alkaloids, terpenoids, reducing sugar and coumarins. The corrosion inhibition efficiency studied showed that the inhibition efficiency increased with increase in the concentration of the extract. RD flower extract showed a maximum efficiency of 99.40% and 96.99% at 2.5 % v/v in 1N HCl and 1N H_2SO_4 respectively.

Keywords: *Musa acuminata* Red Dacca, flower extract, acid medium, mild steel, FTIR.

1. INTRODUCTION

Mild steel finds a wide range of applications in industries, because of its availability, low cost, ease of fabrication and high tensile strength. But mild steel has a high tendency to corrode easily (Vinod Kumar *et al.*, 2010). Use of inhibitors is one of the most practical methods for protection against corrosion. Present study deals with description of methods used in characterization of plant material and corrosion monitoring techniques. Corrosive inhibitive effect of flower extract of *Musa acuminata* Red Decca (Figure 1) in 1N HCl / H_2SO_4, on mild steel was carried out using conventional weight loss method and surface examination analysis (FTIR).

Fig. 1. Photograph of *Musa acuminata* Red Dacca flower

2. MATERIALS AND METHODS

2.1. Phytoanalysis

2.1.1. Collection of plant materials

Study was carried out on *Musa acuminata* Red Dacca flower extract, obtained from cultivated farm in Salem, Tamil Nadu, India. Dried sample was ground into powder (Figure 2) using an electronic blender, sieved and fine powder stored in air tight container.

Fig. 2. RD Flower powder

2.1.2. Phytochemical screening

Extract of *Musa acuminata* Red Dacca flower extract was subjected to preliminary phytochemical screening to identify chemical constituents of plant, as described by various researchers Kotate (1999), Kotate 2010 and Harborne (1984, 1998).

2.2. Corrosion studies

2.2.1. Preparation of the Inhibitor

25 gm of dried powder of flower was boiled in 500ml of 1N HCl / H2SO4 acid with reflux condenser (Figure 3) for three hours and kept overnight to extract its phytonutrients. Extract filtered and filtrate volume made up to 500ml using respective acids. Extract so prepared was taken as 5% stock solution and from this other concentrations were prepared.

Fig. 3. Photograph of Experimental Set up for Obtaining Plant Extract

2.2.2. Weight Loss Method

2.2.2.1. Preliminary treatment of mild steel

Rectangular mild steel coupons of size 5 × 1 × 0.2 cm (Figure 4) cut from a large sheet of mild steel, with a small hole of about 1.0mm diameter near the 1.5cm side end for suspending were polished using silicon carbide emery papers of grade 200, 400, 600, washed with distilled water, dried, degreased with acetone and dried and kept in desiccators to avoid adsorption of moisture.

Fig. 4. Photograph of Mild Steel Coupons

2.2.2.2. Immersion study

Weight loss studies were conducted at room temperature. Mild steel specimens were weighed accurately in electronic balance. After initial weighing, the specimens were fully immersed using glass hooks in beakers containing 100ml of 1N HCl/ H_2SO_4 wihout and with inhibitor of different concentrations (0.1, 0.5, 1.0, 1.5, 2.0, 2.5 % v/v) at various intervals of time (1, 3, 5, 7, 12 hours) (Figure 5). After the specified period of immersion, the specimens were removed, washed with distilled water, dried and reweighed.

2.3. Surface examination studies

Surface analysis studies FTIR of mild steel specimens were done in order to study changes that occur during corrosion of mild steel in presence and absence of inhibitor in acid media (Raja and Sethuraman, 2008, 2009).

Fig. 5. Photograph of specimens fully immersed in acid medium without and with inhibitor

2.3.1. Preparation of the specimen for surface analysis.

Mild steel specimens (5 ×1 × 0.2 cm) were abraded with emery paper of grade 400 and 600 to a mirror finish, washed with distilled water and then rinsed with acetone and dried by hot air drier (Hegazy *et al*., 2011).

2.3.2. Surface morphology studies by Fourier Transform Infrared spectroscopy

Mild steel specimens were immersed in 1N HCl / H_2SO_4 solution in absence and presence of 2% v/v concentration of RD flower extracts for a period of three hours at room temperatue, removed,washed carefully with distilled water without disturbing the suface and dried. FTIR spectra of the samples were taken at Bharathiar University, Coimbatore, India (Model : Shimazdu).

3. RESULTS AND DISCUSSION

3.1. Qualitative phytochemical analysis

Phytochemicals present in aqueous flower extract of *Red Dacca* are summarized in Table 1. Results indicated that reducing sugar, saponins, coumarins and steroids were moderately present in the RD (F) extract. RD (F) extract showed the active presence of alkaloids and terpenoid. Exceptional factor was tannin content seem to be high in RDF extract of *Red Dacca* flower. It is evident from the tabulation that the other phyto chemicals like carbohydrates, flavonids, phlobotannins, cycloglycosides, total phenols, quinones and anthraquinones were absent in extract of *Musa acuminata* Red Dacca flower.

These results suggest the presence of primary bioactive metabolites of commercial importance which acts as the precursors for the synthesis of secondary metabolites. These in turn help in development of new bio products as corrosion inhibitor for future.

Table 1. Phytochemical constituents present in the extract of *Musa acuminata* Red Dacca flower

Phytocompound	RD (F) extract
Carbohydrates	+
Reducing sugars	++
Alkaloids	+
Saponins	++
Tannins	+++
Flavonoids	-
Terpernoids	+
Phlobotannins	-
Coumarins	++
Cycloglycosides	-
Total phenols	-
Quinones	-
Anthraquinones	-
Steroids	++

Key: '+++' active compound copiously present, '++' active compound moderately present, '+' active compound present,'_' active compound absent.

3.2. Weight loss studies

Mild steel was found to corrode in 1N HCl / H_2SO_4 acid solution. This was evidenced by the decrease in the original weight of the metal coupons. With addition of plant extract to the acids, it was found that weight loss decreases with increase in concentration from 0.1 to 2.5% v/v due to adsorption of plant nutrient and protects from dissolution of metal (Loto, 2011).

3.2.1. Effect of Concentration of RD flower Extract on Concentration Rate and Inhibition Efficiency

Variation of inhibition efficiency and corrosion rate with change in concentration of the inhibitor is presented in Table 2 and Table 3. It is obvious from the data that there was decrease in the corrosion rate with increase in the inhibitor concentration for all immersion periods. The decrease in corrosion rate and increase in inhibitor efficiency was usually attributed to the adsorption of flower extract constituents on the surface of mild steel which makes a barrier for mass and charge transfers and protects further attack by the acid (Saratha and Vasudha, 2009).

Table 2. CR of mild steel and IE of RD (F) extract in 1N HCl acid in various concentration and immersion period.

Conc. of Extract (%)	1h		3h		5h		7h		24h	
	CR mm/y	IE (%)	CR mm/y	IE (%)	CR mm/y	IE (%)	CR mm/y	IE (%)	CR mm/y	IE (%)
Blank	32.32	-	35.66	-	37.22	-	44.73	-	83.72	-
0.1	6.68	79.31	4.82	85.08	15.15	59.29	31.84	29.20	2.55	25.65
0.5	5.57	84.56	2.22	93.13	4.68	87.42	3.82	91.37	2.46	28.27
1.0	3.34	89.6	1.85	94.27	3.34	91.02	1.75	94.74	2.32	32.06
1.5	3.34	90.62	1.48	95.40	2.00	94.62	1.91	95.72	2.04	40.52
2.0	2.22	92.1	1.11	96.56	1.33	96.42	1.59	96.44	1.57	54.22
2.5	1.20	93.1	0.74	97.70	0.22	99.40	1.11	97.51	1.48	56.85

Table 3. CR of mild steel and IE of RD (F) extract in 1N H_2SO_4 acid in various concentration and immersion period.

Conc. of Extract (%)	1h		3h		5h		7h		24h	
	CR mm/y	IE (%)	CR mm/y	IE (%)	CR mm/y	IE (%)	CR mm/y	IE (%)	CR mm/y	IE (%)
Blank	42.35	-	75.22	-	79.57	-	79.28	-	83.72	-
0.1	10.03	71.87	22.38	70.74	15.15	80.96	10.50	86.75	27.76	66.84
0.5	7.80	78.12	9.93	88.03	10.47	86.84	7.80	90.16	23.77	71.60
1.0	5.57	86.84	8.08	89.36	6.90	91.32	5.87	92.57	19.87	76.25
1.5	4.45	89.49	7.05	90.64	5.34	93.28	3.98	94.97	16.85	79.87
2.0	3.34	92.11	5.01	93.35	4.01	94.96	3.02	96.19	14.48	82.69
2.5	2.22	94.22	4.17	94.68	3.78	95.24	2.38	96.99	13.83	83.47

3.2.2. Effect of Immersion Time on Corrosion Rate and Inhibition Efficiency

Variation of inhibitor efficiency with inhibitor concentration and immersion time is given in figure 6a and 6b. The inhibition efficiency increased with increase in concentration of the inhibitor from 0.1 to 2.5% at room temperature. The maximum inhibition efficiency was 99.40 % in case of RD (F) extract in 1N HCl for the immersion period of 5h and 96.99 % for RD (F) extract in 1N H_2SO_4 for the immersion period of 7h at a concentration of 2.5 %v/v. The decrease in inhibition efficiency thereafter with increasing time may be due to the shift in adsorption and desorption equilibrium which takes place simultaneously on prolonged exposure to the corrosive media (Putilova, 1960). These results suggest that the adsorption model arrangement and orientation of the constituents present in the *Red Decca* extract on the surface of mild steel may change with time (Rekha Nair, 2010). At low concentration, the aromatic rings of the phyto constituents may be oriented perpendicularly with respect to the metal surface. But at higher concentrations of inhibitor, the molecules may be reoriented to the parallel mode on the surface of mild steel. Therefore for higher concentration of inhibitor, more number of inhibitor molecules gets adsorbed on the surface of mild steel (Patel, 2009).

The adsorption of the phyto constituents on the metal surface makes a barrier for mass and charge transfers and thus protects the metal surface fraction occupied by the adsorbed molecules (Shymala and Arulanantham, 2009).

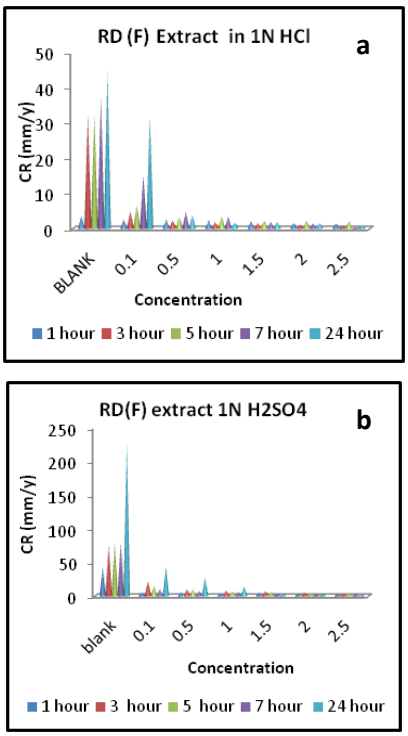

Fig. 6. Effect of concentration on CR of mild steel in (a) 1N HCl (b) 1N H_2SO_4 without and with RD (F) Extract.

3.4. Fourier transform infrared spectroscopy (FTIR) studies

Results of FTIR of Mild steel exposed to 1N HCl and 1N H_2SO_4 in the presence of *Musa acuminata* Red Dacca Flower extract and possible functional group (Harajothi Mazumdar 2010), are represented Table 4 Table 5.

Table 4. FTIR peak values and possible functional group of adsorption layer formed on mild steel surface exposed to 1N HCl without and with RD (F) inhibitor.

Mild Steel in 1N HCl	Possible groups	Mild Steel in 1N HCl with RD(F) extract
	Methylene CH asy/sym (S)	2850.27
	Methoxy O-CH_3	
	Methyl ether C-H(S)	
	Methylamino	2785.67
	N-CH_3,CH(S)	
2678.64		
		2669.00
2608.25		
2508.94		
		2552.33
	N-H ammonium ions	2505.08
2440.47	Multiple broad peaks	
		2400.94
2392.26		
		2366.26
2347.91		
2334.41		

		2316.09
2291.98	Nitrile Isocyanate –N=C=O	2268.84
	Aliphatic cyanide/nitrile Transition metal carbonyl	2046.10
	Fluoro alkanes Skeletal C-C vibration	1056.80
	Primary alcohol C-O (S)	
1015.34	Aromatic CH in plane bend Aromatic fluoro compounds C-F(S)	
554.43	Aliphatic iodo compounds C-I (S)	

Table 5. FTIR peak values and possible functional group of adsorption layer formed on mild steel surface exposed to 1N H_2SO_4 without inhibitor.

Mild steel in 1N H_2SO_4	Possible groups	Mild steel in 1N H_2SO_4 with RD(F) extract
	Primary alcohol / Phenol	3639.02
	OH (S)	3532.95
	Heterocyclic amines N-H (S)	3440.39
	Dimeric OH (S) OH carboxylic acid NH primary amine	
	Normal polymeric OH	3297.68
	Alkynes	3232.11
	N – H, ammonium ions	3156.90
	Multiple broad peaks Methylene C-H (S)	2892.70
	Alkyl C=H Aldehyde CHO Methoxy C-H (S)	2811.70
	Alkyl C=H Aldehyde CHO	
2790.49		
2655.50		
2592.82		
2519.54		
		2491.58
2458.80	N-H Ammonium ions Multiple broad peaks	
		2413.48
2393.23		
		2354.66
		2335.37
2333.45		
2268.20	Nitrile Cyanate OCN, C-OCN Isocyanate –N=C=O asy (S)	
2239.91	Isothiocyanate (-NCS)	
2116.49	C≡C terminal alkynes CH alkynes	707.75
	C-H (b) of aromatic Cis C-H out of plane bend Aliphatic chloro compounds C-Cl (S)	

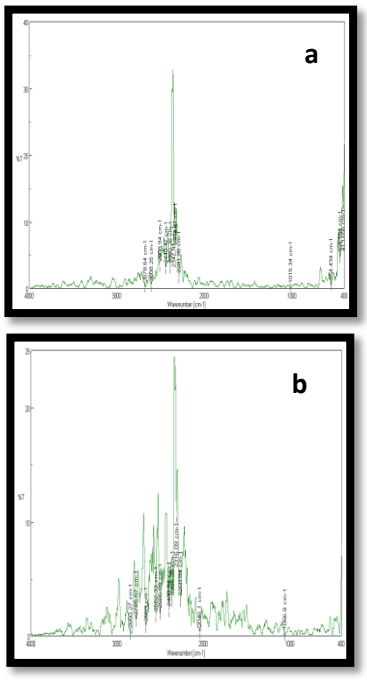

Fig. 7. FTIR spectra of adsorption layer formed on the mild steel surface immersion in 1N HCl acid (a) without (b) with RD (F) extract.

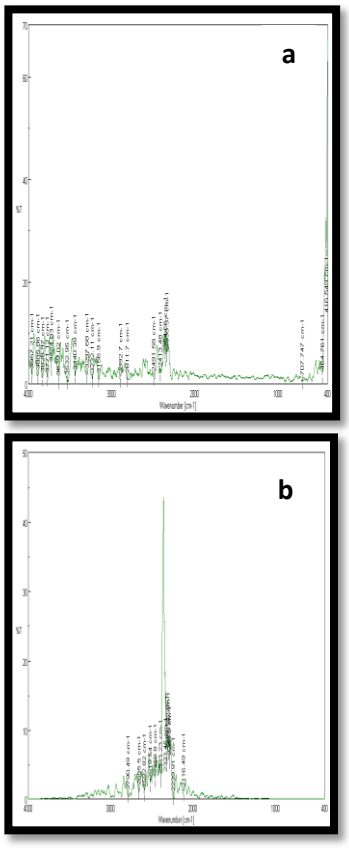

Fig. 8. FTIR spectra of adsorption layer formed on the mild steel surface immersion in 1N H_2SO_4 acid (a) without (b) with RD (F) extract.

3.4.1. Analysis of FTIR spectra

FTIR spectra of mild steel treated with 1N HCl /H_2SO_4 without and with RD flower extract displayed in Figure 7a and 7b and 8a and 8b, showed either a decrease in the transmittance or disappearance of some of the above said bands, giving a strong evidence for the interaction between the metal and the functional groups such as OH, NH_2 and C=O leading to the formation of film of large surface coverage which serve as a barrier between the corrosive acid medium and the metal thereby inhibiting corrosion and also revealing the fact that *Musa acuminata* Red Dacca flower nutrients can absorb on the metal surface on the basis of donor-acceptor interactions between lone-pair electrons of N and the vacant d-orbital of Fe substrate (Deng 2011 a,b).

5. CONCLUSION

Qualitative analysis of *Musa acuminata* Red Decca flower extract showed presence of alkaloids, saponins, tannins, flavonoids, terpenoids, coumarins, phenols and steroids. Corrosion of mild steel in 1N HCl / H_2SO_4 acid medium was significantly reduced upon the additions of RD flower extract. Inhibition efficiency increased with increasing concentration of inhibitor. Maximum inhibitor efficiency was observed at an optimum concentration of 2.5 % v/v. The flower extract of RD showed maximum efficiency of 99.40 % in 1N HCl at 5 hours of immersion. The flower extract of RD showed maximum efficiency of 96.99 % in 1N H_2SO_4 at 7 hours of immersion. RD (F) extract showed better inhibitive effect in 1N HCl when compared to H_2SO_4. Whereas RD (F) had better inhibitive effect spread throughout the various concentrations from 0.1 to 2.5 % v/v in HCl medium. All the results of the present study indicate that the extracts *Red Decca* flower in 1N HCl / H_2SO_4 acid can be used as corrosion inhibitors for mild steel. Further, as these extracts are environmental friendly, they can be considered as green corrosion inhibitors.

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PREPARATION AND CHARACTERIZATION OF THERMAL EVAPORATED $BATIO_3$ THIN FILMS

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ABSTRACT

Thermal evaporated Barium titanate **(**$BaTiO_3$) thin films were prepared on to well cleaned glass substrates under the vacuum of 2 $x10^{-5}$ torr, using 12A4 Hind Hivac coating unit from the $BaTiO_3$ nanoparticles synthesized by using wet chemical method. The thickness of the film was measured by Quartz crystal monitor. From X-ray analysis, it has been found that the deposited film was polycrystalline in nature. SEM analysis revealed that grains of various sizes having tetragonal shape were uniformly distributed throughout the surface of the film. The dependence of capacitance and loss factor on frequency and temperature were investigated and results are discussed.

Keywords: $BATIO_3$, Nanoparticles, X-ray analysis.

1. INTRODUCTION

$BaTiO_3$ thin films are very promising for a wide range of application such as high dielectric capacitors, insulating surface layer, non-volatile memories with low switching voltage, dynamic random access memory (DRAM), positive temperature coefficient of resistance (PTCR) thermistors, infrared sensors and electro-optics devices due to high dielectric constant, low dielectric loss, low leakage current and low temperature coefficient of dielectric constant. Due to the desirable properties and applications, over the last few decades, synthesis of $BaTiO_3$ nanoparticles and their thin film has attracted great attention. A detailed survey of the literature revealed that even though some work on dielectric properties of $BaTiO_3$ thin films prepared by sol - gel method (Maneeshya *et al*., 2013; Hu *et al*., 2003) , r.f. sputtering (Bhattacharya *et al*., 1993), pulsed laser ablation (Yoon *et al*., 1995) and metal-organic chemical vapour deposition (Tahan *et al*., 1996). So far, there is no report on the preparation of thin films of $BaTiO_3$ on glass substrate by vacuum evaporation method. The present work deals with the characterization of thermal evaporated $BaTiO_3$ thin films.

2. EXPERIMENTAL

2.1. $BaTiO_3$ thin film preparation

Using the conventional 12A4 Hind Hivac coating unit, pure (99.99%) aluminium was evaporated from a tungsten filament onto well-cleaned glass substrates through suitable masks to form the bottom electrode. Prepared $BaTiO_3$ nanoparticles were then evaporated from a molybdenum boat to form the middle dielectric layer. An aluminium top electrode was deposited onto the dielectric through suitable masks to complete the aluminium-$BaTiO_3$ -aluminium (Metal-Insulator-Metal) sandwich structure. A working pressure of 2 $x10^{-5}$ torr was maintained in all the evaporation processes. For the structural and surface analysis, the $BaTiO_3$ films were deposited on pre cleaned glass substrates.

2.2. Measurements

Thickness of the prepared films was measured by using Quartz crystal monitor ("Hind Hivac" Digital Thickness Monitor Model–DTM– 101). The structural aspects of the films were analyzed, using X-ray diffractometer with filtered CuKα radiation (λ = 1.5418 Å). Measurements of series capacitance and the dissipation factor in the frequency range 12Hz- 100KHz were carried out at various temperatures (303-483 K) using digital LCR meter (LCR-819, GW instek, Good will Instrument company Ltd., Taiwan). The dielectric constant ε was evaluated from the capacitance data, known area and thickness of the dielectric films.

3. RESULTS AND DISCUSSION

3.1. EDS Analysis

Energy dispersive spectrum (EDS) was carried out to identify the composition of the $BaTiO_3$ thin films prepared by thermal evaporation. Figures 1 shows the EDS spectrum of the $BaTiO_3$ thin film of thickness 160 nm. High intensity peaks corresponding to Ba and Ti elements were clearly noticed in the EDX pattern of the thin films. From the EDS analysis it was found that $BaTiO_3$ did not contain any impurities.

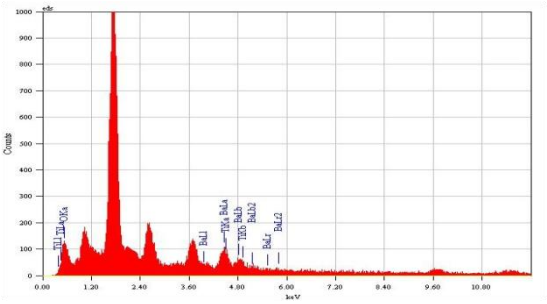

Fig. 1. EDS spectrum of the $BaTiO_3$ thin film of thickness 160 nm.

3.2. SEM analysis

Figure 2 shows the surface morphology of $BaTiO_3$ thin film of thickness 160 nm. No pits and pin holes were seen on the surface. Grains of various sizes having tetragonal shapes are uniformly distributed throughout the surface area.

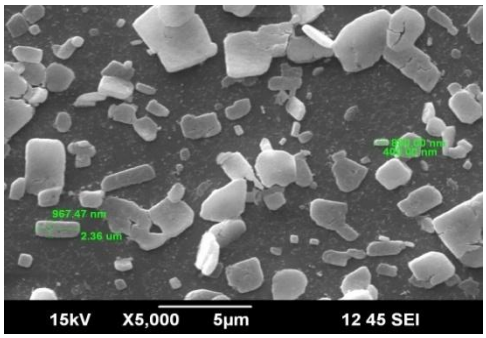

Fig. 2. Surface morphology of $BaTiO_3$ thin film of thickness 160 nm.

3.3. X- Ray diffraction analysis

Fig. 3 shows the X- ray diffraction pattern of the $BaTiO_3$ thin films of thickness 160 nm. The films were found to be polycrystalline with (001), (101), (111) and (200) orientation peaks arising at 2θ values of 22.2˚, 31.4˚, 36.7˚ and 43.8˚ respectively. The intensity of (101) peak was higher than (001), (111) and (200) peaks.

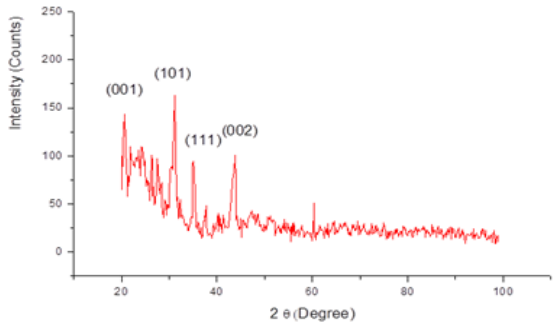

Fig. 3. XRD pattern of the $BaTiO_3$ thin films of thickness 160 nm.

3.4. Frequency effect

The variation of capacitance with frequency for a typical film of thickness 160 nm in the frequency range 12Hz - 100 kHz for different temperatures is shown in Fig. 4. The capacitance value decreases with increase of frequency for all temperature ranges studied. It increases with increase of temperature up to 398 K and then decreases with increase of temperatures. This reveals that phase transition from ferroelectric to paraelectric above 398 K and attain a constant value at higher frequency, which is a characteristics feature of the ferroelectric materials (Lines *et al*., 1979). The decrease of capacitance (C) with increase of frequency is attributed to the trapping of charge carriers due to gap states density in the amorphous films (Budaguan *et al*., 1998). The large increase in capacitance towards the low frequency region may be attributed to the blocking of charge carriers at the electrodes. Actually, the charge carriers present in the film migrate upon the application of the field and because of the impedance to their motion at electrodes resulted in space charge layer leads to a large increase in the capacitance at low frequencies. The observed decrease of capacitance with increasing frequency is also attributed to the increasing inability of the dipoles to orient themselves in a rapidly varying electric field and slow release of charge carriers from relatively deep traps. Increase of capacitance above room temperature is partly due to the expansion of the lattice and partly due to the excitation of charge carriers present at the imperfection sites (Chandar Shekar *et al*., 1999; Beladakere *et al*., 1992).

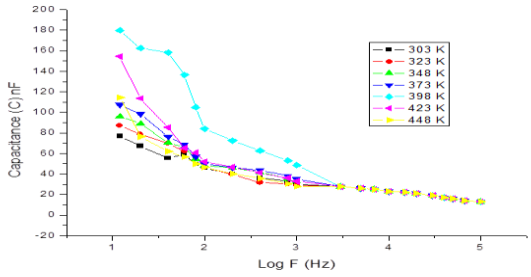

Fig. 4. The variation of capacitance with frequency of the $BaTiO_3$ thin films.

Fig. 5 shows the variation of dielectric constant with frequency for various temperatures. The dielectric constant values decreases with increase of frequency for all temperature ranges studied. It increases with increase of temperature up to 398 K and there after it decreases with the increase of temperature. It exhibit a similar trend as that of the capacitance. The increase of dielectric

constant with temperature was due to an increase of total polarization arising from dipoles and trapped charge carriers (Debye, 1929). It is seen that dielectric constant with frequency curve closely resemble those predicted by the Debye relaxation model for orientation polarization (Chandar Shekar *et al*., 2004).

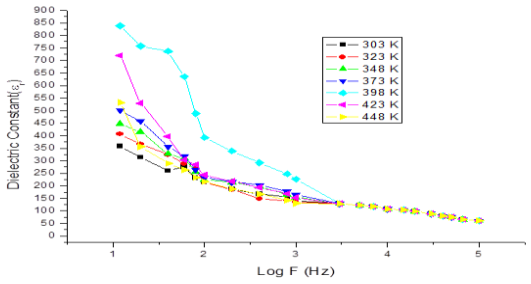

Fig. 5. Variation of dielectric constant with frequency of the $BaTiO_3$ thin film.

5. CONCLUSION

Thin films of few hundred nanometer thickness was prepared on well cleaned glass plate using thermal evaporation method. X-ray analysis showed that the deposited films were polycrystalline in nature. The capacitance is dependent both on temperature and frequency in the lower frequencies and at higher temperatures whereas it is independent of frequency at lower temperature and of high frequencies. The value of dielectric constant depends on frequency and temperature.

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EVALUATION OF ETHNOBOTANICAL PLANTS USED BY THE MALAMUTHANS TRIBAL COMMUNITY IN THE MEDAPPARA FOREST, WESTERN GHATS OF KERALA

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ABSTRACT

Medappara forest of Kerala was surveyed to list out the ethnobotanical plants used by Malamuthans tribal community. Totally 250 plants belonging to 86 families were reported to be present in the study area, in which Fabaceae family was the dominant one contributed 25 species followed by the families, Euphorbiaceae (16 species), Asteraceae (13 species) and Acanthaceae with 12 species and the distribution of species in the study area includes various life-forms *viz.*, trees (81 species), shrubs (53 species), herbs (78 species), climbers (35 species) and epiphytics (3 species) habits respectively. Of the 250 plant species, 237 (95%) were recognized as medicinally important and also cures 127 types of ailments. Of the 250 plant species encounted at all life-form levels. Sixteen ailment categories were classified, among them a higher number of 110 species were prescribed by the Malamuthan ethnic community for Gastro Intestinal Ailment (GIA).

Keywords: Ethnobotany, Malamuthan tribal, Medappara, Kerala.

1. INTRODUCTION

The indigenous people nurture rich knowledge about medicinal plants developed over generations by bold experimentation through trial and error methods (Sahai, 2003). This treasure of knowledge has been passed orally without any written documents and is still retained by them (Perumalsamy and Ignacimuthu, 2000). In the last few years, there has been an exponential growth in the field of herbal or traditional medicine and these drugs are gaining popularity because of their natural origin and exhibit remarkable efficacy in the treatment of various ailments (Siddhiqui *et al*., 1995). A vast knowledge of how to use the plants against different illness may be expected to have accumulated in areas where the use of plant is still of great importance (Diallo *et al.*, 1999).

Ethnomedicinal studies are the suitable source of information regarding useful medicinal plants that can be targeted for domestication and management (Kunwar and Duwadee, 2003). These studies assume great importance in enhancing our traditional skills and technology about the plant grown and used for native or tribal communities for their sustenance. The use of ethnobotanical information in medicinal plant research has gained considerable attention in segment of the scientific community (Heinrich, 2000). Also, traditional medicine and ethnobotanical information play an important role in scientific research, particularly when the literature and field work data have been properly evaluated (Awadh *et al*., 2004). Many reports on ethnobotanical studies in the Nilgiri Biosphere Reserve of Western Ghats are available (Abraham, 1981; Parthasarathy, 1995; Rajan *et al*, 2003; Sivakumar and Murugesan, 2005; Pradheeps and Poyyamoli, 2013; Sharmila *et al*., 2014).

However no studies on ethnobotanical significances have been carried out in the Medappara Forest, Poovaranthode of Kerala, and a part of Nilgiri Biosphere Reserve where rich diversity of plant species is available. Hence the present study is aimed to document the medicinal plants of ethnobotanical importance in the study area of Medappara forest during the period between June, 2014 and February, 2015.

2. MATERIALS AND METHODS

2.1. Study area

The present study was made in Medappara Forest at Poovaranthode, Thamarassery Range which belongs to western parts of Nilgiri Biosphere Reserve, the Western Ghats, Kerala which spreads over an area of about 1400 hectares (Fig.1). It is a tropical moist evergreen forest consisting of multistoreyed structure encompasses high species content. The geographical location of the study forest is 11° 23'45" N and 76° 06' 18" E and its altitude is ranging from 800 m to 2000 m above msl.

2.2. Ethnic community

Malamuthans are one of the primitive tribal groups of Kerala living in Malappuram and Calicut districts including the study area, Medappara Forest. They are also known as Mala Namboothiris (tribal Brahmins) due to their strict beliefs and customs. The Malamuthan consider themselves as a very superior people and prefer to live isolated. They are fearless and loyal and clime to be the Malanamboothiris.

2.3. Data Collection

Ethnobotanical surveys were conducted during March, 2014 – February, 2015 in the Medappara forest of Kerala. The information was gathered through questionnaires, personal interviews and discussions among them (Schultes, 1962; Jain, 1989; Jain and Goel, 1995). The questionnaire contains the details of the plants, parts used, medicinal uses and mode of preparation of remedies. The taxonomic identification of the plant specimens were done with the help of local and regional floras *viz., Flora of Presidency of Madras* (Gamble and Ficsher, 1957) and *Flora of Tamilnadu Carnatic* (Mathew, 1983). The collected plant species were dried for herbarium preparation and the same were maintained in the Department of Botany, Kongunadu Arts and Science College, Coimbatore.

2.4. Ailment Categories

Based on the information obtained from the traditional healers in the study area, all the ailments were grouped into 15 categories *viz.,* Gastro-Intestinal Ailments (GIA), Dermatological Infections/Diseases (DID), Respiratory Systems Diseases (RSD), Genito-Urinary Ailments (GUA), Fever (Fvr), Skeleto-Muscular System Disorders (SMSD), Poisonous Bites (PB), Circulatory System/Cardio-vascular Diseases (CSCD), Endocrinal Disorders (ED), Dental Care (DC), Hair Care (HC), Ear, Nose, Throat problems (ENT), Cooling Agents (CA), Liver Problems (LP), General Health (GH) and Insecticidal(IC).

3. RESULTS

3.1. Documentation of indigenous ethnomedicinal knowledge

The present study revealed the use of 237 species of plants distributed in 196 genera belonging to 86 families which were commonly used by most of the Malamuthan traditional healers for the treatment of 127 types of diseases. The prominent family of medicinal plants was Fabaceae with 25 species followed by Euphorbiaceae, Asteraceae and Acanthaceae with 16, 13 and 12 species respectively. For each reported species, botanical name, family, parts used and ailments treated were provided (Table 1).

The medicinal uses of plants gathered in the present study were compared with the previously published information from various parts of India (Dasture, 1962; Pal and Jain, 1998; Maheswari, 2000; Sahoo *et al.,* 2001; Suresh Babu, 2001; Shiva *et al.,* 2002; Anilkumar, 2003; Kirtikar and Basu, 2005; Alice and Asha Sankar, 2007; Deshpande *et al.*, 2010; Hrudayanath and Rout, 2011; Ayyanar and Ignacimuthu, 2011; Venkatachalapathi *et al.,* 2015). The data showed that no plant was reported as a new medicinal plant as all the plants were reported with different uses elsewhere.

3.2. Ailment Categories

Based on the information obtained from the traditional healers in the study area, all the reported ailments were categorized into 16 categories (Table 2). Among the various ailment categories analyzed, higher number of 110 species was prescribed for a Gastro-Intestinal Ailments (GIA). Next to this, 86 plants were used for the treatment of Dermatological Infections or Diseases (DID) followed by 82 species for Skeleton- Muscular System Disorder (SMSD). For all other ailment category except insecticidal property, generally more than 5 species were used by the Malamuthan tribal communities in the study area, Medappara forest. However, for insecticidal properties (IC) only 2 species *viz., Cycas circinalis* and *Duranta erecta* were used. This data indicates the usefulness of various species for many ailment categories by the tribal community in the study area.

3.3. Life-form and parts used

The percentage of species distribution in various life–forms was varied markedly (Fig 2). Trees were the primary source of medicine (78 species) followed by herbs (71 species) and shrubs (52 species). Among the different plant parts used, the leaves (79 species) were most frequently used for the treatment purposes. Similarly, the root parts were also prescribed mainly (from 71 species) for many ailments. Very less number of species, less than 2 were used for their parts like corm, petiole, tuber, buds, twigs and grains.

4. DISCUSSION

Ethnobotany is perhaps the most important method to identify and study natural plant resources and their management by indigenous people.

Table 1. List of plants with their families, parts used and uses included in major ailment categories.

Sl. No.	Name of Species	Family	Parts used	Medicinal/other economic uses	*Ailment category
1	*Abrus precatorius,*L.	Fabaceae	Roots, seeds, leaves	Roots, seeds and leaves are anti-phlogistic, aphrodisiac and anti-ophthalmic properties. It is used as diuretic tonic and emetic affections of nervous system and hair growth.	ENT ,SMSD, HC
2	*Acacia caesia,*(L.) Willd.	Fabaceae	Bark, leaves	The bark is used to produce a substance for washing hair, which can be used to headlice.It can be also used to stupefy fish. The flowers may be used ornamentally. Tender leaves are used in the treatment of migraine.	HC, SMSD
3	*Acacia catechu* (LF)Willd.	Fabaceae	Bark	The liquid is very good for people suffering from obesity. The extract of the plant is good for curing sore throat, bronchitis and body pains. Bark used as an antipyretic as well as anti-inflammatory substance. It cures psoriasis, anaemia, ulcers, constipation and pain in the chest.	GH, ENT, RSD, DID, GIA,
4	*Acacia concinna* DC.	Fabaceae	Fruit, leaves	The tree is food for the larvae of the butterfly. The "fruit for hair" used as a traditional shampoo. An infusion of the leaves has been used in anti- dandruff preparations. The leaves have an acidic taste and are used in chutneys.	HC,
5	*Achyranthes aspera* L.	Amaranthaceae	Whole plant, leaves, root	Decoction is used in fever and stomach diseases. Root ash is used as tooth powder in pyorrhea. Used as antispasmodic, astringent, Diuretic, odentalgic.	GIA, DC, SMSD
6	*Achyranthes prostrata,*(L.) Blume.	Amaranthaceae	Roots, whole plant	Used by the Malays externally and internally. Decoction used for coughs. Decoction of roots used for desentery. In Cameroon plant used in prescriptions for articular rheumatism.	RSD, GIA, SMSD
7	*Actinodaphne hookeri* ,Bedd.	Lauraceae	Rhizome	The plant is used for aging, atherosclerosis, cancer, diabetes, dysentery, mania, urinary disorders and wound. Rhizome boiled is used for curing dandruff. The plant is used in bronchitis and gynaecological disorders.	GH, ED, GIA, RSD, SMSD, GUA
8	*Adhatoda zeylanica* Medic.	Acanthaceae	Leaves	Leaves are used against glandular tumors. The leaf extract is used in the treatment of asthma for many centuries. It relieves breathesnes	RSD, SMSD
9	*Adiantum philippense* L.	Adiantaceae	Frond, root	Fronds either in decoction or syrup utilized as Adiantum capillus veneries. Roots used for strangury and for fever due to elephantiasis. Used for cough, leprosy, hair falling. Decoction of fresh leaves used as stomachic and diuretic;	GUA, Fvr, RSD, HC, GIA, GH, ED

				used as a cure for dysentery. Fronds extract used in fever, asthma and bronchitis.	
10	*Aegle marmelos* (L.) Correa	Rutaceae	Fruits, root, bark, leaves	Fruits are used in chronic dysentery conditions, accompanied by loose stools alternating with occasional constipation, the ripe fruit is widely used in different formulations. The plant is useful in treating insomnia. Used as cooling agent and in diarrhoea. The leaves are used in the treatment of diabetes.	GIA, GH,CA
11	*Aerva lanata (*L.) Juss.	Amaranthaceae	Whole plant	Used for treatment of snakebite. Leaves used for soup and spinach, plants included in Dasapushpam. The plant also used against cephalalgia and strangury.	PB,
12	*Ageratum conyzoides*, L.	Asteraceae	Whole plant	Whole plant is used as a nervine tonic. Decoction or infusion of whole plant is used in diarrhoea, dysentery, colic with flatulence. Leaves are styptic, vulnerary and useful in haemorrhoids and sores.	SMSD, GIA,
13	*Aglaia elaegnoidea* (Juss.)Benth.	Meliaceae	Fruit	Fruit is antidiarrhoeal, alternative, astringent, tonic, employed in leprosy, burning sensation of the body, inflammations and febrile complaints; seeds used in painful matuaration.	GIA, DID
14	*Albizia falcataria* (L.)Fosberg.	Fabaceae	Leaves, seeds, wood	Leaves and seeds are used for eye problems. Wood is used for making paper pulp.	ENT
15	*Albizia chinensis (*Osbeck.)Merr.	Fabaceae	Wood	Wood is used for making furniture, packing box, tea box etc.	-
16	*Albizia labbeck* (L.)Benth.	Fabaceae	Bark, flower, seed	Bark, flower and seed is used against cough, asthma, leprosy and seminal weakness.	RSD,
17	*Allmania nodiflora* ,(L.)R.Br.exwight in Hook	Amaranthaceae	Ripe fruits, leaves	Ripe fruits are used in the treatment of constipation and dysentery.Leaves;febrifuge	GH, GIA
18	*Alpinia galanga (*Linn.)Willd.	Zingiberaceae	Rhizome	Rhizome is useful in rheumatism, bronchial catarrh, tonic, stomachic,carminative, stimulant expectorant, antispasmodic, anti-amphetamine and diuretic properties.	SMSD,RSD, GIA.
19	*Alstonia scholaris* (L.)R.Br.	Apocynaceae	Bark, milky latex	Bark and milky latex is used in the treatment of malarial fever, ulcer and helminthiasis.	Fvr, GIA
20	*Alstonia venenata* R.Br.	Apocynaceae	Bark, fruit	The plant is used as snake antivenom by the tribals. Roots and fruits are useful for skin diseases, leprosy, cobra and other venomous bites, epilepsy, fatigue, fever, syphilis, insanity, helminthiasis as remedy for impure blood.	PB, DID, Fvr, CSCD, SMSD
21	*Alternanthera brasiliana* ,L.	Amaranthaceae	Leaves	The plant is used against cough and diarrhoea in Brazilian	GIA, DID,

				popular medicine. Also used in inflammation. The leaves used in wounds.	
22	*Alternanthera sessilis*, (L.) R.Br.ex.DC.	Amaranthaceae	Whole plant	The plant is bitter, sweet, astringent, acrid, constipating, depurative, digestive and useful in vitiated conditions of kapha and pitha, burning sensation, diarrhoea, leprosy and skin diseases.	GH, RSD, GIA, DID
23	*Amorphophallus hohenackeri* ,(shott)Engl.&Gehrm.	Araceae	Corm	Corm is used for the treatment of piles, prostatic hyperplasia. Corm is prescribed for bronchitis, asthma, dysentery, enlargement of spleen, elephantiasis etc.	GH, RSD,
24	*Andrographis paniculata (* Burm.)Wall.	Acanthaceae	Whole plant	The plant is used in the treatment of fevers, skin diseases, intestinal worms and flatulence. The decoction of plant is administered against chronic fevers, intestinal worms and dyspepsia.	Fvr, DID, GIA,
25	*Angiopteris helferiana,*C.Presl.	Angiopteridaceae	Frond, rhizome, stem	The stem and rhizome are used in treatment of indigestion and other bowels related problems of cattle and goats. The portion of bark is given orally to cattle in dysentery occurring during rainy seasons.	GIA,
26	*Antidesma montanum* Blume.	Euphorbiaceae	Leaves, fruits	Leaves are considered as an antidote to the sting of cobra. Fruits are edible.	PB,
27	*Aristolochia acuminata,* Roxb.	Aristolochiaceae	Root, leaves	The roots are used to treat malaria, typhus fever, small pox and pneumonia. Poultice of roots are used on open wounds and skin ulcers. Leaves used as antidote for poisonous stings and also to treat skin diseases.	Fvr, DID, GIA, PB
28	*Aristolochia indica* ,L.	Aristolochiaceae	Root , shoot	The root is used for the treatment of snake bite. Shoot extract is used for abdominal pain.	PB,
29	*Artocarpus heterophyllus,* Lamk.	Moraceae	Fruit, latex	Latex applied externally on burns. Fruits are edible, sweet taste used in treatment of cancer. Wall of the young fruit is removed and the inner portion is cooked in goat milk and eaten for ulcers. Juice applied externally to glandular swelling and abscesses to promote suppuration.	SMSD, GIA,
30	*Artocarpus incisus* (Thunb.) L.f;suppl.	Moraceae	Roots, bark, petiole, fruits, exude gum	Exude gum is used for boils. Roots are used for diarrhoea and dysentery. Root bark is utilized in the treatment of fractures.Petiole used for eyesores and irritation. Fruits are edible.	GIA, ENT

31	*Asparagus racemosus*, Willd.	Asparagaceae	Tuberous roots	The roots are bitter, sweet, emollient, cooling, nervine tonic, constipating, ophthalmic and tonic. They are useful in nervous disorders, dyspepsia, diarrhoea, dysentery, tumours, burning sensation, inflammations and ophthalmopathy.	CA, SMSD, GH, GIA,DID, ENT
32	*Asystasia gangetica* (L.) Anders.	Acanthaceae	Whole plant	Juice of the plant administered to children suffering from swellings, worms and rheumatism.	SMSD, GIA
33	*Atlantia racemosa* ,W.& A.	Rutaceae	Leaves	Fresh leaves are used against honey bee sting.	PB
34	*Averrhoa bilimbi* Linn.	Oxalidaceae	Flower, stem, fruit	Flowers and stems are good source of iron, vitamin B and C. Fruit is astringent, stomachai and cooling; in the form of curry useful in piles and scurvy.	GIA, CA, GH,
35	*Baccaurea courtallensis* (Wight.) Muell.Arg.	Euphorbiaceae	Fruits	The fruits are acidic in taste and are edible.	GH
36	*Baliospermum axillarae* Willd.	Euphorbiaceae	Root	Root is used in the treatment of dropsy, constipation, anaemia.	GH
37	*Bambusa bambos (*L.) Voss	Gramineae	Leaves, buds, root	Tender leaves or buds decoction are used to relieve menstrual irregularities. Decoction of tender buds used for leucorrhoea, fever in children and diarrhoea. Natural cure for bronchial ailments for asthma. The dried roots are powdered and prepared into paste with water and externally applied over scabies.	GUA, Fvr, GIA, RSD, DID
38	*Begonia malabarica,* Lam.	Begoniaceae	Leaves	Leaves used for treatment of respiratory infections, diarrhoea, blood cancer and skin diseases. Very few reports on cultivation.	RSD, SMSD, DID, PB,
39	*Beloperon plumbaginifolia* (J.Jacq.) Nees	Acanthaceae	Leaves, root, stem	Snake grass use initial treating snake bites. It is used for cancer. The leaves, root, stem of vishapacha is used kapha, pitha, poison bites and swelling due to viper bite.	SMSD, RSD, PB
40	*Bidens pilosa*, L.	Asteraceae	Whole plant	Leaf paste is applied on cuts and wounds.	DID
41	*Biophytum sensitivum,* DC.	Oxalidaceae	Whole plant	Plant juice is applied on the injured part and also for bleeding. Plant paste is applied on forehead for migraine.	DID, SMSD
42	*Bixa orellana* Linn.	Bixaceae	Fruit, seed, root bark	Fruit used as an astringent, purgative. Root bark- antiper, antipyr. Seeds cordial good remedy for gonorrhea. Leaves used in jaundice and snake bite. Seed pulp used for making dye called “Arnotto” once largely used in dyeing silk and cotton.	PB, LP, GUA

43	*Blumea belangeriana,* DC.	Asteraceae	Whole plant	The plant is used in traditional Chinese medicine. It is also used as decorative dry plant. The plant have pungent, bitter and antipyretic properties	Fvr
44	*Breynia retusa* (Dennst.) Alston,Ann.	Euphorbiaceae	Bark, leaves	Bark is used in diseases of nervous system, oedema, disorders of blood and conjuctivities. Leaves are employed as poultice to hasten suppuration and as a galactagogue.	SMSD, GUA
45	*Bridelia retusa* (L.)Spreng.	Euphorbiaceae	Bark	Bark is used in the treatment of rheumatism.	SMSD
46	*Bryophyllum pinnatum*, (Lam.)Kurz	Crassulaceae	Leaves	Leaves used as astringent, antiseptic and counterirritant against poisonous insect bites. Pounded fresh material is applied as a poultice for a variety of conditions: sprains, eczema, infections. Leaves used for asthma and headache.	DID, PB,RSD, SMSD
47	*Caesalpinia mimosoides*, Lam.	Fabaceae	Leaves	The plant is used as a fresh dietary vegetable.	GH
48	*Calamus pseudotenuis,* Becc.	Arecaceae	Cane, bark	The cane is used for reinforcement of walls, making basket. Barks are used to extract tannin and also for ayurvedic medicinal purposes.	-
49	*Calamus rheedii,* Griff.	Arecaceae	Cane	Used in Ayurvedic system of medicine for curing cough and oedema.	RSD
50	*Calophyllum calaba* L.	Clusiaceae	Root, leaves, flowers	Decoction of root used as a protective medicine after child birth. The leaves applied hot are reported to give relief in rheumatic pains. Preparation of flowers are used as a diuretic for diseases and ailments of the kidneys and the lower urinary tract.	SMSD, ED
51	*Carallia brachiata* (Lour.)Merr.	Rhizophoraceae	Stem bark, wood	The stem bark is evaluated for wound healing activity used in treatment of cuts and wounds. The wood is suitable for general construction, house building, posts, cabinet work, railway sleepers, furniture, musical instrument etc.	DID
52	*Careya arborea* Roxb.	Lecythidaceae	Wood , bark, fruits, flowers, stem	Wood useful; bark and fruits astringent flowers and bark used in the treatment of cough and cold; fruits edible. Stem used for tooth cleaning.	RSD, DC
53	*Caryota urens* L.	Arecaceae	Leaf, seed	The plant is used in hyperdypsia, fatigue and hemicarnia. Leaf bud, seed and toddy are used for diarrhoea, migraine and scorpion- sting poisoning.	GIA, SMSD, PB
54	*Cassia fistula* L.	Fabaceae	Bark, root, fruit	Bark, root and fruit is used in the treatment of syphilis, colic, leprosy, rheumatism, jaundice and cardiac disorder.	SMSD, LP, CSCD

55	*Centella asiatica (*L.) Urb.	Apiaceae	Leaf, whole plant	The plant extract is used in the preparation of hair oils and tonics. It is also used as a vegetable. Leaf paste used for scorpion sting. Whole plant used in the treatment of cardiac debility, abdominal disorders, epilepsy and leprosy.	HC, PB, CSCD, SMSD
56	*Centratherum intermedium*, Less.	Asteraceae	Whole plant	The perennials are best planted in groups suited for rockeries, as well as ground cover.	-
57	*Centrocema virginianum,* L.	Fabaceae	Leaves	The plants extensive root system makes it useful for stabilizing soils and controlling erosion. Make a poultice from the leaves with a pinch of salt and use to reduce swelling.	SMSD
58	*Chassalia curviflora* (Wall.exKurz) Thw.	Rubiaceae	Root	Root decoction used for cough and malaria. It is also used as an adulterant for " sarpagandha".	RSD, Fvr
59	*Chromolaena odorata,* (L.) king & Robins	Asteraceae	Leaves	Leaf juice is applied externally on cuts and wounds to stop bleeding.	DID
60	*Cinnamomum malabatrum* (Burm.f.) Blume.	Lauraceae	Wood, leaves	The wood is used for making furniture. The plant have anti-inflammatory properties. The leaves used to making traditional food.	DID
61	*Cissus latifolia,* Lam.	Vitaceae	Whole plant	Whole plant cooked with jaggery is used for burning fever and pleuritics. Water dripped from the trunk with sugar is useful for cough, purifies blood, cure the ulcer of lungs. Crushed root boild in water is good toothache.	RSD, CSCD, GIA, DC
62	*Citrullus colocynthis (*L.)	Cucurbitaceae	Fruits	Fruits are given in low doses in cases of urticarial, constipation and toxemia.	GH
63	*Cleome burmannii,* wight. &Arn.	Capparaceae	Leaves, seeds	The leaves and seeds are used as a rubefacient and vesicant by traditional medicinal practitioners in Africa and Asia. They are also used to treat infections, fever, rheumatism and headache.	Fvr, SMSD,
64	*Clerodendrum infortunatum* L.	Verbenaceae	Bark, leaves	Bark is used for diabetes. Leaves are used as bitter tonic, vermifuge, laxative and cholagogue; fresh leaf juice used to remove ascarids,leprosy.	GIA,
65	*Clerodendrum paniculatum* L.	Verbenaceae	Leaves, roots	Crushed leaves are used in the treatment of dysentery. Roots contains an antidote for certain snake bites. A paste of the leaves applied to infected burns.	GIA, PB

66	*Clitoria ternatea* L.	Fabaceae	Root, leaf, seed	Root, leaf and seed are used against leucoderma, pulmonary tuberculosis and otalgia.	RSD
67	*Coccinia grandis* (L.) Voigt	Cucurbitaceae	Whole plant	Whole plant is taken orally along with water three times a day for a period of two days to get relief from burning micturition.	DID
68	*Commelina benghalensis*, L.	Commelinaceae	Whole plant	Whole plant is used for haemorrhage, leprosy and rheumatism.	SMSD
69	*Costus speciosus*, (Koenig)J.E.Smith.	Costaceae	Rhizome	Rhizomes are aromatic and used for haemorrhage, fever, cough, and other respiratory diseases, diabetes, blood diseases, leprosy and other skin diseases.	Fvr, RSD, ED, CSCD, DID
70	*Crassocephalum crepidiodes*,(Benth.)S.Moore.	Asteraceae	Leaves, stem	Its fleshy mucilaginous leaves and stems are eaten as a vegetable. A lotion of leaves is used as a mild medicine that strengthens the stomach and excites its action. Leaves used in treatment of wounds, headache.	GIA, DID, SMSD
71	*Crotalaria pallida* (Dryand.)Ait.	Fabaceae	Roots, leaves	Roots and leaves are used for diarrhoea, dysentery, bleeding disorders, swelling, leprosy and other skin diseases.	GIA, SMSD, DID
72	*Crotalaria verrucosa* L.	Fabaceae	Root, leaf, seed	Plant is used for vomiting, diarrhoea, dysentery, bleeding, emetic, swellings, leprosy and other skin diseases.	GIA, SMSD, DID
73	*Cuphea hyssopifolia*, Kunth in HBK , Nov.Gen.	Lythraceae	Whole plant	Oils and serums derived from the plant often used in prevention of organ stones. Subsidence of consumption and fever, curing of infection especially with eyes.	Fvr, ENT
74	*Curculigo orchioides* Gaertn.	Hypoxidaceae	Root stalk	It is used in piles. It also used in the treatment of leucorrhoea, asthma, hydrophobia. The root poultice is applied for itching sensation and rashes.	GH, RSD, DID
75	*Cyathia gigantia*,(Wall.ex Hook)Hottum.	Cyathiaceae	Whole plant	The plant useful in continuous fever. Gum is used as a binder and disintegrater in tablets.	Fvr
76	*Cycas circinalis* Linn.	Cycadaceae	Male cones, bark	Male cone marketed and used to prevent the entry of mosquitoes and other insects in to houses /dwellings including cowsheds. Children are given bath in water soaked with bark and peeling to prevent skin diseases.	IC, DID
77	*Cyclea peltata* (Lam.) Hook.	Menispermaceae	Tuber	Tuber is used for dysentery.	GIA

	&Thoms				
78	*Cyperus cyperinus,(*Retz.) Sur.	Cyperaceae	Rhizome, root	Astringent, appetiser, stomachic, anthelmintic, leprosy, thirst, fever and used for the treatment of blood diseases.	GIA, Fvr, CSCD
79	*Cyperus haspan*, L.	Cyperaceae	Whole plant	The plant is used for making baskets and mats.	-
80	*Dalbergia latifolia* Roxb.	Fabaceae	Leaf, stem bark,root	Paste of the stem bark is internally given for diarrhoea .Decoction of the bark is given in dyspepsia and obesity. Two-three drops of leaf juice are poured in to the ear to get relief from ear pain. Root paste applied on forehead to reduce headache.	GIA, GH, ENT, SMSD
81	*Debregeasia longifolia* (Burm.f.)Wedd.	Urticaceae	Stem, wood, fruits	Stem fibre is used for ropes and cordage. The wood is used for making charcoal. Fruits are edible.	GH
82	*Dendrocalamus brandisii* (Munro)Kurz.	Gramineae	Leaves, nodes	Used as a raw material in paper mills. Leaves are used as forage, and decoction of the leaves, nodes and silicious matter is used in traditional medicine.	GH
83	*Desmodium triquetrum* (L.) DC.	Fabaceae	Whole plant	Extract of herb used in piles.	GH
84	*Desmodium gangeticum* (L.) DC.	Fabaceae	Root	Root is used for the treatment of fever, asthma and dysentery.	Fvr, RSD, GIA
85	*Desmodium heterocarpon*, (L.)DC.	Fabaceae	Root	The boiled roots are used in Malaysia to poultice sore breasts, and a decoction of the plant is regarded as a tonic and bechic. In Taiwan a decoction of root is used against rickets in children.	GUA, SMSD
86	*Desmodium motorium* (Houtt.)Merr.	Fabaceae	Whole plant	A herbal antidote. The decoction prepared by whole plant medicinally used as an antidote.	PB
87	*Dimocarpus longan* Lour.	Sapindaceae	Fruit	The fruit is used in promoting blood metabolism, soothing nerves and relieving insomnia. Pericarp have anti-inflammatory property.	CSCD, SMSD, GH, DID,
88	*Diospyros paniculata* Dalz.	Ebenaceae	Fruits	Dried and powdered fruits are applied to heal burns. A decoction of the fruit is used in gonorrhea, biliousness and blood poisoning.	PB, GUA
89	*Drynaria quercifolia*,(L.)J.Sm.	Polypodiaceae	Fronds, rhizomes	Fronds used for poulticing swellings. Rhizomes astringent, aqueous extract possesses antibacterial properties.	SMSD,
90	*Drypetes roxburghii* (Wall.)Hurus.	Euphorbiaceae	Leaf, fruit	Leaf and fruit are used in the treatment of cold and fever.	RSD, Fvr

91	*Duranta erecta* L.	Verbenaceae	Fruits	Macerated fruits yield a juice diluted in water can be used as a larvicide in ponds and swamps for killing mosquitos.	IC
92	*Eclipta prostrata*,(L.) L,Mant.	Asteraceae	Leaves, roots	It is good for hair and skin, expels intestinal worms, cures cough and asthma. It is specific in night blindness, eye diseases and headache.	HC, DID,GIA, RSD, ENT, SMSD
93	*Elaeocarpus tuberculatus* Roxb.	Elaeocarpaceae	Fruits, bark	The fruits are appetizer and sedative and are useful in cough. Wood used for planking. Decoction of the bark used in haemetemesis, indigestion and biliousness. Nuts used as remedy for rheumatism, typhoid fever and epilepsy.	GIA, RSD, SMSD, Fvr
94	*Elephantopus scaber,* L.	Asteraceae	Root, leaves, flower	Whole plant is used for diarrhoea, hemorrhage, urinary calculi, leprosy, retention of urine, bronchitis, skin disease, intermittent fever, hepatopathy, ophthalmopathy, cough and swellings.	GIA, ED, RSD, DID, Fvr, ENT, SMSD
95	*Eletteria cardamomum, Maton.*	Zingiberaceae	Capsule	The dried capsule is chewed for pleasant aroma and pungent taste.	GH
96	*Embelia ribes* Burm.f	Myrsinaceae	Root bark, fruits, root	Root bark is acrid, astringent, anthelminthic, antifertility, digestive, stomachic and laxative. It is used in treating intestinal parasites and worms, abdominal disorders, skin fungal infection, indigestion and headache. Leaves useful in leprosy. Fruits laxative useful in nervous debility, dyspepsia, tumors and asthma. The root decoction used for heart diseases.	GUA, GIA, DID, SMSD, CSCD
97	*Emilia sonchifolia*, (L.) DC.	Asteraceae	Leaves	The plant is used against dysentery. The decoction of leaves used as febrifuge.	GIA
98	*Epiprinus mallotiformis* (Muell.Arg.) Croizat.	Euphorbiaceae	Leaves, stem	The plant is used to treat digestive problems and dysentery.	GIA
99	*Erythrina indica,* Lam.	Fabaceae	Leaves	The paste of the leaves is applied on the wounds of the cattle for healing.	DID
100	*Euodia lunu-ankeda* Merr.	Rutaceae	Wood, leaves, root	Wood is used in match industries. Root have immune modulatory activity. Aromatic leaves used in cooking. Plant traditionally used for menstrual cramp.	GUA
101	*Euphorbia hirta*, L.	Euphorbiaceae	Leaves, latex, root	Latex is used in eye trouble and plant paste is applied to keep the eye cool. The root is given to allay vomiting. Leaves are used as antidote to snakebite and scorpion sting.	ENT, PB

102	*Euphorbia neriifolia,* L.	Euphorbiaceae	Latex	The milky latex used as purgative rubefacient and expectorant to remove warts and cutaneous eruptions. The latex is drastic purgative used to treat obstinate constipation.	GH, DID
103	*Ficus benghalensis* L.	Moraceae	Aerial root, Bark, latex	Milky latex applied externally for pains in rheumatism and lumbago. Aerial root is used against leucorrhoea, haemorrhages and bruises.	SMSD, GUA
104	*Ficus callosa* Willd.	Moraceae	Wood	The fine wood is usually used for furniture.	-
105	*Ficus hispida* L.	Moraceae	Bark, fruits	Bark and fruits are used for ulcers, leucoderma, psoriasis, anaemia, jaundice, epistaxis and inflammations.	GIA, DID, LP
106	*Ficus racemosa* L.	Moraceae	Bark, fruit	Milky juice is used in piles and diarrhoea. Decoction of the bark is administered orally to cure dysentery. The fruit is given as a tonic for pregnant women.	GH, GIA, GUA
107	*Ficus tictoria* G. Forst.	Moraceae	Root bark	The root bark is stomachic and aperient	GIA
108	*Flacourtia jungomas* (Lour.)Raeusch.	Flacourtiaceae	Leaves, fruits	Dried leaves are used to treat asthma. The fruits and leaves are used against diarrhoea,. Dried leaves used for bronchitis. Also used in treatment of dyspepsia and diabetes.	RSD, GIA, ED
109	*Flemingia macrophylla* (Willd.) Prain ex Merr.	Fabaceae	Roots	Roots used for ulcers and swellings.	GIA, SMSD
110	*Garcinia gummi-gutta (*L.) Roxb.	Clusiaceae	Fruit, root	Fruits are used for the treatment of dysentery. Milky juice contain arabin, essential oil, resin used in treatment of rheumatism and bowel complaints. Root used quire the swelling the body due to viper bite. Plant used in food preparation and preservation. Extract used in traditional medicine as purgative. Kudampuli (dry fruit) helps to promote digestion and a decoction used against arthritis.	GIA, SMSD, PB
111	*Gloriosa superba* L.	Liliaceae	Seeds, tubers	It is used against rheumatism. Plant used in the treatment of infertility, open wounds, snakebite, ulcers, arthritis, cholera, colic, kidney problems, typhus, itching and cancer. Rhizome used in the treatment of ulcer, leprosy and expulsion of placenta.	SMSD, GUA, DID, PB, GIA, LP
112	*Glycosmis pentaphylla*, (Retz.)DC.	Rutaceae	Whole plant	The plant is used as herbal remedy for various ailments. It is used to reduce blood glucose. Leaf fresh juice orally for liver conditions.	CSCD, LP

113	*Gmelina arborea* Roxb.	Verbenaceae	Root, leaf, fruit	Root, leaf and fruit is used against hallucination, piles, ulcer, growth of hair and anaemia.	GH, GIA, HC
114	*Helicteres isora* L.	Sterculiaceae	Root, bark, fruit	Root, bark and fruit is used in the treatment of scabies, diabetes and diarrhoea. The bark used against dysentery. Fruit demulcent, astringent and used in griping and flatulence of bowels and other abdominal complaints.	DID, ED, GIA
115	*Heliotropium indicum*, L.	Boraginaceae	Leaves, flowers	The plant is emollient and diuretic used as local application for ulcers, wounds. Decoction of leaves used in urticaria and fevers. Flowers considered emmenagogue in small doses and abortifacient in large doses.	GIA, DID, Fvr, GUA
116	*Hemidesmus indicus* (L.) R.Br.	Asclepiadaceae	Root, leaf, stem	The roots are bitter, sweet,astringent. They are useful in burning sensation, leucoderma, leprosy, skin diseases, bronchitis, syphilis and rheumatism. The leaves are useful in vomiting, wounds and leucoderma. Stems are laxative useful in inflammations.	DID, RSD, SMSD
117	*Hemigraphis colorata* Hallier f.	Acanthaceae	Leaves, whole plant	In Kerala leaf juice is applied on wounds. In Indonesia the plant is astringent, antidiarrhoeal used in the treatment of diarrhoea, dysentery, kidney stones, dermatoses and wounds. The leaves are used in the treatment of oliguria, haemorrhoids and post- partum bleeding.	DID, GIA, LP,
118	*Hibiscus hispdissimus* Griffith.	Malvaceae	Leaves, roots	Leaves are anthelmintic and improves digestion. Infusion of roots are considered to be useful in inflammations, helminthiasis, dyspepsia and ophthalmopathy.	GIA, DID, ENT
119	*Holarrhena pubescens* (Buch.-Ham)Wall.	Apocynaceae	Bark, leaf	Used in the treatment of amoebic dysentery, asthma, malaria and chronic bronchitis.	GIA, RSD, Fvr
120	*Holigarna arnottiana* Hook.f.	Anacardiaceae	Bark	In Ayurveda, the plant is used in treatment of inflammation, arthritis, hemorrhoids, obesity, tumour, cancer and skin diseases. The dried bark of the plant is used to cure amoebic dysentery. It is febrifugal, stomachic.	DID, SMSD, GH, GIA
121	*Hopea parviflora* Bedd.	Dipterocarpaceae	Wood, resin	Wood brown, close-grained, hard, used extensively in house construction for planking, as piles for bridges, for making platform boards, agricultural implements for making railway sleepers and electric poles. The resin used as a medicine applied to sores and wounds.	DID
122	*Hoya sp.*	Asclepediaceae	Leaves	Used as a treatment for asthma.	RSD
123	*Hypoestes sanguinolenta,* Hook.	Acanthaceae	Whole plant	The plant is used in treatment of headache, diarrhoea and wounds. The plant also used to making bonsai.	SMSD, GIA, DID

124	*Hyptis suaveolens*, (L.) Poit.	Lamiaceae	Leaves	The plant is stimulant, carminative and lactagogue. Infusion used in catarrhal conditions, uterus affections and parasitical cutaneous diseases. Leaf juice given in colic.	GIA, GUA, ENT
125	*Ichnocarpus frutescens* (Linn.) R.Br.	Apocynaceae	Root	Root is used for treatment of fever, seminal weakness and diabetes.	Fvr, ED
126	*Impatiens balsamina*, L.	Balsaminaceae	Leaves, seeds	Plant is useful in amenorrhoea, dysphagia, eye disorders as sores and redness, urinary, rheumatism and vomiting. Juice of leaves and branches is effective against burns, also for snakebite.	ENT, ED, SMSD, PB, GUA
127	*Impatiens cuspidata* (Wight &Arn.)	Balsaminaceae	Whole plant	The plant is used for treatment of skin diseases.	DID
128	*Impatiens hensloviana* Arn.	Balsaminaceae	Whole plant	The plant is used for treatment of blood related diseases.	CSCD
129	*Impatiens minor*, (DC.)Bennet.	Balsaminaceae	Whole plant	The plant is used as an ornamental and cosmetics.	DID
130	*Impatiens scapiflora*, Heyne ex Roxb.	Balsaminaceae	Whole plant	In North America the plant have been used as herbal remedies for the treatment of bee stings, insect bites and stinging nettle rashes.	PB
131	*Ipomoea hederifolia,* L.	Convolvulaceae	Leaf	Leaf juice is applied for cuts and wounds.	DID, RSD
132	*Ipomoea mauritiana* Jacq.	Convolvulaceae	Leaves, root	The leaves and roots are used externally to treat tuberculosis and for the treatment of external and breast infections. The decoction of the tuberous roots are used for the preparation of medicinal wine.	GUA
133	*Ipomoea nil*, (L.) Roth.	Convolvulaceae	Seeds, leaves	Seed is acrid, light, anthelmintic, purgative and blood purifier. It cures inflammations, abdominal diseases. Juice of leaves used for fever, headache and bronchitis.	CSCD, Fvr, SMSD, RSD
134	*Ipomoea obscura,* L.	Convolvulaceae	Leaves	The leaves have a pleasant smell and mucilaginous taste, used as valuable application in aphthous affection. Leaves used for eye diseases.	ENT
135	*Ixora coccinea*, L.	Rubiaceae	Roots, leaves, flowers	Roots, leaves and flowers used as a blood purifier, antiseptic, infantile skin ailments, diarrhoea, dysentery, fever, sores, chronic ulcers and catarrhal bronchitis.	CSCD, DID, GIA, Fvr, ENT, RSD
136	*Justicia gendarussa* Burm.f.	Acanthaceae	Leaves, tender shoots	In the form of decoction it is given in chronic rheumatism.	SMSD
137	*Justicia japonica,*Thunb.	Acanthaceae	Whole plant	Plant extract is used as antiperiodic.	Fvr
138	*Knema attenuata* Hook.f.&Thoms.)Warb.	Myristicaceae	Seed	Medicated ghee is used for treatment of spleen disorders, breathing disorders and tastelessness.	RSD

139	*Kyllinga nemoralis*, (J.R & G.Frost.) Dandy ex Hutch. &Dalz.	Cyperaceae	Whole plant	The plant widely used throughout the world and frequently used for its anti-venom property. It is having analgesic, antidiabetic, anticancer, hepatoprotective, antioxidant and antimalarial properties.The tubers are astringent and febrifuge.	ED, GIA, DID, Fvr,
140	*Lagerstroemia reginae* Roxb.	Lythraceae	Root	Root is stimulant and used in the treatment of fever.	Fvr
141	*Lantana camara* L.	Verbenaceae	Whole plant	Leaves is used for haemorrhage, disease of kapha and diarrhoea. Decoction of whole plant is given in tetanus, rheumatism, malaria and for ataxy of abdominal viscera.	RSD, GIA, PB, SMSD, Fvr
142	*Leea sambucina* (Burm.f.),Merr.	Leeaceae	Leaves, twigs	Leaves and twigs have antiseptic properties and are used for poulticing. Root thirst reliever, cooling properties.	DID, CA
143	*Lepianthes umbellata* (L.)Rafin.	Piperaceae	Leaves	Leaves are used for poulticing.	DID
144	*Leucas aspera* Spr.	Lamiaceae	Leaf, root	The leaf paste is applied on forehead for the relief of headache. The leaf decoction is used in the treatment of scabies. The root decoction is used for the treatment of snakebite, also used as an antidote to poison.	SMSD, DID, PB
145	*Lindernia viscosa*, (Hornem.) Merr.	Scrophulariaceae	Whole plant	The plant is used in traditional medicine.	GH
146	*Lipocarpha chinensis*,(Osbeck) Kern.	Cyperaceae	Whole plant	The plant is used for ornamental purposes.	-
147	*Lobelia nicotianifolia,* Roth ex Roem.&Schult.	Lobeliaceae	Leaves	It is used to treat asthma and bronchitis. It causes irritation of mucous membrane, toxic.	RSD
148	*Ludwigia octovalvis*, (Jacq.)Raven.	Onagraceae	Whole plant	Whole plant is useful in dyspepsia, verminosis, flatulence, strangury,dropsy,cough,asthma and neuropathy.	GIA, RSD, SMSD
149	*Lygodium flexosum* (L.) SW.	Lygodiaceae	Root	Used as an expectorant. Fresh roots used in external applications for rheumatism, sprains, scabies, eczema and wounds.	RSD, SMSD, DID
150	*Macranga indica* Wight.	Euphorbiaceae	Leaves, fruits	Different parts of the plant are used quite frequently in various traditional medicines. Sometimes a gum exuded from the cut branches, petiole bases, young shoots and fruits of the plant are applied externally to get relief from venereal sores. Leaves used in bronchial troubles and consumption.	GUA, RSD
151	*Mallotus philippensis* (Lam.)	Euphorbiaceae	Fruits	Glandular hair from fruits yield a reddish powder used as	GIA

	Muell.Arg.			an anthelmintic and useful in cutaneous affections. It is also used against tapeworms, abdominal disorders, haemopathy andleprosy.	
152	*Mastixia arborea (*Wt.)Bedd.	Cornaceae	Fruits, wood	The plant is used against uterus diseases. Fruits green to be taken as stomach medicine. Small - sized timber used for fuel wood.	GUA, GIA
153	*Melastoma malabathricum* L.	Melastomataceae	Bark, leaves	Bark and leaves are used in diarrhoea, dysentery, mucous discharge, piles and haemorrhages.	GIA, GH
154	*Melia azedarach* Linn.	Meliaceae	Leaf	Leaf paste mixed with rice water taken cures dysentery.	GIA
155	*Merremia vitifolia*, (Burm.f)Hall.f.in Engl.,	Convolvulaceae	Whole plant	Whole plant is used for urethral discharges. Roots are eaten by tribals as a stomachic.	GIA, GUA
156	*Mesua ferrea* L.	Clusiaceae	Root, flower, leaves oil	Root bark is astringent and aromatic used in rheumatism. Leaves are used as poultice for pustular eruptions. Flowers are used in cough attended with expectoration.	RSD
157	*Michelia champaca* Linn.	Magnoliaceae	Wood, flower, bark, fruit	The wood is used as fire wood .The flower is used for the treatment of kapha, pitta. Bark, flower and fruit used in the treatment of amenorrhoea, gastritis and cough.	RSD, GUA
158	*Miconia calvescens* DC.	Melastomataceae	Wood	The plant used for construction or stuck on wheels of bulldozers.	-
159	*Microsorum nigrescens (*Blume) Copel.	Polypodiaceae	Fronds	Used against nasal infections, extract is used as a source of medicinal agents to cure urinary tract infections.	ENT, ED
160	*Microstachys chamaelea*, (L.) Muell. Arg.	Euphorbiaceae	Whole plant	Decoction given with ghee as atonic; also applied in vertigo. Juice used in diarrhoea.	GIA
161	*Mikania micrantha*, Kunth in HBK , Nov. Gen.	Asteraceae	Whole plant	It is used to heal cuts and stop minor external bleeding. Used as a local antiseptic medicine.	DID
162	*Mimosa diplotrica*,C. Wight.	Mimosaceae	Whole plant	In Indonesia, the plant is used as a fodder to buffaloes. It is used as a garden flower.It is used as a herbal medicine	HC
163	*Mimosa pudica*, L.	Mimosaceae	Roots, leaves	Root decoction is used in gravel and urinary complaints. Juice of leaves used in dressings for sinus and also for sores and piles. Whole plant is haemostatic and is used in diarrhoea, uterine disorders and skin diseases.	ED, GH, GIA, GUA, DID

164	*Mimusops elengi* L.	Sapotaceae	Bark, fruit	Bark is used treat diarrhoea and dysentery. A decoction of bark is used as gargle. The fruit and bark possess tonic and astringent properties.	GIA, RSD
165	*Mitracarpus verticillatus* (Schum. &Thonn.)Vatke	Rubiaceae	Whole plant	Extract useful in cosmetics for lightening skin, removing brown patches.	DID
166	*Morinda citrifolia* L.	Rubiaceae	Root	Root used for making dye. "Al-Dye" red, purple, chocolate shades are produced on mordant cotton, silk or wool. The plant have an anti-inflammatory and anti-oxidative property.	DID
167	*Mucuna pruriens* (L.)DC.	Fabaceae	Root, leaf, seed	Root, leaf and seed is used against dropsy, helminthiasis and sterility.	SMSD, GIA
168	*Murdannia spirata*,(L.)	Commelinaceae	Whole plant	It is used as fodder for animals.	-
169	*Mussaenda frondosa*, L.	Rubiaceae	Whole plant	The plant is astringent, useful in bronchitis, cough, fever, inflammation, jaundice, leucoderma. Leaves are useful in inflammation, to expel intestinal worms, ulcers and on swellings and headache.	RSD, Fvr, DID, LP, GIA, SMSD
170	*Myristica beddomei* King.	Myristicaceae	Dried fruits,seeds	It is used in Ayurvedic medicine .And has been shown to have anti-cancer and anti-inflammatory properties.	SMSD, DID
171	*Mytragyna parvifolia (*Roxb.)Korth.	Rubiaceae	Root, bark	The roots and bark are acrid, bitter, stomachic and febrifuge. A decoction of the root is taken to relieve asthma and diorrhoea. The plant is used in menorrhagia, piles, minor skin wounds, fistula and diabetes.	GIA, RSD, GH, DID, ED
172	*Myxopyrum spilacifolium* Blume.	Oleaceae	Stem, oil	Oil is used as a medicine to migraine in siddha medicines. Stem also used as a medicine for migraine.	SMSD
173	*Naregamia alata* W. & A.	Meliaceae	Whole plant	Used for the treatment of rat poison and doing naseum in snake treatment. Also used for rheumatism and inflammations.	PB, SMSD, DID
174	*Naringi crenulata* (Roxb.) Nicolson.	Rutaceae	Root, stem, bark, leaf	Exhibited significant antitumour activity .Root is used as remedy for cobrabite, vomiting and desentery. Bark is used as a remedy for puerperal fever and pitta.	SMSD, PB, GIA, Fvr, RSD
175	*Ochlandra scriptoria*, (Dennst.)Fischer.	Poaceae	Stem	Thin, tough and pliable strips of immature culms are used to make textiles, mats and screens, lashings etc. Stem used to make flute.	-
176	*Ocimum basilicum*, L.	Lamiaceae	Leaves, seed	Whole plant is used for cough, asthma, bronchitis, ophthalmia, giddiness, intermittent and malarial fever, catarrh, otalgia, cephalalgia, dyspepsia and spasmodic affections.	RSD, ENT, Fvr, GIA, SMSD

177	*Oldenlandia auricularia*,(L.)K.Schum.	Rubiaceae	Rhizomes	The rhizomes are stomachic, carminative, stimulant and tonic and are used in dyspepsia in the form of powder and decoction.	GIA
178	*Olea dioica,* Roxb.	Oleaceae	Bark, leaves	Bark and leaves used as a febrifuge and emetic.	FVr
179	*Ophiorrhiza mungos,* L.	Rubiaceae	Whole plant	Whole plant is useful in wounds, ulcers, helminthiasis, snake poison, hydrophobia, cancer, gastropathy and leprosy.	DID, GIA, PB, SMSD
180	*Oroxylum indicum* (L.) Vent.	Bignoniaceae	Bark	Decoction of bark is given in toxemia, rheumatism and cancer.	SMSD, CSCD
181	*Osbeckia aspera*, (L.) Blume.	Melastomataceae	Whole plant	The plant traditionally to treat liver diseases. Aqueous extract should immunomodulatory effects in Taiwan, a decoction of the aerial part is used as a drink to treat dysentery.	LP, GIA
182	*Panjanelia longifolia* (Willd.) K.Schum.	Bignoniaceae	Whole plant	The plant is employed in Malaya medicinally for same purposes a “Syonaka”.Nervine tonic.	SMSD
183	*Parahemionitis cordata ,(Roxb.Ex Hook.&Grev.) Fraser.*	Pteridaceae	Whole plant	Extract used as a source of medicinal agents to cure urinary tract infections. The rabbits ear fern used in the treatment of earaches and as a vermifuge.	ED, ENT, GIA
184	*Paspalum scrobiculatum*, L.	Poaceae	Grains, stem	The grains are sweet, bitter astringent useful in ulcers, flatulence, diarrhoea, hepatopathy, haemorrhages and general debility.	GIA
185	*Persea macrantha* (Nees) Kosterm.	Lauraceae	Stem bark	The plant used for the treatment of asthma and rheumatism. The stem bark used as anti- inflammatory and anti- arthritic.	RSD, SMSD,DID
186	*Persicaria chinensis* (L.) H.Gross	Polygonaceae	Whole plant	Whole plant is used as a tonic, anti-scorbutic and vulnerary.	DID
187	*Phyllanthus amarus,* Schum.&Thonn.	Ephorbiaceae	Whole plant	Plant is used in jaundice, flue, dropsy, diabetes, asthma, bronchial infections, and diseases of liver .In Ayurveda used in problems of stomach, liver and kidney.	LP, Fvr, ED, RSD, GIA
188	*Phyllanthus emblica* L.	Euphorbiaceae	Bark, root bark, leaves, fruits	The root bark is useful in ulcerative stomatitis. Bark is used in gonorrhea and jaundice. Leaves effective for diarrhoea. Fruits are used for cardiac diseases and tuberculosis.	GIA, LP, CSCD,
189	*Pilea micropylla*,(L.)Liebm.	Urticaceae	Leaves	The plant is used in gastric and intestinal troubles. Infusion is given as a diuretic. Crushed leaves are applied to bruises.	GIA, DID

190	*Piper longum,* L.	Piperaceae	Fruit	Fruit used in the treatment of cold and cough. Used to treatment of heart burns, indigestion and diarrhoea.	RSD, CSCD, GIA
191	*Plumbago rosea,* L.	Plumbaginaceae	Roots, root bark	The root and root bark is bitter and dry with stomachic, carminative astringent and anthelmintic used in gastro intestinal diseases, dysentery, diorrhoea and dyspepsia. This tincture is also used for the treatment of haemorrhoids.	GIA, GH
192	*Plumbago zeylanica* L.	Plumbaginaceae	Root	Root is used in the treatment of rheumatism, diarrhoea and piles.	SMSD, GIA, GH
193	*Pogostemon purpurascens,* Dalz.	Lamiaceae	Leaves	Leaves are styptic and used to clean wounds and for promoting granulation. Roots are used in uterine haemorrhage, snake bite and scorpion strings. Leaf juice is given in fever.	DID, GUA, PB, Fvr
194	*Pongamia pinnata (*L.) Prierre.	Fabaceae	Bark, leaf, flower, oil	Bark, leaf, flower and oil is used in the treatment of beriberi, diabetes, scabies and leprosy.	ED, DID
195	*Pothos scandens*, L.	Araceae	Root, stem, leaves	Root bruished and fried in oil for application to abscess. Stem cut smoked with camphor for relief in asthma. Powdered leaves are applied to smallpox pustules.	RSD,
196	*Pouzolzia indica*,(L.)Bennett.	Urticaceae	Roots	Roots mixed with Badra used in gastric problems, sores, boils, ulcers, de-worming and galactagogue.	GIA, GUA
197	*Premna tomentosa*, Willd.	Verbenaceae	Leaves, roots	Used to treat stomach and liver disorders. The leaves are used in treatment of cough, headache and fever. The leaves and roots are used as a diuretic, stomachic and febrifuge.	LP, RSD, SMSD, Fvr, GIA
198	*Psidium guajava,* L.	Myrtaceae	Fruit, seed, leaves, bark	Guava seed oil used for culinary uses, pharmaceuticals or cosmetics. In cosmetic industry, the oil is used in skin care products. A tea made from young leaves useful for diarrhoea, dysentery and fever. Fruits are edible. The entire fruit is key ingredients in punch, and the juice often used in culinary sauces, dried snacks. Bark used for tanning and dyeing purpose.	DID, GIA, Fvr
199	*Pteris confusa,*T.G.Walker.	Pteridaceae	Spores	The fern spores are used to screen the hyper accumulating ferns.	-
200	*Pterocarpus marsupium* Roxb.	Fabaceae	Heart wood, leaf, flower	Heart wood, leaf and flower are used in the treatment of fracture, rheumatoid arthritis, asthma, boils and fever.	DID, SMSD, RSD, Fvr

201	*Rauwolfia serpentine* Benth.	Apocynaceae	Roots	The herb is effective in treating insomnia. It is very useful in lowering the B.P. Used for the treatment of hypertension and nervousness. Root is also used for the treatment of epilepsy and snakebite.	GH, CSCD, SMSD, PB
202	*Rhaphidophora pertusa*, (Roxb.)Schott.	Araceae	Aerial part , stem	Whole plant is used in snake bite and scorpion sting. Stem used in ulcers, pain in the colon, bronchitis and very specific for abdominal tumour.	PB, GIA, RSD, SMSD
203	*Ricinus communis* Linn.	Euphorbiaceae	Leaves, seeds, roots, oil, fruit	Castor oil used on the skin to prevent dryness. The plant is harmless purgative and very effective in treating rheumatic and skin disorders.	DID, SMSD
204	*Rosa multiflora*, Thunb.	Rosaceae	Leaves, fruit	The leaves are poulticed and applied to sores. The fruit is diuretic, antidotal to fish poisoning, hypoglycaemic and laxative. It is used to treat constipation and articular pain and as an application to foul ulcers.	PB, GH, CSCD, GIA
205	*Rotala ritchiei*, (Clarke) Koehne.	Lythraceae	Whole plant	It is used as ornamental for acquaria.	-
206	*Rotheca serrata* (L.) Steane&Mabb.	Verbenaceae	Roots	Root is used in asthma, bronchitis, abdominal disorders, epilepsy, indigestion, respiratory diseases, burning sensation and intermittent fever.	RSD, SMSD, GIA, DID, Fvr
207	*Salacia reticulata,* W.	Celastraceae	Root	Roots are used for treatment of diabetes, bleeding piles, gonorrhea, inflammations, leucorrhoea, leprosy, skin diseases, wounds, ulcers, indigestion, flatulence, colic and spermatorrhoea.	ED, GH, DID, GIA, GUA
208	*Samadera indica* Gaertn.	Simaroubaceae	Seed, leaves, oil	Seed is emetic, purgative; used for bilious fevers. Seed oil applied in rheumatism. Leaves are used as a gargle in sore throat.	Fvr, SMSD, ENT
209	*Saraca asoca (*Roxb.) de Wilde.	Caesalpiniaceae	Bark, flower, seed	Bark, flower and seeds are used against dyspepsia, colic, menorrhagia, hyperdipsia and bone fracture.	GIA, GUA, ED, SMSD
210	*Scoparia dulcis*, L.	Plantaginaceae	Whole plant	The entire plant including the roots, possesses anti-inflammatory, anti-fertile and anti-diuretic properties. It is used in treating coryza, hyperthermia and sore throat.	DID, GUA, ENT
211	*Selaginella involvens,* (Sw.)Spring	Selaginellaceae	Whole plant	The plant is used to treat cirrhosis. It have antimicrobial property. Non-antibiotic source in therapeutic application of the treatment of acne development by reducing the chance of non –specific initiation and augmentation phase of the inflammatory response.	DID, LP

212	*Senna alata*, (L.)Roxb.	Fabaceae	Leaves	Used for treating ringworm and other fungal infections of the skin.	GIA, DID
213	*Senna tora* (L.) Roxb.	Fabaceae	Leaves, seeds	Leaves are purgative, used in ringworm and other skin troubles. Seeds used in leprosy, hemiplegia, skin diseases, constipation, abdominal disorders, obesity and helminthiasis.	GIA, DID, GH,
214	*Sida acuta*, Burm.f.	Malvaceae	Roots, leaves, seeds	Root is astringent, cooling, useful in nervous and urinary diseases, Disorder of blood and bile. Leaves are demulcent, diuretic.	CA, SMSD, ED, CSCD
215	*Sida alnifolia,* L.	Malvaceae	Roots	Used for rheumatism, neurological disorders, general debility, headache, ophthalmia, tuberculosis, diabetes, fever, uterine disorders. It is also promote strength, imparts to the body.	SMSD, ENT, Fvr, ED, GUA
216	*Sida cordifolia* L.	Malvaceae	Roots, leaves	Roots are astringent, diuretic tonic; infusion given in urinary troubles, cystitis, haematuria, rheumatism, neurological disorders. Leaves demulcent and febrifuge; also used in dysentery.	ED, SMSD, GIA
217	*Sida rhomboidea* Roxb.exfleming.	Malvaceae	Stem, leaves, roots	The stems are used to treat rheumatism and tuberculosis. The mucilaginous leaves are used as a demulcent and their stem and roots are used in treatment of wounds and leg ulcers in folkloric medicine.	SMSD, DID, GIA
218	*Sleichera oleosa* (Lour.) Oken	Sapindaceae	Bark, oil	Bark and oil is used in the treatment of malaria, ulcer, burns and scald.	Fvr, GIA, DID
219	*Solanum nigrum* L.	Solanaceae	Whole plant	The plant used in the treatment of asthma, vomiting, dropsy, rat bite and hydrophobia.	RSD, PB
220	*Solanum torvum* SW.	Solanaceae	Fruit , leaves	Part used and uses are similar to “Brihati”.It is useful in liver as well as spleen enlargement.	LP,
221	*Spilanthes calvum* DC.	Asteraceae	Roots, flower heads,leaves	The flower heads are chewed to relieve the toothache and other mouth related troubles. leaves are used externally in treatment of skin diseases. Root decoction is used as	DC, DID

				purgative, diuretic and lithotriptic.	
222	*Spondias pinnata* (L.f.) Kurz.	Anacardiaceae	Root, bark, leaves, fruits	The roots are useful in regulating menstruation, Bark is aromatic, astringent useful in dysentery, diarrhoea, vomiting and muscular rheumatism. Fruit is antiscorbutic and the pulp astringent used in bilious dyspepsia.	GUA, GIA, SMSD,
223	*Stachytarpheta jamaicensis* (L.)Vahl.	Verbenaceae	Whole plant	Whole plant is used for intestinal worms, venereal diseases, purulent, ulcers, dropsy, stomach ailments, vomiting, fevers and rheumatic inflammations.	GIA, GUA, Fvr, SMSD
224	*Sterculia villosa* Roxburg.	Sterculiaceae	Bark,	The bark yields a strong fiber used for rough cordage and the cortex yields a white gum.	-
225	*Strobilanthes heyneanus* Nees.	Acanthaceae	Whole plant	The ethanolic extract of whole plant is known to possess anti-diabetic, anti-implantation, estrogenic and antibacterial activities.	ED, GUA
226	*Strobilanthus ciliates* Nees.	Acanthaceae	Whole plant	Decoction of the whole plant used in the treatment of tooth problem.	DC
227	*Strychnos nux-vomica* L.	Loganiaceae	Bark, leaves, seeds	The root bark is useful in cholera. Leaves are applied as poultice in treatment of chronic wounds and ulcers. Seeds are bitter, acrid,useful in anaemia and asthma.	GIA, DID, RSD
228	*Syzygium munronii (*Wight.) N.P.Balakr.	Myrtaceae	Twigs	The twigs are used against toothache. Twigs are also used in match box and plywood industries.	DC
229	*Tectona grandis* L.f.	Verbenaceae	Bark, leaf, wood	Bark and leaf is used in the treatment of hyper acidity, indolent ulcers, arthritis and eczema. Wood is also used to making furniture.	GIA, DID
230	*Terminalia arjuna* (DC.)Wight & Arn.	Combretaceae	Wood, bark, stem	The wood is used for carts and agricultural implements. Powdered bark is used as appetizer. Stem bark paste is used as an ointment for wounds.	GIA, DID
231	*Terminalia chebula* Retz	Combretaceae	Fruit	Fruit used in the treatment of tridosa, wounds, skin diseases, cardiac disorders and cough. Fruits are powdered and used for preventing cough and fever. Used as an appetizer.	DID, CSCD, RSD, Fvr, GIA
232	*Thottea siliquosa* (Lam.)Ding Hou.	Aristolochiaceae	Roots	Roots are used in ulcers, gonorrhea, leprosy, fever, cholera, rheumatism and antidote to snake-venum.	GIA, Fvr, SMSD, PB
233	*Thunbergia alata* ,Boj.ex Sins.	Acanthaceae	Leaves	The plant mainly used as an ornamental plant. Leaves is used as infusion (internal and external).	CSCD
234	*Thunbergia erecta* Boj.	Acanthaceae	Seeds	It is used in detoxification as the first-aid. Seeds used as	DID

				purgative.	
235	*Tinospora cordifolia,* Miers.	Menispermaceae	Stem, leaf, root	Stem extract is used in fevers. Stem also used for severe mouth ulcers. Leaf decoction when consumed relieve gas problem. Root is prescribed in diarrhoea and diabetes.	Fvr, DID, GIA, ED
236	*Torenia bicolor* Dalz.	Scrophulariaceae	Whole plant	Whole plant is used as an exanthematic ointment. Leaves are used in gonorrhoea.	GUA
237	*Tragia involucrate* L.	Euphorbiaceae	Leaves, stem	The leaves and stem paste is applied to arrest skin diseases.	DID
238	*Tridax procumbens* L.	Asteraceae	Leaf	Leaf juice is applied on cuts and wounds as antiseptic and to stop bleeding.	DID
239	*Triumfetta rhomboidea* Jacq.	Tiliaceae	Roots, bark, leaves, flower	Root is used in dysentery, intestinal ulcers. Bark and leaves are used in diarrhoea. Leaves and flowers are used in leprosy.	GIA
240	*Urena lobata* Linn.	Malvaceae	Roots	Roots are used as folk and siddha medicine.	GH
241	*Uvaria narum* ,(Dunal) Wall.exHook.F.Thoms.	Annonaceae	Root bark, leaves, stem	Decoction of root bark is given to women at the time of delivery to control fits; also used in rheumatism, bowel complaints and eczema. Leaves prescribed in rheumatism, jaundice. Stem is used for gastropathy.	GUA, SMSD, DID, LP, GIA
242	*Vanda tessellata* (Roxb.)Hook.	Orchidaceae	Root	Roots paste is applied one in a day for 5-6 days for rheumatism.	SMSD
243	*Vateria indica,* L.	Dipterocarpaceae	Plant resin	Plant resin burnt for incense, used in varnish,water proofing; essential oil as antibacterial.	DID
244	*Vatica chinensis,* L.	Dipterocarpaceae	Stem	A yellow transparent resin exudes from the stem is used in the manufacture of varnishes. Wood reddish brown used for building piles.	-
245	*Vernonia cinerea*, (L.) Less.	Asteraceae	Whole plant	The plants are bitter, acrid, thermogenic, antivitral, anthelmintic, antifungal. The roots are useful in diarrhoea, inflammations and skin diseases.	DID, GIA
246	*Vigna dalzellliana,*(O.Kzte) Verdc.	Fabaceae	Whole plant	It is used as food and drink. It is also sometimes grown for soil conservation.	GH
247	*Vitex negundo* L.	Verbenaceae	Leaves, root, flower	A decoction is used for steam bath for arthritis and joint pains. Root, leaf and flower is used in the treatment of malarial fever, sprains, odontalgia and otalgia.	SMSD, Fvr,
248	*Wedelia chinensis* (Osbeck)Merr.	Asteraceae	Whole plant	Leaf tonic is used in cough, cephalalgia and alopecia. Decoctions of whole plant are useful in inflammation, otalgia, ulcers, baldness and greyness of hair.	RSD, DID, GIA, HC, SMSD
249	*Zanthoxylum rhetsa* DC.	Rutaceae	Bark, fruits,	Bark and fruits are used in dyspepsia, asthma, bronchitis,	GIA, RSD,

			seeds	heart diseases, toothache, diseases of eye and ear, worm infestation, leprosy and spleenic disorders. Seeds are used in cholera.	CSCD, DC, ENT
250	*Zingiber officinale*, Rosc.	Zingiberaceae	Rhizome	Rhizomes are digestive, dispels cardiac disorders, oedema, coryza, cures vomiting, piles, filariasis, anaemia, cough, dyspnoea, fever, colic, diarrhoea, neurological diseases, diabetes, and eye diseases.	CSCD, GH, RSD, Fvr, GIA, SMSD, ED, ENT

*CSCD – Circulatory system / cardiovascular diseases; CA – Cooling agent; DC – Dental care; ENT – Ear, nose, throat problems; DID – Dermatological infections / diseases; ED – Endocrinal disorders; Fvr – Fever; GIA – Gastro-intestinal ailments; GH- General health; GUA – Genito-urinary ailments; HC – Hair care; LP – Liver problem; PB – Poisonous bite; RSD – Respiratses; SMSD – Skeleto-muscular system disorder; IC – Insecticidal.

Table 2. Ailment categories included with various ailments.

S. No.	Ailment categories	Biomedical terms	Tamil terms
1.	Circulatory system/ cardiovascular diseases (CSCD)	Blood purification	Rattha sutthigarippu
		Memory power	Gnabaga sakthi
		Heart problem	Idhaya noi
		Hypotensive	Rattha alutta noi
2.	Cooling agent (CA)	Body coolant	Udal kulircchi
3.	Dental care (DC)	Tooth ache	Pal vali
4.	Dermatological infections/diseases (DID)	Wound healing	Kaayam
		Skin diseases	Thol noi
		Antiinflammatory	Alargi etirppu
		Antioxidant	
		Scabies	Sori/sirangu
		Antiseptic	Kirumi nacini
		Eczema	Thol alargi
		Itching	Arippu/poocchikadi
5.	Ear, nose, throat problems (ENT)	Eye pain	Kan vali
		Ear pain	Kathu vali
6.	Endocrinal disorders (ED)	Diabetes	Sarkkarai/neerilivu noi
		Kidney stone	Siruneeraga kal
		Urinary problem	Siruneeraga noi
		Cystitis	Siruneerpai alargi
7.	Fever (Fvr)	Fever	Kaichal
		Pneumonia	Jani
		Malaria	Murai/malaria kaichal
8.	Gastro-intestinal ailments (GIA)	Ulcer	Vayitru pun
		Stomachache	Vayitru vali
		Carminative	Iraippai kuṭal vali

		Gastric complaints	Vayvu kolaru
		Digestion/indigestion	Geeranam/ageeranam
		Dysentery	Seedhabaethi
		Dyspepsia	Cerimanaminmai
		Diarrhea	Vayirrup pokku
		Cholera	Kalara
		Vermifuge	Pulukkolli
		Intestinal worms	Kutal pulukkal
		Appetite	Paciyinmai
9.	General health (GH)	Piles	Mula noi
		Tonic	Sathu marunthu
		Constipation	Malaccikkal
		Depression	Mana aluttam
		Insomnia	Tukkaminmai
		Obesity	Udal paruman
10.	Genito-urinary ailments (GUA)	Delivery pain	Pirasava vali
		Menstrual problem	Matavitay thontharavugal
		Abortion	Karu kalaipu
		Male fertility	Anmai sakthi perukkuthal
		Venereal diseases	Paalvinai noi
		Galactagogue	Thaai pallai urpathi pana
		Sexual problem	Paliyal piraccanai
11.	Hair care (HC)	Hair tonic	Mudi valara
12.	Liver problem (LP)	Jaundice	Manajal kaamalai
		Liver infection	Kaleral thotru
13.	Poisonous bite (PB)	Snake bite	Pambukkadi
		Dog bite	Naikkadi
		Poisonous bite	Vishakkadi
		Detoxification	Nachu neeka
14.	Respiratory system diseases (RSD)	Asthma	Moocchu thinaral
		Chest pain	Nenju vali
		Cold	Jalathosam
		Cough	Irumal
		Expectorant	Sali
		Bronchitis	Muccukkulay alarci
		Haemoptysis	Suvacakkulaliruntu irattam varuthal
15.	Skeleto-muscular system disorders (SMSD)	Rheumatism	Moottu vadham
		Arthritis	Kilvatam
		Joint pain	Moottu vali

		Cancer/tumor	Putru noi
		Swelling	Veekam
		Headache/One side headache	Thalai vali/oru paka thalai vali
		Paralysis	Pakavatam
		Nervous/ neuralgia problem	Narambu pathipu
		Epilepsy	
		Antispasmodic	Kaka valipu
			Valipu
16.	Insecticidal (IC)	Mosquito repellent	Kosuviratti

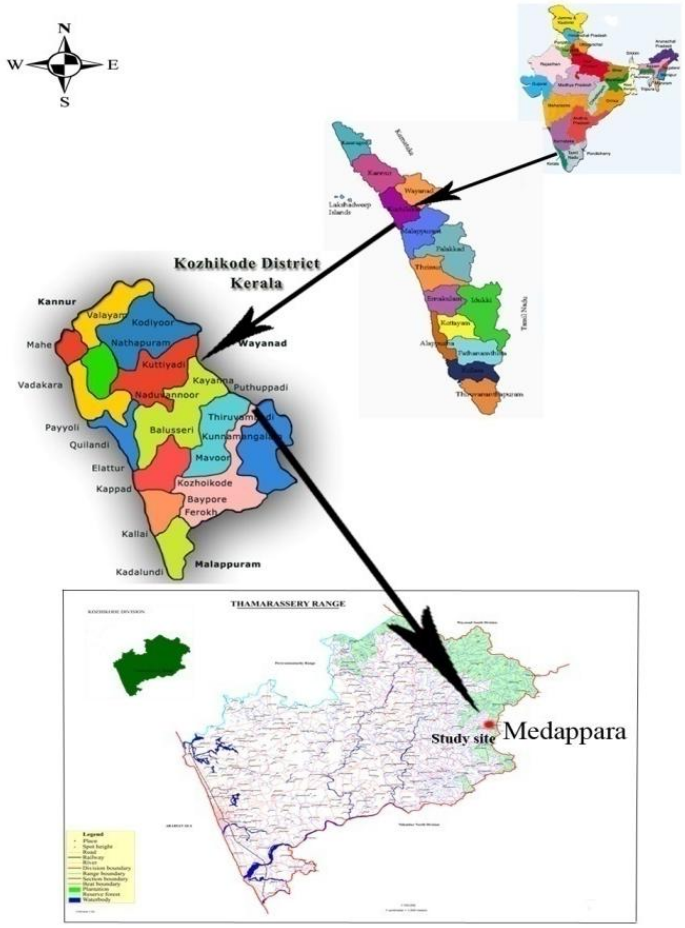

Fig. 1. Location of the study area

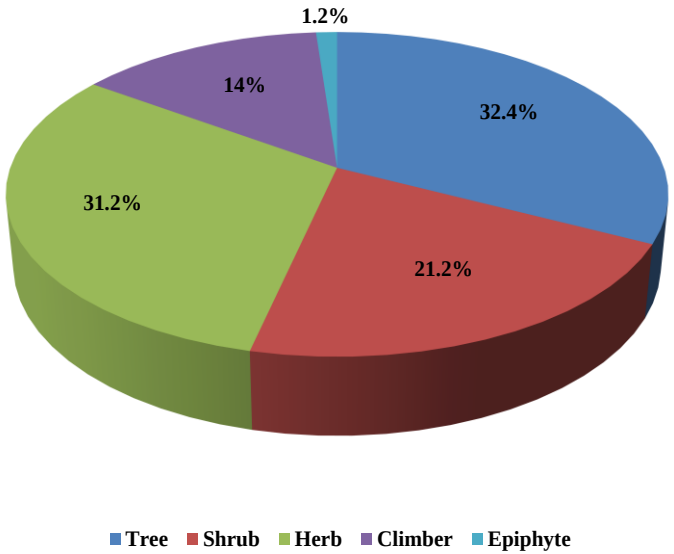

Fig. 2. Pie chart showing the percentage contribution of various life forms in the studied forest at Medappara, Kerala.

It enables us to work with local people to explore knowledge based on experiences and ages. The indigenous population still relies to a great extent on traditional healers and medicinal plants to meet their healthcare needs because of the perceived effectiveness, presumed safety with minimal side effects and affordability (Vliathan, 1998). Of the 16 ailment categories analyzed, a higher number of 110, 86 and 82 species were prescribed for Gastro Intestinal Ailments (GIA), Dermatological Infection (DID) and Muscular System Disorder (SMSD) respectively. It may be explained due to the presence of the respective bioactive compounds in the secondary metabolites produced by the species (Ayyanar and Ignacimuthu, 2011). It has been noted interestingly that a very little number of 2 species *viz., Cycas circinalis* and *Duranta erecta* were used for insecticidal property and also as mosquito repellent species. The presence of certain alkaloids may be the possible reason for this fact (Mayura and Phasomkusolsil, 2014).

The medicinal uses of plants gathered in the present study were compared with the previously published information from other parts of India. It showed that no plants were reported as a new medicinal plant as all the plants were reported with different uses. This fact exhibits that the medicinal plants enlisted in the study area are already prescribed by the healers of various areas in India. When the life-form is considered, higher number of species used for various ailments were trees. It may be explained that the studied forest at Medappara is a climax formation (Champion, 1939) and contains the trees as dominant and most established structures which might aid the sources of medicine consistently. Gonzalez *et al*. (2010) also reported the usage of more tree species for medicinal purpose in the climax forest in the western Spain due to its stable structure and consistency in availability. Among the different plant parts used, the leaves were most frequently used for medicinal purposes. Many indigenous communities elsewhere also utilized mostly leaves for the medicinal purposes (Ignacimuthu *et al*., 2006, 2008; Teklehaymanot *et al*., 2007; Srithi *et al*., 2009; Giday *et al*., 2010; Cakilcioglu and Turkoglu, 2010; Gonzalez *et al*.,2010 and Abdul Latheef *et al*., 2014). The reason why leaves were used mostly is that they are collected very easily than underground parts, flowers, fruits etc. (Giday *et al*., 2009) and in scientific point of view leaves are active in photosynthesis and production of metabolites (Ghorbani, 2005).

5. CONCLUSION

The present study indicated that the study area has numerous medicinal plants to treat a wide range of human ailments. Studies on traditional medicinal plants revealed that the local people from medappara forest prefer traditional medicine due to low cost and sometimes it is a part of their social life and culture so it is necessary to acquire and preserve this traditional system of medical practice. Further, studies by using animal models and subsequent clinical trials are suggested to confirm the traditional knowledge on medicinal plants, thus used for drug manufacturing by pharmaceutical industries.

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IN VITRO ANTI-INFLAMMATORY ACTIVITY OF *VACCINIUM LESCHENAULTII* WIGHT. (VACCINIACEAE) - AN ENDEMIC MEDICINAL PLANT SPECIES IN NILGIRIS, THE WESTERN GHATS, INDIA

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ABSTRACT

Berries of *Vaccinium* species have been a source of food and pharmaceutical ingredients. The ethanol, methanol and chloroform extracts of *Vaccinium leschenaultia* was investigated for anti-inflammatory activity. All the extracts (150 and 300mg/kg each) were found to significantly ($P<0.05$) inhibit paw edema induced by carrageenan in rats. The results indicated that the methanol extract with the dose of 300mgkg^{-1} b.w. and the chloroform extract with the dose of 300mgkg^{-1} b.wt. showed maximum anti-inflammatory activity as compared to the reference drug, Indomethacin. *V. leschenaultia* could be used as a potential source of anti-inflammatory agent.

Keywords: *Vaccinium leschenaultii***,** anti-inflammatory, Indomethacin.

1. INTRODUCTION

Traditional literature shows the use of herbal preparatins in the treatment of inflammation and pain. The search for new anti-inflammatory and analgesic drugs from the medicinal plant resources is intensifying since these shows no side effects (Chatterjee and Pal, 1984). Inflammation is considered as a primary physiologic defense mechanism that helps body to protect itself against infection, burn, toxic chemicals, allergens or other noxious stimuli. An uncontrolled and persistent inflammation may act as an etiologic factor for many of these chronic illnesses (Kumar *et al.*, 2004). Although it is a defense mechanism, the complex events and mediators that are involved in the inflammatory reaction can induce, maintain or aggravate many diseases (Sosa *et al*., 2002). Currently used anti-inflammatory drugs are associated with some severe side effects. Therefore, the development of potent anti-inflammatory drugs with fewer or no side effects from medicinal plants origin is the need of the hour.

Vaccinium is a genus of shrubs or dwarf shrubs in the family Vacciniaceae. Most are edible and some are of commercial importance, including, the cranberry, bilberry, cocoberry and huckle berry. Acetone and methanol extract of *V. leschenaultii* leaf and fruit exhibited higher anti inflammatory and anti analgesic activity (Poornima *et al*.,2005). Among the different species of *Vaccinium* (Bilberries) reported in India, *V. leschenaultii* (Indian cranberry) is considered to be one of the most potent for its medicinal properties. This plant has been used in treatment of several disorders such as mouth ulcer, diarrhea and diabetics. Bilberry fruit extracts have been used for the treatment of diarrhea, dysentery, mouth and throat inflammations (Anon, 2002). Based on the medicinal properties of Vacciniaceae members, the present study was undertaken on *Vaccinium leschenaultia* Wt. an endemic medicinal plant from the Niligiris, the Western Ghats,India.

2. MATERIALS AND METHODS

2.1. Collection and extraction of plant material

Vaccinium leschenaultii was collected during blooming season (January 2012) from nearby sholas of Ebanadu, the Nilgiri Hills, Western Ghats, Southern India, Tamil Nadu. The plant was identified and authenticated by a plant taxonomist.

In the acute toxicity studies the various solvents extracts did not cause any mortality even at the heights dose of 2000 mg/kg. Thus the selected plant extracts were safe and non toxic.

Based on acute toxicity studies two different doses were selected to assess anti inflammatory activity in rat models.

The unadultered powdered material of the whole plant of *Vaccinium leschenaultii* was successively extracted with ethanol, methanol and chloroform in a soxhlet apparatus and concentrated to dryness. These extracts were made free of any solvent by distillation. The various solvent extracts were used as an emulsion in 5% suspension with gum acacia and administered orally at the dose of 150 and 300 mg kg^{-1} . The animals were grouped in cage in an air conditioned room at the temperature of 22±1°C with 12 hour light and dark cycle. The

animals were maintained with pellet diet and water *ad libitum*. They were further segregated in to various groups. This experiment was performed according to ethical guidelines for the investigation of experimental pain in conscious animals (659/02/a/CPCSEA). Intra Gastric Catheter tube (IGC) was used for oral drug administration.

2.2. Anti inflammatory activity

2.2.1. Carrageenan-induced paw oedema in albino rats (Winter and Poster, 1957)

The wister albino rats were divided into 5 groups comprising five animals in each group. In all groups acute inflammation was produced by sub plantar injection of 0.1 ml freshly prepared 1% suspension of carrageenan in normal saline in the right hind paw of the rats and paw volume was measured plethysommetrically at 0 to 180 mins after carrageenan injection. All the animals were premedicated with Indomethacin (10 $mgkg^{-1}$ b.wt.) orally two hour before infection. Mean increase in paw volume was measured and percentage inhibition was calculated for all the extracts. Wister albino rats of 120-180 g were subjected to acute and sub acute toxicity studies. A dose of LD_{50} was determined ideal for pharmacological studies. Percentage inhibition of paw volume was calculated by the following formula

$$\text{Inhibition (\%)} = \frac{Vc - Vt}{Vc} X\ 100$$

Where

Vt- means increase in paw volume in rats treated with test compounds

Vc- means increase in paw volume in control group of rats.

2.3. Statistical analysis

The mean paw volume was expressed in terms of mean ± SEM and evaluated for statistical significance by ANOVA followed by Dunnett's t-test, $P<0.05$ was considered by statistically significant.

3. RESULTS

The anti-inflammatory activity of ethanol, methanol and chloroform extracts of *V. leschenaultii* was evaluated by carrageenan induced rat paw oedema method. The extracts were tested at two different dose levels. Which were found to be statistically significant (Table 1). 150mg/kg b.wt. and 300mg/kg. b.wt. of ethanolic, methanolic and chloroform extracts of *V. leschenaultii* significantly reduced the carageenan induced paw oedema inflammation as compared with that of the standard drug, indomethacin. The dose effect of $300mgkg^{-1}$ b.wt. of the ethanolic, methanolic and chloroform extracts of *V. leschenaultii* was more active than 150 $mgkg^{-1}$ b.wt.

At a dose of $300mgkg^{-1}$ b.wt. the methanol and chloroform extract showed a percent inhibition of 86.89 and 61.71% respectively which are higher than that of reference drug. The ethanol extract at the same dose showed a lower percent inhibition of 39.49% with respect to reference drug. At $150mgkg^{-1}$ b.wt. both the ethanol and chloroform extract shower a lower percent inhibition of 14.68 and 55.98% in comparison to the reference drug. However, the methanol extract at the same dose showed a higher value of 72.50% . Therefore it in clear that the methanol extract at both the doses of 150 and $300mgkg^{-1}$ b.w. showed the maximum percent inhibition as compared to the other extracts of reference drug.

4. DISCUSSION

Anti-inflammatory effect of natural products has been frequently assessed through the method of carragenean induced paw oedema. The inflammatory response is a physiological characteristic feature of vascularized tissues (Rang *et al,* 2007)The inflammatory response is a common feature in many diseases and its control is of relevance in the treatment of these pathologies. There are several herbal drugs used for the anti-inflammatory activity. Plants exhibiting anti-inflammatory activity reveal that species of 96 genera belonging to 56 families have exhibited such potential (Chawla *et al.,* 1987). Keeping in view the growing significance of anti-inflammatory related herbal medicines in global market, the present anti-inflammatory study was been carried out on *V. leschenaultii* (Vacciniaceae) Oedema, which develops after carrageenan inflammation, is a biphasic event. The initial phase is attributed to the release of histamine and serotonin. The oedema maintained between the first and second phase is due to kinin like substances (Vinegar *et al*., 1969). The second phase is said to be promoted by prostaglandins of lysozymes. The second phase of oedema is sensitive to drugs like hydrocortisone, phenylbutazone and Indomethacin (Winter *et al*., 1962). The present results of carragennan induced paw oedema model indicated dose dependent anti-inflammatory activity.

Table 1. Effect of *Vaccinium leschenaultii* extracts on the percentage inhibition of carrageenan induced paw oedema in rats.

Treatment	Dose mg/kg	Oedema volume (ml)				% Inhibition after 180 min
		0 min	60 min	120 min	180 min	
Control (Group-I)	Normal saline	39.63±2.16	85.11±4.15	103±2.33	123.31±9.33	-
Ethanol extract (Group-II)	150 mg/kg	35.08±1.57	74.20±3.60	94.12±3.18	105.46±5.43	14.68 %
	300 mg/kg	22.11±2.18*	39.73±4.05*	63.35±4.18*	74.77±3.58*	39.49 %
Methanol extract (Group-III)	150 mg/kg	28.32±1.95	38.43±2.18*	31.26±3.93*	34.24±2.87*	72.30 %
	300 mg/kg	27.02±1.84*	21.17±1.86**	17.47±2.69**	16.21±1.86**	86.89 %
Chloroform extract (Group-IV)	150 mg/kg	31.37±1.98*	71.37±2.67*	71.16±2.18*	54.41±1.69*	55.98 %
	300 mg/kg	29.31±1.89	53.43±2.68*	54.76±1.98*	47.33±1.90*	61.71 %
Indomethacin (Group-V)	10 mg/kg	25.71±1.69**	28.43±1.94*	49.11±1.69*	51.75±2.15**	58.13%

Value is SEM ± 5 individual observations * P < 0.05; ** P<0.01 Compared paw oedema induced control vs drug treated rats.
Group I : Control rats given normal saline orally by using an Intra Gastric Catheter tube (IGC).
Group II: Rats given ethanol *V. leschenaultii* extract at the dose of 150 and 300 mg/ Kg b.wt. by IGC.
Group III: Rats given methanol *V. leschenaultii* extract at the dose of 150 and 300 mg/ b.wt. by IGC.
Group IV: Rats given chloroform *V. leschenaultii* extract at the dose of 150 and 300 mg/ Kg b.wt. by IGC.
Group V: Rats given Indomethacin at the dose of 10 mg/ Kg b.wt. by IGC.

5. CONCLUSION

In the present study *Vaccinium leschenaultia* methanol extract exhibited maximum and potent anti-inflammatory activity. The importance of this plant as phytotherapeutic for human health especially as an anti-inflammatory agent is proved in this study.

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EVALUATION OF ANTIOXIDANT COMPOUNDS AND FREE RADICAL SCAVENGING ABILITY OF POMEGRANATE FRUIT PEELS

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ABSTRACT

The present study was undertaken to investigate the phytochemical profile and antioxidant activity of pomegranate fruit peels. Qualitative and quantitative phytochemical analyses were made for various solvent extracts of fruit peel of pomegranate and for antioxidant activity, ethanolic extract alone was used. The preliminary phytochemical analysis revealed that higher number of secondary metabolites was found in ethanolic extract of fruit peel than the other solvent extracts. The total phenolics and flavonoids contents of ethanolic fruit peel extract was found to be 246.5 mg GAE/100g extract and 83.95 mg QE/100g extract respectively. The ethanolic fruit peel extracts unveiled highest scavenging ability by quenching the DPPH free radicals with the IC_{50} value, 142.90µg/mL. The present study showed that the tested pomegranate peels exhibited strong antioxidant activity. These results suggest that pomegranate fruit peel could be exploited as a potential source of natural antioxidant agent.

Keywords: Pomegranate, fruit peel, phytochemical analysis, antioxidant activity.

1. INTRODUCTION

Fruits are an important component of a healthy diet. In the recent years, more attention has been paid to the antioxidants contained in fruits. Antioxidants in fruit have been reported to reduce oxidative damage in our body (Halliwell, 2012). The antioxidants are known to play an important role in ameliorating oxidation process by quenching free radicals, chelating metals and scavenging oxygen in foods and biological systems (Anwar and Przybylski, 2012). In general, fruit skin contains a higher concentration of antioxidant substances than the flesh of the fruit (Awad *et al.,* 2001). High fruit intakes reduce the mortality and morbidity of cardiovascular disease and some types of cancer (Guo *et al*., 2003).

Pomegranate (*Punica granatum* L.) is belongs to the family, Punicaceae has gained popularity in recent years due to its multifunctionality and nutritional benefit in the human diet. It is rich in tannins and other biochemicals, predominantly phenolics, which have been reported to reduce disease risk (Martinez *et al.,* 2006; Jaiswal *et al.,* 2010). A paste of its green leaves is applied on eyes in conjunctivitis and their juice is given in dysentery. The bark of the roots and stem is considered astringent and anthelmintic and specially used against tape worm, the fruit juice is considered cooling and refrigerant (Anil kumar, 1999). Pomegranate fruit peel constitutes about 50% of the total fruit weight (Al-Said *et al.,* 2009), and it is often discarded as waste. However, the fruit peel contains higher amounts of polyphenol compounds than the juice, and it possesses stronger biological activities (Li *et al.,* 2006; Hajimahmoodi *et al.,* 2008; Gozlekci *et al.,* 2011). Therefore, the present study was aimed at to elucidate the phytochemical contents and antioxidant activity of various solvent extracts of Pomegranate fruit peels.

2. MATERIALS AND METHODS

2.1. Collection of plant materials

The ripen fruit peel samples of *Punica granatum* was collected at local market, Coimbatore. The peel of the fruits was shade dried and powdered.

2.2. Extracts preparation

Powdered plant samples were extracted using successive solvents *viz.,* petroleum ether, chloroform and ethanol by cold extraction (20g/200ml). After extraction, the extracts were filtered and evaporated under room temperature. The yield of the fruit peel extracts was analysed by following formula

$$\text{Percentage yield} = \frac{\text{Amount of residue taken}}{\text{Amount plant powder taken}} X\ 100$$

2.3. Phytochemical analysis

The ethanolic extract was subjected to preliminary phytochemical analysis as described by Harborne (1998) and Trease and Evan (2002). The

total phenolics and flavonoids content were evaluated and expressed as gallic acid equivalents (GAE) mg/100g (10 to 50µg/ml; R^2 = 0.996) (Siddhuraju and Becker, 2003) and rutin (RE) mg/100g equivalents (10 to 200µg/ml; R^2 = 0.991) (Zhishen *et al.,* 1999) respectively.

2.4. DPPH radical scavenging activity

The ability of pomegranate fruit peel extract to scavenge the 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) radicals was assessed by using Blois (1958) method with some modifications. 0.2mM solution of $DPPH^{\bullet}$ in methanol was prepared and 500µL of this solution was added to different concentrations of the extracts (50-300µg/mL). The mixture was shaken vigorously and allowed to stand for 30min at room temperature. Control was prepared as above but without the sample extracts and methanol was used for the baseline correction. Then changes in the absorbance of the plant samples were measured at 517nm using spectrophotometer. A lower absorbance value indicates the higher radical scavenging activity. Results were compared with the standard antioxidants (rutin, quercetin, BHA and BHT). The ability of DPPH radical scavenging activity was calculated by using the following formula:

$DPPH^{\bullet}$ scavenging effect (% of inhibition) = $[(A_0-A_1)/A_0]$ X100

Where, A_0 is the absorbance of the control, and A_1 is the absorbance of the extracts. The IC_{50} (the microgram of extract to scavenge 50% of the radicals) value was calculated using linear regression analysis. Lower IC_{50} value indicates greater antioxidant activity.

2.5. Statistical analysis

Analysis was carried out in triplicates and mean±SD (Standard Deviation) using Duncan's Multiple Range Test (DMRT) (Duncan, 1955). Statistical significance (p<0.05) were subjected to one way analysis of variance (ANOVA) by using a statistical Package for Social Science (SPSS) (Version 9, SPSS, Inc., Chicago, USA).

3. RESULTS

3.1. Percentage yield

Percentage yield of various solvent extracts of pomegranate fruit peels are given in Table 1. The chloroform and ethanol extracts showed higher percentage yield (9.52 and 4.76%) than the petroleum ether extract (2.15%).

Table 1. Percentage yield of various solvent extracts of pomegranate fruit peels.

Name of the plant	Yield (%)		
	Petroleum ether	Chloroform	Ethanol
Punica granatum	2.15	4.76	9.52

3.2. Phytochemical analysis

3.2.1. Preliminary phytochemical analysis

Table 2 shows the preliminary phytochemical analysis of different solvent extracts of pomegranate fruit peels. Among the three extracts, the ethanol extract revealed the presence of the major phytochemicals *viz*., glycosides, flavonoids, phenols, saponins, steroids, tannins and terpenoids.

Table 2. Qualitative phytochemical analysis of various solvent extracts of pomegranate fruit peels.

S.No.	Phytochemical test	Petroleum ether	Chloroform	Ethanol
1.	**Alkaloids** b) Meyer's Test	+++	++	-
2.	**Cardiac Glycosides** a) Keller killiani Test	-	++	++
3.	**Flavonodis** a) Shinoda Test	-	-	-
	b) Lead Acetate Test	+++	+	+++
4.	**Glycosides** a) Keller Kiliani Test	-	-	-
	b) Legal's Test	+	-	-
5.	**Phenols** a)FreeicChloride Test	+	-	++
6.	Resins Test	-	+	-
7.	**Saponins** a)Frothing/Foam Test	++	+	++
8.	**Steroids** a)Libermann-Burchard's Test	-	++	++
9.	**Tannins** a) Braember's Test	-	-	+++
10.	**Terpenoids** Salkowski Test	-	++	++
11.	**Triterpenoids**Salkowski Test	-	-	-

(-) – Not available; (+) – present; (++) –moderately present; (+++) – highly present.

3.2.2. Quantitative phytochemical analysis

The total phenolics and flavonoids contents of ethanolic extract of pomegranate fruit peels are given in Table 3. The total phenolics content was found to be 246.51±0.17mg GAE/100 g extract and the flavonoids content was 83.95±0.60 mg RE/100g extract.

Table 3. Quantitative phytochemical analysis of ethanolic extracts of pomegranate fruit peels.

Name of the Plant	Total phenolics (mg GAE/100 g extract)	Total flavonoids (mg QE/100 g extract)
Punica granatum	246.51±0.17[a]	83.95±0.60[a]

GAE - Gallic Acid Equivalent, QE - Quercetin Equivalent.
Values are performed in triplicates and represented as mean ± SD (standard deviation).
Mean values followed by different superscripts in a column are significantly different (p<0.05).

3.2.3. DPPH radical scavenging activity

The data of DPPH radical scavenging activity of pomegranate fruit peel ethanolic extracts is exhibited in Table 4. The percentage inhibition of DPPH radicals was increased with the increasing concentration of extracts (50, 100, 150, 200, 250 and 300µg/ml). The IC_{50} value of the pomegranate fruit peel was determined to be 142.9µg/ml. The free radical scavenging activity of the sample was compared with that of the standard (natural and synthetic) antioxidants.

Table 4. DPPH radical scavenging activity of ethanolic extract of pomegranate fruit peels and standard antioxidants.

Sample concentration (µg/ml)	% of Inhibition	Standard antioxidants	IC_{50} (µg/ml)
50	20.00±0.02[d]	Rutin	42.07±0.00[b]
100	44.82±0.03[c]	Quercetin	50.82±4.00[c]
150	51.02±0.04[b]	BHT	52.97±8.23[d]
200	63.48±0.02[ab]	BHA	38.47±1.03[a]
250	74.61±0.10[a]		
300	84.92±0.12[a]		
IC_{50}	142.90±0.45[b]		

Values are performed in triplicates and represented as mean ± SD (standard deviation).
Mean values followed by different superscripts in a column are significantly different (p<0.05).
BHT - ButylatedHydroxy toluene; BHA - ButylatedHydroxy Anisole

4. DISCUSSION

Interest in finding naturally occurring antioxidants for use in foods or medicinal materials to prevent free radical imbalance has increased considerably over the past few year (Mahdavi and Salunkhe, 1995). Use of synthetic antioxidants *viz.,* butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) is restricted due to their carcinogenicity (Mahavi and Salunkhe, 1995). Therefore the need for identifying alternate, natural and safe source of antioxidants (especially of plant origin) has increased in recent years (Zainol*et al.* 2003). The therapeutic benefits of secondary metabolites of plant origin have been researched in several recent studies (Nayak and Lexiey, 2010). For this reason research has been focused on evaluating the antioxidant properties of medicinal plants.

Phytochemical analysis of pomegranate fruit peel extracts showed the presence of the antioxidant compounds *viz.,* total phenolics and total flavonoids. Phenolic compounds have attracted much interest recently because in *vitro* and *in vivo* studies suggest that they have a variety of beneficial biological properties like anti-inflammatory, antitumor and antimicrobial activities (Mbaebae *et al*., 2012; Meenakshi *et al*., 2012). Phenolic studies have attributed that antioxidant properties are due to the presence of phenols and flavonoids (Turkoglue *et al*., 2007). Therefore, it is necessary to determine the total amount of phenols and flavonoids in the fruit peel extract taken for the study. Flavonoids are the most diverse and wide spread group of natural compounds are likely to be the most important natural phenolics. They act as a primary oxidant or free radical terminators. Antioxidant activity of phenolic compounds is based is their ability to donate hydrogen atom to free radicals.

The results of DPPH scavenging activity as in this study indicated that the pomegranate fruit peel was potentially active. The study suggested that pomegranate fruit peel extract contained compounds that were capable of donating hydrogen to a free radical in order to remove odd electron which is responsible for radical's reactivity. The scavenging ability of DPPH radical by the fruit peel extracts was found to be appreciable which this implied that the fruit peel extracts might be useful for treating radical related pathological damages especially at higher concentrations (Wang *et al.,* 1998).

5. CONCLUSION

In this study, a strong correlation between antioxidant activities and their total phenols and flavonoids was found in pomegranate fruit peel extracts. Thus, the pomegranate fruit peels could serve as potential source of natural antioxidants against oxidative stress, which is associated with neurodegenerative disease and biological damage in living tissues. It can be concluded that the species could serve as a natural source of antioxidants in the food industry and with its other pharmacological properties. Hence, further investigation is required to isolate and elucidate the active principles, and evaluate pharmacological properties using animal

models before going for commercial production of drugs by pharmaceutical industries.

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PHARMACOGNOSTIC STUDIES ON *MILLINGTONIA HORTENSIS* L.

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ABSTRACT

The earth is home to a rich and diversity assemblage of living organisms. Natural plant products play a dominant role in the development of novel drug leads for the treatment and prevention of diseases. The phytochemical screening and qualitative estimation of *Millingtonia hortensis* showed that the leaves were rich in steroids, terpenoids, fatty acids, tannins, saponins, anthocyanins, coumarins and emodins. Sulphated ash was higher than the total ash, acid insoluble ash and water soluble ash. The information obtained from the present study is useful during the time of collection and also during extraction process. Using these standards, the plant can be differentiated from other related species. This study will contribute to the existing knowledge over the standardization aspects of the raw drug *M. hortensis.*

Keywords: Adulteration, *Millingtonia hortensis,* sulphated ash and parameters.

1. INTRODUCTION

Nature has provided a complete store house of remedies to cure all ailments of mankind. Plants are a reliable source of potentially important bioactive natural products (Kavitha *et al*., 2004) and are used as traditional medicines and pharmacopoeial drugs. The majority of plants used in the Indian traditional system of medicine have not yet been screened for their pharmacological activities related to their growth condition. Plants may serve as the base for the development of a medicine, natural blue print for the development of new drugs or a phytomedicine. The current estimation of the number of flowering plants ranges between 2, 00,000 and 2, 50,000 species in 300 families and 10,500 genera (Iwu, 1993; Marimuthu *et al*., 2008). Methanolic extracts of *Cassia alata* root showed anthraquinones (Jain *et al*., 2010); methanol extract of rhizome of *Cyperus tagetum* showed the presence of alkaloids, glycosides, proteins, amino acids, phenols, flavonoids, tannins and saponins (Nitai *et al*., 2010). The pharmacognostic studies on leaf of *Merugnkilanzhu* showed the presence of cyclocytic stomata, unicellular trichome and conjoint, collateral vascular bundle (Brinda *et al*., 1981); in *Holoptea integrifolia* leaf, the vessels are present in broken and curved form (Benjamin and Patrick, 2002); numerous calcium oxalate crystals, numerous trichomes on both surfaces, chemo microscopic characters include lignin, starch, cellulose and mucilage were noted in *Mitracarpusscaber*leaf (Abere *et al*., 2007); Presence of pitted xylem vessels and cluster type of oxalate crystals were noted in the leaf of *Amaranthus spinosus* (Chumbhale *et al*., 2009). Rananculaceous type of stomata, cortex are impregnated by schizogenous and laticiferous ducts, vein islets and veinlet termination are characteristics in leaf architecture and uniseriatebicellular, uniseriate multicellular trichomes in the *Gymnema sylvestre* leaf (Deokule and Pokharkar, 2010); glandular trichomes, arch shaped vascular bundle (mid rib), anisocytic type of stomata with three subsidiary cells, large number of fibers, prism type oxalate crystals and lignifiedxylem vessels with bordered pits in the leaf of *Physalis angulate* (Santhya *et al*., 2010). The literature survey shows scanty information available on the pharmacognostic and phytochemical properties of *Millingtonia hortensis*grow in study area. This promoted the present investigation to study the pharmacognostic, phytochemical properties of the above mentioned plant.

2. MATERIALS AND METHODS

2.1. Plant material

Fresh leaves of *Millingtonia hortensis* free from diseases were collected during the month of July 2015 from S.T. Hindu college campus, Kanyakumari District,Tamil Nadu, India (Elevation about 420 meters (Mean Sea Level). *Millingtonia hortensis*is used to carry out the pharmacognostic and phytochemical properties. Taxonomic identification of the plants was carried out with the help of Gamble (1957) and also compared with the herbarium present in Department of Botany, S.T. Hindu College, Nagercoil.

2.2. Extraction

The leaves were washed thoroughly 2-3 times with running tap water, leaf material was then air dried under shade after complete shade drying the plant material was grinded in mixer, the powder was kept in small plastic bags with paper labeling. The grinded leaves material of 5gm weighed using an electronic balance and was crushed in 25 ml of sterile water, boiled at 50-60°C for 30 minutes on water bath and it was filtered through Whatman No.1 filter paper. Then filtrate was centrifuged at 2500 rpm for 15 minutes and filtrate was stored in sterile bottles at 5°C for further use (Harbone,1973)

2.3. Preparation of whole plant dry powder of selected species

The selected species were collected and dried separately at room temperature (30±2°C) for about two weeks to get a constant weight. The dried leaf were ground to powder by mechanical device and stored for further biochemical analysis.

2.4. Preparation of plant extracts for preliminary phytochemical screening

The leafdrypowder samples were extracted with different solvents such as water, methanol, and chloroform at 20 % (w/v) level in a soxhlet apparatus. The extracts were concentrated and used for qualitative phytochemical analysis.

2.5. Phytochemical Screening

Preliminary qualitative phytochemical screening was carried out with the following methods.

2.5.1. Steriods

1 ml of the extract was dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids (Gibbes, 1974).

2.5.2. Terpenoids

2 ml of extract was added to 2 ml of acetic anhydride and concentration of H_2SO_4. Formation of blue, green rings indicates the presence of terpenoids (Ayoola, 2008).

2.5.3. Fatty Acids

0.5 ml of extract was mixed with 5 ml of ether. These extract was allow it for evaporation on filter paper and dried the filter paper. The appearance of transparence on filter paper indicates the presence of fatty acids (Ayoola, 2008).

2.5.4. Tannins

2 ml of extract was added to few drops of 1% lead acetate. A yellowish precipitate indicated the presence of tannins (Paris, 1969).

2.5.5. Saponins

5 ml of extract was mixed with 20 ml of distilled water and then agitated in a graduated cylinder for 15 minutes. Formation of foam indicates the presence of saponins (Gibbes,1974).

2.5.6. Anthocyanins

2 ml of aqueous extract is added to 2 ml of 2N Hcl and ammonia. The appearance of pink-red turn's blue-violet indicates the presence of anthocyanins (Paris, 1969).

2.5.7. Leucoanthocyanins

5 ml of aqueous extract added to 5 ml of isoamyl alcohol. Upper layer appears red in colour indicates for presence of leucoanthocyanins (Gibbes,1974)

2.5.8. Coumarins

3 ml of 10% NaOH was added to 2 ml of aqueous extract formation of yellow colour indicates the presence of coumarins (Rizk, 1982).

2.5.9. Emodins

2 ml of NH_4OH and 3 ml of Benzene was added to the extract. Appearance of red colour indicates the presence of emodins. (Rizk, 1982).

2.5.10. Ash values

Ash values such as total ash, acid insoluble ash, water-soluble ash, and sulfated ash were determined according to Indian pharmacopoeia.

2.6. Pharmacognostic Studies

2.6.1. Macroscopic Studies

Mature and healthy plants of *Millingtonia hortensis*were collected to study the morphological characters. By using hand lens in the field and dissection microscope in the laboratory, micro and macroscopic characters of the plantare recorded.

2.6.2. Microscopic (Anatomy) Studies

2.6.2.1. Collection and Preparation of Specimens

Care was taken to select healthy plants of normal growth. The required samples of different plant parts were cut and fixed in a mixture of FAEA

(5 ml Formalin + 5 ml Acetic acid + 90 ml of 70 % Ethyl Alcohol). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary-butyl alcohol as per the method given by Sass (1940). Infiltration of the specimens was carried out by gradual addition of paraffin wax (melting point 58-60^{o}C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

2.6.2.2. Sectioning

The paraffin embedded specimens were sectioned with the help of Rotary Microtomes. The thickness of the sections was 10-12 mm. De-waxing of the sections was done by customary procedure (Johansen, 1940). The sections were stained with Toluidine Blue as per the method of O'Brien *et al*. **(**1964). Since toluidine blue is a polychromatic stain, the staining results were remarkably good and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies, etc. Wherever necessary, sections were also stained with safranin and Fast-green and IKI (Iodine Potassium Iodide) for starch.

2.6.2.3. Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon Labphoto-2 microscopic unit. For normal observations, bright field microscope was used. For the study of crystals, starch grains and lignified cells, polarized light microscope was employed. Since these structures have birefringent property, under polarized light microscope they appear bright against dark background. Magnifications of the figures are indicated by the scale –bars. Descriptive terms of the anatomical features are as given in the standard anatomy books (Esaa, 1964).

3. RESULTS AND DISCUSSION

3.1. Description

Millingtonia hortensis L. (Family : Bignoniaceae) is a tall deciduous tree grows to a height of about 18 to 25m and can reach a maximum of about 80m, spread to an area of 7 to 11m. The tree bears a straight trunk with corky bark and lesser branches. It flowers at the night and shed flowers early in the morning.

3.2. Leaves

The leaves are large, ornamental, imparipinnate, opposite, tripinnately compound, exstipulate, petiolated**.** The upper tertiary leaflets are sessile, exstipullate and are ovate-lanceolate with a rounded or cuneate base, serrate margins with acuminate tips and 1-3 inches long.

3.3. Inflorescence

Paniculate cymes (terminal or axillary).

3.4. Flowers

Flowers are white, waxy, trumpet shaped and somewhat 2 lipped with 5 sub equal lobes. The tree flowers twice a year and the white flowers come as large panicles which emit a pleasant fragrance. They are bisexual, zygomorphic. The bell-shaped sepals of the flower have five small lobes with four stamens and paralleled anthers. The corolla is a long tube with 5 lobes.The lobes are valvate, ovate-lanceolate, densely pubescent adaxially margin. Ovary sessile, ovoid. Style long; stigma ligulate, compressed, 2-lobed, slightly exerted from corolla tube. Flowering usually takes place from April until the rains and again in October to December.

3.5. Fruits and Seeds

Fruits are smooth, flat, 2 valved, septicidal capsule, oblong, acute at both ends, woody. The seeds are discoid, compressed, winged, except the base; the wing is narrow at the apex and non-endospermic. The fruiting period is November-February.

3.6. Microscopic (anatomical) studies

3.6.1. Microscopic characteristics of Millingtonia hortensis

3.6.1.1. Root

The transverse section of *Millingtonia hortensis* root showed the presence of epiblema with single layer of cuticle. Unicellular root hairs are reported on the epiblema. Next to epiblema, 9-12 layer of parenchymatous cortex are present (homogenous) with intercellular spaces. Next to cortex, single layer of endodermis and pericycle. Then vascular bundles are present. Xylem is star shaped and thick. Phloem is present in the form of patches.

3.6.1.2. Stem

The transversesection of *M. hortensis*stem showed the presence of single layer of epidermis. Epidermis is covered by single layer of cuticle with epidermal hairs. Next to epidermis, heterogenouscortex is present. i.e, outer

parenchymatous, middle chlorenchymatous and inner sclerenchymatous. Medullary rays are traverse in between the xylem. Pith is parenchymatous. Phloem tissues are massive in nature.

3.6.1.3. Leaf

Transverse section of leaf shows a typical dorsoventrally structure. The epidermis of both the structure is single layered. Epidermal cells are rectangular and covered externally with cuticle. The upper epidermal cells are slightly bigger. A number of cells of upper as well as lower epidermis are elongated and are covered by multicellular hairs. Stomata are rananculaceous type and are mostly confined on the lower epidermis.

Mesophyll is differentiated in to two layers, viz. palisade tissue and spongy tissue. Palisade tissue is two rows of elongated chloroplast. Spongy parenchyma cells are loosely arranged with intercellular spaces on the lower side. Mid rib portion is bulged towards adaxial side of the leaf. The vascular bundles are surrounded by the parenchymatous bundle sheath. The xylem is characterized by the presence of small vessels, tracheids and fibers. The xylem lies towards upper epidermis and phloem lies below the xylem i.e. towards lower epidermis.

The phytochemical screening and qualitative estimation of *Millingtonia hortensis* studied showed that the leaves were rich in steroids, terpenoids, fatty acids, tannins, saponins, anthocyanins, coumarins and emodins (Table 1). Preliminary qualitative studies according to Mallikh *et al.,* 2007 is useful in the detection of bioactive principles and subsequently may lead to drug discovery and development. Vaghasiya *et al*., 2011 analyzed fifty three medicinal plants for phytochemical characterization.

Table 1. Preliminary phytochemical analysis of leaf in water, chloroform and methanol extract on *M. hortensis* L.

Phytochemical group	Leaf		
	Water	Chloroform	Methanol
Alkaloids	-	-	-
Steroids	-	+	+
Terpenoids	+	+	+
Fatty acids	-	+	+
Tannins	+	+	+
Saponins	+	+	+
Anthocyanins	-	-	+
Coumarins	+	-	+
Emodins	-	-	+

Table 2. Ash analysisof leaf of *M. hortensis.*

Plant part	Total ash (%)	Acid insoluble ash (%)	Water soluble (%)	Sulphated ash (%)
Leaf	10.5	6.0	2.0	15.0

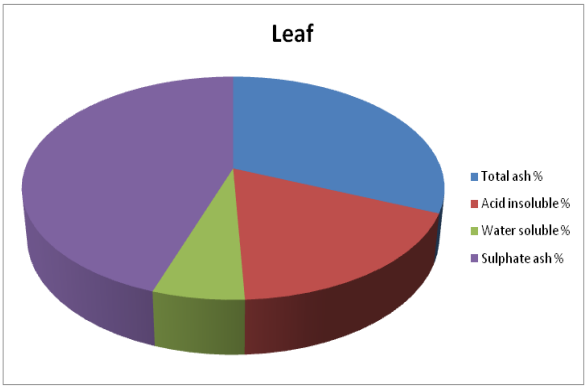

Fig. 1. Ash analysis of leaf of *M.hortensis*

Table 3. Colour analysis of leaf of *M. hortensis*after treated with different chemicals.

Treatment	Leaf
HCL	Brown
H_2SO_4	Brown
HNO_3	Yellow
Acetic acid	Red
Iodine solution	Orange red

Four types of ash were determined of the leaf of *M. hortensis* and their percentage values were recorded in Table 2 and Fig. 1). Sulphated ash was higher than the total ash, acid insoluble ash and water soluble ash. Powders of leaf were treated with concentrated acids, Iodine solution and colour changes in the powder were recorded in Table 3

Ash values, colour under various acids and the qualitative evaluation of extract for the phytochemical groups were parameters used for the characterization of botanical drug, and these are the preliminary steps of the quality control for herbal drugs. Biological activity of crude drug is mainly due to the active chemical constituents, and the constituents may be soluble in different polar, semi polar andnon-polar solvents (Kokate *et al.,* 2005).

Ash value of medicinal plants reflects the carbonate, phosphate, oxides, silicate and silica. Moreover the total ash of a crude drug also reflects the care taken in drug preservation, and the purity of crude and the prepared drug. Acid soluble ash reflects the calcium oxalate content of the drug. In the present investigation considerable amount of

total ash was noticed in leaf, findings can be employed as quality parameter to evaluate *M. hortensis* biomass for any adulteration.

Phytochemical profiling of aqueous, chloroform and methanol extracts of leaf for steroids, terpenoids, fatty acids, tannins, saponins, anthocyanins, coumarins and emodins emerged with noticeable results. Like alkaloids were absent in leaf, same results were reported by Misra, 1909. Such outstanding phytochemical screening results can be good tool for identification of *M. hortensis* biomass particularly when grinded to fine powder.

Various studies have been demonstrated that coumarins are a potential antioxidant and its antioxidant activity is due to its ability to scavenge free radicals and to chelate metal ions (Tseng, 1991). Emodins isolated from a great deal of herbs are an effective constituent with many effects. Lots of pharmaceutical studies have demonstrated that emodin has many biological effects, such as anticancer, antimicrobial and anti-inflammatory effects (Wang *et al.,* 2007). The growth of many fungi, yeasts, bacteria and viruses was inhibitedby tannins (Chung, 1998). Terpenoids and tannins are attributed for analgesic and anti-inflammatory activities. Apart from this, tannins contribute property of astringency (faster the healing of wounds and inflamed mucous membrane). Traditionally saponins have been extensively used as detergents, as pesticides and molluscicides is addition to their industrial applications as foaming and surface active agents and also have beneficial health effects (Shi *et al.,* 2004).

The following previous studies also supports ourpresent study, roots of *Strychnonspotatorum* (*Mallikharjuna et al*., 2007), leaves of *Bauhinia racemose* (Sharanabasappa *et al*., 2007), methanolic extract of roots and leaves of *Hyptissuaveolens* (Nwobu *et al*., 2010), ethanolic extracts of roots of *Rumex vesicarius* (Hari and Rama, 2011).

3.7. Phytochemical studies

Plants can manufacture many different types of secondary metabolites, which have been subsequently exploited by human beings for their beneficial role in a diverse array of applications.Often, plants secondary metabolites may be referred to as plant natural products, which have illicit effects on other organisms.

The results of present study showed that the plant parts of *Millingtonia hortensis* having rich primary and secondary metabolites such as alkaloids, tannins, phenols and starch can be used as industrial raw materials. Therefore, economic use depends partially on the quantitative and qualitative aspects of their organic reserves, specially carbohydrates, proteins, phenols and lipids. These metabolites are further used for biosynthesis of bioactive compounds.

Preliminary phytochemical screening of plants is very useful for the determination of the active constituents in different solvent extracts. Among the phytochemicals tested, terpenoids tannins andsaponins are reported in all the extracts of *Millingtonia hortensis*. Alkaloids are not reported.

However, there are variations in the presence and absence of phytochemical compounds in the various solvent extracts of the selected plant tested.Phytochemical and pharmacological studies of *Bauhinia* species have demonstrated the presence of flavonoids (Silva**,** 2002). Moreover some researchers reported that flavanones, flavones and flavones were the three types of flavonoids found in citrus fruits (Fernandez and Lopez, 2002; Calabro *et al.,* 2004; Ebrahimzadeh *et al.,* 2004; Jayaprakasha and Patil, 2007). Non- polar flavonoids have been isolated from bay leaves (Demo *et al.,* 1998; Elmastas *et al*., 2004).

4. CONCLUSION

The pharmacognostic characters and phytochemical values reported in this plant could be used as the diagnostic tool for the standardization of this plant. Adulterants if any can be easily identified using these parameters. The microscopic features could help in laying down micro morphological standards as per WHO guidelines for authentication of the drug. Macroscopic as well as microscopic studies of any phytodrug are the primary steps to establish its botanical quality control before bring to other studies. As per WHO norms, botanical standards are to be proposed as a protocol for the diagnosis of the herbal drug. The above mentioned parameters are helpful for the future identification and authentication of the plant in the herbal industry and in factories. The ash values, colour under different chemicals will be useful to identify the authenticity of the drug even from the crushed or powdered plant materials. The information obtained from the present study is useful during the time of collection and also during extraction process. Using these standards, the plant can be differentiated from other related species. This study will contribute to the existing knowledge over the standardization

aspects of the raw drug *M. hortensis.*

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ANTIBACTERIAL ACTIVITY LEAF EXTRACTS OF *STRYCHNOS NUX VOMICA* L. - A MEMBER OF LOGANIACEAE

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ABSTRACT

The present study investigates the antibacterial activity of various solvents leaf extracts of *Strychnos nux vomica* against four different bactria strains like *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Salmonella paratyphi b, Alternaria alternate, Aspergillus flavus, Penicillium notatum* and *Cladosporium carrionii.* All the results were compared with respective positive control.

Keywords: *Strychnos nux vomica,* antimicrobial activity.

1. INTRODUCTION

Medicinal plants are rich in traditional knowledge drastically recede in the wake of burgeoning population pressure, acculturation, rapid modernization, multi various human developmental activities. A lot of current research work in ethnobotany is concerned with the loss of traditional knowledge and the preservation of biological diversity in remote parts of the world where cultures and their ecosystems are being destroyed by development (Sereiti *et al*., 1999). The ethnobotanical investigations may become invaluable to rescue knowledge in imminent danger of being lost and to find out new bioactive compounds in plant. The use of plant preparations as foodstuff, insecticides, CNS active, cardio active, antitumor and antimicrobial agents are some examples of immense chemical diversity in plants (Balick *et al*.,2000).

The phytochemical techniques various active principles of many medicinal plants have been isolated and introduced as valuable drugs in morden system of medicine (Dennis,1988). *Strychnos nux vomica* is an evergreen tree native to South East Asia and India belonging to the family Loganiaceae. It is medium sized tree found mostly in open habitats. Two poisonous alkaloids, Strychnine and brucine are found in Strychnos *nux vomica* tree. Traditionally used for treating acute diarrhoea, mixed with lemon juice and made into pills and taken orally during dysentery, arthritis, rheumatism and piles (Dubey *et al*.,2012).

2. MATERIALS AND METHODS

2.1. Plant collection and identification

The plant *Strychnos nux vomica* was collected from the Nilgiris, Tamilnadu, India. The plant was identified and authenticated by a plant taxonomist.

2.2. Extraction of the plant material

About 250 g of freshly collected sample of *Strychnos nux vomica* (leaf) was separately washed 2-3 times with water followed by distilled water and shade dried. All the dried parts were pulverized by mechanical grinder (willey mill) to get the powder through 100 mess sieve and then stored in a refrigerator. It was extracted by cold extraction method with petroleum ether, ethanol, methanol and aqueous. Then all the extracts were concentrated in a rotary evaporator to yield a syrupy residue and used for all the phytochemical analysis.

2.3. Tested Microorganisms

Bacterial strains *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Salmonella paratyphi* b.

2.4. Disc diffusion method

The antibacterial activity of leaves extracts of *Strychnos nux vomica* was evaluated by disc diffusion method. The culture media were prepared and autoclaved at 121^{o}C at 15 psi for 20 minutes and stored in refrigerator. The media were melted before the process of inoculation. The clean dry sterile Petri dishes were poured with nutrient agar medium for bacterial strains. Ten number of 10 ml broths were prepared separately for nutrient agar medium in test tubes and plugged with cotton and autoclaved. The test tubes were labeled according to the microbes to

be inoculated. The bacterial strains were inoculated into the nutrient broth aseptic conditions and incubated at 37+0.5°C for 18 hours. After incubation, the bacteria were smeared on the nutrient agar plate respectively using a sterile cotton swab. A sterile disc of 6 mm diameter was loaded with known quantity of 10 mg of dried crude extracts of aqueous, petroleum ether, ethanol and methanol extracts and dissolved in10 ml of DMSO. These discs were placed on the surface of the media. The positive control antibiotics viz., chloramphenical (10µg) were maintained. Then the Petri dishes were incubated at 37+0.5 °C for 12 to 14 hours. The diameters of inhibition zones were measured. (Bauer *et al.,*1966).

3. RESULTS

Aqueous, petroleum ether, ethanol and methanol leaves extracts of *S. nux vomica* was assessed for antibacterial (Table 1). The results showed that among the four extracts, methanol showed significant result of antibacterial activity. When compared with other extracts, aqueous extract showed minimum level of inhibition. Among the seven bacterial strains, maximum zones were observed in the following bacterial strains such as *B. subtilis, P. aeruginosa* and *E. coli. S. paratyphi- b* were found to be highly susceptible to methanol extract. The inhibition zone of methanol extract was similar to that of the control, chloramphenical.

Table 1. Antibacterial activity of various solvent leaves extracts of *Strychnos nux vomica.*

S. No.	Microorganisms	Zone of Inhibition (mm)				
		Various solvent extracts used (mg/ml)				Control
		Aqueous	Petroleum Ether	Ethanol	Methanol	Chloramphenical
1.	*Bacillus subtilis*	9	11	10	14	15
2.	*Escherichia coli*	10	12	11	17	21
3.	*Pseudomonas aeruginosa*	9	11	9	12	16
4.	*Salmonella paratyphi b*	8	13	7	11	12

4. DISCUSSION

The results showed that the aqueous, petroleum ether, ethanol and methanol leaves extracts of *S. nux vomica* has revealed a significant results of antibacterial activity when compared with other extracts except aqueous showed minimum level of inhibition. Among all the bacterial strains, maximum zones were observed in the following bacterial strains such as *B.subtilis, P. aeruginosa* and *E. coli. S. paratyphi- b* were found to be highly susceptible to methanol extract. Plant extracts are potential sources of novel anti-microbial compounds especially against bacterial pathogens. Phytomedicine can be used for the treatment of diseases as in case of Unani and Ayurvedic system of medicine or it could be the base for the development of medicine, a natural blue print for the development of a drug (Didry *et al*., 1988). The plant extracts were screened against human pathogenic bacteria to check antibacterial activities by agar well diffusion method, which showed valuable zone of inhibition. Specifics zone of inhibition against different types of pathogenic bacteria (Alam Morshed *et al*., 2011). Our study contradicted the earlier reports of Gnanavel *et al* (2012).

5. CONCLUSION

In the present study indicated a significant antibacterial activity effect of the leaf extracts of *Strychnos nux vomica* and exposes the existing potential of the other parts of the tree to be explored for other medicinal benefits of the human kind.

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A SURVEY OF *ISOETES COROMANDELINA* L. F. POPULATIONS IN TIRUNELVELI DISTRICT

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ABSTRACT

The genus *Isoetes* is a cosmopolitan genus found around the world. The genus consists of aquatic or semi-aquatic plants needing water for survival. The distribution of *Isoetes coromandelina* L. f. in the freshwater ponds of Tirunelveli district was studied. The results of the survey show that the population of *Isoetes coromandelina* in Tirunelveli district are healthy and flourish wherever non-polluted, nutrient poor waters are available. Although there is no danger to the populations of *Isoetes* at present, water pollution could endanger the survival of the species.

Keywords: *Isoetes coromandelina,* survey, Tirunelveli, cosmopolitan genus.

1. INTRODUCTION

Isoetes L. is a cosmopolitan genus of heterosporous lycopsids comprising approximately 150 species found in lakes, wetlands (swamps, marshes), and terrestrial habitats (Taylor *et al*., 1993) all over the world. 16 species of *Isoetes* have been reported from different geographical regions of India. Most of the populations are found growing along the margins of small ditches and ponds (Srivastava *et al*., 1993).

Around the world, the genus *Isoetes* is facing a lot of issues that affect its survival due to loss of habitat, an increase in agricultural land use, and invasion of exotic species. *Isoetes* has been declared an endangered species in Korea (Changkyun *et al* ., 2008). In China all the four species present face extinction due to habitat loss, agricultural land use and invasion by exotic species (Fu and Jin, 1992). Local populations of *I. coreana* in Korea growing in marshy areas close to farmland face intense competition from other hydrophytes such as *Eriocaulon sieboldianum*, *Juncus effuses* var. *decipens* Buchen, and *Scirpus triangulates* Roxb. As a result, *I. coreana* plants are now isolated and endangered in South Korea.

Isoetes is an aquatic / amphibious species. The requirement of a large amount of standing water is essential for the survival and reproduction of the species. Tirunelveli district falls in the rain shadow region of the southern Western Ghats. Consequently, while the western parts of the district may receive some rainfall and also some streams dot the countryside, as one moves to the eastern part of the district, the availability of freshwater decreases. The ponds near and east of Tirunelveli/ Palayamkottai depend on the water released from the dams. When the north-east monsoon fails, the ponds in the eastern parts of the district have been observed to be dry and without water for years together.

Though *Isoetes* cannot live without water, it has been observed to regrow vigorously once water is made available either through rain or by release from the dam. Could the survival of *Isoetes* during the rainfall deficient years be due to the perennating structures present in the soil? Could these also account for the rapid growth and colonization of large stretches of a pond's area once water is available?

This survey of *Isoetes* populations in Tirunelveli district aims to answer these questions. The study of the population, population distribution, population structure and genetic diversity of the populations is important to have an idea of the extent of the spread of the population and its genetic health. It will also help us to analyze and understand how the species is coping in the wild to environmental challenges and changes. Such knowledge can give us critical insights and knowledge into the adaptive response of the species and the population as a whole to the stresses facing the populations. It would also help us to understand the long term risks facing the species and the steps needed to be taken to conserve the species. Therefore, this study was undertaken to survey and document the areas in Tirunelveli district where there are *Isoetes coromandelina* L. f. populations

2. MATERIALS AND METHODS

2.1. Area of the study

Tirunelveli District was taken as the area for the study. The *Isoetes* populations at the different sites were studied. The location of the study sites along with their latitude, longitude, altitude and ecological characteristics are given in Table 1.

Fig. 1. Map of the study area

2.2. Plant material

Isoetes coromandelina L. f. was taken for study.

2.3. Methods

The study sites were inspected and the ecological characteristics noted. The plants were collected in the field. The GPS data was collected a Garmin 12XL device. Studies on the distribution of the *Isoetes* populations were conducted and noted. The study period was between November 2014 to March 2015.

3. RESULTS AND DISCUSSION

The characteristics of the different populations surveyed in Tirunelveli District are given in Table 1. Plate 1 shows the different habitats where *Isoetes* coromandelina grows in Tirunelveli District. The map of Tirunelveli District is given in Figure 1. A wide area with St. Xavier's College, Palayamkottai as the base has been surveyed. To the west, the plant populations along the Palayamkottai – Papanasam road up to Ambur railway gate have been surveyed. Along Ambur gate the populations have been surveyed up to Karuthapillaioor and then on to Alwarkurichi and further to Edaikal and then on to Palayamkottai. To the east, the Nanguneri - Uvari road up to Uvari has been surveyed. A total of 18 populations have been surveyed. Due to constraints of time, some taluks in Tirunelveli district i.e. Kadayanallur, Vasudevanallur, Sankarankovil, Kuruvikulam, Shencottah and Melaneelithanallur could not be surveyed.

Table 1. List of *Isoetes* populations in Tirunelveli district surveyed in the current study.

S. No	Location	Latitude	Longtitude	Altitude	Ecology
1	Cheranmahadevi railway gate	N 8. 40.580	E 77 33.570	202 ft	This is a small population of *Isoetes* found in a natural ditch near the railway gate. The soil is a rich loamy reddish clay and the plants are growing in a semi-amphibious state. The growth of the plants is not very vigourous and they are very well spread out in the depression.
2	Veeravanallur	N 8. 41. 026	E 77. 31.714	228ft	The *Isoetes* plants were found to be growing in an irrigation channel filled with rainwater in a semi-amphibious and some plants were submerged in the water. The plants in this area showed vigourous growth. This may be because of the soil which was clay soil with a lot of humus present in it. The plants grew very tall and formed a thick mat on the floor. The population was intertwined with many *Marsilea* plants.

3	Veeravanallur Thadagam	N 8.40.8 30	E 77.29 520	259 ft	A small pool at the base of a hillock. The *Isoetes* are fringing the pool. Plants are separated and present like a fairies ring. Plants not clumped or dense in growth.
4	South Pappankulam – Kallidaikurichi – Manimuthar Road	N8.40.252	E 77.27.808		Small population at the base of a hilling two small clumps. One clump has 40-50 plants and the second one has 7-10 plants.
5	Kalllidaikurichi Railway Station	N8.40.8 01	E77.28.039	255	Sand – red soil, stagnant pool, *Isoetes* –big population in an area 50 x 20 m, *Isoetes* plants at various stages forming a dense carpet at various places
6	Way to Ambur Esakkiamam Temple Pond	N8.44.523	E77.24.880	294	Sand-red soil, big pond covered with *Isoetes,* huge population area50 x 50m rimming the edges of the pond
7	Way to Ambur Kulam	N8.45.383	E77.24.354	332	Sand – red soil, a pit on a sloping hillside, small population, *Isoetes* suppressed by angiosperm weed
8	Puthuparaian kulam	N8.45.358	E77.23.645	342	Big pond –small population seen near northern canal, population size 3 x 5ft, phenotypic differention in populations, population growing in water with tall individuals , population growing in soil with short individuals
9	Kaveri konthankulam	N 8.45.425	E77.23.209	333	A big pond with sandy soil dominated by *Isoetes* at its eastern edges, *Isoetes* present as beds approx. 10 m in width from edge of the pond, length of the *Isoetes* beds>100m. *Isoetes* runs around the entire lake
10	Achan kulam Alwar kurichi	N8.47.276	E77.21.996	409	Base of hill, gentle slope, red soil, tank is dry now, 15m wide x 50m, present as a lush green mat in the process of drying.
11	Vellikulam pottalputhur	N8.47.605	E77.24.840	323	Base of excavated hill in pools of water- *Isoetes* found growing in water and all around the water's edge-plenty of algae for a thick mat on water-water has huge amount of humic content
12	Idaikal kulam – Adaichani	N8.45.533	E77.27.290	300	Big pond sloping to south- *Isoetes* present on edge of the pond –red soil-clear water.
13	Kakkan Nagar	N 8.408794	E 77.752503	-	On the Nanguneri – Uvari Road. Present as a lush green mat along the boundary of INS Vijayanarayanam base
14	South Vijayanarayanam	N 8.40466	E 77.763869	-	The *Isoetes* plants grow along the sides of a local pond. The *Isoetes* plants would cover a few acres atleast fringing the rims of the pond. Very abundant.
15	Tharuvai	N 8.671068	E 77.680560	-	A small population along the edges of the pond
16	Gopalasamudram	N 8.683392	E 77.644780	-	A good population present near the local pond
17	Veeravanallur	N 8.686786	E 77.524472	-	A randomly dispersed population near the

18	Police Station Veeravanallur Petrol Bunk	N 8.687382	E 77.521569	-	police station Sand red soil rich with humus. Stagnant water present in a natural depression. A small population present.

Plate 1. *Isoetes coromandelina* in different environments of Tirunelveli District.

Along side of a hillock

In a irrigation channel.

Growing among rocks

Growing along the edges of the pond.

Growing on land and submerged

Growing in the irrigation channel.

Growing along a hill side

Growing in a stagnant pool of water.

18 populations of *Isoetes* have been surveyed in this study. This study establishes that at the sites investigated i.e. fresh water ponds with an abundant supply of water, there are thriving populations of *Isoetes* . It also seems to suggest that a clean environment with oligotrophic waters is essential for the continued growth and survival of *Isoetes*.

At many sites such as Veeravanallur, Kallidaikurichy Railway Station, Ambur Esakkiamman Temple Pond, Puthuparaiyankulam, Kaverikoonthankulam, Achan Kulam, Vellikulam, Kakkan Nagar, South Vijayanarayanam the populations of *Isoetes* were well established and covered a huge area. At Pappankulam and Veeravanallur Thadagam the populations were very small. In fact, it seems that the population at Pappankulam could be either a newly established population or an old population which was on its way to disappearance locally. At Puthuparayankulam, the lush growth of *Isoetes* was seen. Further, it was seen that some of the *Isoetes* grew in the water while some grew away from the water on higher ground.

It has been reported by Sand – Jensen and Sondergaard (1979) that the quillwort *I. lacustris*, a submerged evergreen perennial, inhabits mainly nutrient - poor lakes. This is confirmed by Boston and Adams (1987) in their study of *Isoetes*. Quilloworts have evolved various adaptations to infertile habitats, including CAM metabolism (Keeley and Busch, 1984), high root biomass and slow leaf turnover (Boston and Adams, 1987; Gacia and Ballesteros, 1994). The adaptation to infertile habitats could explain the abundance of *Isoetes* in nutrient poor freshwater lakes.

4. CONCLUSION

A survey of the populations of *Isoetes coromandelina* in Tirunelveli district has been undertaken. The district received copious rain during 2014 – 15 due to which the ponds were full to the brim either with the rainwater or the water released from the dams. This has helped the growth of *Isoetes*. The growth of *Isoetes* was observed in ponds that had been dry for many years.

Though *Isoetes* cannot survive without water, the species has demonstrated its ability to recover and establish itself when water is available. Vigorous growth of *Isoetes* was observed in many ponds.

Luxuriant growth of *Isoetes* was observed only in environments with stagnant or standing water. However, *Isoetes* is also observed to along the gentle slope of hillock (Achankulam) where there is no chance for the water to stagnate.

The survival and reestablishment of *Isoetes* could be to the perennating structures present in the soil or to the spores present in the soil. The uniform growth of *Isoetes* observed at almost all the ponds at the same time seems to suggest that the growth of *Isoetes* in each pond could have been due to the perennating structures present there. If this is true, then *Isoetes* demonstrates a remarkable ability to overcome drought for long periods of time.

This study is a preliminary study aimed at studying the distribution of *Isoetes* in Tirunelveli district. Further studies on the population structure, population dispersal and genetic relationships of the different populations will give a clearer picture of the nature of *Isoetes* in Tirunelveli district.

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LEMONGRASS OIL - A MAJOR SOURCE OF INCOME FOR THE TRIBALS OF WAYANAD DISTRICT KERALA

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ABSTRACT

Paniyas, Adiyas, Kattunaikan, Kuruman, Karimbalan and Kurchiyas are the native tribal communities and inhibiting the Wayanad district of Kerala. Most of the tribals have engaged in the collecting of minor forest produce and lemongrass cultivation. They have also involved as agriculture labours and casual labours for forest dept etc to meet their demand for basic livelihood. Cultivation of lemongrass for oil distillation is considered to be one of the major sources of income for them. The oil yield was determined on basis of grass biomass distilled and the quantity of oil extracted. Among the study areas, Pulpally registered higher annual biomass production of lemongrass (8380 kg/acre) followed by Ambalavayal (7800 kg/acre), Meppadi (7540 kg/acre) and Mananathavady (6440 kg/acre). Lemongrass cultivation and extraction of lemongrass oil from the host provide better job oppurtunities and fairly good economic return in Wayanad district.

Keywords: Lemongrass oil, Biomass, Tribals, Livelihood, Wayanad.

1. INTRODUCTION

Wayanad is rich in biodiversity with high percentage of endemism in southern Western Ghats. The district has high percentage of tribal communities in Kerala. The native tribal communities *viz;* Paniyas, Adiyas, Kattunaikan, Kuruman, Karimbalan and Kurchiyas are living in biodiversity rich areas of Wayanad district. Most of the tribal people involved in the collection of minor forest produce and casual labours for forest department, agriculture, Reeds and Bamboo processing and lemongrass cultivation are some other major activities for their livelihood. Lemongrass cultivation is generally considered to be a major source of income for tribal people apart from other sources. Encouragement for lemongrass cultivation in fragile ecosystems and rocky slopes may improve the economic status of tribals in Wayanad, besides providing ecological security. Lemongrass oil is known in trade for 200 years; however systematic cultivation and distillation of lemongrass oil commenced only in 1882 in Kerala. Lemongrass oil is of high commercial value and it is a major source of income for a number of cultivators. Though it is important, very little information is available about the nature of the crop and its cultivation practices, socio-economic status of the cultivators, economics of the cultivation and the problems of the cultivators. As tribals are the major cultivators of the crop it is important to examine the status of lemongrass cultivation in tribal economy in the region. As there is no systematic attempt has so far been made to examine the above aspects (Jayapradeepu, 2003) this study focused attention on them

2. MATERIALS AND METHODS

2.1. Study area

Wayanad district is located in the northern part of Kerala state. It lies between the lattitudes 11˚27´ and 12°58´ N and the longitude between 74°52´ and 76° 07´ E. This district is most popular for agriculture and vegetation of high biodiversity in Kerala. The south west monsoon (June – September) brings copious rainfall in this region. The average rainfall in different places of Wayanad ranges between 1000mm and 4000mm per year. The altitude of hill ranges of Wayanad is ranging from 700 msl to 2100 msl. The major native tribal communities of Wayanad are Paniyas, Adiyas, Kattunaikan, Kuruman, Karimbalan and Kurchiyas.

2.2. Methods

A case study has been conducted among the tribals Paniyas, Adiyas, Kattunaikan, Kuruman, Karimbalan and Kurchiyas who settled in different areas of Wayanad districts. These tribals are cultivating lemongrass for oil distillation, which is considered to be one of the major sources of income. Details of the tribal communities like places of their shelter, population, employment opportunities etc were collected from the forest department. The harvested lemongrass biomass was pooled together over a period of one year to arrive the annual

production (Singh and Yadhava, 1974). The harvesting interval is approximately 60 days. The data oil yield was collected from tribals on basis of grass biomass distilled and the quantity of oil extracted.

3. RESULTS AND DISCUSSION

Besides having high endemism and rich biodiversity, Wayanad is having high population of tribals namely, Paniyas, Adiyas, Kattunaikan, Kuruman, Karimbalan and Kurchiyas (Table 1). Due to the high population of tribals and to meet the demand for basic livelihood they were engaged in activities like collection of minor forest produce, casual labours in forest department, lemongrass cultivation etc. Forest is a source of many valuable minor forest produces like different parts of plants and animals for medicinal use, tanning compounds and waxes, extractives such as bark, dyes, fibres, gums, latexes, oils, resins and food like bush meat, flowers, fruits, honey, nuts, leaves, seeds and spices and other products like fuel-wood and bamboo (Shylajan and Mythili 2003). The production of lemongrass biomass is widely varied between the months and also across the places of its cultivation (Table 2 and Fig 1).

Table 1. Habitation, Population and income sources of various tribal communities in Wayanad.

S.No.	Tribe	Places of settlement	Population	Income source
1.	Paniya	Pulpally Mullankolly	69116	Majority are casual labours. Agriculture labours Collection of minor forest product
2.	Adiyan	Thirunelly Thrisselery Vermam	11191	Agriculture labours Collection of minor forest product Cultivators
3.	Kattunaikan	Pookode Muppainadu Muttil Manjoora	17051	Collection of minor forest product Landless agriculture labours Lemongrass cultivation forest labour Small scale cultivation
4.	Kuruman	Cheeral Irulam Kuppadi Noolpuzha Kotathara	20983	Agriculture labours Collection of minor forest products Marginal farmers Lemongrass cultivators
5.	Karimbalan	Edavaka Kaniambetta Mullenkolly Pozhuthana	145	Collection of minor forest produce Shifting cultivation Agriculture labours Lemongrass cultivation
6.	Kuruchiya	Mananthavady Meenangadi Nenmeni Poothadi	25266	Agriculturalist Co-operative farming

Table 2. Annual biomass production and area of lemon grass community, oil content and production and revenue generated in the study sites.

Sites*	Annual production of lemon grass (kg/ha)	Area of lemon grass community (ha)	*Oil content (%)	Oil production (kg/ha/yr)	**Annual revenue (Rs/total area of grass community)
I	6440	2	0.40	25.76	25760
II	8380	3	0.45	37.71	56565
III	7540	2.5	0.42	31.66	39575
IV	7800	3	0.44	34.32	51480

*Site I-Mananthavady, Site II - Pulpally, Site III - Meppadi, Site IV – Ambalavayal.

Generally, the production of grass is peak during rainy season (June- Sept) and falls down to low level during dry months (Mar – April) in all cultivated areas. According to Tothill (1985) the decomposition of dead biomass during the dry season leads to produce higher ammonia since it is too dry for nitrification to occur and at the beginning of wet season ammonia gives way quickly to nitrate which is readily observed by growing vegetation. Among the study areas, Pulpally registered higher annual biomass production of lemongrass (8380 kg/acre) followed by Ambalavayal (7800 kg/acre), Meppadi (7540 kg/acre) and Mananathavady (6440 kg/acre). The percent availability of lemongrass oil extracted from the grass, *Cymbopogan citratus* varied according to climatic and soil conditions of the habitat (Paulsamy 2004, Peter 2012).

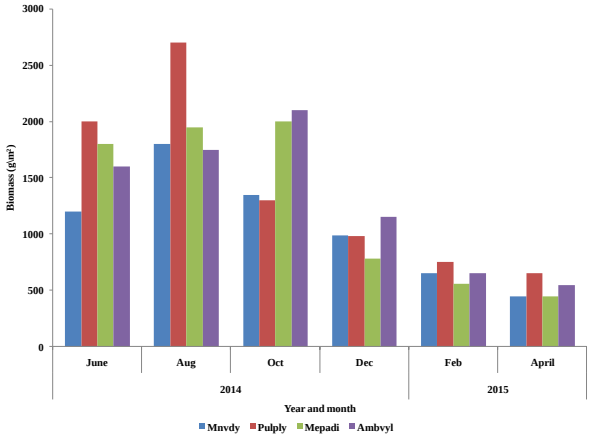

Fig. 1. Above ground biomass of lemongrass in the study sites of Wayanad, Western Ghats, Kerala during the sampling months.

Mnvdy – Manathavady, Pulply – Pulpally, Mepadi – Mepadi, Ambyyl - Ambalavayal

The area of cultivation of lemongrass in the studied site ranged between (2 to 3 hectares Table 2). The yield of lemongrass oil was also varied considerably between the places of cultivation (Table 2). The grass cultivated in Pulpally region yields high rate of 37.71 kg/ha/yr oil production. The lower rate of oil production is found in Mananthavady 25.71 kg/ha/yr oil production. Paulsamy *et al.,* 2000 reported that the relative humidity of air and contents of phosphorus and potassium in the grass are the major factors influencing the lemongrass oil yield in Anaimalais. The annual production of lemongrass oil is higher for Pulpally and it is followed by Ambalavayal, Meppadi and Mananthavady (Table 2).

The economic relation through lemongrass oil is greater in Pulpally (Rs 56565/-) followed by Ambalavayal (Rs 51480/-), Meppadi (Rs 39575/-) and Mananathavady (Rs 25760/-) (Table 2). It is concluded that the lemongrass cultivation and extraction of lemongrass oil from the host provide better job oppurtunities and fairly good economic returns to meet the basic demands for tribal communities in Wayanad. Cultivation by following modern farm practices is supported to get still more revenue for tribal communities.

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ANTI-ANALGESIC ACTIVITY OF METHANOLIC LEAF EXTRACT OF *WATTAKAKA VOLUBILIS* (LINN. F.) BENTH EX. HOOK F. (ASCLEPIADACEAE) - A RARE AND THREATENED MEDICINAL PLANT

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ABSTRACT

The present study, investigates themethanolicleaves extract of *Wattakaka volubilis* (Family: Asclepiadaceae) designated as 'the extract' was evaluated for analgesic activity in mice. The analgesic activity was evaluated in mice models. In the acute toxicity study, it was found that the extract was non-toxic up to 1000mg/kg, p. o. The extract (150, 200 and 400 mg/kg, p. o.) was found to possessanalgesic activities in a dose-dependent manner and the effect was comparable with thatproduced by the standard drug, Diclofenac sodium.

Keywords: *Wattakaka volubilis*, analgesic activity, leaves methanolic extract.

1. INTRODUCTION

Wattakaka volubilis belongs to the family Asclepiadaceaeit is used as an alternate source of the Ayurveda drug Murva, the accepted botanical source of which is *Marsdenia tenacissima* (Roxb.) Moon (Yoganarasimhan, 2000). Murva are used in various diseases like anaemia, fever, diabetes, stomach disorders, typhoid, urinaryinfections and cough (Kolammal, 1978). Leaf juice is used to cure sprain. Leaf paste is taken along with pepper to treat dyspepsia and leaf powder taken orally along with cow's milk is reported to have anti-diabetic activity. It is also used to treat rheumatic pain, cough, fever, severe cold, boils and abscesses. Bark paste is taken along with hot milk for urinary problems. *W. volubilis* is used in the treatment of scorpion and snake bites (Sanyasi *et al.,* 2008; Kumar *et al*., 2007).

2. MATERIALS AND METHODS

2.1. Plant collection and identification

The plant *Wattakaka volubilis* was collected from Velliangiri Hills, a part of Western Ghats Coimbatore, Tamil Nadu. India. The plant was identified andauthenticated by a plant taxonomist.

2.2. Extraction of the plant material

About 250 g of freshly collected sample of *Wattakaka volubilis* (leaf) was separately washed 2-3 times with water followed by distilled water and shade dried. All the dried parts were pulverized by mechanical grinder (willey mill) to get the powder through 100 mess sieve and then stored in a refrigerator. It was extracted by cold extraction method with methanol. The extracts were concentrated in a rotary evaporator to yield a syrupy residue and used for all the phytochemical analysis and pharmacological activity.

2.3. Tail- flick method

The antinociceptive effect of the test substances was determined by the hot tail flick method described by Sewell and Spencer (Sewell and Spencer, 1976). One to two cm of the tail of mice was immersed in warm water bath (Swan scientific instruments) kept constant at 55 ± 1°C. The reaction time was the time taken by the mice to deflect their tails. The first reading is discarded and the reaction time was taken as a mean of the next two readings. Balb-C mice were randomly divided into five groups (six in each). Mice of group I received normal saline (0.1 ml/10 g, p. o.) group II received Diclofenac sodium(150 mg/kg, p. o.) and groups III, IV and V received 150, 200 and 400 mg/kg, p. o. of the extract, respectively. Thirty minutes later, the tail was immersed in the water bath and the tail flick response was recorded. The same experiments were repeated after 60 minutes and 120 minutes again.

2.4. Statistics

Data are presented as arithmetic mean ± S.E.M of at least six experiments. Statistical analysis was performed by one-way analysis of variance (ANVOA) followed by Dunnett's test or by Student's paired t-test. 'P' value of <0.05 was considered as statistically significant.

3. RESULTS AND DISCUSSION

It was found that the methanolic leaf extract was non-toxic up to 1000mg/kg, p. o. body weight up

to 24 hours. Thus one tenth of it, i.e, 1000 mg/kg, p. o. was taken as the initial starting dose and the other two selected doses were 150 mg/kg, p. o. and 200 mg/kg, p. o., respectively.

The leaves methanolic extract of *W. volubilis* produced a dose-dependent analgesic activity in the model and the effect produced by 400 mg/kg, p. o. of the extract was compared to the standard drug Diclofenac sodium (Table 1). Pain is 'an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage' (Nicholas and Moore, 2009). The drug that reduces the nociceptive response indicated by cutaneous thermic stimuli in the hot plate test might exhibit central analgesic properties or supraspinal analgesia (Matheus *et al.,* 2005). In tail flick test, the root extract of *P. corymbosa* produced slower onset of antinociceptive action than the aerial extract. The tail flick model is considered specific test for evaluation of the central pain (Marchioro *et al.,* 2005) at spinal levels (Wong *et al.,* 1994). In case of analgesic study, tail flick method, it showed time- and dose-dependent analgesic activities. The extract also produced a significant antipyretic effect inthe brewer's yeast-induced pyrexia model in rat.

Table 1. Effect of *Wattakaka volubilis* leaves methanolic extract on thermal nociception in mice (Tail flick method)

Drug	Dose	Min. after treatment		
		Response in s (mean± S. E. M)		
		30	60	90
Normal saline	0.9 mg/dl	3.1± 0.20	4.2± 0.51	3.0±0.21
Diclofenac sodium	10 mg/kg	4.2± 0.51	5.0± 0.15*	5.4± 0.40*
Extract	150 mg/kg	3.1± 0.30*	3.6± 0.31*	3.8± 0.52*
Extract	200 mg/kg	2.6± 0.31*	3.0± 0.22*	3.0± 0.21*
Extract	400 mg/kg	4.1± 0.50*	4.3± 0.60*	4.8± 0.56*

The values are expressed as Mean ± SEM; *n* = 6 animals in each group.Data were analysed by Tukey-Kramer multiple comparison test.

4. CONCLUSION

It can conclude from present study that *W. volubilis* leaves methanolic extract can be used for development ofstandardized herbal therapeutic formulation for analgesic activity conditions.

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ANTIBACTERIAL ACTIVITY OF THE MEDICINAL PLANT, *ACALYPHA FRUTICOSA* FORSSK.

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ABSTRACT

The present study is to confirm the antibacterial efficacy of root extracts of the folklore medicinal plant species, *Acalypha fruticosa* by using three alcoholic solvents *viz;* petroleum ether, ethyl acetate and methanol were tested against ten human pathogenic bacteria *viz*., *Pseudomonas aeruginosa, P. stutzeri, Escherichia coli, Micrococcus* sp., *Lactobacillus* sp*., Servatia* sp., *Moraxetta* sp., *Bacillus subtilis*, *B. thuriengensis*, and *Klebsiella pneumoniae* for assessing the antibacterial properties by adapting disc diffusion method. The results of the study revealed that all extracts showed varied degree of antibacterial activity against the tested pathogens. However, the methanol extract exhibited higher inhibition zone (21.83 mm) against the bacterium, *Bacillus subtilis*. This result supports the therapeutic importance of the species, *Acalypha fruticosa* in curing infectious diseases and encourages the extensive use of this species in health care practices.

Keywords: Folklore Medicinal plant, *Acalypha fruticosa*, Antibacterial activity, Microorganisms.

1. INTRODUCTION

Nowadays, an increasing number of infectious agents are becoming more resistant to commercial antimicrobial compounds (Hancock *et al.,* 2012). The necessity to develop new drugs requires varied strategies, among them, the bioprospection of secondary metabolites produced by medicinal plants (Dionisi *et al.,* 2012).Plants have been an essential part of human society since the start of civilization. In Ayurvedic Medicine, there are numerous herbs which have been used historically for treating a large variety ofailments. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. The universal role of plants in the treatment of diseases is established by their employment in all important systems of medicine. There are many herbs on earth which lies unexplored in the field of medicine or Science. Many of the plants used today were known to the people of ancient culture throughout the world for their preservative and medicinal powers (Zaika, 1975).However several plants are used in India in the form of crude extracts. Infusions or plaster to treat common infections without scientific evidence of efficacy (Ahmad *et al*., 1998). *Acalypha fruticosa* Forssk. belongs to the family, Euphorbiaceae is one such folklore plant used in traditional system of medicine in Coimbatore district of Tamil Nadu, India. It is Gregarious bushy shrub, occurring in the tropical regions of India (Matthew, 1995). This plant species has been used as a folk remedy for the treatment ofdigestive troubles such as dyspepsia, colic and diarrhea and even to treat cholera. The root is used for gonorrhea. Leaves and roots are used in siddha system of medicine for the treatment of skin diseases (Pullaiah, 2006). The plant is also used to cure cough, cold and headache. The boiled roots are used to cure cerebral malaria (Sahoo, 2001). The crushed leaves are used to relieve fever for children. A root decoction is drunken to treat convulsions, fever, colds and swellings of the scrotum and to treat whooping cough. Root decoction is taken to snake bites, fever and ulcer of venereal origin. However, no published works are available for the antimicrobial property of root part of this plant. Hence in the present study, an attempt has been made to focus the plant in this angle and hence to assess its therapeutic potency.

2. MATERIALS AND METHODS

2.1. Plant material

Fresh root parts were collected from the population of *A.fruticosa*present in the Maruthamalai Hills of Coimbatore District and washed under running tap water, air dried and then homogenized to fine powder and stored in air tight bottles.

2.2. Preparation of extracts

250g air-dried root powder was subjected to 250ml of methanol in soxhlet extraction for 8 hours (50-85˚C). The extracts were concentrated to dryness in a flask evaporator under reduced pressure and controlled temperature (50-60˚C) to yield crude residue, which was then stored in refrigerator. To obtain petroleum ether and ethyl

acetate extracts, the same method as used to obtain methanol extract was adopted.

2.3. Media used

Freshly prepared nutrient agar medium was used for the culture of bacteria.

2.4. Microorganisms

In vitro antibacterial activity was examined for the chemical extracts of root of the study plant, against ten bacterial species which include the gram positive strains *viz*., *Micrococcus* sp., *Lactobacillus* sp., *Bacillus subtilis*, *B. thuriengensis* and gram negative strains *viz., Pseudomonas aeruginosa, P. stutzeri, Escherichia coli, Klebsiella pneumoniae, Servatia* sp. and *Moraxetta* sp. All these microorganisms were obtained from the Department of Microbiology, Tamil Nadu Agricultural University, Coimbatore. All the microorganisms were maintained at 4˚C on nutrient agar slants until further use.

2.5. Antibacterial assay

The alcoholic extracts were tested for their effect against the growth of pathogenic bacteria by disc diffusion method (Bauer *et al*., 1966). The microrganisms, bacteria tested was inoculated into nutrient agar medium. After an incubation period of 24 hrs at a temperature of 35˚C, three or four colonies isolated from this medium were inoculated into 4ml of nutrient broth and incubated for 2 hrs at 35°C. The cultures were adjusted with sterile saline solution to obtain turbidity. Petri dishes containing Muller- Hinton agar medium was streaked with these bacterial suspensions. Disks of 6mm diameter were impregnated with the extracts of petroleum ether, methanol and ethyl acetate separately. Tetracycline is used as positive control. After equilibrium at 4°C, the plates were incubated overnight at 37˚C and the diameter of any resulting zones of inhibition was measured. Each experiment was repeated at least three times.

3. RESULTS AND DISCUSSION

The antibacterial activity of the all the alcoholic root extracts of the study species, *Acalyphafruticosa*generally showed inhibitory activity against the growth of *Bacillus subtilis, B. thuringiensis, Klebsiella pneumoniae* and *Pseudomonas aeruginosa.* However, towards *Micrococcus* sp., *Lactobacillus* sp., *Escherichia coli, Pseudomonas stutzeri, Moraxetta* sp. and *Serratia* sp., and all these extracts showed activity with less pronounced manner (Table 1).

Table 1. Antibacterial activity of certain alcoholic root extracts of the species, *Acalypha fruticosa*.

Plant extract	Diameter of zone inhibition (mm)									
	Gram positivebacteria				Gram negative bacteria					
	BS	BT	Ms	Ls	KP	EC	PS	PA	Ss	Mos
Standard *	30.83 ± 0.80	30.73 ± 0.67	20.67 ± 0.59	23.63 ± 0.60	12.13 ± 0.71	25.67 ± 0.61	13.73 ± 0.67	21.73 ± 0.70	14.23 ± 0.49	20.83 ± 0.80
Petroleum ether	9.67 ± 0.75	-	-	-	8.13 ± 0.71	-	-	7.32 ± 0.49	-	-
Ethyl acetate	16.13 ± 0.38	15.16 ± 0.38	9.73 ± 0.67	10.73 ± 0.67	8.77 ± 0.71	8.73 ± 0.75	9.77 ± 0.75	11.73 ± 0.67	7.77 ± 0.71	8.77 ± 0.71
Methanol	21.83 ± 0.60	20.63 ± 0.60	8.06 ± 0.31	8.63 ± 0.60	10.16 ± 0.47	9.67 ± 0.58	9.76 ± 0.86	7.67 ± 0.61	-	-

BS - *Bacillus subtilis* ; BT - *B. thuringiensis;* Ms - *Micrococcus* sp.; Ls - *Lactobacillus* sp.; KP - *Klebsiella pneumoniae;* EC - *Escherichia coli;* PS - *Pseudomonas stutzeri;* PA - *P. aeruginosa;* Ss - *Serratia* sp.; Mos - *Moraxetta* sp.* Tetracycline

It is explained that the different phytochemicals like alkaloids, flavonoids, glycosides, saponins, steroids, terpenoids and phenols extracted by different solvents may be responsible for their antibacterial effects (Ananda kumar *et* al., 2009). Further, the methanol extract has determined to have highest inhibitory activity (21.83 mm diameter inhibitory zone) against the bacterium, *Bacillus subtilis*and (20.63 mm diameter inhibitory zone) against the bacterium, *Bacillus thuringiensis* (gram positive) and ethyl acetate extract also showed highest inhibitory activity (16.13 mm diameter inhibitory zone) against the bacterium, *Bacillus subtilis* and (15.16 mm diameter inhibitory zone)

against the bacterium, *Bacillus thuringiensis*. It indicates the presence of effective active principle compounds in the methanol and ethyl acetate extracts of root part of *A. fruticosa* to suppress both gram negative and gram positive bacteria. It has been observed further that the methanol extracts showed significantly higher inhibitory activity against the colonial growth of *Bacillus subtilis* and *B. thuringiensis,* than that of the commercially available antibiotic, tetracycline. This fact shows the higher therapeutic potential of methanol extract of the study species. The ethyl acetate and petroleum ether extracts have comparatively less activity against most of the tested pathogens. It may be attributed to the presence of respective active compounds with insufficient quantities in this crude extract (Taylor *et al*., 2001).

This fact indicats the existence of strong antibacterial activity of rootpart of the study species, *A. fruticosa* and hence its effective healing property against the infectious diseases. The variation in antibacterial activity across the extracts studied may be due to the polarity of the solvents used. Significantly higher inhibitory activity of methanol extract is nearly to the commercially available antibiotic tetracycline against the bacteria, *Bacillus subtilis* and *B. thuringiensis* observed shows the superior healingness of root part of *A. fruticosa.* Proper isolation and purification of active compounds by using methanol solvent would ensure the therapeutic value of this folklore medicinal plant when it will be used commercially.

The overall study on antibacterial activity reports that the study species contains adequate variety of active compounds to reduce or check the growth of microbial colonies. It confirms the therapeutic value and hence the traditional usage of the root part of the study species, *A. fruticosa* against various ailments. Further, the alcoholic extracts of root part of this plant in general and methanol extracts and ethyl acetate in particular are suggested for the therapy of infectious diseases caused by pathogens and further studies are recommended to purify the active compounds for the formulation of new drugs, while go for commercialization.

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REPRODUCTIVE BIOLOGY OF *GNETUM LATIFOLIUM* BLUME (GNETALES) IN TAMIL NADU

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ABSTRACT

The reproductive biology of *Gnetum latifolium* Blume studied in four different forest sites of Tamil Nadu. *G. latifolium* reported to have dioecious plants with less number male population in all the selected areas. Entamophilous cues observed with common anaemophily and pollinator may be flies. Pollination drops are also reported in young ovules. The seed maturation and germination frequency observed with very low frequency in all the selected population whereas in high number of abortive ovules reported in Western Ghats.

Keyword: *Gentum latifolium*, reproductive biology, seed maturation, germination.

1. INTRODUCTION

In Gymnosperms, Gnetales (*Ephedra, Gnetum, Welwitschia*) morphologically distinct true seeded plants and regularly produced bisexual cones and seeds usually with gynodioecious plants. This plant group has been long regarded as insect pollination due to its range-restricted distribution in tropical forests, where wind pollination is supposed to be detrimental. Several accounts reported that *Gnetum* and *Ephedra* produce bisexual cones with sterile or abortive ovules (Endress, 1996; Hufford, 1996). The genus *Gnetum* are woody climbers mostly in tropical Asia of about 40 species; only *G. gnemon* and *G. costatum* are free standing woody trees or shrubs and remaining species occur in mesic habitats. In *Gnetum*, functionally dioecious, male and female cones bear on different individuals. Until now many of the *Gnetum* species never been documented for their reproductive biology and nature of mechanism of dioicy. A few data are available on the internal structure of the male ovule in *Gnetum* (Vasil, 1959; Lata, 1950). Many authors noted reproductive biology of Gnetales and their implications for understanding of the evolution of higher plants (Hufford, 1996; Frohlich, 1999). Reproductive biology of many *Gnetum* species poorly understood (Endress, 1966).It is well known that many Angiosperms produce nectar as an attractant for pollinators, perhaps Gnetales reported that to produce pollination drops. The pollination drops may be produced from nucellus of the ovule and mainly functioned as pollen receptor on the female cone. But in Gnetales which may indicate a variation in the pollination syndrome especially in *Ephedra* has been reported exclusively entomophilic or in combination with anomophily (Meeuse *et al*., 1990; Niklas, 1992). The present study is aimed on the documentation of pollination mechanism and seed development in *Gnetum latifoium*.

2. MATERIALS AND METHODS

The study was conducted in different forest areas of Tamil Nadu such as Sirumalai hills (Dindigul district), Alagar hills (Madurai district), Megamalai hills (Theni district) and Tirunelveli hills (Tirunelveli district). The population survey was initially conducted and locates the population for reproductive biological studies from the year 2010 to 2015. The phonological events of all the selected population were keenly noted in each and every seasons starting from leaf flushing, leaf shedding, cone initiation, maturation, pollination, ovule development, seed production rate are tabulated form all the selected population in a km^2 area.

2.1. Pollination studies

Selected male and female plants from each population noted for pollination studies. The plants were tagged with red threads and information slip tied permanently for observation of pollination events. The time of anther maturation and ovule maturation keenly observed with all the environmental events.To document potential pollinator (if any), eye observations of male and female cones with fully open stamens were conducted at randomly chosen plant of each population. Male and female cones of the individual observed in 30 min intervals. The activities of insects visiting the plants were assessed at a 1 – 5 scale, in which 1 indicates and inactive insect and 5 indicates a very active insect. The insects were photographed,

captured in 70% ethanol and brought to lab for identification.

2.2. Pollen studies

To investigate *Gnetum* pollen was present in the air near the plants, pollen traps consisting of microscopic slides covered with vaseline were placed on pole on one meter above the ground in each direction distances such as 1 m, 5 m, 10, 15m and 20 m for 3 days. During those days, the weather was dry, sunny and windy and the stamens of the male plants were fully open. The slides of the pollen traps were replaced every evening covered with an additional microscopic slide for observation. The slides were transferred to laboratory, observed with compound microscope at 40X enlargement.

3. RESULTS AND DISCUSSION

Gnetum latifolium is an uncommon Gymnosperm sporadically distributed in Tamil Nadu especially in tropical moist deciduous forests of Western Ghats and Eastern Ghats. It is a climbing species with broad leaves and its population located four different forest sites for study purposes. The reproductive characters with pehnological events of four population sites were observed and tabulated (Table 1). The maximum number of individuals observed from Sirumalai hills (76/km^2) followed by Alagar hills (51/ km^2), Megamalai hills (47/ km^2) and Tirunelveli hills (16/ km^2). All these populations have low frequency of male plants which is about 1:15 ratio of male and female plants.Male cone initiation in the month of April in Sirumalai hills and Alagar hills whereas in Megamalai hills and Tirunelveli hills the male cone started produce in the month of June and July. The average pollen density observed in 5- 20 m radius of the male plant of each population showed maximum in Sirumalai hills (15) followed by Megamalai hills (12), Tirunelveli hills (10) and Alagar hills (9).

Period of female cone initiationshowed in the month of May in Sirumalai and Alagar hills, but in Megamalai and Tirunelveli hills it was started in the month of July. It shows well-marked difference in the phonological events of cone formation in Western Ghats and Eastern Ghats. The protandry is noted in the all populations of *G. latifolium* in Tamil Nadu. The average ovule ratio on female cone is calculated in each population, it showed higher number in Sirumalai hills (40/cone), followed by Alagar hills (38/cone), Tirunelveli hills (21/cone) and Megamalai hills (12/cone). In Megamalai population the higher range of abortive ovules observed on the female cone (Fig. 1h). Seed maturation was noted in the month of August in Eastern Ghats and September in Western Ghats respectively. Habitat seed germination frequency is very low in all the selected population sites, usually germination take place in the month of November in Eastern Ghats and January in Western Ghats (Table 1). The different reproductive stages of *Gnetum latifolium* is given in Fig.1.

Table 1. Reproductive characters of *Gnetum latifolium* observed from the four different populations in Tamil Nadu.

Characters	*Gnetum latifolium* population sites			
	Sirumalai hills	**Alagar hills**	**Megamalai hills**	**Tirunelveli hills**
Number of mature individuals / km^2	76 (2 male; 74 female)	51 (6 male; 45 female)	47 (2 male; 45 female)	16 (3 male; 13 female)
Number of seedlings	12	6	15	3
Period of male cone formation	April – May	April – May	June – July	July – August
Average pollen density in microscopic field at 40X (≥ 20 m radius)	15	9	12	10
Period of female cone formation	May – July	May – July	July – August	July – August
Average ovule ratio per female cone	40	38	12	21
Seed maturation	August	August	September	September
Seed germination	November	November	January	January

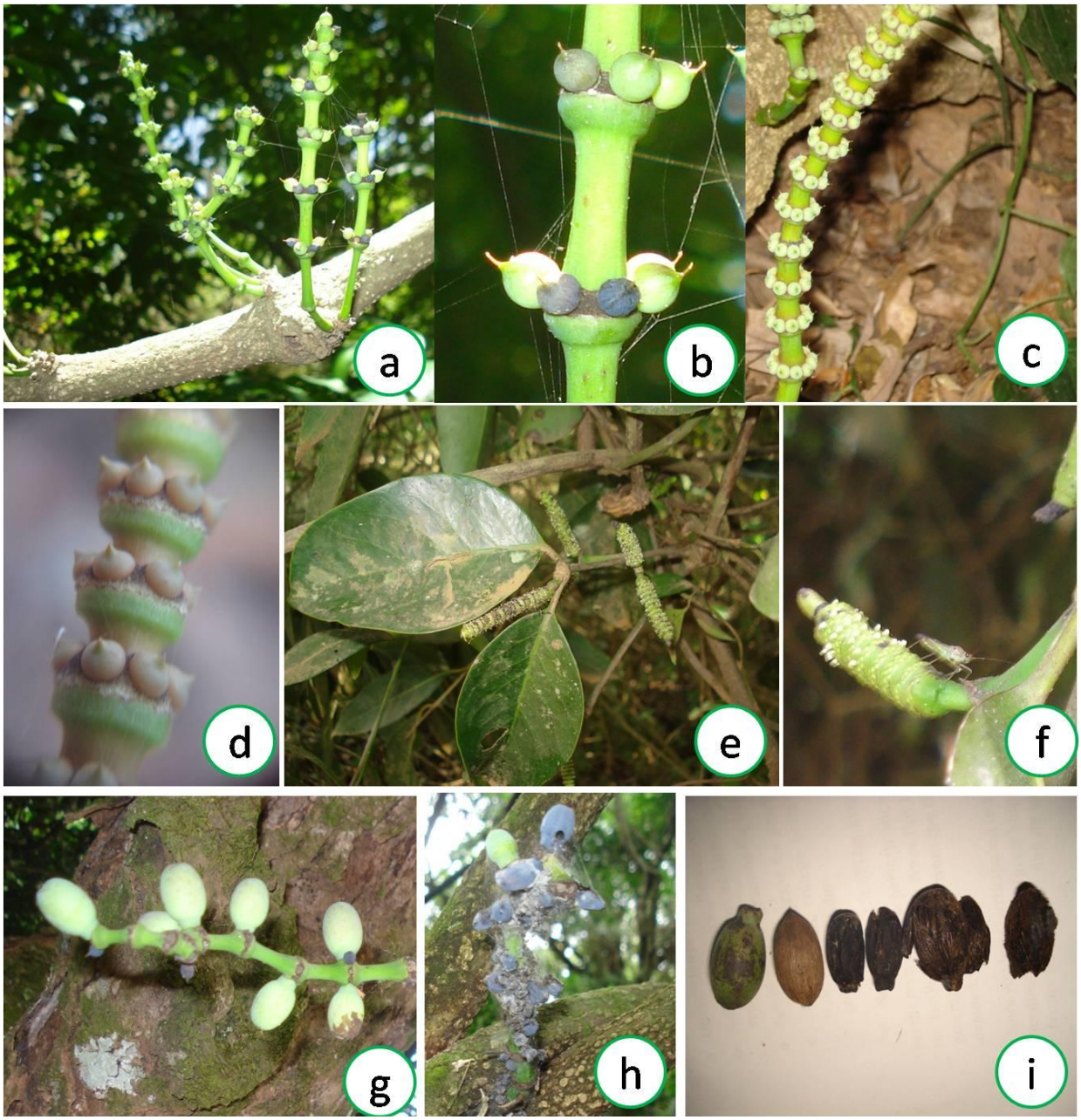

Fig. 1. *Gnetum latifolium* - Reproductive stages.

a) A bunch of female cones on stem; b) Female cone enlarged with elongated papilla on ovules; c) 20 days mature ovules; d) 20 mature ovules enlarged with cupuels; e) Male cones on leafy twig; f) insects sporting on male cone; f) 60 days mature seeds; h) infected female cone; i) different stages of seeds from mature to decomposed wall layers.

There was no report on quantitative distributional account of Gnetales in India or elsewhere in Asia. Usually *Gentum* population have less number of male plants and predominant population of female plants. Pollination drops usually produced in Gnetales from nucellar secretion in young ovules (Haycraft and Carmichael, 2001). *G. latifolium* is found to have unisexual cones in dioecious plants but in *G.gnemon* reported bisexual cones with male ovule. In recent report accounted the role of bisexual cones with sterile ovules in the breeding system of Gnetales remains unclear. Though in *G. latifolium* cones are unisexual, pollination droplets produced even in sterile ovules with rich content of sugars attracts fly pollinators. Similar pollination droplets were reported in *G.*

gnemon (Kato *et al*., 1996). Sterile ovules reported in *G. cuspidatum*, *G. microcarpum*, *G. diminutum* and *G. loerzingii* which are all African species and their ovules hidden among the hairs in cupules. There are no true nectarines found those species (Jorgensen and Catarina, 2015). In several *Gnetum* species of their pollination drops noted mistakenly as nectar. However, African species of *Gnetum* have unisexual male cones similar to *G. latifolium*. Seed germination studies are also not available for any *Gnetum* species, usually it take place year around to germinate the seeds due to hard and fibrous seed coats.The present report is given the base line data for conservation of the species and also the study impressed the seed germination studies needed for these ancestral plant species.

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THERAPEUTICAL IMPORTANCE OF *HYPNEA MUSCIFORMIS* (WULFEN) J.V. LAMOUROUX : A RED ALGAL SEAWEED

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ABSTRACT

Red algae are rich in protein, crude lipid and fibre content and therefore ideal as food in terms of nutritional and biochemical point of view. Currently, red algae have gained importance due to their nutritional composition and various bioactive compounds they produce to accustom to the biodiversity of marine ecosystem. Biologically unique compounds of algal include carrageenan, sulpholipids, and pigments such as phycocyanin. Seaweeds are low in fats but contain vitamins and bioactive compounds such as flavonoids, terpenoids and sulphated polysaccharides, which are potential natural antioxidants not found in the terrestrial plants. *Hypnea*, a common seaweed distributed widely along the tropical and subtropical shores Nutraceutical value of *Hypnea musciformis* includes nutrients, vitamin, ash and large amount of sodium and potassium electrolytes. There were reports that they possess potent antitumour and antimicrobial activity.

Keywords: *Hypnea musciformis,* therapeutical, terpenoids, red algae.

1. INTRODUCTION

Seaweed record states that Japan and China from 4th century BC onwards utilized *Laminaria* species as marine food (Holdt and Karaan,2011). Gupta and Abu-Ghannam (2011), reported the therapeutical references in Traditional Chinese Medicine dates back. Meanwhile, the algal source and usage varies geographically. In East Asia, seaweed usage was mainly nutritional with scanty medicinal uses. *Unnidaria pinnifida* and *Laminaria japonica* popularized as Asian nutritional food for iodine and fiber sources. The Chinese Materia Medica narrates the ancient use for treating goiter, phlegm accumulation, and cleansing heat a key principle to restoring balance in Traditional Chinese Medicine (Yadav *et al*.,2015).Western countries documented dating back to the Greek and Roman empires, where mucilage was used to treat rashes, burns, scurvy treatment and parasite elimination (Shalab,2011) . Seaweeds are consumed as raw or cooked, fresh or dried food. Therapeutical applications ranges from physical application, to different solvent extractions/decoctions utilized for many ailments.

Sea weeds have been one of the promising resources of biologically active metabolites and their extraction has significantly expanded in the last few decades. *Hypnea muscifromis,* a proven candidate among the sea weeds for its rich mucopolysaccharides. More than 150 species of marine algae are commercially important food sources and over $2 billion worth of seaweed is consumed each year by humans, mostly in Japan, China and Korea. Algae have long been recognized as rich and valuable natural resources of bioactive compounds because of their various biological properties (Mayer *et al*., 2002). Since the finding of antimicrobial (antibacterial, antifungal or antiviral) activities in many species of marine algae and the isolation of some active compounds from them, marine algae have become recognized as potential sources of antibiotic substances (Fenical and Paul, 1984; Gonzalez *et al*., 2001; Selvin and Lipton, 2004; Kornprobst, 2005; Salvador *et al*., 2007).

Substances that currently receive the most attention from pharmaceutical companies for use in drug development, or from researchers in the field of medicine-related research include generally: sulphated polysaccharides as antiviral substances, halogenated furanones from Delisea pulchra as antifouling compounds and depsipeptides kahalalide F from a species of *Bryopsis* as a possible treatment of lung cancer, tumours and acquired immune deficiency syndrome (AIDS). Other substances such as macroalgal lectins, fucoidans, kainoids and aplysiatoxins are routinely used in biomedical research and a multitude of other substances have known biological activities (Smit, 2004; Kornprobst, 2005).

Many studies have reported wide array of active molecules from the sea weeds. Phaeophyceous species in particular has combination of active lead molecules. These include phlorotannins, fucoxanthins, and fucoidan. The categorization of seaweed in to brown, red or green algae is based on their photosynthetic pigments, reproductive method, micro and macro morphologies, and its phycopolymers. Seaweeds used cosmetics like body wraps, and baths, with the concept in blood circulation, detoxification, acne treatment, skin moisturizing, purification, exfoliation, or rejuvenating effects. Seaweeds and seaweed-derived products are underexploited bio-resources and source of such natural ingredients for functional foods.

There are reports that marine Rhodophyta from the coast of Morocco have certain inhibitory compounds against Herpes simplex virus type 1 (HSV-1) by cell viability method (Dhivya *et al*., 2012). The aqueous extracts of *Hypnea musciformis* were capable of inhibiting the replication of HSV-1. Fifty-five aqueous, methanolic, chloroform-methanolic and dichloromethanolic extracts derived from sixteen species of marine Rhodophyta from the coast of Morocco have been screened for the presence of inhibitory compounds against Herpes simplex virus type 1 (HSV-1) by cell viability method. The aqueous extracts of *Asparagopsis armata*, *Ceramium rubrum*, *Gelidium pulchellum*, *Gelidium spinulosum*, *Halopitys incurvus*, *Hypnea musciformis*, *Plocamium cartilagineum*, *Boergeseniella thuyoides*, *Pterosiphonia complanata* and *Sphaerococcus coronopifolius* were capable of inhibiting the replication of HSV-1 in vitro at an EC_{50} (Effective Concentration 50%) ranging from < 2.5 to 75.9 µg mL-1. Marine algae from Morocco can be a rich source of potential antiviral compounds**.** The screening showed positive results in orcinol sulfuric acid reaction from extracts *H. musciformis*, suggesting that the main effective components in these extracts could be polysaccharides.

Several reports regarding the metabolic and pharmacological effects of common species of seaweed, *H. musciformis* shows that the blood lipids, cholesterol and triglycerides were shown to be decreased after the administration of *H. musciformis*. This is an important finding since decreased levels of cholesterol and total lipids minimize the incidence of many cardiovascular problems. (Bersot *et al*., 2003). The level of glucose is also increased after the administration of *H. musciformis*, which could be a transient increase only, through action on glucagon, and could also be attributed to the fact that the *H. musciformis* contains many amino acids, which may form glucose. Administration of *H. musciformis* significantly increased the level of dopamine. The possible effect of *H. musciformis* on dopamine and other brain biogenic amines indicate that *H. musciformis* probably have psychotropic and anxiolytic profile. The increased level of dopamine could also be beneficial keeping in view the etiology of Parkinsonism. In present study the level of serotonin was found to be decreased after the administration of *H. musciformis*. The regular use of seaweeds as a diet will relieve the symptoms of anxiety because the known anxiolytics also manifest their effect by decreasing the concentration of serotonin.

Preliminary pharmacological investigation of the algae belonging to the genus *Dictyota* revealed its content of considerable antibacterial, antifungal, antiviral (Nizmuddin, and Campbell, 1995) antimicrobial, antineoplastic, antifungal and cytotoxic activities (Shameel *et al*., 1991; Melo *et al*., 1997). Methanolic extract of *H. musciformis* was reported in regulating serum total cholesterol, triglyceride and low-density lipoprotein cholesterol levels of rabbits and there by mitigate many cardiovascular problems. *Kappaphycus alvarezii* was reported to have antimicrobial activity against certain bacterial and fungal strains by Prabha *et al*.. Antioxidant and free radical scavenging capacity of red seaweed *Hypnea valentiae* was reported by Revathi *et al*.. There are reports of antispasmodic a activity of *Hypnea musciformis* by Salimabi and Das (1980). Studies on *Hypnea musciformis* shows that the level of 5-HT was found to be decreased after its administration. This shows the possibility of the seaweed species having anxiolytic properties may not be ruled out since known anxiolytics are known to produce their effect by decreasing the concentrations of 5-HT. There are reports of gastroprotective activity against ethanolinduced gastric damage in mice and evaluated the role of NO/KATP channels in this effect and the mechanism underlying due to the activity of a sulfated-polysaccharide fraction extracted from the algae *Hypnea musciformis (Wulfen) J.V. Lamour* (Samara *et al*.,2013) .

In Haawai and Indonesia, the red seaweed *Hypnea nidificia* J.Agaardh and *Hypnea musciformis* are used as a vermifuge remedy for stomach troubles caused by parasitic infections. Anti-inflammatory effect of a sulphated polysaccharide fraction extracted from the red algae *Hypnea musciformis* via the suppression of neutrophil migration by the nitric oxide signalling pathway were also reported (Brito *et al.,* 2013). The secondary metabolites of seaweeds

Ulva fasciata and *Hypnea musciformis,* collected form southeast and southwest coast of India, were tested for biotoxicity potential. Both species showed potent activity in antibacterial, brine shrimp cytotoxicity, larvicidal, anti-fouling and ichthyotoxicity assays. The green alga *U. fasciata* exhibited broad-spectrum antibacterial activity whereas the red alga *H. musciformis* showed narrow spectrum antibacterial activity. The brine shrimp cytotoxicity profile indicated that the seaweeds were moderately toxic. The overall activity profile indicated that *U. fasciata* contained more biological potency than *H. musciformis*.

In conclusion, the study underscores the therapeutic potentials of the common seaweed, *H. musciformis*. Further work is planned in this seaweeds to isolate, fractionate terpenoids and to evaluate its biological potentialities.

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PRODUCTION OF ALKALINE PROTEASE ENZYME FROM *BACILLUS SUBTILIS* 168 ISOLATED FROM SOIL SAMPLES COLLECTED FROM A DAIRY FARM

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ABSTRACT

Screening and isolation of protease producing strains of bacteria were carried out from a dairy farm located in Nagarcoil, Tamilnadu.The isolates were positive on skim milk agar (1%) and thus are selected as protease producing strain. The organisms were tested for various biochemical tests, which lead to their identification as *Bacillus subtilis* producing protease enzyme. These *Bacillus subtilis* could group up to 40ºC and pH range 6-9 with optimal growth temperature and pH at 37°C and 8.0 respectively. It was also optimized for carbon test and nitrogen test with optimal growth in dextrose and peptone respectively. Enzyme production was carried in 1 litre of optimized media in the fermenter at 37°C for 48 hours at pH 8.0. This result showed that *Bacillus subtilis* under study is a good producer of extra cellular protease, which can be beneficial for industries.

Keywords: *Bacillus subtilis,* cellular protease, identification.

1. INTRODUCTION

Proteases, one among the three largest groups of industrial enzymes, accounts for about 60% of the total worldwide sale of enzymes from biological sources sincethey possess almost all characteristics desired for their biotechnological applications (Adinarayana*et al*., 2003). Proteases constitute a class of industrial enzymes, which alone form approximately 60% of the total world-wide enzyme production (Chu 2007). Among the various proteases, microbial proteases play an important role in biotechnological processes. Alkaline proteases produced are of special interest as they could be used in manufacture of detergents, food, pharmaceuticals and leather (Saeki *et al.,* 2007, Dias *et al*., 2008). In recent years a number of studies have been conducted to characterize alkaline protease from different microorganisms. However, many of the alkaline proteases applied to industrial purposes face some limitations such as low stability towards surfactants and production cost of the enzymes arisen from growth medium(Joo and Chang,2005). Using of costeffective growth medium for the production ofalkaline proteases from an alkalophilic*Bacillus* sp. is especially important (Joo*et al*. 2002). Therefore, there is a need to the search of new strains of bacteria that produce proteolytic enzymes with novel properties andthe development of low cost media.

2. MATERIALS AND METHODS

2.1. Collection of samples

Protease producing organisms were isolated from soil samples collected from a dairy farm located in Nagarcoil, Tamilnadu. Samples were serially diluted using distilled water and spread plated on the surface of casein agar plates (nutrient agar with 1% casein) and incubated at 30°C for 48hrs (Naidu and Devi, 2005).

2.2. Screening of protease producers

The collected samples were seriallydiluted and streaked on skin milk agarplates. The plates were incubated for 48hat 37 C and protease producers wereselected by observation of zone ofhydrolysis around the colonies (Genkal*et al*., 2006).

2.3. Characterization of protease enzyme

The total protein contents of the sampleswere determined according to the method described by Lowry s method usingBovine Serum Albumin (BSA) asstandard. Enzyme activity was determinedusing culture supernatant collected bycentrifuging culture broth at 10, 000 rpmfor 15min. Protease activity was measuredby standard assay procedure proposed byAkcan and Uyar, 2011. About 0.5ml of0.5% casein and 1.25ml of tris buffer (pH-8.0 to 14.0) was added into 0.2ml of eachof the culture supernatant separately. Mixture was

incubated for 30 min at 370C.About 3ml of trichloroacetic acid wasadded and incubated at 400C for 10 min toform precipitate. The mixture wascentrifuged at 10,000rpm for 15min and0.5ml of supernatant was collected.

Reagent containing sodium carbonate, copper sulphate, sodium potassiumtartarate was mixedwith 1ml of Folinphenolreagent. The mixture wasincubated at dark for 30 minutes to formblue colour. The absorbance was read at660 nm to determine the optical density ofeach sample. The obtained OD wasextrapolated in the standard graph. Thestandard curve was obtained for series ofknown concentrations of bovine serumalbumin. From the graph, the amount ofprotein liberated due to the action ofenzyme protease in the supernatant wasdetermined. One unit of protease activitywas defined as the amount of enzymerequired to liberate 1 g/ml tyrosine underthe experimental conditions.Enzyme activity = OD value X amount ofprotein released (g)/ concentration ofsubstrate X time of incubation X weight ofthe sample.

2.4. Optimization of conditions for enhancedenzyme production (Das and Prasad, 2010)

Standard methods were adopted tooptimize the parameters like culturalconditions, carbon, nitrogen, temperature, pH, inoculum size and substrates.

2.5. Mass production of alkaline protease

The fermentation was carried out in asterile Stirred Bed Reactor (SBR). Thevessel was maintained at optimizedtemperature, pH and other & incubated for48h in a shaking incubator. At the end offermentation period, the whole cultureboth was centrifuged at 10,000 rpm for 15minutes, to remove the cellular debris andthe clear supernatant was used for enzymeanalysis.

2.6. Characterization of partially purifiedalkaline protease

The culture filtrate (crude protease) wascollected aseptically after upstream production in a SBR under controlled conditions. The required volume of the spent media was centrifuged at 10,000 rpmfor 15 min at 4°C in order to obtain a cell free filtrate. About 200 ml of the cell free filtrate containing protease were collected and their proteolytic activity was determined. Protease enzyme was purified by ammonium sulfate fractionation the concentration of ammonium sulphate required for precipitation varies fromprotein to protein and should bedetermined empirically. The two milliliter of the crude protease enzyme was first brought to 20% (w/v) saturation with solid ammonium sulfate (enzyme grade) and 100% saturated dialysis against distilledwater in a dialysis bag (cut off 30) for 3 h, followed by dialysis against phosphatebuffer at pH 7.0. The obtained proteaseenzyme preparation was concentrated against crystals of sucrose and kept in there frigerator at 4°C. The enzyme activityand protein content was determined forsalted out dialyzed enzyme fractions. Theenzyme activity of the purified fractions ofthe alkaline protease after harvesting, ammonium sulfate precipitation anddialysis was determined by the method of Gomori (1955). Separation and sizedetermination of enzyme was performedby SDS-PAGE (Joo *et al*., 2002).

3. RESULTS AND DISCUSSION

3.1. Density of protease producers

In the present study soil samples collected from a dairy farm located in Nagercoil were plated on casein agar medium and the microbial density was found to be in the range of $3.22x10^3$ to $1.6x10^4$ CFU/g(Fig. 1).

3.2. Screening for proteolytic activity

From casein agar plates 151 strains of varying morphology were selected and screened for proteolytic activity adopting well assay method. The zone of clearance was measured and found to be in the range of 4mm-15mm. As most of the strains showed activity with 4mm range, 5 potential strains alone were selected for the further study. Among the five, the one with 15mm of zone was selected for protease production (Fig.3).

3.3 Identification of strains

The potential strains were identified using biochemical methods according to Bergy's manual of determinative bacteriology and identified as *B. cereus*, *E. coli, B. subtilis, B. pumilis*and *P. aeruginosa* and were designated with their strain number as *B. cereus* DF 101, *E. coli* DF 52*, B. subtilis* DF 49,*P. aeruginosa* DF 11and *B. pumilis*DF 78.

3.4. Inoculum concentration

When the log phase culture of *B. cereus* DF 101 was tested for the suitable inoculum concentration in the range of 0.5 – 3%, 1% inoculum resulted in the maximum OD value of 1.202. On further increase in concentration of inoculum decrease in growth of the culture in shake flask was noted. Likewise the protease production also found to be the maximum at this inoculum concentration

(1284U/ml/min.). Surprisingly at 3% inoculum concentration growth was reduced to 0.88 OD at which enzyme production was found to be only 4U/ml/min. (Fig. 4 and 5).

3.5. Static and shaking conditions

The effect of agitation was tested at the range of 50 – 200rpm. At 50rpm the OD value was found to be 0.6 which was in increasing trend on further increase up to 150rpm, where OD value of 1.202 was observed. Further increase in agitation reduced the growth and value of enzyme activity (i.e) 0.89 and 800 U/ml/min. observed at 200 rpm. However least growth and enzyme activity were found when incubation was done in static condition. When cultures were kept static growth attained the level of 0.332 OD, which resulted in only 250 U/ml/min. of enzyme activity (Fig. 6 and 7).

3.6. pH

When a pH range of pH 6 to pH 11 was tested pH 10 resulted in higher OD as well as higher enzyme activity. A maximum of 1249 U/ml/min. was observed at pH 10 at 36 hrswhere it was 802 U/ml/min. at pH 8, 1031 U/ml/min. at pH 9 and 981 U/ml/min. at pH11. However it was only 561 U/ml/min. at pH 7 at 36 hrs. of incubation (Fig. 8 and 9).

3.7. Temperature

At 35^{o}C maximum OD value of 1.05 was obtained in which the protease activity observed was 1057/ml/min. At the end of 42 hrs protease production reduced and the activity was found to be 997U/ml min. with a growth of 0.95 OD. Lower growth and enzyme activity were observed at both extremes (i.e.) 25^{o}C and 45^{o}C (Fig. 10 and 11).

3.8. NaCl concentration

When NaCl concentration of 0 to 2% was tested at an interval of 0.5%, the maximum growth and enzyme activity were observed at 0.5% NaCl. Maximum OD value of 1.14 with an enzyme activity of 1092 was obtained at 36 hrs at that concentration. The minimum was observed at 2% NaCl with an OD value of 0.78 at 36 hrs, at which only 523 U/ml/min. of enzyme activity was observed. Irrespective of concentration after 36 hrs both OD value and enzyme production were decreased (Fig. 12 and 13).

3.9. Carbon sources

In the present study, to select a potential carbon source, glucose, maltose, fructose, sucrose and starch were incorporated in separate flasks at 1% concentration. Among them glucose favoured the maximum growth and protease production respectively with 1.3 OD and 1012U/ml/min. The minimum growth (0.8 OD) and enzyme production (587U/ml/min.) were observed when starch was used as the sole carbon source (Fig. 14 and 15).

3.10. Concentration of carbon source

The ideal carbon source glucose was tested from 0.5% to 2.5% in which 1% resulted in maximum growth as well as the maximum enzyme activity. At 1% glucose concentration the OD value was found to be 1.392 at which maximum enzyme activity of 996U/ml/min. was noted. At 2.5% of glucose growth was reduced to 0.98 OD with corresponding enzyme activity of 759U/ml/min. (Fig. 16 and 17).

3.11. Nitrogen sources

When yeast extract, beef extract and peptone were selected as organic nitrogen sources and ammonium nitrate, ammonium sulphate and potassium nitrate were selected as inorganic nitrogen sources, organic nitrogen sources resulted in more growth as well as enzyme production compared to the inorganic forms. Yeast extract showed a maximum of 1.056 OD of growth and 995U/ml/min. of enzyme production, whereas with potassium nitrate a minimum of 0.509 OD and 412U/ml/min. of enzyme production were observed (Fig. 18).

3.12. Concentration of nitrogen source

The concentration of ideal nitrogen source (i.e.) yeast extract was tested at 0.1 – 1% level, 0.5% favoured the growth of the organism resulting in 1.056 OD at the end of 36 hrsof incubation. At this concentration, the maximum enzyme activity of 878U/ml/min. was observed. When yeast extract concentration was further increased, correspondingly enzyme activity decreased, recording the lowest of 522U/ml/min. at 1%. Even 0.1% resulted in slightly higher enzyme activity (579U/ml/min.).

Bacillus species are attractive industrial organisms for a variety of reasons, including their higher growth rates leading to shorter fermentation cycles, their capacity to secrete proteins as extracellular into the medium, and the GRAS (generally regarded as safe) status with the Food and Drug Administration for most of its species, such as *B. subtilis, Bacillus licheniformis* etc., The present study was on protease production by a *B. cereus* DF 101 strain isolated from a dairy farm soil of

Nagerkoil District. Microbial proteases are produced from bacteria, fungi and yeast using many processes like solid-state fermentation as well as submerged fermentation (Anwar and Saleemuddin, 1998; Kumar and Takagi, 1999 and Haki and Rakshit, 2003). In the present research work, submerged fermentation technique was used. Even fungi like *Aspergillus flavus, A. mellens, A.niger, Chrysosporium keratinophilum, Fusarium graminarum, Pencillium griseofulin, Scedosporium apiosermum* etc., were reported to produce protease.

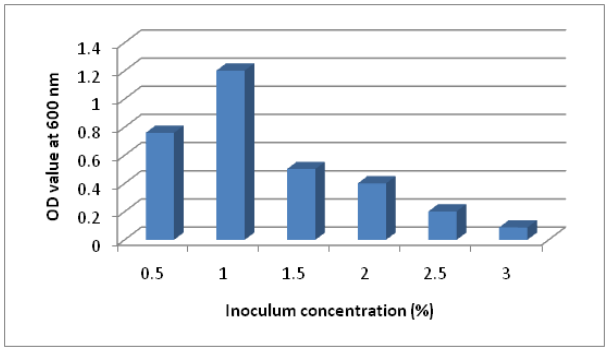

Fig. 1. Effect of inoculum concentration on growth of *B.cereus* DF 101

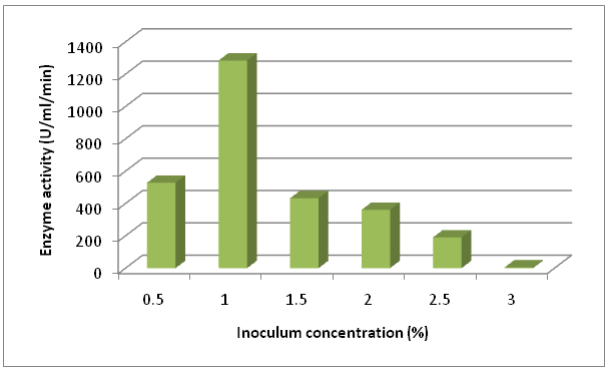

Fig. 2. Effect of inoculum concentration on protease production by *B.cereus* DF 101

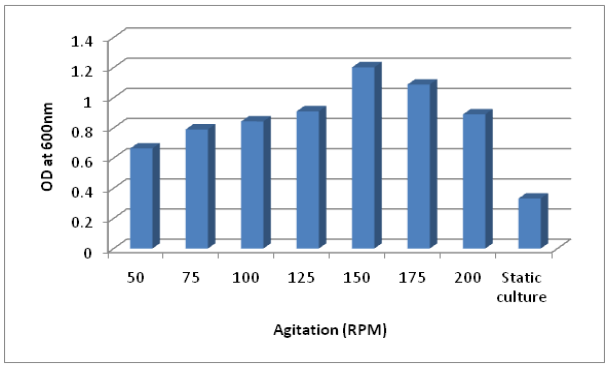

Fig. 3. Effect of agitation on growth of *B. cereus* DF 101

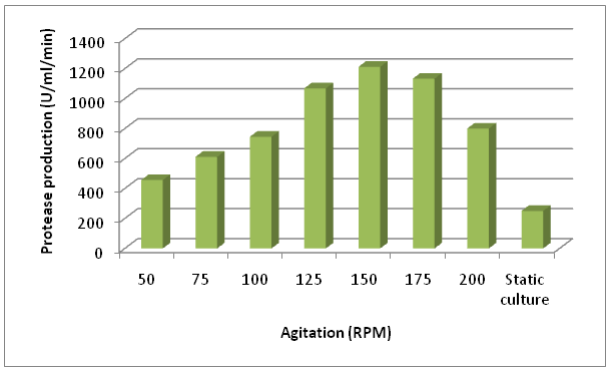

Fig. 4. Effect of agitation on protease production of *B. cereus* DF 101

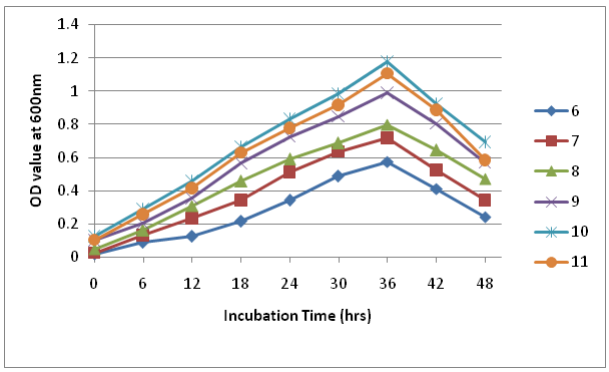

Fig. 5. Effect of pH on growth of *B. cereus* DF 101

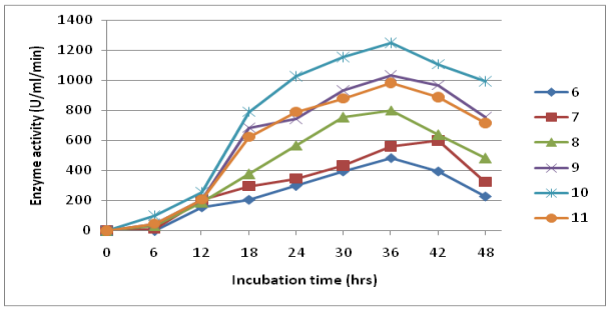

Fig. 6. Effect of pH on protease production of *B. cereus* DF 101

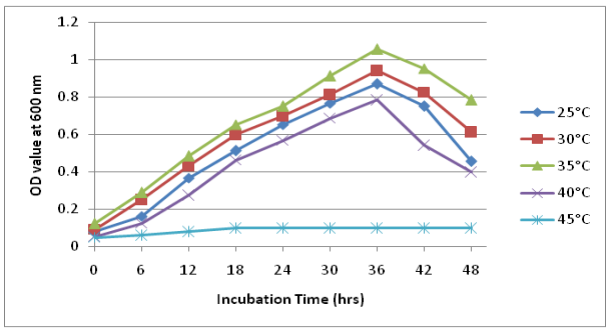

Fig. 7. Effect of temperature on growth of *B. cereus* DF 101

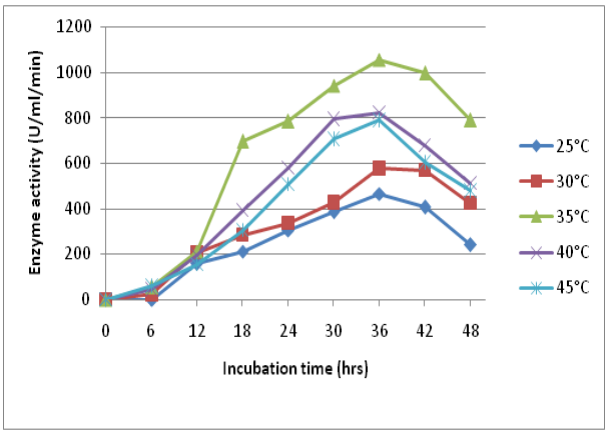

Fig. 8. Effect of temperature on protease production of *B. cereus* DF 101

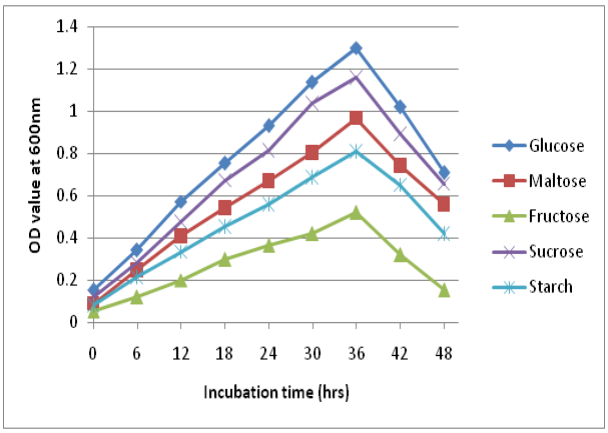

Fig. 11. Effect of carbon source on growth of *B. cereus* DF 101

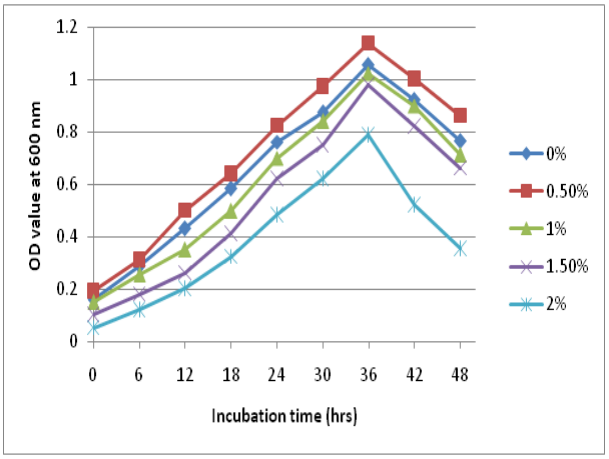

Fig. 9. Effect of NaCl concentration on growth of *B. cereus* DF 101

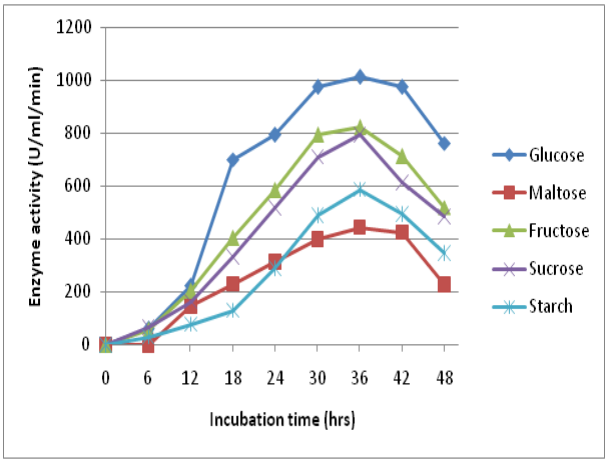

Fig. 12. Effect of carbon source on protease production of *B. cereus* DF 101

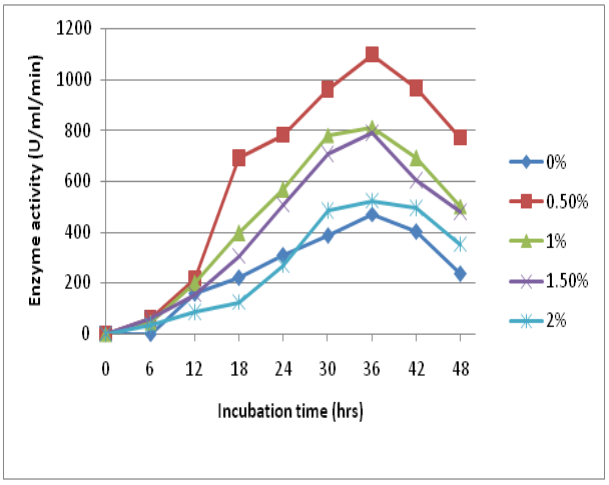

Fig. 10. Effect of NaCl concentration on protease production of *B. cereus* DF 101

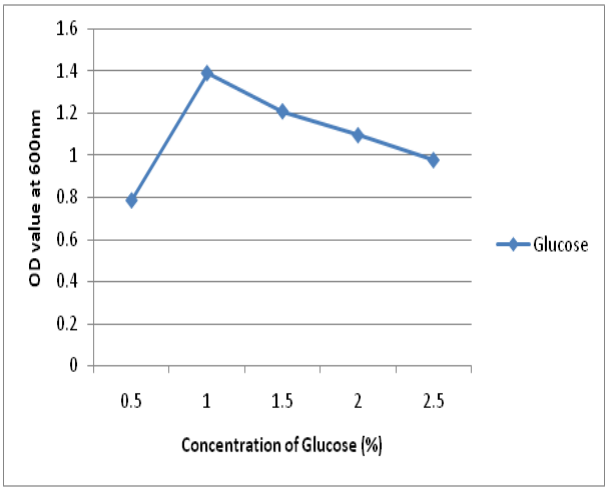

Fig. 13. Effect of glucose concentration on growth of *B. cereus* DF 101at 36hrs of incubation

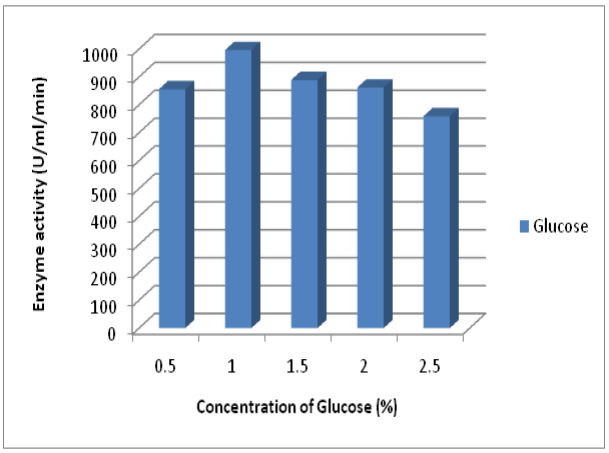

Fig. 14. Effect of glucose concentration on protease production in *B. cereus* DF 101at 36hrs of incubation

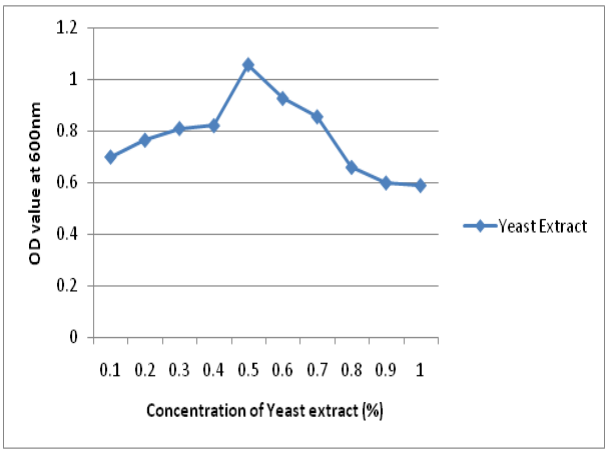

Fig. 17. Effect of yeast extract concentration on growth of *B. cereus* DF 101 at 36hrs of incubation.

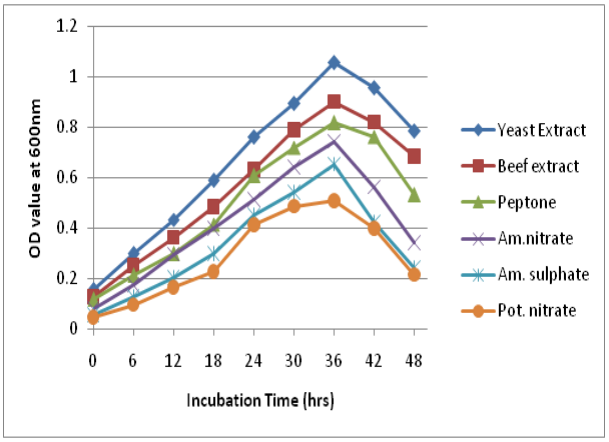

Fig. 15. Effect of nitrogen source on growth of *B. cereus* DF 101

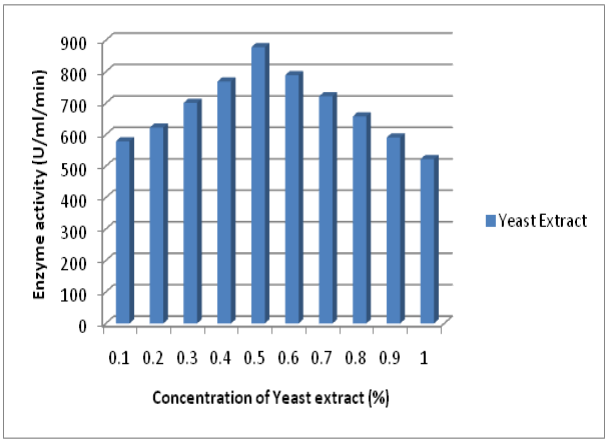

Fig. 18. Effect of yeast extract concentration on protease production in *B. cereus* DF 101at 36hrs of incubation

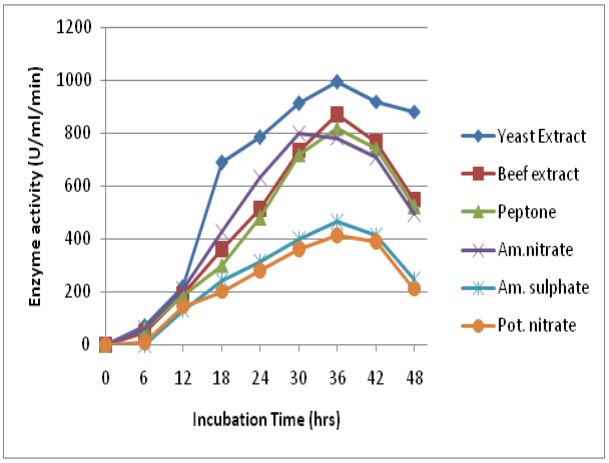

Fig. 16. Effect of nitrogen source on protease production of *B. cereus* DF 101

Bacillus species are considered as major workhorse industrial microorganisms with roles in applied microbiology, which date back more than a thousand years, since the production of natto by solid-state fermentation of soybeans using *Bacillus subtilis* (natto) which was first practiced in Japan (Hara and Ueda, 1982).

4. CONCLUSION

Thus in the present study a dairy farm soil originated *B.cereus* DF 101 strain was found to be an ideal producer of alkaline protease and the study also revealed the potential for the industrial scale production using this strain. The abundance of protease producers in dairy farm soil sample indicated them as a new source for the search of alkaline proteases.

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ANTIBACTERIAL ACTIVITY IN EARTHWORM COELOMIC FLUID *LAMPITO MAURITII*

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ABSTRACT

Earthworms are the first annulated coelomate organism with haemoglobin in the plasma as carrier of oxygen and carbon dioxide. They have the antimicrobial activity against the disease causing micro organisms. In the present study antibacterial activity of earthworm coelomic fluid *Lampito mauritii* were confined against disease causing micro organisms through the inhibition zone formation by well diffusion method as well as disc diffusion method.

Keywords: Earthworm, antibacterial, coelomic fluid, inhibition zone.

1. INTRODUCTION

Earthworms are natural invertebrates of agro ecosystem belonging to the Phylum Annelida, and dominant in the temperate and tropical soils. They are the first group of multicellular eucoelomate invertebrates to have succeeded to inhabit terrestrial environment (Kale and Karmegam, 2010). Being hermaphrodites, both male and female reproductive organs are present in every single earthworm but self-fertilization does not generally occur. Earthworms, as ecosystem engineers, play an important role in many soil ecosystems and are one of the numerous ranges of burrowing organisms, which improve soils (Lavelle, 1997). Due to their relatively large size and characteristic feeding behaviour, certain species have significant impacts on soil structure, soil fertility, plant growth and crop yields.

Earthworm provides optimum conditions for plant growth by reducing both acidity and alkalinity of soil. Plant growth stimulants such as auxins are produced in the earthworm castings. These hormones stimulate roots to grow faster and deeper. A more important beneficial effect of soil is the mixing and changing of soil texture caused by burrowing and improves water filtration rates and absorption rates of soil. The tunneling activity improves soil aeration and permeability.

The swallowed materials are digested and only 10% is absorbed. The remaining 90% us passed out as earthworm casts. In the healthy soil 40 tonnes of soils per acre pass through the bodies of earthworm daily. One earthworm digests 36 tonnes of soil in one year (US soil conservation office)

Now a day the research on earthworm not only focused on agricultural aspects in the therapeutic areas. In Chinese medicine earthworm is considered as complete medicine and used for various therapeutic purposes such as blood purification, blood disorders, against jaundice, wound healing and as antimicrobial, anti inflammatory and anti oxidant agent. The most important characteristics of the immune system of animals in the phylum Annelida are that they have developed a coelom. This body cavity contains coelomic fluid, in which coelomocytes, the worm's leukocytes, are suspended. Coelomocytes are not contained within the circulatory system but are sensitive to perturbations such as infections and are active in defense reaction ranging from phagocytosis to the more complex mechanism of tissue graft rejection. Infection by a pathogen represents one of the major threats to any living organisms. These pathogens are firstly bacteria living in water or soil that are ingested during feeding or introduced into the body following injury. During the course of evolution, earthworms have developed defense strategies against these living pathogens. Earthworms lack true antibodies and hence an adaptive immune response and instead have efficient innate immunity system to defend themselves against invading foreign materials. In living organisms, peptides are an important defense component, many peptides were found in various living organisms. Therefore it can be supposed that earthworm living in the pathogen- abundant environment must have peptides against bacteria. In earthworms innate immunity is maintained by cellular components, different coelomocytes (leukocytes), housed in coelomic cavity whose fluid

also contains many immunological (antimicrobial) active molecules. The component of coelomic fluid of earthworm defenses them in identifying foreign substance and develops potency to fight against them. Studies done by many workers have strongly pointed out that coelomic fluid of earthworms like *Eisenia foetida, Eudrilus euginae, Polypheretima elongata, Perionyx excavatus, Lampito mauritii, Dendrobaena veneta, Lumbricus terresteris, L. rubellus* and *Perionyx sansibaricus* have medicinal and antibacterial properties (Çotuk and Dales, 1984; Dales and Kalaç 1992; Milochau *et al*., 1997; Balamurgan *et al*., 2007). The lysozyme performs lytic activity which can be used to manage the wastes of various organic substances. Moreover, earthworms have been used to treat upper respiratory tract infections, typhoid, and diarrheal pathogenic bacteria as a natural drug in Indonesia more than 50 years. The present study of earthworm antibacterial activity is conducted in the peregrine species *Lampito mauritii.*

2. MATERIALS AND METHODS

2.1. Colection and culture of bacteria

Four different types of bacteria namely *Escherichia coli* (Gram negative), *Klebsiella pneumoniae* (Gram-negative), *Enterococcus faecalis* (Gram-positive) and *Staphylococcus aureus* were purchased from Bioline lab Coimbatore, Tamil Nadu (India) and they were transferred separately on fresh nutrient agar medium at 35°C to 37°C for 24 hours to obtain active culture. Each bacterium was transferred separately to fresh nutrient broth from this active culture was incubated at 35°C to 37°C for 24 hour to get active culture suspension.

Table 1. Composition of nutrient agar medium.

INGREDIENTS	Gms / lit
Peptone	0.5
Nacl	0.5
Beef extract	0.3
Yeast extract	0.3
Agar powder	2.5
Distilled water	100 ml

2.2. Extraction of earthworm coelomic fluid

Fifteen adult earthworms *Lampito mauritii* (average length 10 cm) collected from the garden of Konganadu arts and Science College, Coimbatore, Tamil Nadu (India) and thoroughly washed with tap water and then with sterile distilled water and dried with sterile blotting paper. The coelomic fluid can be directly collected from the body cavity of earthworms without causing any harm to them. In this method of collecting the fluid, three to four earthworms are taken in an approximately 10cm diameter petriplate and holding the plates in a slanting position and keep earthworms pointing downwards. Cold shock is given to earthworm by gently moving a small beaker containing a few ice cubes. The coelomic fluid released due to cold shock drips and gets collected at the lower side of the Petri plate. This fluid can be pipette out using a sterilized pipette with fine nozzle. This is the pure coelomic fluid that can be used for different biological investigations (Radha and Kale, 2006).

2.3. Preparation of Paper Disc

Sterile what man number1 filter papers were used to prepare the discs for absorbing the coelomic fluid of earthworms and standard ampicilline is used for experimental control. This was done, first by punching out several small discs (5 mm in diameter) and then impregnated with coelomic fluid until complete absorption. Several nutrient agar medium were prepared and plate was inoculated with 0.1 ml for 24 hours and spreader over petridish with glass spreader. Then each petridish was inoculated with 5 anti biotic disc, among these one is kept as control and other each one with earthworm pure coelomic fluid, earthworm coelomic fluid and alcohol, coelomic fluid and distilled water, and earthworm paste (it is prepared by cutting the tail region of earthworm and grind them in a mortar and pistle) for the disc diffusion method and these four samples were poured in to the wells of petriplate for well diffusion method. After that each plate is allowed to incubate at 35°C – 37°C for 24 hour. After 24 hour inhibition zone of each plate was measured with a scale.

3. RESULTS AND DISCUSSION

To the antimicrobial activity of coelomic fluid the inhibition zone formation around the coelomic fluid was noted and measured the diameter of that inhibition zone range. The antimicrobial activity of earthworm *Lampito mauritii* were tested against disease causing micro organisms namely *Escherichia coli, Klebssellia pneumonia, staphylococcus aureus,, streptococcus faecalis* through the inhibition formation ranging at 8-15 by well diffusion method and 6-15 by disc diffusion method. The result of well diffusion method showed that sample 'B' (coelomic fluid and alcohol) have the high antibacterial activity against the bacteria *Enterococcus faecalis* and less activity was found in *E.coli* as wella as *Klebseilla pneumoniae*.

Table 2. Measurement of zone formation by well diffusion method.

Sample/Name of bacteria	*E.coli*	*Klebseilla pneumonia*	*Enterococcus faecalis*	*Staphylococcus aureus*
A-Control	8.0mm	8.0mm	9.0mm	8.0mm
B-Coelomic fluid + alcohol	9.0mm	9.0mm	15mm	15mm
C- Pure Coelomic Fluid	15mm	10mm	10mm	10mm
D-Earthworm paste	10mm	12mm	15mm	15mm
E-Coelomic fluid + distilled water	12mm	8.0mm	8.0mm	9.0mm

Earthworm pure coelomic fluid showed the maximum zone formation against the bacteria *E.coli* and minimum zone formation in other three bacteria. Earthworm paste showed the maximum zone formation in two bacteria namely *Enterococcus fecalis* and *Staphylococcus aureus*. The sample E (coelomic fluid and distilled water) forms the minimum zone formation when compared to other three samples. The control forms the less inhibition zone against all bacteria.

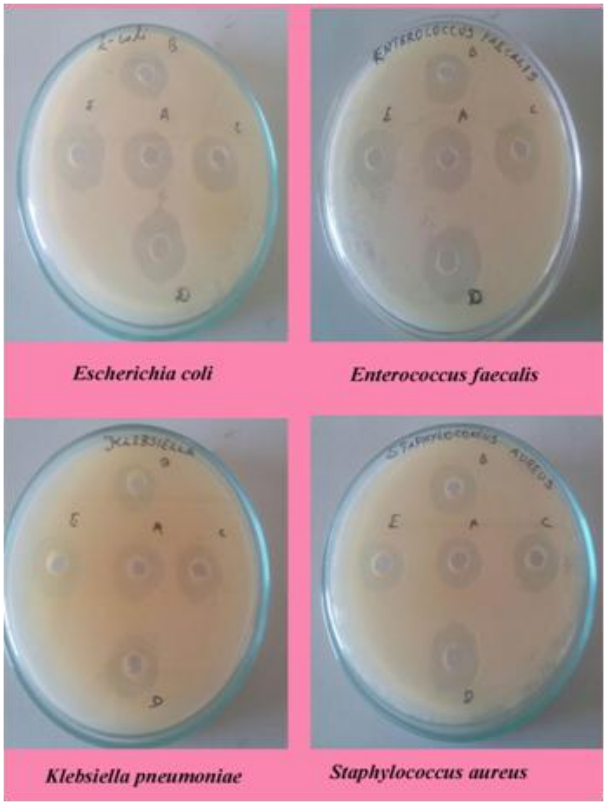

Fig. 1. Measurement of zone formation by well diffusion method

The result of disc diffusion method forms the inhibition zones ranging from 6.0mm to 15mm. The control forms maximum inhibition zone against the bacteria *Enterococcus fecalis* (10mm) and minimum in *Klebseilla pneumonia* (6.0mm). The sample B forms the maximum zone in *Staphylococcus aureus* (9.0mm) and minimum zone in *E.coli* (6.0 mm). The sample C showed the maximum zone formation in *E.coli* (15mm) and minimum zone in two bacteria namely *Klebseilla pneumonia* and *Staphylococcus aureus*. Sample D forms the maximum zones in *Enterococcus fecalis (*15 mm) and minimum In *Klebseilla pneumoniae* (9.0 mm) and sample have the maximum zone formation in *Staphylococcus aureus* (15 mm) and minimum in *E.coli* (9 mm).

Earthworm is a first terrestrial invaded organism and if serving on soil with millions of microorganisms. The antimicrobial activity in the coelomic fluid of earthworm may be because of innate immune mechanism and detect microorganism by recognizing conserved molecular pattern. The Asian countries like China and Korea are aimed at using earthworms in pharmaceutical applications. They have isolated enzymes and other active principles that can serve as antibiotics and anti-tumor agents. They have made detail studies on fibrinolytic enzymes of earthworms have also found their way in the preparation of cosmetics as a factor to delay ageing.

Lampito mauritii belongs to the family Megascolecidae and most of the sopecies of this family have melanin pigment on their body. In the above study we got good antimicrobial activity in Lampito mauritii against four disease causing bacteria namely *E.coli, Klebsiella pnumoniae, Enterocoocus fecalis*, and *staphylococcus aureus. Escherichia coli* (also known as *E. coli*) is a Gram-negative, facultative anaerobic, rod-shaped bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms (endotherms) (Singleton, 1999).

Table 3. Measurement of zone formation by disc diffusion method.

Sample/Name of bacteria	*E.coli*	*Klebseilla pneumonia*	*Enterococcus faecalis*	*Staphylococcus aureus*
A-Control	8.0mm	6.0mm	10mm	7.0mm
B-Coelomic fluid + alcohol	6.0mm	7.0mm	8.0mm	9.0mm
C- Pure Coelomic Fluid	15mm	0.8mm	0.9mm	0.8mm
D-Earthworm paste	10mm	9.0mm	15mm	10mm
E-Coelomic fluid + distilled water	9.0mm	10mm	10mm	15mm

Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination. The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K_2, and preventing colonization of the intestine with pathogenic bacteria. *E. coli* is expelled into the environment within fecal matter. The bacterium grows massively in fresh fecal matter under aerobic conditions for 3 days, but its numbers decline slowly afterwards. *Klebsiella pneumoniae* is a Gram-negative, nonmotile, encapsulated, lactose-fermenting, facultative anaerobic, rod-shaped bacterium. Although found in the normal flora of the mouth, skin, and intestines, (Ryan and Ray, 2004). it can cause destructive changes to human and animal lungs if aspirated (inhaled), specifically to the alveoli (in the lungs) resulting in bloody sputum. In the clinical setting, it is the most significant member of the *Klebsiella* genus of Entero bacteriaceae. *K. oxytoca* and *K. rhinoscleromatis* have also been demonstrated in human clinical specimens. In recent years, *Klebsiella* species have become important pathogens in nosocomial infections. As a general rule, *Klebsiella* infections are seen mostly in people with a weakened immune system. Most often, illness affects middle-aged and older men with debilitating diseases. This patient population is believed to have impaired respiratory host defenses, including persons with diabetes, alcoholism, malignancy, liver disease, chronic obstructive pulmonary diseases, gluco-corticoid therapy, renal failure, and certain occupational exposures (such as papermill workers). Many of these infections are obtained when a person is in the hospital for some other reason (a nosocomial infection). Feces are the most significant source of patient infection, followed by contact with contaminated instruments.

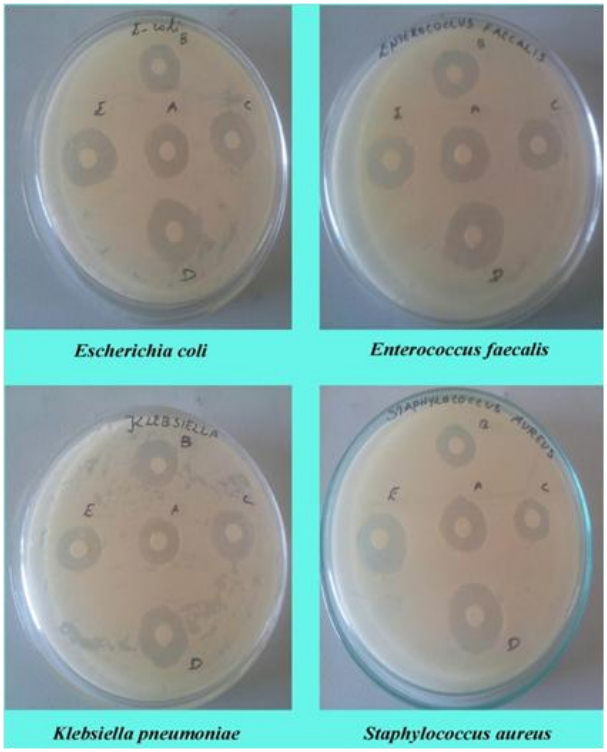

Fig. 2. Measurement of zone formation by disc diffusion method.

Enterococcus faecalis formerly classified as part of the group D *Streptococcus* system – is a Gram-positive bacterium inhabiting the gastrointestinal tracts of humans and other mammals (Ryan and Ray, 2004). Like other species in the genus *Enterococcus*, *E. faecalis* can cause life-threatening infections in humans, especially in the nosocomial (hospital) environment, where the naturally high levels of antibiotic resistance found in *E. faecalis* contribute to its pathogenicity. *E. faecalis* can cause endocarditis and septicemia, urinary tract infections, meningitis, and other infections in humans (Murray, 1990; Hidron *et al*., 2008). *Staphylococcus aureus* is a gram-positive coccal bacterium that is a member of the Firmicutes, and is frequently found in the nose,

respiratory tract, and on the skin. It is often positive for catalase and nitrate reduction. Although *S. aureus* is not always pathogenic, it is a common cause of skin infections such as abscesses, respiratory infections such as sinusitis, and food poisoning. Pathogenic strains often promote infections by producing potent protein toxins, and expressing cell-surface proteins that bind and inactivate antibodies. In this study we have selected the four samples of earthworm *Lampito mauritii*, that include pure coelomic fluid, coelomic fluid and alcohol (methanol), coelomic fluid and distilled water and earthworm paste. These four sample forms the inhibition zones against above mentioned bacteria.

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BIOCHEMICAL CHANGES OF MERCURY CHLORIDE ON BLOOD METABOLITE LEVELS OF A FRESHWATER FISH, *LABEO ROHITA*

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ABSTRACT

Rohu (*Labeo rohita*) is a fish of the carp family Cyprinidae, found commonly in rivers and fresh water lakes in and around South Asia and South - East Asia. The freshwater fish *Labeo rohita* were exposed to Mercury chloride (0.25 ppm) for 10, 20 and 30 days and its effect on blood – bio chemical properties in the form of hyperglycemia, hypoproteinemia and hypercholesteromia. The results of the present study that the blood – biochemical changes may lead to the fish morbidity and mortality.

Keywords: *Labeo rohita,* blood glucose, serum protein and serum cholesterol.

1. INTRODUCTION

Water is the home of the fish and its quality is one of the most over looked aspect of pond management until it affects fish production. Water quality generally means the component of water which must be present for optimum growth of aquatic organisms (Ehiagbonare and Ogundiran, 2010). Heavy metals have been announced to exert a vast range of metabolic, physiological, ecological and behavioral influences on fish (Soengas *et al.,* 1996). Mercury is non biodegradable and non - advantageous heavy metals and their role in the cell is not understood (Bailey *et al.,* 1999). It enters the food was through bacteria, algal and fishes of freshwater and marine water etc. as compared to land animals (Gupta, 1998). Examinations on the toxic effect of metals on fish are joined by the analysis of exchanges in some haematological and biochemical blood indices (Hoyle *et al.,* 2007).

Objectives

- To observe the LC_{50} concentration of mercury chloride to the fish, *labeo rohita*
- To observe the effect of sublethal concentration of Mercury chloride on biochemical characteristics in blood of the fish, *labeo rohita*

2. MATERIALS AND METHODS

2.1. Experimental fish – Labeo rohita

2.2. Test toxicant – Mercury chloride

A static bio - assay test was done to determine LC_{50} of Mercury chloride to *Labeo rohita* following the method of APHA (1985) and sublethal concentration was calculated by adopting the formula of Hart *et al*. (1945). For each experiment the fish (average length 8-8cm and wt. 26-28gm) were exposed to a sublethal concentration of mercury chloride (0.25 ppm) for a period of 10, 20 and 30 days. Side by side a control was also run in equal volume of water. The exposure medium was renewed every 24 hours. At the end of exposure period (10, 20 and 30 days) the fish were anaesthetized with 1:4000 MS 222 (tricane methane sulphonate sandoz).

- Estimation of blood glucose : Sinha (1990)
- Serum protein : Varley *et al*. (1980)
- Serum cholesterol : Karbara's method (1966)

3. RESULTS AND DISCUSSION

The fish *Labeo rohita* under 10, 20 and 30 days exposure of mercury chloride shows hyperglycaemic, hypoproteineric and hypercholesterolenic response. The blood glucose level elevated to 80.62 ± 1.5mg/100ml of blood against the control value of 50.76 ± 3.9 mg/100ml. The amounts to an increase by 58.82 % (Table 1). The protein depletion shows hypoproteinemia which depleted to 3.83 ± 1.4 g/100ml of blood against the control value of 7.15 ± 0.37 g/100ml of blood. The amount decrease by 46.43 % (Table 1). The serum cholesterol elevation shows hypercholesterolemic response which elevates to 289.4 ± 2.08 mg/100ml of blood against the control value of 247.5 ± 0.37 mg/100ml of blood. This amount are increased by 16.92 % (Table 1).

3.1. Blood glucose

Rita & Milton *et al*. (2006) observed hyperglycemia in fish. The increase in the blood sugar level under 30 days treatment might be because of stimulation of a cells of islet of Langerhan's which secrete and much amount of glucagons in turn had an action upon liver glycaogen with consequent enhanced glycogenolysis and the resultant hyperglycemia.

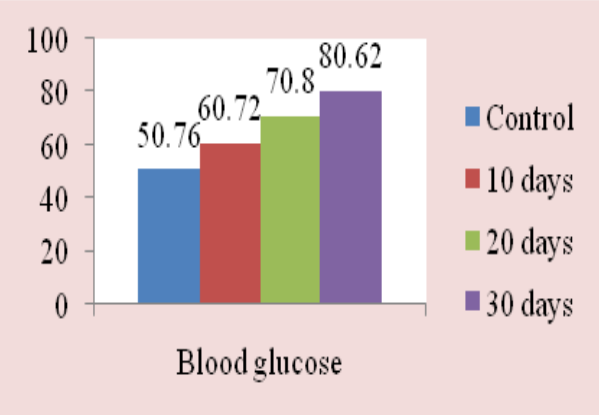

Fig. 1. Changes in the blood glucose levels in *Labeo rohita* exposed to sublethal concentration of mercury chloride.

Table 1. Changes in the blood metabolite levels in *Labeo rohita* exposed to sublethal concentration of mercury chloride.

Parameter	Control	10 days	20 days	30 days
Blood glucose (mg/100ml)	50.76 ± 3.9	60.72 ± 0.8	70.80 ± 0.9	80.62 ±1.5
% change		+19.62%	+39.47%	+58.82%
Serum protein (g/100ml)	7.15 ± 0.37	6.25 ± 0.4	4.45 ± 0.8	3.83 ± 1.4
% change		-12.58%	-37.76%	-46.43%
Serum cholesterol (mg/100ml)	247.5 ± 0.37	265.0 ± 1.6	276.8 ± 1.9	289.4 ± 2.08
% change		+7.07%	+11.83%	+16.92%

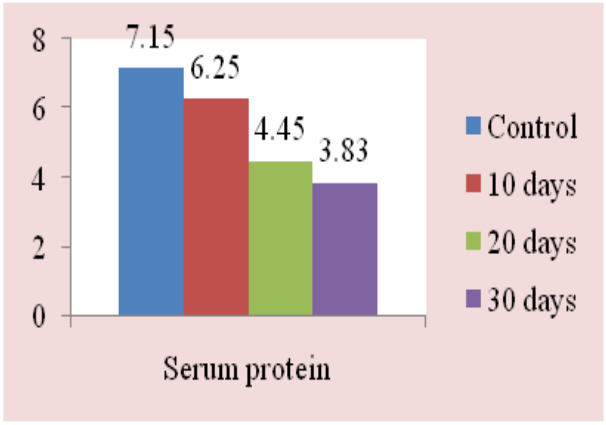

Fig. 2. Changes in the serum protein levels in *Labeo rohita* exposed to sublethal concentration of mercury chloride.

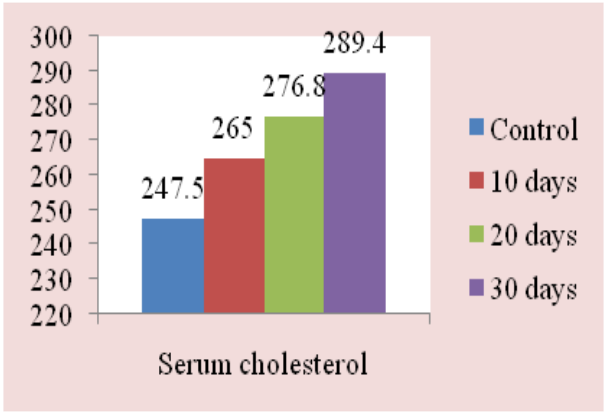

Fig. 3. Changes in the serum cholesterol levels in *Labeo rohita* exposed to sublethal concentration of mercury chloride.

3.2. Serum protein

Rekha Rani *et al*. (2008) observed hypoproteineric in fish. The liver cells might have reduced or stopped the synthesis of serum protein due to direct toxic effects of the mercury chloride and the serum protein would have been utilized under heavy metals induced stress leading to their depletion.

3.3. Serum cholesterol

Rekha Rani *et al*. (2008) observed hypercholesterolenic in fish. This might be attributed to the utilization of the volatile fatty acids by damaged liver parenchyma and ultimate entry to the circulation or to decreased excretion of cholesterol by damaged liver.

4. CONCLUSION

Mercury chloride proved to be highly toxic at sublethal concentration of 0.25ppm where 100% mortality was observed after 60 days exposure. The results of the present study that the blood – biochemical changes may lead to the fish morbidity and mortality.

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MOSQUITOE'S LARVAL GROWTH WITH REFERENCE TO WATER PARAMETERS IN UKKADAM PERIYAKULAM LAKE- COIMBATORE, TAMILNADU, INDIA

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ABSTRACT

Mosquitoes lay eggs in a wide range of habitats with different physicochemical parameters. Ecological data, including physicochemical factors of oviposition sites, play an important role in integrated vector management. Those data help the managers to make the best decision in controlling the aquatic stages of vectors especially using source reduction. To study some physicochemical characteristics of larval habitat waters, an investigation was carried out in Ukkadam periyakullam lake Coimbatore. Water samples were collected during larval collection from three localities. The chemical parameters of water samples were analyzed based on mg/l using standard methods. Water temperature (°C), turbidity (NTU), total dissolved solids (ppm), electrical conductivity (µS/cm), and acidity (pH) were measured using digital testers. Thermotolerant coliforms of water samples were analyzed based on MPN/100ml. In total, 112 mosquito larvae were collected. There was no significant correlation between the abundance of larvae and the different physicochemical and microbial parameters. But fecal coliform was the major impacting factor in the study area.

Keywords: Mosquitoe, water parameters, Ukkadam Periyakulam lake.

1. INTRODUCTION

Mosquitoes are dipteran insects and blood sucking fly pests of man. Mosquitoes are surviving on earth since millions of years. They have always given tough time to men as important carriers of various diseases. People fight globally against mosquitoes and mosquito borne diseases. Malaria, Dengue, Filaria, Japanese encephalitis, West nile virus and Chikungunia are the major diseases spread globally by different mosquito. These diseases challenge the developed and developing countries of the world for irradicaton. Mosquitoes are very well recognized as vectors of protozoan, viruses and other pathogenic organisms, after the discoveries made by Sir Patrick Manson, Sir Ronald Ross and Sir Walter Reed. It is well known also that under the influence of environmental conditions a vector species may show changes in the seasonal distribution in the same area of dominance.

The increase in density of a vector species is very much dependent on climatological factors and water quality favorable for its breeding, and adult survival (Suresh chavathiya.2010). The basic aim of present study is to generate perfect baseline data about mosquito's larva in Ukkadam Lake

2. MATERIALS AND METHODS

2.1. Study area

The ukkadam periyakulam, the life line of many species, provides water for the essential requirements of life. However, over the years it has been subject to tremendous pressure due to untreated sewage and industrial effluents being dumped in to the river at numerous places and the residues of fish wastes servers as a good medium for the growth of mosquitoes larva. Hence that ukkadam periyakulam lake is selected for the study.

2.2. Water sample collection

In the whole lake three location where spotted for the study. And they were named as A, B, and C. Spot A is one end of the lake. It was polluted with the domestic wastes like vegetable wastes from around households; used plastic bottles, waste cloth, plastic papers, and many more wastes were dumped in the lake. Spot B is the other end of lake. It is very much polluted than the other end. This end is polluted with the animal wastes. That is waste parts of fish are directly put into the lake. The worst part is the wastes are put in a plastic carry bags and dumped into the lake. And also the septic sewage is passing through this end. Spot C is the center of the lake where no pollution is found. This spot was clear to see from outside. No physical pollutants were present.

2.3. Methods of analysis for physicochemical parameters in water

Water samples were collected from A, B, and C. In a 1000ml container for testing its physico-chemical parameters (I.O.Oyewole.,*et al*). The collected samples were then taken to SEEDS ENVIRO LAB, COIMBATORE. For testing water parameters like acidity, alkality, ammonia, Ph, temperature, DO, total hardness, nitrate, organic nitrogen, phosphorus, free co2, calcium, magnesium, BOD, COD, total dissolved solids, and faecal coliform. However, a thermometer was used for measuring temperature on the field (Timb.B.M *et.,al*).

2.4. Mosquitoes larval count

The larval stages of mosquitoes were collected with a dipper. This method is called as dipping method. Dippers are the most commonly used tool. Which may vary in size and shape. The capacity of a few hundred milliliters to 1 liter. It is an inexpensive and easily used tool for collecting larvae (Dixon and brust 1972; lemenager *et al*.1986). In this study the larval stages of mosquitoes were collected by dipping which as done within 5 meter square area along the edges (Amerasinghe and Arivasena 1990).

3. RESULTS AND DISSCUSION

To find out the suitable parameter for the mosquito larval growth. The water samples were tested for its physico-chemical properties (Table 1). The parameters of the three water samples were tested for homogeneity. And it was found that the samples were homogeny through correlation matrix (Table 3). Then proceeding to the correlation of each parameter with respect to larval count. This was done using a statistical tool. As a result it was found to be that temperature, total hardness, nitrate, organic nitrogen, phosphorus, megnesium, BOD, COD and fecal coliform were the major parameters which showed a positive correlation with respect to larval count (Table 3).

Further comparing the three samples A, B, and C. A and B were found to be abundance in larval count. This shows that the polluted water is a good source for the larval growth. And also the statistical result showed that fecal coli form is the major factor present in the lake which makes the water unfit for consuming.

Table 1. Physicochemical characteristics of Ukkadam lake at the selected spots.

S. No	Parameters	Results		
		Sample A	Sample B	Sample C
1.	Acidity	BDL	4.0 mg/l	1.50 mg/l
2.	Alkalinity	350.0 mg/l	275.0 mg/l	150.0 mg/l
3.	Ammonia	BDL	BDL	BDL
4.	pH	8.26	7.48	7.18
5.	Temperature	27.5 C	27.7 C	27.4 C
6.	Do	5.80 mg/l	5.40 mg/l	6.10 mg/l
7.	Total hardness	200.25 mg/l	300.43 mg/l	160.20 mg/l
8.	Nitrate	17.43 mg/l	22.56 mg/l	7.42 mg/l
9.	Organic nitrogen	1.0 mg/l	1.0 mg/l	0.02 mg/l
10.	Phosphorus	14.0 mg/l	16.56 mg/l	5.60 mg/l
11.	Free CO_2	BDL	BDL	BDL
12.	Calcium	24.04 mg/l	40.08mg/l	24.04 mg/l
13.	Megnesium	34.07 mg/l	48.68 mg/l	24.34 mg/l
14.	BOD	40.0 mg/l	42.0 mg/l	12.0 mg/l
15.	COD	110.0 mg/l	170.0 mg/l	70.0 mg/l
16.	Total dissolved solids	703.0 mg/l	505.60 mg/l	300.70 mg/l
17.	Faecal coliform	12 MPN/10 ml	14 MPL/10 ml	03 MPL/10 ml

In the present study, 112 mosquito larvae were collected from the three spots A, B, and C. in the ukkadam periyakulam lake. Some physicochemical parameters of the sample in the study area showed significant differences among their localities. Like the total hardness (300.43 mg/l), nitrate (22.56 mg/l), nitrogen(1.0 mg/l), phosphorous (16.51 mg/l), magnesium (48.68 mg/l)BOD 42.0 mg/l, COD 170.0 mg/l, total dissolved solids 703.0 mg/l and the fecal coliform (140 MPL/100ml) (Fig. 1).

Table 2. The number of larvae counted at each spot.

Dippings	Spot A	Spot B	Spot C
1	7	9	3
2	8	7	0
3	5	4	2
4	3	9	0
5	6	7	0
6	2	4	1
7	4	6	0
8	3	5	2
9	5	4	1
10	2	3	0

Total number of larva counted in spot A=45, spot B=58 and spot C=9
Total number of larva counted in the lake=112

Khamala (1971) found that dissolved solids,ph,total nitrogen did not show any significant correlation with the density of some mosquitoes larval species. The same was found in the present study. Hanafi-bojd *et al* (2012). Noted the temperature, ph, total hardness, and dry residue of larval habitat, to that of larval density did not correlate. Both ghanbari *et al.* (2005). And hanafi-bojd *et al.* 2012). Mentioned the habitats in general. None of them provided the exact values of physico chemical features for each species. Piyaratne MK, Amerasinhe FP,Amerasinghe PH, konradsen F (2005). Found a positive correlation of some Anopheles species abundance only to temperature and calcium. Surendran and ramasamy (2003). Observed few anopheles species abundance to dissolved oxygen. Ibrahim AA,El-Monairy OM, El-Sayed, (2011).found that the temperature ,ammonia,and nitrogen are the best predictor for larval density. However no correlation was found between larval density, PH, and dissolved oxygen.

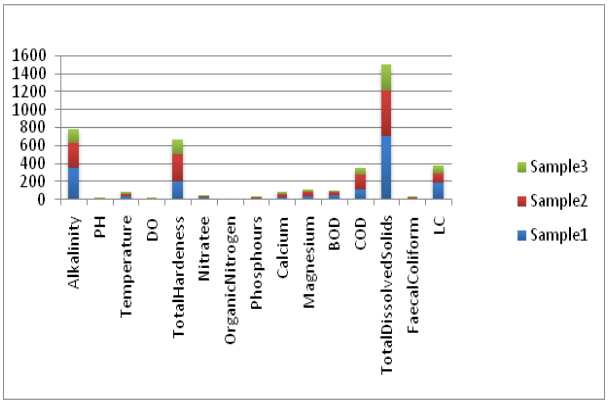

Fig. 1. Statistical diagram showing the relationship between the parameters and larval count of sample A, B and C.

Table 3. Correlation between the parameters of the three water samples.

	ALKALINITY	PH	TEMPERATURE	DO	TOTAL HARDNESS	NITRATE	ORGANIC NITROGEN	PHOSPHORUS	CALCIUM	MEGNESIUM	BOD	COD	TOTAL DISSOLVED SOLIDS	FAECAL COLIFORM	Larwal count
ALKALINITY	1														
PH	0.923177938	1													
TEMPERATURE	0.45895686	0.082199494	1												
DO	-0.551910886	-0.188982237	-0.994191626	1											
TOTAL HARDN	0.411646995	0.029727764	0.998619031	-0.987164522	1										
NITRATE	0.751897882	0.440726356	0.93083128	-0.964755916	0.9103466	1									
ORGANIC NITR	0.928571429	0.714575231	0.755928946	-0.821994937	0.7204921	0.942884978	1								
PHOSPHORUS	0.822274771	0.540362917	0.883002128	-0.928388877	0.857124	0.993456527	0.974761	1							
CALCIUM	0.142857143	-0.248547906	0.944911183	-0.90419443	0.9608027	0.759931551	0.5	0.680721522	1						
MEGNESIUM	0.524139627	0.156529781	0.997197519	-0.999457215	0.99189	0.955563297	0.8027876	0.915642821	0.917774513	1					
BOD	0.904791599	0.671596683	0.793614496	-0.854485824	0.7605545	0.961068149	0.9982212	0.986337203	0.550742713	0.836908705	1				
COD	0.52437882	0.156807201	0.997176465	-0.999466429	0.9918543	0.955646062	0.8029551	0.9157557	0.917662935	0.999999961	0.837062414	1			
TOTAL DISSOL	0.991223536	0.965888429	0.337477771	-0.436828027	0.2875578	0.658144452	0.8713566	0.739826336	0.010762804	0.406956758	0.840554256	0.407213318	1		
FAECAL COLIF	0.851606019	0.584702401	0.856564761	-0.907127959	0.8282712	0.985903519	0.9853293	0.998561462	0.640464031	0.892771148	0.993751471	0.892897656	0.77483687	1	
Larwal count	0.802570119	0.511614402	0.898361938	-0.940418579	0.8740446	0.996749151	0.9666583	0.999428642	0.705091967	0.928706718	0.980205598	0.928810839	0.71666357	0.99617864	1

Among the physicochemical parameters and based on the current investigation,the fecal coliform is the major impacting parameter for larval abundance.As it is obvious, some available data are contradictory and there is not enough information about physicochemical parameters of larval habitats for many mosquitoes species.in addition to the biological differences of different species, the same species has a range of tolerance and sometimes show different correlation with physicochemical parameters.

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THE TOXIC EFFECT OF PESTICIDE CYPERMETHRIN 25% EC ON THE PROTEIN METABOLISM OF THE FRESH WATER FISH *LABEO ROHITA*

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ABSTRACT

The pesticides Cypermethrin 25% EC insecticide has been used for the present study. It is a new highly active pyrethroid insecticide. It is primarily a caterpillar insecticide. Significant differences were observed in protein metabolism of *Labeo rohita* exposed to concentration of 0.4 ppm for 24, 48 and 72 hours respectively. The toxic effect of the pesticide Cypermethrin 25% EC were analysed after each exposure period, fishes were sacrificed and tissues such as liver, gill, muscle and kidney were dissected and removed. Samples were tested for protein analysis. Decreased content of protein was observed when compared to control. The results indicated the toxic nature of the insecticide Cypermethrin 25% EC.

Keywords: Cypermethrin, *Labeo rohita*, Protein, Liver.

1. INTRODUCTION

Environmental pollution, especially water pollution has been increasing at an alarming rate due to rapid industrialization, civilization and green revolution. The pesticide enter into the aquatic ecosystem through various routes affecting adversely to the aquatic biota .Nutritive value of fish is determined by its biochemical composition Glycogen is the only immediately available reserve of blood glucose. Pesticides are a group of heterogeneous compounds with proven toxicity and serious implications for man, animals, and the environment, still they are used regularly world over in agriculture and health programmes. The aquatic ecosystem as greater part of the natural environment is also faced with the threat of a shrinking genetic base and biodiversity due to indiscriminate use of pesticides.

Fishes are among the most vulnerable fauna in the water bodies that are affected by chemical pollutants. They are particularly sensitive to the influence of pesticides and other pollutants because they are able to uptake and retain the dissolved xenobiotic in water and thus a good indicator of the health status of aquatic bodies. However now it has been possible to assess the relative well being of the fishes and use them as indicator of the relative well being of the aquatic system. Assessment can be performed at different levels of organisation, from whole fish communities (*e.g.* fish assemblages) down to the molecular level (*e.g*. gene expression).However, many of the tests conducted are subjective and do not prove sufficient in environmental hazard evaluation. In terms of present utility biochemical tests are ranked higher and crucial in determining changes that may occur in fishes. The changes may be of some value in assessing the impact of exposure under natural conditions and may also serve as tools for biological monitoring.

2. MATERIALS AND METHODS

Cypermethrin 25% EC insecticides enter the freshwater resources and results into aquatic pollution. Pesticides are well known example for causing more toxic effects in teleost. Bulk of sample of fishes (*Labeo rohita*) ranging in weight from 4-5 gms and measuring 4-6 cm in length were procured from Tamil Nadu Fisheries Department, Aliyar, Tamilnadu. Fishes were acclimatized in the laboratory conditions for one month in large cement tank. The tank was washed using 1% KMnO4 to prevent fungal infection prior to stocking. The fishes were fed regularly with conventional diet rice bran and oil cake 1:1 ratio. Feeding was stopped one day prior to the start of the experiment. Fishes about the same size irrespective of sexes were selected for the experiment. The tap water free from contaminants was used as dilution water for the present study.

The physico-chemical analyses of water used in the experiment were carried out using the method (APHA, 2005). Batches of 10 healthy fishes were exposed to different concentration of pesticide Cypermethrin 25% EC to calculate the LC50 value by using the method of Finney, (1971). One more set of

fishes are maintained as control in tap water. Appropriate narrow range of concentration was used to find the median lethal concentration using a minimum of 10 fishes for each concentration and the mortality was recorded for every 24 hours upto 72 hours. In 0.4 ppm out of 10 fishes 5 are died at 72 hours. Thus 0.4 ppm is selected as LC50. Four groups of fishes were exposed in 0.4 ppm concentration of the pesticide Cypermethrin 25% EC for 24, 48 and 72 hours respectively. Another group was maintained as control. At the end of each exposure period, fishes were sacrificed and tissues such as liver, gill, muscle and kidney were dissected and removed. The tissues (10 mg) were homogenized in 80% methanol, centrifuged at 3500 rpm for 15 minutes and the clear supernatant was used for analysis of different parameters. Total protein concentration was estimated by the method (Lowry *et al.,* 1951).

3. RESULTS AND DISCUSSION

Liver tissues showed 1.80, 0.42 and 0.19 mg/g of protein in 0.4 ppm of Cypermethrin 25% EC pesticide and 2.20 mg/g of protein in control after 24,48,72 hours exposures. Table 1 shows the decreased value of protein content in kidney as 0.99, 0.72 and 0.31 mg/g in 0.4 ppm of Cypermethrin 25% EC and 1.57 mg/g in control after 24,48 and 72 hours exposures. In muscle tissues 1.13, 0.79 and 0.39 mg/g of protein in 0.4 ppm of Cypermethrin 25% EC exposures and 1.99 mg/g in control after 24,48,72 hours respectively. The protein level in gill is also reduced. In control the protein level is 3.21 mg/g. It is decreased to 2.65, 1.75, 1.02 mg/g in 0.4 ppm of Cypermethrin 25% EC exposure for 24,48 and 72 hours respectively.

Environmental stress invokes compensatory metabolic activity in the organs of an animal through modification and modulation of the quantity and quality of problems. Gill is an important organ because of its direct contact with water, which allows the pesticides to enter through it and get accumulated in the fish body. The percentage decrease of protein is greater in gill. It is maximum in 72 hours. The percentage decrease is 68.22. It was reported that the alteration in protein value may also be related to some structural changes in the liver, the arrangement of hepatic words leading to the alteration of liver metabolism (Ganeshwade, 2011 and 2012). The decrease in liver protein is also attributed to the inhibition of protein synthesis. The decrease in protein content suggests an increase in proteolytic activity and possible utilization of its products for metabolic purpose. The fall in protein level during exposure may be due to increased catabolism and decreased anabolism of proteins (Sreekala *et al.,* 2013). A significant reduction in the levels of proteins and glycogen (Tilak *et al.,* 1980).

Table 1. Changes in Protein content (mg/g) in the Liver, Kidney, Muscle and Gills of Labeo rohitaexposed to pesticide Cypermethrin 25% EC for different periods.

Tissue mg/g	Exposure Conc. 0.4ppm	Exposure periods		
		24 Hours	48 Hours	96 Hours
Liver	Control	2.20±0.07	2.20±0.07	2.20±0.07
	Experiment	1.80± 0.06	0.42± 0.03	0.19± 0.04
	't' value	10.98**	50.75**	53.95**
	% change	18.18↓	80.70↓	91.36↓
Kidney	Control	1.57±0.03	1.57±0.03	1.57±0.03
	Experiment	0.99±0.01	0.72± 0.04	0.31± 0.04
	't' value	30.28**	33.58**	50.44**
	% change	36.94↓	54.14↓	80.25↓
Muscle	Control	1.99±0.03	1.99±0.03	1.99±0.03
	Experiment	1.13± 0.04	0.79± 0.04	0.39±0.21
	't' value	35.03**	54.28**	17.18**
	% change	43.21↓	60.30↓	80.40↓
Gills	Control	3.21±0.04	3.21±0.04	3.21±0.04
	Experiment	2.65± 0.03	1.75± 0.03	1.02± 0.03
	't' value	21.15**	66.31**	94.54**
	% change	17.44↓	45.48↓	68.22↓

Results are mean (±SD) of 5 observations % = percent increase/decrease over control.
Parenthesis denotes the percentage. C = Control , E = Experiment

4. CONCLUSION

From the present study it is concluded that the above biochemical parameter could be used as a non specific biomarkers with regard to the effects of toxicants on organisms. It is also suggested that the random use of fertilizers and pesticides must be avoided for preserving our aquatic resources.

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FORAGING DYNAMICS AND POLLINATION EFFICIENCY OF *APIS CERANA INDICA* (HYMENOPTERA : APIDAE) ON EGGPLANT, *SOLANUM MELONGENA* L.

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ABSTRACT

Bees are responsible for more than 50% of all successful pollination. The pollination efficiency of *Apis cerana indica* was studied in Eggplant, *Solanum melongena*. The *Apis cerana indica* was found to spend 20 sec./flower/visit (20 ± 1.58). The amount of pollen depleted by *Apis cerana indica* was 563 pollen grains/insect visit (562.8 ± 34.89) and pollen deposition was 197 pollen grains/insect visit (196.8 ± 19.31). Pollen depletion and deposition by *Apis cerana indica* were more during 0800-1000 hrs. The peak activity of bees was recorded at 0900-1100 hrs when the temperature ranged from 28-32^{o}C and humidity ranged from 58-73%. The diurnal activity *Apis cerana indica* showed a significant positive correlation with temperature and negative correlation with humidity. These results showed *Apis cerana indica* is one of the efficient pollinators for *Solanum melongena*. The importance of *Apis cerana indica* colony for the improvement of agriculture is also be discussed.

Keywords: Insects, Bees, *Apis cerana indica,* pollination, pollinators.

1. INTRODUCTION

Insects are very important pollen transporters and they account for 85% of all pollination (Price, 1984; Decourtye *et al*., 2010). Beetle, Bees, wasps, ants, flies and butterflies are the most common insect pollinators and bees account for more than 70% of the insect pollination (Abrol, 1993; Rakesh Kumar *et al*., 1997; Subba Reddy *et al*., 1997). The interaction between the insect pollinators and the plants is a mututulaistic relation (Aguiar *et al*., 2015)

Nectar and pollen are the most common rewards offered by flowering plants to flower visitors in return and providing a pollination service (Simpson and Neff, 1983). Nectar is a phloem sap derivative and is a complex mixture of sugars, aminoacids, proteins, lipids, antioxidants, alkaloids, vitamins and minerals (Kevan and Baker, 1983). It is secreted by locally densely packed groups of specialized cells (nectaries) located on petals or sepals (Richards,1986). The nectar provided by the flowers has been found to be a significant parameter that shapes the pollinator behaviour to their energetic needs (Heinrich, 1975). Nectar is often the only source of energy for the activity, maintenance of metabolism, reproduction and growth of certain pollinators (Heinrich and Raven, 1972). Nectar is a renewable resource. Removal of it by bees visit and by microclimate with continuing secretion and therefore at the end of the day, after frequent bee visits, the nectar available would be of recent origin (Vithanage and Douglas, 1987). As the volume of nectar per flower decreases, the bees can expand its collecting area, but not indefinitely (Pflumm, 1985).

Pollen is considered to be the primitive flower reward. Pollen is highly nutritive and contains essential and quasi-essential aminoacids(Shubharani *et al*., 2012). Pollen is a vital food for many insects especially apidae (larva), flies, beetles, thrips and butterflies (Kevan and Baker, 1983). The amount of pollen presented by each flower is predetermined long before it opens. However, the flowers could adapt themselves in such a way that this non-renewable reward is presented at a time when the other rewards are most attractive (Vithanage and Douglas, 1987). In Macadamia, *Macadamia* Spp. adapt in such a way that the pollen reward is presented at a time when other rewards are most attractive. It helps to attract more number of bees (Vithanage and Doughlas, 1987). In *Antigonon leptopus* L. (polygonaceae), the anthers become dry and turn black by the evening time (Solomon Raju *et al*., 1999). Pollen is the principle source of non-liquid food and is the ultimate source of protein and lipid.

It is responsible for the growth and maturity of honeybees (Saraf, 1987).

Pollen dispersal success in entomophilous plants is influenced by the amount of pollen produced per flower, the fraction of pollen that is exported to other flowers during a pollinator visit, visitation frequency and the complementary between donor and recipients (Galen and Stanton, 1989). Most flowers open in the morning and pollen collector are active then. The pollen availability is high at this time and gradually declines during the day (Vithanage and Douglas, 1987).

Insect pollinators especially bees play a vital role in improving both quantity and quality of crops. In three rabbit eye blueberry cultivars (*Vaccinium ashei* Reade) bee pollinated flowers showed greater fruit numbers (Ne Smith and Krewer, 1999). In litchi, *Litchi chinensis* Sonn. honey bee pollinated flowers produced large sized fruits and 8.17–18.18% increase of fruit weight (Badiyala and Garg, 1990). In white mustard, *Brassica alba*, the plots which remained without access to pollinators (cage without any pollinator) had the lowest yield (966 kg/ha.), plots having free access to all the pollinators showed maximum yield (1620 kg/ha.) followed by plots having honeybees, A*pis cerana* Fabr. (1160 kg/ha.) (Chand and Singh, 1995). In both PSFH-67 and MSFH-8 varieties of sunflower, *Helianthus annuus* L. plots having open access to all the insect visitors had significantly high seed setting (72.36 and 72.50% respectively) followed by the plots caged with bees (*Apis mellifera* L.) (68.90 and 69.86%). In cucumber, *Cucumis sativus* L., *Apis mellifera* L. played an important role in the maximum production of a cucumber crop, and the duration of honey bee visits to flowers was correlated positively to the numbers of cucumber produced, to their weight and to their maximum girth (Gingrass *et al*.,1999). In cherry production, the blue orchard bee, *Osmia lignaria* increased the yield at about 2.68 fold (Bosch and Kemp, 1999). Cross pollination by various pollination agencies plays a pivotal role in increasing the yield of most of the fruit crops (Badiyala and Garg, 1990).

Abrol (1993) stated that the number of insects on a crop is directly related with temperature and inversely with relative humidity. In ridge gourd, *Luffa acutangula*, the diurnal activity of the wasp and *Apis cerana indica* showed insignificant positive correlation with the temperature. The foraging activity of the wasp showed insignificant negative correlation with the humidity, whereas Indian bee (*Apis cerana indica*) showed insignificant positive correlation with the humidity on sunny and cloudy days (Baskaran *et al*., 1997). In Apiaceae plants, bee abundance was shown to be significantly correlated with air temperature, light intensity, solar radiation and nectar concentration but negatively with relative humidity (Koul *et al*., 1993). In almond, *Prunus amygdalus*, the foraging activity of *Apis cerana indica* and *Xylocopa fenestrata* showed a negative correlation with humidity on sunny days (Abrol, 1988). Temperature and humidity influence the foragers activity through the drying of anther and pollen. Anthers dry at high temperature (Freeman and Head, 1990).

The efficiency of pollinator is dependent on number of factors, such as, the number of pollen grains collected at flower visits, the number of pollen grains delivered at subsequent flower visits and the number of pollen grains germinating on the stigma producing pollen tube that reach the ovules (Wiklund *et al*., 1979).

Due to human activities through modernization, natural populations of pollen bees have declined in many years (Batra, 1994; Cameron *et al*., 2011). Thus, since the 1950s it has become necessary to use honeybees for pollination and develop methods to artificially raise or manage pollen bees for use on some crops. Inter-relationship between plants and pollinators has been well studied in temperate zone while little is known from tropical zone (Ram,1980).

In recent years, researchers started collecting limited data on the importance of insect pollinators in commercial crops in tropics. There is an urgent need to undertake such studies in every plant family (Schmitt,1980). Such a need is much more intense in India, where there is a dearth of even a basic data (Reddi and Reddi, 1983). Also efficiency of bees on pollination of the commercial crops is less studied. Hence, it was proposed to study the "Insect Pollination of Eggplant, *Solanum melongena* L." with focused on the pollination efficiency of *Apis dorasata* in *Solanum melongena*

2. MATERIALS AND METHODS

2.1. Study period

For the present study, the of eggplant, *Solanum melongena* L. field situated in the village Anaiyur, Sivakasi Taluk, Virudhunagar District, Tamil Nadu was selected as study areas. The study was conducted during January 2001 to study the following parameters.

2.2. Compositiion and relative abundance of flower visitors.

This parameter was determined following the method of Jyothi *et al*. (1990). The insects that visited flowers during the study period were collected and identified. Relative abundance of each insect visitor was calculated by watching the number of visits of each insect visitor for 10 minutes/hr. from 0600 hrs to 1800 hrs in an area of one square meter. From this data the number of visits per day was calculated.

2.3. Pollen depletion

The amount of pollen carried from a flower by an insect is called as pollen depletion. This was studied following Solomon (1945).

2.3.1. Collection of insect visitors for pollen count

For pollen count the insect visitors were collected by placing a clean specimen tube over the insect while foraging and then closed with a stopper. Then the collected insects were anaesthetized immediately by placing chloroform soaked cotton plug over the mouth of the specimen tube. The specimen tubes were taken to the laboratory and stored in refrigerator until the insects were taken out for examination. In case of bees, the pollen baskets were discarded by removing the hind legs, as the pollen grains present in the pollen baskets are not used for pollination.

2.3.2. Pollen count

For the pollen count, the collected insects were taken out from the refrigerator and each insect was held with forceps over a small petridish. A fine jet of 70% ethyl alcohol was sprayed over the entire surface of the insect, which was then held under the liquid in the petridish and brushed with a small camel hair brush. Finally the insect was taken out from the liquid and sprayed again with a jet of alcohol. Then this sample was poured into a counting chamber. The pollen was allowed to settle and stirred well for uniform distribution of pollen grains in the counting chamber. The number of pollen grains was counted by a compound microscope.

2.3.3. Pollen deposition

Pollen deposition is the amount of pollen deposited on the stigma by an insect. To assess the amount of pollen deposited on the stigma, the flowers bagged with polythene cover just before anthesis (Plates 3 & 4) were opened one by one for the insect to visit following the method of Reddi and Reddi (1983). When such flowers received the first visit, their stigmas were plucked and examined for the number of pollen deposited following the pollen count method as mentioned earlier.

2.3.4. Time spent at flowers

This was calculated for each visitor using a stop watch, when an insect approaches the flower the stop watch was switched on and when it leaves the flower, it was switched off (Reddi and Reddi, 1983).

2.3.5. Diurnal activity

Diurnal activity is the foraging activity of insect visitors during day time from 0600 hrs to 1800 hrs. This was studied following the method suggested by Abrol (1987). The diurnal activity of insect visitors was studied by watching the number visits of insects for 10 minutes/hr. from 0600 hrs to 1800 hrs on the plant population in one square meter. Concurrent with foragers counts, measurements of environmental factors such as temperature and relative humidity were also made in the experimental field by using comfort meter.

2.3.6. Temporal variation in pollen depletion from anthers and pollen deposition on stigma.

Temporal variation is the variation in the pollen depletion from anthers and pollen deposition on stigma in relation to time. This was done following the method suggested by Reddi and Aruna (1990). Here the pollen depletion and pollen deposition by insect visitors were studied at an interval of two hours from 0600 hrs to 1800 hrs.

3. RESULTS AND DISCUSSION

The flowers of egg plant, *Solanum melongena* L. were found visited by Indian bee, *Apis cerana indica*; Little bee, *Apis florea*; Rock bee, *Apis dorsata*; Anthophorid bee, *Anthophora zonata*, Carpenter bees, *Xylocopa violaceous*; and *Xylocopa aestuans*. Among the bees, *Apis cerana indica* (25%) was of the second major visitor next to *Apis dorsata* (Fig. 1). This is due to the fact that hymenoptera is the most important order of anthophilous insects (Kevan and Baker, 1983). Bees are the most important among all angiosperm pollinators (Grissell, 1999; Rosa *et al*., 2015). The honeybees represented about 90% of the total number of insects visiting the watermelon crop (Hadimani *et a*l., 1998). The dominance of hymenopterans is due to their adaptive structures in their body. The body of honeybee is covered with branched hairs for the pollen to adhere (Elzinga, 1987).

Apis cerana indica was found to spend 20 sec./flowers/visit on flowers (Table 1). In apple, *Malus domestica*, the foraging bees such as *A. cerana indica*, *A. mellifera, A. dorsata*, *Epi balteatus*, *E. tenax* and *Orthellia* Spp. spent on average 7.92, 11.40, 6.46, 38.26, 25.80 and 43.60 seconds per flower (Dashad *et al*., 1994).

In guava, *Psidium guajava* L., the time spent per flower by *A. mellifera, A. dorsata* and *A. cerana indica* were 5.8, 5.2 and 4.0 seconds respectively (Rakesh Kumar *et al*., 1996). The varying time spent by different species of thrips in solanaceae plants reflects the degree of host specificity (Annadurai and Noble Marrison, 1987). Honeybees, *Apis cerana indica* and *Apis dorsata* spent the same time (2.3 sec.) on each head for pollen collection but took 3.7sec. and 3.9sec. respectively for nectar collection on niger, *Guizotia abyssinica* (Mohana Rao and Suryanarayana, 1990). In sunflower (*Helianthus annuus* L.), the average time spent per capitulum by *Apis* and *Xylocopa* bees was 47.87 sec. and 34.58 sec. respectively (Abrol, 1996). Mohana Rao and Suryanarayana (1990) reported that in niger, *Guizotia abyssinica*, the honeybee (*Apis cerana indica)* spent 3.7 sec. and 3.9 sec. respectively while collecting pollen and nectar respectively. In scented methi (*Trigonella corniculata* L.), *A. cerana indica* and *Apis florea* spent 5.7 and 10.5 seconds respectively on each flower (Mohana Rao, 1991). The time spent at the flower is an indication of the mobility of an insect which in turn, indicates the effectiveness to utilize the floral resource (Baskaran *et al*., 1997).

The amount of pollen depleted by *Apis dorasata* was 562.8 ± 34.89 pollen grains/insect visit (Table 1). The pollen loads on bees foraging are related to size of the pollinator (Free and Williams, 1972; Elzinga, 1987). Most small insects with smooth bodies carry little or no pollen whereas large hairy insects bear considerable amount of pollen (Kendall and Solomon, 1973).

Pollen carry over is affected by size and hairiness of the pollinator and its cleaning and foraging behaviour. As honeybees move on the flower, pollen is picked up by plumose body hair, the pollen is brushed off by their legs into pollen baskets on the hind legs (Elzinga, 1987). The body of honeybee is covered with branched hairs for the pollen to adhere. This makes the honeybees efficient in pollen depletion. The body of honeybees is covered with branched hairs for the pollen to adhere. This makes the honeybees efficient in pollen depletion. Body setae density of bees determines their pollination efficiency (Free, 1993).

Table 1. Time spent at flowers, Pollen depletion and deposition by *Apis cerana indica* in Eggplant, *Solanum melongena* L.

Parameters	Mean ± SD
Time Spent (Sec./ Flower / Visit)	20.0 ± 1.58
Pollen depletion (Pollen grains / Insect visit)	562.8 ± 34.89
Pollen deposition (Pollengrains / Stigma / Insect visit)	196.8 ± 19.31

Table 2. Pollen depletion and deposition by *Apis cerana indica* in Eggplant, *Solanum melongena* L.

Time in Hours	Pollen Depletion	Pollen Deposition
06.00	NV	NV
08.00	438.6 ± 46.21	176.8 ± 21.55
10.00	473.2 ± 53.28	196.8 ± 15.42
12.00	423.2 ± 29.24	186.6 ± 14.94
14.00	NV	NV
16.00	370.2 ± 38.19	186.2 ± 18.86
18.00	NV	NV

Values represent X ± SD of five readings; NV = No visits.

Table 3. Diurnal activity of *Apis cerana indica* of Eggplant, *Solanum melongena* L. on a sunny day and cloudy day

Time (Hrs.)	Sunny day (29-01-2001)	Cloudy day (13-01-2001)
0600	-	-
0700	1	-
0800	2	2
0900	4	3
1000	6	4
1100	9	6
1200	5	5
1300	4	3
1400	2	2
1500	3	1
1600	2	1
1700	2	-
1800	-	-

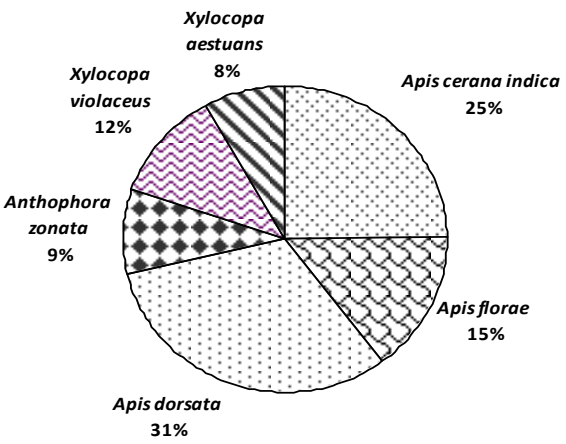

Fig. 1. Composition of bee pollinators of Eggplant, *Solanum melongena*

Pollen depletion and deposition by *Apis cerana indica* was more during 10.00 hrs (Table 2). This may be due to the availability of abundant pollen in morning hours (Spira *et al*., 1992). In morning hours, the *Apis* Spp. were found to deplete and deposit more number of pollen grains in *Brassica compestris* L. Var. toria blossoms in the mid hills of Himachal Pradesh (Jitender Kumar *et al*., 1994). Pollen harvesting rate was more in morning than in evening corresponding to decreasing pollen availability (Buchmann and Shipman, 1990). The hourly observations indicated that bees visited less number of flowers per minute in the morning than at noon and evening time. This may be due to availability of pollen in the flower in large quantities in the morning (Mohana Rao *et al*., 1984). Most flowers open in the morning and pollen collectors are active then. The pollen availability is high at this time and gradually declines during the day (Vithanage and Douglas, 1987). The pollen gathering activity of *Apis mellifera* and *Apis cerana indica* on mustard was more in the morning than in the evening (Thakur *et al*., 1982).

The amount of pollen depleted and deposited was found to be more at 0800 h. due to pollen stickiness and stigma receptivity in guava, *Psidium guajava*. The amount of pollen deposition was found to decrease during successive visits of *Apis cerana indica* (Prakash *et al*., 1993). This decrease may be due to the decreasing receptivity of stigma with time which is influenced by temperature and humidity (Shivanna and Johri, 1985). The pollen loads carried by bees were heavier for the foraging trips in the morning than those of afternoon in sunflower, *Helianthus annuus* L. (Bhuyan and Bhatta charryya, 1998). In cotton, *Gossypium* Spp. the peak time of pollen depletion by *A. mellifera* L. on the stigma was between 1000 and 1200 hours (Mamood *et al*., 1990). Among the flower visitors of onion bloom (*Allium cepa* L.), the maximum number of pollen grains was carried by *A. dorsata* and dipterans carried comparatively less number of pollen grains (Priti, 1998). The size of the pollen loads of bees foraging on polleniferous flowers was larger than that of bees from nectariferous flowers (Neeman *et al*., 1999). The pollen depletion and deposition were 76% during the period of 0800-1400 hours in the flower of *Alangium lamarkii* (Byragi Reddy and Aruna, 1990). In *Delphinium virescens*, the amount of pollen deposited on the body of bees during visit is directly proportional to the amount of anthers. The amount of pollen deposited on the stigma of a flower is directly proportional to the amount of pollen carried by the bees' body (Waddington, 1981).

The diurnal activity of *Apis cerana indica* was mostly found to begin around 0700 hours on sunny days (Table 3). The peak activity of all the visitors was found at 1000-1200 hours when the temperature ranged from 29-31^{o}C and humidity ranged from 65-70%. The activity of these insects was found to cease mostly around 1700-1800 hours on sunny days. In *Zizyphus mauritiana* (Rhamnaceae), bees showed maximum activity between 0900 and 1100 hours then decreased gradually (Ramadevi *et al*., 1989). In the present study, the activity of insect visitors was found to begin around 0700 hours in sunny days and around 0800 hours in cloudy days. The activity was found to cease mostly around 1700 to 1800 hours on sunny days and around 1500 hours during cloudy days. Insects visit were least common during lowlight, moderate winds, lowest temperatures and earliest time of the day. High probabilities of visitiation were found during middle of the day, at high temperature, high light levels, at low humidity and with moderate wind speed (Mc Call and Primack, 1992). Abrol (1993) stated that the number of insects on a crop is directly related with temperature and inversely with relative humidity. In ridge gourd, *Luffa acutangula*, the diurnal activity of the wasp and *Apis cerana indica* showed insignificant positive correlation with the temperature. The foraging activity of the wasp showed insignificant negative correlation with the humidity, whereas Indian bee (*Apis cerana indica*) showed insignificant positive correlation with the humidity on sunny and cloudy days (Baskaran *et al*., 1997).

The diurnal activity of *Apis cerana indica* was mostly found to begin around 0800 hrs during

cloudy day (Table 3). The activity of these insects was found to cease around 1600 hrs. The peak activity of *Apis cerana indica* was recorded at 1100-1200 hrs when the temperature ranged from 29-30°C and humidity ranged from 65-69%. In scented methi (*Trigonella corniculata* L.), the honeybee (*A. cerana indica*) initiated foraging from 0920 and completed at 1830 h. The density was maximum at 1400 h. and gradually their population reduced. *A. florea* foraged from 1020 to 1700 h. (Mohana Rao, 1991). In niger, *Guizotia abyssinica*, the density of *A. cerana indica* was peak at 0900 h. then density gradually decreased. The density of *A. dorsata* was high between 0900 and 1600 h. (Mohana Rao and Suriyanarayana, 1990). The honeybee, *A. cerana indica* showed peak activity during 0700 – 1000 hours and 1400 – 1500 hours. *Xylocopa* was most active during the earlier part of the day at 0700 – 0900 hr. in sunflower, *Helianthus annuus* L. (Abrol, 1996). The *Trigona* bees reached peak of abundance on flower between 1000 and 1200 hr. whereas honeybees reached a peak at 1230 hr. on macadamia flowers (Heard and Exley, 1994). In litchi, *Litchi chinensis*, *A. cerana indica* visits were maximum during 0900 and 1100 hr. The visits were less during 1400 – 1600 hr. (Mahanta and Rahman, 1997). The peak activity of *A. cerana indica* was during 0900 to 1100 hr. on mango flowers (Jyothi, 1994).

Correlation analysis of diurnal activity of *Apis cerana indica* showed significant positive correlation with temperature (r = 0.647 p=0.05) and negative correlation with humidity (r = -0.529 p=0.05). In Apiaceae plants, bee abundance was shown to be significantly correlated with air temperature, light intensity, solar radiation and nectar concentration but negatively with relative humidity (Koul *et al*., 1993). In almond, *Prunus amygdalus*, the foraging activity of *Apis cerana indica* and *Xylocopa fenestrata* showed a negative correlation with humidity on sunny days (Abrol, 1988). Temperature and humidity influence the foragers activity through the drying of anther and pollen. Anthers dry at high temperature (Freeman and Head, 1990). In *Zizyphus mauritiana*, the bee activity was positively correlated with air temperature, light intensity, solar radiation, nectar sugar concentration and negatively correlated with relative humidity (Sihag and Abrol, 1986).

The number of insect visitors on lucerne was inversely related to relative humidity (Cirudarescu, 1971). In ridge gourd, *Luffa acutangula* the foraging activity of wasp showed negative correlation with humidity, whereas, *Apis cerana indica* showed positive correlation (Abrol, 1993). The foraging activity of *Apis cerana indica* and *Apis florea* showed positive correlation with humidity in guava (Prakash *et al*., 1993) The foraging activity of honeybees was maximum at those hours when relative humidity was low in sun flower, *Helianthus annuus* (Panda *et al.*, 1991). Temperature and humidity influence the foragers' activity through the drying of anther and pollen. Anthers dry at high temperature (Freeman and Head, 1990).

Further studies are needed to explore stigma receptivity, temporal variation in floral rewards, influence of light intensity, solar radiations and wind speed on foraging activity of insects which may enlighten new principles on insect visitors of bitter gourd and eggplant.

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VARIATION IN ZOOPLANKTON DIVERSITY AND ITS RELATIONSHIP WITH ABIOTIC ENVIRONMENT OF A VANDIYUR POND TAMILNADU INDIA

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ABSTRACT

Ponds, Lakes and Reservoir are most significant water resources with multiple human utilization andecological relevance in which Zooplankton diversity is one of the most important ecological parameters in water quality assessment. It is good indicator of the changes in water quality because they are strongly affected by environmental conditions. In the present study an attempt has been made to study the seasonal variations in the Zooplankton community, its diversity and hydrological parameters of this water body. In the present study, a total of 24 species of Zooplankton were indentified from different classes during August 2014 to January 2015. Among the identified species, Zooplankton showed the complete dominance, especially, zooplankton belonging to four major groups i.e. 9 species of Rotifera, 6 species each of Cladocera, 4 Copepoda and five species of Ostracoda.

Keywords: Zooplankton, Bioindicator, water quality.

1. INTRODUCTION

Studies on fresh water bodies, natural or manmade have gained much importance in recent years mainly because of their multiple uses. Several workers have attempted to study the hydrobiological profile of varied water bodies with intent of assessing the quality of water zooplankton play a very important role in increasing photosynthesis in some algae which pass through their nutrient rich elementary canal in viable condition. Zooplankton acts as bio-indicator of water quality as well as quantification of primary energy transfer from producer to primary consumer (Dulic *et al*., 2006). Kolhe *et al.,* 2013 also observed the zooplankton communities respond more quickly to environment variations. Therefore the water quality is a major factor in determining the welfare of the society (Dwivedi and Pathak, 2000). It also plays a vital role in governing the production of planktonic biomass. The management of any aquatic ecosystem is a means of conservation of fresh water habitat with an aim to maintain the water quality or to rehabilitate the physico-chemical and biological settling of water (Ravi Kumar *et al*., 2005). Based on the above mentioned facts, it is suggested to make an inventory of the physicochemical parameters and zooplankton diversity of vandiyur pond, located in Madurai.

2. MATERIALS AND METHODS

2.1 Study Area

Vandiyur pond was selected for my research work actually; it is a small aquatic pond with a minimum depth of pond about 25 feet. It is located just east zone of Madurai town. In which possess the common fishes likeCatlacatla, Rohu and kendai. This pond water is used only for the agriculture and fish farm.

2.2 Sample Collection

The water sample was collected from the pond surface once in the early hours of the day from August 2014 to January 2015. The water samples were collected using one litre container for the estimation of water quality parameters. The collected samples were immediately taken to the laboratory for analysis. The estimation was done by using the standard book of Kumar and Kakrani (2000).

2.3 Biological Analysis

Zooplankton samples were collected by filtering 300 litres of water from the surface of the water body through plankton net (40 µm mesh size) and which was fixed immediately with 4% ormalin. The systematic identification of zooplankton was made by using standard keys of Dhanapathi (2000)

and Altaff (2004). The quantitative analysis of planktonic organisms was carried out using Sedgwick Rafter's plankton counting chamber.

3. RESULTS AND DISCUSSION

The seasonal fluctuations of pond water quality parameters have a markedinfluence on the numerical abundance of zooplankton. Jeppesen*et al*. (2002) has stated that the enormous and diversity of zooplankton vary according to limnological features and the trophic state of freshwater bodies.

3.1 Biological parameters

3.1.1 Qualitative study of zooplankton in vandiyur pond

In a pond system, a total of 24 species of zooplankton have been exposed that belongsto four major groups. They were included as follows, Rotifera– 9, species Cladocera– 6, species Copepoda - 4 and species Ostracoda - 5 species.

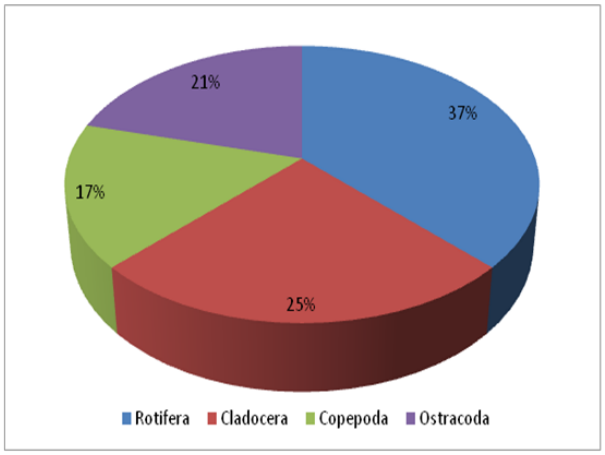

Fig. 1. Zooplankton abundance in vadiyur pond

Table 1. Monthly Variations in the Physico-chemical parameters of the Vandiyur Pond.

Parameter	August 2014	September 2014	October 2014	November 2014	December 2014	January 2015
Temperature (°C) Air	27	28	27	27	29	30
water PH	7.2	7.6	7.8	8.1	7.8	8.0
Total Hardness (ppm)	132.12	142.27	98.56	67.12	58.49	62.10
Dissolved Oxygen (mg/l)	6.2	5.8	6.2	4.2	5.8	6.9
Free carbon dioxide (mg/l)	4.5	3.4	4.9	2.9	3.5	4.1
Total Alkalinity (ppm)	198.00	212.16.	196.14	168.12	182.00	89.17
Salinity (ppm)	222.03	196.15	165.13	145.22	156.28	102.36
Chlorinity (ppm)	121.10	98.27	89.23	123.00	131.00	89.12
Phosphate (mg/l)	0.06	0.04	0.03	0.05	0.03	0.01

3.1.2 Quantitative study of zooplankton in vandiyur pond

In the present work was assessed, *B. calyciflorus* was found to be more in number during the month of August 2014 and *B. forficula*in the month of November 2014 (3units/ml). The other species were recorded in low number. *B. rubens* was observed only during August 2014. The enormous of rotifers and their community characteristics are used as effective indicators of environmental changes, such as, acidity, food level and humidity *etc*. (Attayde and Bozelli, 1998). The number of *cladocerans* recorded was minimum during the study period. *Ceriodaphnia cornuta* was recorded during the entire period of study. Their presence indicates the health of the ecosystem, as it forms the basic food item for fishes. *Mesocyclops aspericornis* was

observed maximum in the month of November 2014 (20 units/ml) and minimum in January 2015 (5 units/ml). Kumar (1999) reported that *cyclops*serve as the most suitable pollution tolerant indicator. *Heliodiaptomusviduus*was found in range from 2 units/ml in August 2014 to 10 units/ml in December 2014. *Diaptomusnauplius* showed variations from 3 units/ml in December to 10 units/ml in January 2015. The ostracod was represented by only one species, *Stenocypris* major was observed in all the months (1 unit/ml) except November 2014.

In the air temperature was ranged from 27 to 30^{o}C and water temperature from 25 to 29^{o} C. Kumar and Kakrani (2000) opined that the rise in temperature of water elevates the metabolic activity of an organisms. It also influences the growth and distribution of plankton. Welch (1952) has observed that smaller the water body, more quickly to react the changes in atmospheric temperature. The pH of the water body showed alkaline in nature ie. 7.2 to 8.0This range is good for growth of aquatic organisms (Lendhe and Yeragi, 2004). Bell (1971) has stated the pH ranges between 6.5 to 9.0 provides an adequate protection to the life of fresh water organisms. Jhingran (1991) reported that pH ranges between 6.0 to 8.5 indicates medium productivity, more than 8.5 highly productive and less than 6.0 low productive nature of water body. Total hardness ranged between 58 ppm in December 2014 and 142.27 ppm in August 2014. Fishes have been found to susceptible to diseases when hardness is below 20 ppm. If it ranged more than 300 ppm, it affects fish production due to more pH as reported by Das (1996). Dissolved oxygen content in the water sample ranged from 4.2 to 6.09 mg/l .Mustafa and Ahmad (1985) opined the partial of O2 dissolved in water depends upon the partial pressure of gas in the air close to water, rate of photosynthesis and oxygen holding capacity of water. Tarzwell (1957) reported that for supporting life, minimum of 3mg/l DO is required. Free Co2 ranged from 2.9 to 4.9 mg/l during the study period. In morning sample, there is an accumulation of free Co2 due to overnight community respiration. Salasker and Yeragi (2003) noted that slightly increased Co2 in winter season. Free Co2 is essential for photosynthesis and its concentration affects the aquatic fauna and its productivity. The total alkalinity was ranged from 89.17 to 212.16 ppm. In the water body, the alkalinity is imparted by number of bases viz., carbonates, bicarbonates, hydroxides, phosphates, nitrates, silicates, borates etc., (Kumar and Kakrani, 2000). The fluctuation in salinity is probably due to fluctuation in total solids (Boyd and Tucker, 1998). The minimum value of chlorides (89.12 ppm) was found in the month of January 2015 and the maximum value of 131.00 ppm during the month of December 2014 was recorted. Chloride content above 250 ppm makes water salty in taste; however a level upto 1000 ppm is safe for human consumption (Kumar and Kakrani, 2000). The phosphatecontent of water sample showed 0.01 to 0.06 mg/l. It is an essential nutrient, play a vital role inbiological activities of aquatic organisms. Lendhe and Yeragi (2004) reported the range ofphosphates from 1.20 mg/l to 3.70mg/l in Phirangekharbav lake.

4. CONCLUSION

An inverse relationship was observed zooplankton abundance. The managed fish culture pond which wasperiodically limed, manured and fertilised showed greater zooplankton being the dominant group. Whereas theunmanaged village pond showed a less diverse and eutrophic condition,zooplankton being the dominant group. It implies that alarge amount of ecological niches are remaining void and unutilized in village ponds. Whereas all the available ecological niches are beingeffectively utilized by the stocked fishes and periodically replenishedby fertilization in the managed fish culture pond. Therefore selectivestocking with appropriate species at low densities and extensive fishculture practices in the village ponds has ample scope. Adoption andtransformation of such village ponds by scientific management practicesinto semi-intensive fish culture ponds may prove to be an ecologicallyefficient, financially feasible and socially viable venture.

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SCIENTOMETRIC ANALYSIS OF "ANNALS OF ONCOLOGY" DURING 2010 – 2014

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ABSTRACT

This paper presents bibliometric analyses of 10681 articles published in ANNALS OF ONCOLOGY (AOO) during 2010-2014. The data was downloaded from web of science database. The analysis covers various parameters like year wise publication, growth pattern, word frequency, ranking of authors, ranking of institution, document types etc., and Histographicanalysis of the datasets has been performed using Histcite software.

Keywords: Oncology, Bibliometric analysis, Content analysis, Citation.

1. INTRODUCTION

Academic journals are utilized by the researchers and experts to share their thoughts, considerations, developments, advancements, and disclosures. So individual journals are the main target of the many bibliometric and scientometric studies. The terms bibliometrics and scientrometrics wereintroduced by Pritchard, Nalimov and Mulchenko in 1969. Bibliometric study is a simple statistic method of bibliography counting to evaluate and quantify the growth of a subject (Tsay *et al.,* 1997).

2. ORIGIN OF THE JOURNAL

Annals of Oncology, the journal of the European Society of Medical Oncology and the Japanese Society of Medical Oncology, provides rapid and efficient peer-review publications on innovative cancer treatments or translational work related to oncology and precision medicine.

Main focuses of interest include: systemic anticancer therapy (with specific interest on molecular targeted agents and new immune therapies), randomized trials (including negatives ones), top-level guidelines, and new fields currently emerging as key components of personalized medicine, such as molecular pathology, bioinformatics, modern statistics, and biotechnologies. Radiotherapy, surgery and pediatrics manuscripts can be considered if they display a clear interaction with one of the fields above or are paradigm-shifting.

With a large international editorial board of experts who are leaders in their fields, Annals of Oncology aims at delivering the best communication on the fast moving, and continually evolving, global oncology landscape.

Annals of Oncology is covered by the following major indexing services CAB Abstracts, Current Contents® /Clinical Medicine, Elsevier BIOBASE - Current Awareness in Biological Sciences (CABS), EMBASE, Journal Citation Reports /Science Edition, PROQUEST, Prous Science Integrity®, PubMed, Science Citation Index Expanded (SciSearch®) and Science Citation Index (http://www.oxfordjournals.org/our_journals/annonc/about.html (09-01-2016)).

3. REVIEW OF LITERATURE

There are several bibliometric studies available in the literature which includes bibliometric studies on single journals, citation studies and subject studies. A study by Nageswara Rao *et al.* (2013) on Bibliometric Analysis of theJournal of Propulsion and Power (1985-2013) showed thatthe highest number (194) articles were published in year 1992 and lowest (81) in 1987. Out of total articles, 1330 were produced by two authors and 1098 by three authors. It is found that 1205 different institutions were involved in publication of articles. 'Purdue University' contributed highest number of 163 articles. Out of ranked list of 21 affiliations which produced more than 50 articles, 18 institutions were from USA, 2 from Japan, and 1 from Germany, etc.

Manoj Kumar and Moorthy (2011) studied Bibliometric Analysis of DESIDOC Journal of Library and Information Technology during 2001-2010. Showed that maximum papers (17.3 per cent each) were published in 2008 and 2009, and minimum papers (3.6 per cent) were published in 2001. And Authors from government research institutions

contributed 110 (40.6 per cent) papers followed closely by 105 papers (38.74 per cent) from universities. Authors from colleges and private research institutions comprised 11.07 per cent and 6.27 per cent, respectively.

Anil Kumar and Prakasan (2008) studies on Pramana - Journal of Physics: A scientometric analysis. Focuses on publishing trend; impact factor; authorship pattern; types of articles; institutional collaboration of authors; affiliated institutions of authors; countries of contributing authors; keyword analysis; and referencing pattern. The number of articles being published in Pramana and its ISI impact factor are increasing. There is an upward trend in number of collaborated papers. Authors from University of Delhi, Delhi; Bhabha Atomic Research Centre, Mumbai; Physical Research Laboratory, Ahmedabad; Institute of Physics, Bhubaneswar; Indian Institute of Science, Bangalore; Tata Institute of Fundamental Research, Mumbaietc. Contributed most number of articles. One fourth of the total articles published in Pramana are from outside India, the host country of the journal and the number of articles submitting from other countries is also increasing. Cosmology; super symmetry; chaos; quantum chromo dynamics; phase transition; and quark-gluon plasma are the leading micro-fields of physics to which maximum number of articles published in Pramana. The average number of references per article is found as 21.85 and it is 104.4 when the average is taken only for review articles.

4. OBJECTIVES

The objectives of this study are as follows:

- ✓ To find out the year wise publication of research output.
- ✓ To analysis the growth rate of the publication using CARG, RGR and Doubling Time.
- ✓ To find out the ranking of authors and Institutions.
- ✓ To examine the contribution of different countries.
- ✓ To analysis the document type of the journal.
- ✓ To identity keyword frequency of the journal.

5. ANALYSIS

The bibliographic records for the analysis are limited to 10681 articles published in ANNALS OF ONCOLOGY (AOO)in the period of 2010-2014. The required bibliographic data have been captured from Web of Science database and analyzed by using Histcite software application. For each articles we identified like year wise publication, growth pattern, word frequency, ranking of authors, ranking of institution, the country of publication and document types.

5. YEAR WISE PUBLICATIONS

Table 1. Year wise distribution of articles

S No	Year	No. of Articles	%	Cumulative	Cumulative %
1.	2010	2931	27.4	2931	27.4
2.	2011	1673	15.7	4604	43.1
3.	2012	3586	33.6	8190	76.7
4.	2013	1048	9.8	9238	86.5
5.	2014	1443	13.5	10681	100
	Total	10681	100	35644	

Table 1 shows the number of papers published in AOO during 2010-2014. Table also shows thatmaximum papers (86.5%) were published in 2014, and minimum papers (27.4%) were published in 2010.

6. COMPOUND ANNUAL GROWTH RATE – CARG

The Compound Annual Growth Rate (CAGR) (http://www.investopedia.com/terms/c/cagr.asp (11-01-2016)) is the mean annual growth rate of an investment over a specified period of time longer than one year.

This can be written as follows:

$$CAGR = \left(\frac{\text{Ending Value}}{\text{Beginning Value}}\right)^{\left(\frac{1}{\#\text{ of years}}\right)} - 1$$

Year	2010	2011	2012	2013	2014	5 Year CARG
Records	2931	1673	3586	1048	1443	-0.13213914

CARG = (1443/2931) ^ (1/5))-1 = -13.21%

Therefore the compound annual growth rate for the period of 5 years is -13.21%

7. RELATIVE GROWTH RATE (RGR) AND DOUBLING TIME

The Relative Growth Rate (RGR) is the increase in 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, 1978), which in turn, had its origin from the study of the rate of interest in the financial investment (Blackman, 1919).The mean Relative Growth Rate(R) over the specific period of interval can be calculated from the following equation:

$$RGR = (\ln W_2 - \ln W_1)/(t_2 - t_1)$$

Where:

$\ln$ = natural logarithm

t_1 = time one (in days)

t_2 = time two (in days)

W_1 = Dry weight of plant at time one (in grams)

W_2 = Dry weight of plant at time two (in grams)

Table 2. Relative growth rate and doubling time of the journal.

S. No.	Year	No. of Records	%	Cumulative	W1	W2	RGR	Doubling Time
1.	2010	2931	27.4	2931	….	7.98	….	…
2.	2011	1673	15.7	4604	7.98	8.43	0.45	1.54
3.	2012	3586	33.6	8190	8.43	9.01	0.58	1.19
4.	2013	1048	9.8	9238	9.01	9.13	0.12	5.775
5.	2014	1443	13.5	10681	9.13	9.27	0.14	4.95
	Total	10681	100	35644				

Table 2 discussed the relative growth rate of the articles during the year between 2010 and 2014. The overall study period has witnessed RGR is decreasing every year, whereas doubling time is increasing every year. The RGR is highest in the year 2012 with 0.58 and lowest in the year 2014 with 0.14. The doubling time is highest in the year 2013 with 5.77 and lowest in the year 2012 with 1.19.

8. RANKING OF AUTHORS

Table 3. Ranking of authors by number of papers published in AOO during 2010-2014(Top 15)

S.No.	Author	Records	TLCS	TGCS
1.	Van Cutsem E	80	16	708
2.	Nakagawa K	74	3	50
3.	Yamamoto N	74	3	70
4.	Tabernero J	64	19	658
5.	La Vecchia C	55	56	1451
6.	Falcone A	54	6	238
7.	Hatake K	53	2	21
8.	Blay JY	48	42	821
9.	Tamura K	48	0	49
10.	Massard C	46	13	329
11.	Soria JC	46	23	374
12.	Takahashi S	46	5	138
13.	Fujiwara Y	45	0	61
14.	Yamada Y	45	0	53
15.	Fizazi K	44	27	554

Table 3 discussed the authors who have published a large number of papers and the table shows only the top 15.

It is clearly seen from the table that Van Cutsem E has published the maximum number of articles with 80 records, having the global citation score of 708 and local citation score of 16, followed by Nakagawa K and Yamamoto N with 74 records, having global citation scores of 50 and 70, local citation scores of 3 respectively. It is also noted that La Vecchia C with 55 records has a global citation score of 1451.

9. RANKING OF INSTITUTIONS

Table 4. Ranked list of organizations which contributed more than 100 articles.

S. No.	Institution	Recs	TLCS	TGCS
1.	InstitutGustave Roussy	333	124	2644
2.	University of Texas:	240	53	1752
3.	National Cancer Centre	213	20	802
4.	Unknown	208	4	35
5.	Memorial Sloan Kettering Cancer Center	174	55	1725
6.	Dana-Farber Cancer Institute	141	22	948
7.	Kinki University	134	11	306

8.	European Institute of Oncology	123	94	2365
9.	Royal Marsden Hospital	122	43	962
10.	University of Milan	120	81	2215
11.	Centre Leon Berard	117	50	894
12.	University Hospitals	117	53	929
13.	NCI	115	18	550
14.	Harvard University	109	79	2424
15.	Medical University of Vienna	103	37	1564

Table 4 shows highly prolific organizations contributing more than 100 articles.From Table 4, it is evident that Institute Gustave Roussy (World's leading cancer-research institutes) contributed highest articles 333 with global citation score of 2644 and local citation score of 124, followed byThe University of Texas: MD Anderson Cancer Center (The original three comprehensive cancer centersin the United States) with 240 articles, having a global citation score of 1752 and local citation score 53 and 213 articles by National Cancer Centre and so on.

10. DOCUMENT TYPES

Table 5. Number of references in different types of articles published in AOO during 2010-2014.

S. No.	Document Type	Recs	%	TLCS	TGCS
1.	Meeting Abstract	8022	75.11	60	1339
2.	Article	1983	18.57	1047	35229
3.	Letter	267	2.5	41	845
4.	Review	209	1.957	121	4691
5.	Editorial Material	145	1.358	33	334
6.	Correction	37	0.346	1	2
7.	Article; Proceedings Paper	18	0.169	7	207
	Total	10681	100	1310	42647

The data presented in Table 5 which gives the types of articles wise distribution of publication and their citation information. It is clearly noticed from the table that the major source of records published in the form of meeting abstracts (75.11%), followed by articles and letter with 1983 (18.57%) and 267 (2.5%) having global citation scores of 35229 and 845, local citation scores of 1047 and 41 respectively.

11. WORD FREQUENCY

Table 6 is the abbreviatedlist of keywords with their number of occurrence in the records analyzed. It is clearly seen from the table that the word "Cancer" has been used in 5590 records by the researchers with global citation score of 25902 and local citation score of 812, followed by the word "Patients" in 3583 records with a global citation score of 13165 and local citation score of 402.

Table 6. Frequently followed 15 keywords connected with the articles published in AOO during 2010-2014.

S. No.	Word	Records	TLCS	TGCS
1.	Cancer	5590	812	25902
2.	Patients	3583	402	13165
3.	Cell	1675	245	7842
4.	Breast	1486	306	8815
5.	Phase	1400	214	7911
6.	Treatment	1369	242	8697
7.	Advanced	1347	140	5475
8.	Chemotherapy	1340	214	5232
9.	Metastatic	1152	146	5879
10.	Colorectal	1062	73	2623
11.	Clinical	921	199	7085
12.	Lung	898	125	4215
13.	Analysis	819	135	3823
14.	Non	799	106	3780
15.	Carcinoma	789	74	3129

12. CONTRIBUTION OF COUNTRIES

Table 7. Countries in the affiliations of the authors of the articles published in AOO during 2010-2014.

S.No.	Country	Records	TLCS	TGCS
1.	USA	2039	440	14404
2.	Japan	1753	55	1857
3.	Italy	1507	448	12547
4.	France	1205	337	9917
5.	UK	1112	282	8743
6.	Germany	974	319	9173
7.	Spain	843	210	6039
8.	Netherlands	576	166	5648
9.	Belgium	553	160	5412
10.	Switzerland	517	257	6461

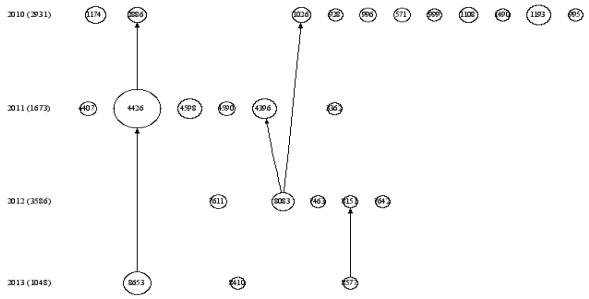
11.	Canada	442	138	3855
12.	Peoples R China	430	51	2143
13.	South Korea	413	47	1306
14.	Australia	291	126	3737
15.	Austria	232	68	2183

Table 7 explain the countries in the affiliations of the authors of the articles published in AOO during 2010-2014. It is clearly observed that all countries multiple participation and the table shows only the top 15.

Hence, It is observed that 'USA' contributed 2039 articlesto the total contributions, followed by 1753 articles by Japan, 1507 articles by Italy and so on.

13. HISTOGRAPH

With using the HistCite Graph Maker,attempt to create "historiographs" of the articles in the collection (10681). A historiograph is a chronological citation network display citation links between articles.



S. No	Paper No		LCS	GCS
1.	571	Arbyn M, 2010, ANN ONCOL, V21, P448	0	122
2.	928	Okines A, 2010, ANN ONCOL, V21, Pv50	2	108
3.	995	Casali PG, 2010, ANN ONCOL, V21, Pv98	6	102
4.	996	Crino L, 2010, ANN ONCOL, V21, Pv103	2	121
5.	999	D'Addario G, 2010, ANN ONCOL, V21, Pv116	0	112
6.	1026	Roila F, 2010, ANN ONCOL, V21, Pv232	8	165
7.	1108	La Vecchia C, 2010, ANN ONCOL, V21, P1323	0	153
8.	1174	O'Day SJ, 2010, ANN ONCOL, V21, P1712	0	177
9.	1193	Reck M, 2010, ANN ONCOL, V21, P1804	13	257
10.	1490	Savagner P, 2010, ANN ONCOL, V21, P89	0	102
11.	2886	Eidtmann H, 2010, ANN ONCOL, V21, P2188	8	147
12.	3362	Posner MR, 2011, ANN ONCOL, V22, P1071	6	117
13.	4396	Bokemeyer C, 2011, ANN ONCOL, V22, P1535	6	240
14.	4407	Witzig TE, 2011, ANN ONCOL, V22, P1622	5	118
15.	4426	Goldhirsch A, 2011, ANN ONCOL, V22, P1736	35	902
16.	4590	Sequist LV, 2011, ANN ONCOL, V22, P2616	2	142
17.	4598	Arbyn M, 2011, ANN ONCOL, V22, P2675	2	266
18.	7463	Cardoso F, 2012, ANN ONCOL, V23, P11	1	96
19.	7611	Peters S, 2012, ANN ONCOL, V23, P56	3	139
20.	7642	Escudier B, 2012, ANN ONCOL, V23, P65	2	110
21.	8083	Schmoll HJ, 2012, ANN ONCOL, V23, P2479	9	223
22.	8151	Mezynski J, 2012, ANN ONCOL, V23, P2943	3	99
23.	8410	Malvezzi M, 2013, ANN ONCOL, V24, P792	7	116
24.	8577	Loriot Y, 2013, ANN ONCOL, V24, P1807	1	104
25.	8653	Goldhirsch A, 2013, ANN ONCOL, V24, P2206	7	341

The above figure illustrates 25 most highly cited papers in the articles published in AOO during 2010-2014 based on their GCS. In this Histography, it is clearly noticed that paper number 4426, Goldhirsch A (2011) has scored the highest global citation scores of 902, followed by paper number 8653, Goldhirsch A (2013) with the global citation scores of 341.

14. CONCLUSION

The investigations exhibited in this study have allowed many conclusions of broad observation on

quantitative research and specifically to ANNUAL OF ONCOLOGY.

- ✓ The study reveals that 10681articles were published in the ANNALS OF ONCOLOGYduring 2010-2014.The highest numbers of articles (86.5 %) were published in 2014, and minimum articles (27.4%) were published in 2010.
- ✓ It has been found that growth trend of (RGR) is highest in the year 2012 with 0.58 and lowest in the year 2014 with 0.14. The doubling time is highest in the year 2013 with 5.77 and lowest in the year 2012 with 1.19.Although this study has identified a decreasing trend of Compound Annual Growth Rate (CAGR), the editorial board should pay much attention to increase it further.
- ✓ The findings of the Authors "Van Cutsem E has published the maximum number of articles with 80 records, having the global citation score of 708 and local citation score of 16, followed by Nakagawa K and Yamamoto N with 74 records, having global citation scores of 50 and 70, local citation scores of 3 respectively.
- ✓ The study shows that the major source of records published in the form of meeting abstracts (75.11%), followed by articles and letter with 1983 (18.57%) and 267 (2.5%) having global citation scores of 35229 and 845, local citation scores of 1047 and 41 respectively.
- ✓ In the frequency of keyword used, the word "Cancer" has been used in 5590 records by the researchers with global citation score of 25902 and local citation score of 812.

The study reveals that 'USA' contributed 2039 articlesto the total contributions, followed by 1753 articles by Japan, 1507 articles by Italy.

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AUTHORSHIP PATTERNS AND COLLABORATIVE RESEARCH OF ONCOLOGY RESEARCH OUTPUT IN INDIA: A SCIENTOMETRICS STUDY

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ABSTRACT

The paper analysis authorship patterns and collaborative research of oncology research in Indiaas reflected by the research papers listed in Web of Science database for a period of 11 years from 2005-2015. The increased trend towards multiple authorship is predominant as compare to single authorship in case of oncology in India.In the study, the degree of collaboration was not a constant value, it reveals varies of 0.03 to 0.16 percent and the mean quality as 0.09. The analysis found that single author papers maintained a low profile among oncology research scientists and the multi authorship pattern is expanding slowly in Indian oncology research.

Keywords: Authorship pattern, Degree of collaboration, Time Series Analysis, Oncology, Scientometrics.

1. INTRODUCTION

One of the preeminent fundamental requirements for advancement in medical and scholarly vocations is the authorship of scientific papers.Collaborative authorship has been a trademark highlight of the present day medical science and there has been a predictable pattern towards expanded collaboration in all the branches of sciences. Multiple authorship and cooperation are among the most critical necessities of exploratory and innovative work today.

A percentage of the writings are evaluated by the authors before conducting the present study.By observing the authorship patterns to be measurably essentially connected with publication in high ranking journals. Consideration of professors, research scholars, and scientists as authors, specifically, were all emphatically connected with publication in high ranking journals.SudhierPillai,K G (2007) have describedThe study revealed that team research is predominant in journal articles while solo research is the trend in the case of books Elango and Rajendran (2012)briefed information that multi authored contributions are dominated in the field of marine sciences. Average collaboration rate (0.57) is better collaboration and mean number of authors per joint authored paper is 3.4. Andras Pinter (2013) examined the authorship patterns in the articles published in the Journal of Pediatric Surgery (JPS) and found that thepercentage of papers with less than 3 authors significantly declined, whereas those with 4 to 5 authors did not change. Manuscripts with more than 6 authors significantly increased. Pallab Pradhan *et al.* (2013) studied the authorship Pattern and Degree of Collaboration in Indian Chemistry Literature. The study found that the researchers in chemistry are keen towards team research or group research rather than solo research.

Goyal *et al.* (2013) have analyzed the tendency in the direction of collaborative research is seen steady during 2002-2011 in the field of Chemical Sciences. Outcome of study clearly show that authorship trend is moving on the way to multiple authorship and degree of collaboration is found to be high.

Michael *et al*. (2014) examined the enrichment of co-authorship patterns with author scientific profiles helps uncover associations between author team characteristics and appearance in high-impact journals. Navaneethakrishnan (2014) the analysis revealed that the majority of the publications are contributed by multiple authors. Degree of collaboration was progressively increased over the study span. Neena Kapoor *et al*. (2014) has described the study demonstrates that the average number of authors per publication dramatically increased from 1980 to 2013 in major radiology journals. Chandran and Natarajan (2015) have briefed the highest number of contributions were multi authored papers. It is found that that the degree of collaboration ranges from 0.36 to 0.77 and the average degree of collaboration is 0.59(9). Mehmet Ali Koseoglu (2016) indicated, the authorship pattern of the SMJ shows multi- authored articles dominated solo work, and this domination increased over the past periods; however, the

growth of multi- authored articles is limited to papers with two or three authors. Senthilkumar and Muthukrishnan (2016) the analysis revealed that the more research papers are being contributed under multiple authorship.

Here, the author has made an attempt to study the authorship pattern, collaborative researchand country of the corresponding author on oncology literature published during the period 2005- 2015 and indexed in web of science database.

2. OBJECTIVES

The objectives of the study are brief as follows:

- ✓ To study the year wise distribution of oncology research output in India.
- ✓ To study authorship pattern in oncology literature
- ✓ To study the year wise single and multi-authored Papers
- ✓ To study the degree of collaboration in the field of oncology
- ✓ To study the time series analysisin the field of oncology

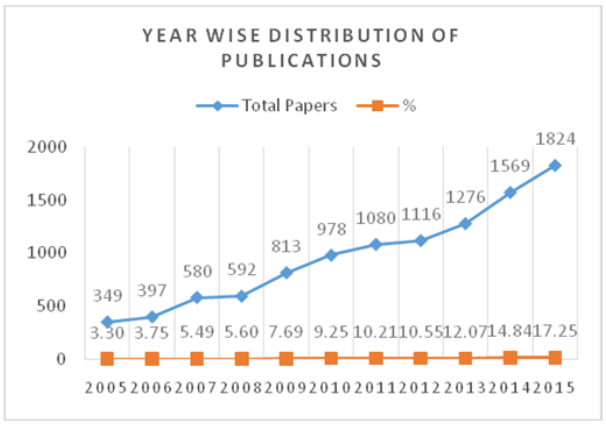
3. DATA ANALYSIS

Data was downloaded on 02nd May 2016 for a period of eleven years (2005-2015) from the Web of Science (WoS) of the Thomson Reuters, USA.The search keyword has 'oncology' has been used for the purpose of collection of data. The size of the sample downloaded for the purpose is 10574. The downloaded records was enriched with different parameters like authors, title, years, research institutions, document type and so on. Further the records analyzed by using Histcite and bibexcel software application.

Table 1. Year Wise Distribution of Publications

S No	Year	Total Papers	%
1.	2005	349	3.30
2.	2006	397	3.75
3.	2007	580	5.49
4.	2008	592	5.60
5.	2009	813	7.69
6.	2010	978	9.25
7.	2011	1080	10.21
8.	2012	1116	10.55
9.	2013	1276	12.07
10.	2014	1569	14.84
11.	2015	1824	17.25
	Total	10574	100.00

Graph 1. Year Wise Distribution of Publications



Here, an effort was made to analyzefor the period of eleven years from 2005-2015. Table-1 and Graph-1 present the year-wise distribution of number of publications indexed in WoS database.The average number of article publication was 961.27 articles per year.It has been realized a steady growth of Indian research output in oncology from 2005 onwards.In the study, the commitment of prior five years (2005-2010) was less than the average publications per year.Out of 10574 articles 1824 (17.25%) articles were published in 2015 and 349 (3.30%) articles were in 2005, which are highest and lowermost in eleven years respectively.

4. AUTHORSHIP PATTERNS

The below table 1 reveals that a total of 61207 authors have contributed the 10574 articles and the average number of authors per article observed to be 5.79. Among 10574 articles, 595(5.63%) articles are written by single author and 9979 (94.37%) articles are written by multiple authors.

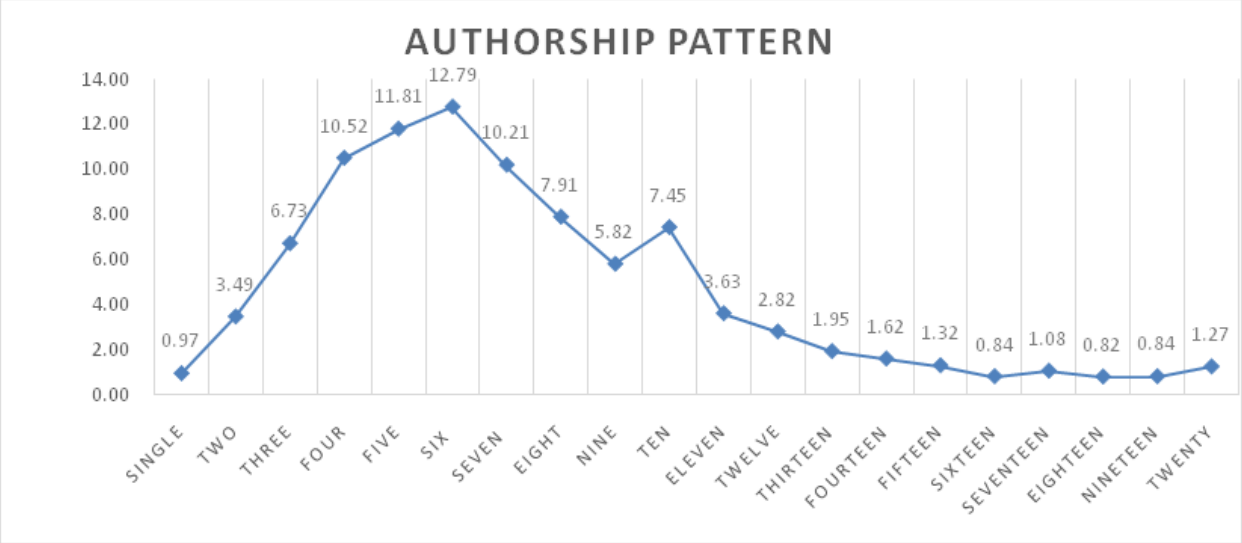
Four authored articles involved highest percentage 1610 (15.23%), after Five authored articles 1446(13.68%) of the aggregate 10574 articles and six to Twelve authored contributions are between 13 to 1 percent. Above Twelve authored contributions are below one percent of articles.

In this manner, showing unmistakably the increased trend towards multiple authorship is predominant as compare to single authorship in case of oncology in India. The above graph demonstrates that the diminishing patterns in the quantity of authors as far as group or team research with respect to more than six authors.

Table 2. Presenting the authorship pattern in oncology research output.

S.No.	No. of Authors	No. of Publications	%	Authorship pattern	%
1.	Single	595	5.63	595	0.97
2.	Two	1067	10.09	2134	3.49
3.	Three	1374	12.99	4122	6.73
4.	Four	1610	15.23	6440	10.52
5.	Five	1446	13.68	7230	11.81
6.	Six	1305	12.34	7830	12.79
7.	Seven	893	8.45	6251	10.21
8.	Eight	605	5.72	4840	7.91
9.	Nine	396	3.75	3564	5.82
10.	Ten	456	4.31	4560	7.45
11.	Eleven	202	1.91	2222	3.63
12.	Twelve	144	1.36	1728	2.82
13.	Thirteen	92	0.87	1196	1.95
14.	Fourteen	71	0.67	994	1.62
15.	Fifteen	54	0.51	810	1.32
16.	Sixteen	32	0.30	512	0.84
17.	Seventeen	39	0.37	663	1.08
18.	Eighteen	28	0.26	504	0.82
19.	Nineteen	27	0.26	513	0.84
20.	Twenty	39	0.37	780	1.27
21.	Twenty+	99	0.94	3719	6.08
	Total	10574	100.00	61207	100

Graph 2. Presenting the authorship pattern in oncology research output



5. DEGREE OF AUTHOR'S COLLABORATION

The degree of collaboration varies from one discipline to another. It is generally high in the intensely collaborative scientific and technical fields, but low in the humanities. The formula given by K Subramanyam (Subramanyam, 1983) is useful for determining the degree of collaboration in quantitative terms. 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 3. Degree of Collaboration

S.No.	Year	Single Author	%	Multi Authors	%	DC
1.	2005-2015	595	5.63	9979	94.37	0.94

Here, Nm = 9979

Ns = 595

$$C = \frac{9979}{9979 + 595}$$

$= 0.94$, Thus, the degree of collaboration (C) is 0.94

The analysis of Table -3 shows that the degree of collaboration during the period of 11 years (2005-2015) is 0.94. The single authored articles are covered only 595 (5.63%) during the years. The multi authored articles 9979 (94.37%) are maximumthroughout 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.

The Table4 speaks to the year wise number of multiauthored articles and their degree ofcollaboration. In the study, the degree of collaboration was not a constant value, it reveals varies of 0.03 to 0.16percent and the mean quality as 0.09.The analysis found that single author papers maintained a low profile among oncology research scientists and the multi authorship pattern is expanding slowly in Indian oncology research.

Table 4. Year Wise Degree of Collaboration

S.No.	Year	No of Articles	No. of Authors	SAP*	%	MAP*	%	DC
1	2005	349	1179	20	0.19	329	3.11	0.03
2	2006	397	1473	28	0.26	369	3.49	0.03
3	2007	580	2004	32	0.30	548	5.18	0.05
4	2008	592	1925	49	0.46	543	5.14	0.05
5	2009	813	2515	37	0.35	776	7.34	0.07
6	2010	978	3245	50	0.47	928	8.78	0.09
7	2011	1080	3578	84	0.79	996	9.42	0.09
8	2012	1116	4112	69	0.65	1047	9.90	0.10
9	2013	1276	4471	45	0.43	1231	11.64	0.12
10	2014	1569	5752	96	0.91	1473	13.93	0.14
11	2015	1824	6349	85	0.80	1739	16.45	0.16
	Total	10574	36603	595	5.63	9979	94.37	0.09 Mean

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

6. TIME SERIES ANALYSIS

Table 5. Time Series Analysis

S.No.	Year	SAP(Y)	X	X^2	XY	MAP (Y)	XY	CP(Y)	XY
1.	2005	20	-5	25	-100	329	-1645	349	-1745
2.	2006	28	-4	16	-112	369	-1476	397	-1588
3.	2007	32	-3	9	-96	548	-1644	580	-1740
4.	2008	49	-2	4	-98	543	-1086	592	-1184
5.	2009	37	-1	1	-37	776	-776	813	-813
6.	2010	50	0	0	0	928	0	978	**0**
7.	2011	84	1	1	84	996	996	1080	1080
8.	2012	69	2	4	138	1047	2094	1116	2232
9.	2013	45	3	9	135	1231	3693	1276	3828

10.	2014	96	4	16	384	1473	5892	1569	6276
11.	2015	85	5	25	425	1739	8695	1824	9120
	Total	595	0	110	723	9979	14743	10574	15466

*SAP = Single Authored Paper, *MAP = Multi Authored Papers, *CP = Collaborative Papers (Y)

7. SINGLE AUTHORED PUBLICATIONS: TIME SERIES ANALYSIS

Straight line equation is applied to arrive at projections for future growth under Time Series analysis. Straight Line equation Yc = a+bX since Σx = 0, a=ΣY/N, ΣY = (Total Number of Paper by Single Author), N = (Number of Years), a = 595/11, a = 54.09, b=ΣXY/Σ, ΣXY = (Total of XY Tables), Σ = (Total of X2 Table), b=723/110, b=6.57.

Estimated literature in 2020 is, When X = 2020-2010(Mid-Year), X = 10,

Apply Straight line equation, Yc = a+bX since Σx = 0, Yc = 54.09+6.57*10, Y

c = 54.09+65.7,

Yc = 119.79

8. MULTI AUTHORED PUBLICATIONS: TIME SERIES ANALYSIS

Straight Line equation Yc = a+bX since Σx = 0, a=ΣY/N, ΣY = (Total Number of Paper by Multi Author), N = (Number of Years), a = 9979/11, a = 907.18, b=ΣXY/Σ, ΣXY = (Total of XY Tables), Σ = (Total of X2 Table), b = 14743/110, b = 134.02.

Estimated literature in 2020 is, When X = 2020-2010(Mid-Year) X = 10,

Apply Straight line equation, Yc = a+bX since Σx = 0, Yc = 907.18+134.02*10, Yc = 907.18+1340.2,

Yc = 2247.38

9. COLLABORATIVE PUBLICATIONS: TIME SERIES ANALYSIS

Straight Line equation Yc = a+bX since Σx = 0, a=ΣY/N, ΣY = (Total Number of Paper by Multi Author), N = (Number of Years), a = 10574/11, a = 961.27, b=ΣXY/Σ, ΣXY = (Total of XY Tables), Σ = (Total of X2 Table), b = 15466/110, b = 140.6.

Estimated literature in 2020 is, When X = 2020-2010(Mid-Year) X = 10,

Apply Straight line equation, Yc = a+bX since Σx = 0, Yc = 961.27+140.6*10, Yc = 961.27+1406,

Yc = 2367.27

On the utilization of the formula of Time Series Analysis for the expectation of oncology research output in India for the year 2020, it was found that the future trend and development in oncology research literature output may take an expanding trend in single authored publications (Yc = 93.51)during the years to come and collaborative publications trends also increasing gradually (Yc = 2367.27).

10. CONCLUSION

In this study directs an authorship patterns towards collaborative research is seen reliable during 2005-2015 in the field of oncologyresearch output in India.The study exposes the following conclusions.

- ✓ The increased trend towards multiple authorship is predominant as compare to single authorship in case of oncology in India.In the study, the degree of collaboration was not a constant value, it reveals varies of 0.03 to 0.16 percent and the mean quality as 0.09.
- ✓ The analysis found that single author papers maintained a low profile among oncology research scientists and the multi authorship pattern is expanding slowly in Indian oncology research.

Time Series Analysis for the expectation of oncology research output in India for the year 2020, it was found that the future trend and development in oncology research literature output may take a positive growth trend.

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