Kong. Res. J. 6(2): 1-3, 2019
Publisher: Kongunadu Arts and Science College, Coimbatore.

DOI:10.26524/KRJ293

RESEARCH ARTICLE

ON DOMINATOR CHROMATIC NUMBER OF RADIAL GRAPH OF SOME GRAPHS

Kalaivani, R*. and D. Vijayalakshmi
Department of Mathematics, Kongunadu Arts and Science College, Coimbatore- 641 029, Tamil Nadu, India.

ABSTRACT

A dominator coloring is a coloring of the vertices of a graph such that every vertex is either alone in its color class or adjacent to all vertices of at least one other class. In this paper, we obtain the Dominator Chromatic number of the Radial graph for the Central graph of Star graph, Super-radial graph for Middle graph of Cycle and Central graph of Path.

Keywords: Domination, Dominator Coloring, Radial Graph, Distance, Radius and Diameter.

1. INTRODUCTION

All graphs considered here are finite, undirected, simple graphs. For graph theoretic terminology refer to D. B. West [1]. Let G be a graph, with vertex set

V (G) and edge set E (G).

In a graph G, the distance d (u, v) between a pair of vertices u and v is the length of a shortest path joining them.

A proper coloring of a graph G is a function from the set of vertices of a graph to a set of colors such that any two adjacent vertices have different colors. A subset of vertices colored with the same color is called a color class. Graph coloring and domination are two major areas in graph theory. A set is a dominating set if every vertex of V(G) \ D has a neighbor in D. An excellent detail of domination is given in the book by Haynes et .al, [2].

A dominator coloring of a graph G is a proper coloring of graph such that every vertex of V dominates all vertices of at least one color class (possibly its own class). i.e., it is coloring of the vertices of a graph such that every vertex is either alone in its color class or adjacent to all vertices of at least one other class. Dominator chromatic number is the minimum number of color classes in a dominator coloring of G, and this concept was introduced by Ralucca Michelle Gera in 2006 [3]. The dominator coloring was studied in [4]. The dominator coloring of Trees, Bipartite graph, Central, Middle graph of Path and Cycle graph were also studied in various papers [5 -10].

The Middle graph of G, denoted by M(G) is defined as follows.

The vertex set of M(G) is . Two vertices x, y in the vertex set of M(G)

*Correspondence: Kalaivani, R., Department of Mathematics, Kongunadu Arts and S
fo Nadu, India. E.mail: kalaivanirm@yahoo.com

i. x, y are in E(G) and x, y are adjacent in G.
ii. x is in V(G), y is in E(G), and x, y are incident in G.

The Central graph [11] C(G) of a graph G denoted by C(G) is formed by adding a new vertex on each edge of G and joining each pair of vertices of the original graph which were previously non-adjacent.

2. DOMINATOR CHROMATIC NUMBER OF RADIAL GRAPHS

Theorem 2.1. [6] Let Pn be any path on n ≥ 5 vertices, then

$$R(P_n) = \begin{cases} (n/2)K_2 \ \textit{if n is even} \\ P_3 \cup ((n-3)/2)K_3 \ \textit{if n is odd} \end{cases}$$

Theorem 2.2. For any n ≥ 2, the dominator chromatic number of radial graph of C(K1,n) = n + 1 i.e.,

$$\chi_d R(C(K_{1,n})) = n+1$$

Proof.

By the definition of the Central graph, the edge vvi, 1 ≤ i ≤ n be subdivided by the vertices u'j, in C(K1,n). Clearly

$$V(C(K_{1,n})) = \{v\} \cup \{v_i : 1 \le i \le n\} \cup \{u_i : 1 \le i \le n\}.$$

Now we define a coloring

$$c : V(R(C(K_{1,n}))) \to \{1,2,3,....n+1\}$$

for

$$c_i = \begin{cases} u_i \ \ for\ 1 \le i \le n \\ v_i \ \ for\ 1 \le i \le n \end{cases}$$

It is not hard to see that above assignment is a proper coloring and also a dominator coloring of the graph R(C(K1,n)). Every vertex in dominates any one color class of ci and the root vertex v dominate itself. Therefore

To Prove

Let us assume that

$$\chi_d R(C(K_{1,n})) < n+1$$

. i.e., $\chi_d R(C(K_{1,n})) = n$.

We assign n colors $\{v_i, u_i : 1 \le i \le n\}$ for proper dominator coloring. Assign n colors for ui because ui forms a clique. If we assign any one the colors from the vertices vi to v then vertices vvi are bicolored, so we have to assign the color n + 1 to the root vertex v. Therefore dominator coloring with n colors is not possible. Hence, $\chi_d R(C(K_{1,n})) \ge n+1$. An easy check shows that $\chi_d R(C(K_{1,n})) = n+1$.

Theorem 2.3. For any n ≥ 6, the dominator chromatic number of radial graph of Path is,

$$R(P_n) = \begin{cases} \lceil n/2 \rceil + 1, \ if\ \ n\ is\ even \\ \lceil n/2 \rceil, \ if\ \ n\ is\ odd \end{cases}$$

Proof.

Let G = R(Pn) and its dominator chromatic number be $\chi_d(G)$. Let D be the minimal dominating set of the given graph G and $V(R(P_n) = \{v_i : 1 \le i \le n\}$. The dominator coloring of G is given in the following cases.

Case 1: If n is even

Now we define a coloring $c : V(R(C(P_n))) \to \{1,2,3,....\lceil n/2 \rceil + 1\}$ for

$$c_i = \begin{cases} v_i \ \ for\ 1 \le i \le n/2 \\ \lfloor n/2 \rfloor + 1\ otherwise \end{cases}$$

It is easy to see that above assignment is a dominator coloring of $R(P_n)$ as the set $D = v_i,\ 2 \le i \le \lceil n/2 \rceil$ dominates itself and the remaining vertices of $V - D$ are dominates atleast any one of the color classes in D.

Case 2: If n is odd.

Now we de ne a coloring $c : V(R(C(P_n))) \to \{1,2,3,....\lceil n/2 \rceil\}$ for

$$c_i = \begin{cases} v_{i-1} \ \ for\ 2 \le i \le \lceil n/2 \rceil \\ \lfloor n/2 \rfloor otherwise \end{cases}$$

Above assignment is a dominator coloring of G as the set $D = v_i,\ 2 \le i \le \lceil n/2 \rceil$ dominate itself and the remaining vertices of $V - D$ dominate any color class in D. This completes the proof of the theorem.

Theorem 2.4. For every $n \ge 6$ the dominator chromatic number of super radial graph of $C(P_n) = n$. ie., $\chi_d R^*(C(P_n)) = n$

Proof. Let $G = R^*(C(P_n))$ and its dominator chromatic number be $\chi_d(G)$. Let D be the minimal dominating set of given graph G, and $V(R^*(C(P_n))) = \{v_i, u_i : 1 \le i \le n\}$ be the vertices of radial graph of central graph of path.

$$c_i = \begin{cases} v_i \ \ for\ 1 \le i \le n \\ u_i \ \ for\ 1 \le i \le n \end{cases}$$

Next we define a coloring $c : V(R(C(P_n))) \to \{1,2,3,....n\}$ for

It is not hard to see that above coloring is proper coloring. Next we have to show that it is a dominator coloring of given graph G. A procedure to obtain a dominator coloring as follows. Here $u_i : 1 \le i \le n$ forms a clique, so that we must assign n colors to u_i. Every vertex in G dominates at least any one color classes of v_i, u_i. Therefore this proper coloring gives rise to a dominator coloring for the respective graphs. Hence $\chi_d R^*(C(P_n)) = n$.

REFERENCES

1. D.B. West, (2001). Introduction to Graph Theory, 2nd ed., Prentice Hall, USA,
2. Haynes, T.W., S.T. Hedetniemi and P.J. Slater, (1998). Fundamentals of Domination in Graphs, Marcel Dekker., New york,
3. Gera, R.M. (2007). On dominator coloring in graphs, Graph Theory Notes N.Y. LII, 947-952.

4. Kavitha, K. and N.G. David, (2012). Dominator Coloring of Central Graphs. *Int. J. Comput. Appl.* **51**(12): 11-14.
5. Kavitha, K. and N.G. David, (2012). Dominator Coloring on Star and Double Star Graph Families. *Int. J. Comput. Appl.* **48**(3): 22-25.
6. Kathiresan, K.M. and G. Marimuthu, (2010). Study on Radial Graphs, Ars Combinatoria, XCVI,
7. Kathiresan, K.M. and G. Marimuthu, (2014). Characterization of Super-Radial Graphs. *Discuss. Math.* 829-848.
8. Manjula, T. and Rajeswari, (2015). Dominator Coloring of Prism Graphs. *Appl. math. sci.* **9**: 1889-1894.
9. Chellali, M. (2012). On The Dominator Coloring in Trees. *Discuss. Math.* **32**: 677-683.
10. Vernold Vivin, J. (2007). Harmonious Coloring of Total Graphs, n- leaf, Central Graphs and Circumdetic Graphs, Ph.D Dissertation, Bharathiar University, India,
11. Gera, R., S. Horton and C. Rasmussen, (2006). Dominator Colorings and Safe Clique Partitions. *Congr. Numer.* 19-32.

DOI:10.26524/KRJ294

Kong. Res. J. 6(2): 4-7, 2019
Publisher: Kongunadu Arts and Science College, Coimbatore.

RESEARCH ARTICLE

BIO-ASSISTED SYNTHESIS OF POTASSIUM DOPED FERRIC SULPHIDE NANOPARTICLES FOR AGRICULTURAL APPLICATIONS

Balaganesh, A.S[1]., N Pavithra[1], R. RanjitKumar[2], K.P.B. Dinesh[3] and B. Chandar Shekar[1,*]
[1]Department of Physics, Kongunadu Arts and Science College, G.N. Mills, Coimbatore. Tamil Nadu, India.
[2]Department of Biotechnology, Kongunadu Arts and Science College, G.N. Mills, Coimbatore. Tamil Nadu, India.
[3]Applied Biology, Higher College of Technology, Muscat, Oman.

ABSTRACT

A simple and cost effective green synthesis (Bio-Assisted) method was used to prepare Potassium doped ferric sulphide nanoparticles. The FTIR spectrum of potassium doped ferric sulphide shows characteristic peak at 617.2 cm^{-1} indicating M-O bond and shows the presence of K and Fe. The XRD analysis revealed the crystalline nature of the NPs. The average crystallite size was found to be 7.02 nm. The observed FESEM images showed the agglomeration of nanoparticles and are sponge like structure. The study revealed that potassium doped ferric sulphide nanoparticles could be used for high yield in agriculture.

Keywords: Green synthesis, Ferric sulphide, Morphology.

1. INTRODUCTION

There is a tremendous research interest in the area of nanotechnology to develop reliable processes for the synthesis of nanomaterials over a range of sizes and chemical composition. Although the conventional methods of synthesis of metal sols, known since the times of Michael Faraday, continue to be used for generating metal nanoparticles, there have been several improvements and modifications in the methods which provide a better control over the size, shape, and other characteristics of the nanoparticles. Nanoparticles (NPs) having one of the dimension in the range of 1–100 nm act as a bridge between bulk materials and atomic or molecular structures [1]. They possess remarkable and interesting properties owing to their small sizes, large surface area with free dangling bonds and higher reactivity over their bulk cousins. Since the nineteenth century scientists have been well aware of the ability of biological entities to reduce metal precursors but the mechanisms are still unexplored.

Synthesis and assembly strategies of nanoparticles mostly accommodate precursors from liquid, solid or gas phase; employ chemical or physical deposition approaches; and similarly rely on either chemical reactivity or physical compaction to integrate the nanostructure building blocks within the final material structure. The variety of techniques can be classified in to top-down and bottom up approaches. These techniques are further classified into three categories namely physical methods, chemical methods and bio-assisted methods. Physical methods are like inert gas condensation, physical vapour deposition, laser pyrolysis, flame spray pyrolysis, electro spraying techniques, melt mixing. Chemical methods are like co-precipitation synthesis, micro emulsion technique, hydrothermal synthesis, polyol synthesis, bio-assisted methods

Bio-Assisted method for the synthesis of nanoparticles:

The NPs synthesized following physico-chemical methods are expensive with many problems including use of toxic solvents, generation of hazardous by-products and the imperfection of the surface structure. Chemical methods are generally composed by more than one chemical species or molecules that could increase the particle reactivity and toxicity and might harm human health and the environment due to the composition ambiguity and lack of predictability [2,3].

The particles produced by green synthesis differ from those using physico -chemical approaches. Green synthesis, a bottom up approach, is similar to chemical reduction where an expensive chemical reducing agent is replaced by extract of a natural product such as leaves of trees/crops or fruits for the synthesis of metal or metal oxide NPs. Biological entities possess a huge potential for the production of NPs. Biogenic reduction of metal

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

precursors to corresponding NPs is eco-friendly [4], sustainable [5], free of chemical contamination [6], less expensive and can be used for mass production [7]. Bio-assisted methods, biosynthesis or green synthesis provides an environmentally benign, low-toxic, cost-effective and efficient protocol to synthesize and fabricate nanoparticles. These methods employ biological systems like bacteria, fungi, viruses, yeast, actinomycetes, plant extracts, etc. for the synthesis of metal and metal oxide nanoparticles [8].

Bio-assisted methods can be broadly divided into three categories: i) Biogenic synthesis using microorganisms ii) Biogenic synthesis using bio-molecules as the templates iii) Biogenic synthesis using plant extracts. The progress of efficient green synthesis utilizing natural reducing, capping and stabilizing agents without the use of toxic, expensive chemicals and high energy consumption have attracted researchers towards biological methods [9-11]. Rapid industrialization, urbanization and population explosion are resulting in deterioration of earth is atmosphere and a huge amount of hazardous and unwanted substances are being released. It is now high time to learn about the secrets that are present in the nature and its natural products which lead to advancements in the synthesis processes of NPs. Furthermore, NPs are widely applied to human contact areas and there is a growing need to develop processes for synthesis that do not use harsh toxic chemicals. Therefore, green/biological synthesis of NPs is a possible alternative to chemical and physical methods. The unique properties of the NPs synthesized by biological methods are preferred over nanomaterials produced from physico-chemical methods.

The present work focuses on the synthesis of K doped Ferric sulphide using a plant extract Simarouba glauca for plant growth applications. The characterization of nanoparticles were carried out by using spectroscopy methods like Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction and field emission scanning electron microscopy (FESEM).

2. **Experimental:**

2.1. Synthesis procedure of Potassium doped Ferric sulphide nanoparticles using plant extract:

Potassium doped Ferric sulphide nanoparticles were prepared via green synthesis by adding ferric chloride (99.99 % pure), Na_2S (99.99 % pure) and KCl (99.9 % pure) to the plant extract Simarouba glauca. Initially, Simarouba glauca leaves collected from Coimbatore region were washed and cleaned with double distilled water and then dried with absorbent paper. Then it was cut into small pieces with an ethanol sterilized knife and crushed with mortar and pestle. The crushed leaves 20 g was dispensed in 100 ml of double distilled water and heated for 1 hour at 80 °C. The extract was then filtered using Whatman filter paper. The filtrate was collected in a clean and dried conical flask by standard sterilized filtration method and was stored. The mother solution was prepared by using 5 ml of plant extract which was added to 20 ml of deionized water. Then, the solution was stirred for 15 minutes using magnetic stirrer. Followed by stirring 1 mol % of ferric chloride was mixed with 40 ml of deionized water which was added drop wise to the mother solution and stirred again for 15 minutes. Then, Na_2S (1 mol %) was added drop wise to the mother solution and stirred for 15 minutes. Finally, 0.125 mol % of KCl dissolved with 40 ml of deionized water was added to the mother solution and continuously stirred for 1 h to get homogenous mixture. The obtained solution was centrifuged and dried in a hot air oven for 1 h. The resultant powder was crushed well to get fine particles and was shown in Fig. 1.

Fig. 1. As prepared Potassium doped Ferric sulphide nanoparticles

The prepared potassium doped Ferric sulfide nanoparticles were characterized by X-ray diffraction analysis, field emission scanning electron microscopy and Fourier transform infrared spectrum analysis.

3. RESULTS AND DISCUSSION

The prepared potassium doped ferric sulphide nanoparticles were then subjected to FTIR spectroscopy measurements. It was recorded by using a Thermo Scientific, Nicolet 10 using a KBr pellet technique. The FTIR spectrum of potassium doped ferric sulphide nanoparticle is shown in Fig. 2. The FTIR spectrum of potassium doped ferric sulphide shows the characteristic peaks at 617.2 cm^{-1} due to the M-O bond and shows the presence of K

and Fe. The peak at 1120.64 cm^{-1} shows the presence of amide or amine. The peak at 3130.40 cm^{-1} is due to C-H stretching. The peak observed at 1402.25 cm^{-1} is due to O-H bond. The peak 1631.78 cm^{-1} is due to the unsaturated nitrogen (N-H bend) compounds from the leaf extract [12,13].

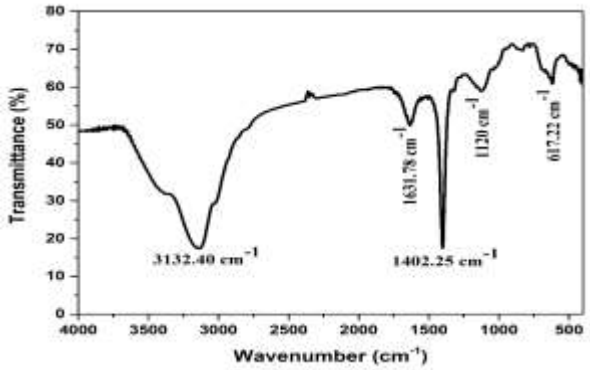


Fig. 2. FTIR spectrum

To understand the structural information of the prepared potassium doped ferric sulphide nanoparticles, the powder X-ray diffraction analysis was done. The powder X-ray diffraction study was carried out in Panalytical X-Pert Pro (Netherlands) X-ray diffractometer using Cu-Kα radiation source (λ=1.5406 Å) operated at 40 kV, and a scan rate of 10°/min.

Figure 3 shows the powder XRD pattern of potassium doped ferric sulphide nanoparticles prepared by using Simarouba glauca leaf extract.

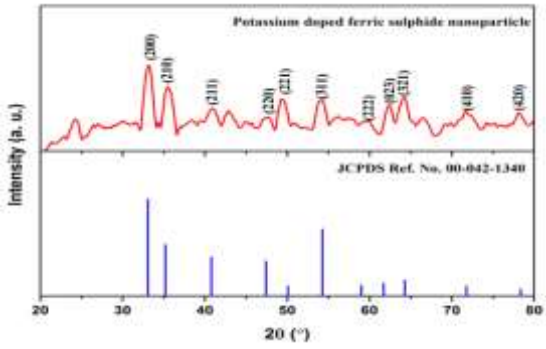


Fig. 3. XRD pattern of potassium doped ferric sulphide nanoparticles

All the diffraction peaks in this pattern were found to be in good agreement with JCPDS card data (Reference code 00-042-1340) which is corresponding to $K:Fe_2S_3$ in cubic geometry. The sample showed the major characteristic peaks for prepared nanoparticles at 2θ values of 33.13°, 35.64° and 49.50° corresponding to (200), (210) and (221) orientation planes, respectively. The average crystallite size of the prepared nanoparticles was found to be 7.01 nm.

The prepared potassium doped ferric sulphide nanoparticles were analysed by field emission scanning electron microscopy to know the morphology of the sample. The morphology of the samples was analyzed through Quanta-200F SEM. Fig. 4 shows the morphology of $K:Fe_2S_3$ nanoparticles.

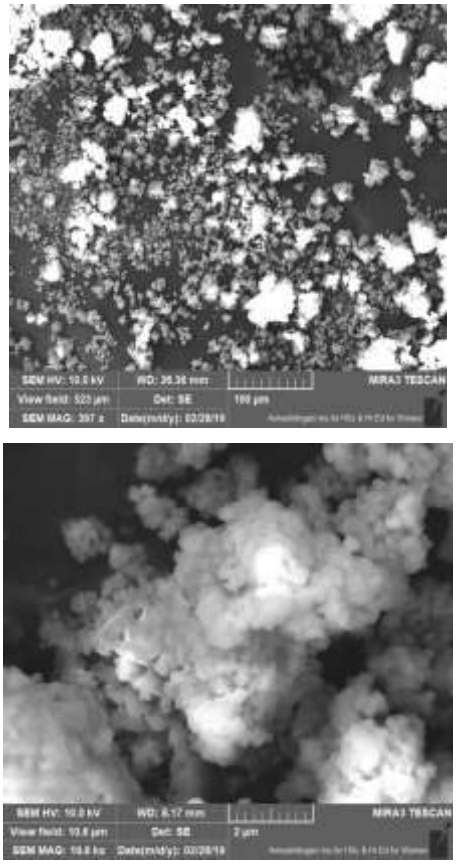


Fig. 4 SEM image a) Magnified at 397 times and b) Magnified at 19600 times

FESEM reveals that the as prepared nanoparticles are agglomerated. The surface seems like almost spongy and sphere shaped. Popcorn like appearance is observed in general.

4. CONCLUSIONS

Potassium doped ferric sulphide nanoparticles were prepared by simple and cost effective Bio assisted co-precipitation (green synthesis) method. The FTIR spectrum of potassium doped ferric sulphide shows the characteristic peaks at 617.2 cm^{-1} which is due to the M-O bond and shows the presence of K and Fe. The peak at 1120.64

cm^{-1} shows the presence of amide or amine. The peak at 3130.40 cm^{-1} is due to the C-H stretching. The peak observed at 1402.25 cm^{-1} due to O-H bond and the peak 1631.78 cm^{-1} is due to the unsaturated Nitrogen (N-H bend) compounds from the leaf extract. The XRD analysis revealed the crystalline nature of the potassium doped ferric sulphide nanoparticles. The structural parameter such as crystalline size was calculated for well resolved XRD peaks. The average crystallite size was found to be 7.02 nm. The observed FESEM images showed the agglomeration of nanoparticles and are sponge like structure. The study revealed that potassium doped ferric sulphide nanoparticles could be used for high yield in agricultural field.

REFERENCES

1. Kaushik N., S.S. Mhatre and R.Y. Parikh, (2010). Biological synthesis of metallic nanoparticles. *Nanomedicine: nanotechnology, biology and medicine* **6:** 257-262.
2. Singh, A., N.B. Singh, I. Hussain, H. Singh and S.C. Singh, (2015). Plant-nanoparticle interaction: an approach to improve agricultural practices and plant productivity. *Int. J. Pharm. Sci. Invent.* **4:** 25-40.
3. Li, X., H. Xu, Z.S. Chen and G. Chen, (2011). Biosynthesis of nanoparticles by microorganisms and their applications. *J. Nanomater.* **2011:** 1-16.
4. Jayaseelan, C., A.A. Rahuman, A.V. Kirthi, S. Marimuthu, T. Santhoshkumar, A. Bagavan, K. Gaurav, L. Karthik and K.B. Rao, (2012). Novel microbial route to synthesize ZnO nanoparticles using *Aeromonas hydrophila* and their activity against pathogenic bacteria and fungi. *Spectrochim. Acta A Mol. Biomol. Spectrosc.* **90:** 78-84.
5. Gopinath, K., V. Karthika, S. Gowri, V. Senthilkumar, S. Kumaresan and A. Arumugam, (2014). Antibacterial activity of ruthenium nanoparticles synthesized using *Gloriosa superba* L. leaf extract. *J. Nanostructure Chem.* **4:** 83.
6. Huang, J., Q. Li, D. Sun, Y. Lu, Y. Su, X. Yang, H. Wang, Y. Wang, W. Shao, N. He and J. Hong, (2007). Biosynthesis of silver and gold nanoparticles by novel sundried *Cinnamomum camphora* leaf. *Nanotechnology* **18:** 105104-105115.
7. Iravani, S. (2011). Green synthesis of metal nanoparticles using plants. *Green Chem.* **13:** 2638-2650.
8. Hasan, S. (2015). A review on nanoparticles: their synthesis and types. *Res. J. Recent Sci.* **4:** 9-11.
9. Mukherjee, P., A. Ahmad, D. Mandal, S. Senapati, S.R. Sainkar, M.I. Khan, R. Parishcha, P.V. Ajaykumar, M. Alam, R. Kumar and M. Sastry, (2001). Fungus-mediated synthesis of silver nanoparticles and their immobilization in the mycelial matrix: a novel biological approach to nanoparticle synthesis. *Nano Lett.* **1:** 515-519.
10. Dhillon, G.S., S.K. Brar, S. Kaur and M. Verma, (2012). Green approach for nanoparticle biosynthesis by fungi: current trends and applications. *Crit. Rev. Biotechnol.* **32:** 49-73.
11. Arumugam, A., C. Karthikeyan, A.S.H. Hameed, K. Gopinath, S. Gowri, and V. Karthika, (2015). Synthesis of cerium oxide nanoparticles using *Gloriosa superba* L. leaf extract and their structural, optical and antibacterial properties.*Mater. Sci. Eng.* **C 49:** 408-415.
12. Paca, A.M. and P.A. Ajibade, (2017). Synthesis and structural studies of iron sulphide nanocomposites prepared from Fe (III) dithiocarbamates single source precursors. *Mater. Chem. Phys.* **202:** 143-150.
13. Lakshmi, H.B., B.J. Madhu, and M. Veerabhadraswamy, (2017). Synthesis and Characterization of Nano-crystaline CaFe2O4 via Solution Combustion Method from Solid Waste Egg Shells as Source of Calcium. *Int. J. Adv. Res. Technol.* **3:** 2454-6135.

DOI:10.26524/KRJ295

Kong. Res. J. 6(2): 8-12, 2019
Publisher: Kongunadu Arts and Science College, Coimbatore.

http://krjscience.com

RESEARCH ARTICLE

EVALUATION OF ANTIOXIDANT AND CYTOTOXICITY PROPERTIES OF AMYGDALIN EXTRACTED FROM *PRUNUS DULCIS*

Sushma, P[1]., Bindu Jacob[1] and R.T. Narendhirakannan[2]*

[1]Department of Biotechnology, School of Agriculture and Biosciences, Karunya Institute of Technology and Sciences, Coimbatore-641114, Tamil Nadu. India.

[2]Department of Biochemistry, Kongunadu Arts and Science College, G.N. Mills (PO), Combatore-641029, Tamil Nadu, India.

ABSTRACT

Free-radical reactions have been implicated in the pathology of many human diseases like atherosclerosis, ischemic heart disease, aging process, inflammation, diabetes, immuno-suppression, neurodegenerative disease etc. Radicals and other reactive oxygen species are formed constantly in the human body and are removed by the enzymatic and non-enzymatic antioxidant defence. The disturbance in 'redox homeostasis' that occurs when antioxidant defences are inadequate can damage lipids, proteins, carbohydrates and DNA. Drugs with multiple protective mechanisms, including antioxidant activity, may be one way of minimizing tissue injury. Phytochemicals with antioxidant property are naturally present in food are of great interest due to their beneficial effects on human health as they offer protection against oxidative deterioration. Amygdalin, also known as vitamin B17 is a cyanogenic glycoside found in several sources mainly in apples, pears, apricots, plums, peaches. Several reports claim amygdalin to be good chemopreventive agent, however these claims are not often backed by proper scientific evidence. Thus the present study is aimed to evaluate the therapeutic potential of amygdalin isolated from Prunus dulcis by studying its in vitro antioxidant and cytotoxic properties.

Keywords: Amygdalin, Antioxidant, Cytotoxicity, Extract, *Prunus dulcis*

1. INTRODUCTION

Antioxidants are radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischemia, anaemia, asthma, arthritis, inflammation, neuro-degeneration, Parkinson's diseases, mongolism, ageing process and perhaps dementias. An increasing number of studies reveal that dietary antioxidants are capable of blocking neuronal death in vitro and many therapeutic properties in animal models of neurodegenerative diseases including Alzheimer's and Parkinson's diseases (1). The antioxidant activity of dietary polyphenols is considered to be much greater than that of the essential vitamins. Hence, studies on the evaluation and exploitation of phyto-nutrient compounds particularly phenolic acids, flavonoids and high molecular weight tannins of legumes as natural antioxidants have assumed great significance (2). Amygdalin also known as "Vitamin B17" is a product of the metabolism of phenylalanine in the bitter almond. The safety levels of amygdalin include the fact that according to researchers, healthy cells break vitamin B17 down into beneficial product and cancerous cells break it down into toxins. Studies regarding indigenous groups which are principally free of cancer have revealed a common diet that is rich in vitamin B17. People of the Abkhazians, the Hunzas, the Eskimaux and the Karakorum obtain their vitamin B17 through variety of foods and may consume about 250-3000 mg of B17 nitriloside a day. When this food habits get changed due to their move into urban from tribe, there is increase drisk of cancer. The average western diet tends to have less than 5 mg a day (3). It has been observed that not much research work has been carried out in elucidating the therapeutic efficacy. Several sources have reported the amygdalin content found in the apricot kernels to possess significant anti-cancer and anti-oxidant properties. However, these reports are not backed up by strong scientific evidence. Thus the present study is aimed to bridge this gap and provide a scientific evidence of the efficacy of amygdalin as a cytotoxic and antioxidant agent.

2. MATERIALS AND METHODS

2.1. Chemicals

Diethyl ether, Ascorbic acid, DPPH-2,2-diphenyl-1-picrylhydrazyl, Sulphuric acid, Ammonium molybdate, Sodium phosphate, Sodium

*Correspondence: Narendhirakannan, R.T., Department of Biochemistry, Kongunadu Arts and Science College, Coimbatore – 641 029, Tamil Nadu, India. E.mail: bionaren_phd@yahoo.co.in

dihydrogen phosphate monohydrate, Disodium hydrogen phosphate anhydrous, Potassium ferricyanide, Trichloroacetic acid, Ferric chloride, Hydrogen peroxide, Rutin, PBS-Phosphate Buffered Saline, 10% FBS-Foetal Bovine Serum, DMEM-Dulbecco's Modified Eagle's Medium, DMSO-Dimethyl Sulfoxide, Trypsin were purchased from Hi Media Laboratories Ltd.,. All chemicals were of analytical grade and were used as such for assays.

2.2. Processing of Prunus dulcis

Powdered almonds were subjected to reflux conditions using ethanol solvent at 78.5°c. The mixture was boiled under reflux for 40, 80, 120 and 180 min. After reflux, the suspension was completely filtered with the help of Whatman filter paper No 1 and the solvent was evaporated using of rotary evaporator. To the dried sample, 10 ml of diethyl ether was added and it was vortexed for 1 min at room temperature (22°C) to get the precipitate. The diethyl ether was made to evaporate overnight in a fume hood. The sample obtained was stored in airtight container and was used for further analysis.

2.3. In vitro antioxidant studies

2.3.1. DPPH scavenging activity

Different concentrations of sample and standard solutions were prepared and made up to 100 µl with methanol. To the samples, 5 ml of DPPH was added and was incubated for 20 mins in dark. The absorbance was recorded at 517 nm. Methanol alone served as blank. A mixture of methanol and DPPH was used as control.

The percentage of inhibition is calculated by the following formula:

%Inhibition = [(control OD-Sample OD)/Control OD]*100. A graph is plotted with % of inhibition on Y-axis and concentration on X-axis.

2.3.2. Reducing Power ability

Different aliquots of standard and sample solutions were prepared in a series of test tubes. The volume in the test tubes was made up to 1ml in all the test tubes. A test tube with 1 ml of methanol act as a blank. In all the test tube, 2.5 ml of phosphate buffer and 2.5 ml of potassium ferricyanide was added including the blank. The solution mixture was incubated at 50°c for 20 min followed by addition of 2.5 ml trichloroacetic acid. The solutions were centrifuged at 1000 rpm form 10 mins at room temperature. After centrifugation, the upper layer of solution was mixed with distilled water and chloride was added. The absorbance of the green colour is read at 700 nm. Increased absorbance of the reaction mixture indicates increased reducing power. A graph is plotted with absorbance in Y-axis and concentration on X-axis.

2.3.3. Hydroxyl radical scavenging activity

Different concentrations of both sample and standard were prepared in a series of test tubes and each tube was made up to 100 µl with the phosphate buffer. Then 1 ml of EDTA, 0.5 of EDTA, 1ml of DMSO and 0.5 ml of ascorbic acid was added to all test tubes followed by uncubation for 15mins at80°-90° C. After the incubation, 1ml of trichloro acetic acid, 3ml of Nash reagent was added and was incubated for 15 mins. The test tube containing Phosphate buffer along with al reagents serves as positive control. The phosphate buffer served as a blank. The absorbance was read at 412 nm. The percentage of hydroxyl radical scavenging activity is calculated by the following formula:

Scavenging activity (%) = [(Control OD-Sample OD)/Control OD]*100. A graph was plotted with percentage of scavenging activity in Y-axis against concentration in X-axis.

2.4. In vitro cytotoxicity studies

The Cell toxicity assays were done by seeding HeLa cells in a 96 well plate and incubating it at 37°C with 5% CO_2 in CO_2 incubator. A series of dilution of the samples were added to the cells containing medium in 96 well plates. Wells containing only media and cells were taken as control wells. After the incubation of 24 h, MTT reagent was added to wells and then the 96 well plates were kept for few hours for the reaction to take place. When purple crystals are clearly visible; DMSO is added and kept in dark for few hours. Optical density of the formazan product was read at 495 nm using scanning multi well spectrophotometer. The results were given as mean of three independent experiments.

3. RESULS AND DISCUSSION

The presence of cyanide peak in a FTIR chromatogram confirms the presence of amygdalinas the major component of amygdalin is the nitrile moiety (4). In recent years, researchers had stated that the anti-oxidants can delay or restrain the oxidation of molecules. The process is mainly done by inhibiting the initiation of the free-radical induced chain reactions (5). Depending upon the assay performed, the results of antioxidant studies are usually scattered and that is to evaluate

the percentage of inhibition. The DPPH assay was performed for the extract, commercial amygdalin and standard.

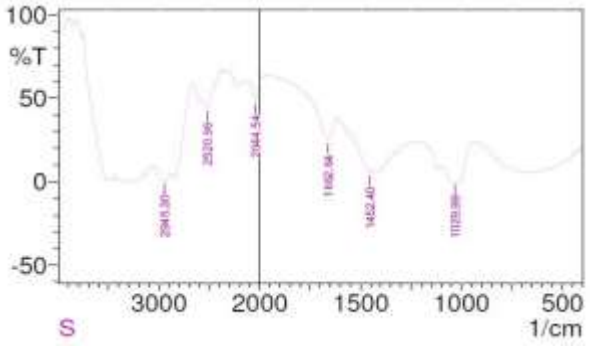


Fig. 1. FTIR for commercial amygdalin

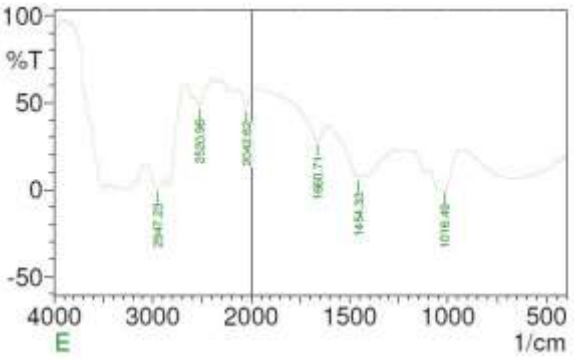


Fig. 2. FTIR for extract (Reflux)

Table 1: FTIR peak values for Std amygdlain

S. No.	Peak values	Groups present
1	2945.3	C-H
2	2520.96	O-H
3	1662.64	α,βUnsaturated
4	1452.4	C=C
5	1029.99	C-N

Table 2: FTIR peak values for extract

S. No.	Peak values	Groups present
1	2947.23	C-H
2	2520.96	O-H
3	1662.71	α,βUnsaturated
4	1454.33	C=C
5	1069.49	C-N

Due to the DPPH radical hydrogen donating ability, the scavenging effects of DPPH was radical was observed in all the samples (6). The scavenging activity of hydroxyl radical is an important index for measuring the antioxidant capacity (7). From the graph, it was concluded that the percentage of inhibition for

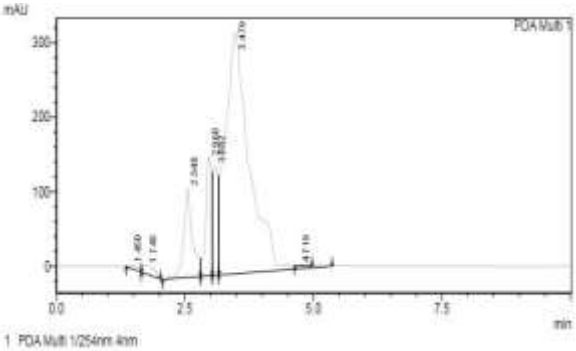


Fig. 3. HPLC for Standard

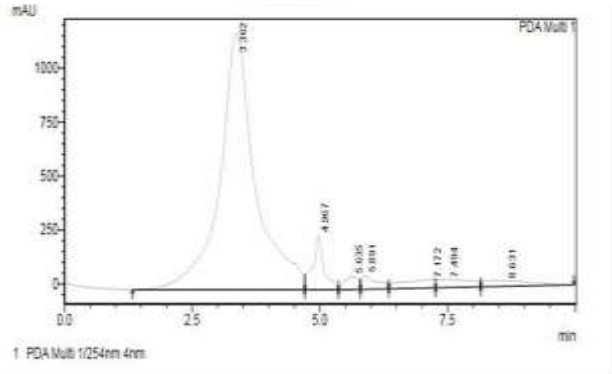


Fig. 4. HPLC for extract (Reflux)

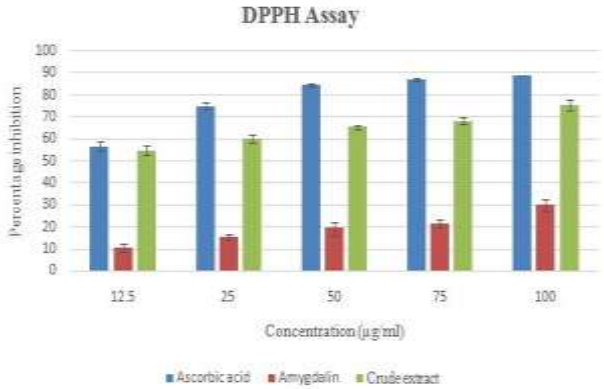


Fig. 5. Graph showing percentage of inhibition by DPPH assay

the extract is high when compared to the commercial amygdalin. In the reducing power assay, with the reduction of the Fe^{3+} cyanide complex to ferrous form, the green/blue is developed from the pale yellow colour. If the absorbance is higher, the reducing power is stronger. The intensity mainly depends upon the reducing power of the anti-oxidants. So the ferrous cation should be monitored by the absorbance at 700 nm (8).

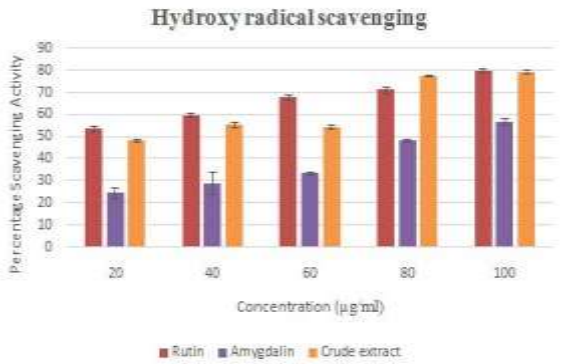


Fig. 6. Graph showing inhibition of free radicals by hydroxyl radical scavenging assay

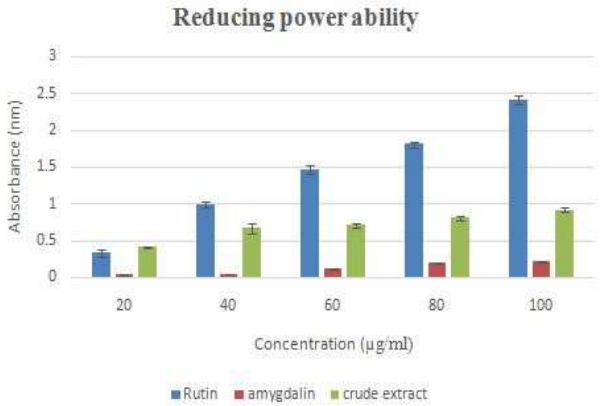


Fig. 7. Graph showing percentage of inhibition by reducing power ability

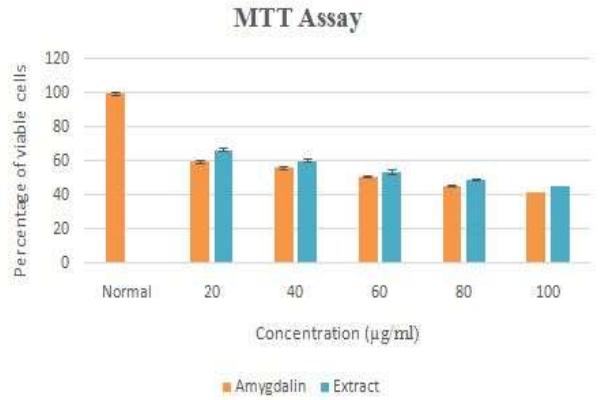


Fig. 8. Effect of commercial amygdalin and extract on percentage of cell viability

The HPLC has been done for the extract and standard and the curve was obtained. The HPLC was the method which was necessary to establish amygdalin in the sample. The ratio of methanol and water should be proper. Incase if it is small, the analytical time will be long. If the methanol is large, the amygdalin would not be separated well. Because of this reason only the gradient elution method should be followed (9).The MTT assay was also performed for the extract in different concentrations and also for the commercial standard also. It is mainly done to check the cell viability. In a study it was stated that amygdalin induces apoptosis in cancer cell lines (10). MTT assay can accurately determine the count of live cells and it is to find the cytotoxicity for cancer drugs (11).

4. CONCLUSION

In the present study extraction of amygdalin from *P. dulcis* demonstrated by the reflux method was easy, fast, cost-effective, eco-friendly and non-toxic. The spectroscopic and chromatographic analysis such as FTIR and HPLC supported the synthesis of amygdalin. The amygdalin extract from *P. dulcis* exhibited a good antioxidant potential which was confirmed by various antioxidant assays. Amygdalin was found to have a significant cytotoxic effect against HeLa cancer cells. Further studies can be carried out to elucidate the biological properties of amygdalin.

REFERENCES

1. Oke, T.R. (2002). Boundary layer climates. *Routledge* 23.
2. Siddhuraju, P. and S. Manian, (2007). The antioxidant activity and free radical-scavenging capacity of dietary phenolic extracts from horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.) seeds. *Food Chem.* **105**(3): 950-958.
3. Stefansson, V. (1960). Cancer: Disease of Civilization?: An Anthropological and Historical Study. New York: Hill and Wang.
4. Volpi, G. (2016). Demonstrating the Presence of cyanide in bitter seeds while helping students visualize metal–cyanide reduction and formation in a copper complex reaction. *J. Chem. Educ.* **93**(5): 891-897.
5. Esfahlan, A.J., R. Jamei and R.J. Esfahlan, (2010). The importance of almond (*Prunus amygdalus* L.) and its by-products. *Food Chem.* **120**(2): 349-360.
6. Kalaivani, T. and L. Mathew, (2010). Free radical scavenging activity from leaves of *Acacia nilotica* (L.) Wild. ex Delile, an Indian medicinal tree. *Food and Chem. Toxicol.* **48**(1): 298-305.
7. Singh, N., and P.S. Rajini, (2004). Free radical scavenging activity of an aqueous extract of potato peel. *Food Chem.* **85**(4): 611-616.

8. Barros, L., M.J., Ferreira, B. Queiros, I.C. Ferreira and P. Baptista, (2007). Total phenols, ascorbic acid, β-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. *Food Chem.* **103**(2): 413-419.
9. Lv, W.F., M.Y. Ding and R. Zheng, (2005) Isolation and Quantitation of Amygdalin in Apricot-kernel and *Prunus tomentosa* Thunb. by HPLC with solid-phase extraction. *J. Chromatogr. Sci.* 43(7): 383-387.
10. Chen, Y., Ma, J., Wang, F., Hu, J., Cui, A., Wei, C., *et al.,* (2013). Amygdalin induces apoptosis in human cervical cancer cell line HeLa cells. *Immunopharmacol. Immunotoxicol.* **35**(1): 43-51.
11. Al-Hazzani, A.A., A.A. Alshatwi, (2011). Catechin hydrate inhibits proliferation and mediates apoptosis of SiHa human cervical cancer cells. *Food and chem. Toxicol.* **49**(12): 3281-3286.

Kong. Res. J. 6(2): 13-19, 2019
Publisher: Kongunadu Arts and Science College, Coimbatore.

DOI:10.26524/KRJ296

RESEARCH ARTICLE

STUDIES ON THE ARBUSCULAR MYCORRHIZAL FUNGAL ASSOCIATION IN THE PLANT SPECIES OF PONNUTHU HILLS, WESTERN GHATS COIMBATORE DISTRICT, TAMILNADU

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

ABSTRACT

The present study analyzed the arbuscular mycorrhizal fungal spores in root colonization and spore population in rhizosphere soils samples in various medicinal plant species at Ponnuthu hills, Western Ghats of Coimbatore district, Tamilnadu, India. Root and rhizosphere soil samples were collected during the month of August, 2018-March, 2019 from the surface to 30 cm depth as well as pH were also recorded. Totally 36 plant species belonging to 21 families were collected and identified. The present result showed arbuscular mycorrhizal spore population in the rhizosphere soil and root colonization of all the plant species. A total of 21 AM fungal spores were recovered from the rhizosphere soil samples in this study region. The *Glomus* was dominant and found in rhizosphere soil samples in all the medicinal plant species. The maximum spore population was found in the rhizosphere soil samples of *Hemidesmus indicus* (573/100g of soil) which belongs to the family Asclepiadaceae and the lowest spore population was observed in the *Abutilon indicum* 145/100g of soil) which belongs to Malvaceae family. The highest 81% AM fungal colonization was found in roots of *Gymnema sylvestre* which belongs to the family Apocynaceae. While the lowest 16 % AM fungal colonization was found in the root of *Tridax procumbens* which belongs to the family Asteraceae.

Keywords: *Glomus aggregatum***,** Medicinal plants, Ponnuthu hills.

1. INTRODUCTION

India is recognized as one of the seventeen mega biodiversity zones of the world. The forest of Western Ghats, in view of their floristic diversity and numerous multipurpose species, are considered as a varietal storehouse of economically important plants and beneficial microbial communities. AM fungi are geographically ubiquitous in occurrence that have a broad range of dissimilar environments (1,2) from the arctic to the tropics and occupy a wide range of ecological niches (3). The fossil record suggests that AM were also present in the subterranean parts of the earliest land regions (4). Mycorrhizas are one such examples of a plant-fungal association that is found in plants under a range of abiotic conditions. Mycorrhizal symbiosis occurs in a vast majority of vascular plants except for members of a few families, including Cruciferae, Brassicaceae and Zygophyllaceae (5,6).

Arbuscular mycorrhizal symbiosis that appeared with the first land plants more than 400 million years ago, is still formed by the large majority of extent plant species with no host specificity (7). Occurrence of AM fungi has been reported from an exceptionally wide range of plant and different ecosystems and plays a major role in better nutrition, species diversity and survival. (8). Almost all higher plants in the terrestrial ecosystems are known to be associated with mycorrhizal fungi (9).

Associations between plants and arbuscular mycorrhizal fungi are common in natural and agricultural ecosystems. Soil microorganisms play important role in plant-soil interactions. Microbes alter nutrient availability, immobilize heavy metals in soils, and bind soil particles into stable aggregates (10). Of the several types of mycorrhizal fungi, the arbuscular mycorrhizal fungi (AMF) form important symbiosis with the flora prevalent in serpentine grasslands.

In developing countries and rural societies, the use of medicineal plants is both a valuable resource and necessity and furthermore it provides real alternative for primary health care systems (11). Globally about 85% of the traditional medicine used for primary healthcare are derived from plants. In many countries scientific investigations of medicinal plants have been initiated because of their contribution to healthcare. Herbal medicines have good values in treating many diseases including infectious diseases, etc.

2. MATERIALS AND METHODS

2.1. Study area Description

Kurudi Malai is the hill at the base of which Ponnuthu Amman temple is situated. It lies between 11.1186° N, 76.8923° E. Few even call it as Ponnuthu Malai. The summit is at a rough elevation of 1200m (3900ft). The terrain is rocky at the beginning and an

*Correspondence: Santhoshkumar, S., Department of Botany, Kongunadu Arts and Science College, Coimbatore – 641 029, Tamil Nadu, India. E.mail: santhosh.biology@gmail.com

abrupt rise in elevation arises as tracks of salt could be clearly seen on those rocks indicating the past traces of waterfalls. After which thick forest cover exists. Thorny shrubs can be found in abundance. Other smaller amphibians like newt were also spotted.

Fig. 1. Study area of Ponnuthu hills.

2.2. Sample collection

In this present study, root and rhizosphere soil samples of 36 plant species were collected for the duration of August, 2018- March, 2019. The collected soil and root samples were placed in the polyethylene bags, labelled and then transported to the laboratory. The root samples were freshly processed, whereas rhizosphere soil samples were analyzed for mycorrhizal spore population and AM fungal root colonization in study species.

2*.3. Estimation of AM fungal root colonization*

The root samples were cleared and stained in tryphan blue with a modified version of following method by Philips and Hayman's). The collected roots samples were cut into 1-2 cm pieces, heated at 90ºC in 10% KOH for about 1 hour. For thicker and older roots, the duration was increased. The root segments were rinsed in water and acidified with dilute HCl. The root pieces were stained with 0.05% tryphan blue in lacto phenol for 5 minutes and the excess stain was removed with clear lacto phenol.
The percentage of AM fungal infection was calculated using the formula:

2.4. Identification of AM fungi

The present study isolation and identification of AM fungal spores based upon their morphological characters such as spore size, color, hyphal attachement, cell wall layer characters, were identified in addition with nomenclature, keys of the following manual authors were used: Raman and Mohankumar (12) Schenk and Perez (13) and Schubler and Walker (14). The Photomicrographs were taken with the help of a Magnus Olympus Microscope.

2.5. Soil pH

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

3. RESULTS AND DISCUSSION

AM fungal colonization and spore population of 36 plant species belongs to 21 families and also analysis of the pH ranges between 4.8 and 6.9 are represented in the (Tables--1 & 2). The maximum temperature was recorded 36.5^{0}C while the maximum rain fall were noted in August, 390 mm.

The maximum spore population was observed in the plant species of *Hemidesmus indicus* (573/100g of soil) belonging to Asclepiadaceae and minimum was observed in *Abutilon indicum* (145/100g of soil) belonging to Malvaceae. In the present investigation the highest AM fungal infection was recorded in *Gymnema sylvestre* (81%) belonging to Apocynaceae and minimum was noticed in *Tridax procumbens* (16%) belonging to Asteraceae.

The plant species like *Acorus calamus* 28% (Acoraceae), *Barleria prionitis* 24% (Acanthaceae), *Catharanthus pusillus* 22% (Apocynaceae), *Commelina benghalensis* 28% (Commelinaceae), *Plectranthus barbatus* 38% (Lamiaceae), *Corchorus aestuans* 33% (Tiliaceae), *Capparis zeylanica* 30% (Capparidaceae), showed 20 to 40 % of infection.

The other plant species like *Areva lanata* 59% (Amaranthaceae), *Azadirachta indica* 55 % (Meliaceae), *Anisomeles malabarica* 43% (Lamiaceae), *Achyranthes aspera* 44 % (Amaranthaceae), *Abutilon indicum* 52 % (Malvaceae), *Argemone mexicana* 58 % (Papaveraceae), *Blepharis maderaspatensis* 48% (Acanthaceae), *Barleria cristata* 46% (Acanthaceae), *Catharanthus roseus* 41% (Apocynaceae), *Datura innoxia* 50% (Solanaceae), *Evolvulus alsinoides* 53% (Convolvulaceae), *Hibiscus micranthus* 46 % (Malvaceae), *Hibiscus vitifolius* 42 % (Malvaceae), *Ipomoea obscura* 58% (Convolvulaceae), *Leucas aspera* 43% (Lamiaceae), *Mimosa pudica* 44% (Mimoseaceae), *Oxalis corniculata* 52% (Oxalidaceae), *Ocimum sanctum* 49% (Lamiaceae) showed above 41 to 60% of infection.

The rest of the species like *Bacopa monnieri* 61% (Plantaginaceae), *Euphorbia hirta* 67% (Euphorbiaceae), *Ficus benghalensis* 64 % (Moraceae), *Oldenlandia umbellate* 69% (Rubiaceae),

Portulaca oleracea 65% (Portulacaceae), showed above 61 to 80% of infection. The plant family like Amaranthaceae and Juncaceae were thought to be mycorrhiza free, most of the species were found to be infected under natural stressed rangeland conditions (15). The plants that do not form mycorrhizas may be related to the presence of fungi toxic compounds in root cortical tissue or in root exudates. It may also be due to interactions between the fungus and the plant at the cell wall and (or) middle lamella level (16). High concentrations of salycilic acid have been found to reduce mycorrhization. But in the in the present study revealed that the plant family Amarathaceae showed the mycorrhizal infection. The research clearly showed that AMF enhanced nutrient uptake and growth of endangered plants (17,18).

Many studies conducted in different ethnic communities, have reported frequently the use of leaves was widely accepted for traditional therapies may be due to large quantity of biologically active components present inside them. Apart from leaves, almost all the other parts of medicinal plants such as flower, bark, stem, seed, fruit are also used. The utilization of leaves in traditional medication may also be due to their easy availability.

Table: 1. List of plants species collected from the Ponnuthu hills, and their medicinal uses.

S. No	Plant species	Family	Habit
1.	*Areva lanata* (L.) Juss. Ex schult.	Amaranthaceae	Herb
2.	*Azadirachta indica* A. Juss.	Meliaceae	Tree
3.	*Anisomeles malabarica* (L.) R.Br.ex Sims	Lamiaceae	Herb
4.	*Achyranthes aspera* L.	Amaranthaceae	Herb
5.	*Abutilon indicum* L.	Malvaceae	Shrub
6.	*Acalypha indica* L.	Euphorbiaceae	Herb
7.	*Acorus calamus* L.	Acoraceae	Herb
8.	*Argemone mexicana* L.	Papaveraceae	Herb
9.	*Blepharis maderaspatensis* (L.) Heyne ex Roth.	Acanthaceae	Herb
10.	*Bacopa monnieri* (L.)	Plantaginaceae	herb
11.	*Barleria cristata* L.	Acanthaceae	Shrub
12.	*Barleria prionitis* L.	Acanthaceae	Shrub
13.	*Crotalaria retusa* L.	Fabaceae	Herb
14.	*Corchorus aestuans* L.	Tiliaceae	Herb
15.	*Capparis zeylanica* L.	Capparaceae	Shrub
16.	*Catharanthus pusillus* (Murray) G. Don	Apocynaceae	Herb
17.	*Catharanthus roseus* (L.) G.Don	Apocynaceae	Shrub
18	*Commelina benghalensis* L.	Commelinaceae	Herb
19	*Datura innoxia* Mill.	Solanaceae	Shrub
20	*Evolvulus alsinoides* (Linn)	Convolvulaceae	Herb
21	*Euphorbia hirta* L.	Euphorbiaceae	Herb
22	*Ficus benghalensis* L.	Moraceae	Tree
23	*Gymnema sylvestre* R.Br.	Apocynaceae	Shrub
24	*Hibiscus micranthus* L.f.	Malvaceae	Shrub
25	*Hibiscus vitifolius* L.	Malvaceae	Herb
26	*Hemidesmus indicus* (L.)	Asclepiadaceae	Shrub
27	*Ipomoea obscura* (L.) Ker Gawl.	Convolvulaceae	Herb
28	*Leucas aspera* Linn.	Lamiaceae	Herb

29	*Mimosa pudica* L.	Fabaceae	shrub
30	*Oxalis corniculata* L.	Oxalidaceae	Herb
31	*Ocimum sanctum* L.	Lamiaceae	Herb
32	*Oldenlandia umbellate* L.	Rubiaceae	Herb
33	*Plectranthus barbatus* Andrews	Lamiaceae	Herb
34	*Phyllanthus amarus* Schumach & Thonn.	Phyllanthceae	Herb
35	*Portulaca oleracea* L.	Portulacaceae	Herb
36	*Tridax procumbens* L.	Asteraceae	Herb

Table 2. Arbuscular Mycorrhizal fungal spore population and root colonization in the plant species of Ponnuthu hills, a part of Western Ghats, Coimbatore district, Tamilnadu, during 2018-2019.

S. No	Plant Species	pH	Types of infection			Spore Population (100g/soil)	(%) root colonization
			Hyphae	Arbuscule	Vesicles		
1.	*Areva lanata* (L.) Juss. Ex schult.	5.2	+	-	+	320	61
2.	*Azadirachta indica* A. Juss.	4.8	+	+	-	280	55
3.	*Anisomeles malabarica* (L.) R.Br.ex Sims	6.2	+	+	-	410	43
4.	*Achyranthes aspera* L.	5.5	+	-	+	185	44
5.	*Abutilon indicum* L.	5.7	+	+	-	387	52
6.	*Acalypha indica* L.	6.0	+	-	+	156	39
7.	*Acorus calamus* L.	5.8	+	+	-	270	28
8.	*Argemone mexicana* L.	5.1	+	-	+	355	58
9.	*Blepharis maderaspatensis* (L.)	4.9	+	+	-	190	67
10.	*Bacopa monnieri* (L.)	5.3	+	-	+	177	65
11.	*Barleria cristata* L.	6.7	+	+	-	445	74
12.	*Barleria prionitis* L.	6.6	+	-	+	390	24
13.	*Crotalaria retusa* L.	5.9	+	+	-	365	43
14.	*Corchorus aestuans* L.	5.4	+	-	+	402	33
15.	*Capparis zeylanica* L.	5.3	+	-	+	339	30
16.	*Catharanthus pusillus* (Murray) G. Don	6.1	-	-	-	130	22
17.	*Catharanthus roseus* (L.) G.Don	6.4	+	-	+	470	60
18.	*Commelina benghalensis* L.	5.2	+	+	-	293	22
19.	*Datura innoxia* Mill.	6.3	+	-	+	245	51
20.	*Evolvulus alsinoides*	5.5	+	+	-	310	53

	(Linn)						
21.	*Euphorbia hirta* L.	6.8	+	-	+	360	67
22.	*Ficus benghalensis* L.	5.7	+	-	+	387	68
23.	*Gymnema sylvestre* R.Br.	6.9	+	-	+	422	81
24.	*Hibiscus micranthus* L.f.	5.6	+	+	-	126	46
25.	*Hibiscus vitifolius* L.	6.1	+	-	+	405	63
26.	*Hemidesmus indicus* (L.)	6.5	+	-	+	573	55
27.	*Ipomoea obscura* (L.) Ker Gawl.	5.8	+	+	-	280	58
28.	*Leucas aspera* Linn.	5.0	+	+	-	410	43
29.	*Mimosa pudica* L.	5.1	+	-	+	185	44
30.	*Oxalis corniculata* L.	5.4	+	+	-	387	52
31.	*Ocimum sanctum* L.	5.2	+	-	+	156	49
32	*Oldenlandia umbellate* L.	5.5	+	+	-	270	49
33.	*Plectranthus barbatus* Andrews	6.2	+	-	+	355	38
34.	*Phyllanthus amarus* Schumach & Thonn.	6.3	+	+	-	190	47
35.	*Portulaca oleracea* L.	5.7	+	-	+	420	65
36.	*Tridax procumbens* L.	5.3	+	+	-	445	16

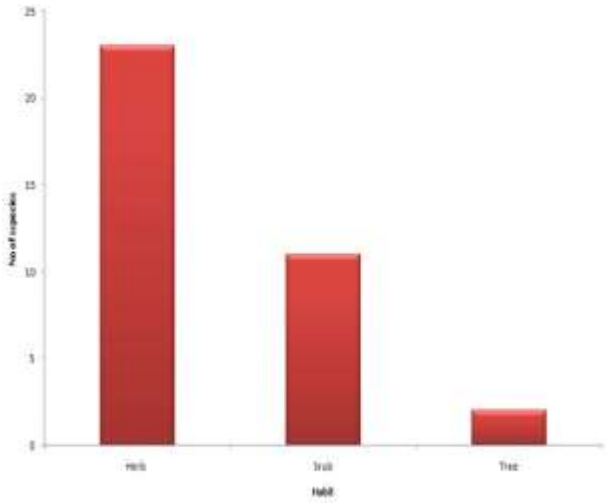


Fig. 2. Habit wise distribution of plant species in Ponnuthu hills.

From the rhizosphere soils sample of Ponnuthu hills, totally 21 AM fungal species were isolated and identified. Of these 4 species of *Acaulospora*, *Aca. delicata, Aca. denticulatum*, *Aca. gdanskensis Aca.levies*, 1 species of *Ambispora*, *Amb. appendicula*, 1 species of *Gigaspora, Gig.candida*, 13 species *of Glomus*, *Gl. aggregatum*, *Gl. albidum*, *Gl. ambisporum*, *Gl. arborense*, *Gl. australe*, *Gl. canadense*, *Gl.citricola*, *Gl. delhiense*, *Gl. deserticola*, *Gl. dimorphicum*, *Gl. radiatum*, *Gl. segmentatum*, *Gl. versiforme*, 1 species of *Redeckera*, *Red*. *fulvum*, 1 species of *Rhizophagus*, *Rhi*. *fasciculatus* were observed.

The names of the species are represented in Table 3. In addition with Santhoshkumar and Nagarajan (19) were studied on AM spore population in the plants species at Sirumalai hills, Eastern Ghats of Dindugul district. Totally 39 AM fungi species belongs to 6 genera were isolated and identified. To isolate and identification of the Am fungal spores in rhizosphere soils samples in different regions such as Yellanahalli hills reported by (20), Kondranghi hills (21), Bargur hills (22).

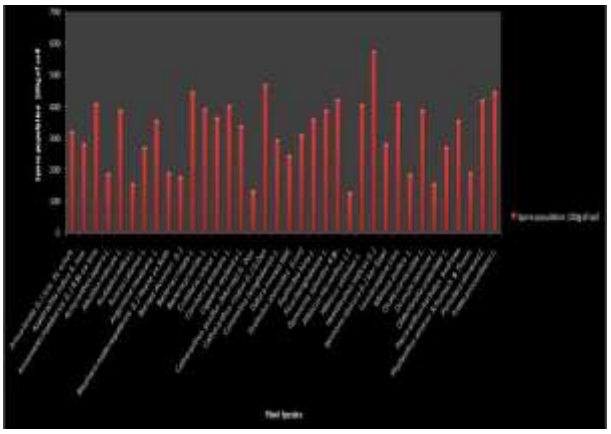


Fig. 3. AM fungal spore population of the plant species of Ponnuthu hills.

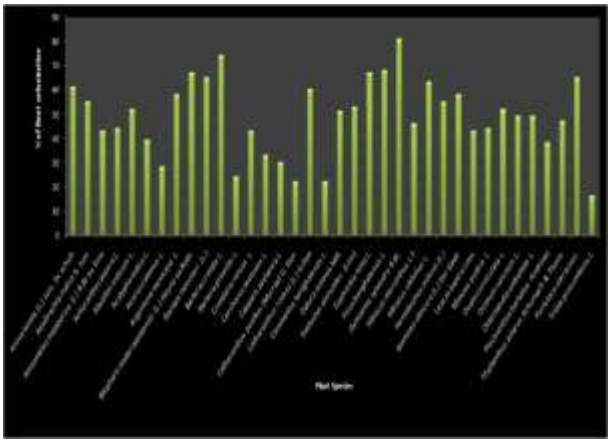


Fig. 4. AM fungal root colonization in collected plant species from Ponnuthu hills.

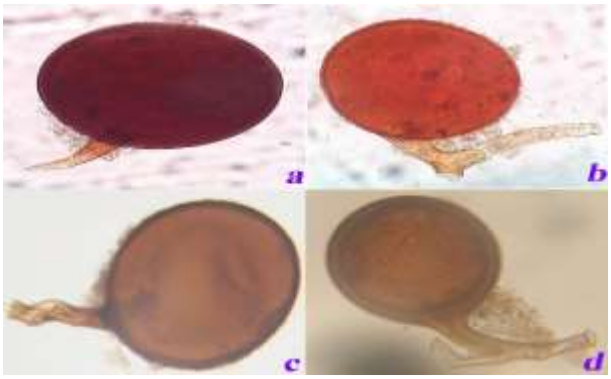


Fig. 5. Isolation and Identification of AM fungal spores in rhizosphere soils of Ponnuthu hills, Western Ghats of Coimbatore district.

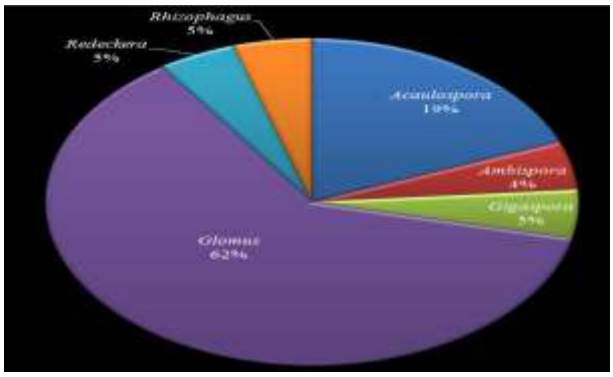


Fig. 6. Dominant genus was recovered from the rhizosphere soils samples in Ponnuthu hills.

4. CONCLUSION

From above results we concluded that the present study the AM fungal root colonization and spore population in all the plant species in Ponnuthu hills. In this symbiotic association of AM fungi in the plant species to absorb the soil nutrients, zinc, copper especially phosphorous and also increased plant resistance to various stresses like drought, salt and heavy metal. In future, the AM fungal spores were cultured under *in vitro* condition for raise agricultural crops plant species growth and development.

REFERENCES

1. Gerdemann, J.W. and J.M. Trappe, (1974). The Endogonaceae in the Pacific Northwest. *Mycological Memoirs* **5**: 1–76.
2. Koske, R.E. (1987). Distribution of VA mycorrhizal fungi along a latitudinal temperature gradient. *Mycologia* **79**: 55-68.
3. Srivastava, D., R. Kapoor, S.K. Srivastava, and K.J. Mukerji, (1996). Mycorrhizal research A priority in agriculture. In: K.G. Mukerji (ed.), Concepts in Mycorrhizal Research. Kluwer Academic Publishers, Dordrecht, The Netherlands.
4. Butler, E.J. (1939). The occurrences and systematic position of the Vesicular arbuscular type of mycorrhizal fungi. *Trans. Br. Mycol. Soc.* **22**: 274-301.
5. Newman, E.I. and P. Reddell, (1987). The distribution of mycorrhizas among families of vascular plants. *New Phytol.* **106**: 745-751.
6. Dhillon, S.S. and C.F. Friese, (1994). The occurrence of mycorrhizas in prairies: application to ecological restora-tion. Thirteenth North American Prairie Conference.

7. Redecker, D., R. Kodner and L.E. Graham, (2000). Glomalean fungi from the Ordovician. *Science* **289:** 1920-1921.
8. Bergelson, J.M., and J.M. Crawley, (1998). Mycorrhizal infection and plant species diversity. *Nature* **334**: 202-204.
9. Smith, S. and D.J. Read (1997). Mycorrhizal symbiosis. Second edition Academic Press, London. 605 pp.
10. Shetty, K.G., B.A.D. Hetrick, D.A.H. Figge and A.P. Schwab, (1994). Effects of mycorrhizae and other soil microbes on revegetation of heavy metal contaminated mine spoil. *Environ. Pollut*. A **86**: 181-188.
11. Alexiades, M.N., and D. Lacaze, (1996). Fenamad program in traditional medicine: an integrated approach to health care in the Peruvian Amazon. In: Balick, M.J., Elisabetsky, E., Laird, S.A. (Eds.), Medicinal Resources of the Tropical Forest. Columbia University Press, New York, pp. 341–365.
12. Raman, N., and V. Mohan Kumar, (1988). Techniques in Mycorrhizal Research. University of Madras, Madras. 279p.
13. Schenck, N.C., and Y. Perez, (1987). A Manual for the Identification of VA-mycorrhizal Fungi, University of Florida, Synergistic Publications, Gainesville, Florida, U.S.A.: 245.
14. Schubler, A., and C. Walker, (2010). The Glomeromycota: a species list with new families and new genera. Online: **1**(58): 41-75.
15. Neeraj, A., J. Shauker Mathew, and A. Varma, (1991). Occurrence of vesicular-arbuscular mycorrhizae with Amaranthaceae in soils of the Indian semi-arid region. *Biol. fertile soils* **11**: 40-144.
16. Tester, M., S.E. Smith, and F.A. Smith, (1987). The phenomenon of "nonmycorrhizal" plants. *Can. J. Bot*. **65**: 419-431.
17. Barroetavena C., S.D. Gisler, D.L. Lumoa and R.J. Meinke, (1998). Mycorrhizal status of the endangered species Asragalus applegatei Peck as determined from a soil bio assay. *Mycorrhiza*, **8**: 117-119.
18. Panwar J., and A. Vyas, (2002). AM fungi: A biological approach towards conservation of endangered plants in Thar desert, India. *Curr. Sci*. **82** (5): 576-578.
19. Santhoshkumar, S., and N. Nagarajan, (2017). Arbucular Mycorrhizal Fungal Association in the Rhizosphere Soil and Root Colonization of Some Medicinal Plant Species in Sirumalai Hills Eastern Ghats of Dindugul District Tamilnadu. American-Eurasian *J. Agric. & Environ. Sci.* **17** (3): 206-212.
20. Santhoshkumar, S., N. Nagarajan and K. Santhoshkumar (2018). Studies on the Arbuscular Mycorrhizal Fungal biodiversity in the Plant species of Kondranghi hills, Dindugul district, Tamil Nadu, India. Kong. Res. J. **5**(2): 34-40.
21. Santhoshkumar, S., N. Nagarajan, R. Prema, R. Kowsaliya, F. Amjath Alikhan and P. Aishwarya (2018). Studies on the Arbuscular Mycorrhizal Fungal biodiversity in the plant species of Yellanahalli hills, Valley View of Nilgiris, Udhagamandalam, Tamil Nadu, India. *Kong. Res. J.* **5**(2): 27-33.
22. Santhoshkumar, S., N. Nagarajan and S. Naveen (2019). Association of Arbuscular Mycorrhizal Fungal species in the plant species of bargur hills, Erode District, Tamil nadu, India. *Kong. Res. J.* **6**(1): 60-65.

DOI:10.26524/KRJ297

Kong. Res. J. 6(2): 20-27, 2019
Publisher: Kongunadu Arts and Science College, Coimbatore.

RESEARCH ARTICLE

SURVEY OF WILD EDIBLE PLANTS OF DHANAKARKULAM PANCHAYATH, TIRUNELVELI DISTRICT, TAMIL NADU, INDIA

Kohila, A. and V. Mary Kensa*
Department of Botany and Research Centre, S.T. Hindu College, Nagercoil – 629002, Kanyakumari District, M.S University, Abishekapatti, Tirunelveli,Tamil Nadu. India.

ABSTRACT

The present study was carried out in the Dhanakarkulam panchayath to document the diversity, indigenous uses and availability status of wild edible plants. The inhabitants of the region are dependent up to a large extent on wild resources for their food and other daily needs.. The study revealed a total of 51 species, 42 genera and 27 families of wild edible plants were recorded in the study area. The total number of plant species recorded as medicinal and wild food plants indicated that the study area has substantial amount of useful plants and diverse source of medicinal and wild food plants. The diversity of these medicinal and wild edible of these medicinal and wild edible plants might be due to the suitability of environmental condition for different types of plant species. This finding is a good indicator for the presence of a considerable diversity of plant species is the study area. The study will be helpful in developing a comprehensive data base on wild plant resources, strengthening the food security in area and in conserving the traditional knowledge for the prosperity of the remote areas.

Keywords: availability, comprehensive, diversity, substantial and remote areas.

1. INTRODUCTION

Edible Wild Plants (EWPs) play an importance role in household livehoods, especially during periods of both natural and manmade stresses. They have significant nutritional economic ecological and socio-cultural values (1,2). EWPs are marketable and provide the opportunity to supplement hold income. People in different parts of the world depend on plant resources for their basic needs for food, clothes and shelter occurring in their environment (3). Wild edible plants (WED) provide stable food for indigenous people, serve as complementary food for non-indigenous people and offer an alternative source of cash income for poor communities (4). Wild edible plants have played an important role in human life since time immemorial. In India, most rural inhabitants depend on the wild plants to meet their supplementary food requirements. Keeping this in view, the present study was conducted as an attempt from the region to explore and identify the wild edible plant resources and indigenous knowledge about their utilization.

The study areas was in Thirunelveli District situated in the southern tip of peninscular India is under strategic location and has rich diversity of plants scattered over the hills and hillocks of the district. However, published data on survey of wild edible plants of Dhanakarkulam panchayath, Thirunelveli district, Tamilnadu are meagre. Reports on the survey of wild edible plants of Dhanakarkulam panchayath, Thirunelveli district, Tamilnadu is very scanty. Hence, the present study was undertaken to survey of wild edible plants of Dhanakarkulam panchayath, Thirunelveli District.

2. METHODOLOGY

Extensive field surveys were made in the study area from December 2018 to February 2019 in different seasons i.e., rainy, winter and summer, to collect the wild edible plants and their indigenous uses. The information on wild plants was collected by interviewing local inhabitants. The informants were men and women working in the fields, priests, medicine-men and birth attendant above the age of 50 years. To determine the authenticity of information collected during field work, repeated verification of data from different informants was done. Thus, only the specific and reliable information cross-checked with informants has been incorporated in the present study. The specimens were identified with the help of existing literature (5,6). The availability status of plants such as abundant, common and uncommon was given based on their occurrence in the study area. I did not collect voucher specimen in cases where field identification of species was certain. In the other cases, field notes and photographs were taken. The specimens were identified with the help of reference collections and expert knowledge.

*Correspondence: Mary Kensa V., Department of Botany and Research Centre, S.T. Hindu College, Nagercoil – 629002, Kanyakumari District, M.S University, Abishekapatti, Tirunelveli,Tamil Nadu. India. E.mail: surejkensa@gmail.com

3. RESULTS AND DISCUSSION

A total of 51 species, 30 genera and 27 families of wild edible plants were recorded in the study area (Table 1). The total number of plant species recorded as medicinal and wild food plants indicated that the study area has substantial amount of useful plants and diverse source of medicinal and wild food plants. The diversity of these medicinal and wild edible of these medicinal and wild edible plants might be due to the suitability of environmental condition for different types of plant species. This finding is a good indicator for the presence of a considerable diversity of plant species in the study area.

The families like Cucurbitaceae (6), Amaranthaceae and Solanaccae contains 5 species, followed by Fabaceae (4), Rutaceae (3), Poaceae (2), Lythraceae (1), Zinginberaceae (2), Arecaceae (2), Phyllanthaceae (2) and Musaceae, Myrtaceae, Sapotaceae, Caricaceae, Moringaceae, Caesalpiniaceae, Vitaceae, Combretaceae, Moraceae, Anonaceae, Talinaceae, Brassicaceae, Liliaceae with single plant species. Similar finding was observed by Getnechokole (7) indicated that Fabaceae accounted the largest species in their study. Among 51 collected plant species, about 12 plant species were used for only medicinal purposes whereas about 39 of plant species are used for only edible food and about 51 plant species were used for both medicinal and food purpose (Table 1).

Fig. 1. Habit wise distribution of plant species in the study area

Number of wild tree species are more 20, followed by herbs 20, shrub 3 and climber8 as is graphically as well as tabulated in Fig. 1. This results also concuss with the work of Teklehaymanot and Giday (8) 2010 and Zemede and Mesin (9). Flowering in most of the enumerated plants start between January and March but fruiting period varies from species to species. Fruits are mostly consumed raw and leafy vegetables are cooked, boiled or fried.

Similarly, Assegid and Tesfaye (10) reported that the collected wild edible plants, trees comprised 18 species, and the remaining 12 species were shrubs. Tilahun and Mirutse (11) also reported that study of wild edible plants in Kara and Kwego agree with this results. However, disagree with the report from Derashe and Kucha district which revealed that wild edible materials are largely collected from shrubs. These differences might be due to environmental differences.

The present study, indicated that in 51 plant species, the fruit part is used by many (28) leaves (2), stem (3), root (1), rhizome (1), seed (6), stem / leaf (6), fruit / seed (3) and flower / fruit (1) respectively (Fig. 4). These results revealed with Assegid and Tesfaye (29), that reported as 80% of consumed edible parts are fruits. The dominance of fruits are edible parts has also been reported in most previous studies that undertaken in Ethiopia (9,10). Roots are most used parts for medicinal and edible purposes. This variation might be due to the variation of plant species is adapting to different ecological zone and culture of the people in different area.

12 species of medicinal plants belonging to Malvaceae, Liliaceae, Sapindaceae, Caricaceae families were identified from the study area (Table 1). Most of the species are reported from Cucurbitaceae family. This indicated that, medicinal plants are more in number as compare to edible plants. The local people had in depth indigenous knowledge on uses of plant remedies for treatment of human diseases and livestock ailments from natural vegetation.

These medicinal plants are used to treat about dysentery, skin disease and eye infection types of human ailments and wounds of mouth and constipation types of livestock ailments. Most of them, about *Alternanthera sesselis* plant species are used to treat fever followed by *Amaranthus spinosus* plant species used to treat internal bleeding and excessive mensuration respectively (Table 2).

The relative high number of edible plants in the study area may be due to the more intensive utilization of plant by the local communities and diverse agroecology. As regards the mode of consumption 16 are consumed raw, 18 boiled, 4 in juice form, 10 either raw/boiled 3 as decoction (Fig. 6).

The present study indicates that the area harbors a high diversity of wild edible plants. Out of 51 plant species, 21 were abundant, 26 common and

4 uncommon to this area. The uncommon plant species to this area are being threatened due to unplanned exploitation. The inhabitants revealed rich presence of many of these species in the area in the past, which has restricted now, to certain patches. If immediate steps for their sustainable utilization and conservation are not taken, these species may reach to the status of threatened in the area.

During this research work, it was found that for most of the herbaceous species, the edible parts were not even sold in local markets. This was due to decline in traditional knowledge, limited availability or availability only in a particular season. Scrutiny of literature revealed that much work have been done to document medicinal uses of plants, but scanty and sporadic researches have been carried out to document the edible wild herbaceous plant species in the tribal inhabited regions of state Jharkhand (12,13).

The study revealed, angiosperms were represented by 27 families comprising of 3.92% of monocotyledons and 96.07% of dicots (Fig. 2). Among the wild edible plants, 14 were obtained from the wild, and 11 of the wild edible plants were obtained both from the wild and home garden while 26 of the wild edible plants were obtained from the home garden (Fig. 3).

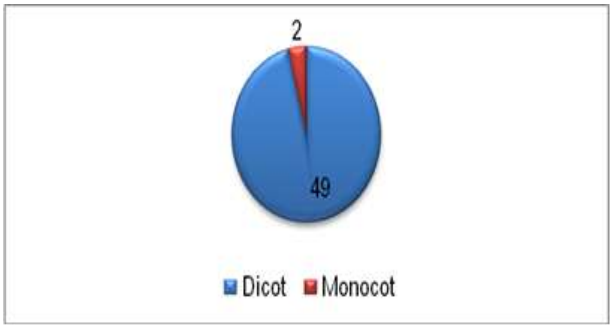


Fig. 2. Cotyledon wise distribution of identified plants

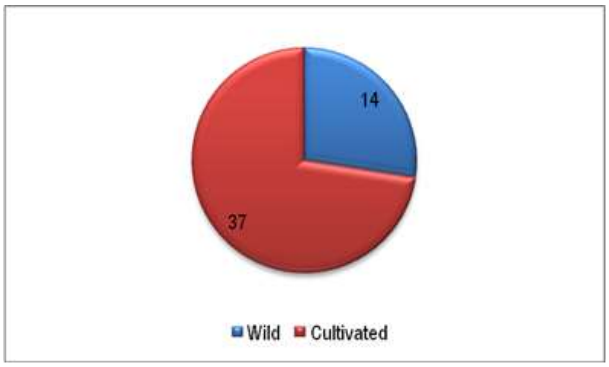


Fig. 3. Distribution of nature of plant

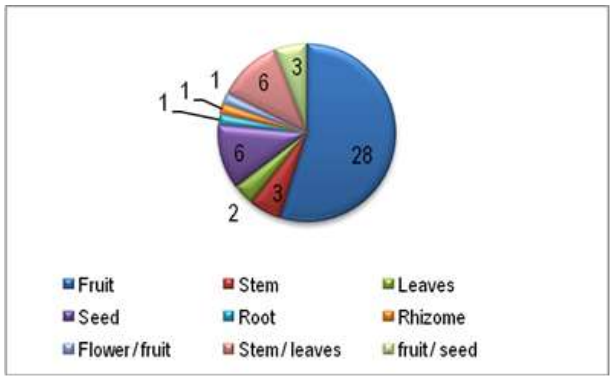


Fig. 4. Parts used from the identified plants

The parts consumed included fruits, seed, stem, leaf, fruits were the dominant edible parts followed by leaves consumed by the people in the study area. The dominance of fruits as edible parts has also been reported in most previous studies (9,14), contrary to this findings. Ali--shtayeh *et al.,* (15) reported leaves and stems as the most widely used parts of wild edible trees and shrubs in the West Bank of Palestine, this difference might be due to variation in the available species and culture of the communities with respect to food preference and preparation.

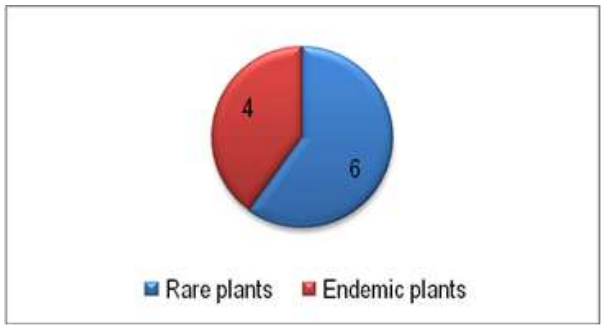


Fig. 5. Identified rare and endemic plants in the study area

The majority of wild edible plants were rare in the study area (Fig. 5), this could be attributed to anthropogenic and natural factors in the study area. Their availability in the study area was also influenced by seasonal variation most of them are scarce during the dry season. Balemie and Kebebew, (176) also reported that the availability of wild edible plants varies depending on ecological and climatic conditions. Wondimu *et al*., (17) reported that the majority of wild edible plants are rare in

their respective study area due to continued destruction of their habitats and over harvesting. Most of the wild edible plants collected during the study period were accessible. They can easily be available on market. Sources are rarely available on market. This could be attributed to season and increase distance to harvest areas. Some examples of commonly available wild edible plants in the study area were *Oryza sativa*, *Amaranthus viridis*, *Amaranthus spinosus*, *Alternanthera ficoidea*, *Cissus quadrangularis,* and *Solanum lycopersicum* (Table 1).

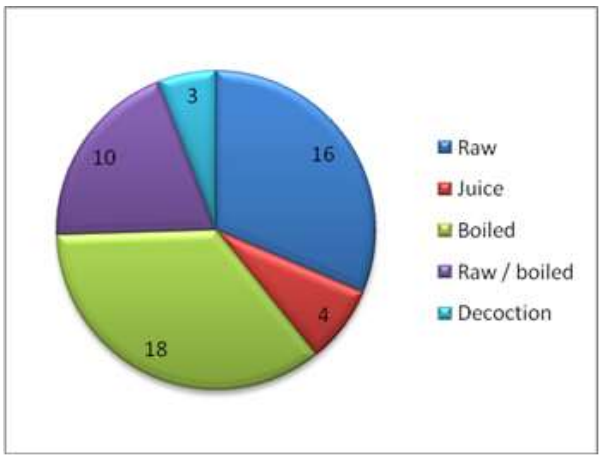


Fig. 6. Mode of utilization of wild edible plants in a selected study area

Among all the edible plants, 4 endemic species were recorded (*Pithecellobium dulce, Brassica nigra, Phyllanthus acidus* and *Talinum fruticosum*). The presence of endemic species illustrates the fact that the informants have a deep knowledge of their environment, since the four of them are not very abundant and can be found only in certain areas with one species. Most of wild species are gathered from waste and uncultivated land or from shrub land and in the roadsides.

Modern crop production of any region is based on only a few plant species. However, many less-recognized plant species continue to be grown, managed, or collected particularly in the rural areas of developing economics. Thus, these less – recognized plant contribute to the live hood soft poor and to the agricultural bio-diversity. Some of the species called underutilized plant species are characterized by the fact that they are locally rare. Their current use is limited relative to their economic potential. Most of the underutilized plant species can benefit from marketing development as a means to support their sustained use and help forester the conservation of agro biodiversity, while generating sustainable income for the native people.

The Mediterranean area shows that the majority of the EWPs used for human consumption belong to the Asteraceae family as they are considered to be particularly appetizing and above all widely – known (18-20). As other studies (21) have also shown, the data demonstrate that collecting and consuming EWPs still is an important local activity. Moreover, many species are also known and collected for their medicinal properties (e.g. 22; 23). In central Italy in particular ethnobotanical knowledge is very much alive (24), while nutraceutical properties have been studied extensively (25,26). With reference to the informants there was a greater number of women, who provided more details than the male informants, probably because collecting and cooking in wild plants is almost exclusively a female occupation and it is them who possess the greatest knowledge of EWPs, in agreement with other studies (18,25).

Table 1. Survey of wild edible plants in the selected study area

S. No.	Name of the plant	Family	Local name	Medicinal uses
1	*Abelmoschus esculentus* (L.) Moench	Malvaceae	Lady's finger	Increase the memory power
2	*Achras sapota* L.	Sapotaceae	Sapota	Treatment of fever, hemorrhage, wounds, ulcers, diarrhea and indigestion
3	*Allium cepa* L.	Liliaceae	Onion	It helps build a better and stronger immunity and control diabetes
4	*Alternanthera ficoidea* (L.) sm.	Amaranthaceae	Ponnanganni	Antiviral agent

S. No.	Name of the plant	Family	Local name	Medicinal uses
5	*Alternanthera sessilis* (L.)R.Br.ex Dc.	Amaranthaceae	Koduppai keerai	Cure fever, diarrhea and dysentry
6	*Amaranthus caudatus* L.	Amaranthaceae	Sigappu thandu keerai	Cure the diuretic
7	*Amaranthus spinosus* L.	Amaranthaceae	Araikeerai	Treatment of internal bleeding, diarrhea and excessive menstruation
8	*Amaranthus viridis* L.	Amaranthaceae	Kuppai keerai	Cure dysentery, purgative and eye infection
9	*Anacardium occidentale* L.	Anacardiaceae	Kollampazham	Cure malaria and reduce blood sugar levels
10	*Annona squamosa*L.	Anonaceae	Seethapazham	Cure fever, diarrohea, skin disease and asthma
11	*Arachis hypogaea* L.	Fabaceae	Groundnut	Cure the inflammatory and rheumatism
12	*Atrocarpus heterophyllus* Lam.	Moraceae	Jack fruit	Ulcer disease
13	*Brassica nigra* L.	Brassicaceae	Black mustard	Relief from rheumatism
14	*Borassus flabellifer*L.	Arecaceae	Panankai	Anti-inflammatory and antioxidant property
15	*Capsicum annuum* L.	Solanaceae	Chilli	Cure the back pain, allergic rhinitis (hay fever), burning mouth syndrome
16	*Cardiospermum helicacabum* L.	Sapindaceae	Mudakathan	Rheumatism
17	*Carica papaya* L.	Caricaceae	Papaya	Skin disease
18	*Cissus quadrangularis* L.	Vitaceae	Pirandai	Regularize menstruation
19	*Citrus limon* (L.) Osbeck	Rutaceae	Lemon	Cure the scurvy
20	*Citrus medica* L.	Rutaceae	Narthangai	Treatment of asthma, arthritis, headache and stomach ache
21	*Coccinia indica* Wight & Arn.	Cucurbitaceae	Covakai	Treatment of antidiabetic patient
22	*Cocos nucifera* L.	Arecaceae	Coconut	Bad odour of mouth, decrease the stone of urinary system
23	*Cucumis sativus* L.	Cucurbitaceae	Cucumber	Soft skin disease increase the water level in a body
24	*Cucurbita pepo* L.	Cucurbitaceae	Pumpkin	Remedy for internal parasites and purgative disorder
25	*Curcuma longa* L.	Zingiberaceae	Turmeric	Cure the skin disease
26	*Lagenaria siceraria* (Mol.) standley	Cucurbitaceae	Suraikai	Treat the purgative, ulcer, stomach acidity
27	*Mangifera indica* L.	Anacardiaceae	Mango	Asthma, diabetes
28	*Manihot esculanta* crantz	Euphorbiaceae	Maravallikizhangu	Cure indigestion, diarrhoea and dysentery

S. No.	Name of the plant	Family	Local name	Medicinal uses
29	*Momordica charantia* L.	Cucurbitaceae	Bitter guard	Antidiabetic, anticancer and antiinflammation
30	*Moringa oleifera* Lam.	Moringaceae	Moringa	Used to antitumor, antipyretic antiulcer, antioxidant
31	*Murraya koenigii* (L.) Sprenge	Rutaceae	Curry leaf	Eye vision, increase hair growth
32	*Musaparadisiaca* L.	Musaceae	Banana	It stimulate the production of hemoglobin in the blood
33	*Oryza sativa* L.	Poaceae	Rice	Dysentery, energy level of the body
34	*Phyllanthus acidus* (L.) skeels	Phyllanthaceae	Gooseberry	Cure skin disease and relief from itching
35	*Phyllanthus emblica* L.	Phyllanthaceae	Gooseberry	Treatment of diarrhea, jaundice and inflammation
36	*Pithecellobium dulce* (Roxb.) Benth	Fabaceae	Kodukapuli	Treat the gum ailments and toothache and bleeding
37	*Psidium guajava* L.	Myrtaceae	Guava	Maintain sugar level, eye vision problem, boost of immunity system
38	*Punica granatum* L.	Lythraceae	Pomegranate	Cure fever and maintain the blood circulation level
39	*Ricinus communis* L.	Euphorbiaceae	Castorbeen	Skin disease
40	*Saccharum officinarum* L.	Poaceae	Sugarcane	Treat to sore throat
41	*Sesbania grandiflora* (L.) Poiret	Fabaceae	Agathi keerai	Treatment of swellings, rheumatism
42	*Solanum lycopersicum* L.	Solanaceae	Tomato	Used to treatment of cancer, high chloestrol, depression, improve vision
43	*Solanum melongena* L.	Solanaceae	Brinjal	Treated with the analgesic, antiasthematic
44	*Solanum nigrum* L.	Solanaceae	Manathakali	Cure skin disease and mouth wounds
45	*Solanum torvum* sw.	Solanaceae	Sundakai	Treat fevers, coughs, asthma and sore throat
46	*Talinum fruticosum* (L.) Juss	Talinaceae	Paruppu keerai	Treatment of diabetes
47	*Tamarindus indica* L.	Caesalpiniaceae	Tamarind	Cure skin disease
48	*Terminalia catappa* L.	Combretaceae	Almond	Treat the jaundice and dysentery
49	*Trichosanthes cucumerina* L.	Cucurbitaceae	Snake guard	Treat the emetic purgative
50	*Vigna mungo* (L.) Hepper	Fabaceae	Black gram	Supportive, cooling and astringent properties
51	*Zingiber officinale* Roscoe	Zingiberaceae	Ginger	Digestion, stomach problem, regulate the

S. No.	Name of the plant	Family	Local name	Medicinal uses
				mensuration

Table 2. Identified rare and endemic plants in the study area

S. No.	Rare plants (6)	Endemic plants (4)
1	❖ *Cardiospermum helicacabum* L. ❖ *Curcuma longa* L. ❖ *Phyllanthus emblica* L. ❖ *Terminalia catappa* L. ❖ *Vigna mungo* (L.) Hepper ❖ *Zingiber officinale* Rose	❖ *Brassica nigra* L. ❖ *Phyllanthus acidus* (L.) Skeels. ❖ *Pithecelloblum dulce* (Roxb) Benth ❖ *Talinum fruiticosum* (L.) Juss

Conclusion

The study revealed that all household members of the study area were involved in the collection and consumption of wild edible plant species. This helps to ensure the maintenance of indigenous knowledge associated with wild edible plant species. The local knowledge about the nutritional composition and side effects of the wild edible plant species is very scanty and little is known about undesirable side effects such as toxicity originating from the wild edible plants. Apart from their food and medicinal value most of the identified wild edible plant species in the study area are used by the community for other different purpose. Thus this has led to a high level of threats of the wild edible plant species in the study area. In addition, many of the wild edible plants found in the study area are found to be under growing pressure, due to anthropogenic and socio economic factors. This has resulted in the dwindling of the species of wild edible plants and the associated indigenous knowledge.

REFERENCES

1. Guijt, I., F. Hinchcliffe and M. Melnyk, (1995). The hidden harvest: the value of wild resources in agricultural systems. A project summary, *International Institute for Environment and Development*, London.
2. Wilson, K.B. (1990). Ecological dynamics and human welfare: A case study of population, health and nutrition in southern Zimbabwe. University College, London.
3. Balick, M.J., and A. Cox, (1996). Plants, People and culture. The science of Ethnobotany. New York: Scientific America.
4. Ju, Y., J. Zhuo, B. Liu, and C. Long (2013). Eating from the wild: diversity of wild edible plants used by Tibetans in Shangri – laregion, Yunnan, China. *J. Ethnobiol. Ethnomed*. **9**: 28.
5. Gamble, J.S., and C.E. Fischer, (1915-1936). Flora of presidency of Madras, Vols. 1-3 Adlard and sons Ltd., London.
6. Hooker, J.D. (1872-1897) .The flora of British India. Reeve & Co. Ltd., London.
7. Getnechokole, (2011). An ethnobotanical study of plants used in traditional medicine and as wild foods in and Tara Gedam and Amba Remnant forests in Libokemkem Wereda, South Gonder Zone, Amhara Region Ethiopia (published thesis)
8. Teklehaymanot, T., and M. Giday, (2010). Ethnobotanical study of wild edible plants of Kara and Kwego semi-pastoralist people in lower Omo River Valley, Debub Omo Zone, SNNPR Ethiopia. *J. Ethnobiol Ethnormed*. **6**: 23.
9. Asfaw, Z., and M. Tadesse, (2001). Prosepects for sustainable use and development of wild food plants in Ethiopia. *Econ. Bot.* **55**: 47-62.
10. Assefa, A., and Tesfaye, (2011). Wild edible trees and shrubs in the semi arid low lands of Southern Ethiopia, Southern Agricultural Research Institute, Hawassa. *Ethiop. J. Sci. Develop.* **1**: 1-15.
11. Tilahun, T., and M. Giday, (2010). Ethnobotanical study of wild edible plants of Kara and Kewegosemipastoralist people in lower Omo River valley, Debubomo Zone, SNNPr, Ethiopia. *J. Ethnobiol. Ethnomed.*
12. Singh, G., and J. Kumar, (2012). Traditional knowledge on some less known wild edible plants used among Munda tribe of Jharkhand. *The Ecoscan* **3&4**: 153-155.

13. Singh, G., and J. Kumar, (2015). Diversity of wild edible yams and its traditional knowledge among Munda tribe of district Khunti, Jharkahand, India. *Int. J. Bioassays* **4**(10): 4440-4442.
14. Addis, G., K. Urga, and D. Dikasso, (2005). Ethnobotanical study of edible wild plants in some selected districts of Ethiopia. *Hum. Ecol.* **3**(1): 83-118.
15. Ali-shtayeh, M.S., R.M. Jamous, J.H. Al-Shafie, W.A., F.A. Elgharabah Kherfan, K.H. Qarariah, I.S., Khdair, *et al.,* (2008). Traditional knowledge of wild edible plants used in Palestine : a comparative study. *J. Ethnobiol. Ethnomed*. **4**: 13.
16. Balmie, K., and Kebebew, F. (2006). Ethnobotanical study of wild edible plants in Derasha and Kucha Districts, South Ethiopia. *J. Ethnobiol. Ethnomed*, 2: 53.
17. Wondimu, T., Z. Asfaw and E. Kelbessa, (2006). Ethnobotanical study of food plants around 'Dheeraa' Town, Arsi, Ethiopia, SINET: *Ethiop. J. Sci*. **29**(1): 71-80.
18. Forbes, M.H.C. (1976). Gathering in the Argolid: a subsistence subsystem in a Greek agricultural community. *Ann. Ny. Acad. Sci*., 251-264.
19. Della, A., D. Paraskeva Hadjichambi and A.C. Hadjichambis, (2006). An ethnobotanical survey of wild edible plants of paphos and Larnaca country side of Cyprus. *J. Ethnobiol. Ethnomed*. **2**: 34
20. Dogan, Y. (2012). Traditionally used wild edible greens in the Aegean Region of Turkey, *Acta. Soc. Bot. Pol*. **81**(4): 245-255.
21. Ghirardini, M.P, M. Carli, N. Del vecchio, A. Rovati, Cova and F. Valigi, (2007). The importance of a taste a comparative study on wild food plant consumption in twenty one local communities in Italy. *J. Ethnobiol. Ethnomed*. **3**: 22.
22. Guarrera, P.M., G. Forti and S. Marignoli, (2005). Ethnobotanical and ethnomedicinal uses of plants in the district of Acquapendente (Latium, Central Italy). *J. Ethnopharmacol*. **96**(3): 429-444.
23. Passalacqua, N.G., P.M. Guarrera and G. De Fine, (2007). Contribution to the knowledge of the folk plant medicine in Calabria region (Southern Italy). *Fitoterapia* **78**(1): 52-68.
24. Gurrera, P.M., and M.L. Leporatti, (2007). Ethnobotanical remarks on central and Southern Italy. *J. Ethnobiol. Ethnomed*. **3**: 23.
25. Ranfa, A., A. Maurizi, B. Romano and M. Bodesmo, (2014). The importance of traditional uses and nutraceutical aspects of some edible wild plants in human nutrition: the case of Umbria (Centrla Italy). *Plant Biosyst.* **148**(2): 297-306.
26. Maurizi, A, A. De Michele, A. Ranfa, A. Ricci, V. Roscini and R. Coli, (2015). Bioactive compounds and antioxidant characterization of three edible wild plants traditionally consumed in the umbria region (central Italy): *Bunis erucago* L. (Corn rocket), *Lactuca perennis* L. (Mountain lettuce) and *Papaver rhoeas* L. (Poppy). *J. Appl. Bot. Food Qual.* **8**: 109-114.

DOI:10.26524/KRJ298

Kong. Res. J. 6(2): 28-36, 2019
Publisher: Kongunadu Arts and Science College, Coimbatore.

RESEARCH ARTICLE

CONTROL OF FUSARIUM WILT DISEASE IN COWPEA PLANT (*VIGNA UNGUICULATA* L.). USING SECONDARY METABOLITES PRODUCED IN *BRADYRHIZOBIUM JAPONICUM*

Kannan, K.[1]*, V. Rajesh Kannan[2], N. Shibinaya[2], and M. Umamaheswari.[2]
[1]Department of Microbiology, The Madura college, Madurai -625 011, Tamil Nadu, India
[2] Rhizosphere Biology Laboratory, Department of Microbiology, School of Life Sciences, Bharathidasan University, Tiruchirappalli - 620 024, Tamil Nadu, India.

ABSTRACT

Rhizobium known for its nitrogen fixation and plant growth promoting capabilities which is symbiotically associative with legume plants. So forth *Rhizobium* used as biofertilizers in the agriculture. The ability of controlling plant diseases by using *Rhizobium* produced secondary metabolites as biocontrol agent is the current open area in the agriculture research. The nodules inhabited *Rhizobium* strains were selected for the production of secondary metabolites and the ability of controlling *Fusarium* was evaluated preliminarily by agar well diffusion assay. Four different *Rhizobium* were isolated, among that S1 cannot showed any inhibition, whereas S2, S3 and S4 were showed 11, 15 and 19mm of inhibition respectively. Among that S4 selected further and DNA isolated and identified using 16S rDNA gene sequencing. The sequences were submitted in genbank and got accession number MH165175. This organism was found to be *Bradyrhizobium japonicum* and mass cultured for compound extraction using organic solvents. The extracted secondary metabolite were purified using different chromatography techniques. The purified fractions were analyzed for the biocontrol of *Fusarium sp.,* isolated from infected cowpea and results showed fraction 4 showed 21mm zone of inhibition. Further the selected fractions were analytically characterized to know the compounds present. Finally the purified compounds were evaluated for its biocontrol behavior against *Fusaium* sp., and plant growth promotion in *in vitro* conditions.

Keywords: Cowpea, Rhizobium, Biocontrol, PGPR and Secondary Metabolites.

1. INTRODUCTION

Cowpea (*Vigna unguiculata* L.), is one of the most important legume crop grown in the tropical belt. This crop provides food, animal feed and cash for the rural population in addition to benefits to farmlands through *in situ* decay of root residues and ground cover due to the spreading habit of the plant. In addition, cowpea grain provides a cheap and nutritious food for relatively poor urban communities. Cowpea's high protein content, its adaptability to different types of soil and intercropping systems, its resistance to drought, and its ability to improve soil fertility and prevent erosion makes it an important economic crop in many developing countries (1). Cowpea due to its wide adaptation across the regions and soils harbours a number of diseases which considerably influence growth and productivity potential. One of the prime cowpea disease is *Fusarium* wilt caused by *Fusarium oxysporum* Schlechtend f.sp. *ciceris* is a major constraint to cowpea production throughout the world and particularly in the Indian subcontinent and the Mediterranean basin (2). *Fusarium oxysporum* can survive in soil several years by means of chlamydospores, which markedly reduce the potential of crop rotation as a disease management strategy. Efforts must be addressed toward developing new alternatives for more effective disease management. In current scenario use of chemical fertilizer for biocontrol of soilborne plant pathogens including *F. oxysporum* has been shifted to the option of green technology that have an agriculture importance.

Biological control agents for plant diseases are currently being examined as alternatives to synthetic pesticides due to their perceived level of safety and minimal environmental impacts. Strains of several bacterial species such as *Bacillus*, *Pseudomonas* and recently the *Rhizobium* group used as the biocontrol agents (1,3). As compared to the other biocontrol agents, Rhizobia offer the great advantage of symbiotic nitrogen fixation by association with legumes. Among the *Rhizobium* group, *Rhizobium leguminosarum*, *Sinorhizobium meliloti* and *Bradyrhizobium japonicum* have been used successfully against fungal pathogens belonging to the genera *Macrophomina, Rhizoctonia* and *Fusarium*. (4-6).

*Correspondence: Kannan, K., Department of Microbiology, The Madura college, Madurai -625 011, Tamil Nadu, India.
E.mail: kannan.bdu.micro@gmail.com

Induction of host defenses can be local and or systemic in nature, depending on the type, source, and amount of stimuli. Induced systemic resistance is one of the mechanisms of disease suppression by fungal pathogens by many microorganisms (7,8). Rhizobia have several mechanisms of action that allow them to control pathogens. In general, competition for nutrients, niche exclusion, induced systemic resistance and antifungal metabolites production are the chief modes of biocontrol activity in PGPR. The aim of the present work is to characterize and select *Rhizobium* isolates with antagonistic activity against wilt disease in cowpea as well as extraction of their secondary metabolites from selected rhizobia and their effects on *Fusarium* and disease development in Cowpea were assessed *in vitro* nursery conditions.

2. MATERIALS AND METHODS

2.1. Sample Collection

Root from young and healthy seedling of cowpea (*Vigna unguiculata* L.) plants was collected from Viralimalai village, Pudukkottai district of Tamil Nadu, India. The collected samples were kept in plastic bags without affecting the root with nodules, brought to the laboratory under sterile conditions and stored in refrigerator for further processing.

2.2. Isolation and Identification of Rhizobium

Healthy cowpea nodules were detached from the root and further isolation of root nodulating rhizobia was carried out. The detached root nodules were washed in tap water to remove the adhering soil particles from nodule surface. Nodules were dipped in 0.1% mercuric chloride ($HgCl_2$) solution for 30 sec and later washed successively ten times with sterilized distilled water to remove the traces of toxic $HgCl_2$. Surface sterilized nodules were transferred in test tube containing 5 ml sterilized distilled water. These nodules were crushed with the help of sterilized glass rod to obtain a milky suspension of bacteroides. These were streaked on YEMA medium containing congo red. The plates were sealed by parafilm to avoid contamination and incubated at 28ºC for 7 days and make daily observations for the appearance of typical colonies of rhizobia (9). After the incubation period from the pool of well grown colonies were picked up and selected four different colonies named as S1, S2, S3 and S4. Further the cultures subjected to gram staining for the shape, size, and arrangement of bacterial isolates (10).

2.3. Biochemical Characterization and Molecular Identification

Various biochemical tests were carried out were indole test, methyl red test, Voges Proskauer test and citrate utilization tests based on standard microbiological manual in order to confirm primarily by the *Rhizobium* genus for all the four isolates. In order to confirm the *Rhizobium* genus secondarily by subjected to Bromothymol blue test and Ketolactose test**,** in which **t**he Yeast Extract Mannitol was enriched with 1% (w/v) Bromothymol blue to selectively identify fast *Rhizobium.* All the four samples S1, S2, S3 and S4 were subjected to grow on BTB added YEM media for 48 hrs at 28^{o}C. Positive sample showed yellow color to acid production after incubation. Whereas in Ketolactose test, a loopful of the inoculums from a fully grown culture slant (7 days old culture) was transferred to a petri plate containing the ketolactose agar medium. After incubation for 5 days at 27^{o}C the plates were flooded with a shallow layer of Benedict's solution. After pouring the plates were incubated for one hour at 30^{o}C without any disturbance. The absence of yellow coloration around bacterial colonies confirms the presence of *Rhizobium* (11).

2.4. Molecular Identification

Overnight fresh bacterial broth cultures (2 ml) were taken and centrifuged at 10000 rpm for 5 minutes and the supernatant was discarded. To the pellet, 570 µl of TE buffer, 30 µl of 10% SDS were added, mixed thoroughly and incubated at 37°C for 1 hour. After incubation, 5M NaCl was added and mixed thoroughly by vortex. 80 µl of CTAB/NaCl solution was added, mixed thoroughly and incubated at 65°C for 10 min. Equal volume (0.7 – 0.8ml) of chloroform or isoamyl alcohol was added, mixed and centrifuged at 10000 rpm for 5 min. The viscous supernatant was transferred to a fresh centrifuge tube (without disturbing the interface); equal volume of phenol/chloroform or isoamyl alcohol was added and spun at 10000 rpm for 5 min. The aqueous supernatant was transferred to a fresh centrifuge tube and 0.6 volume of ice cold isopropanol was added and incubated for 20 min at -20°C. Later, the tubes were centrifuged at 10000 rpm for 5 minutes. To the pellet, 70% of ethanol was added and centrifuged. The pellet was stored at -20°C after adding TE buffer or sterile mQ water. For running the sample, 0.8% of agarose was prepared in TE buffer. To 50 ml of agarose 3 µl of ethidium bromide was added and casted in the boat which contains comb. After cooling, the comb was removed and the sample was loaded. To 5 µl of the sample 2 µl of the gel loading dye was added and loaded in the

respective well, and electrophoresis was done it 50 V (12).

2.4.1. 16S rRNA PCR Amplification

Amplification of the 16S rRNA gene was conducted using universal primers: 27F(5'-GAGAGTTTGATCCTGGCTCAG-3'), 1495R (5'-CTACGGCTACCTTGTTACGA-3'). The final products were analyzed through electrophoresis on 1.2% agarose gel and stained with 0.5μg ml^{-1} ethidium bromide. The PCR products obtained were purified and sequenced using Sanger's dideoxynucleotide chain termination method and were sequences at Amnion sequencing Pvt. Ltd. India (Bangalore). The sequences obtained were aligned with previously published sequences available in NCBI using BLAST (13). The phylogenetic and evolutionary analyses were conducted using MEGA 5 software (14).

2.5. Isolation and Identification of Pathogenic Fungi

Infected stem (Brick red tissue in stem indicates wilt disease) region of cowpea plants were collected and submerged in 5% sodium hypochlorite for five minutes. After this treatment, they were extensively washed with sterile distilled water and placed on Petri dishes containing potato dextrose agar (PDA, Difco) and incubated at 22°C for 48 hrs. Morphological identification are done according to the standard taxonomic key included colony diameter, texture, colour identified in their sporulation state by staining with lactophenol cotton blue.

2.6. Identification of Potential Rhizobium

2.6.1. Antifungal Activity of Rhizobium Extracts against Fungal Pathogen

The *Rhizobium* strain S1, S2, S3, S4 were subjected to antifungal activity using agar well diffusion method against the *Fusarium* sp. on Muller Hinton Agar plates. The test fungi was spread over the agar plate using the sterile cotton swab and well were created on the agar. 100µl bacterial culture was added to into the well and incubated for 4 days at room temperature. Finally plates are observed for zones of inhibition and their diameter was measured with the help of antibiotic zone scale. Among the four isolate S3 and S4 showed better inhibition than the other isolates.

2.7. Extraction and Purification of Secondary Metabolites from Rhizobium Extracts

The *Rhizobium* isolates S4 was mass cultivated in Yeast Extract Mannitol broth and incubated at 37°C for 10 days with periodical shaking at 150 rpm. After the incubation period, the cultures are taken out and then centrifuged at 5000 rpm for 10 mins, the cell free supernatant was transferred to another flask. To this equal volume of different solvents such as ethyl acetate, diethyl ether, chloroform, and hexane was added and the compound was extracted using solvent extractor. The crude extracts were tested for their antimicrobial activity against *Fusarium* sp. The extracted compound was separated by column chromatography using silica gel as the packing material. The fraction was eluted by using chloroform: methanol: water (5:6:4) as the solvent system. Different fractions were eluted from column chromatography and checked for antifungal activity (15).

2.8. Characterization of Pure Compound

The collected fraction from *Rhizobium* isolate (S4) with higher antifungal activity were subjected to FT-IR (Perkin Elmer, Spectrum MRX-1 model) and also analyzed using GC-MS (model Q-Mass 910, Perkin Elmer) (16).

2.9. In vitro Seed Germination

The viability of the seeds were tested by using Tetrazollium chloride test (TZ test). The seeds were soaked in 0.1 % of 2, 3, 5 triphenyl tetrazollium chloride for few minutes and the seeds were evaluated for the viability. Sound tissues produce a normal red colour and resist the penetration of tetrazollium, and those seeds were said to be viable. After the viability test cowpea seeds were sterilized by using 70% ethanol followed by 0.1% mercuric chloride before rinsing with sterilized water. The experiments were carried out in a positive, negative control and preventive method. Positive control (cowpea seed+ pathogen + carboxy methyl cellulose), negative control (cowpea seed+ distilled water + carboxy methyl cellulose) and preventive test using 5 dose of fractions from nodule compound as well as from the *Rhizobium* culture compound (10, 20, 30, 40, and 50 µl) on seed (cowpea seed + pathogen + compound doses + carboxy methyl cellulose). The seeds were coated with respective materials and air dried overnight. The seed were placed on a sterile filter paper in petriplates. Water is sprinkled at regular intervals and kept at a place receiving sunlight for 7 days. The germination rate was assessed in triplicates after 7 days of incubation using the formula below (17).

$$\text{Germination rate (\%)} = \frac{\text{Number of germinated seeds}}{\text{Number of total seed tested}} \times 100$$

3. RESULTS AND DISCUSSION

3.1. Collection of Nodule and Isolation of Rhizobium

Healthy cowpea (*Vigna unguiculata*) plants were carefully uprooted from the cowpea field, Viralimalai village, Pudhukottai district of Tamil Nadu, India. The roots along with nodules were stored in sterile polythene bags at 4^{o} C for further analysis. The surface sterilized root nodules were crushed and the suspension was streaked over the YEMA medium with the addition of congo red. Growth of large opaque mucoid elevated glistening colonies in the medium shows the presence of *Rhizobium.* Four different colonies S1, S2, S3 and S4 were selected for further study (Fig. 1).

Fig. 1. Cowpea plant root with fresh nodules and rhizobium colonies on the YEMA plate

3.2. Identification of Rhizobium

3.2.1. Morphology of Isolates

All the four isolates S1, S2, S3 and S4 were found to be circular, mucoid and raised colonies. The shapes of the isolates were identified by the staining techniques. Colony size, shape, margin, elevation and opacity of all the four bacterial isolates were tabulated (Table 1).

3.3. Biochemical characterization

The biochemical characterization is carried out to identify the genus of the isolated bacteria and along with two confirmatory tests (Table 2). All isolates were streaked on Bromothymol blue added YEM selective media for further confirmation. Strains S1, S3, S4 showed growth in 2 days and turned YEM media from blue to yellow confirming their nature of being fast growers and acid producers whereas strain S2 was a slow grower and acid producer (Fig. 2). Whereas in the ketolactose test the plates were flooded with Benedict's reagent and incubated for one hour. The excess reagent is drained off and the plates were checked for colour change. All the four isolates used lactose from the medium and showed growth. No yellow colourations were found around the colonies after adding Benedict's reagent. Absence of yellow colouration around the colonies denotes the presence of *Rhizobium.*

3.4. Molecular Identification

The Rhizobium isolates having higher antagonistic S3 and S4 were found to be the potential isolates for acting against the fungal pathogen. Hence the genomic DNA was isolated for those particular two isolates (S3 and S4) by using C-TAB method. The isolated DNA was identified using 0.8% agarose gel followed by observation on ultraviolet trans illuminator which revealed sharp high molecular weight bands of DNA. This indicated that the DNA was of good quality and suitable for PCR analysis.

Table 1: Morphological characteristics of the four isolates

S. No.	Strain characters	S1	S2	S3	S4
1.	Colony Shape	Circular	Circular	Circular	Circular
2.	Size	4mm	6mm	6mm	5mm
3.	Colour	Pinkish white	White	White	Milky white
4.	Opacity	Translucent	Transparant	Transparant	Translucent
5.	Motility	Motile	Motile	Motile	Motile
6.	Bacterium shape	Rod	Rod	Rod	Rod
7.	Gram staining	Gram negative	Gram negative	Gram negative	Gram negative

Table 2: Biochemical test of the four isolates

Isolates	Indole	MR	VP	Citrate	Catalase	Oxidase	Urease	Nitrate Reduction	Lipase
S1	-	-	+	-	+	-	+	+	+
S2	-	-	+	+	+	-	+	+	+
S3	-	-	+	+	+	-	+	+	+
S4	-	-	+	-	+	-	-	+	+

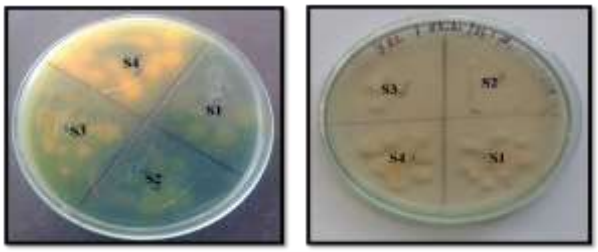


Fig. 2. Bromothymol blue test and Ketolactose test

Notes: In the first plate colour change of the medium from blue to yellow shows the presence of fast growing acid producers and in the second plate addition of Benedict's reagent does not cause any change in the medium.

The DNA of the bacterial isolates S3 and S4 were amplified by performing 16S rRNA gene of the isolated DNA using forward primer 27F (5'-GAGAGTTTGATCCTGGCTCAG-3') and the reverse primer 1495R (5'-CTACGGCTACCTTGTTACGA-3'). The conformation of amplification was done by agarose gel electrophoresis by running 5µl of PCR reaction mixture on 1% agarose gel**.** The amplified product was sequenced in AMNION Pvt. Ltd., Bangalore. The obtained sequences were compared with NCBI databases through BLAST analysis. 16S rRNA gene of the bacterial isolate S4 showed 99.9% similarity with the *Rhizobium* genus. Then the sequence was submitted in Gene Bank for the accession number MH165175 and the organism found to be as *Bradyrhizobium japonicum.*

3.5. Isolation and Identification of Pathogenic Fungi

The wilted stem of cowpea plant showed the presence fungal growth in the PDA plate. The fungal colony is macroscopically and microscopically identified (Fig. 3). Macroscopical colony morphology shows cottony mycelium with pale brown to yellowish brown zonation. The isolated fungi subjected to lactophenol cotton blue staining were observed under microscope. The spore formation and hyphal structures were identified and confirmed to be *Fusarium* strain. The staining shows the presence of sickle shaped conidiospores and septate spores which is the characteristic feature of *Fusarium* sp. The isolated *Fusarium* strains were stored at 5°C in tubes containing PDA.

Fig. 3. Isolation and identification of fungi from infected cowpea plant

Notes: a. Brick red tissue in stem indicates wilt disease, b. Pure culture of plant pathogen on PDA plate showed white cottony colony, c. Lactophenol cotton blue staining showed sickle shaped spore and septate.

3.6. Identification of Potential Rhizobium Antifungal Activity by Agar Well Diffusion Method

All the isolates were cultured in YEM broth and the supernatant was collected by centrifugation. The supernatant was then tested for antifungal activity against *Fusarium* sp., (Fig. 4.). The zone of inhibition was found around the wells containing the supernatants. There was no zone clearance found around the S1 supernatant, whereas the other three cultures showed zone of inhibition against the pathogen. Among them S2 showed 11mm, S3

showed 15mm and S4 showed 19mm of inhibition against *Fusarium* sp.,

Fig. 4. Antifungal test of the culture supernatants, extraction from *Bradyrhizobium japanicum* against *Fusarium* sp.

Notes: a. S1, S2, S3 and S4-Culture supernatants, b. F1-F5-Rhizobium Fractions, C- Ethyl acetate

3.7. Extraction of Secondary Metabolites from Bradyrhizobium japanicum

The collected supernatant from the isolates S3 and S4 were extracted with equal volume of different solvents such as water, ethyl acetate, diethyl ether, chloroform, and hexane. The crude extract is tested for antifungal activity by agar well diffusion method. Among the two isolates the crude extract of S4 isolate obtained from ethyl acetate extraction showed maximum inhibition of 19mm and S3 by 12 mm of inhibition than in the other solvents used (Fig.4). So the ethyl acetate extract of S4 only taken for further purification of the secondary metabolite using column chromatography.

3.8. Purification of the Secondary Metabolite from Bradyrhizobium japanicum by Column Chromatography

In column chromatography 10 different fractions were collected at regular time intervals to get pure compounds. All the obtained fractions were tested for antifungal activity by agar well diffusion method in MHA plates and among them, fractions F4 and F5 showed inhibition against *Fusarium.* The zones of inhibition were measured to be 21 and 18mm for F4 and F5. The pure extract F4 were dried and further subjected for structure elucidation.

3.9. Characterization of extracted compounds

Purified compounds further characterized using FT-IR, IR spectrum and The FT-IR spectrum showed various peaks at different wavelengths which indicate the presence of various chemical groups in the extracted sample (Fig. 5). Several chemical groups also existed in the extracts from *Bradyrhizobium japanicum* which are represented in the FT-IR result showed absorption at 3441.85 (O—H), 2922.0 (COOH), 2851.93 (COOH) broad, 1541.58 (N=O), 1095.00 (C—O), 1462.67 cm^{-1} confirmed the presence of C—C=C (Fig.3). GC-MS results of bacterial extract compound showed that major peak formed at the retention time 4.235, 17.2 and 22.5 were Propenemide , Hexadecanoic acid methyl ester and 1,2 benzenedicarboxylic respectively which has responsible for antimicrobial and antifungal character (Fig.6).

3.10. In vitro Seed Germination in cowpea seeds

Before the seed germination test the seeds subjected to Tetrazolium chloride test (TZ test) to check the viability of the seeds. Color change of seeds from normal to red colour indicates that the seed is viable various color change into brown indicates dead tissue. After 7 days of incubation, the plant indexes such as root length, shoot height, and germination percentage have been observed and compared with positive and negative control (Fig. 7) (Table 3).

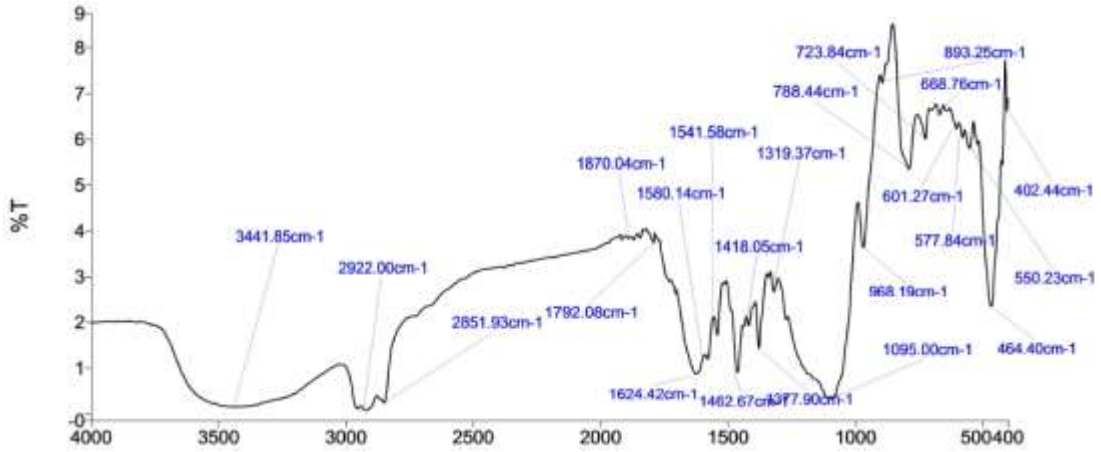


Fig. 5. Detection of compounds from bacterial extract using FT-IR

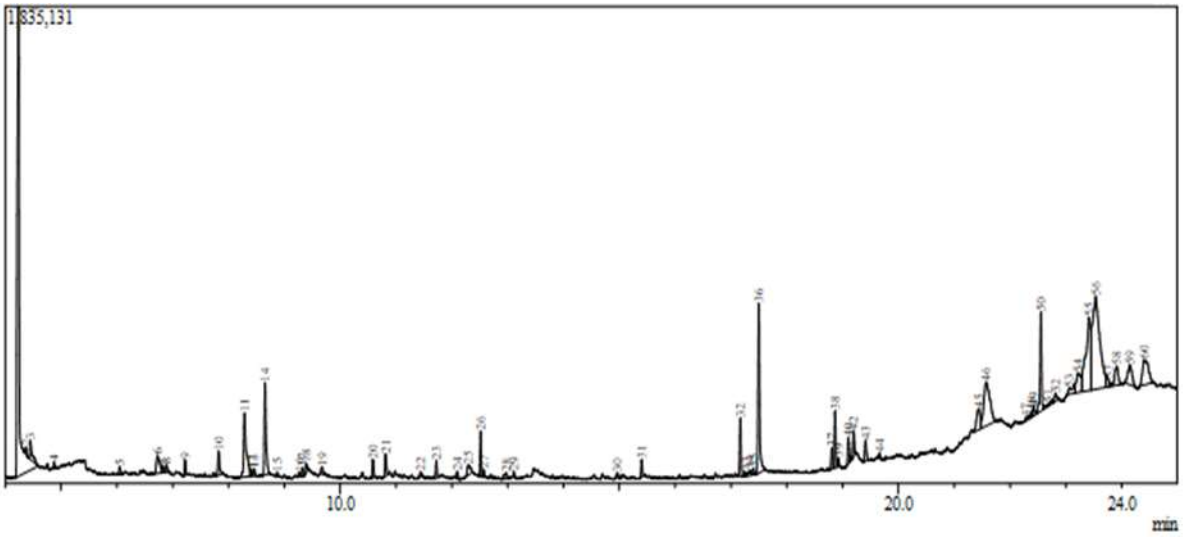


Fig. 6. GC-MS analysis of bacterial extract

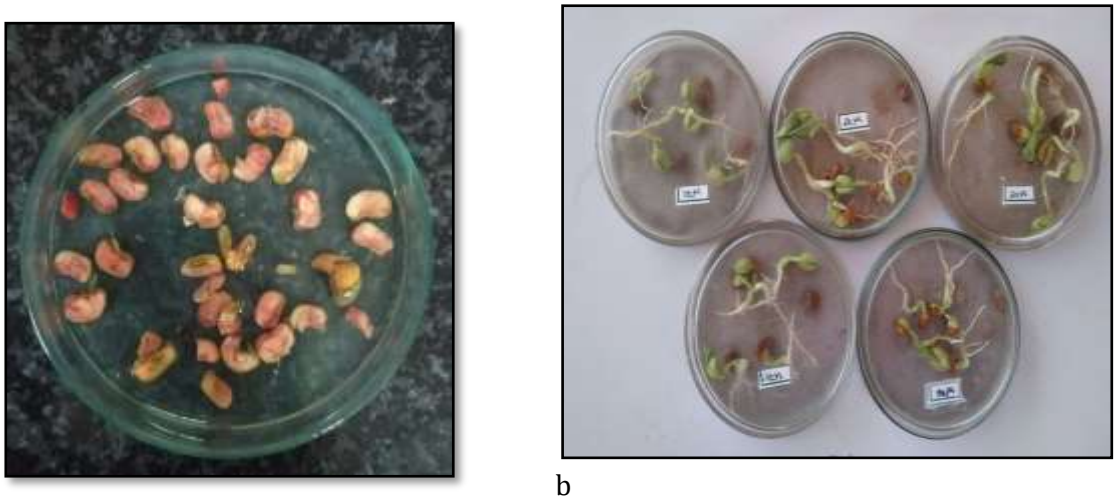


Fig. 7. *In vitro* seed germination in cowpea seeds

Notes : a – Tetrazollium chloride seed viability test, b - Seeds treated with different concentration (10, 20, 30, 40, and 50 µl) of the compound obtained from Rhizobium. c – Positive control (seed + pathogen) and negative control (seed alone), d – Germinated seeds

In comparison it is clearly seen that the compound untreated seeds show less or no growth as they cannot combat with the pathogen. But the compound treated seeds had grown well combating

the pathogen. Moreover as the concentration of the compound increases, the germination also increases to a greater extent. This shows that the compound has a good antifungal activity in *in-vitro* conditions.

4. CONCLUSION

Rhizobium was isolated from the root nodules of cowpea plant and identified using various biochemical and morphological characterization. The isolates were checked for its antagonistic nature by agar well diffusion methods. From those experiments, potential Rhizobium (S4) isolates were identified among the four *Rhizobium* isolates (S1, S2, S3 and S4) and secondary metabolites were extracted from the *Bradyrhizobium japonicum* (S4) using solvent extraction and their antifungal activity checked against *Fusarium* sp., Which causes wilt disease in the cowpea. The pure extracts were then analyzed using FT-IR and GC-MS for the identification of compounds. The identified compounds were treated with seeds and the biocontrol ability was checked by the *in vitro* seed germination methods.

From this study, it is proved that the production of the secondary metabolite from the antagonistic *Rhizobium* strain *Bradyrhizobium japanicum* isolated from cowpea root nodule control the growth of the plant pathogenic fungi *Fusarium* sp., and improves the growth.

The *in vitro* study showed better seed germination in the compound treated seeds to a greater degree of germination when compared to the positive and negative control. The compound extracted from the *Bradyrhizobium japanicum* is not only exhibit biocontrol against *Fusarium* wilt disease but also as improves the growth of the cowpea. These biometabolite compounds from microorganisms act as an alternative for the chemical control agents against wilt diseases in cowpea and which improves the soil fertility for the sustainable agriculture.

REFERENCES

1. Arfaoui, A., B. Sifi, A. Boudabous, I.E. Hadrami and M. Cherif, (2006). Identification of *Rhizobium* isolates possessing antagonistic activity against *Fusarium* oxysporum f. sp. ciceris, the causal agent of *Fusarium* wilt of chickpea. *J. Plant Pathol.* 67-75.
2. Nene, Y.L., M.P. Haware, M.V. Reddy, J.C., Phillips, E.L., Castro, S.R., Kotasthane and R.P. Sah, (1989). Identification of broad based and stable resistance to wilt and root-rots in chickpea. *Indian Phytopathol.* **42**(4), 499-505.
3. Kannan, K., and V. Rajesh Kannan, (2019). Characterization of the bioactive metabolite from a plant growth promoting rhizobacteria *Pseudomonas aeruginosa* VRKK1 and exploitation of antibacterial behaviour against *Xanthomonas campestris* a causative agent of bacterial blight disease in cowpea. *Arch Phytopathol. Plant Protect.*
4. Huang, H.C., and R.S. Erickson, (2007). Effect of Seed Treatment with *Rhizobiumleguminosarum* on *Pythium* Damping-off, Seedling Height, Root Nodulation, Root Biomass, Shoot Biomass and Seed Yield of Pea and Lentil. *J. Phytopathol.* **155**(1): 31-37.
5. Akhtar, M.S., and Z.A. Siddiqui, (2008). Biocontrol of a root-rot disease complex of chickpea by *Glomus intraradices*, *Rhizobium* sp. and *Pseudomonas straita*. *Crop protect.* **27**(3-5): 410-417.
6. Arora, N.K., S.C. Kang and D.K. Maheshwari, (2001). Isolation of siderophore-producing strains of *Rhizobiummeliloti* and their biocontrol potential against *Macrophomina phaseolina* that causes charcoal rot of groundnut. *Curr. Sci.* 673-677.
7. Mavrodi, D.V., R.F. Bonsall, S.M. Delaney, M. Soule, J., G. Phillips, and L.S. Thomashow, (2001). Functional analysis of genes for biosynthesis of pyocyanin and phenazine-1-carboxamide from *Pseudomonas aeruginosa* PAO1. *J. bacterial.* **183**(21): 6454-6465.
8. Yu, X.Q., Y.F. Zhu, C. Ma, J.A. Fabrick and M.R. Kanost, (2002). Pattern recognition proteins in Manduca sexta plasma. *Insect Biochem. Mol. Biol.* **32**(10): 1287-1293.
9. Vincent, J.M. (1970). A manual for the practical study of the root-nodule bacteria. *A manual for the practical study of the root-nodule bacteria.*
10. Graham, P.H. and C.A. Parker, (1964). Diagnostic features in the characterisation of the root-nodule bacteria of legumes. *Plant and soil*, **20**(3): 383-396.
11. Bernaerts, M.J., and I. Deley, (1963). A biochemical test for crown gall bacteria. Nature **197**: 406-7.
12. Saitou, N., and M. Nei, (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**: 406–425.
13. Altschul, S.F., T.L., Madden, A.A. Schäffer, J. Zhang, Z. Zhang, W. Miller and D.J. Lipman, (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* **25**(17): 3389-3402.
14. Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei and S. Kumar, (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* **28**(10): 2731-2739.

15. Raviraja, N.S., G.L. Maria and K.R. Sridhar, 2006. Antimicrobial evaluation of endophytic fungi inhabiting medicinal plants of the Western Ghats of India. *Eng. Life Sci.* **6**: 515-520.
16. Rispail, N., P. Morris and K.J. Webb, (2005). Phenolic compounds: extraction and analysis. In *Lotus japonicus handbook* (pp. 349-354). Springer, Dordrecht.
17. Ng, L.C., M. Sariah, O. Sariam, O. Radziah and M.A. Zainal Abidin, (2012). Rice seed Bacterization for promoting germination and seedling growth under aerobic cultivation system. *Aus. J. Crop Sci.* **6**: 170-175.

DOI:10.26524/KRJ299

Kong. Res. J. 6(2): 37-44, 2019
Publisher: Kongunadu Arts and Science College, Coimbatore.

RESEARCH ARTICLE

SEASONAL VARIATIONS OF NUTRIENTS IN PERUR CHETTIPALAYAM LAKE, COIMBATORE DISTRICT, TAMILNADU, INDIA

Lekeshmanaswamy, M*. and K. Anusiyadevi
Department of Zoology, Kongunadu Arts and Science College, Coimbatore- 641 029, Tamil Nadu, India.

ABSTRACT

The water quality was performed in Perur Chettipalayam Lake, Coimbatore District from July 2015 to June 2016, with specific regard to nutrient content. The nutrients investigated were phosphate, calcium, magnesium, chlorides, nitrates and sulphates. The obtained results showed marked seasonal changes in contamination of the sewage pond. The analyzed nutrient content shows that the lake is extremely contaminated with sewage

Keywords: Perur Chettipalayam Lake, nutrients and seasonal fluctuations.

1. INTRODUCTION

Water is a crucial component of the environment and it retains life on the earth. Due to over extending population increasing and industrial discharges, the demand for fresh water is increasing day by day. In today's situation, rapid industrialization, unplanned urbanization and unselective use of artificial chemicals cause heavy and mixed pollution in aquatic environments leading to degradation of water quality and consumption of aquatic fauna. Even the deterioration of water in ponds and tanks causes extreme problems in aquatic ecosystem and in spoiling the water quality. Physical and chemical parameters play an important role in determining the distribution pattern and quantitative abundance of organisms in a particular aquatic ecosystem (1).

A pond supports a man-made or natural water body with an area of between 2 and 1 m (20, 000 m^2 or ~5 acres) that can catch water for a few months of the year (mainly for four months or may be more) (2). Contamination of rivers and streams has become one of the most significant Environmental issues (3). Water quality usually means the water component that must be present in order for aquatic organisms to grow best (4).

Anthropogenic loads of nutrients may lead to extreme eutrophication, particularly where the circulation is restricted, such as in inlets and coastal lagoons (5). The nitrate ion (NO_3^-) is the general form of combined nitrogen found in natural water. It may be biochemically declined to nitrite (NO_2^-) by denitrification processes, generally under anaerobic conditions. The nitrite ion is promptly oxidized to nitrate. Nitrate is a vital nutrient for aquatic plants and seasonal variations can be caused by plant growth and decay (6).

2 MATERIALS AND METHODS

2.1. Study area and collection of water samples

2.1.1. Perur Chettipalayam Lake

The present attempt was made to analyze the water quality status of Perur Chettipalayam Lake. This study examines the seasonal variation in the different parameters of Perur Chettipalayam Lake between July 2015 and June 2016.

2.1.2. Sample collection

The samples were collected at the Perur Chettipalayam Lake water at the four stations listed below for the current research.

Fig. 1. Station I Before mixing point of sewage in Perur Chettipalayam Lake

*Correspondence: Lekeshmanaswamy, M., Department of Zoology, Kongunadu Arts and Science College, Coimbatore – 641 029, Tamil Nadu, India. E.mail: ml_swamy64@yahoo.co.in

Fig. 2. Station II Mixing point of sewage in Perur Chettipalayam Lake

Fig. 3. Station III After Mixing point of sewage in Perur Chettipalayam Lake

Fig. 4. Station IV Raw sewage in Perur Chettipalayam Lake

Surface water samples were gathered from July 2015 to June 2016 between 11.30 am and 12.30 pm for a 12-months period. Samples in clean, white polythene containers have been collected. It must be able to be sealed tightly with a stopper or a cup. The bottle must be soaked with 10% hydrochloric acid for 24 hours and cleaned and rinsed thoroughly with distilled water. However, all containers should be rinsed with chromic acid solution (35 ml of saturated $Na_2Cr_2O_7$ in 1 liter of concentrated sulphuric acid), tap water and distilled water should be used.All procedures were according to Colorimetric/Titrimetric methods.

STUDY AREA

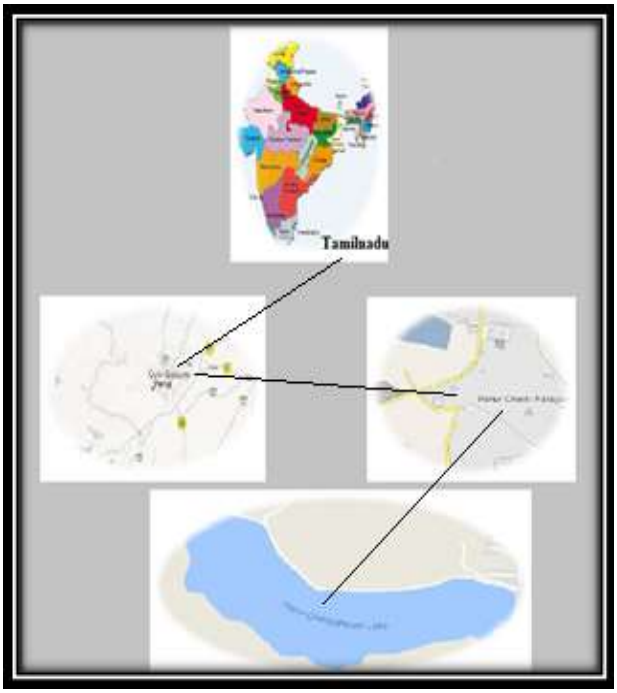


Fig. 5. Shows Map of Perur Chettipalayam Lake

Maps showing

1. Tamilnadu state
2. Coimbatore District and Perur city
3. Perur Chettipalayam (Study area)
4. Perur Chettipalayam Lake

Fig. 6. Google Satellite Map of the Perur Chettipalayam Lake

3. RESULTS AND DISCUSSION

The data for Station I, II and III seasonal variations in Perur Chettipalayam Lake nutrients are shown below (Tables 1 - 3).

3.1 NUTRIENTS

3.1.1 Phosphates

The values of phosphates were recorded as minimum and maximum (0.012 mg/l and 0.955 mg/l) during the month of February and March 2016 at stations I and II respectively. Mishra *et al.* (7) reported earlier high levels of phosphate during the monsoon season. Excessive nutrients in water can also cause contamination and eutrophication of groundwater (8,9).

3.1.2 Calcium

The calcium level was minimum (90.12 mg/l) during the month of March 2016 at station I and maximum (205.12 mg/l) during the month of December 2015 at station II. Hujare (10) has been reported that higher calcium concentrations are not desirable for washing and bathing due to the suppression of soap dither formation.

3.1.3 Magnesium

Minimum value of magnesium was recorded as 50.00 mg/l during the month of June 2016 at station I and maximum was recorded as 154.16 mg/l during the month of December 2015 at station II. Trivedi and Goel (11) and Singh and Mahajen (12) are of the view that the high hardness is suggestive of pollution due to domestic waste and industrial effluents.

3.1.4 Chlorides

The chlorides were minimum value at 40.00 mg/l during the month of September 2015 at station I and maximum as 260.00 mg/l during the month of March 2016 at station I. Prior to the development of bacteriological producers, chloride serves as a basis for detecting groundwater pollution from sewage (13).

3.1.5 Nitrates

The minimum value of nitrates was recorded as 0.22 mg/l during the month of January 2016 at station I and maximum value was recorded as 54.99 mg/l during the month of October 2015 at station II. Nitrates contribute to fresh water by discharging waste and industrial waste from agricultural fields (14).

3.1.6 Sulphates

The minimum value of sulphates was observed as 66.43 mg/l during the month of February 2016 at station I and maximum value was observed as 264.80 mg/l during the month of May 2016 at station II.

Higher concentrations of SO_4 can cause gastrointestinal irritation, especially in drinking water resources (15).

Data on seasonal variations in the different parameters of Perur Chettipalayam Lake for the Station IV (Raw sewage) are represented below (Table 3).

3.2 NUTRIENTS

3.2.1 Phosphates

The values of phosphates were recorded as minimum and maximum (0.4 mg/l and 0.9 mg/l) during summer season (March to June) and winter season (November to February) at station IV.

3.2.2 Calcium

The calcium level was minimum (76.50 mg/l) during rainy season (July to October) at station I and maximum (100.00 mg/l) during summer season (March to June) at station IV.

3.2.3 Magnesium

Minimum value of magnesium was recorded as 20.00 mg/l during rainy season (July to October) at station IV and maximum was recorded as 35.00 mg/l during summer season (March to June) at station IV.

3.2.4 Chlorides

The chlorides were minimum value at 110.00 mg/l during rainy season (July to October) at station IV and maximum as 250.00 mg/l during summer season (March to June) at station IV. 5.1.5

3.2. 5 Nitrates

The minimum value of nitrates was recorded as 40.00 mg/l during summer season (March to June) at station IV and maximum value was recorded as 50.00 mg/l during winter season (November to February) at station IV.

3.2.6 Sulphates

The minimum value of sulphates was observed as 120 mg/l during rainy season (July to October) at station IV and maximum value was observed as 260 mg/l during summer season (March to June) at station IV.

Table 1. Data on seasonal variations in Nutrients of Perur Chettipalayam Lake, Coimbatore for the year 2015 – 2016 at three stations (I, II and III)

Seasons	Parameters	Phosphates (mg/l)			Calcium (mg/l)			Magnesium (mg/l)		
	Months	SI	SII	SII	SI	SII	SIII	SI	SII	SIII
Rainy	Jul - 2015	0.029±0.011	0.708±0.011	0.560±0.061	124.08±1.00	152.00±0.76	145.02±0.11	64.71±0.15	144.45±0.27	140.25±0.20
	Aug	0.028±0.001	0.700±0.011	0.340±0.030	114.06±0.44	159.01±0.28	151.07±0.27	60.05±0.05	148.02±0.05	140.05±0.05
	Sep	0.016±0.005	0.718±0.011	0.700±0.036	117.08±0.21	159.06±0.44	154.04±0.76	60.15±0.12	147.02±0.22	145.02±0.05
	Oct	0.050±0.011	0.712±0.010	0.650±0.050	112.02±0.51	154.08±0.32	150.02±0.22	61.24±0.25	141.04±0.10	130.06±0.21
Winter	Nov	0.016±0.005	0.668±0.002	0.154±0.005	138.54±0.40	192.34±0.82	**190.01±0.22**	76.80±0.15	152.25±0.25	145.07±0.09
	Dec	0.018±0.002	0.672±0.004	**0.151±0.007**	139.67±0.84	**205.12±0.26**	182.04±0.05	78.10±0.10	**154.16±0.10**	144.08±0.10
	Jan - 2016	0.014±0.004	0.614±0.005	0.180±0.010	134.00±1.04	190.78±5.56	170.12±0.26	75.14±0.10	150.00±0.64	**148.06±0.38**
	Feb	**0.012±0.002**	**0.610±0.005**	0.145±0.005	**130.00±2.75**	184.16±0.25	170.00±1.32	**79.10±0.55**	156.14±0.10	144.16±0.20
Summer	Mar	0.017±0.005	**0.955±0.012**	0.774±0.051	**90.12±0.26**	122.24±0.15	100.14±0.25	54.04±0.05	**134.11±0.07**	130.42±0.30
	Apr	0.014±0.004	0.823±0.013	0.800±0.045	92.00±0.50	**120.15±0.21**	110.06±0.10	52.08±0.05	130.50±0.17	**130.00±0.87**
	May	**0.032±0.002**	0.864±0.010	0.820±0.025	98.00±0.76	122.10±0.26	120.00±0.65	50.08±0.06	130.64±2.54	130.30±0.25
	Jun	0.015±0.005	0.943±0.010	**0.834±0.030**	95.00±0.50	121.00±0.76	**100.00±1.32**	**50.00±0.76**	132.86±0.21	131.00±0.41
Standards	WHO	0.5			200			50		
	ICMR	No limit stated			75			30		
	BIS	0.2			200			30 - 70		

Values were expressed as mean ± S. D of three replicates using SPSS statistical package.
Standards: World Health Organization (1963), Indian Council of Medical Research (1975) and Bureau of Indian Standards: IS 10500 (2012).

Table 2. Data on seasonal variations in Nutrients of Perur Chettipalayam Lake, Coimbatore for the year 2015 – 2016 at three stations (I, II and III)

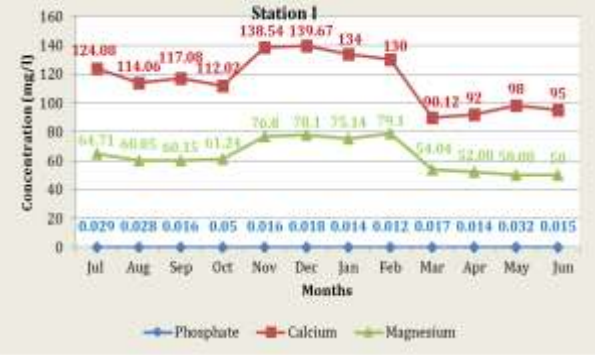
Seasons	Parameters	Chlorides (mg/l)			Nitrates (mg/l)			Sulphates (mg/l)		
	Months	SI	SII	SIII	SI	SII	SIII	SI	SII	SIII
Rainy	Jul - 2015	65±0.30	172±0.26	71±0.65	0.30±0.18	54.40±0.20	21.20±0.05	70.88±0.01	179.56±0.03	175.74±0.05
	Aug	60±0.70	**170±0.40**	65±0.49	0.62±0.03	54.90±0.10	21.15±0.04	74.00±0.43	189.34±0.10	175.45±0.10
	Sep	**40±0.55**	174±0.52	72±0.43	0.42±0.04	54.86±0.03	21.40±0.03	76.18±0.02	179.78±0.02	170.78±0.04
	Oct	48±0.56	174±0.52	**70±2.61**	**0.86±0.02**	**54.99±0.01**	21.25±0.02	78.12±0.02	180.65±0.04	175.60±0.15
Winter	Nov	75±3.27	188±0.65	80±0.47	0.50±0.01	36.30±0.15	**22.20±0.07**	69.55±0.01	**173.74±0.05**	160.60±0.15
	Dec	70±0.65	189±1.00	85±0.62	0.87±0.02	**29.24±0.05**	27.00 ±0.49	84.00±0.41	175.40±0.30	164.72±0.05
	Jan - 2016	78±0.36	186±2.51	75±0.61	**0.22±0.01**	35.25±0.02	25.26±0.02	86.00±0.43	177.89±0.01	166.25±0.16
	Feb	75±3.27	185±1.00	82±0.47	0.40±0.01	32.56±0.03	28.10±0.04	**66.43±0.01**	178.34±0.05	**160.00±0.15**
Summer	Mar	75±3.27	**260±1.52**	110±0.66	0.35±0.02	34.40±0.20	30.18±0.01	**90.68±0.01**	226.54±0.03	215.50±0.05
	Apr	76±0.41	220±2.00	100±0.56	0.50±0.17	36.65±0.05	32.05±0.04	92.72±0.04	228.15±0.05	**225.48±0.02**
	May	75±3.27	218±2.08	120±0.43	0.98±0.01	38.55±0.01	**35.08±0.03**	94.14±0.05	**264.80±0.10**	215.94±0.05
	Jun	**79±0.50**	204±3.05	**130±0.47**	0.65±0.01	36.85±0.04	34.10±0.62	92.10±0.04	221.45±0.15	215.86±0.07
Standards	WHO	250			50			200		
	ICMR	200			45			200		
	BIS	250			45			200		

Values were expressed as mean ± S. D of three replicates using SPSS statistical package.
Standards: World Health Organization (1963), Indian Council of Medical Research (1975) and Bureau of Indian Standards: IS 10500 (2012).

Table 3. Data on seasonal variations in Physico–chemical parameters and Nutrients of Perur Chettipalayam Lake at Station IV (Raw sewage) for the year 2016

Parameters	Units	Station IV (Raw sewage)					
Physico-chemical Parameters and Nutrients		Seasons			Standards		
		Rainy	Winter	Summer	WHO	ICMR	BIS
Water temperature	^{0}C	**25±0.32**	26±1.00	**29±2.64**	35	No Limit stated	No Limit stated
Colour	-	Brownish	**Black**	**Greenish**	No Limit stated	No Limit stated	No Limit stated
Electrical conductivity	µmhos	**280±7.6**	360±0.90	**382±0.38**	300	300	300
Suspended solids	mg/l	**200±5.0**	240±0.45	**390±0.76**	500	No Limit stated	No Limit stated
Dissolved solids	mg/l	**300±10.0**	460±0.87	**540±0.49**	500	500	500-1000
Total solids	mg/l	**500±3.21**	700±0.66	**930±0.42**	30	No Limit stated	No Limit stated
Light penetration	cms	**200±5.0**	245±0.36	**280±0.60**	No Limit stated	No Limit stated	No Limit stated
Phosphates	mg/l	**0.9±0.43**	0.6±0.20	**0.4±0.20**	0.5	No Limit stated	0.2
Calcium	mg/l	**76.5±1.04**	87.0±0.55	**100.0±0.36**	200	75	200
Magnesium	mg/l	**20±0.53**	25±0.49	**35±0.47**	50	30	30-70
Chlorides	mg/l	**110±0.76**	220±0.60	**250±0.80**	250	200	250
Nitrates	mg/l	46±0.80	**50±0.59**	**40±0.61**	50	45	45
Sulphates	mg/l	**120±1.32**	240±0.71	**260±0.36**	200	200	200

Values were expressed as mean ± S. D of three replicates using SPSS statistical package.
Standards: World Health Organization (1963), Indian Council of Medical Research (1975) and Bureau of Indian Standards: IS 10500 (2012).



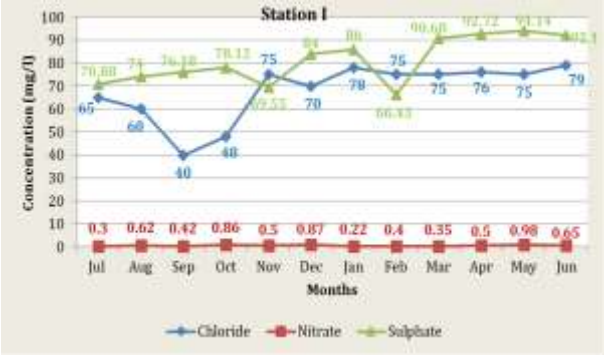
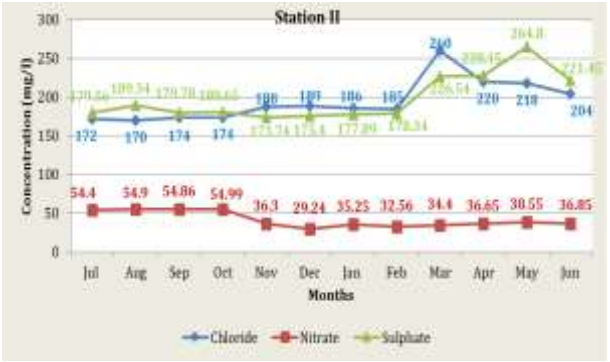


Fig. 7. Data on seasonal variations on nutrients of Perur Chettipalayam Lake, Coimbatore at Station I for the year 2015-2016



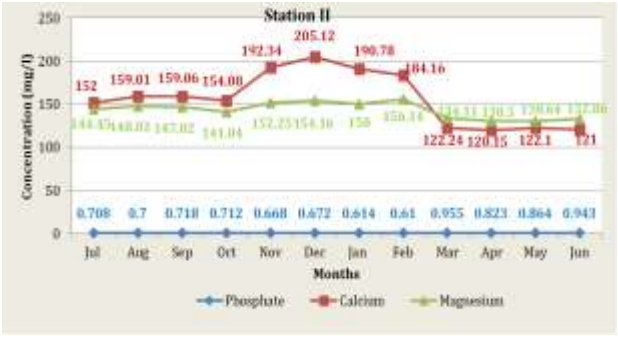
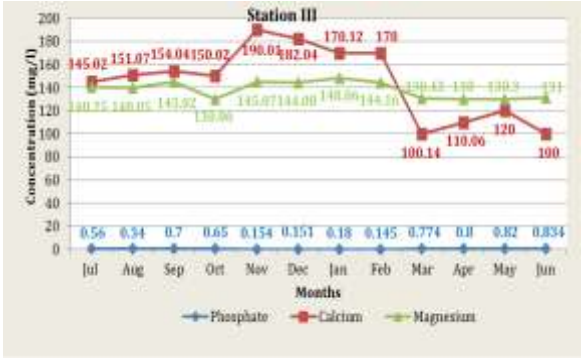


Fig. 8. Data on seasonal variations on nutrients of Perur Chettipalayam Lake, Coimbatore at Station II for the year 2015 – 2016



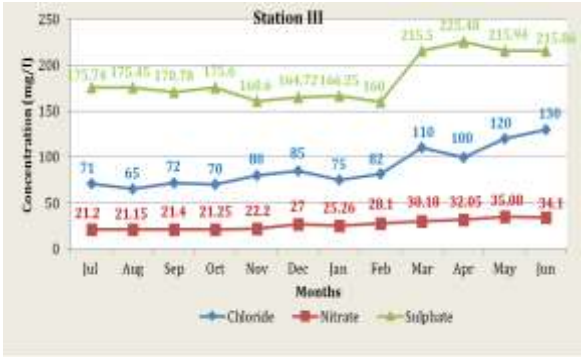


Fig. 9. Data on seasonal variations on nutrients of Perur Chettipalayam Lake, Coimbatore at Station III for the year 2015 – 2016

4. CONCLUSION

The results of the above parameters have shown that the present study clearly shows that the quality of the water depends on the type of pollutant added. Due to the mixture of agricultural pollutants with fertilizers and domestic sewage, the amount of phosphates, calcium, magnesium, chlorides, nitrates and sulphates was found to be high. Therefore, periodic monitoring can help to prevent contamination of the lake water of the region.

REFERENCES

1. Santhokumar Singh, A., D. Dakua and S.P. Biswas, (2009). Physico-chemical parameters and fish enumeration of Maijan Beel (Wetland) of Upper Assam. *Geobios* **36**: 184-188.
2. Rajiv, P., H.A., Salam, M. Kamaraj, R. Sivaraj and R. Balaji, (2012). Comparative Physico-chemical and Microbial Analysis of Various Pond Waters in Coimbatore District, Tamil Nadu, India. *Ann. Biol. Res.* **3**(7): 3533-3540.
3. Otieno, D.S. (2008). Determination of Some Physicochemical Parameters of the Nairobi River, Kenya. *J. Appl. Sci.e Environ. Manage.* **12 (**1): 57-62.

4. Ehiagbonare, J.E. and Y.O. Ogundiran, (2010). Physico - chemical analysis of fish pond waters in Okada and its environs, Nigeria. *African J. Biotech.* **9** (36): 5922-5928.
5. Lin, H.J., X.X., Dai, K.T., Shao, H.M., Su, W.T. Lo, H.L. Hsieh, L.-S. Fang and J.-J. Hung, (2006). Trophic structure and functioning in a eutrophic and poorly flushed lagoon in south-western Taiwan. *Mar. Environ. Res.***62**: 61-82.
6. Bissett, A., J.P. Bowman and J. Burke, (2006). Bacterial diversity in organically-enriched fish farm sediments. *FEMS Microbiol. Ecol.* **55**: 48-56.
7. Mishra, Sugatha, D. Panda and R.C. Panigraphy, (1993). Physico-chemical characteristics of Bahuda estuary (Orissa) east coast of India. Ind. *J. Mar. Sci.* **22**: 75-77.
8. Smith, V.H., G.D. Tilman and J.C., Nekola, (1999). *Environ Pollut*. **100**: 179-196.
9. Ju, X.L., C.L., Kou, F.S. Zhang, and P. Christie (2006). *Environ Pollut*. **143**: 117-125.
10. Hujare, M.S. (2008). Seasonal variation of physico - chemical parameters in the perennial tank of Talsande, Maharastra. *Ecotoxicol. Environ. Monitor.* **18** (3): 233-242.
11. Trivedi, R.K., and P.K. Goel, (1986). Chemical andbiological methods for water pollution studies. *Environmental Publications,* Karad, India, 248p.
12. Singh, R. and I. Mahajan, (1987). Phytoplankton and water chemistry of Rawalsar and Renuka Lake, India. *J. Ecol.* **14** (2): 273-277.
13. Sawyer, C.N. (1960). Chemistry for sanitary engineers. Mc Graw Hill, New York.
14. Solanki, H.A. (2012). Status of Soils and Water reservoirs near industrial areas of Baroda: pollution and soil-water chemistry. Lap Lambert Academic Publishing, Germany, ISBN 376.
15. Suthers, I.M., and D. Rissik, (2009). A Guide to their Ecology and Monitoring for Water Quality. 2nd edition, CSIRO Publishing, Collingwood Victoria, 272p.

DOI:10.26524/KRJ301

Kong. Res. J. 6(2): 51-55, 2019
Publisher: Kongunadu Arts and Science College, Coimbatore.

RESEARCH ARTICLE

EVALUATION OF ANTI-FUNGAL POTENTIAL OF *CINNAMOMUM ZEYLANICUM* BLUME (LAURACEAE) BARKS EXTRACT FOR ONYCHOMYCOSIS

Manivannan, S[1]. and S. Bhuvaneswari[2]

[1]Department of Zoology, Kongunadu Arts and Science College, Coimbatore- 641 029, Tamil Nadu, India.
[2]Department of Pharmaceutics, Periyar College of Pharmaceutical Science, Tiruchirappalli – 620021,Tamil Nadu, India.

ABSTRACT

Onychomycosis is a fungal infection of nails caused by dermatophytes, yeasts of nondermatophyte molds. Far more than being a simple cosmetic problem, infected nail serves as a chronic reservoir, which can give rise to repeated mycotic infections and represents about 30% of mycotic cutaneous infections. The prevalence rate of onychomycosis is determined by age, predisposing factor, social class, occupation, climate, living environment. To evaluation of Anti-fungal potential of *Cinnamomum Zeylanicum* Blume (Lauraceae) Barks Extract for Onychomycosis. The *C. Zeylanicum* barks crude extracts were obtained by soxhlet-solid-liquid extraction using ethanol and aqueous. The dermatophytes namely, *Trichophyton rubrum, Trichophyton mentagrophytes, Trichophyton simii, Epidermophyton floccosum* and *Candida albicans* were recovered from rice farmers in Orathanadu, Thanjavur (Dt), Tamil Nadu. The antifungal activity, minimum inhibitory concentrations and minimum fungicidal concentrations against the onychomycosis of *C. zeylanicum* was determined by the disc diffusion methods in sabouraud dextrose agar using ketoconazole (2mg/disc) as a positive control. Phytochemical analysis revealed the presence of wide range of bioactive constituents like flavonoids, tannins, alkaloids, saponins, terpenes and steroids. The ethanol barks extract inhibited all test organisms at the minimum concentration of 25 mg/ml while fungicidal actions were observed at a concentration of 50 mg/ml for *T. rubrum,* 180 mg/ml for *T. mentagrophytes,* 160 mg/ml for *T. simii,* 120 mg/ml for *E. floccosum* and 60 mg/ml for *C. albicans.* The ability of the extracts to inhibit the growth of fungi likes dermatophytes and yeast is an indication of the antifungal potential of *C. zylanicum*, which makes the candidate for production of antifungal agents.

Keywords: *Cinnamomum zeylanicum*, phytochemical analysis, ethanol extract, antifungal, disc diffusion method, inhibition zone.

1. INTRODUCTION

Onychomycosis is an infection of the nail and may involve the nail bed, nail plate and matrix by fungi that include dermatophytes, nondermatohyte moulds and yeasts. Onychomycosis is the most common condition affecting the nails, accounting for 50% of all nail disorders and 33.3% of all mycotic infections of the skin [1, 2]. The toenails are affected in 80% of all cases of onychomycosis; dermatophyte infection, mostly due to *Trichophyton rubrum*, is the cause in over 90% of cases. 5–10% of these infections are caused by yeasts, especially *Candida albicans*. Onychomycosis occurs in 10% of the general population the incidence of onychomycosis has been increasing, owing to such factors as diabetes, immunosuppression, and increasing age. Population-based studies have found varied estimates of prevalence, ranging from less than 1 % to 8% percent in Europe and the United States and less than 1 % in Central Africa. Various workers have reported the incidence to vary from 0.5 to 5% in the general population in India [3].

Fungal nail infections are not life-threatening, yet they are associated with secondary bacterial infection, chronicity of disease, therapeutic failures and disfigurement like hyperkeratosis, discoloration of nail plate, and brittle nails [4]**.** The causative agents of the disease may vary depending upon geographic or temporal distribution. Even in developed countries, the importance of nail infections has been highlighted only in the last decade. In developing countries, socioeconomic constraints and other common prevalent health issues have led to a low awareness of onychomycosis by physicians and general population. Thus, even in the presence of good personal hygiene, it has continued to persist and spread.

Medicinal plants have been a part of modern life style of a man and these plants are a source of important therapeutic aid for alienating human ailments. With increasing realization of the health hazards and toxicity associated with the indiscriminate use synthetic drugs and antibiotics, interest, in the use of plants and plant based drugs

*Correspondence: MANIVANNAN, S., Department of Zoology, Kongunadu Arts and Science College, Coimbatore – 641 029, Tamil Nadu, India. E.mail: manivannanresearch@gmail.com

revived throughout the world. However, a large number of medicinal plants remain to be investigated, for their possible pharmacological value. Most of the pharmaceutical industry is highly dependent on wild population for the supply of raw materials for extraction of medicinally important compounds. The screening of natural products has been the source for new potential drugs is still largely unexplored and only a small percentage of them has been subjected to phytochemical investigation and the fractions submitted to pharmacological screening. Such screening of various natural organic compounds and identifying active agents is the need of the hour as due to successful prediction of lead molecule and discovery will pay of later in drug development. Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, glycosides, etc., which have been found *in vitro* to have antimicrobial properties [5, 6].

Cinnamomum zeylanicum is a small, tropical, evergreen tree most noted for its bark, which provides the world with the commonly known spice, cinnamon. This species belongs to Lauraceae family, is native to Indonesia and cultivated in various regions of the world. Several biological properties of *C. zeylanicum* have been described such as antiseptic, analgesic, anti-spasmodic, astringent, insecticide and parasiticide properties [7]. Cinnamon is a spice tree contains several bioactive compounds that can be used against a wide range of microorganisms. Cinnamon bark crude extract has constantly been reported to have antifungal activity [8].

Antifungal susceptibility testing remains an area of intense interest. Susceptibility testing can be used for drug discovery and epidemiology. Number of reports is available showing efficacy of *C. zeylanicum* essential oils as antimicrobial agents [9]. The oil extracted from *C. zeylanicum* bark and leaves have been reported to possess fungicidal activity against fungi responsible for causing crown rot disease of banana. The major constituent possessing antifungal activity in *C. zeylanicum* bark and leaf oils were found to be cinnamaldehyde and eugenol, respectively. In addition other compounds having fungicidal property have also been reported to be present in bark and leaves [10]. Cinnamon barks represent important source of compounds like flavonoids, tannins, glycosides, saponines, alkaloids. The objectives of the current investigation to evaluate the anti-fungal activity of *C. zeylanicum* barks extracts is done in order to detect new sources anti-fungal agents.

2. MATERIALS AND METHODS

2.1. Collection of plant materials

The bark of *C. zylanicum* was collected from Spices Research Institute, Kerala in November 2018.

2.2. Preparation of plant extract

Freshly collected plant parts were shade-dried at room temperature for 10–15 days. Dried bark samples were separately crushed and ground into fine powder with mortar and pestle. Powdered plant materials were sequentially extracted with solvents in a Soxhlet apparatus for 8 h [11]. The solvents used for extraction included ethanol and aqueous. The respective extracts were filtered and dried under reduced pressure using rotary evaporator to yield solid/semisolid residues. The residues were lyophilized to get dry solid mass.

2.3. Preliminary phytochemical screening

The extracts were subjected to preliminary phytochemical testing to detect for the presence of different chemical groups of compounds. Air-dried and powdered plant materials were screened for the presence of phenol and polyphenols, flavanoids, terpenoids, tannins, alkaloids and seponins, as described in literatures [12, 13].

2.4. Source of microorganism

Fifty samples were collected from rice farmers in Thanjavur, Tamil Nadu, with lesions suggestive of fungal infections. Dermatophytes and yeast were isolated and identified based on detailed study of their microscopic and macroscopic features [14].

2.5. Determination of anti-fungal activity

Sterilized discs (6 mm) prepared from Whatman No 1 filter paper were impregnated with different concentrations (10 mg, 20 mg, 40 mg, 80 mg) of ethanol extract dissolved in 2% Dimethyl Sulphoxide (DMSO). The disc of the ethanol and aqueous extracts were placed on Sabouraud dextrose agar (SDA) plates seeded with 0.1ml of 10^4 dilution of inoculum preparation [15]. The plates were prepared in triplicate, incubated at room temperature for 7 days and average diameter zone of inhibition recorded. Discs impregnated with 2% DMSO and 2 mg/disc ketoconazole served as negative and positive controls respectively.

2.5. Minimum inhibitory concentration (MIC) and Minimum fungicidal concentration (MFC)

MIC:

Two hundred milligrams of the ethanol extract was dissolved in 2% DMSO and serially diluted two fold in sterile water. Different tubes containing different concentrations (25mg/ml, 50 mg/ml, 100 mg/ml, and 200 mg/ml) of the extracts were inoculated with 0.1ml of 10^4 dilution of the inoculum preparation (standardized suspension of the test organism) and incubated at room temperature for 7 days. These were done in triplicate and the broth medium containing no extract was used as control [16]. MIC was recorded as the tube with the lowest concentration of extract that failed to show any visible macroscopic growth.

MFC:

After determining the MIC, the inhibitory and two following higher concentrations as well as the positive controls were sub cultured on SDA plates in triplicate. After 7 Days of incubation at 30°C, the readings of MFCs were carried out based on growth controls and MFC was the lowest drug concentration that hindered visible growth of the subculture [17].

3. RESULTS AND DISCUSSION

The preliminary phytochemical analysis revealed that different active constituents present in different solvents such as phenol and polyphenols, flavanoids, terpenoids, tannins, alkaloids and seponins (Table.1).

Table: 1. Phytochemical analysis of ethanol and aqueous extracts of *C. zeylanicum* bark

S. No.	Phyto-constituents	RESULTS	
		Ethanol Extract	Aqueous Extract
1.	Phenol and Polyphenols	+	+
2.	Flavanoids	+	-
3.	Terpenoids	+	+
5.	Tannins	+	+
6.	Alkaloids	+	+
7.	Seponins	+	-

+ Presence; - Absence

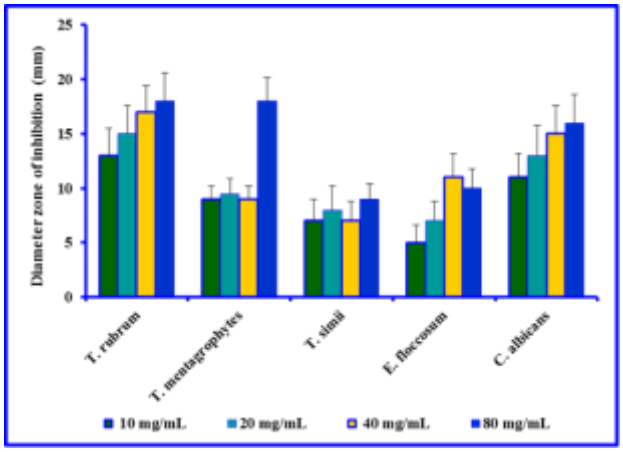


Fig. 1. Anti-fungal activity of ethanol extract of *C. zeylanicum barks*

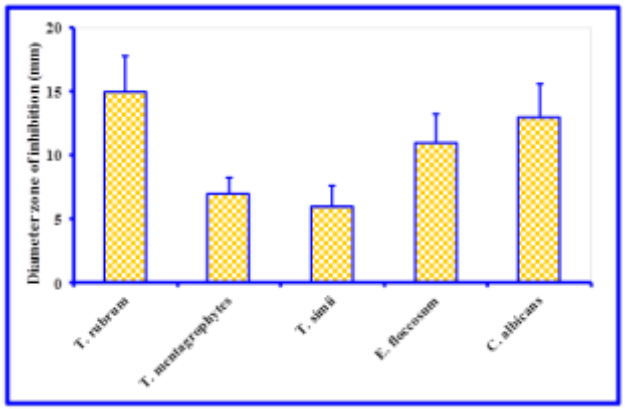


Fig. 2. Anti-fungal activity of aqueous extract of *C. zeylanicum barks*

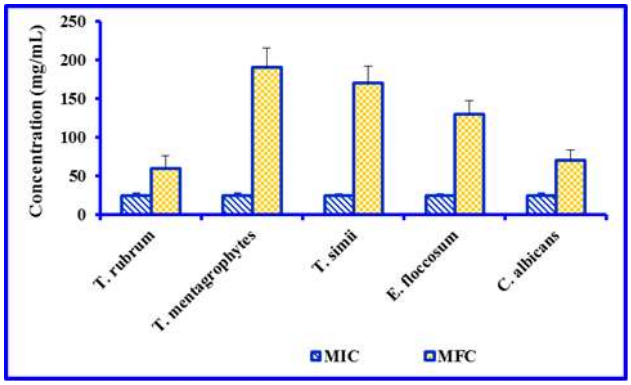


Fig. 3. Minimum inhibitory concentration and minimum fungicidal concentration of ethanol extract of *C. zeylanicum barks*

Abbreviations: MIC- Minimum inhibitory concentration MFC- Minimum fungicidal concentration

The macroscopic and microscopic features of the isolated fungi, a total of 5 species of dermatophytes namely, *Trichophyton rubrum, Trichophyton mentagrophytes, Trichophyton simii, Epidermophyton floccosum* and *Candida albicans* were recovered from rice farmers in Orathanadu, Thanjavur (Dt), Tamil Nadu. The anti dermatophyte and anti yeast activities of crude ethanol barks extract of *C. zeylanicum* tested at four different concentrations with their zones of inhibition is represented (Fig:1). The crude ethanol barks extract of *C. zeylanicum* at a concentration of 10 mg/disc inhibited all test organisms. At that concentration, *T. rubrum* showed diameter inhibition zone of 14 mm, *T. mentagrophytes* (10 mm), *T. simii* (8 mm), *E. floccosum* (6 mm) and *Candida albicans* (12 mm). However, the highest diameter zone of inhibition (20 mm) was observed with *T. rubrum* at a concentration of 80 mg/disc. Based on the detailed study of the macroscopic and microscopic features of the isolated fungi, various species of dermatophytes and non dermatophytes . This result agrees with the work of other researchers who also recorded total inhibition of test dermatophytes by methanol extracts of *L. inermis* [18, 19]. From the results obtained, the diameter zones of inhibition recorded by the different dermatophytes increased as the concentration of the crude extract increases.

The aqueous barks extracts used in this work also showed inhibitory actions against the dermatophytes and *C. albicans*. Represented in Fig.2, is the MIC and MFC of the crude ethanol barks extract of *C. zeylanicum* against the dermatophytes and *C. albicans*. The ethanol barks extract inhibited all test organisms at the minimum concentration of 25 mg/ml while fungicidal actions were observed at a concentration of 50 mg/ml for *T. rubrum,* 180 mg/ml for *T. mentagrophytes,* 160 mg/ml for *T. simii,* 120 mg/ml for *E. floccosum* and 60 mg/ml for *C. albicans.* The results obtained in this work recorded increased bioactivity with ethanol extract than the aqueous extract which is contrary to the reports of other workers. As a general rule, plant extract is considered active against both fungi and bacteria when the zone of inhibition is greater than 6 mm [20]. The range of diameter zones of inhibition by methanol extract (6 mm - 20 mm) and aqueous extract (5 mm - 14 mm) of the leaf of *V. negundo* against the dermatophytes tested, confirms the anti dermatophyte activities of the extracts.

However, the data regarding the use of *C. zeylanicum* extracts as antifungal agents are scanty. Despite serious environmental implications associated with the excessive use of chemical fungicides still remain the first line of defense against fungal pathogens. Moreover, these fungicides when ingested by human beings and animals through food and water cause various ailments in the body. Search of natural fungicidal principles from the plant sources would definitely be a better alternative to these hazardous chemicals. Our study has indicated the anti-fungal potential of plant extracts, as the *C. zeylanicum* bark and leaf extracts displayed complete inhibitory effect on spore germination of aforesaid two dermatiaceous moulds [21].

The relative antifungal activity of *C. zeylanicum* extracts may not be easily correlated with any individual component but with a mixture of compounds present in these extracts. There are reports showing that alkaloids and flavonoids are the responsible compounds for the antifungal activities in higher plants. Moreover, secondary metabolites such as tannins and other compounds of phenolic nature are also classified as active antimicrobial compounds. Phenols, the aromatic compounds with hydroxyl groups are widespread in plant kingdom. They occur in all parts of plants. Phenols are said to offer resistance to diseases and pests in plants. Grains containing high amount of polyphenols are resistant to bird attack [22]. Interestingly, phytochemical screening of the current investigation has revealed that extracts from both the plant parts possess at least three to four of the following classes of secondary metabolites: phenols, flavonoids, terpenoids, tannins, alkaloids and saponins. Therefore, the presence of these phytochemicals could to some extent justify the observed antifungal activities in the current study.

4. CONCLUSION

The results of anti-fungal activities of crude ethanol and aqueous barks extracts of *C. zeylanicum* obtained in this work showed total fungicidal actions against the dermatophytes and yeast. It could be regarded as promising alternative antifungal preparation to be inserted in pharmaceutical formulations for used to treatment mycoses of different clinical severities, particularly, those caused by onychomycosis. The barks could be used in the production of antifungal drugs that will be effective and affordable to the developing countries to benefit from the emerging marks as the developing countries possess most biodiversity of medicinal plants.

ACKNOWLEDGEMENT

The present work has been carried out in the Department of Zoology, Kongunadu Arts and Science College (Autonomous), G.N. Mills post, Coimbatore, Tamil Nadu, India. We express their gratitude to the secretary and Principal for providing the laboratory facilities and encouragement.

REFERENCES

1. Ghannoum, M., and N. Isham, (2014). Fungal nail infections (Onychomycosis): A never-ending story?. *PLoS Pathog.* **10**(6): e1004105.
2. Moreno, G., and R. Arenas, (2010). Other fungi causing onychomycosis. *Clin. Dermatol.* **28**(2): 160-163.
3. Kaur, R., B. Kashyap and P. Bhalla, (2008). Onychomycosis-epidemiology, diagnosis and management. *Indian J. Med. Microbiol.* **26**(2): 108-116.
4. Scher, R.K., (1996). Onychomycosis: a significant medical disorder. *J. Am. Acad. Dermatol.* **35**(3): S2-5.
5. Dahanukar S.A., R.A. Kulkarni, N.N. Rege, (2000). Pharmacology of medicinal plants and natural products. *Indian J. Pharmacol.* **32**(4): S81-118.
6. Cowan, M.M., (1999). Plant products as antimicrobial agents. Clin. Microbiol. Rev. **12**(4): 564-582.
7. Moreira, A.C.P., E.O. Lima, E.L. Souza, M.A.U. Dingenen and V.T. Trajano, (2010). Chemical composition and antifungal activity of *Hyptis suaveolens* (L.) poit leaves essential oil against *Aspergillus* species. *Braz. J. Microbiol.* **38**(1): 33-38.
8. He, Z.D, C.F. Qiao, Q.B. Han, C.L. Cheng, H.X. Xu, R.W. Jiang, *et al.,* (2005). Authentication and quantitative analysis on the chemical profile of cassia bark (cortex cinnamomi) by high-pressure liquid chromatography. *J. Agri. Food Chem.* **53**(7): 2424-2428.
9. Burt, S., (2004). Essential oils: their antibacterial properties and potential applications in foods- a review. *Int. J. Food Microbiol.* **94**(3): 223-253.
10. Delespaul, Q, V.G. Billerbeck, C.G. Roques, G. Michel, C. Marquier-Vinuales and J.M. Bessiere, (2000). The antifungal activity of essential oils as determined by different screening methods. J. Essential Oil Res. **12**(2): 256-266.
11. Pandey, A.K., (2007). Anti-staphylococcal activity of a pan-tropical aggressive and obnoxious weed *Parthenium histerophorus* : An *in vitro* study. *Natl. Acad. Sci. Lett.* **30**(11-12): 383-386.
12. Bhuvaneswari, S., and S. Manivannan, (2014). Anti-diabetic and anti-inflammatory activity of *Caralluma adscendens* var. *adscendens*. *Int. J. Pharm. Bio. Sci.* **5**(1): 42-49.
13. Karthika, C., M. Rafi and S. Manivannan, (2016). Phytochemical analysis and evaluation of antimicrobial potential of *Senna alata* Linn. leaves extract. *Asian J. Pharm. Clin. Res.* **9**(2): 253-257.
14. Ekwealor, C.C, C.A. Oyeka and I. Okoli, (2012). *In vitro* anti dermatophyte activities of crude methanol and aqueous extract of *Lawsonia inermis*. *British Microbiol. Res. J.* **2**(2): 62-70.
15. Duraipandiyan V, M. Ayyanar, S. Ignacimuthu, (2006). Antimicrobial activity of some ehnomedicinal plants used by Paliyar tribe from TamilNadu, India. *BMC Comp. Alter. Med.* **6**:35-41.
16. Dash, D.K., and P.N. Murthy, (2011). Antimicrobial activity of few selected medicinal plants. *Inter. Res. J. Pharm.* **2**(1): 146-152.
17. Cheesbrough, M., (2006). District laboratory practice in tropical countries: 1st Ed. Cambridge UK: Cambridge University press.
18. Saadabi M.A.A., (2007). Evaluation of *Lawsonia inermis* Linn (*Sudanese Henna*) leaf extract as an antimicrobial agent. *Res. J. Biol. Sci.* **2**(4): 419-423.
19. Sharma, K.K, R. Saikia, J. Kotoky, J.C. Kalita and R. Devi, (2011). Antifungal activity of *Solanum melongena* L., *Lawsonia inermis* L and *Justicia gendarussa* B. against dermatophytes. *Inter. J. Pharm. Tech. Res.* **3**(3): 1635-1640.
20. Babu, P.D, R.S. Subhasree, (2009). Antimicrobial activities of *Lawsonia inermis* - a Review. *Acad. J. Plant Sci.* **2**(4): 231-232.
21. Mahomoodally, M.F, A. Gurib-Fakim and A.H, Subratty, (2005). Antimicrobial activities and phytochemical profiles of endemic medicinal plant of Mauritius. *Pharm. Biol.* **43**(3): 237-242.
22. Boussaada, O, J. Chriaa, R. Nabl, S. Ammar, D. Saidana, M.A. Mahjoub, *et al.,* (2008). Antimicrobial and antioxidant activities of methanol extracts of Evax pygmaea (Asteraceae) growing wild in Tunisia. *World J. Microbiol. Biotechnol.* **24**(8): 1289-1296.

DOI:10.26524/KRJ302

Kong. Res. J. 6(2): 56-60, 2019
Publisher: Kongunadu Arts and Science College, Coimbatore.

RESEARCH ARTICLE

WETLAND RESOURCE UTILIZATION BY SPOT-BILLED PELICANS IN COIMBATORE, TAMIL NADU

Theresa Leo and Meiyalagan Velayutham*
Department of Zoology , Kongunadu Arts and Science College, Coimbatore- 641 029, Tamil Nadu, India.

ABSTRACT

The Spot-billed Pelican (*Pelecanus philippensis*), a near threatened bird and one of eight pelican species in the world, can be found only in South and Southeast Asia over an area between 129000 and 181000 km2 with strongholds in India, Sri Lanka, southern Cambodia and coastal areas of Sumatra. In India, it is presently distributed in southern and northeastern India with concentrations in Andhra Pradesh, Tamil Nadu, Karnataka and Assam states. The present study deals how the spot- billed pelicans utilize the wetlands as well as their behaviour is noted in order to plan conservation and management strategies for the species.

Keywords: wetland resources, Spot-billed pelican, threats, conservation.

1. INTRODUCTION

Wetland

Wetlands are defined as 'lands transitional between terrestrial and aquatic eco-systems where the water table is usually at or near the surface or the land is covered by shallow water (1). Wetland ecosystems are important habitats for flora and fauna and hence are of national and international importance for conservation. Wetlands can be defined as areas of high grsoundwater environments that are characterised by permanent (shallow water bodies) or temporary inundation, or soils having hydric properties. They provide a number of critical ecological functions, including the regulation of water regimes, and support a significant percentage of the world's biodiversity that have adapted to life in saturated conditions. Wetland ecosystems depend on water levels and therefore climate change, especially changes in precipitation, is likely to have a significant impact on these habitats and associated species (2).

Wetlands in India occupy 58.2 million hectares, including areas under wet paddy cultivation (Directory of Indian Wetlands). Majority of the inland wetlands are directly or indirectly dependent on the major rivers like, Ganga, Bhramaputra, Narmada, Godavari, Krishna, Kaveri, Tapti. They occur in the hot arid regions of Gujarat and Rajasthan, the deltaic regions of the east and west coasts, highlands of central India, wet humid zones of south peninsular India and the Andaman and Nicobar & Lakshwadeep islands. Wetlands are important to both floral and faunal populations. Wetlands birds provide us with some of nature's wonderful sights. Apart from their beauty and economic importance, these birds are excellent indicators of water quality and measures of biodiversity. A wide variety of birds use wetland habitats for all or part of their life. They form one of the major components of the wetland ecosystems.

Pelican

Pelicans belong to the family Pelecanidae. They are large, web-footed, gregarious birds. They are characterised by large wing- spread, very long bill and a pouch in the upper throat in which they store fish before swallowing. The air sacs serve to keep the pelican remarkably buoyant in the water (3). They roost and loaf communally on beaches, sandbanks, and in shallow water. Adult pelicans rely on visual displays and behaviour to communicate (4). Pelicans are gregarious and nest colonially. Pairs are monogamous for a single season, but the pair bond extends only to the nesting area; mates are independent away from the nest. The location of the breeding colony is constrained by the availability of an ample supply of fish to eat, although pelicans can use thermals to soar and commute for hundreds of kilometres daily to fetch food (5). They feed usually on fish. There are eight living species of pelicans which are American white pelican (North America and Mexico – Least Concern), Brown pelican (Coastal distribution ranging from North America and the Caribbean to northern South America and the Galapagos – Least Concern), Peruvian pelican (Pacific Coast of South America from Ecuador and Peru south through to southern Chile – Near Threatened), Great white pelican (Patchy distribution from eastern Mediterranean east to Indo-China and Malay Peninsula, and south to South Africa– Least Concern), Australian pelican (Australia and New Guinea; vagrant to New Zealand, Solomons, Bismarck Archipelago, Fiji and Wallacea – Least

*Correspondence: Meiyalagan, V., Department of Zoology, Kongunadu Arts and Science College, Coimbatore – 641 029, Tamil Nadu, India. E.mail: meiyazhagan1982@live.com

Concern), Pink-backed pelican (Africa, Seychelles and south-western Arabia – Least Concern), Dalmatian pelican (South-eastern Europe to India and China – Near Threatened) and Spot-billed pelican (Southern Asia from southern Pakistan across India east to Indonesia – Near Threatened). In southern India there are 21 known breeding colonies in the states of Andhra Pradesh, Karnataka and Tamil Nadu (6).

Spot-billed Pelican (*Pelecanus philippensis*)

Spot billed pelican is one of the eight species of Pelicans. Adult pelican has a silver-grey on back with darkish centres to wing-coverts and whiter beneath. Bill pinkish with, in early season, bright yellow margin and bluish-spotted sides (hence name) becoming yellowish towards tip, with orange nail. Pairing pelicans have pale bill-tip. Pouch dull purple or reddish. Legs, feet brown or blackish. Juvenile has pinkish bill at first, not distinctly spotted till 2nd year though spots seen before then. Pouch is pink, grey or pale bluish. Commutes in flocks to and from feeding areas. Both solitary and communal feeding observed latter involving line or semi-circle of birds. These are mainly piscivorous birds. Food includes frogs, lizards, and snakes as well as fish. Twenty types of behaviour were grouped into nine categories: alert, resting, comfort/maintenance, locomotion, foraging, antagonistic, sexual, chic care and feeding and vocalizations (7). The Spot-billed Pelican is not as aggressive to other members of the same species and other species as the White Pelican (Pelecanus erythrorhynchos). In the presence of a perceived threat, both young and adults turn silent. During mating, the pelicans use a number of different social signals, both vocal and visual. Mates also greet each other with neck stretching and a duet of groans. The spot-billed pelican frequents marshes, rivers, estuaries, reservoirs, tanks, flooded fields, large lakes, brackish lagoons, tidal creeks, along the coast, and often feeds in quiet backwaters. As for nesting habitat, it prefers large trees of species in undisturbed areas. The pelicanries are generally found in and around water bodies, including rivers, reservoirs and seasonal ponds and are seen mixed colonies with other water (Kannan & Pandiyan, 2013).

Present status of Spot-Billed pelicans

Spot-billed Pelican is one of the most threatened of the seven species of pelicans in the world (8). The species was first documented in 1789 by Gmelin in the Philippine Islands (9). Most endangered pelican. World breeding population probably not more than 2,500 pairs and total population fewer than 13,000 individuals. Formerly abundant, though 'millions' in Burma perhaps not literal; widespread in Asia. Now mainly if not entirely in Sri Lanka and SE India though perhaps still present in Burma and possibly other sites in Indo-Malaya (Bock and Kikkawa). It's only known present day breeding populations occur in Sri Lanka, India and Cambodia. It was categorized as "Vulnerable" in the IUCN Red Data List in 2001 but according to a recent update from India, its' estimated population has been revised upwards from a low of 5,500-10,000 birds in 2002 to estimated 13,000-18,000 individuals in 2006. Therefore its status has been reviewed as "Near threatened" (10) (Weerakoon and Athukorala). Due to habitat loss and human disturbance, the spot-billed pelican's numbers have declined and many populations in Southeast Asia are now extinct (11).

2. MATERIALS AND METHODS

2.1. Study area

Selected study areas include few of the tanks of Coimbatore district which include Ukkadam-Periyakukam, ValanKulam, and KurichiKulam.

Ukkadam: This Lake is also called as Periyakuam. It receives water supply from the Coimbatore Anicut channel from Noyyal River and from SelvaChinathamani Lake. The lake is used for regular fishing. The lake is used as a regular habitat by many birds. Most species were recorded in March before the start of summer and was the least in the winter months of November and December.

Kurichi Kulam: Kurichi Kulam is one of the major lakes of Coimbatore city. The lake is an excellent example foe the species diversity. It inhabits many insects, reptiles, fishes and birds. It is situated near to the Pollachi road. Slum dwellers living around the lake use it for domestic purposes.

Valankulam: Ukkadam-Valankulam Lake is one of the lakes in Coimbatore, South India. It is situated between Trichy road and Sungam bypass road connecting with Ukkadam. A railway track connecting Coimbatore Junction and Podanur passes over the lake. It serves as an excellent habitat to many local migrant birds.

3. RESULTS

The status and colony size of spot-billed pelicans were studied in Tamil Nadu from the month of July 2018 to December 2018. Bird count and habitat use studies were carried out in the three different wetlands namely Ukkadam- periyakulam,

Kurichikulam and Valankulam, which are used s the foraging sites by the spot-billed pelicans.

Map of three foraging sites

Colony size dynamics was studied with the help of colony size and duration of presence of species. Out of the three sites, the highest number was observed in the Valankulam (73) in the month of July and by the month of November and December the number of spot-billed pelican decreased drastically in all the three wetlands. During the survey, the maximum number of spot-billed pelicans were observed in the month of July; (42), (54) and (73) in Ukkudam-periyakulam, Kurichi kulam and Valankulam respectively whereas the minimum population was observed in the month of December; (6), (0) and (0) in Ukkudam-periyakulam, Kurichi kulam and Valankulam respectively (Fig 1.).

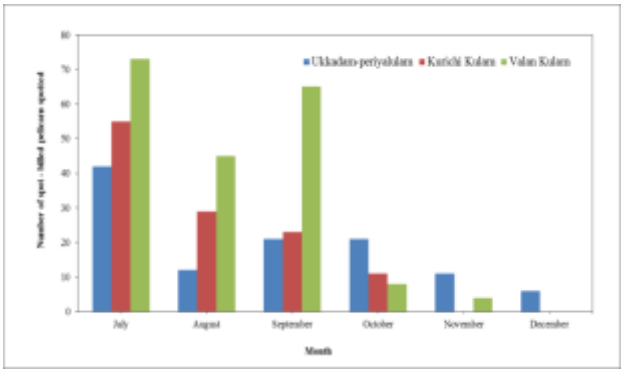


Fig 1. The population of Spot- Billed Pelican observed in different habitat during the ground surveys at three foraging sites from July 2018 to December 2018

The spot-billed pelicans utilized the resources of the three wetlands namely trees, bund, water and tower. In Ukkudam Lake the species were observed utilizing all the four resources compared to the other two wetlands. In the month of July, the maximum population of the spot-billed pelicans were sited on the bund and few numbers in the water followed by August. The species started occupying the trees interestingly and the towers situated in and around the lake slowly by the month of September followed by October. By the month of November and December, the colony size of the spot-billed pelicans reduced drastically in the lake and they were sited only on the bund and in the water (Fig 2.).

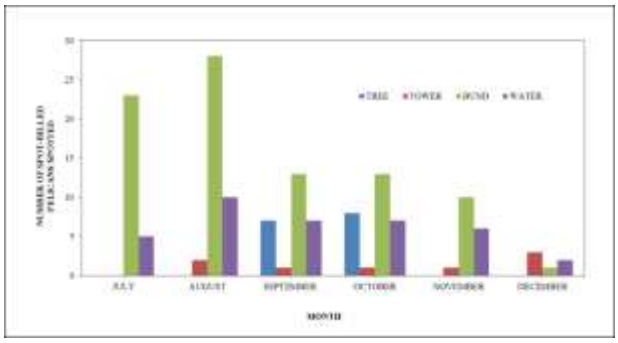


Fig 2. Population of spot-billed pelican observed utilizing the wetland resources during the ground survey at Ukkadam-periyakulam from July 2018 to December 2018

When compared to Ukkadam-periyakulam and Valankulam, Kurichi kulam has no trees and towers situated nearby and therefore the spot-billed pelicans mainly occupied the bund located in the centre of the lake and also utilized the water for different activities. Maximum population was observed on the bund in the month of July which dramatically reduced to zero by the month of December (Fig 3.).

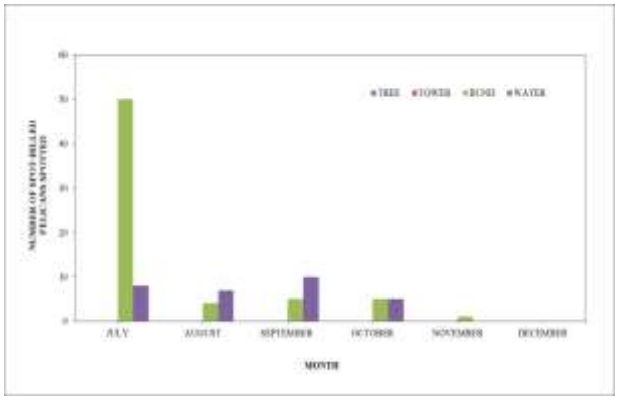


Fig 3. Population of spot-billed pelican observed utilizing the wetland resources during the ground survey at Kurichikulam from July 2018 to December 2018

Valankulam has no towers allocated nearby, which therefore resulted the sightings of the species on the bund, tress and water. In the month of July, the spot-billed pelican colonies were seen on the bund and the species first started appearing on the trees in

August followed by September and October respectively. The colony was restricted only to water in November and by the month of December there were no spot-billed pelicans observed in the lake (Fig 4.).

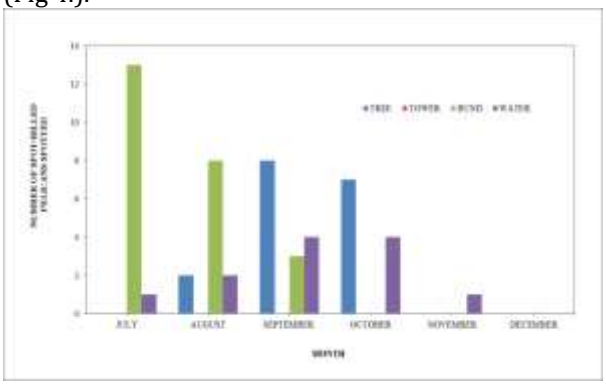


Fig 4. Population of spot-billed pelican observed utilizing the wetland resources during the ground survey at Valankulam from July 2018 to December 2018

4. DISCUSSION

The spot-billed pelicans are considered as the category of Near Threatened species by the IUCN (9). Interestingly the species are observed in South India namely in Karnataka, Andhra Pradesh and Tamil Nadu in varying numbers (8).

The population size of the spot-billed pelican decreased during the study period of six months from July 2018 to December 2018. The population size changed with changes in high quality habitat and broader ranges of habitat. The three wetlands had optimum amount of water and as a result a huge number of avian diversity was observed. Spot-billed pelicans are considered to be the local migratory birds. As pelicans are highly mobile, they can move in and out of the study area in large numbers if conditions are not conducive to feeding. These emigrations create real fluctuations in numbers (12).

The spot-billed pelican population is very high in Valankulam during the months of July to September, whereas the numbers are comparatively high in Ukkadam-periyakulam during October to December. It is noted that the population in the Kurichi kulam gradually decreased from July to December (Fig 1.). It may be suggested that local migration has not been affected in Kurichi kulam during this period, whereas there are changes observed in migration in the other two foraging sites studied. During the month of November and December, none or very few numbers were spotted in the Kurichi kulam (Fig 1.), which also indicates that July to October are the suitable period for the spot-billed pelicans for inhabiting the three wetlands studied.

In Ukkadam Lake the species were observed utilizing all the four resources compared to the other two wetlands. Among the four resources, the spot-billed pelicans occupied mainly the bund all over the study period except in the month of December 2018, wherein the species utilized water resources almost throughout the study period invariably. Very few numbers were spotted on the tower and trees throughout the study period except in the month of December (Fig 2.). Compared to the other two wetlands, trees and towers were not available in the Kurichi kulam and therefore the spot-billed pelicans mainly utilized the bund for roosting throughout the study period except in the month of December. Water resources were well utilized by the species mainly for feeding due to the tremendous availability of fishes in the lake (Fig 3.).

In Valankulam the spot-billed pelicans were observed utilizing the water resources throughout the study period except in the month of December. Compared to the other two study sites the species were recorded utilizing the trees consecutively for three months (August to September). Whereas the bunds were utilized only for the three months from July to September (Fig 4.).

Probably, increased water level was not preferred by this species as they started moving from the three foraging sites to other wetlands which held shallow water. They mostly preferred shallow water for feeding and are voracious feeders. Like other water birds, pelicans are generally found on or near water. Due to its large size and their strong gregarious tendencies, pelicans need an abundant supply of fish, a requirement that servely restricts the potential range of most species (13). Due to the climatic change experienced by south India, it led to the increase in water level in the wetlands which resulted in the drastic variation in the population density of the species. The aquaculture practices in these three wetlands increased as the water level had risen, which marked a threat to the bird. They were being trapped and tangled in the fishing lines which were surrounded all around the lake.

The spot-billed pelicans breeding from the month of October to April (8), in the present study, it was noted that the spot-billed pelicans were migrating to Vellalore lake in for breeding from the foraging sites. Environmental factors can play an important role in influencing pelican population. Weather in particular has various biological and ecological impacts in population of spot-billed pelican (14).

Anthropogenic activities also influenced the declining of the Spot-billed pelicans during the present study period. The pelicans, pelicanaries and their foraging grounds are under multitude of increasing pressures, which, if not addressed, could result in the decline or extinction of the species. Most of the wetland habitats in India face severe and increasing threats from human and human related factors (8). The future of nesting colonies of these birds located in protected areas appears to be safe when looked in isolation, but since breeding success is dependent on food supplies, their future will be assured only if their foraging grounds are in good health.

The reduction in numbers has been attributed to the unscientific desilting, which has damaged native species and blocked the inflow and outflow of water. Rapid urbanization and overgrowth of invasive vegetations like Water Hyacinth, Hydrilla, Prosopis juliflora and Parthenium are also seen as causes.

Based on the observations made in the present study, we concluded that the maximum population were recorded at Valankulam and minimum population at Ukkadam-periyakulam. The spot-billed pelicans mainly utilized wetland resources in the form of bund, tower, trees and water in the three foraging sites. The species preferred shallow water and which was one of the major reasons that spot-billed pelicans were observed in the three selected wetlands. One of the main reasons for the population reduction of Spot-billed pelicans in the studied wetlands is the intense aquaculture practices which are ongoing in the wetlands. It marked a major threat to the species as they tend to get trapped and tangled among the fishing lines spread across the lakes. Local migration for breeding is another reason for the population decline of Spot-billed pelicans.

REFERENCES

1. Mitsch, W.I., and I.G. Gosselink, (1986). Wetlands. Van Nostrand Reinhold, New York.
2. Dawson, T.P., P.M. Berry and E. Kampa, (2003). Climate change impacts on freshwater wetland habitats. *J. Nat. Conserv.* **11**: 25–30.
3. Bumstead, P.E., (2002). Canadian Feathers: A Loon-atics Guide to Anting, mimicry and Dump-nesting. Calgary AB: Simply Wild Publications (100 Lake Lucerne, Close SE, T2J 3H8).
4. Khanna, D.R., (2005). Biology Of Birds. New Delhi, India: Discovery Publishing House. pp. 315-316.
5. Perrins, C.M., and Middleton, A.L.A., eds. (1998) [1985]. Encyclopedia of Birds. New York, New York: Facts on File. pp. 53-54.
6. Subramanya, S., (2006). Pelicans bounce back. *World Birdwatch* **28**: 4
7. Gokula, V., (2014). An ethogram of Spot-billed Pelican (*Pelecanus philippensis*).
8. Kannan, V., and R. Manakadan, (2005). The status and distribution of Spot-billed Pelican *Pelecanus philippensis* in southern India. Forktail, **21**: 9-14.
9. Kannan, V., and J. Pandiyan, (2013). A review on the spot-billed pelican *Pelecanus philippensis* literature. Front Biol (Beijing) **8**: 333-352.
10. Birdlife International. (2007). Species Factsheet: *Pelecanus philippensis*.
11. Crivelli, A.J. and R.W. Schreiber, (1984). "Status of the Pelecanidae". *Biological Conservation* **30(**2): 147-156.
12. Kannan, V., and J. Pandiyan, (2016). Spatial and temporal variations of the density of Spot-billed pelican *Pelecanus philippensis* in Pulica Lake, East coast of Southern India. *Int. J. Pure Appl. Zool.* **3**(3): 33–45.
13. Kannan, V., and J. Pandiyan, (2012). Nesting Ecology of the Spot-Billed Pelican *Pelecanus Philippensis* in Southern India. *World J. Zool.* **7**(4): 295–302.
14. Sparks, T., and P. Tryjanowski, (2005). The detection of climate impacts: some methodological considerations. *Int. J. Climatol.* **25**: 271-277.

DOI:10.26524/KRJ303

Kong. Res. J. 6(2): 61-65, 2019
Publisher: Kongunadu Arts and Science College, Coimbatore.

http://krjscience.com

RESEARCH ARTICLE

MIXED METAL OXIDE MESOPOROUS NANOPARTICLES FOR ENVIRONMENTAL REMEDIATION

Nithya Davis[1], P. Usha Rajalakshmi[1] and T. Sakthivel[2,*]

[1]Department of Physics, Avinasilingam Institute For Home Science and Higher Education For Women1, Coimabatore – 641 043, Tamilnadu, India.

[2]Department of Zoology, Kongunadu Arts and Science College, Coimbatore- 641 029, Tamil Nadu, India.

ABSTRACT

Mesoporous mixed metal oxides ($SnO_{2(x)}$ -$TiO_{2\ (1-x)}$, x= 0.75,0.50 and 0.25) were synthesized by evaporation induced self assembly using cationic surfactant, Cetyl Trimethyl Ammonium Bromide (CTAB) as the structure directing agent. The small angle X-ray diffraction pattern of mesoporous SnO_2 and SnO_2-TiO_2 mixed metal oxides revealed the presence of well defined mesostructure in the metal oxides. The mixed metal oxide system has crystallized in orthorhombic structure, resembling the host lattice. Mesopore channels were collapsed upon calcinations at 550°C. The optical absorption of the SnO_2 has been extended into the visible region upon incorporation of "Ti". A remarkable enhancement of the photocatalytic degradation efficiency (60%) of ($SnO_{2(0.5)}$ -$TiO_{2\ (0.5)}$ was observed against aqueous solution of methylene blue dye.

Keywords: SnO2, TiO2, Metal oxide, Semiconductor, Photocatalysis, Environmetal remediation.

1. INTRODUCTION

Metal oxides are prospective materials for applications in various fields such as solar energy conversion, photocatalysis, electrochemical catalysis, lithium/sodium ion batteries, field effect transistors and super capacitors [1-17] and have been intensively studied due to their inherent chemical stability, abundance, low cost and environmental friendliness. Metal oxide nanostructures are being widely used in place of bulk counterparts as the unique morphology, surface structure and optoelectronic characteristics associated with the nanostructures are uniquely enhancing the performance devices. Rational design and reproducible synthesis of stable nanomaterials of particular shape, size and microstructure is highly desirable. In particular, synthesis of porous metal oxides with ordered pore structures, as required for photocatalytic applications is remaining a challenging task. Notably, tin oxide (SnO_2), a wide band gap semiconductor, has appropriate optoelectronic characteristics suitable for photocatalytic applications but SnO_2 nanostructures produced by solvothermal / hydrothermal methods have always exhibited a fairly low specific surface area(<50 $m^2\ g^{-1}$)[18]. Hence it is imperative to improve the synthesis strategies to produce mesoporous SnO_2 with improved specific surface area. Surfactant templating strategy for the synthesis of non-silica based mesostructures, mainly metal oxides in which both positively and negatively charged low molecular weight surfactants are widely being used for the synthesis of mesoporous metal oxide nanoparticles. It was found that charge density matching between the surfactant and the inorganic species is important for the formation of the organic-inorganic mesophases. In the recent past efforts have been made to employ the potential of mesoporous metal oxides/metal oxide nanocomposites for environmental remediation [19-24].

In the present work, an attempt has been made to synthesize Mesoporous Tin Oxide (SnO_2) by Evaporation Induced Self Assembly and to extend/optimise the synthesis procedure to synthesize SnO_2 –TiO_2 mixed metal oxide system. Attempts have been made to analyse the photocatalytic activity of the mesoporous metal oxides for the degradation of methylene blue.

2. MATERIALS AND METHODS

In the present work following methodology adopted for the synthesis of mesoporous SnO_2 and $SnO_{2(x)}$ -$TiO_{2\ (1-x)}$ mixed metal oxides by Evaporation-Induced Self-Assembly.

2.1. Synthesis of Ordered Mesoporous Titania

Mesoporous tin oxide (SnO_2) is synthesized using cationic surfactant, Cetyl Trimethyl Ammonium Bromide (CTAB) as the structure directing agent and tin tetrachloride (1.0 M in methylene chloride, Sigma Aldrich) and titanium tetrachloride (1.0 M in methylene chloride, Sigma Aldrich) as the source for tin and titanium respectively. The surfactant solution is obtained by dissolving 2.5 g of CTAB in 50 ml of cyclohexanol (Sigma Aldrich) and the solution (Sol A) is

*Correspondence: Sakthivel, T., Department of Zoology, Kongunadu Arts and Science College, Coimbatore – 641 029, Tamil Nadu, India. E.mail: sathivelunom@gmail.com

continuously stirred for 2 h during which 3.5 ml of concentrated HCl is added drop wise. To the resulting solution A, 10 ml of tin tetrachloride is added drop wise and stirred for 4 hours. The resulting solution thus obtained is made as a thin layer and kept in hot air oven maintained at 60°C for 4 days. The solid product obtained is calcined in a tubular furnace at a temperature of 550°C for 6 hours at a heating rate of 1°C / minute with air flow. The sample is coded as MSNO-43. Similarly mixed tin – titanium metal oxides ($SnO_{2(x)}$ -$TiO_{2\ (1-x)}$) are prepared by incorporating suitable amount of tin and titanium precursors. The resulting samples are coded as MSNO - 43 (SnO_2), MSTO - 43 ($SnO_{2(0.75)}TiO_{2(0.25)}$), MSOTO - 43 ($SnO_{2(0.5)}TiO_{2(0.5)}$),MSTO - 43 ($S_2nO_{2(0.25)}TiO_{2(0.75)}$), MTIO - 43 ($TiO_2$) .

2.2 Material characterization

The characteristics of materials prepared in present work were systematically analyzed using X-Ray Diffractometer (Rigaku Miniflex II), High Resolution Transmission Electron Microscope (HRTEM, JEOL JEM 2100, operated at an accelerating voltage of 120 kV), UV-Vis. Spectrophotometer (JASCO, V-650).

2.3 Photocatalytic activity

The synthesized SnO_2 and metal oxide were tested for photocatalytic degradation of methylene blue. Around 0.2g of the catalyst was suspended in quartz cell along with 200ppm, 5ml aqueous solution of the dye. Prior to light irradiation, the suspension was stirred for 30 minutes in dark to attain the absorption- desorption equilibrium. The sample was irradiated using natural sunlight. At periodic intervals, 5ml aliquots were taken from the system and analysed using UV-Vis spectrophotometer.

3. RESULTS AND DISCUSSION

The small angle X-ray diffraction pattern of mesoporous SnO_2 and SnO_2-TiO_2 mixed metal oxides are shown in figures 1-5. The presence of well defined diffraction peak centered at 2θ of 0.7° (Fig.1) is indicative of the formation of long range ordered pore structure and the peaks are arising from (100) reflections associated with 2D hexagonal (*P6mm*) arrays of uniform mesopores [25].

The X-ray diffraction pattern of mesoporous SnO_2 prepared in the present work is shown in figure 6. The samples were found to have crystallized in orthorhombic structure, the formation of which is favored at higher temperatures [26]. XRD pattern of TiO_2 reveals the formation of a mixed phase containing anatase, rutile and brookite.The XRD pattern of mixed metal oxides prominently featured characteristic features of orthorhombic SnO_2 (JCPDS Card No. 78-1063).

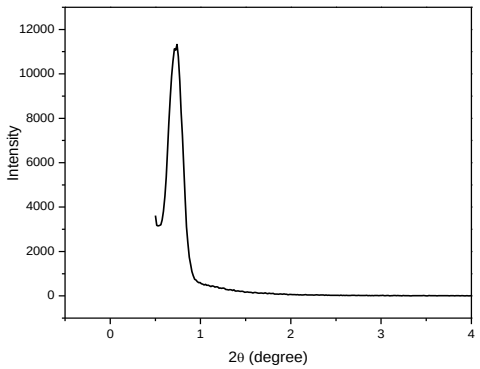


Fig. 1. Small angle XRD pattern of MSNO-43

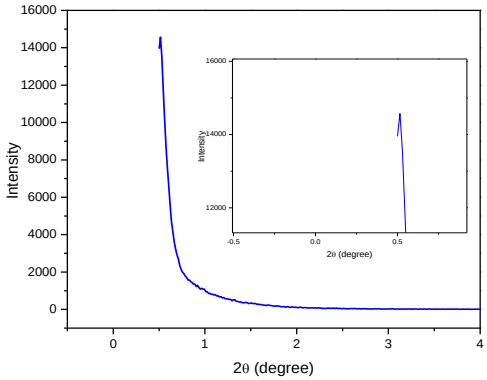


Fig. 2. Small angle XRD pattern of MSTO-43

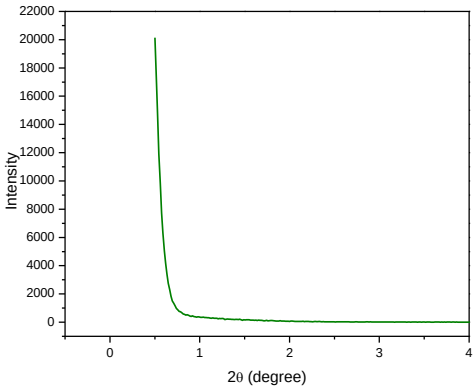


Fig. 3. Small angle XRD pattern of MSOTO-43

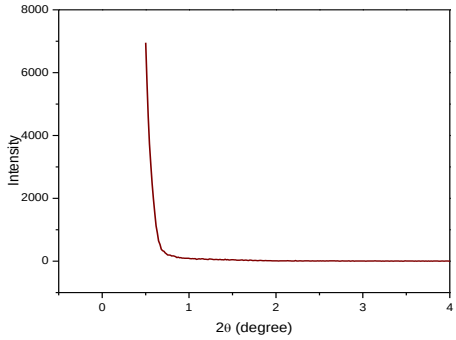


Fig. 4. Small angle XRD pattern of MTSO-43

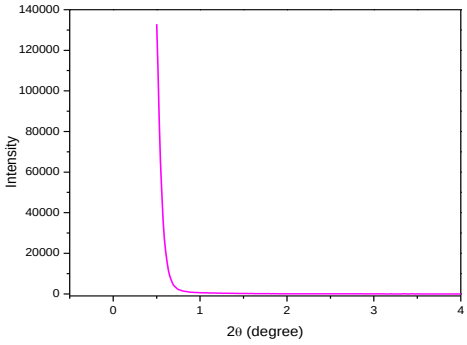


Fig. 5. Small angle XRD pattern of MTIO-43

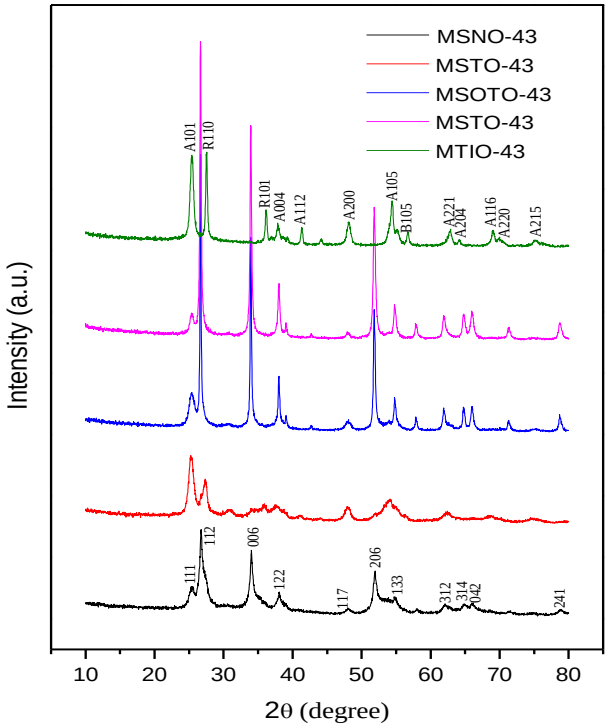


Fig. 6. XRD pattern of mesoporous SnO2 and SnO2-TiO2 mixed metal oxides

The presence of pore channels in the porous metal oxides are noticed from the High Resolution Transmission Electron Micrographs (Figure 7 a and b) of the samples, MSNO-43 and MSOTO-43. The pore structure has been observed to collapse upon calcinations at 550°C.

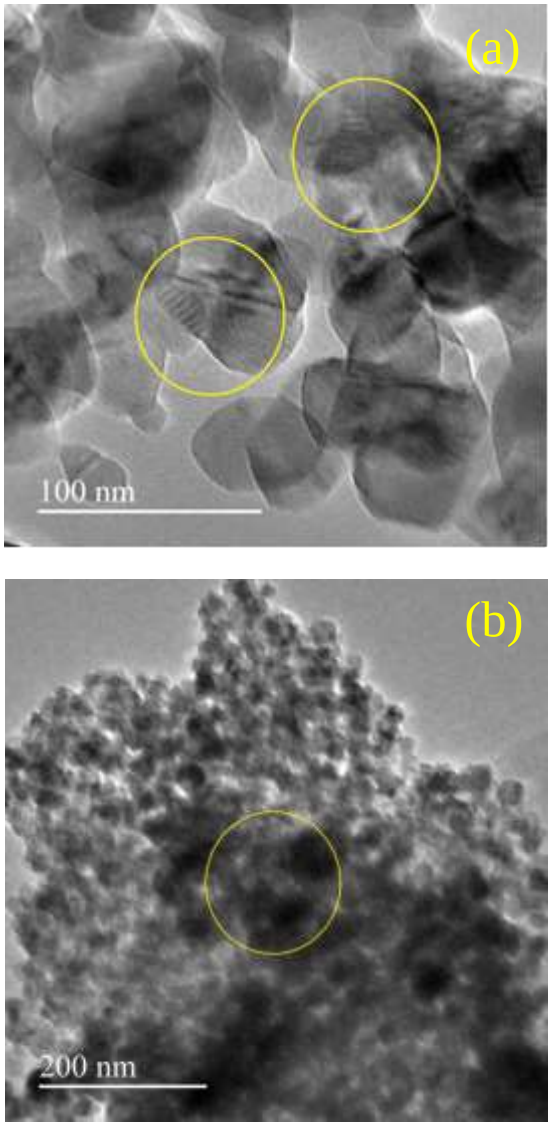


Figure 7. HRTEM images of mesoporous SnO_2 and SnO_2-TiO_2 mixed metal oxides

The diffuse reflectance UV-Vis. spectra of mesoporous SnO_2 and SnO_2-TiO_2 mixed metal oxides are shown in Fig. 8. The mixed metal oxides (MSOTO-43 and MSTO-43) exhibited enhanced visible light absorption which can be ascribed to the presence of defect levels within the band gap of the material.

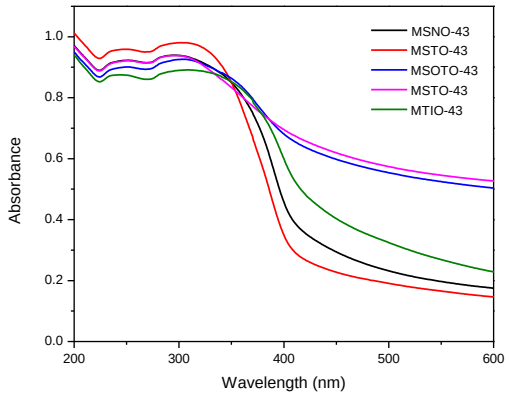


Fig.8. Diffuse reflectance UV-Vis. spectra of SnO_2 and SnO_2-TiO_2 mixed metal oxides

The photocatalytic efficiency of mixed metal oxide nanoparticles are analysed against the photocatalytic degradation of methylene blue. The optical absorption characteristics of the dye without the photocatalyst and with photocatalyst are recorded using UV – Vis spectrophotometer. The photocatalyst is added to the aqueous solution of the dye and the reaction was allowed to proceed under direct sunlight. The concentration of the dye and the absorbance of the reactant at 390 nm (where has it absorbance maxima) are evaluated from Beer – Lambert law after specified reaction time of 20, 40 and 60 minutes. The photocatalytic efficiency of the dye is calculated by noting the variation in the concentration of the dye after a specified reaction time (C) with respect to the concentration of the dye at time t = 0 (C_0).

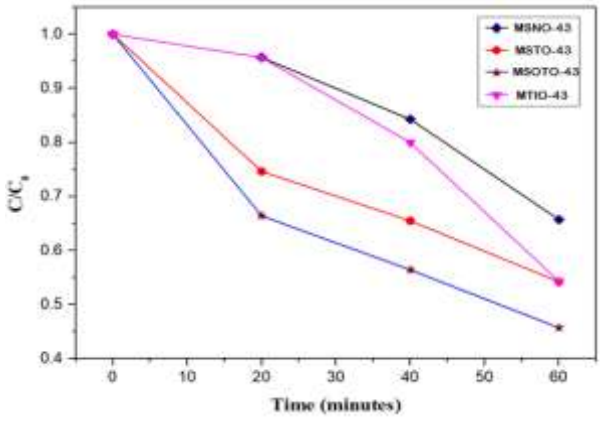


Figure 9. Photocatalytic Performance of SnO_2 and SnO_2-TiO_2 mixed metal oxides

Fig. 9 shows the variation of $C/C_0$with respect to time. SnO_2 particles photocatalytically degraded methylene blue (MB) under visible light radiation by 30%. Since the mesoporous structure enhances the surface area of the semiconductor widely, remarkable enhancement in the photocatalytic efficiency of the mesoporous photocatalyst was observed. Photocatalytic efficiency of mesoporous $SnO_{2(0.5)}$ -$TiO_{2\,(0.5)}$ (MSOTO-43) nanoparticles was the highest (60%) and further doping has been found to decrease the photocatalytic efficiency. The creation of defect level in the host metal oxide due to formation of mixed metal oxide system plays a pivotal role in enhancing the visible light absorption and in increasing the lifetime of photogenerated charge carriers.

4. CONCLUSION

Mesoporous tin oxide and $SnO_{2(x)}$ -$TiO_{2\,(1-x)}$ mixed metal oxides were synthesized by evaporation induced self assembly method. Systematic analysis on the characteristics of the material revealed the formation of crystalline and mesoporous nanoparticles. The $SnO_{2(x)}$ -$TiO_{2\,(1-x)}$ exhibited visible light activity which originates from the creation of electronic states in the band gap of the material. The enhanced optoelectronic characteristics of the system $SnO2_{(0.5)}$ -$TiO_{2\,(0.5)}$ extends the potential of material for environmental remediation through the treatment of organic pollutants such as 4-Chlorophenol and synthetic dyes.

REFERENCES

1. Kim, H.R., K.I. Choi, J.H. Lee and S.A. Akbar, (2009). Highly Sensitive and Ultra-fast Responding Gas Sensors Using Self-assembled Hierarchical SnO_2 Spheres. *Sens. Actuators B* **136**: 138-143.
2. Kuang, Q., C. Lao, Z.L. Wang, Z. Xie and L. Zheng, (2007). High-Sensitivity Humidity Sensor Based on a Single SnO_2 Nanowire. *J. Am. Chem. Soc.*, **129**: 6070-6071.
3. Liu, Y., Y. Jiao, Z. Zhang, F. Qu, A. Umar and X. Wu, (2014). Hierarchical SnO_2 Nanostructures Made of Intermingled Ultrathin Nanosheets for Environmental Remediation, Smart Gas Sensor, and Supercapacitor Applications. *ACS Appl. Mater. Interfaces* **6**: 2174-2184.
4. Lipeng, Q., X. Jiaqiang, D. Xiaowen, P. Qingyi, C. Zhixuan, X. Qun and L. Feng, (2008). The Template-free Synthesis of Square-shaped SnO_2 Nanowires: the Temperature Effect and Acetone Gas Sensors. *Nanotechnology* **19**: 185705.
5. Dong, Z., H. Ren, C.M. Hessel, J. Wang, R. Yu, Q. Jin, M. Yang, Z. Hu, Y. Chen, Z. Tang, H. Zhao and

D. Wang, (2014). Quintuple-Shelled SnO_2 Hollow Microspheres with Superior Light Scattering for High-Performance Dye-Sensitized Solar Cells. *Adv. Mater.* **26**: 905-909.
6. Gubbala, S., V. Chakrapani, V. Kumar and M.K. Sunkara, (2008). Band-Edge Engineered Hybrid Structures for Dye-Sensitized Solar Cells Based on SnO_2 Nanowires. *Adv*. *Funct*. *Mater*. **18**: 2411-2418.
7. Hong, Y.J., M.Y. Son and Y.C. Kang, (2013). One-Pot Facile Synthesis of Double-Shelled SnO_2 Yolk-Shell-Structured Powders by Continuous Process as Anode Materials for Li-ion Batteries. *Adv. Mater.* **25**: 2279-2283.
8. Ding, J., Z. Li, H. Wang, K. Cui, A. Kohandehghan, X. Tan, D. Karpuzov and D. Mitlin, (2015). Sodiation vs. Lithiation Phase Transformations in a High Rate-high Stability SnO_2 in Carbon Nanocomposite. *J. Mater. Chem. A,* **3**: 7100-7111.
9. Jahel, A., C.M., Ghimbeu, A. Darwiche, L. Vidal, S. Hajjar-Garreau, C. Vix-Guterl and L. Monconduit, (2015). Exceptionally Highly Performing Na-ion Battery Anode Using Crystalline SnO_2 Nanoparticles Confined in Mesoporous Carbon. *J. Mater. Chem. A.* **3**: 11960-11969.
10. Chen, J.S., and X.W. Lou, (2013). SnO_2-Based Nanomaterials: Synthesis and Application in Lithium-Ion Batteries. *Small* **9**: 1877 - 1893.
11. Wang, Z., D. Luan, F.Y.C., Boey and X.W. Lou, (2011). Fast Formation of SnO_2 Nanoboxes with Enhanced Lithium Storage Capability. *J. Am. Chem. Soc.* **133**: 4738-4741.
12. Su, D., C. Wang, H. Ahn and G. Wang, (2013). Octahedral Tin Dioxide Nanocrystals as High Capacity Anode Materials for Na-ion Batteries. *Phys. Chem. Chem. Phys.* **15**: 12543-12550.
13. Han, Y., X. Wu, Y. Ma, L. Gong, F. Qu and H. Fan, (2011). Porous SnO_2 Nanowire Bundles for Photocatalyst and Li ion Battery Applications. *Cryst. Eng. Comm.* **13**: 3506-3510.
14. Wu, H.C., Y.-C. Huang, I.K. Ding, C.-C. Chen, Y.-H. Yang, C.-C. Tsai, C.-D. Chen and Y.-T. Chen, (2011). Photoinduced Electron Transfer in Dye-Sensitized SnO_2 Nanowire Field-Effect Transistors. *Adv. Funct. Mater.* **21**: 474-479.
15. Shin, G., C.H. Yoon, M.Y. Bae, Y.C. Kim, S.K. Hong, J.A. Rogers and J.S. Ha, (2011). Stretchable Field-Effect-Transistor Array of Suspended SnO_2 Nanowires. *Small* **7**: 1181-1185.
16. Oh, H.S., H.N. Nong and P. Strasser, (2015). Preparation of Mesoporous Sb, F, and In-Doped SnO_2 Bulk Powder with High Surface Area for Use as Catalyst Supports in Electrolytic Cells. *Adv. Funct. Mater.* **25**: 1074-1081.
17. Shinde, D.V., D.Y. Lee, S.A. Patil, I. Lim, S.S. Bhande, W. Lee, M.M. Sung, R.S. Mane, N.K. Shrestha and Han, S.-H. (2013). Anodically Fabricated Self-organized Nanoporous Tin Oxide Film as a Supercapacitor Electrode Material. *RSC Adv.* **3**: 9431-9435.
18. Bian, H., Y. Tian, C. Lee, M.F. Yuen, W. Zhang and Y. Yang, (2016). Mesoporous SnO_2 Nanostructures of Ultrahigh Surface Areas by Novel Anodization. *ACS Appl*. *Mater*. *Interfaces* **8**: 28862-28871.
19. Akhila, A.K., P.S., Vinitha and N.K. Renuka, (2018). Photocatalytic Activity of Graphene-Titania Nanocomposite Materials Today: *Proceedings* **5**: 16085-16093.
20. Wang, D., S.C., Pillai, S.-H. Ho, J. Zeng, Y. Li and D.D. Dionysiou, (2018). Plasmonic-based nanomaterials for environmental remediation**.** *Applied Catalysis B: Environmental* **237**: 721-741.
21. Chen, Y., B. Zhai, Y. Liang, Y. Li and Li, J. (2019). Preparation of CdS/ g-C_3N_4/ MOF composite with enhanced visible-light photocatalytic activity for dye degradation. *J. Solid State Chem.* **274**: 32-39.
22. Jiang, L., D. Chen, L. Qin, J. Liang and Y. Huang, (2019). Enhanced photocatalytic activity of hydrogenated $BiVO_4$ with rich surface-oxygen-vacancies for remarkable degradation of tetracycline hydrochloride. *J. Alloys Compd.* **783**: 10-18.
23. Yousaf Khan, M., M. Ahmad, S. Sadaf, S. Iqbal, F. Nawaz and Iqbal, J. (2019). Visible light active indigo dye/graphene/ WO_3 nanocomposites with excellent photocatalytic activity. *J. Mat. Res. Tech,* **8**: 3261-3269.
24. Zhong, N., M. Chen, Y. Luo, Z. Wang, X. Xin and B.E. Rittmann, (2019). A novel photocatalytic optical hollow-fiber with high photocatalytic activity for enhancement of 4-chlorophenol degradation. *Chem. Eng. J.* **355**: 731-739.
25. Wang, T., X. Meng, P. Li, S. Ouyang, K. Chang, G. Liu, Z. Mei and J. Ye, (2014). Photoreduction of CO_2 over the well-crystallized ordered mesoporous TiO_2 with the confined space effect. *Nano Energy* **9**: 50-60.
26. Zhang, Q., P. Liu, C. Miao, Z. Chen, C.M.L. Wu and C. Shek, (2015). Formation of orthorhombic SnO_2 originated from lattice distortion by Mn-doped tetragonal SnO_2. *RSC Adv.* **5**: 39285-39290.

DOI:10.26524/KRJ304

Kong. Res. J. 6(2): 66-71, 2019
Publisher: Kongunadu Arts and Science College, Coimbatore.

RESEARCH ARTICLE

PLANT INSECT INTERACTION AND CROP PROTECTION: A DYNAMIC ANALYSIS

Bharathi, P*. and Dylin Raja Sheribha
Department of Zoology, Kongunadu Arts and Science College, Coimbatore- 641 029, Tamil Nadu, India.

ABSTRACT

Integrating supporting and regulating ecosystem functions provided by several components of biodiversity into cropping systems has been prepared as a promising way to decrease agrochemical inputs and negative environmental impacts while maximizing crop productivity and food security. The co-evolution of plants and insects in very intriguing and plays vital role in the crop protection. Plants have developed efficient mechanisms to protect them against herbivore while insects have found diverse ways of avoiding negative effects of their host plant defense mechanism. Even though many workers have attempted to study plant - insect interaction, still our knowledge is limited. A changing climate, growing pest have given uncertain impacts on crop protection so, the present study address the key question that Is it possible to find alternative to meet these challenges by studying the plant-insect interaction and formulating integrated pest management? The study was conducted at Dharapuram, Dindugal district as this area is riched with the variety of crop cultivation. The study concludes that the biological control of insect pests with natural products by the development of new plant varieties with enhanced chemical defenses should be followed for the better crop protection.

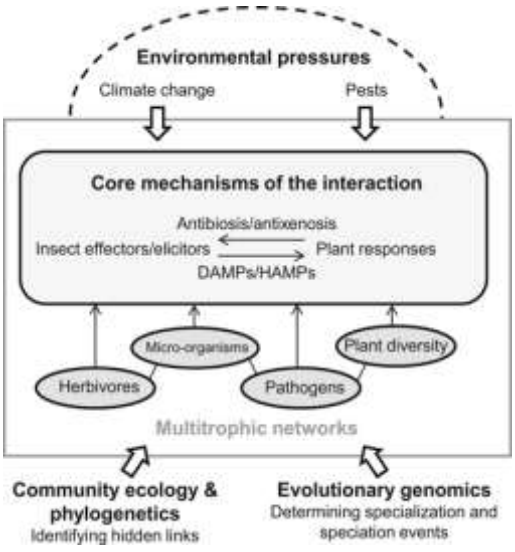
Keywords: Insect, Crop protection, Interaction, Biological control.

1. INTRODUCTION

In nature, most plants are fed upon by insects. Some herbivorous insects are very particular in their choice of food plants, whereas others are more generalist feeders. Plants are not passive by standers, however, as they have evolved resistance to most potential insect attackers (1). The world is mostly green. Domesticated crops are also inherently resistant to most insects (2). although we are sensitive to any insect damage that reduces yield, quality and profits to the farmer, and certain insects can indeed devastate their crop host leaving nothing to harvest. The ancestors of modern-day crop plants coevolved with insects and through natural selection accumulated many physical and chemical traits that formed a core defense against attackers (3) . Plant domestication and breeding involving selection for improved yield and quality has generally made crops more susceptible to pest damage (4).

The co-evolution of plants and insects is very intriguing. Plants have developed efficient mechanisms to protect them against herbivory while insects have found diverse ways of avoiding negative effects of their host plants defense mechanisms (4,5). The better understanding of this process will allow us to achieve more effective methods for the biological control of insect pests with natural products by the development of new plant varieties with enhanced chemical defenses (6).

Current investigations of plant–insect interactions hold promise for us to gain a better understanding of the functional, ecological, and evolutionary impacts of insect–plant interactions, with implications and relevance for both applied and fundamental research (7,8).



Promises and challenges in insect-plant interaction. Damage-Associated Molecular Patterns (DAMPs); Herbivore-Associated Molecular Patterns (HAMPs)

Over the coming years, a changing climate, growing pest have given uncertain impacts on crop

*Correspondence: Bharathi, P., Department of Zoology, Kongunadu Arts and Science College, Coimbatore – 641 029, Tamil Nadu, India. E.mail: pkbharathi@gmail.com

protection so, is it possible to find alternative to meet these challenges by studying the plant-insect interaction and formulating integrated pest management? To address these issues, the main objective of this study is to screening of insects for a ten high yielding plants and to study its impact over it to find the alternative for high yield.

2. MATERIALS AND METHODS

The present study was carried out from august 2018 to January 2019 in. The insect pests were indentified up to family/genus/species levels wherever possible. The host plants also were identified. The insect pests in the field were recognized and observed by using mobile camera, And also by the picture downloaded from the website. During the study insect pests were identified also classified according to their family. And also the plant part attacked by insect pest.

AREA OF STUDY:

- The study was conducted at Dharapuram, Dindugal district.
- The local farm of 7 acres with various plantation is selected and the pests were identified.
- The interaction of insect with plants was observed externaly.
- The damage caused by the insect was noticed and finding the alternate for the better yield of the host plant.

3. RESULTS

Table 1. Screened insects with their host plant

	HOST PLANT	PEST	PART OF ATTACK	ORDER AND FAMILY
1	Coconut (*Cocos nucefera*)	*Oryetes rhinoceros*	Tender crown	Coleoptera Scarabaeidae
2	Paddy (*Oriyza sativa*)	*Leptocorisa acuta*	Flowers and leaves	Hemiptera Alydidae
3	Sugarcane (*Saccharum* sp.)	*Chilo infuscatellus*	leaves	Lepidoptera Crambidae
4	Brinjal (*Solanum melongena*)	*Leucinodes orbanalis guen*	Fruit and shoot	Lepidoptera Crambidae
5	Ground nut (*Arachis hypogaea*)	*Aphis craccivora* koch	shoot	Hemiptera Alydidae
6.	Cotton (*Gossypium* sp.)	*Dysdercus cingulatus*	Flower and seeds	Hemiptera Pyrrhocoridae
7.	Drumstick (*Moringa oleifera*)	*Noorda moringae*	Flowers and Buds	Lepidoptera Crambidae
8.	Mango (*Mangifera indica*)	*Orthaga euadrusalis*	Tender shoot and leaves	Lepidoptera Pyralidae
9.	Sorghum (*Sorghum bicolor*)	*Chinavia hilaris*	Leaves and shoot	Hemiptera Pentatomidae
10.	Corn (*Zea mays*)	*Agrotis segetum*	Leaf, Bud and Stem	Lepidoptera Owlet moths

COCONUT PEST

- PEST COMMON NAME: Rhinoceros Beetle
- SCIENTIFIC NAME: *Oryetes rhinoceros*
- The rhinoceros beetle, well known for their unique shapes and large size, is one of the major pest of the coconut.
- It burrows the tender crown leaving behind the series of holes in the leaflets.

Fig. 1. *Oryetes rhinoceros*

PADDY PEST

- PEST COMMON NAME: Rice ear head bug.
- SCIENTIFIC NAME: *Leptocorisa acuta*
- This pest attacks during the flowering stages of the rice crop.
- It is distributed in Australia and south Asian countries.
- The excessive feeding reduces the rate of photosynthesis and cause the discoloration of the grains which reduces the market quality.

Fig. 2. *Leptocoris aacuta*

SUGARCANE PEST

- PEST COMMON NAME: yellow top borer
- SCIENTIFIC NAME: *Chiloinfus catellus*
- The pest belongs to moth family & attacks 1-3 month old crops.
- Widely distributed in south Asian countries.
- The larva feeds on the midrib and cause "death heats", which makes the central leaf sheath dry.

Fig 3. *Chiloinfus catellus*

BRINJAL PEST

- PEST COMMON NAME: Root borer
- SCIENTIFIC NAME: *Leucinodes orbanaliguen*
- It is the most serious pest of Brinjal & it is found throughout the country.
- The larve bores into tender shoots in the early stage & cause "dead hearts".
- It also attacks the buds & developing fruits.

Fig. 4. *Leucinodes orbanalisguen*

GROUNDNUT PEST

- PEST COMMON NAME : Aphids
- SCIENTIFIC NAME: *Aphis craccivora* koch

- They are the black species of aphids attacking the leguminous crops.
- They are present in large number and completely drain the plant sap.
- Due to the mass attack, the plant succumbs quickly then the larger plants.
- It also the vector of the virus that cause rosette disease of groundnut.

Fig. 5. *Aphidoidea*

COTTON PEST

- PEST COMMON NAME :Red cotton bug
- SCIENTIFIC NAME : *Dysdercus cingulatus*
- Like other true bugs,Dysdercus cingulatus also has the piercing and sucking type of mouth.
- The part of the cotton plant affected by this pest is the flower and seeds capsule or boll.
- As this develops,the insect thrusts its rostrum between the carpel s and sucks fluids from the still soft seeds inside.
- Apart from the cotton, Ladies Finger also acts as the hast plant for this bug.

Fig. 6. *Dysdercus cingulatus*

DRUMSTICK PEST

- PEST COMMON NAME :Bud worm of drumstick
- SCIENTIFIC NAME : *Noorda moringae*
- Adult is small in size with dark brown fore wings and white hind wings with dark brown border.
- Oval creamy white eggs in clusters or singly on flower buds.
- Caterpillars are dirty brown with a prominent mid-dorsal stripe and black head and pro-thoracic shield.
- Destructive and specific pest of drumstick in South India.
- Larva bores into flowers buds and causes shedding

Fig. 7. *Noorda moringae*

MANGO PEST

- PEST COMMON NAME : Leaf webber
- SCIENTIFIC NAME: *Orthaga euadrusalis*
- The caterpillars feed on the leaf surfsce by gregariously srappuing and later they make the web of the tender shoots and leaves and feed within.
- Several caterpillars may be found in a single webbed up cluster of leaves.
- The male is slightly smaller than the female.

Fig. 8. Leaf Webber

SORGHUM PEST

- PEST COMMON NAME: Stink Bug

- SCIENTIFIC NAME: *Chinavia hilaris*
- The abdomen is made of scutellum, giving the family name "Shield Bug".
- It causes the wide spread damages in many vegetables and fruits, especially in sorghum.
- It mainly affects the leaves and shoot of the plant.
-

Fig. 9. Stink bug

CORN PEST

- PEST COMMON NAME: Cut worm
- SCIENTIFIC NAME: *Agrotis segetum*
- The term cutworm mainly applies to larvae of various species in the Nocutidae, a large family of moths.
- They are the voracious leaf, bud and stem feeders and can destroy entire plants
- Cutworms are not worms, biologically speaking they are caterpillars.

Fig.10. Cut Worm

4. DISCUSSION

The study of plant - insect interaction continues to be an exciting and fast moving field that build upon the more extensive literature available in plant insect interaction and offers new and significant insights into both unique molecular determinants of plant-insect interactions and the wider ecological context.

Global change is resetting the spatial and ecological equilibrium of complex co- evolutionary relationships between plants and their insect's herbivores (9). We distinguish between the direct effect of global changes on each partner's from indirect impacts on insects via the response of plants. The indirect effects include a change in the nutritional quality of the plant tissues for herbivore insects as well as changes in the microclimatic condition at the leaf surface (10).

Pollinators are involved in a close symbiotic relationship with their favourite. Plants and any depression caused by climate stress lead to pollination deficit. Pollinators are indeed quite sensitive to global changes, furthermore, although species are connected by trophies links, but species respond differently to global changes (11).

REFERENCES

1. Mescher, M.C., and C.M. De Moraes, (2015). Role of plant sensory perception in plant–animal interactions. *J. Exp. Bot.* **66**: 425-433.
2. Bruce, T.J.A. (2015). Interplay between insects and plants: dynamic and complex interactions that have coevolved over millions of years but act in milliseconds. *J. Exp. Bot.* **66**: 455-465.
3. Sugio, A., G. Dubreuil, D. Giron and J-C. Simon, (2015. Plant–insect interactions under bacterial influence: ecological implications and underlying mechanisms. *J. Exp. Bot.* **66**: 467-478.
4. Kerchev, P.I., B. Fenton, C.H. Foyer and R.D. Hancock, (2012). Plant responses to insect herbivory: interactions between photosynthesis, reactive oxygen species and hormonal signalling pathways. *Plant Cell Environ.* **35**: 441-453.
5. Harris, M.O., T.L. Friesen, S.S. Xu, M.S., D. Chen and J. Giron Stuart, (2015). Pivoting from Arabidopsis to wheat to understand how agricultural plants integrate responses to biotic stress. *J. Exp. Bot.* **66**: 513–531.
6. Rasmann, S., T.G. Köllner, J. Degenhardt, I. Hiltpold, S. Toepfer, U. Kuhlmann, J. Gershenzon, and T.C.J. Turlings (2005). Recruitment of entomopathogenic nematodes by insect-damaged maize roots. *Nature* **434**: 732–737.

7. Hiltpold, I., G. Jaffuel and T.C.J. Turlings, (2015). The dual effects of root-cap exudates on nematodes: from quiescence in plant-parasitic nematodes to frenzy in entomopathogenic nematodes. . *J. Exp. Bot.* **66**: 603–611.
8. Ryalls, J.M.W., B.D. Moore, M. Riegler, A.N. Gherlenda and S.N. Johnson, (2015). Amino acid-mediated impacts of elevated carbon dioxide and simulated root herbivory on aphids are neutralized by increased air temperatures. . *J. Exp. Bot.* **66**: 613–623.
9. Panda, N., and G.S. Khush, (1995). Host plant resistance to insects. CAB International, Wallingford.
10. Nishida, R., (2002). Sequestration of defensive substances from plants by lepidoptera. *Annu. Rev. Entomol.* **47**: 57-92.
11. Bernays, E.A., (2001). Neural limitations in phytophagous insects: Implications for diet breadth and evolution of host affiliation. *Annu. Rev. Entomol.* **46**: 703-727.

DOI:10.26524/KRJ305

Kong. Res. J. 6(2): 72-75, 2019
Publisher: Kongunadu Arts and Science College, Coimbatore.

REVIEW ARTICLE

ROLE OF C- PEPTIDE AS A BIOMARKER FOR DIABETES – A MINI REVIEW

Subburaj Bhuvaneshwari and Ramasamy Amsaveni*
PG and Research Department of Biotechnology, Kongunadu Arts and Science College, Coimbatore- 641 029, Tamil Nadu, India.

ABSTRACT

Diabetes is accumulation of higher glucose content in the blood. When the glucose level is increased it indicates the low insulin secretion or no response to the insulin production in the body. C-peptide is cleaved from the proinsulin chains A and B during insulin synthesis. C- peptide produced in the same amount to insulin because; C-peptide is removed when insulin is formed. As per the amount is same for both the C-peptide and insulin, the C-peptide works as a biomarker for finding the insulin secretion and to finding of diabetes type accurately. In this review it has been focused on C-peptide and its role as a biomarker in diabetes and predictor of complications and other risks caused by the diabetes.

Keywords: Diabetes, Insulin, Biomarker, C-peptide.

1. INTRODUCTION

Diabetes is caused by the way our body makes or uses insulin. Insulin is made to move blood glucose into the cells, where it is stored and then used for the energy. Diabetes mellitus is one of the most burdensome chronic diseases and is associated with shorter lifetime, diminished quality of the life and economic burdens on the patient and the society as a result of healthcare, medication (1). Type1 diabetes mellitus originated from an immune-mediated destruction of insulin-producing-cells found in the pancreatic islets of Langerhans. Type 1 is the most common form of diabetes. It is characterized by the destruction of pancreatic beta cells resulting in the absence of insulin secretion, thus requiring exogenous insulin for the survival. The activation of auto reactive lymphocytes and the cytokine induced apoptosis of pancreatic-cells play a major role in the etiology of type1 diabetes.

There are clear differences in the immunogenetic predisposition to type1 diabetes between countries and the disease incidence seems to vary along with the differences in the predisposition (2). In type 2 diabetes mellitus, endogenous insulin secretion may be insufficient to maintain glucose homeostasis during additional, stress-induced insulin resistance as occurs during critical illness (3). Diabetes mellitus imposes a considerable burden on the health systems and the societies, leading to a variety of disabling, life-threatening and expensive complications such as cardiovascular disease, retinopathy, neuropathy, and nephropathy (1). The most common biomarkers used for the diagnosis of diabetes are oral glucose, Glycated Albumin (HbA1c), C-peptide, insulin, fructosamine. One of the early detection of diabetes is done by the oral glucose in which false positive results are seen in normal subjects. Similarly, the common test used is HbA1c which shows the effective of drug in the diabetes subjects rather than the insulin levels. As mention with the fluctuations in the biomarkers in the diabetic subjects C- Peptide shows the exact insulin secretion levels which are a potential biomarker for early diagnosis of diabetes.

C-peptide is cleaved from the proinsulin chains A and B during insulin synthesis. C-peptide is produced in same quantity to the insulin and is the best measure of insulin secretion in the patients with diabetes. Measurement of insulin secretion using C-peptide will be helpful in clinical practice: differences in insulin secretion are fundamental requirements in the treatment of diabetes. C-peptide level may be used as a good predictor of the diabetes. Range of C-peptide is 0.8-3.1 ng/mL or 0.26-1.03 nmol/L (SI). C-peptide can be used to assist in patient selection for islet cell transplantation and post-transplant monitoring. High uncorrected fasting C-peptide in the presence of hyperglycemia may suggest insulin resistance (4). The C-peptide, product of proinsulin proteolysis, is a chaperone for insulin during its storage in the transport vesicles of pancreatic beta-cells and further after its secretion into the bloodstream. Along with this, C-peptide functions as regulator of the intracellular effector proteins, including phospholipase Cβ, phosphatidylinositol 3-kinase, mitogen-activated protein kinases, non-receptor tyrosine kinases, and controls cAMP- and cGMP-dependent cascades (5). C-peptide also works as an important regulator of physiology and biochemical processes (6).

This review reveal about the functions of C-peptide in diabetes and other complications or risk caused by the diabetes. The C-peptide is used a

*Correspondence: Amsaveni, R., PG and Research Department of Biotechnology, Kongunadu Arts and Science College, Coimbatore – 641 029, Tamil Nadu, India. E.mail: amsabiotech@gmail.com

biomarker for the diabetes and also it predict the risk or onset of other disease. It works as a good marker for the detection of insulin level, insulin resistance and corrects diagnosis of the diabetes and type and differentiates those from the Latent autoimmune diabetes in adult (LADA).

2. C-PEPTIDE AS A BIOMARKER IN PREDICTION OF TYPE IN DIABETES

Becht *et al.* (7) worked on correct diagnosis of diabetes type and the insulin requirement using the fasting C-peptide level. C-peptide allows estimation of insulin secretion even in the presence of insulin treatment. Relating ambient glucose levels to C-peptide concentrations can improve the diagnostic potential. The study included 303 patients with type1 diabetes and 841 patients with type2 diabetes. As a result they have got low C-peptide concentrations were associated with a high odds ratio for type1 diabetes and vice versa. C-peptide/glucose ratios or HOMA-ßC-Peptide did not perform better. By the result it have been concluded that fasting C-peptide and derived parameters help to differentiate type1 from type2 diabetes. Relating C-peptide to glucose did not improve diagnostic accuracy and also C-peptide does not help predicting a need for insulin treatment in patients with type2 diabetes.

2.1. C-Peptide and type1 diabetes

Shpakov and Granstrem, (6) studied the C-peptide and its physiological effect. C-peptide is one of the key regulators of physiological processes. Type1 diabetes complication can be prevented by C-peptide replacement therapy. It can also prevent from some other complications such as atherosclerosis, diabetic peripheral neuropathy, and nephropathy. By the replacement therapy the c-peptide interacts with the insulin hexamer complexes and indicates its dissociation, as a result it regulates the functional activity of the insulin signalling system. From this study, it has been concluded that C-peptide is an important regulator of physiological and biochemical processes.

Kuhtreiber *et al,* (8) determined whether the low C-peptide levels produced by the pancreas for decades after onset of type1 diabetes and also to study the relationship between C-peptide and HbA1c control as well as diabetic complications and presence of hypoglycaemia. It has been concluded that C-peptide levels and the full range of C-peptide levels was compared with 1,5-anhydroglucitol, a glucose responsive marker low C-peptide levels may be used as a biomarker for characterizing at-risk patients with type 1 diabetes as there was low insulin secretion.

Buckingham *et al.* (9) estimated that the association between HbA1c, insulin-dose-adjusted HbA1c and C-peptide responses to the diagnosis of type1 diabetes. It included 67 participants. As a result it has been concluded that in the first 2 years after diagnosis of type1 diabetes, higher C-peptide levels are associated with increased glucose levels in the target range and with lower glucose variability.

Wahren *et al.* (10) worked on type1 diabetes with including the long acting C-peptide and neuropathy. A total of 250 patients with type1 diabetes and peripheral neuropathy included in the study and it received long-acting C-peptide in weekly dosages of 0.8 mg for 52 weeks. As a result, plasma C-peptide rose during the study to 1.8-2.2 nmol/L (low dose) and to 5.6-6.8 nmol/L (high dose). It is concluded that, once-weekly subcutaneous administration of long-acting C-peptide for 52 weeks did not improve bilateral sural nerve conduction velocity (SNCV)

2.2. C-Peptide and type2 diabetes

Shklovskiĭ *et al.* worked on C-peptide and its role as a predicator of the cardiovascular complications in type 2 diabetes patients. It is found that C-peptide leads to different capillary actions and macrovascular complications in patients with type 2 diabetes mellitus. As a result C-peptide as a possible predicator of cardiovascular complications in patients with type2 diabetes mellitus and without diabetes.

Beliakin *et al.* (11) the aim of the study was to investigate the relationship of C-peptide levels with insulin, resistance; components of metabolic syndrome and cardiovascular disease in patients with type 2 diabetes mellitus. The study included 98 patients with type 2 diabetes mellitus. The patients with elevated C-peptide level found to have all components of metabolic syndrome and also the high incidence of arterial hypertension and ischemic heart disease. This study concluded that the detection of C-peptide level with the comparison with insulin for the assessment of insulin resistance, metabolic syndrome, risk of cardiovascular disease in type 2 diabetes patients.

Pikkemaat *et al.* (12) examined about the C-peptide concentration and cardiovascular risk in type 2 diabetes patients. The study included 399 patients. As a result it has been concluded that measurement of C-peptide concentration at diagnosis could help identify patients with high risk of cardiovascular disease.

Mallipedhi *et al.* (13) investigated on resolution of type 2 diabetes 6 months following bariatric surgery with the association between the preoperative fasting and postprandial C-peptide. The study included 24 participants with type 2diabetes undergoing bariatric surgery. C-peptide levels for both fasting C-peptide and 2-hour C-peptide had a sensitivity and negative predictive value of 100% to predict type 2 diabetes mellitus remission. This work provides insight into C-peptide dynamics as a predictor of response to bariatric surgery.

Chung *et al.* (14) studied that whether the C-peptide level would relate to the risk of diabetic retinopathy in type 2 diabetic patients independently of estimated glomerular filtration rate (eGFR). 2,062 patients with type 2 diabetes were studied with the measures of fasting C-peptide, 2-hour postprandial C-peptide, and ΔC-peptide (postprandial C-peptide minus fasting C-peptide) levels. As a result the patients with and without renal impairment and with diabetic retinopathy showed lower levels of fasting C-peptide, postprandial C-peptide and ΔC-peptide and so it has been concluded that serum C-peptide levels are inversely associated with the prevalence of diabetic retinopathy in type 2 diabetic patients independently of eGFR.

Sonoda *et al.* (15) had done study on type 2 diabetes patients along with the C-peptide, HbA1c, and pooled urine. Monitored 202 diabetes patients. In univariate analysis, fasting plasma C-peptide immunoreactivity (F-CPR) and pooled urine CPR (U-CPR) were significantly associated with HbA1c, in contrast to ΔCPR and C-peptide index (CPI). This study indicated that patients with type 2 diabetes mellitus, F-CPR and U-CPR may predict improved glycemic control after six months.

2.3. C-Peptide as a potential biomarker for other risks

Dickson *et al*. (16) worked on, C-peptide concentrations used to develop insulin-secretion for the purposes of glycemic control. The study included 41 hyperglycemic infants. C-peptide kinetics was used to estimate insulin secretion. Insulin secretion was examined with respect to nutritional intake. As a result, insulin secretion was found to be highly variable and could not be predicted with respect to age, weight, or protein or dextrose intake. Insulin secretion was increase with blood glucose, with a stronger association it is found in female infants. This means nutritional intake was not a correct predictor for the insulin secretion.

Shpakov (5) studied the structural-functional organization of C-peptide and the molecular mechanisms of its action on the cell and identified the specific receptor GPR146 for C-peptide, which belongs to the superfamily of G protein-coupled receptors. The decrease in the level of C-peptide and the activity of signalling cascades in diabetes mellitus leads to a wide range of complications of this disease including diabetic nephropathy, cardiomyopathy, angiopathy, and neuropathy. The change in C-peptide level is also found in non-diabetic patients with cardiovascular system disorder and renal failure.

Gonzalez-Mejia *et al.* (17) worked on the C-peptide and the insulin as a marker for metabolic syndrome. They studied it with 156 females and 144 males, they were determined with anthropometrics, glucose, insulin, C-peptide, triglycerides, and high-density lipoproteins. Insulin resistance was determined by the HOMA2 calculator using insulin or C-peptide. As a result the C-peptide and insulin correlated with all components of MetS, for waist circumference, waist-to-hip ratio, and fasting plasma glucose, C-peptide correlated better than the insulin. HOMA2-IR calculated with C-peptide was more accurate than HOMA2-IR calculated with insulin at determining MetS. Therefore, C-peptide is a strong indicator of MetS than the insulin.

Taylor *et al.* (18) worked on the intact of C-peptide and insulin. The insulin and C-peptide were collected from the serum using the monoclonal antibodies immobilized on magnetic beds. As a result, an analytical measurement range of 3 to 320 µIU/ ml (18 to 1920 pmol/ l) for insulin and 0.11 to 27.2 ng/ml (36 to 9006 pmol/l) for C-peptide, only the recommended drug insulin lispro caused significance for the determination of endogenous insulin.

G protein-coupled receptors (GPCRs) are the most common receptor family encoded by the human genome. Kolar *et al.* (19) worked on the therapeutic potential of orphan GPCRs with a special focus on C-peptide and GPR146. It is the target of drugs for the treatment of diseases such as diabetes and its associated complications and found to have regulating function of the retinal pigment epithelium, a monolayer of cells in the retina that serves as part of the blood-retinal barrier and is disrupted in diabetic macular oedema.

3. CONCLUSION

From this review it has been highlighted about the role of C-peptide as a biomarker for diabetes. C-peptide can be also used as a predictor of risk of heart disease and also some other complications caused by diabetes. C-peptide may be used as a diagnostic tool mainly for diabetes.

REFERENCES

1. Bermudez-Tamayo, C., S. Besanc¸ M. Johri, S. Assa, J. Betz Brown, K. Ramaiya, (2017). Direct and indirect costs of diabetes mellitus in Mali: A case-control study. PLOS one tenth anniversary.
2. Kamel, M.M., S.A. Fouad, O. Salaheldin, A.E.A. Abd El-Razek and A.I.A. El-Fatah, (2014). Impact of vitamin D receptor gene polymorphisms in pathogenesis of Type-1 diabetes mellitus. *Int. J. Clin. Exp. Med.* **7**(12): 5505-5510.
3. Crisman, M., L. Lucchetta, N. Luethi, L. Cioccari, Q. Lam, G. M. Eastwood, R. Bellomo and J. Mårtensson, (2017). The effect of insulin administration on c-peptide in critically ill patients with type 2 diabetes. *Ann. Intensive Care* **7**: 50.
4. Otto-Buczkowska, E., (2015). The clinical utility of C-peptide measurement in diabetology. *Pediatr. Endocrinol. Diabetes Metab.* **20**(2): 63-68.
5. Shpakov, A.O., (2015). New achievements in the study of the mechanisms and targets of action of proinsulin c-peptide. *Tsitologiia* **57**(6): 405-414.
6. Shpakov, A.O., and O.K. Granstrem, (2013). C-peptide physiological effects. *Ross Fiziol Zh Im I M Sechenova* **99**(2): 196-211.
7. Becht, F.S., K. Walther, E. Martin and M.A. Nauck, (2016). Fasting C-peptide and Related Parameters Characterizing Insulin Secretory Capacity for Correctly Classifying Diabetes Type and for Predicting Insulin Requirement in Patients with Type 2 Diabetes. *Exp. Clin. Endocrinol. Diabetes* **124**(3): 148-156.
8. Kuhtreiber, W.M., S.L. Washer, E. Hsu, M. Zhao, P. Reinhold, D. Burger, H. Zheng, D.L. Faustman, (2015). Low levels of C-peptide have clinical significance for established Type 1 diabetes. *Diabet Med.* **32**(10): 1346-1353.
9. Buckingham, B., P. Cheng, R.W. Beck, C. Kollman, K.J. Ruedy, S.A. Weinzimer, *et al.,* (2015). Diabetes Research in Children Network (DirecNet) and Type 1 Diabetes TrialNet Study Groups, (2015) CGM-measured glucose values have a strong correlation with C-peptide, HbA1c and IDAAC, but do poorly in predicting C-peptide levels in the two years following onset of diabetes. *Diabetologia* **58**(6): 1167-1174.
10. Wahren, J., H. Foyt, M. Daniels and J.C. Arezzo, (2016). Long-acting c-peptide and neuropathy in type 1 diabetes: a 12-month clinical trial. *Diabetes Care* **39**: 596-602.
11. Beliakin, S.A., V.N. Serebrennikov, B.L. Shklovskiĭ and M.B. Patsenko, (2014). C-peptide level as an early diagnostic marker of metabolic syndrome and predictor of cardiovascular disease in patients with diabetes mellitus type 2. *Voen Med. Zh.* **335**(10): 46-49.
12. Pikkemaat, M., O. Melander, S. Mölstad and K.B. Boström, (2015). C-peptide concentration, mortality and vascular complications in people with Type 2 diabetes. *Diabet. Med.* **32**(1): 85-89.
13. Mallipedhi, A., T. Min, S.L. Prior, C. MacIver, S.D. Luzio, G. Dunseath, *et al.,* (2015). Association between the preoperative fasting and postprandial C-peptide AUC with resolution of type 2 diabetes 6 months following bariatric surgery. *Metabolism* **64**(11): 1556-1563.
14. Chung JO, Cho DH, Chung DJ, Chung MY, (2015). Relationship between serum C-peptide level and diabetic retinopathy according to estimated glomerular filtration rate in patients with type 2 diabetes. J Diabetes Complications. 29(3):350-5.
15. Sonoda, R., K. Tanaka, T. Kikuchi, Y. Onishi, T. Takao, T. Tahara, *et al.,* (2016). C-Peptide Level in Fasting Plasma and Pooled Urine Predicts HbA1c after Hospitalization in Patients with Type 2 Diabetes Mellitus. *PLoS One* **11**(2): e0147303.
16. Dickson, J.L., J. Alsweiler, C.A. Gunn, C.G. Pretty and J.G. Chase, (2015). A C-Peptide-Based Model of Pancreatic Insulin Secretion in Extremely Preterm Neonates in Intensive Care. *J. Diabetes Sci. Technol.* **10**(1):111-118.
17. Gonzalez-Mejia M.E., L.M. Porchia, E. Torres-Rasgado, G. Ruiz-Vivanco, P. Pulido-Pérez, B.G. Báez-Duarte and R. Pérez-Fuentes, (2016). C-Peptide Is a Sensitive Indicator for the Diagnosis of Metabolic Syndrome. *Metab. Syndr. Relat. Disord.* **14**(4): 210-216.
18. Taylor, S.W., N.J. Clarke, Z. Chen and M.J. McPhaul, (2016). A high-throughput mass spectrometry assay to simultaneously measure intact insulin and C-peptide. *Clin. Chim. Acta* **455**: 202-208.
19. Kolar, G.R., S.M. Grote and G.L. Yosten, (2017). Targeting orphan G protein-coupled receptors for the treatment of diabetes and its complications: C-peptide and GPR146. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **307**(7): R862-868.

DOI:10.26524/KRJ306

Kong. Res. J. 6(2): 76-80, 2019
Publisher: Kongunadu Arts and Science College, Coimbatore.

RESEARCH ARTICLE

ISOLATION AND IDENTIFICATION OF LUMINESCENT BACTERIA FROM MANGROVE AREAS IN PARANGIPETTAI COASTAL AREAS, SOUTHEAST COAST OF INDIA

Arulmoorthy, M.P. [1], and G. Anbarasi[2,*]

[1] Annamalai University, Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Parangipettai-608 502, Tamilnadu, India.

[2] Department of Biotechnology, Kongunadu Arts and Science College, Coimbatore- 641 029, Tamilnadu, India.

ABSTRACT

The present study was carried out to isolate luminescent bacteria from the sediment sample collected from mangrove areas in Parangipettai. The luminous bacteria density was found to be 5 X 10^4 CFU/g in sediment samples. Totally 10 luminous bacteria selected for biochemical analysis and genus level identification. The results shows all ten bacterial isolates were belonging to the genus Vibrio sp. Vibrio sp are the good indicator of the environmental conditions. Hence, it is useful to assess the environmental conditions of an aquatic environment.

Keywords: Luminescent bacteria, mangrove, *Vibrio* sp..

1. INTRODUCTION

Microorganisms are essential parts of all ecosystem which includes viruses, bacteria, fungi, diatoms, algae, protozoa etc. The two fundamental reasons for the indigenous microorganisms are used for biologically indicator of coastal environment are (i) they are responsible for the regeneration of nutrients (ii) the transfer of primary production from phytoplankton to micro zooplankton and to larger organisms (1). There are many biologically relevant and common general indicators of the quality of the coastal water available to assess the effect of human activities on the functioning of the ecosystems, particularly indicators of chemical contamination (2). Last few decades the population densities in coastal regions are estimated to be nearly three times higher than the global average density (3) which directly increase the level of pollution in the coastal ecosystem.

The majority of bioluminescent organisms reside in the ocean because more than 700 genera of living organisms known to contain luminous species (4). These occupy a diverse range of habitats, from polar to tropical and from pelagic waters to the benthic sea floor (5). It is exhibited by a diverse group of organisms although their number is very less compared to the total number of known species. It has been estimated that luminous organisms may have come from about 30 different evolutionarily distinct origins (6,7). However some animals, including crustaceans, squid, jellyfish and fish, release their light-emitting chemicals into the water, producing clouds or particles of light that serve to distract or blind a predator (8).

Luminescent bacteria are distributed widely in shallow coastal environments and deep pelagic water (9,10). Certain bioluminescent species are established as species-specific symbionts with marine fish and squids and are harboured in highly specialized light organs (11). The most luminous bacteria are classified into three major genera such as *Photobacterium* spp, *Vibrio* spp and *Photorhabdus* spp. Among them the species *Photobacterium* spp and *Vibrio* spp are exist in marine environment and the *Photorhabdus* spp are considered to be a terrestrial species (12).

The *Vibrio* species are a diverse group of bacteria found in abundance in the marine environment and associated with aquatic plants and animals to which they may provide a chemical defence for the host and some of them are showing bioluminescence (13). Research on isolation of bioactive compounds from the 74 species of this group has already shown promise with the isolation and identification of many antibiotic compounds (14,15). This study aimed to isolate and identify the bioluminescent bacterial strains from sediments collected from mangrove areas in Parangipettai.

2. MATERIALS AND METHODS

2.1. Sample collection

The pond Sediment samples were collected at bottom of the pond using the sterile polyvinyl corer (10cm diameter) and these samples were transported to sterile vials and tightly sealed. The collected samples brought to the lab in an ice-box for further analysis.

*Correspondence: Anbarasi, G., Department of Biotechnology, Kongunadu Arts and Science College, Coimbatore – 641 029, Tamil Nadu, India. E.mail: anbarasi.gk08@gmail.com

2.2. Viable count for enumeration of cell

Sediment samples in each pond were pooled, weighed and pulverized with a mortar and pestle. Dilution plate method was used to isolate bacteria, 1 g sediment sample mixed with 9 ml of sterile distilled water and shaken well. It was assumed that the bacteria were evenly distributed between solid and liquid. Use the pipette to remove 1 ml and delivered into the next dilution tube. Discarded the pipette, and continued for the required number of dilutions (10-, 100-, 1000-fold dilution). Pipetted 0.1 ml of each dilution of sediment samples into center of a plate agar. A separate plate for each dilution was used. Spread each inoculum using sterile bent glass rod over the plate and incubated for 24 to 48 h and then observed the growth. The number of colonies on a plate were counted and calculated.

2.3. Identification of bacteria

The bacterial strains were identified up to genus level based on the morphological and biochemical feature of the bacteria (16,17)

2.4. Morphological characteristics

2.4.1. Gram staining

Thin smear was prepared and gram stained. The slide was observed under 10, 45 and 100X magnification.

2.4.2. Motility determination

The isolated bacterial cultures were stab inoculated into the motility agar in a test tube. Then tubes were incubated at room temperature for 24 to 48 hrs. After incubation, the tubes were observed under the light source.

2.4.3. Biochemical characteristics

The bacterial isolate was subjected to various biochemical tests such as oxidase, catalase triple sugar iron test, gelatinase, arginine decarboxylase, growth on gelatin agar with Nacl, growth on TCBS agar all the tests were done by standard procedure as follows.

2.4.4. Oxidase test

The fresh growth from the plate was scraped with a disposable loop and the colony was touched with the edge of the oxidase disc (Himedia). The disc was examined with blue colour within 10 seconds.

2.4.5. Catalase test

A loop full of 24 hrs fresh bacterial culture was touched with the drop of 3% hydrogen peroxide on slide. The formation of air bubbles was taken as positive reaction.

2.4.6. Triple sugar iron test

Triple sugar iron agar medium was prepared, sterilized and inoculated with the isolates and incubated at room temperature for 48 hrs. A formation of acid (Yellow), alkaline (Red) and gas were noted at the end of the incubation period.

2.4.7. Gelatin hydrolysis test

Nutrient gelatin medium was prepared, sterilized and inoculated with the isolates and incubated at room temperature for 24 hrs. After incubation period tubes were further incubated at 4°C for 1 min and liquefying the medium in test tubes were considered as a positive reaction.

2.4.8. Amino acid decarboxylase test

A decarboxylase broth (Moeller broth) containing 1% L-arginine monohydrochloride, 1% L-lysine dihydrochloride and 1% L-ornithine dihydrochloride amino acids and pH indicator was prepared. After inoculating bacterium, test tubes were incubated for 24 – 48 hrs at room temperature. Development of yellow to dark purple colour was considered as positive reaction.

2.4.9. Grwoth of bacteria in gelatin agar medium

Gelatin agar medium was prepared with 3% NaCl and without 3% NaCl, sterilized and inoculated with isolates and incubated at room temperature for 24 hrs. After incubation period growth were observed in the medium

2.4.10. Growth of luminescent bacteria in TCBS medium

The TCBS (Thiosulfate citrate bile salt sucrose agar) medium was prepared, sterilized and inoculated with the isolates and incubated at room temperature for 24 hrs. Appearance of yellow and green colour colonies in this medium indicates the bacterial strain like *Vibrio* spp.

3. RESULTS

The total bacterial density enumerated from sediment is given in table and figure. Only 10 bacterial strains were selected and screened for identification. The isolated bacterial strains were given designated codes from SD.

Table 1: Total bacterial count in sediment

Sample	No of bacteria
Sediment	5 X 10^4 CFU/g

3.1. Identification of bacterial strains

All the isolated bacterial strains were identified as *Vibrio* spp. the results are represented in Table (2).

3.2. Grams characterization

Microscopic observations have confirmed that all the 10 bacteria were pink in colour and were identified as gram negative rod.

3.3. Test for motility

All the 10 bacteria were found to be actively motile in motility agar.

3.4. Catalase test

In Catalase test, all the strain was positive to the formation of bubbles against hydrogen peroxides.

3.5. Oxidase test

In Oxidase test, all strain showed positive and oxidase disc was changed to blue colour.

3.6. Triple sugar iron test

In Triple sugar iron test, nine strains fermented triple sugar and produced acid butt and acid slant (yellow colour), expect strains SD2 produced acid butt (yellow colour) and alkaline slant (red colour) and there was no formation of gas.

3.7. Growth of luminescent bacteria on tcbs agar

All bacteria were produced green colour colonies in TCBS Agar which is very selective for *Vibrio* spp. So, it has been confirmed that all the 10 strains were belonging to the genera *Vibrio.*

3.8. Tentative identification of the strains

Reference to the keys provided in the Bergeys Manual of Determinative Bacteriology and the results of biochemical tests that are summarized in the table 5, all the 10 strains were tentatively identified as *Vibrio* spp.

Table 2. Morphological and biochemical characters of isolated bacterial strains

Strain code	Gram stain	Motility	Oxidase	Catalase	TSI	Growth on TCBS agar	Tentative identification
SD1	Rod /gram negative	Motile	+	+	Acid butt & Acid slant	Yellow color	*Vibrio* sp
SD2	Rod /gram negative	Motile	+	+	Acid butt & Alkaline slant	Green color	*Vibrio* sp
SD3	Rod /gram negative	Motile	+	+	Acid butt & Acid slant	Yellow color	*Vibrio* sp
SD4	Rod /gram negative	Motile	+	+	Acid butt & Acid slant	Green color	*Vibrio* sp
SD5	Rod /gram negative	Motile	+	+	Acid butt & Acid slant	Yellow color	*Vibrio* sp
SD6	Rod /gram negative	Motile	+	+	Acid butt & Acid slant	Yellow color	*Vibrio* sp
SD7	Rod /gram negative	Motile	+	+	Acid butt & Acid slant	Yellow color	*Vibrio* sp
SD8	Rod /gram negative	Motile	+	+	Acid butt & Acid slant	Yellow color	*Vibrio* sp
SD9	Rod /gram negative	Motile	+	+	Acid butt & Acid slant	Yellow color	*Vibrio* sp
SD10	Rod /gram negative	Motile	+	+	Acid butt & Acid slant	Yellow color	*Vibrio* sp

Fig. 1. Culture of bacteria from sediment

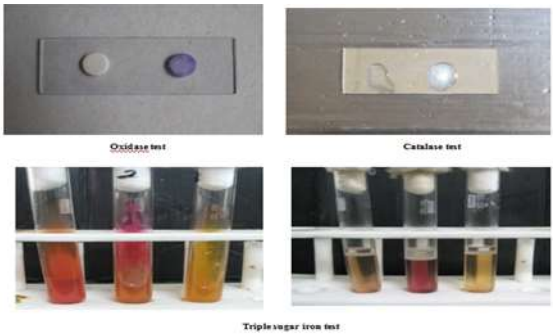


Fig. 2. Morphological and Biochemical Characters of Isolated Bacteria

Fig. 3. Growth of bacteria on TCBS agar

4. DISCUSSION

Luminous bacteria are the most common of all bioluminescent organisms' presents in all environment. The marine luminescent bacteria are presents in either as free living bacteria or in symbiotic association with other marine organisms such as squids, deep water fishes, octopus etc. The occurrence of luminescent bacteria considered as indicative of the physico chemical conditions and indicates the presents increased availability of nutrients. These associated bacterial luminescent genes are considered as biosensors for marine environmental studies, with special emphasis on the micro toxicity assay (18). In this study the average density of luminous bacteria was 5 X 10^4 CFU/ml. The present observation of luminous bacterial population was higher when compared with Abraham *et al.* (19) who reported 20 to 1050 cells/ml of luminous bacteria from seawater of Tuticorin bay, 3.0 X 10^3 CFU/ml in port Harcourt in Nigeria and 1.3 X 10^4 to 3.0 X 10^4 in Bonny estuary Nigeria (20), 20 to 90 cells/100 ml in surface seawater of the Seto Inland sea (21).

These results noted that the luminous bacteria found on sediment samples from mangrove environment of Parangipettai probably shows the presence of low level of toxicants. The present observation concurrent with the Ramaiah and Chandramohan (22) reported that luminous bacteria loose their ability to emit light in the presence of a toxicant even at very low concentration. As the presence of luminous bacteria in high numbers in coastal waters indicated healthy and pollution free condition.

Globally, the*Virbrio* sp., are reported to be most common luminescent bacteria. In the present study that all luminous bacterial strains were identified as *Vibrio* spp and according the emission of light the prominent active strain (SD5) was further identified as *Vibrio mediterranei* based on the morphological and biochemical characteristics. This obtained result is backed by the observation of Abraham *et al.* (19,23) who reported different species of Vibrio such as *Vibrio harveyi, V. orientalis, V.splendidus, V. fischeri* and *V. mediterranei* in seawater and sediment samples from Tuticorin bay. The results of the present study are in close agreement with the observation of Yang *et al.* (24) who isolated and identified some luminous bacteria such as *Vibrio harveyi, Vibrio orientalis V. splendidus, Photobacterium phosphorium* and *photobacterium leiognathi*. The present study has revealed the distribution of luminous bacteria in sediments, suggested the healthy environment in Mangrove areas of Parangipettai.

REFERENCES

1. Sherr, E., and B. Sherr, (2000). Marine microbes: An overview, p. 13-46. In D.L. Kirchman (ed.) Microbial ecology of the oceans. Wiley- Liss, New York.
2. Frischer, M.E. and P.O. Verity, (2005). Alternatives to coliform bacteria as indicators of human impact on coastal ecosystems. In Kleppel, O.S., M.R. DeVoe and M. Rawson, (ed.) Implications of land use change to coastal

ecosystems challenges to effective resource management. Springer-Verlag, Berlin (in press).
3. Small, C., and R.J. Nicholls, (2003). A global analysis of human settlement in coastal zones. *J. Coastal Res*. **19**: 584-599.
4. Shimomura, O., (2006). Bioluminescence: chemical principles and methods (World Scientific, Toh tuck link, Singapore), pp 500.
5. Haddock, S.H.D., M.A. Moline and J.F. Case, (2010). Bioluminescence in the sea. *Annu. Rev. Mar. Sci*., **2**: 443-493
6. Hastings, J.W., C.J. Potrikas, S.C. Gupta, M. Kurfurst and J.C. Makemson, (1985). Biochemistry and physiology of bioluminescent bacteria. *Ado. Microbiot Physiol.* **26**: 235-291.
7. Alldredge, A., T. Cowles, S.S. Macintyre, J. Rines and P. Donaghay, (2002). Occurrence and mechanisms of formation of a dramatic thin layer of marine snow in a shallow Pacific fjord. *Mar. Ecol. Prog. Ser*. **233**: 1-12
8. Aguilar, J.A., A. Albert, F. Ameli, M. Anghinolfi and G. Anton, (2006). First results of the Instrumentation Line for the deep-sea ANTARES neutrino telescope. *Astropart. Phys*. **26**: 314-324
9. Reichelt, J.L., and P. Baumann, (1973). Taxonomy of the marine luminous bacteria. *Arch Mikrobiol.* **94**: 283-330.
10. Ramesh, A., E.G. Loganathan and K. Venkateswaran, (1990). Ecological dynamics of marine luminous bacteria*. J. Basic Microbiol,* **30**: 689-703.
11. Dunlap, P.V., and K. Kita-Tsukamoto, (2001). Luminous bacteria. The Prokaryotes (Dworkin M, Falkow S, Rosenberg E, Schleifer KH & Stackebrandt E, eds). Academic Press, New York.
12. Campbell, A.K., (2008). Jean-Marie Bassot (1933–2007): a life of unquenched curiosity—Obituary. *Luminescence,* **23**: 187-190.
13. Engel, S., P.R. Jensen and W. Fenical, (2002). Chemical Ecology of marine microbial defense. *J. Chem. Ecol*. **28 (**10): 1971-1985.
14. Baranova, N.A., and N.S. Egorov, (1989). Production of antibiotic substances by natural variants of the marine bacterium *Vibrio fischeri*. *Antibiot. Khimioter*. **34**(1): 7-10.
15. Kobayashi, M., S. Aoki, K. Gato, K. Matsunami, M. Kurosu and I. Kitagawa, (1994). Marine natural products. XXXXIV. Trisindoline, a new antibiotic indole trimer, produced by a bacterium of Vibrio sp., separated from the marine sponge *Hyrtios altum*. *Chem. Pharm. Bull*. (Tokyo). **42** (12): 2449 – 2451.
16. Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley and S.T. Williams, (1994). Bergey's manual of detminative bacteriology, Ninth edition, (Williams and Wilkins, Baltimore), 260
17. Abraham, T.J., R. Manley, R. Palaniappan and K. Dhevendaran, (1998). Luminous bacteria as indicator of penaeid shrimp grow out phase abnormalities, in: Proceeding of the national symposium on Frontiers in Applied environmental Microbiology, edited by IS brightSingh, (School of environmental sciences, Cochin University of Science and Technology, Cochin) pp: 205-210.
18. Halldorson, V.S.N., and N.L. Duran, (2003). Bioluminescent Bacteria: Lux Genes as Environmental Biosensors. *Braz. J. Microbiol.* **34**: 91-96.
19. Abraham, T.J., S.A. Shanmugam, R. Palaniappan and K. Dhevendran, (2003). Distribution and abundance of luminous bacteria with special reference to shrimp farming activities. *Indian J. Mar. Sci.* **32**(3): 208-213.
20. Adoki, A.A., and L.O. Odokuma, (2007). Bioluminescent hydrocarbonclastic bacteria of the Niger Delta. *Afr. J.f Biotechnol.* **6**(4): 393-399.
21. Venkateswaran, K., H. Nakano and H. Hashimoto, (1989). Occurrence and distribution of bioluminescent bacteria in the seto inland sea. *Bull. Jap. Soc. Sci. Fish.,* **55**: 467-473.
22. Ramaiah, N., and D. Chandramohan, (1993). Ecological and laboratory studies on the rate of luminous bacteria and their luminescence in coastal pollution surveillance. *Marine Poll. Bull*., **26**: 190-201.
23. Abraham, T.J., R. Palaniappan and K. Dhevendaran, (1999). Simple taxonomic key for identifying marine luminous bacteria. *Indian J. Mar. Sci.* **28**: 35-38
24. Yang, Y., L.P. Yeh, Y. Cao, L. Baumann, P. Baumann, J.S. Tang and B. Beaman, (1983). Characterization of marine luminous bacteria isolated off the coast of china and description of *Vibrio orientalis* sp. Nov, *Curr. Microbiol.,* **8**: 95-100.

DOI:10.26524/KRJ307

Kong. Res. J. 6(2): 81-86, 2019
Publisher: Kongunadu Arts and Science College, Coimbatore.

http://krjscience.com

RESEARCH ARTICLE

FRUIT BIOWASTE MEDIATED GREEN ROUTE APPROACH SILVER NANOPARTILCES - AS ANTIBACTERIAL MATERIAL

Ranjith Kumar, R[1]., J. Manikantan[2], A.S. Balaganesh[3], K.P.B. Dinesh[4] and B. Chandar Shekar[3,*]

[1]Research Department of Biotechnology, Kongunadu Arts and Science College, Coimbatore-641029 Tamilnadu, India.

[2]Department of Physics, Sri Ranganathar Institute of Engineering and Technology, Coimbatore, Tamilnadu, India.

[3]Department of Physics, Nanotechnology Research Lab, Kongunadu College of Arts and Science, Coimbatore, Tamilnadu, India.

[4]Applied Biology, Higher College of Technology, Muscat, Oman.

ABSTRACT

Environmental free approach or green chemistry synthesis of metallic nanoparticles has become new growing branch of nanobiotehnology. In this present work a simple and environmental free biosynthesis silver nanoparticles (AgNPs) were prepared using Musambi Peels (MPs) aqueous extract as the reducing agent guided by the principles of green chemistry. The fruit waste aqueous extract was challenged with silver nitrate solution for the production of AgNPs in room temperature. The crystalline phase and morphology of AgNPs were determined from UV-Vis spectroscopy, Fourier transform infrared (FTIR) spectra, X-ray diffraction (XRD), Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDS). The UV-Vis spectrum indicated that the surface plasmon broad peak was observed nearby 450 nm throughout the reaction 30min-24h. XRD spectrum revealed that the average size of biowaste mediated AgNPs obtained approximately 46 nm by using the Debye-Scherrer equation. SEM image of AgNPs showed uniformly distributed on the surface of the cell with high agglomeration. EDS analysis revealed that the presence of silver was confirmed from the Ag peaks at 2.8-3.7 keV. In addition, the biowaste mediated AgNPs loaded disk were tested for antibacterial properties against *Escherchia coli* and *Staphylococcus aureus* and found that the obtained metallic AgNPs have been good antibacterial material for biological applications.

Keywords: Musambi Peels, silver nanoparticles, XRD, SEM, antibacterial.

1. INTRODUCTION

Nanoparticles frequently expression unique and considerably changed physical, chemical and biological properties compared to their macro scaled counterparts (1). Nanomaterials are naturally described as materials smaller than 100 nm in at least one dimension. The nanoparticles utilization is the most progressive at present, both in scientific knowledge and in commercial applications including water treatment process (2). Numerous noble metal nanoparticles for example copper, gold, silver and platinum were usually synthesized by using various methods including chemical, physical and biological route. Generally, the physical and chemical method nanoparticles synthesis have many disadvantages and this approach not consider as eco-friendly approach due to toxic effect that adversely effects the ecosystem. Later, researcher across the world has searched for novel and eco-friendly approach for the synthesis of biocompatible nanoparticles (3). Hence, biological route preparation of nanoparticles is less costly, less time consuming, l and more environmentally free materials, therefore nowadays researcher are looking forward to the possible biological approach to synthesis nanoparticles (4).

Recently many researcher reported synthesis of silver and gold nanoparticles via eco-friendly methods using a wide range of biological resources like fungi (5,6), bacterial (7,8) marine algae (9), marine yeast (10) Actinomycete (11) and plants (12,13). Conversely, production of metal nanoparticles using plants medicated green route approach, the reduction rate of metal salts is very fast and preparation routes itself requires no specific conditions unlike the physical and chemical method (14, 15). Hence, owing of the present results focuses on the green synthesis of silver nanoparticle (AgNPs) using aqueous extract of musambi peels (*Citrus limetta*) as reducing agent in aqueous solution of silver nitrate. The synthesized AgNPs were characterized by using UV-visible spectrophotometer (UV-vis), Fourier transform infrared (FTIR) spectra, X-ray diffraction (XRD), Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDS). Additional, the antibacterial properties of green synthesized AgNPs

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

were test against human pathogens by disk diffusion method.

2 MATERIALS AND METHODS

2.1. Preparation of peel extract

Musambi Peels (MPs) were collected from local market nearby our college, Tamilnadu, India, on the basis of cost effectiveness and ease of availability. The fresh and healthy MPs were rinsed thoroughly first with tap water 5-10 min followed by distilled water 10-20 min to remove all the dust and unwanted visible particles, MPs were cut into small pieces and dried at room temperature. To prepare the aqueous extract of MPs about 20g of finely incised MPs were weighed and transferred in to 200 mL beakers containing 100 ml of distilled water and boiled for about 30 min at 80°C using heating mantel. After heating treatment, the colour of the MPs aqueous solution changed from watery to light yellow colour. The MPs aqueous extract was separated by filtered through normal filter paper followed by Whatman No.1 filter paper to remove particulate matter and to get clear aqueous extract solution. The MPs aqueous extract was stored at refrigerated (4°C) in 100 ml Erlenmeyer flaks to be used for biosynthesis of silver nanoparticles from silver nitrate. The effectiveness and accuracy in results without any contamination, each and every steps of the experiment were maintained under sterility conditions.

2.2. Synthesis of silver nanoparticles

In a typical reaction procedure, aqueous solution of silver nitrate ($AgNO_3$) at the concentration of 1 mM was prepared in 250 ml Erlenmeyer flasks and 10 ml MPs aqueous extract was added for reduction into Ag^+ ions at room temperature. The colour change of the reaction mixture from faint light to yellowish brown to reddish brown to colloidal brown was monitored occasionally for maximum 2 h using UV-visible spectrophotometer (JASCO V670, Japan) at the intervals of 30 min. The experimental reaction was carried out in darkness to avoid photo activation of $AgNO_3$ at room temperature. After 2 h incubation, the reduction of reaction mixture (MPs extract + $AgNO_3$) to Ag^+ ions was confirmed by the color change followed by the bioreduction, the reaction mixture was kept aside 24 h at room temperature for complete bioreducation. Then, the AgNPs solution obtained by MPs extract was centrifuged at 8,000 rpm for 30 min, the supernatant was discarded and final pellet was washed with distilled water 5-10 min, the pellet was dried at 60°C using hot air oven to get fine powder form of AgNPs.

2.3. Characterization of Silver nanoparticles

The sample (2mL) of the suspension was collected periodically to monitor the completion of bioreduction of Ag^+ in aqueous solution scan in UV-visible spectrophotometer (JASCO, V-670, Japan) between wavelengths of 300 to 800 nm, having a resolution of 1 nm. UV-vis spectra were recorded at intervals of 30min, 1h, 2h and 24h. Fourier transform infrared (FTIR) spectra for green synthesis silver nanoparticles was recorded on a Shimadzu FTIR spectrometer 8000 series, with a sample as KBr pellet method in the wavenumber region of 4,000 - 400 cm^{-1}. Crystalline nature of the nanoparticles was analyzed by XRD at 2θ ranges from 20 to 80°C (Philips PW 1830).The morphology and elemental composition of green synthesis silver nanoparticles was identified by Scanning Electron Microscopy (SEM, JEOL JSM-6390) along with Energy Dispersive X-ray Spectroscopy (EDS, Model No. 9582, Oxford Instruments) operating at an accelerating voltage of 20 kV.

2.4. Antibacterial activity

The green synthesized silver nanoparticles using musambi peels aqueous extract were tested for antibacterial activity by disc diffusion methods against human pathogenic organisms such as *Escherchia coli* and *Staphylococcus aureus*. Pure culture of bacteria Mueller Hinton, the bacterial test organisms were grown in nutrient broth at 37°C for 24 h. About 200 L of aliquot of each strain (1×106 cfu/mL) was spread uniformly onto the individual pre-sterilized petridishes plates using sterile cotton swabs and allowed to dry for 10 to 15 min. On other side, Whatman No. 1 filter paper discs (3 mm in diameter) were prepared and coated with 50µl of silver nanoparticles. The silver nanoparticles coated filter paper dics were placed on the surface of each cultured plate, streptomycin dics was used as positive control. Finally, the petridishes were incubated at 37°C for 24 h to find antibacterial properties of green synthesized silver nanoparticles coated filter paper inhibition zones were measured in millimeters.

3. RESULTS AND DISCUSSION

Due to the extensive applications of nanoparticles, a large number of methods have been developed for the controllable synthesis of nanoparticles. However, precise control on the size and distribution of nanoparticles remains a great task (6). In the present study, silver nanoparticles

(AgNPs) were rapidly synthesized using MPs aqueous extract as bio-reductants. The bio-reduction of $AgNO_3$ into AgNPs was completed within 24h of incubation turned the colour to dark brown (Fig. 1).

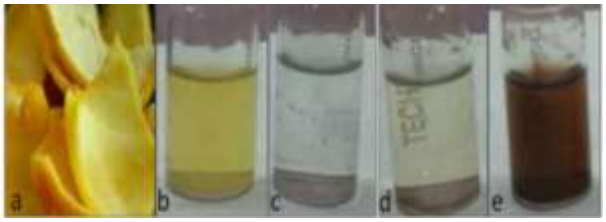


Fig. 1. Biosynthesis of AgNPs using MPs aqueous extract a) Musambi peels, b) Aqueous xtract of MPs, c) 1mM $AgNO_3$, d) Reaction mixture 0^{th} min and e) Reaction mixture after 24h.

3.1. UV-Vis Spectrum Studies

The green route synthesized AgNPs using MPs aqueous extract was confirmed by the UV-Vis spectrum analysis at different nm scale. The colour changed into light yellowish brown to dark brown was due to excitation of Surface Plasmon Vibration which indicated the formation of AgNPs at room temperature.

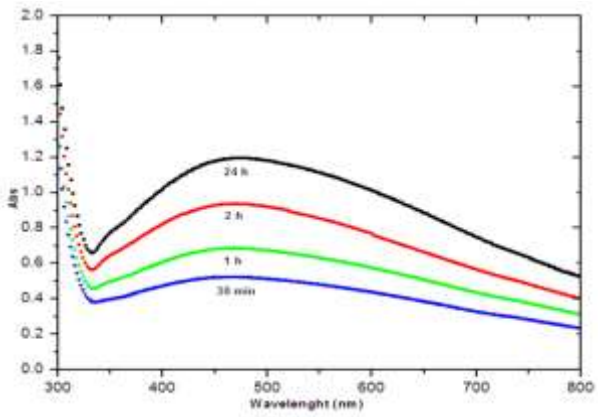


Fig. 2. UV Spectrum of biosynthesized AgNPs

3.2. FTIR study

FTIR spectrum analysis has helped to understand the nature of biomolecules present in the MPs extract that involved in the formation of AgNPs. The FT-IR spectrum of MPs extract mediated green synthesized AgNPs showed sharps peak located at 3371.54, 1635.64, 1373.32 and 1256.87 cm^{-1} and light peaks located at 2121.70, 2314.58, 2546.04, 2638.62, 2723.49 and 2846.93 cm^{-1}. The sharp peak at 3371.54, 1635.64, 1373.32, 1256.87 cm^{-1} may be assigned to the O-H, C-N and C-O stretch bonding function group of secondary alcohols respectively. The light peaks at 2121.70 and 2314.58 cm^{-1} indicated C-O bonding function group of phenols and alcohols and the peaks at 2546.04, 2638.62, 2723.49 and 2846.93 indicated the presence of N-H primary amine and O-H stretch the function group of carboxylic acid (Fig.3).

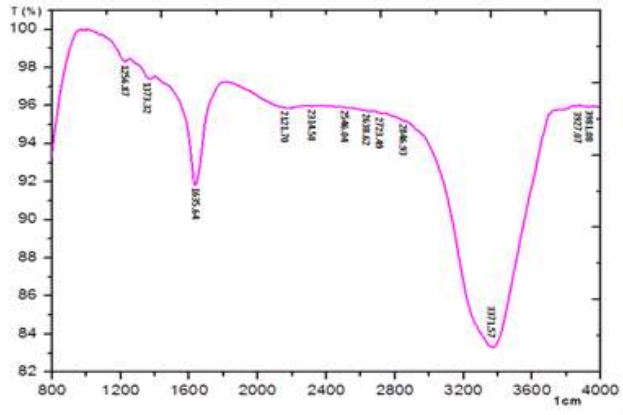


Figure 3: FTIR Spectrum of MPs extract mediated AgNPs

The FTIR spectrum revealed that the different functional groups present at different position and functional biomolecules like phenols, alcohols and carboxylic acid are involved in the reduction of silver ions (16).

3.3. X-ray diffraction study

Green synthesized MPs extract mediated AgNPs were further analysis through X-ray diffraction was carried out to confirm the crystalline nature of the AgNPs. The XRD pattern indicates numbers of Bragg reflections that may be indexed on the basis of the face centered cubic structure of silver. A comparison of obtained XRD spectrum with the standard confirmed that the silver particles formed in present experiment were in the form of nanocrystals, as evidenced by the peaks at 2θ values of 38.14°, 44.33°, 64.48° and 77.44° corresponding to (111), (200), (220) and (311) Bragg reflections,

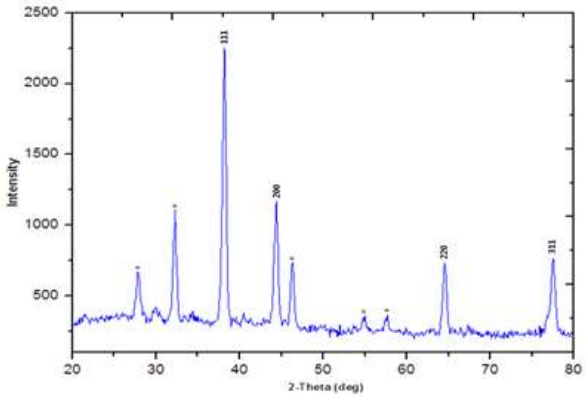


Fig. 4. XRD analysis of AgNPs

respectively, which may be indexed based on the face-centered cubic structure of silver (JCPDS file nos. 04-0783). X-ray diffraction results clearly show that the silver nanoparticles formed by the reduction of Ag+ ions by the carob leaf extract are crystalline in nature. The unassigned peaks at 2θ = 27°, 32°, 46° 54° and 57° denoted by (*) were also observed signifying that the crystallization of bioorganic phase occurs on the surface of the nanoparticles (Fig. 4).

The average particle size of AgNPs synthesized by the green route method can be calculated using the Debye-Scherrer equation D ¼ Kλ=β cosθ; where D is the crystallite size of AgNPs, λ is the wavelength of the X-ray source (0.1541 nm), β is the full width at half maximum of the diffraction peak, K is the Scherrer constant with a value from 0.9 to 1, and θ is the Bragg angle. From the XRD analysis the MPs aqueous extract mediated green synthesized AgNPs was found that the average size found to be approximately 46 nm using the Debye-Scherrer equation.

3.4. SEM-EDS analysis

Scanning electron microscopy images shows the shape of the green synthesized AgNPs using MPs extracts (Fig. 5a). The shape of surface morphology of the AgNPs was observed at different magnification and revealed that the SEM images of MPs extracts mediated AgNPs shown uniformly distributed on the surface of the cell with high agglomeration was noted. The large particles may be due to the aggregation of the smaller once (16). The SEM was equipped with energy dispersive spectroscopy (EDS) analysis indicated that the presence of silver was confirmed from the Ag peaks (Fig. 5b).

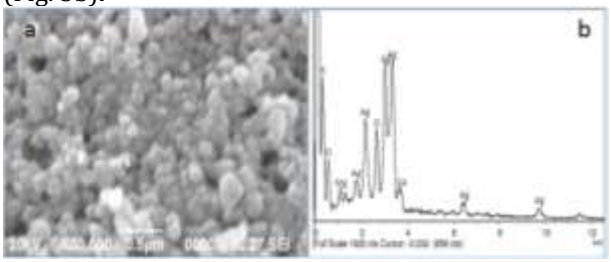


Fig. 5. SEM-EDS image of biosynthesis AgNPs

Silver nanoparticles revealed a strong signal of metallic silver at 2.8-3.7 keV and presence of oxygen, calcium and Mg in the EDS spectrum indicates that organic moieties exits in the aqueous extract of MPs (17). Therefore, these organic constituents are partially involved in the reduction of silver metallic nanoparticles at room temperature.

3.5. Antibacterial properties

The antibacterial properties of the silver nanoparticles and silver nanoparticles coated product have been exploited for a long time the biomedical field (18,15). In this present study, antibacterial properties of MPs aqueous extract mediated AgNPs material was evaluated by using standard Zone of Inhibition (ZOI) microbiology assay against *E. coli* and *S. aureus* and found MPs extracted mediated AgNPs achieved significant antibacterial activity against tested pathogens (Fig. 6). MPs extract mediated AgNPs loaded disk maximum ZOI was found to be 17mm for *S. aureus* and 14mm for *E. coli*, whereas, standard antibiotic disk streptomycin 19, 20mm against *S. aureus* and *E. coli* respectively. The AgNPs shown inhibition zone against both studied bacteria and the present study achieved the MPs extract medicated AgNPs loaded disk showed maximum activity against *S. aureus*. Pervious study by Subbaiya *et al.* (19) reported that the *Nerium oleander* mediated biological synthesized silver nanoparticles the zone of inhibition was found more at *B. subtilis* and *E. coli*. Another study by Ranjithkumar *et al.* (2013b) reported that the areca nut mediated biogenic synthesized silver nanoparticles coated cotton fabric showed significant antibacterial properties against both Gram positive and Gram negative pathogens.

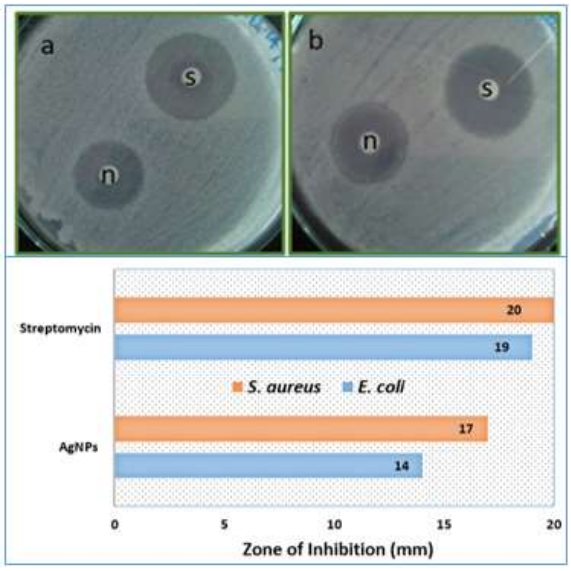


Fig. 6. Antibacterial activity of AgNPs by disk diffusion method. a) *E. coli* and b) *S. aureus*

Likewise, our present results suggested that the plant mediated green approach obtained metallic silver nanoparticles have been good antibacterial material for biological applications.

4. CONCLUSION

Green chemistry nanoparticles is gaining important due to the free form toxic chemicals and provides effective synthesis of expected products in an economic manner. In this present work, we developed an environmental free and convenient green chemistry method of the synthesis of silver nanoparticles from fruit waste as reducing agent and found MPs aqueous extract is found suitable for the production of AgNPs at room temperature by green approach. Production of AgNPs after inhibition the colour change occur due to surface Plasmon resonance during the reaction with the organic compounds present in the MPs extract resulting in the formation of AgNPs which was confirmed by UV-vis spectrum and Surface Plasmon broad peak was observed nearby 450nm. The FTIR spectrum indicated the different functional biomolecules present at different position such as phenols, alcohols and carboxylic acid are involved in the reduction of silver ions. XRD and SEM-EDS indicated the MPs extract mediated AgNPs shown uniformly distributed on the surface of the cell with high agglomeration. In addition, antibacterial activity of this green route synthesized AgNPs showed potential antibacterial activity against test pathogens. The overall graphical abstract of present work shown in figure 7.

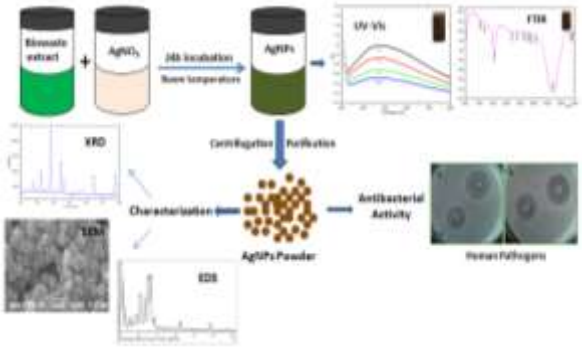


Fig. 7. Graphical abstract of prepartion and characterization and applications nanoparticles

The growing need to develop environment friendly processes through green synthesis and other biological approaches to preparation of zero toxicity nanoparticles for biomedical applications. Hence, "Green synthesis" of nanomaterials makes use of environment friendly and non-toxic reagents.

Acknowledgment

We acknowledge our management of Kongunadu Arts and Science College and Department of Biotechnology for providing facilities and encouragement.

Conflict of Interest

We, the authors declare that they have no conflict of interests.

REFERENCES

1. Sharma, V.K., R.A. Yngard and Lin, Y., (2009). *Adv. Colloid Interfac*. **145**: 83.
2. Geeta, G. and A.R. Choudhury, (2019)., A review on the biosynthesis of metal and metal salt nanoparticles by microbes. *RSC Adv.* **9**: 12944-12967.
3. Kumar, V., and S.K. Yadav, (2009). Plant-mediated synthesis of silver and gold nanoparticles and their applications. *J. Chem. Technol. Biotechnol.* **84**: 151-157.
4. Karthick Raja, S., S. Ganesh and Avimanyu, (2011), Evaluation of antibacterial activity of silver nanoparticles synthesized from Candida glabrata and *Fusarium oxysporum*. *Int. J. Medicobiological Res.* **1**(3): 130-136.
5. Chauhan, A., S. Zubair, S. Tufail, A. Sherwani, M. Sajid, S.C. Raman, A. Azam and M. Owais, (2011). Fungus-mediated biological synthesis of gold nanoparticles: potential in detection of liver cancer. *Int. J. Nanomedicine* **6**: 2305-2319.
6. Li, G., D. He, Y. Qian, B. Guan, Y. Cui, S. Gao, K. Yokoyama and L. Wang, (2012). Fungus medicated green synthesis of silver nanoparticles using *Aspergillus terreus*. *Int. J. Mol. Sci.* **13**: 466-476.
7. Sriram, M.I., K. Kalishwaralal and S. Gurunathan, (2012). Biosynthesis of silver and gold nanoparticles using *Bacillus licheniformis*, *Methods Mol. Bio.* **906**: 33-34.
8. Du, L., J. Jiang, X. Liu and E. Wang, (2007). Biosynthesis of gold nanoparticles assisted by *Escherichia coli* DH5α and its application on direct electrochemistry of hemoglobin. *Electrochemistry Communications* **9**(5): 1165-1170.
9. Singaravelu, G, J.S. Arockiamary, V.G. Kumar and K. Govindaraju, (2007). A novel extracellular synthesis of monodisperse gold nanoparticles using marine alga, *Sargassum wightii* Greville. *Colloids Surf. B* **57**: 97-101.
10. Agnihotri, M., S. Joshi, A.R. Kumar, S. Zinjarde and S. Kulkarni, (2009). Biosynthesis of gold nanoparticles by the tropical marine yeast *Yarrowia lipolytica* NCIM 3589. *Mater. Lett.* **63**(5): 1231-1234.
11. Sukanya, M.K., K.A. Saju, P.K. Praseetha and G. Sakthivel, (2013). Therapeutic potential of biologically reduced silver nanoparticles from actinomycete cultures. *J. Nanosci.* **2013**: 1-8.

12. Ali, D.M., N. Thajuddin, K. Jeganathan, M. Gunasekaran, (2011). Plant extract mediated synthesis of silver and gold nanoparticles and its antibacterial activity against clinically isolate pathogens. *Colloids. Surf. B.* **85**: 360-365.
13. Krishnaraj. C., P. Muthukumaran, R. Ramachandran and M.D. Balakumaran and P.T. Kalaichelvan, (2014). *Acalypha indica* Linn: Biogenic synthesis of silver and gold nanoparticles and their cytotoxic effects against MDA-MB-231, human breast cancer cells. *Biotechnol. Rep.* **4**: 42-49.
14. Narayanan, K.B. and N. Sakthivel, (2008). Coriander leaf mediated biosynthesis of gold nanoparticles. *Mater. Lett.* **62**: 4588-4590.
15. Shanmugavadivu. M, Selvam Kuppusamy and R. Ranjithkumar, (2014). Synthesis of Pomegranate Peel Extract Mediated Silver Nanoparticles and its Antibacterial Activity, *Am. J. Adv. Drug Deliv.* **2**(2): 174-182.
16. Ranjithkumar R., S.Kuppusamy and M. Shanmugavadiu, (2013). Green synthesis of silver nanoparticles using areca nut extract for enhanced antibacterial activity. *J. Green Sci. Technol.* **1**(2): 102-106.
17. Palaniselvam K., S.J.A. Ichwan, N.R. Parine, M.M. Yusoff, G.P. Maniam and N. Govindan, (2015). Intracellular biosynthesis of Au and Ag nanoparticles using ethanolic extract of *Brassica oleracea* L. and studies on their physicochemical and biological properties. *J. Environ. Sci.* **29**: 151-157.
18. Ranjithkumar R., S. Kuppusamy and M. Shanmugavadiu, (2013). Antibacterial textile finishing via green synthesized silver nanoparticles. *J. Green Sci. Technol.* **1**(2): 111-113.
19. Subbaiya, R., M. Shiyamala, S. Revathi, R. Pushpalatha and M. Masilamani Selvam, (2014). Biological synthesis of silver nanoparticles from *Nerium oleander* and its antibacterial and antioxidant property. *Int. J. Curr. Microbiol. App. Sci.* **3(**1): 83-87.

Kong. Res. J. 6(2): 87-93, 2019
Publisher: Kongunadu Arts and Science College, Coimbatore.

DOI:10.26524/krj308

RESEARCH ARTICLE

RESEARCH RESPONSE ON "PEDIATRIC": A SCIENTOMETRIC STUDY ON THE LITERATURE OUTPUT FROM WOS

Abirami, S.[1] and R. Senthilkumar[2,*]
[1]Bharathiar University, Coimbatore, Tamilnadu, India
[2]Department of Library and Information Science, Kongunadu Arts & Science College, Coimbatore, Tamilnadu, India.

ABSTRACT

The hit rate of publications even more grew on the basis of everyday development in each and every department. To study the growth and publication trend in "PEDIATRIC" decided to use scientometric tools. The desired data were downloaded from web of science (WOS) on pediatric. The analysis was based on the year wise growth on publications, publications based on languages, document wise publications and top ten journals; the Lotka's Law has been analyzed. The scope and hypothesis are framed for the present study. The research topic was "Research Response on "Pediatric": A Scientometric Study on the Literature Output from WOS".

Keywords: Scientometric, Pediatric, Hypothesis, WOS, Lotka's Law.

1. INTRODUCTION

The word "Pediatric" derived from two Greek words that are Pais means child and iatros meaning doctor or healer. Pediatric is a division of medicine exclusively for infants, children and also for adolescents up to the age of 18. This pediatric department has been developed only in the mid of 19th century and "Abraham Jacobi (1830-1919) is known as the father of pediatric" (1-3). Scientometric is one of the most significant methods for evaluating the scientific productions. In recent years, Scientometric study has been showing the gradual improvement by using its techniques to scrutinize the research output of researchers and the development of a range of disciplines in sciences. "Modern Scientometrics is mostly based on the work of Eugene Garfield creator and founder of the Science Citation Index and the Institute for Scientific Information (ISI) which is greatly used for Scientometric analysis"(4-5).

2. REVIEW OF RELATED LITERATURE

Elango (2017) the "Science Citation Index-expanded" (Sci-E), in tribology research the science citation index-expanded is used to get back the bibliographic records. A relative funding index, this is a new relative indicator is also introduced in this study. The higher number of cited reference will go to the funded research publications after that international collaborative papers than the papers published through non-funded publications. Comparatively the publications by single authored productivity are lower than non-funded ones. The funded and non-funded researches do not have any difference in citation impact.

Senthilkumar and Muthukrishnan (2017) analysis of "Scientific publication research productivity" published in the British Journal of Cancer for the period of 11 years from 2005 to 2015. The purpose of using different types of scientometric parameters like; author productivity, country wise distribution, degree of collaboration, annual growth rate, Institution wise distribution, and the research document type are done for analyzing the data. The study concludes with the publications totally 264 issues in the journal and 6818 by records.

Maity and Hatua (2015) Library and Information Science [LIS] gives the importance on research as doctoral level. In the year 1957 the first doctoral degree was given, and then slowly it sped up in the year 1990s. During 1950-2012 according to "INDCAT, Vidyanidhi, Inflibnet and University News databases it was found that about 1058 doctoral thesis have been produced through different universities in India. Current study is an effort in finding the trend in research field of LIS and analyzing the research activity during 1950-2012. This study results declare that 1058 numbers of doctoral thesis awarded in various universities for the past 63 years.

Aswathy and Gopikuttan **(**2012**)** in the list of premier journals in the field of space technology, the top positioned journal is "Journal of Spacecraft and Rockets". It is published by American Institute of Aeronautics and Astronautics. This analysis provides the details of bibliometric analysis of the

*Correspondence: Senthilkumar, R., Department of Library and Information Science, Kongunadu Arts and Science College, Coimbatore – 641 029, Tamil Nadu, India. E.mail: kasclibrary@yahoo.com

journal during the period of 2006-2010, which contains "780 papers and 15648 references. This analysis contains different parameters like growth pattern, authorship pattern and distributions with respect to subject, year, institution and geographical area". The pattern of citing the authors is also analyzed from the given reference to each research paper. The most productive year also has been studied in this analysis.

Bar-Ilan (2008) the results observed from a research of google scholar is considerably different from the results which based on web of science and scopus. In the previous years the most single comprehensive source was ISI citation index. There were no other sources to rely on, although the citation index was often criticized for many reasons. The promotion committees of universities in world-wide will be using the data from the ISI citation index and the journal citation report (JCR) regularly. Freshly two options to the ISI citation indexes have come for the usage. One of the names is scopus maintained by Elsevier and the other one is google scholar [freely available]. "The science citation index [SCI] first published in print in the year of 1963, with citation data from 1961 [Garfield, 1963]"

Costas and Bordons (2006) the data downloaded from web of science (WOS) for the period 1994-2004, are used to find the relationship of the h-index with other bibliometric tools at the micro level is scrutinized for the scientists from Spanish CSIC in natural resources. The research performance described by different activity and impact tools, the h-index cited through factor analysis in a quantitative dimension extremely correlated with the absolute number of productivity and citations. The remaining proportions to be included in the analysis of research presentation of scientists and the threats are only rely on h-index are pressured. When the h-index is tested the hypothesis that the attainments of few strongly visible but intermediate productive authors are also underestimated when compared with other scientists.

Rahm and Thor (2005) the analysis on two important database [SIGMOD, VLDB] conferences on citation frequencies, the three database journals [TODS, VLDB Journal, Sigmod Record] over 10 years. The citation data is achieved by put together and clean-up data from "DBLP and Google Scholar". This study consists of various relevant comparative metrics for each publication venue, in specific the sum and average count of citations and the impact factor which is measured for journals alone so far. This study established another fact that the "most cited papers, authors, author institutions and their countries".

Brookes (1990) the word "Scientometrics" was coined by Vassily V and Nalimov in the year 1960. This term has full-fledged in popularity and top position in describing the study about science. The scientometrics indistinguishable from bibliometric and many research papers published in the journal scientometrics. In public domain output is literature that is papers, patents etc., The Bibliometrics, scientometrics and informetrics literatures towards science and technology are (Wilson, 2001) Nagpaul et al. (1999) given 13 papers on the scientometric emerging trends. This is categorized in 3 different parts ie. Scientometrics, Policy of Science & Technology and the Structure & Dynamics.

3. OBJECTIVES OF THE STUDY

- To analyze the year wise publication
- Assess the language wise percentage analysis
- To identify the document wise percentage analysis
- Identifying the top ten journals
- Scrutinizing the country wise publications.

4. METHODOLOGY

The worldwide data about pediatric are allowed access and also indexed in web of science (WOS), which were downloaded from the year 2001 to 2016 (16years).

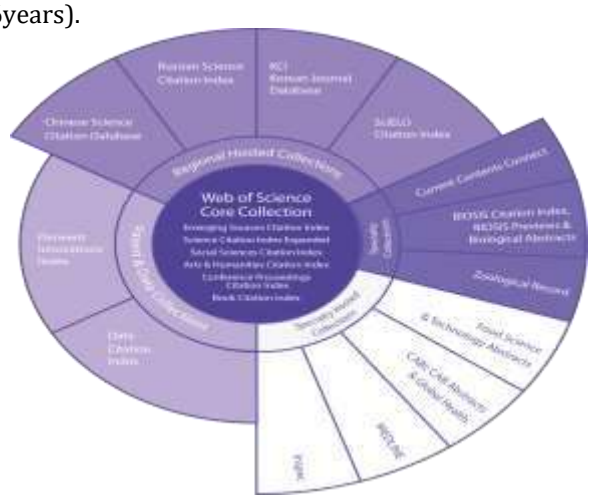


Fig. 1. The model of WOS (Web of science) platform with literature databases

In the web of science platform many fields will be available to extract the data, in that "Number of Records" to be filled by 1 to 500. [Only 500 records can be downloaded at a time] it means the total number of records for this study 20615, so 41 times

the data to be downloaded for the whole required records. Next in the field of record content to be filled as full record and cited references with plain text, at last by clicking sent option the data will be downloaded.

5. RESULTS

5.1.. The analysis of yearwise publications

In the yearwise analysis on pediatric contains the period of 2001 to 2016 the data reveals that 20615 and the following table shows the yearwise publications on pediatric in global level.

Table 1. Year wise productivity

S.No	Year	Records	%	Rank
1	2001	478	2.3	16
2	2002	493	2.4	15
3	2003	546	2.6	14
4	2004	591	2.9	13
5	2005	716	3.5	12
6	2006	839	4.1	11
7	2007	875	4.2	10
8	2008	1105	5.4	9
9	2009	1697	8.2	8
10	2010	1828	8.9	5
11	2011	1950	9.5	3
12	2012	2068	10	1
13	2013	1866	9.1	4
14	2014	1764	8.6	7
15	2015	1995	9.7	2
16	2016	1804	8.8	6
	TOTAL	20615	100	

The table 1 indicates that the growth of the publication during the study period are seems to be neither in a firm constant growing trend nor declining trend. It is analyzed that, the year 2012 shows the publications as 2068 records which is holds the first place and the most productive year. The year 2015 took the second place with 1995 publications and the year 2011 holds the third place with 1950 research records.

5.2. The analysis of document wise publications

Totally 20615 research records are published during the study period on pediatric over the world and totally fifteen types of research documents are involved. Out of fifteen types of documents "Article" took the first place with 15913 (77.2%). Second place goes to "Review" document with 1846 (9%), "Editorial Material" document holds the third place with 1196 (5.8%).

Table 2. Document wise publications

Sl.No	Document Type	Records	%
1	Article	15913	77.2
2	Review	1846	9
3	Editorial Material	1196	5.8
4	Article : Proceedings Paper	747	3.6
5	Meeting Abstract	608	2.9
6	Letter	153	0.7
7	Book Review	31	0.2
8	News Item	29	0.1
9	Biographical-Item	26	0.1
10	Reprint	26	0.1
11	Correction	24	0.1
12	Review: Book Chapter	10	0
13	Article: Book Chapter	4	0
14	Article: Retracted Publication	1	0
15	Poetry	1	0
	TOTAL	**20615**	

5.3. The analysis of language wise publications

The following table-3 denotes that totally nineteen languages were involved towards the publication of 20615 research records on "Pediatric", in which the language "English" plays the major role in publishing 19425 (94.2%) records and holds the first place among all the other 18 languages. The second place holds by "Spanish" language with 361 (1.8%) records and the third place took by the language of "German" with 341(1.7%) research records.

Table 3 – Analysis of Language wise publications

S. No.	Language	Records	%
1	English	19425	94.2
2	Spanish	361	1.8
3	German	341	1.7
4	French	339	1.6
5	Portuguese	61	0.3
6	Turkish	34	0.2
7	Italian	13	0.1
8	Croatian	9	0
9	Korean	9	0
10	Russian	6	0
11	Polish	5	0
12	Hungarian	3	0
13	Icelandic	2	0
14	Slovene	2	0
15	Arabic	1	0
16	Czech	1	0
17	Georgian	1	0
18	Japanese	1	0
19	Serbian	1	0
	Total	**20615**	

5.4. The analysis of journal wise publications

The following table 4 shows the leading top ten journals are identified, in which the journal "Pediatrics" holds the first place with 4083 research records. The journal entitled as "Journal of pediatric infectious" took the second place with 385 publications. Third place took by the journal called "Circulation" with 324 records. The journal named "Pediatric critical care medicine" with 300 records, "Journal of urology" with 254 records, "Clinical pediatric" with 214 records, "Pediatric infectious disease journal" with 194 records, "Archives de pediatric" with 183 records, "Pediatric blood & cancer" with 171, "Monatsschrift kinderheilkunde" with 169 records placed 4th, 5th, 6th, 7th, 8th, 9th and 10th respectively.

Table 4 – Analysis of Journal wise publications

S. No.	Journal	Records
1	Pediatrics	4083
2	Journal of pediatric infectious	385
3	Circulation	324
4	Pediatric critical care medicine	300
5	Journal of urology	254
6	Clinical pediatric	214
7	Pediatric infectious disease journal	194
8	Archives de pediatric	183
9	Pediatric blood & cancer	171
10	Monatsschrift kinderheilkunde	169

5.5. The analysis of country wise publications

The following table – 5 indicates the country wise publications in which the "United States of America" stands first in publishing most number of research records on "Pediatric" with 11754, secondly "Canada" holds the place with 1636 records. Third place took by "United Kingdom" with 827 research records.

Table 5. Analysis of Country wise publications

S.No	Country	Records
1	United States	11754
2	Canada	1636
3	United Kingdom	827
4	Germany	703
5	France	698
6	Italy	674
7	Australia	527
8	Netherlands	503
9	Spain	418

Table 6. Application of Lotka's Law

No. of contribution (x)	No. of contributors (y)	∑x=log x	∑y=log y	x2	XY	y/∑y	∑(y/∑y)	$1/x^n$	Fe=C($1/x^n$)	CT	D
1	50421	0.000	10.828	0.000	0.000	0.761	0.761	1.000	0.6100	0.61	-0.151
2	8791	0.693	9.081	0.480	6.295	0.133	0.894	0.250	0.1525	0.8	-0.131
3	3077	1.099	8.032	1.207	8.824	0.046	0.940	0.111	0.0678	0.8	-0.110
4	1471	1.386	7.294	1.922	10.111	0.022	0.962	0.063	0.0381	0.9	-0.094
5	771	1.609	6.648	2.590	10.699	0.012	0.974	0.040	0.0244	0.9	-0.081
6	527	1.792	6.267	3.210	11.229	0.008	0.982	0.028	0.0169	0.9	-0.072
7	333	1.946	5.808	3.787	11.302	0.005	0.987	0.020	0.0124	0.9	-0.065
8	229	2.079	5.434	4.324	11.299	0.003	0.990	0.016	0.0095	0.9	-0.059
9	159	2.197	5.069	4.828	11.138	0.002	0.993	0.012	0.0075	0.9	-0.053
10	106	2.303	4.663	5.302	10.738	0.002	0.994	0.010	0.0061	0.9	-0.049
11	86	2.398	4.454	5.750	10.681	0.001	0.996	0.008	0.0050	1.0	-0.045
12	58	2.485	4.060	6.175	10.090	0.001	0.996	0.007	0.0042	1.0	-0.042
13	41	2.565	3.714	6.579	9.525	0.001	0.997	0.006	0.0036	1.0	-0.039
14	45	2.639	3.807	6.965	10.046	0.001	0.998	0.005	0.0031	1.0	-0.036
15	30	2.708	3.401	7.334	9.211	0.000	0.998	0.004	0.0027	1.0	-0.034
16	15	2.773	2.708	7.687	7.508	0.000	0.998	0.004	0.0024	1.0	-0.032
17	14	2.833	2.639	8.027	7.477	0.000	0.999	0.003	0.0021	1.0	-0.030
18	14	2.890	2.639	8.354	7.628	0.000	0.999	0.003	0.0019	1.0	-0.028
19	13	2.944	2.565	8.670	7.552	0.000	0.999	0.003	0.0017	1.0	-0.027

20	12	2.996	2.485	8.974	7.444	0.000	0.999	0.003	0.0015	1.0	-0.026
21	9	3.045	2.197	9.269	6.689	0.000	0.999	0.002	0.0014	1.0	-0.024
22	10	3.091	2.303	9.555	7.117	0.000	1.000	0.002	0.0013	1.0	-0.023
23	6	3.135	1.792	9.831	5.618	0.000	1.000	0.002	0.0012	1.0	-0.022
25	5	3.219	1.609	10.361	5.181	0.000	1.000	0.002	0.0010	1.0	-0.021
26	7	3.258	1.946	10.615	6.340	0.000	1.000	0.001	0.0009	1.0	-0.020
27	5	3.296	1.609	10.863	5.304	0.000	1.000	0.001	0.0008	1.0	-0.020
28	5	3.332	1.609	11.104	5.363	0.000	1.000	0.001	0.0008	1.0	-0.019
29	4	3.367	1.386	11.339	4.668	0.000	1.000	0.001	0.0007	1.0	-0.018
30	3	3.401	1.099	11.568	3.737	0.000	1.000	0.001	0.0007	1.0	-0.018
31	4	3.434	1.386	11.792	4.761	0.000	1.000	0.001	0.0006	1.0	-0.017
32	3	3.466	1.099	12.011	3.808	0.000	1.000	0.001	0.0006	1.0	-0.017
33	2	3.497	0.693	12.226	2.424	0.000	1.000	0.001	0.0006	1.0	-0.016
34	1	3.526	0.000	12.435	0.000	0.000	1.000	0.001	0.0005	1.0	-0.016
35	4	3.555	1.386	12.640	4.929	0.000	1.000	0.001	0.0005	1.0	-0.015
36	1	3.584	0.000	12.842	0.000	0.000	1.000	0.001	0.0005	1.0	-0.015
38	1	3.638	0.000	13.232	0.000	0.000	1.000	0.001	0.0004	1.0	-0.014
39	1	3.664	0.000	13.422	0.000	0.000	1.000	0.001	0.0004	1.0	-0.014
42	1	3.738	0.000	13.970	0.000	0.000	1.000	0.001	0.0003	1.0	-0.014
50	2	3.912	0.693	15.304	2.712	0.000	1.000	0.000	0.0002	1.0	-0.013
52	1	3.951	0.000	15.612	0.000	0.000	1.000	0.000	0.0002	1.0	-0.013
97	1	4.575	0.000	20.928	0.000	0.000	1.000	0.000	0.0001	1.0	-0.013
Total	**66289**	**116.02**	**122.40**	**363.08**	**247.45**	**1.000**	**40.46**	**1.62**	**1.0**		

5.6. Application of Lotka's Law

It was identified that the value of n in pediatric research output in global is 2.86, the Lotka's law, finding was n= 2 while the outcome of Lotka's law result in our study was found to be 2.86. Hence the present analysis invalidates the Lotka's results. So that based on the observation the Lotka's Law will not fit in to the authors' productivity of this study.

The hypothesis framed as H0- "No significant relationship between the Lotka's Law and the author productivity".

6. CONCLUSION

In this study during the year 2001 to 2016 the total research records were 20615. It is observed that in the analysis of yearwise publications the year 2012 shows the publications as 2068 records which is holds the first place and the most productive year. The year 2015 took the second place with 1995 publications and the year 2011 holds the third place with 1950 research records. In the analysis of document wise publications, out of fifteen types of documents "Article" took the first place with 15913 (77.2%). Second place goes to "Review" document with 1846 (9%), "Editorial Material" document holds the third place with 1196 (5.8%). In the language wise analysis language "English" plays the major role in publishing 19425 (94.2%) records and holds the first place among all the other 18 languages. The second place holds by "Spanish" language with 361 (1.8%) records and the third place took by the language of "German" with 341(1.7%) research records. In the observation of journal wise publications journal "Pediatrics" holds the first place with 4083 research records. The journal entitled as "Journal of pediatric infectious" took the second place with 385 publications. Third place took by the journal called "Circulation" with 324 records. In the analysis of country wise publications the "United States of America" stands first in publishing most number of research records on "Pediatric" with 11754, secondly "Canada" holds the place with 1636 records. Third place took by "United Kingdom" with 827 research records.

REFERENCES

1. Arsenova, I.. (2016). Bibliometric Analysis of Lead-acid batteries Publications: Trends and Characteristics. *Journal of Scientometric Research,* **5**(2).
2. Bibasha Biswas. (2016). The Bottom Line Journal 2011-2015: A Bibliometric study, *International Journal of Library and Information Science,* **5**(3): 141-148.
3. Chellapandi, P., and L. Sangeetha, (2016). A Bibliometric Analysis of Literature on Marine Science Research. Shanlax International Journal of Arts, Science & Humanities. **4**(2): 132-139.
4. Gogoi M., and P.K. Barooach, (2016). Bibliometric analysis of Indian Journal of chemistry, section B to study the usage pattern of information in the field of material science.
5. Shafiullah, Yasinullah (2015) The Electronic Library Journal: A Bibliometric Study (2010-2014), *International Journal of Academic Library and Information Science* **3**(8): 226-235.