Kong. Res. J. 2(1) : 1-3, 2015 ISSN 2349-2694
Kongunadu Arts and Science College, Coimbatore.

PREPARATION OF MESOSCOPIC STRUCTURE POLY METHYL METHACRYLATE THIN FILMS FOR AFM DATA STORAGE DEVICES.

Chandar Shekar, B[a*]., S. Sathish[a], Sulana Sundari[b] , S.Sunnitha[b] and C. Sharmila[c]
[a] Department of Physics, Kongunadu Arts and Science College, Coimbatore, Tamil Nadu.
[b] NEAR foundation, The Nilgiris, Tamil Nadu.
[c] Department of Physics, PSGR Krishnammal College for Women, Coimbatore, Tamil Nadu.
*E-mail: chandar.bellan@ gmail.com

ABSTRACT

Poly methyl methacrylate (PMMA) thin films were prepared by dip coating method. Benzene was used as a solvent to prepare PMMA thin films for the time periods ranging from 1 min. to 1 h. The thickness of the films deposited was measured by using an electronic thickness measuring instrument (Tesatronic-TTD-20). Fourier Transform Infrared spectrum was used to identify the above said films. X-ray diffraction spectra indicated the predominantly amorphous nature of the films. Surface morphology of the coated films studied by using scanning electron microscope (SEM) indicated the absence of any pits, cracks and pin holes in the surface. Both as grown and annealed films showed smooth and amorphous structures. The closer SEM inspection revealed the presence of self assembled mesoscopic cells. The mesoscopic structure PMMA thin films could be used as an AFM-based data storage which is promising alternative to conventional magnetic data storage because it offers great potential for considerable storage density improvements.

Key words: Polymethyl methaacrylate, Dip coating, Morphology, Mesoscopic, FTIR, SEM .

1. INTRODUCTION

Thin films of polymer have attracted the attention of researchers mainly because of their unique properties, resistivity, electrical properties and their ease of processing and fabrication. The important advantages of polymer thin films are that they can be prepared easily and at low cost. Poly methyl methacrylate (PMMA) is one of the promising representatives of polymeric materials and there are numerous proposals for its application as dielectric in organic thin film transistors (OTFTs) (Puigdollers *et al.,*2004; Uemura *et al.,* 2003; Chandar Shekar *et al.,* 2004), sensors (Ponelyte and Palevicius, 2014) as optical lenses in cameras and optical fibers (Nakata *et al.,* 2004; Yang *et al.,* 2004) . Extensive work has been carried out on synthesis, preparation and various properties such as morphology, dielectric, optical and aging behavior of PMMA films (Sakai *et al.,* 2009; Konno *et al.,* 2009; Kim *et al.,* 2009; Mabrook *et al.,* 2009) . In the present work an attempt has been made to prepare PMMA thin films of suitable thickness by a simple dip coating method which could be used in AFM data storage devices.

2. MATERIALS AND METHODS

PMMA polymer obtained from Sigma-Aldrich was used without further purification to form the insulator layer. The cleaned glass plates were held vertically above the PMMA solution (concentration of 2.5% with benzene as a solvent) by means of mechanical arrangement capable of slow and steady vertical movement. The substrates were immersed in the solution at room temperature for different time period (1 min to 1h). After withdrawal from the solution, the substrates with the deposited film was dried in the atmosphere for 45 minutes and then kept inside an oven at 373 K for 1 h. The thickness of the coated films was measure by using an electronic thickness measuring instrument (Tesatronic-TTD-20). The PMMA films coated were identified by using FTIR spectrometer. The structure of the deposited PMMA films was studied by using XRD. The surface morphologies of the deposited PMMA films were investigated by using Scanning Electron Microscope.

3. RESULTS AND DISCUSSION

The functional groups present in the deposited polymer film were identified by FTIR spectrum. Figs 1a, b shows the FTIR spectrum of PMMA thin films of thickness 360 nm and 1810 nm respectively.

The bands observed at 677 cm^{-1} and 750 cm^{-1} are assigned to OH bending. The bands at 1060 cm^{-1}, 1245 cm^{-1}, 1730 cm^{-1} and 2926 cm^{-1} are respectively assigned to υ(C-O) stretching vibration, wagging vibration of C-H, C=O stretching and C-H stretching.

The X-ray diffraction pattern of PMMA thin films of thickness 360 nm and 1810 nm are presented in the Figs. 2a, b.

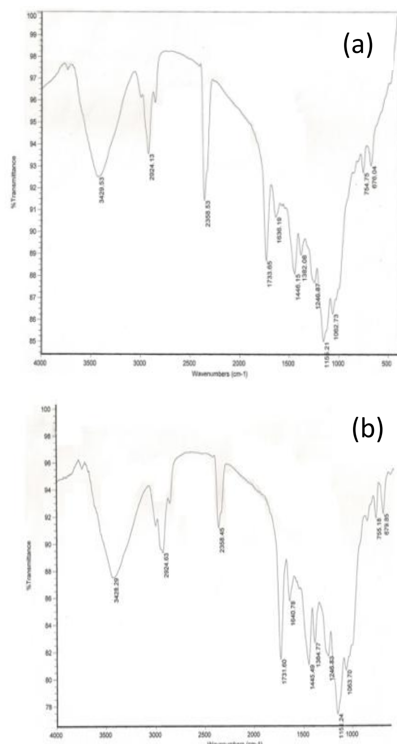


Fig. 1. FTIR spectrum of PMMA film of thickness a) 360 nm and b) 1810 nm.

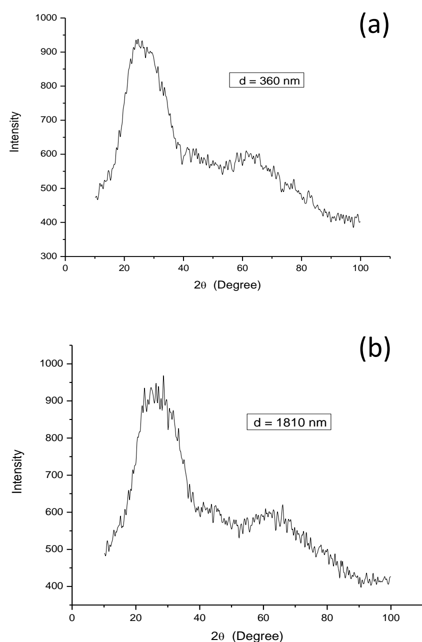


Fig 2. XRD pattern of PMMA thin film of thickness a) 360 nm and b) 1810 nm

The x-ray diffraction pattern shows large diffraction maximum that decreases at large diffraction angles indicates the amorphous nature of the film. The shape of the first main maximum indicates the ordered packing of the polymer chains. The intensity and shape of the second maxima are related to the effect inside the main chains (Chandar Shekar *et al.,* 2013) . The broad humps observed in the XRD spectrum indicate the presence of crystallites of very low dimensions. The absence of any prominent peaks in the spectrum indicates the predominantly amorphous nature of the films.

Figs. 3a, b shows the SEM image of PMMA films of thickness 360 nm and 1810 nm respectively.

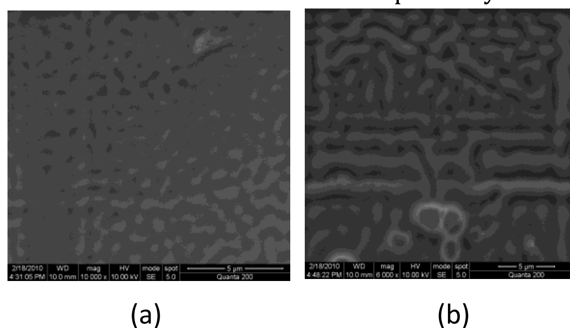


Fig. 3. SEM Micrographs of PMMA thin films of thickness a) 360 nm and b) 1810 nm.

The SEM analysis revealed self assembled mesoscopic structured films as presented in the figure, when deposited under dip coating process. The formation of the mesoscopic structures may be described with the help of the considerations presented below. Due to fast external drying, all solvents which leave the solution are immediately removed from the film. Then at an early stage of the evaporation process, a polymer rich layer is formed. The thickness of such layers may be of the order of mesoscopic scale range. The dip coating process presented in this work is simple, highly reproducible and permits fabrication of large areas of mesoscopic structured films, which have a potential as membranes, long period gratings and photonic molecules. The surface morphology is quite homogenous and amorphous in nature without any pits, cracks and pinholes. The mesoscopic structures obtained for the very thin film of PMMA could be used as an AFM-based data storage which is promising alternative to conventional magnetic data storage because it offers great potential for considerable storage density improvements.

4. CONCLUSION

The FTIR analysis indicated the absence of any impurity in the PMMA thin films. The X-ray diffraction pattern revealed the amorphous nature of

the films studied. The SEM analysis revealed the phenomenon of self assembly on the mesoscopic scale. The dip coating process presented in this work is simple, highly reproducible and permits fabrication of large areas of mesoscopic structured films. The mesoscopic structured films have the potential as membranes, long period gratings, photonic molecules and AFM based data storage system.

REFERENCES

Chandar Shekar, B., M. Na, J. Lee and S.W. Rhee, (2004). *Mol. Cryst. Liq. Cryst.* **424**: 43-53.

Chandar Shekar, B., S. Sathisha and R. Sengoden, (2013). *Physics Procedia.* **49**: 145 – 157.

Kim, T.G., E.H. Jeong, S.C. Lim, S.H. Kim, G.H. Kim, S.H. Kim, H.Y. Jeon and J.H. Youk, (2009). *Synthetic Metals* **159**: 749-753.

Konno, K., H. Sakai, T. Matsushima and H. Murata, (2009). *Thin Solid Films* **518**: 534-536.

Mabrook, M.F., A.S. Jombert, S.E. Machin, C. Pearson, D. Kolb, K.S. Coleman, D.A. Zeze and M.C. Petty, (2009). *Materials Science and Engineering: B* **159-160**: 14-17.

Nakata, K., M. Ohji, Y. Ikuno, S. Kusaka, F. Gomi, M. Kamei, D.F. Ross and Y. Tano, *Am. J. Ophthalmol.* **137**: 760-762.

Ponelyte, S. and A. Palevicius, (2014). *Sensors* **14**: 6910-6921.

Puigdollers, J., C.V.I. Martin, A. Orpella, M. Vetter and R. Alcubilla, (2004). *J. Non-Crystalline Solids* **338-340**: 617-621.

Sakai, H., K. Konno and H. Murata, (2009). *Thin Solid Films* **518**: 510-513.

Uemura, S., M. Yoshida, S. Hoshino, T. Kodzasa and T. Kamata, (2003). *Thin Solid Films* **438-439**: 378 – 381.

Yang, D.X., J. Yu, X. Tao and H. Tam, (2009). *Materials Science and Engineering A* **364**: 256-259.

Kong. Res. J. 2(1) : 4-6, 2015 ISSN 2349-2694
Kongunadu Arts and Science College, Coimbatore.

STRUCTURAL STUDIES OF FERROELECTRIC BATIO$_3$ NANO PARTICLES AND VACUUM EVAPORATED BATIO$_3$ NANO SCALE THIN FILMS

Chandar Shekar, B[1*]., R. Sengodan[2], S. Sathish[1] and Sulana Sundari[3]
[1]Departments of Physics, Kongunadu Arts and Science College, Coimbatore, Tamil Nadu, India.
[2]Department of Physics, Kumaraguru College of Technology, Coimbatore, Tamil Nadu, India
[3]The NEAR Foundation, The Nilgiris, Tamil Nadu, India.
*E-mail: chandar.bellan@gmail.com

ABSTRACT

Barium titanate ($BaTiO_3$) nanoparticles were prepared by wet chemical method using commercially available materials barium chloride, titanium dioxide and oxalic acid. Nano scale thin films of different thickness were coated on pre - cleaned glass substrate by using vacuum evaporation technique under a vacuum of $2x10^{-5}$ Torr. The X - ray analysis showed that the particles have tetragonal structure. The deposited films of a lower thickness were found to be amorphous in nature, whereas the crystallinity increases with increase of thickness. The estimated value of grain size (D), strain and dislocation density (δ) were also reported in this paper.

Key words: $BaTiO_3$, Thermal Evaporation, XRD

1. INTRODUCTION

The discovery of ferroelectric barium titanate ($BaTiO_3$) opens the present era of ceramic dielectric materials. $BaTiO_3$ having the perovskite structure with tetragonal symmetry at room temperature, possesses a relatively large dielectric constant (ε') and electro optic coefficient. Now-a-days $BaTiO_3$ has become the basic capacitor material in semiconductor technology. $BaTiO_3$ ceramics have a strong piezoelectric effect. These ceramics find wide applications in devices such as microphones, ultrasonic and underwater transducers, sensors and actuators, electro – optic device, multilayer capacitors and spark generators. $BaTiO_3$ is one of the ABO_3 type (A = mono or divalent, B = tri-hexavalent ions) ceramic materials which have been examined in search of ferroelectric applications. Due to the desirable properties and applications, over the last few decades, synthesis of $BaTiO_3$ nanopowder and thin film has attracted great attention. Various chemical methods could be employed for the production of these fine particles like sol-gel techniques (Tangwiwat and Milne, 1988), co-precipitation, alkoxide hydrolysis (Kirby *et al.,* 1988), metal-organic processing (Shaikh and Vest, 1986), hydrothermal treatment (Boulos *et al.,* 2005) and mechanochemical synthesis (Stojanovic *et al.*, 2005). Wet chemical method is a promising technique that offers relative low cost, uniform size, homogenous powder and high purity of the ceramics. In addition to that different techniques have also been applied to prepare thin film of barium titanate such as r.f.- sputtering (Bhattacharya *et al.,* 1993), pulsed laser ablation (Yoon *et al.,* 1995) and metal-organic chemical vapour deposition (Tahan *et al.*, 1996).

Dent et al., have successfully optimized high velocity oxy-fuel (HVOF) spraying for the deposition of barium titanate as dense thick dielectric layer (25-150 µm) and compared the dielectric constant (k) values of these deposits with those (k) values of $BaTiO_3$ layers produced by plasma spraying. The maximum dielectric constant values achieved by HVOF method of deposition are in the range 70-115. Plasma spraying of these materials has produced layers with k values close to 200. However, considerable success has been achieved for both process, some of the problems inherent in each type of deposition are still to be overcome. Despite several techniques have been explored to deposit thin film of $BaTiO_3$, less attention has been devoted to thermal evaporation. In this paper, we have reported about the preparation of nanoscale $BaTiO_3$ thin films by thermal evaporation technique from $BaTiO_3$ nanoparticles synthesised by wet chemical method. Structure parameters of $BaTiO_3$ nanopartilces and vacuum evaporated nano scale thin films have also been reported.

2. MATERIALS AND METHODS

2.1. Synthesis of $BaTiO_3$ nanoparticles

$BaTiO_3$ nanoparticles were synthesized using wet chemical method. The starting materials used were barium chloride ($BaCl_2.2H_2O$), titanium dioxide (TiO_2) powder and oxalic acid. A solution of barium chloride, titanium dioxide and oxalic acid having mole ratio1: 1: 1 was stirred and evaporated at 80°C

till a clear, viscous resin was obtained and then dried at 100° C for 20 hours. The precursor formed was heated at 1000° C for 2 hours to form $BaTiO_3$ nanoparticles.

2.2. $BaTiO_3$ nanoscale thin film preparation

Nano scale thin films of $BaTiO_3$ were prepared by thermal evaporation of $BaTiO_3$ nanoparticles evaporated onto pre-cleaned glass substrate under a vacuum of $2x\ 10^{-5}$ torr, using a Hind High vacuum coating unit. The growth rate and thickness were measured during growth process by using a quartz crystal oscillator thickness monitor attached inside the vacuum evaporation chamber. The growth rate was adjusted to be as low as 1 Å/sec to avoid the differential evaporation of elements of the alloy.

2.3. Structural studies of $BaTiO_3$ nanoparticles and $BaTiO_3$ nanoscale thin films

The XRD patterns of the $BaTiO_3$ nanoparticles and their nanoscale thin films were obtained from X-ray powder diffraction with CuKα radiation (λ = 1.5418 Å).

3. RESULTS AND DISCUSSION

3.1. X – Ray Diffraction Analysis

Fig.1 shows the X – ray diffraction pattern of the $BaTiO_3$ nanoparticles. The spectrum obtained exhibits a tetragonal polycrystalline structure with the preferred orientation along (101), (111), (002), (112), (003) and (113) and the value of interplanar spacing d were evaluated and compared with the standard JCPDS values of $BaTiO_3$ (Pattern: 00 – 003 – 0725) and are found to be in good agreement with the standard data [Hu et al.,].

The grain size is calculated from the full with half – maximum (FWHM) of the XRD peaks by using Scherrer formula

$$D = 0.94\lambda/\beta\cos\theta \qquad (1)$$

Where k is the wavelength of the X-rays used, 2θ is the angle between the incident and scattered X-rays, and β is the full width at half maximum. The strain (ε) was calculated from the formula

$$\varepsilon = \beta\cos\theta/4 \qquad (2)$$

The dislocation density (δ) is defined as the length of dislocation lines per unit volume of the crystal and is given by

$$\delta = 1/D^2 \qquad (3)$$

Table.1 shows the calculated interplanar spacing (d), grain size (D), strain (ε) and dislocation density (δ) and the standard'd' values of corresponding predominant peaks. The calculated 'd' values are found to be in agreement with the standard values. The average grain size (D), strain (ε) and dislocation density (δ) were found to be 26 nm, $1.40x10^{-3}$ $lin^{-2}m^{-4}$ and $1.58x10^{15} lin/m^2$.

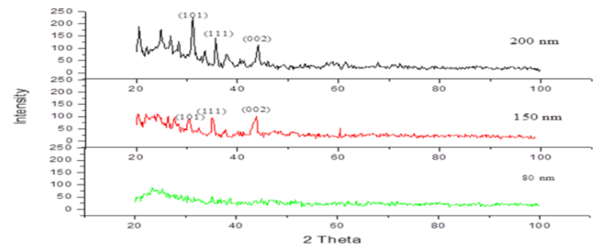


Fig. 1. XRD Spectrum of $BaTiO_3$ nanoparticles

The X – ray diffraction pattern of the $BaTiO_3$ nano scale thin films of different thickness are shown in figure 2. It reveals that the films of lower thickness (80 nm) appear to be amorphous in nature without well-defined peaks, whereas the films of higher thickness are polycrystalline in nature. It is observed from diffactogram that the crystallites are preferentially oriented along (002) plane of the tetragonal structure. The intensity of the predominant peaks increases with increase in film thickness, indicating the high degree of preferential orientation towards these directions (12). This means that, at the initial state of film formation, i.e.; during the atomistic condensation of the film formation, the deposited atoms are at random orientations. As the film thickness increases, the polycrystalline grains begin to orient along their direction which is evident from the diffractograms of thickness 150 nm and 200 nm. The lattice parameter values determined for the peaks in the diffractograms coincide fairly well with the standard JCPDS data (pattern: 00 – 003 - 0725).

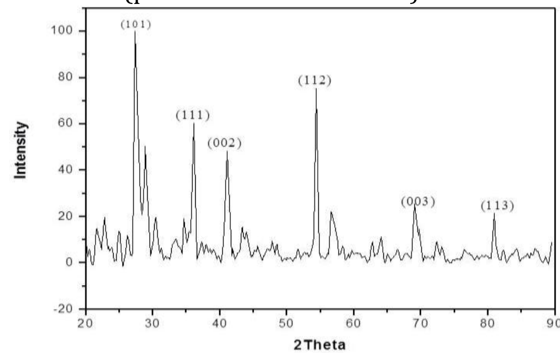


Fig. 2. XRD spectrum of $BaTiO_3$ nano thin film at different thickness

Table. 2 show a comparative look of the grain size, strain and dislocation density of the $BaTiO_3$ films of different thickness. It is observed that the grain size increases with film thickness and attained a value of 23 nm for 200 nm thickness film. Due to the increase in grain size with film thickness, the

defect in the lattice are decreased, which is turn reduce the internal microstrain and dislocation density or the columnar grain growth is increased. The strain and dislocation density decreases with increase of thickness, which may be due to increase in crystallinity.

Table 1. Structural parameters of $BaTiO_3$ nano particle.

2θ	Standard JCPDS d- values (A^0)	hkl	Calculated d- values(A^0)	D(nm)	ε x10^{-3} lin^{-2} m^{-4}	δx10^{15} lin/m^2
28.80	2.83	101	2.94	29	1.18	1.18
36.08	2.31	111	2.39	24	1.46	1.73
41.19	1.8	102	1.88	23	1.62	1.89
54.28	1.64	112	1.73	25	1.38	1.6
68.91	1.34	003	1.36	25	1.52	1.73
80.88	1.21	113	1.18	27	1.25	1.37

Table 2. Structural parameter of $BaTiO_3$ nano scale thin films

Thickness (nm)	2θ	hkl	D(nm)	ε x10^{-3} lin^{-2}m^{-4}	δx10^{15}lin/m^2
	30.50	101	21.69	1.59	2.12
150	35.19	111	21.06	1.64	2.25
	43.79	002	12.06	2.75	6.29
	31.13	101	24.54	1.41	1.66
200	35.65	111	22.73	1.52	1.93
	44.24	002	20.43	1.69	2.39

4. CONCLUSION

Nanoparticle of $BaTiO_3$ were successfully synthesised by low cost wet chemical method using commercially available chemicals such as oxalic acid, TiO_2 and $BaCl_2$. Thin films of few hundred nanometer thickness were prepared on well cleaned glass plate for the first time using thermal evaporation method. X-ray analysis showed that the nanoparticles have tetragonal nature and the deposited films at lower thickness have amorphous structure, whereas film of higher thicknesses showed increase in crystallinity. The crystallinity increases with increase of film thickness where as strain and dislocation density decreases with increase of film thickness. The improved crystallinity with temperature and thickness indicated the feasibility of utilising them for sensor applications.

REFERENCES

Bhattacharya, P., T. Komeda, D. Park and Y. Nishioka, Japan. (1993). *J. Appl. Phys.* **32**: 4103.

Boulos, M., S. Fritsch, F. Mathieu, B. Durand, T. Lebey and V. Bley. (2005). *Solid State Ionics* **176**: 1301.

Dent, A.H., A. Patel, J. Gutleber, E. Tormey, S. Sampath, H. Herman. (2001). *Mater. Sci. Eng. B* **87**: 23.

Hu, Z.G., G.S. Wang, Z.M. Huang and J.H. Chu. (2003). *Journal of Physics and Chemistry of solids* **64**: 2445-2450.

Kaur, J., R.K. Kotnala, K.C.Verma. (2012). *Journal of optoelectronics and advanced materials* 14: 219–223.

Kirby, K.W., Mater. (1988). *Res. Bull* **12**: 881.

Shaikh, A.S. and G.M. Vest. (1986). *J. Am. Ceram. Soci.* **69:** 682.

Stojanovic, B.D., A.Z. Simoes, P.C.O. Santos, C. Jovalekic, V.V. Mitic and J. A. Varela. (2005). *J. Euro Ceram. Soci.* **25**: 1985.

Tahan, D., A. Safari, L.C. Klin. (1996). *J. Am. Ceram. Soci.* **79**: 1593.

Tangwiwat, S. and S.J. Milne. (1988). *J. Non-Crystalline Solids* **351**:

Yoon, S.G., L. Lee and A. Safari. (1995). *Integrated Ferroelectrics* **7**: 329.

Kong. Res. J. 2(1) : 7-10, 2015 ISSN 2349-2694
Kongunadu Arts and Science College, Coimbatore.

PREPARATION AND CHARACTERIZATION POLY (VINYLIDENE FLUORIDE-TRIFLUOROETHYLENE) COPOLYMER THIN FILMS FOR ORGANIC FERROELECTRIC FIELD EFFECT THIN FILM TRANSISTORS.

Chandar Shekar, B[1*]., Sulana Sundari[2], S. Sunnitha[2] and C. Sharmila[3]
[1]Department of Physics, Kongunadu Arts and Science College, Coimbatore, Tamil Nadu, India.
[2]The NEAR Foundation, The Nilgiris, Tamil Nadu, India.
[3] Department of Physics, PSGR Krishnammal College for women, Coimbatore, Tamil Nadu, India.
*E-mail: chandar.bellan@gmail.com

ABSTRACT

Polyvinylidene fluoride (PVDF) and Trifluoroethylene ((TrFE) are potential polymers which are used in acoustic transducers and electromechanical actuators because of their inherent piezoelectric response, as heat sensors because of their inherent pyroelectric response and as dielectric layer in organic thin film transistors. In the present study thin films of copolymer Poly(vinylidene fluoride-trifluoroethylene) were prepared by spin coating method for two different concentrations 2% to 8% and for various spin speeds from 2000 RPM to 5000 RPM. A P-type Si wafer was used as a substrate to deposit P(VDF-TrFE) thin films. 2-butanone was used as a solvent to prepare P(VDF-TrFE) solution. To study the annealing effect, the films were annealed for three different temperatures 50°C, 100°C and 175°C. Ellipsometry was used to measure the thickness of the films. The identification of the films prepared was done by using FTIR spectrophotometer. The structure of the films was studied by using small angle XRD. The morphology of the coated surface was investigated using SEM. It is observed that the thickness of the film coated depends on concentration, spin speed and annealing temperature. The XRD spectrum indicated the amorphous nature with crystallites of very low dimension. SEM micrographs also conforms the predominantly amorphous nature of the film surface. The observed smooth surface with amorphous structure indicated that these films could be used as dielectric layer in organic ferroelectric field effect thin film transistors.

Keywords: PVDF -TrFE, Morphology, XRD,FTIR, SEM

1. INTRODUCTION

Ferroelectric field effect devices offer the possibility of non-volatile active memory elements. In the metal-ferroelectric-semiconductor field effect transistor, it is important for a ferroelectric material to have a suitable dielectric constant to enable the application of sufficient electric field to a ferroelectric film. Polyvinylidene fluoride (PVDF) and Trifluoroethylene ((TrFE) are potential polymers which are used in acoustic transducers and electromechanical actuators because of their inherent piezoelectric response, as heat sensors because of their inherent pyroelectric response , as an hole injecting interlayer in organic light emitting diodes (Jeon *et al.,* 2010; Mahdi *et al.,* 2014) as an interlayer in organic solar cells to improve the efficiency through fluorinated interlayer induced crystallization (Jeon *et al.,* 2009; Xiao *et al.,* 2013) and as dielectric layer in organic ferroelectric thin film transistors (Chang *et al.,* 2009; Jung *et al.,* 2010; Yoon *et al.,* 2010). As a dielectric layer in organic ferroelectric field effect transistor structure along with suitable dielectric constant value it need to satisfy various constraints concerning band offsets, limits on charge traps, processability, reproducibility, stability against degradation, small leakage current, high breakdown potential, smooth surface and amorphous nature. In the present study effect of annealing on the structure and morphology of Polyvinylidene fluoride-trifluoroethylene (50/50) copolymer thin films prepared by spin coating was carried out with a view to use P(VDF-TrFE) film as an efficient dielectric layer in organic ferroelectric thin film transistors.

2. MATERIALS AND METHODS

Conventional P(VDF-TrFE) (50/50) copolymer was dissolved in 2-2-butanone to form the solution of concentrations 2 % and 8 % . The solution was spun on Boron doped Si (100) substrate (p-type Si) for 20 Sec. at room temperature to prepare P(VDF-TrFE) thin films. Before depositing P(VDF-TrFE), the surface preparations of silicon wafers were done by degreasing with organic solvents such as trichloroethylene (TCE) and ethanol and then followed by a rinse in deionized (DI) water. After spin coating process, the samples were dried in the vacuum chamber to evaporate the solvent remained

in the film. The films were annealed in Ar ambient. The P(VDF-TrFE) films coated were identified by using a FTIR spectrometer. Spectroscopic ellipsometry (SE, J.A. Woolam Co., Model WVASE32) was used to measure the thickness of the films coated on Si substrate. The surface morphologies of the as deposited and annealed P(VDF-TrFE) films were investigated by means of SEM (FEI company, XL-305).

3. RESULTS AND DISCUSSION

To determine the amount of coating, P(VDF-TrFE) (50/50) solution of 2 % to 8 % concentrations was chosen. Fig.1 shows the variation of film thickness with spin speed ranging from 2000 RPM to 5000 RMP for various concentrations (wt.%) 2, 4, 6, 8 and 10. For the concentrations of 2 % to 8%, the thickness of the film decreases with the increase in spin speed. For 2 % concentration the variation in thickness is less when the spin speed increases above 3000 RPM. As the concentration increases, the variation in thickness coated with spin speed increases and it is much pronounced at 10 % concentration.

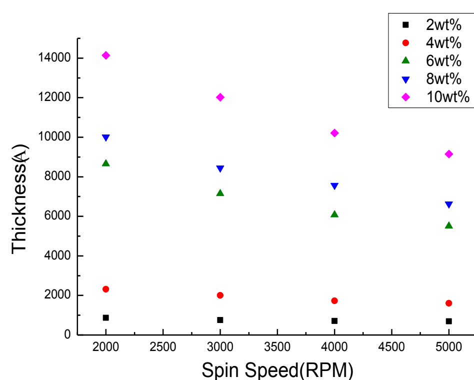


Fig. 1. Variation of thickness with spin speed for various concentrations.

Fig. 1 shows the thickness of the as grown and annealed films for various concentrations ranging from 2 % to 8 %. The figure shows that the thickness of the as grown films increases from 752 Å to 14000 Å as the concentration increases from 2 % to 8 %. For all the concentration range studied, the film thickness decreases with the increase of annealing temperature.

Fig.2 shows the FTIR spectrum PVDF-TrFE thin film of as grown (8450Å) and annealed at 50^{0}C, 100^{0}C and 175^{0}C.

The peak observed at 840 cm^{-1} is assigned to C-F stretching where as the peaks observed at 890 cm^{-1} is assigned to C-H bending.

The X-ray diffraction pattern of P(VDF-TrFE) film of thickness 8450 Å is as shown in the Fig.3. The diffraction pattern indicates the predominantly amorphous nature with large diffraction maxima that decreases at large diffraction angles. The shape of the first main maximum indicates the ordered packing of the polymer chains. The intensity and shape of the second maxima are related to the effect of ordering inside the main chains. The prominent peaks observed around 19^0 indicates the crystalline structure of spin coated PVDF-TrFE film (Nguyen et al., 2007). The observed broad humps in the XRD spectrum also indicate the presence of crystallites of very low dimensions.

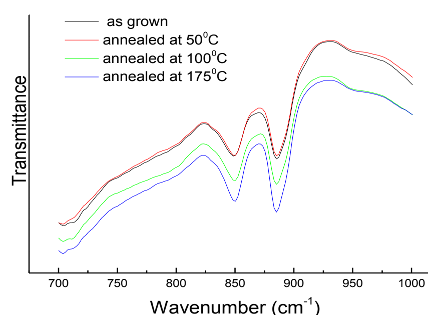


Fig. 2. FTIR spectrum of PVDF-TrFE thin film of thickness 8450 Å.

The crystal structure of P(VDF-TrFE) is normally related to the composition (mole ratio of P(VDF/TrFE) of the copolymer and the annealing process. In the β crystalline phase of P(VDF-TrFE), the unit cell is orthorhombic, with each chain aligned and packed with the CF2 groups parallel to the b-axis. Figure 3 shows the XRD pattern of P(VDF-TrFE) for different annealing temperatures (not annealed (27 ^{0}C) to 175 °C) to obtain information on the degree of the crystalline structure of the copolymer thin films. Characteristic peaks, associated with the β phase, appearing at 2θ = 19°, are assigned to (110/200) reflection planes. An elevated diffraction peak indicates a high percentage of the crystalline structure in the β phase. One can note from the XRD result that the peak intensity at unannealed (27 ^{0}C) was one of the lowest, because the annealing temperature was below the Curie temperature of the materials, which is not enough to align the chains. However, the highest intensity peak was achieved above 100 °C, which means that the crystalline structure of the copolymer thin film is the highest. The Curie temperature of P(VDF-TrFE) is around 90 °C, where the β phase starts to change above 100 °C. The crystalline structure of the thin film obtained

when annealed above 100 °C will be more favorable for the ferroelectric, pyroelectric and dielectric properties. This is due to the high crystalline structure exhibited by the composite thin film.

Surface morphology of dielectric layer is very important because it affects the property of the semiconductor layer coated over it. Fig. 4a-d shows the SEM image of the P(VDF-TrFE) films annealed at different temperatures. The film surface of as grown and films annealed at 50^{0}C and 100^{0}C is compact. Few pits are found on the surface. No pin holes, cracks and dendritic features are found in the surface. Macroscopic granular chains appear at the surface in the stretching direction of P(VDF-TrFE) film annealed at 175^{0}C. The granular structures (grooves) vary in size from approximately few nanometers to twenty nanometers.

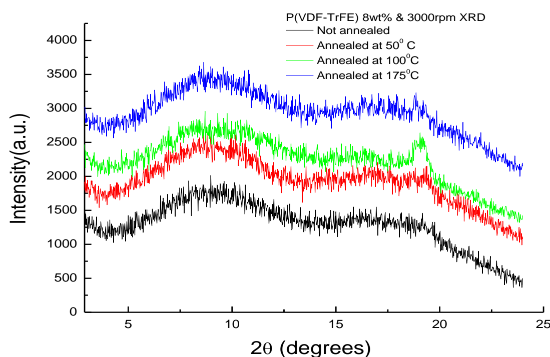
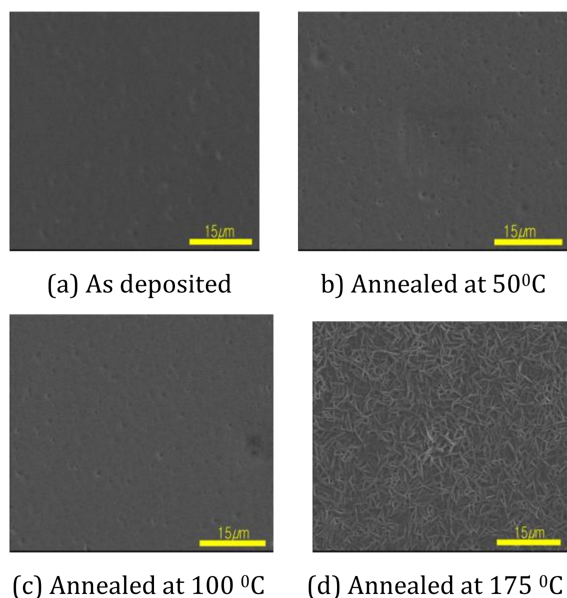


Fig. 3. XRD pattern of as grown and films annealed at 50^{0}C, 100^{0}C & 175^{0}C for 60 seconds.



(a) As deposited b) Annealed at 50^{0}C

(c) Annealed at 100 ^{0}C (d) Annealed at 175 ^{0}C

Fig. 4. SEM image of a) as grown, b) 50 ^{0}C annealed , c) 100 ^{0}C annealed and d) 175^{0}C annealed samples.

The surface morphology of both as grown (unannealed) and films annealed at 50^{0}C and 100 ^{0}C is quite homogeneous and amorphous nature. Only films annealed at 175^{0}C showed randomly ordered needle-like crystal domains of the PVDF-TrFE layer with a width of approximately 5nm to 20 nm. This indicates crystalline phase formation during thermal annealing process. As the annealing temperature increases, intrinsic changes in the microstructure of PVDF-TrFE as well as in interface are expected. These intrinsic changes may lead to the rougher and corrugated interfaces at the p-Si substrate sides. Similar needle like structure for PVDF-TrFE film with a domain width of 40 nm to 200 nm was reported by Chang *et al.,* 2009.

As deposited and films annealed up to 100 °C produced the morphology of the undefined crystalline structure. Significant elongated crystalline structures were clearly observed when the annealing temperature was increased to 175 °C. In general, P(VDF-TrFE) thin films are annealed between the Curie and melting temperatures in order to induce the crystalline structure. In the paraelectric phase, the chain mobility is higher compared to that of the ferroelectric phase. It favors the lowest energy conformation (all trans), because as the temperature increases, the chain mobility increases as a function of temperature. As a result, the molecular chains prefer being oriented in parallel to the substrate, and rod-like crystals are observed when the annealing temperature reached 175°C, as shown in Figure 4d. This suggests that small crystallites undergo a transition into a paraelectric phase. They grew by incorporating surrounding non-crystalline molecules and thus contributed to an increase in the crystalline structure. On the other hand, annealing above 100 °C resulted in the formation of acicular grains (needle-like crystals) in edge-on lamellae, as shown in Figure 4d. Near the melting point, the morphology changes drastically, due to the small crystals being partially fused and recrystallized. In such a way, the chain axis is reoriented normal to the substrate surface. This implies that annealing above 100 °C induced growth in the crystalline structure. The significant change in the morphology of the surface for the thin films annealed above 100°C, can be clearly observed with the combination of some defects, such as a crack in the surface, as shown in Figure 4d. The defects will lead to a decrease in the electrical properties of the material.

4. CONCLUSIONS

P(VDF-TrFE) thin films of around 50 nm to 1200 nm have been prepared by spin coating with a

spin speed between 2000 RPM and 5000 RPM. Surface morphology and structure of the PVDF-TrFE thin films have been studied by SEM and XRD. No pin holes were found in the surface. Both as grown and films annealed up to 100^0C showed smooth surface. The observed topographical features of the as grown and annealed films indicated the predominantly amorphous nature of the films studied. XRD indicated the presence of crystallites of very low dimension in the predominantly dominated amorphous phase. The effect of annealing temperature on the crystalline structure of P(VDF-TrFE) thin films has been investigated with a wide range of treated temperature (27 –175 °C) below and above the Curie temperature to optimize the crystalline structure. The annealing temperature is an essential parameter that can be used to enhance the crystalline structure of the thin films and the related electrical properties (pyro-, ferro- and dielectric). From the obtained results, we found that the optimized crystalline structure was achieved at around 175 °C when the thin films were annealed in a vacuum chamber oven. The thin films show very sharp and high intensity XRD peaks with a dominant β phase.

If the thin films were annealed with an oven, it required a higher optimum annealing temperature, which is about 175 °C. This annealing temperature is much closer to the melting point of the material and, thus, can cause many defects in the sample. The lamella length increases at 175 °C with cracks appearing on the surface of the thin film. The thin films also had poor ferroelectric and pyroelectric properties with high dielectric loss when annealed below the Curie temperature. Thus, the technique of annealing the thin film above 100 °C with a vacuum chamber oven can be used to achieve P(VDF-TrFE) thin film with improved ferroelectric, dielectric and pyroelectric properties, which is suitable for many applications, such as energy storage, sensors and actuators. The observed thermal stability, amorphous and smooth surface implies that thin films of P(VDF –TrFE) formed by spin coating can be used as sensors, solar cells and an efficient dielectric layer in ferroelectric organic thin film transistors.

REFERENCES

Chang, J., C.H. Shin, Y.J. Park, S.J. Kang, H.J. Jeong, K.J. Kim, C.J. Hawker, T.P. Russell, D. Y. Ryu and C. Park. (2009). *Organic Electronics* **10**: 849-856.

Jeon, S.O., K.S. Yook and J.Y. Lee. (2009). *Organic Electronics* **10**: 1583-1589.

Jeon, S.O., K.S. Yook and J.Y. Lee. (2010). *Journal of Luminescence* **130**: 1708-1710.

Jung, S.W., J.K. Lee, Y.S. Kim, S.M. Yoon, I.K. You, B.G. Yu and Y.Y. Noh. (2010). *Current Applied Physics* **10**: 58-61.

Maude, R.I., W. C. Gan and W.H.A. Majid. (2014). *Sensors* **14**: 19115-19127.

Nguyen, C.A., P.S. Lee and S.G. Mhaisalkar. (2007). *Organic Electronics* **8**: 415-422.

Xiao, Z., Q. Dong, P. Sharma, Y. Yuan, B. Mao, W. Tian, A. Gruverman and J. Huang. (2013). *Advanced Energy Materials* **3**: 1581- 1588.

Yoon, S.M., S.W. Jung, S.H. Yang, C.W. Byun, C.S. Hwang, S.H.K. Park and H. Ishiwara. (2010). *Organic Electronics* **11**: 1746-1752.

Kong. Res. J. 2(1) : 11-14, 2015 ISSN 2349-2694
Kongunadu Arts and Science College, Coimbatore.

ANTIBACTERIAL ACTIVITY OF A COPPER(II) COMPLEX OF 1-(1*H*-BENZIMIDAZOL-2-YL)-*N*-(TETRAHYDROFURAN-2-YLMETHYL) METHANAMINE

Elayaperumal, R[1], M. Kiruthika[2] and P. Dharmalingam*
[1]Department of Chemistry, J. J. College of Engineering and Technology, Tiruchirappalli.
[2]Department of Chemistry, Kongunadu Arts and Science College, Coimbatore.
*Department of Chemistry, Urumu Dhanalakshmi College, Tiruchirappalli.
*E-mail: relaya82@gmail.com

ABSTRACT

A mononuclear copper(II) complex of 1-(1*H*-benzimidazol-2-yl)-*N*-(tetrahydrofuran-2-ylmethyl)methanamine) was synthesized and characterized by various physicochemical techniques like cyclic voltammetry and elemental analysis, ESI-MS, UV–Visible, Infra red and EPR spectroscopy. The antibacterial activities of the ligand and its complex were screened by disc diffusion method and found that the metal complexes have higher antimicrobial activity than the free ligand.

Keywords: Copper(II), tetrahydrofuran, disc diffusion, antimicrobial.

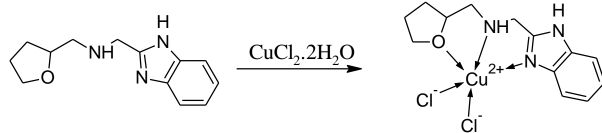
1. INTRODUCTION

Copper is an essential trace element in plants and animals, but not some microorganisms. Copper is essential to all living organisms as a trace dietary mineral because it is a key constituent of the respiratory enzyme complex cytochrome c oxidase. Copper proteins have diverse roles in biological electron transport and oxygen transportation, processes that exploit the easy interconversion of Cu(I) and Cu(II) (Lippard and Berg, 1994). It is well known that the derivatives of thiosemicarbazones possess the antibacterial activity. In addition, there are many studies that show the antimicrobial activity of copper(II) complexes with these ligands (Rauf *et al.,* 2009). Common anti-bacterial agents have been also used as ligands to complex with copper ions. It was noticed that the antimicrobial activity against *M. Smegmatis* of a metal ion complex in comparison to free ciprofloxacin, a bacterial gyrase inhibitor, increased three times. It may result from facilitated diffusion of the drug through the cell membrane, presumably by an increase in the lipophilicity of the drug. The activity against *Streptococcus* can also be influenced by the slow release of the ligands inside the bacterial cell. This was noticed with the copper complex of isoniazide and ethambutol. It seems that intercellular reduction of Cu(II) into Cu(I) can activate the oxygen which is toxic for bacteria. Moreover, a copper(II) complex of sulfacetamide, (N-[4-(amino-fenil)sulfonil]acetamide), has been intensively used in treatment of ophthalmic and dermatologic infections (Mistra and Pandey, 1992). Further studies of the copper(II) complexes of sulfacetamide and sulfanilamide and sulfisoxazole have shown promising results. The biological studies of metal complexes highlighted the potential of antioxidant activity of copper(II) complex with bioactive ligand (Rao *et al.,* 2010). In the present work, we synthesized and characterized a copper(II) complex of the ligand 1-(1*H*-benzimidazol-2-yl)-*N*-(tetrahydrofuran-2-ylmethyl)methanamine and also the antimicrobial activity was explored. The synthetic route for the present complex is shown in scheme 1.

2. MATERIALS AND METHODS

1-(tetrahydrofuran-2-yl)methanamine and copper(II) chloride procured from Sigma Aldrich, USA and used as received. Other materials like sodium borohydride and solvents like methanol, acetonitrile and dichloromethane were of reagent grade. Benzimidazole carbaldehyde was prepared using published procedure (Sathyaraj *et al.,* 2010). UV–visible spectrum of the complex was recorded on a Perkin–Elmer Lambda 35 double beam spectrophotometer at 25°C. Electron paramagnetic resonance spectrum of the copper(II) complex was obtained on a Varian E 112 EPR spectrometer. IR spectrum was recorded as KBr pellets in the 400-4000 cm^{-1} region using a Shimadzu FT–IR 8000 spectrophotometer. Cyclic voltammetry study of the complex was carried out by using three electrode system in a single compartment comprising of glassy-carbon working electrode and potentials were referenced to standard calomel electrode. Minimum quantity of the complex was dissolved in DMSO and decimolar solution of tetra butyl ammonium perchlorate was added. Positive ion electrospray

ionization mass spectrum of the complex was obtained by using Thermo Finnigan LCQ 6000 advantage max ion trap mass spectrometer.



Scheme 1. Synthesis of Copper(II) Complex.

2.1. Synthesis of 1-(1H-benzimidazol-2-yl)-N-(tetrahydrofuran-2-ylmethyl)methanamine (L1)

Benzimidazole-2-aldehyde (0.767 g, 5 mmol) and tetrahydrofurfuryl amine (0.505 g, 5 mmol) were mixed in methanol (20 mL) and stirred well for one day. Sodium borohydride (0.28 g, 7.5 mmol) was added to the above solution at 0˚C and the reaction mixture was stirred overnight at room temperature. The reaction mixture was rotaevaporated to dryness and the residue was dissolved in water (15 mL) and extracted with dichloromethane. The organic layer was dried and the solvent was evaporated to give the ligand as a brown oil, which was used as such for the preparation of complex. Yield: 0.1.016 g (88 %).

2.2. Synthesis of [Cu(L1)(Cl)]Cl (1)

The complex was prepared in good yield from the reaction of $CuCl_2{\cdot}2H_2O$ in methanol with L1. The ligand, L1 (0.68 g, 3 mmol) and $CuCl_2{\cdot}2H_2O$ (0.5 g, 3 mmol) were dissolved in methanol individually and the solutions were warmed. To the hot solution of L1, copper chloride was added slowly and stirred for 3 hours. The resulting solution was cooled to room temperature and the green colored copper–L1 complex separated out was filtered and dried. Yield: 0.921 g (84 %). Anal. Calc. for $C_{13}H_{17}Cl_2CuN_3O$: C, 42.69; H, 4.68; N, 11.49; Cu, 17.37; Found: C, 42.67; H, 4.62; N, 11.43; Cu, 17.31 %. FT-IR (KBr pellet) cm^{-1}: 3248, 2954, 1620, 1452, 752, 631. ESI-MS: m/z = 367.27 $[M – L{\cdot}Cl]^+$.

2.3. Antibacterial Assay

2.3.1. Micro-organisms used

Five species of bacteria, two gram positive (*Streptococcus faecalis & Bacillus subtilis*) and three gram negative (*Escherichia coli, Klebsiella pneumonia & Salmonella paratyphi*) were obtained from KMCH, Coimbatore.

2.3.2. Preparation of Inoculum

A loopful of strain was inoculated in 30 mL of nutrient broth in a conical flask and incubated on a rotary shaker at 37°C for 24 hours to activate the strain.

2.3.3. Bioassay

The bioassay used was the standard Agar Disc Diffusion assay. Mueller Hinton Agar was prepared for the study. Mueller Hinton agar plates were swabbed with a suspension of each bacterial species, using a sterile cotton swab. Subsequently, the sterilized filter paper discs were completely saturated with the test compound. The impregnated dried discs were placed on the surface of each inoculated plate. The plates were incubated overnight at 37°C. Each compound was tested against each organism in triplicate. Methanol was used as negative control. Standard discs of Ampicillin served as positive antibacterial control. The test materials having antimicrobial activity inhibited the growth of the micro organisms and a clear, distinct zone of inhibition was visualized surrounding the disc. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition in mm.

3. RESULTS AND DISCUSSION

3.1. Synthesis and Characterization

Ligand L1 was prepared by condensing tetrahydro furfuryl amine with benzimidazol-2-aldehyde to form Schiff base followed by reduction with Sodium borohydride. The copper(II) complex(1) was synthesized by the reaction between copper(II) chloride and L1 in equimolar quantities using methanol as solvent. The present complex was obtained in good yield and characterized by using elemental analysis, UV-Vis, ESI-MS and EPR spectral techniques. The analytical data obtained for the new complex agree well with the proposed molecular formula. The synthetic scheme for the present complex is shown in scheme 1. The ESI mass spectrum of [Cu(L1)(Cl)](Cl) displayed the molecular ion peak at m/z 367.27 which is reliable with the proposed molecular formula of the copper (II) complex.

3.2. Electronic Spectral Analysis

The electronic spectrum of the present complex shows two bands shows two bands at 270.8 and 277.4 nm, which can be attributed to intra ligand transitions of the ligand. Broad metal to ligand charge transfer (MLCT) transition has been observed at 364.6 nm. Complex 1 also exhibits its ligand field transition as broad band at 682 nm. Three d–d transitions are possible for copper (II) complex. They are d_{xz},d_{yz}- $d_{x^2-y^2}$, d_{z^2}-$d_{x^2-y^2}$ and d_{xy}-$d_{x^2-y^2}$. However, only a single broad band is observed for the copper (II) complex. This indicates the total sum of all the above transitions. The broadness associated with the d–d bands is generally taken as

an indication of the geometrical distortion of the complex from perfect planar symmetry.

3.3. IR and EPR Spectral Analysis

IR spectrum of complex 1 supports the coordination of N-H group of ligand (L1) as there is lowering of ν_{N-H} from 3360-3248 $cm^{-1.}$ The absorption found at 1091-1042 cm^{-1} is attributed to the presence of the coordinated furan ring through oxygen atom. The absorption in low frequency region at 752-630 cm^{-1} is attributed to the positive shifting of in-plane ring deformation vibration of the imidazole ring indicating the imidazole nitrogen coordination. For the complex 1, the calculated g_{II} and $g\perp$ values are 2.274 and 2.0871 respectively. The g_{II} value is $g\perp$ predicting the presence of the unpaired electron in the $d_{x^2-y^2}$ orbital. The g_{II} value is slightly less than 2.3 indicating that the metal-ligand bonding is predominantly covalent in nature. The G value is found to be 3.225 which is much less than 4 indicating that there is a strong interaction between two copper centres in the solid state. The g_{II} value is higher than 2.2 predicting the complex to have a pseudo-tetrahedral geometry.

3.4. Electrochemical Behavior of Copper Complex

The redox behavior of copper complex is studied with the help of cyclic voltammetry. Cyclic voltammogram of the copper complex was recorded in DMSO (Dimethyl sulphoxide) solution at 300 K using tetrabutyl ammonium perchlorate (TBAP) as supporting electrolyte. Complex 1, records E_{pc} and E_{pa} values at -0.523 and -0.2824 V respectively. These peak potential values signify the existence of of Cu^{II}/Cu^{I} redox couple. The reduction of Cu^{II} to Cu^{I} occurs at the cathodic peak potential of -0.523 V and reoxidation of Cu^{I} occurs at the anodic peak potential of -0.2824 V on scan reversal. The peak separation is found at 240 mV. This indicates a quasi-reversible one electron redox process. The $E_{1/2}$ value is measured at 0.12 V. This positive value of $E_{1/2}$ shows that the reduction of Cu^{II} in the complex is difficult as it is stabilized by the sigma bonding ligands. The peak current ratio for this complex is measured at 1.09. This value is slightly higher than unity and it indicates that the electron transfer is not followed by chemical reaction.

3.5. Antibacterial Activity

The *in vitro* biological screening effects of the investigated compounds were tested against the bacteria: Salmonella *paratyphi*, Streptococcus *faecalis*, Escherichia *coli*, Klebsiella *pneumonia* and Bacillus subtilis by the disc diffusion method. Antibacterial activity shown in Table 1 clearly indicates that the inhibitions are much larger by copper complexes as compare to the metal free ligand. The observed zone of inhibition order of complex 1 was S. *faecalis* > B. *subtilis* > K. *pneumonia* > S. *paratyphi* > E. *coli*. The increased activity of the metal complex can be explained on the basis of chelation theory. Also activity increases with concentration of the metal complexes. The chelation tends to make the ligands act as more powerful and potent bacterial agents, thus killing of more bacteria than the ligand. It is observed that in complexes the positive charge of the metal partially shared with the donor atoms present in the ligand and there may be π-electron delocalization over the whole chelate ring. Such an electron delocalization enhances the penetration of the complexes into lipid membranes and blocking of the metal binding sites in the enzymes of microorganisms. These complexes also disturb the respiration process of the cell and thus block the synthesis of proteins, which restricts further growth of the organism (Arjmand *et al.,* 2005).

Table 1. Antibacterial Activity of Complex 1.

S. No.	Bacteria	Zone of inhibition (mm)		
		Ampicillin	L1	Complex 1
1	*Streptococcus faecalis*	13.2±0.51	10.17±0.57	17.3±0.9
2	*Bacillus subtilis*	14.5±0.4	7.49±0.34	13.6±0.29
3	*Klebsiella pneumonia*	15.0±0.04	8.64±0.31	9.0±0.38
4	*Salmonella paratyphi*	15.0±0.57	6.71±0.23	8.0±0.13
5	*Escherichia coli*	16.3±0.15	5.44±0.05	6.0±0.26

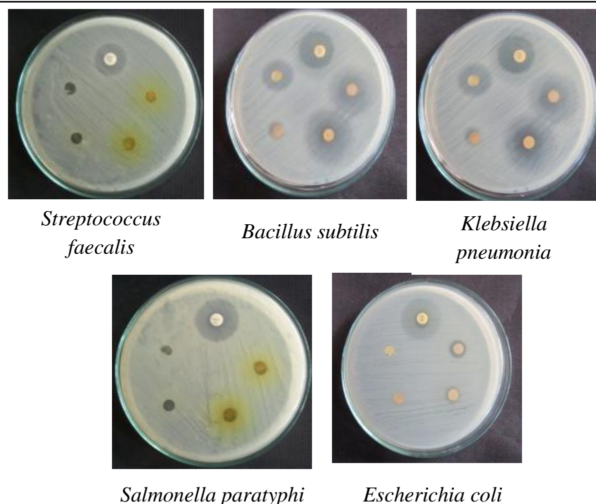


Fig. 1. Antibacterial Activity of Complex 1.

4. CONCLUSION

In the present work mononuclear copper(II) complex of tridentate reduced Schiff base ligand 1-(1*H*-benzimidazol-2-yl)-*N*-(tetrahydrofuran-2-ylmethyl)methanamine has been isolated and

characterized by various physico-chemical techniques. The antibacterial activity of the complex has also been evaluated and found that the Cu(II) complex showed better biological activity when compared to that of the ligand.

ACKNOWLEDGEMENT

The authors thank the Head, Department of Biotechnology, Kongunadu Arts and Science College, Coimbatore for antibacterial studies.

REFERENCES

Arjmand, F., B. Mohani and S. Ahmed, (2005) Synthesis, antibacterial, antifungal activity and interaction of CT-DNA with a new benzimidazole derived Cu(II) complex. *Eur. J. Med. Chem.* **40**: 1103-1110.

Lippard, S.J. and J.M. Berg, (1994). Principles of bioinorganic chemistry, University Science Books: Mill Valley, CA.

Mistra, L. and A.K. Pandey, (1992). Spectroscopic, electrochemical and antifungal studies of transition metal complexes with macrocycles containing nitrogen and sulphur donor atoms. *Polyhedron* **11**: 423-430.

Rao, Y.S., B. Prathima, S.A. Reddy, K. Madhavi and V.A. Reddy, (2010). Complexes of Cu(II) and Ni(II) with Bis(phenylthiosemicarbazone): Synthesis, spectral, EPR and *in vitro* - antibacterial and antioxidant Activity. *J. Chin. Chem. Soc.* **57**: 677-682.

Rauf, M.K., I. Din, A. Badshah, M. Gielen, D. Ebishara, D. Vos and S. Ahmed, (2009). Synthesis, structural characterization and in vitro cytotoxicity and anti-bacterial activity of some copper(I) complexes with N,N'-disubstituted thioureas. *J. Inorg. Biochem.* 1135-1144.

Sathyaraj, G., T. Weyhermuller and B.U. Nair, (2010). Synthesis, characterization and DNA binding studies of new ruthenium (II) bisterpyridine complexes. *Eur. J. Med. Chem.* **45**: 284-291.

Kong. Res. J. 2(1) : 15-17, 2015 ISSN 2349-2694
Kongunadu Arts and Science College, Coimbatore.

STARCH MEDIATED SYNTHESIS OF HYDROXYAPATITE NANOPARTICLE FOR BIOMEDICAL APPLICATIONS

Indira, J*.
Department of Chemistry, Vellalar College for Women (Autonomous), Erode-12
*E.mail: jayabal.indira@gmail.com

ABSTRACT

Hydroxyapatite (HAP) nanoparticles with uniform morphologies and controllable size have been synthesized by template directed method. The environment and eco-friendly polysaccharide soluble starch is used as a template to regulate size and shape of the nanoparticles synthesized. Structural and morphological properties of as-synthesized hydroxyapatite nanoparticles have been examined through the techniques like Fourier Transform Infrared Spectroscopy (FT-IR), X-ray Diffraction (XRD) and Scanning Electron Microscopy(SEM), respectively. The results indicate that the obtained particles are uniform discrete spherical nanoparticles. The average size of the hydroxyapatite nanoparticles were ranged from 45 to 60 nm.

Keywords: Hydroxyapatite, Ecofriendly, starch, template, biomedical

1. INTRODUCTION

Hydroxyapatite $[Ca_{10}(PO_4)_6(OH)_2]$ is the principal inorganic constituent of bones and teeth (Dorozhkin *et al.*, 2002). It is widely applied in biomedical engineering as bone substitutes, bone cavity filler, bone cement, coating for implant due to its good biocompatibility, excellent ability to form chemical bond with living bone tissue (i.e., bioactivity), and suitable osteoconductivity (Hench *et al.*,1998 and Dorozhkin *et al.*, 2002). Considering the copious application of hydroxyapatite in biomedical field, various synthesis techniques have been developed. These include mechanochemical synthesis (Liou *et al.*, 2003), combustion preparation (Hench *et al.*, 1994), and wet chemistry such as direct precipitation from aqueous solution (Raman *et al.*, 2002), sol–gel procedure (Hwang *et al.*, 1999; Bezzi *et al.*, 2003), and hydrothermal synthesis (Yan *et al.*, 2001). The size and shape of hydroxyapatite alter the applicability of this material in various biomedical fields. In this perspective, controlling the size and shape of HAP nanoparticles is a fascinating area of research in recent trends. Complemently, template assisted synthesis method is proved to be a convenient method to control the size and morphology of nanoparticles (Gopi *et al.*, 2012). Moreover, an increasing awareness towards green chemistry and other biological processes has led to the development of an eco-friendly approach for the synthesis of HAP nanoparticles. The use of environmentally benign and renewable materials like soluble starch offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and biomedical applications. In this outlook, herein we report synthesis of hydroxyapatite nanoparticles using starch as template by green synthesis route. The addition of starch of various concentrations how can alter the purity, crystallinity, size and shape of HAP nanoparticles were also discussed.

2. MATERIALS AND METHODS

Calcium nitrate tetrahydrate ($CaNO_3.4H_2O$) and phosphoric acid (H_3PO_4) are used the precursors for calcium and phosphate respectively. Aqueous ammonia and soluble starch are the other chemicals in analar grade. In a typical experimental procedure, 0.05 M of $CaNO_3.4H_2O$ was mixed with 0.01 wt. % of starch, and the solution was stirred for about 1 h to ensure the co-operative interaction and the self assembly process was completed. At the same time, 0.03 M of H_3PO_4 was added slowly to the above mixture and the stirring was extended to about 16 h. The pH of the solution was maintained at 9 by using aqueous ammonia.

A milky white precipitate was obtained which is kept in an oven for 24 h at a temperature to 80°C. The precipitate is washed with water and ethanol to remove the insoluble precursors and other organic residues. Followed by, the precipitate is dried in an oven for 40°C. Then it was calcined at 600°C for 6 h to remove the starch then sintered at 900°C for 2 h in a muffle furnace to obtain nanohydroxyapatite powders. The experiment is repeated for the other two different concentration of starch (0.03 wt. % and 0.05 wt. %) also to compare the effect of its addition on the purity, crystallinity and morphology of HAP.

To characterize the synthesized nanohydroxyapatite, Fourier transform infrared spectra were recorded using Nicolet 380 FT-IR

spectrophotometer over the range from 4000 – 400 cm^{-1} with a number of scans 32 and resolution 4 cm^{-1}. For this, small amount of HAP powders were blended with KBr and then pressed into discs for the measurement.

The phase composition and the crystallinity of the HAP powders were determined by X-ray diffraction (XRD) Bruker D-8 Advance-Germany Spectrometer, with CuKα radiation λ=1.5406Å generated at 35 kV and 25 mA. Data were collected over the 2θ range 20-60° with a step size of 0.010° and a count time of 0.2s. The morphology of the nano-HAP powders was examined by scanning electron microscopic (JSM 840A Scanning Microscope, JEOL-Japan.) technique.

3. RESULTS AND DISCUSSION

3.1. Fourier Transform Infra red Spectroscopic Studies

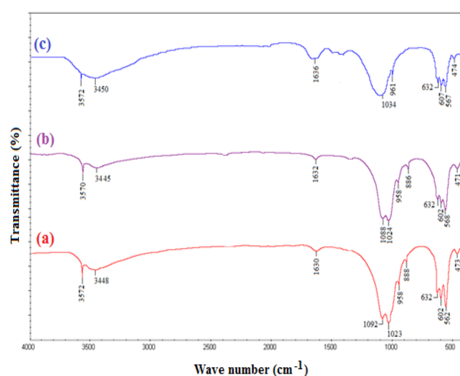


Fig. 1. FTIR spectra of HAP nanoparticles synthesized by template method using starch at three different concentration (a) 0.01 wt.% (b) 0.03 wt.% (c) 0.05 wt. %.

Fig. 1(a-c) shows the FT-IR spectra of HAP nanoparticles synthesized by template method using aqueous starch at three different concentrations. From the fig. 1 (a-c), it is evident that the formation of apatite in all the cases by the observed fundamental vibrational modes of the phosphate peaks at around 1092&1023 cm^{-1} (υ_3), 602 & 562 cm^{-1}(υ_4), 473 cm^{-1} (υ_2) and 958 cm^{-1}(υ_1) respectively. The peaks at 3572 cm^{-1} and 632 cm^{-1} corresponding to the stretching and bending vibration of the hydroxyl (OH^-) group and are considered as the characteristic peaks of stoichimetric hydroxyapatite. Apart from that the peaks observed at around 3448 cm^{-1} and 1630 cm^{-1} is due to the stretching and bending modes of adsorbed water. These findings were well coincide with earlier results (Gopi *et al.*, 2010). There are no impurities formed as evident from the FTIR results.

3.2. X-ray Diffraction Studies

The X-ray diffraction patterns for the HAP nanoparticles synthesized by template method using starch at various concentrations are shown in Fig. 2 (a-c).

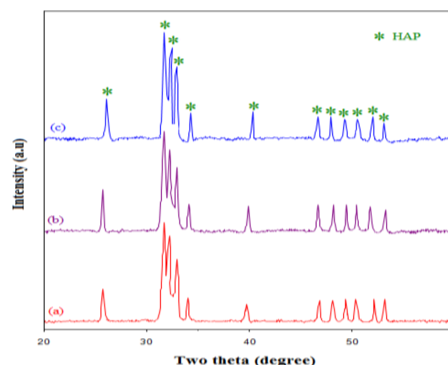


Fig. 2 XRD pattern of HAP nanoparticles synthesized by aqueous starch as template at three different concentrations (a) 0.01 wt.% (b) 0.03 wt.% (c) 0.05 wt.%.

All the peaks in the XRD pattern shown in Fig. 2(a-c) are attributed to stoichimetric HAP which is well consistent with the ICDD No.09-0432 and no other calcium phosphate peaks were detected (Gopi *et al.*, 2012). On increasing the starch concentration, the intensity of the XRD peaks shown in Fig. 2(a-c) seems to be increased thus suggesting the increased crystallinity of the HAP particles.

3.3. Scanning Electron Microscopic Studies

Fig. 3 (a-c) shows the SEM images of HAP nanoparticles synthesized by template directed method using aqueous starch at three different concentrations.

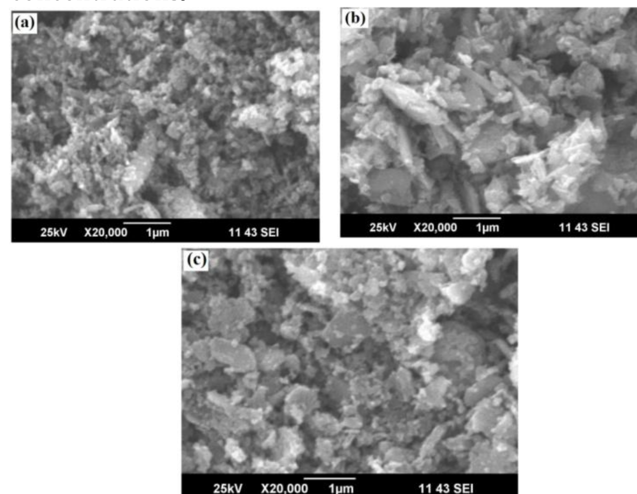


Fig. 3. SEM images of HAP nanoparticles synthesized by template directed aqueous starch method at three different concentrations (a) 0.01 wt.% (b) 0.03 wt.% (c) 0.05 wt.%.

When the concentration of the starch was 0.01 wt. % for the synthesis of HAP, the micrograph shown in Fig. 3(a) depicts nanosized discrete particles with uniform in size distribution without any agglomeration. On increasing t he starch concentration to 0.03 wt. % (Fig. 3(b)) the particles seems feather like shape but the size is somewhat higher than previous. On further increasing of the starch addition to 0.05 wt. % (Fig. 3(c)), the particles is in the nanometer range but unclear discrete manner were seen. Some agglomerated particles are also found in this image. Hence the optimum concentration was found to be 0.01 wt. %.

4. CONCLUSIONS

Hydroxyapatite nanoparticles were successfully synthesized by template directed method using aqueous starch. The FT-IR results have shown that the obtained powders were free from any organic impurities. As the addition of starch is increased, the crystallinity of the particles increased as evident from the XRD. The SEM result evident the formation of discrete spherical nanoparticles with reduced size and uniform throughout was obtained when the starch concentration was lower. The size of the particles was seen in the range around ~60 nm. Hence the developed protocol will be a convenient one as it works without complicated apparatus and chemicals. The HAP nanoparticles synthesized by this method can serve as a good biomaterial for various biomedical applications.

ACKNOWLEDGEMENT

The author acknowledges the financial support from UGC, Hyderabad, India in the form of minor research project.

REFERENCES

Bezzi, G., G. Celotti, E. Landi, T.M.G. La Torretta, I. Sopran and A. Tampieri, (2003). A novel sol-gel technique for hydroxyapatite preparation. *Mater. Chem. Phys*. **78**: 816–824.

Dorozhkin, S.V. and M. Epple, (2002). Biological and medical significance of calcium phosphates. *Angew. Chem., Int. Ed. Engl*. **41**(17): 3130–3146.

Gopi, D., J. Indira, L. Kavitha, M. Sekar and U. Kamachi Mudali, (2012). Synthesis of hydroxyapatite nanoparticles by a novel ultrasonic assisted with mixed hollow sphere template method. *Spectrochimica Acta Part A.***93**:131–134.

Gopi, D., J. Indira, L. Kavitha, S. Kannan and J.M.F. Ferreira, (2010). Spectroscopic characterization of nanohydroxyapatite synthesized by molten salt method. *Spectrochimica Acta Part A.* **77A**(2): 545–547.

Hench, L.L. (1991). Bioceramics - From concept to clinic. *J. Am. Ceram. Soc.* **74**(7): 1487–1510.

Hench, L.L. (1998). Bioceramics. *J. Am. Ceram. Soc.* **81**(7): 1705–1728.

Hwang, K. and Y. Lim, (1999). Chemical and structural changes of hydroxyapatite films by using a sol–gel method. *Surf. Coat. Technol.* **115**: 172 – 175.

Liou, S.C., S.Y. Chen and D.M. Liu, (2003). Synthesis and characterization of nanocomposite with controlled aspect ratios. *Biomaterials* **24**(22): 3981–3988.

Raman, R.E., W.L. Suchanek, K. Byrappa, C.W. Chen, P. Shuk and C.S. Oakes, (2002). Solution synthesis of hydroxyapatite designer particulates. *Solid State Ionics.* **151**(1-4): 393–402.

Yan, L., L.Y.D. Li, Z.X. Deng, J. Zhuang and X.M. Sun, (2001). Surfactant-assisted hydrothermal synthesis of hydroxyapatite nanorods. *Int. J. Inorg. Mater.* **3**: 633–637.

Kong. Res. J. 2(1) : 18-23, 2015 ISSN 2349-2694
Kongunadu Arts and Science College, Coimbatore.

SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL STUDIES ON DIVALENT TRANSITION METAL COMPLEXES OF HYDRAZINE WITH ARYL SUBSTITUTED ACETIC ACIDS

Manimekalai, R*, K. Kalpanadevi, N. Anjutha Prabha and O. Raina
Department of Chemistry, Kongunadu Arts and Science College, Coimbatore, Tamil Nadu, India
*E-mail: manimekalair@ymail.com

ABSTRACT

Metal phenylacetate Sesquihydrazinate hydrates of the formula $M(PhAc)_2.1.5N_2H_4.H_2O$ where M=Co,Ni or Cd and Metal phenoxyacetate Sesquihydrazinate hydrates $M(PhOAc)_2.1.5N_2H_4.H_2O$ Where M=Co, Ni , Cd or Mn have been prepared and characterized by analytical, IR spectral and thermal studies. The composition of complexes have been determined by analytical studies. Infrared spectral data indicate that the bidentate bridging by hydrazine molecules and monodentate coordination by carboxylate ions to the cental metal ion. Thermogravimetry (TG) and differential thermal analyses (DTA) in air have been used to study the thermal behaviour of the complexes. The simultaneous TG-DTA curves of all the complexes in air resultedin the formation of respective metal or metal oxide as final residue. These complexes decompose exothermically either in single step or decompose through respective metal carboxylate intermediates. The antibacterial activity of the prepared complexes screened against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Proteus mirabilis.*

Keywords: Phenylacetate Sesquihydrazinate hydrates, phenoxyacetate Sesquihydrazinate hydrates.

1. INTRODUCTION

Hydrazine is a versatile ligand and forms a wide variety of complexes with various metal ions. The monodentate and bridging bidentate coordination of the hydrazine molecule on complexation has been well documented in the literature (Braibanti *et al.,* 1968). The emerging interest in these hydrazine complexes is mainly due to their structure and thermal behaviour. Hydrazine carboxylates of the transition metal ions with variety of acids have been reported. These include simple aliphatic monocarboxylic acid (Ravindranathan and Patil, 1983; Sivasankar and Govindrajan, 1994a; Sivasankar and Govindrajan, 1995a; Sivasankar and Govindrajan, 1997; Vikram and Sivasanka, 2007) aliphatic dicarboxylic acids (Gajapathy *et al.,* 1983; Sivasankar and Govindrajan, 1994b; Govindrajan *et al.,* 1995; Yasodhai and Govindrajan, 2000; Sivasankar, 2006) aromatic mono and dicarboxylic acids (Kuppusamy and Govindrajan, 1996; Sivasankar, 2006). Most of these formed as mono, bis-hydrazine or hydrazinium corboxylates and few tris-hydrazine carboxylates are known with aliphatic substituted acetic acid (Kuppusamy and Govindrajan, 1995) and other Inorganic anions (Sivasankar and Govindrajan, 1995; Nicholls *et al.,* 1996). Generally all phenolic derivatives show antibacterial property. Particularly 2,4-dichlorophenoxyacetic acid altered envelope properties of the bacteria Escherichia coli, such as hydrophobic index (Blague *et al.,* 2001). This prompted us to make antibacterial study of hydrazinium salt of dichlorophenoxyacetic acid against Escherichia coli.

In this context, we present some new metal hydrazine complexes with aromatic carboxylic acids namely phenylacetic acid and phenoxyacetic acid.

2. EXPERIMENTAL

2.1. Preparation of $M(PhAc)_2.1.5N_2H_4.H_2O$ where M=Co,Ni or Cd

The Cobalt, Nickel and Cadmium complexes were prepared by the addition of an aqueous solution (50 ml) of hydrazine hydrate (0.2 ml, 0.004 m) and phenylacetic acid (0.5 g, 0.0036 m) to the corresponding aqueous solution (50 ml) of metal nitrate hydrates ($Co(NO_3)_2.6H_2O$,0.5 g, 0.0017 m, $Ni(NO_3)_2.6H_2O$,0.5 g, 0.0017 m, $Cd(NO_3)_2.4H_2O$, 0.5 g, 0.0016 m). The mixture was stirred well to get a clear solution. This solution was concentrated on a water bath to 20ml and it was kept for complexation. After 15 minutes complex was formed. It was filtered and washed by using water, alcohol and diethyl ether and air dried.

2.2. Preparation of $Mn(PhAc)_2.1.5N_2H_4.H_2O$

The manganese complex was also prepared by the same procedure with aqueous solution (50 ml) of phenylacetic acid (0.5 g, 0.0036 m), hydrazine

hydrate (0.4 ml, 0.008 m) and aqueous solution of manganese acetate hydrate (0.5 g, 0.002 m).

2.3. Preparation of $M(PhOAc)_2.1.5N_2H_4.H_2O$ Where M=Co, Ni , Cd or Mn

The same procedure was followed with aqueous solution of Phenoxyacetic acid (0.5 g, 0.0032 m), hydrazine hydrate (0.2 ml, 0.004 m) and metal nitrate hydrates (0.5 g, 0.0017 m) (or) Manganese acetate hydrate (0.5 g, 0.002 m).

2.4. Quantitative methods

The hydrazine content in the complexes was determined by titration using KIO_3 as the titrant (Von Burg and Stout, 1991). The percentage of metals in the complexes was estimated by the standard methods given in the Vogel's textbook (Von Burg and Stout, 1991).

2.5. Physico-chemical techniques

2.5.1. Infrared spectrum

The infrared spectrum of the solid precursor sample was recorded by the KBr disc technique using a Perkin Elmer 597/1650 spectrophotometer.

2.5.2. Thermal analysis

The simultaneous TG-DTA experiment was carried out in Shimadzu DT40, Stanton 781 and STA 1500 thermal analyzer. Thermal analysis was carried out in air at the heating rate of 10°C per minute using 5-10 mg of the sample. Platinum cups were used as sample holders and alumina as reference. The temperature range was ambient to 700°C.

2.5.3. Biological assay

The antibacterial activities of the prepared complexes were determined by the disc diffusion method. The bacteria were cultured in nutrient agar medium and used as inoculum for the study. The antibacterial activity of the synthesized compounds of 25μg, 50μg, 100μg and 200μg concentrations were tested against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Proteus mirabilis.* The inhibition zones were calculated and recorded.

3. RESULTS AND DISCUSSION

3.1. Chemical formula determination of the complexes

The chemical formula $[M(PhAc)_2.1.5N_2H_4.H_2O$ (M=Ni, Co, Mn, Cd) and $M(PhOAc)_2.1.5N_2H_4.H_2O$ (M = Co, Ni, Cd, Mn) has been assigned to the prepared complexes, based on the observed and calculated percentage of hydrazine and metals, which are found to match closely with the calculated values (Table 1).

Table 1. Compositional analysis data of the prepared complexes.

Compound	Molecular weight (gm)	Hydrazine (%)		Metal (%)	
		Obsd value	Calcd value	Obsd value	Calcd value
$Co(PhAc)_2.1.5 N_2H_4.H_2O$	397.35	12.25	12.16	14.55	14.92
$Ni(PhAc)_2.1.5 N_2H_4.H_2O$	395.08	12.66	12.17	14.50	14.86
$Cd(PhAc)_2.1.5 N_2H_4.H_2O$	449.31	9.93	10.70	26.2	25.24
$Mn(PhAc)_2.1.5 N_2H_4.H_2O$	391.3	12.25	12.28	14.00	14.03
$Co(PhOAc)_2.1.5 N_2H_4.H_2O$	427.39	10.09	11.25	13.00	13.80
$Ni(PhOAc)_2.1.5 N_2H_4.H_2O$	427.1	10.65	11.25	13.00	13.74
$Cd(PhOAc)_2.1.5 N_2H_4.H_2O$	481.33	9.40	9.99	24.46	23.46
$Mn(PhOAc)_2.1.5N_2H_4.H_2O$	423.32	10.65	11.36	12.00	12.79

PhAc - Phenylacetate

3.1.1. PhOAc –Phenoxyacetate

These are in good agreement with proposed formulae of the complexes.

3.2. FT-IR spectral analysis

The hydrated derivatives displayed a broad band in the region 3625-3282 cm^{-1} due to O-H stretching shows the presence of water molecule. The absorption band in the region 3246-3224 cm^{-1}.This is due to the N-H stretching frequency of N_2H_4. The complexes show asymmetric and symmetric stretching frequencies of COO^- in the region 1610-156cm^{-1} and 1392-1338 cm^{-1} respectively. The $\Delta\gamma(\gamma_{asym}-\gamma_{sym})$ of COO^- in range >222 cm^{-1} confirms the monodendate coordination of carboxylate anion. In the complexes, the N-N stretching is seen in the range 983-943 cm^{-1} confirming the bridging bidentate coordination of hydrazine (Sivasankar and Govindrajan, 1996). The IR spectra of the prepared complexes are displayed in Fig. 1- 8.

Table 2. FT-IR spectral data of the prepared complexes.

Compound	$\gamma_{(O-H)}$	$\gamma_{(N-H)}$	$\gamma_{asy(OCO)}$	$\gamma_{sym(OCO)}$	$\Delta\gamma$	$\gamma_{(N-N)}$
$Co(PhAc)_2.1.5N_2H_4.H_2O$	3294	3226	1606	1382	224	970
$Ni(PhAc)_2.1.5N_2H_4.H_2O$	3292	3224	1604	1382	222	975
$Cd(PhAc)_2.1.5N_2H_4.H_2O$	3303	3246	1610	1384	226	958
$Mn(PhAc)_2.1.5N_2H_4.H_2O$	3625	-	1566	1392	-	943
$Co(PhOAc)_2.1.5N_2H_4.H_2O$	3282	3228	1598	1342	256	948
$Ni(PhOAc)_2.1.5N_2H_4.H_2O$	3282	3244	1585	1344	247	983
$Cd(PhOAc)_2.1.5N_2H_4.H_2O$	3326	3242	1608	1346	262	968
$Mn(PhOAc)_2.1.5N_2H_4.H_2O$	3330	3240	1583	1338	245	964

$\Delta\gamma = (\gamma_{asy(OCO)} - \gamma_{sym(OCO)})$

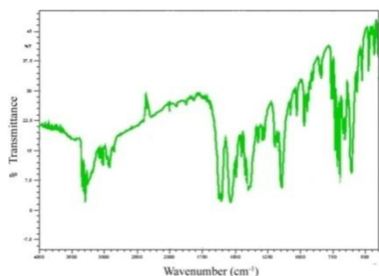


Fig. 1. Infrared spectrum of $Co(PhAc)_2.1.5N_2H_4.H_2O$.

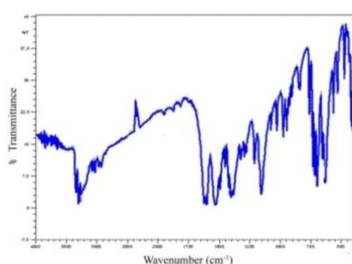


Fig. 2. Infrared spectrum of $Ni(PhAc)_2.1.5N_2H_4.H_2O$.

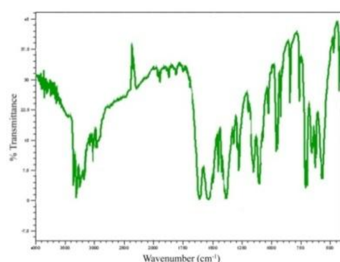


Fig. 3. Infrared spectrum of $Cd(PhAc)_2.1.5N_2H_4.H_2O$

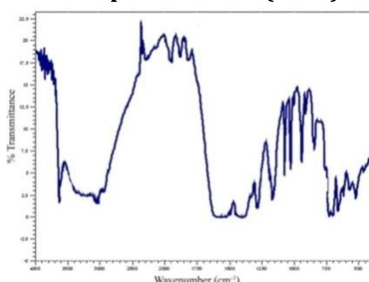


Fig. 4. Infrared spectrum of $Mn(PhAc)_2.1.5N_2H_4.H_2O$

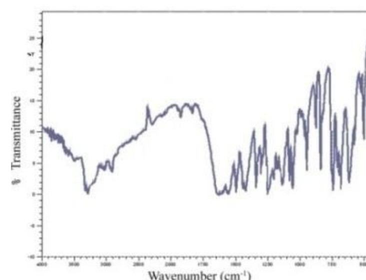


Fig. 5. Infrared spectrum of $Co(PhOAc)_2.1.5N_2H_4.H_2O$

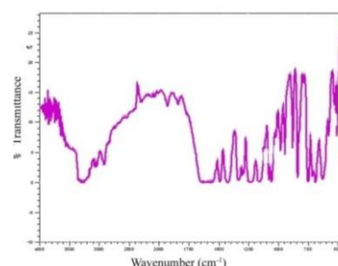


Fig. 6. Infrared spectrum of $Ni(PhOAc)_2.1.5N_2H_4.H_2O$

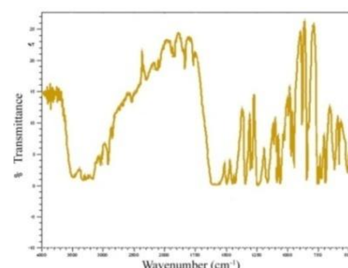


Fig.7. Infrared spectrum of $Cd(PhOAc)_2.1.5N_2H_4.H_2O$

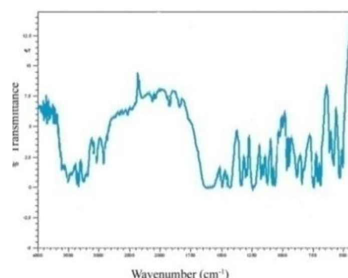


Fig. 8. Infrared spectrum of $Mn(PhOAc)_2.1.5N_2H_4.H_2O$

Table 3. The thermal decomposition pattern of some complexes are given.

Compound	DTA -Peak Temp/ ^0C	TG - Temp Rang/ ^0C	Mass Loss %		Decomposition product
			Found	Calcd	
$Co(PhAc)_2.1.5N_2H_4.H_2O$	220(+)	175-245	14.55	16.71	$Co(PhAc)_2$
	440(+)	245-500	80.92	81.03	CoO
	140(-)	105-160	3.6	3.7	$Cd(PhOAc)_2.1.5N_2H_4$
$Cd(PhOAc)_2.1.5N_2H_4.H_2O$	205(-)	170-230	12	13.72	$Cd(PhOAc)_2$
	520(+)	400-580	73.46	73.21	CdO
$Ni(PhAc)_2.1.5N_2H_4.H_2O$	230(+)	195-250	16.37	16.72	$Ni(PhAc)_2$
	465(+)	370-480	83.32	81.08	NiO

Exothermic - (+)　　Endothermic - (-)

3.3. Thermal analysis

3.4. $Co(PhAc)_2.1.5N_2H_4.H_2O$

This complex undergoes two step decomposition. The TG curve shows 14% mass loss in the temperature range 175-245°C which coincides with the calculated mass loss for the formation of $Co(PhAc)_2$ with loss of hydrazine and water molecule. The higher temperature decomposition indicates that coordinated water molecule. This intermediate further decomposes in the temperature range 245-500°C to give CoO,as the final product. DTA shows exotherm corresponding to the above two stages at 220 and 440°C, respectively. The TG-DTA pattern of this complex is given in Fig.9.

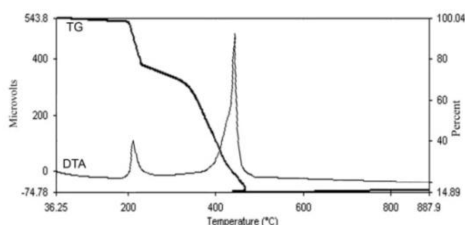


Fig. 9. $Co(PhAc)_2.1.5N_2H_4.H_2O$

3.5. $Cd(PhOAc)_2.1.5N_2H_4.H_2O$

This DTA curves reveal three peaks corresponding to three step decomposition of the complex as shown by TG. The first endothermic peak at 140°C is assigned to the loss of molecule of water. The second step corresponds to the decomposition of the intermediate, $Cd(PhOAc)_2.1.5N_2H_4$ to yield $Cd(PhOAc)_2$ which is observed as an endotherm at 205°C, in DTA. The $Cd(PhOAc)_2$ further decomposes exothermically at 520°C in the final step to form CdO as the end product. The thermogram of the complex is given in Fig. 10.

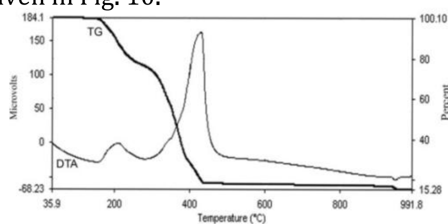


Fig. 10. $Cd(PhOAc)_2.1.5N_2H_4.H_2O$

3.6. $Ni(PhAc)_2.1.5N_2H_4.H_2O$

This complex undergoes two step decomposition. The TG curve shows 16% mass loss in the temperature range 195-250°C which coincides with the calculated mass loss for the formation of $Ni(PhAc)_2$ as an intermediate. This intermediate further decomposes in the temperature range 370-480°C to give NiO, as the final product. DTA shows exotherm corresponding to the above two stages at 230 and 465°C, respectively. The TG-DTA pattern of this complex is given in Fig. 11.

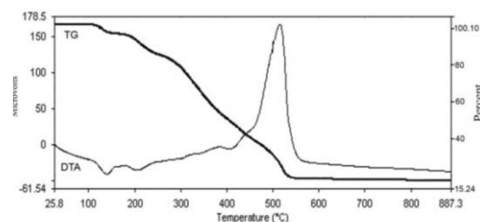


Fig. 11. $Ni(PhAc)_2.1.5N_2H_4.H_2O$

3.7. Coordination geometry

The analytical and physico chemical studies suggest that, in these complexes ,the hydrazine molecules are present as a bidentate bridging ligand. The phenylacetate and phenoxyacetate ions are seen to present as a monodentate ligand (Figs. 12 and 13) as evidented from IR spectra. From TG-DTA analysis we confirmed that water molecule is present as a coordinated one. The complexes are isolated only as a polycrystalline powders. Hence, without crystal structure, it is very difficult to predict the environment of the metal in the complexes. Six-coordination has been tentatively proposed for all the complexes with octahedral stereochemistry. The insoluble nature of these complexes conforms to the polymeric structure.

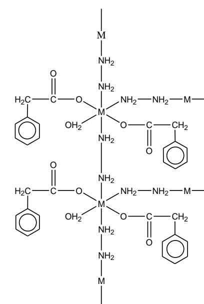


Fig. 12. Polymeric structure $M(PhAc)_2.1.5N_2H_4.H_2O$
where M = Co, Ni, Cd and Mn

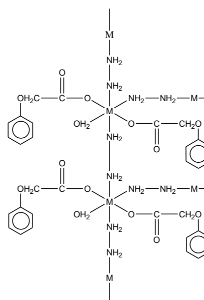


Fig. 13. Polymeric structure $M(PhOAc)_2.1.5N_2H_4.H_2O$
where M = Co, Ni, Cd and Mn

3.7. Antibacterial studies

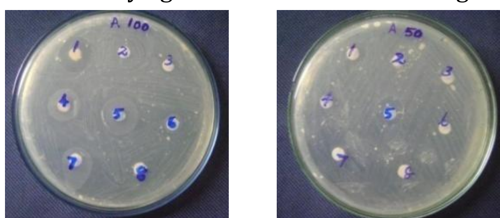
The complexes of phenylacetic and phenoxyacetic acid have been screened to evaluate their antibacterial activities against (A) *Staphylococcus aureus,* (B) *Escherichia coli,* (C) *Pseudomonas aeruginosa,* (D) *Proteus mirabilis,* respectively at two different concentrations (Fig. 14). The radius of the zone of inhibition was measured in millimeter. *Cefepimetazobactum* were used as a standard control and is tabulated as follows.

Table 4. Antibacterial activities of the complexes are given. (the test solutions were prepared in dil HCl).

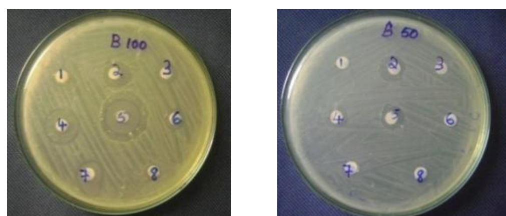
S. No	Compound	Diameter of inhibition zone (mm)							
		S. aureus		*E.coli*		*P. aeruginosa*		*P. mirabilis*	
		50 %	100 %	50 %	100 %	50 %	100 %	50 %	100 %
1	$Co(PhAc)_2.1.5N_2H_4.H_2O$	10	18	-	-	-	18	-	17
2	$Ni(PhAc)_2.1.5N_2H_4.H_2O$	-	-	12	12	-	-	-	-
3	$Cd(PhAc)_2.1.5N_2H_4.H_2O$	-	-	-	-	-	-	-	12
4	$Mn(PhAc)_2.1.5N_2H_4.H_2O$	-	15	-	15	-	-	-	-
5	$Co(PhOAc)_2.1.5N_2H_4.H_2O$	-	17	8	18	-	20	-	14
6	$Ni(PhOAc)_2.1.5N_2H_4.H_2O$	-	-	-	-	-	8	-	10
7	$Cd(PhOAc)_2.1.5N_2H_4.H_2O$	-	7	-	8	-	6	12	12
8	$Mn(PhOAc)_2.1.5N_2H_4.H_2O$	-	-	-	-	-	-	-	-
9	*Cefepimetazobactum*		24		24		20		20

(9) – standard (-) – no activity

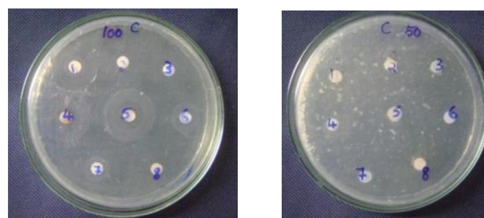
From the result, it has been observed that there is a concentration dependent percentage inhibition in the tested compounds. The activities of the complexes have been compared with the activity of standard antibiotics (*Cefepimetazobactum*) and it has been found that all the complexes showed good activities but compound $Mn(PhOAc)_2.l.5N_2H_4.H_2O$ has no activity against all the four microorganisms.



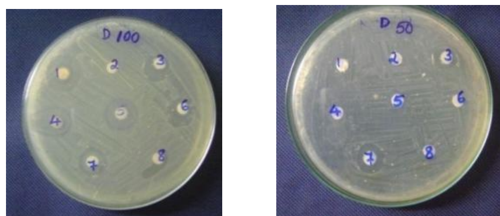
A – *Staphylococcus aureus*



B – *Escherichia coli*



C – *Pseudomonas aeruginosa*



D – *Proteus mirabilis*

D – *Proteus mirabilis*

9 – *Cefepimetazobactum* (Standard)

Fig. 14. Screening for the antibacterial activity at 100% and 50% concentration

From the Table 3.4, it can be seen that the compounds $Co(PhoAc)_2.1.5N_2H_4.H_2O$ showed remarkable activity against *Pseudomonas aeruginosa,* and *Escherichia coli.* Compound $Co(PhAc)_2.1.5N_2H_4.H_2O$ showed remarkable activity against *Pseudomonas aeruginosa*, and *Staphylococcus aureus*.

The results suggest that the antibacterial activity of complexes prepared from both phenylacetic acid phenoxyacetic acid are almost same. This may be due to structural relaitivity between them.

4. CONCLUSION

Transition metal hydrazine complexes of the type $M(PhAc)_2.l.5N_2H_4.H_2O$ where M = Co, Ni, Cd (or) Mn, $M(PhOAc)_2.1.5N_2H_4.H_2O$ where M = Co, Ni, Cd (or) Mn were prepared in aqueous medium by using respective metal nitrate hydrate or metal acetate hydrate, hydrazine hydrate and phenylacetic acid or phenoxyacetic acid. Among the hydrazine derivatives, sesquihydrazinates are unique complexes, and also it is appropriate to mention here that these types of complexes are separated only with these acids except cadmium oxalate sesquihydrazinate[126].

The prepared complexes were characterized by IR and thermogravimetric analysis. The antibacterial

activity of the complexes against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Proteus mirabilis* were also carried out.

The IR spectral data indicates that the binding of hydrazine to a metal ion is a bidentate fashion. Carboxylate ligands are monodentatively coordinated to the central metal ion. The broad peak around 3625-3282 cm^{-1} indicates the presence of water molecule. The prepared complexes undergo two or three step decomposition to form metal oxide as the final product. The higher temperature dehydration indicates that the presence of water molecule as coordinated one.

The antibacterial screening shows that $Co(PhOAc)_2.l.5N_2H_4.H_2O$, $Cd(PhOAc)_2.l.5N_2H_4.H_2O$ are active against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Proteus mirabilis.* $Co(PhAc)_2.l.5N_2H_4.H_2O$ active against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Proteus mirabilis.* $Ni(PhOAc)_2.l.5N_2H_4.H_2O$ active against *Pseudomonas aeruginosa* and *Proteus mirabilis.* $Mn(PhAc)_2.1.5N_2H_4.H_2O$ active against *Staphylococcus aureus,* and *Escherichia coli.* $Ni(PhAc)_2.l.5N_2H_4.H_2O$ and $Cd(PhAc)_2.l.5N_2H_4.H_2O$ shows active against only *Escherichia coli* and *Proteus mirabilis* respectively. $Mn(PhOAc)_2.l.5N_2H_4.H_2O$ does not show any antibacterial activity.

REFERENCES

Balague, C., N. Stirtz, R. Duffard and A.M. Evangelista de Duffard, (2001). Effect of 2, 4-dichlorophenoxyacetic acid herbicide in Escherichia Coli growth chemical composition and cellular envelope. *Environ. Toxicol*. **16**: 43-53.

Braibanti, A., F. Dallavalle, M.A. Pellinghelli and E. Laporti, (1968). The nitrogen-nitrogen stretching band in hydrazine derivatives and complexes. *Inorg. Chem.* **7**: 1430-1433.

Gajapathy, D., S. Govindrajan, K.C. Patil and H. Monohar, (1983). Synthesis, characterisation and thermal properties of Hydrazinium metal oxalate hydrates. Crystal and molecular structure of hydrazinium Copper oxalate monohydrate. *Polyhedron* **2**: 865-873.

Govindrajan, S., S.U. Nasrin Banu, N. Saravanan and B.N. Sivasankar, (1995). Bis-Hydrazine metal maleates and fumarates: Preparation, spectral and thermal studies. *Proc. Indian. Acad. Sci. (Chem. Sci.)* **107**: 559-567.

Kuppusamy, K. and S. Govindrajan, (1995). Hydrazinium cation as a ligand: Preparation and spectral, thermal and XRD studies on hydrazinium metal phthalates. *Eur. J. Solid state Inorg. Chem.* **32**: 997-1005.

Kuppusamy, K. and S. Govindrajan, (1996). Benzoate complexes of di positive first row transition metal ions with hydrazine. *Synth. React. Inorg. Met. Org. Chem.* **26**: 225-231.

Nicholls, D., M. Rowley and R. Swindells, (1996). Hydrazine complexes of Cobalt(II) chloride. *J. Chem. Soc.* **5**: 950-953.

Ravindranathan, P. and K.C. Patil, (1983). Thermal reactivity of metal acetate hydrazinates. *Thermochim. Acta.* **71**: 153-160.

Sivasankar, B.N. (2006). Cobalt(II), Nickel(II) and Zinc(II) dicarboxylate complexes with hydrazine as bridged ligand characterization and thermal degradation. *J. Therm. Anal. Calorim.* **86**: 385–392

Sivasankar, B.N. and S. Govindrajan, (1994b). Bis-Hydrazine metal maleates and fumarates: Preparation, spectral and thermal studies. *Z. Naturforsch*. **49**: 950-956.

Sivasankar, B.N. and S. Govindrajan, (1994a). Studies on bis(hydrazine) metal malonates and succinates. *Synth. React. Inorg. Met. Org. Chem.* **24**: 1573-1580.

Sivasankar, B.N. and S. Govindrajan, (1995a). Formato complexes of Cobalt(II), Nickel(II) and Zinc(II) with the hydrazinium(1+) Cation. *Synth. React. Inorg. Met. Org. Chem.* **25**: 31-37.

Sivasankar, B.N. and S. Govindrajan, (1995b). Tris-hydrazine metal glycinates and glycolates: Preparation, spectral and thermal studies. *Thermochim. Acta.* **244**: 235-242.

Sivasankar, B.N. and S. Govindrajan, (1996). Hydrazine mixed metal malonates-New preursors for metal cobaltites. *Mater. Res. Bull.* **31:** 47-53.

Sivasankar, B.N. and S. Govindrajan, (1997). Actate and malonate complexes of Cobalt(II), Nickel(II) and Zinc(II) with hydrazinium cation. *J. Therm. Anal.* **48**: 1401-1408.

Vikram, L. and B.N. Sivasankar, (2007). Spectral, thermal and X-ray studies on some new bis-hydrazine metal glyoxylates and bis- hydrazine mixed metal glyoxylates, *Thermochim. Acta.* **452**: 20-28.

Von Burg, R. and T. Stout, (1991). Toxicology Update: Hydrazine. *J. Appl. Toxicol.* **11**: 447-456.

Yasodhai, S. and S. Govindrajan, (2000). Hydrazinium oxydiacetates and oxydiacetatedianion complexes of some divalent metals with hydrazine. *Synth. React. Inorg. Met. Org. Chem.* **30**: 745-752.

Kong. Res. J. 2(1) : 24-28-, 2015 ISSN 2349-2694
Kongunadu Arts and Science College, Coimbatore.

SYNTHESIS, CHARACTERIZATION, DNA BINDING AND ANTIBACTERIAL ACTIVITY OF RUTHENIUM(III) COMPLEX CONTAINING MIXED LIGANDS

Sampath[1] K. and C. Jayabalakrishnan[2]*
[1]Department of Chemistry, Kumaraguru College of Technology, Coimbatore – 641 006, Tamil Nadu, India.
[2]P.G & Research Department of Chemistry, Sri Ramakrishna Mission Vidyalaya College of Arts and Science, Coimbatore-641020.
*Email-drcjbstar@gmail.com

ABSTRACT

Ruthenium(III) complex, $[RuBr_2(AsPh_3)_2L]$ (where L = (E)-2-(2-chlorobenzylidene)-N-methylhydrazinecarbothioamide) have been synthesized. Structural features of the complex were determined by various physico-chemical and spectral techniques. DNA binding of the complex was investigated by absorption spectroscopy which indicated that the complex bind to DNA *via* intercalation and this complex bind strongly than ligand. The complex has shown significant growth inhibition activity against a panel of bacteria which indicating the pharmacological significance of the ruthenium(III) complex.

Keywords: Ruthenium(III) complexes, Thiosemicarbazones, DNA binding, Cytotoxicity.

1. INTRODUCTION

Though possible remedial measures are available at present to tackle any disease, continuous search in trying to find better and more effective drugs is on the increase. This is particularly true in the case of cancer where cisplatin and its analogs such as carboplatin, and oxaliplatin during chemotherapy result in drawbacks, including intrinsic or acquired resistance, and toxicity. So, the efforts to mitigate the drawbacks have prompted chemists to synthesize a variety of analogs, which is valuable not only for providing better drugs but also for offering useful tools in the study of the molecular mechanisms underlying tumor development. In this connection, ruthenium based complexes have shown enormous impact. Though a wide range of ruthenium complexes have been described in the literature, only a few of them show outstanding anticancer activity (Yan *et al.,* 2005; Ang and Dyson, 2006; Ronconi and Sadler, 2007) and two of them, for instance NAMI-A and KP1019, are currently involved in clinical trials (Rademaker-Lakhai *et al.,* 2004; Hartinger *et al.,* 2006; Kostova, 2006). A combination of such ruthenium with the potent cytotoxic thiosemicarbazone pharmacophore has been shown to produce synergistic effects on the antiproliferative activity of the parent steroidal ligands (Murugkar *et al.,* 1999). The above discussions have inspired us to synthesize a new ruthenium complex containing thiosemicarbazone ligand and triphenylarsine, and bromine as ancillary ligands. The biological properties of the complex were investigated by interaction with DNA. DNA is considered to be one of the major pharmacological targets of anticancer drugs because metal complexes exert their anticancer effects through binding to DNA, thereby changing the replication of DNA and inhibiting the growth of the tumor cells (Pyle *et al.,* 1990; Friedaman *et al.,* 1991). So the objective of the present work is to understand in detail the interaction of the new complexes with DNA. Further, the *in vitro* cytotoxicity of the synthesized complex was also been determined.

2. EXPERIMENTAL

2.1. Materials and methods

All the chemicals used were chemically pure and AR grade. Solvents were purified and dried according to the standard procedure (Vogel, 1989). Calf-thymus (CT-DNA) was purchased from Bangalore Genei, Bangalore, India. The metal precursor, $[RuBr_3(AsPh_3)_3]$ and the ligand were prepared by literature method (Natarajan *et al.,* 1977; Sampath *et al.,* 2013). Micro analyses (C, H, N & S) were performed on a Vario EL III CHNS analyser at STIC, Cochin University of Science and Technology, Kerala, India. IR spectra were recorded as KBr pellets in the 400-4000 cm^{-1} region using a Perkin Elmer FT-IR 8000 spectrophotometer. Electronic spectra were recorded in DMSO solution with a Systronics double beam UV-vis spectrophotometer 2202 in the range 200-800 nm. Magnetic susceptibility measurements of the complexes were recorded using Guoy balance. EPR

spectra were recorded on a varian E-112 ESR spectrophotometer at X- band microwave frequencies for powdered samples at room temperature. EI mass spectra of the complexes were recorded on a JEOL GCMATE II mass spectrometer. Melting points were recorded with Veego VMP-DS heating table and are uncorrected.

2.2. Synthesis of ruthenium(iii) thiosemicarbazone complex, [rubr₂(asph₃)₂l]

A methanolic solution (20 mL) containing thiosemicarbazone ligand (0.110 g, 0.5 mmol) was added to $[RuBr_3(AsPh_3)_3]$ (0.630g, 0.5 mmol) in benzene (20 mL). The resulting solution was refluxed for 8 h. The reaction mixture was then cooled to room temperature, which results in the formation of precipitate. It was filtered off and the purity of the complex was checked by TLC. This solid was recrystallized from CH_2Cl_2/Hexane mixture. Our sincere effort to obtain single crystal of the complex went unsuccessful. Yield: 57 %. M.P: 268 °C. Anal. calcd. for $C_{45}H_{39}As_2Br_2ClN_3RuS$ (%): C, 49.13; H, 3.57; N, 3.82; S, 2.91. Found (%): C, 49.41; H, 3.88; N, 4.15; S, 2.17. EI-MS: Found m/z = 1100.18 (M^+) (calculated m/z = 1100.05 for M^+). IR (KBr, cm^{-1}): 1571 ν(C=N); 750 ν(C–S); 548 ν(Ru–N); 1439 ν(Ru–triphenylarsine). UV-vis (DMSO), λmax (nm): 315, 365 (ILCT), 415 (LMCT). EPR (300 K, 'g' value): 2.14. μ_{eff} (300 K): 1.65 μ_B.

2.3. DNA binding - Titration experiments

All the experiments involving the binding of complex with CT-DNA were carried out in a doubly distilled water buffer with tris(hydroxymethyl)-aminomethane (Tris, 5 mM) and sodium chloride (50 mM) and adjusted to pH 7.2 with hydrochloric acid. A solution of CT-DNA in the buffer gave a ratio of UV absorbance of about 1.8-1.9 at 260 and 280 nm, indicating that the DNA was sufficiently free of protein. The CT-DNA concentration per nucleotide was determined spectrophotometrically by employing an extinction coefficient of 6600 M^{-1} cm^{-1} at 260 nm. The complex was dissolved in a mixed solvent of 5 % DMSO and 95 % Tris-HCl buffer. Stock solution was stored at 4 °C and used within 4 days. Absorption titration experiments were performed with fixed concentrations of the complex (25 μM) with varying concentration of DNA (0-50 μM). While measuring the absorption spectra, an equal amount of DNA was added to both the test solution and the reference solution to eliminate the absorbance of DNA itself.

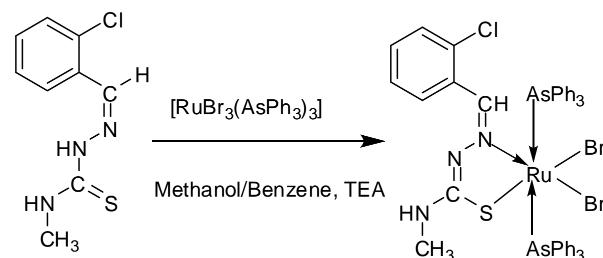
2.4. Cytotoxic study

2.4.1. Antibacterial activity

The *in vitro* antibacterial screenings of the complex was tested for their effect on certain human pathogenic bacteria by disc diffusion method. The complex was stored dry at room temperature and dissolved in dimethyl sulfoxide. The bacteria (*Escherichia coli* and *Staphylococcus aureus*) was grown in nutrient agar medium and incubated at 37 ºC for 24 h followed by frequent subculture to fresh medium, and was used as test bacteria. Then the petriplates were inoculated with a loop full of bacterial culture and spread throughout the petriplates uniformly with a sterile glass spreader. To each disc the test sample and reference antiobiotic (*Cotrimazole*) were added with a sterile micropipette. The plates were then incubated at 35 ºC for 24 h and at 27 ºC for bacteria. Plates with disc containing respective solvents served as control. Inhibition was recorded by measuring the diameter of the inhibitory zone after the period of incubation.

4. RESULTS AND DISCUSSION

Analytical and spectroscopic data for the complex indicate a 1:1 metal-ligand stoichiometry for the complex. The synthetic route of the complex and the proposed structure of the complex are shown in Scheme 1. The complex is soluble in most common organic solvents like CH_2Cl_2, $CHCl_3$, DMF, DMSO, etc.



Scheme 1- Synthetic route of the ruthenium(III) thiosemicarbazone complex

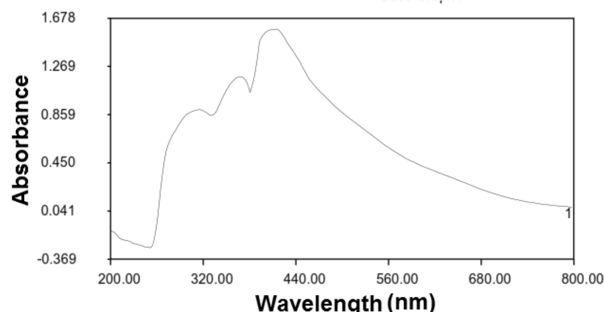
4.1. Infrared spectra

The complex shows absorption at 1571 cm^{-1} assigned to azomethine C=N which is lower wavelength compared to ligand (1614 cm^{-1}). This is due to the interaction of ligand with metal ion and confirms the coordination through N atom (Raja and Ramesh, 2010). A band at 851 cm^{-1} for the ligand due to the vibration of C=S double bond was disappeared in the spectrum of the complex and a new band, C–S appeared at 750 cm^{-1} indicating that the other coordination is through thiolate sulphur after enolization followed by deprotonation on sulphur (Thangadurai and Natarajan, 2001). The complex

shows bands at 548 cm^{-1} is attributed to Ru–N (Manivannam *et al.,* 2007). Moreover, the characteristic absorption band due to triphenylarsine was also observed for all the complexes at 1439 cm^{-1}.

4.2. Electronic spectra

The electronic spectrum of the complex (Fig. 1) was recorded in DMSO. The intense bands were observed at 315 and 365 nm are characterized as ligand centered transitions, π-π* and n-π* transitions, occurring within the ligand orbitals (Sharma *et al.,* 1980). The ground state of ruthenium(III) (t^5_{2g} configuration) is ^2T_2g, while the first excited doublet levels in the order of increasing energy are ^2A_2g and ^2T_1g, which arise from t_{2g}^4 e_g^1 configuration. Since in a d^5 system, and especially in ruthenium(III) that has relatively high oxidising properties, the charge transfer bands of the type $L_{\pi y}\rightarrow t_{2g}$ are prominent in the low energy region obscuring the weaker bands due to d-d transition. Therefore, it becomes difficult to assign conclusively the bands of ruthenium(III) complexes appearing in the visible region. Hence, the band that appear at 415 nm have been assigned to charge transfer transitions, which are in conformity with the assignments made for similar ruthenium(III) complexes (Raja and Ramesh, 2010; Sampath *et al.,* 2013).



ig. 1. UV-visible spectrum of the complex.

4.3. Magnetic moment and EPR spectra

The room temperature magnetic susceptibility measurements of the ruthenium(III) complex shows that it is paramagnetic. The magnetic moment value, μ_B = 1.65 corresponds to single unpaired electron in a low-spin $4d^5$ configuration and confirms that ruthenium is in +3 oxidation state in the complex (Manivannan *et al.,* 2007).

The complexes is uniformly paramagnetic with magnetic moments corresponding to one unpaired electron at room temperature (low-spin Ru(III), t_{2g}^5). The X-band EPR spectrum of powdered sample of the complex was recorded at room temperature and the EPR spectrum of the complex is shown in Fig. 2. The nature of the spectrum revealed the absence of any hyperfine splitting due to interactions with any other nuclei present in the complex. The complex exhibits a single isotropic resonance with *g* values at 2.14. Although the complex have some distortion in their octahedral geometry, the observation of isotropic line in the EPR spectrum may be due to the occupancy of the unpaired electron in a degenerate orbital (Khan *et al.,* 1990). The nature of spectrum obtained is in good agreement with that of previously reported ruthenium(III) complexes (Khan *et al.,* 1990; Sampath *et al.,* 2013).

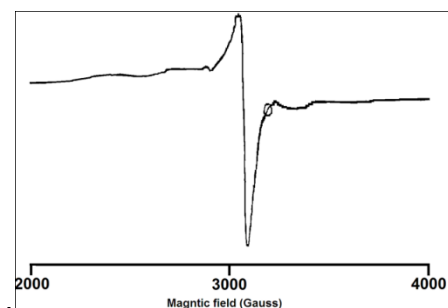


Fig. 2. ESR spectrum of the complex.

4.4. Mass spectral analysis

The mass spectrum of the ruthenium(III) complex is in good agreement with the proposed molecular structure. The molecular ion peak, [M^+] appears at m/z = 1100.48 confirm the stoichiometry of the complex.

4.5. DNA binding – Titration experiments

In the present investigation the binding of the complex has been characterized classically through absorption spectral titrations by following the changes in absorbance and shift in the wavelength as a function of added concentration of DNA. The results of absorption spectra of the complex in the absence and presence of CT-DNA are given in Fig. 3. As shown in Fig. 3, when titrated with CT-DNA the absorption bands of the complex exhibited hypochromism of 17.55 % with red shifts of 2 nm at 358 nm. This result suggested an intercalate binding of complex with DNA helix. After the complex intercalate to the base pairs of DNA, the π* orbital of the intercalated complexes could couple with π orbitals of the base pairs, thus decreasing the π → π* transition energies, hence resulting in hypochromism (Raja *et al.,* 2011). In order to compare quantitatively the binding strength of the complex, the intrinsic binding constants (K_b) of them with CT-DNA was determined from the following equation.

$$[DNA]/(\varepsilon_a - \varepsilon_f) = [DNA]/(\varepsilon_b - \varepsilon_f) + 1/K_b (\varepsilon_b - \varepsilon_f)$$

where [DNA] is the concentration of DNA in base pairs and the apparent absorption coefficient ε_a, ε_f and ε_b correspond to A_{obs}/[complex], the extinction coefficient of the free complex and the extinction coefficient of the complex when fully bound to DNA, respectively. The plot of $[DNA]/(\varepsilon_a - \varepsilon_f)$ *versus* [DNA] gave a slope and the intercept which are equal to $1/(\varepsilon_b - \varepsilon_f)$ and $1/K_b (\varepsilon_b - \varepsilon_f)$, respectively; K_b is the ratio of the slope to the intercept. The magnitudes of intrinsic binding constant (K_b) was calculated to be 2.5 X 10^4 M^{-1}. The observed value of K_b revealed that the ruthenium(III) complex bind strongly than the ligand (1.7 X 10^4 M^{-1}) to DNA *via* intercalative mode.

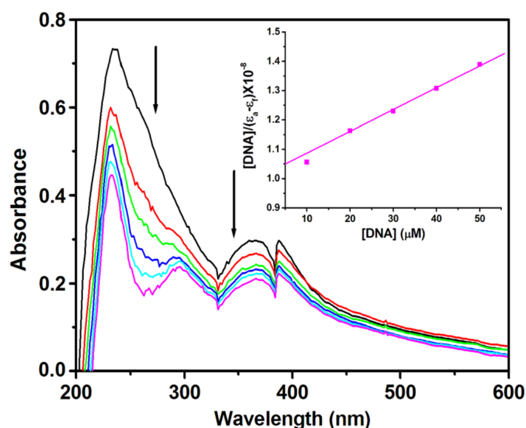


Fig. 3. Electronic spectra of complex in Tris-HCl buffer upon addition of CT-DNA. [Complex] = 25 μM, [DNA] = 0-50 μM. Arrow shows the absorption intensities decrease upon increasing DNA concentrations (Inset: Plot between [DNA] and [DNA]/ [ε_a-ε_f] X 10^{-8}).

4.6. Cytotoxic activity

4.6.1. Antibacterial activity

The antibacterial activity of the complex was tested against the human pathogens (*Escherichia coli* and *Staphylococcus aureus*). The screening data are reported in Table 1. The inhibition activity of ruthenium complex against the bacteria suggesting that chelation facilitates the ability of a complex to cross a cell membrane which leads alteration in the cell permeability and subsequent injury to the cell membrane (Perez *et al.,* 1990; Leelavathy *et al.,* 2009). Moreover, the mode of action of the complex may involve the hydrogen bond through >C=N group with active centers of all cell constituents resulting in interference with normal cell process (Thangadurai and Natarajan, 2001). Even though the complex possess significant activity they could not reach the effectiveness of standard drug *Cotrimazole*.

Table 1. Antibacterial activity of the ligands and complexes.

Complex	Diameter of inhibition zone (mm)[a]	
	E. coli	***S. aureus***
$[RuBr_2(AsPh_3)_2L]$	17	19
Ciprofloxacine	23	26
DMSO	No activity	

[a]Values are an average of triplicate runs.

5. CONCLUSION

The present contribution describes the synthesis of new ruthenium(III) complex comprising mixture of ligands and, were characterized by various spectroscopic techniques. An octahedral geometry has been tentatively assigned for the complex. The DNA binding ability of the complex assessed by absorption spectroscopy suggested an intercalative mode of binding with binding constant 2.5 X 10^5 M^{-1}. The complex possesses significant antibacterial activity against panel of bacteria. At this juncture, it is notable to mention that the major chemical and biological findings of this study throw some light on the potential of this complex in a reasonable range of concentrations under *in vitro* conditions.

REFERENCES

Ang, W.H. and P.J. Dyson. (2006). *Eur. J. Inorg. Chem.* 4003-4018.

Friedman, A.E., C.V. Kumar, N.J. Turro and J.K. Barton. (1991). *Nucleic Acids Res.* **19**: 2595–2602.

Hartinger, C.G. S. Zorbas-Seifried, M.A. Jakupec, B. Kynast, H. Zorbas and B.K. Keppler. (2006). *Inorg. Biochem.* **100**: 891-904.

Khan, M.M.T., D. Srinivas, R.I. Kureshy and N.H. Khan. (1990). *Inorg. Chem.* **29**: 2320-2326.

Kostova, I. (2006). *Curr. Med. Chem.* **13**: 1085-1107.

Leelavathy, L., S. Anbu, M. Kandaswamy, N. Karthikeyan and N. Mohan. (2009). *Polyhedron* **28**: 903-910.

Manivannan, S., R. Prabhakaran, K.P. Balasubramanian, V. Dhanabal, R. Karvembu, V. Chinnusamy and K. Natarajan. (2007). *Appl. Organometal. Chem.* **21**: 952-957.

Murugkar A., B. Unnikrishnan, S. Padhye, R. Bhonde, S. Teat, E. Triantafillou and E. Sinn. (1999). *Met. Based Drugs* **6**: 177–182.

Natarajan, K., R.K. Poddar and U. Agarwala. (1977). *J. Inorg. Nucl. Chem.* **39**: 431-435.

Perez, Z., M. Pauli and P. Bezerque. (1990). *Acta Biol. Med. Exp.* **15**: 113-115.

Pyle, A.M., T. Morii and J.K. Barton. (1990). *J. Am. Chem. Soc.* **112**: 9432–9434.

Rademaker-Lakhai, J.M. D. Van Den Bongard, D. Pluim, J.H. Beijnen and J.H.M. Schellens. (2004). *Clin. Cancer Res.* **10**: 3717-3727.

Raja, D.S., N.S.P. Bhuvanesh and K. Natarajan. (2011). *Eur. J. Med. Chem.* **46**: 4584-4594.

Raja, N. and R. Ramesh. (2010). *Spectrochim. Acta A* **75**: 713-718.

Ronconi, L. and P.J. Sadler. (2007). *Coord. Chem. Rev.* **25**: 1633-1648.

Sampath, K., S. Sathiyaraj and C. Jayabalakrishnan. (2013). *J. Mol. Struct.* **1046**: 82-91.

Sharma, R.K., R.V. Singh and J.P.J. Tandon. (1980). *Inorg. Nucl. Chem.* **42**: 1382-1384.

Thangadurai, T.D. and K. Natarajan. (2001). *Transition Met. Chem.* **26**: 717-722.

Vogel, A.I. (1989). *Textbook of Practical Organic Chemistry.* Longman, London.

Yan, Y.K., M. Melchart, A. Habtemariam and P.J. Sadler. (2005). *Chem. Commun.* 4764-4776.

Kong. Res. J. 2(1) : 29-31, 2015 ISSN 2349-2694
Kongunadu Arts and Science College, Coimbatore.

SYNTHESIS OF ISOXAZOLO AND PYRAZOLINO ANNELATED CARBAZOLES FROM 2-(3'-(2'-CHLORO) QUINOLIDINE)-1-OXO-1, 2, 3, 4-TETRAHYDROCARBAZOLE AND 2-CHLORO-3-FORMYLQUINOLINE.

Sangeetha, V.
Department of Chemistry, Kongunadu Arts and Science College, Coimbatore 641 029, India.
*E.mail: vsangeetha@rediffmail.com

ABSTRACT

1-Oxo-1,2,3,4-tetrahydrocarbazole (**1**) on mixed aldol condensation with 2-chloro-3-formylquinoline (**2**) yielded 2-(3'-(2'-chloro)quinolidine)-1-oxo-1,2,3,4-tetrahydrocarbazole (**3**), which was further treated with hydroxylamine hydrochloride and hydrazine hydrate in separate reactions to afford 4,5-dihydro-3-3'-(2'-chloro) quinoline -isoxazolo [3,4-*a*]carbazole (**4**) and 3'-(2'-chloro)- quinoline- 3,3a,4,5- tetrahydro- 2*H*-pyrazolino [3,4-*a*]carbazole (**5**). The prepared compounds were elaluated for their invitro antibacterial and antifungal activities against certain pathogenic fungal and bacterial strains.

Key words: 1-Oxo-1,2,3,4-tetrahydrocarbazole, 2-chloro-3-formylquinoline, 2-(3'-(2'-chloro)quinolidine)-1-oxo-1,2,3,4-tetrahydrocarbazole, 4,5-dihydro-3-3'-(2'-chloro)quinoline-isoxazolo[3,4-*a*]carbazole, 3'-(2'-chloro)-quinoline-3,3a,4,5-tetrahydro-2*H*-pyrazolino[3,4-*a*]carbazole.

1. INTRODUCTION

Among the nitrogenous plant constituent's carbazole derivatives, a special class of indole alkaloids has attracted considerable attention owing to their diverse physiological activities. There has been a great deal of intrest in annelated heterocycles for designing novel structures capable of performing multiple functions. Among the numerous indole alkaloids those containing *b*-fused cycloalkanes (Benoit *et al.,* 2001), unit in general and *b*-fused cyclopentane (Elisabeth *et al.,* 2008), unit in particular is reported to possess potential pharmacological activities (Julien *et al.,* 2010). Indole is main constituent unit in many of the alkaloids of the natural origin. Indole and its derivatives are shown to exhibit antitumour, antiinflammatory, antibacterial (Sangeetha and Jayaram Pillai, 2003) and antifungal activities (Sangeetha and Jayaram Pillai, 2003). Pyrazolines have also been reported to possess excellent antibacterial (Sangeetha and Jayaram Pillai, 2003), antifungal (Sangeetha and Jayaram Pillai, 2003), and antiviral activities (Thomas *et al.,* 2009). These compounds owe their activities to the heterocyclic ring present in the structure. The structural and biosignificance of indoles as well as pyrazolines (Youssef *et al.,* 2010), has infused interest in us to synthesize some unknown 3'-(2'-chloro)-quinoline-3,3a,4,5-tetrahydro-2*H*-pyrazolino [3,4-*a*] carbazole and 4,5-dihydro-3-3'-(2'-chloro) quinoline-isoxazolo [3,4-*a*] carbazole utilizing 2-(3'-(2'-chloro) quinolidine)-1-oxo-1,2,3,4-tetrahydro carbazole as synthons to construct pyrazolino- and oxazolo-annelated rings on the carbazole skeleton. The new products have been characterized by C, H, N analysis, IR, 1H NMR and mass spectral studies.

2. RESULTS AND DISCUSSION

Mixed aldol reaction of 1-oxo-1,2,3,4-tetra hydrocarbazole (1a) with 2- chloro- 3-formyl quinoline (2) under basic condition gave the expected 2- (3'- (2'-chloro) -quinolidine) -1- oxo-1,2,3,4- tetrahydrocarbazole (3a) in a good yield. The IR spectrum of 3a exhibited two sharp and strong absorption bands at 3253 and 1643 cm^{-1} characteristic of –NH group and α,β-unsaturated carbonyl group respectively. Its ^1H NMR spectrum showed the disappearance of C_2 proton signal and appearance of quinolinic proton signal as a singlet at δ 7.71 proved the validity of mixed aldol reaction of 1a with 2-chloro-3-formyl quinoline to give 3a. The C_3 and C_4 protons resonated as two multiplets centered at δ 3.09 and δ 3.16 respectively and a broad singlet at δ 11.87 for carbazole-NH. Further it exhibited a multiplet at δ 7.07 - 8.12 due to eight aromatic protons. A sharp singlet appeared at δ 8.60 shows presence of C_4' proton. The mass spectrum (m/z = 358) and the elemental analysis was compatible with the molecular formula $C_{22}H_{15}N_2OCl$. A series of similar compounds 3b-3d were realized with 1b-1d. (Scheme 1)

2.1. Synthesis of 4,5-dihydro-3-(3'-(2'-chloro)quinolin)isoxazolo[3,4-a]carbazole (4a-4d)

In an another experiment, 2-(3'-(2'-chloro)quinolidine)-1-oxo-1,2,3,4-trahydrocarbazole

3a was condensed with hydroxylamine hydrochloride in dry pyridine. The reaction mixture, after work up, afforded the compound 4a, as a solid mass which was purified by column chromatography. The IR spectrum of 4a of this compound exhibited two absorptions at 3222 cm^{-1} and 1604 cm^{-1} which were ascribable to –NH and >C=N stretching vibrations respectively. Its ^{1}H NMR spectrum registered a multiplet at δ 3.30 – 3.32 corresponding to C_4 and C_5 protons in addition, a multiplet appeared at δ 7.15 – 8.96 for nine aromatic protons and a broad singlet at δ 12.01 corresponding for carbazole – NH proton. Its mass spectrum and the elemental analysis agreed well with the molecular formula $C_{22}H_{14}N_3OCl$. Based on the above mentioned spectral data the structure of the compounds was proposed as 4,5-dihydro-3-(3′-(2′-chloro) quinoline) isoxazolo[3,4-*a*]carbazole 4a. Similarly following the above condition, the compounds 8b-8d afforded the corresponding isoxazalo[3,4-*a*]carbazoles (4b-4d).

2.2. Synthesis of 3'-(2'-chloro)-quinoline-3,3a,4,5-tetrahydro-2H-pyrazolino-[3,4-a]carbazole (5b-5d)

Reaction of 2-(3'-(2'-chloro)quinolidine)-1-oxo-1,2,3,4-tetrahydrocarbazole (8a) with hydrazine hydrate in ethanol afforded the expected 3-(3'-(2'-chloro)quinolin)-3,3a,4,5-tetrahydro-2*H*-pyazolino [3,4-*a*]carbazole 9a in a moderate yield. Its IR spectrum (Fig. 4) revealed the formation of >C=N (1637cm^{-1}), thereby indicating the absence of carbonyl absorption. The ^{1}H NMR spectrum (Fig. 5) of 9a in DMSO showed as a multiplet resonated at δ 2.48 and δ 2.49 for C_4 and C_5 protons. C_3 and C_{3a} protons appeared as a multiplet at δ 2.50. A broad singlet at δ 3.50 accounts for pyrazolino-NH proton and the nine aromatic protons resonated at δ 7.05-8.60 as a multiplet. Carbazole-NH appeared as a broad singlet at δ 8.65. Further the mass spectrum m/z =372 (fig.6) and elemental analysis agreed well with the molecular formula $C_{22}H_{17}N_4Cl$. On the basis of the aforesaid data, the product was attested to 3-(3'-(2'-chloro)quinolin)-3,3a,4,5-tetrahydro-2*H*-pyrazolino[3,4-*a*]carbazole 9a. Similarly following the above conditions the compounds 8b-8d afforded the corresponding pyrazolino[3,4-*a*]carbazoles, 9b-9d (Scheme 5).

2.3. Preparation of 2-(3'-(2'-chloro)quinolidene)-1-oxo-1,2,3,4-tetrahydrocarbazoles (8a-8d)

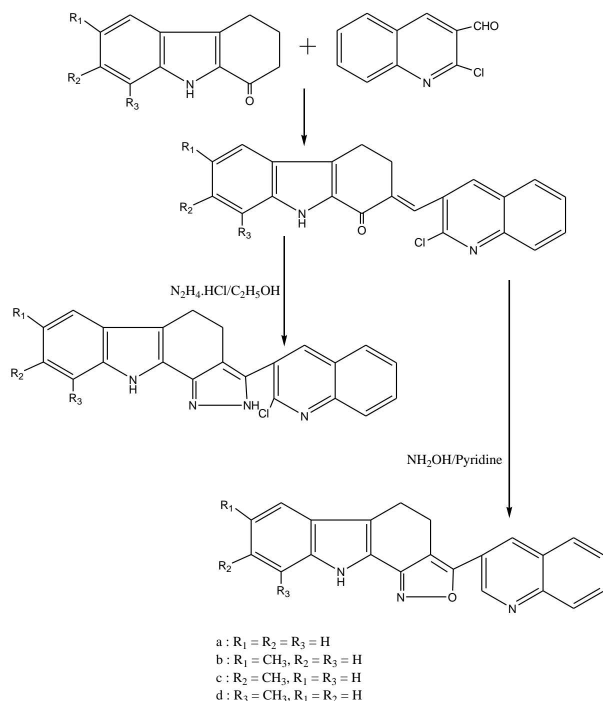
2.3.1. General Procedure

A mixture of the respective 1-oxo-1,2,3,4-tetrahydrocarbazole (6a-6d) 0.002 mol and 2-chloro-3-formyl quinoline 0.002 mol was treated with 4% alcoholic KOH (20 mL) and stirred for 12 hours at room temperature. The product precipitated as crystalline mass was filtered off and washed with 50% aqueous ethanol. Another quantum of the same crystalline compound was obtained from the filtrate on neutralization with acetic acid followed by dilution with water. Pure crystals of the respective products (8a-8d) were obtained by recrystallization with methanol.

2.4. Preparation of 4,5-Dihydro-3-(3'-(2'-chloro)quinolin)isoxazolo[3,4-a]carbazole (9a-9d)

2.4.1. General Procedure

A mixture of the respective 2-(3'-(2'-chloro) quinolidene) -1 -oxo- 1,2,3,4- tetrahydro carbazole (8, 0.001 mol) with hydroxylamine hydrochloride (0.139g, 0.002 mol) in pyridine (5 mL) was refluxed at 130ºC for 10 hours. The resulting reaction mixture was poured into crushed ice and stirred. The product separated as semisolid was extracted twice with chloroform (2×15mL), combined organic layers were washed with dilute hydrochloric acid and water successively and dried over anhydrous sodium sulphate. Evaporation of the excess solvent yielded a crude product and chromatographed over silica gel and eluted with petroleum ether-ethyl acetate mixture (80:20) to obtain the compound 4,5-dihydro-3-(3'-(2'-chloro)quinolin)isoxazolo[3,4-*a*]carbazole (9).



2.5. Preparation of 3-(3'-(2'-chloro)quinolin)-3,3a,4,5-tetrahydro-2H-pyrazolino [3,4-a] carbazoles (9a-9d)

2.5.1. General procedure

To an ethanolic solution of 2-(3-'(2-'chloro) quinolidene) -1 -oxo- 1,2,3,4- tetrahydrocarbazole (0.001mol) (20mL), hydrazine hydrate (1.5mL) was added and mixture was refluxed. After a period of 3h the solvent was removed under reduced pressure and the residue was washed with water and extracted with chloroform (3x15ml), and the combined organic layers were dried over anhydrous sodium sulphate. Evaporation of the solvent followed by crystallization yielded the desired product and chromatographed over silica gel and eluted with petroleum ether – ethylacetate mixture (80:20) to obtain the compound 3-(3'-(2'-chloro)quinolin)-3,3a,4,5-tetrahydro-*2H*-pyazolino[3,4-*a*] carbazole.

REFERENCES

Benoit, J., M. Facompre, H. Da Costa, S. Routier, J.Y. Merour, P. Colson, C. Houssier and C. Bailly, (2001). Synthesis, Cytotoxicity, DNA Interaction and Topoisomerace II Inhibition Properties of Tetrahydropyrrolo[3,4-*a*]carbazole-1,3-dione and Tetrahydropyrido-[3,2-*b*]pyrrolo[3,4-*g*]indole-1,3-dione Derivatives. *Bioorg. Med. Chem.* **9**: 1533-1541.

Elisabeth, C., F. Anizone, B. Aboab, R.M. Golsteyn, S. Leonce, B. Pfeiffer and M. Prudhomme, (2008). Synthesis, in vitro antiproliferative activities, and Chk1 inhibitory properties of pyrrolo[3,4-*a*]carbazole -1, 3- diones, pyrollo [3, 4 -*c*] -carbazole-1,3-diones, and 2-amino pyridazino [3,4-*a*] pyrrolo- [3,4-*c*] carbazole- 1,3,4,7-tetraone. *Euro. J. Med. Chem.* **43**: 82-292.

Julien, D., W. Zeghida, B. Baldeyrou, C. Mahieu and A. Lansiaux, (2010). Montmorillonite K-10 catalyzed cyclization of *N*-ethoxycarbanyl-*N'*-arylguani-dines: Acess to pyrimido [4,5-*c*]carbazole and pyrimido [5,4-*b*] indole derivatives. *Bioorg. Med. Chem. Lett.* **20**: 4244-4247.

Sangeetha, V. and R.K. Jayaram Pillai, (2002). Tetracyclic compounds from cyclopent [b] indoles. Synthesis of isoxazolo [3',4':5,4] cyclopent [*b*] indoles. *Heterocy. Communicat.* **8**: 65.

Sangeetha, V. and R.K. Jayaram Pillai, (2003). Synthesis of novel pyrazolino [3',4':5,4] cyclopent [*b*] indoles. *Indian J. Chem.* **42**B: 2109.

Thomas, L., U. Pindur, G. Lenglet, S. Depauw, C. Dassi and M.H. David-Cordonnier, (2009). Photochemical electrocyclization of 3-vinylindoles to pyrido [2,3-*a*]carbazoles: Design, synthesis, DNA binding and antitumour cell cytotoxicity. *Euro. J. Med. Chem.* **44**: 3235-3252.

Youssef, H., C. Neagoie, B. Biannic, A. Chilloux, E. Vedrenne, B. Baldeyrou, C. Bailly, J.Y. Merour, S. Rosca and S. Routier, (2010), Synthesis and biological activities of new furo-[3,4-*b*]carbazoles: Potential topoisomerase II inhibitors. *Euro. J. Med. Chem.* **45**: 5428-5437.

Kong. Res. J. 2(1) : 32-35, 2015 ISSN 2349-2694
Kongunadu Arts and Science College, Coimbatore.

EXISTENCE OF SOLUTIONS FOR NEUTRAL FUNCTIONAL VOLTERRA-FREDHOLM INTEGRODIFFERENTIAL EQUATIONS

Dhanalakshmi, S. and G. Deepa
Department of Mathematics (UA), Kongunadu Arts and Science College, Coimbatore.
*E.mail: dhana_bala16@yahoo.co.in

ABSTRACT

In this paper, we study the existence of mild solutions of nonlinear neutral functional Volterra-Fredholm integrodifferential equations with nonlocal conditions. The results are obtained by using fractional power of operators and Sadovskii's fixed point theorem.

Keywords: Integrodifferential equations, fixed point theorem, Banach space.

MSC subject classification : 45J05, 47H10, 46Bxx.

1. INTRODUCTION

Many phenomena in several branches have mathematical model in terms of differential equations. Differential equations are like a bridge which links mathematics and science with applications. It is a rightly considered as a language of sciences. Many branches of science have led to some kind of differential equations.

The study of integrodifferential equations has emerged in recent years as an independent branch of modern research because of its connections to many applied fields such as elasticity, biology, epidemics and other branches of science and engineering. Neutral differential equations arises in many areas of applied mathematics and for this reasons this equations have received much attention in the last few decades.

The advantages of using nonlocal conditions is that measurable at more places can be incorporated to get better models. The nonlocal Cauchy problem for abstract evolution differential equation was first considered by Byszewski (Byszwski, 1991) Subsequently, several authors have investigated the problem for different types of nonlinear differential equations and integrodifferential equations including functional differential equations in Banach spaces (Balachandran, 1998; Byszwski and Acka, 1998; Balachandran and Park, 2001a, b; Fu and Ezzinbi, 2004).

In the past several years theorems about existence, uniqueness and stability of differential and functional differential abstract evolution Cauchy problem have been studied by Byszewski and Lakshmikantham (1990), Byszewski (1997, 1998), Balachandran and Chandrasekaran (1996), Lin and Liu (1996) and Murugesu and Suguna (2010).

In this paper, we extend this problem to neutral functional Volterra-Fredholm type integro differential equations with nonlocal conditions and discuss the existence of solutions for nonlinear neutral functional Volterra-Fredholm integro differential equations with nonlocal conditions of the form

$$\frac{d}{dt}[x(t)+F(t,x(t),x(b_1(t)),\cdots,x(b_m(t)))] = Ax(t)+G(t,x(t),x(a_1(t)),\cdots,x(a_n(t))) + K\left(t,x(t),\int_0^t k(t,s,x(s))ds,\int_0^a h(t,s,x(s))ds\right),\quad 0\le t\le a,$$
$$x(0)=x_0+g(x)$$

------------------(1)

where –A generates an analytic semigroup and F, G, K, k, h are given functions to specifed later.

This paper has the following subsections. In section 2, we present some preliminary lemmas and definitions which will be used to prove our main results. In section 3, we present the existence of mild solution of the system (1) using Sadovskii's fixed point theorem (Sodovskii, 1967).

2 PRELIMINARIES

Throughout this work, let –A is the infinitesimal generator of a compact analytic semigroup of uniformly bounded linear operators T(t) defined in the Banach spaces X. Let $0\in\rho$ then define the fractional power A^{α}, for $0 \le \alpha \le 1$, as a closed linear operator on its domain $D(A^{\alpha})$ which is dense in X. Further, D(A) is a Banach space under the norm

$$\|x\|_{\alpha} = \|A^{\alpha}x\|,\ x\in D(A^{\alpha})$$

Which we denote by X_{α}. Then for each $0 < \alpha \le 1$, $X_{\alpha}\to X_{\beta}$ for $0 < \beta < \alpha \le 1$ and the imbedding is

compact whenever the resolvent operator of A is compact. We assume that

a) There is a $M \geq 1$ such that $\| T(t) \| \leq M$, for all $0 \leq t \leq a$.

b) For any a > 0, there exists a positive constant C_α,

$$\| A^{\alpha} T(t) \| \leq \frac{C_\alpha}{t^\alpha}, 0 < t \leq a \qquad \text{--------(2)}$$

For our convenience, let us take

$$F(0, x(0), x(b_1(0)), \ldots x(b_m(t))) = 0.$$

Let $M_0 = \| A^{-\beta} \|$ and also assume the following hypotheses:

(H1) F : $[0,a] \times X^{m+1} \rightarrow X$ is a continuous functions and there exists a $\beta \in (0,1)$ and L, $L_1 > 0$ such that the function $A^\beta F$ satisfies the Lipschitz condition:

$$\| A^\beta F(s_1, x_0, x_1, \cdots, x_m) - A^\beta F(s_2, \bar{x}_0, \bar{x}_1, \cdots, \bar{x}_m) \| \leq L\left(| s_1 - s_2 | + \max_{i=0,1,\cdots,m} \| x_i - \bar{x}_i \| \right)$$

for any $0 \leq s_1, s_2 \leq a$, $x_i, \bar{x}_i \in X$, i = 0, 1, ..., m and the inequality

$$\| A^\beta F(t, x_0, x_1, \cdots, x_m) \| \leq L_1 (\max\{\| x_i \| : i = 0, 1, \cdots, m\} + 1) \qquad \text{---------------- (3)}$$

holds for any $(t, x_0, x_1, \ldots, x_m) \in [0,a] \times X^{m+1}$.

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

(i) For each $t \in [0,a]$, the function G(t, .) : $\times X^{n+1} \rightarrow X$ is continuous and for each $(x_0, x_1, \ldots, x_n) \in X^{n+1}$ the function $G(., x_0, x_1, \ldots, x_n) : [0,a] \rightarrow X$ is strongly measurable.

(ii) For each positive number $n \in N$, there is a positive function $\phi_n \in L^1([0,a])$ such that

$$\sup_{\|x_0\|, \cdots, \|x_n\| \leq n} \| G(t, x_0, x_1, \cdots, x_n) \| \leq \phi_n(t)$$

$$\lim_{n \to \infty} \frac{1}{n} \int_0^a \phi_n(s) ds = \gamma < \infty$$

(H3) The function K : $[0,a] \times X \times X \times X \rightarrow X$ satisfies the following conditions:

(i) For each $t \in [0,a]$, the function K(t, ., ., .): $X \times X \times X \rightarrow X$ and for each x, y, z $\in X$, K(., x, y, z) : $[0,a] \rightarrow X$ is strongly measurable.

(ii) For each positive number $n \in N$, there exists a positive function $q_n \in L^1([0,a])$ such that

$$\sup_{\|x\| \leq n} \left\| K\left(s, x(s), \int_0^s k(s, \tau, x(\tau)) d\tau, \int_0^a h(s, \tau, x(\tau)) d\tau \right) \right\| \leq q_n(s)$$

$$\text{and} \qquad \lim_{n \to \infty} \frac{1}{n} \int_0^a q_n(s) ds = \gamma_1 < \infty$$

(H4) $a_i, b_j \in C([0,a]; [0,a])$, i = 1, ..., n, j = 1, ..., m. $g \in C(E; X)$, here after E =

C([0,a]; X) and g is completely continuous.

(H5) There exist positive constants M_3 and M_4 such that

$$\| g(x) \| \leq M_3 \|x\| + M_4 \text{ for every } x \in E.$$

DEFINITION : 2.1 (Pazy, 1983)

Let X be a Banach space, a one parameter family T(t), $0 \leq t < +\infty$, of bounded linear operators from X to X is a semigroup of bounded linear operators on X, if

(i) T(0)=I, where I is the identity operator on X,

(ii) T(t+s)=T(t)T(s) for every t, s ≥ 0, (the semigroup property)

A semigroup of bounded linear operator T(t) is uniformly continuous if

$$\lim_{t \downarrow 0} \| T(t) - I \| = 0$$

THEOREM : 2.1. (SADOVSKII'S FIXED POINT THEOREM) (Sodovskii, 1967)

Let ψ be a condensing operator on a Banach space X, that is ψ is continuous and takes bounded sets into bounded sets and $\mu(\psi(B)) \leq \mu(B)$ for every bounded set B of X with $\mu(B) > 0$. If $\psi(T) \subset T$ for a convex closed and bounded set γ of X, then ψ has a fixed point in X.

3. EXISTENCE OF MILD SOLUTION

DEFINITION 3.1.

A continuous function x(.) : $[0,a] \rightarrow X$ is said to be a mild solution of the Cauchy problem (1), if the function $AT(t-s)F(s, x(s), x(b_1(s)), \ldots, x(b_m(s)))$, $s \in [0,a)$ is integrable on [0,a) and the integral equation is satisfied.

$$\begin{aligned} x(t) = {} & T(t)[x_0 + F(0, x(0), x(b_1(0)), \cdots, x(b_m(0))) + g(x)] - F(t, x(t), x(b_1(t)), \cdots, x(b_m(t))) \\ & - \int_0^t AT(t-s) F(s, x(s), x(b_1(s)), \cdots, x(b_m(s))) ds \\ & + \int_0^t T(t-s) G(s, x(s), x(a_1(s)), \cdots, x(a_m(s))) ds \\ & + \int_0^t T(t-s) K\left(s, x(s), \int_0^s k(s, \tau, x(\tau)) d\tau, \int_0^a h(s, \tau, x(\tau)) d\tau \right) ds \end{aligned}$$

------------------- (4)

THEOREM 3.1

If the assumptions (H1) – (H5) are satisfied and $x_0 \in X$, then the Cauchy problem (1) has a mild

solution provided that

$$L_0 := L[M_0 + \frac{1}{\beta} C_{1-\beta} a^{\beta}] < 1 \qquad ------------(5)$$

$$(\gamma + \gamma_1 + M_3)M + M_0 L_1 + \frac{1}{\beta} C_{1-\beta} a^{\beta} L_1 < 1 \qquad ------------(6)$$

Where $M_0 = \| A^{-\beta} \|$.

Proof:

For the sake of brevity, we write that

$(t, x(t), x(b_1(t)), \ldots, x(b_m(t))) = (t, v(t))$

and $(t, x(t), x(a_1(t)), \ldots, x(a_m(t))) = (t, u(t))$.

Define the operator Q on E by the formula

$$(Qx)(t) = T(t)[x_0 + g(x)] - F(t, v(t)) - \int_0^t AT(t-s)F(s, v(s))ds + \int_0^t T(t-s)G(s, u(s))ds$$
$$+ \int_0^t T(t-s)K\left(s, x(s), \int_0^s k(s,\tau,x(\tau))d\tau, \int_0^a h(s,\tau,x(\tau))d\tau\right)ds$$

For positive integer r, let

$$B_r = \{x \in E : \| x(t) \| \le r, 0 \le t \le a\}$$

then for each r, Br is clearly a bounded closed convex set in E. Since by (2) and (3) the following relation holds:

$$\| AT(t-s)F(s, v(s)) \| \le \| A^{1-\beta} T(t-s) A^{\beta} F(s, v(s)) \|$$
$$\le \frac{C_{1-\beta}}{(t-s)^{1-\beta}} L_1 (r+1)$$

then from Bochners theorem (Marle, 1974) it follows that AT(t-s)F(s,v(s)) is integrable on [0,a], so Q is well defined on B_r.

Claim : there exists a positive integer r such that $QB_r \subseteq B_r$:

If it is not true, then for each positive integer r, there is a function $x_r(.) \in B_r$, but $Qx_r(t) \notin B_r$, that is $\|Qx_r(t)\| > r$ for some $t(r) \in [0,a]$, where t(r) denotes t is dependent of r. However, on the other hand, we have

$$r < \| Qx_r(t) \|$$
$$= \| T(t)[x_0 + g(x)] - F(t, v_r(t)) - \int_0^t AT(t-s)F(s, v_r(s))ds + \int_0^t T(t-s)G(s, u_r(s))ds$$
$$+ \int_0^t T(t-s)K\left(s, x(s), \int_0^s k(s,\tau,x(\tau))d\tau, \int_0^a h(s,\tau,x(\tau))d\tau\right)ds \|$$
$$\le M \| x_0 \| + MM_3 r + MM_4 + M_0 L_1 (r+1) + \frac{1}{\beta} C_{1-\beta} a^{\beta} L_1 (r+1) + M\int_0^a \phi_n(s)ds + M\int_0^a q_n(s)ds$$

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

$$(\gamma + \gamma_1 + M_3)M + M_0 L_1 + \frac{1}{\beta} C_{1-\beta} a^{\beta} L_1 \ge 1$$

This contradicts (6). Hence for some positive integer r, $QB_r \subseteq B_r$.

Next we will show that the operator Q has a fixed point on B_r :

Let us decompose Q as $Q = Q_1 + Q_2$ where the operators Q_1 and Q_2 are defined on B_r respectively by

$$(Q_1 x)(t) = -F(t, v(t)) - \int_0^t AT(t-s)F(s, v(s))ds$$

$$(Q_2 x)(t) = T(t)[x_0 + g(x)] + \int_0^t T(t-s)G(s, u(s))ds$$
$$+ \int_0^t T(t-s)K\left(s, x(s), \int_0^s k(s,\tau,x(\tau))d\tau, \int_0^a h(s,\tau,x(\tau))d\tau\right)ds$$

for $0 \le t \le a$, and we will verify that Q_1 is contraction and Q_2 is a compact operator.

Claim : Q_1 is a contraction

Let $x_1, x_2 \in B_r$. Then for each $t \in [0,a]$ and by condition (H1) and (5), we have

$$\| (Q_1 x_1)(t) - (Q_1 x_2)(t) \| \le \| F(t, v_1(t)) - F(t, v_2(t)) \| + \left\| \int_0^t AT(t-s)[F(s, v_1(s)) - F(s, v_2(s))]ds \right\|$$
$$\le M_0 L \sup_{0 \le s \le a} \| x_1(s) - x_2(s) \| + \int_0^t \frac{C_{1-\beta}}{(t-s)^{1-\beta}} L ds \sup_{0 \le s \le a} \| x_1(s) - x_2(s) \|$$
$$\le L\left[M_0 + \frac{1}{\beta} C_{1-\beta} a^{\beta}\right] \sup_{0 \le s \le a} \| x_1(s) - x_2(s) \|$$
$$\le L_0 \sup_{0 \le s \le a} \| x_1(s) - x_2(s) \|$$

Thus $\| Q_1 x_1 - Q_1 x_2 \| \le L_0 \| x_1 - x_2 \|$

So by assumption $0 < L_0 < 1$, we see that Q_1 is a contraction.

Claim : Q_2 is compact

To prove this we have to prove that Q_2 is continuous on B_r.

Let $\{x_n\} \subseteq B_r$ with $x_n \to x$ in B_r, then by (H2) (i), we have

$$G(s, u_n(s)) \to G(s, u(s)), \quad n \to \infty$$

$$K\left(t, x_n(t), \int_0^t k(t,s,x_n(s))ds, \int_0^a h(t,s,x_n(s))ds\right) \to K\left(t, x(t), \int_0^t k(t,s,x(s))ds, \int_0^a h(t,s,x(s))ds\right) \ as\ n \to \infty$$

Since $\| G(s, u_n(s)) - G(s, u(s)) \| \le 2\phi_n(s)$,

$$\left\| K\left(t, x_n(t), \int_0^t k(t,s,x_n(s))ds, \int_0^a h(t,s,x_n(s))ds\right) - K\left(t, x(t), \int_0^t k(t,s,x(s))ds, \int_0^a h(t,s,x(s))ds\right) \right\| \le 2q_n(s)$$

by the dominated convergence theorem, we have

$$\| Q_2 x_n - Q_2 x \| = \sup_{0 \le t \le a} \| T(t)[g(x_n) - g(x)] + \int_0^t T(t-s)[G(s, u_n(s)) - G(s, u(s))]ds$$
$$+ \int_0^t T(t-s)\left[K\left(s, x_n(s), \int_0^t k(s,\tau,x_n(\tau))d\tau, \int_0^a h(s,\tau,x_n(\tau))d\tau\right)\right.$$
$$\left. - K\left(s, x(s), \int_0^t k(s,\tau,x(\tau))d\tau, \int_0^a h(s,\tau,x(\tau))d\tau\right)\right]ds \|$$
$$\to 0 \quad as \quad n \to \infty$$

(i.e) Q_2 is continuous.

Next, we prove that $\{Q_2 x : x \in B_r\}$ is a family of equicontinuous functions. To see this we fix $t_1 > 0$ and $t_2 > t_1$ and $\varepsilon > 0$ be enough small. Then

$$
\begin{aligned}
\|(Q_2x)(t_2)-(Q_2x)(t_1)\| \le{}& \|T(t_2)-T(t_1)\|\|x_0+g(x)\| + \int_0^{t_1-\varepsilon}\|T(t_2-s)-T(t_1-s)\|\|G(s,u(s))\|ds \\
&+ \int_{t_1-\varepsilon}^{t_1}\|T(t_2-s)-T(t_1-s)\|\|G(s,u(s))\|ds \\
&+ \int_{t_1}^{t_2}\|T(t_2-s)\|\|G(s,u(s))\|ds \\
&+ \int_0^{t_1-\varepsilon}\|T(t_2-s)-T(t_1-s)\|\|K\left(s,x(s),\int_0^s k(s,\tau,x(\tau))d\tau,\int_0^a h(s,\tau,x(\tau))d\tau\right)\|ds \\
&+ \int_{t_1-\varepsilon}^{t_1}\|T(t_2-s)-T(t_1-s)\|\|K\left(s,x(s),\int_0^s k(s,\tau,x(\tau))d\tau,\int_0^a h(s,\tau,x(\tau))d\tau\right)\|ds \\
&+ \int_{t_1}^{t_2}\|T(t_2-s)\|\|K\left(s,x(s),\int_0^s k(s,\tau,x(\tau))d\tau,\int_0^a h(s,\tau,x(\tau))d\tau\right)\|ds
\end{aligned}
$$

Note that $||G(s,u(s))|| \le \phi_n(s)$ and $\phi_n(s) \in L^1$, we see that $||Q_2x(t_2)-Q_2x(t_1)||$ tends to zero independently of $x \in B_r$ as $t_2-t_1 \to 0$. Since the compactness of T(t), t>0 implies the continuity of T(t), t>0 in t in the uniform operator topology.

We can prove that the function Q_2x, $x \in B_r$ are equicontinuous at t=0. Hence Q_2 maps B_r into a family of equicontinuous function.

Claim : $V(t)=\{(Q_2x)(t) : x \in B_r\}$ is relatively compact in X.

Let $0<t\le a$ be a fixed and $0 < \varepsilon < t$. For $x \in B_r$, we define

$$
\begin{aligned}
(Q_{2,\varepsilon}x)(t) &= T(t)[x_0+g(x)] + \int_0^{t-\varepsilon} T(t-s)G(s,u(s))ds \\
&\quad + \int_0^{t-\varepsilon} T(t-s)K\left(s,x(s),\int_0^s k(s,\tau,x(\tau))d\tau,\int_0^a h(s,\tau,x(\tau))d\tau\right)ds \\
&= T(t)[x_0+g(x)] + T(\varepsilon)\int_0^{t-\varepsilon} T(t-\varepsilon-s)G(s,u(s))ds \\
&\quad + T(\varepsilon)\int_0^{t-\varepsilon} T(t-\varepsilon-s)K\left(s,x(s),\int_0^s k(s,\tau,x(\tau))d\tau,\int_0^a h(s,\tau,x(\tau))d\tau\right)ds
\end{aligned}
$$

Then from compactness of $T(\varepsilon)$, $\varepsilon>0$ we obtain $V_\varepsilon=\{(Q_{2,\varepsilon}x)(t) : x \in B_r\}$is relatively compact in X for every ε, $0 < \varepsilon < t$. Moreover, for every $x \in B_r$, we have

$$
\begin{aligned}
\|(Q_2x)(t)-(Q_{2,\varepsilon}x)(t)\| &= \int_{t-\varepsilon}^{t}\|T(t-s)G(s,u(s))\|ds \\
&\quad + \int_{t-\varepsilon}^{t}\|T(t-s)K\left(s,x(s),\int_o^t k(s,\tau,x(\tau))d\tau,\int_o^a h(s,\tau,x(\tau))d\tau\right)\|ds \\
&\le M\int_{t-\varepsilon}^{t} g_k(s)ds + M\int_{t-\varepsilon}^{t} q_r(s)ds
\end{aligned}
$$

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

Thus, by Arzela-Ascoli theorem, Q_2 is a compact operator. Those arguments enable us to conclude that $Q=Q_1+Q_2$ is a condensing map B_r, and by the Sadovskii's fixed point theorem there exist a fixed point x(.) for Q on B_r.Therefore, the Cauchy problem (1) has a mild solution, and the proof is completed.

REFERENCES

Balachandran, K. and J.Y. Park, (2001a). Existence of mild solution of a functional integrodifferential equation with nonlocal condition. *Bull. Korean Math. Soc.* **38**: 175-182.

Balachandran, K. and J.Y. Park, (2001b). Existence of solution of second order nonlinear differential equations with nonlocal conditions in Banach spaces. *Ind. J. Pure Appl. Math.* **32**: 1883-1892.

Balachandran, K. and M. Chandrasekaran, (1996). Existence of solutions of a delay differential equations with nonlocal condition. *Ind. J. Pure Appl. Math.* **27**: 443-449.

Balachandran, K., (1998). Existence and uniqueness of mild and strong solutions of nonlinear integrodifferential equations with nonlocal conditions. *Diff. Equ. Dyn. Sys.* **6**: 159-165.

Byszwski, L. and V. Lakshmikantham, (1990). Theorem about the existence and uniqueness of a solution of a nonlocal abstract cauchy problem in a Banach space. *Appl. Anal.* **40**: 11-19.

Byszwski, L. and Acka H., (1998). Existence of solutions of semilinear functional differential evolution nonlocal problems. *Nonlinear Anal.* **34**: 65-72.

Byszwski, L., (1991). Theorems about the existence and uniqueness of solutions of semi linear evolution nonlocal Cauchy problem. *J. Math. Anal. Appl.* **162**: 494-505.

Byszwski, L., (1997). On weak solutions of functional differential abstract nonlocal cauchy problem. *Ann. Polonici. Math.* **65**: 163-170.

Byszwski, L., (1998). Application of properties of the right hand sides of evolution equation to an investigation of nonlocal evolution problem. *Nonlinear Anal.* **33**: 413-426.

Fu, X. and K. Ezzinbi, (2004). Existence of solutions for neutral functional differential evolution equations with nonlocal conditions. *Nonlinear Anal.* **54**: 215-227.

Lin, Y. and H. Liu, (1996). Semilinear integrodifferential equations with nonlocal cauchy problem. *Nonlinear Anal.* **26**: 1023-1033.

Marle, C.M., (1974). Measures of Probabilities, Hermam, Paris,.

Murugesu, R. and S. Suguna, (2010). Existence of solutions for neutral functional integer differential equations. *Tamkang J. Math.* **41**(2): 117-128.

Pazy, A., (1983). Semigroups of Linear Operators and Applications to Partial Differential Equations. Applied Mathemati- cal Sciences, Springer, New York, USA.

Sodovskii, B.M., (1967). On a fixed point principle. *Fun. Anal. Appl.* **1**: 74-76.

Kong. Res. J. 2(1) : 36-39, 2015 ISSN 2349-2694
Kongunadu Arts and Science College, Coimbatore.

SUPRA bT^{μ} - CLOSED SETS IN MINIMAL STRUCTURES

Krishnaveni, K. and M. Vigneshwaran*
Department of Mathematics, Kongunadu Arts and Science College (Autonomous), Coimbatore.
*E.mail: vignesh.mat@gmail.com

ABSTRACT

We introduce a new set called mbT^{μ} - closed set in a supra topological spaces which are defined on a family of sets satisfying some minimal conditions.

Mathematics subject classification: 54A05

Keyword: mbT^{μ}-closed set

1. INTRODUCTION

In Mashhour *et al.* (1983) introduced Supra topological spaces and studied S-continuous maps and S^*-continuous maps . Popa and Noiri (2000) introduced concept of minimal structure on a nonempty set. Also they introduced the notation m_X-open set and m_X- closed set and characterize these sets using m_X-cl and m_X- int operators respectively.

In this paper, we introduced a new class m_X-structures set called minimally bT^{μ} - closed set called as mbT^{μ} - closed set in supra topological spaces. Further, we study the properties of mbT^{μ} - closed sets in supra topological spaces.

2. PRELIMINARIES

Let (X,μ) be a supra topological space and A be a subset of X. The closure of A and interior of A are denoted by $cl^{\mu}(A)$ and $int^{\mu}(A)$ respectively in supra topological spaces. Let (X,m_X) be an m-space where X is a nonempty set and m_X is the minimal structure defined on X. The m_X-cl^{μ} and m_X-int^{μ} denotes the m_X-closure and m_X- interior on (X,m_X) respectively on supra topological space.

Definition 2.1 (Mashhour *et al.,* 1983; Sayed and Noiri, 2010)

A subfamily of μ of X is said to be a supra topology on X, if

(i) $X,\phi\in\mu$

(ii) if $A_i\in\mu$ for all $i\in J$ then $\cup A_i\in\mu$.

The pair (X,μ) is called supra topological space. The elements of μ are called supra open sets in (X,μ) and complement of a supra open set is called a supra closed set.

Definition2.2 (Sayed and Noiri, 2010)

(i) The supra closure of a set A is denoted by $cl\mu(A)$ and is defined as

$cl\mu(A)=\cap\{B:B$ is a supra closed set and $A\subseteq B\}$.

(ii) The supra interior of a set A is denoted by $int\mu(A)$ and defined as

$int\mu(A)=\cup\{B:$ B is a supra open set and $A\supseteq B\}$.

Definition 2.3 (Mashhour *et al.,* 1983)

Let (X,τ) be a topological spaces and μ be a supra topology on X. We call μ a supra topology associated with τ if $\tau\subset\mu$.

Definition 2.4 (Andrijevic, 1996)

Let (X,μ) be a supra topological space. A set A is called a supra b-open set if $A\subseteq cl\mu(int\mu(A))\cup int\mu(cl\mu(A))$. The complement of a supra b-open set is called a supra b-closed set.

Definition 2.5 (Arockiarani and Pricilla, 2011a)

Let (X,μ) be a supra topological space . A set A of X is called supra generalized b – closed set (simply $g\mu b$ – closed) if $bcl\mu(A)\subseteq U$ whenever $A\subseteq U$ and U is supra open. The complement of supra generalized b-closed set is supra generalized b-open set.

Definition 2.6 (Arockiarani and Pricilla, 2011b)

A subset A of (X,μ) is called $T\mu$-closed set if $bcl\mu(A)\subseteq U$ whenever $A\subseteq U$ and U is $g\mu b$- open in (X,μ).The complement of $T\mu$-closed set is called $T\mu$-open set.

Definition 2.7 (Arockiarani and Pricilla, 2012)

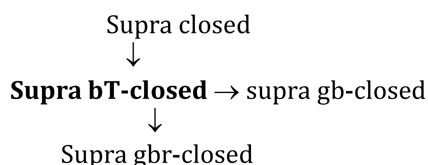
A subset A of a supra topological space (X,μ) is called supra generalized b-regular closed set if

$bcl\mu(A) \subseteq U$ and whenever $A \subseteq U$ and U is supra regular open of (X,μ). The complement of supra generalized b-regular closed set is called supra generalized b-regular open set.

Definition 2.8 (Krishnaveni and Vigneshwaran, 2013)

A subset A of a supra topological space (X,μ) is called $bT\mu$-closed set if $bcl\mu(A) \subseteq U$ whenever $A \subseteq U$ and U is $T\mu$ -open in (X,μ).The complement of supra $bT\mu$ -closed set is called supra $bT\mu$ -open set.

Remark 2.9 (Krishnaveni and Vigneshwaran, 2013) The following relations are well known



Definition 2.10 (Popa and Noiri, 2000)

Let X be a nonempty set and P(X) the power set of X. A subfamily m_X of P(X) is called a minimal structure (m- structure) on X if $\varphi \in m_X$ and $X \in m_X$. The pairs (X, m_X) is called a minimal space (or m-space).

Definition 2.11 (Popa and Noiri, 2000)

A minimal structure m_X on a nonempty set X is said to have property B if the union of any family of subsets belongs to m_X.

Lemma 2.12 (Popa and Noiri, 2000)

Let X be a nonempty set and m_X a minimal structure on X satisfying property B. For a subset A of X, the following properties hold:

(i) $A \in m_X$ if and only if m_X- int(A) = A
(ii) A is m_X-closed if and only if m_X-cl(A) = A
(iii) m_X-int(A) $\in$ m_X-open and m_X-cl(A) is m_X-closed.

Definition 2.13

Let (X,m_X) be an m-space. A set A is called a mb^{μ}-open set if $A \subseteq m_X\text{-}cl\mu(m_X\text{-}int\mu(A)) \cup m_X\text{-}int\mu(m_X\text{-}cl\mu(A))$. The complement of a mb^{μ}-open set is called a mb^{μ}-closed set.

Definition 2.14

Let (X,m_X) be an m-space. A set A is called a m supra regular-open set if $A= m_X\text{- } cl\mu(m_X\text{- } int\mu(A))$. The complement of a m supra regular-open set is called a m supra regular-closed set.

Definition 2.15

Let (X,m_X) be an m-space. A subset A of X is said to be minimal supra g b-closed($mg^{\mu}b$- closed) if $m_X\text{-}bcl^{\mu}(A) \subseteq G$ whenever $A \subseteq G$ and G is m supra-open.

Definition 2.16

Let (X,m_X) be an m-space. A subset A of X is said to be minimal supra gbr-closed ($mg^{\mu}br$- closed) if $m_X\text{- } bcl^{\mu}(A) \subseteq G$ whenever $A \subseteq G$ and G is m supra regular-open.

Definition 2.17

Let (X,m_X) be an m-space. A subset A of X is said to be minimal supra T-closed(mT^{μ}- closed) if $m_X\text{-}bcl^{\mu}(A) \subseteq G$ whenever $A \subseteq G$ and G is m supra gb-open.

Definition 2.18

Let (X,m_X) be an m-space. A set A is called a m supra regular open set if $A = m_X\text{-}int\mu(m_X\text{-}cl\mu(A))$. The complement of a m supra regular open set is called a m supra regular closed set.

3. mbT^{μ}-CLOSED SETS IN MINIMAL STRUCTURES

Definition 3.1

Let (X,m_X) be an m-space. A subset A of X is said to be minimal supra bT closed (mbT^{μ}- closed) if $m_X\text{-}bcl^{\mu}(A) \subseteq G$ whenever $A \subseteq G$ and G is mT^{μ}-open.

Remark 3.2

Let(X,μ) be a supra topological space and m_X be minimal structure on X. If $m_X = \mu$, then an mbT^{μ}-closed set is bT^{μ}-closed set in X.

In this section, let (X,μ) be a supra topological space and m_X be an m-structure on X. We obtain several basic properties and some characterizations of mbT^{μ} - closed sets and mbT^{μ} - open sets on m-space.

Theorem 3.3

Let m_X have the property B. A subset A of X is mbT^{μ} - closed in (X,m_X) iff $m_X\text{-}bcl^{\mu}(A) - A$ contains no non empty mT^{μ} - closed set in X.

Proof Suppose that F is a nonempty mT^{μ}-closed subset of $m_X\text{-}bcl^{\mu}(A) - A$. Now $F \subseteq m_X\text{-}bcl^{\mu}(A) - A$. Then $F \subseteq m_X\text{-}bcl^{\mu}(A) \cap A^c$, since $m_X\text{-}bcl^{\mu}(A) - A = m_X\text{-}bcl^{\mu}(A) \cap A^c$. Therefore $F \subseteq m_X\text{-}bcl^{\mu}(A)$ and $F \subseteq A^c$. Since F^c is mT^{μ}- open set and A is mbT^{μ} - closed, $m_X\text{-}bcl^{\mu}(A) \subseteq F^c$. That is $F \subseteq m_X\text{-}bcl^{\mu}(A)^c$. Hence $F \subseteq m_X\text{-}bcl^{\mu}(A) \cap m_X\text{-}bcl^{\mu}(A)^c = \varphi$. That is $F = \varphi$. Thus $m_X\text{-}bcl^{\mu}(A) - A$ contains no nonempty mT^{μ} - closed set.

Conversely, assume that $m_X\text{-}bcl^{\mu}(A) - A$ contains no nonempty mT^{μ} - closed set. Let $A \subseteq G$, G is mT^{μ}-open. Suppose that $m_X\text{-}bcl^{\mu}(A)$ is not contained in G. Then $m_X\text{-}bcl^{\mu}(A) \cap G^c$ is a nonempty mT^{μ} - closed set

of m_X-$bcl^{\mu}(A) - A$, which is a contradiction. Therefore m_X-$bcl^{\mu}(A) \subseteq G$ and hence A is mbT^{μ} - closed.

Theorem 3.4

For subsets A and B of X, the following properties hold:

(i) If A is m_X-supra closed, then A is mbT^{μ}-closed.
(ii) If m_X has the property B and A is mbT^{μ}-closed and mT^{μ}-open then A is m_X-supra closed.
(iii) If A is mbT^{μ}-closed and $A \subseteq B \subseteq m_X$-$bcl^{\mu}(A)$, then B is mbT^{μ}-closed.

Proof (i) Let A be an m_X-supra closed set in (X,m_X). Let $A \subseteq G$, where G is mT^{μ}-open in (X,m_X). Since A is m_X-supra closed, m_X-$cl^{\mu}(A) = A$,we know that m_X-$bcl^{\mu}(A) \subseteq m_X$-$cl^{\mu}(A) = A$, m_X-$bcl^{\mu}(A) \subseteq G$. Therefore A is mbT^{μ}-closed.

(ii) Since A is mT^{μ}-open and mbT^{μ}-closed, we have m_X-$bcl^{\mu}(A) \subseteq A$. Therefore A is m_X-supra closed.

(iii) Let A is mbT^{μ}-closed, m_X-$bcl^{\mu}(B) - B \subseteq m_X$-$bcl^{\mu}(A) - A$, and since m_X-$bcl^{\mu}(A) - A$ contains no nonempty mT^{μ}- closed set, neither does m_X-$bcl^{\mu}(B)$-B. By theorem 3.3, the result follows.

Theorem 3.5

Union of two mbT^{μ}-closed sets is mbT^{μ}-closed.

Proof Assume that A and B are mbT^{μ}- closed sets in X. Let G be an mT^{μ}-open set in X such that $A \cup B \subseteq G$. Then $A \subseteq G$ and $B \subseteq G$. Since A and B are mbT^{μ}-closed, m_X-$bcl^{\mu}(A) \subseteq G$ and m_X-$bcl^{\mu}(B) \subseteq G$. Hence, m_X-$bcl^{\mu}(A \cup B) \subseteq m_X$-$bcl^{\mu}(A) \cup m_X$-$bcl^{\mu}(B) \subseteq G$. Therefore $A \cup B$ is mbT^{μ}-closed.

Theorem 3.6

Every m_X-supra closed set in X is mbT^{μ}-closed in X .

Proof Let G be an mT^{μ}- open set such that $A \subseteq G$. Since A is m_X- supra closed, m_X-$cl^{\mu}(A) = A$, then -$cl^{\mu}(A) \subseteq G$. We know that m_X-$bcl^{\mu}(A) \subseteq m_X$-$cl^{\mu}(A)$, then m_X-$bcl^{\mu}(A) \subseteq G$. Therefore A is mbT^{μ}-closed.

The converse of the above theorem need not be true as seen from the following example.

Example 3.7

Consider the m-space X = {a,b,c} with minimal structure m_X = {X,φ,{a},{a,b}}. mbT^{μ}-closed are { X,ϕ, {b}, {c},{b,c}}.The set {b} is mbT^{μ}-closed but not m_X-supra closed set.

Theorem 3.8

Every mbT^{μ}-closed in X is $mg^{\mu}b$-closed in X but not conversely.

Proof Let $A \subseteq G$ and G is m- supra open set in X. We know that m-supra open set is mT^{μ}- open set. since A is mbT^{μ}- closed,we have m_X-$bcl^{\mu}(A) \subseteq G$. Therefore A is $mg^{\mu}b$-closed set in X.

The converse of the above theorem need not be true as seen from the following example.

Example 3.9

Consider the m-space X = {a,b,c} with minimal structure m_X = {X,φ,{a}}. $mg^{\mu}b$- closed are { X,ϕ,{b}, {c}, {a,b},{a,c},{b,c}} and mbT^{μ}- closed are { X,ϕ,{b},{c},{b,c}}.The set {a,b} is $mg^{\mu}b$- closed but not mbT^{μ}- closed set.

Theorem 3.10

Every mbT^{μ}-closed in X is $mg^{\mu}br$-closed in X but not conversely.

Proof

Let $A \subseteq G$ and G is m- supra regular open set in X. We know that m-supra regular open set is mT^{μ}-open set. Since A is mbT^{μ}- closed, we have m_X-$bcl^{\mu}(A) \subseteq G$. Therefore A is $mg^{\mu}br$-closed set in X.

The converse of the above theorem need not be true as seen from the following example.

Example 3.11 Consider the m-space X = {a,b,c} with minimal structure m_X = {X,φ,{a}}.The set {a,b} is $mg^{\mu}br$- closed but not mbT^{μ}- closed set.

Theorem 3.12

For each $x \in X$, {x} is mT^{μ}- closed in X or {x} is mbT^{μ}- closed set in X.

Proof If {x} is not mT^{μ}- closed. Then the only mT^{μ}-open set containing $\{x\}^c$ in X. Also, the m_X-$bcl^{\mu}(\{x\})^c$ is contained in X and hence {x} is mbT^{μ}- closed set in X.

Theorem 3.13

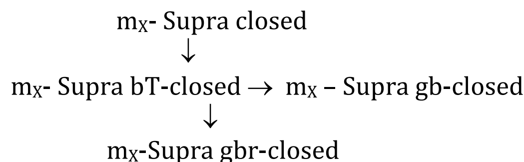
Let m_X have property B. Let A be a subset of X ,then A is mbT^{μ}- closed iff m_X-$bcl^{\mu}(A)$-A does not contain any nonempty m_X-supra closed set.

Proof Suppose that F is nonempty mbT^{μ}- closed subset of m_X-$bcl^{\mu}(A)$-A. Now $F \subseteq m_X$-$bcl^{\mu}(A) - A$. Then $F \subseteq m_X$-$bcl^{\mu}(A) \cap A^c$, since m_X-$bcl^{\mu}(A) - A = m_X$-$bcl^{\mu}(A) \cap A^c$. Therefore $F \subseteq m_X$-$bcl^{\mu}(A)$ and $F \subseteq A^c$. Since F^c is mbT^{μ}- open set and A is mbT^{μ} - closed, m_X-$bcl^{\mu}(A) \subseteq F^c$. That is $F \subseteq [m_X\text{-}bcl^{\mu}(A)]^c$. Hence $F \subseteq m_X$-$bcl^{\mu}(A) \cap [m_X\text{-}bcl^{\mu}(A)]^c = \varphi$. That is $F = \varphi$. Thus m_X-$bcl^{\mu}(A) - A$ contains no nonempty mbT^{μ} - closed set.

Conversely, assume that m_X-$bcl^{\mu}(A) - A$ contains no nonempty m_X- supra closed set. Let $A \subseteq G$, G is mbT^{μ}- open. Suppose that m_X-$bcl^{\mu}(A)$ is not contained in G. Then m_X-$bcl^{\mu}(A) \cap G^c$ is a nonempty

mbT^{μ} - closed set of m_X-$bcl^{\mu}(A) - A$, which is a contradiction. Therefore m_X-$bcl^{\mu}(A) \subseteq G$ and hence A is mbT^{μ} - closed.

Remark 3.14 From the above observation we get the following implications



REFERENCES

Andrijevic D., (1996).On b-open sets, mat.Vesnik **48**(1-2): 59-64.

Arockiarani I. and M.T. Pricilla, (2012). On generalized b-regular closed sets, In supra topological spaces, *Asian J Curr Eng Math* **1**(1): 1-4.

Arockiarani, I. And M.T. Pricilla, (2011b). On supra T-closed sets, *Int J Mathematics Arch* **2**(8): 1376-1380 .

Arockiarani, I. and M.T. Pricilla, (2011a). On supra generalized b-closed sets. *Antartica J Math* **8**.

Krishnaveni, K. and M. Vigneshwaran, (2013). On bT-Closed sets in supra topological Spaces, *Int J Math Arch* **4**(2): 1-6.

Mashhour, A.S., A.A. Allam, F.S. Mohamoud and F.H. Khedr, (1983). On supra Topological spaces, *Indian J Pure Appl Math*No.4, 14(1983),502-510.

Noiri, T., (2006). 11th meetings on topological spaces Theory and its Applications, Fukuoka University Seminar House. 1-9.

Parimelazhagan, R., K. Balachandran and N. Nagaveni, (2009). On Weakly Generalized Closed Sets in Minimal Structure *Int J Contemp Math Sci* **4**(27): 1335-1343.

Popa V. And T. Noiri, (2000). On M-continuous functions, Anal.Univ. Dunarea De Jos Galati.Ser.Mat.Fiz.Mec.Teor.(2). **18**(23): 31-41.

Sayed, O.R. and T. Noiri, (2010). On b-open sets and supra b-Continuity on Topological spaces, *Eur J Pure Appl Math* **3**(2): 295-302.

Kong. Res. J. 2(1) : 40-46, 2015 ISSN 2349-2694
Kongunadu Arts and Science College, Coimbatore.

INTUITIONISTIC FUZZY ψ-CONTINUOUS MAPPINGS

Parimala, M. and C. Indirani*
Department of Mathematics, Bannari Amman Institute of Technology, Sathyamangalam, Erode(District), Tamilnadu, India.
*E-mail: indirani2012@gmail.com

ABSTRACT

In this paper we introduce intuitionistic fuzzy ψ-continuous mappings and intuitionistic fuzzy ψ-irresolute mappings. Some of their properties are studied.

Keywords: Intuitionistic fuzzy topology, intuitionistic fuzzy ψ-closed set, intuitionistic fuzzy ψ-continuous mappings and intuitionistic fuzzy ψ-irresolute mappings.

1. INTRODUCTION

The concept of fuzzy sets was introduced by Zadeh (1965), is a framework to encounter uncertainity, vagueness and parital truth and it represents a degree of membership for each member of the universe of discourse to a subset of it. By adding the degree of non-membership to fuzzy set and later Atanassov (1986) proposed intuitionistic fuzzy set in 1986 which appeals more accurate to uncertainity quantification and provides the opportunity to precisely model the problem, based on the existing knowledge and observations. On the other hand Coker (1997) introduced intuitionistic fuzzy topological spaces using the notation of intuitionistic fuzzy sets. In this paper we introduced intuitionistic fuzzy ψ-continuous mappings and studied some of their basic properties. We provide some characterizations of intuitionistic fuzzy ψ-continuous .

2. PRELIMINARIES

2.1. Definition (Atanassov, 1986)

Let X be a non empty fixed set and I be the closed interval $[0,1]$. In intuitionistic fuzzy set (IFS) A is an object of the following form $A=\{\langle x,\mu_A(x),\nu_A(x)\rangle : x\in X\}$

Where the mapping $\mu_A : X\to I$ *and* $\nu_A : X\to I$ denote the degree of membership (namely $\mu_A(x)$) and the degree of non membership (namely $\nu_A(x)$) for each element $x\in X$ to the set A, respectively and $0\leq \mu_A(x)+\nu_A(x)\leq 1$ for each $x\in X$.

Obviously, every fuzzy set A on a nonempty set X is an IFS of the following form

$$A=\{\langle x,\mu_A(x),1-\mu_A(x)\rangle : x\in X\}$$

2.2. Definition (Atanassov, 1986)

Let A and B be IFSs of the form $A=\{\langle x,\mu_A(x),\nu_A(x)\rangle : x\in X\}$ *and* $A=\{\langle x,\mu_B(x),\nu_B(x)\rangle : x\in X\}$ and $B=\{\langle x,\mu_B(x),\nu_B(x)\rangle : x\in X\}$. Then

(i) $A\subseteq B$ *if and only if* $\mu_A(x)\leq\mu_B(x)$ *and* $\nu_A(x)\geq\nu_B(x)$;

(ii) $\overline{A}=\{\langle x,\nu_A(x),\mu_A(x)\rangle : x\in X\}$;

(iii) $A\cap B=\{\langle x,\mu_A(x)\wedge\mu_B(x),\nu_A(x)\vee\nu_B(x)\rangle : x\in X\}$

(iv) $A\cup B=\{\langle x,\mu_A(x)\vee\mu_B(x),\nu_A(x)\wedge\nu_B(x)\rangle : x\in X\}$

(v) A = B iff $A\subseteq B$ *and* $B\subseteq A$;

(vi) [ ] A= $\{\langle x,\mu_A(x),1-\mu_A(x)\rangle : x\in X\}$

(vii) $\langle\rangle A=\{\langle x,1-\nu_A(x),\nu_A(x)\rangle : x\in X\}$

(viii) $1_{\sim}=\{\langle x,1,0\rangle, x\in X\}$ and $0_{\sim}=\{\langle x,1,0\rangle, x\in X\}$

We will use the notation $A=\langle x,\mu_A,\mu_A\rangle$ instead of A= $\{\langle x,\mu_A(x),\nu_A(x)\rangle : x\in X\}$

The intuitionistic fuzzy sets $0_{\sim} = \{ \langle x, 1, 0\rangle, x \in X \}$ and $1_{\sim} = \{ \langle x, 1, 0\rangle, x \in X \}$ are respectively the empty set and the whole set of X.

2.3. Definition (Coker, 1997)

An intuitionistic fuzzy topology (IFT in short) on X is a family τ of IFSs in X satisfying the following axioms.

(i) $0_{\sim}, 1_{\sim} \in \tau$

(ii) $G_1 \cap G_2 \in \tau$ for any $G_1, G_2 \in \tau$

(iii) $\cup G_i \in \tau$ for any family $\{ G_i / i \in J\} \subseteq \tau$.

In this case the pair (X,τ) is called an intuitionistic fuzzy topological space (IFTS in short) and any IFS in τ is known as an intuitionistic fuzzy open set (IFOS in short) in X.

The complement A^C of an IFOS A in IFTS (X,τ) is called an intuitionistic fuzzy closed set (IFCS in short) in X.

2.4. Definition (Coker, 1997)

Let (X,τ) be an IFTS and $A = \langle x, \mu_A, \mu_A\rangle$ be an IFS in X. Then the intuitionistic fuzzy interior and intuitionistic fuzzy closure are defined by

int(A) = $\cup\{G/G$ is an IFOS in X and $G \subseteq A\}$

cl(A) = $\cap\{K/K$ is an IFCS in X and $A \subseteq K\}$

Note that for any IFS A in (X,τ), we have $cl(A^C) = [\text{int}]^C$ *and* $\text{int}(A^C) = [cl(A)]^C$.

2.5. Definition

An IFS A = $\{\langle x, \mu_A, \mu_A\rangle\}$ in an IFTS (X,τ) is said to be an

(i) Intuitionistic fuzzy semi open set (Joung Kon *et al.,* 2005) (IFSOS in short) if $A \subseteq cl(\text{int}(A))$,

(ii) Intuitionistic fuzzy $\alpha -$ open set (Joung Kon *et al.,* 2005) (IFα OS in short) if $A \subseteq \text{int}(cl(\text{int}(A)))$,

(iii) Intuitionistic fuzzy semi pre open set (Young Bae and Seok-Zun, 2005) (IFSPOS in short) if $A \subseteq cl(\text{int}(cl(A)))$,

(iv) Intuitionistic fuzzy pre open set (Young Bae and Seok-Zun, 2005)(IFPOS in short) if $A \subseteq \text{int}(cl(A))$.

(v) Intuitionistic fuzzy regular open set (Joung Kon *et al.,* 2005) (IFROS in short) if $A = \text{int}(cl(A))$.

The family of all IFOS (respectively IFSOS, IFα OS, IFSPOS, IFPOS, IFROS) of an IFTS (X,τ) is denoted by IFO(X) (respectively IFSO(X), IFα O(X), IFSPO(X), IFPO(X), IFRO(X)).

2.6. Definition

An IFS A = $\{\langle x, \mu_A, \mu_A\rangle\}$ in an IFTS (X,τ) is said to be an

(i) Intuitionistic fuzzy semi closed set (Joung Kon *et al.,* 2005) (IFSCS in short) if $\text{int}(cl(A)) \subseteq A$,

(ii) Intuitionistic fuzzy α -closed set (Joung Kon *et al.,* 2005) (IFα CS in short) if cl($\text{int}(cl(A))) \subseteq A$,

(iii) Intuitionistic fuzzy semi pre closed set (Young Bae and Seok-Zun, 2005) (IFSPCS in short) if int($cl(\text{int}(A))) \subseteq A$,

(iv) Intuitionistic fuzzy pre closed set (Young Bae and Seok-Zun, 2005) (IFPCS in short) if $cl(\text{int}(A)) \subseteq A$.

(v) Intuitionistic fuzzy regular closed set (Joung Kon *et al.,* 2005) (IFRCS in short) if $A = cl(\text{int}(A))$.

The family of all IFCS (respectively IFSCS, IFα CS, IFSPCS, IFPCS, IFRCS) of an IFTS (X,τ) is denoted by IFC(X) (respectively IFSC(X), IFα C(X), IFSPC(X), IFPC(X), IFRC(X)).

2.7. Definition (Young Bae and Seok-Zun, 2005)

Let A be an IFS in an IFTS (X,τ). Then

sint(A) = $\cup\{G/G \textit{ is an IFSOS in } X \textit{ and } G \subseteq A\}$

scl(A) = $\cap\{K/K \textit{ is an IFSCS in } X \textit{ and } A \subseteq K\}$.

Note that for any IFS A in (X,τ), we have $scl(A^C)$ = (sint $(A))^C$ and sint (A^C) = (scl($A))^C$.

2.8. Definition

An IFS A in an IFTS (X,τ) is an

(i) Intuitionistic fuzzy generalised closed set (Thakur and Rekha, 2006) (IFGCS in short) if cl(A) $\subseteq$U whenever A $\subseteq$U

and U is an IFOS in X.

(ii) Intuitionistic fuzzy generalised semi closed set (Santhi and Sakthivel, 2009) (IFGSCS in short) if scl(A) $\subseteq$ U whenever A

$\subseteq$ U and U is an IFOS in X.

(iii) Intuitionistic fuzzy semi generalised closed set (Santhi and Arun Prakash, 2010) (IFSGCS in short) if scl(A) $\subseteq$ U whenever

A $\subseteq$ U and U is an IFSOS in X.

(iv) Intuitionistic fuzzy α - generalised closed set (Sakthivel, 2010) I (IF α GCS in short) if α cl(A) $\subseteq$ U whenever

A $\subseteq$ U and U is an IFOS in X.

(v) Intuitionistic fuzzy generalised α - closed set (Gowri *et al.,* 2012) (IFG α CS in short) if α cl(A) $\subseteq$ U whenever A

$\subseteq$ U and U is an IF α OS in X.

(vi) Intuitionistic fuzzy generalised semi pre closed set (Young Bae and Seok-Zun, 2005) (IFGSPCS in short) if spcl(A) $\subseteq$ U

whenever A $\subseteq$ U and U is an IFROS in X.

2.9. Definition

Let f be a mapping from an IFTS (X,τ) into an IFTS (Y,σ). Then f is said to be

(i) Intuitionistic fuzzy semi continuous (Joung Kon *et al.,* 2005) (IFS continuous in short) if $f^{-1}(B) \in IFSO(X)$ for every $B \in \sigma$.

(ii) Intuitionistic fuzzy α continuous (Joung Kon *et al.,* 2005) (IF α continuous in short) if $f^{-1}(B) \in IF\alpha O(X)$ for every $B \in \sigma$.

(iii) Intuitionistic fuzzy pre continuous (Joung Kon *et al.,* 2005) (IFP continuous in short) if $f^{-1}(B) \in IFPO(X)$ for every $B \in \sigma$.

(iv) Intuitionistic fuzzy semi pre continuous (Young Bae and Seok-Zun, 2005) (IFSP continuous in short) if $f^{-1}(B) \in IFSPO(X)$ for every $B \in \sigma$.

2.10. Definition (Thakur and Rekha, 2006)

Let f be a mapping from an IFTS (X,τ) into an IFTS (Y,σ). Then f is said to be

(i) Intuitionistic fuzzy generalised continuous (IFG continuous in short) if $f^{-1}(B) \in IFGCS(X)$

for every IFCS B in Y.

(ii) Intuitionistic fuzzy semi generalised continuous (IFSG continuous in short) if

$f^{-1}(B) \in IFSGCS(X)$ for every IFCS B in Y.

(iii) Intuitionistic fuzzy generalised semi continuous (IFGS continuous in short) if

$f^{-1}(B) \in IFGSCS(X)$ for every IFCS B in Y.

(iv) Intuitionistic fuzzy generalised α - continuous (IFG α continuous in short) if

$f^{-1}(B) \in IFG\alpha CS(X)$ for every IFCS B in Y.

(v) Intuitionistic fuzzy α - generalised continuous (IF α G continuous in short) if

$f^{-1}(B) \in IF\alpha GCS(X)$ for every IFCS B in Y.

(vi) Intuitionistic fuzzy generalised semi pre continuous (IFGSP continuous in short) if

$f^{-1}(B) \in IFGSPCS(X)$ for every IFCS B in Y.

2.11. Theorem (Parimala *et al.,*)

Let (X,τ) be an intuitionistic fuzzy topological space. Then the following are hold

(i) Every IFCS in X is an IF ψ CS in X.

(ii) Every IFRCS in X is an IF ψ CS in X.

(iii) Every IF α CS and hence IFSCS in X is an IF ψ CS in X.

(iv) Every IF ψ CS in X is an IFSPCS in X.

(v) Every IF ψ CS in X is an IFGSPCS in X.

(vi) Every IF ψ CS in X is an IFGSCS and hence IFSGCS in X.

INTUITIONISTIC FUZZY ψ - CONTINUOUS MAPPINGS

In this section we introduce intuitionistic fuzzy ψ - continuous mapping and studied some of its properties.

3.1. Definition

A function $f:(X,\tau) \rightarrow (Y,\sigma)$ function intuitionistic fuzzy ψ - continuous (IF ψ -continuous in short) if $f^{-1}(B)$ is an IF ψ CS in (X,τ) for every IFCS B of (Y,σ).

3.2. Example

Let $X=\{a,b\},\ Y=\{u,v\}$ and $T_1=\langle x,\ (0.5,0.3),(0.4,0.3)\rangle$, $T_2=\langle y,\ (0.5,0.7),(0.5,0.3)\rangle$. Then $\tau=\{0_\sim,T_1,1_\sim\}$ and $\sigma=\{0_\sim,T_2,1_\sim\}$ are IFTs on X and Y respectively. Define a mapping $f:(X,\tau)\to(Y,\sigma)$ by f(a) = u and f(b) = v. Then f is an IF ψ - continuous mapping.

3.3. Theorem

Every IF continuous mapping is an IF ψ -continuous mapping but not conversely.

3.3.1. Proof.

Let $f:(X,\tau)\to(Y,\sigma)$ be an IF continuous mapping. Let A be an IFCS in Y. Since f is an IF continuous mapping, $f^{-1}(A)$ is an IFCS in X. Since every IFCS is an IF ψ CS by Theorem 2.11, $f^{-1}(A)$ is an IF ψ CS in X. Hence f is an IF ψ -continuous mapping.

3.4. Example

Let $X=\{a,b\},\ Y=\{u,v\}$ and $T_1=\langle x,\ (0.5,0.3),(0.4,0.3)\rangle$, $T_2=\langle y,\ (0.5,0.7),(0.5,0.3)\rangle$. Then $\tau=\{0_\sim,\ T_1,\ 1_\sim\}$ and $\sigma=\{0_\sim,\ T_2,\ 1_\sim\}$ are IFTs on X and Y respectively. Define a mapping $f:(X,\tau)\to(Y,\sigma)$ by f(a) = u and f(b) = v. The IFS $A=\langle y,\ (0.5,0.3),(0.5,0.7)\rangle$ is IFCS in Y. Then $f^{-1}(A)$ is IF ψ CS in X but not IFCS in X. Therefore f is an IF ψ - continuous mapping but not IF continuous mapping.

3.5. Theorem

Every IF semi continuous mapping is an IF ψ - continuous mapping but not conversely.

3.5.1. Proof.

Let $f:(X,\tau)\to(Y,\sigma)$ be an IFsemi continuous mapping. Let A be an IFCS in Y. Since f is an IF semi-continuous mapping, then $f^{-1}(A)$ is an IFSCS in X by Theorem 2.11. Since every IFSCS is an IF ψ CS in X. Therefore f is an IF ψ - continuous mapping.

3.6. Example

Let $X=\{a,b\},\ Y=\{u,v\}$ and $T_1=\langle x,\ (0.2,0.2),(0.4,0.5)\rangle$, $T_2=\langle y,\ (0.5,0.5),(0.1,0.1)\rangle$. Then $\tau=\{0_\sim,T_1,1_\sim\}$ and $\sigma=\{0_\sim,T_2,1_\sim\}$ are IFTs on X and Y respectively. Define a mapping $f:(X,\tau)\to(Y,\sigma)$ by f(a) = u and f(b) = v. The IFS $A=\langle y,\ (0.1,0.1),(0.5,0.5)\rangle$ is IFCS in Y. Then $f^{-1}(A)$ is IF ψ CS in X but not IFSCS in X. Therefore f is an IF ψ - continuous mapping but not IF semi continuous mapping.

3.7. Theorem

Every IF α -continuous mapping is an IF ψ - continuous mapping but not conversely.

3.7.1. Proof.

Let $f:(X,\tau)\to(Y,\sigma)$ be an IF α -continuous mapping. Let A be an IFCS in Y. Then by hypothesis $f^{-1}(A)$ is an IF α CS in X. Since every IF α CS is an IF ψ CS in X by Theorem 2.11. Therefore f is an IF ψ - continuous mapping.

3.8. Example

Let $X=\{a,b\},\ Y=\{u,v\}$ and $T_1=\langle x,\ (0.2,0.2),(0.4,0.5)\rangle$, $T_2=\langle y,\ (0.5,0.5),(0.1,0.1)\rangle$. Then $\tau=\{0_\sim,T_1,1_\sim\}$ and $\sigma=\{0_\sim,T_2,1_\sim\}$ are IFTs on X and Y respectively. Define a mapping $f:(X,\tau)\to(Y,\sigma)$ by f(a) = u and f(b) = v. The IFS $A=\langle y,\ (0.1,0.1),(0.5,0.5)\rangle$ is IFCS in Y. Then $f^{-1}(A)$ is IF ψ CS in X but not IF α CS in X. Then f is an IF ψ - continuous mapping but not IF α - continuous mapping .

3.9. Theorem

Every IF ψ -continuous mapping is an IFSP continuous mapping but not conversely.

3.9.1. Proof.

Let $f:(X,\tau)\to(Y,\sigma)$ be an IF ψ -continuous mapping. Let A be an IFCS in Y. Then by hypothesis $f^{-1}(A)$ is an IF ψ CS in X. Since every IF ψ CS is an IFSPCS by Theorem 2.11, $f^{-1}(A)$ is an IFSPCS in X. Therefore f is an IFSP continuous mapping.

3.9. Example

Let $X=\{a,b\}$, $Y=\{u,v\}$ and
$T_1=\langle x,\ (0.5,\ 0.7),\ (0.4,\ 0.3)\rangle$,
$T_2=\langle y,\ (0.5,\ 0.3),\ (0.5,\ 0.7)\rangle$. Then $\tau=\{0_\sim,T_1,1_\sim\}$ and $\sigma=\{0_\sim,T_2,1_\sim\}$ are IFTs on X and Y respectively. Define a mapping $f:(X,\tau)\to(Y,\sigma)$ by f(a) = u and f(b) = v. The IFS $A=\langle y,\ (0.5,\ 0.7),(0.5,\ 0.3)\rangle$ is IFCS in Y. Then $f^{-1}(A)$ is IFSPCS in X but not IFψ CS in X. Then f is an IFSP- continuous mapping but not IFψ - continuous mapping .

3.10. Theorem

Every IFψ -continuous mapping is an IFSG continuous mapping but not conversely.

3.10.1. Proof.

Let $f:(X,\tau)\to(Y,\sigma)$ be an IF ψ- continuous mapping. Let A be an IFCS in Y. Then by hypothesis $f^{-1}(A)$ is an IFψ CS in X. Since every IFψ CS is an IFSGCS by Theorem 2.11 , $f^{-1}(A)$ is an IFSGCS in X. Therefore f is an IFSG continuous mapping.

3.11. Example

Let $X=\{a,b\}$, $Y=\{u,v\}$ and
$T_1=\langle x,\ (0.6,\ 0.5),\ (0.3,\ 0.4)\rangle$,
$T_2=\langle y,\ (0.2,\ 0.2),\ (0.6,\ 0.6)\rangle$. Then $\tau=\{0_\sim,T_1,1_\sim\}$ and $\sigma=\{0_\sim,T_2,1_\sim\}$ are IFTs on X and Y respectively. Define a mapping $f:(X,\tau)\to(Y,\sigma)$ by f(a) = u and f(b) = v. The IFS $A=\langle y,\ (0.6,\ 0.6),(0.2,\ 0.2)\rangle$ is IFCS in Y. Here IFOS $G=\langle x,\ (0.7,\ 0.6),(0.2,\ 0.2)\rangle$, clearly $A\subseteq G$. Therefore A is an IFSGCS in X. Then $f^{-1}(A)$ is IFSGCS in X but not IFψ CS in X. Then f is an IFSG continuous mapping but not IFψ - continuous mapping .

3.12. Theorem

Every IFψ - continuous mapping is an IFGS continuous mapping but not conversely.

3.12.1. Proof.

Let $f:(X,\tau)\to(Y,\sigma)$ be an IF ψ- continuous mapping. Let A be an IFCS in Y. Then by hypothesis $f^{-1}(A)$ is an IFψ CS in X. Since every IFψ CS is an IFGSCS by Theorem 2.11, $f^{-1}(A)$ is an IFGSCS in X. Therefore f is an IFGS continuous mapping.

3.13. Example

Let $X=\{a,b\}$, $Y=\{u,v\}$ and
$T_1=\langle x,\ (0.6,\ 0.5),\ (0.3,\ 0.4)\rangle$,
$T_2=\langle y,\ (0.2,\ 0.2),\ (0.6,\ 0.6)\rangle$. Then $\tau=\{0_\sim,T_1,1_\sim\}$ and $\sigma=\{0_\sim,T_2,1_\sim\}$ are IFTs on X and Y respectively. Define a mapping $f:(X,\tau)\to(Y,\sigma)$ by f(a) = u and f(b) = v. The IFS $A=\langle y,\ (0.6,\ 0.6),(0.2,\ 0.2)\rangle$ is IFCS in Y. Here IFOS $G=\langle x,\ (0.7,\ 0.6),(0.2,\ 0.2)\rangle$, clearly $A\subseteq G$. Therefore A is an IFGSCS in X. Then $f^{-1}(A)$ is IFGSCS in X but not IFψ CS in X. Then f is an IFGS continuous mapping but not IFψ - continuous mapping .

3.14. Theorem

Every IFψ -continuous mapping is an IFGSP continuous mapping but not conversely.

3.14.1. Proof.

Let $f:(X,\tau)\to(Y,\sigma)$ be an IF ψ-continuous mapping. Let A be an IFCS in Y. Then by hypothesis $f^{-1}(A)$ is an IFψ CS in X. Since every IFψ CS is an IFGSPCS by Theorem 2.11, $f^{-1}(A)$ is an IFGSPCS in X. Therefore f is an IFGSP continuous mapping.

3.15. Example

Let $X=\{a,b\}$, $Y=\{u,v\}$ and
$T_1=\langle x,\ (0.6,\ 0.5),\ (0.3,\ 0.4)\rangle$,
$T_2=\langle y,\ (0.3,\ 0.4),\ (0.6,\ 0.5)\rangle$. Then $\tau=\{0_\sim,T_1,1_\sim\}$ and $\sigma=\{0_\sim,T_2,1_\sim\}$ are IFTs on X and Y respectively. Define a mapping $f:(X,\tau)\to(Y,\sigma)$ by f(a) = u and f(b) = v. The IFS $A=\langle y,\ (0.6,\ 0.5),(0.3,\ 0.4)\rangle$ is IFCS in Y. Here IFOS $G=\langle x,\ (0.7,\ 0.6),(0.3,\ 0.2)\rangle$, clearly $A\subseteq G$. Therefore A is an IFGSPCS in X. Then $f^{-1}(A)$ is IFGSPCS in X but not IFψ CS in X. Then f is an IFGSP continuous mapping but not IF ψ - continuous mapping .

3.16. Remark

IF ψ -continuity and IFG-continuity are independent of each other.

3.17. Example

Let $X=\{a,b\},\ Y=\{u,v\}$ and $T_1=\langle x,\ (0.4,0.5),(0.5,0.6)\rangle$, $T_2=\langle y,\ (0.4,0.7),(0.2,0.3)\rangle$. Then $\tau=\{0_\sim,T_1,1_\sim\}$ and $\sigma=\{0_\sim,T_2,1_\sim\}$ are IFTs on X and Y respectively. Define a mapping $f:(X,\tau)\to(Y,\sigma)$ by f(a) = u and f(b) = v. Then f is an IFG-continuous but not an IF ψ -continuous mapping since $A=\langle y,\ (0.2,0.3),(0.4,0.7)\rangle$ is an IFCS in Y but $f^{-1}(A)$ $=\langle x,\ (0.2,0.3),(0.4,0.7)\rangle$ is not IF ψ CS in X.

3.18. Example

Let $X=\{a,b\},\ Y=\{u,v\}$ and $T_1=\langle x,\ (0.2,0.2),(0.4,0.4)\rangle$, $T_2=\langle y,\ (0.5,0.5),(0.1,0.1)\rangle$. Then $\tau=\{0_\sim,T_1,1_\sim\}$ and $\sigma=\{0_\sim,T_2,1_\sim\}$ are IFTs on X and Y respectively. Define a mapping $f:(X,\tau)\to(Y,\sigma)$ by f(a) = u and f(b) = v. Then f is an IF ψ -continuous but not an IFG-continuous mapping since $A=\langle y,\ (0.1,0.1),(0.5,0.5)\rangle$ is an IFCS in Y but $f^{-1}(A)$ $=\langle x,\ (0.1,0.1),(0.5,0.5)\rangle$ is not IFGCS in X.

3.19. Remark

IF ψ -continuity is independent from IF α G-continuity, IFG α -continuity and pre-continuity.

3.20. Example

Let $X=\{a,b\},\ Y=\{u,v\}$ and $T_1=\langle x,\ (0.4,0.5),(0.5,0.6)\rangle$, $T_2=\langle y,\ (0.2,0.3),(0.4,0.7)\rangle$. Then $\tau=\{0_\sim,T_1,1_\sim\}$ and $\sigma=\{0_\sim,T_2,1_\sim\}$ are IFTs on X and Y respectively. Define a mapping $f:(X,\tau)\to(Y,\sigma)$ by f(a) = u and f(b) = v. Then f is an IF α G-continuous but not an IF ψ -continuous mapping since $A=\langle y,\ (0.4,0.7),(0.2,0.3)\rangle$ is an IFCS in Y but $f^{-1}(A)=\langle x,\ (0.4,0.7),(0.2,0.3)\rangle$ is not IF ψ CS in X.

3.21. Example

Let $X=\{a,b\},\ Y=\{u,v\}$ and $T_1=\langle x,\ (0.2,0.2),(0.3,0.4)\rangle$, $T_2=\langle y,\ (0.5,0.4),(0.2,0.2)\rangle$. Then $\tau=\{0_\sim,T_1,1_\sim\}$ and $\sigma=\{0_\sim,T_2,1_\sim\}$ are IFTs on X and Y respectively. Define a mapping $f:(X,\tau)\to(Y,\sigma)$ by f(a) = u and f(b) = v. Then f is an IF ψ -continuous but not an IF α G-continuous mapping since $A=\langle y,\ (0.2,0.2),(0.5,0.4)\rangle$ is an IFCS in Y but $f^{-1}(A)=\langle x,\ (0.2,0.2),(0.5,0.4)\rangle$ is not IF α GCS in X.

3.22. Example

Let $X=\{a,b\},\ Y=\{u,v\}$ and $T_1=\langle x,\ (0.6,0.5),(0.3,0.4)\rangle$, $T_2=\langle y,\ (0.2,0.2),(0.6,0.6)\rangle$. Then $\tau=\{0_\sim,\ T_1,\ 1_\sim\}$ and $\sigma=\{0_\sim,\ T_2,\ 1_\sim\}$ are IFTs on X and Y respectively. Define a mapping $f:(X,\tau)\to(Y,\sigma)$ by f(a) = u and f(b) = v. Let $A=\langle y,\ (0.6,0.6),(0.2,0.2)\rangle$ is an IFCS in Y. Here IFOS $G=\langle x,\ (0.9,0.9),(0.1,0.1)\rangle$, clearly $A\subseteq G$. Therefore A is an IFG α CS in X. Then f is an IFG α -continuous but not an IF ψ -continuous mapping since but $f^{-1}(A)$ $=\langle x,\ (0.6,0.6),(0.2,0.2)\rangle$ is not IF ψ CS in X.

3.23. Example

Let $X=\{a,b\},\ Y=\{u,v\}$ and $T_1=\langle x,\ (0.8,0.8),(0.2,0.1)\rangle$, $T_2=\langle y,\ (0.1,0.3),(0.9,0.7)\rangle$. Then $\tau=\{0_\sim,T_1,1_\sim\}$ and $\sigma=\{0_\sim,T_2,1_\sim\}$ are IFTs on X and Y respectively. Define a mapping $f:(X,\tau)\to(Y,\sigma)$ by f(a) = u and f(b) = v. Then f is an IF ψ -continuous but not an IFG α -continuous mapping since $A=\langle y,\ (0.9,0.7),(0.1,0.3)\rangle$ is an IFCS in Y but $f^{-1}(A)=\langle x,\ (0.9,0.7),(0.1,0.3)\rangle$ is not IFG α CS in X.

3.24. Example

Let $X=\{a,b\},\ Y=\{u,v\}$ and $T_1=\langle x,\ (0.4,0.5),(0.5,0.6)\rangle$,

$T_2 = \langle\, y,\ (0.4, 0.7), (0.2, 0.3) \rangle$. Then $\tau = \{0_{\sim}, T_1, 1_{\sim}\}$ and $\sigma = \{0_{\sim}, T_2, 1_{\sim}\}$ are IFTs on X and Y respectively. Define a mapping $f : (X, \tau) \rightarrow (Y, \sigma)$ by f(a) = u and f(b) = v. Then f is an IFP-continuous but not an IF ψ -continuous mapping since $A = \langle\, y,\ (0.2, 0.3), (0.4, 0.7) \rangle$ is an IFCS in Y but $f^{-1}(A)$ $= \langle\, x,\ (0.2, 0.3), (0.4, 0.7) \rangle$ is not IFPCS in X.

3.25. Example

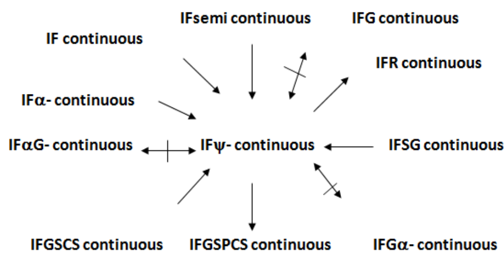
Let $X = \{a, b\}$, $Y = \{u, v\}$ and $T_1 = \langle\, x,\ (0.3, 0.4), (0.7, 0.6) \rangle$, $T_2 = \langle\, y,\ (0.4, 0.5), (0.6, 0.5) \rangle$. Then $\tau = \{0_{\sim}, T_1, 1_{\sim}\}$ and $\sigma = \{0_{\sim}, T_2, 1_{\sim}\}$ are IFTs on X and Y respectively. Define a mapping $f : (X, \tau) \rightarrow (Y, \sigma)$ by f(a) = u and f(b) = V. Then f is an IF ψ -continuous but not an IFP-continuous mapping since $A = \langle\, y,\ (0.6, 0.5), (0.4, 0.5) \rangle$ is an IFCS in Y but $f^{-1}(A)$ $= \langle\, x,\ (0.6, 0.5), (0.4, 0.5) \rangle$ is not IFPCS in X.

3.26. Theorem

A mapping $f : X \rightarrow Y$ is an IF ψ -continuous if and only if the inverse image of each IFOS in Y is an IF ψ OS in X.

3.26.1. Proof.

Let A be an IFOS in Y. This implies A^C is an IFCS in Y. Since f is an IF ψ -continuous, $f^{-1}(A^C)$ is IF ψ CS in X. Since $f^{-1}(A^C)$ = $(f^{-1}(A))^C$, $f^{-1}(A\ )$ is an IF ψ OS in X.



REFERENCES

Atanassov, K. (1986), Intuitionistic fuzzy sets. *Fuzzy Sets Syst.* **20:** 87-96.

Coker, D. (1997). An introduction to fuzzy topological space. *Fuzzy Sets Syst.* **88**: 81-89.

Gowri, C.S., K. Sakthivel and K. Kalamani, (2012). Generalised Alpha continuous mapping in intuitionistic fuzzy topological spaces. *Global J. Math. Sci.* **4**: 407-420.

Joung Kon, J., J. Young Bae and J. Han Park, (2005). Intuitionistic fuzzy alpha continuity and intuitionistic fuzzy pre continuity. *Inter J. Math. Math. Sci.* **19**: 3091-310.

Parimala, M., C. Indirani, and A. Selvakumar. Annal of Fuzzy Mathematics and Informatics, On intuitionistic. *Fuzzy ψ -Closed Sets And Its Application,* (Accepted).

Sakthivel, K. (2010). Intuitionistic fuzzy Alpha generalised Continuous mappings and Intuitionistic fuzzy alpha generalised Irresolute Mappings. *Appl. Math. Sci.* **4**: 1831-1842.

Santhi, R. and Sakthivel, (2009). Intuitionistic fuzzy generalised semi continuous mappings. *Advanc. in Theoret. Appl. Math.* **5**: 73-82.

Santhi, R., K. Arun Prakash, (2010). On intuitionistic fuzzy semi-generalised closed sets and its Applications. *Int. J. Contemp. Math. Sciences,* **5**: 1677-1688.

Thakur, S.S. and C. Rekha, (2006). Regular generalised closed sets in intuitionistic fuzzy topological spaces, *Universitatea Din Bacau, Studii Si Ceretari Stiintifice, Seria, Mathematica,* **16**: 257-272.

Young Bae, J. and S. Seok-Zun, (2005). Intuitionistic fuzzy semi-preopen sets and intuitionistic fuzzy semi-pre continuous mappings. *J. Appl. Math. & computing* **19:** 467-474.

Zadeh, L.A. (1965). Fuzzy sets. *Inform. Control* **8**: 338-353.

Kong. Res. J. 2(1) : 47-49, 2015 ISSN 2349-2694
Kongunadu Arts and Science College, Coimbatore.

ON TOTALLY SUPRA N-CONTINUOUS FUNCTION AND TOTALLY SUPRA N-CLOSED MAP

Vidyarani, L. and M. Vigneshwaran*
Department of Mathematics, Kongunadu Arts and Science College(Autonomous), Coimbatore-641029, Tamilnadu, India.
*E.mail: vignesh.mat@gmail.com

ABSTRACT

In this paper, we introduce the concept of totally supra N-continuous function and totally supra N-closed map and investigated the relationship of these functions with other functions.
Mathematics subject Classification: 54C05, 54C10.

Keywords: Totally supra N-continuous function, totally supra N-closed map.

1. INTRODUCTION

In 1983, Mashhour *et al.* (1983) introduced the notion of supra topological spaces and studied, continuous functions and s* -continuous functions. Jamal M.Mustafa (2012) introduced and studies a class of functions called totally supra b-continuous and slightly supra b-continuous functions in supra topological spaces.

In this paper, we introduce the concept of totally supra N-continuous function and totally supra N-closed map and investigated the relationship of these functions with other functions in supra topological spaces.

2. PRELIMINARIES

Definition 2.1(Mashhour *et al.*,1983)

A subfamily μ of X is said to be supra topology on X if

i) $X, \phi \in \mu$

ii)If $A_i \in \mu \ \forall i \in j$ then $\cup A_i \in \mu$. The pair (X,μ) is called supra topological space.

The element of μ are called supra open sets in (X, μ) and the complement of supra

open set is called supra closed sets and it is denoted by μ^c.

Definition 2.2 (Mashhour *et al.*, 1983)

The supra closure of a set A is denoted by clμ (A), and is defined as supra cl(A) = ∩ {B : B is supra closed and A ⊆ B}.

The supra interior of a set A is denoted by intμ(A), and is defined as supraint(A) = ∪ {B : B is supra open and A ⊇ B}.

Definition 2.3 (Mashhour *et al.*, 1983) Let (X, τ) be a topological space and μ be a supra topology on X. We call μ a supra topology associated with τ , if τ ⊆ μ.

Definition 2.4 Let (X, μ) be a supra topological space. A set A of X is called

(i)supra semi- open set (Levine, 1991), if A ⊆ clμ(intμ(A)).

(ii) supra α -open set (Devi *et al.,* 2008), if A ⊆ intμ(clμ (intμ (A))).

(iii) supra Ω closed set (Noiri and Sayed, 2005), if sclμ(A) ⊆ intμ (U),whenever A ⊆ U , U is supra open set.

(iv) supra N-closed set (Vidyarani and Vigneshwaran, 2013), if Ωclμ (A) ⊆ U , whenever A ⊆ U , U is supra α open set.

The complement of above supra closed set is supra open and vice versa.

Definition 2.5

A map f:(X, τ) → (Y, σ) is said to be

(i) supra N-continuous (Vidyarani and Vigneshwaran, 2013a), if $f^{-1}(V)$ is supra N-closed in (X, τ) for every supra closed set V of (Y, σ).
(ii) Perfectly supra N-continuous (Vidyarani and Vigneshwaran, 2013a), if $f^{-1}(V)$ is supra clopen in (X, τ) for every supra N-closed set V of (Y, σ).
(iii) strongly supra N-continuous (Vidyarani and Vigneshwaran, 2013a), if $f^{-1}(V)$ is supra closed in (X, τ) for every supra N-closed set V of (Y, σ).
(iv) supra N-closed map (Vidyarani and Vigneshwaran, 2013b), if f(V) is supra N-closed in (Y, σ) for every supra closed set V of (X, τ).

(v) strongly supra N-closed map (Vidyarani and Vigneshwaran, 2013b), if f(V) is supra N-closed in (Y, σ) for every supra N-closed set V of (X, τ).

3. TOTALLY SUPRA N-CONTINUOUS FUNCTIONS

Definition 3.1 A map f:(X, τ) → (Y, σ) is called totally supra continuous function if the inverse image of every supra open set in (Y, σ) is supra clopen in (X, τ).

Definition 3.2 A map f:(X, τ) → (Y, σ) is called totally supra N-continuous function if the inverse image of every supra open set in (Y, σ) is supra N-clopen in (X, τ).

Theorem 3.3 Every strongly supra N-continuous function is totally supra N-continuous function.

Proof Let f:(X, τ) → (Y, σ) be a strongly supra N-continuous function. Let V be supra open set in (Y, σ). Then V is supra N-open set in (Y, σ), since every supra open set is supra N-open set. Since f is strongly supra N-continuous function $f^{-1}(V)$ is both supra open and supra closed in (X, τ). Implies $f^{-1}(V)$ is supra N-clopen in (X, τ). Therefore f is totally supra N-continuous function.

The converse of the above theorem need not be true. It is shown by the following example.

Example 3.4 Let X=Y={a, b, c} and τ = {X, φ, {a},{b},{a,b},{b, c}} , σ = {Y, φ, {a} }. N-closed set in (X, τ) are {X, φ, {a},{b},{a,b},{b, c}}. N-closed set in (Y, σ) are {Y, φ, {a},{b},{c},{a,b},{b, c},{a,c}}. f:(X, τ) → (Y, σ) be the function defined by f(a)=a, f(b)=c, f(c)=b. Here f is totally supra N-continuous but not strongly supra N-continuous, since V={a,c} is supra N-closed in (Y, σ) but $f^{-1}(\{a,c\})$ = {a,b} is supra open but not supra closed set in (X, τ).

Theorem 3.5 Every totally supra N-continuous function is supra N-continuous function.

Proof Let f:(X, τ) → (Y, σ) be a totally supra N-continuous function. Let V be supra open set in (Y, σ). Since f is totally supra N-continuous function, then $f^{-1}(V)$ is supra N-clopen in (X, τ). Implies $f^{-1}(V)$ is supra N-open in (X, τ). Therefore f is supra N-continuous function.

The converse of the above theorem need not be true. It is shown by the following example.

Example 3.6 Let X=Y={a, b, c} and τ = {X, φ, {a},{b},{a,b},{b, c}} , σ = {Y, φ, {a},{b,c} }. N-closed set in (X, τ) are {X, φ, {a},{b},{a,b},{b, c}}. N-closed set in (Y, σ) are {Y, φ, {a},{b},{c},{a,b},{b, c},{a,c}}. f:(X, τ) → (Y, σ) be the function defined by f(a)=c, f(b)=b, f(c)=a. Here f is supra N-continuous but not totally supra N-continuous, since V={a} is supra open in (Y, σ) but $f^{-1}(\{a\})$ = {c} is supra N-closed but not supra N-open set in (X, τ).

Theorem 3.7 Every totally supra continuous function is supra N-continuous function.

Proof Let f:(X, τ) → (Y, σ) be a totally supra continuous function. Let V be supra open set in (Y, σ). Since f is totally supra continuous function, then $f^{-1}(V)$ is supra clopen in (X, τ). Implies $f^{-1}(V)$ is supra N-clopen in (X, τ). Hence $f^{-1}(V)$ is supra N-open in (X, τ). Therefore f is supra N-continuous function.

The converse of the above theorem need not be true. It is shown by the following example.

Example 3.8 Let X=Y={a, b, c} and τ = {X, φ, {a},{b},{a,b},{b, c}} , σ = {Y, φ, {a},{b,c} }. N-closed set in (X, τ) are {X, φ, {a},{b},{a,b},{b, c}}. N-closed set in (Y, σ) are {Y, φ, {a},{b},{c},{a,b},{b, c},{a,c}}. f:(X, τ) → (Y, σ) be the function defined by f(a)=c, f(b)=b, f(c)=a. Here f is supra N-continuous but not totally supra continuous, since V={b,c} is supra open in (Y, σ) but $f^{-1}(\{b,c\})$ = {a,b} is supra open but not supra closed set in (X, τ).

Theorem 3.9 Every totally supra continuous function is totally supra N-continuous function.

Proof Let f:(X, τ) → (Y, σ) be a totally supra continuous function. Let V be supra open set in (Y, σ). Since f is totally supra continuous function, then $f^{-1}(V)$ is supra clopen in (X, τ). Implies $f^{-1}(V)$ is supra N-clopen in (X, τ). Therefore f is totally supra N-continuous function.

The converse of the above theorem need not be true. It is shown by the following example.

Example 3.10 Let X=Y={a, b, c} and τ = {X, φ, {a}} , σ = {Y, φ, {a},{b},{a,b},{b,c} }. N-closed set in (X, τ) are {X, φ, {a},{b},{c},{a,b},{b, c},{a,c}}. N-closed set in (Y, σ) are {Y, φ, {a},{b},{b, c},{a,c}}. f:(X, τ) → (Y, σ) be the function defined by f(a)=b, f(b)=a, f(c)=c. Here f is totally supra N-continuous but not totally supra continuous, since V={a,b} is supra open in (Y, σ) but $f^{-1}(\{a,b\})$ = {a,b} is not supra clopen set in (X, τ).

Theorem 3.11 If f:(X, τ) → (Y, σ) is totally supra N-continuous and g: (Y, σ) →(Z,η) is supra continuous then gof: (X, τ) →(Z,η) is totally supra N-continuous.

Proof Let V be supra open set in Z. Since g is supra continuous, then $g^{-1}(V)$ is supra open set in Y. Since f is totally supra N-continuous, then $f^{-1}(g^{-1}(V))$ is supra N-clopen in X. Hence gof is totally supra N-continuous.

Theorem 3.12 If f:(X, τ) → (Y, σ) is perfectly supra N-continuous and g: (Y, σ) →(Z,η) is totally supra N-continuous then gof: (X, τ) →(Z,η) is totally supra N-continuous.

Proof Let V be supra open set in Z. Since g is totally supra N-continuous, then $g^{-1}(V)$ is supra N-closed and supra N-open set in Y. Since f is perfectly supra N-continuous, then $f^{-1}g^{-1}(V)$ is supra clopen in X. Implies f^{-1} $g^{-1}(V)$ is supra N-clopen in X. Hence gof is totally supra N-continuous.

4. TOTALLY SUPRA N-CLOSED MAP

Definition 4.1 A map f:(X, τ) → (Y, σ) is said to be totally supra N-closed map, if f(V) is supra clopen in (Y, σ) for every supra N-closed set V of (X, τ).

Theorem 4.2 Every totally supra N-closed map is supra N-closed map.

Proof Let f:(X, τ) → (Y, σ) be a totally supra N-closed map. Let V be supra closed set in (X, τ), then V is supra N-closed set in (X, τ), since every supra closed set is supra N-closed set. Since f is totally supra N-closed map, then f(V) is supra clopen in (Y, σ). Implies f(V) is supra closed in (Y, σ). Therefore f(V) is supra N-closed in (Y, σ). Therefore f is supra N-closed map.

The converse of the above theorem need not be true. It is shown by the following example.

Example 4.3 Let X=Y={a, b, c} and τ = {X, φ, {a,b}} , σ = {Y, φ, {a,b},{b,c} }. N-closed set in (X, τ) are {X, φ, {c},{a,c},{b, c}}. N-closed set in (Y, σ) are {Y, φ, {a},{c},{a,c}}. f:(X, τ) → (Y, σ) be the function defined by f(a)=c, f(b)=b, f(c)=a. Here f is supra N-closed map but not totally supra N-closed map, since V={b,c} is supra N-closed in (X, τ) but f ({b,c}) = {a,b} is supra open but not supra closed set in (Y, σ).

Theorem 4.4 Every totally supra N-closed map is strongly supra N-closed map.

Proof Let f:(X, τ) → (Y, σ) be a totally supra N-closed map. Let V be supra N-closed set in (X, τ). Since f is totally supra N-closed map, then f(V) is supra clopen in (Y, σ). Implies f(V) is supra closed in (Y, σ). Therefore f(V) is supra N-closed in (Y, σ). Therefore f is strongly supra N-closed map.

The converse of the above theorem need not be true. It is shown by the following example.

Example 4.5 Let X=Y={a, b, c} and τ = {X, φ,{a},{a,b}} , σ = {Y, φ, {a},{c},{a,c} }. N-closed set in (X, τ) are {X, φ, {b},{c},{a,c},{b, c}}. N-closed set in (Y, σ) are {Y, φ, {a},{b},{c},{a,b},{b,c}}. f:(X, τ) → (Y, σ) be the function defined by f(a)=a, f(b)=c, f(c)=b. Here f is strongly supra N-closed map but not totally supra N-closed map, since V={b} is supra N-closed in (X, τ) but f ({b}) = {c} is supra open but not supra closed set in (Y, σ).

Theorem 4.6 If f:(X, τ) → (Y, σ) is totally supra N-closed map and g: (Y, σ) →(Z,η) is totally supra N-closed map then gof: (X, τ) →(Z,η) is totally supra N-closed map.

Proof Let V be supra N-closed set in X, then f(V) is supra clopen in Y, since f is totally supra N-closed map. Implies f(V) is supra closed in Y. Then f(V) is supra N-closed in Y, since every supra closed set is supra N-closed set. Since g is totally supra N-closed map g(f(V)) is supra clopen in Z. Hence gof is totally supra N-closed map.

REFERENCES

Devi, R., S. Sampathkumar and M. Caldas, (2008). On supra α open sets and sα-continuous maps, *Gen. Math.* **16**(2): 77-84.

Jamal M. Mustafa, (2012). Totally supra b-continuous and slightly supra b-continuous functions, stud. *Univ. Babes. Bolyai Math.* 57(1): 135-144.

Levine, N., (1991). Semi-open sets and Semi-continuity in topological spaces, *Amer. Math.* **12**: 5-13.

Mashhour, A.S., A.A. Allam, F.S. Mahmoud and F.H. Khedr, (1983). On supra topological spaces, *Indian J. Pure and Appl.Math.***14**(A): 502-510.

Noiri, T. and O.R. Sayed, (2005). On Ω closed sets and Ωs closed sets in topological spaces, *Acta Math*., **4**:307-318.

Vidyarani, L. and M. Vigneshwaran, (2013). N-Homeomorphism and N*-Homeomorphism in supra topological spaces. *Int. J. Math. Stat. Inv.* **1**(2): 79-83.

Vidyarani, L. and M. Vigneshwaran, (2013a). Some forms of N-closed maps in supra Topological spaces. *IOSR J. Math.* **6**(4): 13-17.

Vidyarani, L. and M. Vigneshwaran, (2013b). On supra N-closed and sN-closed sets in supra Topological spaces. *Int. J. Math. Arch.* **4**(2): 255-259.

Kong. Res. J. 2(1) : 50-52, 2015 ISSN 2349-2694
Kongunadu Arts and Science College, Coimbatore.

A STUDY ON FUZZY SHORTEST ROUTE ALGORITHM FOR TELEPHONE LINE CONNECTION

Vijayalakshmi, D. and R. Kalaivani*
Department of Mathematics, Kongunadu Arts & Science College, Coimbatore-641 029, India
*E-mail: kalaivanirm@yahoo.com

ABSTRACT

In computer science, there are many algorithms that finds a minimum spanning tree for a connected weighted undirected fuzzy graph. The minimum length (or cost) spanning tree problem is one of the nicest and simplest problems in network optimization, and it has a wide variety of applications.The problem is to find a minimum cost (or length) spanning tree in G. Applications include the design of various types of distribution networks in which the nodes represent cities, centers etc.; and edges represent communication links (fiber glass phone lines, data transmission lines, cable TV lines, etc.), high voltage power transmission lines, natural gas or crude oil pipelines, water pipelines, highways, etc. The objective is to design a network that connects all the nodes using the minimum length of cable or pipe or other resource.In this paper we find the solution to the problem is to minimize the amount of new telephone line connection using matrix algorithm with fuzzy graph.

Keywords: Spanning tree, minimum spanning tree, fuzzy graph, Matrix algorithm.

1. INTRODUCTION

A minimum cost of the fuzzy spanning tree is spanning tree, but it has weight or length associated with the edges and total weight of the tree is minimum. A fuzzy tree for that graph would be a subset of those paths that has no cycles but still connects to every vertex. There might be several spanning trees possible. A minimum fuzzy tree would be one with the lowest total cost. A less obvious application is that the minimum fuzzy spanning tree can be used to approximately solve the traveling salesman problem (Hassan, 2012).

In this paper, we find the solution for the problem, (Abhilasha, 2013) that (A.V.V.M. Sri Pushpam College, (Autonomous), Poondi, Thanjavur District, South India) needs to connect updated intercom lines connecting all the departments using a fuzzy shortest route algorithm (Amit Kumar and Manjot Kaur, 2011). The problem is to minimize the amount of new line using matrix Algorithm with fuzzy graph.

2. Definition

2.1. Definition

A ***fuzzy graph*** with V as the underlying set is a pair G: (A, Γ) where A: V --� [0,1] is a fuzzy subset, Γ : VxV ------> [0,1] is a fuzzy relation on the fuzzy subset A, such that Γ(u,v) ≤ A(u) ∩ A(v) for all u,v є V (Antony Xavior *et al.,* 2013).

2.2. Definition

A **fuzzy Hamiltonian circuit** is a circuit that visits every vertex in a fuzzy graph once with no repeats, being a fuzzy Hamiltonian circuits must start and end at the same vertex.

2.3. Definition

A **fuzzy Hamiltonian path** is a path that passes through each of the vertices in a fuzzy graph exactly once.

2.4. Definition

A fuzzy spanning tree is a fuzzy tree which covers all the vertices of a fuzzy graph.

NOTE 2.1: Fuzzy tress has no circuits, and it is fine to have vertices

2.5. Definition

A fuzzy graph $\hat{G} = (V, \sigma, \mu)$ is called a complete fuzzy graph (Nivethana and Parvathi, 2013)

if $\mu(uv) = \sigma(u) \cap \sigma(v)$ for all $u, v \in V$ and $uv \in E$. We denote this complete fuzzy graph by $\hat{G}k$.

3. MATRIX ALGORITHM

Let G = (V, E) be an undirected connected weighted graph with n vertices, where V is the set of vertices, E is the set of edges and W be the set of weights (cost) associated to respective edges of the graph. Where

e_{ij}, the edge adjacent to vertices v_i and v_j.

w_{ij}, the weight associated to the edge e_{ij}.

The Weight Matrix M of the graph G is constructed as follows:

If there is an edge between the vertices v_i to v_j in G then Set,

$M_{[i,j]} = w_{ij}$

Else

Set, $M_{[i,j]} = 0$

Input: the weight matrix $M = [w_{ij}]_{n \times n}$ for the undirected weighted graph G

Step 1: Start

Step 2: Repeat Step 3 to Step 4 until all (n-1) elements matrix of M are marked or set to zero or in other words all the nonzero elements are marked

Step 3: Search the weight matrix M by row-wise to find the unmarked nonzero minimum element $M_{[i,j]}$, which is the weight of the corresponding edge e_{ij} in M.

Step 4: If the corresponding edge e_{ij} of selected $M_{[i,j]}$ forms cycle with the already marked elements in the elements of the M then Set $M_{[i,j]} = 0$ else mark $M_{[i,j]}$

Step 5: Construct the graph T including only the marked elements from the weight matrix M which shall be the desired Minimum cost spanning tree of G.

Output: Minimum Cost Spanning Tree T of G.

Example:

In this example we applied the matrix algorithm to plan and connect an efficient fuzzy route telephone line connection for the problem (Nirmala and Uma, 2012). Consider each department as vertex such as x_1 –office room, x_2- mathematics, x_3 – economics, x_4 – History, x_5 – Computer Science, x_6 – Library, x_7– Physics, x_8 – Chemistry, x_9 – Botany and x_{10} – Physical education. The distance between them are represented as fuzzy weights matrix shown in the fig.3.8.

In this section, we find the solution for the below matrix the table is taken from (Nirmala and Uma, 2012) needs to connect updated intercom lines connecting all the departments exactly once. The problem is to minimize the amount of new line using Matrix Algorithm (Vijayalakshmi and Kalaivani, 2014) with fuzzy graph. The step by step procedure is given below.

Distance between the departments is shown in below matrix:

-	x_1	x_2	x_3	x_4	x_5	x_6	x_7	x_8	x_9	x_{10}
x_1	-	0.37	0.1	0.22	0.07	0.17	0.25	0.28	0.24	0.35
x_2	0.37	-	0.25	0.16	0.5	0.19	0.07	0.1	0.13	0.01
x_3	0.1	0.25	-	0.12	0.27	0.12	0.09	0.16	0.13	0.24
x_4	0.22	0.16	0.12	-	0.43	0.03	0.04	0.08	0.02	0.15
x_5	0.07	0.5	0.27	0.43	-	0.45	0.47	0.34	0.38	0.42
x_6	0.17	0.19	0.12	0.03	0.45	-	0.06	0.11	0.06	0.18
x_7	0.25	0.07	0.09	0.04	0.47	0.06 0	-	0.11	0.08	0.11
x_8	0.28	0.1	0.16	0.08	0.34	0.11	0.11	-	0.03	0.05
x_9	0.24	0.13	0.13	0.02	0.38	0.06	0.08	0.03	-	0.11
x_{10}	0.35	0.01	0.24	0.15	0.42	0.18	0.11	0.05	0.11	-

Fig. 2.2: Weight matrix.

From the above fig 3.7 the minimum weight is selected and colored, their corresponding edges were drawn repeat the process until the algorithm terminates. While drawing the graph if its forms fuzzy circuit, we remove that and in the table the weight is marked as 0.

x_2	to	x_{10}	0.01
x_4	to	x_9	0.02
x_4	to	x_6	0.03
x_4	to	x_7	0.04
x_8	to	x_{10}	0.05
x_7	**to**	**x_6**	**(forms fuzzy circuit) its shown in belowFig.3.7)**
x_1	to	x_5	0.07
x_4	to	x_8	0.08
x_3	to	x_7	0.09
x_1	to	x_3	0.1

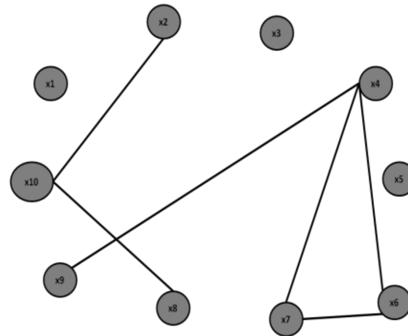


Fig. 2.3: Fuzzy circuit {x_4,x_6,x_7}

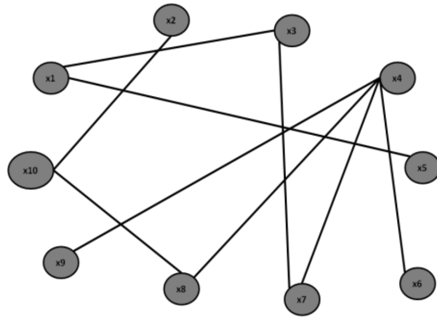


Fig 2.4: Updated fuzzy route telephone line connection

Using the Matrix Algorithm

The length of the cable

$=\{(x_2,x_{10})+(x_4,x_9)+(x_4,x_6)+(x_4,x_7)+(x_8,x_{10})+(x_1,x_5)+(x_1,x_3)+(x_4,x_8)+(x_3,x_7)\}$

$= 0.01+0.02+0.03+0.04+0.05+0.07+0.08+0.09+0.1$

$= 0.49.$

4. CONCLUSION

In this paper we apply the Matrix Algorithm for the problem that connecting distances to plan and visit an efficient fuzzy spanning tree route for the intercom land line (phone) to reach it all the department exactly once. So we conclude that Matrix Algorithm is the best to adopt for these types of problems.

REFERENCES

Abhilasha, R. (2013). Minimum cost spanning tree using prim's Algorithm. *Int. J. Adv. Res. Comp. Sci. Manag. Stru.* **1(**1).

Amit Kumar and Manjot Kaur (2011). A new algorithm for solving network flow problems with fuzzy arc lengths. *Turk. J. Fuzzy Sys.* **2(**1).

Antony Xavior, D., D. Florence Isido and V.M. Chtitra (2013). On Domination in fuzzy graphs. *Int. J. Comp. Alg.* **2**: 248-250.

Hassan (2012). An efficient method to solve least cost minimum spanning tree. *Comp. Inform. Sci.* **24**: 101-105.

Nirmala, G. and K. Uma (2012). Fuzzy Shortest Route Algorithm for Telephone Line Connection, *Int. J. Sci. Res. Pub.* **2**.

Nivethana, V. and A. Parvathi (2013). Fuzzy Total Coloring and Chromatic Number of a Complete Fuzzy Graph. *Int. J. Emerg. Trends Eng. Dev.* **6**(3).

Vijayalakshmi, D., R. Kalaivani (2014). Minimum Cost Spanning tree using Matrix Algorithm. *Int. J. Sci. Res. Pub.* **4**(9).

Kong. Res. J. 2(1) : 53-55, 2015 ISSN 2349-2694
Kongunadu Arts and Science College, Coimbatore.

ANTIMICROBIAL ACTIVITY OF *SYZYGIUM GARDNERI* Thw. (MYRTACEAE).

Asha devi, V., A. Shalimol*, K. Arumugasamy, R. Nantha kumar and H. Abdul kaffoor
PG and Research Department of Botany, Kongunadu Arts and Science College, Coimbatore-641029.
*Email id: shalimol.anamika@gmail.com

ABSTRACT

The present study was evaluated the antibacterial and antifungal activity of various extracts of *S. gardneri* against four different bactria and fungal strains like *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Salmonella para typhi b, Alternaria alternate, Aspergillus flavus, Penicillium notatum* and *Cladosporium carrionii.* All the results were compared with respective positive control.

Keywords: *Syzygium gardneri,* antimicrobial activity.

1. INTRODUCTION

Infectious diseases account for high proportion of health problems in the developing countries like India. Microorganisms have developed resistance to many antibiotics and created immense clinical problem in the treatment of infectious diseases. The resistance of the organism increased due to the indiscriminate use of commercial antimicrobial drugs commonly used.This situation forced the scientist to search for new antimicrobial substances from various sources including medicinal plants (Sashikumar *et al.,* 2003). However several plants are used in India in the form of crude extracts, infusions or plaster to treat common infections without scientific evidence of efficacy (Ahmad *et al*., 1998).

In India, the floristic accounts suggest that *Syzygium gardneri* occur evergreen forests in the country. It is used in treatment of diabetes mellitus, inflammation, ulcers and diarrhea, dysentery possessing also anti-neoplastic, chemopreventive and radio-protective properties. The seeds and bark are well known in the Far East for the treatment of dysentery and in control of hyperglycemia and glycosuria in diabetic patients. The astringent bark may be used as a gargle. Fruits are used as a relief for colic, while the wood yields a sulphate pulp that has medicinal uses.

2. MATERIALS AND METHODS

2.1. Plant collection and identification

The Plant *Syzygium gardneri* Thw. was collected from Valparai, Coimbatore district, Tamilnadu. The plant was identified and authenticated by a plant taxonomist.

2.2. Extraction of the plant material

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

2.3. Tested Microorganisms

Bacterial strains *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Salmonella para typhi b,* and fungal strains *Alternaria alternate, Aspergillus flavus, Cladosporium carrionii,* and *Penicillium notatum,* were employed.

2.4. Disc diffusion method

The antimicrobial activity of leaves and bark extracts of *Syzygium gardneri* was evaluated by disc diffusion method. The culture media were prepared and autoclaved at 121˚C at 15 psi for 20 minutes and stored in refrigerator. The media were melted before the process of inoculation. The clean dry sterile Petri dishes were poured with nutrient agar medium for bacterial strains and potato dextrose agar medium for fungal strains. Ten number of 10 ml broths were prepared separately for nutrient agar medium and potato dextrose agar medium in test tubes and plugged with cotton and autoclaved. The test tubes were labelled according to the microbes to be inoculated. The bacterial strains were inoculated into the nutrient broth and fungi were inoculated on to potato dextrose broth under aseptic conditions and incubated at 37+0.5 ˚C for 18 hours. After

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

3. RESULTS

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

The results of fungal study showed that among the four extracts, methanol showed significant result of antifungal activity. The results of the extracts, aqueous extract showed minimum level of inhibition. *In vitro* anti-fungal studies of different solvent extracts of *S. gardneri* revealed that the methanol extract had significant activity against most of the organism tested (Table-2). Methanol extract has exhibited the maximum inhibitory effect against fungal strain, *A. alternata* and moderate activity against *A. flavus* and *P. notatum*. Whereas, in aqueous extract has no activity. The positive control chloramphenical showed highest activity against all organisms tested. *C. carrionii* found to be highly susceptible to methanol extract.

4. DISCUSSION

The results showed that the aqueous, petroleum ether, ethanol and methanol extracts of *Syzygium gardneri* has revealed a significant results of antibacterial activity when compared with other extracts except aqueous showed minimum level of inhibition. Among all the bacterial strains, maximum zones were observed in the following bacterial strains such as *B.subtilis, P. aeruginosa* and *E. coli. S. paratyphi- b* were found to be highly susceptible to methanol extract. Plant extracts are potential sources of novel anti-microbial compounds especially against bacterial pathogens. Phytomedicine can be used for the treatment of diseases as in case of Unani and Ayurvedic system of medicine or it could be the base for the development of medicine, a natural blue print for the development of a drug (Didry *et al*., 1988).

In Myrtaceae, family plants showed the efficient antimicrobial effect on microbial organisms. The anti-microbial activity of ethanol, methanol and aqueous extracts of leaf, stem and root of *Acanthus ilicifolius* were studied the strains like *Bacillus megaterium, Lactobacillus plantarum, Salmonella paratyphi B, Shigella dycentreae, Escherichia coli, Streptococcus mutans, Klebsiella pneumoniae, Candida albicans, Aspergillus flavus, Staphylococcus albus* and *Lactobacillus acidophilus,* revealed that the solvent used in extractions had significant effect on the level of significant. Aqueous and acetone extracts of bark, leaves and seeds of *Syzygium jambos* was tested for anti-microbial activity *in vitro* by the agar well diffusion method in petri dishes. Both extracts showed moderate activity against all the tested microorganisms. Among the three different parts, aqueous extract of bark has exhibited a minimum inhibitory effect against *S. aureus*, *E. coli* and *S. typhi*, whereas seeds inhibited the growth of *P. aeruginosa* and *V. cholerae*, and leaves exhibited inhibitory effect only against *S. typhi*. Among the acetone extracts, bark was found to be effective against the entire test microorganisms. Leaf extract has inhibited only *S. aureus*, whereas seed extracts failed to exhibit any inhibitory effect against the test organisms (Murugan *et al*., 2011).

Table 1. Antibacterial activity of various solvent extracts of *Syzygium gardneri.*

S. No.	Microorganisms	Zone of Inhibition (mm)				
		Various solvent extracts used (mg/ml)				Control*
		A	PE	E	M	
1	*Bacillus subtilis*	10	11	11	13	15
2	*Escherichia coli*	10	12	11	12	14
3	*Pseudomonas aeruginosa*	9	10	10	12	16
4	*Salmonella para typhi b*	8	10	9	11	12

A-Aqueous; PE-Petroleum Ether; E-Ethanol; M-Methanol; *Chloramphenical

In vitro anti-fungal studies of different solvent extracts of *Syzygium gardneri* revealed that the methanol extract had significant activity against most of the organism tested. Methanol extract exhibited the maximum inhibitory effect against fungal strain, *Alternaria alternata* and moderate activity against *A.flavus* and *P.notatum*. The positive control chloramphenical showed highest activity

against all organisms tested. *C. carrionii* found to be highly susceptible to methanol extract.

Table 2. Antifungal activity of various solvent extracts of *Syzygium gardneri.*

Sl. No.	Microorganisms	Zone of Inhibition (mm)				
		Various solvent extracts used (mg/ml)				Control
		A	PE	E	M	
1	*Alternaria alternata*	8	10	10	12	15
2	*Aspergillus flavus*	9	10	10	13	17
3	*Cladosporium carrionii*	9	11	12	14	16
4	*Penicillium notatum*	10	11	11	12	14

A-Aqueous; PE-Petroleum Ether; E-Ethanol; M-Methanol; *Chloramphenical

Myrtaceae family plants showed the efficient effect on microbial organisms. The anti-microbial activity of ethanol, methanol and aqueous extracts of leaf, stem and root of *Acanthus ilicifolius* was studied against different strains like *Bacillus megaterium, Salmonella paratyphi-B, Escherichia coli, Candida albicans, Aspergillus flavus,* and *Lactobacillus acidophilus.*The inhibitory effect of the extracts on *Lactobacillus acidophillus* showed no significant difference (P>0.05) between extract concentration. The most active anti-microbial parts were aqueous root, ethanol stem and leaf. Aqueous and acetone extracts of bark, leaves and seeds of *S. jambos* was tested for anti-microbial activity *in vitro* by the agar well diffusion method. Among the three different parts, aqueous extracts of bark have exhibited a minimum inhibitory effect against *S. aureus*, *E. coli* and *S. typhi*, whereas seeds inhibited the growth of *P. aeruginosa* and *V. cholerae*, and leaves exhibited inhibitory effect only against *S. typhi*. Among the acetone extracts, bark was found to be effective against the entire test microorganisms. Leaves inhibited only *S. aureus*, whereas seed extracts failed to exhibit any inhibitory effect against the test organisms (Murugan *et al*., 2011).

REFERENCES

Ahmad, I., Z. Mehmood and F. Mohammad, (1998). Screening of some Indian medicinal plants for their antimicrobial properties. *J. Ethanopharmacol*. **62**:183-193.

Bauer, A.W., W.M.M. Kirby, J.C. Sherris and M. Turck, (1966). Anti-biotic susceptibility testing by a standardized single disk method*. Am. J. Clin. Pathol*. **45:** 493-496.

Didry, N., L. Dubreuil, F. Trotin and M. Pinkas, (1988). Anti-microbial activity of the aerial parts of *Drosera pellata* smith on oral bacteria. *J. Ethnopharmacol*. **60**: 91-96.

Murugan, S., P. Uma Devi, N. Kannika Parameswari and K.R. Mani. (2011). Anti-microbial activity of *Syzygium jambos* against selected human pathogens. *Int. J. Pharm. Sci*. **3**(2): 44-47.

Sashikumar, J.M., M. Remya and K. Janardhanan, (2003).Antimicrobial activity of ethanol medicinal plants of Nilgiri Bioshpere reserve and Western Ghats. *Asian J. Microbial. Biotechnol*. **5**: 183-185.

Kong. Res. J. 2(1) : 56-62, 2015 ISSN 2349-2694
Kongunadu Arts and Science College, Coimbatore.

PHYTOSOCIOLOGICAL ANALYSIS OF THE MEDICINAL PLANT SPECIES, *THALICTRUM JAVANICUM* BLUME IN THOTTABETTA, NILGIRIS, THE WESTERN GHATS.

Abinaya .G. and S. Paulsamy*
Department of Botany, Kongunadu Arts and Science College, Coimbatore-641 029, Tamil Nadu, India.
*E-mail: paulsami@yahoo.com

ABSTRACT

Phytosociological study is the most essential in any community to know its structure and organization. The various qualitative characters obtained are used to determine the level of distribution, numerical strength and degree of dominance exhibited by the constituent species in the community. *Thalictrum javanicum* belongs to the family Ranunculaceae family, it is medium sized erect herb, found in the temperate Himalayas from Kasmir to Sikkim in Khasi hills, and Kodaikanal and Nilgiri hills of Western Ghats in Tamil Nadu, India. At global level, it is generally distributed in the hilly tracts of India, Srilanka, China and Java at the altitude of around 2400 m above msl. The present study was undertaken in Thottabetta , the Nilgiris by sampling using belt transects of 10x1000m size which further divided into 100 segments each which 10x10m size. The total number of species encountered in the study area is 45 which includes 5 grasses and 40 forbs. The quantitative ecological characters of the study species, *T. javanicum* is a detailed below: frequency 11%, abundance 3.82 individuals/m^2, density 0.42 individuals/m^2, basal cover 172.20/mm^2/ m^2, relative frequency 0.55% and relative density 0.08%, relative dominance 0.16%. Based on the ecological attributes it is determined that the species, *T. javanicum* is less perpetuated in the community studied. Hence, further studies on the determination of propagation strategies for population enhancement and conservation of wilds are suggested.

Keywords: Phytosociological study, qualitative characters and quantitative ecological characters.

1. INTRODUCTION

Phytosociology is the study of the characteristics, classification, relationships and distribution of plant communities. These studies are most essential to understand the species diversity, community organization and to select out useful plant species from natural communities (Daubenmire, 1970), and to know the ecological status of the constituent species in the communities (Katsuno, 1977). Ecological status of species in a habitat is determined by assessing the biodiversity of a region, the environmental conditions prevailing there and their interactions. Ecological assessment is done by evaluation of the components along with its functional abilities of an ecosystem. This includes the assessment of species diversity, frequency and their importance value index and threat to their habitat by anthropogenic activities which may alter the physical, chemical and biological integrity of the system (Manoj *et al.,* 2012). In light of this fact, certain ecological tools were employed to determine quantitative ecological characters such as frequency, abundance, density, basal cover and their importance value index for all the constituent species along with the study species, *Thalictrum javanicum* to know the current ecological position of *T. javanicum* in its major area of occurrence, Thottabetta, Nilgiris, the Western Ghats.

2. MATERIALS AND METHODS

2.1. Study area

The study was carried out in Thottabetta, Nilgiris, the Western Ghats, Tamil Nadu, India situated at 11°24' 08.7" N and 76°44'12.2"E. The Nilgiri plateau covers an area of ca. 2,000 Km^2. The climate is of the tropical montane type (Meher-Homji, 1967). The mean monthly temperature varies between 5°C (January) and 24°C (April) (Von Lengerke, 1977). The data on climatic factors of the study area was given in Table 1. During the study period, the lowest and highest minimum temperatures have varied between 9.1 (December, 2012) and 15.8°C (May, 2013). On the other hand, the lowest and highest maximum temperatures have recorded as 11.5 (January, 2013) and 26.3°C (May, 2013) respectively. The dry season lasts from January to April and less rainfall occurred during November to June. The mean annual rainfall recorded at Thottabetta between June, 2012 and May, 2013 was 1688.2 mm. The area is exposed to southwest monsoon during June-September and to the northeast monsoon during October-November.

The altitude of the study area is 2400 m above msl. The relative humidity was ranging from 73% (April, 2013) to 93% (October, 2012).

The vegetation is mainly sholas with grasslands and extensive private tea plantations and exotic tree plantations (*Acacia mearnsii, A. dealbata, Eucalyptus globulus, Pinus longifolia,* etc.) managed by the Forest Department. The largest resident mammalian herbivore is the Sambar (*Cervus unicolor*). Elephants (*Elephas maximus*) visit the area particularly in January-March, during the dry season. Large predators include tiger (*Panthera tigris*) and leopard (*Panthera pardus*) are also sighted.

2.2. Methods

In order to study the phytosociological characters of the traditional medicinal herb, *Thalictrum javanicum,* Thottabetta of the Nilgiri District was selected as it is inhabiting better than the other areas of Nilgiris. For this, a one hectare rectangular plot (10X1000m) was established during Febraury, 2012. Each plot was subdivided into 100 subplots of 10X10m size. The occurrence with the individuals of *T. javanicum,* was recorded in each subplot to determine its frequency, density and abundance in the communities (Cottom and Curtis, 1956). Importance value index (IVI) was computed by summing up relative frequency, relative density and relative basal area. Distribution levels were also noted for other associated species available in the study area.

Frequency, density and abundance were calculated using the following formulae:

$$\text{Frequency (\%)} = \frac{\text{Number of quadrats in which the species present}}{\text{Total number of quadrats studied}} \times 100$$

$$\text{Density} = \frac{\text{Total number of individuals of the species in all quadrats}}{\text{Total number of quadrats studied}}$$

$$\text{Abundance} = \frac{\text{Total number of individuals of the species in all quadrats}}{\text{Number of quadrats of occurrence of the species}}$$

To calculate average basal area of individuals, the stem circumference at 30 cm high from soil for each species was measured. Then the formula, πr^2 was used to derive the average basal area. The average basal area was multiplied with the density to obtain the basal cover.

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

$$\text{Relative frequency (\%)} = \frac{\text{Number of occurence of the species}}{\text{Number of occurence of all specie}} \times 100$$

$$\text{Relative density (\%)} = \frac{\text{Number of individuals of the species}}{\text{Number of individuals of all species}} \times 100$$

$$\text{Relative dominance (\%)} = \frac{\text{Total basal area of the species}}{\text{Total basal area of all species}} \times 100$$

The importance value index (IVI) and the relative value of importance (RVI) were calculated as per the method of Curtis and Mc Intosh, (1950).

Importance value index (IVI) is the sum of quantities of relative frequency, relative density and relative dominance expressed per 300.

Relative value of importance (RVI) was calculated by using the formula: $RVI = \frac{IVI}{3}$

3. RESULTS AND DISCUSSION

3.1. Climatic data of the study area

The climatic data of the study area is presented in Table 1. The range of temperature over the study period was existing between 9.1 (December, 2012) and 26.3°C (May, 2013). The annual rainfall during the study period of June, 2012 - May, 2013 was 1688.2 mm. Rainfall was mostly through south-west and north-east monsoons (June - November) and the remaining months in the year were generally dry. The relative humidity ranged from 73% (April, 2013) to 93% (October, 2012). The climatic data particularly the temperature (maximum of 26.3°C) showed that the study area is experiencing temperate climate. The rainfall and humidity data exhibited that the study area is also having favorable environmental factors for plant growth.

3.2. Floristic analysis

A total number of 45 plant species was enlisted in the study area which includes 5 grasses (11.11%) and 40 forbs (88.89%) (Table 2).

3.3. Family-wise composition

A total number of 24 plant families was recorded (Table 2). Overall, the family, Poaceae has the highest number of individuals 43,851/ha (*Agrostis peninsularis* -11628 individuals/ha, *Cynodon dactylon* - 9855 individuals/ha, *C. barberi*-9688 individuals/ha, *Brachiaria semiverticillatta*-8365 individuals/ha and *Digitara ternata* - 4315 individuals/ha). On the other hand, the families Geraniaceae (*Impatiens leschenaultii*- 13 individuals/ha and *I. modesta* - 9 individuals/ha), Boraginaceae (*Cynoglossum zeylanicum* - 11 individuals), Acanthaceae (*Strobilanthes kunthiana* -

13 individuals/ha) and Ranunculaceae (*Thalictrum javanicum* - 42 individuals/ha) have least number of individuals. In the present study a total number of 45 plant species which includes 5 grasses and 40 forbs. The Poaceae was predominant plant family in the present study site, while Geraniaceae, Boraginaceae, Acanthaceae and Ranunculaceae constituted only least number of individuals. Michael and Boodram, (2006) found the similar trend of dominance of Poaceae members in the similar climatic dry forest vegetation communities of little Tobago Island, West Indies.

3.4. Frequency

Frequency is usually expressed in terms of percentage occurrence of individual species in an area. The highest frequency of 100% in the studied community was registered by the grass species *viz; Agrostis peninsularis*, *Brachiaria semiverticillatta, Cynodon barberi, C. dactylon,* and *Digitara ternata* and the forbs, *Erigeron karvinskianus* has highest frequency (93%). It indicates that these six species have fitted well with the environment of Thottabetta region. The higher seed output and greater reproductive potential exist in these species may be the possible reasons for this fact (Khoshoo and Mahal, 1967; Manorama, 1996). The species such as *Cynoglossum zeylanicum, Hypochaeris radicata, Impatiens leschenaultii, I. modesta, Strobilanthes kunthiana* and *Thalictrum javanicum* were present with lower frequencies of 6, 8, 6, 5, 8 and 11% respectively (Table 3). It may be attributed to its microclimatic preference for the appearance of these species only in the margins of the forests and not the entire stretch of the forests.

3.5. Abundance

Abundance refers to the number of individuals per unit area on basis of the number of quadrats of occurrence and it is not like the density where the number of all quadrats studied in the community is taken into account. Among the different species analyzed the highest abundance value was obtained by the species, *Agrostis peninsularis* (116.28/m^2) followed by *Cynodon dactylon* (98.55/m^2), *C. barberi* (96.88/m^2) and *Brachiaria semiverticillatta* (83.65/m^2), and the lowest abundance was obtained by the species *Pteris cretica* (1.05/m^2), *Psoralea pinnata* (1.07/m^2), *Pteris quadriauriata* (1.09/m^2), *Anaphalis elliptica* (1.10/m^2) and *Thalictrum javanicum* (3.82/m^2) (Table 3). This indicates the higher concentration of these species in limited area which in tern may be influenced by dispersal mechanism of the respective species. However, the study species, *Thalictrum javanicum* obtained the less abundance of 3.82/m^2 which indicates slightly wider distribution with lower numerical strength. Therefore, despite the existence of good dispersal mechanism, the fitness to the habitat in terms of population size is not appreciable in comparison to that of other species.

3.6. Density

The density is the most important quantitative character of any species in a community to know its structural and functional contribution to the ecosystem. In addition, determination of density for a species is more useful to know its distribution and microclimatic preferences in a common macroclimatic condition. The density of studied community showed that the species, *Agrostis peninsularis* has recorded higher density of 116.28/m^2. However, the study species, *Thalictrum javanicum* has present with very lower value of density (0.42/m^2) in the community studied (Table 3) and it is not comparable to that of the other species. The poor reproductive potential with less seed output and weaker competitive ability may lead the species with low density in the communities (Chandrasekaran and Swamy, 1995). Further, it is known that because of the medicinal properties, the species, *T. javanicum* is being exploited by the local people severely (Paulsamy, 2007), though the sholas are being given intensive protection by *in situ* conservation of the valuable species present in the study area. This fact may also be a reason for the lower density of *T. javanicum* in the study area.

3.7. Basal cover

On the basis of increasing or decreasing values of basal cover and importance value index, species have been classified as 'increasers' and 'decreasers' (Daubenmire, 1940; Weaver and Hansen, 1941; Dyksterhuis, 1949; Weaver and Albertson, 1956). The basal cover of the constituent species in the studied community was varied widely (Table 3). It was determined to be higher for the forbs, *Cyperus digitatus* (2873.90/mm^2/ha) and *C. esculentus* (2343.60/mm^2/ha). The basal cover occupied by the study species, *Thalictrum javanicum* (172.20/mm^2/ha) was not comparable to that of the forbs mentioned above (Table 3) which indicates its less role in community metabolism. It may be explained due to herbaceous nature of this species which naturally expected to have very small sized stem. This feature results in lesser basal area of individuals of the study species. Secondly, the less density value obtained by this species may also leads to the occupation of less basal cover, since this character is the manipulation of the attribute, density for the species of homogenous communities. In general, based on the basal cover, it is known that

all the species were mere present in the forest margins of the study area and they have no major functional role in the community metabolism.

3.8. Relative values of frequency, density and dominance

The values of relative frequency, relative density and relative dominance are the magnification of the quantitative characters such as frequency, density and basal area respectively. Hence, the studied community exhibited the similar trend of values for these characters as exhibited by the characters, frequency, density and basal cover for all the species including *Thalictrum javanicum* (Table 3).

3.9. Importance value index (IVI)

In order to express the ecological success of any species with a single value, the concept of importance value index has been developed. The quantitative values of relative frequency, relative density and relative dominance are added to get the IVI. It gives the idea on ecological success of any species with a single value. The IVI of the constituent species in the studied community was ranging between 0.29 and 47.31. The Poaceae members *viz;* as *Agrostis peninsularis, Cynodon dactylon*, *Brachiaria semiverticillatta* and *Cynodon barberi* have scored the highest IVI of 47.31, 42.65, 42.27 and 41.13 respectively. The presence of higher ecological importance for these species in the study area showed that they are having well adaptive mechanism against the disturbance. The higher seed output with greater germination percentage and survivability rate may also assist these species for their stronger perpetuation (Paulsamy, 2005). On the other hand, the lowest IVI value was scored by the Geraniaceae member, *Impatiens modesta* (0.29). The study species, *Thalictrum javanicum* has also registered lower IVI value of 0.79 only. It showed that this species has less perpetuation in the community of Thottabetta than the other associated species (Table 3). The absence of adequate micro-sites for this species may also be pointed out as reason for the lower IVI of these species (Paulsamy, 2006). Further, it is known that the impact of environment on this species was also not noteworthy.

3.10. Relative value of importance (RVI)

RVI is an ecological character, which showed the importance of particular species in relation to other constituent species present in the community by considering the attributes, frequency, density and dominance. Since it is a manipulated character of IVI, it exhibited the values in the same trend as shown by the IVI. The RVI of the constituent species in the studied community was ranging between 0.10 and 15.77%. The Poaceae members such as *Agrostis peninsularis* followed by *Cynodon dactylon, Brachiaria semiverticillatta* and *C. barberi* have scored the highest RVI of 15.77, 14.22, 14.09 and 13.71% respectively. On the other hand, the lowest RVI value was scored by the Geraniaceae member, *Impatiens modesta* (0.10%) and the studied Ranunculaceae member, *Thalictrum javanicum* (0.26%) (Table 3).

4. CONCLUSION

From the phytosociological studies it is concluded that the species, *Thalictrum javanicum* is considered to have very less ecological importance. Based on the quantitative ecological characters in terms of frequency, density and basal cover, it is investigated that this species is less perpetuated in the community. Further studies on autecological aspects will be carried out to diagnose factors responsible for its poor status and so to device new strategies of propagation to establish the species.

Table 1. Climatic data of the study area, Thottabetta, the Nilgiris, Western Ghats.

Year and Month	Temperature (°C)		Rainfall (mm)	Rainy Days	Relative humidity (%)
	Max.	Min.			
2012 Jun	20.4	15.3	271.3	17	75
Jul	18.4	14.0	290.2	21	85
Aug	17.6	14.2	271.0	18	80
Sep	18.2	15.0	190.2	14	92
Oct	16.8	14.1	315.1	20	93
Nov	14.5	9.3	230.5	15	90
Dec	13.2	9.1	44.8	3	83
2013 Jan	11.5	10.0	0.0	0	85
Feb	13.2	10.4	7.8	1	85
Mar	19.4	10.9	43.2	3	90
Apr	22.0	15.2	12.8	2	73
May	26.3	15.8	11.3	2	80

Table 2. Species composition: species and their included family, number of individuals and number of quadrats of occurrence of constituent species in the studied community at Thottabetta, Nilgiris, the Western Ghats.

Name of the species	Family	Number of individuals /ha	Number of quadrats of occurrence
Grasses			
Agrostis peninsularis H&F.	Poaceae	11628	100
Brachiaria semiverticillatta Alston.	Poaceae	8365	100
Cynodon barberi Rang& Tad.	Poaceae	9688	100
C. dactylon Pers.	Poaceae	9855	100
Digitara ternata Stapf.	Poaceae	4315	100
Forbs			
Adiantum concinnum Wild.	Adiantaceae	369	48
A. raddianum Presl.	Adiantaceae	218	43
Anaphalis elliptica Dc.	Asteraceae	65	59
A. subdecurrens, Gamb.	Asteraceae	94	84
Anemone rivularis Ham.	Ranunculaceae	84	59
Arisaema leschenaultti Bl.	Araceae	15	13
Cardamine africana L.	Brassicaceae	21	15
Crotalaria laevigata Lam.	Fabaceae	19	15
Cynoglossum zeylanicum cl.	Boraginaceae	11	6
Cyperus digitatus Roxb	Cyperaceae	991	89
C. esculentus Linn.	Cyperaceae	868	83
Eria sp.	Orchidaceae	33	19
Erigeron alpinus L.	Asteraceae	698	89
E. karvinskianus Dc.	Asteraceae	715	93
Euphorbia rothiana Spr.	Euphorbiaceae	49	35
Fragraia indica Andr.	Rosaceae	36	30
Fimbristylis tetrogana RBr.	Cyperaceae	210	69
Hypochaeris radicata L.	Asteraceae	15	8
Impatiens leschenaultii Wall.	Geraniaceae	13	6
I. modesta W.	Geraniaceae	9	5
Microlepia manjuscula (Lowe) Moore Ind. Fill.	Dennstaedtiaceae	26	18
Myriactis wightii Dc.	Asteraceae	46	21
Odontosoria chinensis (L).	Lindsaeaceae	35	19
Oxalis corniculata L.	Oxalidaceae	319	63
O. pubescens L.	Oxalidaceae	405	71
Pilea trinervia W.	Utricaceae	30	21
P. wightii Wedd.	Utricaceae	41	23
Pogostemon travancoricus Bedd.	Lamiaceae	65	36
Polygonum chinense Linn.	Polygonaceae	28	23
Psoralea pinnata L.	Fabaceae	45	42
Pteris cretica L.Mant	Pteridaceae	63	60
P. quadriauriata .Retz	Pteridaceae	93	85
Rubus racemosus Roxb.	Rosaceae	41	30
R. ellipticus Sm.	Rosaceae	53	41
Solanum nigrum L.	Solanaceae	31	19
Strobilanthes kunthiana T. And.	Acanthaceae	13	8
Thalictrum javanicum Blume.	Ranunculaceae	42	11
Ulex europaeus L.	Fabaceae	38	16
Viola serpens Wall.	Violaceae	46	31
V. patrini Dc.	Violaceae	55	35

Table 3. Frequency, abundance, density and basal cover with their relative values, importance value index (IVI) and relative value of importance (RVI) of constituent species in the community at Thottabetta, Nilgiris, the Western Ghats.

S.No	Name of the species	F (%)	A (individuals /m²)	D (individuals /m²)	BC (mm²/ha)	R.F (%)	R.De (%)	R.Do (%)	IVI	RVI (%)
	Grasses									
1.	*Agrostis peninsularis*	100	116.28	116.28	20930.40	4.97	23.30	19.04	47.31	15.77
2.	*Brachiaria semiverticillatta*	100	83.65	83.65	22585.50	4.97	16.76	20.54	42.27	14.09
3.	*Cynodon barberi*	100	96.88	96.88	18407.20	4.97	19.42	16.74	41.13	13.71
4.	*C. dactylon*	100	98.55	98.55	19710.00	4.97	19.75	17.93	42.65	14.22
5.	*Digitara ternata*	100	43.15	43.15	10787.50	4.97	8.65	9.81	23.43	7.81

	Forbs									
6.	*Adiantum concinnum*	48	7.69	3.69	1033.20	2.38	0.74	0.94	4.06	1.35
7.	*A. raddianum*	43	5.07	2.18	654.00	2.14	0.44	0.59	3.17	1.06
8.	*Anaphalis elliptica*	59	1.10	0.65	201.50	2.93	0.13	0.18	3.24	1.08
9.	*A. subdecurrens*	84	1.12	0.94	291.40	4.17	0.19	0.27	4.63	1.54
10.	*Anemone rivularis*	59	1.42	0.84	277.20	2.93	0.17	0.25	3.35	1.12
11.	*Arisaema leschenaultti*	13	1.15	0.15	57.00	0.65	0.03	0.05	0.73	0.24
12.	*Cardamine Africana*	15	1.40	0.21	58.80	0.74	0.04	0.05	0.83	0.28
13.	*Crotalaria laevigata*	15	1.27	0.19	55.10	0.74	0.04	0.05	0.83	0.28
14.	*Cynoglossum zeylanicum*	6	1.83	0.11	22.00	0.30	0.02	0.02	0.34	0.11
15.	*Cyperus digitatus*	89	11.13	9.91	2873.90	4.42	1.99	2.61	9.02	3.01
16.	*C. esculentus*	83	10.46	8.68	2343.60	4.12	1.74	2.13	7.99	2.66
17.	*Eria* sp.	19	1.74	0.33	99.00	0.94	0.07	0.09	1.10	0.37
18.	*Erigeron alpinus*	89	7.84	6.98	1884.60	4.42	1.40	1.71	7.53	2.51
19.	*E. karvinskianus*	93	7.69	7.15	2073.50	4.62	1.43	1.89	7.94	2.65
20.	*Euphorbia rothiana*	35	1.40	0.49	98.00	1.74	0.10	0.09	1.93	0.64
21.	*Fragraia indica*	30	1.20	0.36	93.60	1.49	0.07	0.09	1.65	0.55
22.	*Fimbristylis tetrogana*	69	3.04	2.1	651.00	3.43	0.42	0.59	4.44	1.48
23.	*Hypochaeris radicata*	8	1.88	0.15	30.00	0.40	0.03	0.03	0.46	0.15
24.	*Impatiens leschenaultii*	6	2.17	0.13	32.50	0.30	0.03	0.03	0.36	0.12
25.	*I. modesta*	5	1.80	0.09	24.30	0.25	0.02	0.02	0.29	0.10
26.	*Microlepia manjuscula*	18	1.44	0.26	54.60	0.89	0.05	0.05	0.99	0.33
27.	*Myriactis wightii*	21	2.19	0.46	133.40	1.04	0.09	0.12	1.25	0.42
28.	*Odontosoria chinensis*	19	1.84	0.35	115.50	0.94	0.07	0.11	1.12	0.37
29.	*Oxalis corniculata*	63	5.06	3.19	988.90	3.13	0.64	0.90	4.67	1.56
30.	*O. pubescens*	71	5.70	4.05	1215.00	3.53	0.81	1.11	5.45	1.82
31.	*Pilea trinervia*	21	1.43	0.3	84.00	1.04	0.06	0.08	1.18	0.39
32.	*P. wightii*	23	1.78	0.41	127.10	1.14	0.08	0.12	1.34	0.45
33.	*Pogostemon travancoricus*	36	1.81	0.65	195.00	1.79	0.13	0.18	2.10	0.70
34.	*Polygonum chinense*	23	1.22	0.28	112.00	1.14	0.06	0.10	1.30	0.43
35.	*Psoralea pinnata*	42	1.07	0.45	139.50	2.09	0.09	0.13	2.31	0.77
36.	*Pteris cretica*	60	1.05	0.63	157.50	2.98	0.13	0.14	3.25	1.08
37.	*P. quadriauriata*	85	1.09	0.93	251.10	4.22	0.19	0.23	4.64	1.55
38.	*Rubus racemosus*	30	1.37	0.41	196.80	1.49	0.08	0.18	1.75	0.58
39.	*R. ellipticus*	41	1.29	0.53	238.50	2.04	0.11	0.22	2.37	0.79
40.	*Solanum nigrum*	19	1.63	0.31	96.10	0.94	0.06	0.09	1.09	0.36
41.	*Strobilanthes kunthiana*	8	1.63	0.13	54.60	0.40	0.03	0.05	0.48	0.16
42.	*Thalictrum javanicum*	11	3.82	0.42	172.20	0.55	0.08	0.16	0.79	0.26
43.	*Ulex europaeus*	16	2.38	0.38	133.00	0.79	0.08	0.12	0.99	0.33
44.	*Viola serpens*	31	1.48	0.46	96.60	1.54	0.09	0.09	1.72	0.57
45.	*V. patrinii*	35	1.57	0.55	99.00	1.74	0.11	0.09	1.94	0.65

F-Frequency; A-Abundance; D-Density; B-Basal cover; R.F- Relative frequency, R.De- Relative density, R.Do- Relative dominance, IVI- Importance value index , RVI- Relative value of importance.

ACKNOWLEDGEMENT

The authors graciously acknowledge Dr.M. Aruchami Research Foundation for providing financial support to carry out this work.

REFERENCES

Chandrasekaran, S. and P.S. Swamy, (1995). Changes in the herbicious vegetation following disturbances due to biotic interference in natural and man-made ecosystems in Western Ghats. *Trop. Ecol.* **36**: 213-220.

Cottom, G. and J.T. Curtis, (1956). The use of distance measure in phytosociological sampling. *Ecol.* **37**(3): 451-460.

Curtis, J.T. and R.P.M.C. Intosh, (1950). The interrelations of certain analytic and synthetic phytosocioligal characters. *Ecol*. **31**(3): 434-455.

Daubenmire, R. F, (1970). Plants and Environment. 2nd Edition. Wiley Eastern Private Limited, New Delhi. p. 422 .

Daubenmire, R. F. (1940). Plant succession due to overgrazing in the *Agropyron* bunch grass prairie in the southeastern Washington. *Ecol.*. **21**(1): 55-64.

Dyksterhuis, E. J, (1949). Conditions and management of rangeland based on quantitative ecology. *J. Range Management*. **2**: 104-115.

Katsuno, T. (1977). *Phytosociological studies on the road side vegetation*. Part 1. Bull. Coll. Agric. Vet. Med. Nihon Uty. **34**: 311-343.

Khoshoo, T N. and C. Mahal, (1967). Versatile reproduction in *Lantana camara. Curr. Sci.* **36**: 201-203.

Manoj, K., M.R. Nandakumar, M.R. Remya, K. Shinila and P. Divya Lakshmi, (2012). Phytosociological analysis of riparian tree species of Alakyam stream; Pariyaram, Kerala, India. *Inter J Environment. Sci.* **2**(4): 1895-1903.

Manorama, S. (1996). Studies on the natural regeneration of lime mined sitesat Madukkarai, Coimbatore District, Tamil Nadu, India. Ph.D., Thesis, Bharathiar, Coimbatore, India.

Meher-Homji, V.M, (1967). Phytogeography of the South Indian hill stations. *Bulletin of the Torrey Botanical Club*. **94** (4): 230-242.

Michael, P.O. and N. Boodram, (2006). The dry forest vegetation communities of little Tobago island, West Indies: floristic affinities. *Trop. Ecol*. **47**(2): 211-228.

Paulsamy, S. (2005). *Annual progress report of the project, "Evaluation of conservation strategies for the sustainable utilization of herbaceous bioresources in the sholas of Nilgiris, the Western Ghats*, sponsored by Ministry of Environment and Forest, Govt. of India, New Delhi.

Paulsamy, S. (2006). Evaluation of conservation strategies for the sustainable utilization of herbaceous bioresources in the sholas of Nilgiris, the Western Ghats. *Annual Progress Report, Ministry of Environment and Forests Scheme,* Govt. of India, New Delhi.

Paulsamy, S. (2007). *Annual Progress Report, Ministry of Environment and Forests, Government of India*, New Delhi sponsored project, "Evaluation of conservation strategies for the sustainable utilization of herbaceous bioresources in the sholas of Nilgiris, the Western Ghats" (File No. 08/ 16/03.CS /BR).

Von lengerke, H.J. (1977). The Nilgiris: *Weather and climate of a mountain area in South India*. Franz steiner verlag GMBH, Wiesbaden, Germany.

Weaver, J.E. and F.W. Albertson, (1956). *Grasslands of the great plains.* Johnson Publ.Co., Lcoln, Nbraska. p. 395.

Weaver, J.E. and W.W. Hansen, (1941). Native mid-western pastures; their origin; composition and degradation. *Agronomy and Horticulture-Faculty publications* **22**: 93.

Kong. Res. J. 2(1) : 63-64, 2015 ISSN 2349-2694
Kongunadu Arts and Science College, Coimbatore.

BIOSORPTION STUDIES OF CHROMIUM AND NICKEL BY *PSEUDOMONAS* AND *BACILLUS* SP.

Anupriya, P., V. Jacob Vargheese and S. Saravanan Babu*
Department of Botany, Chikkah Naciker College, Erode.
*E.mail: ssbabuflora@yahoo.com

ABSTRACT

In the present investigation, an attempt has been made to study the effect of biosorption of heavy metal such as Chromium and Nickel using *Pseudomonas* and *Bacillus*. The resistance of bacterial isolates to heavy metals such as chromium and Nickel has been studied. The Percentage of removal of chromium and nickel was analysed. The Percentage of removal of chromium was found using immobilization techniques. It has been found that as the concentration increases the resistance to the metal also increases. At 1000ppm concentration the resistance was found maximum. The uptake of chromium and Nickel was 90.7% and 80.60% by *Pseudomonas* respectively. Among the *Pseudomonas* and *Bacillus*, *Pseudomonas* proved be an efficent strain.

Keywords: Biosorption studies, chromium, nickel, *Pseudomonas* sp., *Bacillus* sp.

1. INTRODUCTION

The unprecedented population increase and anthropogenic activities such as industrialization and urbanization of the twentieth century in the name of modernization have not only increased conventional solid and liquid waste pollutants to critical levels but also produced wide range of previously unknown contaminants in the form of xenobiotics which are released to the environment.The persistence of toxic metals and organic pollutants in the surroundings causes pollution and deteriote the environment. Contamination of heavy metal to the environment is the major global concern because of their toxicity and threat to human life (Sharma, 2000)

The term "heavy metal" commonly refers to metals either with a specific weight higher than 5g/cm3 or an atomic number above 20.All of them may not be toxic at relatively low concentrations, classified as heavy metals. So,the heavy metals are a very heterogeneous group of elements, which greatly differ in their chemical properties and heterogenous group of elements, which greatly differ in their chemical properties and biological functions. For this reason, the term "Heavy metal " is discredited and terms "trace elements " are preferred by numerous authors(Phipps,1981). Heavy metals are non-biodegradable pollutants on environment and some are even hazardous to human and animals. Heavy metals in excessive quality cause toxicity and death to most living organisms. Contamination of soil and soil water results from the presence of undisturbed organisms. Contaminated soil and water results from the presence of undisturbed metal near the soil surface or from the actual mining of ores. The heavy metals are often used as fungicides, pesticides or disinfectants which are responsible for toxicity.As the metal pollutants are non degrable and are readily taken up by plants, these are likely to enter easily in to food chain (Prasad,1995).

Bio sorption is defined as a nondirected physicochemical interaction occurs between metal or radionuclide sps and microbial cells(shumate and stranberg,1985). It is a biological method of environmental control and can be an alternative to conventional contaminated water treatment facilities. A successful biosorption process required preparation of good biosorbent.The process starts with selecting various types of biosorbent. Choosing the biosorbent for metal absorption depends on origin of the adsorbents(Regin and volesky,2000). In the present study the removal of Nickel and chromium is studied using the bacterial bioadsorbents *Pseudomonas* and *Bacillus*.

2. MATERIALS AND METHODS

With a view to study the biosorption of Nickel and Chromium, the present study was made considering the environmental parameters which affect the update of Nickel and chromium by *Bacillus* and *Pseudomonas* .The glassware were washed thoroughly in running tap water, rinsed in distilled water and dried in a hot air oven(Yorco vertical;autoclave,Mumbai) at 121^{0}C at 15 lbs pressure for 15minutes.The sample was collected in a sterile container from the heavy metal contaminated sites in and around sivakasi. The

isolation was done by inoculating 1gm of soil in 100ml of nutrient broth. It was incubated in a shaker in 150rpm for 24 hours at 37^0C.The isolated were subcultured in fresh medium and maintained at 40C.This bacterial isolates were tested to determine the resistance for heavy metals such as chromium and nickel at various concentrations. Resistance to heavy metals is determined by plane method and well method. Cells of *Bacillus* and *Pseudomonas* were harvested during the exponential phase of growth and re-suspended in 4% sodium alginate solution. The resulting mixture was dropped in to the $% calcium chloride solution and incubated at 4^0C for 2hours with constant and gentle stirring for complete gel formation. The immobilized beads were then transferred to fresh nutrient broth and incubated for 12hours for further cell growth of *Pseudomonas* .The beads were harvested by filtration and washed with sterile distilled water. The immobilized cell beads were prepared under aseptic conditions. The beads were transferred to 100ppm of chromium and nickel in 100ml sterile distilled water in separate flasks and incubated at 30^0C in a rotary shaker at 120rpm for 24hours.Two controls without cell were maintained for chromium and nickel respectively. After the incubation the beads were collected and suspended in 50ml of 0.05M nitric acid with gentle stirring for 1hr for the desorption of metal ions. Finally the metal concentration of present in the filtrate was analysed in shimadzu AA-6300 Atomic Absorption Spectrophtometer.

3. RESULTS AND DISCUSSION

The following results were obtained in the comparative study of Nickel and Chromium by *Bacillus* and *Pseudomonas*. The resistance of bacterial isolates to chromium and nickel was checked qualitively by inoculating the isolates in nutrient agar plates containing various concentrations of Chromium and Nickel. It was found that as the concentration increases the growth of bacteria decreases for both Chromium and Nickel. At 500ppm, it exhibits maximum resistance for both Chromium and Nickel. The Zone of inhibition at various concentration is determined. As the concentration increases, the zone of inhibition also increases. The isolates *Pseudomonas* and *Bacillus* Strains exhibits maximum resistance. Hence these two strains are selected for further study. Chromium and Nickel reduction by was also observed by using immobilized biobeads using calcium alginate gel. Immobilzed biobeads were inoculated at various concentrations of nickel and chromium. After incubation the supernatent was digested with acid and analysed in AAS.

Table 1

S.No	Isolates	Percentage of Removal	
		Chromium	Nickel
1	*Pseudomonas*	90.70%	80.60%
2	*Bacillus*	78.00%	75.30%

Table 2

S.No	Isolates	Concentration of Chromium(mm)			
		250	500	750	1000
1	*Pseudomonas*	1.50	2	3	2.2
2	*Bacillus*	1.70	2	3	3.2

ACKNOWLEDGEMENT

The authors are greatful to the principal chikkah naicker college,Erode for providing laboratory facilities.The authors are also thankful to the Biogenic lab,Namakal.

REFERENCES

Phipps, D.A., (1981). Chemistry and Biochemistry of trace elements in biological systems. In N.W.Leep Effect of heavy metal pollution on plants. Applied science publications London **1**: 1-54.

Prasad, M.N.Y., (1995). Inhibition of maize leaf chlorophyll,carotenoids and gas exchange funcations by cal. Photosynthesis. *App. Environ. Microbiol.* **31**: 635-640.

Regine and Volesky, (2000). Isoution and characterization of chromate resistanct bacteria from tannery effluent. *J. Environ. Biol.* **28**(22): 399-403

Sharma., (2000). Mircobiol Bioremediation. P. Rajendran, P. Gunasekaran. MJP Pubilshers **344**: 47-49.

Shumate, S.E., and G.W. Strangberg, (1985). Accumlation of metal by microbial cells in comprehensive Biotechnol. M.M. Young Pergamom Press New York. 235-247.

Sultan, S. and S. Hasnain, (2005). Chromate reduction capability of a Gram-positive bacterium isolated from effluent of dying industry. *Bull. Environ. Contam. Toxicol.* **75**: 699-706.

Kong. Res. J. 2(1) : 65-70, 2015 ISSN 2349-2694
Kongunadu Arts and Science College, Coimbatore.

WINE PRODUCTION IN WILD FRUITS OF *OPENTIA STRICTA* HAW.

Ganesan, C.M.*[1], R. Ravichandran[2], R. Deepthi[1] and S. Paulsamy[1]
[1]Department of Botany, Kongunadu Arts and Science College, Coimbatore.
[2] Department of Botany, The Madura College, Madurai.
*E.mail: bioganesan@gmail.com

ABSTRACT

The present study was attempted for using the wild species present in Nilgiri Biosphere Reserve, the Western Ghats for wine production. A wild species *Opuntia stricta* has been identified as an alternative source of grapes for wine production. The results of the study report that the carbohydrate content (sugar and pectic substances) was higher in the fruits and it was comparable to that of grapes. The various kinds of minerals, vitamins and other nutrients are also found to be higher in this species. This enriched status of nutrients provide favourable environment for the fermentation of carbohydrates. The percentage of ethanol determined in the fruit of *Opuntia stricta* was also higher as in grapes. Further it has been observed that 30 days of incubation for fermentation is found to be optimum for higher alcohol production (1.8%). Hence, *Opuntia stricta* can be served as prominent source for wine making.

Keywords: *Opentia stricta,* wine production.

1. INTRODUCTION

Wine is as old as history and has historically been regarded as a necessary, even sacred, part of our lives. Over 2,500 years ago, Cyrus the Great required wine to be carried by his troops. Jesus turned water into wine for the wedding feast. Fruit wine is an undistilled, low alcoholic beverage containing all the natural ingredients of the fruit like vitamins, amino acids, polyphenols, flavanoids, tannins, anthocyanins and minerals in it. Which is together makes it a nutritive health drink of high commercial value. The red wines are abundant source of natural antioxidants and polyphenols and represent an important dietary component for some populations. The polyphenols in the red wines are the major components which are responsible for the taste, colour, astringency, bitterness and mouth feel.

In India, the indigenous fruits collected from wild play significant role in the food and nutrient security of rural poor and tribal. Some wild fruits have been identified to have better nutritional value than cultivated fruits (Eromosele *et al.,* 1991; Maikhuri *et al.,* 1994). As a result, in recent years, a growing interest has emerged to evaluate various wild edible plants for their nutritional features (Slikkerveer, 1994; Arora and PandeyAnjula, 1996; Sundriyal *et al.,* 1998; FAO, 1999; Grivetti and Ogle Britta, 2000; Katewa, 2003; Glew *et al.,* 2005; Musinguzi *et al.,* 2007; Nkafamiya *et al.,* 2007; Aberoumand and Deokule, 2009; Nazarudeen, 2010)

The wild fruits form an important additional food source for the people of Nilgiri District. The forest wealth of edible fruits/plants is exploited by these people to quench their huger during adverse environmental conditions. (Nayakam *et al.,*1993). The Nilgiri District is blessed by nature with its diversified and rich flora (Sharma, 1977).

Opuntia stricta belongs to the family Cactaceae. It is a succulent shrub, native of the new world, now widely grown in the warmer parts of the world on account of its unique appearance and attractive flowers. *Vitis vinifera* (grape) belongs to the family, Vitaceae is the major do area of wine served as standard.

2. MATERIALS AND METHODS

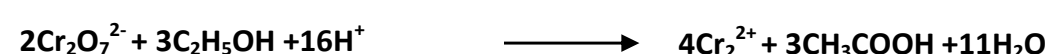
Wine is a product of the natural fermentation of the fruit juices, by the action of yeast cells, *Saccharomyces cerevisiae.* This fermentation process involves a biochemical conversion of juice into wine when baker's yeast enzymatically degrade the fruit sugars like glucose into acetaldehyde and then into ethanol and carbon dioxide.

$$\text{Glucose} \xrightarrow[\text{Enzymes}]{\text{Glycolytic}} \underset{\text{Pyruvic acid}}{CH_3\text{-}\overset{\overset{\text{O}}{||}}{C}\text{-}COOH} \xrightarrow{\text{Decarboxylation}} \underset{\text{Acetaldehyde}}{CH_3\text{-}CHO} + Co_2 \uparrow$$

$$\text{Acetaldehyde} \xrightarrow[\text{Reduction}]{4H^+} \text{Ethanol}$$

For wine production in a laboratory scale, as a first step, the fruits are washed, crushed and extracted. Baker's yeast is inoculated and incubated for 10 days under aerobic conditions at room temperature. During this time, the wine is clarified of any turbidity, thereby producing volatile esters that are responsible for characteristic flavours in the wine.

The major product in wine is the ethanol. The quality of the wine is checked by estimating the ethanol content of the wine employing dichromate oxidation method. This method is based on the quantity of acid dichromate required to oxidize the primary or secondary alcohols to acetic acid.



The optimum temperature for oxidation is 60-65˚C. The intensity of color developed by the oxidation reaction is read colorimetrically at 570 nm.

2.1. Materials required

1. Fruits
2. Sugar
3. Baker's yeast
4. 25% Chromic acid

2.2. Procedure

2.2.1. Wine production

1. 1 kg of the fruits were weighed and washed with distilled water.
2. It was crushed by hand and transferred into a conical flask.
3. Sugar was added to the final concentration of 20% on the weight by weight basis to that of the fruits taken.
4. The flask was plugged air tight.
5. It was incubated for 21 days at room temperature.

2.2.2. Analysis

1. The ethanol content was estimated by dichromate oxidation method.
2. Absolute alcohol was used as standard and 1% acid was prepared as working standard.
3. Different concentrations of standard solutions of 0.2%, 0.4%, 0.6%, 0.8% and 1% were prepared in separate test tubes.
4. Unknown sample of equal volume was taken. Water was used as blank.
5. 25% chromic acid was added in equal volume to all the standards and also to the unknown sample.
6. The tubes were incubated in a 70˚C water bath for about 10 min.
7. The absorbance values were taken at 570 nm using a colorimeter.
8. A calibration curve was drawn by plotting the concentration of alcohol on X- axis and the absorbance values on Y- axis for the standard. The ethanol content of the wine sample was determined from the fruits.

3. RESULTS AND DISCUSSION

The results of fermentation experiments and nutrition are given table number 1-3 and graph 1 and 2. Wine is an alcoholic bewarage typically made of fermented fruit juice (Okafor, 2007). In addition to grape *Opuntia stricta* Haw. Has been identified as the source for wine production*.* The contents of nutrients complied from literature sources, absorbance level during different period of fermentation and content of ethanol in the fermented fruits with different time intervals are noted in the present study. The results of the study revealed that *Opuntiastricta* fruit containing the carbohydrates 12-17%.The minerals and vitamins estimated in the studied wild fruit also known to be higher and most adequate.

The carbohydrates are the primary sources for the production of wine throughout world since many centuries. Soluble carbohydrates are the major nutritional reward in the pulp of most vertebrate-dispersed fruits (Corlett 1996; Herrera, 1987; Johnson *et al.,* 1985; Jordano, 1995). However, although detailed analyses have been made of the carbohydrate content of many cultivated fruits (e.g. Nagy *et al.,* 1990; Widdowson and McCance, 1935), most studies of wild fruits have only quantified total soluble carbohydrates (e.g. Conklin and Wrangham, 1994; Corlett, 1996; Foster and McDiarmid, 1983; Herrera, 1987; Izhaki, 1992). This is unfortunate because relatively minor differences in the chemical structure of nutrients can have profound implications for frugivorous animals (Martinez del Rio and Restrepo, 1993), and the fruit choices of these animals may, in turn, have major implications for the abundance and distribution of plant species.

The absorbance value at 570nm for the determination of ethanol content in various time, old series of fermented fruits of*Opuntiastricta*. Ajay and Harsh (2013) also be reported wild berriesuses as a cheap source of herbal wine. Generally the absorbance value was increased progressively from the 10 days old fermented product to 30 days old fermented products range between 0.77 and 1.50 value.It is of common fact that any fruit material with lot of minerals and vitamins can be fermented

easily with high efficiency and produce large quality of ethanol in wine (Sawaya *et al.,* 1983; El kossori *et al.,* 1998; Ramadan and Morsel, 2003). The present study it is evident that quantitatively of many kind of nutrients and ascorbic acid. This is the most favourable factor for the activity of yeast type of fermentor to produce necessary enzyme for the production of alcohol. So the intrinsic factor available in these fruit sources is known to be more favourable for alcohol production.

The 40 and 50 days old fermented fruit showed absorbance value in declined condition. It indicates that 30 days fermentation is the most appropriate and optimum for higher production of ethanol (from 0.51 to 1.10 Ab).The content of ethanol in the fruits of the time old series in fermented juice contained high content of 1.80% of ethanol. The production of ethanol was declined in 40 and 50 days of fermentation.

Opuntia stricta produced more ethanol than that of the grapes, despite the higher content of carbohydrate and sugar in grape fruits. This may be attributed to be presence of many kinds of nutrients including vitamins in higher content. Which can support the yeast nutritionally for better activity of fermentation *Opuntia stricta* (Saenz, 1995; Piga 2004; Stintzing and Carle, 2005) through the alcohol producing efficiency is greater than grapes, the availability of fruits of these species in a constraint to utilize the species for alcoholic production. Therefore cultivation of this species in large scale in degraded sholar, waste lands and other cultivable lands in Nilgiri Biosphere Reserve will solve this problems, the large scale production through cultivations can also give good economic return to the down trodden formers. This species can be used as an alternative source of wine production to the grapes. In addition raising of these plants would also ensure ecological security through enhancing green cover.

4. CONCLUSION

Opuntia stricta Haw.has been identified an alternative source of grapes for wine production. The results of the study report that the carbohydrate which include the sugar and pectic substances in higher and comparable to that of grapes. The various kinds of minerals, vitamins and other nutrients are also founded to be higher in this species. This enriched status of nutrients is providing favourable environment for the fermentation of carbohydrates.

The percentage of ethanol determined in *O. stricta* is also comparable to that of grapes. This fact clearly ensured the usage of the fruits of these species as an alternative source for wine production. Further it has been observed that 30 days duration of fermentation is found to be optimum for higher alcohol production. Hence this species can be served as prominent source for wine making.

For better utilization, this species are suggested for cultivation is degraded shola and other waste lands of Nilgiri Biosphere Reserve. This can improve the status of farmers and can meet the demand as well.

Table 1. Nutrient content in the fruits of *Opuntia stricta* (Feugang *et al.,* 2006).

S. No.	Nutrients	Percentage (%)
1.	Water	84-90
2.	Carbohydrate	12-17
3.	Ash	0.3 -1
4.	Protein	(0.5)
5.	Fibers	0.02-3.15
6.	Iron (mg/100 g)	4.28
7.	Protein	0.21-1.6
8.	Lipids	0.09-0.7
9.	Ascorbic acid	12-81 mg 2
10.	Total vitamin E	111-115 μg
11.	Calcium (Ca)	12.8 -59
12.	Magnesium (Mg)	16.1 -98.4
13.	Potassium (K)	90-220
14.	Phosphorus (P as PO4)	15-32.8

Table 2. Absorbance at 570nm for the ethanol content present in the wild fruits of various duration of fermentation

AA	Species	Days under fermentation (O.D)				
		10	20	30	40	50
1.	*Opuntia stricta,* Haw.	0.80	1.10	1.50	1.10	0.72
2.	*Vitis vinifera,* L.	0.77	0.94	1.28	0.93	0.51

Table 3. Concentration of alcohol (%)in the fermented fruits of wild plants during various time intervals.

S. no	Species	Days under fermentation				
		10	20	30	40	50
1.	*Opuntia stricta,* Haw	0.97	1.22	1.80	1.22	0.87
2.	*Vitis vinifera,* L.	0.92	1.15	1.55	1.12	0.62

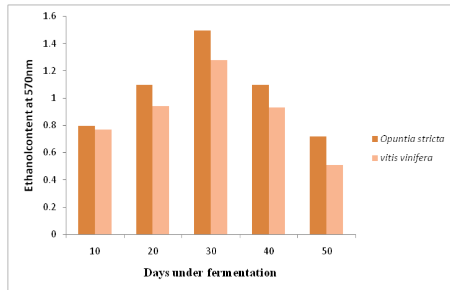


Fig. 1: Ethanol content present at various duration of fermentation

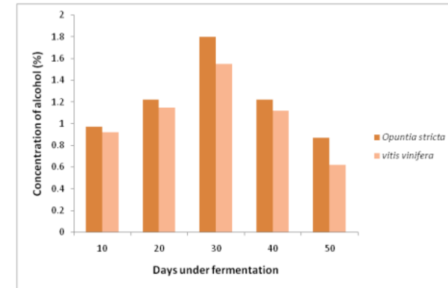


Fig. 2: Concentration of alcohol (%) in the fermented fruits during various time intervels

FRUITING STAGE

OPENTIA STRICTA

VITIS VINIFERA

30 DAYS FERMENTATION

OPENTIA STRICTA

VITIS VINIFERA

REFERENCES

Aberoumand, A. and S.S. Deokule. (2009). Studies on Nutritional Values of Some Wild Edible Plants from Iran and India. *Pak. J. Nutri*. **8**(1): 26-31.

Ajay Rana and HarshPratap Sing. (2013). Bio-utilization of wild berries for preparation of high valued herbal wines.*Indian J. Nat. Prod. Resour*. **4**(2): 165-169.

Arora, R.K. and PandeyAnjula. (1996). Wild edible plant of India- Diversity, Conservation and Use, (National Bureau of Plant Genetic Resources, ICAR, New Delhi).

Conklin, N.L. and R.W. Wrangham. (1994). The value of figs to a hind-gut fermenting frugivore: a nutritional analysis. *Biochem. Syst. Ecol.* **22**: 137–151.

Corlett, R.T. (1996). Characteristics of vertebrate-dispersed fruits in Hong Kong. *J. Trop. Ecol.* **12**: 819–833

Nayagam, C.M., M.S. Pushparaj and S. Rajan. (1993). Less known edible fruit-yielding plants of Nilgiris. *Ancient Sci Life* **14**: 363-376.

El Kossori, R.L., C. Villaume., E. El Boustani., Y. Sauvaire and L. Mejean. (1998). Composition of pulp, skin and seeds of prickly pears fruits (*Opuntiaficus indica* sp.). *Plant Food Human Nutri*. **52**: 263-270.

Eromosele, I.C., C.O. Eromosele and D.M. Kuzhkzha. (1991). Evaluation of mineral elements and ascorbic acid contents in fruits of some wild plants. *Plant Food Human Nutri.* **41**: 151-154.

FAO. (1999). Use and potential of wild plants (Information Division, Food and Agricultural Organization of the United Nations, Rome, Italy).

Feugang, J.M., P. Konarski, D. Zou, F.C. Stintzing and C. Zou. (2006). Nutritional and medicinal use of cactus pear (*Opuntia spp*.) cladodes and fruits. *Front Bioscience*. **11**: 2574-2589.

Foster, M.S. and R.W. McDiarmid. (1983). Nutritional value of the aril of *Trichiliacuneata*, a bird-dispersed fruit.*Biotropica.* **15**: 26–31.

Glew, R.S., D.J. VanderJagt, R. Bosse., Y.S. Huang., L.T. Chuang and R.H. Glew. (2005). The nutrient content of three edible plants of the Republic of Niger. *J. Food Compos. Anal.* **18**: 15-27.

Grivetti, L.E. and M. Ogle Britta. (2000). Value of traditional foods in meeting macro-micronutrient needs: the wild plant connection. *Natl. Res. Rev.* **13**: 31- 46.

Herrera, C.M. (1987). Vertebrate-dispersed plants of the Iberian Peninsula: a study of fruit characteristics. *Ecol. Monogr.* **57**: 305–331.

Izhaki, I. (1992). A comparative analysis of the nutritional quality of mixed and exclusive fruit diets for yellow-vented bulbuls. *Condor*. **94**: 912–923.

Johnson, R.A., M.F. Willson and J.N. Thompson. (1985). Nutritional values of wild fruits and consumption by migrant frugivorous birds. *Ecology.* **66**: 819–827.

Jordano, P. (1995). Angiosperm fleshy fruits and seed dispersers: a comparative analysis of adaptation and constraints in plant-animal interactions. *Amer. Nat*. **145**: 163–191.

Katewa, S.S. (2003). Contribution of some wild food plants from forestry to the diet of tribal of Southern Rajasthan. Ind. *Forest.* **129**(9): 1117-1131.

Maikhuri, R.K., R.L. Semwal., A. Singh and M.C. Nautiyal. (1994). Wild fruit as a contribution to sustainable rural development: A case study from the Garhwal Himalaya. *Int. J. Sust. Dev. World.***1**: 56-68.

Martinez del RIO, C., and C. Restrepo. (1993). Ecological and behavioral consequences of digestion in frugivorous animals. *Vegetatio.* **107**(108): 205–216.

Musinguzi, E.L, J.K. Kikafunda and B.T. Kiremire. (2007). Promoting indigenous wild edible fruits to complement roots and tuber crops in alleviating vitamin A deficiencies in Uganda. Proceedings of the 13th ISTRC Symposium, p. 763-769.

Nagy, S., P.E. Shaw and W.F. Wardowski. (1990). Fruits of tropical and subtropical origin: composition, properties and uses. Florida Science Source, Florida p. 391.

Nazarudeen, A. (2010). Nutritional composition of some lesser-known fruits used by ethnic communities and local folks of Kerela. *I. J. T. K.* **9**(2): 398-402.

Nkafamiya, I.I., U.U. Modibbo., A.J. Manji and D. Haggai. (2007). Nutrient content of seeds of some wild plants. *Afric. J. Biotech.* **6**(14): 1665-1669.

Okafor, N. (2007). Microbiology and biochemistry of oil palmwine. *Advanced Application of Microbiol*ogy. **24**: 237-255.

Piga, A. (2004) Cactus pear: A fruit of nutraceutical and functional importance. *J. Professional Association of Cactus Development*, p. 9-22.

Ramadan, M.F. and J.T. Morsel. (2003). Oil cactus pear (*Opuntiaficus-indica* L.). *Food Chem.* **82:** 339–345.

Saenz, C. (1995). Food Manufacture and by-products. In: Agroecology, cultivation and uses of cactus pear. Eds: Barbera G, Inglese P, Pimienta-Barrios E, FAO. *Plant Product and Protection Paper*, *Rome*. **132**: 137–143

Sawaya, W.N., J.K. Khalil and M.M. Al-Mohammad. (1983). Nutritive value of prickly pear seeds, *Opuntiaficus indica*. *Plant Foods Human Nutr.* **33:** 91–97

Sharma, B.M. (1977). Ph.D. Thesis, Banaras Hindu University, Varanasi, India.

Slikkerveer, L. (1994). Indigenous agricultural knowledge systems in developing countries: a bibliography. Project Report on Indigenous Knowledge Systems Research Development, Studies no.1, Special Issue: INDAKS in collaboration with the European Commission DG XII, Leiden, and the Netherlands.

Stintzing, F.C. and R. Carle. (2005). Cactus stems (*Opuntia* sp.): A review on their chemistry, technology, and uses*. Mol. Nutr. Food Res.* **49**: 175-94.

Sundriyal, M. (1998). Wild edibles and other useful plants from the Sikkim Himalaya, India. *O. Ecology. Mont.* **7**: 43- 54.

Widdowson, E.M. and R.A. McCance. (1935). The available carbohydrate of fruits: determination of glucose, fructose, sucrose and starch. *Biochem. J.* **29**: 151–156.

Kong. Res. J. 2(1) : 71-80, 2015 ISSN 2349-2694
Kongunadu Arts and Science College, Coimbatore.

SPECIES DIVERSITY, UTILIZATION AND CONSERVATION IN HOME GARDENS OF SOME RESIDENTIAL AREAS, COIMBATORE, INDIA.

Jamuna, S., R. Subhasree, K. Karthika, S. Paulsamy* and K. Thenmozhi.
Department of Botany, Kongunadu Arts and Science College, Coimbatore-641 029, Tamil Nadu, India.
*E-mail: paulsami@yahoo.com

ABSTRACT

The present study was aimed at documenting species composition, utilization and conservation of plant species growing in home gardens of 10 residential areas of Coimbatore city, India. Household interviews and home garden surveys revealed that all the 109 plant species included in 60 families included have some economic uses or with ornamental significance. Higher number of species was herbs followed by shrubs, trees, climbers, succulent herbs, vines and sub-shrubs. The families *viz.,* Asteraceae, Apocynaceae and Acanthaceae contributed higher number of plant species than the other families to the home gardens. The species namely, *Celosia cristata, Chrysanthemum odoratum* and *Ocimum basilicum* have registered 50% frequency among the home gardens sampled which indicates that these species have distributed and maintained in comparatively higher number of home gardens. The home garden species are mainly under the categories of vegetables, fruits, ornamentals, economic important species and medicinal. These results further report revealed that homegardens satisfy various household needs like food, ornamentals, medicines, building material, religious and ceremonial uses.

Keywords: Home gardens, species diversity, species usage, frequency, use value.

1. INTRODUCTION

Home garden is generally accepted to be economically efficient, ecologically sound and biologically sustainable agroforestry system (Fernandez and Nair, 1986). It also serves as sink of carbon, thereby, playing an ecological role in the current global climate change scenario (Saha *et al*., 2009). Home garden maintained in many places not only to meet out the need of day to day life but also to provide ecological security to some extent. Seasonal gardens in many residential areas of developing countries offer adequate economical return to the people (Eliotcoleman, 2000). Home gardens have recently been recognized for their potential for biodiversity conservation (Raheem *et al.,* 2008; Kabir and Webb, 2008) and for their social and cultural significance (Buchmann, 2009; Rowe, 2009). Increasing attentions have been focused on the potential of home garden to harbor genetic diversity, which is a key component of conservation efforts associated with population management (Hollingsworth *et al.,* 2005; Lengkeek *et al.,* 2006; Miller and Schaal, 2006).

Coimbatore is the leading industrial city in southern India, endowed with huge human population of 3,458,045. The industrial areas, educational institutions and residential areas are maintaining home gardens almost in all parts of the city. In addition to several industries like textile mills, boundaries etc. residential areas are well designed in terms of maintaining home gardens and according to availability of the land area, the residents developed and established home gardens. People of upper economic and educated maintained their home gardens mainly with many ornamental plants and some plants of red listed categories also. Despite the well establishment of home gardens no taxonomical and ecological studies have been made so far in Coimbatore city. To address this lacuna, the present study was aimed to document the flora of the home garden and to categorize the plants into medicinal/other economical important species at different locations in Coimbatore city.

2. MATERIALS AND METHODS

2.1. Study site

A total number of 10 home gardens with different sizes located in places *viz.,* Ganapathy, Race course, Onampalayam, Avinashi, Cheran nagar, Saravanampatti, G.N. Mills, Thudiyalur, Vadavaalli and Vinayagapuram were selected for present study.

2.2. Species analysis

The home gardens selected were explored for the plant species for the information on habit or life form, medicinal other economic uses, parts used. Family-wise contribution of species has also been

enumerated. The degree of distribution of various plant species among the home gardens was determined as per the following formula:

$$\text{Frequency (\%)} = \frac{\text{Number of home gardens in which the species present}}{\text{Total number of home gardens sampled}} \times 100$$

2.3. Species usage patterns in home gardens

The medicinal and other economic uses of the plant species present in the home gardens of sampling places were documented on the basis of personal interview with the respective gardeners and the owners of the residential homes and by literature. The red listed and endemic species were enlisted according to Ahmedullah and Nair (1987).

3. RESULTS AND DISCUSSION

For the present study, all over the city of Coimbatore, 10 home gardens in 10 different residential areas have been selected to enumerate the species taxonomically and to evaluate ecologically (Table 1 and Fig. 1). The sizes of the home gardens sampled were also varied much between ca. 25 m^2 and 225 m^2. The species richness noted to be varied between the home gardens studied. The number of species was not in accordance with the size of the home gardens studied in Coimbatore city. Studies of home gardens in Mexico (Rico Gray *et al*., 1991) and Indonesia (Abdoella, 2006) indicated that the number of species or individuals is not related to home garden size.

The higher species richness of 32 was present in the home gardens of Ganapathy followed by 30 in Cheran Nagar and Vinayagapuram residential areas each. On the other hand, the lower species richness of 3 was noted in the residential area of Vadavalli. Altogether, 109 plant species belongs to different life-forms were noted to be present in the studied home gardens. Kumar and Nair (2004) aptly regarded home gardens with high species richness above 20 'as the glorious examples of species diversity in cultivated and managed plant communities.

The variation in life-form among the species noted in the home garden of Coimbatore city was mostly herbs (49.54%) followed by shrubs (22.02%), trees (18.35%), climbers (3.67%), succulent herbs, vines and sub-shrub species (1.83%). This may be due to the need and individual option. The most grown herbs in the gardens are mainly for the purpose of supplying of requirements to their day to day life as vegetables, greens and medicinal plants.

In addition to higher species diversity, the diversity of families was noted to be most noteworthy (Table 2). A total number of 60 families with the contribution 109 species were present in the home gardens. The family, Asteraceae contributed the higher number of 7 species (11.67%) followed by the family, Apocynaceae with 6 species (10%), Acanthaceae with 5 species (8.33%), Moraceae, Solanaceae and Fabaceae with 4 species each (6.67%) and Araceae, Amaranthaceae, Myrtaceae, Asparagaceae, Rubiaceae and Malvaceae with 3 species each (5%) to the communities of home gardens. The remaining families have contributed 1 or 2 species only to home garden communities. The higher number of species in the families of Asteraceae, Apocynaceae and Acanthaceae indicates the diverse utility of the plant resources particularly the preferences towards medicinal uses. The present findings of 109 plant species belong to various life-form categories with different utilities indicate the biological richness of home gardens in Coimbatore city (Kumar *et al.,* 1994). The high floristic diversity is perhaps the reflection of the potential of home gardens to serve as repositories of genetic diversity as well. Kabir and Webb (2008) also reported the predominance of herbs and trees in the home gardens of south-western Bangladesh.

The degree of distribution of the various enumerated plant species was very low and it was ranging between 10 and 50% only (Table 1). The species namely, *Celosia cristata, Chrysanthemum odoratum* and *Ocimum basilicum* have registered 50% frequency which indicates that these species have distributed comparatively in higher number of home gardens (Fig. 2). The other species have recorded below 50% frequency only and hence they have restricted in distribution in few home gardens only. The overall distribution level indicates that each home garden owner has their own preference over the species. Pandey *et al*. (2006) also reported the lower distribution level of many home garden plants in South Andaman and he explained that the selective cultivation of species with the home gardens is mainly due to the utility value of the species.

The total number of individuals maintained for the constituent species in the home gardens was widely varied (Table 1 and Figs. 3 and 4). Few species like *Calliandra cyanometroides, Canna indica, Catharanthus roseus, Chrysanthemum odoratum, Cosmos bipinnatus* and *Coriandrum sativum* were registered with higher density when compared to other species (Fig. 3). The endemic plant species, *Saraca indica* was represented by only one

individual at Vinayagapuram residential area. Similarly, the species such as *Artocarpus heterophyllus, Callistemon citrinus, Cereus pterognus, Hamelia patens, Mangifera indica, Momordica charantia, Ravenala madagascariensis, Robinia pseudoacia, Salvinia officinalis, Scindapsus variegate, Saraca indica* and *Thuja mysorensis* have also been represented by only one individual in very less number (Fig. 4). It has been recognized that most of the mentioned above are economically important. The maintenance of this species with higher standing crop individuals in the home gardens may be due to economic security provided by these species to the respective home.

Table 1. Number of individuals of constituent plant species and their frequency in the sampled home gardens of sampling places in Coimbatore city.

S.No	Species	Habit	Home gardens*										Frequency (%)
			1	2	3	4	5	6	7	8	9	10	
1	*Acalypha wilkesiana* hort.	Shrub	5	8	-	-	4	-	-	-	-	-	30
2	*Adenium obesum* (Forsk.) Roem. et Schult.	Shrub	1	-	3	-	4	-	-	-	6	-	40
3	*Anthurium spathiphyllum* Schott	Herb	7	-	-	-	-	5	-	6	-	-	30
4	*Allamanda cathartica* L.	Shrub	3	-	-	-	-	1	-	-	-	-	20
5	*Aloe vera* (L.) Burm.f.	Succulent herb	-	-	-	-	-	-	5	-	2	-	20
6	*Antigonon leptopus* Hook & Arn	Vine	-	-	-	-	-	-	17	-	-	25	20
7	*Aphelandra squarrosa* Nees.	Herb	-	-	-	-	-	-	-	-	-	6	10
8	*Aralia* sp. L	Herb	-	-	-	-	-	-	9	-	-	-	10
9	*Arctotis hirsuta* (Harv.) Beauverd	Herb	-	-	12	-	-	-	-	-	6	8	30
10	*Araucaria excelsa* R.Br	Tree	-	-	-	2	-	5	-	-	-	-	20
11	*Achyranthes aspera* L.	Herb	-	-	-	-	23	-	-	-	-	-	10
12	*A. caudatus* L.	Herb	-	-	-	-	-	-	-	-	-	24	10
13	*Azardica indica* A. Juss	Tree	1	-	-	-	-	-	-	-	1	-	20
14	*Artocarpus heterophyllus* Frost.	Tree	1	-	1	-	-	-	1	-	-	-	30
15	*Basella rubra* L.	Vine	9	-	-	-	-	-	-	-	-	-	10
16	*Bougainvillea glabra* Choisy	Creeper	2	-	-	-	3	-	-	-	-	-	20
17	*Callistemon citrinus* (Curtis) Skeels	Shrub	-	-	-	-	-	1	-	-	-	-	10
18	*Calliandra cyanometroides* Bedd	Herb	-	-	-	-	30	11	-	16	10	-	40
19	*Calathea* sp. R.Br	Herb	-	-	-	-	-	5		-	-	-	10
20	*Canna indica* L.	Herb	-	-	-	-	15	-	-	21	10	5	40
21	*Capsicum annuum* L.	Shrub	-	3	-	-	-	-	-	-	2	-	20
22	*Catharanthus roseus* Linn.	Sub shrub	18	-	-	-	12	-	-	15	-	10	40
23	*Celosia cristata* L.	Shrub	2	-	1	3	-	-	-	5	2	-	50
24	*Cereus pterogonus* Lem.	Herb	-	-	-	-	-	-	-	1	-	-	10
25	*Cestrum nocturnum* L.	Herb	-	-	-	-	-	-	29	-	-	-	10
26	*Chlorophytum variegatum* Ker	Herb	4	-	-	7	-	-	-	10	2	-	40
27	*Chrysanthemum carinatum* L	Herb	-	-	3	-	-	-	-	7	-	-	20
28	*C. grandiflorum* L.	Herb	-	21	-	-	-	-	-	-	-	-	10
29	*C. odoratum* L.	Herb	70	-	41	55	63	-	-	-	84	-	50
30	*Clitoria ternatea* L.	Herb	-	-	-	-	-	25	-	-	-	-	10
31	*Coleus aromaticus* Benth	Herb	-	20	-	6	9	-	-	-	-	-	30
32	*Cordyline stricta* L.	Herb	-	-	-	6	-	-	-	-	-	-	10
33	*Cosmos bipinnatus* Cav	Herb	63	-	-	-	-	-	-	-	-	82	20
34	*Crassula* sp.L.	Herb	-	-	5	-	-	8	-	-	-	-	20
35	*Crossandra infundibuliformis* L Salib	Herb	18	-	10	-	-	-	-	-	7	-	30
36	*Cucumis pepo* DC.	Climber	-	-	-	-	-	3	-	-	-	-	10
37	*Curcuma longa* L.	Herb	2	-	5	-	-	-	-	-	-	-	20
38	*Calotropis gigantea* R.Br.	Shrub	-	-	-	-	-	-	2	-	-	-	10
39	*Carica papaya* L.	Tree	-	-	-	-	1	-	-	1	-	-	20
40	*Coriandrum sativum* Linn.	Herb	35	-	-	-	-	19	-	-	28	-	30
41	*Citrus lemon* L.	Tree	1	-	-	-	3	-	5	-	-	-	30
42	*Cardiospermum halicacabum* L	Herb	-	-	-	-	5	-	-	-	-	-	10
43	*Cycas siamensis* Miq	Tree	1	2	1	-	-	1	6	-	-	-	50
44	*Dracaena* sp. Lam	Shrub	-	-	-	-	5	-	2	-	-	-	20
45	*Duranta repens* L.	Shrub	-	-	-	-	-	-	4	-	-	6	20
46	*Damascus carota* Nayeem Ket	Herb	4	-	-	-	-	-	2	-	-	-	20
47	*Ficus benghalensis* Linn.	Tree	-	-	-	-	-	1	1	-	-	-	20
48	*F. benjamina* Linn.	Tree	1	-	2	-	-	-	-	-	-	-	20
49	*F. microspora* Wight	Tree	-	-	4	-	-	3	-	-	-	-	20

50	*Geranium domesticum* Roxb.	Herb	-	-	-	-	-	2	5	6	-	-	30
51	*G. peltatum* Roxb	Herb	-	-	-	-	-	-	-	5	-	-	10
52	*Grevillea robusta* A. Cunn. ex R. Br	Shrub	2	-	-	-	-	-	-	-	-	-	10
53	*Hamelia patens* Jacq	Shrub	-	-	-	-	-	-	-	1	-	-	10
54	*Hibiscus rosa sinensis* L.	Shrub	-	-	-	-	6	-	-	8	-	9	30
55	*H. mutabilis* L.	Shrub	-	8	-	-	4	6	-	2	-	-	40
56	*H. syriacus* L.	Shrub	2	-	-	-	-	-	-	-	-	2	20
57	*Hydrangea macrophylla* (Thunb.) Ser.	Shrub	-	-	-	-	-	-	-	-	2	-	10
58	*Inga cyanocetroides* Linn.	Shrub	4	-	2	-	1	-	-	-	3	-	40
59	*Ixora coccinea* L.	Shrub	1	-	-	-	4	4	-	-	-	-	30
60	*Jacquemontia pentantha* Choisy	Herb	-	-	-	-	37	-	-	-	-	-	10
61	*Jasminum angustifolium* Vahl	Herb	-	-	-	-	-	3	-	2	-	-	20
62	*J. grandiflorum* L	Herb	-	-	-	-	-	2	1	1	-	1	40
63	*J. sambac* Ait	Herb	-	-	-	-	2	-	-	-	-	3	20
64	*Jatropha peltata* Wight	Herb	-	-	-	-	-	-	-	-	-	2	10
65	*Kalanchoe fentchokoi* Adans	Herb	-	-	10	-	-	-	-	-	-	14	20
66	*Knoxia* sp. L.	Herb	-	-	-	-	-	-	-	20	16	-	20
67	*Lablab purpureus* (L) Sweet	Vine	-	-	3	-	-	-	1	2	4	-	40
68	*Lantana viscose* L.	Shrub	1	-	-	-	-	-	-	1	-	-	20
69	*Lawsonia inermis* L.	Tree	-	-	-	-	-	-	-	1	-	1	20
70	*Madhuca longifolia* J. Konig J.F.Macbr.	Tree	-	-	-	-	-	-	-	2	1	2	30
71	*Mangifera indica* L.	Tree	-	-	-	-	-	-	-	-	1	-	10
72	*Miranda leucophyllum* Harts	Tree	7	-	-	-	-	-	-	-	-	-	10
73	*Momordica charantia* L.	Herb	-	-	-	1	-	-	-	-	-	-	10
74	*Moringa oleofera* Lam	Tree	-	-	-	-	2	-	-	-	-	-	10
75	*Murraya paniculata* L.	Herb	-	-	-	-	4	-	-	2	-	-	20
76	*Musa paradisiaca* L.	Tree	-	-	-	-	-	1	4	5	-	-	30
77	*Nephrolepis* sp. Schot	Herb	-	-	-	-	-	-	-	-	-	5	10
78	*N. tuberosa* Bory ex Willd	Herb	-	-	1	-	-	-	-	-	-	6	20
79	*Nerium oleander* Linn.	Shrub	-	-	-	-	-	-	3	-	2	-	20
80	*Ocimum basilicum* Linn.	Herb	-	-	-	5	4	4	3	2	-	-	50
81	*Oxalis radicosa* Linn.	Herb	-	-	-	-	-	-	-	-	-	42	10
82	*O. corniculata* Linn.	Herb	-	-	-	37	-	-	-	-	-	-	10
83	*Phyllanthus emblica* Linn.	Tree	-	-	-	-	1	-	1	-	1	-	30
84	*Pistia stratiotes* Linn.	Succulent herb	-	-	-	-	-	-	10	-	-	22	20
85	*Plumbago auriculata* Lam	Herb	-	-	3	-	-	-	-	10	3	1	40
86	*Plumeria rubra* Linn.	Shrub	2	-	-	-	1	-	-	-	-	-	20
87	*Punica granatum* Linn.	Sub shrub	-	-	-	3	2	-	1	-	-	3	40
88	*Piper betle* Linn.	Creeper	-	-	-	-	-	-	-	-	15	10	20
89	*Ravenala madagascariensis* Sonn	Herb	-	-	1	-	-	-	-	-	-	-	10
90	*Robinia pseudoacacia* L.	Herb	-	-	-	-	-	1	-	-	-	-	10
91	*Tiarella grandiflora* Roxb	Herb	1	-	-	-	-	-	-	-	-	-	10
92	*Rosa* sp. W.	Shrub	1	-	4	-	-	-	-	-	3	1	40
93	*Salvinia officinalis* L.	Herb	-	-	-	-	-	-	-	-	1	-	10
94	*Sansevieria roxburghiana* Schult	Herb	-	-	-	-	-	-	-	-	-	2	10
95	*Scindapsus variegate* (Hayata) Kanehira	Creeper	1	-	-	-	-	-	-	-	-	-	10
96	*S. melongena* Pr.	Herb	-	-	-	-	1	-	3	-	2	-	30
97	*S. lycopersicum* Linn.	Herb	-	-	-	5	-	-	-	-	-	-	10
98	*Saraca indica* Linn.	Tree	-	-	-	-	-	-	-	-	-	1	10
99	*Tagetes erecta* B.	Herb	-	-	-	-	-	1	-	-	2	3	30
100	*Tradescantia discolor* S .W	Herb	-	-	-	-	10	-	-	-	-	-	10
101	*Thuja occidentalis* L.	Shrub	-	-	-	-	1	-	-	1	-	1	30
102	*T. mysorensis* T. and Roxb	Shrub	1	-	-	-	-	-	-	-	-	-	10
103	*Tabernaemontana divaricata* R.Br. ex Roem. & Schult.	Shrub	4	-	-	-	-	5	1	1	-	-	40
104	*Tecoma grandis* L.f	Tree	-	-	-	-	1	2	-	-	-	2	30
105	*Terminalia catappa* Linn.	Tree	-	-	-	-	-	1	-	-	-	1	20
106	*Taxus wallichiana* Linn	Tree	-	-	-	-	2	1	-	-	-	1	30
107	*Ursinia cerevisiae* (Thunb.) N.E.Br	Herb	-	-	-	-	1	-	-	-	2	1	30
108	*Zephyranthes carinata* Herb	Herb	-	-	-	-	20	12	-	-	-	-	20
109	*Zinnia grandiflora* Linn.	Herb	-	-	-	6	-	-	2	-	4	-	30

*1 - Ganapathy; 2 - Race course; 3 – Onampalayam; 4 – Avinashi; 5 - Cheran nagar; 6 – Saravanampatti; 7 - G.N. Mills; 8 – Thudiyalur; 9 – Vadavaalli; 10 - Vinayagapuram.

Fig. 1. The sampled home gardens of Coimbatore city.

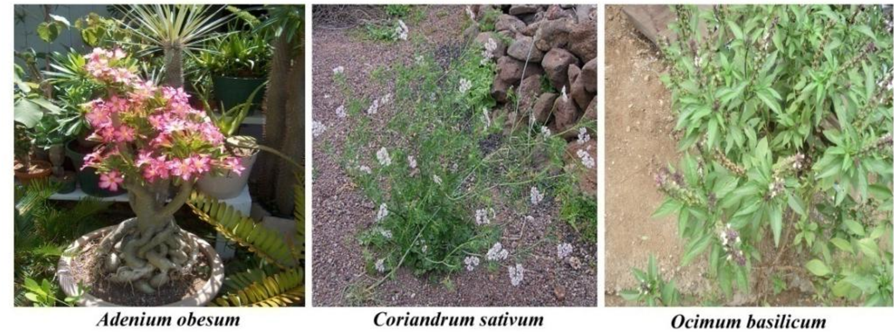


Fig. 2. The species of higher degree of distribution among the home gardens.

Fig. 3. Some species of relatively high density.

Fig. 4. Certain species of relatively low density.

Table 2. Family, useful part, medicinal and other economic uses of various plant species in the home gardens of Coimbatore city.

S. No.	Species	Family	Parts used	Medicinal/other economic uses
1	*Acalypha wilkesiana*	Acanthaceae	Leaf	The extract of the flower inhibits the ovarian function and stimulate the uterine. Roots are used in the treatment of diabetes, antipyretic, abortifacient, demulcent, lessens inflammation and heat of the body; useful to relieve chest pain.
2	*Adenium obesum*	Apocynaceae	Bark and sap	The plant sap and bark are used as remedy for bone dislocation, rheumatism, sprains, paralysis, swellings, wounds and skin infections.
3	*Anthurium spathiphyllum*	Araceae	Whole plant	Cleans indoor air of many environmental contaminants, including benzene, formaldehyde and other pollutants.
4	*Allamanda cathartica*	Apocynaceae	Flower	Flower has been used to treat liver tumors, jaundice, splenomegaly and malaria.
5	*Aloe vera*	Liliaceae	Leaf	Aloe has been marketed as a remedy for coughs, wounds, ulcers, gastritis, diabetes, cancer, headaches, arthritis, immune-system deficiencies, and many other conditions when taken internally. The lower leaf is sliced open, the gel obtained can be applied on the affected area of the skin.
6	*Antigonon leptopus*	Polygonaceae	Leaf, bark and seed	The leaves and barks are protective against bronchial asthma and other allergic disorders. Barks and seeds are astringent and are given in piles and diarrhoea.
7	*Aphelandra squarrosa*	Acanthaceae	-	Ornamental plant.
8	*Aralia* sp.	Araliaceae	-	Ornamental plant.
9	*Arctotis hirsuta*	Acanthaceae	-	Ornamental plant.
10	*Araucaria excelsa*	Araucariaceae	Leaf	It reduces the bacterial contaminants.
11	*Achyranthes aspera*	Amaranthaceae	Leaf and seed	It is used to improve appetite and to cure various types of gastric disorders. It is useful in haemorrhoids, leaves and seeds are emetic, hydrophobia, carminative, resolve swelling, digestive and expel phlegm.
12	*A. caudatus*	Amaranthaceae	Leaf, stem and root	The roots are used to cure kidney stones. The leaves used to cure cuts, leprosy, boils, burns, fever and decoction of the stem used in jaundice.
13	*Artocarpus heterophyllus*	Meliaceae	Whole plant	Leaves, bark, flowers, fruits, seed, gum, oil and neem cake are used to have antiallergenic, antidermatic, antifeedent, antifungal, antiinflammatory, antipyorrhoeic, antiscabic, cardiotonic, diuretic, insecticidal, larvicidal, nematicidal, spermicidal and other biological activities.
14	*Azardirachta indica*	Moraceae	Leaf and fruit	The leaves are useful in fever, ulcers, boils wounds, skin diseases, antidiarrhoeal, analgesic and as immuno modulator. The ripe fruits are sweet cooling, laxative, aphrodisiac, and tonic. The seeds used for are sweet, diuretic, aphrodisiac and constipation.
15	*Basella rubra*	Basellaceae	Root	Decoction of the root relieves bilious vomiting. Spinach extracts has beneficial effects such as chemo and central nervous system protection, anticancer and antiaging function.
16	*Bougainvillea glabra*	Nyctaginaceae	Flower	The leaves used for a variety of disorders such as diarrhoea, and to reduce stomach ulcers, cough, sore throat, hepatitis, a decoction of dried stems and flower used as treatment for low blood pressure.
17	*Callistemon citrinus*	Myrtaceae	Leaf	It is used for treating hemorrhoids.
18	*Calliandra cyanometroides*	Myrtaceae	-	Ornamental plant.
19	*Calathea* sp.	Marantaceae	-	Ornamental plant.
20	*Canna indica*	Cannaceae	Root and seed.	The root decoction is used for the treatment of fever, dropsy, and dyspepsia. Seed juice is used to relieve ear aches.
21	*Capsicum annuum*	Solanaceae	Fruit	It is used as carminative, an appetizer and a stomachic. Externally, it is used as a counter irritant and also in the treatment of rheumatism, lumbago and neuralgia.
22	*Catharanthus roseus*	Apocynaceae	Whole plant	Minimizing the adverse effects of cheamotheraphy, caricinogenic agents and prolonging longevity types possesses known antibacterial, antifungal, antidiabetic and antiviral activities.
23	*Celosia cristata*	Amaranthaceae	Leaf and flower	It is used in the treatment of diarrhoea, piles, bleeding nose, disinfectant, inflammation, haematological and gynaecologic disorders.
24	*Cereus pterogonus*	Cactaceae	-	Ornamental plant.
25	*Cestrum nocturnum*	Solanaceae	Leaf	Leaves are used for their pharmacological significance in burns and swellings. It is also used for treating epilepsy and as stupefying charm medicine. It is used to prevent malaria.
26	*Chlorophytum variegatum*	Liliaceae	-	Ornamental plant.
27	*Chrysanthemum*	Asteraceae	Flower	The leaves are used medicinally to cure influenza symptoms, liver and

	carinatum			menstrual disorders and have antiinflammatory and antispasmodic effects.
28	*C. grandiflorum*	Asteraceae	Leaf	It is used for anticancer activity.
29	*C. odoratum*	Asteraceae	Flower	Flowers are used for antihypertensive, hypertropic scar fibroblast inhibiting, antidepressive, serotonin antagonist, anticancer, antispasmodic, antioxidative and antimicrobial activities roselle can prevent cancer and lower blood pressure as well as improve the digestive system in human
30	*Clitoria ternatea*	Fabaceae	Whole plant	The herb is effective in curing fever and acts as asthma and bronchitis etc. The extract gives neuropharmacological value. A paste of the whole plant can be applied over the infected area and decoction of the plants is very effective in cleaning the wound.
31	*Coleus aromaticus*	Lamiaceae	Leaf	The leaves are used for the treatment of cough, throat infection and nasal congestion.
32	*Cordyline stricta*	Asparagaceae	Leaf	It is used to treat dysentery and skin diseases. It breaks fever and to assuage headache. The leaves consumed as vegetable.
33	*Cosmos bipinnatus*	Asteraceae	Flower and leaf	Leaves are used for fever, flue, cough, asthma, digestive troubles, piles, diabetes, urinary diseases, male sexual diseases, gynecological diseases, joints pain/rheumatic pains and inflammation, ear diseases, tooth problems, cuts and wounds, skin diseases, cooling agents and miscellaneous uses.
34	*Crassula* sp.	Crassulaceae	-	Ornamental plant.
35	*Crossandra infundibuliformis*	Acanthaceae	Leaf and latex	In the treatment of infectious diseases while simultaneously mitigating many of the side effects.
36	*Cucumis pepo*	Cucurbitaceae	Leaf, fruit and seed.	Seeds are used as a diuretic. Leaves are used as a painkiller, a treatment for nausea, and a boost to haemoglobin content of the blood. The fruit is used for cooling and astringent to the bowels, increases appetite, cures leprosy and purifies the blood.
37	*Curcuma longa*	Zingiberaceae	Rhizome	A fresh juice is commonly used in many skin conditions, including eczema, chicken pox, shingles, allergy and scabies. The active compound curcumin have antiinflammatory, antioxidant, antitumour, antibacterial and antiviral activities.
38	*Calotropis gigantea*	Asclepiadaceae	Leaf and latex	The powdered root is used to treat bronchitis, asthma, leprosy, eczema, elephantiasis while the latex is used to treat vertigo, baldness, hair loss, toothache, intermittent fevers, rheumatoid/joint swellings and paralysis.
39	*Carica papaya*	Caricaceae	Leaf, fruit and root	It increase appetite, ease menstrual pain, meat tenderizer and relieve nausea
40	*Coriandrum sativum*	Apiaceae	Seed, root and leaf	The seeds were included in a host of prescriptions for fever, diarrhoea, vomiting, indigestion as in stomach and carminative. Leaves are given for biliousness, intestinal irritations, heartburn, thirst and nausea. Volatile oil is carminative.
41	*Citrus lemon*	Rutaceae	Fruit and leaf	It has also been found useful in the treatment of hepatobiliar, dyski-nesia, oxiurasis, varicose veins, haemorrhoids, phlebitis and urolithiasis.
42	*Cardiospermum halicacabum*	Sapindaceae	Leaf and fruit	The tender, young shoots are used as a diuretic, stomachic and rubefacient. It is used in rheumatism, lumbago, nervous diseases and as a demulcent in arthritis and in dropsy.
43	*Cycas siamensis*	Cycadaceae	-	Ornamental plant.
44	*Dracaena* sp.	Asparagaceae	Fruit	The fruits are used in the treatment of malarial and intestinal worms.
45	*Duranta repens*	Verbenaceae	Leaf and fruit	The roots are stimulant for indolent ulcer. Different parts of the carrot have been used in medicine for the treatment of kidney dysfunction, asthma, dropsy, inflammation, leprosy, worm troubles, etc.
46	*Damascus carota*	Apiaceae	Leaf and latex	The latex for a depilatory, pain relief, antibacterial and emetic. Remedies for skin, warts and toothache. Regarding safety and efficacy in pregnancy and lactation is lacking
47	*Ficus benghalensis*	Moraceae	Leaf	It is used for the treatment of skin diseases and enlargement of liver.
48	*F. benjamina*	Moraceae	Leaf, bark and root	The treatment of certain skin disorders, stomachic, hypotensive and antidysentery. Leaf, bark and fruits are used as antimicrobial, antitumor, antiinflammatory, antinociceptive, antipyretic and cytotoxic activity.
49	*F. microspora*	Moraceae	Leaf and bark	It has been used for intestinal problems, wounds and respiratory ailment. Oil is considered a relaxant in aroma therapy and in recent years it is used as respiratory.
50	*Geranium domesticum*	Geraniaceae	Seed and leaf	Ornamental plant.
51	*G. peltatum*	Geraniaceae	Flower	It is used to treat athlete's foot, skin lesions, rashes, insect bites, nervous shock, inflammation, rheumatism, headache, asthma, and dysentery.

52	*Grevillea robusta*	Proteaceae	-	Ornamental plant.
53	*Hamelia patens*	Rubiaceae	Leaf and flower	It regulates menstruation and stimulate blood circulation. The flower extract has been traditionally used for liver disorders, high blood pressure and as an aphrodisiac. Young leaves and flowers are used in case of headache.
54	*Hibiscus rosasinensis*	Malvaceae	Flower, root and leaf	They are used in antiinflammatory.
55	*H. mutabilis*	Malvaceae	Leaf, flower and seed	The leaves are diuretic, expectorant and stomachic. Decoction of the flowers is used for ophthalmic and stomachic. It is also used in the treatment of itch and other skin diseases.
56	*H. syriacus*	Malvaceae	Leaf and flower	It cures skin diseases.
57	*Hydrangea macrophylla*	Hydrangeaceae	-	Ornamental plant.
58	*Inga cyanometroides*	Fabaceae	Leaf	It is used for hepatic disorder, cancer, microbial infection, antioxidant, pain, inflammation. The flowers were used for the treatment of cancer, leucorrhoea, dysentery, dysmenorrhoea, haemoptysis and hypertension.
59	*Ixora coccinea*	Rubiaceae	Leaf, root and flower	The leaves are used as an emetic in cases of poisoning. The roots are bitter, acrid and are useful for external application in ringworm and herpes infestations and are recommended for ophthalmopathy, ulcerative stomatitis, leprosy, pruritus and wounds.
60	*Jacquemontia pentantha*	Convolvulaceae	-	Ornamental plant.
61	*Jasminum angustifolium*	Oleaceae	Leaf and root	Leaves are used in the treatment of leprosy, skin disease, ulcers, wounds and corns.
62	*J. grandiflorum*	Oleaceae	Leaf and root	Leaves are used in the treatment of leprosy, skin disease ulcers wounds and corns.
63	*Jatropha peltata*	Oleaceae	Leaf, flower and root	The plant is used for cooling, skin disorders, leprosy, ulcers, in cases of insanity, weakness of sight and affections of mouth and opium for gangrenous ulcers of the gums.
64	*Jasminum sambac*	Euphorbiaceae	Leaf	It is used to treat gastric ulcer and allied stomach ailments and malarial disease.
65	*Kalanchoe fentchokoi*	Rubiaceae	-	Ornamental plant.
66	*Knoxia* sp.	Juncaceae	-	Ornamental plant.
67	*Lablab purpureus*	Fabaceae	Leaf	It has been used an antioxidant, anticancer, antiviral and antiinflammatory activities.
68	*Lantana viscose*	Verbenaceae	Leaf	The leaves are used to treat rheumatism, gout, hemorrhoids, fractures and snake bites and also in the treatments of anesthetic and smooth muscle relaxant antidiabetic, antiulcer, antiinflammatory and antimicrobial.
69	*Lawsonia inermis*	Myrtaceae	Stem bark, root and leaf	Henna leaves, flowers, seeds, stem bark and roots are used in traditional medicine to treat a variety of ailments as rheumatoid arthritis, headache, ulcers, diarrheoa, leprosy fever, leucorrhoea, diabetes, cardiac disease, hepatoprotective and colouring agent.
70	*Madhuca longifolia*	Sapotaceae	Fruit and latex	The flowers are used as tonic, analgesic and diuretic, used as cooling agent, tonic, aphrodisiac, astringent, demulcent and for the treatment of helminthes, acute and chronic tonsillitis, bronchitis. Madhuka can be used to treat gastro intestinal ulcers.
71	*Mangifera indica*	Anacardiaceae	Fruit and leaf	Fruit is proposed as nutritional supplement (antioxidant) and an antiinflammatory, analgesic and immunomodulatory treatment to prevent disease progress or increase the patient's quality of life in gastric and dermatological disorders, AIDS, cancer and asthma. Root bark is a bitter aromatic and used for diarrhea and leucorrhea.
72	*Miranda leucophyllum*	Scrophulariaceae	-	Ornamental plant.
73	*Momordica charantia*	Cucurbitaceae	Leaf, Fruit and root	Leaves are used for ulcers and as a bitter digestive aid for intestinal gas, bloating, stomachache and sluggish digestion. Fruit pulp, leaf juice and seeds are used for antihelimintic. Leaf act as galactogogue. Roots are used for astringent.
74	*Murraya paniculata*	Moringaceae	Leaf and fruit	The leaves and fruit possesses antiinflammatory, antimicrobial, antioxidant and anticancer activity and also used for cardiovascular, hepatoprotective, antiulcer, diuretic, antiurolithiatic and antihelmintic activities.
75	*Musa paradisiaca*	Rutaceae	Leaf and root bark	Their root bark is used as an anodyne or local anesthetic for the treatment of gout, contusion and bone ache.
76	*Moringa oleofera*	Musaceae	Whole plant	Unripe bananas and plantain fruits are astringent, and used to treat diarrhea. The leaves are used for cough and bronchitis. The roots can

				arrest hemoptysis and posses strongly astringent and anthelmintic properties.
77	*Nephrolepis* sp.	Nephrolepidaceae	-	Ornamental plant.
78	*N. tuberosa*	Nephrolepidaceae	Whole plant	Used as healing agents in inflammation, leucorrhoea, piles and as antidote. It possesses antiviral, antibacterial, antiparasitic, antiinflammatory, antiulcer and antioxidant activity and used as diuretic.
79	*Nerium oleander*	Apocynaceae	Flower and leaf	The flowers are used as blood purifier and also used in the treatment of jaundice, diabetes, cancer, inflammation and eye disorders.
80	*Ocimum basilicum*	Lamiaceae	Leaf	It cures cold, cough and having high medicinal value.
81	*Oxalis radicosa*	Oxalidaceae	-	The leaves cure dysentery, diarrhea and skin disease
82	*O. corniculata*	Oxalidaceae	Leaf and flower	It is used to cure blood pressure high cholesterol hardening of the arteries atherosclerosis, pain and swelling of the pancreas and pancreatitis cancer. Leaves are applied to sore eyes.
83	*Phyllanthus emblica*	Euphorbiaceae	Fruit and leaf	Juice of the plant is useful in eye and ear diseases. Leaves are considered antiseptic, antitubercular, antidysenteric and anthelmintic and also used in eczema, leprosy, piles, ulcers, syphilis, cough and asthma. It is also used as a poultice in hemorrhoid.
84	*Pistia stratiotes*	Araceae	Leaf	The root juice is used for gastric acidity before each meal for a weak.
85	*Plumbago auriculata*	Plumbaginaceae	Root and leaf	The latex has been used for the treatment of itches, swellings and fevers, inflammations, arthritis and constipation. In the Guinas medicines are produced from root and bark and used for the treatment of skin eruptions and abscesses, dysentery, herpes, syphilis, cough and as a purgative.
86	*Plumeria rubra*	Apocynaceae	Root, bark and latex	The fruits are used in the treatment and prevention of cancer, cardiovascular disease, diabetes, dental problems, erectile dysfunction, bacterial infections, antibiotic resistance and ultraviolet radiation induced skin damage.
87	*Punica granatum*	Punicaceae	Fruit	Fruits are used in the treatment of diabetes and kidney stone problems, arteriosclerosis, diabetic nephropathy, diabetic retinopathy in addition to the control of blood glucose level.
88	*Piper betle*	Piperaceae	Leaf	It cures cold and cough.
89	*Ravenala madagascariensis*	Strelitziaceae	Leaf	Leaves are used for metrorrhagia, hemoptysis, large intestine hemorrhage, rheumatic arthritis and gynecologic disease.
90	*Robinia pseudoacacia*	Fabaceae	Leaf	Leaves are used to cure skin diseases and scabies.
91	*Tiarella grandiflora*	Brassicaceae	-	Ornamental plant.
92	*Rosa* sp	Rosaceae	Flower	It has been used for maintaining health, boosting immune system function and remission of cancer.
93	*Salvinia officinalis*	Lamiaceae	Leaf	The leaf sap is applied directly to sores, cuts and grazes and it include treatment for abdominal pains, ear ache, diarrhea and hemorrhoids.
94	*Sansevieria roxburghiana*	Asparagaceae	Flower	Paste of leaves can be applied to relieve pains. Seeds act as expectorant in cough and asthma. The roots are expectorant and diuretic, useful in the treatment of catarrhal fever, coughs, asthma and chest pain.
95	*Scindapsus variegata*	Araceae	-	Ornamental plant.
96	*S. melongena*	Solanaceae	Fruit and leaf	Decoction of leaf is used to cure diabetes, leprosy, gonorrhea, cholera, bronchits, dysentery, asthenia and haemorrhoids.
97	*S. lycopersicum* .	Solanaceae	Fruit	It is used in women related problems, such as leucorrhoea, menorrhagia, dysfunctional uterine bleeding and bleeding hemorrhoids.
98	*Saraca indica*	Caesalpiniaceae	Leaf	It cures the diseases of eyes cold conjunctivitis, cough, bleeding piles and ulcers bronchitis.
99	*Tagetes erecta*	Asteraceae	Flower	It is used for anticancer.
100	*Tradescantia discolor*	Commelinaceae	-	Ornamental plant.
101	*Thuja occidentalis*	Cupressaceae	Leaf	The essential oil within the plant has been used for cleansers, disinfectants, hair reparations, insecticides, liniment, room sprays and soft soaps.
102	*T. mysorensis*	Acanthaceae	-	Ornamental plant.
103	*Tabernaemontana divaricata*	Apocynaceae	Leaf	Ornamental plant.
104	*Tecoma grandis*	Bignoniaceae	Flower	The leaves were widely used as medicine for dermatosis and hepatitis. Leaves and fruits have anticancer, antioxidant, anti HIV, antiinflammatory, anti diabetic and hepatoprotective activities.
105	*Terminalia catappa*	Combretaceae	Fruit	It has unique property of preventing the growth of cancerous cells, and being used in the treatment of breast and ovarian cancer.
106	*Taxus wallichiana*	Taxaceae	Leaf and	The stem bark is used as a anticancer. This species is also used as fuel

			bark	wood by the local communities.
107	*Ursinia cerevisiae*	Asteraceae	Leaf	Used for the treatment of vermifuge and astringent.
108	*Zephyranthes carinata*	Amaryllidaceae	Flower	Ornamental plant.
109	*Zinnia grandiflora*	Asteraceae	-	Ornamental plant

Among the 109 species enlisted in the studied home gardens, the economic importance including the medicinal uses of the various plant species present in the sampled home gardens is depicted in Table 2. In the account of 109 species, 86 (78.90%) were recognized as medicinally important and 23 (21.10%) as ornamentals. The medicinal uses of the plant species are multidimensional. A greater number of 20 species are used to treat skin diseases and a sizeable number of 12 species are precribed for anticancer activities. In addition, 11 species have been known for antidiabetic properties and 2 species each for gynecological disorders and for the treatment of dysentery respectively. Interestingly it has been noted that the 2 species namely *Tecoma grandis* and *Mangifera indica* are having antiviral property suggested for AIDS patients. The results of present study exhibited a considerable array of plant species in the home gardens of Coimbatore city with different medicinal and other economic uses. Presently many home gardens show a shift from subsistence oriented agriculture to market (Peyre *et al.,* 2006).

4. CONCLUSION

In conclusion, home gardens in Coimbatore city appear to be supplementary agricultural production systems, which are managed and controlled by household members. Involvement of family members in home gardening activities empowers them to become self-reliant and simultaneously making a contribution to household food security. In addition, the home gardens can save species from the risk of extinction and thus, home gardens can be considered as a tool for conservation of medicinal plants.

ACKNOWLEDGEMENT

The authors graciously acknowledge Dr.M. Aruchami Research Foundation for providing financial support to carry out this work.

REFERENCES

Abdoellah, O.S. (1990). Home gardens in the Java and their future development In: K Landaeurand M. Brazil (eds.) *Tropical Home gardens*. United Nations University Press Tokyo, Japan. 69-79.

Ahmedullah, M. and M.P. Nayar, (1987). Endemic plants of the Indian Region. Vol. 1. Botanical Survey of India, Howrah.

Buchmann, C. (2009). Cuban home gardens and their role in socio ecological resilience. *Human Ecol.* **37**: 705-721.

Eliotcoleman, (2000). A Gardens for all seasons, St6.yahoo.com/cooks garden.

Fernandez, E.C.M. and P.K.R Nair, (1986). An evaluation of the structure and functions of tropical Home gardens. *Agroforestry systems* **2**: 279-310.

Hollingsworth, P.M., I.K. Dawson, W.P. Goodall-Copestake, J.E. Richardson, J.C. Weber, C. Sotelo and R.T. Pennington, (2005). Do farmers reduce genetic diversity when they domesticate tropical trees? A case study from Amazonian. *Mol Ecol.* **14**: 497-501.

Kabir, M.E and E.L. Webb, (2008). House hold and homegardens characteristics in south western Bangladesh. *Agro. syst.* **24**: 203-213.

Kumar, B.M and P.K. Nair, (2004). The enigma of tropical home gardens. *Agro. Syst.* **61**:135-152.

Kumar, B.M., S.J. George and S. Chinnamani, (1994). Diversity, structure and standing stock wood in the home gardens of Kerala Peninsular India. *Agro. syst.* **25**: 243-262.

Lengkeek, A.G., A.M. Mwangi, C.A.C. Agufa, J.O. Ahenda, I.K. Dawson, (2006). Comparing genetic diversity in agroforestry systems with natural forest: a case study of the important timber tree *Vitex fischeri* in central Kenya. *Agro. Syst.* **67**: 293-300.

Miller, A.J and B.A. Schaal, (2006). Domestication and the distribution of genetic variation in wild and cultivated populations of the Mesomerican fruit tree, *Spondias purpurea* L. (Anacardiaceae). *Mol. Ecol.* **15**: 1467 -1480.

Pandey, C.B., R.B. Rai, L. Singh and A.K. Singh, (2006). Home gardens of Anadaman and Nicobar, India. *Agricultural systems* (press).

Peyre A., A. Guidal, K.F. Wiersum and F. Bongers, (2006). Dynamics of home garden. Structure and function in Kerala India *Agro. Syst.* **66**: 101.

Raheem, D.C., F. Naggs, R.C. Preece, Y. Mapatuna, L. Kariyaswam, P. Eggleon, (2008). Structure and conservation of Sri lankan land –snail assemblages in fragmented lowlands rain forest and village home gardens. *J. Appl. Ecol.* **45**: 1019 -1028.

Rico Gray.V., A. Chemas and S. Mandujano, (1991). Uses of tropical deciduous forest species by the Yucataean Maya, *Agro. Syst.* **14**: 149-161.

Rowe, C.W. (2009). Kitchen gardens in Tajikistahan: The economic and cultural importance of small-scale private property in a post-soviet society. *Human Ecol.* **37**: 691-703.

Saha, S.K., P.K.R. Nair, V.D. Nair and B.M. Kumar, (2009). Soil carbon stock in relation to plant diversity of home gardens in Kerala, India. *Agro. syst.* **76**: 53-65

Kong. Res. J. 2(1) : 81-87 2015 ISSN 2349-2694
Kongunadu Arts and Science College, Coimbatore.

MICROMORPHOLOGY, KARYOLOGY AND PHYTOCHEMICAL STUDIES ON THE *CARALLUMA ADSCENDENS* (ASCLEPIADACEAE) COMPLEX IN TAMIL NADU, INDIA

Karuppusamy, S*.
Department of Botany, The Madura College, Madurai – 625 011, Tamilnadu.
*E.mail: ksamytaxonomy@gmail.com

ABSTRACT

Caralluma adscendens (Roxb.) R. Br. (Apocynaceae) is a wild growing variable succulent herb distributed frequently in drier regions of Peninsular India especially more diversified in Tamilnadu. Seven varieties under the taxa recognized and they have given detail morphological and distributional notes for these varieties. Local Floras are not treating these varieties properly and still exhibits controversy in the identification on these taxa. The present study emphasizes on the floral micro-morphology, chromosome numbers and amount of flavonoid compounds in each variety, resulted that clear differences showed between each varieties. Specifically rounded angle varieties namely var. *adscendens, attenuate, bicolor* and *fimbriata* are possessed uniform chromosome number (2n = 22), whereas acute angled varieties such as var. *carinata* and var. *gracilis* are having tetraploid (autoploid 2n = 44). Var. *geniculata* is reported only 2n = 22 chromosomes but it has acute angled with phytogeographically segregated in nature when compared to all other varieties. The present study provided separate manual keys for *Caralluma adcendens* and their varieties based on the stem morphology, corolla epidermal micromorphology, karyology and chemical compounds present in them. This study would be supported that the elevation of var. *geniculata* into species level as *Caralluma geniculata* mentioned by Meve and Leide (2002).

Keywords: *Caralluma adscendens,* Micromorphology, Karyology Phytochemical studies.

1. INTRODUCTION

Caralluma was first described in 1810 by Robert Brown to include an Indian species with very characteristic elongated flowering stems. A number of other related genera were consequently described: *Desmidorchis* (1829), *Boucerosia* and *Hutchinia* (1834), *Aptheranthes* (1835), *Sarcodon* (1878), *Orbeobsis* and *Pachycymbium* (1978), *Quaqua* (1879), *Tridentea* (1980), *Pectinaria* (1981), *Urmalcala* and *Rhytidocaulon* (1990), *Australluma, Boreallluma, Caudanthera, Crenulluma, Cryptolluma, Cylindrilluma, Monolluma, Sanguilluma, Saurolluma, Somalluma, Spiralluma* and *Sulcolluma* (1995). Indian *Caralluma*, with over 14 species and perhaps 4 genera, have been much less studied and are more diverse than is generally realized (Karuppusamy *et al*., 2013). Many new species have been described since Gravely and Mayuranathan (1931), Kumari and Subba Rao (1976), Lavranos and Frandsen (1978), and Sarkaria (1980). However, only very tentative primitive steps have been taken towards untangling the problems of overall species relationships and generic delimitation. After getting revision of the genus *Caralluma* by Gilbert (1990) and Plowes (1995), the genus is attracting the botanists in many countries. But a still vast lacuna in the taxonomy of Indian *Caralluma* is great controversy.

Caralluma adscendens (Roxb.) R.Br. (Syn. *Stapelia adscendens* Roxb.) is originally described from coast coromandal area of Peninsular India without indicate precise locality by Roxburgh. Gravely and Mayuranathan (1931) recognized a total of six varieties within this species. Recently one more new species *Caralluma bicolor* described from Tamil Nadu and which is now consider under the variety of *Caralluma adscedens* (Karuppusamy *et al*., 2013). Mainly these varieties can be divided into 2 major groups based on whether the stems have rounded or acute angles. But Bruyns (1984) has drawn attention to the very great similarity between one of these variety *fimbriata*, and *Caralluma subulata* from Arabia and *Caralluma dalzielii* from West Africa. This could be taken to indicate that the two groups such as round and acute angles varieties merit formal recognition at least as subspecies. The status of the varieties within *C. adscendens* seems more questionable as Gravely and Mayuranathan indicated that intermediates are apparently common (Gilbert, 1990).

The earlier accounts indicates that *Caralluma adscendens* is variable and widely distributed species

at least seven distinct morphoforms have recognized. In the three varieties, viz. var. *adscendens s.str*., *attenuata* and *fimbriata*, that have long been known and have hitherto been regarded as distinct species (Gamble, 1919). The stem is squarish in section with the sides usually slightly hollowed and the angles always rounded. Another three varieties are acute angles i.e. var. *carinata, gracilis* and *geniculata* which are distributed in narrow ranges in Tamilnadu. Gravely and Mayuranathan (1931) have suggested to use classification of three varieties partly in the flower morphology and partly in the stem morphology. But these characters unable to hold in herbarium specimens of these groups, because of these are succulent genera. There is considerable floral (but also vegetative) variability in *C. adscendens*, quite often even between plants of single populations, but almost always between different populations. These circumstances have stimulated many taxonomists and regional florists to describe morphotypes, ecotypes or varieties or forms. The diversity of *C. adscendens* in Tamilnadu is indeed higher than other states of Peninsular India. Even though in many places, overlapping populations are exhibiting among these varieties. Bruyns (1987) suggested that variable forms of African *Caralluma* complex taxonomically still needs to be answered because of this complex has never been revised as a whole. Egyptian *Caralluma europaea* complex have been revised based on the morphology and karyology studies by Meve and Heneidak (2005). Meve and Liede (2002) tried molecular taxonomy of this group, they treated on variety under *C. adscendens* var. *geniculata*, is raised to species level (*Caralluma geniculata*) based on their molecular data.

With special reference to micromorphological, karyological and chemical data, this study is the first attempt to present a detailed picture of the infraspecific differentiation within the *Caralluma adscendens* complex.

2. MATERIALS AND METHODS

2.1. Plant specimens

The materials used for morphological, karyological and chemical investigations are summarized in Table 1.

2.2. Morphological study

Floral parts viz. corolla epidermis, hairs, corona, pollinaria have been investigated under the stereo microscope.

2.3. Chromosome counts

Chromosome numbers were established from adventitious root tip squash preparations. The root tips were pretreated in 0.02 M hydroxyquinoline for 4 h at 20^{0}C (Meve and Heneidak, 2005), fixed in Carnoy's solution for 24 h at 20^{0}C and stained with carmine for 24 h at 60^{0}C (Snow, 1963).

2.4. Chemicals analysis

For extraction of flavonoids 1 g of crushed fresh stem material was extracted in 3ml absolute ethyl alcohol, boiled for about 2 min, cooled and left for 24 h. Samples were filtered, dried and rehydrated im 3 ml of 70% ethyl alcohol. About 20µl were used for analytical High Performance Liquid Chromatography (HPLC) analysis. They were identified by means of UV spectroscopy using shift reagents (Markham, 1982), acid hydrolysis and analysis of aglycone and sugar moieties (Harbone, 1998), cochromatography with authentic standards (FLC, 2006) were compared for the amount and types of flavonoid subgroups.

Table 1. Voucher specimens collected and used in this study.

Name of the variety	Place of collection	Voucher number
Caralluma adscendens		
var. *adscendens*	Madurai (Madurai district)	S. Karuppusamy, 118
var. *adscendens*	Nagamalai (Madurai district)	S. Karuppusamy, 265
var. *attenuata*	Oddanchatram (Dindigul district)	S. Karuppusamy, 259
var. *attenuata*	Palani (Dindigul district)	S. Karuppusamy, 75
var. *attenuata*	Tirumayam (Tanjaore district)	S. Karuppusamy, 752
var. *attenuata*	Madhukkarai (Coimbatore district)	S. Karuppusamy, 824
var. *fimbriata*	Barigam (Dharmapuri district)	S. Karuppusamy, 53
var. *fimbriata*	Bhavani (Erode district)	S. Karuppusamy, 325
var. *fimbriata*	Kolli hills (Namakkal district)	S. Karuppusamy, 68
var. *carinata*	Nagamalai (Madurai district)	S. Karuppusamy, 329
var. *carinata*	Sirumalai (Dindigul district)	S. Karuppusamy, 783
var. *geniculata*	Maruthuvamalai (Kanyakumari dt.)	S. Karuppusamy, 589
var. *gracilis*	Varathamanathi (Dindigul district)	S. Karuppusamy, 673
var. *gracilis*	Vaguthumalai (Madurai district)	S. Karuppusamy, 89
var. *gracilis*	Pudukottai (Pudukottai district)	S. Karuppusamy, 435

3. RESULTS AND DISCUSSION

3.1. Habit and habitat

As is typical for most Carallumas, *Caralluma adscendens* prefers shaded stands such as the base of thorny bushes (Jonkers and Walker, 1993). The individuals nevertheless vary considerably in their general habit depending on the edaphic situation. In deep, sandy soil there is a strong tendency to form diffuse stem base especially variety *attenuata*. On rocks the plants grows usually from compact clumps of rather thick stems (var. *geniculata*). Typically, the species grows on rocky slopes among rocks under stunted bushes in Salem, Dharmapuri and Dindigul districts. Var. *carinata* attains more than a meter height in Nagamalai in Madurai district and Sirumalai hills in Dindigul districts (Fig.2a).

3.2. Vegetative morphology

Stem surfaces and angels are variable in all six varieties. The colour varies from uniform bright green (var. *gracilis*) to dark blue green (var. *geniculata*). Varieties *adscendens, attenuata* and *fimbriata* are rounded angles and varieties *carinata, gracilis* and *geniculata* are acute angled stems.

3.3. Floral morphology

Variability in flower morphology of *C. adsendens* is higher than typically found in widespread stapeliads, and has therefore hampered a sound taxonomic treatment. The increasing availability of plant material has demonstrated infraspecific variability, where almost every population shows its own features (the many illustration published by Gravely and Mayuranathan, 1931). Especially with regard to frequently occurring differences in corolla structure, size and ciliation, a wealth of taxa has been described (see floral key). However, several distinct tendencies that support infraspecific differentiation have to be considered. Inner coloration of the corolla surface is predominantly purple, but creamy yellow developed in var. *gracilis*. Dense purple-red transversal stripes or streaks are found in all the six varieties of *C. adscendens* but each variety it has differentiated by amount and patterns of striation (Fig 1&2). Flower size is small in var. *gracilis* but rather larger in var. *carinata.* Amount of hairiness of the petals are also varied in all six varieties (Fig. 1& 2).

Microscopic studies of the corolla epidermis of samples (each voucher from Table 1) were conducted that revealed a characteristic separation between the verities and its populations. In the varieties *attenuata, fimbriata, adscendens* showed homogeneously isodiametric with slightly convex to dome-shaped papillate (bottle-neck like) cells (Fig.2e). But in the varieties of *gracilis, geniculata* and *carinata* have observed heterogeneous cells mingled with papillate-apiculate cells on the corolla epidermis. The very smooth and tiny periclinals lacks a pleated cuticle but they are topped by mighty papilla-like structure showing a verrucose to prickly surfaces. Corona structures and their coloration are rather similar over all the varieties. The corona is normally purplish; however varieties *fimbriata* and *attenuata* is occasionally yellowish-brown. Shape and size of pollinaria are also greatly differing in all six varieties.

3.4. Chromosome numbers

All 14 samples of six varieties investigated (Table 2) possess basic somatic 2n = 22 chromosomes in the varieties *adscendens, attenuata, fimbriata* and *geniculata* and the chromosome number tetraploid (2n = 44) in the varieties *carinata* and *gracilis*. This is the standard situation in Asclepiadaceae, where ca 96% of the investigated taxa have a basic chromosome number of n = 11, and over 90% are euploid diploids (Albers and Meve, 2001). Karyotypic analysis revealed a homogeneous gemone of predominantly meta- to sub-metacentric chromosomes for *C. adscendens*. One pair of chromosome shows secondary constrictions with satellites in all the varieties. This feature is already mentioned by Albers and Meve (2001).

3.5. Chemical analysis

The flavonoid profiles of all the six varieties were investigated (Table 3). The HPLC retention time and UV spectral maxima of the flavonoid glycoside compounds analyzed and given. The overall flavonoid compounds rich in the variety *carinata* when compared with all other five varieties.

Table 2. Chromosome number of *Caralluma adscendens* complex.

Variety	Voucher used	Chromosome number	Karyo type
var.*adscendens*	S. Karuppusamy, 165	22	28.23
var.*attenuata*	S. Karuppusamy, 75	22	27.31
var.*bicolor*	S. Karuppusamy 1026	22	27.58
var.*fimbriata*	S. Karuppusamy, 68	22	28.12
var.*carinata*	S. Karuppusamy, 329	44	28.51
var.*gracilis*	S. Karuppusamy, 673	44	27.92
var.*geniculata*	S. Karuppusamy, 589	22	22.15

Morphologically, there are quantitative differences between all the six varieties. Typically the corolla epidermis of those samples is more strongly sculptured with markedly convex outer epidermal wall specifically in each variety. The amount of papillate sculpture serves as demarcation line between the varieties. The present study is

comparable with *Caralluma europaea* complex (Meve and Heneidak, 2005).

Table 3. HPLC retention times and UV spectral maxima and positive flavonoid compounds found in all six varieties of *C. adscendens* studied.

Variety	HPLC		Possible flavonoid compounds in percentage (%)
	Rt (min)	UV λmax (nm)	
Var. *adscendens*	13.55	274, 329	2.92
Var. *attenuata*	12.59	270, 331	2.96
Var. *bicolor*	13.22	272, 253	4.85
Var. *carinata*	14.07	274, 329	14.79
Var. *fimbriata*	13.52	271, 329	12.62
Var. *geniculata*	13.28	271, 328	5.28
Var. *gracilis*	13.92	254, 356	7.51

Taxonomically significant variation in chromosome sizes in stapeliads have been reported by Albers and Meve (2001) at the tribe level, Meve and Liede (2001) at genus and species level and Meve and Heneidak (2005) at infraspecific level. Even within species, however, chromosome length high varies around 0-2-%. The ca. 10% deviation found between the two varieties *C. adsecendens* is then not significant at al, and falls within the variation range which can be expected on species level.

All six varieties of *C. adscendens* investigated and collected in different places in Tamilnadu only in the amount of their flavonoid compounds. Flavonoids, so far known, generally are of limited occurrence and diversity in Apocynaceae-Asclepiadaceae (Meve and Heneidak, 2005). Mostly, aglycone and glycosides occur in limited diversity and quantity in comparison to many angiosperms. Especially from the *Caralluma*, glycosides are mostly known as pregnane glycosides (Halim and Khalil, 1996). This study were restricted to the easily investigated aglycone parts the flavonoid glycosides. In the presence of Luteolin-4'-O-neohesperidoside was already reported from *Caralluma adscendens* var. *attenuata* (Ramesh et al, 1999). All other varieties are also possessed the same compounds in different amount in their plant parts. The present study indicated the presence of the higher amount of flavonoid compounds in the var. *carinata* when compared to other varieties tested. Possibly this flavonoid is characteristic for this group and which is having more taxonomic significance.

Plowes (1995) suggested that the *C. adscendens* complex in India may segregate species level. This has not been questioned by succeeding *Caralluma* investigators. Considering the high vegetative, floral and chemical variability, the diploidy and tetraploidy, and the narrow and nearly discontinuously distribution, the treatment of the complex as variable species. The acute angled varieties (var. *carinata* and *gracilis*) is having tetraploid (autoploid) chromosomes, which is taxonomically significant but these populations are not overlapping anywhere in natural condition. Another acute angled variety *gneiculata* might have originated independently with its habitats due to geographical isolation. Hence, this variety may be considered as separate species as supported by Meve and Liede (2002). All other rounded angle varieties might be considered as ecotypes which are exhibiting overlapping populations sometimes. The differences between the varieties are really pronounced between rounded angled and sharp angled. However, the fairly high number of characters concerned point to a considerable degree of separation.

3.6. Taxonomy

3.6.1. Caralluma adscendens (Roxb.) R.Br.

Stems fleshy, quadrangular; internodes four-angled, 6–18 mm long and 2–6 mm across, slender above, glabrous. Latex watery. Leaves present only on young branches, reduced to scales, opposite, decussate, sessile, 1–2 mm long, subulate, tip acute, glabrous.

Flowers terminal or subterminal, solitary or paired, axillary; pedicel terete, 3–5 mm long and ca 1.0 mm in diam., brown, glabrous. Calyx 5-lobed, divided up to base, lobes ca 2.5 × 1.0 mm, ovate, apex acuminate, margin thin, glabrous. Corolla campanulate, ca 8 mm long; corolla tube ca 1.5 mm long; lobes 5, 6–7 × 2.5 mm, lanceolate-oblong, apex apiculate, hairy or glabrous. Corona staminal, biseriate; outer annualar, with two filiform filaments, ca 1.5 mm long, alternating with anther-lobes, glabrous; inner 5-lobed, flap-like, overlapping anther-lobes, ca 1.0 × 0.4 mm long, apex truncate or crenate. Stamens 5, ca 1.6 mm long; pollinia 5, pollen masses solitary in each anther cell, yellow, waxy, caudicles light brown, corpuscle dark brown. Follicles usually solitary, 5.5 × 2.3 cm long and 3.5–4.5 mm in diam., cylindrical, peaked with curved tip, base acute, glabrous; seeds many, ca 9.5 × 4.5 mm, oblong to obovate, base rounded, dark brown with light brown margin ca 1.0 mm wide; coma silky white, 2.0–3.0 mm long.

Gravely and Mayuranathan (1931) classified these varieties based on their stem and flower characters. The distribution of these varieties occupied different degree of frequency in Peninsular India. But most dominant and older varieties are var. *attenuata* and var. *fimbriata*, other varieties are

evolved from degree of reduction in hairiness of flower and roundness of stem.

Key to the varieties of *Caralluma adscendens* based on stem and floral morphology:

1. Corolla-lobes hairy …………………………... 2

1. Corolla-lobes glabrous ……var. *adscendens*

2. Stems rounded towards the bas…………..3

2. Stems acute towards the bas…………………5

3. Distal portion of the stem attenuate; 25–50 cm high ………. var. *attenuata*

3. Distal portion of the stem not attenuate; 12–20 cm high ………………4

4. Flowers large, deep purple hairs along the margin; pedicel pendulous …var. *fimbriata*

4. Flowers small, greenish purple; hairs not developed; pedicel erect ………var. *gracilis*

5. Pedicel abruptly bent; hairs on corolla not strongly developed; stems not attenuate ……………………………………………………………………… var. *geniculata*

5. Pedicel never bent; hairs strongly developed; stems obviously attenuate ...var. *carinata*

Key to the varieties of *C. adscendens* based on corolla epidermal morphology:

1. Corolla epidermal cells homogeneous ……………………………………………… (2)

1. Corolla epidermal cells heterogeneous …………………………………………... (3)

2. Epidermal cells with distinct apiculate or papillate growth on the top ……………… (4)

2. Epidermal cells without apiculate process on the surface ………………... var. *fimbriata*

3. Papillary growth sharply mucronate, cells intermingled with long hairs … var. *gracilis*

3. Papillary growth with blunt tip, cells without intermingled hairs ………………… (5)

4. Apiculate process distinctly bottle-neck shaped ……………………… var. *attenuata*

4. Apiculate process simply elongate, not constricted below the papilla .. var. *adscendens*

5. Papilla uniformly elongated, cells hexagonal …………………………... var. *carinata*

5. Papilla differently elongated, cells polygonal …………………………... var. *geniculata*

3.6.2. Caralluma adscendens var. *adscendens*

Stems often with reddish streaks, not attenuate, but slightly tapering in distal end, their angles rounded, acute; leaf-scars raised on more or less distinct especially in older stems and outwardly directed tubercles; flowers hairless, more or less pendulous; pale base of inner side of petals finely spotted with purple spots or striped transversely, only on basal half of inner side.

Flowers: March–February; Fruits: July–September

Gravely and Mayuranathan noted that this variety occurs from northeast of the Godavari to south end of Attapady valley of Malabar. Dense populations of this variety occur in Coimbatore, Salem, Pudukottai and Madurai districts of Tamilnadu. Present study specimens were collected from Madurai and Pudukottai districts of Tamilnadu, but a few populations have been observed in Nagamalai hills near Madurai, where they have flowers with short hairs on the margins of the petals and are prominently striped purple on the inner side of basal part. In the same district some populations do not have these hairs, and petals are glabrous with a greenish yellow background. All of these populations grow close to cultivated land and exposed dry scrub forests of the foothills. In dry areas they grow about 75 cm tall, but in fertile areas it can reach a height of 1 m.

Distribution: Discontinuous population in Peninsular India. Endemic.

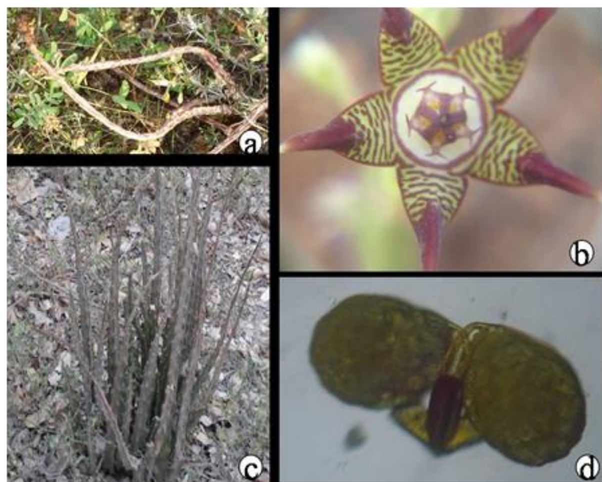
3.6.3. Caralluma adscendens var. *attenuata* (Wight) Gravely & Myuranathan

Stems bushy growth, strongly attenuate, usually much branched distally, angles streaked reddish, rounded; flowers hairy, pendulous, dark purple, flowering throughout the year.

This is the most widely distributed of the varieties of *C. adscendens*. The present study found it at Coimbatore, Dindigul, Madurai, Salem and Pudukottai districts of Tamilnadu. It was first described from the foothills of Nilgiris, and Hooker quotes Cochin as another locality, but at that time *Caralluma stalagmifera* unknown and seems often to have been confused with *C. attenuata*. This variety has more hybridizable potency with neighboring *Caralluma* varieties, hybridized forms having been recorded by Gravely and Mayuranathan (1931) from the Madurai and Pudukottai districts of Tamilnadu.

Distribution: Distributed all over the Deccan Peninsula.

Figure 1



a. *Caralluma adscendens* var. *adscendens* – habit from Nagamalai hills of Madurai.
b. *Caralluma adscendens* var. *adscendens* – Flower enlarged.
c. *Caralluma adscendens* var. *attenuata* – habit from Kuniyamuthur near Coimbatore.
d. *Caralluma adscendens* var. *attenuata* - Pollinarium

3.6.4. Caralluma adscendens var. *carinata* Sarkaria

Stems acute angled, green, distally strongly attenuate, rarely branched; flowers purplish, hairy, usually pendulous and campanulate but sometimes semi-erect and rotate; pedicels not bent. Maximum height a little over 1m.

Distribution: Very few populations known in Tamilnadu (endemic). This variety has been observed only in the Nagamalai and Azhargar hills of the Madurai district and Sirumalai hills of Dindigul district of Tamilnadu. This variety is threatened due to habitat modification.

3.6.5. Caralluma adscendens var. *geniculata* Gravely & Mayuranathan

Stems acute angled, green, slightly attenuate and unbranched distally, but more slender secondary stems frequently branched distally; flowers hairy but less conspicuously so than the other varieties, widely opened, facing upwards, dark chestnut brown markings, pedicel bent at an angle just below the flower; flowering throughout the year.

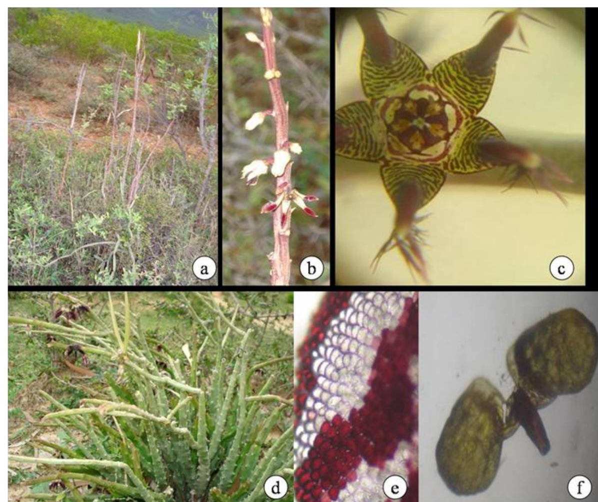
This variety is endemic to Tamilnadu. The present study recorded from Marthuvamalai hills of Kanyakumari district in Tamilnadu.

Distribution: A small population located in Tamilnadu.

3.6.6. Caralluma adscendens var. *fimbriata* Wallich

Stems small, not definitely attenuate, equal thickness throughout, creamy reddish distally, not streaked, angles rounded; flowers dark purple, densely hairy along the margin of petals, usually pendulous, flowering throughout the year.

Figure – 2



a. *Caralluma adscendens* var. *carinata* – natural habit growing in Sirumalai foot hills.
b. *Caralluma adscendens* var. *carinata* – flowering stem apex.
c. *Caralluma adscendens* var. *carinata* – Flower enlarged.
d. *Caralluma adscendens* var. *fimbriata* – natural habit.
e. *Caralluma adscendens* var. *fimbriata* – corolla epidermal surface view.
f. *Caralluma adscendens* var. *fimbriata* - Pollinarium

Distribution: This variety is described originally from Myanmar, but it has since been recorded from India and Sri Lanka. It exists in discontinuous populations in drier regions of peninsular India. Large numbers of natural populations have suddenly declined due to the increased demand in pharmaceutical market for its glycosides. It is not currently cultivated for this purpose, markets relying on wild collected material.

3.6.7. Caralluma adscendens var. *gracilis* Gravely & Mayuranathan

Stems greenish, purple streaks absent on stem, acute angled, slightly attenuate distally; flowers hairy but less than previous varieties, small, axillary paired below the distal apex, pedicel 1.5 cm; corolla chestnut brown. Maximum height of stems at least 75 cm; flowering throughout the year.

Gravely and Mayuranathan (1931) originally collected and described this variety from the town of Pudukottai forests of Tamilnadu. It is distributed throughout Tamilnadu and occurs in dense populations in Pudukottai forests. In the present investigation specimens were collected from the

forests of Palni hills. This variety also hybridizes with *C. stalagmifera* and other neighboring varieties presenting intermediate forms (Gravely & Mayuranathan, 1931).

Distribution**:** South Tamilnadu to north of Palni hills to Pudukottai. This variety is endemic to Tamilnadu.

REFERENCES

Albers, F. and U. Meve, (2002). A karyological survey of Asclepiadaceae, Periplocaceae and Secamonoideae, and Evolutionary considerations within Apocynaceae *s.l. Ann. Missouri Bot. Garden* **8**: 624-656.

Bruyns, P.V., (1987). The genus *Caralluma* R.Brown (Asclepiadaceae) in Israel. *Israel J. Bot.* **36**: 73-86.

Bruyns, P.V., (1988). The genus *Caralluma* R.Brown in Israel. *Asklepios* **45**: 32-44.

Gamble, G.S., (1919). *Flora of Presidency of Madras*. Vol.2. London.

Gilbert, M.G., (1990). A review of *Caralluma* R.Br. and its segregates. *Bradleya* **8**: 1-32.

Gravely, F.H. and P.V. Mayuranathan, (1931). The Indian species of the genus *Caralluma* (Fam. Asclepiadaceae). *Bull. Madras Govn. Museum* **8**:1-28.

Halim, A.F. and A.T. Khalil, (1996). Pregnane glycosides from *Caralluma reterospiciens*. *Phytochem.* **42**: 1135-1139.

Harborne, J.B., (1998). *Phytochemical methods*, 3rd Edition, Chapman & Hall, London.

Jagtap, A.P. and N.P. Singh, (1999). *Fascicles of Flora of India. Fascicles 24*. Botanical Survey of India, culcutta, India.

Jonkers, B. and C.C. Walkers, (1993). The Asclepiads of Morocco – a short commentary. *Asklepios* **59**: 14-21.

Karuppusamy, S., A. Ugraiah, and T. Pullaiah, (2013). *Caralluma* (sensu lato) Antiobesity plants. Astral Publication, New Delhi, India.

Kumari, G.R. and G.V. Subba Rao, (1976). A new species of *Caralluma* (Asclepiadaceae) from India. *J Bombay Nat. His. Soc.* **73**: 194-196.

Lavranos, H. and L. Frandsen, (1978). *Caralluma sarkariae*. *Cactus and Succulent Journal (U.S.A)* **50**: 211.

Markham, K.R., (1982). *Techniques of flavonoid indentification*. Academic Press, London.

Meve, U. and S. Heneidak, (2005). A morphological, karyological and chemical study of the *Apteranthes* (*Caralluma*) *europaea* complex. *Bot. J. Linn. Soc.* **149**: 419-432.

Meve, U. and S. Liede, (2002). A molecular phylogeny and generic rearrangement of the stapeliad Ceropegiae (Apocynaceae – Asclepiadaceae). *Pl. Sys. Evol.* **234**: 171-209.

Plowes, D,C.H., (1995). A reclassification of *Caralluma* R.Brown (Stapeliae: Asclepiadaceae). *Haseltonia* **3**: 49-70.

Ramesh, M., Y.N. Rao, M.R. Kumar, G.K. Mohan, B.R. Kumar, A.V.N. Rao, M.R. Krishna, and B.M. Reddy, (1999). Flavone glycosides from three *Caralluma* species. *Biochem. Sys. Ecol.* **27**: 85-86.

Sarkaria, J., (1980). *Caralluma bhupinderiana*. *Nat. Cactus Succulent J.* **35**: 68-72.

Snow, R., (1963). Alcoholic hydrochloric acid-carmine as stain for chromosomes in squash preparations. *Stain Technol.* **38**: 9-13.

Kong. Res. J. 2(1) : 88-93, 2015 ISSN 2349-2694
Kongunadu Arts and Science College, Coimbatore.

SURVEY OF CLIMBERS IN ATCHANKULAM, KOTTARAM PANCHAYAT, KANYAKUMARI DISTRICT, TAMILNADU, INDIA.

Mary Kensa, V.[1*], BeemaJainab, S.J.[2], Kavitha, A.[1], Rejitha,S.[1], Anusha M.[1] and Vinitha, R.[1]
[1]Department of Botany and Research Centre, S.T. Hindu College, Nagercoil-629 002, Tamil Nadu, India
[2]J.B.A.S.College for women (Autonomous), Chennai-18.
*E.mail: surejkensa@gmail.com

ABSTRACT

Climbing plants are one of the most interesting group but a much neglected group of plants. But, they also play a part in historical importance of our ancient buildings which owe their attraction to the green veil which covers up their architectural or structural defects making them assume perfect beauty in our eyes. The present survey reveals that angiospermic climbers of the study area are represented by 94 species under 63 genera belonging to 32 families. Among all families, Convolvulaceae, Papilionaceae (7 species) and Vitaceae are the most dominating family species as well as genera wise. The dominant families are Convolvulaceae, Papilionaceae, Vitaceae, Apocynaceae, Menispermaceae and Oleaceae. The most abundant liana species include the thorny stragglers *Pterolobium hexapetalun* (Caesalpiniaceae), *Lantana camara* (Verbenaceae), and the twiners *Jasminum angustifolium* (Oleaceae), *Gymnena sylvestre* (Asclepiadaceae) and *Aganosmacymosa* var. *cymosa* (Apocynaceae). The enumerated climbing modes were classified into woody vines, the lianas (75) and herbaceous vines (19). Six climbing modes of lianas were recognized as stem twiners (37) followed by stragglers-unarmed (28), stragglers unarmed (10), tendril climbers (17), root climbers (1) and hook climber (1).

Key words: Climbing plants, Atchankulam, Survey.

1. INTRODUCTION

Climbing plants are defined as plants incapable of autonomous vertical support once they reach a certain height and depend on other plants for support in their natural environment (Gentry, 1991). The climbing habit has arisen several times in the evolutionary history of Angiosperms, and this has resulted in a great taxonomic diversity of climbing plants (Gentry, 1985). Families such as Smilacaceae, Menispermaceae, Passifloraceae, Cucurbiaceae and Convolvulaceae are essentially entirely composed of or dominated by species with a climbing habit. Climbing plants are an interesting but much neglected group. This group consists of plants that are rooted in the ground but need support for their weak stems, and both herbaceous (vines) and woody (lianas) climbing plants can be found. Recent reviews of the role of climbers in forest ecosystems (Putz and Mooney, 1991; Schnitzer and Bongers, 2002; Wright *et al.,* 2004; Phillips *et al.,* 2005) have highlighted the abundance, competitive abilities, and contribution to disturbance regimes. Today, climbing plants typically contribute 2-15% of the leaf biomass and about 5% of the wood biomass to forests (Fearnside*et al.,* 1999; Gerwing and Farias, 2000; Clark *et al.,* 2008). In climber-rich areas, they can contribute as much as 40% of the estimated total biomass (Hegarty and Caballe, 1991; Perez-Salicrup*et al.,* 2001). Climbers not only form important structural components but also play an important ecological role in forest dynamics, diversity and nutrient recycling (Gentry and Dodson 1987; Schnitzer and Bongers, 2002). No comprehensive work is available for climbers in the study area. Therefore, the objective of the present study is to document the angiospermic climbers of Atchankulam, KottaramPanchayat, Kanyakumari District.

2. MATERIALS AND METHODS

2.1. Description of the Study Area

The present study was carried out in Atchankulam of KottaramPanchayat and AgastheeswaramTaluk of Kanyakumari District. This District constitute the southernmost step of India, with Kerala on the West-North, Tirunelveli District in the North-East Arabian sea in the South – West, Bay of Bengal in the south-East and Indian ocean in the south. The annual rainfall of this area is low when compared to other areas of the Kanyakumari District. There are nearly 1450 families are live in this panchayat. Most of the people are coolies or farmers.

2.2. Floristic Survey

The present study was carried out through intensive and extensive field visits during January 2014-July 2014. During field survey, the plants have been collected in their flowering and fruiting stages as far as possible from the natural habitats. They are identified with the help of local floras (Gamble and Fischer 1015-1936; Matthew 1983; Nair and Henry 1983; Henry *et al.,* 1987; 1989; Chandrabose and Nair 1988). Further the identities were confirmed is referring authentic specimens and the voucher specimens deposited in the Herbarium of Department of Botany, South Travancore Hindu College, Nagercoil.

Table 1: Angiosperm climbing plants enumerated from the study area, binomial, family, category and climbing mode.

S. No.	Species / Family	Category	Climbing Mode
	Annonaceae		
1	*Desmosviridiflora*(Bedd.) Safford	WV	Str-UA
	Apocynaceae		
2	*Aganosmacymosa*(Roxb) G. Don var. Cymosa	WV	ST
3	*Anodendronpaniculatum*A.DC.	WV	ST
4	*Carissa gangetica*Stapf	WV	Str-A
5	*Carissa salicina*Lam.	WV	Str-A
6	*Carissa spinarum*L.	WV	Str-A
	Aristolochiaceae		
7	*Aristolochiaindica*L.	HV	ST
	Asclepiadaceae		
8	*Gymnemasylvestre*(Retz.) R.Br.ex Roemer &Schultes	WV	ST
9	*Hemidesmusindicus*(L) R. Br.	HV	ST
10	*Pergulariadaemia*(Forssk.) Chiov.	HV	ST
11	*Sarcostemmaacidum*(Roxb.) Voigt	WV	Str-UA
	Caesalpiniaceae		
12	*Caesalpinia crista* L.	WV	Str-A
13	*Caesalpiniacucullata*Roxb.	WV	Str-A
14	*Pterolobiumhexapetalum*(Roth) Sant. &Wagh	WV	Str-A
	Capparaceae		
15	*Capparisbrevispina*DC.	WV	Str-A
16	*Capparissepiaria*L.	WV	Str-A
17	*Capparizeylanica*L.	WV	Str-A
	Celastraceae		
18	*Celastruspaniculatus*Willd.	WV	Str-UA
19	*Maytenusheyneana*(Roth) Raju&Babu	WV	Str-A
20	*Maytenusroyleanus*(Wallich ex M. Lawson) M.A.Rau.	WV	Str-A
21	*Salaciachinensis*L.	WV	Str-UA
	Combretaceae		
22	*Combretumacuminatum*Lam.	WV	TC
23	*Combretumalbidum*G. Don	WV	ST
	Convolvulaceae		
24	*Argyreiaelliptica*(Roth) Choisy	WV	ST
25	*Argyreiainvolurata*Clarke	WV	ST
26	*Ipomoea asarifolia*(Desr.) Roem. &Schultes	HV	ST
27	*Ipomoea companulata*L.	WV	ST
28	*Ipomoea eriocarpa*R. Br.	WV	ST
29	*Ipomoea staphylina*Roem&Schultes	WV	ST
30	*Merremiavitifolia*(Burm. F.) Hall. F.	HV	ST
	Cucurbitaceae		
31	*Cocciniagrandis*(L) J. Voigt	WV	TC
32	*Kedrostiscourtallensis*(Arn.) Jeffrey	WV	TC
33	*Trichosanthesanaimalaiensis*Bedd. L	WV	TC
	Dioscoreaceae		
34	*Dioscoreaoppositifolia*L.	HV	ST
35	*Dioscoreapentaphylla*L.	HV	ST
36	*Dioscoreatomentosa*J. Koenig ex Sprengel	HV	ST
	Euphorbiaceae		
37	*Phyllanthusreticulatus*poir.	WV	Str-UA
38	*Tragiainvolucrata*L.	WV	ST
39	*Tragiaplukenetii*R. Smith	HV	ST
	Liliaceae		
40	*Asparagus racemosus*Willd.	HV	Str-A
	Linaceae		
41	*Hugoniamystax*L.	WV	HC
	Malpighiaceae		
42	*Hiptagebenghalensis*(L.) Kurz	WV	Str-UA
	Menispermaceae		
43	*Anamirtacocculus*(L) Wight &Arn.	WV	ST
44	*Cissampelospareira*L.	HV	ST
45	*Cocculushirsutus*(L) Diels	WV	ST
46	*Cycleapeltata*(Lam.) Hook.f. &Thoms.	HV	ST
47	*Pachygoneovata*(Poir.) Miers ex Hook.	WV	ST
	Mimosaceae		
48	*Acacia pennata*(L.) Willd.	WV	Str-A
49	*Acacia sinuata*(Lour.) Merr.	WV	Str-A
50	*Acacia tortao*(Roxb) Craib	WV	Str-A
51	*Mimosa intsia*L.	WV	Str-A
	Nyctaginaceae		
52	*Pisonia aculeate* L.	WV	Str-A
	Oleaceae		
53	*Jasminumangustifolium*(L.) Willd.	WV	ST
54	*Jasminumauriculatum*Vahl	WV	ST
55	*Jasminumcuspidatum*Rottl.	WV	ST
56	*Jasminummalabaricum*Wight	WV	ST
57	*Jasminumtrichotomum*Heyne ex Roth	WV	ST
	Papilionaceae		
58	*Abrusprecatorius*L.	WV	ST
59	*Buteaparviflora*Roxb.	WV	Str-UA
60	*Dalbergiacongesta*Graham ex Wight &Arn.	WV	ST
61	*Dalbergiarubiginosa*Roxb.	WV	ST
62	*Mucunamonosperma*DC.ex Wight	WV	ST
63	*Mucunapruriens*(l.)DC	WV	ST
64	*Pseudarthria viscid* (l.) Wight &Arn.	HV	ST
	Passifloraceae		
65	*Passiflorafoetida*L.	HV	TC
66	*Passiflorasubpeltata*Ortega	HV	TC
	Piperaceae		
67	*Piper nigrum* L.	WV	RC
	Ranunculaceae		
68	*Naraveliazeylanica*(l.) DC.	WV	TC
	Rhamnaceae		
69	*Sageretiafiliformis*(Schultes) Don	WV	Str-A
70	*Scutiamyrtina*(Burm.f.) Kurz	WV	Str-A
71	*Ziziphushorrida*Roth	WV	Str-A
72	*Ziziphusrugosa*Lam.	WV	Str-A
	Rosaceae		
73	*Rubusellipticus*Smith	WV	Str-A
	Rubiaceae		

74	*Morindaumbellata*L.	WV	ST
75	*Mussaendahirsutissima*(Hook.f.)ex Gamble	WV	ST
76	*Rubiacordifolia*L.	HV	Str-UA
	Rutaceae		
77	*Paramignyabeddomel*Tanaka	WV	Str-A
78	*Toddaliaasiatica*(L.) Lam.	WV	Str-A
79	*Zanthoxylumovalifolium*Wight	WV	Str-A
	Sapindaceae		
80	*Cardiospermumcanescens*Wall.	HV	TC
81	*Cardiospermumhalicacabum*L.	HV	TC
	Smilacaceae		
82	*Smilax zeylanica*L.	HV	TC
	Tiliaceae		
83	*Grewiaflavescens*juss.	WV	Str-UA
84	*Grewia obtuse* Wall	WV	Str-UA
	Verbenaceae		
85	*Lantana camara*L.	WV	Str-A
86	*Premnacorymbosa*(Burm.f.) Rottler&Willd.	WV	Str
87	*Premnavillosa*clark	WV	Str
	Vitaceae		
88	*Ampelocissusaraneosa*(Dalz. &Gibs.).	WV	TC
89	*Ampelocissustomentosa*(Heyne ex Roth) Planch.	WV	TC
90	*Cayratiapedata*(Lour.) A.L. Juss. Ex Gagnep.	WV	TC
91	*Cayratiaroxburghii*(Wight &Arn.) Gagnep.	WV	TC
92	*Cissusgigantea*(Bedd.) Planch.	WV	TC
93	*Cissusquadrangularis*L.	WV	TC
94	*Cissusvitiginea*L.	WV	TC

WV: Woody vines; HV: Herbaceous vines; ST: Stem twiners, Str-A: Stragglers –armed; Str-UA: Stragglers – unarmed, TC: Tendril climbers, RC: Root climbers, and HC: Hook climber.

Table 2: Family wise and Taxonomic data of distribution of identified plants.

Sl.No	Family	% composition	Number of species	Number of genus
1	Annonaceae	1.06	1	1
2	Apocynaceae	5.31	5	3
3	Aristolochiaceae	1.06	1	1
4	Asclepiadaceae	4.25	4	4
5	Caesalpiniaceae	3.19	3	2
6	Capparaceae	3.19	3	2
7	Celastraceae	4.25	4	1
8	Combretaceae	2.12	2	2
9	Convolvulaceae	7.44	7	3
10	Cucurbitaceae	3.19	3	3
11	Dioscoreaceae	3.19	3	3
12	Euphorbiaceae	3.19	3	2
13	Liliaceae	1.06	1	1
14	Linaceae	1.06	1	1
15	Malpighiaceae	1.06	1	1
16	Menispermaceae	5.319	5	5
17	Mimosaceae	4.25	4	2
18	Nyctaginaceae	1.06	1	1
19	Oleaceae	5.31	5	1
20	Papilionaceae	7.44	1	1
21	Passifloraceae	2.12	2	1
22	Piperaceae	1.06	1	1
23	Ranunculaceae	1.06	1	1
24	Rhamnaceae	4.25	4	3
25	Rosaceae	1.06	1	1
26	Rubiaceae	3.19	3	3
27	Rutaceae	3.19	3	3
28	Sapindaceae	2.12	2	1
29	Smilacaceae	1.06	1	1
30	Tiliaceae	2.12	2	1
31	Verbenaceae	3.19	3	2
32	Vitaceae	7.44	7	3

Table 3. Dominant families of identified plants

Sl.No	Family	Number of Plants
1.	Convolvulaceae	7
2.	Papilionaceae	7
3.	Vitaceae	7
4.	Apouynaceae	5
5.	Menispermaceae	5
6.	Oleaceae	5

Table 4. Distribution of identified plants under climbing mode

Sl.No	Climbing mode	Number of plants	%
1	Woody vines	75	79.8
2	Herbaceous vines	19	20.2

3. RESULTS AND DISCUSSION

The present survey reveals that angiospermic climbers of the study area are represented by 94 species under 63 genera belonging to 32 families (Table-1 and 2) Among all families, convolvulaceae, papilionaceae (7 species) and vitaceae are the most dominating family species as well as genera wise (Table- 3). The dominant families are Convolvulaceae, Papilionaceae, Vitaceae, Apocynaceae, Menispermaceae and Oleaceae (Table 3).

The most abundant liana species include the thorny stragglers *Pterolobium hexapetalun* (Caesalpiniaceae), *Lantana camara*(Verbenaceae), and the twiners *Jasminum angustifolium* (Oleaceae), *Gymnena sylvestre* (Asclepiadaceae) and *Aganosma cymosa* var. *cymosa* (Apocynaceae). The enumerated climbing modes were classified into woody vines, the lianas (75) and herbaceous vines (19) (Table - 4). Six climbing modes of lianas were recognized as stem twiners (37) followed by stragglers-unarmed (28), stragglers unarmed (10), tendril climbers (17), root climbers (1) and hook climber (1).

Plasticity in eco-physiological traits has been related to the ecological breadth of forest forms (Saldana *et al.*, 2005) and shrubs (Valladares*et al.,* 2000) but this issue has not been addressed for climbing plants. It is verified that climbers and rest supporting species would share functional strategies to successfully cope with light heterogeneity, despite the intrinsic differences between these growth forms (Rowe and speck, 2005). It has been earlier shown that climbing plants exhibit life history trade-offs along forest light environments similar to those of trees (Gilbert *et al.,*2006) and that the relationship between photosynthetic rate and dark respiration is

comparable among lianas and trees (Domingues*et al.,* 2007). Because earlier work has suggested possible differences in the ecology of climbing plants in tropical and temperate rain forests.

This result is consistent with the conclusion of Rundel and Franklin (1991), who in their study on vines of arid and semiarid environments, reported that the great majority of arid zone climbers are herbaceous (Vines) .while woody climbers are rare. Even though *Olaxscandens* (Oleaceae), *Chilocarpusatrovinens* (Apocynaceae), *Artabtryszeylamicus* (Annonaceae) and *Calamusgamblei* (Arecaceae) were reported as most abundant species in the western Ghats and *Strychnos minor* (Loganiaceae) in the tropical dry evergreen forests on the colonnade coast of India (Parthasarathy*et al.,* 2004) these species did not occur in the study site. Only one climbing mode, the grapnel-like climbing (rattans) which was reported from Indian Western Ghats sites (Muthuramkumar and Parthasarathy, 2000) did not occur in our study sites.

Presently, plant vegetation's are subjected to various anthropogenic pressures and the data so plant diversity such as this on lianas will be useful in highlighting the importance of this vegetation in species conservation and management.

Contrary to findings from tropical forests (Balfour and Bond, 1993, Sridhar Reddy and Parthasarathy, 2003, Dewalt*et al.,* 2006, Yan *et al.,* 2006) trees were not represented among the 94 climbing plants of study area. According to EL Hadidi*et al.,* (1992) some climbing plants were considered endangered, including *Cadapafarinosa, Maeruaoblongifolia, Ephedra foemina* and *Plicosepaluscurviflorus.* Tackholm (1974) considered another climbingg plant species to be very rare (eg. *Podostelma schimperi, Merremiasemis agittata, Corallocarpus suhimperi, Kedrostis foetidissima, Corallocarpus schimperi, Kedrostis foetidissima, Cissus quadragularis, Peatatropis rivalis* and *Pergularia daemia*)

The comparison between the members of desert climbing plants in Egypt and those of deserts in other continents revealed that ConvolvulaceaeLeguminosae, Cucurbitaceae and Asclepiadaceae were the dominant plant families (Parsons, 2005). In the present survey Convolvulaceae, Papilionaceae and Vitaceae are the dominant families Speciation in the family Convolvulaceae, has been more prolific in the Desert of India where it is the fourth largest family of vascular flora (Shmida, 1985). Vitaceae, the fifth largest vine family in the North American deserts were poorly represented in the Egyptian deserts but not known at all in Australian deserts. Australia has only about 34 species of the approximately 700 species of vitaveae found worldwide (Morley and Toelken, 1983), the family being considered Laurasian (Krings, 2000).

It has been reported that woody vines are increasing in dominance, relative in both tropical (Philips *et al.,* 2002; Wright *et al.,* 2004 and Swaine and Graace, 2007) and temperate forests (Allen *et al,* 2007). This pattern has been related to climate change (Malhi and Wright, 2004, Vander *et al.,* 2008). One of the global change drivers (Matesaaz*et al.,* 2010) but more comprisal evidence is needed. Schnitzer, (2005) reported that the abundance of woody vines in tropical forests is correlated negatively with precipitation and positively with seasonality. He further proposed that this pattern may be explained by the greater efficiency in water uptake and transport of woody climbers as compared to trees. Our study area is a wet, cold (Dorsch, 2003) where light availability is the major ecological factor affecting distribution and abundance of trees (Lusk *et al.,* 2006, Lusk, 2002 and Saldana of Lusk, 2003) but not woody vines (Gianoli*et al.,* 2010, Carrasco *et al.,*(2009) in the temperate rainforest, where the potential evapotranspiration is very low, water availability is not a limiting factor and therefore water use features are was likely to determine plant distribution and abundance. From an applied perspective, the results of the present study suggest that the dominant climbers in the southern temperate rainforest could be able to cope with another global change driver, and use change if forest clearing occurs due to human activities.

Because climbers are present in so many ecosystems today, the morphological characteristics of the climber communities in disturbed versus stable, wetland versus well drained and open versus shaded forest ecosystems should help us recreate the distribution and importance of climbers in ecosystems throughout the last 30 million years. However, destruction of habitat through deforestation and over exploitation for commercial purposes and changes in cultural attitude threatens to constrain many of these climbers in to extinction.

Over exploitation of some climber species particularly collection of roots and underground parts from the climbers causes damage to these plants. Therefore, there are a people for the importance as well as conservation of these climbers in their original habitat. Climber abundance is dependent on climate and forest structure. Site with short or absent dry season have. We propose that a great heterogeneity of potential sites for climbers, thus also increasing, their richness. It is possible that sites with different dry season combined with

tree heterogeneity can enhance the rates of climber speciation.

REFERENCES

Allen, B.P., R.R. Sharitz and P.C. Goebel, (2007). Are lianas increasing in importance in temperate floodplain forests in the south-eastern United States? *Forest Ecology Management* **242**: 17–23.

Balfour, A.D. and W.J. Bondm, (1993). Factors limiting climber distribution and abundance in a Southern African forest. *Journal of Ecology* **81**: 93-99.

Carrasco-Urra, F. and E. Gianoli, (2009). Abundance of climbing plants in a southern temperate rainforest: host-tree characteristics or light availability? *Journal of Vegetaion Science* **20**: 1155–1162.

Chandrabose, M. and N.C. Nair, (1988). Flora of Coimbatore. Bishen Singh Mahendra Palsingh, Dehra Dun.

Clark, D.B., P.C. Olivas, S.F. Oberbauer, D.A. Clark and M.G. Ryan, (2008). First direct landscape-scale measurement of tropical rain forest leaf area index, a key driver of globl primary productivity. *Ecology letters* **11**: 163-172.

Dewal, S.J., K. Ickes, R. Nilus, K.E. Harms and D.F. Burslem, (2006). Liana habitat associations and community structure in a Bornean Lowland tropical forest. *Plant Ecology* **186**: 203-216.

Domingues, T.F., L.A. Martinelli and J.R. Ehleringer, (2007). Ecophysiological traits of plant functional groups in forest and pasture ecosystems from eastern Amazônia, Brazil. *Plant Ecology* **193**: 101–112.

Dorsch, K., (2000). Hydrogeslogischeuntersuchungan der Geothermalfelder Von Puyehue and Cordon Caudle, Chile, Ph.D. Dissertation. Ludwing – Maximilians – Universitat, Munchen.

EL Hadidi, M.N., M.M. Abd E1-Ghani and A.G. Fahmy, (1992). The plant Red Data Book of Egypt. I Woody perennials. Cairo: Palm Press and Cairo University Herbarium. *Ecology* **5**: 1345-1353

Feamside, P.M., P.M.L.A. Graça, N. Leal Filho, F.J.A. Rodrigues and J.M. Robinson, (1999). Tropical forest burning in Brazilian Amazonia: measurements of biomass loading, burning efficiency and charcoal formation at Altamira, Para. *Forest Ecology and Management* **123**(1): 65-79.

Gamble, J.S. and C.E.C. Fisher, (1915-1936). Flora of the presidency Madras. Vol. I-III, Adlard& Co. London (Reprinted 1956). Botanical Survey of India. Calcutta.

Gentry, A.H., (1985). An ecotaxonomic survey of Panamanian lianas In: D'Arey, W. and Correa M. (Eds.), Historia natural de panama Monograph of systematic Botany, Missouri Botanical garden saint Louis pp.29-42

Gentry, A.G., (1991). The distribution and evolution of climbing plants. InF.E. Putz and H.A. Mooney [eds.], The biology of vines, 73-97. Cambridge University Press. Cambridge, UK.

Gentry, A.H. and C. Dodson, (1987). Contribution of nontrees to species richness of a tropical rain forest. Biocropica, **19**: 149-156.

Gerwing, J.J. and D.L. Farias, (2000). Integrating liana abundance and forest stature in to an estimate of total above ground biomass for an eastern Amazonian forest. *Journal of Tropical Ecology* **54**:5-9.

Gianoli, E., A. Saldana, M. Jimenez-Castillo and F. Valladares (2010). Distribution and abundance of vines along the light gradient in a southern temperate rainforest. *Journal of Vegetation Science* **21**: 66–73.

Gilbert, B., S.J. Wright, H.C. Muller-Landau, K. Kitajima and A. Hernández, (2006). Life history trade-offs in tropical trees and lianas. *Ecology* **87**: 1281–1288.

Hegarty, E.E. and E. Caballe, (1991). Distribution and abundance of vines in forest communities. In: F.E. Putz& H.A Mooney (eds.) The biology of vines, Cambridge university Press. p. 313:335

Henry, A.N., V. Chithra and N.P. Balakrishnan, (1987). Flora of Tamil Nadu, India, Series –I: Analysis, Vol.2. Botanical survey of India Coimbatore.

Krings, A. (2000). A phytogeographical characterization of the vine flora of the sonoran and chihuahuan deserts. *Journal of Biogeography* **27**: 1311.1319.

Lusk, C.H. (2002). Leaf area accumulation helps juvenile evergreen trees tolerate shade in a temperate rainforest. *Oecologia* **132**: 188–196.

Lusk, C.H., R.L. Chazdon and G. Hofmann, (2006). A bounded null model explains juvenile tree community structure along light availability gradients in a temperate rain forest. *Oikos* **112**: 131–137.

Malhi, Y. and J. Wright, (2004). Spatial patterns and recent trends in the climate of tropical rainforest regions. Philosophical Transactions of the Royal Society: *Biological Sciences* **359**: 311-329.

Matesanz, S., E. Gianoli and F. Valladares, (2010). Global change and the evolution of phenotypic

plasticity in plants. *Annals of the New York Academy of Sciences* **1206**: 35–55.

Mathew, K.M., (1983). The flora of Tamil Nadu Carnatic (Madras, Diosceesam Press).

Morley, B.D. and H.R. Toelken, (1983). Flowering plants in Australia. Adelaide. Rigby

Muthuramkumar, S. and N. Parthasarathy, (2000). Alpha diversity of lianas in a tropical evergreen forest in the Anamalais, Westtern Ghats, India. Diversity and Distributions 61-14.

Nair, N.C. and A.N. Henry, (1983). Flora of Tamil Nadu, India, Series I. Analysis Vol.I, Botanical Survey of India, Coimbatore.

Parsons, R.F., (2005). Notes on the origins of Australian desert vines. Adansonia **28**: 405-412

Parthasarathy, N., S. Muthuramkumar and M.S. Reddy, (2004). Patterns of liana diversity in tropical evergreen forests of peninsular India. *Forest Ecology and Management* **190**: 15-31.

Perz- Salicreep, D.R., V.L. Sork and F.E. Putz, (2001). Lianas and trees in a liana forest of Amazonian Bolivia. *Biotropica* **33**: 34-47.

Phillips, O.L., R.V. Martinez, L. Arroyo, T.R. Baker and T. Killeen, (2002). Increasing dominance of large lianas in Amazonian forests. *Nature* **418**: 770–774.

Phillips, O.L., R.V. Martínez, A.M. Mendoza, T.R. Baker and P.N. Vargas, (2005). Large lianas as hyperdynamic elements of the tropical forest canopy. *Ecology* **86**: 1250–1258.

Putz, F.E. and H.A. Mooney, (1991). The biology of vines, Cambridge, UK: Cambridge University Press.

Rowe, N. and T. Speck, (2005). Plant growth forms: an ecological and evolutionary perspective. *New Phytologist* **166**: 61–72.

Rundel, P.W. and T. Franklin, (1991). Vines in arid and semi-arid ecosystems. In: Putz FE & Mooney HA (eds.) The Biology of vines p. 337-356. Cambridge: Cambridge university press.

Saldana, A. and C.H. Lusk, (2003). Influencia de lasespecies del dosel en la disponibilidad de recursos y regeneraciónavanzada en un bosquetempladolluvioso del sur de Chile. *Revista Chilena de Historia Natural* **76**: 639–650.

Schnitzer, S.A. and F. Bongers, (2002). Lianas and gap phase regeneration: implications for forest dynamics and species diversity, In: F. Bongers: M.P>E>parren& D. Trasore (eds.) Forest climbing plants of West Africa Diversity, Ecology and Management CABI Publishing p. 59-72.

Shmida, X., (1985). Biogeography of the desert flora, In: M. Evenari M. Noy Meir. I & Goodall DW (eds) Ecosystems of the World, Vol.12A, p.23-77, Amsterdam Elsevier.

Sridhar Reddy, M. and N. Parthasarathy, (2003). Liana diversity and distribution in four tropical dry evergreen forests on the Coromandel Cost of south India. *Biodiversity and Conservation* **12**: 1609-1627.

Swaine, M.D. and J. Grace, (2007). Lianas may be favoured by low rainfall: evidence from Ghana. *Plant Ecology* **192**: 271–276.

Tackholm, V., (1974). Student's Flora of Egypt, 2nd ed. Cairo: Cairo University Press.

Vander Heijden G.M.F., J.R. Healey and O.L. Phillips, (2008). Infestation of trees by lianas in a tropical forest in Amazonian Perú. *Journal of Vegetation Science* **19**: 747–756.

Wright S.J., O. Calderón, A. Hernández and S. Paton, (2004). Are lianas increasing in importance in tropical forests? A 17-year record from Barro Colorado Island, Panamá. *Ecology* **85**: 484–489.

Kong. Res. J. 2(1) : 94-96, 2015 ISSN 2349-2694
Kongunadu Arts and Science College, Coimbatore.

EVALUATION OF *IN VTRO* ANTI BACTERIAL ACTIVITY OF THE *BREYNIA PATENS (ROXB.) BENTH. & HOOK. F.* EXTRACTS

Muthuraj, K*., H Abdul Kaffoor and N. Nagarajan
PG and Research Department of Botany, Kongunadu Arts and Science College, Coimbatore-641 029, Tamilnadu, India
*E-mail: rajfzyama@gmail.com

ABSTRACT

The plants provide a source of inspiration for novel drug compounds, as plant derived medicines made large contributions to human health and well being. The Present investigation is about the antibacterial activity of *Breynia patens* (Roxb.) Benth. & Hook.f. against five bacterial strains using the disc diffusion agar method. The whole plant extracted with ethanol, chloroform, petroleum ether and Aqueous. The sensitivity order of the Methanol crude extracts was illustrated by the corresponding inhibition zone diameter to be *Salmonella paratyphii*(20±009mm zone of incubation) and followed by other bacterial strains. The results indicated that the plant extract exhibited antibacterial properties, thus justifying scientifically their traditional uses as medicinal plants.

Key word: antibacterial activity, *Breynia patens, Salmonella paratyphii.*

1. INTRODUCTION

World turned towards the herbal products which are ecofriendly. We are unaware of biomass produced by wild plants, Most of the plants are having antimicrobial property. Susceptibility to antibiotics is continuously increasing. This increase has been attributed to random use of broad-spectrum antibiotics. The increasing occurrence of antimicrobial resistance represents a worldwide major concern for both human and veterinary medicine (Delamare, 2007). Now a days, drug resistance to human pathogenic bacteria has been commonly and widely reported (Brinda *et al.,* 1981) and many scientists have been paid attention to herbal extracts for biologically active compounds from the medicinal plants,because of the side effect and the resistance of the pathogenic microorganism of synthetic drug (Essawi and Srour 2000). Still now, opposing to common belief, drugs from plants to occupy an important position in modern medicine. At least 130 drugs in single chemical entities from higher plants extract, or customized further synthetically (Newman, 2000). *Breynia patens* (Roxb.) Benth. & Hook. f. is a shrub up to 2m tall. It belongs to the family Euphorbiaceae. The plant bark and stem juice were used as the general tonic. The folklore used this plant because of the medicinal properties like Antipyretic, antitoxic, antiswelling, and antipiruritic; also used for fever, headache, hemorrhage, mumps, puerperium, stomach pain; antiseptic for cuts and sores, bruises, syphilis, abscesses, suppurating sores, lactagogue. Oxidative stress is believed to be a most important provider to the pathogenesis of a number of chronic diseases (Ames *et al*., 1993: Abirami and Muthuswamy, 2013). This study was to evaluate the antibactreial activity of medicinal plant *Breynia patens* (Roxb.) Benth. & Hook. f. used in Ayurveda and traditional medicinal system for treatment. Therefore, extract of the following plant were tested for their potential activity against microbial pathogens.

2. MATERIALS AND METHODS

2.1. Preparation of plant extracts

Fresh plant material was washed under running tap water, air dried and powdered. About 50g of coarsely powdered plant materials (50g/250ml) were extracted in a soxhlet extractor for 8 to 10 hours, with ethanol, chloroform and petroleum ether. The extract obtained was then concentrated using vacuum evaporator and weighted. For stock solutions, 1mg/ml of extract was dissolved in ethanol.

2.2. Aqueous extraction

Ten grams of dried powder was extracted in distilled water for 6 h at slow heat. Every 2 h, it was filtered through 8 layers of muslin cloth and centrifuged at 5000 g for 15 min. The supernatant was collected. This process was repeated twice and after 6 h, the supernatant was concentrated to make the final volume one-fourth of the original volume. It

was then autoclaved at 121ºC and 15 lbs pressure and then stored at 4 ºC.

2.3. Bacterial Strains

The microbial strains are identified strains and were obtained from the department of microbiology, Bharathidasan university, trichirappalli, Tamilnadu, India. The studied bacterial strains were G- E.coli, Proteus mirabilis and G+ Salmonella paratyphii, Streptococcus pyogenes and Euterococcus faccalis

2.4. Media Preperation and Antibacterial Activity

The antimicrobial assay of whole plant was performed by methods of agar disc diffusion method (Parekh, 2005). Two colonies of a 24-hour plate culture of each organism were transferred aseptically into 10 ml nutrient broth in a test tube and mixed thoroughly using an electric shaker for uniform distribution. Petri dishes were plated with Nutrient agar medium were prepared according to the manufacturer's manual and allowed for 30 minutes to solidify. The test organisms were then spread on the surface of the media using a sterile swap stick. The different solvent extracts of plants were (10mg/ml) was introduced on the disc (0.7cm) and then allowed to dry. Then the disc was impregnated on the agar plates and chloramphenical used as reference drug for the bacteria. The plates were then incubated at 37º C for 24 h. Microbial growth was determined by measuring the diameter of zone of inhibition. The composition of NAM medium is given below.

Composition of Nutrient Agar Medium for bacterial culture

Composition	**Quantity (g)**
Peptone	5.0
Beef extract	3.0
Sodium chloride	5.0
Agar	15.0
Distilled water	1000 ml
pH	7

3. RESULTS AND DISCUSSION

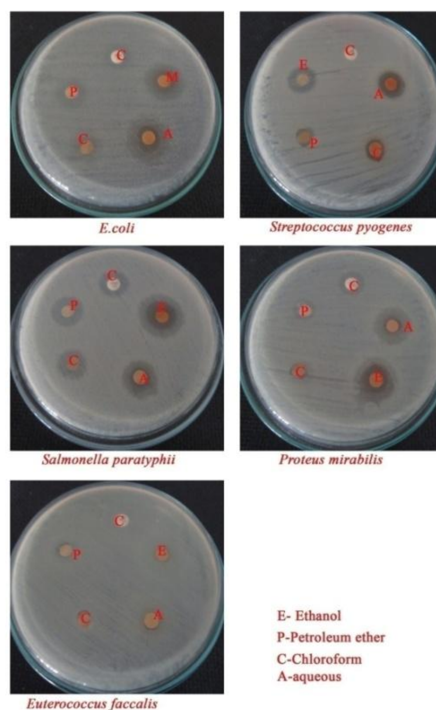
Herbal medicines are a valuable and readily available resource for primary health care and complementary health care systems. A considerable scientific interest is exhibited nowadays to the antimicrobial screening of bioactive herbal extracts or constituents, due to their unique and complex biological potential (Niculae *et al*., 2009). The present study revealed that *Breynia patens* extracts posses potential antibacterial activity in *Proteus mirabilis*, *E. coli*, *Salmonella paratyphii*, *Streptococcus pyogenes* and *Euterococcus faccalis* (Table 1). When tested by the disc diffusion method, the methanol leaf extract showed significant activity against all the five bacterial strains. The highest antibacterial activity of 20 mm zone of the incubation in *Salmonella paratyphii* and least activity recorded in *Streptococcus pyogenes* measured 8mm. similarly Pandey and Verma (2013) reported Methanolic extract of *Euphorbia hirta* leaves showed maximum zone of inhibition against *E.coli, S.aureus* and *P.aeruginosa*. The other extracts also have some considerable activity against the bacterial species. The results summarized in fig.1 extracts from *Breynia patens* prevented the growth of all the tested microorganisms with an inhibition zone diameter variation depending on the extract, concentrations and the type of the bacterium

Table 1: Antibacterial Activity of *Breynia patens* extracts.

S. no	Bacteria	Control (mm)	PE (mm)	C (mm)	A (mm)	E (mm)
1	*E.coli*	17 ±002	5±002	6±03	10±012	11±005
2	*Proteus mirabilis*	24±001	-	4±07	12±008	18±021
3	*Salmonella paratyphii*	18±006	6±032	8±041	13±009	20±009
4	*Streptococcus pyogenes*	26±004	4±05	5±12	14±01	8±02
5	*Euterococcus faccalis*	18±002	5±06	7±012	8±013	12±013

PE-Petrolium ether; C- Chloroform; A-Aqueous; E-Ethanol

Fig. 1. Antibacterial Activity of *Breynia patens* extracts.



4. CONCLUSION

The result of Antibacterial susceptibility assay showed promising evidence for the antibacterial effect of, *Breynia patens* (Roxb.) Benth. & Hook. f. which have the wide spectrum of antimicrobial activity on the bacteria. It has various medicinal values and have been used since earliest time as a medicine for curing various diseases. It is now considered as a valuable source of several unique medicinal products against various diseases and also for the development of some industrial products.

REFERENCES

Abirami, M.S. and Muthuswamy, (2013). Antioxidant potential, total phenolic and total flavonoids content of various extracts from whole plant of *Polycarpaea corymbosa* lam. *Asian J. Pharmaceu. Clini. Res.* **6**(4):121

Ames, B., N.M.K. Shigenaga and T.M. Hagen, (1993). Oxidants, antioxidants, and the degenerative diseases of aging. *Proc. Nat. Acad. Sci. USA*. **90**: 7915–7922.

Brinda P., Sasikala B. and Purushothaman K.K. 1981. Pharmacognosic studies on merugan kilzhangu Bull. Med- Ethno-Bot Res. **3**(1):84-96.

Delamare, A.P.L., I.T.M. Pistorello, I.T. Moschen-Pistorello, L. Artico, L. Atti-Serafini and S. Echeverrigara, (2007). Antibacterial activity of the essential oils of Salvia officinalis L. and Salvia triloba L. cultivated in South Brazil. *Food Chem.* **100**(2): 603-608.

Essawi T. and Srour M. 2000 Screening of some palestnian medicinal plants for antibacterial activity. *Ethnopharmacol*. **70**:343 – 349.

Newman, D.J., G.M. Cragg and K.M. Snader, (2000). The influence of natural products upon drug discovery. *Nat. Prod. Res*. **17**: 215-234.

Niculae, M., M. Spinu, C. Dana Sandru, F. Brudasca, D. Cadar, B. Szakacs, I. Scurtu, P. Bolfa and C.I. Mate, (2009). Antimicrobial potential of some lamiaceae essential oils against animal multiresistant bacteria. *Lucrari Stiinłifice Medicina Veterinara*. **42** (1): 170-175.

Pandey, A. and N. Verma, 2013. Evaluation of antimicrobial activity of euphorbia hirta and calotropis procera against mdr pathogens. *Inter. J. Plant, Animal Environment. Sci*. **3**(3): 17-24.

Parekh, J., R. Nair. and S. Chanda, (2005). Preliminary screening of some folklore medicinal plants from western India for potential antimicrobial activity. *Indian J. Pharmacol*. **37**:408-409.

Kong. Res. J. 2(1) : 97-101, 2015 ISSN 2349-2694
Kongunadu Arts and Science College, Coimbatore.

IN VITRO CALLUS INDUCTION OF *SMILAX WIGHTII* A.DC AN ENDEMIC MEDICINAL PLANT

Nantha kumar, R*., H. Abdul kaffoor, K. Arumugasamy, A. Shalimol and V. Asha devi
PG and Research Department of Botany, Kongunadu Arts and Science College, Coimbatore-641029.
*E.mail: nanthabio@gmail.com

ABSTRACT

Smilax wightii A.DC is an endemic medicinal plant belongs to the family smilacaceae and distributed in Kodanadu, The Nilgiri Hills, The Western Ghats, Southern India. The callus was obtained at the concentration of 1.5+0.05 mg/I TDZ with NAA. Highest number of shoots was observed in 2.0+0.04 mg/l BAP + Kn and followed by 2.0+0.04mg/l. The multiplied shoots were harvested and used for rooting on half strength MS medium containing indole-3-butyric acid and naphthalene acetic acid within 45 days. The best rooting response was achieved on half-strength MS medium supplemented with 2.0 mg/l IBA. The well rooted plantlets were acclimatized and successfully transferred to natural condition, where 85% plantlets were survived.

Key words: Callus, endemic medicinal plant, *Smilax wightii.*

1. INTRODUCTION

Plant tissue culture is the technique of growing plant cells, tissues and organs in an artificially prepared nutrient medium static or liquid, under asceptic conditions. Tissue culture is used for conservation of biological diversity by multiplication of plant species that have extremely small populations, for species with restricted reproductive capabilities and for recovery and reintroduction (Bramwell, 1990). The main areas of research in plant tissue culture viz. micropropagation, anther and microspore culture, somaclonal variations and mutagenesis, protoplast culture and somatic hybridization are some of the effective tools for regeneration and conservation of endangered plants (Bapat *et al*., 2008).

Smilacaceae comprises of 10 genera with more than 300 species, found on temperature zones, tropic and subtropics worldwide (Fnaec, 2002). *Smilax wightii* A.DC. is a rare endemic generally distributed in Shola forests at high altitudes in Nilgiri Biosphere Reserve, the Western Ghats, Southern India.The roots of *S. wightii* have been reported to cure dysentery, amoebiasis, veneral diseases, leucorrhoea, urinary complaints, fever, spermatorrhoea, antifertility, anaemia, rheumatic-arthritis, veterinary amoebiasis and gastric complaints (Adhikari *et al*., 2010).

2. MATERIALS AND METHODS

2.1. Collection of plant material

The whole plant *Smilax wightii* was collected from Kodanadu, The Nilgiri Hills, The Western Ghats, Southern India, Tamil Nadu. The plant was identified and authenticated by a plant taxonomist, Coimbatore.

2.2. In vitro study

2.2.1. Explant sources

The fresh leaf samples of *S. wightii* was collected from its original habitat, and maintained in green house condition and one year old young, healthy, disease free leaves were used as explants, sources.

2.2.2. Explant sterilization

Freshly excised leaf explants of *S. wightii* was surface sterilized and washed in tap water for 2-5 minutes, and again rewashed with liquid detergent (Rankleen, Ranbaxy India) and Tween 20 (Himedia Laboratories, India) for 2-5 minutes with vigorous shaking. These explants were again washed with running tap water to remove any traces of detergent and kept in 1% (w/w) solution of Bavistin (BASP India Limited) for one hour and transferred to 1% (v/v) Savlon (Johnson and Johnson, USA) for 1-2 minutes. In the laminar hood there washed again 2-3 times with sterile distilled water. All these explants were taken and dipped in 70% ethyl alcohol for 30 seconds, surface sterilized with freshly prepared 0.1% (w/v) aqueous solution of mercuric chloride for 20-30 seconds and thoroughly washed with 3-4 times with sterile water and removed all traces of mercuric chloride.

2.2.3. Culture media

The basal medium used for the culture is Murashige and Skoog medium with sucrose 3% (Analytical grade, Himedia, India) and 0.8% agar (Bacteriological grade, Himedia, India) was used the

composition of this medium consist of growth homones, 6 -benzylamino purine (BA), Thiadiazuron (TDZ), 3 -Indole butyric acid (IBA), Kinetin (Kn), adenine sulphate were added to the basal medium either singly or in various combinations. Cytokinins were dissolved first with the respective acidic solution (1N NHcl) and auxins in few drops of basic solutions (1NKOH). The final volume was made up with distilled water and kept in refrigeration. (Growth regulators are from Sigma, USA). Generally, after adding all the ingredients in required amounts, the final volume was made up with distilled water. The pH of the medium was adjusted to 5.8 by using 1N KOH or 1NHcl (Cyber scan 510, Eutech Instruments, Singapore), agar (Himedia Labs Limited, India) added to the medium at the rate of 0.8% (w/v) for solidification of the medium, The media (50 ml/300 ml bottle) were transferred in to the bottles which were tightly capped and labelled properly in to the media was autoclaved (Equitron, Medical Instuments, India) at 121^{0}C for 20 minutes at 15 psi.

2.2.4. Inoculation of the explants

All the explants were sterilized, trimmed and inoculated in culture bottles aseptically and transferred to culture tubes with the help of sterile forceps under strict aseptic conditions. The mouths of the tubes were quickly flamed and culture tubes tightly capped and the mouth of the tubes were properly sealed with cotton plug to avoid entry of external air. All the tubes were properly labelled and transferred to aseptic culture room.

2.2.5. Culture conditions

All cultures were incubated and maintained under 16 hours photoperiod with light intensity of 2000-2500 luX (PolyluX XL, GE Britain, 36 W and temperature of 25±10^{0}C).

3. RESULTS

An *In vitro* propogation method has been developed for *S. wightii*, an endemic medicinal plant from Kodanadu, the Nilgiri Hills, In the present study, the leaf explants of *S. wightii* were excised from their natural habitat and maintained in green house were cultured on MS basal medium supplemented with various concentrations of BA, TDZ and 2,4-D and combinations of NAA with BA, NAA with 2,4-D and NAA with 2,4-D at various concentrations for the induction of callus.

3.1. Callus proliferation

The leaf explants were cultured on MS basal medium supplemented with BA, 2-4D and TDZ with regulators separately in different concentrations (0.1, 0.5, 1.5 and 2.0 mg/L) and NAA with a concentration of 0.05 mg/L was combined with BA, 2,4-D and TDZ at different concentrations (0.1,0.5,1.5 and 2.0 mg/L). In the above combinations callus formation from leaf explants were achieved by using MS medium supplemented with BA (2.0 mg/L), TDZ with (2.0 mg/L), NAA with BA (0.05 + 2.0), NAA with 2,4-D (0.05 + 2.0) and NAA with TDZ (0.05 + 2.0) respectively (Table-1).

The callus formation was observed on MS basal medium with TDZ in the concentration of 2.0 mg/L with respect to 73% in leaf explants. MS medium with TDZ + NAA combinations (1.5+0.05 mg/L) showed 83% callus formation. BA and NAA in the concentration of 1.5+0.05 (mg/L) developed 74% callus formation. Among these concentrations of growth regulators, maximum callus formation for leaf explant was observed on MS basal medium with TDZ+NAA (1.5+0.05 mg/mL) (Table- 2). It was observed that leaf explants cultured in the basal medium containing 2,4-D appeared to be white green coloured and friable callus. The BA, BA in combination with NAA, and TDZ, TDZ in combination with NAA developed light green coloured and compact callus. Crystallized callus mass which turned to hard, compact green solid mass of callus growth within 20-27 days (Table- 2).

4. DISCUSSION

Plant tissue culture techniques enable high efficiency and quick production of ethic and pathogens-free plants besides provoke a considerable interest as a potential alternative to produce bioactive compounds. Plant growth regulators are fundamental to integrate and regulate plant development and their addition in culture media have been widely employed to improve morphogenesis and plant development. In vitro propagation techniques offer an option for the conservation of rare, threatened and endangered medicinal plants (Mallon *et al.,* 2010). These techniques used for rapid and large-scale propagation of medicinal, aromatic, crop, ornamental plants and for the isolation and enhancement of more medicinal secondary products (Sahooy *et al.,* 1997). Plant tissue culture production of medicinal plants offers a number of unique advantages like possibility of year-round continuous production of phytochemicals under controlled conditions (Kayser and Quax, 2007).

The present study is described the *in vitro* regeneration of an endemic medicinal plant, *Smilax wightii* of Smilacaceae from the leaf explants. These explants were cultured on MS basal medium supplemented with various concentrations of, BA

TDZ, 2,4- D and with combinations of NAA, for the induction of callus and subsequent development of plantlets. MS medium has showed the highest percentage and dry weight of the callus in *S. wightii*. Khatun *et al.* (2003) have reported that MS was the most suitable media for callus induction. *S. tetrandra* grown on different basal salt media developed maximum callogenesis with one fold MS media. Addition of peptone into MS the culture medium has strongly improved the growth of tobacco callus. In the study, the addition of peptone (2g/L) has promoted callus growth. Because peptides could be more efficient source of nitrogen for sustaining *in vitro* callus proliferation. The texture of callus varied according to the nature of cytokinin and also on auxin and cytokinin ratio (Martin, 2002). Morphological and phytochemical differences in callus are attributing to culture conditions, composition of the medium orientation of the explants, and growth and morphogenesis ability of plants itself. Various types and concentration of hormones present in medium directly affect the nature, colour and biochemical composition of callus (Skoog and Miller, 1967).

It may be explained that the specific growth hormones at appropriate concentrations can play major role to induce callus besides the other factors (Ananthi *et al.,* 2011). Baskar and Jayabalan (2005) suggested that the differential response of same or different explants for callus formation could be due to the nature of tissue, degree of totipotency and composition of medium with respect to micronutrients and hormones. Further, it is explained that the variation in response of leaf discs in terms of callus formation may be due to the variation in distribution of endogenous level of growth regulators as observed in plants (Farternale et al., 2002). The present results of *S. wightii* leaf explants indicated that individual growth regulators are not developed any organs or callus induction. However, several combinations of growth regulators have shown to induce the formation of callus. The young moderate sized leaf explants were well responded for rapid callogenesis after incubation period of 4 weeks. A pale green colour and healthy compact calli were observed on first week of inoculation at the trimmed regions on MS media supplemented with 1.5 mg/l TDZ concentrations. The highest callus fresh weight was obtained in the medium containing 1.5mg/l TDZ + 0.02mg/l NAA combinations.

Variations in the callus forming ability of different explant types have reported in many plants. Callus from leaf segments showed initiation of vigorous, proliferating, soft and green colored tissue (Chand and Roy, 1980). TDZ, a synthetic phenylurea is considered to be one of the most active cytokinins for callus and shoot induction in plant tissue culture. TDZ-induced callus induction from different explants of many recalcitrant species as well as from medicinal plants has been reported. Several reports suggest that TDZ results in callus induction better than other cytokinins.

Aditi Singh *et al.* (2009) have explained callus formation in *Tinospora cordifolia* developed from nodal, inter-nodal and leaf explants when planted on the MS medium containing the combination of BAP and NAA. Of these, callusing response was seen only on leaf explants study have showed a combination of 2,4-D, BAP and Kin (2.0, 2.0 and1.0µM) in MS medium enhances the callus production in *Coscinium fenestratum*. Our results are similar to Kuo *et al.* (2005) investigations, where an efficient in vitro callus induction system in *S. Wightii* was established on MS medium supplemented with 3% sucrose and different concentrations of plant growth regulators i.e. auxins (2,4-D, IAA and NAA) and cytokinins (BA, kinetin, TDZ and zeatin) in the dark. MS medium supplemented with 1.0mg/L BA and 0.5 mg/L TDZ supported callus growth and its proliferation. A maximum amount of dry biomass (7.8 fold) was produced 45 days after culture. Similarly in *Taxus wallichiana* the stem explants developed brown friable callus in different basal media, supplemented with 2mg/l of 2,4-D. Callus induction was found to be best in MS media solidified with 10g/l agar and supplemented with 1-5mg/l NAA. Further, addition of kinetin (1-5mg/l) has resulted in more active callus formation. The colour of calluses ranged from brown to green, greenish yellow and yellow.

Callus potential has reported to vary from species to species and often differs in varieties of same species (Pradeep Kumar and RanjithaKumari, 2010). Karappusamy and Pullaiah (2007) have reported an effective callus formation from the leaf explant in the medium containing high quantity of NAA for *Bupleurum distichophyllum*. Mariani *et al.* (2011) have suggested the requirement of the cytokinin like compounds, TDZ for callus formation in *Aglaonema spp*. The cytokinin is known major growth hormone involved in shoot formation of plant species (Sujatha *et al.,* 2008).

The highest number of shoots from leaf explants of *S. wightii* were observed in TDZ+NAA (1.5+0.03 and 2.0+0.04mg/l) and followed by 2.0+0.04mg/l of BA+NAA combinations when the callus cultured in MS medium. The regeneration frequency was high in TDZ and BA combined with NAA. These results were similar to Kannan *et al.*

(2007) who have established an in vitro propagation of *Excaum travancoricum*. The internodal explant of *E. travancoricum* was cultured on MS with different levels of Thidiazuron or BA showed gradual bulging. They have observed the direct shoot morphogenesis on the cut ends of the explants cultured on MS medium with TDZ or BA. Here, TDZ was superior to BA in the induction and proliferation of shoots. The shoots induced on TDZ enriched medium were healthier than the shoots raised on BAP. Culture of shoot clumps on MS medium having TDZ favoured proliferation of shoots. The high efficient of TDZ over BA has been documented in several woody plants (Vidya *et al.,* 2005).

Table 1. Effect of different concentrations of plant growth regulators on callus induction from leaf explants of *Smilax wightii.*

Plant growth regulators	Concentration of plant growth regulators mg/L	Leaf explant		
		Intensity of callus formation	Mean callus weight ± SD	Nature of callus
Control	--	--	--	--
BA	0.1	+	0	No callus formed
	0.5	+	0.32 ± 0.03	White green friable
	1.5	+	0.45 ± 0.17	White green, friable
	2.0	++	0.74 ± 0.26	Light green, compact
TDZ	0.1	+	0.33 ± 0.12	White green friable
	0.5	+	0.49 ± 0.23	White green, friable
	1.5	+	0.68 ± 0.06	White green, friable
	2.0	++	0.85 ± 0.34	Light green, compact
2,4-D	0.1	--	0	No callus formed
	0.5	+	25 ± 0.11	White green, friable
	1.5	+	0.32 ± 0.23	White green, friable
	2.0	++	0.38 ± 0.04	White green, friable
BA + NAA	0.1+0.05	--	0	No callus formed
	0.5+0.05	+	0.34 ± 0.16	White green, friable
	1.5+0.05	+	0.51 ± 0.32	White green, friable
	2.0+0.05	++	0.87 ± 0.26	Light green, compact
2,4-D + NAA	0.1+0.05	--	0	No callus formed
	0.5+0.05	+	0.27 ± 0.41	White green, friable
	1.5+0.05	+	0.39 ± 0.17	White green, friable
	2.0+0.05	+	0.42 ± 0.08	White green, friable
TDZ + NAA	0.1+0.05	+	0.76 ± 0.12	White green, friable
	0.5+0.05	+	0.84 ± 0.23	White green, friable
	1.5+0.05	+	0.92 ± 0.08	Light green, compact
	2.0+0.05	+++	1.42 ± 0.22	Light green, compact

Intensity of callus: (+) low; (++) moderate; (+++) high.
Values are expressed as Mean ± Standard Error of 3 replicates. Means within a column followed by a common letter aren't significantly different at 5% level by SPSS.

Table 2: Effect of different concentrations of plant growth regulators on the duration and percentage of callus formation from leaf explants of *Smilax wightii.*

Plant growth regulators	Concentration of plant growth regulators mg/L	Days required for callus formation after inoculation	Callus formation (%)
BA	0.1	0	0
	0.5	27 ± 0.02	50 ± 0.02
	1.5	25 ± 0.05	65 ± 0.12
	2.0	23 ± 0.04	53 ± 0.01
TDZ	0.1	24 ± 0.02	61 ± 0.08
	0.5	23 ± 0.01	66 ± 0.02
	1.5	24 ± 0.02	71 ± 0.14
	2.0	22 ± 0.13	73 ± 0.04
2,4-D	0.1	0	0
	0.5	26 ± 0.06	43 ± 0.05
	1.5	25± 0.11	40 ± 0.02
	2.0	24 ± 0.01	57 ± 0.08
BA + NAA	0.1+0.05	0	0
	0.5+0.05	23 ± 0.13	62 ± 0.16
	1.5+0.05	21 ± 0.02	74 ± 0.08
	2.0+0.05	20 ± 0.12	68 ± 0.01
2,4-D + NAA	0.1+0.05	0	0

	0.5+0.05	23 ± 0.13	48 ± 0.06
	1.5+0.05	23 ± 0.05	56 ± 0.12
	2.0+0.05	22 ± 0.13	64 ± 0.04
TDZ + NAA	0.1+0.05	21 ± 0.04	65 ± 0.06
	0.5+0.05	20 ± 0.18	70 ± 0.02
	1.5+0.05	25 ± 0.06	83 ± 0.14
	2.0+0.05	20 ± 0.11	74 ± 0.18

Values are expressed as Mean ± Standard Error of 3 replicates. Means within a column followed by a common letter aren't significantly different at 5% level by SPSS.

REFERENCES

Adhikari, B.S., M.M. Babu, P.L. Saklani and G.S Rawat, (2010). Medicinal Plants Diversity and their Conservation Status in Wildlife Institute of India (WII) Campus, Dehradun. *Ethnobotanical Leaflets* **14**: 46-83.

Aditi Singh, S., K. Sah, A. Pradhan, S. Rajbahak and N. Maharaja, (2009). *In vitro* study of *Tinospora cordifolia* (Willd.) Miers (Menispermaceae). *Botanica Orientalis – J. Plant Sci.* **6**: 103–105.

Ananthi, P., B.D. Ranjitha Kumari and A. Ramachandran, (2011). *In vitro* propagation of *Rorippa indica* L. from nodaland shoot tip explants. *Inter. J. Biotechnol. Molecular Biol. Res.* **2**(3) 51-55.

Bapat, V.A., S.R. Yadav and G.B. Dixit, (2008). Rescue of endangered plants through biotechnological applications. *National Academy Sci. Letters,* **31**: 201-210.

Baskar, P. and N. Jayabalan, (2005). An efficient micropropagation system for *Eclipta alba* a valuable medicinal herb. *In Vitro Cellular and Develop. Biol. Plant*. **41**(4): 532-539.

of Sourthern India. *Plant Tissue Cult. Biotechnol.* **17**(2): 115-124.

Kayser, O., and W.J. Quax, (2007). Medicinal Plant Biotechnology. From Basic Research to Industrial Applications. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

Khatun, A., S. Nasrin and M.T. Hossain, (2003). Large scale multiplication of ginger (*Zingiber Officinale* Rosc.) from shoot-tip culture. *J. Biological Sci*. **3**: 59-64.

Kuo, P.L., Y.L. Hsu, T.C. Lin, L.T. Lin, J.K. Chang and C.C. Lin, (2005). Induction of cell cycle arrest and apoptosis in human non-small cell lung cancer A549 cells by casuarinin from the bark of *Terminalia arjuna* Linn. *Anticancer Drugs*, **16**: 409-415.

Mallon, R., J. Rodriguez-Oubina, and M.L. Gonzalez, (2010). *In vitro* propagation of the endangered plant *Centaurea ultreiae*: assessment of genetic stability by cytological studies, flow cytometry and RAPD analysis. *Plant Cell Tiss. Org.* **101**: 31–39.

Mariani, T.S., A. Fitriani, J.A. Teixeira da Silva, A. Wicaksono. And T.F. Chia, (2011). Micropropagation of *Aglaonemausing* axillary shoot explants. *Inter. J. Basic and Appl. Sci.* **11**(1):46-53.

Martin, K.P. (2002). Rapid propagation of *Holostema ada-kodien* Schult. a rare medicinal plant, through axillary bud multiplication and indirect organogenesis. *Plant Cell. Rep.* **21**: 112-117.

Bramwell, D. (1990). *The Role of in vitro cultivation in the conservation of endangered species.* Conservation Techniques in Botanic Gardens - Koeningstein, Germany, Koeltz Scientific Books.

Chand, S. and S.C. Roy, (1980). Effect of different auxins on chromosome behaviour of leaf callus tissues of *Nigela sativa*. *Caryologia* **33**:387-392.

Fnaec, (2002). Flora of North America editorial committee. *Flora of North America North of Mexico* **26**: 14–46.

Fraternale, D., L. Giamperi, D. Ricci and M.B.L. Rocchi, (2002). Micropropagation of *Bupleurm fruticosum*: The effect of triacontanol. *Plant Cell, Tissue and Organ Cult.* **69**: 135-140.

Kannan, P., A. Premkumar and S. Ignacimuthu, (2007). Thidiazuron induced shoot regeneration in the endangered species, *Exacum travancoricum* Beedi. *Indian J. Biotech*. **6**: 564-566.

Karuppusamy, S. and T. Pullaiah, (2007). *In vitro* shoot multiplication of *Bupleurum distichophyllum* Wight.-A native medicinal plant

Paulsamy, S., P. Senthilkumar and M. Shivashanmugam, (2008). Clonal multiplication strategies for medicinal plants inhabiting Nilgiri Biosphere Reserve, the Western Ghats, India. *J. Theoret. Experiment. Biol.* **4**(3):115-119.

Pradeep Kumar, S. and B.D. Ranjitha Kumari, (2010). Effect of Amino Acids and Growth Regulators on Indirect Organogenesis in *Artemisia vulgaris* L. *Asian J. Biotechnol.* 1-9.

Sahooy, Pattnaik, S.K. and P.K. Chand, (1997). *In vitro* clonal propagation of an aromatic medicinal herb *Ocimum basilicum* L. (sweet basil) by axillary shoot proliferation*. In Vitro Cell. Development. Biol. – Plant* **33**: 293-296.

Skoog, F. and C.O. Miller, (1957). Chemical regulation of growth and organ formation in plant tissue cultures *in vitro . Symp. Soc. Exp. Biol.* **11**: 118–131.

Sujatha, G., B.D. Ranjitha Kumari, P.L. Cioni, and G. Flamini, (2008). Mass propagation and essential oil analysis of *Artmeisia vulgaris*. *J. Biosci. Bioengineer*. **105**(3): 176-183.

Vidya, S.M., V. Krishna, B.K. Manjunath and Shankarmurthy (2005). Micro propagation of Entada pursaetha DC-An endangered Medicinal plant of Western Ghats. *Indian. J. Biotech.* **4**: 561-564.

Kong. Res. J. 2(1) : 102-104, 2015 ISSN 2349-2694
Kongunadu Arts and Science College, Coimbatore.

BIOMASS PRODUCTION AND OIL YIELD OF LEMONGRASS IN PUNALUR FOREST DIVISION, KERALA, INDIA.

Nisha Raj, S[1]. S. Sekaran[2*], and S. Paulsamy[3]
[1]PG Department of Biotechnology, SAS, SNDP Yogam College, Konni- 689691, Kerala, India.
[2]Department of Botany, Sree Narayana College, Kollam-691 001, Kerala, India.
[3]Department of Botany, Kongunadu Arts and Science College, Coimbatore – 641 029, Tamil Nadu, India.
*E-mail: drsekhar72@gmail.com

ABSTRACT

Lemongrass (*Cymbopogon citratus*) cultivation and oil distillation from its biomass are some of the important sources of earning for the rural people and tribal communities of sourthern Western Ghats in certain specific localities. Punalur Forest Division of Kerala is one such region where some local public involved in these practices. However, no data are available on the biomass production and oil yield of lemongrass for this region. Therefore, the present study has been carried out in this line. The results of the study reports that annual biomass production was greater (9250kg/ha/yr) and comparable to that of the other lemongrass cultivated areas. Similarly, the oil yield was also higher (46.7kg/ha/yr) and the concentration of oil varied significantly across the harvesting times (0.43-0.63%). The statistical processing of data elucidates that soil pH and calcium content were having significant positive correlation to lemongrass biomass production. Oil concentration in the biomass was negatively correlated to relative humidity. Therefore, soil nutrient status can be considered as a key factor while preparing the management plan for lemongrass cultivation in Punalur Forest Division.

Keywords: Lemongrass, biomass production, oil yield, Punalur Forest Division, Kerala.

1. INTRODUCTION

Lemongrass (*Cymbopogan citratus* Stapf.) belongs to the family, Poaceae is a native aromatic tall sedge grass (Rangari Vinod, 2009) and has distributed in low hills of southern Western Ghats of Kerala and Tamilnadu in peninsular India. Due to its diverse medicinal values, it is being cultivated commercially in these regions for the past few decades. In Punalur Forest Division, Pathanamthitta district of Kerala state, at fragile ecosysytems in Western Ghats around 100-200m altitude, this grass is cultivated for oil distillation. Local people those involved in this practice are getting a sizable economic return also. However, no ecological data are available on lemongrass production status and oil yield for this grass in this region. Therefore, the present study was aimed at to obtain the data on biomass production and oil yield of lemongrass in Punalur Forest Division, Kerala.

2. MATERIALS AND METHODS

2.1. Study area

Punalur Forest Division situated in Pathanamthitta district, Kerala is mainly covered by teak plantation forests in addition to natural forests, comprising mainly *Artocarpus* sp. and *Pterocarpus* sp. The sloppy areas where the forests area cleared are used for the utilization of lemongrass by the people of local panchayath. The soil of the area is sandy loam with alkaline pH around 8.5. Climatic data of the study area was collected from Punalur Forest Division for the study period. The annual rainfall for the past 15 year's average is 1400mm. The temperature is varying between 20 and 39°C and the relative humidity was ranging between 61 and 91%.

2.2. Soil analysis

Soil samples from A_1 layer (0-10cm) collected during each harvesting time were mixed thoroughly to obtain composite sample. Working samples were obtained to analyze the soil for pH (Ghosh *et al*. 1983), total nitrogen (Jackson, 1962), available phosphorous (Bray and Kurty, 1945) and available potassium, calcium and magnesium (flame photometric method, Standford and English, 1949).

2.3. Biomass production and oil yield

An one hectare plot was established in the lemongrass community. Sampling for biomass was made 5 times in the study year, 2013-2014 when the farmers harvested the grass for oil distillation. The data on dry weight of lemongrass was collected from the farmers directly after harvested on hectare basis. Similarly, data on oil yield was collected from the

farmers on hectare basis. As the grass was completely scraped during harvest, the biomass harvested at every time is its production. The biomass data collected for all the five harvesting times were added together to arrive the annual production (Singh and Yadhav, 1974).

3. RESULTS AND DISCUSSION

The variations in climatic and soil factors between the harvesting periods of lemongrass are present in Table 1. Both maximum and minimum temperatures were higher during April, 2014 (39 and 28°C respectively) and lower during January, 2014 (22 and 18°C respectively). As a general pattern, the rainfall during south-west monsoon (June – August, 2013) was higher (810mm) in the study year which shared 58% in the total annual rainfall. The relative humidity was also higher during south-west monsoon period (90 - 91%). The soil pH was always alkaline during the study period (8.0 – 8.5). The contents of soil nutrients such as N, P, K, Ca and Mg were also showed much variations between the sampling times (N – 0.90 to 1.10%, P – 0.05 to 0.07%, K – 0.25 to 0.45%, Ca – 0.08 to 0.14% and Mg – 0.02 to 0.04%). All the climatic and soil factors studied were found to be more suitable for plant growth, particularly the lemongrass (Nambiar and Matela, 2012).

Biomass production of lemongrass in Punalur Forest Division was much varied across the periods of harvesting (Table 2). The growth of the lemongrass in the study area, Punalur was higher during August - November, 2013 as the biomass was greater during that period (2600kg/ha). The winter and summer months were characterized by less biomass of 1800kg/ha during January and 1200kg/ha during summer. It may be attributed to the fact that with the onset of monsoon in June, there was an active biomass build up and it attained maximum during August after two months of vegetative growth. Singh and Krishnamurthy (1981) reiterates that the temporal variation exhibited in the attainment of peak biomass in grassland communities of tropical climate is related to quantity of rainfall. Paulsamy *et al.* (2000) showed an enhanced biomass production of lemongrass at the end of south-west monsoon season in some parts of Anaimalais, the Western Ghats. The lemongrass production decreased progressively towards summer from August. It may be explained due to the influence of water as limiting factor. The total annual net primary production of lemongrass in the study area was estimated as 9250kg/ha.

The percentage of oil in the lemongrass biomass was significantly varied between the sampling times (Table 2). Higher oil concentration of 0.63% was noted during summer and that of the lower 0.43% was during the rainy month of August, 2013. Oliveros and Aureus (1977) explained that the variation in oil content is the function of weather conditions like dry environment with higher incidence of sunlight which promotes the formation of such chemicals for resisting the adverse conditions.

Table 1. Climatic and soil factors of the study area, Punalur Forest Division during the samplings of lemongrass biomass.

Year and month	Climatic factors				Soil factors					
	Temperature (°C)		Rainfall (mm)	Relative humidity (%)	pH	Nutrient content (%)				
	Max	Min				N	P	K	Ca	Mg
2013										
Jun	33	29	145	91	8.1	0.90	0.06	0.35	0.10	0.02
Aug	31	28	665	90	8.3	0.95	0.05	0.30	0.12	0.03
Nov	29	24	435	85	8.5	1.10	0.05	0.25	0.14	0.02
2014										
Jan	22	18	80	70	8.0	1.05	0.07	0.35	0.10	0.03
Apr	39	28	60	61	8.0	0.95	0.06	0.45	0.08	0.04

Table 2. Biomass production and oil yield of lemongrass in Punalur Forest Division, Kerala.

Year and month	Lemongrass biomass (kg/ha)	Oil concentration (%)	Oil yield (kg/ha/yr)
2013			
Jun	1600	$0.47^{a}\pm0.05$	7.52
Aug	2030	$0.43^{a}\pm0.06$	8.82
Nov	2600	$0.51^{b}\pm0.05$	13.26
2014			
Jan	1800	$0.53^{b}\pm0.07$	9.54
Apr	1200	$0.63^{c}\pm0.05$	7.56
Total annual production	9250		46.7

*Mean followed by various letter for the column 'oil concentration' are significantly varied at 5% level.

Table 3. Correlation coefficient (*r*) between the variables of lemongrass production and certain environmental factors in Punalur Forest Division, Kerala.

Lemongrass production attributes	Climatic variables				Soil variables					
	Ma.t	Mi.t	Rf	RH	pH	N	P	K	Ca	Mg
Lemongrass biomass	-0.525	-0.295	0.678	0.566	0.904*	0.707	-0.565	-0.967**	0.986**	-0.663
Oil concentration	0.378	-0.137	-0.700	-0.926*	-0.484	0.129	0.412	0.725	-0.605	0.650

Ma.t – Maximum temperature; Mi.t – Minimum tempertature, Rf – Rain fall; RH – Relative humidity. **Correlation is significant at $P<0.01$ level.
*Correlation is significant at $P<0.05$ level.

An analysis of data elucidated several functional relationships among the variables of lemongrass production (biomass and oil yield) and certain climatic and soil factors (Table 3). The soil pH and calcium content were significant positive correlation to biomass production (r = 0.904, $P<0.05$ and 0.986, $P<0.01$). Similar kind of observations on the requirement of nutrients like Ca for better growth of *Cymbopogon* spp. was made already (Jayalakohmimituala and Mohanaraopuli, 2013; Singh *et al.,* 2014). The level of alkalinity is reported to be a most preferable factor for the growth of lemongrass (Jayasinha *et al.,* 1999; Paulsamy *et al.,* 2000). Oil concentration in the biomass of lemongrass has no significant positive correlation to any environmental factors studied.

In Pearson's correlation, potassium content of soil was significant negative correlation to lemongrass biomass (r = -0.967, $P<0.01$). The available potassium content in soil generally has inverse relationship with rainfall as this nutrient leaches out rapidly than any other elements in the soil by rain water percolation (Salisbary and Ross, 1991). Therefore, biomass enhancement was coupled with rain water and thus by negatively influenced by soil potassium content. The oil concentration in lemongrass was also has significant negative correlation to relative humidity of atmosphere (r = -0.926, $P<0.05$). It is explained that it is a defence mechanism to produce more biochemical compounds including essential oils to resist high humidity for certain plant species (Castelo *et al.,* 2012).

The present study clearly suggests that lemongrass production and oil concentration in biomass are the functions of soil factors like pH and calcium content but their intensity of influence vary according to other climatic variables like relative humidity and soil variables like potassium content. Therefore, the soil nutrient status can be considered as a key factor by preparing management plan for lemongrass cultivation in Punalur Forest Division, because rainfall is not a limiting factor in the landscape.

ACKNOWLEDGEMENT

The authors acknowledge the UGC, Bangalore, India for providing financial support.

REFERENCES

Bray, R.H. and L.T. Kurtz, (1945). Determination of total, organic, and available forms of phosphorus in soils. *Soil Sci.* **59**: 39-45.

Castelo, A.V.M., C.H.S. Del Menezzi and I.S. Resck, (2012). Seasonal variation in the yield and the chemical composition of essential oils from two Brazilian native abusive species. *J. Appl. Sci.* **12**: 753-760.

Ghosh, A.B., J.C. Bajaj, R.Hasan and D. Singh, (1983). Soil and Water Testing Methods, Laboratory Manual. pp. 21-22. (ICAR Publication, New Delhi).

Jackson, M.L. (1962). *Soil Chemical Analysis*. Asia Publishing House, Bombay.

Jayasinha, P., D. Warnaswriya and H. Dissanayake, (1999). *Medicinal and aromatic plant series*. No. 9. Lemongrass survey. Information Service Centre, Industrial technology Institute, Colombo, Sri Lanka.

Nambiar, V.S. and H. Metala, (2012). Potential functions of Lemongrass (*Cymbopogon citratus*) in health and disease. *Int. J. Pharmaceu. Biol. Arch.* **3**: 1035-1043.

Oilveros, B.L. and E. Aureus, (1977). Essential oil from (*Cymbopogon citratus* Stapf.) growing wild in Philippines. *Perfume. Flavour.* **2**: 59.

Paulsamy, S., T.N. Rangarajan, K. Arumugasamy, S. Manian, K. Udaiyan, R. Sivakumar and D. Senthilkumar, 2000. Effect of habitat variation on the structure, herbage production and oil yield of *Cymbopogon flexuosus* Stapf. dominated grasslands in Anamalais, the Western Ghats. *J. Environ. Biol.* **12**: 85 – 94.

Rangari Vinod, D, (2009). *Pharmacognosy and Phytochemistry*, Carrer Publication, Vol. 1st, 2nd ed. pp. 380-381.

Salisbury, F.B. and C.W. Ross, (1991). *Plant Physiology*. Wordworth Publishing Com., California, USA. pp. 682.

Singh, J.S. and L. Krishnamoorthy, (1981). Analysis of structure and function of tropical grassland vegetation of India. *Indian Rev. Life Sci.* **1**: 225-270.

Singh, J.S. and P.S. Yadava. 1974. Seasonal variation in composition, plant biomass and net primary productivity of tropical grassland at Kurukshetra, India. *Ecol. Monogr.* **44**: 351-375.

Stanford, S. and L. English (1949). Use of flame photometer in rapid soil test of K and Ca. *J. Agro.* **41**: 446-447.

Kong. Res. J. 2(1) : 105-110, 2015 ISSN 2349-2694
Kongunadu Arts and Science College, Coimbatore.

ESTIMATION OF MICROBIAL BURDEN DUE TO DIFFUSED AND POINT SOURCES AT SPATIAL SCALE IN MIDDLE STRETCH OF RIVER BHAVANI, COIMBATORE, TAMILNADU

Nithya Jeniffer, P[1]*., and Manish Kumar[2], and K. Logan Kumar[3]
[1]PG and Research Department of Zoology, Nirmala College for Women, Red fields Coimbatore - 641 029.
[2]Environmental Impact Assessment Division, Sálim Ali Centre for Ornithology and Natural History (SACON), Anaikatti (PO), Coimbatore - 641 108
[3]PG and Research Department of Zoology, Kongunadu Arts and Science College, Coimbatore - 641 029.
*E-mail: jenii@rediffmailcom

ABSTRACT

This study was aimed to estimate the microbiological status of river Bhavani which is the major life line of the surrounding area. The river water is used mainly for drinking, washing and swimming purposes. Water samples were collected from five sites (Pilloor, Effluent, Sweage I & II, Bhavanisagar reservoir) and the results obtained were compared with WHO and EP standards for drinking and recreational water. In the present investigation station III and IV (which is Sweage I & II respective points) showed more numbers of microbes were present which cause the deadly diseases to human being as well as in aquatic, terrestrial organism due the sewage discharges from the nearby towns. But in the case of lower stream (Bhavanisagar reservoir) that the counts of microorganism present in the reservoir was slightly reduced. The major groups of microorganisms were isolated and identified are *E. coli, P. aerugionosa*, *E. aerogenes*, *S. aureus*, *Salmonella sp*, *Bacillus sp*, *Proteus sp*, *Klebsiella sp*, *Flavo bacterium*, *Acinetobacter sp*. The results are alarming and show that alternative measures to reduce the pollution should be taken in favor of society.

Key words: Microbial pollution, river Bhavani, Bhavanisagar reservoir, Effluent, Pilloor reservoir

1. INTRODUCTION

Fresh water resources are becoming deteriorated day-by-day at a very faster rate. Now water quality is a global problem (Mahananda *et al*., 2005). Water is a resource that has many uses, including recreation, transportation, and hydroelectric power, domestic, industrial and commercial uses. Water also supports all forms of life and affects our health, lifestyle, and economic wellbeing. Although more than three quarters of the earth's surface is made up of water, only 2.8 percent of the earth's water is available for human consumption (Iskandar, 2010). Water is available in the universe in huge quantity in the order of 1400 x 106 km^3 and only 3% of the waters in the universe as freshwater. Among the freshwaters, only about 5% of them or 0.15% of the total world waters are readily available for beneficial use. The total water resource available in India is 1850 km^3, which is roughly 4% of the world's fresh water resources. Water can be obtained from a number of sources, among which are streams, lakes, rivers, ponds, rain, springs and wells. Rivers are vital and vulnerable freshwater systems that are critical for the sustenance of all life. However, the declining quality of the water in these systems threatens their sustainability and is therefore a cause for concern. Rivers are waterways of strategic importance across the world, providing main water resources for domestic, industrial, and agricultural purposes (Fathi, 2006). Rivers play a major role in assimilation or carrying off of municipal and industrial wastewater and runoff from agricultural land, the former constitutes the constant polluting source whereas the later is a seasonal phenomenon.

Pollution of river in India has now reached to a point of crisis due to unplanned urbanization and rapid growth of industrialization. Waste comprises liquid waste discharged by domestic residences, commercial properties, industry or agriculture and can encompass a wide range of potential contaminants and concentrations. Discharge of organic wastes human excreta, sewage waste, polythenes, municipal garbage and toxic discharge from the factories increasing bacterial pollution. These wastes flow into the storm drains, mixing with common water and subsequently posing a serious threat to the water ecology, animal and human society.

In the most common usage, it refers to the municipal wastewater that contains a broad spectrum of contaminants resulting from the mixing

of wastewater from different sources. Sewage is created by residences, institutions, hospitals and commercial and industrial establishments (APHA, 1998). Raw influent includes household waste liquid from toilets, baths, showers, kitchens, sinks, and so forth that is disposed of via sewers. In many areas, sewage also includes liquid waste from industry and commerce. As rainfall runs over the surface of roofs and the ground, it may pick up various contaminants including soil particles and other sediment, heavy metals, organic compounds animal waste and oil and grease (FWPCA, 1998). Consequently, the problem was taken up when effluents of these industries go into the water system and change the physicochemical quality of water and make it unfit for drinking and other uses. This effect of water pollution results in transmission of infectious diseases such as cholera, diarrhoea and typhoid (FWPCA, 1998).

Microorganisms are widely distributed in nature and diversity of microorganisms may be used as an indicator for organic pollution (Okpokwasili and Akujobi, 1996). Bacteriological quality of drinking water usually shows concentration of particular species of bacteria and their occurrence (Sandy and Richard, 1995). Cryptosporidiosis, typhoid fever, Cholera, dysentery and hepatitis are some of the common waterborne diseases that spread through contaminated water. Contaminated water can cause eye, ear, nose and throat infections also.

Bacteriological examination offers the most sensitive test for the detection of recent and therefore potentially dangerous faecal pollution thereby providing a hygienic assessment of water quality with a sensitivity and specificity that is absent from routine chemical analysis. It is essential that water is examined regularly and frequently as contamination may be intermittent and may not be detected by the examination of a single sample. For this reason, it is important that drinking water is examined frequently (Mane, 2005).

2. MATERIALS AND METHODS

2.1. Description of the study area

River Bhavani is one of the major tributaries of the river Cauvery originated from Western Ghat region, Nilgiris biosphere, and second largest river in Tamilnadu. It crosses the distance of about 217 km through Mettupalayam and Sirumugai before merging with Cauvery spread over Tamil Nadu (87%), Kerala (9%) and Karnataka (4%). About 90 percent of the river water is used for irrigation, culturing of fishes, drinking purposes and hydroelectric power. Around six million people are directly or indirectly depending and living along its banks. The study area divided in a broad sense in three parts namely upper stream (station I, Pilloor reservoir), middle stream (station II, effluent mixing point; station III, sewage 1, station IV sewage mixing point 2) and downstream (station V, Bhavanisagar reservoir) and water samples were collected aseptically and transported to the laboratory for analysis the rapid survey was carried our between 2011-12 session to estimate the microbial load and possible impacts of microorganisms on the people receiving end.

Pilloor reservoir (N 11.27° & E 76.80°) located on River Bhavani, 88 m high has a catchment area of 1,191 km^2; the dam is 357 m long and the mean annual flow is 685 MCM. Mettupalayam (11.30°N & 76.95°E) is situated on the bank of Bhavani River at the foot of the Nilgiri hills. It has an average elevation of 314 meters (1033 feet). The Bhavanisagar reserviour (11° 26' 45.09" N, 77° 4' 25.37" E) is located in Bhavanaisagar at 50 Km North East from Mettupalayam city, Tamilnadu. It is the longest earthen reservoir of its kind in Tamilnadu state measuring 8780 mt and ranking second largest reservoir with a capacity of 929 million cubic meters; the total catchment area is1621.50 Sq. miles.

At Mettupalayam, numerous slaughter houses, domestic sewage, dyeing and United Bleaching factory (UBL) effluents mix with river Bhavani. Numerous industries are located along the stretch of river causing severe environmental pollution and ecological imbalance to the river.

Map of Study area (Station I-V)

2.2. Media used and their preparation

The media used for this present analysis are given in below. All the media were prepared according to the manufacturer's instruction and

adequately sterilized in an autoclave at 121°C for 15 min.

Media used	Remarks
Nutrient Agar (NA)	For isolation of gram negative
Eosine Methylene Blue (EMB)	bacteria and screens them
MacConkey broth	(isolates)
Sugars like glucose, mannitol, lactose, fructose and galactose	For fermentation test
Indole Ornithine fluid media	For motility and indole test

2.3. Enumeration and detection of bacteria

The aliquot of the specimens to be cultured was placed in the bottom of an empty, sterile petri dish and melted and cooled agar was poured over it. The plate was swapped to allow proper mixing. The agar was allowed to gel (solidified) after which the plate was incubated in an incubator at 37ºC for 24 h. Sub-culturing of isolates and stock cultures Nutrient Agar (NA) was poured aseptically into plates and allowed to solidify Specific colonies on the samples. When primary isolation of the plates has been properly streaked, individual colonies was picked and incubated on fresh NA. Subsequent sub-culturing was carried out until pure cultures of the different isolates were obtained. These pure isolates were transferred onto agar slants in McCartney bottles and kept in the refrigerator as stock culture for subsequent tests during identification.

2.4. Total plate counts

The heterotrophic plate count (HPC)/total count was carried out to provide an estimate of the total number of bacteria in each of the samples that would develop into colonies during the period of incubation on Nutrient Agar and Eosine methylene blue agar plates. This test detects a broad group of bacteria including the pathogens, nonpathogenic and opportunistic pathogens. The laboratory procedure involves making serial dilution of the sample in sterile distilled water and cultivating 10^{-3} and 10^{-5} then 10° and 10^{-2} dilution factor into the center of petri dish. The prepared media were allowed to cool to about 40°C before they were added to the dilution factors. The plates were incubated at 37°C for 24 h in inverted position to prevent condensation from the lid to the agar, after which the number of the colonies formed was counted. The acceptable value of the total number of Colony Forming Units (CFU) during the plate count for potable water was a total of less than 102 / ml.

2.5. Coliform count

Most probable number (MPN) method: This was done as recommended by standard method (APHA, 1998). The materials and media used for the analysis consisted of fermentation tubes with aluminum caps, Durham tubes, MacConkey Broth (Single and double strength) inoculating loop, Bunsen burner and syringes (10, 5 and 2 ml). The most probable number tube fermentation technique is performed in three stages: Presumptive test, confirmative test and completed test.

2.6. Biochemical tests and identification of microbial isolates

Morphological and biochemical characteristics of the microbial isolates were used for the identification of the isolates according to Baron *et al*. (1990), Benson (1990) and Bitton (1994). The Bergey's Manual of determinative bacteriology by Buchanan and Gibbons (1974) was used to compare the characteristics with the results obtained.

3. RESULTS

The public health significance of water quality cannot be over emphasized. Many infectious diseases are transmitted by water through the fecal-oral route. In human health, water has a profound influence and quality of the water supplied is important in determining the health of individuals and whole communities.

The quality of river water was deteriorated by the presence of bacterial population. In the present study microorganisms were analyzed in the following stations of Pilloor reservoir, effluent mixing point, sewage I, sewage II and Bhavanisagar reservoir. The viable counts for all water samples were quite high.

The mean Total bacterial count of different water samples are Pilloor reservoir ($25X10^{-6}$ CFU), effluent mixing point (45 $X10^{-6}$ CFU), sewage I (70 $X10^{-6}$ CFU), sewage II ($65X10^{-6}$ CFU), Bhavanisagar reservoir ($53X10^{-6}$ CFU). But in station III and IV the total bacterial count found to be higher when comparing to other stations.

Comparing the results with (WHO, 1993) the sewage mixing point I and II are highly contaminated, and also denotes the potential public health hazards. In the present study station III (sewage II) shows the maximum 24% of TBC (total bacterial count) and in the case of TC (total coliform) station II showed the maximum 27%. Various groups of microorganisms were isolated and identified are *E. coli*, *P. aerugionosa*, *E. aerogenes*, *S. aureus*, *Salmonella sp*, *Bacillus sp*, *Proteus sp*, *Klebsiella sp*, *Flavo bacterium*, *Acinetobacter sp*.

Table 1. Morphological Characteristics of identified microorganisms present in river Bhavani at different sampling sites.

S.No	Morphological Characteristics	Identified micro organism
1	Non-spore forming and non-motile, Gram positive cocci, circular, low convex with entire margin, smooth, medium, opaque, golden yellow colony on Nutrient Agar, grown at pH 7 and 37^{0}C	*S. aureus*
2	Dark centered, gram negative, non endospores forming colony on Salmonella-Shigella Agar	*Salmonella* sp.
3	Gram negative, circular, low convex, with entire margin, mucoid, opaque, small, non endospores forming rod shaped, pinkish glistering with metallic sheen colony on Eosin Methylene Blue (EMB) Agar; grown at pH 7, 37and 45OC.	*E. coli*
4	Non-spore forming, Gram negative short rods, colourless colony on Nutrient Agar, grown at 4and 42OC	*P. aerugionosa*
5	Gram negative, non endospores forming rod, light-yellow colony with feather-like margin.	*E. aerogenes*
6	Spore forming, Gram positive rods, creamy white colony on Nutrient Agar with entire margin	*Bacillus* sp.
7	Non-spore forming and non-motile gram negative rod colony on Nutrient Agar that appeared translucent with serrated or feather-like margins	*Proteus* sp
8	Gram negative rods on Nutrient Agar	*Klebsiella* sp.
9	Gram negative rods that appeared yellowish with entire margin on Nutrient Agar	*Flavobacterium* sp.
10	Gram positive rod that appear grey-white with undulated margin on Nutrient Agar	*Acinetobacter* sp.

Table 2: Total bacterial and coliform counts of water samples in river Bhavani.

Sample source	Total bacterial count		Total coli form counts	
	CFU X10^{-6}ml	CFU X10^{-7}ml	CFU X10^{-6} ml	CFU X10^{-7} ml
Pillur reservoir	25	16	10	8
Effluent	45	12	34	12
Sewage I	70	25	48	30
Sewage II	65	22	45	25
Bhavanisagar reservoir	53	18	38	10

Table 3: Isolated microorganisms from different water samples of river Bhavani

Identified microorganism	Pillur reservoir	Effluent	Sewage I	Sewage II	Bhavanisagar reservoir
Echerichia coli	-	+	+	+	+
Pseudomonas sp	+	+	+	+	+
Enterobacter aerogenes	+	+	+	+	+
Salmonella sp	-	+	+	+	+
S. aureus	-	+	+	+	+
Flavobacterium	-	+	+	+	+
Proteus sp	+	+	+	+	+
Klebsiella sp	+	+	+	+	+
Acinetobacter sp.	+	+	+	+	+
Bacillus subtills	+	+	+	+	+

4. DISCUSSION

The assessment of microbiological quality of water from different sources was essential for detecting the presence or absence of organisms that might constitute health hazards in water, which could be used as a guide to monitor and protect the water sources. The total bacterial counts for all the samples were generally high, exceeding the limit of 1.0 ×102 CFU/ml which was the standard limit of heterotrophic count for drinking water (EPA, 2002).

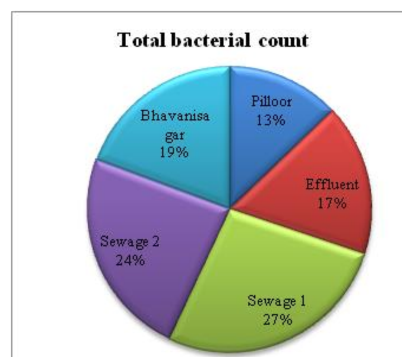


Fig. 1: Total bacterial count in different sampling stations of river Bhavani

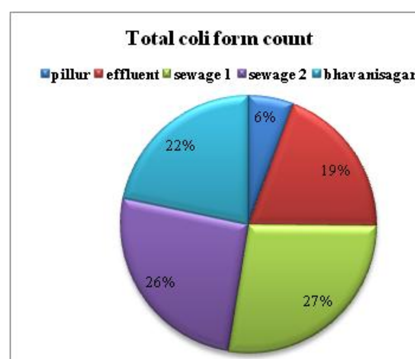


Fig. 2: Total coilform count in different sampling stations of river Bhavani

The reason for high number of bacterial colonies might be due to inadequate maintenance of reservoir water and the percolation of sewage into river (Krishnan, 2007). The high total plate counts observed in sewage water indicated the presence of

high organic matters and related nutrient sources. The primary sources of bacterial contamination might include the surface runoff, sewage treatment facilities, natural soil/plants, bacteria and improper management activities of the inhabitants like washing, refuse dumpage, faecal droppings, dipping of different materials inside the water sources. These contaminants are reflected in the river water. Omezuruike *et al*. (2008) revealed that the original source of any drinking water is rich in aquatic microbes, some of which could be dangerous if they enter the human body.

The water samples from sewage point the total coliform were exceedingly high as against the EPA maximum contamination level (MCL) for coliform bacteria in drinking water of zero total coliform per 100ml of water (EPA, 2002). The high coliform count obtained in the samples may be an indication that the water sources were faecal contaminated (EPA, 2002; Osunide and Enuezie, 1999). The same results of the high number of total coliforms were observed in the Umian lake water in both pre monsoon and post monsoon seasons (Krishnan, 2007; Ajayi, 2011).

Various groups of microorganisms were isolated and identified during the study. They include *Escherichia coli*, *Streptococcus sp*., *Enterobacter sp*., *Pseudomonas sp*., *S. aureus*, *Flavobacterium sp*., *Pseudomonas sp*., *Proteus sp*., *Klebsiella sp*., and *Bacillus sp*. The presence of coliforms group in water samples generally suggests that a certain selection of water may have been contaminated with faeces either of human or animal origin (Richman, 1997).

5. CONCLUSION

River Bhavani is lifeline of the Tamilnadu which feeds the more than twelve thousands acre agricultural lands and supports the livelihoods of millions of peoples residing along the banks. The river water is mainly used for the drinking, irrigation, recreational and industrial uses. In India almost 70% of the water has become polluted due to the discharge of domestic sewage and industrial effluents into natural water source. The improper management of water systems may cause serious problems in availability and quality of water. The wastes discharge from sewage, industry, agriculture and anthropogenic activities are seriously polluting the water which promotes the growth of pathogenic microorganisms. Microbial examination of water is therefore a powerful and foremost tool in order to foreclose the presence of health hazard microorganisms. Commonly used indicators of water quality include Coliforms, faecal streptococci, *Clostridium perfringes,* and *Pseudomonas aeroginosa* etc. In the present study also these indicators are recorded in different concentration at different stations. The higher amount of microorganisms are found near more populous area and lowered in diminish. The continuous accumulation in down flow may create serious problem in future for stakeholders. So it is a high time to take the serious step regarding pretreatment of sewage and effluents, setup of STPs and ETPs in local levels, make awareness program regarding the wise use of water and its wastage, contaminants and future impacts on our health.

REFERENCES

Ajayi, O. and T.O. Adejumo, (2011). Microbiological assessment and some physico-chemical properties of water sources in Akungba-Akoko, Nigeria. *A. J. Toxi. and Envir. Heal. Scie.* **3**(13): 342-346.

American Public Health Associated (APHA). (1998). *Standard method for examination of water and waste water,* 20th ed. American Public Health Associated Inc, New York. American Water Work Associated (AWWA). Research Foundation, 1993. Bacterial examination of water. West Quincy Avenue Denver, p. 81-85.

Baron, J., M.F. Sydney, (1990). *Bailey and Scott's Diagnostic Microbiology.* The C.V. Mosby Co., St. Louis, p. 861

Benson, H.J. (1990). *Microbiological Applications: A Laboratory Manual in General Microbiology.* Wm, C, Brown Publishers, Dubuque, p. 459.

Bitton, G. (1994). *Waste Water Microbiology.* Gainesvill, New York Wiley-Liss, p. 118.

Buchanan, R.E. and N.E. Gibbons, (1974). *Bergey's Manual of Determinative Bacteriology.* 8th ed. The Williams and Wilkins Co., Baltimore, p. 124.

Environmental Protection Agency US (US-EPA) (2002). *Safe Drinking Water Act Amendment*. http://www.epa. gov/safe/mcl/Html.

Fathi, A.A., H.M.A. Abdelzaher, R.J. Flower, M. Ramdani, M.M. Kraïem, (2001). Phytoplankton communities of North African wetland lakes: the CASSARINA Project. *Aquatic Ecol.*, **35**: 303-318.

FWPCA (Federal Water Pollution Control Administration), (1968). Water Quality Criteria: Report of the National Technical Advisory Committee to the Secretary of the Interior. U.S. coastal Cities: FWPCA, p. 32-34

Iskandar, M.B. (2010). *The effectiveness of biofilter as a treatment for domestic wastewater.* University Malaysia Pahang (thesis).

Krishnan, R.R, K. Dharmaraj and B.D.R. Kumari, (2007). A comparative study on the physicochemical and bacterial analysis of drinking bore well and sewage water in the three different places of Sivakasi. *J. Environ. Biol.* **28**(1): 105-108.

Mahananda, M.R. (2010). “Physico-Chemical analysis of surface water and ground water of Bargarh District, Orissa, India.” *Inter. J. Res. Review in Appl. Sci.* **2**(3): 284-295.

Mane, V.R., A.A. Chandorkar and R. Kumar, (2005). Prevalence of pollution in surface and ground water sources in the rural areas of Satara Region. *Asian J. Water Environ. Pollut*. **2**: 81-87

Omezuruike, O.I., A.O. Damilola., O.T. Adeola., A. Enobong and B.S. Olufunke, (2008). Microbiological and physicochemical analysis of different water samples used for domestic purposes in Abeokuta and Ojota, Lagos State, Nigeria. *Afri. J. of Biotech*. **7** (5): 617-621.

Okpokwasili, G.C. and T. C. Akujobi, (1996). Bacteriological indicators of tropical water quality. *Environ. Toxicol. Water Quality.* **11**: 77–81.

Osunide, M. I. and N.P. Eneuzie, (1999). Bacteriological analysis of ground water. *Nig. J. Microbiol.* **13**: 47-54.

Richman, M. (1997). Industrial Water Pollution. *Wastewater.* **5**(2): 24-29.

Tebutt, T.H.Y. (1983). *Principles of Quality Control Pergamon.* England, p. 235.

Sandy, C. and F. Richard, (1995). Quality and Standard for Drinking Water Chapter 3 Environmental Health Engineering in the Tropics. And Introductory Textbook Wiley Inter Science. 2nd ed. ISBN 0471938858, p. 294.

WHO, (1999). Guidelines for Drinking Water Quality.2nd ed. Geneva: WHO.

Kong. Res. J. 2(1) : 111-114, 2015 ISSN 2349-2694
Kongunadu Arts and Science College, Coimbatore.

PARTIALLY PURIFIED EXTRACT OF *RICINUS COMMUNIS* AGAINST DEVELOPMENTAL STAGES OF *AEDES AEGYPTI* (CULICIDAE: DIPTERA)

Rajmohan, D*, K. Logankumar, V. Sabitha, D. Saranya and T. Sujila
PG and Research Department of Zoology, Kongunadu Arts and Science College, Coimbatore-641 029.
*E-mail: rajmohandevadass@gmail.com

ABSTRACT

The present study has been taken to study the insecticidal properties of botanical,*Ricinus communis* on the growth and development of the Chikungunya vector mosquito, *Aedes aegypti*. Mortality value of egg, larvae and pupae treated with different concentration of the partially purifiedseed extract of *Ricinus communis* was observed at the end of 24 hours of exposure. The results revealed that the plant, *Ricinus communis*showed better response against the developmental stages on the mosquito. All of these studies of the effect of plant extracts on the mosquito susceptibility showed that *Ricinus communis* may cause death by acting as neurotoxicants on respiratory toxicants by inhibiting the flow of nerve impulse and decrease in oxygen uptake ultimately resulting in death. The preliminary phytochemical analysis shows the presence of certain secondary metabolites like alkaloids, flavonoids in the extracts of experimental plant,*Ricinus communis*. As the alkaloids, flavonoids are known to have effective mosquitocidal properties. It indicates that the extract of*Ricinus communis*is most reliable and effective in terms of mosquitocidal properties.

Keywords: *Ricinus communis, Aedes aegypti*, mosquito control, LC50.

1. INTRODUCTION

Vector control is of serious concern in developing countries like India. Due to lack of awareness, development of resistance and socio economic reasons, every year a large part of the population is affected by one or more vector borne diseases. *Aedes aegypti* is a tropical mosquito. It is believed that *Aedes aegypti* originated from Central Africa, where it is found in greatest abundance. Being a domestic breeder, it found breeding places on sailing ships on those days, where it has been distributed to all parts of the world.

The mosquitoes not only annoy us by their noise and painful bites but also transmit human diseases such as malaria, yellow fever, filariasis, encephalitis etc. In the present study much attention has been focused on the *Aedes aegypti* since it plays a major role in the transmission of nocturnal periodic form of Chikungunya all over the world. Mosquitoes transmitdiseases like malaria, filariasis,dengue fever and Japanese encephalitis are among the most serious vector- born diseases in developing countries.

The castor oil plant (*Ricinus communis*) is a species of flowering plant in the spurge family, Euphorbiaceae. It belongs to a monotypicgenus, *Ricinus*, and subtribe, Ricininae. The evolution of castor and its relation to other species are currently being studied using modern genetic tools.

Its seed is the castor bean, which, despite its name, is not a true bean. Castor is indigenous to the southeastern Mediterranean Basin, Eastern Africa, and India, but is widespread throughout tropical regions (and widely grown elsewhere as an ornamental plant Castor seed is the source of castor oil, which has a wide variety of uses. The seeds contain between 40% and 60% oil that is rich in triglycerides, mainly ricinolein. They also contain ricin, a poison, which is also present in lower concentrations throughout the plant. The toxicity of raw castor beans is well-known, and reports of actual poisoning are relatively rare. Children could conceivably die from as few as three beans; adults may require eight or more. As an example of the rarity of castor bean poisoning, in recent years there have only been two cases reported in all of England, and in both the victims recovered uneventfully.

The approach to combat these diseases largely relies on interruption of the disease transmission cycle by either targeting the mosquito larvae at breeding sites through spraying of stagnant water or by killing or repelling the adult mosquitoes (Corbel *et al*., 2004; Joseph *et al*., 2004).

Phytochemicals are botanicals which are naturally occurring insecticides obtained from floral resources. Applications of phytochemicals in

mosquito control were in use since the 1920s (Shahi, *et al*., 2010). The extract of this plant was administrated on egg and larval stages because the best option for mosquito control is to target these aquatic stages rather than the adults

2. MATERIALS AND METHODS

2.1. Selection of mosquito species

An important vector species of mosquito *Aedes aegypti* is selected for the presented study. *Aegypti* is the principle vector of chikungunya, dengue fever and dengue hemorrhagic fever and it is reported to infect more than hundred million people every year and more than 110 countries in the tropics (Halstead, 2000).

2.2. Mosquito rearing

Mosquito colony maintained at 28 ± 2°C, 70 ± 10% Relative humidity and a photoperiod of 12:12, L: D at the Zoology Research Department, Kongunadu Arts and Science College, Coimbatore.

2.3. Mosquito feeding

Larval forms are maintained in trays by providing dog biscuits and yeast powder in the 3:1 ratio. Adult are provided with 10% sucrose solution and one week old chick for blood meal.

2.4. Selection of the plants

The experimental plant was collected from The Nilgiri district, Tamilnadu, India. The experimental plant is *Ricinus communis*

2.5. Preparation of seed extract of experimental plant

The experimental plant was collected from the Nilgiri district and brought to the laboratory. The separated seeds were dried under shade at room temperature (28±1°C) for about 20 days. The completely dried seeds were powdered and sieved to get fine powder of seed. The seed extract were obtained by using Soxhlet apparatus.

Two hundred fifty grams of seed powder were dissolved in 200 ml of solvent separately and extract in the Soxhlet apparatus for 8 hours over a mantle heater at 55°C. The extracts were concentrated using a vacuum evaporator at 45°C under low pressure. After complete evaporation of the solvent, the concentrated extract was collected and stored in a refrigerator for later use.

2.6. Preparation of stock solution and different concentration of seed extract

One gram of the concentrated extract of dried seeds of experimental plant wasdissolved in 100 ml of water separately and kept as stock solutions. These stock solutions were used to prepare the desiredconcentrations of the extract for exposure of the mosquito egg, larvae and pupae.

2.7. Partial purification of plant extracts

Different parts of the plants were taken based on the effect of crude extracts tested to purify on silica gel column. Sufficient quantity of powdered plant materials were dissolved in 60% acetone and extracted for 8 hrs. Clear supernatant was air dried concentrated and dissolved in acetone. Column was packed with silica gel (60x120 mesh) and washed with 1% acetone several times. Sample was centrifuged at 5000 rpm for 2 minutes. The clear supernatant was applied over the column eluted with 1% acetone. Fraction collected 3 ml per minute and were air dried and used for bioassay

To obtain the different concentration of test medium for crude 1 to 10 gm of stock powder and foe silica gel fractions 1 mg of dried powder was dispersed in 100 ml of 0.02% acetone. The effect of crude and silica gel fractions on the development was noticed for a period of 24 hrs.

2.8. Treatment of egg, larvae and pupae with different concentration of the experimental plants extracts

In the present study, for treatment of egg, larvae and pupae with the extracts of different experimental plants, 100 ml of tap water was kept in a series of glass beakers (200 ml of capacity). Required quantity of stock solution (containing 10 mg/ml) was added into each beaker (containing 100 ml of tap water) to obtain a particular concentration of the extract

Control medium was also maintained with 100ml of tap water added with the maximum quantity of solvent present in the stock solution of the extract. Separate series of exposure medium with desired concentration of extracts were kept for *Aedes aegypti*. The egg hatchability, larval mortality, pupal mortality and adult emergence of *Aedes aegypti* was observed separately in control and different concentration of the seed and leaf extracts of experimental plant, and in both the victims recovered uneventfully.

3. RESULTS

3.1. Effect of partially purified plant extracts on the egg hatchability of Aedes aegypti

Eggs of *Aedes aegypti* were treated with the partially purified plants extract for 24 hrs.The order of LC50(ppm) concentrationswere 50.46, 38.49, 25.74, 58.94, 39.83, 72.27, 72.65, 88.46, 82.53 and

82.73 ppm in the plantRicinus communis (Table 1, Fig 1).

3.2. Effect of partially purified plant extracts on the I instar larvae of Aedes aegypti

The first instar larvae of *Aedes aegypti* were exposed to plant extracts and the LC50(ppm) values noticed were 4.57, 3.38, 4.15, 7.86, 7.35, 18.68, 22.38, 16.18, 19.66 and 24.17 ppm .The effect of plant extract were found to be in the order of *Ricinus communis* (Table 1,Fig 1).

3.3. Effect of partially purified plant extracts on the II instar larvae of Aedes aegypti

The second instar larvae of *Aedes aegypti* were exposed to plant extracts and the LC50(ppm) values noticed were 7.25, 7.24, 4.93, 23.84, 15.54, 31.19, 26.73, 24.24, 26.16 and 31.54 ppm .The effect of plant extract were found to be in the *Ricinus communis* (Table 1, Fig. 1)

3.4. Effect of partially purified plant extracts on the III instar larvae of Aedes aegypti

The third instar larvae of *Aedes aegypti* were exposed to plant extracts and the LC50(ppm) values noticed were 7.93, 10.21, 10.21, 32.37, 23.76, 35.18, 36.87, 29.16, 38.46 and 47.28 ppm .The effect of plant extract were found to be in the*Ricinus communis* (Table 1, Fig 1).

3.5. Effect of partially purified plant extracts on the IV instar larvae of Aedes aegypti

The fourth instar larvae of *Aedes aegypti* were exposed to plant extracts and the LC50(ppm) values noticed were 25.26, 18.28, 23.22, 32.43, 41.09, 47.05, 48.35, 43.64, 43.64 and 61.47 ppm .The effect of plant extract were found to be in the*Ricinus communis* (Table 1, Fig 1).

3.6. Effect of partially purified plant extracts on the pupae of Aedes aegypti

When the pupae of *Aedes aegypti* were exposed to plant extracts,the order of the LC 50(ppm) values noticed were 29.18, 29.18, 48.17, 53.59, 51.45, 82.43, 78.94, 83.56, 83.56 and 82.25 ppm in the plant *Ricinus communis* (Table 1, Fig 1).

4. DISCUSSION

In the present study efficacy of partially purified plant extracts on the life cycle (egg, larvae and pupa) of *Aedes aegypti* and biochemical parameters such as β-N-acetylglucosaminidase,phosphatases and phenoloxidases (embryonic egg and IV Instar larva) were observed. The plant extracts contain active principles that the protein content of the larvae, pupae and ovary of *Aedes aegypti* were recorded. β -N-acetylglucosaminidase and phosphatases are involved in moulting and the enzyme phenoloxidase may be involved in the synthesis of cuticle during embryogenesis. Preliminary phytochemicals of plant extracts were observed and GC-MS of the plant.

Botanical insecticides have been used for centuries for crop protection. Only with the development of synthetic insecticides in the mid 1900's did their use drop as more effective products took their place .Within a relatively short time, problems arise with the synthetic products.Environmental contamination, poisoning of non-target species and resistance. This led many to reconsider botanical formulations as natural alternatives because they are less toxic (Scott and Kaushik, 2004).Plants with and established record for culinary or medicinal use that therefore offer a safer starting material have been evaluated in terms of their potential application as mosquitoes .

For the current study the plant compounds were considered not as leads for synthetic insecticides, but for extract based formulations that combine all the co-occurring secondary plant compounds.Keeping the above said factors, ten medicinal plants were screened plant biopesticidal activity.Eggs, I, II, III, IV instar larvae and pupae were treated with the plant of medicinal value of *Ricinus communis.*

Unhatched eggs, mortality of larvae and pupae were recorded at 24 hours intervals. Dead larvae,pupae,partially emerged or deformed adults were regularly removed and counted. Live pupae were collected and observed till emergence. The inhibition of these plant compounds against eggs, I, II, III, IV instar larvae and pupae were estimated on the corresponding mortality values and LC50 values were calculated. Morphogenetic abnormalities were studied from the dead larvae,pupae, larval pupalintermediates and partly enclosed adults.

In the present study the morphogenetic abnormalities noted in the life cycle of the treated *Aedes aegypti* includes extension of developmental period. Enlarged pupae, partly exuviated adults with part of abdomen still in pupal case and attachment of pupal case by legs, deformities on the abdominal wings, wing deformities consisted of twisting unevenness, incomplete development and disorientation of fore and hind wings, deformed adults failed to detach themselves from their pupal cases.

After the introduction of larvae in the test compound of different concentration,the larvae showed mortality and the surviving larvae in the treatment were seen resting in S or U shaped postures and stretching frequently.This behavior was not noticed in the control.Such U shaped postures and frequent stretching has been described previously (Mwangi and Rembold, 1988) as characteristic of mosquito larvae reared in water treated with *Melia volkensii* fruit extracts.

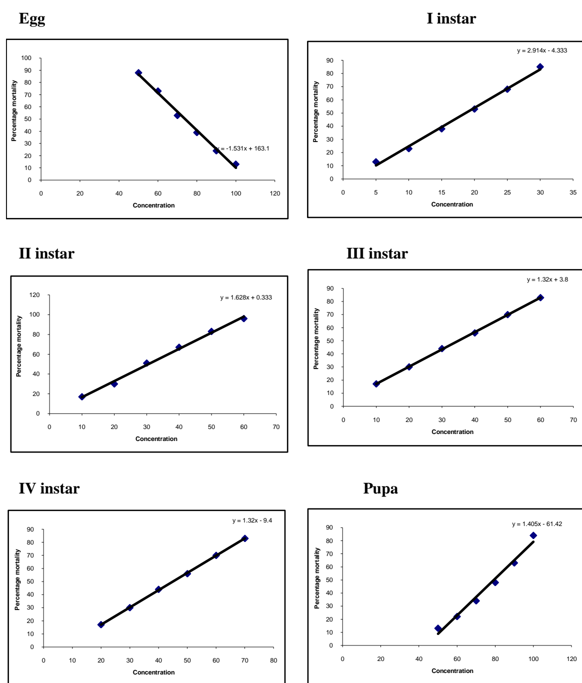


Fig. 1. Probit regression line for response of *Aedes aegypti* to *Ricinus communis* partially purified leaf extract in laboratory test

Table 1. LC_{50} (ppm) of the partially purified leaf extract of *Ricinus communis* on the different stages of *Aedes aegypti*.

Plant	Stages	LC_{50} (ppm)	95% Fiducial limit (ppm)		X^2	$\bar{x}$	SD	SE
			Upper	Lower				
Ricinus communis	Egg	72.27	75.60	69.54	2.26	55.4	26.24	2.60
	I Instar	18.68	20.46	16.73	1.17	39.0	24.99	2.09
	II Instar	31.19	34.37	28.49	2.54	49.6	27.87	2.23
	III Instar	35.18	38.39	32.16	1.92	43.4	22.54	0.44
	IV Instar	47.05	50.33	44.28	1.91	43.4	22.54	0.44
	Pupa	82.43	85.29	79.54	1.45	36.0	24.22	3.99

REFERENCES

Cheng, S.S., H.T. Chang, S.T. Chang, K.H. Tsai and W.J. Chen, (2003). Bioactivity of selected plant essential oils against the yellow fever mosquito *Aedes aegypti* larvae*. Bioresource Technol.* **89**: 99-102.

Cheng, S.S., J.Y. Liu, K.H. Tsai, W.J. Chen and S.T. Chang, (2004). Chemical composition and mosquito larvicidal activity of essential oils from leaves of different *Cinnamomum osmophloeum* provenances. *J. Agric Food Chem.* **52**: 4395-4400.

Rembold, F.L. and R.W. Mwang, (1989). Compounds from/ MeKa volkensi and their growth inhibitory effect on *Aedes aegypti* larvae. Proc second symposium of host regulated developmental mechanisms in vector arthropods at Vero Beach. *Florida.* 3-8.

Sagar, S.K. and S.S. Sehgal, (1996). Effects of aqueous extract of deoiled neem *(Azadirachta Indica* A. juss) seed kernel and karanja (Pongamia Glabra vent) seed kernel against *Culex quinquefasciatus. J. Commun. Dis.* **28**: 260-269.

Shahi, M., A.A. Hanafi-Bojd, M. Iranshahi, H. Vatandoost and M.Y. Hanafi-Bojd, (2010). Larvicidal efficacy of latex and extract of *Calotropis procera* (Gentianales: Asclepidaceae) against *Culex quinquefasciatus* and *Anopheles staphensi* (Dipptera: Culicidae). *J. Vector Borne Dis.* **47**: 185-188

Singh, N., A.K. Mishra and A. Saxena, (1996). Use of neem cream as a mosquito repellent in tribal areas of central India. *Indian J. Malariol.* **33**: 99-102.

Singh, R.K., R.C. Dhiman and S.P. Singh, (2003). Laboratory studies on the predatory potential of dragonfly nymphs on mosquito larvae. *Common. Dis.* **35**: 96-101.

Singh, R.P. (1984). Effects of water extract of deoiled neem kernel on second instar larvae of *Culex fatigans* Wiedemann. *Neem Newsletter.* **1**: 16.

Kong. Res. J. 2(1) : 115-117, 2015 ISSN 2349-2694
Kongunadu Arts and Science College, Coimbatore.

IMPACT OF PESTICIDE METHOXYCHLOR ON PROTEIN CONTENT IN THE FRESH WATER FISH, *CYPRINUS CARPIO*

Vasanthi. J*. and S. Binukumari
Department of Zoology, Kongunadu Arts and Science College, Coimbatore.
*E.mail: vasanthijraman@gmail.com

ABSTRACT

Fishes are very sensitive to a wide variety of toxicants in water, various species of fish show uptake and accumulation of many contaminants or toxicants such as pesticides. The fresh water fish, *Cyprinus carpio* was exposed to methoxychlor and the median lethal concentration was found to be 0.42 ppm. After sublethal exposure tissues such as gill, liver, kidney, muscle and brain were sacrificed and analysed for protein content.

Key words: *Cyprinus carpio,* pesticide, fresh water fish.

1. INTRODUCTION

Pesticides have brought tremendous benefits to mankind by increasing food production and controlling the vectors of man and animal diseases. At the same time use of these pollutants has posed potential health hazards to the life of fishes. Pesticides are major cause of concern for aquatic environment because of their toxicity, persistency and tendency to accumulate in the organisms (Joseph and Raj, 2010). Pesticides are mostly non-selective, widespread applied, possess toxic properties and in some cases are very refractory. These features entitle pesticides to be one of the most fearful group substances as far as biological communities and humans are concerned.

Most of the pesticides of both plant origin and chemical applied in agricultural field reach water bodies through runoff affecting aquatic flora and fauna specially fishes. The lipophilicity of synthetic pesticides indicates that these chemicals will be absorbed by fish even from very low concentrations in water (Hill, 1989). Persistent chemical molecules with long half-life periods found in chemicals pose a threat to fish and also to the human population consuming the affected fish (Bouregeois *et al*., 1993; Nayak *et al*., 1995; Kalavathy *et al*., 2001; Saravanan *et al*., 2003; Selvarani and Rajamanickam, 2003 and Park *et al*., 2004).

2. MATERIALS AND METHODS

They were collected from the Mettur fish farm stocked and acclimatized for a time period of 10-15 days in the laboratory conditions in glass aquaria containing dechlorinated water. The water of the aquarium was aerated continuously through stone diffusers connected to a mechanical air compressor. Water temperature ranged between 22 ± 6^0C and the pH was maintained between 6.1 and 8.4. Fish were fed twice daily alternately with rice bran and oil cakes.For the present study, matured adult fishes were exposed to different concentrations viz LC_{50} of Methoxychlor for short term (24, 48, 72 and 96 hours) and long term (10, 20 and 30 days) continuously. Seven groups of ten fishes for each concentration of the pesticides were used. In these aquaria water was replaced daily with fresh treatment of pesticides. Each experiment was accompanied by its respective control.

Methoxychlor is an organochlorine pesticide that was developed as a substitute for DDT. Methoxychlor has been used as a pesticide on pets, livestock, crops, gardens and in animal feed (Palanza *et al*., 2001; Magliulo *et al*., 2002). Appropriate narrow range of concentration was used to find the median lethal concentration, using a minimum of 10 fishes for each concentration and the mortality was recorded for every 24hrs upto 96hrs. It was found as 0.42 ppm for 96 hrs, using probit analysis method (Finney, 1971). From the stock solution various sublethal concentrations were prepared for bioassay studies.

At the end of each exposure period, fishes were sacrificed and tissues such as gill, liver, kidney, muscle and brain and were dissected and removed. The tissues (10 mg) were homogenized in 80% methanol, centrifuged at 3500 rpm for 15 minutes and the clear supernatant was used for the analysis of protein content. Total protein concentration was estimated by the method of Lowry *et al.* (1951).

3. RESULTS AND DISCUSSION

Proteins occur in the body in the form of amino acids and other metabolites, which serve as building blocks of the body. Hence, protein content of the cell is considered to be an important tools for

evaluation of the physiological standards. Several authors have reported alterations in total proteins and their metabolites in aquatic organisms exposed to toxicants (Ahmed *et al*., 1997).

The reduction in protein content under effluent stress noticed in the present study may be attributed to the utilization of amino acids in various catabolic reactions. The reduction in protein content might be due to the blocking of protein synthesis or protein denaturation or interruption in the amino acid synthesis by metals (Jha, 1991).

Table 1. Changes in the protein content in the tissues of *Cyprinus carpio* on short term exposure due to methoxychlor.

Sample (mg/g wet tissue)	Exposure Periods				
	Control	24 hrs	48 hrs	72 hrs	96 hrs
Gill	23.50±0.09	21.36±0.10	19.11±0.02	18.36±0.06	15.77±0.05
% change		9.10↓	18.68↓	21.87↓	32.89↓
Liver	25.61±0.06	25.35±0.05	24.87±0.10	22.90±0.05	22.76±0.07
% change		1.01↓	2.88↓	10.58↓	11.12↓
Kidney	19.38±0.03	19.06±0.04	18.32±0.30	17.45±0.06	17.20±0.05
% change		1.65↓	5.46↓	9.95↓	11.24↓
Muscle	29.80±0.03	29.31±0.09	28.54±0.14	28.09±0.09	27.35±0.04
% change		1.64↓	4.22↓	5.73↓	8.22↓
Brain	14.59±0.05	13.97±0.11	13.45±0.08	12.93±0.01	12.66±0.18
% change		4.24↓	7.81↓	11.37↓	13.22↓

Values are mean ± SD, n=5, Figures in Parenthesis are percentage decrease over control.
* - Significant at 5% (t<0.05) ** - Significant at 1% (t<0.01) NS – Non Significant

Table 2. Changes in the protein content in the tissues of *Cyprinus carpio* on long term exposure due to methoxychlor.

Sample (mg/g wet tissue)	Exposure Periods			
	Control	10 days	20 days	30 days
Gill	23.50±0.09	13.98±0.01	10.45±0.07	9.78±0.13
% change		40.51↓	55.53↓	58.38↓
Liver	25.61±0.07	20.76±0.04	20.07±0.03	18.32±0.16
% change		18.93↓	21.63↓	28.46↓
Kidney	19.38±0.11	16.54±0.05	16.11±0.01	15.08±0.08
% change		14.65↓	16.87↓	22.18↓
Muscle	29.80±0.12	25.89±0.03	25.20±0.04	24.56±0.04
% change		13.12↓	15.43↓	17.58↓
Brain	14.59±0.05	12.08±0.02	11.75±0.06	11.00±0.03
% change		17.20↓	19.46↓	24.60↓

Values are mean ± SD, n=5, Figures in Parenthesis are percentage decrease over control.
* - Significant at 5% (t<0.05) ** - Significant at 1% (t<0.01) NS – Non Significant

REFERENCES

Ahmed, R., M. Elumalai and M.P. Ezhilarasi Balasubramanian, (1997). Individual and combined effect of copper and chromium on oxygen consumption and phophatases of a marine edible crab, *Scyil serrate. Biomed*. **55**: 147-179.

Bouregeois, D.P., J. Gaudet, P. Deveau and N. Maller, (1993). Micro extraction of organophosphorus pesticides from environmental water and analysis by gas chromatography. *Bull. Environ. Contam. Toxicol*. **50**: 433-440

Finney, D.J. (1971). Probit analysis, 3rd edition. (London: Cambridge University Press). 20.

Hill, J.R. (1989). Aquatic organisms and pyrethroids. *Pesticide Sci*. **27**: 429-465.

Jha, B.S. (1991). Alterations in the protein and lipid contents of intestine, liver and gonads in the lead exposed fresh water murrel, *Channa punctatus* (Bloch). *J. Ecobio*. **12**: 9-34.

Joseph, B. and S.J. Raj, (2010). Effect of curacron toxicity on the total serum protein content of *Cyprinus carpio. Toxicol. Environ. Chem*. **92**: 1889-1893.

Kalavathy, K., A.A. Sivakumar and R. Chandran, (2001). Toxic effect of the pesticide Dimethoate on the fish, *Sarotherodon mossambicus. J. Ecol. Res. Biocons*. **2**(1-2): 27-32.

Lowry, O.H., N.J. Rose Brough, L. Farr and R.J. Randall, (1951). Protein measurements with the folin phenol reagent. *J. Biol. Chem*. **193**: 265-275.

Magliulo, L., M.P. Schreibman, J. Cepriano and J. Ling, (2002). Endocrine disruption caused by two common pollutants at ''acceptable' concentrations. *Neurotoxicol. Teratol*. **24**: 71-79.

Nayak, A.K., R. Raha and A.K. Das, (1995). Organochlorine pesticides in middle stream of the Ganga River, India. *Bull. Environ. Contam. Toxicol*. **54**: 68-76.

Palanza, P., S. Parmigiani and F.S. Vomsaal, (2001). Effects of prenatal exposure to low doses of diethylstilbestrol, o.p'-DDT and methoxychlor on postnatal growth and neurobehavioural development in male and female mice. *Horm. Behav.* **40**: 252-265.

Park, D., M.D. Minor and C.R. Propper, (2004). Toxic response of endosulfan to breeding and non breeding female mosquito fish. *J. Environ. Biol*. **25**: 119-124.

Saravanan, T.S., M.A. Mohamed, R. Chandrasekhar and M. Sundramoorthy, (2003). Fresh water fishes as indicators of Cauvery River pollution. *J. Environ. Biol*. **24**: 381-389.

Selvarani, D. and C. Rajamanickam, (2003). Toxicity of PCB 1232 on mitochondria of fish, *Arius caelatus* (Valenciennes). *Indian. J. Exp. Biol*. **41**: 336-340.

Kong. Res. J. 2(1) : 118-120, 2015 ISSN 2349-2694
Kongunadu Arts and Science College, Coimbatore.

STANDARDIZATION OF MEDIUM FOR SYNTHETIC SEED GERMINATION OF *SALVIA SCLAREA* L.

Durgha.H., G. Ramya, M. Gogul Ramanth and R. Thirugnanasampandan*
PG and Research Department of Biotechnology, Kongunadu Arts and Science College, Coimbatore
*E.mail: rtsampandan@yahoo.com

ABSTRACT

Young nodal explants (0.5-1cm) of *Salvia sclarea* L. was used for synthetic seed preparation. Synthetic seeds were prepared using 5% sodium alginate and 1.11% calcium chloride. Seed germination was observed on MS medium fortified with 1.4µM GA3+4.4µM BA after twenty days of culture. Further multiple shoot induction was observed after fifteen days of shoot induction.

Keywords*:* Synthetic seed, sodium alginate, calcium chloride

1. INTRODUCTION

Salvia the largest genus in the family Lamiaceae contains more than 1000 species distributed mainly in Central and South America (500 sp.), Central Asia/Mediterranean (250 sp.) and Eastern Asia (100 sp.). Most species are perennial herbs but annuals, shrubs, a few trees and vines also exist (Alziar, 1993). The name *Salvia* comes from two latin words "*salvare*" meaning "to heal" and "*salveo*" which means "to save" or "to recover" (Aktas *et al*., 2009). Like other members of Lamiaceae, *Salvia* species are aromatic and rich in essential oils which have been used in food, cosmetics, perfumes and pharmaceuticals (Baratta *et al.,* 1998).

Salvia sclarea L. an aromatic perennial herb belonging to Lamiaceae is considered economically and one of the most cultivated species for medicinal purpose and extraction of active constituents (Pierik, 1987). The plant is known for its high value essential oil, the oil is used to reduce stress, tension, depression, insomnia and wild colic etc (Grieve, 1974). Traditionally *S. sclarea* has been used to promote blood circulation, remove stagnation, tranquilize the mind, clear heat from the blood and resolves swelling. The most important and frequent clinical use has been in the treatment of coronary heart disease for the alleviation of angina pectoris, coronary artery spasm and myocardial infarction (Zhou *et al*., 2005).

Since poor seed set and germination leads to the extinction of such valuable species. So the present study was aimed to prepare the synthetic seed using nodal explants for immediate regeneration and storage for long term use.

2. MATERIALS AND MEHODS

2.1. Collection of plant material

The leaves of *Salvia sclarea* L. was collected from Cinchona, Nilgiri hills, Tamil Nadu, India

2.2. Explant selection and mode of sterilization

Nodal explant was collected from actively growing plants and washed thoroughly in running tap water followed by Teepol treatment for 5-10 min. The explants were subsequently surface sterilized with 0.01% (w/v) mercuric chloride solution for 2-3 min and washed 3-4 times with sterile double distilled water for duration of 15 min with an interval of five min for each wash.

2.3. Method of medium preparation

MS (Murrashige and Skoog, 1962) medium was employed in the present study and the composition of the medium is given in Table-1. The nutrient medium basically consists of inorganic salts, carbon source, vitamins and amino acids. Stock solutions were prepared separately for macronutrients, micronutrients, iron, potassium iodide and vitamins. All the chemicals were weighed accurately in electronic weighing machine. All the stock solutions were poured into well stoppered sterilized bottles and preserved in a refrigerator at 4°C. Specific quantity of the stock solutions and growth regulators were pipetted onto a little beaker. The final volume was made up with distilled water.

Table 1. Chemical composition of MS medium (Murashige and Skoog, 1962)

S.NO	Components	mg/L
Major salts		
1.	NH_4NO_3	1650
2.	KNO_3	1900
3.	$CaCl_2.2H_2O$	440

4.	$MgSO_4.7H_2O$	370
5.	KH_2PO_4	170
Minor salts		
6.	KI	0.83
7.	H_3BO_3	6.20
8.	$MnSO_4.4H_2O$	22.3
9.	$ZnSO_4.7H_2O$	8.6
10.	$Na_2Mo_4.2H_2O$	0.25
11.	$CuSO_4.5H_2O$	0.025
12.	$CoCl_2.6H_2O$	0.025
13.	Fe/Na- EDTA	0.73
Vitamins and Organics		
14.	Myoinositol	100
15.	Thiamine HCl	0.10
16.	Pyridoxine HCl	0.50
17.	Nicotinic acid	0.50
18.	Glycine	2.0
19.	Agar	8g
20.	Sucrose	30g
21.	pH	5.8

To the above said medium, 3% sucrose was added and pH was adjusted to 5.8 with either 0.1N NaOH or 0.1N HCl using a pH meter, further 0.8% agar was added, melted in a water bath and the medium was dispensed into 100mL culture flask (50 mL medium) or to 25 mL (25 x 150 mm) test tubes (10-15 mL medium). The tubes or the flask after covering with cotton plug or screw caps were autoclaved at 1.06 kg pressure/sq cm for about 20 min at 121°C. The autoclaved medium in the culture tubes were cooled and allowed to solidify as slants or straights and were stored at 25ºC in the dark for future use. The inoculation was done after 5days to ensure that the tubes were free from contamination.

2.4. Artificial seed preparation

For encapsulation purpose 5% sodium alginate and 100mM calcium nitrate (w/v) were prepared using sterile distilled water. The nodes were transferred to the sodium alginate solution. The node along with sodium alginate was dropped into calcium nitrate solution and left for atleast 15 min for bead formation. The beads were recovered by discarding the sodium nitrate solution and washed twice with sterile distilled water.

2.5. Culture medium and condition

The encapsulated nodes were cultured on MS medium supplemented with various concentrations of cytokinins individually and in combination of BA + GA3. All cultures were maintained in the culture room at 25±2°C under 16h photoperiod.

3. RESULTS AND DISCUSSION

3.1. In vitro synthetic seed germination

Synthetic seeds were prepared from the young and tender nodal explants of *S. sclarea* (Plate 1A). The inoculated synthetic seeds were germinated on MS medium containing 1.4µM GA_3 and 4.4 µM BA after 20 days of culture (Plate 1B). Further shoot induction, elongation and multiple shoot formation was observed on the same medium after 15 days of culture (Plate 1 C-D). The increasing demand for useful secondary metabolites has intensified the application of biotechnological methods to reproduce high yielding plants under controlled growing conditions and or to obtain homogenous and stable genotypes. A growing interest in the development of efficient protocols to micropropagate certain species of *Salvia* has also been increased (Arikat *et al.*, 2004). In the present study, synthetic seeds were prepared using nodal explants of *S.sclarea*. Further the seeds were cultured on GA_3+BA combination showed seed germination and shoot elongation. The shoot induction and elongation is associated with the presence of both GA_3 and BA, since both growth regulators are reported to be best suited for shoot induction and elongation for number plant species. GA_3+ BA and GA_3+ kin showed marked effect on shoot induction from the nodal explants of *Salvia officinalis* (Pinarosa Avato *et al.,* 2005). Young nodal segments of *Salvia santolinifolia* cultured on MS+BA (1-3mg/L) showed maximum number of shoot production (Javanmardi and Khalida, 2014). Rahmani *et al*. (2014) reported higher seed germination percentage in medium fortified with GA_3 in *Salvia sahendica* and *S. hypoleuca*.

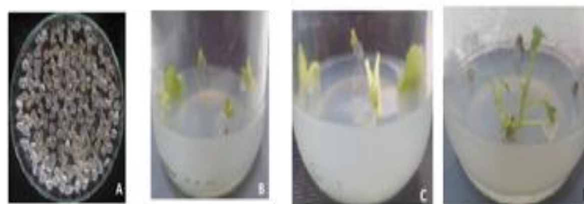


Plate 1:(A) Synthetic seeds prepared from nodal explants (B) Synthetic seed germination on MS medium supplemented with 1.4µM GA3 and 4.4µM BA (C) Shoot induction on the same medium (D) Shoot elongation on the same medium after 15 days of culture

4. CONCLUSION

The present study revealed that 5% soldium alginate and 100mM calcium chloride are best suited for synthetic seed preparation of *salvia sclarea* L. and MS medium supplemented with 1.4µM GA3 and 4.4µM BA showed appreciable shoot induction from the synthetic seeds.

REFERENCES

Aktas, K., C. Ozdemir, M. Ozkan, Y. Akyol and P. Baran, (2009). Anatomoical characteristics of *Salvia tchihatcheffii* endemic to Turkey. *Afr. J. Biotechnol.* **8**(18): 4519-4528.

Alziar, G. (1993). Catalogue synonymique des Salvia L. du monde (Lamiaceae), I–VI. Biocosme Mesoge´en. 5:3–4, 87–136; 6:1–2, 4, 79–115, 163–204; 7:1–2, 59–109; 9:2–3, 413–497; 10:3–4, 33-117.

Arikat, N.A., F.M. Jawad, N.S. Karam, and R.A. Shibli, (2004). Micropropagation and accumulation of essential oils in wilde sage (*Salvia fruticosa* Mill.). *Sci. Hort.* **100**: 193–202.

Baratta, M.T., H.J.D. Dorman, S.G. Deans, A.C. Figueiredo, J.G. Barroso and G. Ruberto, (1998). Antibacterial and anti-oxidant properties of some commercial essential oils. *Flav. Frag. J.* **13**: 235–244.

Grive, M. (1974). A Modern Herbal, Jonathan Cape Ltd. 30- Bedford Square, London, 705-707.

Javanmardi, J., A. Khalighi, A. Kashi, H.P. Bais and J.M. Vivanco, (2002). Chemical characterization of Basil (*Ocimum basilicum* L.) found in local accessions and used in traditional medicines in Iran. *J. Agri. Food Chem.* **50**: 5878-5883.

Murashige, T. and F. Skoog, (1962). A revised medium for rapid growth and bio-assays with tobacco tissue cultures. *Physiol. Pl.* **15**(3): 473-497.

Pierik, R.L.M. (1987). *In Vitro* Culture of Higher Plants. Martinus Nijhoff, Dordrecht, The Netherlands.

Pinarosa Avato, Irene Morone Fortunato, Claudia Ruta. and Roberta D'Elia, (2005). Glandular hairs and essential oils in micropropagated plants of *Salvia officinalis* L. *Pl. Sci.* **169**: (2005) 29–36.

Rahmani, M., Y. Hamidoghli and H. Zakizadeh (2014). Micropropagation of two Iranian endemic salvia species. *Pl. cell biotechnol. Molecul. Biol.* **15**: 43-50.

Zhou, L., Z. Zuo and M.S.S. Chow, (2005). Danshen: an overview of its chemistry, pharmacology, pharmacokinetics and clinical use. *J. Clin. Pharmacol.* **45**: 1345–1359.

Kong. Res. J. 2(1) : 121-124, 2015 ISSN 2349-2694
Kongunadu Arts and Science College, Coimbatore.

DISTRIBUTION OF HAEMOLYTIC THIOBACILLI IN SEWAGE SAMPLES

Midhusha Johny, S. Ramapriya, MinuVenugopal, A. Sivaranjini and R. Subashkumar*
PG and Research Department of Biotechnology, Kongunadu Arts and Science College, Coimbatore – 641 029, Tamil Nadu, India.
*E.mail: rsubashkumar@gmail.com

ABSTRACT

Sulphur is one of the essential plant nutrients and it contributes to yield and quality of crops. *Thiobacillus* play an important role in sulphur oxidation in soil and domestic wastewater.The present study is sought to understand the frequency of *Thiobacillus* in sewage water samples collected from hospital and domestic area.Distribution of sulphur oxidizing bacteria were analysed by using modified Thiobacilli agar. Five isolates were selected and analysed their selected putative virulence properties phenotypically.Also they were showed biocompatible within the group of the *Thiobacilli*.The extracellular products (culture supernatant) of *Thiobacilli* showed a substantial level of haemolytic activity and antagonistic activity. Among the isolates from sewage sources, TB-D1 and TB-D2 produced higher level of bacteriocin against pathogenic bacteria due to the formation of maximum inhibition zone. It reveals that the bacteriocin from *Thiobacillus* showed the elevated antagonistic activity against most of the pathogenic bacteria, as we tested.The strains TB-D4 and TB-D5 showed beta and alpha haemolysis, it might have virulent properties and cause infection in human and animals.The strain TB-D5 showed the maximum inhibitions while compared with others. It is visibly showing presence of virulence factors in the extracellular metabolites of *Thiobacilli.*

Key words: Extracellular products, Biocompatibility, Antagonistic activity, Haemolytic activity

1. INTRODUCTION

Sulphur is now considered the fourth major plant nutrient after N, P and K and is one of the sixteen elements which are essential for the growth and development of plants. The role of chemolithotrophic bacteria of the genus *Thiobacillus* in this process is essential.Organisms belonging to the group of colorlesssulfur bacteria oxidize sulfide to elemental sulfur under oxygen limiting conditions. Based on this feature many researchers worked for biological oxidation using various types of microorganisms (Gadre, 1989). The advantage of this biological sulfide oxidation system is that, no chemicals are required except oxygen (Buismanet *al*., 1990).

*Thiobacilli*can use reduced inorganic sulphur compounds as an energy source (Kelly and Harrison, 1989) and are therefore used for removing sulphide from industrial outlets, domestic outlets and also from wastewater (Kim *et al*., 2002; Kleerebezem and Mendez, 2002 and Martin *et al*., 2002; Cha *et al* 1999) have also studied the removal of organic sulphur compounds.Various workers have proposed the assignment of some species to other genera or the creation ofnew genera for most of the former species of *Thiobacillus* (Katayama *et al*., 1995; Moreira andAmils,1997; Hiraishi*et al*., 1998; Kelly and Wood, 2000).

Considerable interest has been shown in *Tferrooxidans* because of its use in industrial mineral processing and its unusual physiology. The major contribution of *T. ferrooxidans* to metal extraction is its ability to attack sulphide containing minerals and convert the insoluble sulphides of metals such as copper, lead, zinc or nickel to their soluble metal sulphates.An alternative way to replace chemical methods in removing heavy metals is microbial leaching with *Acidithiobacillus*sp. (Tyagi and Couillard, 1989; Tyagi *et al*., 1993; Blasis *et al*., 1993; Couillard and Mercier, 1993; Sreekrishnan *et al*., 1996). This method shows several advantages over chemical methods extraction, lower acids and alkali consumption and minimum reduction in sludge nutrients such as N and P (Tyagi*et al*., 1988; Couillard and Mercier, 1993).

2. MATERIALS AND METHODS

2.1. Collection of samples

Sewage samples were collected from in and around Kongunadu College campus. Sewage discharge received from the hospitals, house, hotels, bakery and college hostel premises. Sterile bottles were taken to collect the samples from the

respective places and transported to laboratory for further analysis.

2.2. Isolation and enumeration of total heterotrophic bacteria

All the sewage samples were subjected to enumerate the bacterial population in respective samples by total plate count method. The dilutions 10^{-4} and 10^{-5} of each sample were selected and 1 mL of suspension was transferred to *Thiobacillus* agar medium and incubated at 37°C for 24 h. After incubation colonies were counted and tabulated. Different colonies were selected and inoculated into *Thiobacillus*agar slants, and maintained it by frequent sub culturing and stored in refrigerator at 4˚C.

2.3. Presumptive and Biochemical Identification of Thiobacilli

Identification of *Thiobacillus*was performed based on morphological, cultural and biochemical identification methods. The isolates were selected from *Thiobacillus* agar based on colony morphology with whitish blue colour.The presumptive colonies were subjected to Gram's stain;spore stain; motility test and biochemical test such as catalase test and oxidase test are followed.

2.4. Biocompatibility assay

Symbiotic relationship between the *Thiobacilli* were assessed by inhibiting within groups. *Thiobacillus* agar plates were prepared, the loop full of *Thiobacillus*isolates (n=5) were streaked on the agar plates. The plates were incubated at 37°C for 24 hrs. The growth and inhibitory activity of each organism were noticed.

2.5. Antagonistic activity

Thiobacilli isolates (n=5) were qualitatively tested for the production of antimicrobial compounds like bacteriocin at 37°C for 24hrs. Overnight culture of indicator bacterium *Aeromonashydrophila, Pseudomonas aeruginosa, Acinetobactorbaumanii, Serratiamarcescens* and *Bacillus subtilis)* approximately 5mm×10^{-7}cells were swabbed over nutrient agar plate and 0.5mm×0.5 mm sized wells were made using well cutter and kept it for incubation for 30 min. About 500µL filter sterilized (Sartorius) bacterial supernatant (culture supernatant) loaded on to the wells of plates seeded with indicator bacterium (pathogenic bacterium) obtained from Kongunadu College Culture, Kongunadu Arts and Science College, Coimbatore. After incubation of 24hrs at 37°C the plates were checked for the zone of inhibition. The inhibition as positive if the width of clear zone around the colonies of producer strains is 0.5 mm or larger. Inhibition zone could be around the wells, thus demonstrating bacteriocins-mediated inhibition of the sensitive microorganism.

2.6. Hemolytic activity

Haemolytic activity was determined as a zone of haemolysis around the colonies on blood agar plates containing 5 % (v/v) human blood, after 24 hrs incubation at 37°C (Brenden and Janda, 1987).Blood agar plates were prepared and the loop full of *Thiobacillus*isolates [n=5] were streaked on the blood agar plates and the growth and inhibition zone was noticed in all the plates tested.

2.7. Determination of haemolysin production

Extracellular products: Brain heart infusion broth (BHIB) (15mL) was prepared and loop full of *Thiobacillus*isolates [n=5] were inoculated. The tubes were incubated at 37°Cat 24 hrs. After 24 hrs, broth was taken and centrifuged at 12,000 rpm for 15 min. After centrifugation, collect the supernatant in fresh tubes and ammonium sulphate (5mL) was added to it and kept the content for overnight in cold room. The content was then centrifuged at 15,000rpm for 30 minutes and discards the supernatant immediately. Phosphate buffer saline [1X concentration] (1mL) was addedto the pellet and stored at cold room.

*Haemolysin assay: Thiobacillus*was subjected to haemolytic efficiency by well diffusion method. About 10μl of each extracellular protein was loaded on plates and incubated at 37°C for 24 hrs. After 24 hours incubation the zone was observed.

3. RESULTS AND DISCUSSION

3.1. Total heterotrophic population in sewage samples

Collected samples were serially diluted and the total heterotrophic bacterial population were noticed on the respective dilutions. Sewage samples and Hospital sewage/discharges were labelled as TB-D1 to D5 and TB-H1 to TB-H2 respectively. The population count was maximum (68X10^{-7} cfu mL^{-1}) in sewage samples collected from Saibaba colony, Coimbatore. Hospital samples showed too numerous countable numbered colonies which indicates the existence of bacteria is more in hospital sewage.

Table 1. Estimation of *Thiobacillus*

S.No.	Source	Sample label	*Thiobacillus* Population (cfu/ml)
1.	Kavundamplayam	TB-D1	43 X 10^3
2.	Cheran Nagar	TB-D2	71 X 10^3
3.	G. N. mills	TB-D3	19 X 10^3
4.	Thudialur	TB-D4	26 X 10^3
5.	Saibaba Colony	TB-D5	49 X 10^4

*TB – *Thiobacillus; D* – sewage samples / isolates

3.2. Preliminary and biochemical identification of Thiobacillus

All the five isolates showed Gram negative, motile and endospore production positive by performing Gram's staining; motility assay and Malachite green staining method respectively. The biochemical tests such as catalase, hydrogen suphide production ferrous ion oxidation and nitrate respiration were studied and noticed that results were favour for *Thiobacillus*.

Table 2. Biochemical properties of *Thiobacillus*

Colony on MW-agar plate	Whitish-yellow with sulfur Deposited 1-1.5 mm in diameter
Morphology	Short rod, 0.5 × 1-1.5
Motility	Positive
Gram-staining	Negative
Intracellular sulfur	Negative
Autotrophic growth with	
Hydrogen sulfide	Positive
Elemental sulfur	Positive
Thiosulfate	Positive
Tetrathionate	Positive
Hetrotrophic growth	Negative
Ferrous iron oxidation	Negative
Nitrate respiration	Negative

3.3. Biocompatibility of the thiobacillus

The results of biocompatible assay showed the significant level of symbiotic relationship with in the group of sewage isolates by showing the non-inhibitory growth on *Thiobacillus* agar after 24 and 48 hrs growth. It exposes the biocompatibility of the isolates and provides an evidence for supporting the growth of each other.

3.4. Antagonistic Activity

Influence of environmental factor on the production of extracellular products that could account for the differences found in virulence for trout and mile of the strains studied, a comparative study was made of the enzymatic and toxic activities contained in culture supernatant fluids growth at 28°C and 37°C after incubation for 24 to 48 hrs (Mateos*et al*., 1993).

Bacteriocin as extracellular metabolites, which has a potential inhibitory activity against pathogenic microorganisms. In the present study, we qualitatively measured the efficiency of bacteriolytic activity of sulphur oxidizing bacteria, *Thiobacillus*, which produces bacteriocin, were used to analyse bacteriolytic activity by the method of antagonistic activity with pathogenic microorganisms (*Aeramonashydrophila, Pseudomonas aeruginosa, Acinetobactorbaumanii, Serratiamarcescens*and *Bacillus subtilis*).

Table 3. Antagonistic activity of *Thiobacillus.*

Pathogens	TB-D1	TB-D2	TB-D3	TB-D4	TB-D5
Aeromonas hydrophila	++	+++	+	+	+
Pseudomonas aeruginosa	+++	++	+++	+	++
Acinetobactorbaumanii	+++	+++	+	+	++
Serratiamarcescens	++	+++	+	-	++
Bacillus subtilis	+++	+	++	+	+

*TB – *Thiobacillus; D* – sewage samples / isolates; +++ - High; ++ Medium; + Low

3.5. Haemolytic Activity

*Thiobacillus*is known to produce a variety of virulence factors. Among them, haemolysin is the important one, also considered as the primarytoxin, produced by most of the pathogenic strains of *Thiobacillus*. On blood agar plate, all the *Thiobacillus* isolates were showed growth after 24 hrs incubation. *Thiobacillus* were showed inhibition zone around the colonies due to the RBC's lysis effectively. The strains TB-D4 and TB-D5 were showed beta and alpha haemolysis; it might have virulent properties and cause infection in human and animals.

In a study, Wong *et al*. (1996) reported bacterial haemolysin – positive genotype was virulent in the suckling mouse model assay. They also observed that after 24 hours at 37° C, the production of hemolysin was found high, whereas Wretlind*et al*. (1973) and Riddle *et al*., (1981) observed the haemolytic activity during the exponential growth phase, reaching a maximum before maximal growth, and then falling on prolonged incubation.

Table 4.Haemolytic activity of *Thiobacillus* on blood agar.

Isolates	α	β	γ	Inhibition zone of Extracellular metabolites
TB-D1	-	-	+	1.2 cm
TB-D2	-	-	+	1.1 cm
TB-D3	-	-	+	1.0 cm
TB-D4	-	+	-	1.1 cm
TB-D5	+	-	-	1.4 cm

*TB – *Thiobacillus; D* – sewage samples / isolates

3.6. Hemolytic activity in extracellular protein

Allen and Stevenson (1981) reported that haemolytic activity appeared extracellularly during the early stages of growth, reading a peak just before an increase in the haemolytic activity. The cell free culture supernatant was showed significant level of haemolytic activity on blood agar. The extracts were showed inhibition zone around the well with clear inhibition zone and it was revealed the RBC lytic

exists in culture supernatant. The strain TB-D5 showed the maximum inhibitions while compared with others. It is visibly showing presence of virulence factors in the extracellular metabolites of *Thiobacilli.*

REFERENCES

Allan, B.J., and R.M.W. Stevenson, (1981). Extracellular virulence factors of *Aeromonas hydrophila* in fish infections. *Can. J. Microbiol*. **27**(10): 1114-1122.

Blais, J.F., R.D. Tyagi, and J.C. Auclair, (1993). Metals removal from sewage sludge by indigenous iron-oxidizing bacteria. *J. Environ. Sci. Health Part A: Environ. Sci. Eng. A* **28**: 443–467.

Buisman, C.J.N., B.G. Geraats, P. Ijspreet and G. Lettinga, (1990). Optimization of sulfur production in a biotechnological sulfide removing reactor. *Biotechnol. Bioeng.* **35**: 50-56.

Cha, J.M., W.S. Cha and Jh. Lee, (1999). Removal of organo-sulphur odour compounds by *Thiobacillus novellas* SRM, sulphur-oxidizing microorganisms. *Process Biochem.* **34**: 659-665.

Couillard, D. and G. Mercier, (1993). Removal of metals and fate of N and P in the bacterial leaching of aerobically digested sewage sludge. *Water Res*. **27**: 1227–1235.

Gadre, R.V. (1989).Removal of hydrogen sulfide from biogas by chemoautotrophic fixed film reactor. *Biotechnol. Bioeng.* **34**: 410-414.

Hiraishi, A., K.V.P. Nagashima, Y. Katayama, (1998). Phylogeny and photosynthetic features of *Thiobacillus acidophilus* and related acidophilic bacteria: its transfer to the genus *Acidiphilium*as *Acidiphiliumacidophilum* Comb. Nov. *Int. J. Syst. Bacteriol.* **48**: 1389-1398.

Katayama, Y., A. Hiraishi and H. Kuraishi, (1995). *Paracoccusthiocyanatus*sp. nov., a new species of thiocyanate-utilizing facultative chemolithotroph, and transfer of *Thiobacillusversutus*to the genus *Paracoccus*as *Paracoccusversutus*comb. nov.with emendation of the genus. *Microbiology* **141**: 1469-1477.

Kelly, D.P. and A.P. Wood, (2000). Genus *Thiobacillus*Beijerinck. In *Bergey's Manual of Systematic Bacteriology*, 2nd ed, vol. 2.

Kelly, D.P. and A.H. Harrison, (1989).Genus *Thiobacillus*. In *Bergey's Manual of Systematic Bacteriology*, 1st ed, **3**: 1842-1858.

Kim, H., Y.J. Kim, J.S. Chung and X. Quan, (2002). Long-term operation of a biofilter for simultaneous removal of H2S and NH3. *J. Air Waste Manag Assoc*. **52**(12): 1389-1398.

Kleerebezem, R. and R. Mendez, (2002). Autotrophic denitrification for combined hydrogen sulphide removal from biogas and post-denitrification. *Water Sci. Technol.* **45**(10): 349-356.

Martin, R.W.Jr., H. Li, J.R. Mihelcic, J.C. Crittenden, D. R. Lueking and P. Ball, (2002). Optimization of biofiltration for odor control: model calibration, validation and applications. *Water Environ. Res*. **74**(1): 17-27.

Mateos, D., Anguita, J., Naharro, G. and C. Paniagua, (1993) Influence of growth temperature on the production of extracellular virulence factors and pathogenicity of environmental and human strains of *Aeromonas hydrophila*. *J. Appl. Bacteriol.* **74**: 111–118.

Moreira, D.R. and R. Amils, (1997). Phylogeny of *Thiobacilluscuprinus*and other mixotrophic *Thiobacilli*: proposal for *Thiomonas*gen. Nov. *Int. J. Syst. Bacteriol.* **47**: 522-528.

Riddle, L.M., T.E. Graham, R.L. Amborski, (1981). Medium for the accumulation of extracellular hemolysin and protease by *Aeromonas hydrophila*. *Infection and Immunity* **33**: 728–733.

Sreekrishnan, T.R. and R.D. Tyagi, (1996). A comparative study of the cost of leaching out heavy metals from sewage sludges. *Process Biochem*. (Oxford) **31**: 31–41.

Tyagi, R.D., D. Couillard and F.T. Tran, (1988). Heavy metals removal from anaerobically digested sludge by chemical and microbiological methods. *Environ. Pollut*. **50**: 295–316.

Tyagi, R.D. and D. Couillard, (1989). Bacterial leaching of metals from sludge. *In* P.E. Cheremisinoff (ed.) *Encycloped.Environ. Cont. Technol.,* Gulf Publ. Co., Houston. p. 537–591

Tyagi, R.D., J.F. Blais, J. Auclair and N. Meunier, (1993). Bacterial leaching of toxic metals from municipal sludge: Influence of sludge characteristics. *Water Environ. Res*. **65**: 196–204.

Wong, H.C., K.T. Lu, T.M. Pan, C.L. Lee, C.L. and D.Y.C. Shih, (1996). Subspecies typing of *Vibrio parahaemolyticus*by pulsed-field gel electrophoresis. *J. Clin. Microbiol*. **34**: 1535-1539.

Wretlind, B., L. Hede´n, T. Wadstro¨m, (1973). Formation of extracellular haemolysin by *Aeromonas hydrophila* in relation to protease and staphylolytic enzyme. *J. Gen. Microbiol.* **78**: 57–65.

Kong. Res. J. 2(1) : 125-127, 2015 ISSN 2349-2694
Kongunadu Arts and Science College, Coimbatore.

CHARACTERIZATION OF ANTIFUNGAL ACTIVITY OF THE VARIOUS ACTIVE CONSTITUENTS OF *COLEUS FORSKOHLII*

Ranjith Santhosh Kumar, D.S.* and B. Lakshman Kumar
Department of Biotechnology, Kongunadu Arts& Science College, Coimbatore.
*Email: ranjith.deva87@gmail.com

ABSTRACT

The aim of this study was to evaluate the anti-fungal effect of different solvent extracts of *Coleus forskohlii* which contains minor diterpenoids, deacetylforskohlin, 9-deoxyforskohlin, 1,9-deoxyforskohlin, 1,9-dideoxy-7-deacetylforskohlin, and four other diterpenoids, along with alkaloids and volatile oils. The solvent extracts included both polarized and non-polarized solvents like methanol, ethylacetate, benzene, chloroform, water, hexane, acetone and ethanol. As a result of this study, the antifungal effect was best observed with the methanolic extract against Candida albicans in comparison to the other extracts. This study also revealed that apart from forskohlin, which was confirmed by Thin Layer Chromatography, other compounds that were extracted with methanol are required to produce the antifungal effect and can be taken forward to pharmaceutical preparations.

Keywords: Antifungal, active constituents, *Coleus forskohlii.*

1. INTRODUCTION

Coleus forskohlii also known as *Plectranthus barbatus* (Lamiaceae) is one of the most significant potential medicinal crops of the future, as its pharmacopoeial properties have been discovered only recently. The tuberous roots have been identified as a rich drug for glaucoma, congestive, cardiomyopathy, asthma and certain cancers. Forskolin primary mode of action is to increase cyclic adenosine monophosphate (cAMP) and cAMP mediated functions, via activation of the enzyme adenylatecyclase (Metzger,*et al* 1981).Forskolin has been shown to increase cAMP formation in all eukaryotic cells except sperm, without hormonal activation of adenylatecyclase (Seamon, *et al*1981). Forskolin's potentiation of cAMP in turn inhibits basophil, mast cell degranulation and histamine release (Marone, *et al*1986); lowers blood pressure (Dubey,*et al* 1981), intraocular pressure inhibits platelet aggregation (Agarwal, 1982), promotes vasodilation (Wysham *et al1986*), bronchodilation (Lichey,*et al*1984), thyroid hormone secretion (Roger, 1987) and stimulates lipolysis in fat cells (Okuda,1992).

Forskolin has been shown to enhance lipolysis, regulated by cAMP, also inhibits fat storage. Forskolin is responsible for virtually all pharmacological activities attributed to *Coleus forskohlii* and the extracts of this constituent have been used in nearly all existing studies though other plant constituents, such as volatile oils and other diterpenoids and coleonols, contribute to the pharmacological activity and adsorption of forskolin.

Candida albicans causes Candidiasis which is an increasingly important disease that has a worldwide distribution due to the fact that it is a frequent opportunistic pathogen in patients. It is a fungus normally present on the skin and in mucous membrane such as vagina, mouth or rectum. The immune system keeps Candida proliferation under control, but when immune responses are weakened, Candida growth can precede unhindered. The uncontrolled growth of Candida is called Candida over growth. Solid media is generally employed for fungal culture, as the broths are not usually recommended except for fungal blood cultures were bi-phasic medium is used. The media commonly employed is Emmon's modification of Sabouraud dextrose agar. The media may be supplemented with antibiotics, such as gentamicin and chloramphenicol to minimize bacterial contamination and cyclohexamide to inhibit saprobic fungi (Jagdishchander, 1999).

The study of Senff et al., (1990) showed the presence of Candida albicans in samples with scalp psoriasis or seborrhoic dermatitis of the scalp (psoriasis capillitii). Yeasts were found in half of the tongue cultures and in 1/8th of the scalp scales. Further proof that Candida is one of the causes of psoriasis was seen in the study analyzed in more than 40,000 patients which showed that patients with atopic dermatitis and psoriasis had elevated levels of Candida in the intestines (Henseler et al.,

1997).The analysis showed that Candida colonization in the digestive tract can be one of the causes of psoriasis.

2. MATERIALS AND METHODS

2.1. Collection of raw drugs

The plants of *Coleus forskohlii* was collected randomly from Tamil Nadu Agricultural University (TNAU) Coimbatore, Coimbatore and Salem regions. The taxonomic identities of these plants were confirmed at Botanical survey of India and the voucher specimen numbers of the plants were preserved. Fresh plant material was washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles.

2.2. Extraction procedure

3g of air-dried powder was extracted in water and all the solvents in Bio Sox apparatus and then kept on a rotary shaker at 190-220 rpm for 2h. It was then filtered through 8 layers of muslin cloth and centrifuged at5000g for 10 min. The supernatant was collected. This procedure was repeated twice. The supernatant collected was pooled together and concentrated by evaporating in a vacuum drier to make the final volume one fourth of the original volume and stored at room temperature in airtight bottles.

2.3. Anti-fungal activity

2.3.1. Fungal Strains

In vitro antimicrobial activity was examined for aqueous and solvent extracts of *Coleus forskohlii*. The culture of *Candida albicans* was obtained from the PSG Institute of Medical Sciences and Research, Coimbatore (ATCC11778) and maintained at 4° C on nutrient agar slants.

The nutrient broth and nutrient agar were prepared as per the standard composition.

2.3.2. Method

Nutrient broth was prepared as per the standard composition and consisted of peptone 5 gm, NaCl 5 gm, Beef extract 3 gm, yeast extract 2 gm, pH 6.8 per 1 litre. Nutrient Agar- Peptone 5 gm, NaCl 5 gm, Beef extract 3 gm, Yeast extract 2 gm, Agar 20 gm, pH6.8 per 1 litre. Nutrient broth prepared for each culture and was sterilized in an autoclave at 1210C for 15 minutes. After sterilization the drug, different concentrations of the extracts were added. Further the cultures Candida sps. was inoculated in the tubes and subcultured weekly. Control sample was prepared along with culture in the absence of the drug. Only medium served as blank.

2.3.3. Media preparation and antifungal activity

The antimicrobial assay was performed by agar well diffusion method for all the extracts. The molten Mueller Hinton agar was inoculated with 100 µl of the inoculum (1 x 10^8 cfu/ml) and poured into the Petri plate (Hi-media). For agar well diffusion method, a well was prepared in the plates with the help of a cork-borer (0.85 cm). Different concentrations of the test compound were introduced into the well. The plates were incubated overnight at 37°C. Microbial growth was determined by measuring the diameter of zone of inhibition. Controls were maintained where pure solvents were used instead of the extract. The result was obtained by measuring the zone diameter. The experiment was done in triplicate and the mean values recorded.

3. RESULTS AND DISCUSSION

In the present study the different solvent extracts of *Coleus forskohlii* (methanol, ethyl acetate, benzene, chloroform, water, hexane, ethanol and acetone) at different concentrations 75µg and 150 µg was tested for its antimicrobial property against the growth of *Candida albicans.* The zone of inhibition observed after 72 hrs showed potent activity at 150µg of the methanol extract (20 mm) and benzene extract (13mm) and water (12mm), whereas with chloroform it was 6mm and other solvents like acetone, ethylacetate , ethanol , hexane did not reveal any zone of inhibition(Table 1 and Figure 1).

The anti microbial activity of plant oils and extracts has been recognized for many years and the significance of the microbial activity of oils and extracts of plants against *Candida albicans*, *Enterococcus faecalis and E. coli* has been reported. The oil of lemon grass, oregano and bay leaves were observed to inhibit the growth of most microorganisms (Naveens, 2008). The plant extracts, as methanolic extracts of *Peltophorum pterocarpum and Punica granatum* exhibited highest antifungal activity against C*andida albicans* (Duraipanidyan *et al.,* 2006).

The above results imply that the compounds from *Coleus forskohlii* that was extracted into methanol had the property to inhibit the growth of *Candida albicans* compared to the other solvents extracts. There was no antagonistic effect on *Candida albicans* by the *Coleus forskohlii* extracted in chloroform, petroleum ether and other solvents. However, maximum inhibition was observed with the reference drug clopidogriel, where as DMSO, used as vehicle control showed no inhibitory effect. The studies of screening results show a correlation with the antibiotic uses of the plant and the active

compounds present in extracts that may be responsible for the antimicrobial activity.

4. CONCLUSION

In the present study, methanolic *Coleus forskohlii* extracts exhibited high level of antifungal activity against *Candida albicans.*They have an excellent safety profile and are generally without toxicity or side effects at the recommended dosage. In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents. Thus, it is anticipated that phytochemicals with adequate efficacy can be used for the treatment and prevention of various ailments. The potential for developing therapeutic drugs from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against safely in humans. Plant-based drugs have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic drugs. Continued further exploration of plant-derived products is needed today.

Further research is necessary to determine the identity of the compounds from within these plants and also to determine their full spectrum of efficacy. However, the present study of *in vitro* evaluation of *Coleus forskohlii* forms a primary platform for further phytochemical and pharmacological studies.

Table 1

S.No	Forshkolin extracts	Zone of inhibition in (mm)
1	Methanol	20
2	Ethyl acetate	-
3	Benzene	13
4	Chloroform	-
5	Water	12
6	Hexane	-

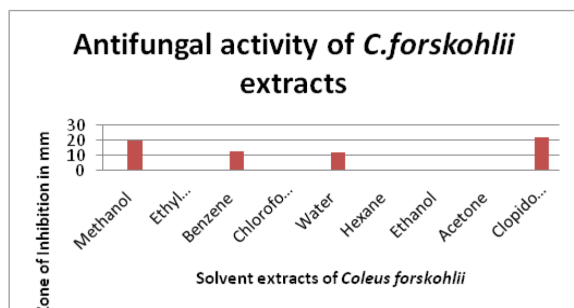


Figure 1

REFERENCES

Agarwal, K.C. and R.E. Jr. Parks, (1982). Synergistic inhibition of platelet aggregation by forskolin plus PGE1 or 2-fluoroadenosine: effects of 2',5'-dideoxyadenosine and 5'-methylthioadenosine. *Biochem. Pharmacol.* **31**: 3713-3716

Buslau, M., I. Menzel and H. Holzmann, (1990). Fungal flora of human faeces in psoriasis and atopic dermatitis. *Mycoses* **33**: 90–94

Dubey, M.P., R.C. Srimal, S. Nityanand and B.N. Dhawan, (1981). Pharmacological studies on coleonol, a hypotensive diterpene from *Coleus forskohlii*. *J. Ethnopharmacol.* **3**: 1-13.

Marone, G., M. Columbo and M. Triggiani, (1986). Forskolin inhibits the release of histamine from human basophils and mast cells. *Agents Actions* **18**: 96-99.

Metzger, H. and E. Lindner, (1981). The positive inotropic-acting forskolin, a potent adenylate cyclase activator. *Arzneimittelforschung* **31**: 1248-1250.

Naveena, B.M., M. Muthukumar, A.R. Sen, Y. Babji and T.R.K. Murthy, (2006). Improvement of shelf-life of buffalo meat using lactic acid, clove oil and vitamin C during retail display. *Meat Sci.* **74**: 409–415.

Okuda, H., C. Morimoto and T. Tsujita, (1992). Relationship between cyclic AMP production and lipolysis induced by forskolin in rat fat cells*. J. Lipid Res.* **33**: 225-231

Roger, P.P., P. Servais and J.E. Dumont, (1987). Regulation of dog thyroid epithelial cell cycle by forskolin, and adenylate cyclase activator*. Exp. Cell Res.* **172**: 282-292.

Seamon, K.B., W. Padgett and J.W. Daly, (1981). Forskolin: unique diterpene activator of adenylate cyclase inmembranes and intact cells. *Proc. Natl. Acad. Sci. USA* **78**: 3363-3367

Senff, H., C. Bothe, J. Busacker and D. Reinel, (1990). Studies on the yeast flora in patients suffering from psoriasis capillitii or seborrhoic dermatitis of the scalp. *Mycoses* **33**: 29–32.

Srinivasan, S., M. Anitha, S. Mwangi and R.O. Heuckeroth, (2005). Enteric neuroblasts require the phosphatidylinositol 3-kinase/Akt/Forkhead pathway for GDNF-stimulated survival. *Mol. Cell. Neurosci.* **29**: 107–119

Veeramuthu Duraipandiyan, Muniyappan Ayyanar and Savarimuthu Ignachimuthu, (2006). *Complemen. Alter. Med.* 1-7.

Wysham, D.G., A.F. Brotherton and D.D. Heistad, (1986). Effects of forskolin on cerebral blood flow: implications for a role of adenylate cyclase. *Stroke* **17**: 1299-1303.

Kong. Res. J. 2(1) : 128-132, 2015 ISSN 2349-2694
Kongunadu Arts and Science College, Coimbatore.

PHYTOCHEMICAL ANALYSIS AND *IN VITRO* FREE RADICAL SCAVENGING ACTIVITIES OF *MEDICAGO SATIVA* SEEDS

Gomathi, R[1]., S.Banu[2] and K. Usha[3]
[1]Department of Biochemistry, [2]Biotechnology and [3]Bioinformatics
Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore.

ABSTRACT

Phytochemical analysis and *in vitro* free radical scavenging activities were analyzed in the various extracts of *Medicago sativa* seeds*.* The phytochemical analysis showed the presence of alkaloids, carbohydrates, flavonoids, glycosides, saponins, phytosterols, tannins, terpenoids and phenols. Among the various extracts, phytochemicals were extracted best in ethanol. Free radical scavenging activities such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), hydroxyl, superoxide, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), ferrous ion chleating activity and non radicals such as hydrogen peroxide and nitric oxide were analyzed in the various extracts of *Medicago sativa* seeds and were compared with standard antioxidant ascorbic acid. All the extracts of *Medicago sativa* seeds scavenged the free radicals in a concentration dependent manner. The antioxidative activity of all the extracts was found to be more pronounced than that of the standard antioxidant ascorbic acid. Among the various extracts, the antioxidant activity was found to be more pronounced in ethanolic extract of *Medicago sativa* seeds.

Keywords: *Medicago sativa*, Alfalfa, phytochemicals, DPPH, ABTS, free radicals and antioxidant.

1. INTRODUCTION

Plant materials remain an important resource to combat serious diseases in the world. The traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries. The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on the human body. The most important bioactive constituents of plants are alkaloids, tannin, flavonoid and phenolic compounds (Edeoga *et al.,* 2005).

Reactive oxygen species such as superoxide anions ($O_2^{\bullet -}$), hydroxyl, nitric oxide radicals and hydrogen peroxide (H_2O_2) play an important role in oxidative stress related to the pathogenesis of various important diseases (Halliwell and Gutteride, 1999; Finkel and Holbrook, 2000). Antioxidants act as a major defense against radical mediated toxicity by protecting the damages caused by free radicals. Antioxidant based drugs/formulations for the prevention and treatment of complex diseases, like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer, have appeared in the last three decades (Devasagayam *et al.,* 2004).

Medicago sativa L., or Alfalfa is a member of Leguminosae or Fabaceae family, which has a long history of dietary and medicinal uses in traditional herbal medicine in China, America, India and many Middle Eastern countries for the treatment of a variety of ailments and is also used as a fodder for animals (Al-Dosari, 2012). *Medicago sativa L*., sprouts are often consumed as vegetable salad and their leaves or seeds are also sold as bulk powdered herb, capsules and tablets for nutritional supplement in health food stores. The extracts from alfalfa sprouts, leaves and roots have been indicated to be helpful in lowering cholesterol levels in animal and human studies (Hong *et al.,* 2009). In this regard, the present investigation was aimed to analyze the phytochemical constituents and free radical scavenging activity of *M. sativa* raw seeds and germinated seeds. The present study was aimed to analyze the phytochemical constituents and *in vitro* free radical scavenging activities of *M. sativa* seeds.

2. MATERIALS AND METHODS

2.1. Plant material

The *M.sativa* seeds were collected from Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India and authenticated (BSI/SRC/5/23/2014-15/Tech/394) by the authority of the botanical survey of India (BSI), Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.

2.2. Preparation of the plant sample

After that, the seeds were washed with water to remove contamination and shade dried at room

temperature. The dried samples were ground into coarse powder with the help of mechanical grinder and stored in airtight containers for further studies. 20 grams of powdered samples were weighed and wrapped separately with whatmann No.1 filter paper and extracted using various solvents such as petroleum ether, benzene, chloroform, ethyl acetate, ethanol, methanol and aqueous with the help of soxhlet extractor. Then, the various plant extracts were concentrated using rotary evaporator and then preserved for future analysis.

2.3. Qualitative analysis of phytochemicals

The phytochemical constituents of various extracts of *Medicago sativa* seeds were qualitatively analyzed using standard procedure (Iyengar, 1995; Siddiqui and Ali, 1997; Raaman, 2006).

3.4. Determination of free radical scavenging activity

Free radical scavenging activities such as DPPH, hydroxyl, hydrogen peroxide, nitric oxide, superoxide, ABTS and ferrous ion radical scavenging activities were analyzed using standard procedures (Carter, 1971; Misra and Fridovich, 1972; Green *et al.,* 1984; Ruch *et al.,* 1989; Elizabeth and Rao, 1990; Mensor *et al.,* 2001; Shirwaiker *et al.,* 2006.

3. RESULTS AND DISCUSSION

Table1 indicates the phytochemical analysis of *Medicago sativa* seeds. The alkaloids and flavonoids were found to be present in all the extracts including petroleum ether, benzene, ethanol, methanol and aqueous extracts. The anthraquinones were found to be present in all the extracts except petroleum ether and aqueous extract. Saponins were present in chloroform, ethanol and methanol extracts. Phenols were present in all the extracts except ethyl acetate. Steroids were present in petroleum ether, benzene, ethyl acetate, and aqueous extracts. Tannins were found in chloroform, ethyl acetate, ethanol and methanol extracts. Terpenoids were found in petroleum ether, chloroform, ethanol, methanol, and aqueous extracts. Glycosides were found to be present in ethanolic extract alone. Phenols were found to be present in petroleum ether, benzene and chloroform extracts. Saponins were found to be present in chloroform, ethanol and methanolic extracts. Steroids were found to be present in petroleum ether, benzene, ethyl acetate and aqueous extracts. Tannins were found to be present in chloroform, ethyl acetate, ethyl acetate, ethanol and methanolic extracts. Terpenoids were found to be present in all the extracts except benzene and ethyl acetate extracts.

Table 1: Qualitative analysis for the presence of phytochemicals.

Phytochemical constituents	PE	B	C	EA	E	M	A
Amino acids	-	-	-	+	+	+	-
Anthraquinones	-	+	+	+	+	+	-
Alkaloids	+	+	-	-	+	+	+
Carbohydrates	+	+	-	+	+	-	-
Flavonoids	+	-	+	-	+	+	+
Glycosides	-	-	-	-	+	-	-
Phenols	+	+	+	-	-	-	-
Saponins	-	-	+	-	+	+	-
Steroids	+	+	-	+	-	-	+
Tannins	-	-	+	+	+	+	-
Terpenoids	+	-	+	-	+	+	+

(+ Presence; - Absence, PE - Petroleum ether; B - Benzene; C - Chloroform; EA - Ethyl acetate; E - Ethanol; M – Methanol, A - Aqueous)

Among the various extracts, the ethanolic extract of *Medicago sativa* seeds showed maximum phytochemical constituents. So, the ethanolic extract of *Medicago sativa* seeds were taken for further study.

The phytochemical screening of *C. lanatus* seeds showed the presence of phytochemical constituents such as alkaloids, flavonoids, tannins, aminoacids, carbohydrates, cardioglycosides, terpenoids, oils and fats in the ethanolic extract of plant material when compared with other solvents (Varghese *et al.,* 2013).

3.1. Free radical scavenging activity

The scavenging of stable DPPH radical model is a widely used method to evaluate antioxidant activities in a relatively short time compared with other methods. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability. DPPH radical is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares *et al.,* 1997).

Figure 1 indicates the DPPH radical scavenging activity of various extracts of *Medicago sativa* seeds. Ethanolic extract of *Medicago sativa* seeds showed the highest DPPH scavenging activity which was followed by standard antioxidant ascorbic acid, methanol and chloroform extracts. This may due to the extraction of maximum phytochemicals in ethanolic extract. The least activity was noted in aqueous extract.

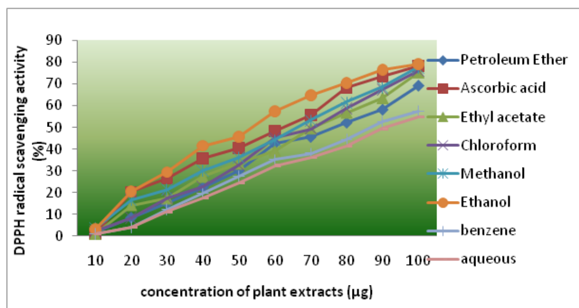


Fig. 1. Free radical scavenging activity of various extracta of *M. sativa* seeds by 2,2-diphenyl-1-picryl hydrazyl radicals (DPPH).

3.2. Hydroxyl radical scavenging activity

The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology capable of damaging the biomolecules of living cells (Zhang *et al.,* 2009).

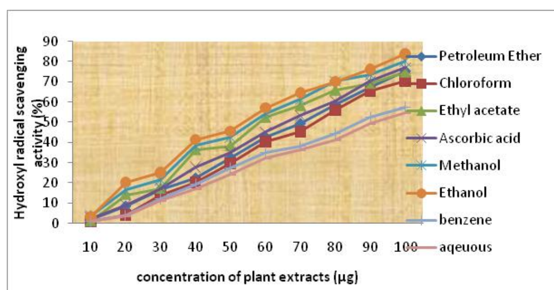


Fig. 2. Hydroxyl radical (OH) scavenging effects of various extracts of *M. sativa* seeds.

Figure 2 illustrates the hydroxyl radical scavenging activity of various extracts of *Medicago sativa* seeds. Ethanolic extract of *Medicago sativa* seeds showed the highest radical scavenging activity which was followed by standard antioxidant ascorbic acid, methanol and ethyl acetate extracts. The least activity was noted in aqueous extract.

3.3. Scavenging of hydrogen peroxides (H_2O_2)

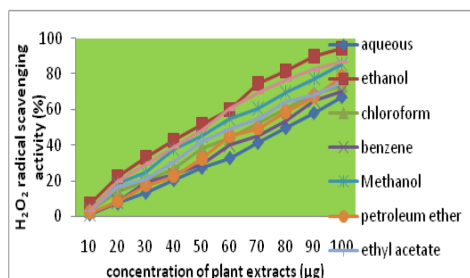


Fig. 3. Hydrogen peroxide (H2O2) scavenging effects of various extracta of *M. sativa* seeds.

H_2O_2 is highly important because of its ability of penetrate biological membranes. H_2O_2 itself is not very reactive, but it can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cells. Thus, removing H_2O_2 is very important for the protection of living systems.

Figure 3 illustrates the hydrogen peroxide radical scavenging activity of various extracts of *Medicago sativa* seeds. Ethanolic extract of *Medicago sativa* seeds showed the highest radical scavenging activity which was followed by standard antioxidant ascorbic acid and methanol. The least activity was noted in aqueous extract.

3.4. Nitric oxide scavenging activity

Nitric oxide (NO) is a free radical produced in mammalian cells, involved in the regulation of various physiological processes. However, excess production of NO is associated with several diseases Nitric oxide is a very unstable species under aerobic condition. It reacts with O_2 to produce stable product nitrate and nitrite through intermediates NO_2, N_2O_4 and N_3O_4 (Jayakumari *et al.,* 2012).

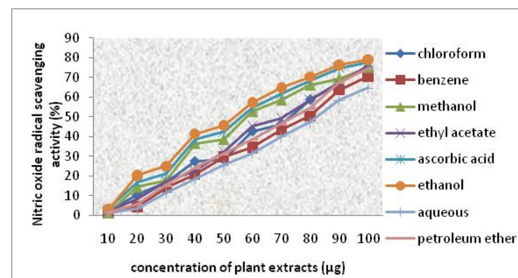


Fig. 4. Nitric oxide radical scavenging activity of various extracts of *Medicago sativa* seeds

Figure 4 illustrates the nitric oxide radical scavenging activity of various extracts of *Medicago sativa* seeds. Ethanolic extract of *Medicago sativa* seeds showed the highest radical scavenging activity which was followed by standard antioxidant ascorbic acid, methanol and ethyl acetate extracts. The least activity was noted in aqueous extract.

3.5. Superoxide anion radical scavenging activity

Superoxide anion radicals are produced endogenously by flavoenzymes like xanthine oxidase, which converts hypoxanthine to xanthine and subsequently to uric acid in ischemia-reperfusion (Bora and Sharma, 2010). Superoxide is generated *in vivo* by several oxidative enzymes, including xanthine oxidase. In PMS-NADH-NBT system, superoxide anion derived from dissolved oxygen by PMS-NADH coupling reaction reduces NBT. The decrease of absorbance at 560 nm with

antioxidants indicates the consumption of superoxide anion in the reaction mixture.

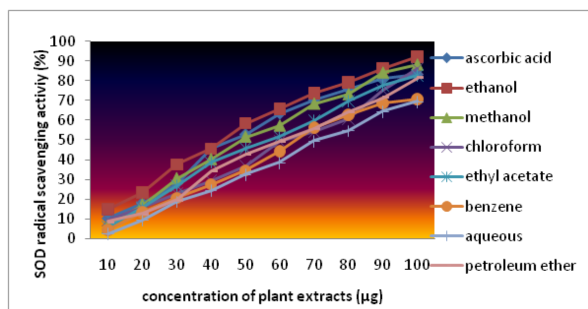


Fig. 5. superoxide anion radical scavenging activity of various extracts of *Medicago sativa* seeds

Figure 5 illustrates the inhibition of superoxide radical generation of various extracts of *Medicago sativa* seeds. Ethanolic extract of *Medicago sativa* seeds showed the highest radical scavenging activity which was followed by standard antioxidant ascorbic acid, methanol and ethyl acetate extracts. The least activity was noted in aqueous extract.

3.6. ABTS radical scavenging activity

ABTS is frequently used by the food industry and agricultural researcher to measure the antioxidant capacities of foods (Peiyuan *et al.,* 2010). Proton radical scavenging is an important attribute of antioxidants. ABTS, a protonated radical, has characteristic absorbance maxima at 734 nm which decreases with the scavenging of the proton radicals. ABTS is a blue green chromogen and this assay is based on the ability of the antioxidants to scavenge long – life radical cation ABTS.

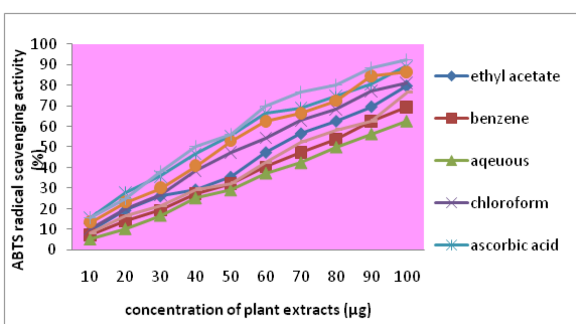


Fig. 6. ABTS radical scavenging activity of various extracts of *Medicago sativa* seeds.

Figure 6 illustrates the ABTS radical scavenging activity of various extracts of *Medicago sativa* seeds. Ethanolic extract of *Medicago sativa* seeds showed the highest radical scavenging activity which was followed by standard antioxidant ascorbic acid, methanol and ethyl acetate extracts.

The scavenging effect of all the extracts of *Pouzolzia zeylanica* increased with increasing concentration of Trolox has shown higher antioxidant activity (% inhibition) in ABTS (2,2-Azino-bis 3-ethyl benothiazoline-6-sulfonic acid diammonium salt) radical scavenging assay (Lobo *et al.,* 2010).

3.7. Ferrous metal ion chelating activity

The chelating of ferrous ions by various extracts of Medicago sativa seeds were estimated by the method of Carter. Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of chelating agents, the complex formation is disrupted with the result that the red color of the complex is decreased. Measurement of colour reduction, therefore, allows estimation of the chelating activity of the coexisting chelator. In this assay MMS and standard antioxidant compounds interfered with the formation of ferrous and ferrozine complex, suggesting that they have chelating activity and capture ferrous ion before ferrozine. Iron can stimulate lipid peroxidation by Fenton reaction, and also accelerates peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation (Halliwell, 1991; Gulcin *et al.,* 2003).

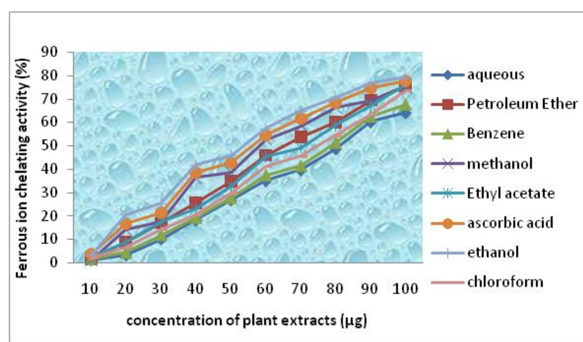


Fig. 7. Ferrous ion chleating activity of various extracts of *Medicago sativa* seeds.

Figure 7 illustrates the ferrous ion radical scavenging activity of various extracts of *Medicago sativa* seeds. Ethanolic extract of *Medicago sativa* seeds showed the highest radical scavenging activity which was followed by standard antioxidant ascorbic acid and methanol. The least activity was noted in aqueous extract.

5. CONCLUSION

From the results, it clearly indicates that *M. sativa* seeds possess powerful *in vitro* antioxidant activity. The encouraging results of various extracts *M. sativa* seeds with the various *in vitro* antioxidant tests proved the plant as a metal chelator, its

hydrogen donating ability, and effectiveness as scavengers of free radicals, superoxide anions, hydroxyl and hydrogen peroxide. Further work will be carried out to isolate and elucidate the bioactive principle(s) responsible for the antioxidant activity.

REFERENCES

Al-Dosari, M.S. (2012). *In vitro* and *in vivo* antioxidant activity of Alfalfa (*Medicago sativa* L.,) on carbon tetrachloride intoxicated rats. *The American J Chinese Med* **40**(4): 779–793.

Bora, K.S. and A. Sharma. (2010). *In vitro* antioxidant and free radical scavenging potential of *Medicago sativa* Linn. *J Pharm Res* **3**(6): 1206-1210.

Carter, P. (1971). Spectrophotometric Determination of serum iron at the sub microgram level with a new regent – ferrozine. *Anal. Biochem* **40**: 450-458.

Devasagayam, T.P.A, J. Tilak, C. Boloor, K.K. Sane, K.S. Ghaskadbi and R.D. Lele. (2004). Review: Free radicals and antioxidants in human health: Current status and future prospects. *J Assoc Phys India* **52**: 794-804.

Edeoga, H.O, D.E. Okwu and B.O. Mbaebie. (2005). Phytochemical constituents of some Nigerian medicinal plants. *Afr J Biotech* **4**:685-688.

Elizabeth, K. and M.W.A. Rao. (1990). Oxygen radical scavenging activity of *Curcumin. Int. J. Pharm.* **58**: 237-240.

Finkel, T. and N.J. Holbrook. (2000). Oxidants, oxidative stress and the biology of aging. *Nature* **408**: 239-247.

Green, M.J. and H.A.O. Hill. (1984). *Chemistry of Dioxygen methods.* Enzymology. 105.

Gulcin, I., M.E. Buyukokuroglu and O.I. Kufrevioglu. (2003). Metal chelating and hydrogen peroxide scavenging effects of melatonin. *J. Pineal Res.* **34**: 278-281.

Halliwell, B. (1991). Reactive oxygen species in living systems: source, biochemistry and role in human disease. *Am J Med.* **91**: 14-22.

Halliwell. B. and J.M.C. Gutteridge. (1999). *Free radicals in biology and medicine.* 3rd ed., Oxford University Press, Oxford.

Hong, Y.H. W.W. Chao, M.L. Chen and B.F. Lin. (2009). Ethyl acetate extracts of alfalfa (*Medicago sativa* L.) sprouts inhibit lipopolysaccharide induced inflammation *in vitro* and *in vivo. J Biomed Sci* **16**: 64.

Iyengar, M.A. (1995). *Study of crude drugs*, 8th edn Manipal power press.

Jayakumari, S., A. Arthanareswaran, A. Vijayalakshmi, M. Velraj and V. Ravichandran. (2012). Free radical scavenging activity of *Pisonia grandis* R.Br Leaves. *Indian J Pharmaceu Edu Res* **46**(1): 34-37.

Lobo, V.C., A. Phatak and C. Naresh. (2010). Antioxidant and free radical scavenging activity of *Hygrophila schulli (Buch.-Ham.*) Almeida and Almeida Seeds. *Advance in Bio Research* 72-78.

Mensor, L.I., F.S. Menezes, G.G. Leitao, A.S. Reis, T. Dos- Santos, C.S. Coube and S.G. Leitao. (2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytotherapy Res* 127-130.

Misra, H.P. and Fridovich. (1972). The role of superoxide anion in the antioxidation of epinephrine and a simple assay for superoxide dismutase. *J. Biol. Chem.,* **247**: 3170 - 3171.

Peiyuan, L., H. Lini, S. Wei, L. Rumei, D. Chaocheng, L. Liangquan, D. Yongkun, G. Nana, L. Chengsheng and H. Chunling. (2010). Free radical-scavenging capacity, antioxidant activity and phenolic content of *Pouzolzia zeylanica. J Serbian Chem Soc* **76**(5): 709–717.

Raaman, N. (2006). *Phytochemical Techiques*. (New Publishing Agency), **19**(24), 32-40.

Ruch, R.J., S.J. Cheng and J.E. Klaunig. (1989). Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogen* **10**: 1003-1008.

Shirwaiker, A., H.N.A. Ram and F. Mohanpatra. (2006). Antioxidant and antiulcer activity of aqueous extract of a polyherbal formulation. *Indian J. Exp. Bio.* **44**: 474-480.

Siddiqui, A.A. and M. Ali. (1997). *Practical Pharmaceutical Chemistry*, 1st edn, CBS Publishers and Distributors, 126-131.

Soares, J.R. T.C.P. Dins, A.P. Cunha and L.M. Almeida. (1997). Antioxidant activity of some extracts of *Thymus zygis. Free Radic Res.* **26**: 469-478.

Varghese, S., R. Narmadha, D. Gomathi, M. Kalaiselvi and K. Devaki. (2013). Phytochemical screening and HPTLC finger printing analysis of *Citrullus lanatus* (Thunb.) seed. *J Acute Disease* 122-126.

Zhang, W., B. Li, L. Han and H. Zhang. (2009). Antioxidant activities of extracts from Areca (Arecacatectu L.) flower, husk and seed. *Afr J Biotechonol* **8**(16): 3887-3892.

Kong. Res. J. 2(1) : 133-137, 2015 ISSN 2349-2694
Kongunadu Arts and Science College, Coimbatore.

THE EFFECT OF BATTERY RECYCLING INDUSTRY SOLID WASTE LEACHATE ON ANTIOXIDANT STATUS OF *TRIGONELLA FOENUM-GRAECUM*

Jeba Sweetly, D*[1]., K. Sangeetha[2] and B. Suganthi[2]
[1]Department of Biochemistry, [2]Department of Biotechnology,
Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore.
*E-mail: sweetly5647@gmail.com

ABSTRACT

Effect of battery recycling industry solid waste leachate on the growth and antioxidant *status of Trigonella foenum-graecum* was investigated. Plants have played a crucial role in maintaining human health and improving the quality of human life for thousands of years. Plants were grown in four dilutions of leachate and the results were compared with the control plants grown simultaneously in the same condition. Enzymic and Non-enzymic antioxidants were analyzed in the coriander leaves. The concentration of Superoxide dismutase, Glutathione peroxidase, Ascorbic acid, Reduced glutathione were studied in the leachate and control growing plants. This study proves that coriander should not be grown in the soil having highest level of battery recycling industry solid waste for maintaining human health and the environment.

Keywords: Antioxidant, battery recycling, solid waste leachate, coriander

1. INTRODUCTION

Heavy metals induce oxidative stress by generating free radicals and toxic reactive oxygen species. These species react with lipids, proteins, pigments and nucleic acids and cause lipid peroxidation, membrane damage and inactivation of enzymes, thus affecting the cell viability. The deleterious effects resulting from the cellular oxidative state may be alleviated by the enzymatic and non enzymatic antioxidant machinery of the plant (Sharma and Agrawal, 2005).

One of the major mechanisms behind heavy metal toxicity has been attributed to oxidative stress. Toxic metals increase production of free radicals and decrease availability of antioxidant reserves to respond to the resultant damage. A growing amount of data provide evidence that metals are capable of interacting with nuclear proteins and DNA causing oxidative deterioration of biological macromolecules (Leonard, 2004).

The battery industry represents one of the most important and growing sectors where the use of non-toxic and non-hazardous substitute material has not rapidly developed. As regulations increase and concern for the environment and human health becomes more prevalent, the fate of toxic and hazardous materials in the environment should be more carefully considered. Exide's network spreads throughout India and its factories are geographically distributed at strategic locations around the country. Exide industry is one of the leading companies towards manufacturing of lead-acid batteries nationally and internationally (Rahangdale *et al*., 2012).

The maximum allowable concentration of lead is 0.05 mg L-1 for drinking water and 0.2 mg L-1 for effluent discharge. Lead is used for manufacturing of storage batteries, due to its characteristic properties: conductivity, corrosion resistance and reversibility of the reaction between lead, lead oxide and sulfuric acid (Dermentzis *et al*., 2012).

Plants are rich sources of natural antioxidants that play a vital role in the prevention or progression of the degenerative diseases. The consumption of fruits, vegetables and herbs rich in antioxidants is associated with a decline in the incidence of degenerative diseases and cancer (Harish *et al*., 2005).

Plants possess two very efficient antioxidant defense systems: the enzymic which includes catalase, peroxidase, superoxide dismutase, polyphenol oxidase, glutathione reductase and the non enzymic antioxidant defense systems such as ascorbic acid, glutathione, tocopherol and carotenoids. Both allow scavenging of reactive oxygen species leading to protection of plant cells from oxidative damage (Blokhina *et al*., 2003; Gratao *et al*., 2005). Indeed, activities of antioxidant enzymes have been detected in various cellular organelles of various plant species. These antioxidant enzymes were found in various compartments of the plant leaf cell, e.g: superoxide

dismutase (SOD) found in chloroplasts (Mittova *et al*., 2000). It is found that people who eat fruits and vegetables rich in polyphenols and anthocyanins have a lower risk of cancer, heart disease and some neurological diseases (Stanner *et al*., 2004). Antioxidants can cancel out the cell damaging effects of free radicals. These compounds might prevent conditions such as macular degeneration, suppressed immunity to poor nutrition and neurodegeneration which is caused by oxidative stress (Wang *et al*., 2005).

The term antioxidant originally was used to refer to a chemical that prevented the consumption of oxygen. Research into how vitamin E prevents the process of lipid peroxidation led to the identification of antioxidants as reducing agents that prevent oxidative reactions, often by scavenging reactive oxygen species before they can damage cells (Wolf, 2005). Heavy metals induce oxidative stress by generating free radicals and toxic reactive oxygen species. These species react with lipids, proteins, pigments and nucleic acids and cause lipid peroxidation, membrane damage and inactivation of enzymes, thus affecting the cell viability. The deleterious effects resulting from the cellular oxidative state may be alleviated by the enzymatic and non enzymatic antioxidant machinery of the plant (Mittler 2002; Sharma and Agrawal, 2005).

Fenugreek is one of the oldest medicinal plants, originating in India and Northern Africa. An annual plant, fenugreek grows to an average height of two feet. This plant use for blood lipids and sugar decreasing in diabetic and non diabetic people and has antioxidant and antibacterial activity. This plant decreases body fats and effective on obesity. This plant are used in therapy atherosclerosis (Nandini *et al*., 2007), rheumatism (Amit *et al*., 2010), sugar lowering (Gupta *et al*., 2001), blood lipids lowering (Xue *et al*., 2007), appetizer and contain antioxidant activity (Bukhari *et al*., 2008).

A major defence mechanism involves the antioxidant enzymes, which includes SOD, catalase and glutathione peroxidase (GPx), which converts active oxygen molecules into non toxic compounds (Merlin and Parthasarathy, 2011).

Hence, in the present study antioxidant status of *Trigonella foenum-graecum* plant was investigated under different concentration of battery recycling industry solid waste leachate.

2. MATERIALS AND METHODS

2.1. Collection of solid waste

The Battery recycling industry solid waste was collected from Industrial area near kuruchi, Coimbatore, Tami Nadu.

2.2. Preparation of leachate from battery recycling industry solid waste

Leachates were prepared by adding 100 g of solid waste to 1L of demineralized water (10% w/v), followed by continuous shaking for 24 h at ambient room temperature (25±1 10°C) following the were removed by centrifugation at 3000 rpm for 15 min and the leachate samples were preserved in screw-capped bottles at 4± 10°C (Coya *et al*., 2000) for further use.

2.3. Randomized block design

Pot culture experiment was conducted for a period of 60 days. Red soil and sand free from pebbles and stones were mixed in the ratio of 3:1. 10 kg of the mixture was filled it the individual pots. The seeds of coriander selected for the study were collected form Department of pulses, Tamil Nadu Agricultural University, Coimbatore. The study carried out at Avinashilingam University was laid out in a complete randomized block design consisting of 4 treatments with a control. Each experiment was replicated thrice. The seeds were sown in different pots in five different dilutions as follows and used for the pot culture study.

Control - plants grown in normal water
25% - 25ml leachate + 75 ml water
50% - 50ml leachate + 50 ml water
75% - 75ml leachate + 25 ml water
100% - 100ml leachate

There was no germination observed when seeds were grown with 100% leachate. Hence the present study continued with 25%, 50% and 75% leachate. The biochemical parameters in the leaves of selected plant which were subjected to the various treatments as indicated in table 1 were analyzed for a period of 20th, 40th and 60th days.

Table 1. Biochemical parameters analysed

Characteristics	Method of analysis	References
Superoxide dismutase	Spectrophotometry	Misra and Fridovich, 1972
Glutathione peroxidase	Spectrophotometry	Rotruck *et al*., 1973
Ascorbic acid	Spectrophotometry	Roe and Kuether, 1953
Reduced glutathione	Spectrophotometry	Moron *et al*., 1979

2.4. Statistical analysis

The results for biometric and biochemical parameters were expressed as mean±S.D. The results were subjected to TWO WAY ANOVA using Sigma Stat Version 3.0. A value of $p < 0.05$ considered to be highly significant.

3. RESULTS AND DISCUSSION

Antioxidants are compounds that dispose, scavenge and suppress the formation of free radicals or oppose their actions. There are two main categories of antioxidants (enzymic and non enzymic) whose role is to prevent the generation of free radicals that are generated (Priya and Surapaneni 2008; Kaur *et al.*, 2008).

Heavy metals induce oxidative stress by generating free radicals and toxic reactive oxygen species. These species react with lipids, proteins, pigments and nucleic acids and cause lipid peroxidation, membrane damage and inactivation of enzymes, thus affecting the cell viability. The deleterious effects resulting from the cellular oxidative state may be alleviated by the enzymatic and non enzymatic antioxidant machinery of the plant (Sharma and Agrawal, 2005).

A major defence mechanism involves the antioxidant enzymes, which includes SOD, catalase and glutathione peroxidase (GPx), which converts active oxygen molecules into non toxic compounds (Merlin and Parthasarathy, 2011). In the present study, selected enzymic and non enzymic antioxidants were analysed in the plants grown with untreated leachate.

Table 2. Superoxide dismutase activities in the leaves of *Trigonella foenum-graecum.*

	Superoxide dismutase (Units/g)		
	20th day	**40th day**	**60th day**
Control	0.062±0.002	0.068±0.004	0.052±0.001
25% Leachate	0.059±0.002	0.067±0.001	0.043±0.001
50% Leachate	0.054±0.001	0.055±0.001	0.039±0.001
75% Leachate	0.045±0.001	0.050±0.001	0.036±0.001
CD (*P<0.05*)		0.0018	

From t he table 2 and figure 1 shows the superoxide dismutase activity in the leaves of Fenugreek grown in red soil with and without the various leachate samples. Among the various treatments, control recorded higher enzymic activity followed by 25% leachate, while 50 and 75% leachate exhibited lower activity of the enzyme.

He and Goa, 2008 have also reported a decline in the activity of SOD, CAT and POD after flowering stage in the plant *Chimonathus praecox*, which is similar to our observation.

Table 3. Glutathione peroxidase activities in the leaves of *Trigonella foenum-graecum*

	Glutathione peroxidase (Units/g)		
	20th day	**40th day**	**60th day**
Control	0.157±0.001	0.163±0.006	0.142±0.002
25% Leachate	0.139±0.001	0.152±0.002	0.132±0.006
50% Leachate	0.133±0.008	0.146±0.003	0.130±0.002
75% Leachate	0.120±0.002	0.127±0.001	0.112±0.002
CD(*P*<0.05)		0.0018	

From table 3 and figure 2 it is shown that glutathione peroxidase activity was maximum in fenugreek grown with 25% leachate. There was a decrease in plant grown with 50% and 75% leachate. However the enzymic antioxidant levels were found to be reduced on 60th day in plants grown with various leachate concentrations.

Ascorbic acid has antioxidant activity and it reduces oxidizing substances such as hydrogen peroxide (Duarte and Lunec, 2005). It can also reduce metal ions which lead to the generation of free radicals through the Fenton reaction (Valko *et al.*, 2005).

Table 4. Ascorbic acid content in the leaves of *Trigonella foenum-graecum*

	Ascorbic acid (mg/g)		
	20th day	**40th day**	**60th day**
Control	0.036±0.002	0.038±0.001	0.033±0.001
25% Leachate	0.026±0.002	0.033±0.001	0.024±0.001
50% Leachate	0.022±0.001	0.028±0.002	0.021±0.001
75% Leachate	0.020±0.001	0.024±0.002	0.019±0.001
CD(*P*<0.05)		0.002	

From Table 4 and figure 3, the ascorbic acid content was maximum in the control group followed by 25% leachate, whereas a significant reduction was observed in 50% and 75% leachate.

Table 5. Reduced glutathione content in the leaves of *Trigonella foenum-graecum*

	Reduced glutathione (nmoles)		
	20th day	**40th day**	**60th day**
Control	0.151±0.001	0.164±0.002	0.139±0.001
25% Leachate	0.146±0.001	0.162±0.002	0.123±0.004
50% Leachate	0.139±0.002	0.151±0.002	0.107±0.003
75% Leachate	0.105±0.006	0.133±0.001	0.091±0.003
CD(*P*<0.05)		0.198	

From table 5 and figure 4 it is clear that fenugreek plant grown with normal water showed elevated levels of reduced glutathione in both the plants with treated and 25% leachate. 50% and 75% leachate showed minimum amount of glutathione reductase when compared to normal control showing a negative correlation between the non enzymic antioxidant content and leachate concentration.

4. CONCLUSION

Based on these results the farmers around the locality should be properly educated about the beneficial effect of battery recycling industry leachate in agriculture and it would facilitate reduction in pollution load on environment. It is therefore conceivable that illegal dumpsite should be closed and landfills with effective leachate collection system should be built and they should be located in the outskirts of city far away from dwelling houses. A pot culture experiment was carried out to study the effect of the untreated and control samples of leachate on the growth attribute of the selected plant fenugreek. Various biochemical parameters such as enzymic and non enzymic antioxidants were determined. The plants grown with 25% leachate samples showed better growth characters which were comparable with that of control. Since the application of 25% leachate samples resulted in better growth of the selected green leafy vegetable coriander. Hence these leachates on a large scale can be used for irrigation purposes.

REFERENCES

Amit V.S., P.G. Nailesh, P.H. Aashish, P.K. Rameshwar and P.M. Madhabhai, (2010). Antiarthritic and vascular protective effects of fenugreek, *Boswellia Serrata* and *Acacia Catechu* alone and in combinations, *Pharma Sci Monitor* **1**(2): 95-111.

Blokhina, O.E., E. Virolainen, and K.V. Faqerstedt, (2003). Antioxidants, oxidative damage and oxygen deprivation stress: A review. *Ann Bot* **91**: 174-194.

Bukhari, B.S., M.L. Bhanger and S.H. Memon, (2008). Antioxidative activity of extracts from fenugreek seeds (*Trigonella foenumgraecum*). *Pak J Anal Environ Chem* **9:** 78-83.

Coya, B., E. Maranon and H. Sastre, (2000). Ecotoxicity assessment of slag generated in the process of recycling lead from waste batteries. *Resource Conservation and Recycling* **29**: 291–300.

Dermentzis, K., E. Valsamidou, and D. Marmanis, (2012). Simultaneous removal of acidity and lead from acid lead battery wastewater by aluminum and iron electrocoagulation, *J Engineer Sci Tech Rev* **5**(2): 1-5.

Duarte, T.L. and J. Lunec, (2005). Review: when is an antioxidant not an antioxidant, a review of novel actions and reactions of vitamin C. *Free Radi Res* **39**(7): 671 – 686.

Gratao, P.L., A. Polle, P.J. Lea and R.A. Azevedo, (2005). Making the life of heavy metal – stressed plants a little easier. *Functional Pl Biol* **32**: 481-494.

Gupta, A., Gupta, R., and Lal, B, (2001) Effect of *Trigonella foenum-graecum* (fenugreek) seeds on glycaemic control and insulin resistance in type 2 diabetes mellitus: a double blind placebo controlled study. *J Ass Physicians of India.* **49**: 1057-61.

Harish, R., S. Divakar, A. Srivastava, and T. Shivanandappa, (2005), Isolation of antioxidant compounds from the methanolic extract of the roots of Decalepis hamiltonii (Wright and Arn). *J Agric Food Chem* **53**: 7709-7714.

Leonard, S.S, G.K, Harris, X.L. Shi, (2004), Metal-induced oxidative stress and signal transduction*. Free Rad Biol Med* **37***:* 1921-42.

Merlin, N.J. and V. Parthasarathy**,** (2011)**,** Antioxidant and hepatoprotective activity of chloroform and ethanol extracts of *Gmelina asiatica* aerial parts**.** *J Med Plants Res* **5**(4): 533-538.

Misra, M.P. and I. Fridovich, (1972), The role of superoxide anion in the autoxidation of epinephrine and simple assay for superoxide dismutase. *J Biol Chem* **247**, 31-70.

Mittler, R. (2002). Oxidative stress antioxidants and stress tolerance. *Trends Plant Sci* **7**: 405-410.

Mittova, V., M. Volokita, M. Guy and M. Tal, (2000). Activities of SOD and ascorbate glutathione cycle enzymes in subcellular compartments in leaves and roots of the cultivated tomato and its world salt – tolerant relative Lycopersicon pennellii, *Physiol Plant* **110**: 42-51.

Moron, M.S., J.W. Bepierre and B. Mannerwick, (1979). Levels of glutathione reductase and glutathione -S-transferase in rat lung and liver. *Biochemica Biophysica Acta* 582.

Nandini, V.S., N. Devaraj, and H. Devaraj, (2007). A fiber cocktail of fenugreek, guar gumand wheat bran reduces oxidative modification of LDL induced by an atherogenic diet in rats, *Mol Cell Biol* **294**: 145–153.

Priya, V.V. and K.M. Surapaneni, 2008. Erythrocyte lipid peroxidation, glutathione, ascorbic acid, vitamin E, antioxidant enzymes and serum homocysteine levels in patients with coronary artery disease. *JCDR* **8**: 338-384.

Rahangdale, R.V., S.V. Kore and V.S. Kore, (2012). Waste Management in Lead-Acid Battery Industry: A Case Study. *World J App Environ Chem* **1**(1): 7-12.

Roe, J.H. and A. Keuther, (1953). The determination of ascorbic acid in whole blood and urine through 2, 4 di-nitrophenyl hydrazine derivative of Dehydro ascorbic acid. *J Biol Chem* **147**: 399-404.

Rotruck, T.T., H.E. Ganther, A.B. Swanson, D.G. Hafeman and W.G. Hoeckstra, (1973). Selenium: Biochemical role as a component of glutathione peroxidase. *Science* **179**: 588-590.

Sharma, R.K. and M. Agarwal, (2005). Biological effects of heavy metals. *J Environ Biol* **26**: 301-313.

Stanner, S.A., J. Hughes, C.N. Kelly and J. Buttriss (2004). A review of the epidemiological evidence for the antioxidant hypothesis, *Public Health Nutr* **7**(3): 407-22.

Valko, M., H. Morris, and M.T. Cronin, (2005). Metals toxicity and oxidative stress. *Curr Med Chem* **12**(10): 1161 – 1208.

Wang, C., X. Hu, M.L. Chen and Y.H. Wu, (2005). Total concentration and fraction of Cadmium, Chromium, Lead, Copper, Nickel and Zinc in sewage sludge form municipal and industrial wastewater treatment plants, *J Hazardous Matter* **119**: 245-249.

Wolf, G. (2005). The discovery of the antioxidant function of vitamin E: the contribution of Wenry A mattil, *J Nutr* **135**(3): 363 – 6.

Xue, W., X. Li, J. Zhang, Y. Liu, Z.H. Wang and R.J. Zhang, (2007). Effects of *Trigonella foenum-graecum* (fenugreek) extract on blood glucose, blood lipid and hemorheological properties in streptozotocin-induced diabetic rats, *Asia Pac J Clin Nutr* **16**(1): 422-426.

Kong. Res. J. 2(1) : 138-140, 2015 ISSN 2349-2694
Kongunadu Arts and Science College, Coimbatore.

THE JOURNAL OF ENTREPRENEURSHIP- A BIBLIOMETRIC STUDY

Senthilkumar, R[1*]. and G. Ulaganathan[2]
[1]Department of Library and Information Science, Kongunadu Arts & Science College (Autonomous), Coimbatore.
[2]Department of Library and Information Science, Dr.SNS.Rajalakshmi College of Arts & Science, Coimbatore.
*E.mail: kasclibrary@yahoo.com

ABSTRACT

This paper presents a bibliometric analysis of the journal "The Journal of Entrepreneurship" for the period between 2007 to 2013.The analysis cover mainly the distribution of articles volume wise, Year wise authorship pattern, geographical distribution of articles, distribution of citations, forms of documents cited, length of issues length of articles. All the studies point towards the merits and weakness of the journal which will be helpful for its further development. The study reveals that journals accounted highest number of journal citation with 1525 (51.87%) Followed by books with 798 (27.14%) and Seminar/conference Proceedings /Thesis sources 233(7.93%) Citations ,Reports 221(7.52%) citations, and Web sites with163(5.54%) citations it was seen that the majority of the authors use mostly journal articles for their article publications.

Key words: Bibliometrics, Bibliography, Citation analysis, Authorship pattern, Geographical distribution.

1. INTRODUCTION

Bibliometric analysis is the quantitative description of literature and helps in the measurement of the patterns of all forms of recorded information and their producers. It has extensive applications in the field of library and information science particularly with regards to studying the trends in a particular subject. The subject of bibliometrics was first defined by Pritchard (1969) as "the application of mathematical and statistical methods to books and other media". It involves the analysis of a set of publications characterized by bibliographic variables such as the author(s), the subject keywords and the citations. The methods of Bibliometrics (and the closely related specialism of informatics, Scientometrics and Webometrics (Hood and Wilson 2001) are used to investigate an increasing range of topics. According to Sengupta (1985) Bibliometrics is the organization classification and quantitative evaluation of publication patterns of all macro and micro communication along with their authorships by mathematical and statistical calculus. The British standard Institution (1976) defines that the word bibliometric is used to study of documents and patterns of publications in which mathematical and methods have been applied.

The current study is a bibliometric analysis of the journal "The Journal of Entrepreneurship "for the period 2007 to 2013. The Journal of Entrepreneurship is a multidisciplinary forum for the publication of articles and research and discussion of issues that bear upon and enfold the field of entrepreneurship. Topics appropriate and related to entrepreneurship include intrapreneurship, manager ship, organizational behavior, leadership, motivation, training and ethical / moral notions guiding entrepreneurial behavior. Disciplinary boundaries that straddle entrepreneurship theory and research include economics, psychology, sociology, anthropology, history, management and others. The journal of entrepreneurship is peer-reviewed Journal with ISSN 0971-3557. The journal is published by SAGE publications, Los Angeless, London, New Delhi, Singapore and Washington DC –in March and September. In this study, the articles published during the period 2007 to 2013(7 Year Issues) have been analyzed.

2. METHODOLOGY

Seven volumes (volume 16to 22 each 2 issues) containing 12 issues of The Journal of Entrepreneurship Published during the year 2007 - 2013 have been taken up for the study. The details with regard to each published article such as distribution of articles volume-wise, year wise authorship pattern, year wise geographical distribution of articles by authors, year wise distribution of citations, Forms of documents cited, length of issues, length of articles were recorded and analyzed for making observations.

Table 1. Distribution of articles volume-wise (Volume number 16-22)

Month	16	17	18	19	20	21	22	Total	volume wise %
March	10	9	10	8	9	9	10	65	48.51
September	9	10	9	12	10	10	9	69	51.49
Total	19	19	19	20	19	19	19	134	100
Volume Wise %	14.18	14.18	14.18	14.92	14.18	14.18	14.18	100.00	100

The table 1 reveals distribution of articles (volume –wise). Volume number 19 shows the highest number of total articles 20 published. The other volumes are same number of publications 19 articles.

Table 2. Year wise authorship pattern

Authorship	Year							Total	Percentage
	2007	2008	2009	2010	2011	2012	2013		
Single	14	14	10	16	12	13	10	89	66.42
Joint	5	5	9	4	7	6	9	45	33.58
Total	19	19	19	20	19	19	19	134	100.00

The above Table - Showed that out of 134 articles single author 89(66.42%) articles while the 45 (33.58%) articles were contributed by joint authors.

Table 3. Year wise geographical distribution of articles by authors

Year	Indian	Percentage	Foreign	Percentage	Total	percentage
2007	11	5.53	16	8.04	27	13.57
2008	13	6.53	13	6.53	26	13.06
2009	21	10.55	9	4.52	30	15.07
2010	13	6.53	12	6.03	25	12.56
2011	11	5.53	19	9.55	30	15.08
2012	7	3.52	23	11.56	30	15.08
2013	14	7.04	17	8.54	31	15.58
Total	90	45.23	109	54.77	199	100.00

Table 3 showed that maximum number of articles by authors in the year 2013 articles 31 (15.58%) followed by the year 2009, 2011, 2012 number of articles 30 each (15.08%) and the year2010 minimum number of articles25 (12.56%).

Table 4. Year wise distribution of citations

Year	Number of Citations	Percentage
2007	393	13.37
2008	243	8.27
2009	327	11.12
2010	332	11.29
2011	605	20.58
2012	544	18.50
2013	496	16.87
Total	2940	100.00

The above Table 4 showed that maximum number of citations 605(20.58%) produced as 2011 followed by 544 (18.50%) citations in 2012 and 496(16.87%) in the year 2013.

Table 5. Forms of documents cited

Forms of Document	Total Number of citation	Percentage
Journals	1525	51.87
Books	798	27.14
Seminar/ Conference Proceedings /Thesis	233	7.93
Reports	221	7.52
Websites	163	5.54
Total	2940	100

The table 5 above showed that majority of the contributors preferred journals as the source of information which occupied the top position with the highest number of citations 1525 (51.87%) of the 2940 total citations followed by books with 798 (27.14%) citations, Seminar/Conference Proceedings/ Thesis sources 233(7.93%) citations, Reports 221(7.52%) citations, and Websites with163(5.54%) citations. It is found that

the researchers preferred journal articles more frequently for their research work, than any other types of communication channels.

Table 6. Distribution length of issues year-wise

Year/ Month	2007	2008	2009	2010	2011	2012	2013	Total	Issue wise %
March	130	101	137	98	158	171	133	928	51.58
September	100	103	102	145	144	156	121	871	48.42
Total	230	204	239	243	302	327	254	1799	100
Year Wise %	12.78	11.34	13.29	13.51	16.78	18.18	14.12	100	

The above table 6 shows that highest number of 327(18.18%) pages was published during the year 2012 followed by the years 2011 number of pages 302 (16.78%) and 2013 with 254(14.12%) pages.

Table 7. Length of articles

Pages	Year							Total	Percentage
	2007	2008	2009	2010	2011	2012	2013		
1-5	7	7	9	8	2	5	7	45	33.58
6-10	4	1	2	2	6	2	2	19	14.18
11-15	1	7	--	3	--	--	1	12	8.96
16-20	3	2	3	4	3	3	3	21	15.67
21-25	3	2	2	1	6	3	3	20	14.93
26-30	--	--	3	2	1	4	3	13	9.70
31-35	1	--	--	--	1	2	--	4	2.98
Total	19	19	19	20	19	19	19	134	100

The table7 reveals that the majority of articles 45(33.58%) have the length of 1-5 pages followed by 21(15.67%)articles with 16-20 pages,20(14.93%) articles with 21-25 pages ,19(14.18%) articles 6-10 pages,13(9.70%) articles 26-30 pages,12(8.96%) articles 11-15 pages and remaining 4 (2.98%) articles have the length 31-35 pages.

3. CONCLUSION

Bibliometric techniques are being used for a variety of purposes like determination of various scientific indicators, evaluation of scientific output, selection of journals for libraries and even forecasting the potential of a particular field. The popularity in the adaptation of bibliometric techniques in various disciplines stimulated stupendous growth of literature of bibliometrics and its areas. The journal has published 134 articles during the period of study. The maximum number of contributions is single authors with 89(66.42%). The present study reveals that the most of the articles are published by Academic profession authors 190(95.48%). The most of the contributors are from Foreign with 54.77%% while Indian contributions are 45.23% only. The study revealed that the highest contributions were from universities with 110(55.28%). Majority of the authors preferred journals 1525(51.87%) as the source of information.

REFERENCES

Pritchard Alan, (1959). A Statistical Bibliography: An Interim Bibiliography. North-western Polytechnic School of Librarianship, London.

Hood and C.S. Wilson, (2001). Annual Review of Information Science and Technology. **34**: 3–143.

Sengupta, I.N., (1985). Bibliometrics, Informetrics, Scientometrics and Libraries: An overview Libri: International Library Review. **42**(2): 75-98.

British Standards Institution, (1976). British standards of documentation terms. BSI; London. p7.