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ANTIMICROBIAL PROPERTIES OF LANTHANUM ALUMINATE NANOPARTICLES

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ABSTRACT

The sol-gel route synthesized LA-NPs were tested for antimicrobial properties against different human pathogenic bacteria and fungi. The test organisms used were clinical isolates *viz., Streptococcus pyogenes, Staphylococcus aureus, Escherichia coli, Klebsiella nemoniae* and the human fungal pathogens like *Candida albicans* and *Trichoderma viride.* The LA- NPs achieved maximum activity against *S. aureus* compared with other three tested organisms such as *S. pyogenes, E. coli* and *K. pneumonia.* It also showed very good antimicrobial properties against studied fungi. At the concentration 1 mg/ml LA-NPs impregnated filter paper disk achieved maximum activity against human pathogen.

Keywords: Antimicrobial activity, Lanthanum aluminate nanoparticles, human pathogens.

1. INTRODUCTION

The application of nanomaterials in biotechnology merges the fields of material science and biology. Nanoparticles provide a mostly useful platform, demonstrating distinctive properties with potentially wide-ranging applications in therapeutic field (Gao *et al.,* 2004). The advancements in the area of nanoparticles technology and nanotechnology have offered an understanding and controlling of the materials at atomic and molecular levels. It has also assisted in fabricating advanced materials with added optical, electrical, magnetic and biological properties for pharmaceutical and biomedical applications (Iconaru *et al*., 2012). Nanovectors in the field of delivery are promising novel tools for controlled release of drug (Maya *et al*., 2015). Bio macromolecule external recognition by nanomaterials as artificial receptors provides a potential tool for controlling cellular and extracellular processes for numerous biological applications such as enzymatic inhibition, transcription regulation delivery and sensing. The biological application of nanoparticles depending on the core size of material providing a suitable platform for the interaction of nanomaterial with biomolecules (Hostetler *et al*., 1998). Nanomaterials have already been used for a wide range of applications both *in-vitro* and *in-vivo*. The surface and core properties of nanomaterials can be engineered for individual and multimodal applications, including biomolecular recognition, therapeutic delivery such as antimicrobial, anticancer, biosensing and bio imaging (Mrinmoy *et al.,* 2008).

In this world of emerging nanotechnology, one of the primary concerns is the potential environment impact of nanoparticles. An efficient way to estimate nanotoxicity is to monitor the response of bacteria exposed to these particles. Resistance of bacteria to bactericides and antibiotic has increased in recent years due to the development of resistant strains. Some antimicrobial agents are extremely irritant and toxic and there is much interest in finding ways to formulate new type of safe and cost-effective biocidal materials (Brayner, 2008). Earlier studies have been shown that antimicrobial formulations in the form of nanoparticles could be used as effective bactericidal materials. Recently, it has been reported that highly reactive metal oxide nanoparticles exhibit excellent biocidal active against Gram positive and Gram negative bacteria (Kim *et al*., 2007; Savithramma *et al.,* 2011; Kagan *et al*., 2002).

Bacteria are generally characterized by a cell membrane, cell wall, and cytoplasm. The cell wall lies outside the cell membrane and is composed mostly of a homogeneous peptidoglycan layer. The cell wall maintains the osmotic pressure of the cytoplasm as well the characteristic cell shape. Gram positive bacteria have one cytoplasmic membrane with multilayer of peptidoglycan polymer and a thicker cell wall (20-80 nm).Whereas gram-negative bacteria wall is composed of two cell membranes, an outer membrane and a plasma membrane with a thin layer of peptidoglycan with a thickness of 7-8 nm. Nanopartilces size within such ranges can readily pass through the peptidoglycan and hence are highly susceptible to damage (Fu *et al*., 2005;

Amna *et al*., 2015). Hence, the preparation, characterization and surface modification of nanosized particles open the possibility of formulation of a new generation of bactericidal materials (Duncan, 2011). The nanoparticles present a highly attractive platform for a diverse array of biological applications. Hence the present study focuses the antimicrobial properties of sol gel route synthesized Lanthanum Aluminate Nanoparticles (LA-NPs) against different human pathogenic bacteria and fungi.

2. MATERIALS AND METHODS

2.1. Lanthanum Aluminate Nanoparticles (LA-NPs)

Our research group already reported synthesis of LA-NPs by sol-gel method and obtained nanoparticles were characterized by X-Ray Diffraction (XRD), Scanning Electron Microscope (SEM) and Energy Dispersive Spectrum (EDS) (Gayathri and Chandar Shekar, 2015). The synthesized LA-NPs were tested for antimicrobial properties against different human pathogenic bacteria and fungi.

2.2. Test microorganisms

The test organisms used were clinical isolates *viz., Streptococcus pyogenes, Staphylococcus aureus, Escherichia coli* and *Klebsiella nemoniae.* The human fungal pathogens like *Candida albicans* and *Trichoderma viride,* which were obtained from Department of Microbiology, Raja Muthaiyah medical college, Annamalai University. The bacterial and the fungal cultures were maintained on nutrient agar medium and potato dextrose agar (PDA) medium respectively. The bacterial cultures were maintained on nutrient broth (Table I) at 37°C and fungus was maintained on Potato dextrose agar (Table. II) at 28°C.

Table 1. Composition of Nutrient Broth (NA) medium

Peptone	5.0 g
Beef extract	3.0 g
Agar	15.0 g
Distilled water	1000 ml
pH	7.0

Table 2. Composition of Potato Dextrose Agar (PDA) medium

Potato	200.0 g
Dextrose	20.0 g
Agar	15.0 g
Distilled water	1000 ml
pH	6.2

2.3. Preparation of inoculum

The gram positive bacteria *Streptococcus pyogenes* (*S. pyogenes*), *Staphylococcus aureus* (*S. aureus*) and gram negative bacteria *Escherichia Coli* (*E. coli*), *Klebsiella pneumoniae* (*K. pneumoniae*) were pre-cultured in Nutrient Broth (NB) over night in a rotary shaker at 37°C, centrifuged at 10,000 rpm for 5 min, pellet was suspended in double distilled water and the cell density was standardized spectrophotometrically (A_{610} nm). The fungal inoculums *Candida albicans* (*C. albicans*), *Trichoderma viride* (*T. viride*), were prepared from 5 to 10 day old culture grown on Potato Dextrose Agar (PDA) medium. The Petri dishes were flooded with 8 to 10 ml of distilled water and the conidia were scraped using sterile spatula. The spore density of each fungus was adjusted with spectrophotometer (A_{595}nm) to obtain a final concentration of approximately 10^5 spores/ml.

2.4. Anti-bacterial activity

The antibacterial activities of sol-gel route synthesized nanoparticles were tested against both gram positive and gram negative human pathogens by the standard disk diffusion method. In brief, different concentration nanoparticles were prepared by reconstituting with distilled water. The test human pathogens were seeded into respective medium by spread plate method 10 µL (10 cells/ml) with the 24h cultures of bacteria growth in nutrient broth. After solidification the filter paper disks (4 mm in diameter) were impregnated with different concentration of LA-NPs (0.5 mg/ml and 1mg/ml). Followed by this step, the nanoparticles impregnated filter papers were placed on test organism-seeded plates. The antibacterial assay plates were incubated at 37°C for 24h. The diameters of the inhibition zones were measured in mille meter (mm).Chloramphenicol (10 µg) used as standard for antibacterial test.

2.5. Anti-fungal activity

The antifungal activities of sol-gel route synthesized nanoparticles were tested against two pathogens by the standard disk diffusion method. In brief, the potato dextrose agar plates were inoculated with each fungal culture (10 days old) by point inoculation. The filter paper wells (4 mm in diameter) impregnated with different concentrations (0.5 mg/ml and 0.1 mg/ml) of LA-NPs. Followed by this step, different concentration nanoparticles impregnated filter paper disk were placed on test organism-seeded plates. The activity was determined after 72 hrs of incubation at 28°C. The diameters of the inhibition zones were

measured in mm. Chloramphenicol (10 µg) used as positive control.

3. RESULTS AND DISCUSSION

3.1. Antibacterial activity of LA-NPs

The sol-gel route synthesized LA-NPs were tested against clinically isolated both gram positive and gram negative human pathogenic microorganisms. The zone of inhibition was measured for both standard and LA-NPs coated filter paper and the results depicted in figure 1. It was found that LA-NPs coated filter paper disk shown maximum activity against gram positive organisms compared with gram negative organisms. At the concentration of 0.5 and 1mg/ml LA-NPs impregnated filter paper disk achieved maximum activity around 16 and 18 mm against *S. aureus* respectively**.** For LA NPs impregnated filter paper disk shown significant activity against gram negative bacteria *E. coli* around 15 and 16 mm for 0.5 and 1 mg/ml concentration.

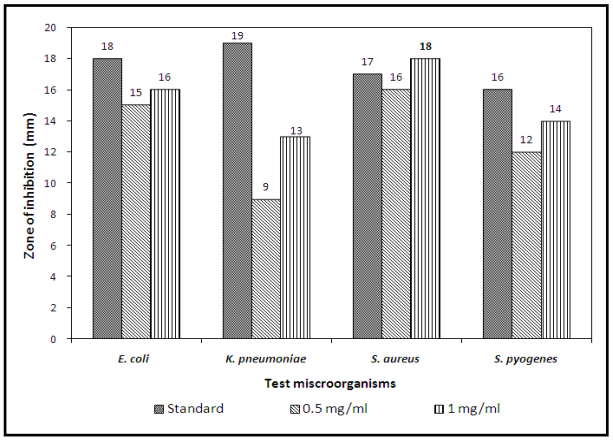

Fig. 1. Antibacterial activity of LA-NPs against pathogenic bacterium

LA-NPs impregnated filter paper disk shown significant antibacterial properties against various pathogens investigated and were compared with control. The diameter of inhibition zones increased for the test pathogen (Fig. 2). Whereas, other two clinically isolated bacteria strains of *K. pneumoniae* and *S. pyogenes* showed zone of inhibition of 9 and 12 mm at the concentration of 0.5 mg/ml and 13 and 14 mm at the concentration of 1mg/ml respectively. Whereas, standard antibiotic disk Chloramphenicol obtained 18, 19, 18 and 14 mm against *E. coli, K. pneumoniae, S. aureus* and *S. pyogenes* respectively. The sol-gel route synthesized LA-NPs showed inhibition zone against all the studied bacteria and we found that the synthesized LA-NPs have good antibacterial action against both gram positive and gram negative bacteria.

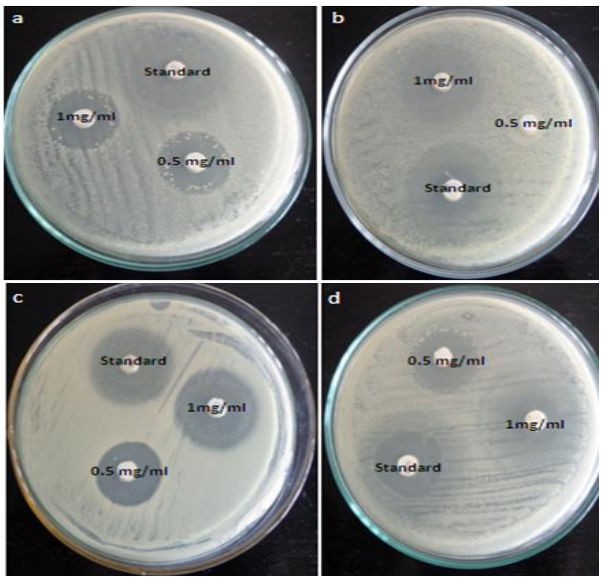

Fig. 2. Antibacterial activity of LA-NPs against human pathogenic
a) *E. coli* b) *K. pneumoniae* c) *S. aureus* and d) *S. pyogenes*

3.2. Antifungal activity of LA-NPs

The sol-gel route synthesized LA-NPs were tested against clinically isolated human pathogenic fungus by standard disk diffusion method. The zone of inhibition was measured for both standard and lanthanum aluminate coated filter paper and the zone of inhibition was shown in figure 3. It was found that lanthanum aluminate coated filter paper disk shown maximum activity against *T. viride* compared with *C. albicans*. At the concentration of 0.5 and 1mg/ml lanthanum aluminate impregnated filter paper disk achieved maximum activity around 5 and 20 mm against *T. viride* respectively**.** Whereas, lanthanum aluminate impregnated filter paper disk shown significant activity against *C. albicans* around 3 and 19 mm for 0.5 and 1 mg/ml concentration.

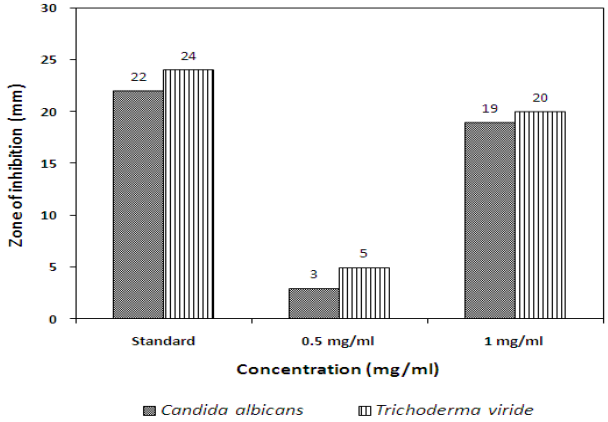

Fig. 3. Antifungal activity of LA-NPs against human pathogenic fungi

LA-NPs impregnated filter paper disk shown significant antifungal properties against test pathogens investigated and were compared with control. The diameter of inhibition zones increased for the test pathogen at the maximum concentration of 1mg/ml. whereas, standard antibiotic disk Chloramphenicol obtained 22, and 24 mm against *C. albicans* and *T. viride* respectively (Fig. 4). The present experiment clearly revealed that the sol-gel route synthesized lanthanum aluminate showed inhibition zone against the studied human pathogenic fungi and we found that the synthesized lanthanum aluminate have good antifungal action.

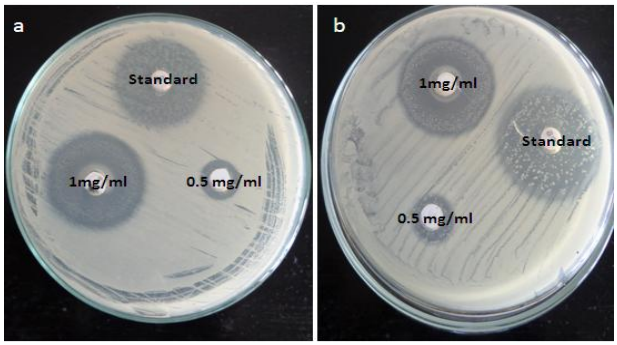

Fig. 4. Antifungal activity of LA-NPs against human pathogenic

In general, nanoparticles have the ability to anchor to the microorganisms such as bacteria or fungi cell wall and subsequently penetrate it, thereby causing structural changes in the cell membrane like the permeability of the cell membrane and death of the cell. There is formation of 'pits' on the cell surface, and there is accumulation of the nanoparticles on the cell surface (Sondi *et al.,* 2004). Developing novel antibacterial agents against bacteria strains, mostly major food pathogens, such as *Escherichia coli* O157: H, *Campylobacter jejuni*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Salmonella*, and *Clostridium perfringens*, has become utmost demand (Hafez *et al*., 2014). The inhibition of microorganisms growth reported in this present study is dependent on the concentration of LA-NPs in the disk.

Nano-sized particles exhibit varying morphologies and show significant antibacterial activity over a wide spectrum of bacterial species explored by a large body of researchers (Buzea *et al.,* 2007). The exact mechanism which nanoparticles employ to cause antimicrobial effect is not clearly known. However, various theories have been proposed on the action of nanoparticles on microbes to cause the microbial effect against human pathogenic bacteria and fungi.

4. CONCLUSION

In the present study, sol-gel route synthesized LA-NPs impregnated filter paper disk achieved maximum activity against *S. aureus* compared with other three tested organisms such as *S. pyogenes, E. coli* and *K. pneumonia.* The synthesized LA- NPs showed very good antimicrobial properties against studied fungi. At the concentration 1 mg/ml LA-NPs impregnated filter paper disk achieved maximum activity against human pathogen.

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VIRTUAL SCREENING OF *GINKGO BILOBA* FOR THERAPEUTIC POTENTIALS AGAINST PARKINSON'S DISEASE

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ABSTRACT

Parkinson's disease (PD) is a neurodegenerative disorder that affects 2% of the population older than 60 years. Monoamine Oxidase B (MAO-B) inhibitors improve the symptoms of Parkinson's disease and can delay the progress. Inhibition of MAO-B, further prevent breakdown of dopamine in the brain and reduce the motor symptoms associated with PD. *Ginkgo biloba* has a number of therapeutic properties and contains phytonutrients that helps in improvement of neurological disorders. In present study, phytonutrients of *Ginkgo biloba* namely Myricetin, Quercetin, Isorhamnetin, Kaempferol, Ginkgolides A-C, and Ginkgolide J were selected for Molecular docking against Monoamine Oxidase-B enzyme. The Molecular Docking studies were performed using Autodock 4.2 and interaction between MAO-B and compounds were analyzed. The efficiency of the compound was screened based on the binding energy existing between the protein and inhibitor. The docking studies show that the phytochemicals of *Ginkgo biloba* against MAO-B were quite effective. The potential compound can be subjected to further clinical trials and can be an alternative in the future treatment of Parkinson's disease.

Keywords: Virtual Screening, Molecular Docking, Auto Dock, *Ginkgo biloba,* Phytonutrients.

1. INTRODUCTION

The age dependent neurodegenerative diseases include Parkinson's disease and Alzheimer's disease (Arumo *et al*., 2003), which are caused by genetic and environmental influences (Jenner and Olanow, 1998) and lead to the accumulation of protein aggregation thereby causing oxidative stress and inflammation (Behl, 1999). Abnormal action of the monoamine oxidase B isoform has been associated with neurological dysfunctions including parkinson´s disorder and alzheimer´s disorder whereas the monoamine oxidase A isoform seems to be associated with psychiatric considerations including depression and cardiac cellular degeneration (Bortolato *et al*., 2008). MAO-B inhibitors are used for the treatment of Parkinson's disease and for symptoms associated with Alzheimer's disease (Binda *et al*., 2004; Terud and Langston, 1989). In the present work, our purpose was to distinguish correct poses of inhibitor in the binding pocket of monoamine oxidase B and to predict the affinity between the inhibitor and monoamine oxidase B.

In other words, in this study docking procedure describes a process by which two molecules fit together in three-dimensional space (Kitchen *et al*., 2004). Computer aided drug design is an applicable method that can study these interactions and describe significant characteristics for monoamine oxidase binding site recognition (Delogu *et al*., 2011; Harkcom and Bevan, 2007). Extracts of *Ginkgo biloba* leaves produce reversible inhibition of rat brain monoamine oxidase (MAO) (White *et al*., 1996). Mao inhibition was due to the phytonutrients present in *Ginkgo biloba*. The computer aided drug design is an attempt to study the interaction of phytonutrients with MAO-B which in turn helps to treat the symptoms of Parkinson's disease.

The use of computers to predict the binding of libraries of small molecules to known target structures is an increasingly important component in the drug discovery process (Schoichet, 2004; Koppen, 2009). There is a wide range of software packages available for the conduct of molecular docking simulations like, Auto Dock, GOLD, and FlexX (Collignon *et al*., 2011). Auto Dock 4.2 is the most recent version which has been widely used for virtual screening, due to its enhanced docking speed (Dykstra, 2007). Docking is applied to predict the binding orientation of small molecular drug candidates to protein targets, subsequently predicting the affinity and activity of the drug candidates (Goodsell, 2009; Morris *et al*., 2009). Docking is often applied to predict binding affinities of drug candidates in virtual screening experiments and in considering structure-activity relationships to prioritize synthesis of new drugs (Wu *et al*., 2003). The present study deals with the examination of the

interactions between potentials from *Ginkgo biloba* and MAO-B protein by molecular docking method in order to calculate the minimum binding energy (kcal/mol) between them. Molecular docking determines the binding affinity between the protein and ligands which aims to determine the 3D conformation and binding interactions.

2. MATERIALS AND METHODS

2.1. Protein structure

The high-resolution crystal structure of monoamine oxidase-B, co-crystalized with its irreversible inhibitor 6-hydroxy-N-propargyl-1(R)-aminoindan, was obtained from the Protein Data Bank (PDB entry code 1S3E, 1.6A° resolution). The study was carried out on only one subunit of the enzyme protein (Yelekçi *et al*., 2007) shown in **Figure 1**. The water molecules were removed during modeling. The energy minimized protein structure was included prior to docking to accommodate hydrogen atoms.

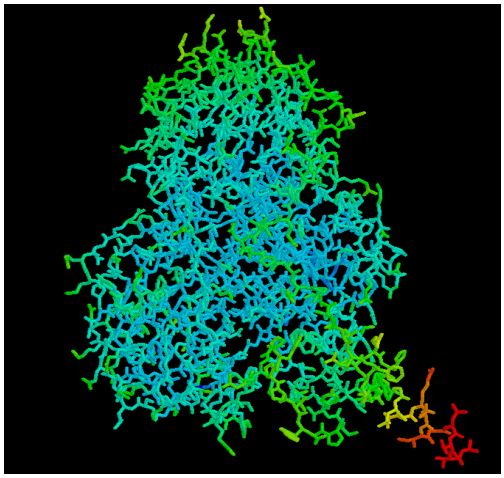


Fig. 1. 3D structure of Monoamine oxidase – B molecule (PDB ID: 1S3E)

2.2. Phytochemicals

The structures of phytochemicals namely Myricetin, Quercetin (Oyama *et al*., 1994), Ginkgolide A, Ginkgolide B, Ginkgolide C, Ginkgolide J, Bilobalide (Teris, 2002), kaempferol, Isorhamnetin (Xu *et al*., 2012) used in this study were retrieved from Pubchem compound database. The 2D structures of molecules were converted to 3D structures using Open Babel software (O'Boyle *et al*., 2011). These phytochemicals satisfied Lipinski's rule of 5 and ADME properties.

2.3. Binding site Prediction

The binding site in MAO-B was determined using Computer Atlas of Surface Topology of Proteins (CASTp) (Dundas *et al*., 2006). CASTp helps in identifying the geometric properties of protein pockets which are assumable positions on protein surface. The residues within the binding site were identified. Potential active site of protein calculated by CASTp in Fig. 2. Showed there are several pockets which fit in the role of active site.

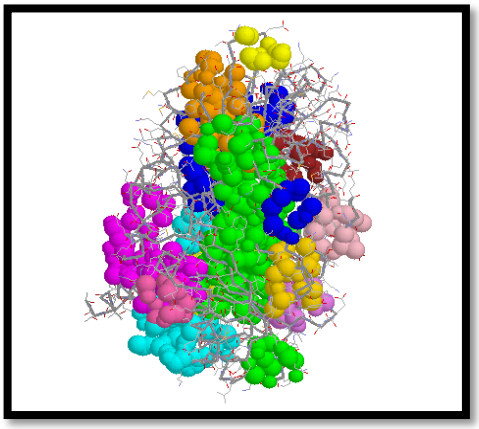


Fig. 2. Active sites predicted in the MAO-B using CASTp server

2.4. Molecular docking

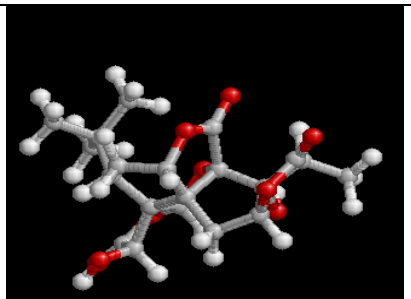
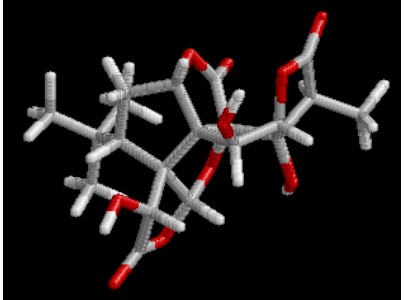
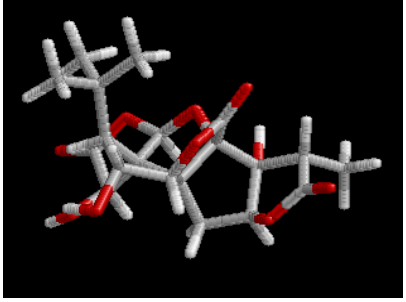
Molecular docking combined with a scoring function can be used to screen potential drugs insilico to identify molecules that are likely to bind to protein target of interest. To perform the docking model, the Auto Dock 4.2 suite molecular-docking tool was used and the methodology was followed (Gowthaman *et al*., 2008). AutoDock was employed to perform a docking simulation using a Lamarckian genetic algorithm (Morris *et al*., 1998). Auto Dock 4.0 is widely distributed molecular docking software which performs the flexible docking of the ligands into a known protein structure. The default parameters of the automatic settings were used. Each docking experiment consisted of 10 docking runs with 150 individuals and 500,000 energy evaluations. The size of the grid box is key parameter in Auto Dock. The volume of the box was fixed to 27000Å to have large search space. The Auto Dock results indicated the binding position and bound conformation of the protein, as well as hydrogen bond interactions between the protein and ligand molecule. The docked conformation which had the minimum binding energy was selected to analyze the mode of binding.

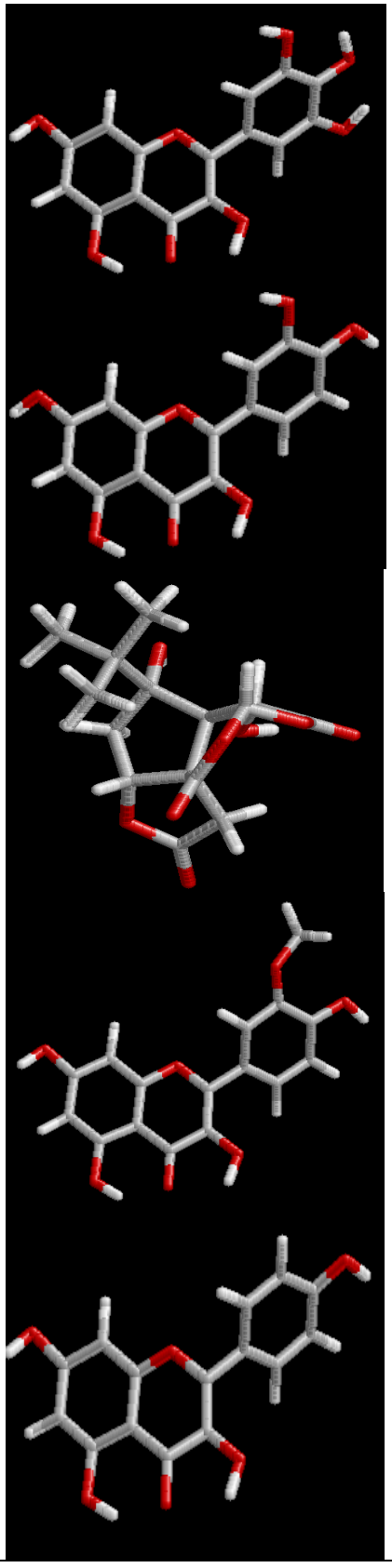
3. RESULTS AND DISCUSSION

The phytochemicals were docked using Auto dock 4.2 successfully. The interactions and binding energy of the phytochemicals are listed in Table 1. Good interactions were observed between the amino

acid residues of the protein and phytochemical molecules. The phytochemicals showed binding energy between -7.78 to -8.70 kcal/mol. The results were analyzed based on the binding energy of the complex. The number of H-bonds was calculated between atoms of protein-ligand docked complex. Quercetin and Kaempferol illustrate high affinity for the protein molecules with score of –8.70 and -8.68 shown in Fig. 3 and 4. Quercetin and Kaempferol showed six and five hydrogen bond interactions with MAO-B respectively. Quercetin illustrated interactions with GLN206, GLY434, SER59, TYR435 and TYR60 residues of the protein. Kaempferol showed five hydrogen bond interactions with SER59, TYR60, TYR188, and GLY434 residues of the MAO-B protein and bound to the active sites. The compounds were bound to the active site of the MAO-B receptor.

Table 1. Docking results of *Ginkgo biloba* phytochemicals with MAO-B protein.

Phytochemicals	**Compound structure**	**Binding energy kcal/mol**	**Hbonds**	**Residues interacting with ligand (Hbonds)**
Ginkgolide A		-8.33	2	SER59, LYS296
Ginkgolide B		-8.65	4	SER59, LYS296, GLN206, TYR398
Ginkgolide C		-7.78	3	SER59, LYS296, TYR398
Ginkgolide J		-8.57	3	LYS296, TYR398, TYR60

Myricetin		-8.06	3	ILE14, TYR398, TYR60
Quercetin		-8.70	6	GLN206, GLY434, SER59, TYR435, TYR60
Bilobalide		-8.42	2	SER59
Isorhamnetin		-8.50	2	TYR398, TYR60
Kaempferol		-8.68	5	SER59, TYR60, TYR188, GLY434

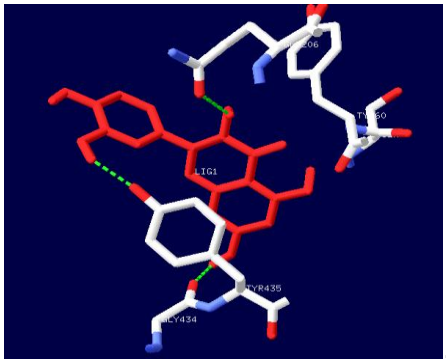


Fig. 3a. Quercetin interacting with residues of MAO-B protein

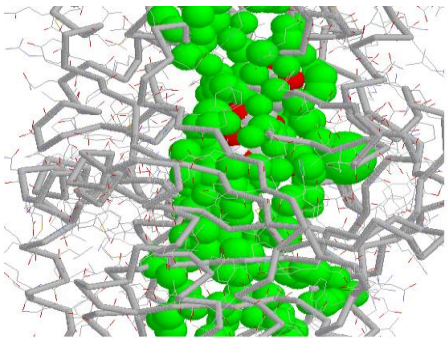


Fig. 3b. Quercetin bound to the active site of protein molecule.

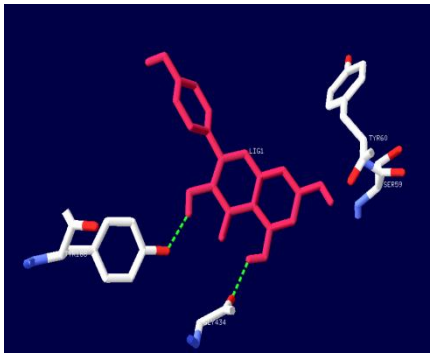


Fig. 4a. Kaempferol interacting with residues of MAO-B protein.

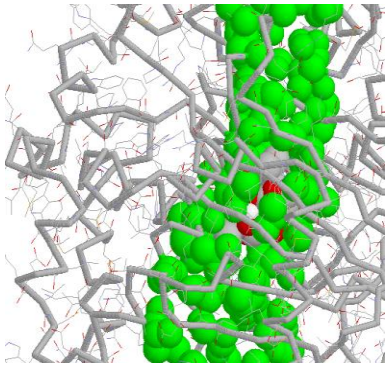


Fig. 4b. Kaempferol bound to the active site of protein molecule.

The screening of phytochemicals from *Ginkgo biloba* against MAO-B is carried out using molecular docking methods. Screening of Phytonutrients compounds showed the binding affinity towards MAO-B receptor. The Quercetin and Kaempferol were screened with least binding energy of – 8.70 and -8.68 and were selected as Lead molecule. The molecular docking of the two compounds showed the binding mode and interaction energy. H-bond pattern was analyzed and confirmed the inhibition of MAO -B target and show the molecular activity of phytochemicals. This work based on Insilico studies, concluded that Quercetin and Kaempferol possess better activity against MAO-B. Further In vivo studies on these compounds can be done to confirm the inhibition and used in the treatment of Parkinson's disease.

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MICROWAVE ASSISTED SYNTHESIS AND STRUCTURAL CHARACTERIZATION OF NICKEL OXIDE NANOPARTICLES

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ABSTRACT

Nickel oxide (NiO) nano-particles were produced via a simple microwave method from the $Ni(OH)_2$ precursor, which was obtained by slow drop-wise addition of 0.1M sodium hydroxide to 0.1M nickel nitrate. The mixture was vigorously stirred until the pH reached 7.2. The mixture was then irradiated with microwave to deposit $Ni(OH)_2$ at a better precipitation rate. Drying the precipitate at 320°C resulted in formation of NiO nanoparticles. High Resolution Transmission Electron Microscope (HRTEM), Scanning Electron Microscope (SEM) and X-ray diffraction (XRD), employed for the structural characterization of the as-prepared NiO nanoparticles, revealed their good crystallinity and high-purity. Microwave irradiation increased homogeneity and decreased the mean particle size of the produced NiO particles.

Keywords: NiO, microwave synthesis, nanoparticles, HRTEM, SEM, XRD.

1. INTRODUCTION

Nano-particle oxides of transition metals have attracted materials scientists. These materials have exceptional properties which stimulate many advanced applications (Duran *et al*., 2003; Wang *et al*., 2005 Mazaheri *et al*., 2008). Nano-structured nickel oxide is a prominent example having a large exciton binding energy and a wide band gap ranging from 3.6 to 4.0eV.4,5 This p-type semiconductor can be used in optical, electronic, catalytic and super-paramagnetic devices like transparent conductor films, gas sensors, alkaline battery cathodes, dye-sensitized solar cells and solid oxide fuel cells (SOFC) (Bhadur *et al*., 2008; Sato *et al*., 1993). Versatile methods such as sol–gel (Ghosh *et al*., 2006; Wu *et al*., 2007), chemical precipitation (Bhadur *et al*., 2008; Bahari Molla Mahaleh *et al*., 2008) and anodic arc plasma method (AAPM) (Hongxia *et al*., 2009) have been used to produce nanomaterials. Microwave heating has such advantages as high-efficiency, nanoparticle rapid-formation, narrow crystallite size distribution and agglomeration decrease when compared to the conventional methods (Krishnakumar *et al*., 2009). Microwave methods apply electromagnetic waves having 0.001 to 1m wavelength to accelerate the chemical reaction of interest. These wavelengths correspond to frequencies between 0.3 to 300GHz. Synthesis via microwave routes is simple, energy efficient, time saving and produce great of samples (Krishnakumar *et al*., 2009). Production of nickel oxide nanoparticles by microwave chemical synthesis, their morphological characterization and their structural study are discussed in this paper.

2. MATERIALS AND METHODS

2.1. Preparation of NiO nanoparticles

Microwave synthesis of NiO nanoparticles comprised three stages: (1) formation of $Ni(OH)_2$ precursor, (2) microwave irradiation of $Ni(OH)_2$ and (3) annealing of $Ni(OH)_2$ to convert into NiO. $Ni(OH)_2$ precursor was obtained by drop-wise slow addition of 0.1M NaOH to 0.1M $Ni(NO_3)_2$ while vigorous stirring of the solution continued until the pH reached 7.2. The mixture was then irradiated by microwave (2.45GHz, 900W, SAMSUNG) until a dry green precipitate formed. Simultaneous thermal analysis (TG-DTA) was carried out using (Universal V4.5A TA Instrument) to determine the $Ni(OHs)_2$ to NiO conversion temperature under air. After determining the temperature of nickel hydroxide to nickel oxide conversion by thermal analysis, the oven-dried cake was heated up to 320ºC for 1hour to form dark grey particles. The resulting powder was filtered and washed several times with distilled water and finally with ethanol to remove the residual by products.

2.2. Characterization of the prepared nanoparticles

X-ray diffraction (X'per PRO model) was used for structural study and characterization of the sample. Phase purity of the initial powder was also investigated by XRD. Morphological study was carried out by Scanning Electron Microscope (HITACHI Model S-3000H). High Resolution

Transmission Electron Microscope (Jeol Gem Model) was used to analyse the particle size of the NiO nanoparticles.

3. RESULTS AND DISCUSSION

3.1. Thermal Analysis

Fig. 1 shows TG-DTA curves of the $Ni(OH)_2$ precursor. It can be seen that two endothermic reactions take place between ambient temperature and 600°C in the sample $Ni(OH)_2$. Both reactions accompanied the mass reduction due to H_2O removal from the powder, to form NiO as the end product (Kim *et al.,* 2006).

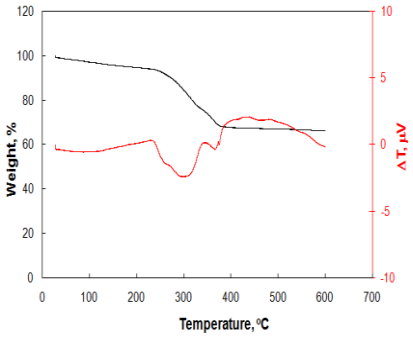

Fig. 1. TG-DTA Curves of $Ni(OH)_2$

3.2. XRD analysis

XRD pattern (Fig. 2) confirms the formation of nickel oxide (JCPDS card No. 22- 1189). No other components were detectable in the final product. The mean crystallite size was calculated by application of the Debye-Scherer equation, $D = K\lambda / \beta \cos\theta$, where, θ is Bragg diffraction angle, K is Blank's constant, λ is the source wavelength (1.54), and β is the width of the XRD peak at half maximum height, is 19 nm (Needham *et al.,* 2006).

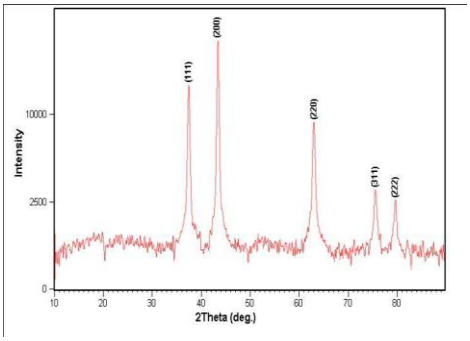

Fig. 2. XRD pattern of NiO nanoparticles

3.3. SEM Analysis

Aggregated particles around 50 to 300nm in diameter are observable in the SEM images (Fig. 3). Their creation may be due to the influence of the interfacial energies and intraparticle magnetic interactions.

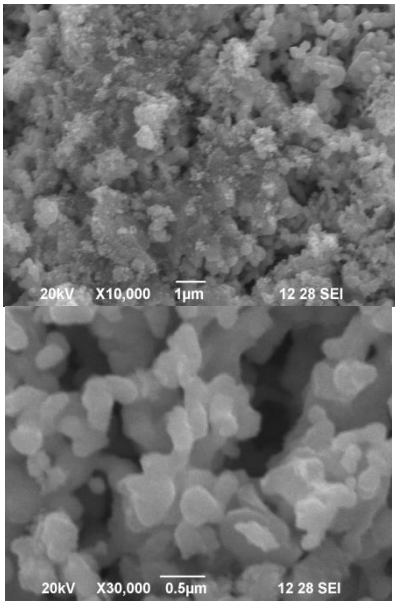

Fig. 3. SEM images of NiO nanoparticles

3.4. HRTEM Analysis

HRTEM images are shown in Fig. 4. They exhibit NiO nanoparticles having mean crystallite size of ~20nm.

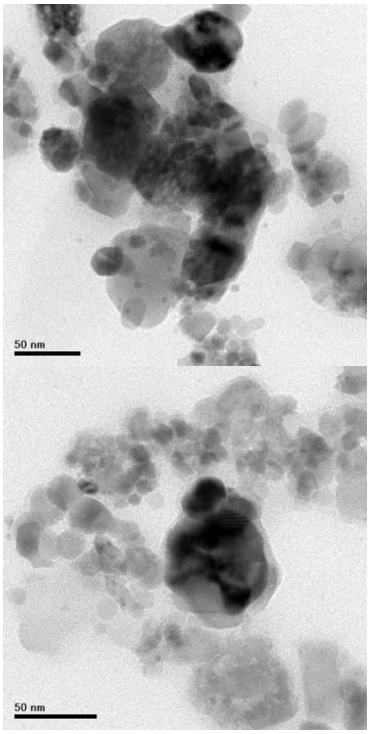

Fig. 4. HRTEM images of NiO nanoparticles

4. CONCLUSION

Highly-crystallized pure nickel oxide nanoparticles with a mean crystallite size of around 20 nm were synthesized by a microwave chemical approach. Morphology of the produced sample showed well-shaped homogenously crystallized particles. Microwave irradiation has been found to be efficient in speeding up of the production rate and reduction of the size of particles.

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ON *gα-FUZZY CLOSED SETS IN FUZZY TOPOLOGICAL SPACES

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ABSTRACT

In this paper we introduce the concept of *gα- fuzzy closed sets in fuzzy topological spaces and study some of its properties.

Keywords: *gα- fuzzy closed sets.

1. INTRODUCTION

Levine introduced generalized closed sets (Levine, 1970) in topological spaces. The concept of fuzzy closed set (Chang, 1968) is an important role in fuzzy topological spaces. The concept of gα-closed sets (Maki *et al.,* 1993) in a topological space was introduced.

Throughout this paper X and Y are represents fuzzy topological spaces. For a fuzzy set A of a topological spaces X, the notations cl(A), Int(A) and 1-A will respectively stand for the fuzzy closure, fuzzy interior and fuzzy compliment of A.

2. *gα- FUZZY CLOSED SETS IN FUZZY TOPOLOGICAL SPACES

DEFINITION 2.1 (Balasubramanian and Sundram, 1997)

Let X be a fuzzy topological space. A fuzzy set p in X is called fuzzy generalized -closed if cl(p)≤ q, whenever p≤ q and q is fuzzy open.

DEFINITION 2.2 (Devi and Bhuvaneswari, 2006)

Let X be a fuzzy topological space. A fuzzy set p in X is called fuzzy gα-closed if αcl(p)≤ q, whenever p≤ q and q is fuzzy α-open.

DEFINITION 2.3

A fuzzy set p in X is called *gα-fuzzy closed if cl(p)≤ q, whenever p≤ q and q is fuzzy gα-open.

THEOREM 2.4

Every *gα-fuzzy closed set is fuzzy g-closed.

PROOF

Let p≤ q and q is fuzzy open. But every fuzzy open set is fuzzy gα-open. Since p is *gα-fuzzy closed, cl(p)≤ q and q is fuzzy gα-open. Therefore p is fuzzy g-closed.

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

EXAMPLE 2.5

Let X = {a, b, c}. Define the fuzzy sets A,B,C : X [0, 1] as follows.

A(a) = 0.2 B(a) = 0.6 C(a) = 0.3

A(b) = 0.3 B(b) = 0 C(b) = 0.2

A(c) = 0.7 B(c) = 1 C(c) = 1

Consider the fuzzy topology τ = {0, 1, C}. Here A and B are fuzzy g-closed set. but not *gα-fuzzy closed set.

THEOREM 2.6

If A and B are *gα-fuzzy closed set in X, then A∨ B is a *gα-fuzzy closed set in X.

PROOF

Assume that A and B are *gα-fuzzy closed set in X. Let q be a fuzzy gα -open set in X such that A ≤ q and B ≤ q . Then A∨ B ≤ q. Since A and B are *gα-fuzzy closed cl(A) ≤ q and cl(B) ≤ q . Therefore

$$cl(A \vee B) = cl(A) \vee cl(B)$$
$$\leq q \vee q$$
$$= q.$$

Implies cl(A∨ B) ≤ q. Hence A∨ B is *gα-fuzzy closed set in X.

THEOREM 2.7

Let A is *gα-fuzzy closed set in a fuzzy topological space X, and A ≤ B ≤ cl(A), then B is *gα-fuzzy closed set in X.

PROOF

Let q be a fuzzy gα-open set such that B≤ q. Then A ≤ q, since A is *gα-fuzzy closed set in X, cl(A)≤

q. Now B≤ cl(A) implies cl(B)≤ cl(cl(A)) = cl(A) ≤ q. Hence B is *gα-fuzzy closed set in X.

THEOREM 2.8

Let X be a fuzzy topological space. A fuzzy set A of X is *gα-fuzzy open if and only if B≤ Int(A), whenever B is fuzzy gα -closed set and B ≤ A.

PROOF

Let A be a *gα-fuzzy open set and B is fuzzy gα-closed such that B ≤ A implies 1-B ≥ 1-A is *gα-fuzzy closed. So cl(1-A) ≤ 1-B implies (1-cl(1-A)) ≥ (1-(1-B)) = B. But (1-cl(1-A)) = Int(A). Thus B≤ Int(A).

Conversely, suppose that A is fuzzy set such that B≤ Int(A), whenever B is fuzzy gα -closed set and B ≤ A. We show that 1-A is *gα-fuzzy closed set. Let 1-A ≤ B, where B is fuzzy gα-open. Since 1-A ≤ B implies that 1-B ≤ A. By assumption that we must have 1-B ≤ Int(A) or 1-Int(A) ≤ B. Now 1-Int(A) = cl(1-A) which implies that cl(1-A) ≤ B and 1-A is *gα-fuzzy closed set.

THEOREM 2.9

Let A is *gα-fuzzy open set in a fuzzy topological space X and Int(A) ≤ B ≤ A, then B is *gα-fuzzy open set in X.

PROOF

Given that Int(A) ≤ B ≤ A, we have 1-A≤ 1-B ≤ 1-Int(A). Since A is *gα-fuzzy open in X, 1-A is *gα-fuzzy closed in X and so by theorem 2.7, 1-B is *gα-fuzzy closed in X. Hence B is *gα-fuzzy open in X.

THEOREM 2.10

Let X be a fuzzy topological space and gα-f-open(X) stand for the family of all gα-fuzzy open set of X and gα-f-closed(X) stand for the family of all gα-fuzzy closed set of X. If every fuzzy subset of X is a *gα-fuzzy closed set then gα-f-open(X) = gα-f-closed(X).

PROOF

Let us assume that every fuzzy set p is *gα-fuzzy closed set in X. Let p∈ gα-f-open(X). Since p ≤ p and p is *gα-fuzzy closed set, we have cl(p) ≤ p, but p ≤ cl(p). Therefore cl(p) = p implies p is gα-f-closed(X). Therefore

gα-f-open(X) ⊆ gα-f-closed(X)…….**(1)**

Assume that p is gα-f-closed(X) then 1-p is gα-fuzzy open. By(1) gα-f-open(X) ⊆ gα-f-closed(X). Implies 1-p is gα-f-closed(X) implies p is gα-f-open(X). Hence

gα-f-closed(X) ⊆ gα-f-open(X)…….**(2)**

From **(1)** and **(2)** we get gα-f-open(X) = gα-f-closed(X).

REMARK 2.11

A and B are *gα-fuzzy closed set, but A ∧ B is not *gα-fuzzy closed set.

It can be seen by the following example.

EXAMPLE 2.12

Let X = {a, b, c}. Define the fuzzy sets A,B,C : X [0, 1] as follows.

A(a) = 0.7	B(a) = 0.3	C(a) = 0.3
A(b) = 0.8	B(b) = 1	C(b) = 0.2
A(c) = 1	B(c) = 1	C(c) = 1

Consider the fuzzy topology τ = {0, 1, C}. It is clear that A and B are*gα-fuzzy closed set. But A ∧ B is not a *gα-fuzzy closed set.

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VARIATION IN SEED CHARACTERS OF *TERMINALIA CHEBULA* RETZ. FROM THE SOUTHERN WESTERN GHATS OF TAMIL NADU

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ABSTRACT

Seed is an important aspect of a plant's life history. The successful regeneration of a plant in its habitat and the ability of the plant to disperse across ecosystems depend upon the seed. The number of seeds produced and the size of the seed are factors which affect the survival of a plant. The size of the seed determines the amount of reserve food available to the developing seedlings. In stressful environments, larger seeds may have a higher establishment rate, as they may provide more reserve food for the seedlings. From the *Terminalia chebula* populations in the Southern Western Ghats of Tamil Nadu, 100 seeds each were collected from twelve populations. The seed weight, seed length and seed width were noted individually. The seed characters were noted and compared between the populations. The mean values were also calculated and compared. The inter-population variation was studied with respect to the seed characters of *Terminalia chebula.*

Keywords: *Terminalia chebula,* Southern Western Ghats, seeds.

1. INTRODUCTION

Seed size is an important aspect of the reproductive biology of a plant. It is traditionally considered that seed mass within a species is a remarkably constant characteristic (Harper *et al*., 1970; Silvertown, 1981). Schaal (1980) has shown that there is considerable variation in the seed mass between the individuals of a species or the seed mass of an individual plant can vary greatly (Schaal, 1980). It has also been recognized that seed size is correlated with habitat (Salisbury, 1942). Seed size affects the seedling biomass. Usually the seedlings from larger seeds are larger than those from small seeds, especially in the early stages of growth (Schaal, 1980).

Evolution has led to variation in the seed weight between or within plant species and populations. The variation in seed size and weight are a means to produce a large number of seeds which can ensure the fitness of the plant populations. A greater allocation of the resources from the mother plant also ensures a greater chance of establishment of the resulting seedling (Zhang, 1998).

There is however, a *quid pro quo* in that the massive production of seeds and allocation of resources to individual seeds is dependent on the resources available with the maternal plant. Thus the compromise between the two different strategies results in the variation in the seed weights even within a species. Various biological and environmental factors play a crucial role in determining the seed weight. Thus seed weight is dependent on the genetic constitution of the maternal plant and the environmental and evolutionary processes operating in the environment (Zhang, 1998).

Plant species in closed or dry habitats produce larger seeds than those in open or moisture-rich habitats (Salisbury, 1942; Herbert, 1972). The seed weight also varies with the height, growth, form and mode of dispersal (Zhang, 1998). Therefore the study of the relationship between seed characters and other traits in natural populations is important (Sivakumar *et al*., 2002).

This study investigated the variation in seed characters in natural populations of *Terminalia chebula* of the Southern Western Ghats in Tamil Nadu. Therefore the seed length, seed width and seed mass (seed weight) of the above species was investigated.

2. MATERIALS AND METHODS

2.1. Plant material

Terminalia chebula Retz. is a tropical tree, 15-20m high, which has yellow-white flowers. The fruit is a dry drupe. It is widely distributed in the greater part of India and Burma in mixed deciduous

forests of comparatively dry types. It grows well in laterite, clayey and sandy soils. In peninsular India, it is found in mixed deciduous forests to dry deciduous forests and extends up to an elevation of 3000'. It survives well with the temperature between minimum 30° - 60° F. and 100° - 180° F and rainfall from 30" to 130". It is fairly hardy against frost as well as drought. It withstands fire well and shows good recovery from burning. The fruits, known as myrobalans, contain 3.5% chebulic acid, 37% fatty oil, 27-39% tannin and ellagic acid. The drug is highly astringent (Keys, 1976). The fruits are used for their astringent, anti-diarrhoeal and haemostatic properties.

2.2. Seed collection and data analysis

Seeds of *Terminalia chebula* were collected randomly from natural populations and labeled. The seeds were dried in shade in the lab. One hundred seeds were taken randomly from each population to represent the population. Two collections were made at Chanan Parai and Veerapuli forests from two different populations. The seed length and width were measured using a Vernier calliper and the seed weight was measured using an electronic balance (SHIAMDZU Model BL- 6205). The data was tabulated and analyzed.

3. RESULTS AND DISCUSSION

The results of the study are given in Table 1-4 and Fig. 1-3.

3.1. Seed length

The maximum seed length among all the populations was observed to be 3.98 cm (S1) and the minimum seed length was 1.27 cm (S10). The average seed length was found to be maximum at 3.21 am in Chanan Parai I population (S1) and the average seed length was minimum in Kadukkatheri population (S9) (2.73). The mean seed lengths of the populations varied from 2.73 cm to 3.21 cm although individual seeds showed a much higher variation. However as a population the mean seed weight does not show much variation.

3.2. Seed width

The maximum average seed width was 1.94 cm in Chanan Parai I population (S1) and the minimum average seed width was 1.48 cm represented by two populations (Courtallam (S5) and Kadukkatheri, Courtallam (S9). The maximum seed width was observed in two populations (2.94 cm width at S6 and S10). The minimum seed width was observed in Mylar with seed width of 1.00 cm. The mean seed width too shows a variation of 1.48cm to 1.94 cm. The range of variation too is not very high for the seed width. Therefore the mean seed width too does not show much variation.

3.3.Seed weight

The average maximum seed weight was 3.66g at Chanan Parai II (S2) and the minimum average seed weight was 2.91g at Kadukkatheri (S9). The minimum seed weight was 1.12 g at Courtallam (S5). The maximum seed weight was 6.62 g at Chanan Parai II (S2). The mean seed weight varied from 3.15g to 3.66 g although individual seeds did show quite a large variation. When considered as a group however, the variation between the groups is not significant.

There is variation in the seed sample collected from the given area. However, the results of the ANOVA show that the variation in the seed length, seed width and seed weight are not statistically significant. This could be because the seeds have been collected over a small geographical area.

The difference in seed size may have important ecological implications. The variation in seed mass within a species may affect the seed germination (Weis, 1982) and the germination rate (Zhang and Maun, 1990). Large seeds may have more reserve food which could enable them to have a greater percent germination than smaller seeds (Hendrix, 1984). On the other hand, smaller seeds may germinate more quickly than larger seeds. They may be better able to exploit the given conditions in an environment and may thus have a competitive edge over larger seeds (Howell, 1981). Seedlings from smaller seeds, particularly of fast-growing species, would be able to cope with mild drought by morphogenetic and physiological plastic response in a better way than those from large seeds. However, seedlings from large seeds had greater survival than those from smaller seeds under intense water stress. (Ekta and Singh, 2004).

For a plant, decreased seed weight may be a disadvantage as small seeds are often associated with lower germination percentage and smaller seedlings would lead to decreased chances of seed germination and seedling establishment (Stanton, 1984; Krannitz *et al.*, 1991). However, Stamp (1990) found that with increase in the size of the seed, there is a decrease in the seed germination rate. Hendrix (1984) reported that the increase or decrease in rate of germination with increase in seed size was dependent on the environmental conditions.

Several studies have documented the variation in seed weight both within and between plant populations (Hawke and Maun, 1988; Michaels *et al*., 1998, Zhang and Hamill, 1996; Cordazzo, 2002). Populations with different seed weights are expected to have evolved under different selection pressures (Westoby *et al*., 1990; 1992). Moreover, seed traits do not evolve in isolation but are shown to be correlated with other plant traits such as plant height and growth form (Mazer, 1989; Leishman *et al*; 1995). Variability studies are the prerequisite for genetic improvement of any tree species under various agro climatic conditions, (Sharma *et al.,* 1994). In case of *Grewia optiva,* the seed length and the 100 seed weight were found to be the best predictors of germination (Tyagi *et al.,* 1999).

Table 1. Seed characters in *Terminalia chebula* seeds.

Characters	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12
Avg. seed length (cm)	3.21± 0.77	3.00± 0.90	2.98± 0.72	2.96± 0.54	2.90± 0.87	3.01± 0.6	3.04± 0.67	2.92± 1.03	2.73± 0.4	2.88± 1.61	2.94± 0.91	2.93± 0.81
Range for seed length	2.49 - 3.98	2.12 - 3.90	2.34 - 3.70	2.42 - 3.43	2.14- 3.77	2.41 - 3.56	2.37 - 3.67	2.12 - 3.95	2.34 - 3.13	1.27 - 2.65	2.14 - 3.85	2.12 – 0.3.67
Avg. seed breadth (cm)	1.94± 0.96	1.64± 0.54	1.57± 0.45	1.69± 0.64	1.48± 0.42	1.64± 1.3	1.57± 0.3	1.65± 0.48	1.48± 0.36	1.59± 3.35	1.55± 0.77	1.56± 0.56
Range for seed breadth	1.35 - 2.90	1.10 - 2.10	1.22 - 2.02	1.12 - 2.33	1.06 - 1.75	1.16 - 2.94	1.26 - 1.87	1.17 - 2.10	1.12 - 1.73	1.90 - 2.94	1.31 - 2.32	1.00 - 1.95
Avg. seed weight (g)	3.25± 1.66	3.66± 2.96	3.35± 1.91	3.15± 2.24	3.15± 1.27	3.46± 1.93	3.47± 1.80	3.29± 1.53	2.91± 1.68	3.28± 1.66	3.21± 3.24	3.33± 1.95
Range for seed weight	1.94 - 4.91	1.36 - 6.62	2.15 - 5.26	1.41 - 5.39	1.12 - 4.42	1.53 - 4.97	1.67 - 4.65	1.76 - 4.77	1.23 - 4.59	1.90 - 4.94	1.82 - 6.45	1.38 - 4.87

S1- Chanan Parai I, Karayar; S2 - Chanan Parai II, Karayar; S3 - Talaianai, Manimuthar; S4 - Below Manjolai; S5 - Courtallam; S6 - Old Courtallam; S7 - Veerapuli I; S8 - Veerapuli II; S9 - Kadukka theri, Courtallam; S10 - Foothills of Kadukka theri, Courtallam; S11 – Servalar; S12 – Mylar.

Table 2. Results of ANOVA for seed length.

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	14.39304	11	1.308458	7.22926	4.98E-12	1.796696
Within Groups	215.0218	1188	0.180995			
Total	229.4148	1199				

Table 3. Results of ANOVA for seed width.

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	16.15565	11	1.468696	18.45258	2.18E-34	1.796696
Within Groups	94.55643	1188	0.079593			
Total	110.7121	1199				

Table 4. Results of ANOVA for seed weight.

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	39.715	11	3.610454	3.370128	0.000132	1.796696
Within Groups	1272.717	1188	1.071311			
Total	1312.432	1199				

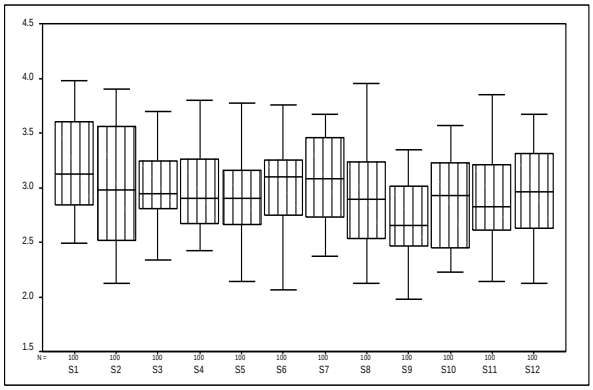

Fig.1. Boxplot of mean seed length in *Terminalia chebula* from the different populations

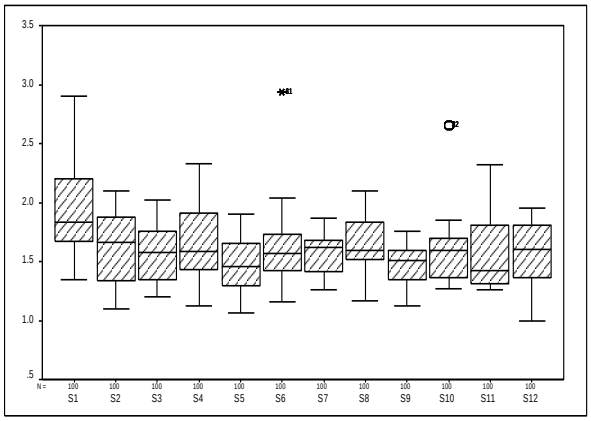

Fig. 2. Box plot for mean seed width in *Terminalia chebula* from the different populations

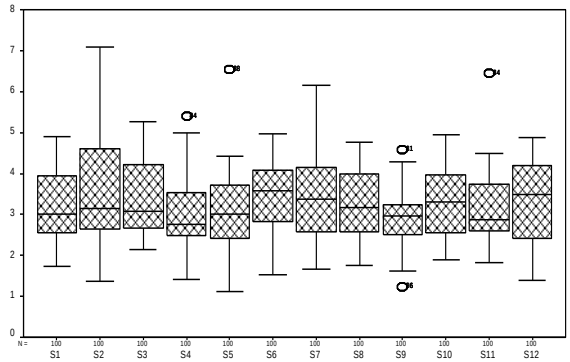

Fig. 3. Box plot of mean seed weight in *Terminalia chebula* from the different populations

4. CONCLUSION

Apart from the variations in the seed characteristics such as seed size, seed length, seed breadth and seed weight a number of other factors too play a crucial role in the establishment of a seed. These include ecological factors such as seed dispersal, seed dormancy and viability, seed predation and the ability of a seed to uptake water. The requirements of a seed may also vary from site to site (Winn, 1985). Thus the variation in seed size is of great ecological significance in the establishment and maintenance of plant populations. The seed also plays an important role in the perpetuation of populations in harsh environments which in turn are important to determine the evolutionary success of a species.

Further studies should be conducted on a wider scale to understand the variation in seed characteristics of *Terminalia chebula.*

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BRYOPHYTES, THE IGNORED MEDICINAL HERBALS OF THE BIOLOGICAL WORLD - A SEARCH AT NEYYAR WILDLIFE SANCTUARY, TRIVANDRUM, KERALA

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ABSTRACT

Currently, the medicinal potential of bryophytes has received immense value in pharmaceutical industries and many phytochemicals have been isolated, characterized from this group. Native North Americans and Chinese are in forefront in bryological research compared to India. Traditional and ethnic uses of Indian bryophytes with description and illustration were seen in *Hortus Malabaricus*. Information about the medicinal use of bryophytes in terms of microbicidal, anti-inflammatory and antitumour are traced in developed countries. Despite, the long history of medicinal bryophytes used by tribal and local peoples their significant utilization in medicines is still scanty. Present report is an attempt to create awareness about this group of plants from Neyyar Wild Life sanctuary, Trivandrum, Kerala.

Keywords: Bryophytes, Kerala, *Hortus malabaricus.*

1. INTRODUCTION

Bryophytes are the ancient terrestrial green spore-forming small imaged plants with amphibian mode of life cycle. The evidence of similarity of the present-day liverworts with the first land plant fossil spores record almost 400 million years ago (Kaur *et al*.,2010). They are categorized between thallophytes and the pteridophytes. The group comprises 14000 species of bryophyta, 6000 of liverworts, 300 hornworts and 7700 moss species (Asakawa, 2012; Meenu Krishnan *et al*., 2014).

Phytochemicals are secondary metabolites produced by the plants with diverse biological potentialities. Botanical products are gaining significance due to their microbicidal activity against pathogens. Ethnically, bryophytes have been trailed as medicinal in traditional healers. They are employed for treatment of many skin disorders and wounds. Diverse kinds of biological activities are reported from bryophytes (Asakawa, 2003). Traditionally, Chinese initiated this sort of works more than 400 years ago. For instance, *Polytrichum* and *Fissidens* species were used as diuretic and hair growth stimulating drugs in China (Asakawa, 2003). North American Indians used *Polytrichum juniperinum*, *Bryum, Mnium* and *Philonotis* mosses to heal burns, bruises and wounds (Ilhan *et al*., 2006). Many others show microbicidal effects against fungi and bacteria (Dülger *et al*., 2009). Liverwort like *Marchantia* exhibited antifungal, antibacterial and antitumour activity (Veljic *et al*., 2010). It has also been shown that *Ptilidium pulcherrimum* have antibacterial and antifungal activity (Pejin and Pristov, 2012; Veljic *et al*., 2010).

Flavonoids, phenolics, monoterpenes and sesquiterpenes were proven phytochemicals with medicinal potentialitiesetc. The documented bryophyte chemistry opens many avenues in the field of biological research that can be used to establish future medicine. Knowledge of the bryophyte chemistry has recently expanded at an logarithmic phase. For example 49 monoterpenes, 389 sesquiterpenes, 112 diterpenoids, 69 steroids and several other saponins from liverworts were reported by various bryologists. Despite that it was estimated that only 6% of the species have been investigated chemically. The limitations are difficulty of collecting sufficient pure plant material for chemical studies and its identification (Lubaina *et al*., 2014). Some complex lipids and fatty acids have been reported from the mosses which change in response to environmental conditions. Asakawa *et al*. (2012) have studied about 1000 liverworts species from different parts of the world with respect to their chemistry, pharmacology and application as cosmetics, medicinal or agricultural drugs. They suggested that most of the hepaticeae contain lipophilic mono-, sesqui-, diterpenoids, aromatic compounds (bibenzyls, benzoates, cinnamates, long chain alkyl phenols, naphthalenes, phthalides, isocoumarins), and acetogenins which constitute the oil bodies (Alam *et al*., 2012**;** Alam *et al*., 2015**)**. The biological activities of bryophytes are mainly due to the presence of these compounds

(Dulger *et al*., 2009; Huai *et al*., 2010). Some species of Hepaticae cause allergic contact dermatitis (*Frullania* sp.) and allelopathy. The allergy inducing substances are sesquiterpene lactones (+)-frullanolide and (-)- frullanolide and their related α-methyl-γ-butyrolactones which has been isolated from *Frullania dilatata* and *F. tamarisii* respectively. Frullanolide, the best known constituents but the related lactones, constunolide are also reported. The liverwoet *Chiloscyphus pallescens* proved to be of interest as it contained a large amount of sesquiterpenoid ketol, Chiloscypholone, whereas *Conocephalum conicum* contain large amount of conocephalenol. Xie and Lou (2009) reported sesquiterpenoids namely isobicyclogermacremal, lepidozenal and vitrenal from *Lepidozia vitrea*. The first representative of large group of aromatic and phenolic compounds of bibenzyls derivatives is lunularic acid isolated by Sabovljevic *et al*., (2001) from *Lunularia cruciata*. This compound was also reported from several other liverworts in small amounts.

Many bryophyte species in fact manufacture broad-range antibiotics and are employed in dressings, diapers production, and other human medicinal applications in various parts of the world such as Asia, Germany, Brazil, England, China, and India. Occurrence of unique odors is one such hint which confirmed the presence of exclusive and potentially pharmaceutically important chemicals in bryophytes. This is particularly spot on for liverworts e.g. *Isotachis japonica, Geocalyx graveolens* has a turpentine-like smell, *Leptolejeunea* and *Moerckia* are specifically aromatic,62 species of *Solenostoma* scent like carrots, *Lophozia bicrenata* has a pleasing odor, *Conocephalum conicum* scents like mushrooms and *Plagiochila rutilans* smells like mint due to numerous menthane monoterpenoids. These are basically a combination of many compounds, including monoterpene hydrocarbons such as α-pinene, ß-pinene, myrcene, alpha-terpinene, camphene, sabinene, limonene, fatty acids, and methyl esters (benzyl benzoate, benzyl cinnamate, and -phenylethyl cinnamate) of low molecular weight.

Generally bryophytes are not infected by pathogens, insects, snails, slugs and mammals, however, studies on their chemical constituents have been limitted. Bryophytes are considered to be useless for human diets. Some of the isolated terpenoids from liverworts show characteristic scents, pungency and bitterness, allergenic contact dermatitis, cytotoxicity, anti-HIV inhibitory, antimicrobial, antifungal, insect antifeedant and mortality, nematocidal activity, superoxide anion radical release.

2. MATERIALS AND METHODS

Neyyar Wildlife Sanctuary in the southern state of Kerala in India is spread over the southeast corner of the Western Ghats, and covers a total area of 128 km^2 (49 sq mi). It is located between 77° 8’ to 77° 17’ East Longitude and 8° 29’ to 8° 37’ North Latitude, central location 8°33’N 77°12.5’E. Although it was declared as a sanctuary in 1958, not much was done about wildlife conservation, until 1985, when a separate wildlife wing was set up and as a result, conservation efforts have gathered momentum. Summer temperature is around 35 degrees Celsius and the winter being around 16 degree Celsius. The average rainfall from the Southwest monsoon between May and July and the Northeast monsoon between October and November, is about 2800 mm. The tourist season here is between the months of November and March. This is the drainage basin for the Neyyar River and its tributaries - Mullayar and Kallar. The towering peak of Agasthyamalai at an elevation of 1868 meters is a prominent landmark.

2.1. Methodology

Traditional knowledge of bryophytes in medicinal field was gathered mainly through individual interviews with selected informants using a semi-structured interview format from 2013 to 2015 (Huai *et al*., 2010). Interviews were largely conducted in local language with the help of a local translator and responses were recorded in English. During interview with each informant, information regarding the type of ailments managed by the ethnic group against the reported ailments, the plant parts used, ways of remedy preparations, route of administration, precautions if any and dosage was gathered. Ethnobotanical data related to habitat and abundance, threat and local marketability of claimed medicinal plants as well cultivation practice were also collected. The information was also discussed with different lada vaidhyan in the localities to validate the claims as far as possible. Specimens for most of the reported bryophytes were collected, dried, properly identified and authenticated with the reference herbarium from Department of Botany, University of Calicut. Voucher specimens are deposited at the Herbarium of University College, Trivandrum.

3. RESULTS AND DISCUSSION

The results show that 112 households were surveyed and provided 277 citations of medicinal

bryophytes. The number of citations per household ranged from 1 to 12. The 277 citations were identified 14 species of medicinal bryophytes distributed in 13 genera and 11 families (Table 1). The identity of the bryophytes related to medicinal potential was confirmed with the local inhabitants through oral interview and field survey with their full concern. The high usage among the people could be an indication of their abundance as it was witnessed during visits to the study sites that areas very close to houses were well covered with herbs. The study area remains humid for most months of the year creating a favorable condition for the growth of herbs. The common use of herbaceous medicinal plants was also reported in studies carried throughout the world. *Marchantia polymorpha* thallus resembles the liver so for curing liver ailments. It is commonly used to treat the jaundice of hepatitis and as an external cure to reduce inflammation and has gained the reputation of cooling and cleansing the liver. For curing leucorrhoea, thalli are chewed and the sap is swallowed. During diarrhoea, the leafy extract is administered daily thrice for three days and in abdominal diseases, leaf paste is administered orally twice in a day. The plants reported by the locals are employed for curing mist of their local ailments.

Rhodobryum giganteum, a traditional medicine for heart trouble. During menstrual pain, the leafy extract crushed with seven peppers is administered daily once for 4-5 days. The herb is eaten raw for heart problems, nervous prostration and cardio-vascular diseases and nervous tension. *Fissidens asperisectus*, a moss used as a bactericidal agent to treat sore throats. During fever, decoction of tender tips is administered daily twice; during paralysis, slightly warmed leaf decoction is poured over the affected area daily twice. To make bandages for dressing the wound this plant is commonly employed. *Philonotis fontana* is a wetland moss to relieve pain of burns. Mixture of the thallose liverwort *Marchantia polymorpha* with vegetable oils is used on bites, boils, burns, cuts, eczema, and wounds. During rheumatic pain, flower extract is administered daily once for a fortnight; similarly, the paste is also administered daily once for a month as general tonic; during insect bite, the leaf paste is applied over inflammated area. Soothing a wound of a different sort i.e., burned, to put on their heads to encourage hair growth. *Riccardia* is a thallose liverwort known for its antileukemic activity. During rheumatic swellings in cattle, slightly warmed leaf juice is used as a lotion. During stomachache, leaves crushed with peppers are taken orally. Leafy liverwort *Barbula* is active against human epidermoid carcinoma. For curing constipation in cattle, plant extract is administered twice or thrice daily. During menstrual pain, leaf extract is administered twice a day for three days; during intermittent fever, whole plant decoction is administered daily once for five days. *Frullania* species with both allergic and medicinal properties. In case of burning micturition, extract of aerial parts crushed with cumin seeds is taken; pus oozing in ears, aerial parts crushed with cumin seeds and sugar is administered. During ephemeral fever, paste prepared from whole plant crushed with turmeric and common salt is fermented in two litres of toddy for 12 h, the fermented toddy is administered daily once for four days.

Table 1. Medicinal bryophytes along with their ethnic use

Sl.No	Name	Family	Uses
1	*Bryum argentums*	Bryaceae	Extract to cure angina Can increase aorta blood transit
2	*Dumortiera hirsute*	Marchantiacea	Exhibits antileukemic/antimicrobial activity
3	*Frullania muscicola*	Jubulaceae	Skin ailments
4	*Marchantia polymorphya*	Marchantiacea	Used as diuretics, for liverailments, insect bites, boils and abscesses, treat pulmonary tuberculosis; Used to cure cuts, poisonous snake bites, burns, for cardiovascular disease. To treat boils and abscess As a source of antibiotic
5	*Marchantia sp.*	Marchantiacea	Used as diuretics, for liverailments, insect bites, boils and abscesses, treat pulmonary tuberculosis; Used to cure cuts, poisonous snake bites, burns, for cardiovascular disease To treat boils and abscess As a

			source of antibiotic
6	*Pallavicinia lyellii*	Pallaviciniaceae	
7	*Rhodobryum giganteum*	Bryaceae	Treating cardiovascular problem and nervous prostration, anti-hypoxia, antipyretic, diuretic and antihypertensive
8	*Barbula sp.*	Potiaceae	Boiled as tea for treating fever and body ache
9	*Riccardia sp.*	Aneuraceae	Exhibits anti-leukemic activity
10	*Thudium sp.*	Thuidiaceae	relief from stress
11	*Brachythecium sp.*	Brachytheciaceae	Relief from stress
12	*Fissidens nobilis*	Fissidentaceae	Diuretics and hair growth stimulation tonic
13	*Philonotis sp.*	Bartramiaceae	Heal burns for adenopharyngitis, antipyretic and antidotal
14	*Riccia sp.*	Marchantiaceae	Liver disorders

4. CONCLUSION

Bryophytes are source of novel interesting bioactive drugs. The majority of the phytochemicals reported in the bryophytes are lipophilic terpenoids (mono-, sesqui-, and diterpenoids) and fragrant compounds. Few of them are nitrogen- or sulfur-containing compounds. It is remarkable that most of the sesqui- and diterpenoids reported in liverworts are the enantiomers of those reported in angiosperms. Mono- and sesquiterpenoids are very unusual in mosses and hornworts, however, di- and triterpenoids have been isolated from certain mosses. Hence, there is an insightful call for their proper assessment regarding their useful chemical constituents and activities. Kerala, being one of the main centers of bryo-diversity is still at the back in applied bryological research and therefore need some serious efforts.

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HERBICIDAL EFFICACY OF ROOT EXUDATES OF RICE (*ORYZA SATIVA* L.) ON BARNYARD GRASS (*ECHINOCHLOA CRUS-GALLI* L.)

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ABSTRACT

A laboratory study was conducted to assess the herbicidal potential of root exudates of three rice cultivars (ADT-36, BPT and IR-20) against germination and growth of common crop field weed, barnyard grass (*Echinochloa crus-galli L.*). Various concentrations (5, 10, 15 and 20%) of root exudates were prepared from the underground part of rice cultivars from the postharvest rice fields. The studies revealed that all the three rice cultivars were exhibited significant inhibition on growth and development of weed species. Among the rice cultivars, ADT-36 exhibited the greatest inhibition on the seed germination (86%), seedling growth (83%), dry weight (81%) of barnyard cross than BPT and ADT-36. The percentage of inhibition on concentration depends. The order of inhibition of the rice cultivars was ADT-36 > BPT > IR-20 on barnyard grass.

Keywords: Herbicidal potential, rice cultivars, barnyard grass, *Echinochloa-crus-galli.*

1. INTRODUCTION

In modern agriculture, various methods of weed control have been studied. In particular, the exploitation of allelopathic properties in plants may give promising results for controlling the weed (Chung *et al.*, 2003). Allelopathy was defined by Rice (1984) to mean the direct or indirect harmful or beneficial effects of one plant on another through the production of chemical compounds that escape into the environment. Certain plant species or their residues selectively inhibit the development of particular species. This differential sensitivity observed in field, green house and laboratory experiments with residues, extracts and purified allelochemicals (Djurdjevic *et a*l 2004 and Leslie, A.W.1996). Plants are thought to produce about 200,000 natural products (Dixon and Strack 2003). These products are transferred to leaf, stem, seeds and roots. Plant roots exude metabolites as much as 10%, or even more, of photosynthetically fixed carbon into the soil (Werner, 1992). Root exudates contain sugars, amino acids, organic acids, flavonoids, enzymes, and nucleotides (Rovira 1969).Rice residues and their aqueous extracts suppressed the growth of lettuce and *Phalaris minor* (Khan *et al.,*2001) and aqueous and organic solvent extracts of rice plants inhibited the growth of several plant species (Das and Goswami, 2001;Kato-Noguchi, 2002). Several phenolic acids, such as p-hydroxybenzoic acid, vanillic acid p-coumaric acid and ferulic acid were found in aqueous extracts of rice straws, roots and residues (Chung *et al.,* 2001).Phenolic acids were also found in rice root exudates. Although phenolic acids are often mentioned as putative allelochemicals, it is unclear whether concentrations of phenolic acids measured in rice ecosystems are sufficient for causing growth inhibition of neighboring plant species (Olofsdotter, 2001). Rice has been extensively studied with respect to its allelopathy as part of a strategy for sustainable weed management, such as breeding allelopathic rice strains (Takeuchi *et al.,* 2001). A large number of rice varieties were found to inhibit the growth of several plant species when grown together under field and or laboratory conditions (Azmi *et al.,* 2000).These findings suggest that rice may produce and release allelochemical(s) into the neighboring environment, thus encouraging the exploration of allelochemicals in rice.

Hence the purpose of this study was to determine the allelopathic effects of aqueous rice root exudates of some rice cultivars (ADT-36, BPT and IR-20) on germination and seedling growth of weed barnyard grass (*Echinochloa crus-galli L.*) as an bioherbicidal tool for controlling the weed.

2. MATERIALS AND METHODS

Root parts of all three rice cultivars (IR-20, BPT and ADT-36) were collected from the post harvest rice fields of Annamalai Nagar, Tamil Nadu and thoroughly washed with tap water by 3 to 4 times and soaked in 5% hydrogen peroxide solution for providing maximum sterilization then allow to wash by tap water and finely chopped into small

pieces, About 5kg root parts of each cultivar were soaked with tap water of 10 litters at 7 days and leaching were collected through draining. Various concatenations (5, 10, 15 and 20%) of exudates were prepared from stock solution for studying their effect on seed germination and growth of barnyard grass.

Seeds of barnyard grass were surface sterilized with water:bleach solution (10:1) and were placed evenly on filter paper in sterilized 9cm Petri dishes. Ten milliliters of extract solution of exudates was added to Petri dishes and distilled water was used as a control. All Petri dishes were placed in a lighted room at 25ºC. After 7 days, germination percentage. Seedling length lengths, fresh weight and dry weight of barnyard grass were recorded. All data were analyzed one way ANOVA at 0.05 probability levels.

3. RESULTS AND DISCUSSION

The differences among rice root exudates were highly significant in all parameters. Rice cultivars root exudates exhibited different allelopathic potential on barnyard grass seedling growth. The original stock solution showed higher inhibitory activities as compared with 5, 10, 15 and 20% concentration and the control. Result showed that barnyard grass root growth was more sensitive to rice root exudates than shoot growth.

Table-1 shows that all the three rice cultivars have inhibitory effect on the seed germination of barnyard grass. At low concentration of root exudates inhibitory effect was not so much pronounced. But as soon as concentration increased, inhibitory effects were also increased. ADT-36 showed maximum negative effect than BPT and IR-20. ADT-36 caused 86% of germination retardation at 20% root exudates over control. BPT caused 75% germination failure compare with control at 20% aqueous exudates and IR-20 caused least inhibitory effect (57%).

Root exudates shown phytotoxic effect over the growth of weed seedling. Root showed highest reduction percentage than the shoot of weed seedlings. ADT-36, BPT and IR-20 caused significantly inhibitory effect respectively 75%, 65% and 56% on root length of barnyard grass seedlings.The allelopathic effect was less pronounced in the case of shoot length. Though, results were similar as in the case of root length of barnyard grass. ADT-36 showed 76% negative effect over control on weed seedling shoot length at 20% aqueous concentration. BPT caused 65% reduction over control and IR-20 caused least reduction on shoot length and exhibited 51% negative effect over control seedlings of barnyard grass. This finding is supported by Zohair *et al., (*2007) who mentioned that plumule length decreased when treated by aqueous extract of barley. Allelopathic potential can be a valuable trait to incorporate into rice cultivars to improve weed control. Greater inhibitory effects on roots as compared with shoots may be due to the direct contact of the root system to the extract solution in the growth media.

Table 1. Roots exudates of rice cultivars on germination percentage on root length (cm/plant) and shoot length (cm/plant) of barnyard grass.

Exudates Conc.	Germination %			Root Length			Shoot Length		
	ADT-36	BPT	IR-20	ADT-36	BPT	IR-20	ADT-36	BPT	IR-20
C	98	98	98	2.15	2.15	2.15	3.18	3.18	3.18
5	82 (-16.32)	86 (-12.24)	89 (-9.18)	1.60 (-25.58)	1.71 (-20.46)	1.93 (-10.23)	2.68 (-15.72)	2.78 (-12.57)	2.92 (-8.17)
10	64 (-34.49)	69 (-29.59)	75 (-23.46)	1.24 (-42.32)	1.41 (-34.41)	1.55 (-27.90)	2.29 (-27.98)	2.49 (-21.69)	2.61 (-17.92)
15	24 (-75.51)	29 (-70.40)	52 (-46.93)	0.96 (- 55.34)	1.11 (-48.37)	1.19 (-44.65)	1.82 (-42.76)	2.11 (-33.69)	2.28 (-28.30)
20	13 (-86.73)	24 (-75.51)	42 (-57.14)	0.53 (-75.34)	0.75 (-65.11)	0.85 (-60.46)	1.18 (-62.89)	1.45 (-54.40)	1.51 (-52.51)
Avg.	56.2	61.2	71.2	6.48	7.13	7.67	11.05	12.01	12.5
F	26.128			50.74075			57.11101		

(-) indicates percentage of phytotoxicity over control.

Table 2. Roots exudates of rice cultivars on fresh and dry weight (g/plant) of barnyard grass.

Exudates concentration	ADT-36		BPT		IR-20	
	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight
C	2.14	1.31	2.14	1.31	2.14	1.31
5	1.84 (-14.01)	1.03 (-21.37)	1.97 (-7.94)	1.24 (-5.34)	1.98 (-7.47)	1.16 (-11.45)
10	1.63 (-23.83)	0.87 (-33.58)	1.72 (-19.62)	0.93 (-29)	1.72 (-19.62)	1.12 (-14.50)
15	0.98 (-54.20)	0.68 (-48.09)	1.16 (-45.79)	0.85 (-35.11)	1.30 (-39.25)	0.98 (-25.19)
20	0.56 (-73.83)	0.41 (-68.70)	0.64 (-70.09)	0.63 (-51.90)	0.92 (-57)	0.81 (-38.16)
Average	1.43	4.3	1.526	4.96	1.612	5.69
F	FW.-73.768:DW.-11.867					

(-) indicates percentage of phytotoxicity over control .

Phytotoxic effects of root exudates were negatively pronounced on shoot than the root fresh and dry weights of barnyard grass. ADT-36 root exudate was retarding highly on the fresh weight of weed seedling than BPTand IR-20. Negative allelopathic was more pronounced in ADT-36 followed by BPT and IR-20. ADT-36. Root exudates shown maximum inhibitory effect (73%) at 20% root exudates. BPT shown inhibitory effect above 70% in the higher concentrations of aqueous root exudates. IR-20 had least inhibitory effect (57%) at 20% root exudates. Root exudates of ADT-36 shown maximum phytotoxic effect at 20% aqueous exudates and caused 68% dry weight loss over control. BPT showed 51% dry weight loss by 20% aqueous root exudates treatments over control. These results are agree with other studies reporting that water extracts of allelopathic plants had more pronounced effects on radical growth than on plumule growth (Turk and Tawaha, 2002, 2003 and chung and miller, 1995). A number of secondary metabolites, phenolic acids, phenylalkanoic acids, hydroxamic acids, fatty acids, terpenes and indoles, were identified in rice extracts (Rimando & Duke, 2003). The results showed that the maximum reduction in germination was by ADT-36 followed by BPT than IR-20 rice cultivars. Similar results also found by Agnes Rimando (2010) who mentioned that allelopathic potential may be differ from variety to variety. The results of this study are in agreement with those of Ahn *et al.* (2000), Chung *et al*. (2003), Asghari and Dilday *et al*. (1991). From the present investigations,it can be concluded that variation in detrimental allelopathic activity exists among cultivars. Among the three rice cultivars used in this study, ADT-36 and BPT exudates were highly reduced the germination percentage, seedling length, total dry weight and fresh weight than IR-20 cultivar.

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AESCHYNANTHUS PERROTTETII A.DC. (GESNERIACEAE) – A NEW RECORD FOR EASTERN GHATS

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ABSTRACT

Aeschynanthus perrottetii A.DC. (Gesneriaceae) were collected and reported in Kolli hills of Eastern Ghats for its new distributional record. The detailed description and photographs, flowering and fruiting period, distribution and ecological notes are given for its easy location and identification.

Keywords: *Aeschynanthus perrottetii,* Gesneriaceae, Kolli hills of Eastern Ghats.

1. INTRODUCTION

The Eastern Ghats, one of the nine floristic zone in southern India extending over 2000 km stretch of north to south isolated hillocks covering the area under 11^0 30' – 21^0 00' N and 77^0 22' – 85^0 20' E. The hill ranges rise from almost sea level to about 1572 m altitude and spread over three states of India namely Orissa, Andhra Pradesh and Tamil Nadu between the rivers of Mahanathi and Vaigai along the east coast. Ganjam and Mahendragiri ranges in Orissa; Nallamalais, Araku and Sesachalam ranges in Andhra Pradesh; Servarayans, Javadhu, Chitheeri, Pachamalais and Kolli hill ranges in Tamil Nadu are well known biodiversity spots for Eastern Ghats. Many earlier studies are recorded the floristic wealth of Eastern Ghats ranges time to time by various authors (Gamble, 1927; Matthew, 1983-86; Anand *et al*., 2006; Pullaiah *et al*., 2007).

During the recent botanical visits of Kolli hills of Eastern Ghats, we collected an interesting epiphytic plant species *Aeschynanthus perrottetii* (Gesneriaceae). The species is critically analysed with pertinent literature available for Eastern Ghats, it is hitherto unreported by any earlier floristic accounts (Karuppusamy *et al*., 2001; Pullaiah and Muralidhara Rao, 2002; Pullaiah *et al*., 2007, Reddy, 2008). These plant species are phytogeographically significance in the Eastern Ghats. Hence the description, phonological data, distribution details, specimen examined, and ecological notes are given here for easy identification. The voucher specimens are deposited in the herbarium of the Department of Botany (Sri Ganesan Herbarium Madura College - SGHMC), The Madura College, Madurai.

2. OBSERVATIONS

2.1. Aeschynanthus perrottetii A. DC. Prodr. 9: 261. 1865. Gamble, Fl. Madras Pres. 2: 985. 1927 (Fig.1).

Epiphytic, trailing subshrub, stem terete, rooting at swollen nodes. Leaves simple, opposite, fleshy, linear-lanceolate to elliptic, 7 - 8 x 1.5 – 2 cm, entire, lateral nerves obscure, mid vein conspicuous, rounded at base, acute at apex. Flowers solitary, rarely paired, terminal or axillary peduncles; bracts minute, linear-lanceolate, up to 3 mm long. Calyx 5-lobed, lobes linear, 0.5 – 0.8 mm long, greenish. Corolla tubular, 4.5 cm long, tube curved, glabrous, mouth bilabiate, oblique, upper lip of 2 small, the lower lip of 3 larger lobes, scarlet with purple lines or spots on the lobes, glandular ciliate. Stamens 4, all perfect, inserted at the middle of the corolla tube, anther 2, oblong, connate at the tips. Ovary oblong or linear, stipitate, 1-celled, ovules on the margins; stigma dilated. Fruits linear, ca 15 cm long capsule, 2-valved. Seeds many, small, oblong or linear with hairs at the end and near hilum.

2.2. Flowering & Fruiting

September – February.

2.3. Specimen examined

Tamil Nadu: Namakkal district, Kolli hills, Kulivalavu shola, 1.09.2012. (11^0 19.35' N – 78^0 21.49') *S. Karuppusamy & V. Ravichandran* 15142 (SGHMC).

2.4. Distribution

Western Ghats of south India (Endemic), Kolli hills of Eastern Ghats.

2.5. Ecological notes

Aschynanthus is a genus of about 150 species of evergreen subtropical plants in the family Gesneriaceae. They are usually trailing epiphytes with brightly coloured tubular flowers that are pollinated by sunbirds. *A. perrottetii* is an endemic species of Western Ghats and it was not recorded for

Eastern Ghats so far. This is a first report on the extended distribution of this species into Eastern Ghats range. The plant is growing on *Myristica dactyloides, Celtis tymoriensis, Mallotus tetracoccus, Olea glandulifera*, *Macranga indica* and *Neolitsea ceylanica*.

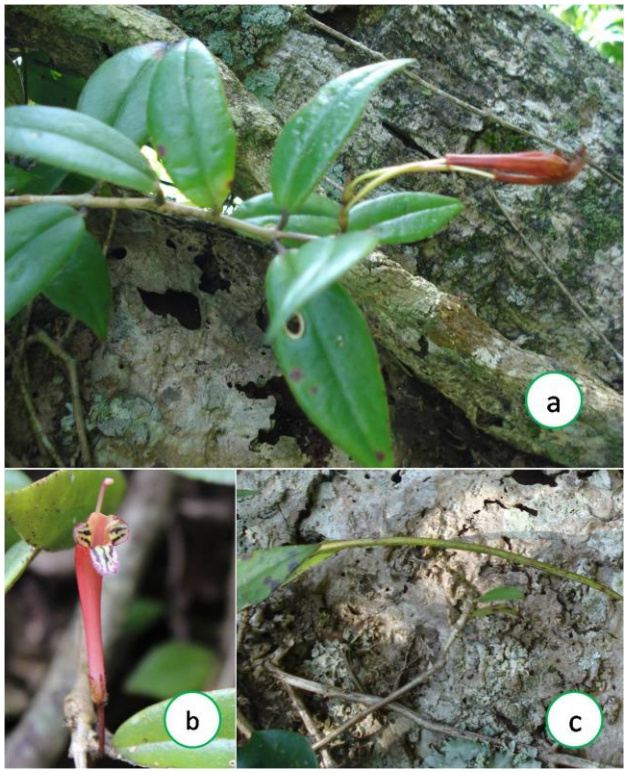

Fig. 1. *Aeschynanthus perrottetii* A. DC.

a- Plant in epiphytic habitat; b – Flower; c- Fruit

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ETHNO-MEDICO-BOTANICAL STUDIES ON AQUATIC PLANTS IN RURAL AREAS OF CUDDALORE DISTRICT, TAMILNADU, INDIA

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ABSTRACT

The present work was undertaken to explore the ethno-medico-botanical values of aquatic plants from rural areas of Cuddalore District in Tamilnadu, India. Traditional knowledge of 22aquatic plants was identified as medicinally important species from local residents, vaidya, other medicine men and from other sources. Collected information's are arranged in an alphabetical order followed by the sequence of scientific name, family, vernacular name, prescription and usage. Documentation of traditional knowledge of ethno-medico-botanical values of aquatic plant species will provide baseline information for investigating new bio-dynamic compounds of potential therapeutic uses in future.

Keywords: Documentation, Medicinal properties, aquatic plants, Cuddalore district.

1. INTRODUCTION

The knowledge of medicinal property of plants has been accumulated in the course of many centuries (Kirthikar and Basu, 1980). The local inhabitants have inherited rich traditional knowledge on the use of many plants or plant parts for treatment of common diseases (Vedavathy, 2003; Jain, 2004; Maity, *et al.,* 2004). The remedies based on these plants often have minimal side effects (Lambert *et al.,* 1997). Recently there is a paradigm shift from over use synthetic drugs to herbal medicines. The medicinal value of a particular species of plant differs from one locality to another or from one community to another. Hence, it is highly imperative to document local knowledge on the medicinal properties of plants to gain wider and in-depth knowledge on their curative abilities. It play a significant role in the primary production, nutrient cycling, and serve as bioindicators for eutrophication processes (Thangam *et al.,* 2010; Regini Balasingh, 2011)

Hydrophytes grow profusely in lakes and waterways all over the world and have in recent decades their negative effects magnifies by man's intensive use of natural water bodies. Eradication of this water plants are has proved almost impossible and even reasonable control is difficult. The potential of aquatic plants as food and feed has been emphasized by several authors (Indirani, 2010; Lawrence, 2010). Large growths of hydrophytes in lakes and waterways of tropical countries, although a menace, represent a natural resource of green leaves (Lawrence, 2010). With increasing interest in finding new drugs, the wild or unutilized plants receive more attention which offers a good scope to meet the increasing demand for novel drug discovery.

Local people use a wide variety of wetland / wetland –associated plants as ingredients of traditional herbal medicinal preparations. Often the information on the composition of a specific medicinal preparation or the knowledge on the use and medical value of particular plant is restricted to a few members of a community or even to one or two individuals of a household. Since most of this vital system of knowledge is transmitted orally, the local extinction of a plant results in the gradual loss of knowledge related with the medicinal value of such species.

Documentation of ethno-botanical importance of terrestrial plant species was more than aquatic plant species. Maya *et al*. (2003) analyzed the economic importance of river vegetation of Kerala and gave the uses of 35 species including the bank specie apart from the aquatic/wetland species. Panda and Misra (2011) provided information about ethno medicinal uses of 48 wetland plant species of South Orissa and discussed their conservation. Swapna *et al.* (2011) made a review on the medicinal and edible aspects of 70 aquatic and wetland plants of India. Though the aquatic situations of India are rich repositories of various plant species, not much work has been under taken to explore the medicinal uses of them.

Hence, the present study was carried out to document the ethno-medico-botanical values of aquatic plants in Cuddalore District of Tamilnadu, India.

2. MATERIALS AND METHODS

The present study, the data collection and survey have been made by field visits during June 2012- April 2013 and focused mainly on the aquatic plant species used by different local vaidhyas and medicinemen in Cuddalore District (11.75°N 79.75°E) of Tamilnadu, India for primary healthcare needs as reported by the informants/traditional healers. The large number of local people, medicine men, herbal informants and women chieftains were personally interviewed and requested to answer a few questions about the (i) local aquatic plants and their availability in the area; (ii) application of these plants in healthcare and the data were recorded time to time. The collected information of ethno-medico-botanical values of aquatic plants was arranged according to their alphabetical sequence such as scientific name, families, voucher specimen number, family, vernacular names, parts used, the therapeutic uses and method of usage of herbal preparations.

Table 1. Particulars regarding the name of the species, morphology of useful part, diseases, method of preparation and their mode of administration

Sl.No	Botanical Name and Family	Morphology of useful part	Disease cured	Method of preparation and mode of administration
1.	*Alternanthera philoxeroides* (Mart.) Griseb. (Amaranthaceae)	Shoot	Dysentery	The decoction of the young shoot is taken in empty stomach twice a day.
2.	*Alternanthera sessilis* (L.) R.Br. ex DC. (Amaranthaceae)	Leaves, twigs	Fever	Decoction is taken with(30-50ml) two principal meals.
		Root	Cataract	Root of the fresh plant touched in the eyes five times a day.
3.	*Ammania baccifera* L. (Lythraceae)	Leaves	Oedema Skin abcess	Leaves are ground in water and the paste applied on the area and repeated for 3 days.
		Whole plant	Gonorrhoea	Two teaspoonful decoction of the entire plant is taken orally twice a day for three week.
4.	*Bacopa monnerii* (L.) Pennell (Scrophulariaceae)	Leaves	Dysentery	The paste of the leaves are taken with rice.
			Gastritis	Leaf juice is taken orally to treat gastritis and as liver stimulant.
		Whole plant	Malarial fever	Plant juice together with black pepper give twice a day for three days.
5.	*Centella asiatica* L. (Apiaceae)	Leaves	Scabies Spermatorrhoea Mouth sores	Leaf paste is applied on the affected portion. Extract of fresh leaves (one teaspoon) is given. Five to six leaves are chewed four times a day.
6.	*Commelina bengalensis* L. (Commelinaceae)	Leaves	Fever	5-10 leaves were fed into patient early morning for 2-3 days.
		Whole plant	Herpes	Whole plant paste is applied externally.

			Haemorrhoids	Leaves crushed and applied over.
7.	*Cyperus rotundus* L. (Cyperaceae)	Tuber	Intestinal worms, Colic complaints	Crushed tubers are given with milk.
			Snake bite	Tuber powder mixed with cow butter is given to patients .
			Spermatorrhoea	Two tubers per day are given with water for 5 days.
8.	*Eichhornia crassipes* (Mart) Solms (Pontederiaceae)	Whole plant	Bone fracture	Plants are pounded and paste then applied .
9.	*Hygrophila auriculata* (Schumacher) Heine (Acanthaceae)	Leaves	Anaemia	The decoction of the young leaves are taken orally for two consecutive weeks in empty stomach.
		Whole plant	Body swellings	Whole plant paste in applied over it.
		Leaves	Leucorrhoea Infantile diarrhea	Powdered leaf is given with water. A handful of leaves pounded together with black pepper and eaten twice daily.
10.	*Ipomoea aquatica* Forster (Convolvulaceae)	Twigs	Blood dysentery, Indigestion	Special type of curry is pre pared with young twigs and taken with rice.
		Leaves	Piles	Leaf paste is given topically.
		Whole plant	Itching	Plant paste is applied over the body.
		Leaves	Snanke bite	About 25g leaves are ground and taken with 250g curd for a week as an antidote for snake bite.
11.	*Ipomoea carnea Jacq.* var.*fistulosa* (Mart.exChoisy) Austin (Convolvulaceae)	Leaves	Wounds and boils	Leaves are warmed with edible oil and tied on wounds and boils.
		Leaves, Root	Bone fracture	Root and leaf paste is plastered over the fractured area.
12.	*Ludwigia adscendens* (L.) Hara (Onagraceae)	Whole plant	Stomach pain, Intestinal worms	Leaf decoction with black pepper is taken orally
		Seeds	Rheumatism	Grounded seeds are taken orally with hot water

13.	*Marsilea minuta* L. (Marsileaceae)	Sporocarp	Throat inflammation	Sporocarps are crushed and applied on throat with the help of finger to cure throat inflammation in children
14..	*Nasturitium officinale* R.Br. (Brassicaceae)	Whole plant	Improve eyesight	Whole plant is used as vegetable to improve eyesight
15.	*Nelumbo nucifera* Gaertn. (Nymphaeceae)	Fruit	Check vomitting	One fruit is crushed and given with 20 ml of water three times a day for check vomiting in children
16.	*Neptunia prostrata* (Lamarck) Baillon (Mimosaceae)	Leaves	Dysuria	Half glass of leaf decoction is taken orally about a fortnight
		Tender shoots	White discharge	The decoction of the young twigs are taken with common salt
		Whole plant	Jaundice	The whole plant is a very good tonic particularly for those who are suffering from jaundice
		Root	Dysentery	Root extract is taken with curd
17.	*Nymphaea nouchalli* Burm.f. (Nymphaeceae)	Roots	Check conception	10g roots and 3g of seed of *Crotalaria juncea* are ground into paste and taken with water on the date of menstruation
		Whole plant	Inducing puberty	Paste of whole plant in water is applied around the navel
18.	*Nymphaea stellata* Burm.f. (Nymphaeceae)	Leaves	Toothache	Leaves given fresh
19.	*Nymphoides indica* (L.) Kuntze (Gentianaceae)	Whole plant	Jaundice	Decoction of the plant is drunken three teaspoonfuls every morning.
			Dysentery	Root power is used in dysentery
20.	*Monochoria vaginalis* Presl (Pontederiaceae)	Whole	Nausea	Decoction of fresh root given
21.	*Plantago major* L. (Plantaginaceae)	Leaves	Cuts, wounds	Powdered leaves are applied to cuts and wounds. Decoction of seeds, roots, and leaves are taken to treat stomach disorder.
		Seeds, roots	Stomachic	
		Root	Piles	Root paste is applied to cure piles.
22.	*Sagittaria guyanensis* Kunth (Alismatacceae)	Whole plant	Fever	Plant juice is drunk to cure fever

3. RESULTS AND DISCUSSION

During the field survey, ethno medicinal data of 22aquatic plant species under 19 genera belonging to 17 families have been documented. Among the ethno-medico-botanical values of the species, the family Nymphaeaceae was most frequently represented with a total of 3 species, followed by Pontederiaceae, Amaranthaceae and Convolvulaceae having 2 species. Whole plants part and leaves are predominantly used when compare to other parts of plants. The data on the medicinally important plants indicate that the observed species were used to treat 37 ailments including fever, gynaecological complaints, stomach disorders, jaundice, snake bite, skin diseases, rheumatism, ulcer, wounds, boils, cuts and wounds, diseases of blood, and other diseases. (Table 1).

Local communities and vaidhyas in District living with the day to day practices and there are no written documents. Moreover, the existing knowledge on traditional uses of plants are destroying in fast pace, because the lack of interest of local youth to learn the traditional knowledge from the old herbal healer. It is also felt that the valuable and time-tested knowledge on the medicinal uses of plants are also is appearing due to modernization, acculturation, forests destruction, urbanization, industrialization, etc. Scientific investigations through the evaluation of these aquatic plants for their biological activity and isolation of active constituents responsible for their medicinal properties which will give a lead to develop new natural drug molecules so as to reach he benefit of research for the welfare of human beings.

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A COMPARATIVE ANATOMICAL CHARACTERISTICS OF THE STEMS OF CLIMBING PLANTS IN ARALAM WILD LIFE SANCTUARY, KANNUR

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ABSTRACT

Climbing plants differ from self-supporting plants, such as shrubs and trees, in a range of characteristics. The most notable is the mechanical properties of the stem Comparison of the differentiated anatomical structures recorded in ten species of the climbing plants. The plants selected for the present study are *Ampelocissus latifolia,* (Vitaceae), *Lygodium flexuosum* (Lygodiaceae), *Centrosema virginianum* (Fabaceae), *Tinospora cordifolia,* (Menispermaceae), *Wattakakka volubilis* (Asclepiadaceae) *Cyclea peltata* (Menispermaceae), *Calycopteris floribunda* (Combretaceae) *Pothos scandens* (Araceae) *Ipomoea separia* (Convolvulaceae) *and Piper nigrum* (Piperaceae). The stems of climbing plants are characterized by the scarcity of supporting cells (fibers) and an increase in the diameter of the xylem vessels. The study con firms that they show a greater diversity of organization than other plant life forms. This anatomical radiation could probably not exist without the achievement of a wide range of secondary growth processes. Many dicotyledons, notably those with a climbing habit, show interesting secondary structure which differs from the more usual type described, therefore, sometimes termed anomalous. The variant secondary growth is particularly widespread in tropical climbers. It is speculated that variant growth can increase stem flexibility, protect the phloem, increase storage parenchyma, aid in clinging to supports, limit physical disruption of vascular tissues during twisting and bending, and promote wound healing after girdling.

Keywords: Climbing plants, Anatomy.

1. INTRODUCTION

Climbing plants differ from self-supporting plants, such as shrubs and trees, in a range of characteristics. The most notable is the mechanical properties of the stem. Considering the tremendous number of possibilities for the functions and structures of stems, it is truly remarkable that there is only one single basic type in all of the vascular plants. In cross section, there is an outermost epidermis that overlies the cortex; the cortex in turn surrounds the vascular tissues. Climbing stems in primary growth with this basic type seems to be common. However, many variations of stem structure are usually called anomalies, can be seen in many climbers.

Climbing plants are found in numerous ecosystems, but are more abundant in low elevation tropical forests than in any other habitat. According to Gentry (1991), climbing plants in temperate forests represent on average 7% of the local flora, while in tropical forests this number reaches 20%. Lianas are characteristic of tropical forests, where at least 50% of the trees contain lianas.

Comparison of the differentiated anatomical structures recorded in 10 species of the climbing plants confirms that they show a greater diversity of organization than other plant life forms. This anatomical radiation could probably not exist without the achievement of a wide range of secondary growth processes. Many dicotyledons, notably those with a climbing habit, show interesting secondary structure which differs from the more usual type described, therefore, sometimes termed anomalous. The anomalous or unusual structure may be a consequence of

(1) a cambium of normal type which gives rise to unusual arrangements of secondary xylem and phloem, or

(2) a cambium which itself is abnormally situated and so gives rise to abnormal arrangements of tissues, or

(3) the formation of accessory or additional cambial zone

The variant secondary growth is particularly widespread in tropical climbers. It is speculated that variant growth can increase stem flexibility, protect

the phloem, increase storage parenchyma, aid in clinging to supports, limit physical disruption of vascular tissues during twisting and bending, and promote wound healing after girdling. Fisher & Ewers (1992) consider that the major benefits of variant xylem arrangements to climbers are not in their influence upon transport pathways, but rather in their mechanical and regeneration effects.

However, most of the information about cambial variants is based on the mature structure, and only a few developmental studies have been made (Nair 1993, Araújo & Costa 2006). Besides, as suggested by Caballé (1993), the study on the anatomical structure of liana stems should provide a highly efficient descriptive tool for the identification of taxa (families, genera or species)

The present study carried out a) To analyse the comparative stem structure of ten selected climbing species and b) To answer the inquiry whether these species present cambial variants or not and to verify the modes of cambial activities.

2. MATERIALS AND METHODS

In the present study the following ten climbing plants were selected from Aralam Wildlife Sanctuary.

1.*Centrosema virginianum (L) Benth*

Family: Fabacee

2. *Piper nigrum L*.

Family: Piperaceae

3. *Ipomoea separia Roxb*.

Family: Convolvulaceae

4. *Pothos scandens L*

Family: Araceaea

5. *Calycopteris floribunda (Roxb.) Poir*.

Family: Combretaceae

6. *Cyclea peltata (Lam).Hook.f.&Thoms*.

Family: Menispermaceae

7. *Wattakaka volubilis Stapf*

Family: Asclepiadaceae

9. *Lygodium flexuosum L*. **(**climbing fern**)**

Family: Lygodiaceae

10. *Ampelocissus latifolia (Roxb.)Planch.*

Family:Vitaceae

The stems of above mentioned plants were collected from nearby locality of Aralam wild life sanctuary, Kannur. The stems were sectioned by freehand. The cross-sections were stained using safranin (Souza *et al*. 2005) in agreement with usual techniques in plant anatomy (Gerrits 1991). The illustrations were made by drawings (diagrams), obtained in light microscope equipped with camera lucida, and photomicrographs. Photomicrographs were obtained by processing the image captured in Olympus microscope with Cannon digital camera.

3. RESULTS

3.1. Centrosema virginian (L) Benth

The plants have hairs in the epidermis. But hairs are absent at the mature region of the stem. Cortex is sclerenchymatous. The stem have 13-17 vascular bundles, their distribution goes like follows, 13 vascular bundles at the 7th internodes, 17 vascular bundles at 3rd internodes, and 16 vascular bundles at the twisted portion. Large pith can be seen but a great controversy is seen within the pith and also in the stem. The stem of older region appears like two stems which are merged together and new branch is arises from the node as single stem and pith also shows variation in this plant. At older region the pith have a slit as it separates the two merged stems anatomically. But this slit is not seen at the younger stages. This splitting comes larger towards the base.

3.2. Piper nigrum L.

It is irregular in outline under microscopic observation. Ridges and grooves can be seen in the epidermis. Epidermis is followed by cortex which contains three layered collenchyma and four to five sclerenchyma then parenchyma, below it two to three layers of chlorechyama also seen. Vascular bundles are seen only in grooves, small and large vascular bundles are seen alternatly which is surrounded by wavy sclerechyama. The pith is delimited from the xylem by a wavy band of thick walled fibers. Pith is homogenous, it contain hexagonal parenchyma with medullary vascular bundles and schizogenous secretory canals are seen.

3.3. Ipomoea separia Roxb.

Hairs are seen in the epidermis. Heterogenous cortex. Succcessive rings of cambia can be seen. Interxylery phloem can be seen. Secondary xylem towards inner side is large. Large, homogenous, parenchymatous pith. The connective tissue is wide almost equal to vascular bundes. In mature stem vascular bundles are in different diameter.

Fig. 1. ***Centrosema virginianum***

Fig. 2. ***Piper nigrum***

Fig. 3 ***Ipomoea separia***

Fig. 4. ***Pothos scandens***

Fig. 5. ***Calycopteris floribunda***

Fig. 6. ***Cyclea peltata***

Fig. 7. ***Wattakakka volubilis***

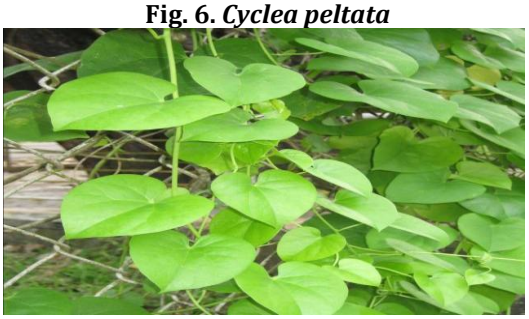


Fig. 8. ***Tinospora cordifolia***

Fig. 9. ***Lygodium flexuosum***

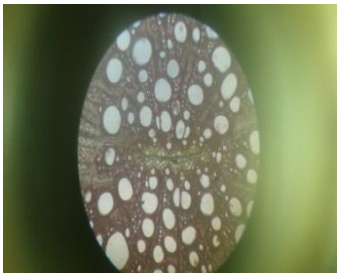
Fig. 10. ***Ampelocissus latifolia***

PLATES

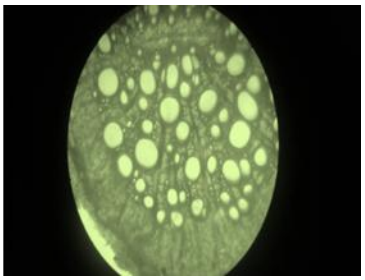
DETAILS OF STEM IN CROSS –SECTIONS

1.*Centrosema virginianum* (L) Benth

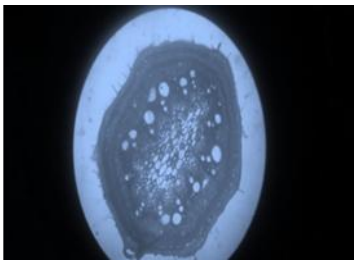
First internode



Third internode

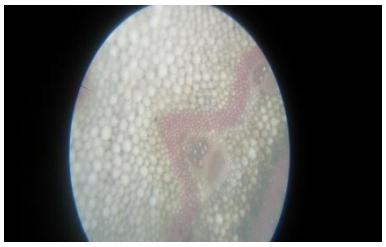


Seventh internode

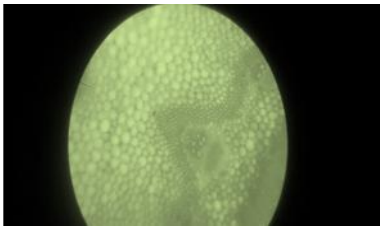


2. *Piper nigrum* L.

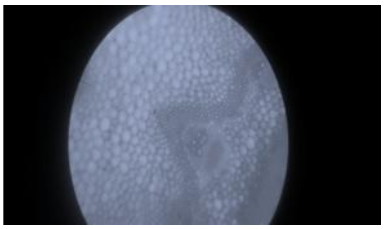
First internode



Third internode

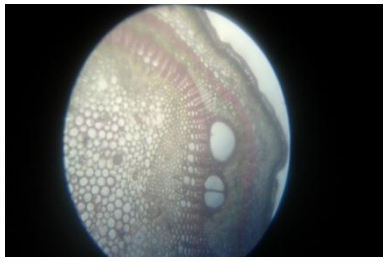


Seventh internode

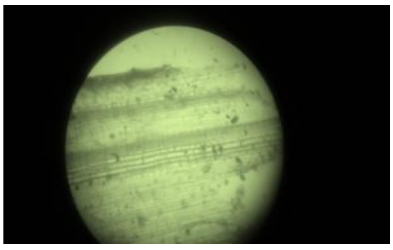


3. *Ipomoea separia* Roxb.

First internode



Third internode



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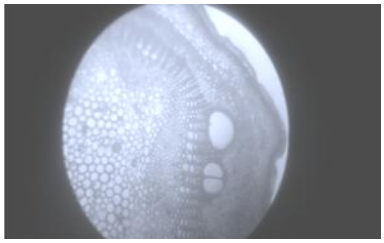
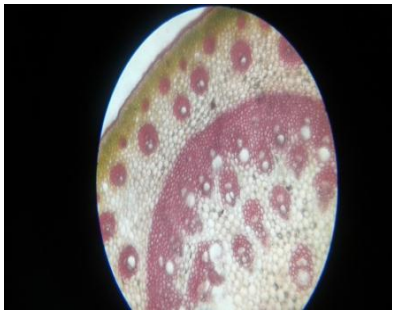
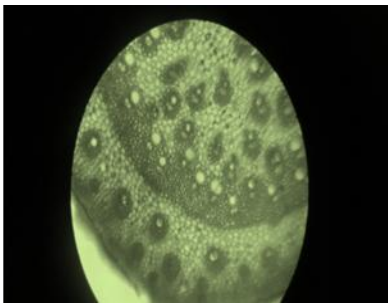


4. *Pothos scandens* L.

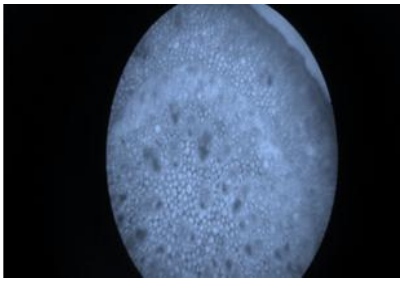
First internode



Third internode

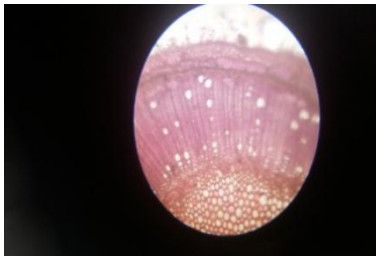


Seventh internode

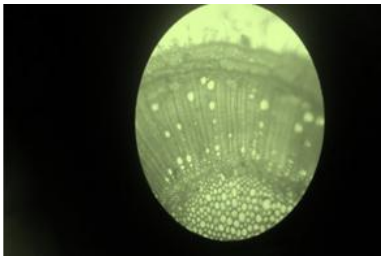


5. *Calycopteris floribunda* (Roxb.) Poir.

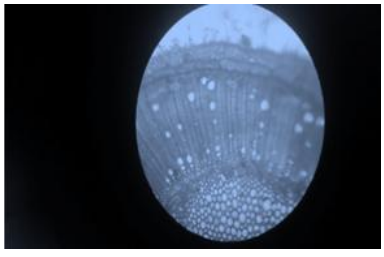
First internode



Third internode

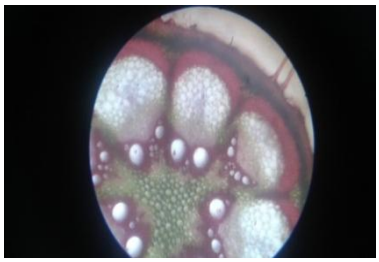
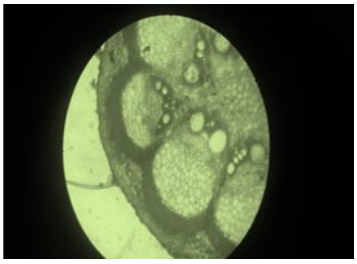
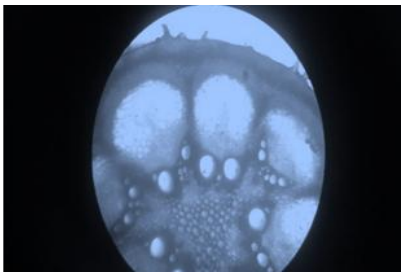


Seventh internode



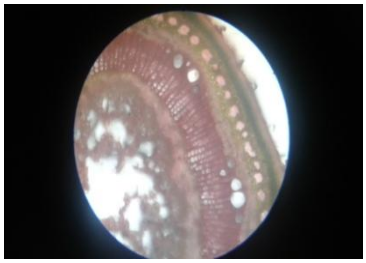
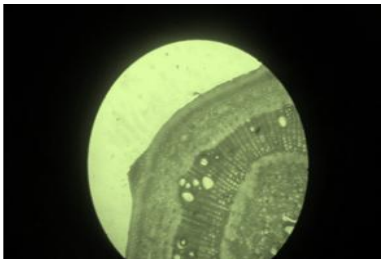
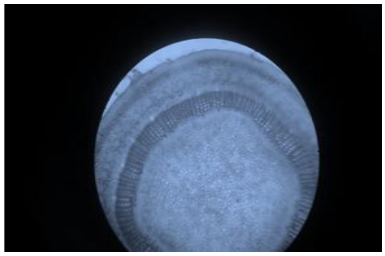
6. *Cyclea peltata* (Lam).Hook.f.&Thoms.

First internode **Third internode** **Seventh internode**

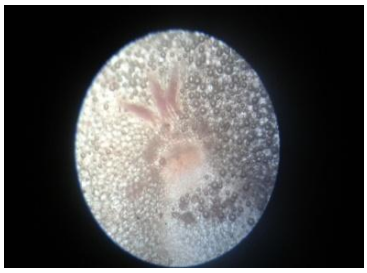
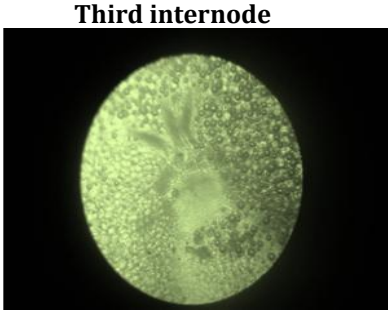
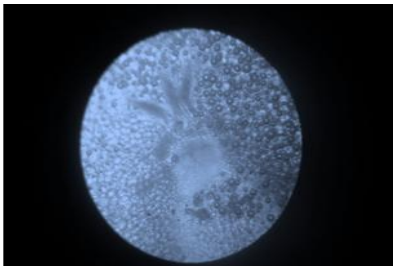
7. *Wattakaka volubilis* Stapf.

First internode **Third internode** **Seventh internode**

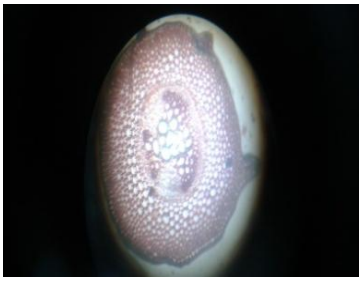
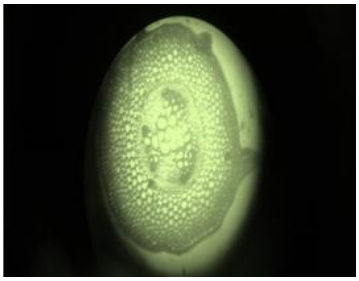
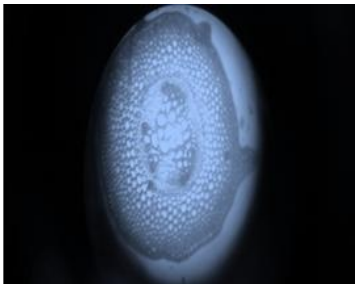
***8.Tinospora cordifolia* Miers.**

First internode **Third internode** **Seventh internode**

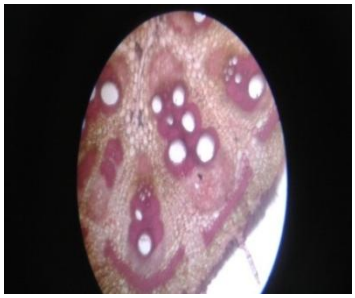
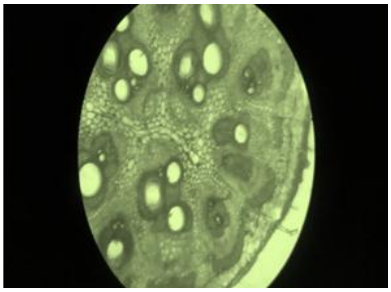
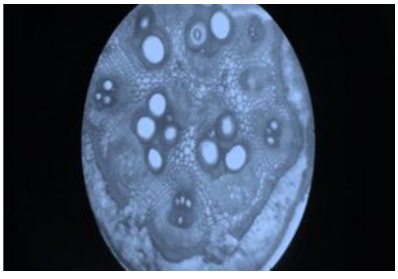
9. *Lygodium flexuosum* L.

First internode **Third internode** **Seventh internode**

***10.Merremia vitifolia* (Burm.f.) Hallier f.**

First internode **Third internode** **Seventh internode**

3.4. Pothos scandens L

Epidermis is seen more or less irregular with flat lateral sides and thick walled with lignified cells,circular in shape. Circular or parenchymatous ground tissue fills the cotex. Distinct layer of less thivk walled endodermis with two whorls of vascular bundles. Stellar vascular bundles scattered and densely occupied the stele and since pith cannot be differentiated.

3.5. Calycopteris floribunda(Roxb.)Poir.

Hairy epidermis. Cortex is differentiated into 5-6 layers of parenchyma,sclerenchyma and chlorenchyma . In vascular bundles outer and inner cambial segment seen at certain places. In secondary xylem sclereids are seen more than vessels. Homogenous large parenchymatous pith.

3.6. Cyclea peltata (Lam).Hook.f.&Thoms.

Single layered hairy epidermis seen. Cortex is differentiated with three types of cells, two layers of collenchyma and chlorenchyma, wavy sheath of sclerenchyma present at the end. 8-10 vascular bundles seen. Secondary xylem is enormous. Pith is small in primarystructure but large in secondary structure.

3.7. Wattakaka volubilis Stapf

Wavy outline,hairs are present. Cortex have three layers of collenchyma, three layers of chlorenchyma and three layers of sclerenchyma. About 10-12 vascular bundles are seen, large and small vascular bundles are seen alternately. In secondary structure smallround bundles of phloem is seen in secondary cortex which is 12-15 layered chlorenchyma cells. In secondary xylem morevessels are seen in protoxylem region but in metaxylem region sclereids are seen.

3.8. Tinospora cordifolia Miers

Single layered epidermis . Below the epidermis collenchymatous cells seen which is starting of the cortical layer. Collenchyma cells are followed by three to four layered wavy sclerenchymatous cells, which foam a continuous ring. Parenchymatous cells are seen below this, the elongated cells become widened as we go towards pith. In mature stem pericyclic sclerenchyma and phloem persist in original. Arround 9 vascular bundles can be seen in primary structure. Vascular bundles differ in size alternatly. The fascicular and interfascicular cambium seen between the two vascular bundles. Vascular bundles. Vascular bundles increas in size and number as the stem grows older. Parenchymatous large pith with suberin deposition.

3.9. Lygodium flexuosum L.

Outermost epidermis, followed by sclerenchymatous cells. Parenchymatous cortex is present. And stele is protostel. The vascular bundle are arised from supernumerary cambial tissue. In secondary structure it has numerous vascular bundles. Large round pith, and pith is constricted in twisted rwgion.

3.10. Ampelocissus latifolia (Roxb.)Planch.

Hairy epidermis which is irregular in outline. Trichomes are also seen. Epidermis is followed by collenchyma. Sclerenchymatous cells, which is six layered is seen just below the grooves and this is followed by chlorenchymatous cells and rounded parenchyma. Bicollateral vascular bundles seen. Large vessels are seen at the innermost vascular bundles but small vessels are seen in the peripheral vascular bundles. Pith is almost absent in mature portion.

4. DISCUSSIONS

Climbing plants present numerous morphological and anatomical characteristics that distinguish them from other forms of plant life. Among these characteristics are the anatomical structure of the stems and the climbing and attachment mechanisms. Vines have long and flexible stems that depend on external support to maintain themselves erect or to reach illuminated areas in their habitat.

The stems of climbing plants are characterized by the scarcity of supporting cells (fibers) and an increase in the diameter of the xylem vessels, which may be visible to the naked eye. The increase in the diameter of the xylem vessels triplicates the conduction of water, making climbers able to maintain a great quantity of leaves in relation to the total diameter of their stems. (Ampelocissus latifolia) These stems that are specialized for the conduction of water are known only in plants that possess xylem vessels (elements with perforated walls), and are absent in those that have only tracheids or imperforate elements. Imperforate elements obviously represent an obstacle to the free flow of water, slowing it down and making water transport over great distances difficult.

The stems of climbing plants face structural challenges that differ from those experienced by trees and shrubs. They are subjected to tensile and

compacting forces, due to the movement of the structures (usually small trees) that support them. For this reason their stem construction, with an alternation of vascular and parenchymatous tissues, gives them considerable flexibility to withstand these types of pressure (*Ampelocissus latifolia, Tinospora cordifolia and Cyclea peltata*). In addition, the stems of climbing plants are subject to friction against the host trees that can cut or tear irregularly their bark and thus wound the phloem tissue. Many lianas have encountered a solution to this problem by having phloem tissue inside the xylem. (*Tinospora cordifolia* and *Calycopteris floribunda*). The arrangement of phloem tissue in relation to the xylem can produce patterns sometimes considered anomalous, which serve to characterize families or genera of climbers.

Alternation of bands of vascular tissue with connective tissue. This pattern is the result of the activity of successive bands of cambium that produce a band of vascular tissue (xylem and phloem) accompanied by a band of connective tissue (parenchyma). The cambial activity is repeated to produce successive concentric bands of vascular tissue and connective tissue. The connective tissue can be as wide as the vascular tissue, thus producing a conspicuous pattern of alternating bands. This pattern can be observed in *Ipomoea separia* of the family Convolvulaceae.

Non-concentric bands. This pattern, like the previous one, is the result of the activity of successive bands of cambium. In this case, however, the activity of the cambial tissue gives rise to asymmetric bands, which develop primarily toward only one sector of the stem, thus producing a stem whose pith is not in a central position. Examples of this pattern are seen in the *Centranthera* of the family Fabaceae, and in the *Cyclea peltata* of the family Menispermaceae.

Discrete vascular bundles. This pattern is the result of the activity of successive bands of cambium, which produce discrete bundles of xylem and phloem surrounded by parenchyma cells. The resulting pattern is that of collateral bundles dispersed in connective tissue (parenchyma). Examples of this type of pattern are found in *Tinospora cordifolia*

In one pattern, the peripheral vascular cylinders are of a smaller diameter than the central cylinder and can be seen both in young stems and in mature ones. Vascular Bundles are of different diameters. This pattern is visible only in mature stems. Examples are found in the genus *Ipomoea separia*

Compressed stem pattern, with the vascular cylinder in a central position, is obtained through asymmetrical secondary growth, in which the stem grows laterally in two opposing directions. Examples of this pattern are found in *Centrosema virginianum* .

In the climber, *Ampelocissus latifolia*, the aerial stem includes young stems with parenchymatous cortex and a cylinder vascular, as well as older stages with significant secondary growth of the vascular cambium and periderm.

Structure and development of included phloem was investigated in the stems of *Calycopteris floribunda*. After the definite period of cambial activity, cells in the middle of the cambial zone began to differentiate into thin walled cambial derivatives which separated the cambium into outer and inner cambial segment at certain places. Rest of the cambium along with separated outer segment remained functionally active while inner segment became temporarily nonfunctional. Original circular outline of the cambial cylinder was restored by joining of outer segment with existing one whereas inner cambial segment got embedded resulting in production of an islands of included phloem in the secondary xylem. This process was repeated several times resulting in a number of phloem islands surrounded within thick walled secondary xylem. Differentiation of phloem elements was initiated only after the formation of thick walled xylem derivatives from the outer cambial segment. The segments of the cambium producing the phloem island remained active for fairly long time. Sieve tube elements of the phloem islands situated deep inside the older stem became non-functional and underwent obliteration after heavy accumulation of callose. Secondary xylem was diffuse porous with indistinct growth rings and composed of vessels (both wider and fibriform vessels), nucleated xylem fibers, axial and ray parenchyma.

ACKNOWLEDGEMENT

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DYNAMICS OF SUSTAINABLE LIVESTOCK AND NATURAL RESOURCES MANAGEMENT IN PACHAIMALAI HILLS, EASTERN GHATS, TAMIL NADU

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ABSTRACT

Pachaimalais situated at the North West border of Thiruchirapalli District, the Pachaimalais (Green Hills) extend into the Salem District (Attur Pachaimalai). The traditional communities (Malayalis) derive both their socio-cultural and spiritual identity from land and forest for which a dynamic body of traditional knowledge were evolved to sustain and manage the natural resources. Looking to the importance of this dynamics, an attempt has been made to explore the knowledge and practices pertaining to livestock and natural resources management governed by traditional knowledge. To achieve the objective, the livestock owners from different ethnoculture from different villages of Pachaimalai hills, Tamilnadu were selected purposively. Various ethnographic tools, conventional methods like personal interview and participatory tools were adopted to explore and interpret the data. A range of locally available plant and other materials are widely used for curing many diseases and ailments of livestock. The indigenous biodiversity including local grasses, shrubs and trees are dynamically associated with local feeds, forages and are over all part of natural resources management of livestock owners. Still more preference is given to rear the local breeds of different livestock on account of their socio-cultural and spiritual compatibility resulting in conservation of biological diversity. The ecological diversities in which pasture lands are categorized in to private and common property and associated with customary rules and culture play a significant role in sustainable use, conservation and management of the natural resources.

Keywords: Natural resources management, Pachaimalai hills.

1. INTRODUCTION

The indigenous knowledge (IK) tuned to local culture, social system, need based, tested over the centuries, dynamic in nature allow the local people to adapt to social and ecological attributes and play an important role for food security and overall enhancement of the sustainability of natural resources. In relation to natural resources management, various aspects of ethnoveterinary medicine, vegetation taxonomy, water and forestry resources, tenure arrangements, mobility patterns and breeding concepts have been described (Geetha *et al.,* 1996). The local knowledge pertaining to ethnoveterinary and natural resources deals with folk belief, culture, knowledge, skills and methods and practices pertaining to the healthcare of livestock. However, many IK systems and social institutions are currently at risk of extinction and threat because of rapidly changing natural environments and economic, political and cultural changes on a global scale. Practices can vanish, as they become inappropriate for new challenges or because they adapt too slowly.

2. MATERIALS AND METHODS

Pachaimalai hills are well known for its ethnocultural diversity and role of different tribal society based institutions in natural resources management. The livestock owners and practitioners of IK belong from tribal community and resource poor classes of villagers. The study area lies in the rainfed agroclimatic zones of Pachaimalai hills. These villages are rich in forest having bamboo, redwood, santalwood, teak and other local biodiversity used for medicine against both human and animals. Most of them have cows, bullocks, goat, poultry and pigs as a subsidiary source of income and major components of their farming system for sustainable livelihood. They adopt indigenous backyard gardening for obtaining necessary local vegetables, fruits, fodder, medicinal plants and to some extent food grains.

In keeping with the socio-cultural, political and ecology approach, the research applies historical and social analysis to understand dynamics of users (livestock owners) and managers of local ecosystems. During five trips of 10 months in 2010 living in selected villages. A survey questionnaire was applied to a conventional mixed sample and open-ended closed questions were asked.

Informants were chosen to include three different generations from the community. The outcome was an oral eyewitness account of a systematized portion of reality.

3. RESULTS AND DISCUSSION

The tribal people were mostly dependent on the indigenous crops ranging from cereals to pulses and oil seeds in combination with forest products. Diversification process adopted by local people is more dynamic than to sustain life. Local livestock owners still prefer and depend on the locally available indigenous plants for ethnoveterinary practices for curing different diseases and disorders of their animals like diarrhoea, maggot, puberty problem, ephemeral fever, wounds, insect bites, foot and mouth disease (FMD), worms infection, sores and blain and influenza. These locally available ethnoveterinary practices are more compatible to their sociocultural & economic values, based on years of experience, without side effects, cost effective. It not only important for animals but were also found to cure many diseases in human.

3.1. Biological and cultural diversity

The major factors responsible for conserving and using the local breeds of animals were found to be more or less similar (Galay *et al.,* 2010). The local breeds are compatible with the socio-cultural and biophysical conditions, useful in making variety of ethnic foods & cloths, providing manure & fuel, draught power, compatible to access natural resources, and helpful in developing knowledge & cultural network among livestock owners. Human cultures and languages are also vanishing rapidly and if a culture disappears, it irretrievably takes along a wealth of knowledge and the domestic animals and plants that had been the basis of its livestock and food production system.

3.2. Livestock rearing and natural resource management

Pastoralists' traditional ecological knowledge of the landscapes and the local livestock resources provides invaluable information resources for efficient livestock production involving nutrition, breeding and veterinary practices (Mathias, 2004). These factors include rapid deforestation by influential farmers to expand the agricultural land resulting in the loss of valuable local fodder biodiversity, nutritious indigenous perennial grasses replaced by less nutritious annual grasses and loss of grazing land has resulted in overgrazing of the more palatable grass species. Availability of crop residues of both local grains and pulses are declining.

Table 1. Particulars regarding the Botanical name, habit, plant parts used and their therapeutic uses of the species studied.

Sl.No	Botanical name	Habit	Plant parts used	Therapeutic uses
1.	*Abrus precatorius* L.	Climber	B	Anthrax,
			L	Insect bite,
			W	Retained placenta,
			Se	Liver disorder
2.	*Achyranthes aspera* L.	Herb	L	Opacity of cornea
			W	Retained placenta
			I	Boil, ulcers, Wounds
3.	*Ailanthes excelsa* Roxb.	Tree	B	Anorexia
			L	Body Lice
			L	Tympanites and fever
			B	Skin diseases
4.	*Alangium salvifolium* (Linn.f.) Wang	Tree	B	Oedema
5.	*Albizia lebbeck* (L.) Willd.	Tree	B	Fever
6.	*Aloe barbadensis* Mill.	Herb	F	Swellings, mastitis and wounds
7.	*Anacardium occidentale* L.	Tree	N	Infection of Housefly
8.	*Andrographis paniculata* (Burm.f.) Wall. ex Nees	Herb	L	Ephimeral Fever
			L	Epilepsy
9.	*Argyreia nervosa* (Burm.f.) Boj	Climber	L	Trypanosomiasis
			L	Wounds and skin diseases
10.	*Atalantia monophylla* (L.) DC.	Tree	L	Wounds

11.	*Azadirachta indica* A. Juss.	Tree	L	Suppuration
			L	Ulcer
12.	*Balanites aegyptiaca* (L.) Delile	Tree	L	General Opacity
			F	Expulsion of Placenta
13.	*Barringtonia acutangula* (L.) Gaertn.	Tree	St	Rheumatism
			L	Dysentery
14.	*Breynia retusa* (Dennst.) Alston	Shrub	L	Cough
			L	Maggots of Infect Sores
18.	*Cissus quadrangularis* L.	Climber	S	Bone fracture
			S	Sprains and swellings
19.	*Coccinia grandis* (L.) Voigt	Climber	F	Dizziness
			L	Dysentery
20.	*Crotalaria verrucosa* L.	Herb	L	Ephemeral Fever
			L	Insect bite
21.	*Curculigo orchioides* Gaertn.	Herb	R	Khuri disease
			T	Anthrax
			R	Impaction
			T	Eye Disorders
22.	*Curcuma longa* L.	Herb	Rh	Sprains, swellings and mastitis
23.	*Cynodon dactylon* (L.) Pers.	Herb	W	Diarrhea
			W	Snake bite
24	*Dodonaea viscosa* (L.) Jacq.	Shrub	L	Bone fracture
25.	*Enicostemma axillare* (Lam.) Raynal	Herb	R	Wound
			L	Ephemeral Fever
			L	Horn cancer
26.	*Euphorbia antiquorum* L.	Shrub	La	Bone fracture
27.	*Evolvulus alsinoides* (L.) L.	Herb	L	Boils, Blisters, ulcers and
			L	wounds
				Ephemeral Fever
28.	*Gmelina asiatica* L.	Tree	L	Epitaxis
			F	Insecticide
29.	*Gymnema sylvestre* (Retz.) R.Br.	Climber	L	Ephermeral fever
			L	Galactogogue
			L	Diarrhea
	Justicia adhatoda L.	Shrub	L	Panting
30.			L	Anthrax
			L	Epitaxis
31.	*Litsea glutinosa* (Lour.) Robins	Tree	L	Indigestion
32.	*Martynia annua* L.	Shrub	L	Boils, Blisters, ulcers and
			L	wounds
				Epilepsy
34.	*Ricinus communis* L.	Shrub	L	Swellings and wounds
			Oil	Gout
35.	*Senna auriculata* L.	Shrub	L	Boils, swellings and wounds
			L	Maggot infected sores
			Se	Skin diseases
36.	*Terminalia bellirica* (Gaertn.) Roxb.	Tree	F	Foot and mouth Diseases
			F	Diarrhea
37.	*Tinospora cordifolia* (L.) Merr.	Climber	St	Poultry disease
			L	Bone fracture
38.	*Wrightia tinctoria* R.Br.	Tree	St	Anthrax
			St	Snake bite

3.3. Ecological diversities

The major preconditions are ethno-ecological variability of the pasturelands, utilization patterns, indigenous institutional framework and socio-economic equity. The pastoralists experiential wisdom on pasture land use and management showed the distinct ecological zones in accordance with the characteristics of the natural resources. The accessibility for the outside boundary of the villages is restricted. The local forests also provide the spiritual and cultural significance for ceremonies and are important for the local community, consequently resulting in sustainability of local biodiversity (Ponnusamy *et al.,* 2009).

3.4. Local feed resources

Tribal livestock owners use a wide variety of leaves and other locally available materials for the healthcare of livestock. For feeding the animals, they have identified many plants, trees, shrubs, and grasses, according to the season and choices and nature of animals (Karethikeyani and Janardhanan, 2003). During the drought period, additional wild shrubs, leaves and grasses are used to supplement dietary requirements and to sustain health. These resources are naturally found and conserved on common and barren or fallow land.

4. CONCLUSION

Livestock owners and communities usually pass on their indigenous knowledge of resource management to the next generation through oral transmission. Hence, the continuity and transmission of that knowledge and its associated culture from one generation to another and its more effective distillation into practical applications that are socially and economically viable, are critical factors in survival of culture and dynamics of natural resources (Jain and Srivastava, 2003). The local people use and conserve locally available plants relevant to cure different diseases and disorders of animals. These locally available practices are cost effective and easy in operation for the first hand remedies. The local plants based feed materials are identified as an alternative to maintain the health of animals.

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ANTIMICROBIAL ACTIVITY OF THE FOLKLORE MEDICINAL PLANT, *ACACIA CAESIA* (L.) WILD.

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ABSTRACT

The aim of this study is to evaluate the antimicrobial efficacy of stem bark extracts of the folklore plant species, *Acacia caesia* L. by using three alcoholic solvents *viz;* petroleum ether, ethyl acetate and methanol were tested against ten human pathogenic bacteria *viz*., *Pseudomonas aeruginosa, P. stutzeri, Escherichia coli, Micrococcus* sp., *Lactobacillus* sp*., Servatia* sp., *Moraxetta* sp., *Bacillus subtilis*, *B. thuriengensis*, and *Klebsiella pneumoniae* and ten human pathogenic fungi *viz., Aspergillus niger, A. flavus, A.baumannii, Fusarium oxysporum, F. solani, Mucor rouxii, Alternaria alternata, Candida albicans, Cladosporium* sp. and *Rhizopus* sp*.* for assessing the antimicrobial properties by adapting disc diffusion method. The results of the study revealed that all extracts showed varied degree of antimicrobial activity against the tested pathogens. However, the ethyl acetate extracts exhibited higher inhibition zone (17.23 mm) against the bacterium, *Klebsiella pneumoniae* and the fungus, *Mucor rouxii* (30.77 mm). These results support the therapeutic importance of the species, *Acacia caesia* in curing infectious diseases and encourage the extensive use of this species in health care practices.

Keyword: Folklore Medicinal plant, *Acacia caesia*, Antimicrobial activity.

1. INTRODUCTION

Plants have been an essential part of human society since the start of civilization. Around 250 drugs have been identified from plants during Rig veda and Atharvana veda descriptions of the veda period. The rural population in different part of the world is more disposed to traditional ways of treatment because of easy availability and cheaper cost. It is estimated that 80% of the population is consulting with traditional healers (Iris *et al*., 2007). The universal role of plants in the treatment of diseases is established by their employment in all important systems of medicine. There are many herbs on earth which lies unexplored in the field of medicine or Science. Many of the plants used today were known to the people of ancient culture throughout the world for their preservative and medicinal powers (Zaika, 1975). However several plants are used in India in the form of crude extracts. Infusions or plaster to treat common infections without scientific evidence of efficacy (Ahmad *et al*., 1998). Among them, many species of *Acacia* are found to have diverse photochemical compounds of medicinal properties (Lee *et al*., 2000; Readel *et al*., 2001; Seo *et al*., 2002; Sathishkumar *et al*., 2009). *Acacia caesia* L. belongs to the family, Mimosaceae is one such folklore plant used in traditional system of medicine in Coimbatore district of Tamil Nadu, India. It is an armed woody shrub occurring throughout the tropical and sub-tropical regions of India (Krishnamurthy, 1993). This plant species has been used as a folk remedy for the treatment of skin diseases, asthma, bronchitis, scabies, cold, menstrual disorders and antiseptic also. The leaves of this plant are used as vegetable and the powdered bark and pod are used as substitute for soap and their decoctions as lice killer (Thammanna and Narayana, 1990). Woody branches of this species are used as tooth brushes by tribal folk and the shrub is used as fuel wood. However, no published works are available for the antimicrobial property of stem bark of this plant. Hence in the present study, an attempt has been made to focus the plant in this angle and hence to assess its therapeutic potency.

2. MATERIALS AND METHODS

2.1. Plant material

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

2.2. Preparation of extracts

250g air-dried stem bark powder was subjected to 250ml of methanol in soxhlet extraction for 8 hours (50-85^{0}C). The extracts were concentrated to dryness in a flask evaporator under reduced pressure and controlled temperature (50-60˚C) to yield crude residue, which was then

stored in refrigerator. To obtain petroleum ether and ethyl acetate extracts, the same method as used to obtain methanol extract was adopted.

2.3. Media used

Freshly prepared nutrient agar medium and PDA medium were used for the culture of bacteria and fungi respectively.

2.4. Microorganisms

In vitro antimicrobial activity was examined for the chemical extracts of stem bark of the study plant, against ten bacterial species which include the gram positive strains *viz*., *Micrococcus* sp., *Lactobacillus* sp., *Bacillus subtilis*, *B. thuriengensis* and gram negative strains *viz., Pseudomonas aeruginosa, P. stutzeri, Escherichia coli, Klebsiella pneumoniae, Servatia* sp. and *Moraxetta* sp. and fungal species *viz., Aspergillus niger, A. flavus, A. baumannii*, *Fusarium oxysporum, F. solani, Mucor rouxii, Alternaria alternata, Candida albicans, Cladosporium* sp. and *Rhizopus* sp*.* All these microorganisms were obtained from the Department of Microbiology, Tamil Nadu Agricultural University, Coimbatore. All the microorganisms were maintained at 4^0C on nutrient agar slants (for bacteria) and PDA slants (for fungi) until further use.

2.5. Antimicrobial assay

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

3. RESULTS AND DISCUSSION

The antibacterial activity of the all the alcoholic stem bark extracts of the study species, *Acacia caesia* generally showed inhibitory activity against the growth of *Bacillus subtilis, Bacillus thuringiensis, Klebsiella pneumoniae* and *Moraxetta* sp., However, towards *Micrococcus* sp., *Lactobacillus* sp., *Escherichia coli, Pseudomonas aeruginosa, P. stutzeri* and *Serratia* sp., and all these extracts showed activity with less pronounced manner (Table 1). It is explained that the different phytochemicals like steroids, cardiac glycosides, anthraquinone, flavonoids and phenolics extracted by different solvents may be responsible for their antibacterial effects (Tambekar and Khante, 2010). Further, the ethyl acetate extract has determined to have highest inhibitory activity (17.23 mm diameter inhibitory zone) against the bacterium, *Klebsiella pneumoniae* (gram negative) and (16.93 mm diameter inhibitory zone) against the bacterium, *Pseudomonas stutzeri* followed by the methanol extract against the bacterium, *Bacillus thuringiensis,* (gram positive) (16.63 mm diameter inhibitory zone). It indicates the presence of effective active principle compounds in the ethyl acetate and methanol extracts of stem bark part of *A. caesia* to suppress both gram negative and gram positive bacteria. It has been observed further that the ethyl acetate extracts showed significantly higher inhibitory activity against the colonial growth of *Bacillus subtilis*, *B. thuringiensis, Escherichia coli, Pseudomonas aeruginosa* and *Serratia* sp. than that of the commercially available antibiotic, tetracycline. This fact shows the higher therapeutic potential of ethyl acetate extract of the study species. The petroleum ether extract has comparatively less activity against most of the tested pathogens. It may be attributed to the presence of respective active compounds with insufficient quantities in this crude extract (Taylor *et al*., 2001).

The antifungal activity of various alcoholic stem bark extracts of the study species, *Acacia caesia* against the ten studied fungal species is given in Table 2. The results of the study report that the ethyl acetate extract has the highest inhibitory activity (30.77 mm diameter inhibitory zone) against the fungus, *Mucor rouxii*. The petroleum ether and methanol extracts were also found to be better with respect to inhibitory function against the two fungal species, *Mucor rouxii* (20.73 and 24.73mm diameter inhibitory zone respectively) and *Alternaria alternata* (15.77 and 18.67mm diameter inhibitory zone respectively).

Table 1. Antibacterial activity of certain alcoholic stem bark extracts of the species, *Acacia caesia*.

Plant extract	Diameter of zone inhibition (mm)									
	Gram positive bacteria				Gram negative bacteria					
	Bacillus subtilis	***B. thuringiensis***	***Micrococcus* sp.**	***Lactobacillus* sp.**	***Klebsiella pneumoniae***	***Escherichia coli***	***Pseudomonas stutzeri***	***P. aeruginosa***	***Serratia* sp.**	***Moraxetta* sp.**
Standard *	24.77 ± 0.20	30.76 ± 0.56	22.63 ± 0.57	25.27 ± 0.64	12.33 ± 0.42	18.53 ± 0.61	12.97 ± 0.25	28.83 ± 0.67	32.63 ±0.56	25.36 ±0.82
Petroleum ether	12.87 ±0.15	11.16 ± 0.57	-	-	9.16 ± 0.37	-	8.93 ± 0.40	-	-	6.97 ± 0.25
Ethyl acetate	14.13 ± 0.61	12.13 ± 0.61	11.73 ± 0.56	8.77 ± 0.59	17.23 ± 0.58	12.16 ±0.66	16.93 ±0.40	12.87 ± 0.85	12.67 ± 0.36	10.03 ± 0.45
Methanol	11.16 ±0.47	16.63 ± 0.60	8.77 ± 0.56	8.77 ±0.32	10.73 ± 0.75	8.06 ± 0.30	-	14.13 ± 0.61	7.93 ±0.31	13.16 ±0.47

*Tetracycline

Table 2. Antifungal activity of certain alcoholic stem bark extracts of the species, *Acacia caesia*.

Plant extract	Diameter of zone inhibition (mm)									
	Aspergillus niger	***A. flavus***	***A. baumanii***	***Fusarium oxysporum***	***F. solani***	***Mucor rouxii***	***Alternaria alternata***	***Candida albicans***	***Cladosporium* sp.**	***Rhizopus* sp.**
Standard *	27.67 ± 0.48	28.17 ± 0.67	26.73 ± 0.67	30.73 ±0.67	23.73 ± 0.67	25.73 ±0.67	27.67 ± 0.61	10.73 ± 0.67	15.77 ± 0.75	40.83 ± 0.85
Petroleum ether	-	-	8.73 ±0.66	-	9.73 ±0.70	20.73 ±0.67	15.77 ± 0.75	-	-	10.77 ±0.75
Ethyl acetate	10.63 ±0.53	12.77 ± 0.71	10.77 ± 0.71	12.67 ±0.59	12.77 ± 0.75	30.77 ±0.71	17.73 ±0.70	7.67 ± 0.59	8.67 ±0.65	14.77 ±0.71
Methanol	7.73 ± 0.54	10.73 ± 0.70	11.63 ± 0.65	8.17 ± 0.38	10.76 ± 0.71	24.73 ±0.67	18.67 ±0.65	13.77 ± 0.75	8.03 ±0.91	-

*Tetracycline

This fact indicats the existence of strong antifungal activity of stem bark part of the study species, *A. caesia* and hence its effective healing property against the infectious diseases. The variation in antifungal activity across the extracts studied may be due to the polarity of the solvents used. Significantly higher inhibitory activity of ethyl acetate extract is nearly to the commercially available antibiotic tetracycline against the fungus, *Mucor rouxii* observed shows the superior healingness of stem bark part of *A. caesia.* Proper isolation and purification of active compounds by using ethyl acetate solvent would ensure the therapeutic value of this folklore medicinal plant when it will be used commercially.

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

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A NEW REPORT ON THE AGGREGATION CUM FORAGING SITE OF PAINTED STORK (*MYCTERIA LEUCOCEPHALA*) AND SPOT BILLED PELICAN (*PELICANUS PHILIPPENSIS*) IN MADURAI, TAMIL NADU, INDIA

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ABSTRACT

For the conservation of birds, especially birds like migratory waders, documentation and protection of foraging sites cum aggregation sites along with breeding sites are crucial. These birds also use many sites of urban wetlands and shallow water too as foraging sites at extreme conditions. The present work is to report a new foraging cum aggregation site of a near threatened species such as Painted Stork (*Mycteria leucocephala*) and Spot billed Pelican (*Pelicanus philippensis*) in Madurai, Tamilnadu. Painted Stork was recorded more (74±33.01) when compared to Spot billed Pelican (49±26.32). Maximum of 120 individuals, a minimum of 35 individuals of Painted Stork was recorded per day. A detailed community level study on migratory waders at these sites is crucial for the conservation of these birds.

Keywords: *Mycteria leucocephala*, *Pelicanus philippensis*, Foraging site, Aggregation site.

1. INTRODUCTION

Wetlands are the most valuable ecosystems in the world and are useful for improving water quality and storing flood waters and releasing it slowly as they travel down-stream. A very few natural and artificial wetlands in India have been systematically surveyed to understand their importance for birds (Abhisheka, 2013). India being a megadiversity centre, harbours 1,200 species of birds, which amounts to 13 percent of the bird species of the world 9,600 species. (Nazeema and Nirmala, 2015). Wetlands harbour large number of threatened species of birds in addition to a variety of wildlife. "Waterbirds" refers to the bird species that entirely depend on wetlands for a variety of activities such as foraging, nesting, loafing, and moulting (Rajpar and Zakaria, 2009). Aquatic birds are excellent bioindicators of wetland ecosystems, because they quickly respond to any changes in vegetation composition and water level fluctuation as compared to other animals (Siriwardena *et al.,* 1998; Krebs *et al.,* 1999).

Painted stork (*Mycteria leucocephala*) is a large size wading bird of the stork family. It is found in the wetlands of the plains of tropical Asia, South of the Himalayas in the Indian subcontinent and extending into south east Asia (Hancock *et al.,* 1992). The IUCN Red list status report states that Painted Storks is a near threatened species. Due to loss of natural habitats, the numbers of these storks are declining in recent years (Yee *et al.,* 2013).

The Spot billed Pelican or Grey Pelican (*Pelecanus philippensis*) is a member of the pelican family. It breeds in southern Asia from, southern Pakisthan across India east to Indonasia. This species is most threatened species of the world. The population of Spot Pilled Pelican is estimated about 2,500–5,000 individuals in southern Asia (BirdLife International 2001; Wetlands International 2002). Tamil Nadu has been a traditional home of Spot-Pilled Pelican for centuries in Koonthakulam which appears to have been in existence from well over a century (Grubh, 2004; Rhenius, 1907).

In Southern India, Spot billed Pelican and Painted Stork population have been declined in the recent past years, because of the climate change, destruction of natural habitats urbanization, lack of adequate water in their feeding grounds, and many of the wetlands disappeared or modified to fast growing city. The cutting trees for commercial use of woods, sewage water runoff and urbanization activities causes many species of birds including waders to inhabit in the urban areas and constrain them to breed there. In Tamil Nadu, majority of the wetlands were dried during 2012 due to the reason of poor monsoon rain or failure of monsoon in some areas.

For the conservation of birds, documentation and protection of foraging sites along with breeding sites are crucial for the conservation. However, the foraging areas of Spot billed Pelican and Painted Stork are yet to be explored in many parts of southern India. These birds use many sites of urban

wetlands and shallow water too as foraging sites. The present work is to report a new forging cum aggregation site of these birds at Madurai, Tamil Nadu. The shallow water body situated near kilathikulam wetland that attracts the near threatened species of Painted Stork (*Mycteria leucocephala*) and Spot billed Pelican (*Pelicanus philippensis*) population. The analysis of water quality parameters of this wetland is another objective of this study.

2. MATERIALS AND METHODS

2.1. Study area

A large congregation of Painted Stork and Spot billed Pelican was observed in the second week of May 2013 at the study site (Plate 1). Hence we made an attempt to count and monitor of these birds. This study was carried out in Madurai south taluk region of Iyonpapakudi village. It is very close to Airport- Mattuthavani ring road highways (NH 45B) (Fig. 1). The study area is a shallow revenue land contains housing plots. This area is known as Theiva nagar (9.853800^{o}N, 78.115443^{o}E) and very close to kilathikulam wetland. The water of the study area is overflown from the Thavaranenthal pond, which received water from vellakkal sewage treatment plant.

A total count method was used to count the birds. This method was used by walking around the wetlands or from specific vantage points to count the birds (Vijayan, 1991). The census was conducted between 0700 hours and 1000 hours. The census was carried out in May, 2013. Birds were identified with the help of field guides and standard reference books (Ali and Ripley, 1983; Grimmet *et al*., 2000). The scientific name of the birds was given as in Grimmet *et al.,* 2000.

The study period is the hottest season of the Tamilnadu. The major precipitation of the study area is northeast monsoon which usually brings rain during October-December.

3. RESULTS AND DISCUSSION

We counted the birds for five days and a total of 369 Painted Stork (74 $\pm$ 33.01) (*Mycteria leucocephala*) and 245 Spot billed Pelican (49 $\pm$ 26.32) (*Pelicanus philippensis)* were recorded. We have also observed that the number of birds increased in the study period and reached the maximum of 120 per day on 26th May 2013 (Table1). Painted stork (*Mycteria leucocephala*) and Spot billed Pelican (*Pelicanus philippensis*) were present in more population at the study site than compared to nearby wetlands of Madurai (plate 1).

Our study report's the new foraging cum aggregation site for Painted stork and Spot billed pelicans at Madurai. More over the study site is a shallow revenue land and most of the area contains housing plots. The present study also concluded that water level in a wetland is a major factor for the selection as aggregation cum foraging site by the birds. The water level could be the major cause for the present observation. Because the water level of the nearby wetlands were very low and mostly absent (Thangalakshimi *et al*., 2013). The study area received water from Thavaranenthal pond, which receives the treated effluent from vellakkal sewage treatment plant of Madurai Corporation. So abiotic and biotic factors such as temperature, rainfall, plants, fishes, frogs, small snakes and optimum quality water influenced in determining diversity, abundance of birds in the study area. The results of this study indicated that water level during drought period is a major factor along with the quality. In addition, it also effects on the dynamics of aquatic vegetation composition such as, emergent, submerged, and grasses in this wetland.

Table 1. Number of Painted Stork (*Mycteria leucocephala*) and Spot billed Pelican *(Pelicanus philippensis*) recorded form the study area.

Days	Painted Stork *(Mycteria leucocephala*)	Spot Pilled Pelican *(Pelicanus philippensis*)
14.05.13	50	75
18.05.13	35	25
21.05.13	79	20
23.05.13	85	50
26.05.13	120	75

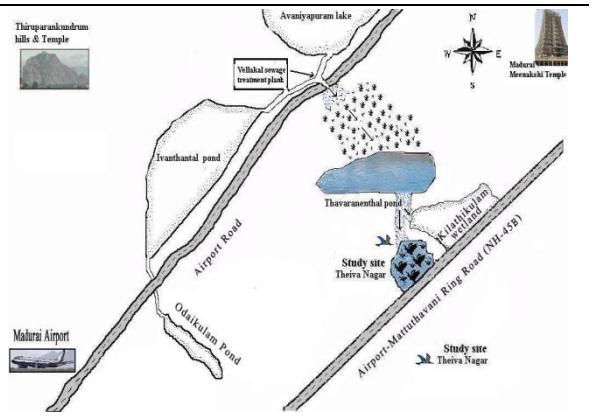

Fig. 1. Map showing the study area.

Map is not to the scale.

Plate 1. Painted Stork (*Mycteria leucocephala*) and Spot billed Pelican (*Pelicanus philippensis*) at the study site.

The analysis of water quality parameters and other habitat qualities of these areas are crucial for the assessment of the habitat. The inventory and mapping of foraging or aggregation sites during summer and drought year, like the present one, are essential for the conservation of Painted stork and Spot billed pelicans of India.

4. CONCLUSION

The present study has reported a new aggregation cum foraging site of Painted Stork (*Mycteria leucocephala*) and Spot billed Pelican (*Pelicanus philippensis*) at Madurai. During extreme conditions like drought, birds especially waders are forced to visit new available foraging sites, also select urban wetlands with meager disturbance too. The results of the present study has led to the conclusion that a detailed study on birds of these sites with community perspective will shed light on status of bird diversity and impacts of unsustainable anthropogenic practices on birds. Hence inventory, documentation and protection of foraging sites cum aggregation sites similar to the site reported in the present study are crucial for the conservation of birds.

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SURVEY ON THE NARROW ENDEMIC THREATENED PLANTS IN MADURAI DISTRICT OF TAMIL NADU, INDIA

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ABSTRACT

Medicinal plant survey was conducted and assessed the narrow endemic, endangered and threatened plants in Madurai district of Tamil Nadu during the year 2013-2014. There are 9 narrow endemic threatened plant species enumerated and further analysed their distribution with various threat categories both global and regional scale. *Hygrophila madurensis* is only one species were identified critically endangered and other species are not evaluated so for IUCN categories but they are distributed narrowly to Madurai and adjacent districts of Tamil Nadu. The data provide the information for diversity of threatened plant species to design the sustainable utilization and conservation measures.

Keywords: Endemic plants, threatened species, conservation.

1. INTRODUCTION

Among the plant diversity of India, endemic medicinal plants are an important source which have been used all over the world. It has been widely used by all sections of the population, and country is richly endowed with a wide variety of plants of medicinal value, which represents the great national resource (Myers *et al*., 2000). It is approximately estimated that at least 70 per cent of country's population relay on herbal medicines for primary health care. In India, different classical medicinal systems such as Ayurveda, Siddha and Unani are being practiced since time immemorial in the country and in addition to these, innumerable local folk medicinal traditions exist. In total about 8000 plant species are in medicinal use. It constitutes around 45 per cent of 17,500 known flowering plant species of India (Ravikumar and Ved, 2000). This rich medicinal wealth is mainly distributed in two hot spots diversity that is north eastern region and Western Ghats. The Western Ghats comprises of a hill range running about 1500 km long the western edge of Indian sub-continent. Although it covers a mere 5 per cent of the country's total land area in the country, it is believed to be more than 27 per cent of country's plant species remarkably high level of endemism ranging from 25 to 60 per cent of recorded species (Pascal, 1992). Narrow endemic plants are important components of the biodiversity of the Western Ghats. The high anthropogenic pressures and associated fragmentation of natural forests have resulted in loss of habitat and species. Several endemic plants species are also under constant threat due to over exploitation from natural habitats for its commercial values in the absence of cultivation. Biogeographically, the Western Ghats have long been isolated from the vast south-east Asian humid forest tract and thus protect a relict pocket of evolutionarily distinct biota. Geology, soil and climate also contribute to promote high biodiversity in these regions.

Peninsular India has a centre of flowering plant endemism, due to diversity of climate and vegetation (Ahmedullah and Nayar, 1987; Nayar, 1996). The Western Ghats of India is one of the 34 global biodiversity hotspots of the world (Myers *et al*., 2000) and over one-third of its angiosperms are endemic (Kaveriappa and Shetty, 2001). It is a chain of mountains of 1600 Km in length running parallel to west cost of Peninsular India from the river Tapthi to Kanyakumari, the southern tip of peninsular India. Many of these endemics are threatened due to human impacts and figure in the threatened categories of the International Union for the Conservation of Nature (IUCN, 2015). Madurai is one of the small district in Tamil Nadu and has medium proportion of its landscape under tree cover (30 %). The reserve forests and protected areas are owned and managed by the forest department and they constitute about 18 per cent of geographical area. The study area of Madurai district is an attractive spot for taxonomist over past centuries, as possessed a part of rich plant diversity in both Western Ghats and Eastern Ghats.

2. MATERIALS AND METHODS

The study area of Madurai district is lying between 9^0 58' N – 78^0 10' E to 9^0 95'N - 78^0 13' E with different forest vegetation types. It covers an area of 3,742 km^2 with rich diversity of potential endemic plants which are many of them are medicinal. Frequent field visits conducted to the area for surveying medicinal plants and collecting the data if local medicinal uses of plants from the study area. The plant specimens were collected in non-destructive manner. The specimens were made into herbarium for identification with standard traditional method. The primary identification of plant specimens done with help of local and regional Floras (Gamble, 1915-1936; Henry *et al*., 1987; Matthew, 1983; Hooker, 1872-1897) and the conformity of identification compared with authentic herbarium deposited Botanical Survey of India, Southern Circle, Coimbatore. The list of medicinal species prepared and analysed the endemic, endangered and threatened plants with pertinent literature (Henry *et al*., 1979; Nayar, 1982 and 1996; Nayar and Sastry 1987 – 1990; Ramesh and Pascal, 1997; http//www.iucnredlist.org). The available narrow endemic medicinal plants enumerated from the study area for the preparation of conservation measured to ensure the survival of potential medicinal plants for posterity.

3. RESULTS AND DISCUSSION

In Madurai district composed of scrub forest, dry deciduous to moist deciduous teak forest and dispersed with semievergreen forests at Alagar hills. There are 9 plant species identified as narrow endemic plants which are distributed only in Madurai district and adjacent areas (Table 1). Out of 9 species, one species is critically endangered, 8 species are not assessed so far. Many of these species are highly exploited for trade purpose either medicinal or some other purposes that are *Caralluma adscendens, C. sarkariae* are largely extracted for both timber and medicinal value. Of these narrow endemic plants, 8 species are restricted to Madurai and surrounding areas, though these species are facing great problem for its survival due to high value utilization in Indian medicine.

Table 1. Narrow endemic threatened plants of Madurai district, Tamil Nadu.

S.No.	Plant name	Family	Distributional range
1.	*Caralluma adscendens* (Roxb.) R.Brown var. *bicolor* V.S.Ramach.,S.Joseph, H.A.John & Sofiya	Apocynaceae	Dindigul, Coimbatore
2.	*Caralluma adscendens* (Roxb.) R.Brown var. *carinata* Gravely et Mayuranathan	Apocynaceae	Theni
3.	*Caralluma sarkariae* Lavranos et Frandsen	Apocynaceae	Dindigul
4.	*Crotalaria digitata* Hook.	Leguminosae	Dindigul
5.	*Fimbristylis paupercula* Boeckeler	Cyperaceae	Dindigul, Tirunelveli
6.	*Fimbristylis rugosa* Govind.	Cyperaceae	Dindigul, Theni
7.	*Fuirena pubescens* (Poir.) Kunth var. *pergamentaceae* C.E.C.Fisch	Cyperaceae	Virudhunagar
8.	*Henckelia gambleana* (C.E.C.Fisch.) A.Weber & B.L.Burtt	Gesneriaceae	Dindigul, Coimbatore
9.	*Hygrophila madurensis* (N.P. Balakr. & Subr.) Karthik. & Moorthy	Acanthaceae	Pudukottai

This is the preliminary survey of the narrow endemic plant diversity in Madurai district of Tamil Nadu. It provides some base line data for sustainable utilization and conservation measures for potential national bioresources. Ecological amplitude is the ability of a growing medicinal plants species in habitat with environmental gradients. Several scientists have referred to this feature by various terms like niche width, habitat preferences or habitat versatility. Until now there are no exclusive studies on the population biology or ecological amplitude of narrow endemic threatened plants. Airi *et al*. (2000) have assessed the habitat ecology of *Nardostachys jatamansi* a critically endangered herbaceous plant species of western Himalaya. Varghese *et al*. (1999) have studied the ecological status of different tree species including some medicinal tree like *Artocarpus hirsutus* in Peppara Wildlife Sanctuary, Kerala. In Tamil Nadu, the studies need for the effect of distribution levels, forest types and association on the regeneration of important narrow endemic plant species. A few studies have

reported for regeneration and distribution of narrow endemic trees like *Palaquium ellipticum* (Ganesh *et al*., 1996), *Myristica malabarica* (Mali *et al*., 2001) and *Embelia ribes* (Rajanna *et al*., 2001). More recently the Madura swampweed (*Hygrophila madurensis*) rediscovered after the type collection from Pudukottai district of Tamil Nadu (Raja *et al*., 2015).

In many cases, the declining habitats of native plants can no longer supply the expanding market for medicinal plant products. In the case of rare, endangered or over-exploited plants, cultivation is the only way to provide material without further endangering the survival of those species. The best means of conservation is to ensure that the populations of species of plants continue to grow and evolve in the wild in their natural habitats. The study gives basic knowledge about documentation of niches and amplitude of rare, threatened and endemic species in a regional scale. This documentation can help locate areas and habitats of high concentration of these species so that critical habitat/habitat sites would get priority for conservation.

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EXPLORATION OF ORNAMENTAL FLORAS IN THE CAMPUS OF S.T. HINDU COLLEGE, NAGERCOIL, KANYAKIMARI DISTRICT, TAMILNADU, INDIA

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ABSTRACT

Most of the present day flowers have come from the wild progenitors, a few of which still exist in natural habitat.Ornamental flowers are highly promising and unutilized resources having tremendous and prover economic importance.Ornamental plants accompany people, since their birth to death and they co-exist with almost all happy events in life such birthday celebrations, weddings, carrier progress etc. In addition, they form our best partners in our everyday life in our flats, offices, different public spaces, parks, gardens and elsewhere.An extensive floristic survey was conducted during the year 2015. Taxonomic identification, photographic documentation and ornamental characterization of each species with potential for use on floral art were recorded. The methodology used is based on observation method for the determination of flora. All the specimens collected were identified with the help of recent literature.The field expeditions of study area gave interesting results concerning floristic diversity.

Keywords: Ornamental flowers, domestication, floristic diversity and methodology.

1. INTRODUCTION

Ornamental flowers are highly promising and unutilized resources having tremendous and prover economic importance (Jenomics, 2014). Ornamental plants accompany people, since their birth to death and they co-exist with almost all happy events in life such birthday celebrations, weddings, carrier progress etc. In addition, they form our best partners in our everyday life in our flats, offices, different public spaces, parks, gardens and elsewhere (Arora, 2013). They are an inseparable part of the culture of all nations and nationalities. This is the reason, why people since time immemorial have tried to improve or change flowers and other ornamental plants according to their imagination, dreams and practical aspects of planting.

2. MATERIALS AND METHODS

2.1. Floristic Survey

An extensive floristic survey was conducted during June - November 2015. Taxonomic identification, photographic documentation and ornamental characterization of each species with potential for use on floral art were recorded. The methodology used is based on observation method for the determination of flora. All the specimens collected were identified with the help of recent literature by local floras authored by Hooker,(1972-1987), Gamble and Fischer, (1915-1935)and Henry *et al*., 1989.

3. RESULTS AND DISCUSSION

The field expeditions of study area gave interesting results concerning floristic diversity. A total of 108 plant species are present in this study area (Table -I). Among the species, dicots were distributed in 36 families with 74 species, monocots in 13 families with 28 species; pteriophytes are in 4 families with 4 species and gymnosperms in 2 families with 2 species, 22 plant species are wild, 72 species are significantly ornamental and 14 plant species are wild or cultivated.When the percentage distributions are calculated it is found that 68.5% of dicots, 25.09% of monocots, 3.7% of pteridophytes, 1.8% of gymnosperms are present. This profiling indicated that the maximum ornamentals are dicots following monocots gymnosperms, pteridophytes and cacti. Among the total identified plants, Acanthaceae is dominant family 14.28.Whenthe percentage distribution is calculated it is found that 68.5% of dicots, 25.09% of monocots, 3.7% of pteridophytes, 1.8% of gymnosperms are present.

The classification of the ornamental flora based on the diversity of its utilization indicating that the maximum 4 of the plant can be used as aquatic ornamentals followed by 22 species as

ornamentals trees, 24 species as ornamental shrubs, 27 species as ornamental herbs, 16 species as ornamental hedges and fencing, 4 species as ornamental succulents and cacti, 11 species as ornamental climbers 2. Regarding the habit wise distribution of identified plants, 22 species as trees, 48 species as herbs, 26 species as shrubs, 4 species as climbers and 8 species as succulents,2 species can be used as ornamental foliage followed by 1 species as carpet bedding, 7 species as mixed borders, 6 species as bushy and upright foliage, 3 species used in topiary formations and also, 3 species are used as ornamental palms, 2 species ornamental ferns, 15 species are recommended for railway lines 18 species are recommended for town roads, 2 species as dry wall, 3 species as topiary and 3 species as a roof gardening (Table 2-4).

Table 1. Number of plants and its family, habits in the study area.

S.No.	Plant Names	Family	Habits	Wild/ Cultivated
1*	*Adathoda vasica Nees.*	Acanthaceae	S	W
2.*	*Allamanda cathartica* L.	Apocynaceae	S	C
3.*	*Albizzi ajulibrissin* L.	Mimosaceae	T	C
4.**	*Aloe sps*	Liliaceae	Su	C
5.*	*Albizzi alebbeck* L.	Mimosaceae	T	C
6.**	*Alocasi amacrorhiza* L.	Araceae	H	C
7.**	*Anthurium sps*	Araceae	H	C
8. ⬡	*Araucaria sps*	Araucariaceae	T	C
9.*	*Aristalochia indica* L.	Aristolocaceae	H	W
10.**	*Asparagus racemosus* Willd.	Liliaceae	CL	C
11.*	*Azadirachta indica* A.Juss	Meliaceae	T	C
12.*	*Balsam impatiens* Royle.	Balsamnaceae	H	C
13.*	*Barleria prionitis* L.	Acanthaceae	H	W
14.**	*Beaureare curvata* Lem.	Agavaceae	S	C
15.*	*Begonia floccifera* L.	Bignoniaceae	H	C
16.*	*Bougainvillea spectabis* Wild.	Nyctaginaceae	CL	C
17.*	*Caesalpinia pulcherrima* L.	Caesalpiniaceae	S	C
18.**	*Caladium bicolour* Vent.	Araceae	H	C
19.*	*Callistemon citrinus* L.	Myrtaceae	T	C
20.*	*Calotropis gigantean* L.	Asclepiadaceae	S	W
21.**	*Caryota urens* L.	Arecaceae	T	W
22.*	*Cassia biflora* L.	Caesalpiniaceae	S	W
23.*	*Cassia fistula* L.	Caesalpiniaceae	T	C
24.**	*Casuarina equisetifolia* L.	Casurinaceae	T	C
25.*	*Catharanthus roseus* L.	Apocynaceae	S	C
26.**	*Chlorophytum cosmosum* Thumb.	Liliaceae	H	C
27.**	*Cissus quadrangularis* L.	Vitaceae	Su	C
28.*	*Clerodendrum speciosum* L.	Verbenaceae	H	C
29.*	*Clitoria ternatea* L.	Fabaceae	CL	C
30.*	*Codiaeum variegatum* L.	Euphorbiaceae	S	C
31.*	*Coleus amboinicus* Lour.	Lamiaceae	H	C
32.*	*Coleus blumei* Benth.	Lamiaceae	H	C/W
33.**	*Commelina benghalensis* L.	Commelinaceae	H	W
34.**	*Cordyline* sps	Liliaceae	S	C
35.*	*Crescentia cujete* L.	Bignoniaceae	T	C
36.**	*Crinum amboinensis* L.	Amaryllidaceae	H	C/W
37.**	*Crinum powellii* Baker.	Amaryllidaceae	H	C/W
38.*	*Crossandra nilotica* Oliv.	Acanthaceae	H	C/W
39.*	*Cryptostegia grandiflora* Roxb.	Asclepiadaceae	S	C/W
40. ⬡	*Cycas revolute* L.	Cycadaceae	T	C
41.*	*Delonix regia* Hook.	Caesalpiniaceae	T	C/W

42.**	*Dieffenbachia picta* Schott.	Araceae	S	C/W
43.**	*Dracaena sps*	Liliaceae	S	C
44. △	*Dryopteris sps*	Dryopteridaceae	H	C
45.*	*Duranta plumeri* Jacq.	Verbenaceae	S	C
46.**	*Eichhornia crassipes* Solms.	Pontederiaceae	H	W
47.*	*Eranthemum tricolor* W.Bull	Acanthaceae	S	C
48.*	*Euphoria antiquorum* L.	Euphorbiaceae	Su	C
49.*	*Euphorbia heterophylla* L.	Euphorbiaceae	H	W
50.*	*Euphorbia milli* Moul.	Euphorbiaceae	H	C
51.*	*Galphimia glauca* Bartl.	Malphiginaceae	CL	C
52.**	*Gompherena globosa* L.	Amaranthaceae	H	W
53.*	*Heliotropium indicum* L.	Boraginaceae	H	W
54.*	*Hemigraphis alternate* Burm .(f)	Acanthaceae	H	C
55.*	*Hibiscus rosasinensis* L.	Malvaceae	S	C/W
56.*	*Hibiscus schizopetalous* L.	Malvaceae	S	C
57.*	*Hippestrum hybridum* L.	Acanthaceae	H	C
58.*	*Ixora coccinea* L.	Rubiaceae	H	C
59.*	*Jacaranda mimosifolia* D.Don	Mimosaceae	T	W
60.*	*Jasmium sambac* L.	Oleaceae	H	C
61.*	*Jatropa hastate* Jacq.	Acanthaceae	S	C
62.**	*Kalanchoebioss feldiana* L.	Crassulaceae	H	C/W
63.*	*Kleinia grandiflora* L.	Asteraceae	H	C
64.*	*Lantana camera* L.	Verbenaceae	H	C/W
65.*	*Lawso niainermis*L.	Lythraceae	S	C/W
66. △	*Microsorum pustulatum* Coper.	Polypodiaceae	H	C
67.*	*Mirabillis jalaba* L.	Nyctaginaceae	H	C
68.**	*Mamilaria baumii* L.	Cactaceae	Su	C
69.*	*Morinda coriea* L.	Rubiaceae	T	W
70.*	*Muntingia calabura* L.	Tiliaceae	T	C
71.*	*Mussaenda frondosa* L.	Rubiaceae	S	C
72.*	*Nymphae* sps	Nymphaeaceae	H	C
73.*	*Opuntia dillenii* L.	Opuntiaceae	Su	C
74*	*Opuntia rhodantha* Mill.	Opuntiaceae	Su	C
75.*	*Orthosiphon spiralis* Lour.	Lamiaceae	H	C
76.*	*Oxalis corniculata* L.	Oxalidaceae	H	W
77.*	*Passiflora foetida* L.	Passifloraceae	CL	W
78.*	*Peltophorum pterocarpum* Roxb.	Fabaceae	T	W
79.**	*Phoenix sps*	Palmae	T	C
80.**	*Pistia stratiotes* L.	Araceae	S	C
81.*	*Plumeriarubra* L.	Apocynaceae	S	C
82.*	*Podranea brycei*L.	Bignoniaceae	H	C
83.*	*Polyalthia longifolia* L.	Annonaceae	T	C
84.**	*Polyscias bulfourana* Andre.	Araliaceae	H	C
85.*	*Quiqualis indica* L.	Combretaceae	S	C
86.*	*Pongamia pinnata* L.	Fabaceae	T	W
87.**	*Rhoeo spathacea* (sw)	Commelinaceae	H	C
88.*	*Ruelia tuberosa* L.	Acanthaceae	H	W
89.*	*Rueliat weediana* Griseb.	Acanthaceae	H	C
90.*	*Russeli aequisetiformis* L.	Scorphulariaceae	H	C
91. △	*Salvinia molesta* L.	Salvinaceae	H	C
92.**	*Sanseveria trifurcate* L.	Convolvulaceae	H	C
93.**	*Sanseveria roxburgiana* Schult.	Convolvulaceae	Su	C
94.*	*Saraca asoka* Roxb.	Fabaceae	T	C/W
95. △	*Selaginella sps*	Selaginellaceae	H	C

96.*	*Stylosanthe shamata* L.	Fabaceae	T	W
97.**	*Setcreasea purpurea* Boom.	Commelinaceae	H	C
98.*	*Swienteni amahagoni* L.	Meliaceae	T	W
99.*	*Santalum album* L.	Santalaceae	T	C
100.*	*Tectona grandis* L.F	Verbenaceae	S	C/W
101.*	*Tecoma stans* L.	Bignoniaceae	S	C
102.*	*Tecomaria capensis* Thumb.	Bignoniaceae	S	C
103.*	*Terminalia catappa* L.	Combretaceae	T	C
104.*	*Thespesia populnea* L.	Malvaceae	T	W
105.*	*Thunber giaerecta* Benth.	Acanthaceae	H	C
106.*	*Tabernaemontana coronaria* Schut.	Apocynaceae	S	C
107.*	*Trapanatans* L.	Trapaceae	H	W
108.*	*Tridax procumbens* L.	Asteraceae	H	W

H = Herb; * Dicot; S = Shrub; **Monocot; T = Tree; △Pteridophytes; Cl = Climber;
⬡Gymnosperms; W = Wild; C = Cultivated; Su= Succulent

Table 2. Cotyledons wise distribution

Sl. No	Nature of the plant	No. of Plants	%
1.	Monocots	28	25.9
2.	Dicots	74	68.5
3.	Pteridophytes	4	3.7
4.	Gymnosperms	2	1.8

Table 3. Characterization of the recorded flora according to the ornamental utilization

S.No	Ornamental Utility	Name of the plants
1.	Aquatic ornamentals	*Trapanatans, Salviniamolesta, Nymphaea* sps*, Pistia stratiotes, Eichhornia crassipes.(4)*
2.	Ornamental trees	*Azadirachta indica, Caryota urens, Araucaria sps, Cassia fistula, Cycas revoluta, Casuarina equisetifolia, Callistemon citrinus, Cresentiacujete, Delonix regia, Jacaranda mimosifolia, Thespesia populnea, Morinda coriea, Polyalthia longifolia, Pongamia pinnata, Peltophorum pterocarpum, Plumeria rubra, Muntingia calabura, Albizzia julibrissin, Albizzia lebbeck, Terminala catappa, Santalum album, Swietenia mahagoni (22)*
3.	Ornamental shrubs	*Tectona grandis, Tecomaria capensis, Tecomastans, Russeliae quisetiformis, Phoenix sps, Polyscias bulfourana, Mussaenda frondosa, Lantana camera, Lawsonia inermis, Jatropaha stata, Ixora coccinea, Hibiscus schizopetalous, Hibiscus rosasinesis, Hippestrum hybridum, Hemigraphis alternate, Eranthemum tricolor, Dracaena sps, Duranta plumerie, Dieffenbachia pictata, Calotropis gigantea, Chlolrophytum cosmosum, Cordylinesps, Cassia biflora, Adathoda vasica (24).*
4.	Ornamental herbs	*Alocasia macrorhiza, Aristalochaia indica, Barleria prionitis, Balsam impatiens, Begonia fiocifera, Caladium bicololur, Cathranthus roseus, Commelinea benghalensis, Crinum powellii, Crinum amboinensis, Coleus blumei, Coleus amboinicus, Euphorbia heterophylla, Clerodendrum speciosum, Gompherena globosa, Hippestrum hybridum, Keleinia grandiflora, Mirabilis jalaba, Oxalis corniculata, Stylosanthus hamatus, Rhoeospa thacea, Ruelia tweediana, Ruelia tuberosa, Orthosiphon spiralis, Tridax procumbens, Setrea seapurpurea, Sanseveria roxburghiana, Sanseveria trifuscata. (27)*
5.	Ornamental Hedge	*Polyalthia longifolia, Casuarina equisetifolia,Albizzia lebbeck, Bougainvillea spectabilis, Delonixregia, Callistemon citrinus, Azadiracta indica, Jacaranda mimosifolia, Plumeria rubra, Pongamia pinnata, Muntingia calabura, Santalam album, Thespesia populnea, Hibiscus rosasinensis, Tecomastans, Tecomaria capensis. (16)*
6.	Ornamental	*Euphorbia antiquorum, Mamillaria baumii, Opuntia dilleni, Opuntia*

	succulent & cacti	*rhodantha.(4)*
7.	Ornamental climber	*Allamanda carthartica, Asparagus race mosus, Cryptostegia grandiflora, Bougainvillea spectabilis, Clitoriaternatea, Cissus quadrangularis, Galphimia glauca, Jasminum sambac, Passiflora foetida, Quiqualis indica, Thunber giaerecta.(11)*
8.	Ornamental foliage	*Alocasia macrorhiza, Coleus blumei, Cordyline sps, Codiaeum variegatum, Caladium bicolour, Dieffenbachia picta, Dracenasps, Dryopteris sps, Chlorophytum cosmosum, Kleinia grandiflora, Phoenix sps, Micorosorum pustulatum, Sanseveria trifuscata, Sanseveria roxburgiana, Begonia flocifera, Asparagus racemosus, Cycasrevoluta, Setreasea purpurea, Commelina benghalensis, Anthurium sps. (20)*
9.	Carpet bedding	*Coleus blumei.(1)*
10.	Mixed Border	*Euphorbia heterophylla, Tecomastans, Thunber giaerecta, Catharathus roseous, Crossandra nilotica, Caesalpinia pulcherrima, Tecomaria capensis. (7)*
11.	Trailers	*Setcrea seapurpurea, Chlorophytum comosum.(2)*
12.	Bushy & upright foliage	*Aracucaria sps, Begonia flocifera, Cordyline sps, Dieffenbachia picta, Dracena sps, Setcreasea purpurea.(6)*
13.	Topiary	*Bougainvillea spectabilis, Clerodendrum speciosum, Durantaplumerri.(3)*
14.	Ornamental palms	*Cycasrevoluta, Phoenix, Caryotaurens.(3)*
15.	Ornamental ferns	*Microsorum pustutatum, Selaginella sps.(2)*
16.	Ornamental trees recommended for town roads	*Polyalthia longifolia, Saracaasoca, Delonix regia, Tecoma stans, Azodirachta indica, Muntingia calabura, Albizzi ajulibrissin, Cassia fistula, Callistemon citrinus, Thespesia populnea, Pongamia pinnata, Jacaranda mimosifolia, Cresentia cujete, Albizzia lebbeck, Peltophoruminerne, Terminalia catappa, Santalum album, Swietenia mahagoni.(18)*
17.	Ornamental trees recommended for Railway line.	*Albizzia lebbeck, Peltophorump terocarpum, Terminalia catappa, Santalum alum, Swietenia mahagoni, Delonix regia, Cassia fistula, Pongamia pinnata, Thespesia populnea, Albizzia julibrissin, Saracaasoca, Azadiracta indica, Polyalthia longifolia, Jacaranda mimosifolia, Cresentiacujete.(15)*
18.	Dry wall	*Oxalis corniculata, Selaginella sps.(2)*
19.	Hanging baketes	*Alocasia macrorhiza, Anthurium sps, Mamillaria baumii, Caladium bicolour, Chlorophytum comosum, Cissus qudrangularis, Commelina benghalensis, Dryopteris sps, Microsorum pustalatum (9)*
20.	Roof gardening	*Bougainvillea spectabilis, Plumeriarubra, Passiflora foetida.(3)*

Table 4. Habitat wise distribution of identified plants

Sl. No	Habitat	No. of Plants
1.	Climber	4
2.	Succulent	8
3.	Shrub	26
4.	Herb	48
5.	Tree	22

Landscape gardening and bio-aesthetic planning is a recent trend to establish eco-friendly human habitats. Exploration of collection and conservation of wild and cultivated ornamental species is also one of the cultural methods to maintain the diversity of the species and conserve to endemic and endangered species of ornamental interest. There is a lot of significance in recent year for the ornamental species in the utilization of various kinds and is the income generation among poor also in the export market of India. Wild ornamental species are also the sources for the medicinal significance (Asati and Yadav, 2010) so the ornamental germplasm relatives are to be conserved. In the development for new hybrids, polyploidy mutation of ornamental interest it is essential to know ornamental species. The dynamic floriculture industry is constantly looking for new products, technology and market riches.

Over-exploitation by humans, both for direct consumption and also for botanical and horticulture value, also threatens wild ornamentals. Grassland reclamation programmes and overgrazing by cattle have had a debilitating effect on these wild species. Fragmentation of extensive habitats into small isolated patches can mean that they become too limited to maintain their plant populations. Fragmentation seems to reduce genetic variation and seedling vigour. Natural disasters have also played a role in species extinction. Even protected areas cannot be expected to safeguard plants from the effects of disasters such as volcanoes, fires, airborne pollutants, droughts and landslide.

This process is largely based on research and development and requires strong collaboration between many links of the production chain most modern scientific research in the field of new ornamental crops deals with the adaptability of new species to the environmental and the regulation of their life cycle or propagation systems. New ornamental products can be developed by researches and breeders only in collaboration with efficient produces and satisfied consumes, linked together in mutually beneficial ways. It is very easy for the propagation of wild species by traditional propagation methods. The cost of domestication and maintenanceofornamental species is also very less in comparison. We hope this work will help the researches and people who are interested in ornamental plants.

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ANTIBACTERIAL ACTIVITY OF METHANOLIC RHIZOME EXTRACT OF *ALPINIA CALCARATA* ROSC. (ZINGIBERACEAE)

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ABSTRACT

The methanolic rhizome extract of *A. calcarata* was evaluated for its antibacterial activities against five bacterial strains *Pseudomonas aeuroginosa, Proteus vulgaris, Salmonella paratyphi, Bacillus thurungiensis* and *Staphylococcus faccealis.* The extract has inhibited all the tested bacterial species with different manner at various concentration. However the higher level zone of inhibition in 400 (mg/ml) is significant against all the above said bacterial strains of these *Salmonella paratyphi.* Based on the present study it can be conculuded that the plant rhizome possess potent anti bacterial activity.

Keywords: *Alpinia calcarata,* antibacterial, *Salmonella paratyphi*.

1. INTRODUCTION

Natural products, such as plants extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity (Cosa *et al.,* 2006). The use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases (Duraipandiyan *et al.,* 2006). Due to the development of adverse effects and microbial resistance to the chemically synthesized drugs, mankind turned to ethnopharmacognosy. They found literally thousands of phytochemicals from plants as safe and broadly effective alternatives with less adverse effect.

Alpinia calcarata Rosc. (Zingiberaceae) is an important medicinal plant among the seven species of Alpinia that occur in Bangladesh, India, Myanmar, Indonesia, Thailand. It is a perennial herb with non tuberous pungent root stock. Its rhizomes showed antinociceptive activities (Arambewela *et al.,* 2004).

2. MATERIALS AND METHODS

Freshly collected rhizome of *A. calcarata* was cut in to small pieces and shade dried. All the dried parts were pulverized by mechanical grinder to get the powder through 100 mesh sieve and stored in a air tight container. Required quantity of powder was weighed and transferred to a conical flask. The powder was treated with various solvents like petroleum ether, methanol, chloroform, ethanol and aqueous. This process was repeated for a week and the extract was filtered through Whatman No.1 filter paper. The filtrate was collected and evaporated to dryness. The concentrated residue was used for various phytochemical and biological studies.

2.1. Tested organisms

The bacterial strains *Pseudomonas aeuroginosa, Proteus vulgaris, Salmonella paratyphi, Bacillus thurungiensis, Staphylococcus faccealis* employed in this study were purchased from Department of microbiology, Bharathidasan University, Trichirappali. All these cultures were maintained on nutrient and potato dextrose agar plates at 4°C in lab.

2.2. Antibacterial assay

Anti bacterial activity of various concentration of *A. calcarata* rhizome was determined by the disc diffusion method (Bauer *et al.,* 1966*).* All petridishes were plated with nutrient agar prepared according to the manufacturer's manual given below.

Chemical composition of nutrient agar medium for bacterial culture

S.No.	Composition	Quantity (g)
1.	Peptone	5.0
2.	Beef extract	3.0
3.	Sodium chloride	5.0
4.	Agar	15.0
5.	Distilled water	1000ml
6.	pH	7.0

Sterile liquid nutrient agar medium (pH 7.4±2) was poured (15-20ml) into each sterile petriplate. The test organisms were inoculated with the help of a sterile cotton swap soaked in respective bacterial culture grown in peptone broth. The disc containing plant extract was placed on the solidified agar plate in such a way that there is no overlapping of zone of inhibition. Chloramphenicol antibiotic disc (10mg)

was used as standard. Plates were kept at room temperature for half an hour for the diffusion of the sample into agar media. The organisms inoculated in the petridishes were incubated in thermostat at 37°C for 24hrs. The zone of inhibition produced by plant extract on different organisms were measured and recorded by using zone reader. Antibacterial activity was evaluated by measuring the zone of inhibition against the test organisms. Each assay was conducted in triplicate.

3. RESULTS

Antibacterial activity of *A.calcarata (*Zingiberaceae) in methanol extract was carried out against selected negative bacterial strains *Staphylococcus aureus*, *Pseudomonas aeroginosa*, *Proteus vulgaris*, *Salmonella paratyphi* and positive strain *Bacillus subtilis* and these are compared with reference antibiotic, Ampicillin. Generally, methanol extract with the concentration of 400(mg/ml) showed a significant zone of inhibition against all the above said bacterial strains. of these *Salmonella paratyphi,*has showed highest degree of inhibition followed by*, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeroginosa,and Proteus vulgaris*. Methanol extract with the concentrations of 300 (mg/ml) and 200 (mg/ml) showed a moderate activity against all the bacteria.Thus the methanolic extract of *A. calcarata* in significant level shows antibacterial activity and it could be used for controlling bacterial diseases.

4. DISCUSSION

The crude extracts from rhizome of *A.calcarata* in methanolic solvent were subjected to antimicrobial screening against selected Gram positive and Gram negative bacteria. All the extracts showed varying degree of inhibitory potential against all the tested bacteria. Acetone and chloroform extracts of leaf had higher inhibitory action against *Salmonella typhi* and *Streptococcus subtilis* respectively. Acetone extracts of stem showed maximum inhibitory action against *S. typhi* and benzene extracts of stem had moderate inhibitory action against *Escherichia coli* (Viji and Murugaesan, 2010). Ripa *et al.* (2010) have explained *Nephelium longan* has significant antimicrobial activity. Chloroform extracts of leaf and stem showed excellent activity with the average zone of inhibition of 13-21mm among the tested bacteria. The ethyl acetate crude extracts showed good activity against the growth of *Sarcina lutea*, *Vibrio mimicus*, *Salmonella typhi*, *E.coli* and *Staphylococcus aureus*. Susceptibility of various microbes to the methonalic extract of the plant sample in our study suggest the scope for developing antimicrobial natural herbal drugs on the it is concluded that the promising antimicrobial properties of the plant extract could be exploited in herbal preparation against bacterial infection justifying their use in traditional medicine.

Table 1. Antibacterial activity of various concentration extract of *A. calcarata*

S.No.	Microorganism	Zone of inhibition(mm)				
		Various concentration of extracts used (mg/ml)				Ampicillin
		100 mg/ml	200 mg/ml	300 mg/ml	400 mg/ml	10 mg/ml
1.	*Pseudomonas aeuroginosa*	-	-	6	6	22
2.	*Proteus vulgaris*	-	-	-	-	29
3.	*Salmonella paratyphi*	7	8	8	9	30
4.	*Bacillus thurungiensis*	-	-	-	7	15
5.	*Staphylococcus faccealis*	-	-	-	8	16

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ANALYSIS OF MILK QUALITY, ADULTERATION AND MASTISIS IN FOUR DIFFERENT MILK SAMPLES

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ABSTRACT

The present study is aimed to analyze the milk quality, adulteration and mastitis infection in milk Samples available in market. Four different milk samples were analyzed for physical appearance, quality, adulterants and mastitis infection. 90% milk samples were white in color and 10 % were yellowish white. pH ranged between 6.5 – 6.9. Analysis of milk quality showed that Arokya milk appears to be better than the other three milk samples. Out of 4 milk samples analyzed for adulteration, adulterants found were glucose (80%), skim milk powder (58%), salt (51%) and urea (35%) while found negative for formalin, salicylic acid, boric acid, starch, soap and ammonium sulphate. All the samples were free from mastitis infection. The adulterants decrease the nutritive value of milk and may also cause serious human health related problems.

Keywords: Raw milk samples, Milk quality, Adulteration, Mastitis.

1. INTRODUCTION

Milk is the fluid normally secreted by female mammals for the nourishment of their young ones. It is a part of daily diet for the expectant mothers as well as growing children. Milk provides a near perfect diet for most young animals and in most cases forms the sole source of diets for most mammals in their first few weeks or months of life. Milk is one of the complete foods which there seems to be no adequate substitute. Milk has good quality protein and is a unique substance in that it is consumed as fluid milk with minimal processing and also it is the raw material used to manufacture a wide variety of products. Milk may be modified by condensing, drying, flavouring, fortifying, demineralization and other treatments Milk being a major constituent of human diet, can serve as a good medium for the growth of many microorganisms especially bacterial pathogens, therefore its quality control is considered essential to the health and welfare of a community. As reported by Foster, the threat posed by diseases spread through contaminated milk is well known and the epidemiological impact of such diseases is considerable. The presence of these pathogenic microorganisms in milk has emerged as a major public health concern especially for those individuals who still drink raw milk. The aim of this work therefore is to determine the microbiological quality of raw milk from different locations in Abia state and also to compare the microbial quality of the milk from a controlled environment (Michael Okpara University of Agriculture, Umudike) to others from commercial source .

2. MATERIALS AND METHODS:

2.1. Collection of the samples

Four different milk samples were collected from market and to be transported easily without any delay. The milk samples are Aavin, Raw milk, Arokya millk, Komatha milk samples were collected in autoclaved sterilized containers, All the possible precautions were taken to avoid external contamination at the time of collection of samples and during processing.

2.2. Analysis of milk samples

The raw milk samples were analyzed for physical appearance, quality, presence of adulterants and Mastitis infection. Color and pH of all samples were checked and milk quality was analysed by Methylene Blue Reduction test (MBRT), Alcohol test, Phosphatase test and Clot on boiling (COB) test. The adulteration tests were done using the HiMedia Adulteration Testing Kit protocol. Tests included were Urea test, Salt test, Soap test, Skim milk powder test, Glucose test, Formalin test, Salicylic Acid test, Boric Acid test, Starch test and Ammonium Sulphate test. The mastitis tests were White Side test, Chloride test, Catalase test, Strip Cup test.

2.3. Methylene blue reduction time (MBRT) test

The MBRT test was performed according to American Public Health Association (APHA, 1960). 1 ml of methylene blue solution (1 : 25,000) was added to sterilized and labelled test tubes, each containing 10 ml of raw milk sample. The tubes were sealed with rubber stopper and carefully inverted three to four times to mix up the dye with the milk sample.

All the tubes were incubated in a water bath at 37°C and examined at intervals of 30 min to 1 h for 8 h. The time taken for the methylene blue dye to decolorize was recorded between last inversion and complete de-colourization when four-fifths of the colour had disappeared (Duangpan and Suriyaphan, 2009).

3. RESULTS AND DISCUSSION

The color of milk observed was white in appearance (90%) to yellow (10%). The pH of milk samples ranged from 6.5 to 6.9. The adulterants analysed were glucose (80%), skim milk powder (58%),salt (51%) and urea (35%) while found negative for formalin, salicylic acid, boric acid, starch, soap and ammonium sulphate . All the samples showed negative result for mastitis infection. Milk is one of the most complete foods available in nature for human consumption. Milk contains all nutrients in balanced proportions to meet the demand of humans. Good quality milk is required for quality dairy products. The adulterated raw milk with adulterants is taken as defective and cannot be processed. Recently Chakravorty and Chakravarty (2011) showed that milk distributed in different localities of Varanasi city is highly adulterated and impure.

In the present study, out of the four different milk samples Arokya found to be better than other milk samples based on the quality of milk. The milk samples have white or yellow color with pH ranges from 6.5-6.9. These findings agreed with the reports of Judkins and Mack (1955), who reported that normal milk has a yellowish color due to presence of fat, casein. These differences in color may be due to differences in nature of feed consumption or the breed of cow or the fat and solid contents of the milk. As per the present data, only Arokya milk sample were under better quality standards while compared to other milk samples. Milk, as it is secreted from the udder of a healthy cow is very low in bacterial numbers. Bacteria can increase in raw milk due to poor milking methods, inadequate cleaning of milk equipment, poor cooling and in some cases, as a result of mastitis (Khan *et al.,* 2008).

Table 1. Physical properties of milk.

S.No.	Nature of milk sample	Colour	Odour	Taste
1	Raw milk	Yellowish colour	Sour odour	Salty taste
2	Arokya milk	Creamy white colour	Less sour odour	Sweet taste
3	Aavin milk	Creamy white colour	Less sour odour	Sweet taste
4	Komatha milk	Creamy white colour	Less sour odour	Sweet taste

Table 2: Methylene blue reduction test.

S.No.	Class of milk samples	Time taken for Decolourisation in hours	Quality of milk
1	ClassI(Arokya milk)	Decolourisation takes more than 8 hours	Excellent
2	Class II (Aavin milk)	6 to 8 hours	Good
3	Class III (Komatha milk)	2 to 6 hours	Fair
4	Class IV (Raw milk)	Less than 2 hours	Poor

These differences in color may be due to differences in nature of feed consumption or the breed of cow or the fat and solid contents of the milk. As per the present data, only Arokya milk sample were under better quality standards while compared to other milk samples. Milk, as it is secreted from the udder of a healthy cow is very low in bacterial numbers. Bacteria can increase in raw milk due to poor milking methods, inadequate cleaning of milk equipment, poor cooling and in some cases, as a result of mastitis. Out of four milk samples tested for presence of adulterants, Glucose was highest (80 %), skim milk powder (58%) followed by salt and urea with 51% and 35% respectively. Wadekar Sanjeevani *et al.* (2011) observed that maximum milk samples adulterated with sugar were 20.00 per cent in summer, 12.00 per cent in rainy and 3.00 per cent in winter seasons. Mastitis, an infection of the udder, is one of the most common heard concerns. Mastitis in dairy cows, which is most often result of a bacterial infection (contagious or environmental), causes an increased somatic cell levels in milk. Unhealthy cow's milk has the potential to yield milk that is lower in quality. However, in the present study no positive samples were found for mastitis.

It was assumed that most of these bacteria isolates have the capacity to cause diseases like food poisoning, gastroenteritis and mastitis. However some of these microorganisms can be prevented from causing disease in humans since milk is usually pasteurized or treated before consumption. The

presence of pathogens in milk emerges as a public health concern, especially for those individuals who still drink raw milk. The presence of fungal isolates suggests that not only bacteria can be found in milk. Mucor species which has a world-wide distribution but mostly found in soil, dung etc was isolated. Mucor spp are known to cause diseases in man and animal. Mucor racemosus is often found in milk and other food products. *Candida* spp also found in milk sample causes many human and animal diseases, despite being the normal flora of the skin. This yeast has been implicated in some milk related diseases by some other researchers. Their presence in milk though in low numbers, might be a public health concern Also adequate care, treatment of the animals, and regular check up of the animals in the campus by veterinary doctors and animal scientists goes a long way to reduce infections in these animals.

According to differences in feeding and housing strategies of cows may influence the microbial quality of milk. is generally proportional to pollution degree produced by Listeriosis is a sporadic disease which is often feces associated with the consumption of contaminated milk (Aggad *et al.,* 2010). The presence of high numbers of coliforms in milk and dairy products (Aygun and Pehlivanlar, 2006). The important characteristics of Listeria production of milk, as unclean udder and teats can spp. contributing to food-borne transmission are the contribute to the presence of coliforms from a variety of ability to grow at a low temperature (Rahimi *et al.,* 2010). Sources such as manure, soil, food, personnel and even there was one contamination with L. monocytogenes in water (Bille *et al.,* 2009).

4. CONCLUSION

On the basis of data obtained in the present study, conclusion may be drawn that milk quality is not completely as per standards and adulteration in milk is still in practice and has not been checked completely. It is increasing very fast . Consumption of lower quality milk may lead to serious human health problems. To eradicate this malpractice by local dairy owners which is deep rooted in the cities more than rural areas, steps should be taken from the door steps of local consumers. The consumers must be more active against milk adulteration going on in whole country. It is important to have a quality control system that regularly check and ensure that only good quality milk is sold. The consumers and the milk sellers combined effort will help to decrease the adulteration practice. In conclusion, high microbial counts and the occurrence of pathogens are likely to affect the keeping quality and safety of raw milk as well as products derived from it. The achievement of hygiene in dairy farm directly influences the production oriented economic results and health safety perspective in human beings. It is therefore recommended that the animals be treated by experts to ensure the health of the animals. Also public health training and guidance should be given to farmers, and their workers. Meanwhile, information on health hazards associated with contaminated raw milk should be extended to the public so that consumption of untreated/improperly treated raw milk could be avoided.

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STUDIES ON THE BACTERIAL CONTAMINATION OF SANGANUR CANAL FROM NANJUNDAPURAM ORIGIN TO NOYYAL END POINT, COIMBATORE DISTRICT, TAMILNADU, INDIA

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ABSTRACT

The water quality with particular reference to bacterial analysis was carried out in the river Sanganur canal from Nanjundapuram origin to Noyyal end point, Coimbatore District from January 2014 to June 2014. The obtained results showed that running contamination of streams from agricultural areas was not extremely high, but it showed marked seasonal fluctuations. The bacterial parameters analyzed reveal that the canal is highly polluted with faecal material.

Keywords: Sanganur canal, Coliform bacterial count, *E.coli* count

1. INTRODUCTION

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 well being. 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). The most commonly used indicators for bacteriological contamination are the coliforms: total and fecal coliforms and fecal streptococci. *E. coli* is a subgroup of fecal coliform group, and its presence indicates the fecal pollution of groundwater (Viessman and Hammer, 2005). Oinam Jayalakshmi devi and Belagali (2005) conducted water quality assessment from different districts of southern Karnataka. The results of the study showed that a few samples have objectionable bacterial coliforms.

2. MATERIALS AND METHODS

2.1. Study area and collection of water samples

2.1.1. Sanganur canal

The present attempt was made to analyze the water quality status of Sanganur canal. It's a natural water body from Kurudi malai hills (Nanjundapuram origin) to Noyal river situated at a distance of just 45 km from the Coimbatore city.

2.1.2. Sample collection

For the present study, the samples were collected water Sanganur canal at below mentioned three stations.

Station I: Nanjudapuram in kurudimalai hills sample as Sample I, the origin of Sanganur canal

Station II: Rathinapuri water sample as Sample II of Sanganur canal.

Station III : Noyyal river end point of Sanganur canal water sample as Sample III.

Surface water samples were collected between 11.30 am and 12.30 pm for a period of 6 months from January 2014 to June 2014 every fortnight. Samples were collected in a 300ml Borosil bottles with ample air space were transported in an ice basket and all analysis were carried out within 2hr of collection. All procedures were according to methods outlined and following the guideline of Anon (1976) and the American Public Health Association (APHA 1981). The most probable number of coliform bacterial count and E.*coli* counts (MPN/100ML) were read from the table (APHA 1981).

3. RESULTS AND DISCUSSION

3.1. Standard plate counts

The analysis of the samples for standard plate count during the study period. The populations of bacterial colonies were gradually increased during the study period at all stations. The maximum of 3.5 x 10^3/ ml was recorded during the first fortnight collection of June 2014 in station III and minimum of 0.8 x 10^3/ ml during the first fortnight collection of June 2014 in Station I. The counts of bacterial population increased with the onset of rains and can be attributed to subsoil movement of water carrying pollutants from the catchment area into the river.

3.2. Coliform bacterial counts (MPN)

Variation in coliform bacterial counts (410 - 750/100 ml) of the river water observed in the present study. Mean values in coliform bacterial counts for station III was higher (750/100ml) than those of the other two. The coliform bacterial counts in the residential areas were relatively very high, indicating that their occurrence for the most part was influenced by the residential activities. The minimum coliform bacterial counts were observed during January 2014. This reduction may be due to dilution and death of coliform due to their retention in the river (Holden 1970; Johari *et al* 1999).

3.3. E.coli counts (MPN)

Counts of Escherichia coli varied widely from 30/100 ml to 36/100ml at Station I, 26/100 ml to 30/100 ml at Station II, 37/100 ml to 48/100 ml at Station III. *E.coli* can be used as bio-indicators of aquatic ecosystem dynamics and determination of their occurrence may help to assess the water quality.

Table 1. Standard plate count population in the surface water at different stations

SAMPLE	JAN	FEB	MAR	APR	MAY	JUN
SAMPLE I	0.7	0.9	0.5	0.8	0.7	1.0
SAMPLE II	2.9	2.8	3.1	2.9	3.1	3.5
SAMPLE III	1.7	2.0	1.8	2.0	2.2	2.1

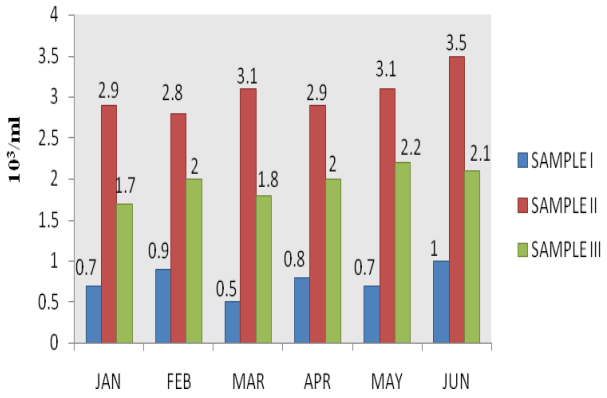

Fig. 1. Standard plate count population in the surface water at different stations

Table 2. Coliform bacterial population in the surface water at different stations

SAMPLE	JAN	FEB	MAR	APR	MAY	JUN
SAMPLE I	410	430	450	420	470	500
SAMPLE II	550	620	590	620	640	670
SAMPLE III	690	680	720	710	730	750

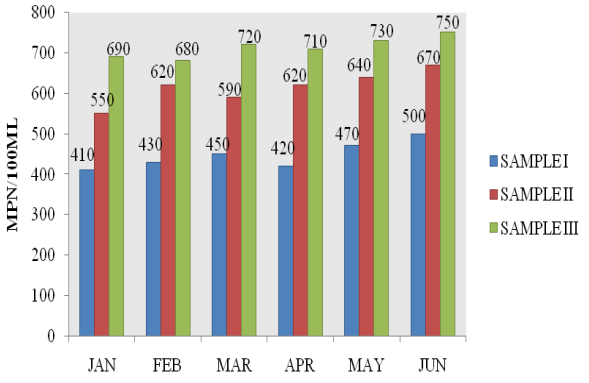

Fig. 2. Coliform bacterial population in the surface water at different stations

Table 3. *Escherichia coli* population in the surface water at different stations

SAMPLE	JAN	FEB	MAR	APR	MAY	JUN
SAMPLE I	33	32	35	36	31	30
SAMPLE II	26	29	30	28	27	25
SAMPLE III	37	40	41	42	45	48

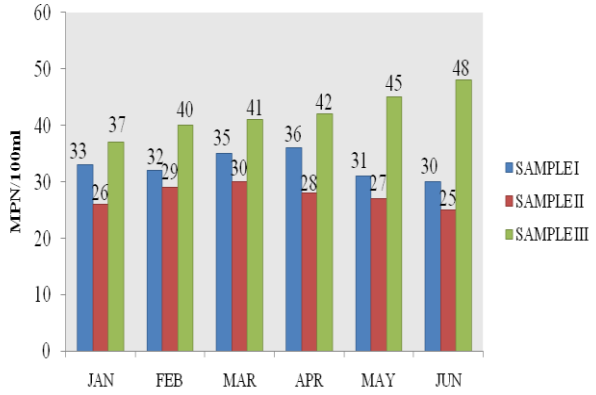

Fig. 3. *Escherichia coli* population in the surface water at different stations

In the present study, it has been found that the Sanganur canal waters were polluted by human and animal wastes. Therefore conservation of this canal are important to protect them from further contamination and for the human needs. This is historic earth water source for Coimbatore city. This is our duty of every individual of Coimbatore for the preservation of the canal.

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GREEN ROUTE SYNTHESIZED SILVER NANOPARTICLES: AS POTENTIAL ANTIBACTERIAL MATERIAL

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ABSTRACT

The green-synthesized method is rapid, superficial, toxic free, suitable, less time consuming, environmental safe and can be applied in a variety of applications in medicinal field. In the present study demonstrated antibacterial action of *Vinca rosea* Leaf extract medicated Silver Nanoparticles (VrL-AgNPs) tested against both Gram positive and Gram negative pathogens such as *Staphylococcus aureus, Escherchia coli* and *Pseudomonas aeruginosa.* The obtained results indicate the VrL-AgNPs achieved maximum zone of inhibition against test pathogens *P. aeruginosa* and significant action against *E.coli* and *S. aureus.* These green routes synthesized silver nanoparticles using biological sources like plant and plant extract which makes them a potent source of antibacterial agent.

Keywords: AgNPs, VrL, Antibacterial, ZOI, pathogenic.

1. INTRODUCTION

Nanotechnology is fast growing by producing nanoproducts and nanoparticles that can have novel and size-associated physico-chemical properties differing considerably from larger matter. The new properties of nanoparticles have been subjugated in a broad range of potential applications in cosmetics, renewable energies material, medicine, biomedical devices and environmental remediation (Tran *et al*., 2013). Metals like gold, silver, platinum and zinc have been used for the biosynthesis of nanoparticles having greater potentials application in field of nanotechnology. Among the all metal nanoparticles, the silver nanoparticles (nanosilver or AgNPs) have increasing attention due to their unique chemical, physical and biological properties. Silver nanoparticles are very important and most widely used nanoparticles with potential applications in biomedical nanotechnology (Arunachalam *et al*., 2013).

The emerging infectious diseases and the development of drug resistance in the pathogenic bacteria and fungi at an alarming rate is a matter of serious concern. Despite the increased knowledge of microbial pathogenesis and application of modern therapeutics, the morbidity and mortality associated with the microbial infections still remains high (Kolar *et al*., 2001). Therefore, there is a pressing demand to discover novel strategies and identify new antimicrobial agents from natural and inorganic substances to develop the next generation of drugs or agents to control microbial infections. Prior to the extensive use of chemotherapeutics in modern health care system, inorganic antimicrobials such as silver and copper were used since ancient times to treat microbial infections (Moghimi, 2005). In the recent times, the advances in the field of nanosciences and nanotechnology has brought to fore the nanosized inorganic and organic particles which are finding increasing applications as amendments in industrial, medicine and therapeutics, synthetic textiles and food packaging products (Gajjar *et al*., 2009; Silvestre *et al*., 2011). This is present study deals with antibacterial properties *Vinca rosea* Leaf (VrL) extract medicated Silver Nanoparticles (VrL-AgNPs) using *Vinca rosea* plant extract tested against both Gram positive and Gram negative pathogens.

2. MATERIALS AND METHODS

2.1. Green Synthesis VrL-AgNP

Our earlier study reported that the simple and environmental free green route was used to synthesis AgNPs from silver nitrate using aqueous extract of *Vinca rosea* (VrL) leaf. VrL extract mediated synthesized AgNPs was characterized using by UV visible spectroscopy (UV-Vis), X-ray Diffraction Spectrum (XRD), Scanning Electron

Microscopy (SEM) and Energy Dispersive X-ray Analysis (EDX) and obtained VrL-AgNPs showed spherical in shape with average particles size round around 30- 70 nm (Rajmohan *et al*., 2015). These green synthesized VrL-AgNPs powder sample was tested for antibacterial activity against human pathogens.

2.2. Microorganisms used

Human pathogenic bacteria culture such as *Staphylococcus aureus (S. aureus) Escherchia coli* (*E. coli*) and *Pseudomonas aeruginosa (P. aeruginosa)* were obtained from Kovai Medical Center Research and Hospital (KMCH) Coimbatore, Tamilnadu, India.

2.3. Antibacterial Activity of VrL-AgNPs

Green route synthesized VrL-AgNPs were tested for antibacterial activity by standard disc diffusion method against three different human pathogenic bacteria. In brief, pure culture of bacteria strain on Mueller Hinton Agar (MHA), the bacterial test organisms were grown in Nutrient Broth (NB) at 37°C for 24 hours. Followed by this step, about 100 µl of aliquot of each strain (1 X 10^5 cfu/mL) was spread uniformly onto the individual plates using sterile cotton swabs and allowed to dry for 15-30 minutes. On other hand, VrL-AgNPs stock prepare at concentration of 0.5mg/mL followed this, sterile filter paper discs around 5 mm in diameter were loaded with 100 µl of VrL-AgNPs (Fig.1) and reference drug were placed on the each cultured plate and incubated at optimum temperature (37°C) for 24 hours to 48 hours and the diameters of the inhibition zones were measured in millimeters.

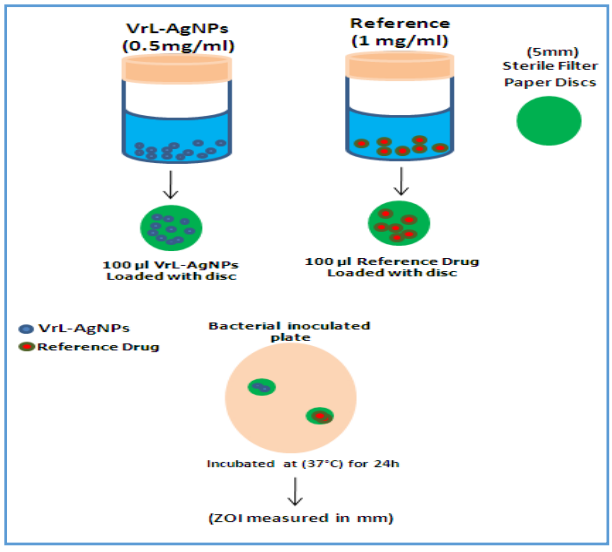

Fig. 1. Schematic diagram of preparation of VrL-AgNPs

3. RESULTS AND DISCUSSION

3.1. Antibacterial Activity

Drug resistant pathogens are more pathogenic microorganisms with high mortality rate than that of wild strain. To overcome microbial drug resistant, researcher are looking forward for the development of alternative, low cost and environmental free novel drugs. Nanobiotechnology is filed of biology with nanotechnology expected to open some new aspects to fight and prevent diseases using atomic scale tailoring of materials (Afreen *et al*., 2011). Antibacterial activity of silver is well-known for many years (Kamyar *et al.,* 2012). The inhibitory action of silver compounds and silver ions had been historically recognized and applied as a useful therapeutic agent for preventing wound infections (Gordon *et al.,* 2010). In this study, antibacterial activity of green route synthesized VrL-AgNPs was evaluated by using standard Zone of Inhibition (ZOI) microbiology assay. Significant antibacterial properties against various pathogens was investigated and compared with control, the diameter of inhibition zones increased for the test pathogen (Fig. 2).

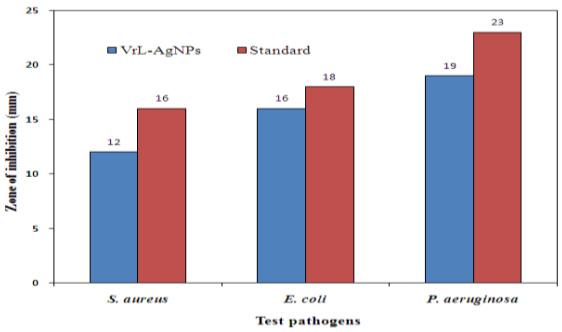

Fig. 2. Antibacterial activity of VrL-AgNPs against different human pathogens

VrL-AgNPs shown maximum ZOI was found to be 19 mm for *P. aeruginosa*, whereas, the other two bacteria strains of *a. aureus* and *E. coli* showed ZOI of 12 and 16 mm. The standard antibiotic streptomycin obtained 16, 18 and 23 mm against *S. aureus, E. coli* and *P. aeruginosa* respectively (Fig. 3). Ranjithkumar *et al*. (2013) study reported that the nanocomposite (BN-AgNPs) showed excellent antibacterial activity against both gram negative *E. coil* and Gram-positive *S. aureus*. In this present study indicated the good antibacterial activity of plant mediated VrL-AgNPs showed inhibition zone against all the studied bacteria and achieved maximum activity against *P. aeruginosa* (19 mm).

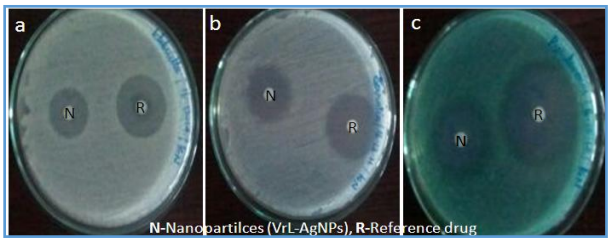

Fig. 3. Antibacterial activity of VrL-AgNPs.
a) *S. aureus, b) E. coil and c) P. aeruginosa*

Many researchers demonstrated the green route synthesis of silver nanoparticles by using bacteria, actinomycetes, fungi and plants. However, the plant materials (leaf, stem, seeds, fruit, roots and peels) have been successfully used for silver nanoparticles production because to their potential medicinal property, huge availability, faster rate of synthesis and toxicity free (Ranjithkumar *et al*., 2013; Shanmugavadivu *et al*., 2014). The inhibitory mechanisms of silver on bacterial cells are related to the interaction of AgNPs with thiol groups present in respiratory enzymes in bacteria. Whereas, Nano size crystalline AgNPs shows the most effective inhibitory properties with a rapid inhibition rate against human pathogenic bacteria (Wright *et al*., 1998). In the present study results indicated that the plant extract mediated VrL-AgNPs have potential antibacterial action against test human pathogens.

4. CONCLUSION

The antibacterial activities of green route synthesized silver nanoparticles were evaluated against human pathogenic bacteria such as *S.aureus*, *E.coli* and *P.aeruginosa*. The VrL-AgNPs showed excellent antibacterial action against *P.aeruginosa* and moderated action against other test human pathogens. Present study results demonstrated that biosynthesized silver nanoparticle, as a kind of antibacterial material, has a great promise for application in a wide range of biomedical applications.

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EVALUATION OF ANTIOXIDANT POTENTIAL OF *CASSIA FISTULA* (LINN.) BARK EXTRACT

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ABSTRACT

The agro-climatic and traditional atmosphere of India provides a vital deposit of various herbs available in many parts of the country which are used as traditional medicine in Ayurveda, Unani, Siddha and Homeopathy. The over dosage and irregular practice of consuming various antibiotics has produced many side effects and also helped in evolving multidrug resistant organisms. In this research we have focused on the usage of bark extracts of *Cassia fistula* (L.) for evaluating their antioxidant scavenging activity. With the traditional practice of shade drying and cold percolation method, the methanolic bark extract was chosen for various antioxidant activity like DPPH, $ABTS^{+}$, FRAP, Hydrogen peroxide and hydroxyl radical scavenging activity. The plant extract demonstrated higher activity and IC_{50} value for extract in all the tests showed nearly 60µg/ml. The graphical representation also correlated with the spectrum analysis when plotted using the percentage of inhibition scavenging activity vs the concentration taken for each plant extracts.

Keywords: *Cassia fistula,* DPPH, $ABTS^{+}$, FRAP, Hydrogen peroxide and hydroxyl radical.

1. INTRODUCTION

A country with vital deposit of various herbs growing in the tropical climate like India provides a historical reference and traditional usage of these herbs as food sources, spices and various medicinal preparations from time immemorial. It has been estimated by the World Health Organization (WHO) that nearly 80% of the world population are dependent on the plant or their extracts which are used in the traditional folk medicine (WHO report, 2009). In this 21st century, the survey shows that with an increase rate of over exposure towards various antibiotics, the evolution of multidrug resistance microbes have increased such as *Escherichia coli, Klebsiella pneumoniae, Aeromonas* sp., *Mycobacterium tuberculosis, M. leprae*, *Candida* sp., etc. (Waters and Basseler, 2005). Due to over growing scenario, the scientists are involved in the process of exploring new drugs either using plant sources like medicinal herbs or their parts or in combination using chemical sources. Wide range of population have made the regular usage of medicinal plants and many are on their path of transition towards the traditional treatment like Ayurveda, Siddha, Unani and Homeopathy to get devoid of the side effects by the usages of chemical drugs.

In the present research, the bark extract of *Cassia fistula* (L.) has been chosen based on their source and medicinal property recorded in the Ayurvedic texts (Misra and Dixit, 1978; Misra *et al.,* 1997; Perumal Samy *et al.,* 1998; Phongpaichit *et al.,* 2004; Prashanth *et al.,* 2006; Duraipandiyan and Ignacimuthu, 2007; Sangetha *et al.,* 2008). For understanding and evaluating any medicinal plant, the antioxidant potential has to be determined because our body as well as various products which we consume tends to release higher amounts of free radicals (Abraham *et al.,* 1993; Gupta and Ray, 2004; Kumar *et al.,* 2010; Arawwala *et al.,* 2011). These free radicals remain as an adjuvant thereby induces cancer in our body like breast cancer and also various trouble related to gastric problems as well as imbalance in the metabolism. Due to the irregular food habits as well as usage of chemical agents for skin application led us to evaluate the scavenging potential of these plants extracts commonly used in India as a source for treating various skin ailments, allergies and for internal health.

2. MATERIALS AND METHODS

2.1. Collection of herbal plants

Cassia fistula bark was collected from the areas in and around Coimbatore. The bark was selected based on the appearance and cut into small pieces. The plant barks along with leaves, fruit and flowers were subjected for plant authentication at Botanical Survey of India, Coimbatore, Tamil Nadu.

2.2. Processing of plant bark using cold percolation method

Based on the method described by Adonizio *et al.*, in 2008, the plant bark was processed using cold percolation method which helps to avoid the medicinal property. The bark pieces were weighed and kept for completed dehydration in dark for a period of 4-6 weeks. They were powdered, sieved and stored in dark bottle. About 3g of powdered plant bark was separately weighed and added to 30 ml of different solvents in increasing order of polarity such as non-polar solvents (hexane, petroleum ether, chloroform) and polar solvents (ethanol, methanol and distilled water). They were kept in shaking at 120rpm for 3 consecutive days and the extraction was done using muslin cloth. The filtrate was evaporated in dark, scrapped and stored in dark bottle. They were subjected to antioxidant scavenging activity and based on the results they were used for future research against pathogens.

2.3. Evaluating the various antioxidant properties of extracted Cassia fistula (L.) by in vitro free radical scavenging activity

2.3.1. DPPH scavenging activity

Based on the method described by Blois (1958), the scavenging activity of *C. fistula* was determined by using 2,2-diphenyl-1picryl hydrazyl (DPPH) method. The sample was distributed in various concentrations and as standard Vitamin C was used. The volume was adjusted to 500 µl by adding methanol. 5 ml of 0.1 mM methanolic solution of DPPH was added to these test tubes and vortexed. The tubes were allowed to stand at room temperature for 20 min. The control was prepared as above without any extract and methanol was used for the baseline correction. Changes in the absorbance of the samples were measured at 517 nm. The percentage of inhibition radical scavenging activity was measured by subtracting the values of sample from the control and divided by the values of the control and also taking the percentage for evaluation. The percentage inhibition vs. concentration was plotted and the concentration required for 50% inhibition of radicals was expressed as IC_{50} value.

2.3.2. $ABTS^+$ radical scavenging activity

Using the method described by Re *et al.* (1999), the test was based on the relative activity of antioxidants to quench the radical cation $ABTS^+$. The reaction was initiated by the addition of 1.0 ml of diluted ABTS to 10 µl of different concentration of bark extract wit higher activities against the pathogen or 10 µl of methanol serve as control. The absorbance was read at 734 nm and the percentage inhibition was calculated by subtracting the values of sample from the control and divided by the values of the control and also taking the percentage for evaluation.

2.3.3. Hydrogen peroxide scavenging activity

Based on the spectrophotometry analysis with a decrease in the absorbance at 230 nm, the hydrogen peroxide scavenging activity was measured using the method described by Ruch *et al.,* 1989. A solution of H_2O_2 was prepared in phosphate buffer. H_2O_2 concentration was determined using spectrophotometer from its absorption at 230 nm. Various concentrations of plant extracts were added to H_2O_2 and incubated for 10 min. The absorbance at 230 nm was determined against a blank containing phosphate buffer without H_2O_2. The percentage of scavenging of H_2O_2 and standard compound Vitamin C was calculated using the formula as mentioned earlier.

2.3.4. Hydroxyl radical scavenging activity

The scavenging activity of the plant extracts were determined using the method described by Klein *et al.,* (1991) by taking hydroxyl radicals which were generated from ferrous ammonium sulphate and EDTA. Various concentration of plant extracts were added with 1 ml of iron-EDTA solution (0.13% ferrous ammonium sulphate and 0.26% EDTA), 0.5 ml of EDTA solution (0.018%), and 1 ml of DMSO (0.85% v/v in 0.1 M phosphate buffer, pH 7.4). The reaction was initiated by adding 0.5ml of ascorbic acid (0.22%) and incubated at 80-90^{o}C for 15 minutes in a water bath. After incubation the reaction was terminated by the addition of 1 ml of ice-cold tri-chloro acetic acid (TCA) (17.5% w/v). 3 ml of Nash reagent was added and left at room temperature for 15 minutes. The reaction mixture without sample was used as control. The intensity of the color formed was measured spectrophotometrically at 412 nm against reagent blank. The percentage of hydroxyl radical scavenging activity is calculated by the same formula which was used in previous activity.

2.3.5. Ferric reducing antioxidant power (FRAP) assay

Using the method described by Benzie and Strain (1996), the total antioxidant potential of sample was determined using ferric reducing antioxidant power (FRAP) method. The stock solution of 10 mM 2, 4, 6-tripyridyl-s-triazine (TPTZ)

in 40 mM HCl, 20 mm $FeCl_3$, $6H_2O$ and 0.3 M acetate buffer (pH 3.6) were prepared. The FRAP reagent contained 2.5 ml TPTZ solution, 2.5 ml ferric chloride solution and 25 ml acetate buffer. It was freshly prepared and warmed to 37°C. 900 µl FRAP reagent were mixed with 90 µl water and 30 µl test sample/ ethanol /distilled water/ standard antioxidant solution. The reaction mixture was then incubated at 37°C for 30 min and the absorbance was recorded at 595nm. An intense blue color complex were formed when ferric tripyridyl triazine (Fe^{3+}-TPTZ) complex were reduced to ferrous (Fe^{2+}) form. The absorption at 540 nm was recorded. The calibration curve was plotted with absorbance at 595 nm vs concentration of ferrous sulphate in the range 0.1mM ethanol solutions. The concentrations of $FeSO_4$ were in turn plotted against concentration of standard antioxidants.

$$\text{Percentage of FRAP scavenging activity} = \frac{\text{Control - Sample}}{\text{Control}} \text{ X } 100$$

3. RESULTS AND DISCUSSION

The collected *Cassia fistula* (L.) barks were taken to Botanical Survey of India, Coimbatore and authentication no. for the plant is BSI/SRC/5/23/2013-14/Tech./1814. The processing and extraction of the plants bark were done accordingly and the methanolic extract of both the plants were subjected for further scavenging activity analysis based on the work done by Gupta and Ray (2004).

Methanolic extracts of *C. fistula* (L.) were carried out with different antioxidant scavenging activity and observed that the free radical of the extract were found to have high percentage of inhibition against DPPH, $ABTS^+$, hydrogen peroxide, hydroxyl and FRAP. Vitamin C served as the standard for all the antioxidant assays and when compared with the methanolic extracts of *C. fistula* (L.), it has been determined that the bark extract of this plant has higher scavenging activity than the control. The results were observed with higher percentage of inhibition for the extracts (as shown in table 1, 2, 3, 4 and 5) and the IC_{50} value was observed as 60µg/ml in all the tests (figure 1, 2, 3, 4, and 5).

This shows that *C. fistula* (L.) has higher scavenging activity as compared with the results of Gupta and Ray, in 2004 and Kumar *et al.,* 2010. Based on the similar experimental outcome of Ahmad and Aqil (2007), the antioxidant activities of the bark extract of *C. fistula* (L.) showed the majority of the active compounds such as phenolics, vitamin C, vitamin E, tannins and carotenes. Antioxidant activities were measured using FRAP, DPPH, superoxide anion, nitric oxide and hydroxyl radical scavenging assays was also found to higher as the result obtained in the current research.

Table 1. DPPH radical scavenging activity

Concentration µg/mL	% of Inhibition of Standard	% of Inhibition of Sample
20	17.69	8.88
40	28.31	27.84
60	45.09	40.11
80	56.21	58.88
100	75.22	85.82

Table 2. $ABTS^+$ radical scavenging activity

Concentration µg/mL	% of Inhibition of Standard	% of Inhibition of Sample
20	11.17	7.28
40	27.27	35.76
60	52.45	53.96
80	61.17	76.55
100	69.65	89.18

Table 3. Hydroxyl radical scavenging activity

Concentration µg/mL	% of Inhibition of Standard	% of Inhibition of Sample
20	7.11	6.21
40	17.22	23.28
60	36.28	57.40
80	54.42	79.76
100	77.49	89.68

Table 4. Hydrogen peroxide scavenging activity

Concentration µg/mL	% of Inhibition of Standard	% of Inhibition of Sample
20	20.44	16.93
40	39.65	31.42
60	58.61	45.26
80	67.02	58.10
100	74.19	78.68

Table 5. Ferric Reducing Antioxidant Power Assay

Concentration µg/mL	% of Inhibition of Standard	% of Inhibition of Sample
20	22.3	18.0
40	40.0	36.2
60	59.0	62.0
80	67.3	71.3
100	74.5	96.0

Figure 1. DPPH radical scavenging activity of *Cassia fistula* (L.) bark extract

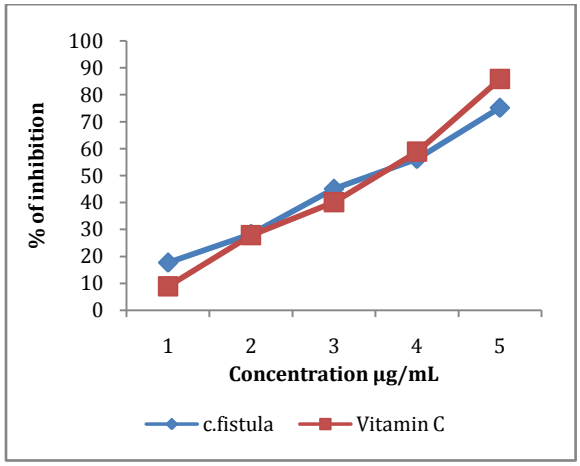


Figure 2. ABTS⁺ radical scavenging activity of *Cassia fistula* (L.) bark extract

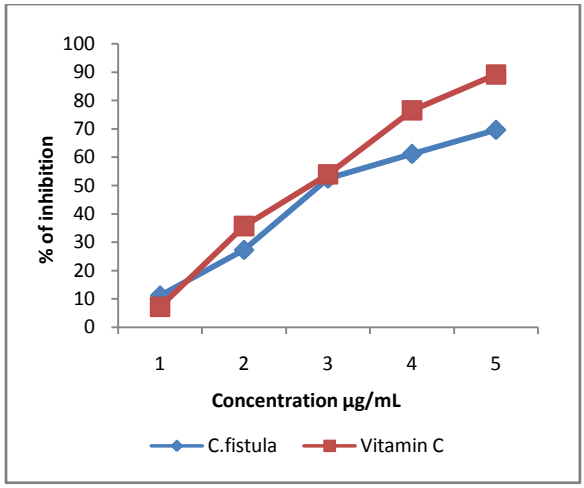


Figure 3. Hydroxyl radical scavenging activity of *Cassia fistula* (L.) bark extract

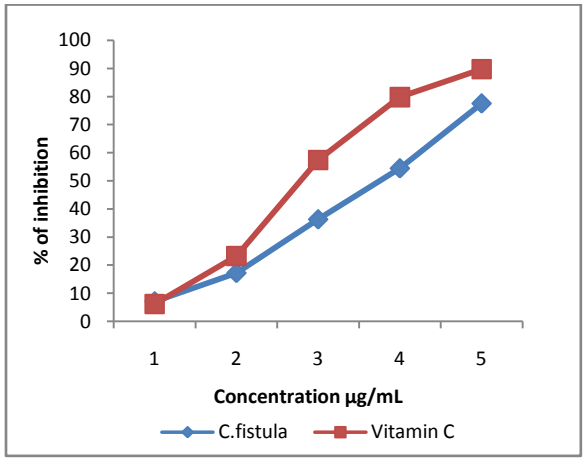


Figure 4. Hydrogen Peroxide radical scavenging activity of *Cassia fistula* (L.) bark extract

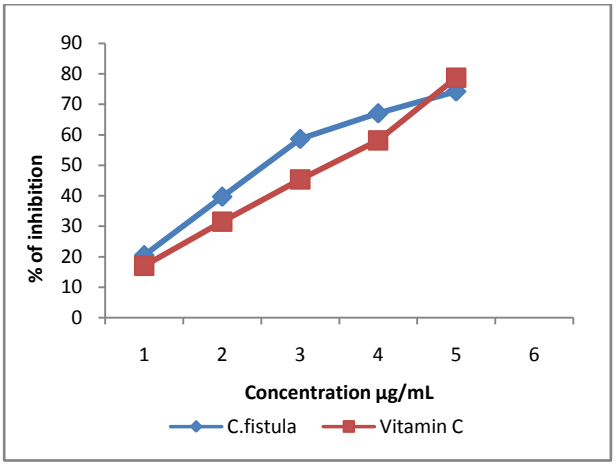
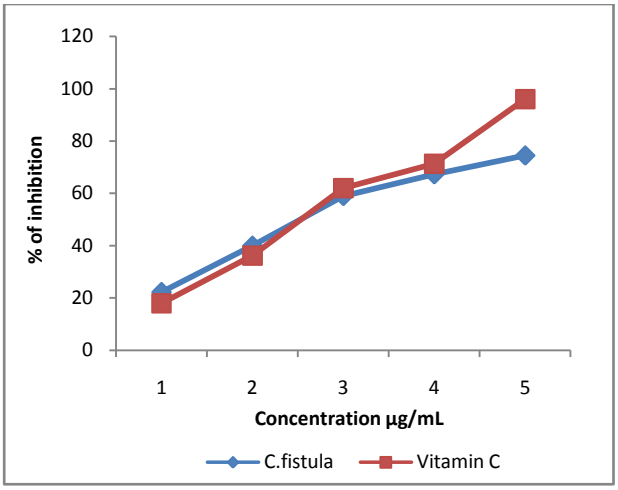


Figure 5. FRAP radical scavenging activity of *Cassia fistula* (L.) bark extract



4. CONCLUSION

This proves that the plant *Cassia fistula* (L.) has a higher scavenging activity using the methanolic extract of plant bark. This shows that these plant extracts can be further studied in future to understand the antibacterial activity as well as can serve as a potent drug in future pharmaceutical research.

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AN EFFICIENT FUZZY BASED ANOMALY DETECTION USING COLLECTIVE CLUSTERING ALGORITHAM

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ABSTRACT

Anomaly detection is a significant problem that has been researched within various research areas and application domains. Many anomaly detection methods have been particularly examined for certain application domains, as others are more standard. This present study describes an anomaly detection technique for unsupervised data sets accurately reduce the data from a kernel Eigen space performing a batch re-computation. For each anomaly behavior activities is to identify the key factors, which are used by the methods to differentiate between normal and abnormal actions. This present study provides a best and brief understanding of the techniques belonging to each anomaly and kernel mapping category. Further, for each grouping, to identify the improvements and drawbacks of the techniques in that category. It also provides a discussion on the computational complexity of the techniques since it is an important issue in real application domains hope that this survey will provide a good understanding of the many directions in which research has been done on this topic.

Keywords: Adaptive, non-stationary, anomaly detection, outlier detection, kernel principal component analysis, kernel methods.

1. INTRODUCTION TO DATA MINING

Data Mining, "The Extraction of hidden predictive information from large databases", is a powerful new technology with great potential to help companies focus on the most important information in their data warehouses. Data mining tools predict future trends and behaviours, allowing businesses to make proactive, knowledge-driven decisions. The automated, prospective analyses offered by data mining move beyond the analyses of past events provided by retrospective tools typical of decision support systems. Data mining tools can answer business questions that traditionally were too time consuming to resolve. The databases for hidden patterns are finding predictive information that experts may miss because it lies outside their expectations.

Data Mining consists of more no of collecting and managing data; it also includes analysis and prediction. Data Mining can be performed on data represented in quantitative, textual, or multimedia forms. Data Mining applications can use a variety of parameters to examine the data. They include association, sequence or path analysis, classification, clustering, and forecasting. Many simpler analytical tools utilize a verification-based approach, where the user develops a hypothesis and then tests the data to prove or disprove the hypothesis.

Data Mining techniques are the result of a long process of research and product development(Kriegel, Et al,2008). This evolution began when business data was first stored on computers, continued with improvements in data access, and more recently, generated technologies that allow users to navigate through their data in real time. Data mining takes this evolutionary process beyond retrospective data access and navigation to prospective and proactive information delivery.

2. MATERIALS AND METHODS

The proposed architecture accepts the user parameters as input which contains the MATLAB simulation where the Fuzzy based kernel mappings with adaptive Neighboring Splitting and Merging algorithm is applied to the datasets. This architecture in figure 3.1 follows a path from the start to end state. The users initialize the number of *k*-value as cluster parameters in which the anomaly detection process is to be evaluated(Zhang,et al.,2010).

Anomaly detection holds great potential for detecting previously unknown outliers (Barnett and Lewis, 1994). In order to be effective in a practical environment, anomaly detection systems have to be capable of online learning and handling concept. Clustering becomes difficult due to the increasing

sparsity of such data, as well as the increasing difficulty in distinguishing distances between data points. It has been widely recognized that consensus clustering can help to generate robust clustering results, find bizarre clusters, handle noise, outliers and sample variations, and integrate solutions from multiple distributed sources of data or attributes. An presents an optimal perspective on the problem of kernel principal component analysis in high-dimensional data (Ding and Kolaczyk,2013). The proposed method called "Fuzzy based kernel mappings with adaptive Neighbouring Splitting and Merging" (FKANSM), which takes as key measures of correspondence between pairs of data points. The proposed method is to establish a unified framework for FKANSM on both supervised and unsupervised data sets. Also, we examine some important factors, such as the clustering quality and assortment of basic partitioning, which may affect the performances of FKANSM. Experimental results on various synthetic and real world data sets demonstrate that FKCNC is highly efficient and is equivalent to the state-of-the-art methods in terms of clustering index quality. In addition, FKANSM shows high robustness to incomplete basic partitioning with many anomaly values.

3. RESULTS

To find the clusters of a data set sampled from a certain unknown distribution is important in many machine learning and data mining applications. Probability density estimate may represent the distribution of data in a given problem and then the modes may be taken as the representatives of clusters. As a nonparametric method, the kernel density estimation is mostly applied in practice to model the unknown probability density function (Fig. 1).

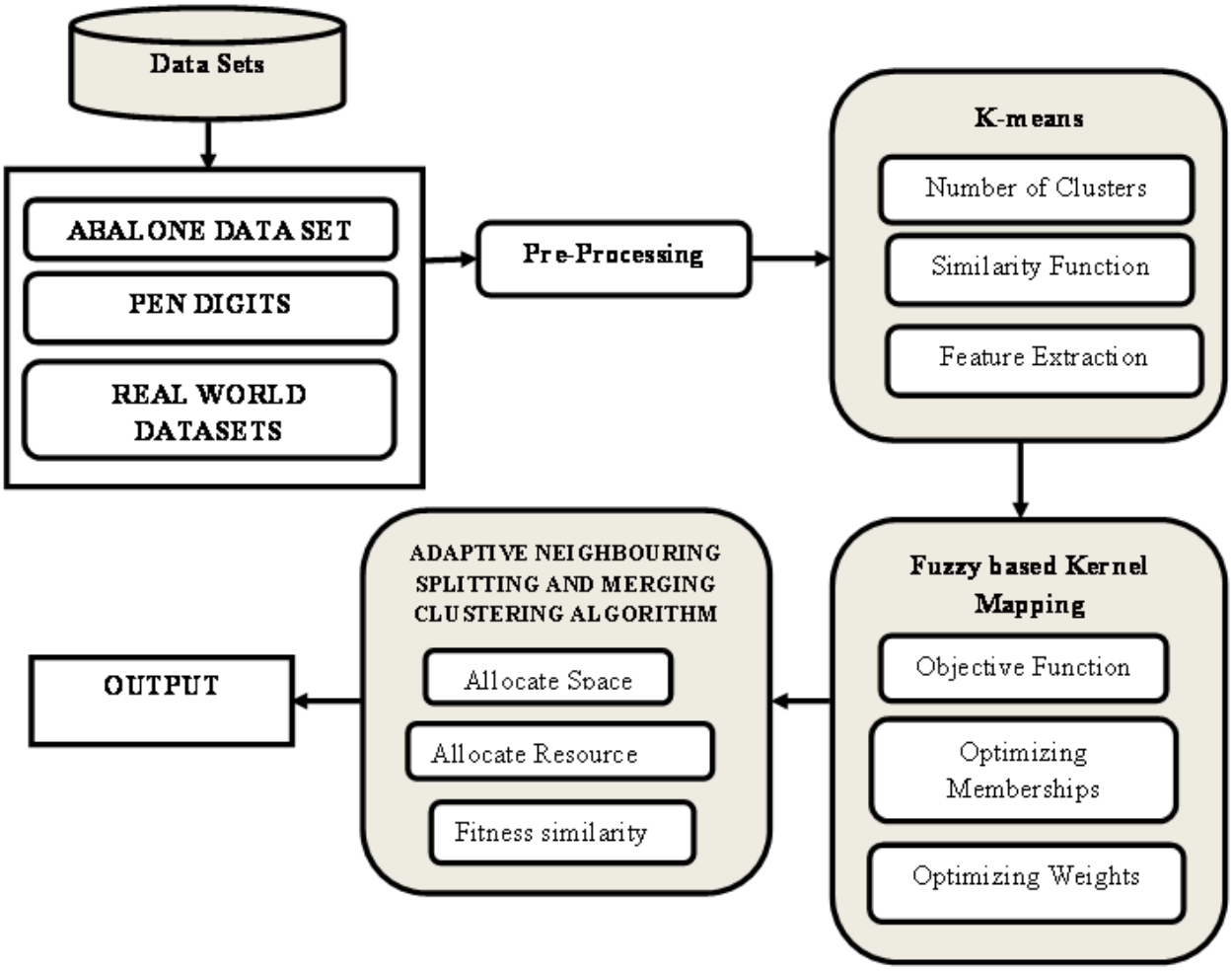
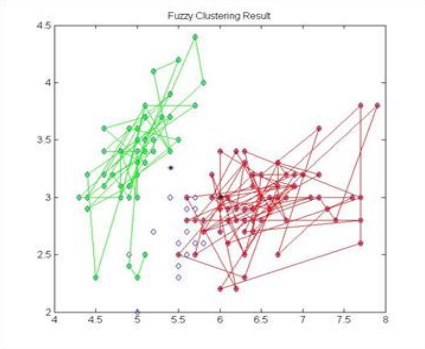


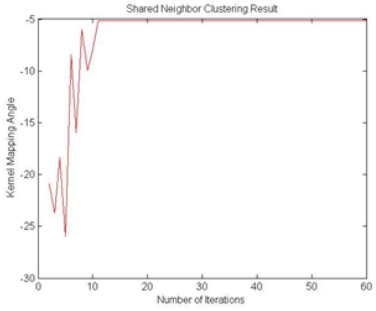
Fig. 1. Architecture of Proposed System

3.1. Abalone Dataset

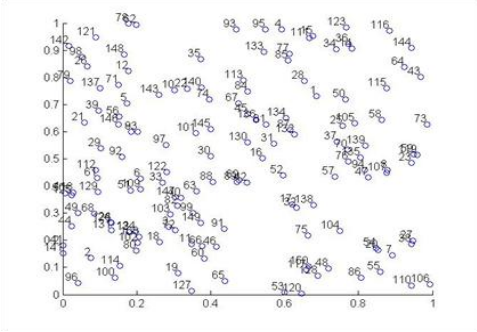
The abalone dataset describes the predicting age of abalone from physical measurements. The age of abalone is determined by cutting the shell through the cone, staining it, and counting the number of rings through a microscope - a boring and time-consuming task. Other measurements, which are easier to obtain, are used to predict the age. Further information, such as weather patterns and location (hence food availability) may be required to solve the problem. From the original data examples with missing values were removed (the majority having the predicted value), and the ranges of the continuous values have been scaled for use with an "Artificial Neural Network" (ANN) (by dividing by 200).



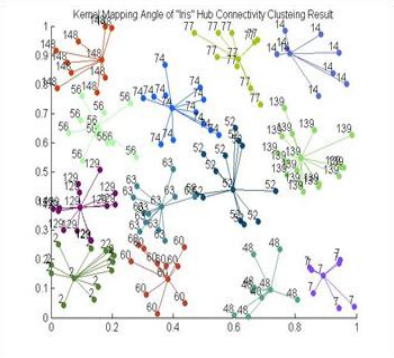
(a) Outliers (unwanted data)



(b) Outlier range



(c) Data sets



(d) Cluster data

```
Press appropriate keys for required operation........: 4
Strength of Connectivity (Fitness Similarity): -5.153254
**********************************************************************
The Fuzzy based Cluster Unique Cluster Data Points
  Columns 1 through 12

     2     7    14    48    52    56    60    63    74    77   129   139

  Column 13

   148

**********************************************************************
```

(e) Fitness similarity for data points

Fig. 2 (a-e). Abalone datasets

4. CONCLUSION

Anomaly detection in data mining area is efficient and effective task to ensure the quality and right decisions. A selection of anomaly detection models was proposed in an Efficient Fuzzy Based Anomaly Detection; however, most of them suffer from high dimensional datasets effectiveness or high outliers. This result shows the challenges that face the design of an efficient and effective anomaly detection model for synthetic and real-world data sets in data mining domain that should be satisfied to design such models.

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SCIENTOMETRIC ANALYSIS OF ASTROPHYSICS RESEARCH OUTPUT IN INDIA

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ABSTRACT

Astrophysics is a branch of space science that applies the laws of physics and chemistry to explain the birth, life and death of stars, planets, galaxies, nebulae and other objects in the universe. Astrophysics creates physical theories of small to medium-size structures in the universe. Astrophysicists seek to understand the universe and our place in it. At NASA, the goals of astrophysics are "to discover how the universe work, explore how it began and evolved, and search for life on planets around other stars." This study analyzes the Astrophysics research output in India from the year 1989-2014. The data was downloaded from web of science database which was maintained by Thomson Reuters. The findings of the study revealed that two authors has the maximum of contribution with 3673 (28.81%) publication followed by three authors with 2875 (22.55%).

Keywords: Scientometrices, Web of Science, Astrophysics. Histcite, Authorship pattern.

1. INTRODUCTION

Scientometric study is a statistical method of counting to evaluate and quantify the growth of a subject. The research trend during the said time span would be clearly understood from this study and a predictive projection may be made for anticipatable future. There are several areas in science, social science and arts for which scientometric studies were carried out. A number of studies have been accomplished to evaluate research output and productivity in different areas of physics. In 2009, Kumara (2009) carried out scientometric studies in major areas of physics and engineering sciences. Some other scientometric studies in different subject domains include Jain and Garg (1992) (Laser research), Kademani (2006) (Thorium research), Stanhill (2001) (climatology), Garg and Padhi (1998) (Laser patent literature), Upadhye (2004) (physics Noble lectures), Lee (2003) (molecular and cell biology), Schummer (1997) (chemistry), Braun *et al.* (2000) and Gupta (1999) (Fullerene research) et al. A number of scientometric studies in the areas of astronomy and astrophysics have also been executed. Basu and Lewison (2005) evaluated research output of global astronomy and astrophysics by an analysis of papers in the Science Citation Index identified with a special filter and found out leading Indian institutions and authors. Jamali and Nicholas (2010) attempted scientometric analysis from a new angle. The results presented by him revealed interdisciplinary differences within physics and astronomy in terms of reading behaviour. Leta (2005) executed a comparative analysis of Brazilian research trend in astronomy, immunology and oceanography. Davoust and Schmadel (1969) studied publishing activities of the astronomers since 1969. Fernández (1998) studied transitional steps from individual science to collectivization in astronomy during twentieth century. Uzun and Ozel (1996) studied publication pattern of Turkish astronomers. Marx and Bornmann (2009) showed the transition from the static view of the universe to the big bang theory in cosmology through citation analysis. Sen (2004) discussed definition and scope of scientometrics for all major science subjects in the context of web resources (cybermetrics).

Objectives

- To analyze the year wise publication of Astrophysics research output in India.
- To analyze the Half period comparison.
- To find out the Authorship pattern.
- To determine the Document wise distribution of publication.

2. MATERIALS AND METHODS

The data for the study were retrieved from web of science database which is a scientific and indexing service maintained by Thomson Reuters. The Astrophysics research output of India was

analyzed. The bibliographic details such as Astrophysics research ouput,authors, document types etc. were analyzed using Histcite which is a software package used for bibliometric analysis and information visualization.

3. ANALYSIS

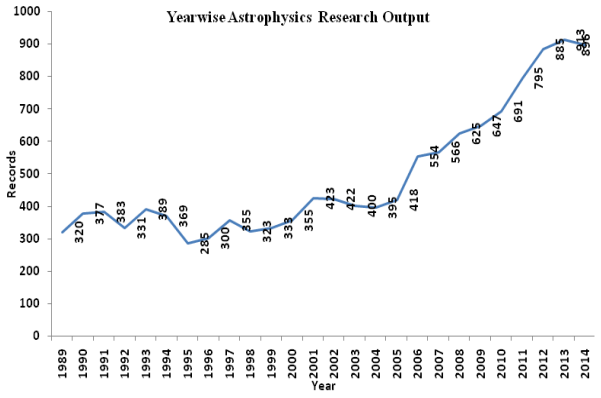
A total of 12750 astro physics records were published in India. The research output was analyzed using various scientometric indicators.

4.1. Yearwise publications

Table 1. Year wise distribution of astrophysics research output

S.No.	Publication year	RECS	TLCS	TGCS
1.	1989	320	572	2361
2.	1990	377	758	3434
3.	1991	383	702	3616
4.	1992	331	554	2804
5.	1993	389	963	4904
6.	1994	369	985	5997
7.	1995	285	878	4245
8.	1996	300	977	6688
9.	1997	355	884	5200
10.	1998	323	904	6647
11.	1999	333	900	5297
12.	2000	355	1011	6967
13.	2001	423	1042	7771
14.	2002	422	992	11018
15.	2003	400	1113	10796
16.	2004	395	1035	9109
17.	2005	418	1080	8766
18.	2006	554	1543	12066
19.	2007	566	1009	8861
20.	2008	625	1034	9813
21.	2009	647	1133	10575
22.	2010	691	865	9049
23.	2011	795	657	9055
24.	2012	885	483	6902
25.	2013	913	324	4342
26.	2014	896	88	3287
	Total	12750	22486	179570

Table 1 shows the year wise distribution of Astrophysics research output in India from the year 1989-2014. A total of 12750 records were published during the given period. The highest number of publications is in the year 2013 with 913 records, having a Global Citation score 4342 and Local Citation Score of 324,followed by 896 papers in the year 2014 with Global Citation score of 3287 and a Local Citation Score of 88. The year 2006 has scored the maximum Global Citation Score of 12066 with 554 publications. The lowest number of publications is in the year 1995 with 285 records, having a Global Citation Score of 878.It is also observed from the table that even minimum numbers of records have scored higher Global Citation Scores.

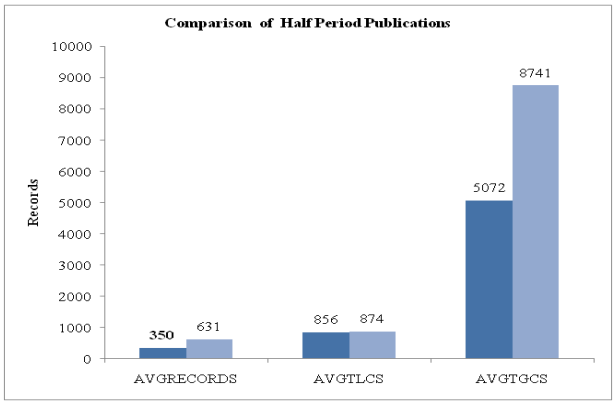


4.2. Half period comparison

Table 2. Half Period Comparison

Period	Average Records	Average TLCS	Average RGCS
I Half (1989-2001)	350	856	5072
II Half (2002-2014)	631	874	8741

The above table shows that in the second half period more number of articles is published compare to first half period. It shows growth rate of astrophysics research output in India.

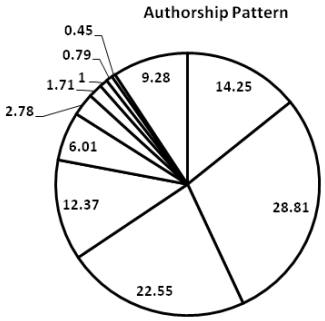


4.3. Authorship pattern of astrophysics research output

Table 3. Authorship pattern of astrophysics research output

S.No.	Authorship Pattern	No. of Publications	%
1.	Single Author	1817	14.25
2.	Two Author	3673	28.81
3.	Three Author	2875	22.55
4.	Four Author	1577	12.37
5.	Five Author	766	6.01
6.	Six Author	355	2.78
7.	Seven Author	218	1.71
8.	Eight Author	127	1.00
9.	Nine Author	101	0.79
10.	Ten Author	58	0.45
11.	More than Ten Author	1183	9.28
	Total	12750	100

The above table indicates authorship pattern of Astrophysics research output by India from the period 1989-2014.It is clearly noticed from the table that two authors has the maximum of contribution with 3673 (28.81%) publication. It is also noted that out of 12750 publications only 58(0.45%) publications are contributed by ten authors.

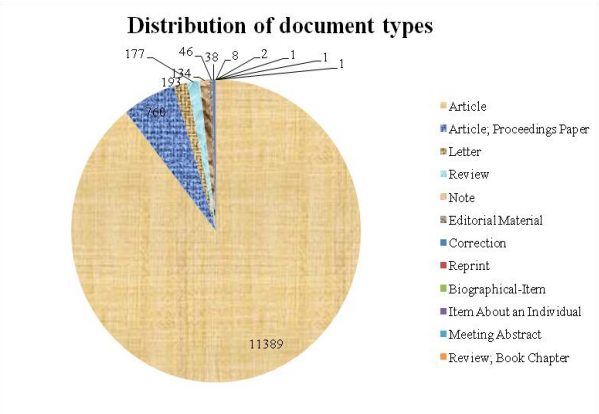


4.4. Document types

Table 4. Distribution of Document Types

S.No	Document Type	Recs	TLCS	TGCS
1.	Article	11389	20340	155123
2.	Article; Proceedings Paper	760	539	3458
3.	Letter	193	462	2318
4.	Review	177	930	17196
5.	Note	134	198	1262
6.	Editorial Material	46	2	99
7.	Correction	38	5	69
8.	Reprint	8	10	36
9.	Biographical-Item	2	0	0
10.	Item About an Individual	1	0	1
11.	Meeting Abstract	1	0	0
12.	Review; Book Chapter	1	0	8

The above table provides the distribution of publication on Astrophysics research by document types. It is clearly noticed from the table that the major source of publication in Astrophysics research comes in the form of articles with 11389 records, followed by proceedings paper and letter 760 and 193 publications respectively.



4. CONCLUSION

The Astrophysics research output in India as evidenced from the study has the highest publication of 913 papers in 2013 with 4342 Global Citation Scores followed by 896 papers in 2014 with 3287 Global Citation Score and 885 papers in 2012 with 6902 Global Citation Scores. The study shows that in the second half period average number of records is 631 with average Global Citation Score 8741 .It reveals that second half period (55%) increase in average number of publications compare to the first half period. The majority of the articles are contributed by multiple authors. Especially Two authors' contribution is the highest among the other collaborative productivity. It indicates that the single authored work is less than that of the multiple authored contributions. The research has identified the factor; the three or two authored has been leading their research work to a winning triumph in the every year output in Astro physics. The major source of publication in Astrophysics research comes in the form of articles with 11389 records, followed by proceedings paper and letter 760 and 193 publications respectively.

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CITATION ANALYSIS OF THE "JOURNAL OF DIGITAL INFORMATION MANAGEMENT"

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ABSTRACT

This paper presents a Citation Analysis of the Journal of Digital Information Management for the period between 2010 to 2014. The analysis covers mainly the Volume-wise Distribution of Citations, Distribution of Citations According to Bibliographic Forms, Authorship Pattern of Citations, Chronological Distribution of Citations, Author self citation. All the studies point towards the merits and weaknesses of the Journal which will be helpful for its further development. The study reveals that the average citations per article are 17.64. The study also found that journals/serial publications remain the most useful source of information 1896 (41.28%) out of a total 4593 citations.

Keywords: Citation analysis, Bibliography, Authorship pattern.

1. INTRODUCTION

The essence of Journal publishing is to report research findings and to contribute to the field of knowledge. Journals are the most current channel of dissemination of new ideas, knowledge and breakthroughs in scientific development. Academic journals play a significant role in academic scholarship (Xiao and Smith, 2006) (Chandy and William, 1994). Citations appearing in journals of particular disciplines provide an objective measure of the contributions of other knowledge systems to the development and progress of that particular discipline (Chandy and Williams, 1994). According to Gao, Yu, and Luo (2009) librarians have used several different quantitative methods to identify patrons' needs, including circulation and shelving data, the analysis of inter library loan requests, as well as citation analysis. Edward (1999) asserts that citation analysis can be used to determine a core collection of journals critical to local users and representative of the research needs of the collection. Gooden (2001) opines that citation analysis has been used by Librarians in various disciplines to eliminate costly, low used/unused journals, purchase needed materials and ascertain core journals needed for patron use, and to reveal the most active research in a particular field. Ching and Chennupati (2002) opine that citation analysis is a form of checklist approach, and basically compares a library's holdings to an authoritative list for the purpose of assessing the quality of all or part of the collection.

The current study is a citation analysis of the Journal of Digital Information Management for the period between 2010-2014.The journal of Digital Information Management (JDIM), a Bi monthly Journal of Digital Information Science and Technology, has been published since March 2003.Sponsored by the Digital Information Research Foundation, it concentrates on all aspects of digital information management, and covers digital information processing, digital content management, digital world structuring, digital libraries, metadata, information management and other related fields. An International peer-reviewed journal, it acts as a portal to the digital information world.

2. RESULS

Five volumes (Volumes 8 to 12) each containing 6 issues of Journal of Digital Information Management have been taken up for the study. The details with regard to each published article such as Volume-wise Distribution of Citations, Distribution of Citations According to Bibliographic Forms, Authorship Pattern of Citations, Chronological Distribution of Citations, Author Self citation were recorded and analyzed for making observations.

The Journal published 266 articles during the period of study i.e. from Vol.8.-12 (Year 2010-2014). The Journal publishes on an average of 17.64 citations per article. The above Table shows that the maximum number of citations per article was in the volume number 8 with 1055(21.10%) out of 4593 total citations.

Table 1. Volume-wise distribution of citations

Volume Number	Number of Articles	Number of Citations	Average Citations per Article
8	50	1055	21.10
9	41	762	18.59
10	53	967	18.25
11	72	972	13.50
12	50	837	16.74
Total	266	4593	17.64

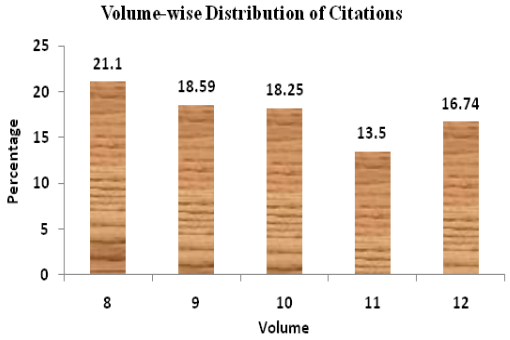


Table 2. Distribution of citations according to bibliographic forms

Bibliographic Form	Number of Citations	Percentage	Ranking
Journals/Serial Publications	1896	41.28	I
Conference/Proceedings	1583	34.46	II
Web sites	404	8.80	III
Books	365	7.95	IV
Reports	196	4.27	V
Others	149	3.24	VI
Total	4593	100	

Table 2 shows the analysis of citations according to bibliographic forms of the 4593 citations, as many as 1896(41.28%) are from Journal/Serial publications, followed by Conference/Proceedings 1583(34.46%), Websites 404(8.80%), Books 365 (7.95%), Reports 196 (4.27%) and Others 149(3.24%).

Table 3 shows the authorship pattern of citations as appended to the References Section of 266 research articles of Journal of Digital Information Management, the source journal. The Table indicates that out of the total of 4593 citations, 1727 (37.60%) are three-authored, followed by four-authored contributions totaling 1339 (29.15%), and two –authored contributions totaling 671 (14.61%), five or six authors contributed the remaining articles. However, there are 73 (1.59%) citations with incomplete details on the authorship.

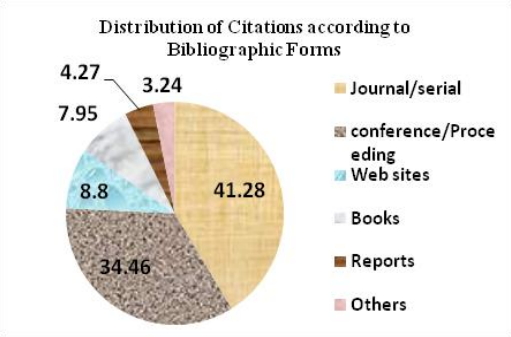


Table 3. Authorship Pattern of Citations

Number of Authors	Number of Citations	Percentage	Ranking
One	294	6.40	V
Two	671	14.61	III
Three	1727	37.60	I
Four	1339	29.15	II
Five	351	7.64	IV
Six	138	3.01	VI
Incomplete	73	1.59	VII
Total	4593	100	

Table 4. Chronological Distribution of Citations

Span of Period	Number of Citations	Percentage	Rank
1930-1939	3	0.07	IX
1940-1949	4	0.09	VIII
1950-1959	4	0.09	VIII
1960-1969	13	0.28	VII
1970-1979	44	0.96	VI
1980-1989	146	3.18	IV
1990-1999	518	11.28	III
2000-2009	2744	59.74	I
2010-2014	1035	22.53	II
Unknown period	82	1.78	V
Total	4593	100	

Table 4 gives the chronological distribution of citations. The result indicates that the period 2000-2009 received the most citations, 2744 (59.74%) of the total citations in terms of chronological distribution of citations. It is noticed, however, that of the 4593 citations counted, 82 (1.78 %) citations had incomplete data pertaining to the year of publication.

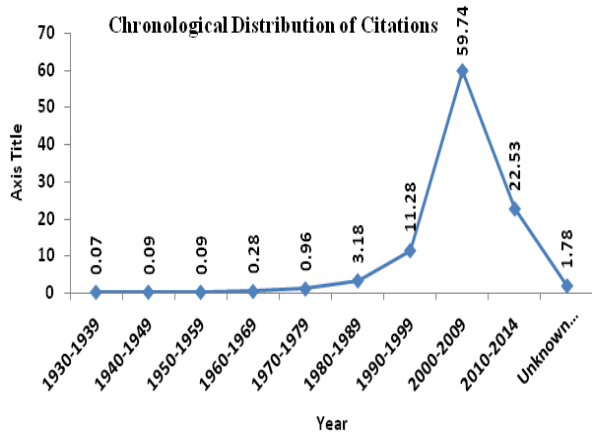
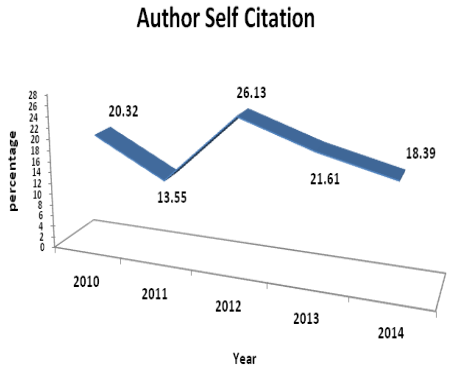


Table 5. Author Self citation

Year	Number of Author Self citation	Percentage
2010	63	20.32
2011	42	13.55
2012	81	26.13
2013	67	21.61
2014	57	18.39
Total	310	100



Self-citation occurs when an author cites any of his articles written singly or jointly with others. In this study we found 310 author self citations that amount to 6.75% of total citations. The above Table shows the year wise author self citations during the year 2012, maximum number of author self citations 81(26.13%).

CONCLUSION

Citation Analysis reveals that between 2010-2014 every issue of Journal of Digital Information Management published an average citation per article 17.64. The study reveals that journals/serial publications remain the most useful source of information 1896 (41.28%) out of a total 4593 citations. The authorship pattern of citations reveals very clearly that scientists are moving towards collaborative research, as the majority of citations 1727 (37.60%) are three-authored. The chronological distribution of citations indicates that scientists are quite up-to-date as references cited are fairly recent with 2744 (59.74%) published between 2000-2009. During this study we found 310 author self citations that amount to 6.75% of total citations.

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