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Green Synthesis of Nanoparticles (Ag, Cu and Zn) from *Emilia sonchifolia*, *Tabernaemontana divericata* and *Clerodendrum infortunatum* Leaves Extract: Evaluation of Antibacterial Activity

Prem Jose Vazhacharickal* and Sruthi S. Nair

Department of Biotechnology, Mar Augusthinose College, Ramapuram-686576, Kerala, India

**Corresponding author*

Abstract

Nanoparticles are particles with dimension on the range 10^{-9} and 10^{-10}. Green synthesis is the new method developed for the synthesis of nanoparticles which is small in size, large surface area and eco-friendly. In India plant and tree leaves are used as medicine for the treatment of various diseases. They are rich source of antimicrobial agents. Silver, copper and zinc nanoparticles are synthesised from the leaf extract of different plants. Leaf extracts of *Emilia sonchifolia* (Muyalcheviyan), *Tabernaemontana divericata* (Nandhiarvattam) *and Clerodendrum infortunatum* (Peravam) are used for the synthesis of nanoparticles. Leaf extract is added to the prepared stock solution of1mM silver nitrate, 100mM copper sulphate and100mM zinc sulphate. Synthesised nanoparticles were characterized by UV-VIS spectrophotometry to confirm the formation of nanoparticles. Anti-bacterial are used to treats bacterial infections. The leaf extract and silver nitrate, copper sulphate, zinc sulphate has antibacterial activity. The antibacterial activity of synthesised nanoparticle is determined using agar well diffusion method. The synthesised nanoparticles exhibit anti- bacterial activity by inhibiting the growth of gram negative and gram positive bacteria.

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Nanoparticles, Green synthesis, Antibacterial activity, *Emilia sonchifolia, Clerodendrum infortunatum.*

Introduction

In India plant and tree leaf are used as medicine for the treatment of various disease including bacterial disease. They are rich source of anti-microbial agents. India is called the botanical garden of the world and is the largest producers of herbs. For thousands of years, plants have been used in Ayurveda, Siddha, Unani (Aiswarya *et al.*, 2011). Plants are used as traditional medicine and pharmaceutical drugs by a large population of the world because of scarcity and high cost of orthodox medicine. Natural products have a dominant role in the development of drugs.

Leaf extracts of *Emilia sonchifolia (Muyalcheviyan), Tabernaemonana divericata* (Nanthiarvattam) and *Clerodendrum infotumatum* (Peravam) were used to synthesis nanoparticles. These plants have medicinal as well as antibacterial activity. Use of plant source offers several advantages such as cost effectiveness, Eco friendliness and toxic chemicals necessary in the traditional synthesis methods (Su and Chiu, 2007).

Nanotechnology is an emerging field of science. It has increased applications in diverse area for the development of new materials at nanoscale levels (Paul *et al.*, 2015). Nano-technology mainly consists of the

processing of, separation, consolidation, and deformation of materials by one atom or one molecule. Nanoparticles has 1-100 nm in size and they possess novel physical and chemical properties (Sajeshkumar *et al.,* 2015a; Sajeshkumar *et al.,* 2015b). Nanoparticles bear antibacterial properties (Hajipour *et al.,* 2012).

Nanoparticle are particles of any shape with dimensions on the $1*10^{-9}$and $1*10^{-10}$ (Carlos *et al.,* 2015). Metals like silver, copper and zinc has inhibitory effect on microbes. Nano particles synthesized by physical and chemical methods. They have draw back like expensive re-agent, hazards reaction condition, longer time, tedious process to isolate nanoparticles. These lead to the development of new method for the synthesis of nanoparticles which should be required, non-expensive re-agent, less drastic reaction condition and Eco friendly (Kulkarni *et al.,* 2004).

Green synthesis of nanoparticles

Synthesis of nanoparticles using biological methods is referred as greener synthesis of nanoparticles. Green synthesis provides advancement over chemical and physical method as it is cost effective, environment friendly, and safe for human therapeutic use (Kumar *et al.,* 2009). Metals like silver, copper and zinc has inhibitory effect on microbes. Biological synthesis of metallic nanoparticles is inexpensive single step and eco-friendly methods. The plants and seeds are used successfully in the synthesis of various greener nanoparticles such as copper, silver, and zinc oxide (Kuppusamy *et al.,* 2014; Mishra *et al.,* 2014).

Application of nanoparticles

Nanoparticles has various applications. Nanoparticles have been used for constructing electrochemical and biosensors. Metal nanoparticles embedded paints have good antibacterial activity (Kumar *et al.,* 2008). Current research is going on regarding the use of magnetic nanoparticles in the detoxification of military personnel in case of biochemical warfare (Salata, 2005).

One of the major opportunities for nanoparticles in the area of computers and electronics is their use in a special polishing process, chemical-mechanical polishing or chemical mechanical planarization, which is critical to semiconductor chip fabrication (Euzeby *et al.,* 1997).

Magnetic nanoparticles are also used in targeted therapy where a cytotoxic drug is attached to a biocompatible nanoparticle for tumour cell treatment. Porous nanoparticles have been used in cancer therapy.

Bioremediation of radioactive wastes from nuclear power plants and nuclear weapon production such as uranium has been achieved using nanoparticles (Duran *et al.,* 2007).

Silver nanoparticles

Silver has long been recognized as having inhibitory effect on microbes present in medical and industrial process. The most important application of silver and silver nanoparticles is in medical industry such as topical ointments to prevent infection against burns and open wound.

Silver ions (Ag^{+}) and its compounds are highly toxic to microorganisms exhibiting strong biocidal effects on many species of bacteria but have a low toxicity towards animal cells (Prema, 2011).

In past ten years silver Nano particles have been one of the extensively studied Nano materials. It have physical, chemical, optical biological application and application in bio medicine, drug delivery, topical oilmen's and creams (Patcharaporin *et al.,* 2006).

The main objectives of this study were

Synthesis of silver, copper, zinc nanoparticles using aqueous leaf extract.

Characterization of nanoparticles by UV- Vis spectroscopy.

Analyse antimicrobial properties against gram-positive and gram-negative bacteria

Scope of the study

The study would enlighten the medical and pharmaceutical applications various green synthesised nanoparticles applications against different microorganism which could be further explored.

Tabernaemonta divaricata (Crape jasmin)

Tabernaemonta divaricata is a shrub which is commonly seen in India it generally grows into a height of six feet, and it has the capability to grow in to a small tree with a thin crooked stump and the stump exude a milky latex

when broken. Its flower are commonly used for pooja in south and north India.

The plant is used in ayrvedic, Chinese and Thai traditional medicine. The plant has anti-oxidant, anti-tumour, anti-infection effect. In Ayurveda the plant is considered to Paafyvata and pitta vedas (Plant data base, 2015).

Taxonomical classification *Tabernaemonta divaricata* (Crape jasmin)

Kingdom: Plantae--planta, plantes, plants, vegetal
Subkingdom: Virdaeplantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Gentianales
Family: Apocynaceae
Genus: Tabernaemantana
Species: *Tabernaemonta divaricata*

Emilia sonchifolia (Muyalcheviyan)

Emilia sonchifolia is a shrub very common in India. Generally grows into a height of one foot. It is seen in wet area mostly in paddy fields. It is medicinal plants which have been used for a wide variety for purposes. They have potential for use against general food spoilage and human pathogens.

It is used in the treatment of dysentery, eye inflammation, and night blindness. From this plant new food preservatives may be developed. It help in the development of new pharmaceuticals in food preservation as well as natural plant based medicine.

Taxonomical classification of *Emilia sonchifolia* (Muyalcheviyan)

Kingdom: Plantae-- planta, plantes, plants, vegetal
Subkingdom: Tracheobionta
Division: Magnoliophyta
Class: Magnoliopsida
Order: Asterales
Family: Asteraceae
Genus: Emilia
Species: *Emilia sonchifolia*

Clerodendrum infortunatum (Perruvalam)

Clerodendrum infortunatum is flowering shrub or small tree and is so named because of its rather ugly leaf. Leafs are simple opposite, both surface sparsely villous, puhescent elliptic broadly elliptic, 35 to 20 cm broad and 1.4-7.9 cm in wide, 6 to 25 cm long.

Major compounds of the plant are sterols, sugars, flavonoid and saponins. Saponins is one of the major compound of the leaf. It is a traditional herbal medicine used in Ayurveda, siddha (Duke *et al.,* 2010). Fresh leaves are used for treating diarrhoea, liver disorder and head ache. It is also used as anti-dandruff, anti-pyretic, ascaricide, laxative, vermifuge, anti-convulrant, anti-diabetic (Sharma *et al.,* 2001).

Taxonomical classification of *Clerodendrum infortunatum* (Perruvalum)

Kingdom: Plantae-- planta, plantes, plants, vegetal
Subkingdom: Viridaeplantae
Division: Magnoliophyta
Class: Magnoliopsida
Subclass: Lamiidae
Order: Laminales
Family: Lamiaceae
Genus: Clerodendrum
Species: *Clerodendrum infortunatum*

Nanoparticles

Nano particles are particles of any shape with dimension on the $1*10^{-9}$ and $1*10^{-10}$ (Carlos *et al.,* 2015). They exhibit size related properties which is different from fine particles or bulk materials. Nano particles are a bridge between bulk materials and atomic or molecular structure (Mac Naught *et al.,* 1997). They possess unexpected optical properties they are small enough to confine their electrons and produce quantum effect (Hewakuruppu *et al.,* 2013). Nanoparticles have higher specific surface area appropriate for catalysis. Nanoparticles synthesized by physical and chemical methods are highly expensive; require longer time need tedious process to isolate nanoparticles. Thus new method was developed for the synthesis of Nano particles, green synthesis which is small in size, large surface area and eco-friendly (Kulakarni *et al.,* 2004).

Sliver nanoparticles

Silver has inhibitory effect on microbes. They prevent infection against burn and open wounds. They are highly toxic to micro-organism exhibiting strong biocidal effect. Application of plant extract for the synthesis of silver nanoparticles is more advantageous because of its

resource availability, security, reaction rate and convenience. Factors including pH, dosage of plant extracts, dosage of silver ions, reaction temperature and time affect synthesis of Silver nanoparticles. Plant extracts act as a reducing agent has an important role in capping and stabilizing of nanoparticles (Rao *et al.*, 2017).

Copper nanoparticles

Copper nanoparticles have high optical, catalytic, mechanic and electrical properties. They are cheap high yielding and have short reaction time under normal reaction condition. Copper nanoparticles have anti-microbial activities against various bacterial and fungal strain from any researchers (Kulkarni *et al.*, 2004). It is used in various fields including agricultural, industrial, engineering and technical fields. Effective anti-bacterial activities are exhibited by copper nanoparticles. They are cost effective and have efficient bio synthesize techniques. Copper nanoparticles have less cost than silver and gold nanoparticles.

Zinc nanoparticles

Zinc nanoparticles have wide application; various synthetic methods have been employed to produce ZnNps. Zinc nanoparticles can produced from zinc oxide and zinc sulphate. Zinc nanoparticles has several medicinal uses, which harm skin, stomach, intestine and lymphastic system and they probably induces tumours. Zinc nanoparticles has antibacterial effect on microbes, and it mainly depends up on the size and the presence of visible light. Zinc nanoparticles are used in the optical devices, sensors, catalysis, biotechnology, DNA labelling, drug delivery, medical, chemical and biological sensors (Devasenan *et al.*, 2016).

Anti-microbial activity

Anti-microbial agent is a substance that kills microorganisms or stops their growth. Anti-microbial medicines are grouped according to the micro-organisms they act. Antibiotic are used against bacteria, antifungal are used against fungi. They are also classified on the basis of their function. The agents that kill microbes are called microbicidal; those that merely inhibit their growth are called biostatic (Kingston *et al.*, 2008). The use of anti-microbial agents for the treatment of infection is known as anti-microbial therapy. The use of antimicrobial medicines for the prevention of infection is known as antimicrobial prophylaxis.

Anti-bacterial activity

Antibacterials are used to treat bacterial infections. The drug toxicity to humans and other animals from antibacterials is generally low. The continuous use of certain antibacterials can decrease the number of gut flora, which may have a negative impact on health.

The consumption of probiotics and reasonable eating can help to replace the destroyed gut flora (Mohanty, 2010). The discovery, development and the use of antibacterials started during the 20th century and it has reduced mortality from bacterial infection.

The antibiotic era began with pneumatic application of nitroglycerine drugs followed by a golden period of discovery from about 1945-1970 (Sanu *et al.*, 2013). Antibacterials are among the most commonly used drugs by physician. As a consequence of widespread and injudious use of antibacterials there has been an increased emergence of antibiotic resistant pathogens, which resulting in a serious threat to public health. Antibacterial activities potentially offer solution to the problem of antibiotic resistance (Brown *et al.*, 1975).

Agar well diffusion

Agar well diffusion test is used for antibacterial assay. The well that cut on the solidified agar act as pour for loading sample. The agar that is inoculated with test organism after overnight incubation may show zone of inhibition. The sample that is diffused in the agar inhibits the growth of microbes.

Hypothesis

The current research work is based on the following hypothesis

Leaf extracts of *Emilia sonchifolia* (muyalcheviyan), *Tabernaemonana divericata* (nanthiarvattam), *Clerodendrum infotumatum* (peravam) could be used as antibacterial agents.

These leaves extracts could be used in formulating different kind of nanoparticles (silver, copper and zinc) and their antibacterial activity of the nanoparticles vary widely.

Materials and Methods

Study area

Kerala state covers an area of 38,863 km^2 with a population density of 859 per km^2 and spread across 14

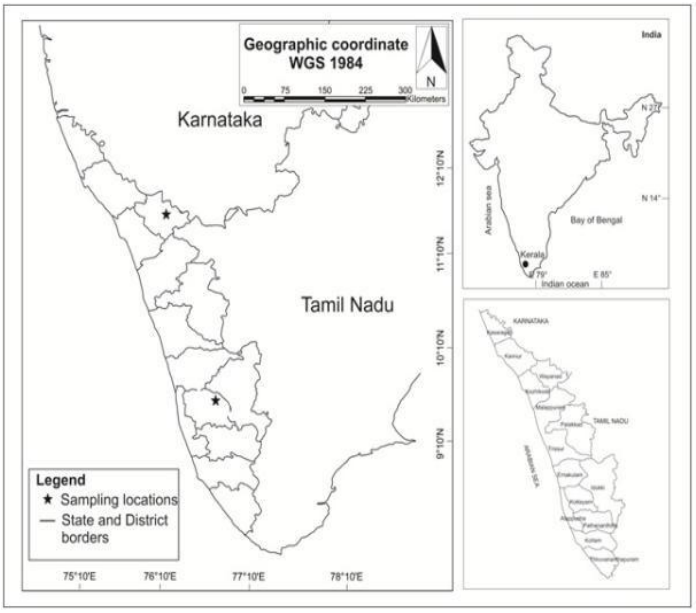
districts. The climate is characterized by tropical wet and dry with average annual rainfall amounts to 2,817 ± 406 mm and mean annual temperature is 26.8°C (averages from 1871-2005; Krishnakumar *et al.,* 2009). Maximum rainfall occurs from June to September mainly due to South West Monsoon and temperatures are highest in May and November.

Sample collection

Fresh leaves of *Emilia sonchifolia, Clerodendrum infortunatum* and *Tabanaemontana divericata* are collected from Ezhakkaranad, Ernakulum district of Kerala state, India.

The fresh leaves were collected in poly ethylene zipper bags, later washed two times with distilled water and stored in polyethylene zipper bags and processed in the laboratory. The samples were dried in hot air oven at 60ºC for 48hrs. The samples were finely powdered using a kitchen blender (Prestige Nakshatra plus, Prestige industries Mumbai) and later stored in air tight polyethylene zipper bag for analysis.

Fig.1 Map of Kerala showing the various sample collection points.



Leaf extract is prepared with 10 g of fresh leaves (*Emilia sonchifolia, Clerodendrum infortunatum* and *Tabanaemontana divericata*) thoroughly washed with tap water and then with DH_2O for at least two times and cut in to small pieces.

It is then crushed in a pistil and motor by adding 50 ml of DH_2O.it is then filtered using a filter paper in to a conical flask. It was then stored at 4°C after covering the beaker with aluminum foil for further use. The obtained leaves extract which appeared light green in color was stored 4°C for further use.

Synthesis of nanoparticles

Sliver nanoparticles

Stock solution was prepared by dissolving 1mMsliver nitrate ($AgNO_3$; Merck, Mumbai, India) and volume made up to 250 ml with distilled water.1ml of leaf extract of different concentration was added to 100 ml of 1mM $AgNO_3$ solution and allowed to react at room temperature. The formation of nanoparticle increases in the presence of sun light. Dark brown colour indicates the formation of $AgNO_3$.

Copper nanoparticles

Stock solution was prepared by dissolving 2.49 g Copper sulphate ($CuSO_4$). 1ml of leaf extracts of (*Emilia sonchifolia, Clerodendrum infortunatum, Tabanaemontana divericata*) is added to the100ml of 100mM $CuSO_4$ solution and allowed to react in room temperature. The $CuSO_4$ nanoparticles will be formed after 2-3 hours.

Zinc nanoparticles

Stock solution was prepared by dissolving 2.87 g Zinc sulphate ($ZnSO_4$). 1ml of leaf extracts of (*Emilia sonchifolia, Clerodendrum infortunatum, Tabanaemontana divericata*) are added to the100ml of 100mM $ZnSO_4$ solution and allowed to react in room temperature.

Test microorganisms

The organisms used comprise of two gram-negative organisms (*Klebsiella* and *E.coli*) and three gram-positive organisms (*Staphylococcus, Bacillus* and *Micrococcus*). The test organisms were obtained from the Department of Biotechnology, Mar Augusthinose College, Ramapuram.

Escherichia coli

These are gram negative, facultative or anaerobic rods (commonly abbreviated *E.coli*) commonly found in the lower intestine of warm blooded organisms. The

organisms are relatively heat sensitive and are readily destroyed at high temperature. The optimal temperature for growth is 37°C. *E. coli* is responsible for intestinal tract infection and diarrhoea.

Staphylococcus species

These are spherical in shape, non-motile, gram positive and facultative anaerobes which are positive in the catalase test. The coagulase test is used to broadly demarcate Staphylococcus species into coagulase positive and coagulase negative species.

Staphylococus species grow readily on ordinary media with a temperature range of 10 to 40°C, the optimum being 37°C and a pHof 7.4-7.6. *S. aureus* strains have emerged resistant to the penicillinase-stable penicillins (cloxacillin, dicloxacillin, methicillin, nafcillin, and oxacillin).

Klebsiella species

The genus Klebsiella consists of non-motile, capsulated rods that grow well on ordinary media forming large, dome shaped, mucoid colonies of varying degrees of stickiness. *Klebsiella species* are widely distributed in nature, occurring both as commensals in the intestines and as saprophytes in soil and water. *Klebsiella species* can cause diseases like pneumoniea, ozena and rhinoscleroma.

Micrococcus species

These are positive cocci which occur mostly in pairs, tetrads or irregular clusters. They are catalase and oxidase positive. They are aerobic with a strictly respiratory metabolism. They are parasitic on mammalian skin and are ordinarily non-pathogenic.

Bacillus species

The genus Bacillus consists of anaerobic bacilli forming heat resistant spores. They are gram positive but tend to be decolourised easily so as to appear gram variable, or even frankly gram negative.

They are generally motile with peritrichous flagella. *Bacillus anthracis*, the causative agent of anthrax, is the major pathogenic species. *B. cereus* can cause food borne gastroenteritis. Some species may be responsible for opportunistic infections.

Characterization of nanoparticles

UV-Vis spectroscopy

The periodic scans of the optical absorbance between 345 and 700nm with a UV- Vis spectrophotometer (Model 118, Systronics, Mumbai, India) at a resolution of 1 nm were performed to investigate the reduction rate of silver ions by neem leaf extract. The reaction mixture was diluted 20 times and used for UV-Vis spectrophotometry. Deionised water was used to adjust the baseline.

The reduction of Ag^{+}, Cu^{2+} and Zn^{2+} was monitored periodically by using a UV- Vis Spectrophotometer and the UV- Vis spectra of the reaction solutions were measured in the range of 375–760 nm.

SEM-XRD analysis

SEM-EDX Analysis was carried out in instrument JSM 6390 with acceleration voltage 20kV. SEM reveals information about the sample including external morphology, chemical composition and crystalline structure and orientation of materials making up the sample. SEM provides detailed high-resolution images of the sample by rastering a focused electron beam across the surface and detecting secondary or back scattered electron signal. The XRD imaging of the silver, copper and zinc nanoparticles was performed to confirm the presence of elemental metal signal and provides quantitative compositional information.

Antibacterial assay

Antimicrobial assay was performed by agar well diffusion method. The broth cultures of each organism were aseptically swabbed on Muller Hinton agar plates using sterile cotton swabs. Wells of 7 mm diameter were made in the inoculated plates using sterile cut tips and wells are filled with 20, 40 and 60 µl of nanoparticle solution and 20 µl of control (stock solution) and sample (leaf extract). The plates were incubated at 37^{0}C for 24 hours after which the diameter of zones of inhibition were measured.

Statistical analysis

The survey results were analyzed and descriptive statistics were done using SPSS 12.0 (SPSS Inc., an IBM Company, Chicago, USA) and graphs were generated using Sigma Plot 7 (Systat Software Inc., Chicago, USA).

Results and Discussion

Synthesis of nanoparticles

Nanoparticles were synthesized from the leaf extract of *Emilia sonchifolia, Clerodendrum infortunatum* and *Tabarnaemontana divaricata.*

Silver nanoparticles

Silver nanoparticles were synthesized from leaf extracts of different plants (*Emilia sonchifolia, Clerodendrum infortunatum* and *Tabarnaemontana divaricata*). Leaf extract was added to 1mM silver nitrate solution and kept to reaction to take place. A colour change was observed from colourless to dark brown. This occurred as a result of the reduction of silver ions present in the solution.

Copper nanoparticles

Copper nanoparticle were synthesized from leaf extract of different plants (*Emilia sonchifolia, Clerodendrum infortunatum* and *Tabarnaemontana divaricata*). Leaf extract was added to 100 mM copper sulphate solutions and kept to reaction to take place. A colour change was observed from blue to pale yellow. This occurred due to the reduction of copper ions present in the solution.

Zinc nanoparticles

Zinc nanoparticles were synthesized from leaf extract of different plants (*Emilia sonchifolia, Clerodendrum infortunatum* and *Tabarnaemontana divaricata*). Leaf extract was added to 100 mMzinc sulphate solution and kept to reaction to take place. A colour change was observed from colourless to pale brown. This occurred due to the reduction of zinc ions present in the solution.

Characterization of nanoparticles

Silver nanoparticles

UV spectrometry

Synthesized silver nanoparticles were characterized by UV-VIS spectrophotometry. The maximum peak was found to be 435 nm for *Glycosmis pentaphylla*. The intensity of the peak at 435 nm was increased with time until the reduction completes. The maximum peak was found to be 385 nm for *Emilia sonchifolia, Clerodendrum infortunatum* and *Tabarnaemontana divaricata.* The intensity of the peak at 385 nm was increased with the time until the reduction completes.

Copper nanoparticles

UV spectrometry

Synthesized copper nanoparticles were characterized by UV-VIS spectrophotometry. The maximum peak was found to be 680 nm for *Emilia sonchifolia, Clerodendrum infortunatum* and *Tabarnaemontana divaricata.* The intensity of the peak at 680 nm was increased with the time until the reduction completes.

Zinc nanoparticles

UV spectrometry

Synthesized zinc nanoparticles were characterized by UV-VIS spectrophotometry. The maximum peak was found to be 350 nm for *Emilia sonchifolia, Clerodendrum infortunatum* and *Tabarnaemontana divaricata.* The intensity of peak at 350 nm was increased with time until the reduction completes.

Antibacterial assay

The leaf extract of *Emilia sonchifolia, Clerodendrum infortunatum* and *Tabarnaemontana divaricata* showed growth inhibitory effects against *salmonella, pseudomonas, staphylococcus, E.coli* and *Klebsiella.*

Tabarnaemontana divaricata

Zinc and copper Nano particles shows observable range of activity rather than silver nanoparticles on the other hand copper nanoparticles shows more activity in *Klebsiella*. Interestingly *Pseudomonas* and *Salmonella* resembles their measurement of zone of inhibition.

Emilia sonchifolia

Zinc nanoparticles has highest range of activity in case of *E.coli, Klebsiella, Salmonella* and *Staphylococcus* respectively. Irrespective of slight change in zone of inhibition at their higher concentration *Pseudomonas* has major activity in copper nanoparticles rather than zinc nanoparticles.

Clerodendrum infortunatum

Zinc nanoparticles showed upper hand in anti-bacterial activity against *Pseudomonas* and Staphylococcus. *E.coli, Klebsiella* and *Salmonella* showed increasing level of anti-bacterial activity for silver nano particles

and copper nano particles respectively. They have antibacterial activity against *Salmonella species*, Pseudomonas, *Staphylococcus species*, *E.coli*, and *Klebsiella species*. when antibacterial activity of silver, copper and zinc nanoparticles from different concentration were observed, nanoparticles have 60µl concentration shows maximum activity against these microbes. Silver nanoparticles shows greater antibacterial activity compared to silver nitrate and leaf extract. Copper nanoparticles shows greater antibacterial activity compared to copper sulphate and leaf extract.

Zinc nanoparticles show greater antibacterial activity to zinc sulphate and leaf extract. Maximum zone of inhibition was at 60 µl for all the bacterial cultures. It indicates that zone of inhibition increases as the concentration of nanoparticles increased.

Table.1 Different vernacular names of *Tabernaemonta divaricata* around the globe and India.

Language	Names
Scientific names	*Tabernaemonta divaricata*
Name in various global languages	
French	
German	
English	Crape jasmin
Name in various Indian languages	
Sanskrit	Nandivrksa
Hindi	Tagar
Urdu	
Marathi	Ananta
Kannada	Nandi battalu
Gujarati	Sagar
Malayalam	Nandhiarvattam
Tamil	Nandhiarvattai

Table.2 Different vernacular names of *Emilia sonchifolia* around the globe and India.

Language	Names
Scientific names	*Emilia sonchifolia*
Name in various global languages	
French	
German	
English	lilac tasselflower
Name in various Indian languages	
Sanskrit	Sasasruti
Hindi	Hirankhuri
Urdu	
Marathi	Panom
Kannada	Elikivigida
Gujarati	
Malayalam	Muyalcheviyan
Tamil	Mayarcevi

Table.3 Different vernacular names of *Clerodendrum infortunatum* around the globe and India.

Language	Names
Scientific names	*Clerodendrum infortunatum*
Name in various global languages	
French	
German	
English	Clerodendrum
Name in various Indian languages	
Sanskrit	Bhandirah
Hindi	Titabhamt
Urdu	Bharangi
Marathi	Bandira
Kannada	Basavanapada
Gujarati	Bharangee
Malayalam	Perivalam
Tamil	Perukilai

Table.4 Zone of inhibition against various bacteria (*E. coli*, *Klebsiella species*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococus aureus*) using nanoparticles produced by *Cleorodendrum infortunatum* leaves extract.

Microorganism	Nanoparticles	Control	Sample	Measure of zone of inhibition in cm		
				20	40	60
E.coli	Silver	1.0	1.0	1.1	1.2	1.3
	Copper	1.1	1.0	1.3	1.5	1.8
	Zinc	2.1	1.0	2.3	2.6	2.8
Klebsiella species	Silver	1.1	1.0	1.2	1.3	1.4
	Copper	1.4	1.0	1.6	1.8	2.5
	Zinc	2.1	1.0	2.3	2.5	3.0
Pseudomonas aeruginosa	Silver	1.4	1.0	1.6	2.0	2.6
	Copper	1.1	1.0	1.3	1.4	1.6
	Zinc	1.6	1.0	1.8	2.0	2.5
Salmonella typhi	Silver	1.0	1.0	1.1	1.2	1.3
	Copper	1.2	1.0	1.4	1.6	1.8
	Zinc	1.8	1.0	2.0	2.2	2.6
Staphylococcus aureus	Silver	1.1	1.1	1.2	1.3	1.4
	Copper	2.0	1.1	2.6	2.8	3.3
	Zinc	1.8	1.1	2.0	2.6	3.0

Table.5 Zone of inhibition against various bacteria (*E. coli*, *Klebsiella species*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococus aureus*) using nanoparticles produced by *Emilia sonchifolia* leaves extract.

Microorganism	Nanoparticles	Control	Sample	Measure of zone of inhibition in cm		
				20	40	60
E.coli	Silver	1.1	1.0	1.2	1.3	1.4
	Copper	1.1	1.0	1.2	1.4	1.8
	Zinc	2.0	1.0	2.2	2.5	3.3
Klebsiella species	Silver	1.1	1.1	1.2	1.3	1.4
	Copper	1.0	1.1	1.2	1.3	1.4
	Zinc	1.4	1.1	1.8	2.0	2.2
Pseudomonas aeruginosa	Silver	1.1	1.0	1.1	1.2	1.3
	Copper	2.0	1.0	2.4	2.7	3.2
	Zinc	2.1	1.0	2.2	2.5	3.0
Salmonella typhi	Silver	1.0	1.0	1.1	1.2	1.3
	Copper	0.9	1.0	1.0	1.1	1.2
	Zinc	0.9	1.0	1.9	2.1	2.3
Staphylococcus aureus	Silver	1.1	1.0	1.2	1.3	1.4
	Copper	1.2	1.0	1.4	1.7	2.0
	Zinc	1.4	1.0	1.8	2.0	2.2

Table.6 Zone of inhibition against various bacteria (*E. coli*, *Klebsiella species*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococus aureus*) using nanoparticles produced by *Tabernaemontana divericata* leaves extract.

Microorganism	Nanoparticles	Control	Sample	Measure of zone of inhibition in cm		
				20	40	60
E.coli	Silver	1.0	1.1	1.2	1.3	1.5
	Copper	1.2	1.1	1.3	1.5	2.1
	Zinc	1.6	1.1	1.8	2.2	2.6
Klebsiella species	Silver	1.1	1.0	1.2	1.3	1.4
	Copper	1.2	1.0	1.4	1.9	2.6
	Zinc	1.3	1.0	1.4	1.7	2.0
Pseudomonas aeruginosa	Silver	1.0	1.2	1.2	1.3	1.4
	Copper	1.2	1.2	1.4	1.6	2.0
	Zinc	1.8	1.2	2.1	2.3	2.6
Salmonella typhi	Silver	1.0	1.0	1.1	1.2	1.3
	Copper	1.2	1.0	1.3	1.6	2.0
	Zinc	1.8	1.0	2.0	2.2	2.4
Staphylococcus aureus	Silver	1.1	1.1	1.2	1.3	1.4
	Copper	1.3	1.1	1.4	1.6	2.0
	Zinc	1.8	1.1	2.1	2.3	2.6

Table.7 UV absorption spectrum of Silver nanoparticles formed from *Emilia sonchifolia* during different time of incubation.

Time	385 nm	435nm	560nm	680nm
½ hr	1.220	0.968	0.665	0.490
1 hr	1.238	1.001	0.709	0.524
1 ½ hr	1.262	1.027	0.752	0.552
2 hr	1.270	1.050	0.798	0.596
2 ½ hr	1.283	1.065	0.821	0.606
Blank	0.000	0.000	0.000	0.000

Table.8 UV absorption spectrum of Copper nanoparticles formed from *Emilia sonchifolia* during different time of incubation.

Time	385 nm	435nm	560nm	680nm
½ hr	0.201	0.178	0.265	0.616
1 hr	0.317	0.254	0.277	0.663
1 ½ hr	0.380	0.348	0.282	0.726
2 hr	0.388	0.385	0.339	0.751
2 ½ hr	0.398	0.419	0.351	0.762
Blank	0.000	0.000	0.000	0.000

Table.9 UV absorption spectrum of Zinc nanoparticles formed from *Emilia sonchifolia* during different time of incubation.

Time	350 nm	385 nm	435 nm	560 nm
½ hr	0.754	0.574	0.406	0.258
1 hr	0.762	0.577	0.409	0.280
1 ½ hr	0.814	0.589	0.448	0.283
2 hr	0.866	0.640	0.459	0.306
2 ½ hr	0.893	0.682	0.487	0.318
Blank	0.000	0.000	0.000	0.000

Table.10 UV absorption spectrum of Silver nanoparticles formed from *Cleodendrum infortunatum* during different time of incubation.

Time	385 nm	435nm	560nm	680nm
½ hr	0.443	0.429	0.352	0.309
1 hr	0.661	0.601	0.486	0.386
1 ½ hr	0.702	0.623	0.519	0.414
2 hr	0.731	0.682	0.531	0.432
2 ½ hr	0.906	0.838	0.601	0.582
Blank	0.000	0.000	0.000	0.000

Table.11 UV absorption spectrum of Copper nanoparticles formed from *Cleodendrum infortunatum* during different time of incubation.

Time	385 nm	435nm	560nm	680nm
½ hr	0.143	0.132	0.149	0.627
1 hr	0.153	0.180	0.208	0.638
1 ½ hr	0.176	0.192	0.215	0.768
2 hr	0.201	0.202	0.220	0.861
2 ½ hr	0.350	0.262	0.276	0.873
Blank	0.000	0.000	0.000	0.000

Table.12 UV absorption spectrum of Zinc nanoparticles formed from *Cleodendrum infortunatum* during different time of incubation.

Time	350 nm	385 nm	435 nm	560 nm
½ hr	0.312	0.147	0.132	0.086
1 hr	0.333	0.186	0.135	0.091
1 ½ hr	0.339	0.202	0.139	0.094
2 hr	0.358	0.210	0.151	0.097
2 ½ hr	0.365	0.211	0.162	0.108
Blank	0.000	0.000	0.000	0.000

Table.13 UV absorption spectrum of Silver nanoparticles formed from *Tabernaemontana divaricata* during different time of incubation.

Time	385 nm	435nm	560nm	680nm
½ hr	1.356	1.201	0.918	0.713
1 hr	1.370	1.221	0.926	0.744
1 ½ hr	1.387	1.252	0.951	0.771
2 hr	1.426	1.316	0.980	0.773
2 ½ hr	1.478	1.394	1.051	0.808
Blank	0.000	0.000	0.000	0.000

Table.14 UV absorption spectrum of Copper nanoparticles formed from *Tabernaemontana divaricata* during different time of incubation.

Time	385 nm	435nm	560nm	680nm
½ hr	0.157	0.150	0.175	0.598
1 hr	0.168	0.165	0.257	0.709
1 ½ hr	0.286	0.256	0.268	0.726
2 hr	0.327	0.274	0.276	0.736
2 ½ hr	0.422	0.363	0.279	0.748
Blank	0.000	0.000	0.000	0.000

Table.15 UV absorption spectrum of Zinc nanoparticles formed from *Tabernaemontana divaricata* during different time of incubation.

Time	350 nm	385 nm	435 nm	560 nm
½ hr	0.779	0.673	0.571	0.439
1 hr	0.855	0.719	0.601	0.445
1 ½ hr	0.902	0.791	0.676	0.513
2 hr	0.923	0.794	0.691	0.516
2 ½ hr	1.013	0.879	0.723	0.529
Blank	0.000	0.000	0.000	0.000

Table.16 Biochemical characterization of the organisms used in the study.

Organisms	I	MR	VP	C	GS	U	O	CL	COG	NR
Salmonella typhi	-VE	+VE	-VE	-VE	-VE	-VE	-VE	+VE	_	+VE
Pseudomonas aeruginosa	-VE	-VE	-VE	+VE	-VE	-VE	+VE	+VE	-VE	+VE
Staphylococcus aureus	-VE	+VE	+VE	+VE	+VE	+VE	-VE	+VE	+VE	+VE
E. coli	+VE	+VE	-VE	-VE	-VE	-VE	-VE	+VE	_	+VE
Klebsiella pneumoniae	-VE	-VE	+VE	+VE	-VE	+VE	-VE	+VE	_	+VE

(I- Indole, MR- Methyl Red, VP- Voges Proskauer, C- Citrate, GS- Gram Staining, U- Urease, O- Oxidase, CL- Catalase, COG- Coagulase, NR- Nitrogen Reductase).

Table.17 Antibiotic susceptibility test of the organisms used in the study.

Organisms	Zone of Inhibition (mm)							
	AMP	CHL	ENO	ERY	GEN	KAN	PEN	TET
Salmonella typhi	1.7	3.2	_	_	_	_	_	1.17
Pseudomonas aeruginosa	_	_	22-28	_	16-21	-	_	_
Staphylococcus aureus	27-35	19-26	22-28	22-30	19-27	19-26	26-37	24-30
E. coli	16-22	21-27	28-36	_	19-26	17-25	_	18-25
Klebsiella pneumoniae	32	_	_	16	_	_	16	14

AMP: Ampicillin; CHL: Chloramphenicol; ENO: Enonacin; ERY: Erythromycin; GEN: Gentamycin; KAN: Kanamycin; PEN: Penicillin; TET: Tetracycline.

Fig.2 Description of *Emilia sonchifolia* a) plant with young and mature leaves, b) flowers, c) mature flowers ready for seed dispersal, d) plant in natural habitat, e) fully developed flowers. Photo courtesy: Wikipedia.

Fig.3 Description of *Tabanaemontana divericata* a) plant from garden, b) plant with leaves flowers and developing buds, c) flower, d) flower cluster, e) and f)plant with flowers and leaves. Photo courtesy: Wikipedia.

Fig.4 Description of *Clerodendrum infortunatum* a) plant in natural habitat, b) flower inflorescence, c) and d)flowers, e) plant with developing flowers and leaves. Photo courtesy: Wikipedia.

Fig.5 Description of various nanoparticle formations a) Silver nitrate solution, b) Copper sulphate solution, c) Zinc sulphate solution, d) Silver nanoparticle formation, e) Copper nanoparticle formation, f) Zinc nanoparticle formation.

Fig.6 Antibacterial activity study using well diffusion method of *Tabernaemontana divertica* leaf extract nanoparticles (Cu), (Ag) and (Zn) a) to e) control plates of *Salmonella typhi*, *Pseudomonas aeruginosa*, *E. coli*, *Klebsiella species and Staphylococcus species*, f) to t) above mentioned organisms plate in same order for green synthesised nanoparticles Cu, Ag and Zn (20, 40 and 60 µl).

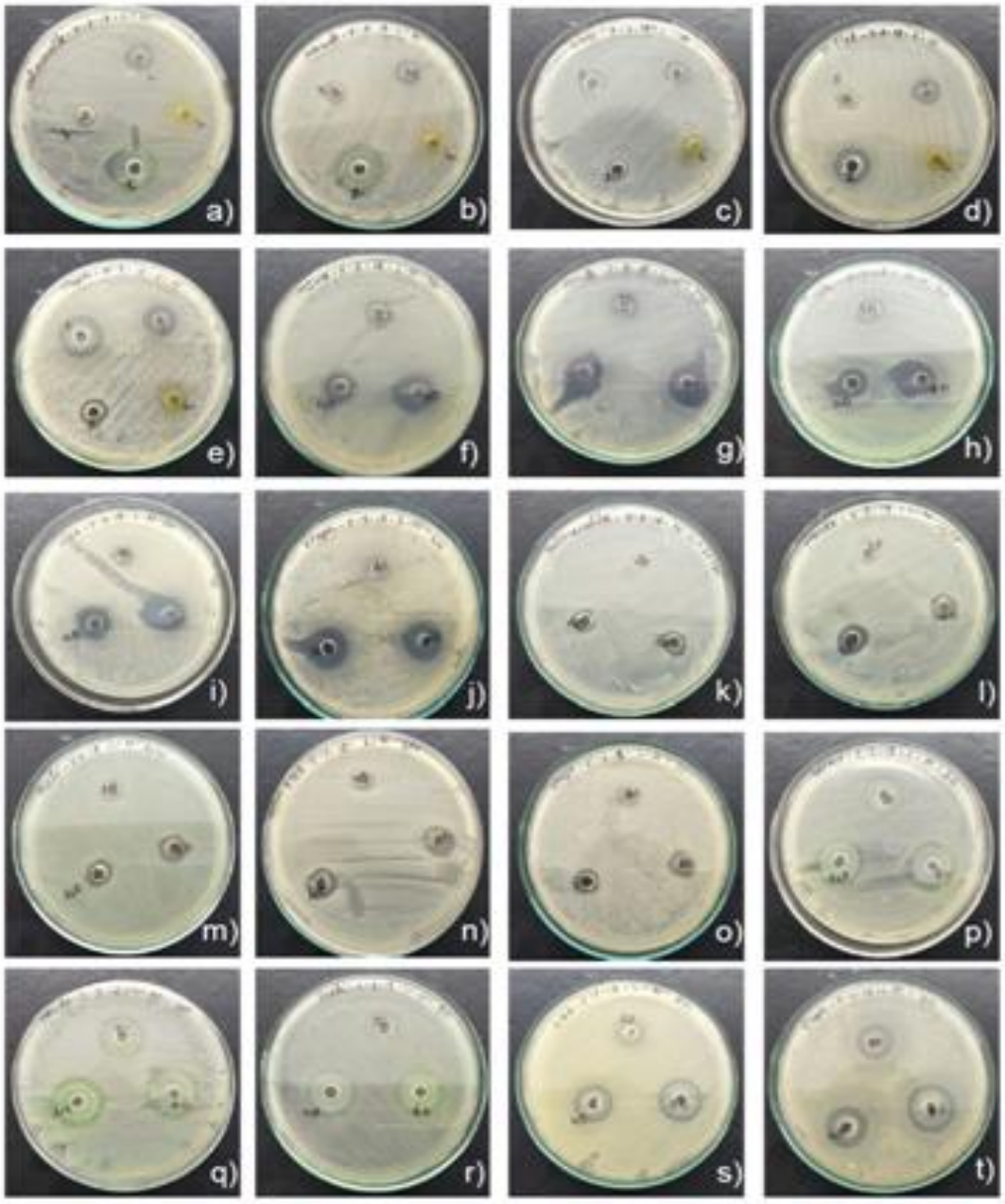


Fig.7 Antibacterial activity study using well diffusion method of *Glycosmis pentaphylla* and *Clerodendrum infortunatum* leaf extract nanoparticles (Zn) a) *Salmonella typhi* control plate (Zn), b) *Salmonella typhi* green synthesised nanoparticle test plate (Zn; 20, 40 and 60 µl), c) *Pseudomonas aeruginosa* control, d) *Pseudomonas aeruginosa* test, e) *E. coli* control, f) *E. coli* test, g) *Klebsiella species* control, h) *Klebsiella species* test, i) *Staphylococcus species* control, j) *Staphylococcus species* test, k) to t) above mentioned organisms plate in same order for Zn nanoparticles (*Clerodendrum infortunatum*).

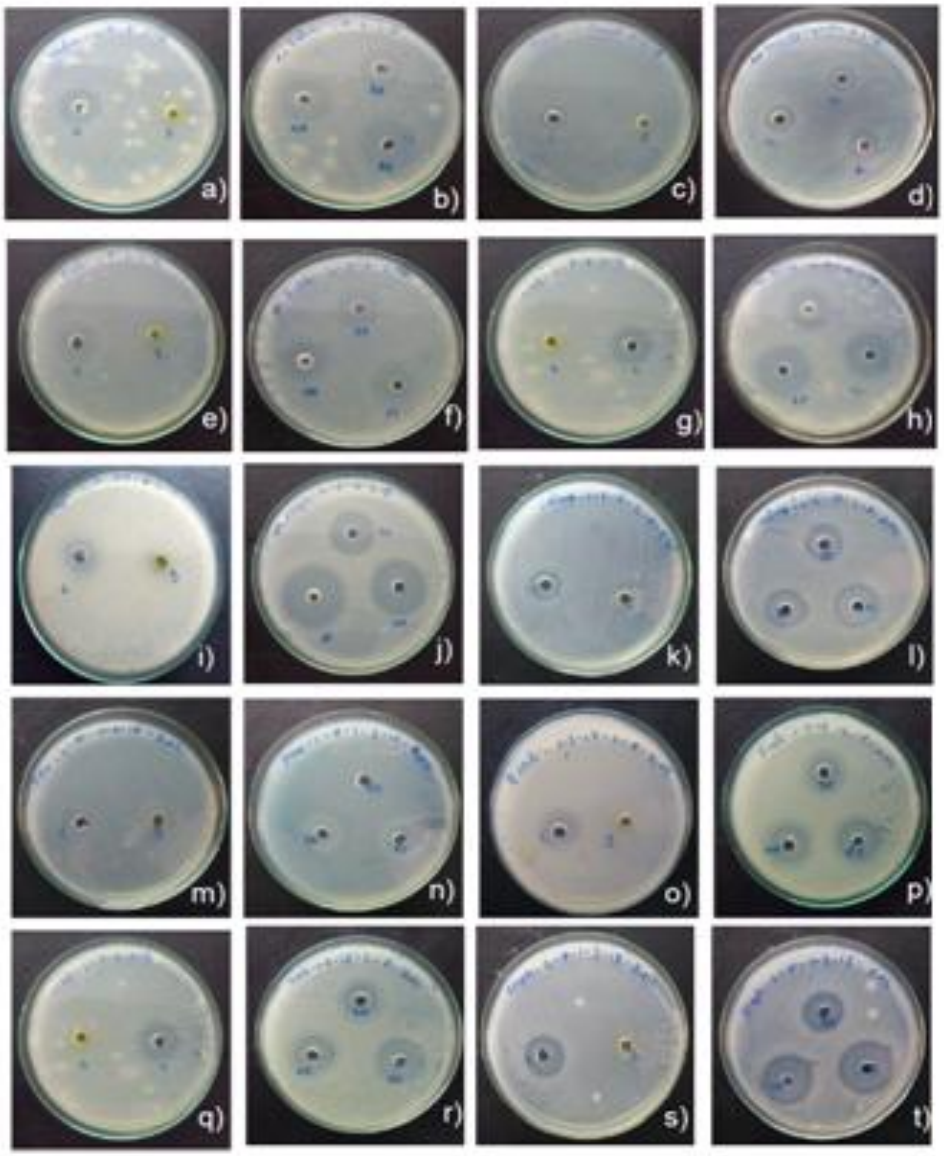


Fig.8 Antibacterial activity study using well diffusion method of *Clerodendrum infortunatum* leaf extract nanoparticles (Cu) and (Ag)a) *Salmonella typhi* control plate (Cu), b) *Salmonella typhi* green synthesised nanoparticle test plate (Cu; 20, 40 and 60 µl), c) *Pseudomonas aeruginosa* control, d) *Pseudomonas aeruginosa* test, e) *E. coli* control, f) *E. coli* test, g) *Klebsiella species* control, h) *Klebsiella species* test, i) *Staphylococcus species* control, j) *Staphylococcus species* test, k) to t) above mentioned organisms plate in same order for Ag nanoparticles.

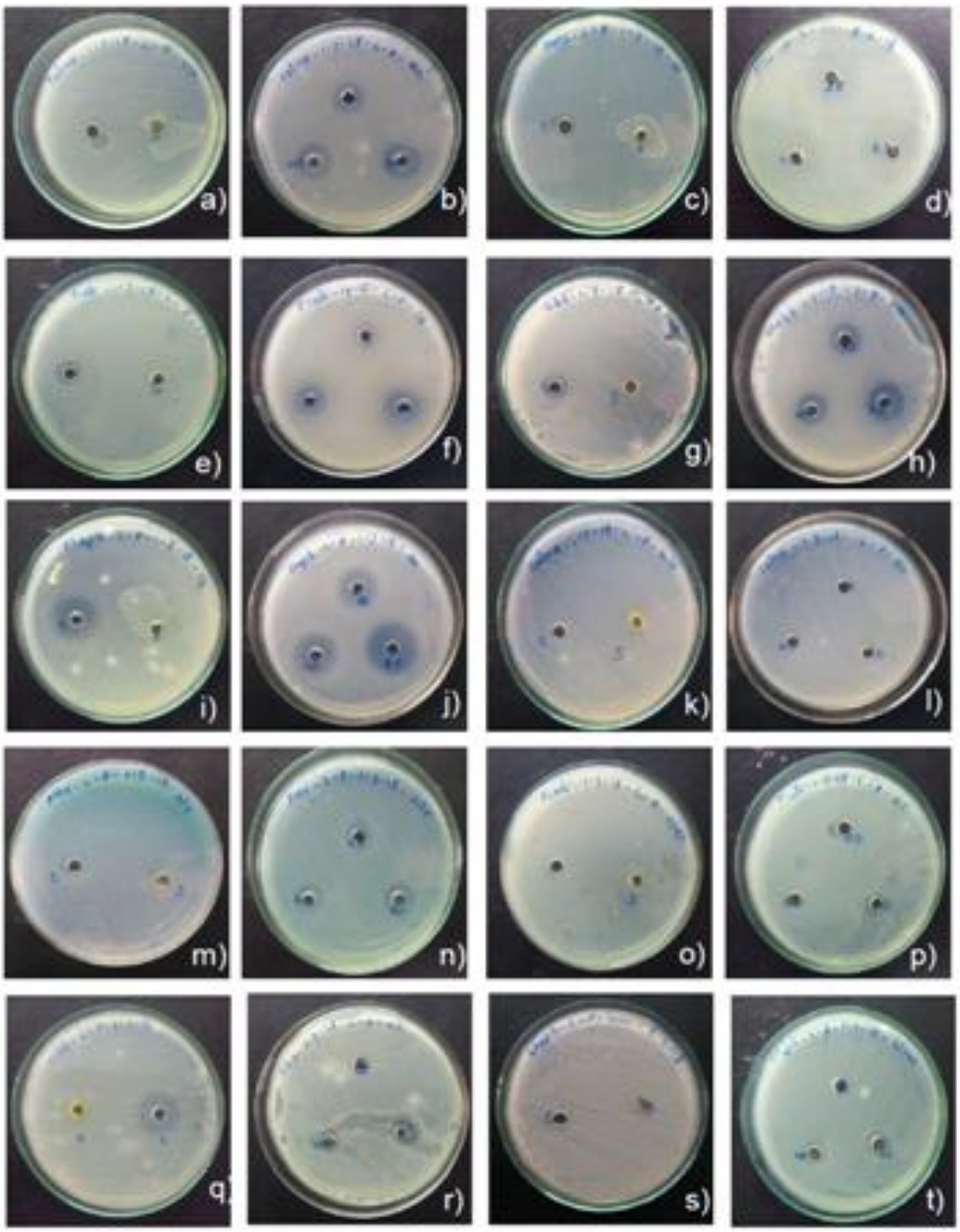


Fig.9 Antibacterial activity study using well diffusion method of *Emilia sonchifolia* leaf extract nanoparticles (Cu) and (Ag) a) *Salmonella typhi* control plate (Cu), b) *Salmonella typhi* green synthesised nanoparticle test plate (Cu; 20, 40 and 60 µl), c) *Pseudomonas aeruginosa* control, d) *Pseudomonas aeruginosa* test, e) *E. coli* control, f) *E. coli* test, g) *Klebsiella species* control, h) *Klebsiella species* test, i) *Staphylococcus species* control, j) *Staphylococcus species* test, k) to t) above mentioned organisms plate in same order for Ag nanoparticles

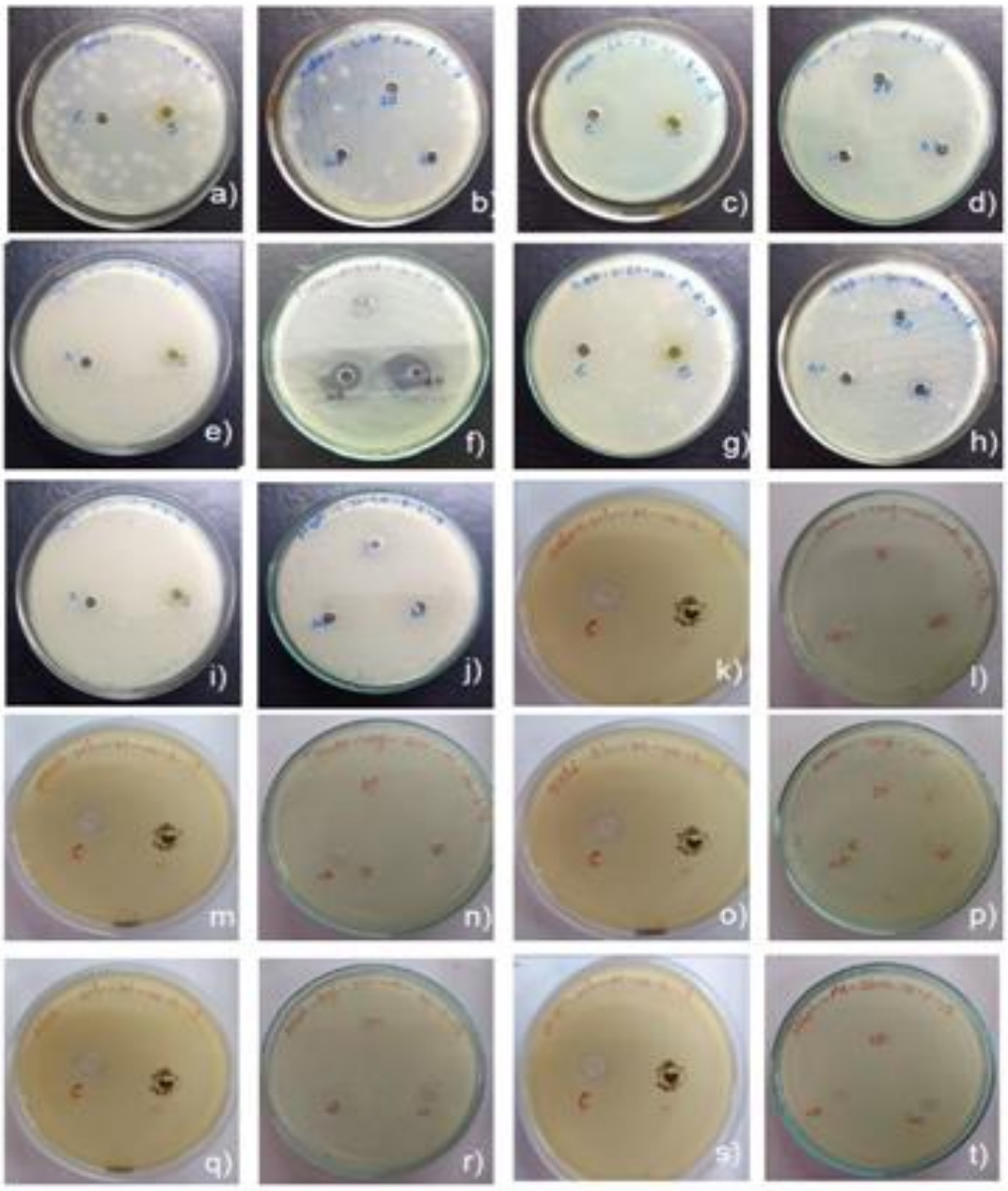


Fig.10 Antibacterial activity study using well diffusion method of *Emilia sonchifolia* leaf extract nanoparticles (Cu) and (Ag)a) *Salmonella typhi* control plate (Cu), b) *Salmonella typhi* green synthesised nanoparticle test plate (Cu; 20, 40 and 60 µl), c) *Pseudomonas aeruginosa* control, d) *Pseudomonas aeruginosa* test, e) *E. coli* control, f) *E. coli* test, g) *Klebsiella species* control, h) *Klebsiella species* test, i) *Staphylococcus species* control, j) *Staphylococcus species* test, k) to t) above mentioned organisms plate in same order for Ag nanoparticles.

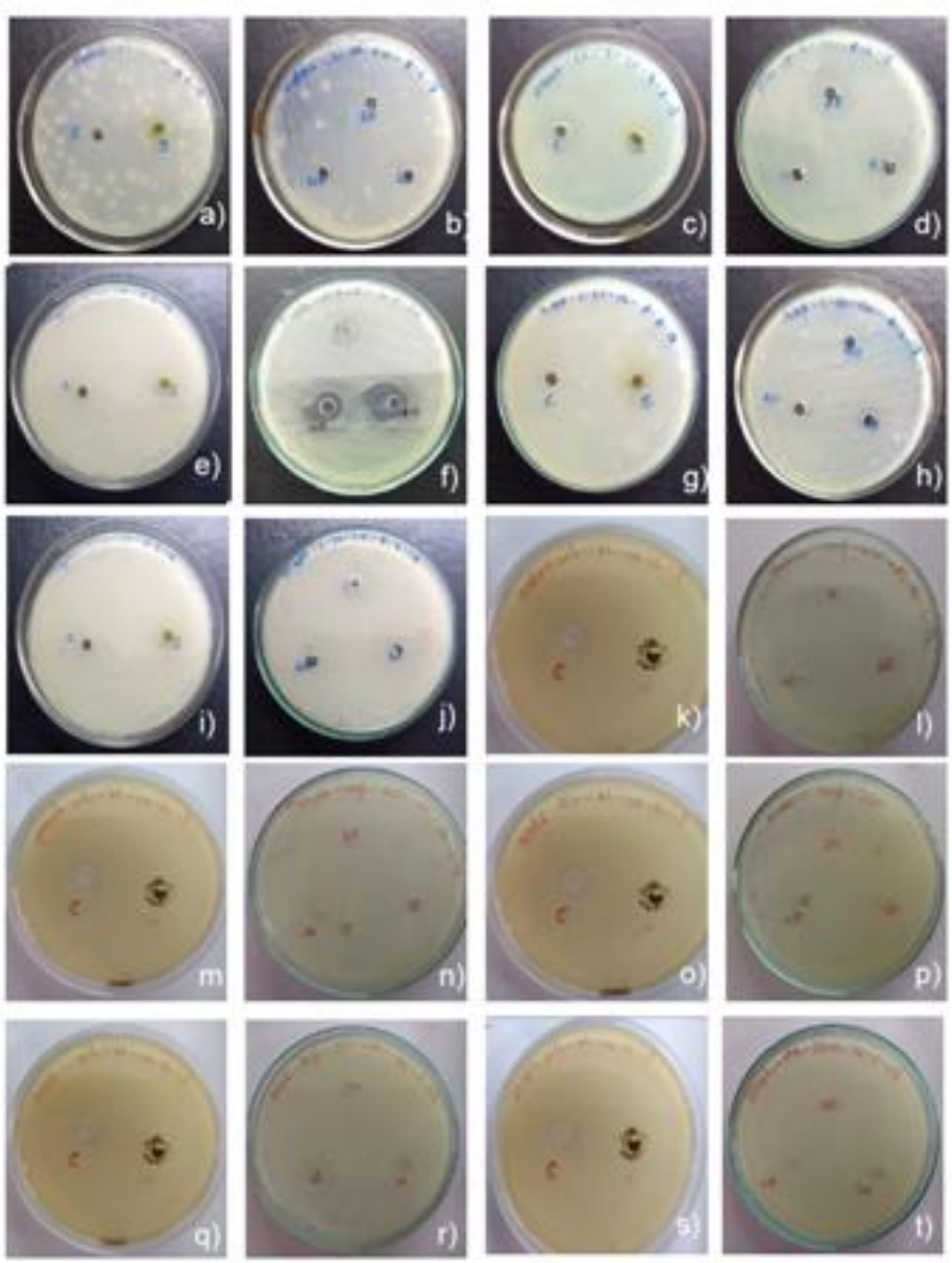
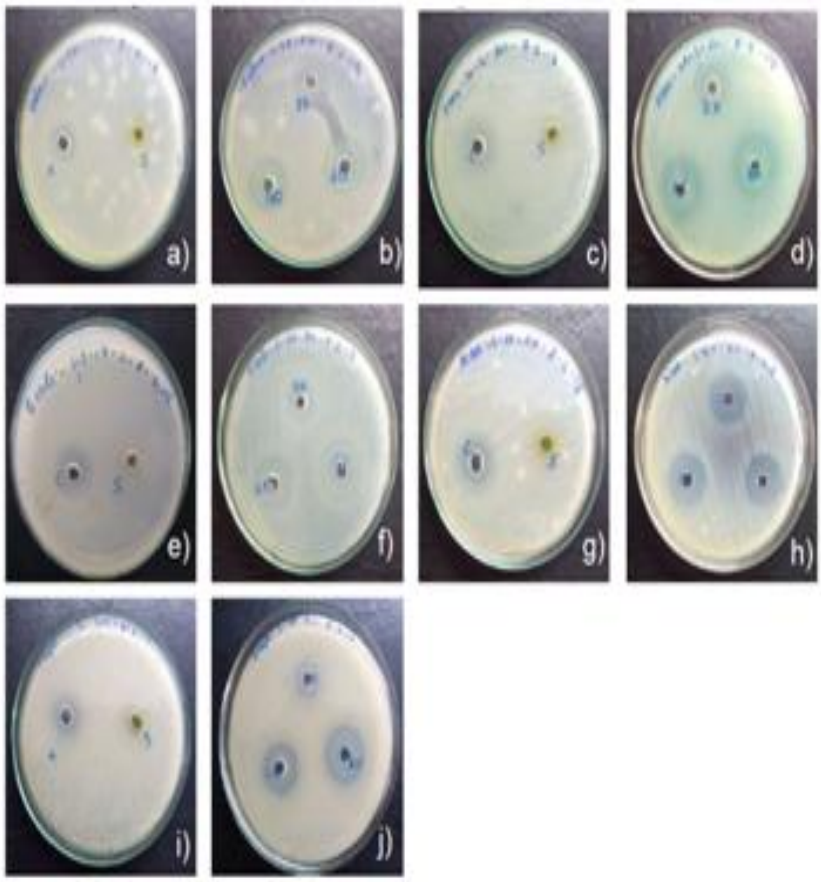


Fig.11 Antibacterial activity study using well diffusion method of *Emilia sonchifolia* leaf extract nanoparticles (Zn) a) *Salmonella typhi* control plate (Zn), b) *Salmonella typhi* green synthesised nanoparticle test plate (Zn; 20, 40 and 60 µl), c) *Pseudomonas aeruginosa* control, d) *Pseudomonas aeruginosa* test, e)*E. coli* control,f) *E. coli* test, g) *Klebsiella species* control, h) *Klebsiella species* test, i) *Staphylococcus species* control, j) *Staphylococcus species* test



Antibacterial assay

The SEM-XRD analysis proved the effective formation of silver, copper and zinc nanoparticles in all the samples.

The results shows that leaf extracts of *Emilia sonchifolia*, *Clerodendrum infortunatum* and *Tabarnaemontana divaricata* are used to the synthesis of silver, copper and zinc nanoparticles. The synthesised silver, copper and zinc nanoparticles shows antibacterial activity on both gram positive and gram negative bacteria. This green synthesis of nanoparticle is cost effective, pollution free and easy to synthesis.

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References

Aiswarya G, Reza K H, Radhika G, and Farook S. M. (2011). Study for antibacterial activity of cashew apple (*Anacardium occidentale*) extracts. *Der Pharmacia Lettre*, 3 (1) 193-200.

Carlos, Batista A. Silvera; Larson, Ronald G; Kotov, Nicholas A.(2015). Non additivity of nanoparticles interactions. *Science*. 350.

Brown D F, Kothari D (1975). Comparison of antibiotic discs from different sources.779-83

Devasenan S, N. HajaraBeevi, S. S. Jayanthi (2016). Green synthesis and characterization of zinc nanoparticle using *Andrographis paniculata* leaf extract. *International Journal of Pharmaceutical Sciences Review and Research*, 243-247.

Duke J A. (2010). Ethnobotanical uses *Clerodendrum infortunatum*; Dr Dukes Phytochemical and Ethnobotanical Database.

Euzeby J P (1997). List of bacterial names with standing in nomenclature a folder available on the internet. Int J SystBacteriol 47 (2) 590-2.

Hajipour, M. J., Fromm, K. M., Ashkarran, A. A., Aberasturi, D. J., Larramendi, I. R., Rojo, T., Serpooshan, V., Parak, W. J., & Mahmoudi, Morteza (2012). Antibacterial properties of nanoparticles. "*Trends in Biotechnology*doi: 10.1016/j.tibtech.2012.06.004, 2012".

Hewakuruppu Y L; Dombrovsky, L A, Chen C; Timchenko V, Jiang X; Baek S, Tayler R A (2013). Plasmionic pump prob method to study semi transparent nanofluids.

Kingston W (2008). Irish contributions to the origins of antibiotics. Irish Journal of Medical Science. 177 (2): 87-92.

Kulkarni. D and Pramod. S. Kulkarni (2004). Green synthesis of copper nanoparticle using *Ocimum sancium* leaf extract.

Kumar, A., Kumar, P., Ajayan, M. P., & John, G. (2008). Silver nanoparticles embedded antimicrobial paints based on vegetable oil. *Nature Materials*, 7(3), 236-241.

Kumar, V., & Yadav, S. K. (2009). Plant mediated synthesis of silver and gold nanoparticles and their applications. *Journal of Chemical Technology and Biotechnology*, 84(2), 151-157.

Kuppusamy, P., Yusoff, M. M., Maniam, G. P., & Govindan, N. (2014). Biosynthesis of metallic nanoparticles using plant derivatives and their new avenues in pharmacological applications-An updated report. *Saudi Pharmaceutical Journal*, 24, 473-484.

MacNaught, Alan D.; Wilkinson, Andrew R., (1997). *Compendium of chemical Terminology:IUPAC Recommendations* (2nd ed.). Blackwell Science.

Mishra, Vijayalaxmee., Sharma, Richa., Jasuja, N. D., & Gupta, D. K. (2014). A review on green synthesis of nanoparticles and evaluation of antimicrobial activity. *International Journal of Green and Herbal Chemistry*, 3(1), 081-094.

Mohanty, A. (2010): physiochemical and antimicrobial study of polyherbal. Pharmacleglobale. Vol 4,1-3.

Patcharaporn Tippayawat, Nuthakrittaphromviyo, and ApiwatChrompoosor (2006). Green synthesis of silver nanoparticles in aloe vera plant extract prepared by a hydrothermal method and their synergistic antibacterial activity.

Paul, N. S., & Yadav, R. P. (2015). Biosynthesis of silver nanoparticles using plant seeds and their antimicrobial activity. *Asian Journal of Biomedical and Pharmaceutical Sciences*, 5(45), 26-28.

Sahu B K (2013). Antimicrobial properties of Aerial Part of *Sesbania grandifloria*. The pharmaceutical college Barpali, India.

Sajeshkumar, N. K., Vazhacharickal, P. J., Mathew, J. J., & Joy, J. (2015b). Synthesis of silver nanoparticles from neem leaf (*Azadirachta indica*) extract and its antibacterial activity. CIB Tech Journal of Biotechnology, 4(2), 20-31.

Sajeshkumar, N. K., Vazhacharickal, P. J., Mathew, J. J., & Sebastian, A. (2015a). Synthesis of silver nanoparticles from curry leaf (*Murraya koenigii*) extract and its antibacterial activity. CIB Tech Journal of Pharmaceutical Sciences, 4(2), 15-25.

Sharma H K, Chhangte L, Dolai A K. (2001). Traditional medicinal plants in mizoram India. Fitoterapia 72(2): 146-161.

Su L H, Chiu C H (2007). Salmonella clinical importance and evolution of nomenclature. Chang gung Medical Journal. 30: 210-9

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