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Green Synthesis of Nanoparticles (Ag, Cu and Zn) from *Glycosmis pentaphylla* and *Macaranga peltata*: Evaluation of Antibacterial Activity

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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 *Glycosmis pentaphylla* (Pannal) and *Macaranga peltata* (Vatta)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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Keywords

Nanoparticles, Green synthesis, Antibacterial activity, Glycosmis pentaphylla.

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 (Valli *et al.*, 2016). Leaf extracts of *Glycosmis pentaphylla* (Pannal) and *Macaranga peltata (Vatta)* 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 (Sun and Xia, 2002).

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 (Prasad *et al.*, 2008). 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, hazard's 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.,* 2016; Mishra *et al.,* 2014).

Application of nanoparticles

Nanoparticles has various applications. Nanoparticles have been used for constructing electrochemical and biosensors (Luo *et al.,* 2006). 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 (Elechiguerra *et al.,* 2005). Magnetic nanoparticles are also used in targeted therapy where a cytotoxic drug is attached to a biocompatible nanoparticle for tumour cell treatment (Pankhurst *et al.,* 2003). 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 (Morones *et al.,* 2005; Lok *et al.,* 2007). 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.

Macaranga peltata (Vatta)

Macaranga peltata is found mainly in northern Thailand, Sri Lanka and India. It is seen in low country wet zone. It is a resinous tree grows up to ten meters. The young parts are velvet hairy in appearance. Leafs as a measure up to 20 to 50 cm. They are alternately arranged circular or broadly ovate. The leaf stalk is attached on the lower surface of the leaf, not on the hair. It is used for serving food in ancient time. The leaf many medicinal use and have anti-bacterial activity.

Taxonomical classification of *Macaranga peltata* (Vatta)

Kingdom: Plantae-- planta, plantes, plants, vegetal

Division: Magnoliophyta

Class: Magnoliopsida

Order: Malpighiales

Family: Euphorbiaceae

Genus: Macaranga

Species: *Macaranga peltata*

Glycosmis pentaphylla (Panal)

Glycosmis pentaphylla is a shrub which grow up to 5m. it is found in western Ghats, throughout Kerala grow as a wood. Its branches grow up to three meter in tall, husk is smooth and dark brown. It is used for food and traditional medicine by the people in Kerala. It is also an ingredient of various medicinal mixtures. Leaf and stem husk extract has healing effect on damaged liver tissue. The plant is used in the treatment of deceases like diarrhoea, coughs, sheumats, anaemia and jaundice. (Data base 2014).

Taxonomical classification of *Glycosmis pentaphylla* (Panal)

Kingdom: Plantae-- planta, plantes, plants, vegetal

Subkingdom: Tracheobiota

Division: Magnoliophyta

Class: Magnoliopsida

Order: Sapindales

Family: Rutaceae

Genus: Glycosmis

Species: *Glycosmis pentaphylla*

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 (Tippayawat *et al.,* 2016). 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 (Song *et al.,* 2007). 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 (Saxon *et al.*, 2014).

Anti-bacterial activity

Anti-bacterials 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 *Glycosmis pentaphylla* (pannal) and *Macaranga peltata* (vatta), 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 *Macranga peltate* and *Glycosmis pentaphylla*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.

Extraction method

Leaf extract is prepared with 10 g of fresh leaves (*Maranga peltate* and *Glycosmis pentaphylla*) 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 (*Macranga peltata*, *Glycosmis pentaphylla*, *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 (*Macranga peltata*, *Glycosmis pentaphylla*, *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 rasteringa 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 *Glycosmis pentaphylla* and *Macranga peltate.*

Silver nanoparticles

Silver nanoparticles were synthesized from leaf extracts of different plants (*Glycosmis pentaphylla* and *Macranga peltata*). 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 (*Glycosmis pentaphylla* and *Macranga peltata*). 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 (*Glycosmis pentaphylla, Macranga peltata, Emilia sonchifolia, Clerodendrum infortunatum, 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.

Table.1 Different vernacular names of *Macaranga peltata* around the globe and India.

Language	Names
Scientific names	*Macaranga peltata*
Name in various global languages	
French	
German	
English	Macaranga
Name in various Indian languages	
Sanskrit	Pat kenda
Hindi	Chanda
Urdu	
Marathi	Chanda
Kannada	Batlachamdrike
Gujarati	
Malayalam	Vatta
Tamil	Vattakkanni

Table.2 Different vernacular names of *Glycosmis pentaphylla* around the globe and India.

Language	Names
Scientific names	*Glycosmis pentaphylla*
Name in various global languages	
French	
German	Mangopfaume
English	Orangeberry/Gin berry
Name in various Indian languages	
Sanskrit	Ashbashakota
Hindi	Ban nimbu
Urdu	
Marathi	Kirmira
Kannada	Guruvade
Gujarati	
Malayalam	Pannal
Tamil	Kattukonchi

Table.3 Zone of inhibition against various bacteria (*E. coli*, *Klebsiella species*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococus aureus*) using nanoparticles produced by*Glycosmis pentaphylla* leaves.

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	1.3	1.4	1.5
	Zinc	1.2	1.0	1.8	2.1	2.3
Klebsiella species	Silver	0.9	0.9	1.0	1.1	1.2
	Copper	1.0	0.9	1.2	1.3	1.4
	Zinc	1.7	0.9	1.9	2.1	2.5
Pseudomonas aeruginosa	Silver	1.0	1.0	1.1	1.2	1.3
	Copper	1.0	1.0	1.1	1.2	1.3
	Zinc	1.3	1.0	1.4	1.5	1.6
Salmonella typhi	Silver	1.0	1.0	1.1	1.2	1.3
	Copper	1.2	1.0	1.4	1.6	2.0
	Zinc	1.6	1.0	1.8	2.0	2.3
Staphylococcus aureus	Silver	1.0	1.0	1.0	1.1	1.2
	Copper	1.2	1.0	1.4	1.5	1.7
	Zinc	2.1	1.0	2.3	2.7	3.0

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

Microorganism	Nanoparticles	Control	Sample	Measure of zone of inhibition in cm		
				20	40	60
E.coli	Silver	1.1	0.9	1.2	1.3	1.4
	Copper	0.9	0.9	1.0	1.1	1.2
	Zinc	0.9	0.9	1.0	1.1	1.2
Klebsiella species	Silver	1.0	0.9	1.1	1.2	1.3
	Copper	0.9	0.9	1.0	1.1	1.2
	Zinc	0.9	0.9	1.0	1.1	1.2
Pseudomonas aeruginosa	Silver	1.1	1.1	1.2	1.4	1.6
	Copper	2.8	1.0	3.0	3.2	3.6
	Zinc	1.0	1.0	1.1	1.2	13
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	1.0	1.1	1.2
Staphylococcus aureus	Silver	1.1	1.0	1.3	1.4	1.5
	Copper	1.2	1.0	1.4	1.6	2.0
	Zinc	1.0	1.0	1.2	1.4	1.6

Table.5 UV absorption spectrum of Silver nanoparticles formed from *Glycosmis pentaphylla* during different time of incubation.

Time	385 nm	435nm	560nm	680nm
½ hr	1.085	1.364	0.535	0.283
1 hr	1.786	1.980	1.216	0.621
1 ½ hr	2.148	2.492	1.342	0.654
2 hr	2.488	2.867	1.432	0.698
2 ½ hr	2.684	3.010	1.841	0.885
Blank	0.431	0.365	0.243	0.128

Table.6 UV absorption spectrum of Copper nanoparticles formed from *Glycosmis pentaphylla* during different time of incubation.

Time	385 nm	435nm	560nm	680nm
½ hr	0.456	0.317	0.182	0.621
1 hr	0.520	0.320	0.189	0.596
1 ½ hr	0.568	0.345	0.209	0.633
2 hr	0.730	0.449	0.267	0.678
2 ½ hr	0.792	0.498	0.288	0.698
Blank	0.037	0.035	0.047	0.470

Table.7 UV absorption spectrum of Zinc nanoparticles formed from *Glycosmis pentaphylla* during different time of incubation.

Time	350 nm	385 nm	435 nm	560 nm
½ hr	0.454	0.247	0.100	0.043
1 hr	0.510	0.306	0.156	0.078
1 ½ hr	0.564	0.304	0.169	0.080
2 hr	0.569	0.319	0.150	0.069
2 ½ hr	0.504	0.284	0.130	0.062
Blank	0.000	0.000	0.000	0.000

Table.8 UV absorption spectrum of Silver nanoparticles formed from *Macaranga peltata* during different time of incubation.

Time	385 nm	435nm	560nm	680nm
½ hr	0.540	0.579	0.420	0.303
1 hr	0.547	0.581	0.443	0.309
1 ½ hr	0.585	0.592	0.446	0.312
2 hr	0.589	0.611	0.453	0.337
2 ½ hr	0.590	0.643	0.469	0.350
Blank	0.000	0.000	0.000	0.000

Table.9 UV absorption spectrum of Copper nanoparticles formed from *Macaranga peltata* during different time of incubation.

Time	385 nm	435nm	560nm	680nm
½ hr	0.133	0.124	0.071	0.463
1 hr	0.172	0.131	0.110	0.510
1 ½ hr	0.208	0.147	0.119	0.516
2 hr	0.229	0.159	0.122	0.519
2 ½ hr	0.235	0.163	0.124	0.523
Blank	0.000	0.000	0.000	0.000

Table.10 UV absorption spectrum of Zinc nanoparticles formed from *Macaranga peltata* during different time of incubation.

Time	350 nm	385 nm	435 nm	560 nm
½ hr	0.126	0.094	0.070	0.039
1 hr	0.134	0.099	0.073	0.047
1 ½ hr	0.142	0.104	0.076	0.049
2 hr	0.149	0.110	0.088	0.073
2 ½ hr	0.220	0.167	0.124	0.086
Blank	0.000	0.000	0.000	0.000

Table.11 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.12 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.1 Map of Kerala showing the various sample collection points.

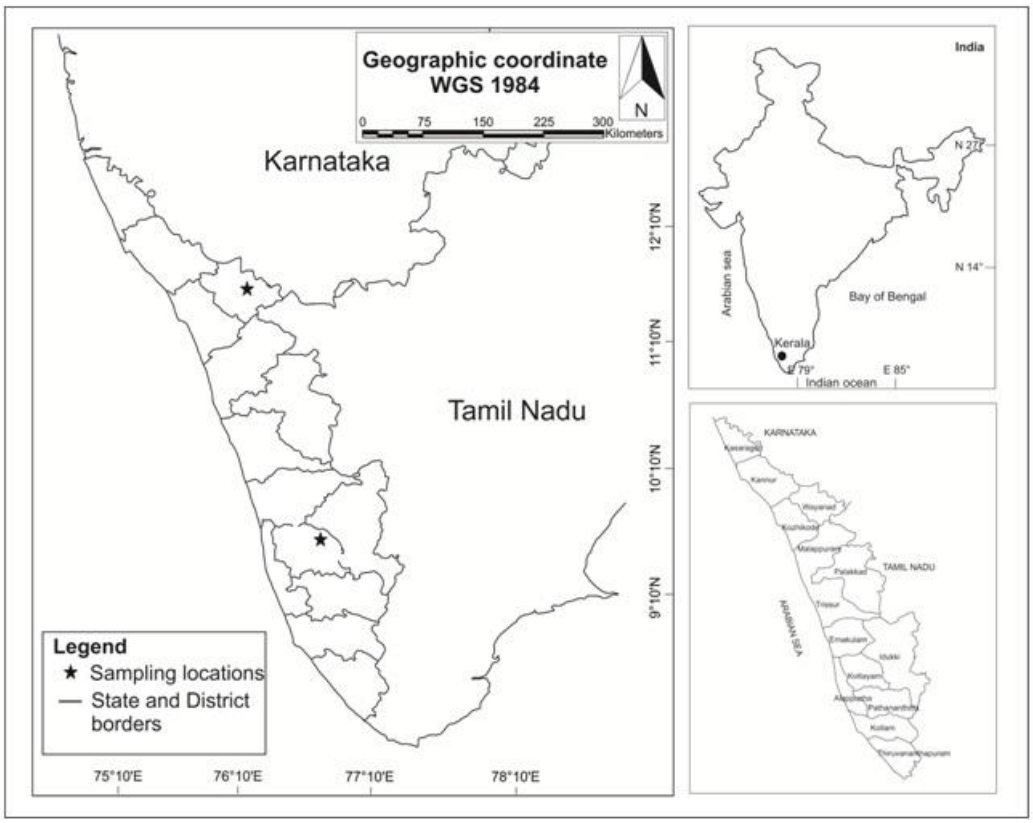


Fig.2 Description of *Macaranga peltata* a) leaves, b) leaf nodes, c) tree with flowers, d) fruits, e) young and mature leaves. Photo courtesy: Wikipedia.

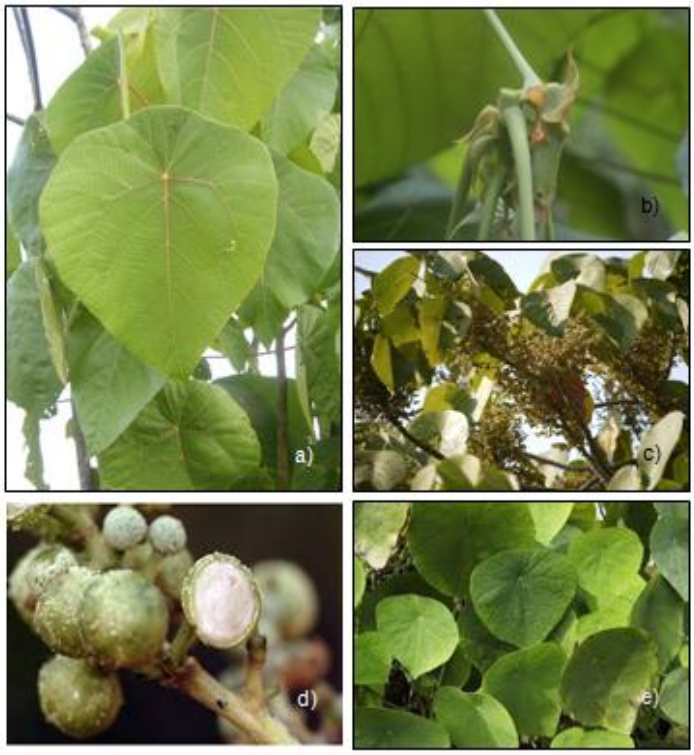


Fig.3 Description of *Glycosmis pentaphylla* a) tree in natural habitat, b) fully developed berries, c) partially mature berries, d) developing flower inflorescence. Photo courtesy: Wikipedia.

Fig.4 Description of *Clerodendrum infortunatum* a) plant in natural habitat, b) flower inflorescence, c) and d) flowers, e) [illegible] ipedia.

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 *Glycosmis pentaphylla* 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.

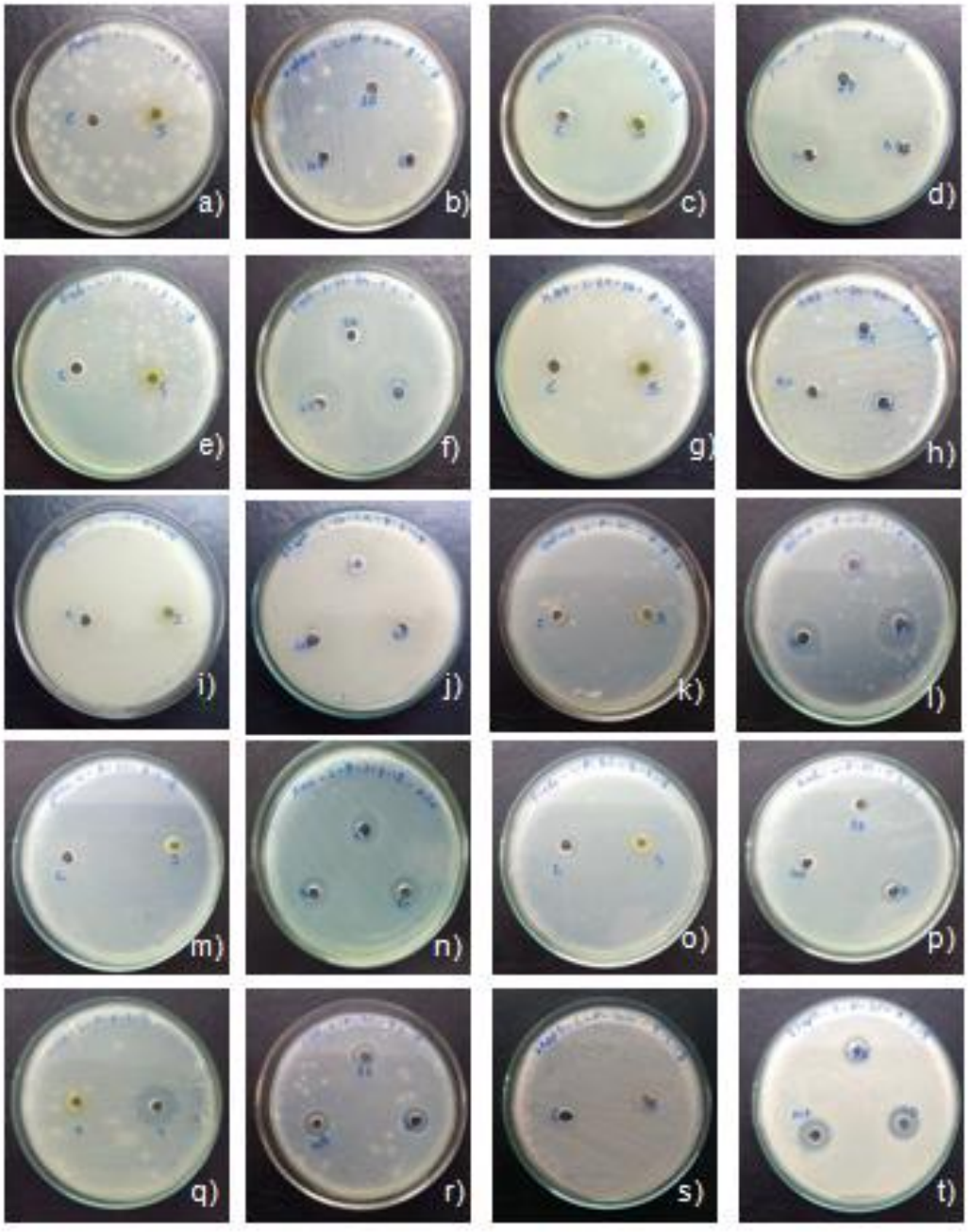


Fig.7 Antibacterial activity study using well diffusion method of *Glycosmis pentaphylla* and *Clerodendrum infortunatum* leaf extract nanoparticles (Zn) a) *Salmonella typhi* control plate (Zn), b) *Salmonella typhi* green synthesised nanoparticle test plate (Zn; 20, 40 and 60 µl), c) *Pseudomonas aeruginosa* 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*).

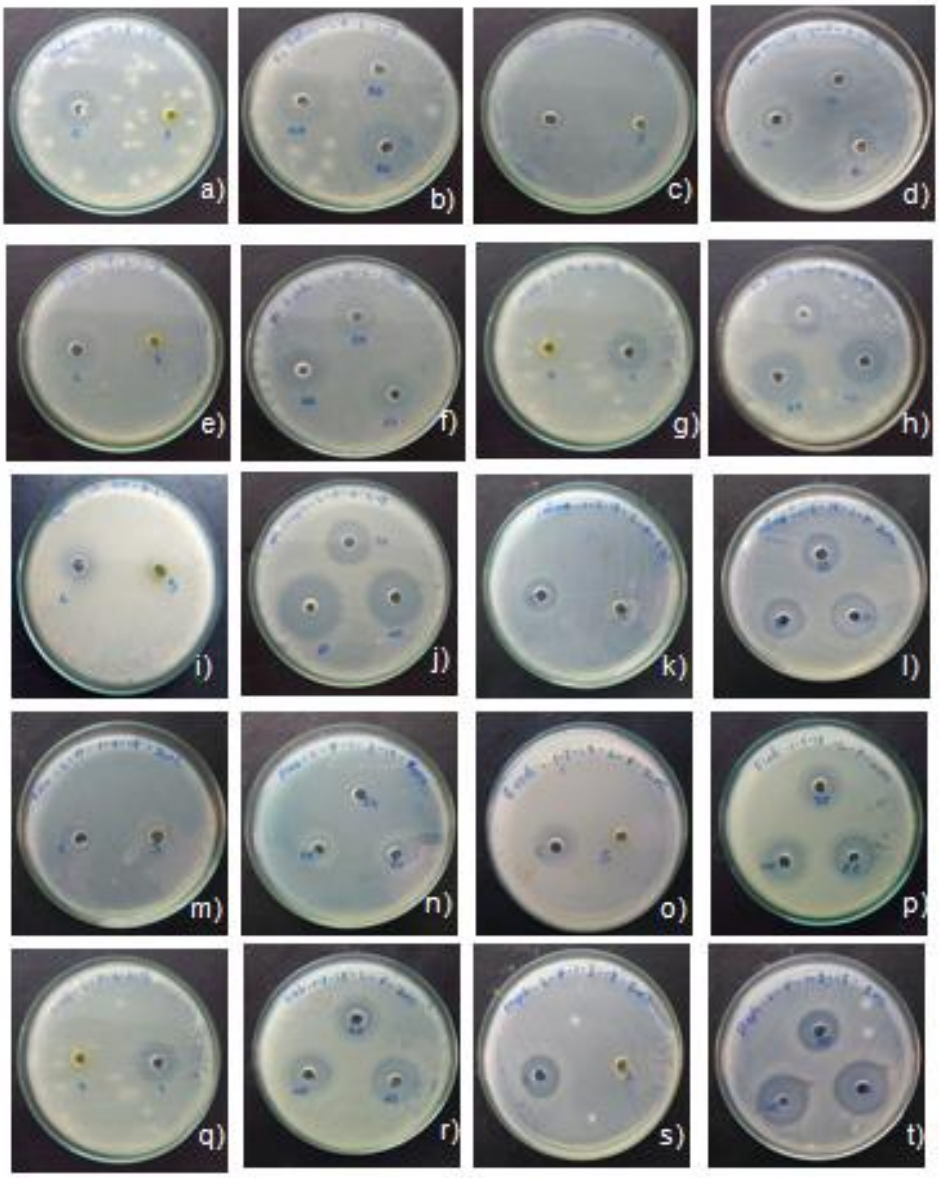


Fig.8 Antibacterial activity study using well diffusion method of *Macaranga peltata* leaf extract nanoparticles (Cu), (Ag) and (Zn)a) to e) control plates of *Salmonella typhi*, *Pseudomonas aeruginosa*, *E. coli*, *Klebsiella species and Staphylococcus species*, f) to t) above mentioned organisms plate in same order for green synthesised nanoparticles Cu, Ag and Zn (20, 40 and 60 µl).

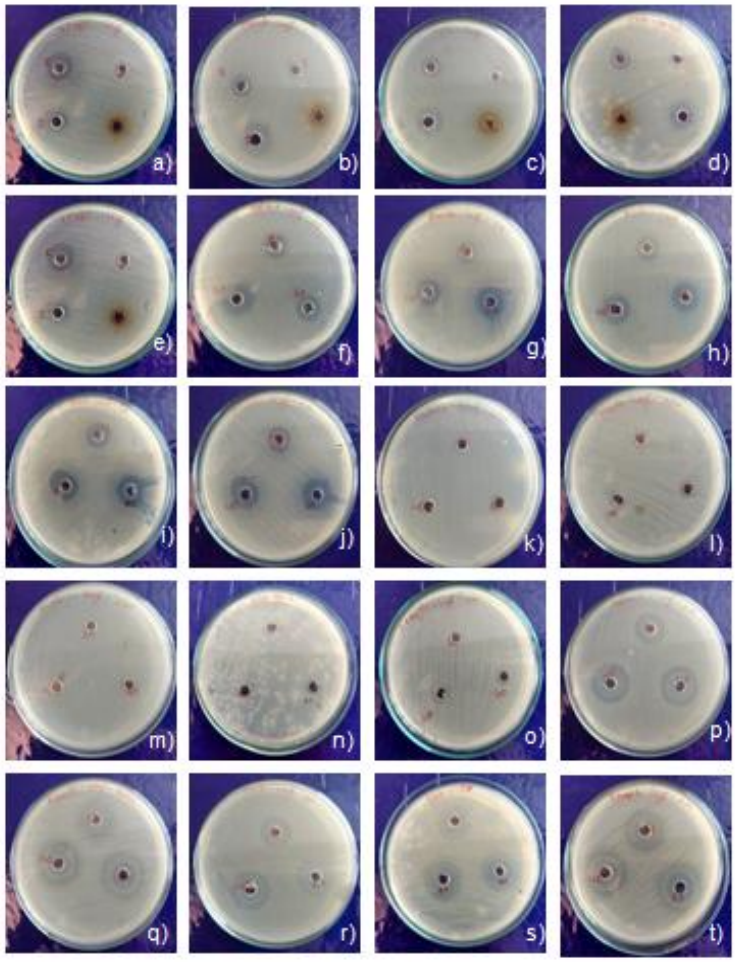


Fig.9 Silver nanoparticle formation of *Glycosmis pentaphylla* leaf extract under SEM imaging system with various resolutions.

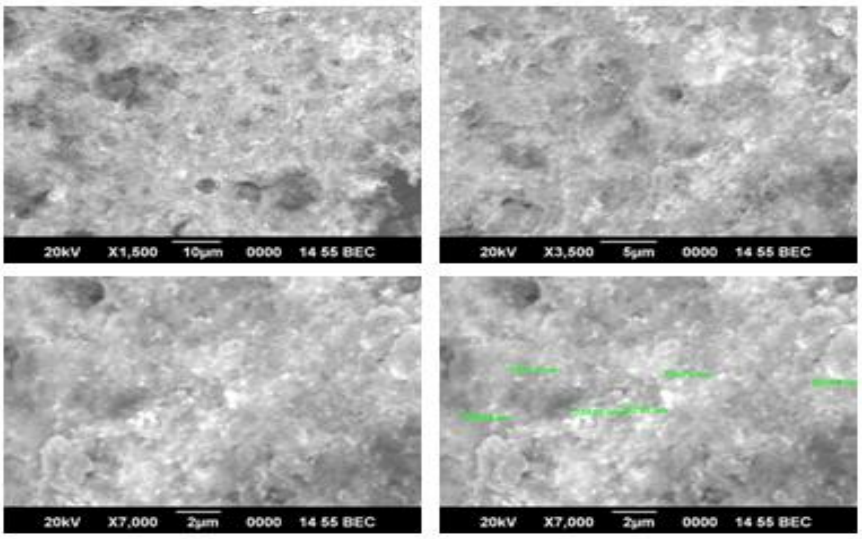


Fig.10 Copper nanoparticle formation of *Glycosmis pentaphylla* leaf extract under SEM imaging system with various resolutions.

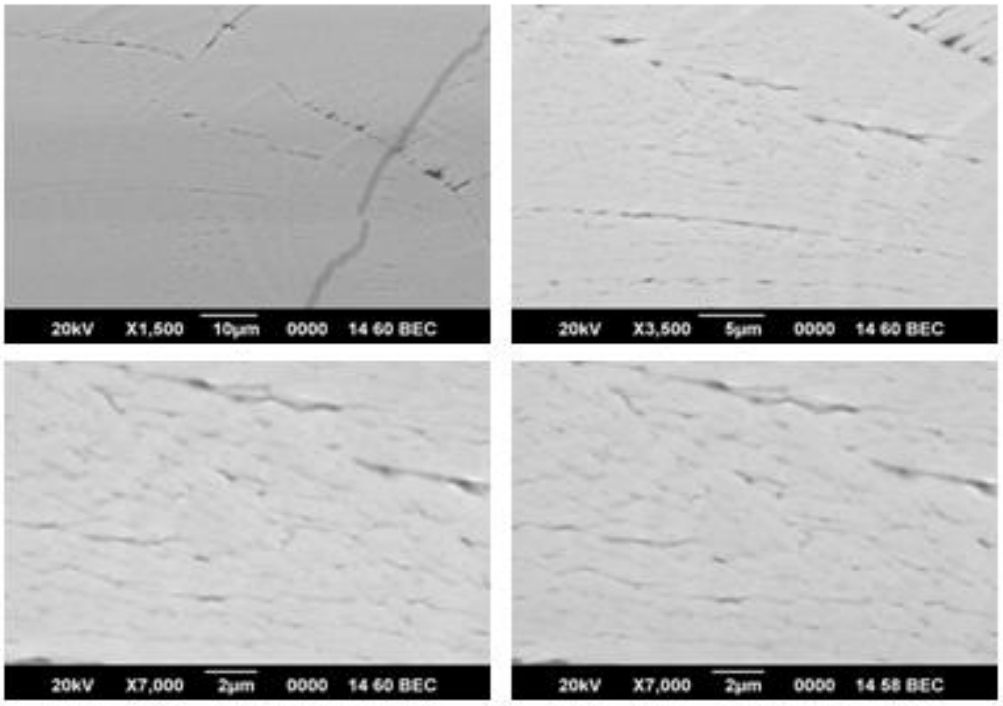


Fig.11 Zinc nanoparticle formation of *Glycosmis pentaphylla* leaf extract under SEM imaging system with various resolutions.

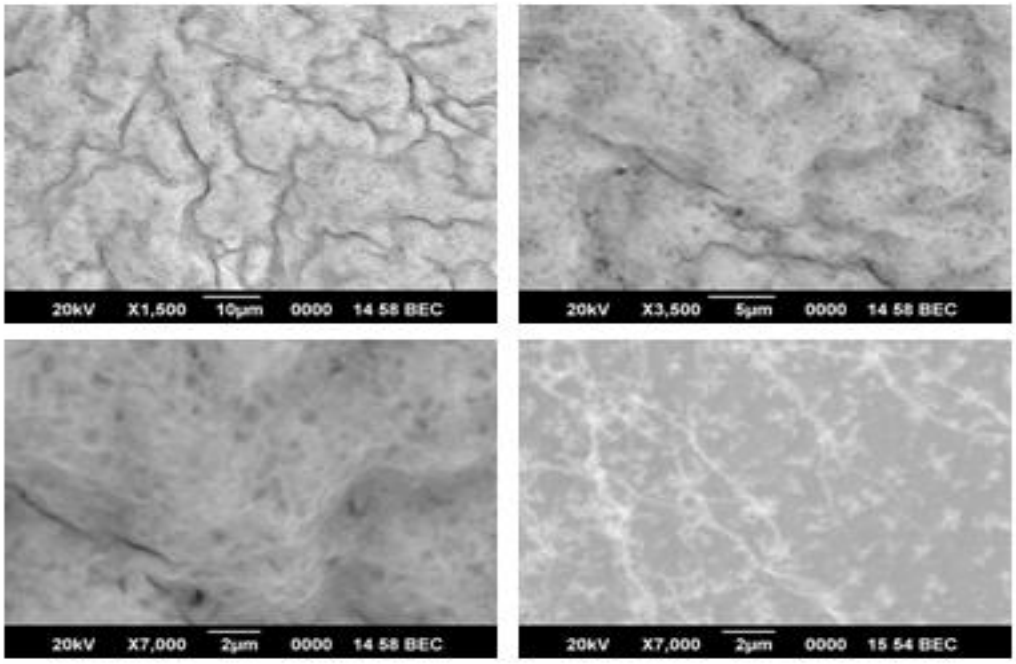
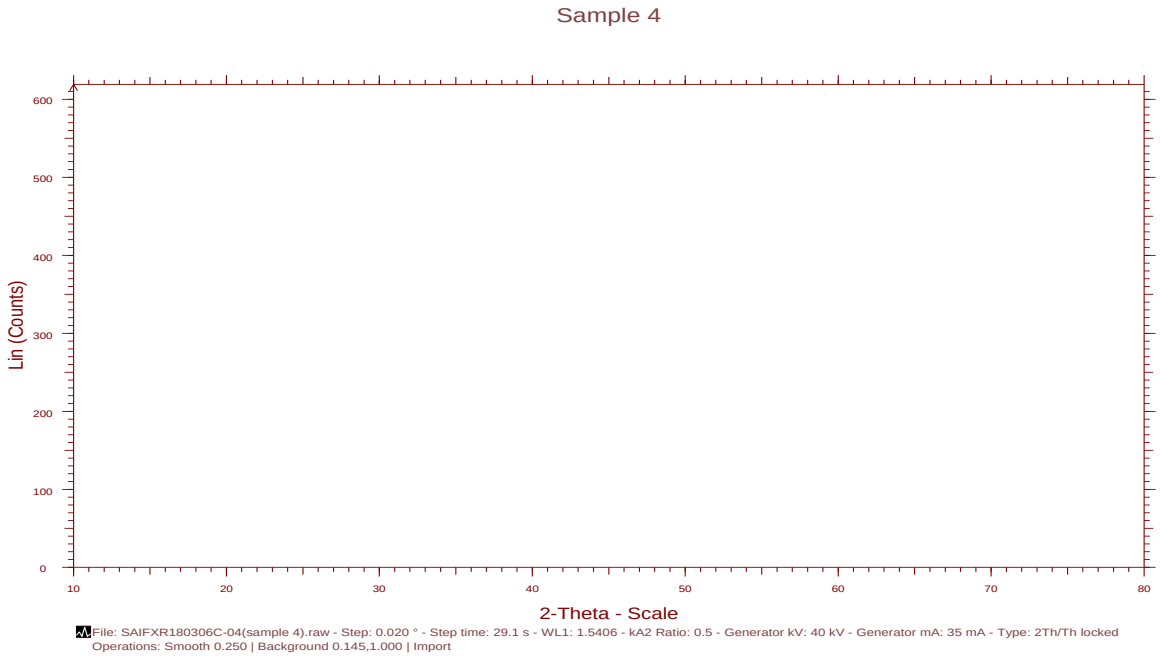


Fig.12 Silver nanoparticle formation of *Glycosmis pentaphylla* leaf extract under XRD imaging system.



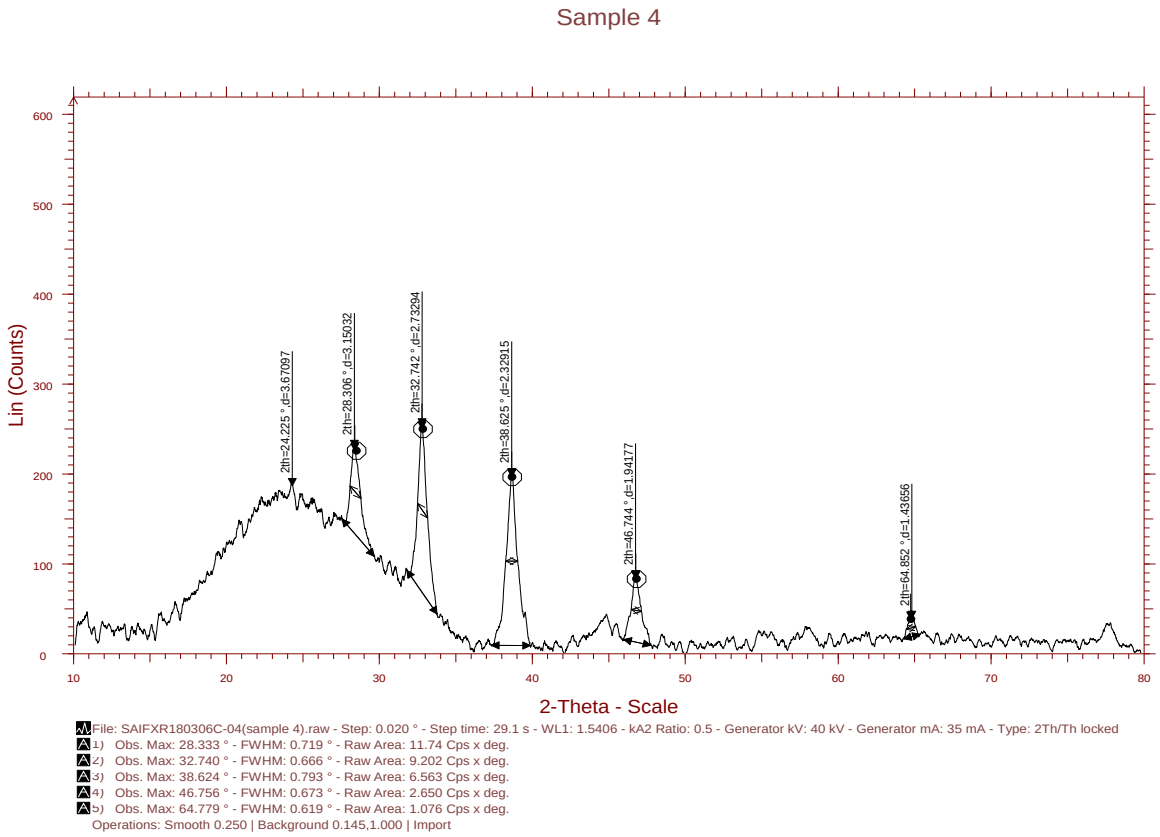
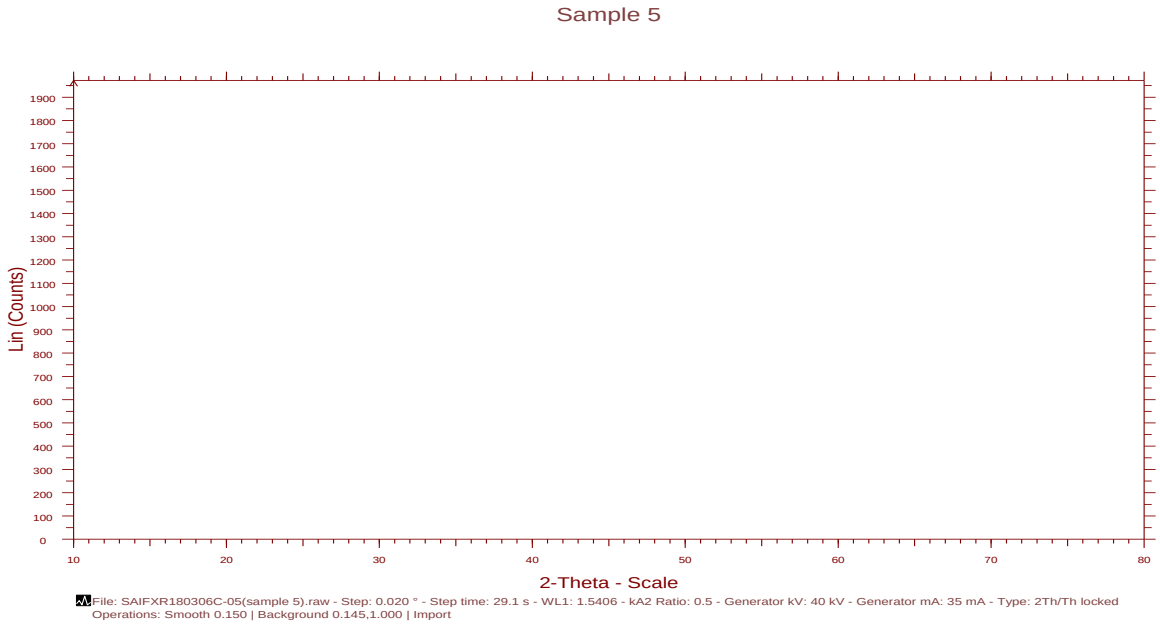


Fig.13 Copper nanoparticle formation of *Glycosmis pentaphylla* leaf extract under XRD imaging system.



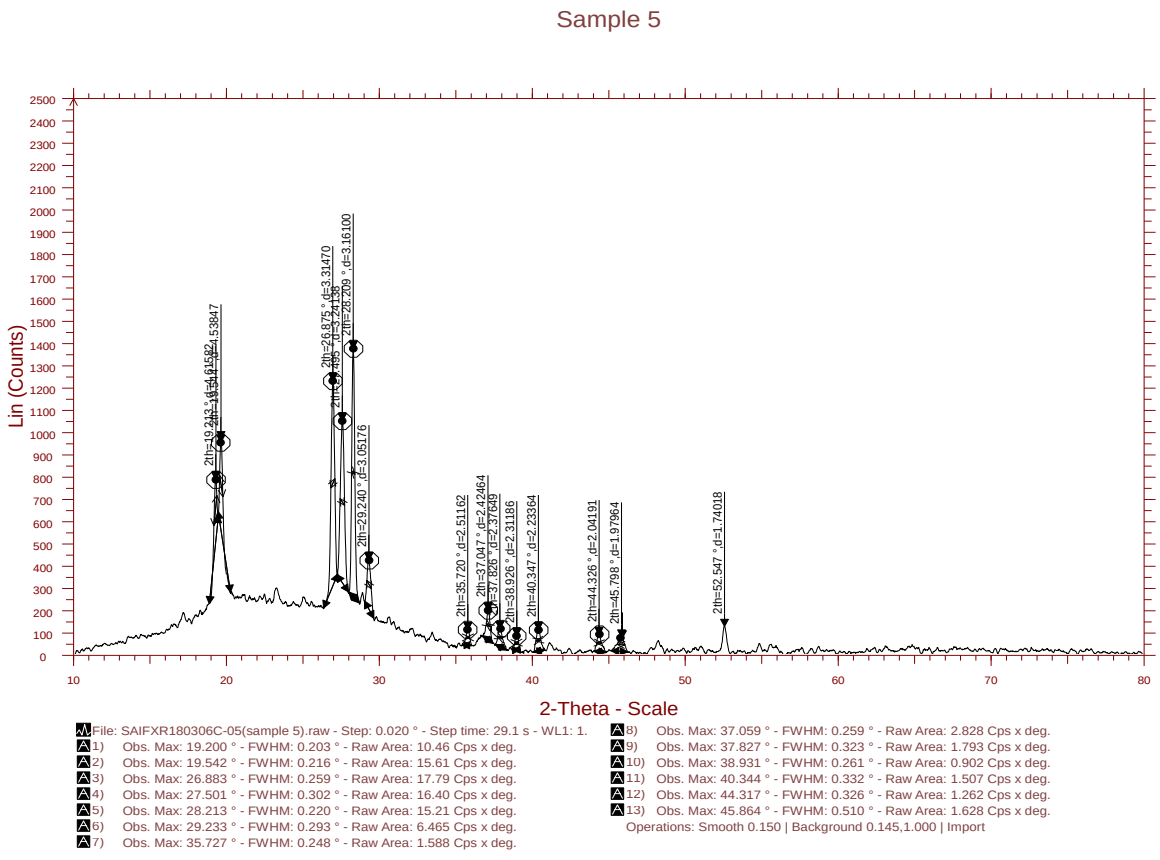
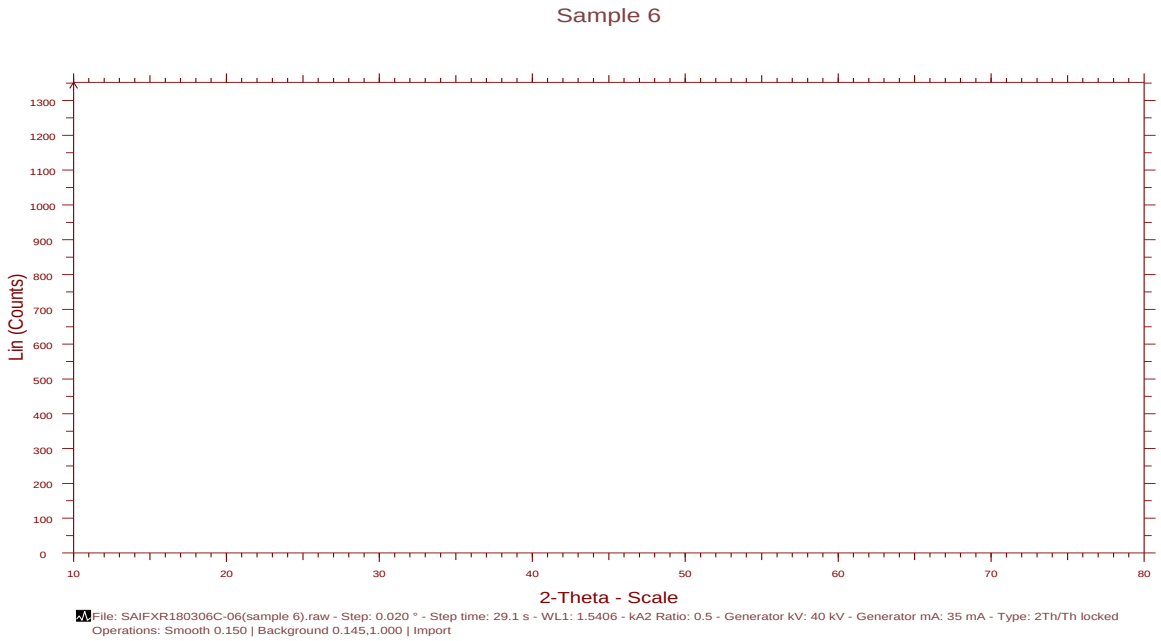
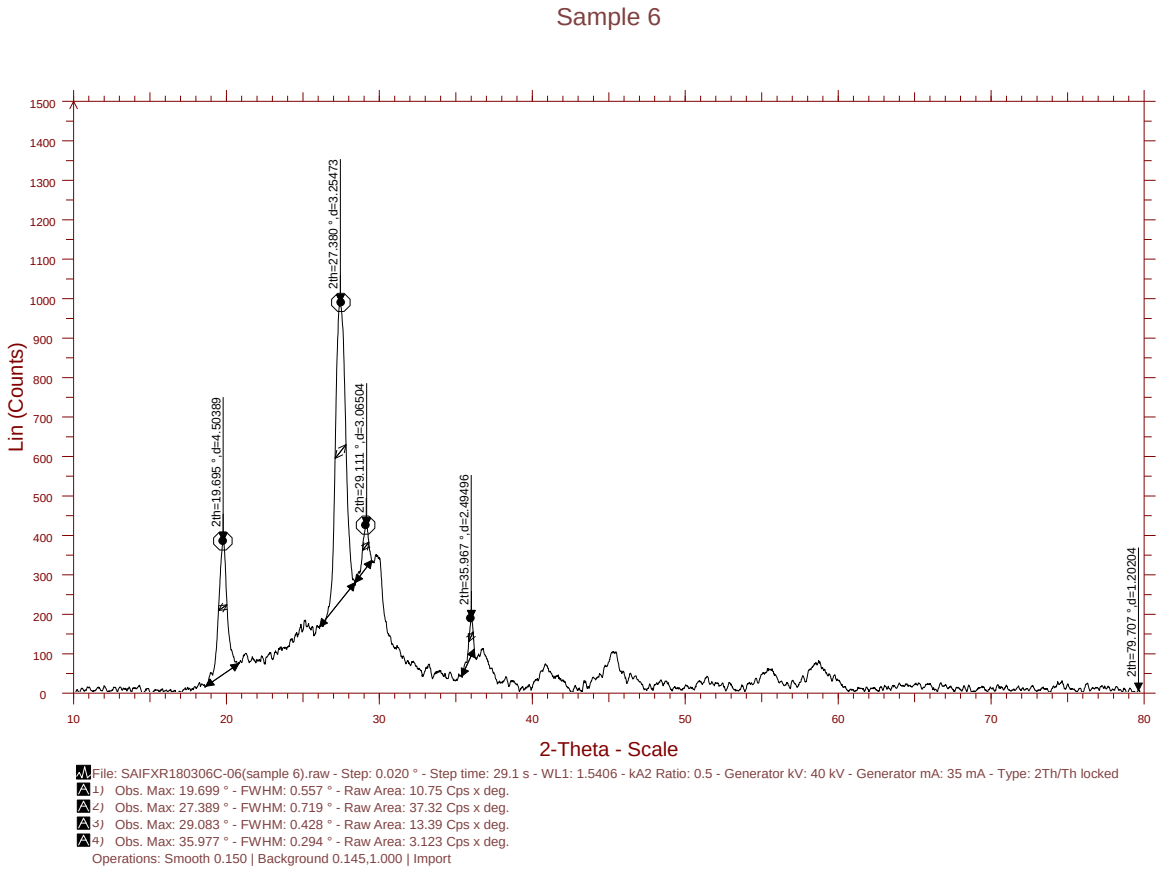


Fig.14 Zinc nanoparticle formation of *Glycosmis pentaphylla* leaf extract under XRD imaging system.





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 *Macrangapeltate*. 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 *Glycosmis pentaphylla* and *Macranga* peltate. 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 *Glycosmis pentaphylla* and *Macranga* peltate. The intensity of peak at 350 nm was increased with time until the reduction completes.

Antibacterial assay

The leaf extract of *Glycosmis pentaphylla* and *Macranga peltate* showed growth inhibitory effects against *salmonella, pseudomonas, staphylococcus, E.coli* and *Klebsiella.*

Glycosmis pentaphylla

Zinc nanoparticles showed more anti-bacterial activity rather than copper and silver nanoparticles respectively in *E.coli*. In Klebsiella the Zinc nanoparticles itself shows comparative activity in correspondence to sample Nano particles. Higher zone zinc Nanoparticles samples showed nearly doubled values in zone of inhibition than copper and silver respectively.

Pseudomonas resembled *E.coli* inhibition range by a slight variation in 60µl nanoparticles of zinc, copper and silver. Salmonella shows intermediate effect for copper nanoparticles, with zinc showing the highest and silver the lowest zone of inhibition.

Staphylococcus showed highest antibacterial activity for zinc sample nanoparticles other than activity in the other four test organism with increase in effectively from silver nanoparticles to copper nanoparticles.

Macranga peltata

Anti-bacterial activity of *E.coli* and Klebsiella shows similar range zone of inhibition. In *Pseudomonas* copper Nano particles shows steep increase in anti-bacterial activity with a zone of inhibition of 3.6 cm of higher zone taken. *Salmonella* and *Staphylococcus* resembles action with *E.coli* with copper nanoparticles being the major anti-bacterial agent.

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 µlfor all the bacterial cultures. It indicates that zone of inhibition increases as the concentration of nanoparticles increased.

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 *Glycosmis pentaphylla* and *Macranga peltata,* 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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