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Green Synthesis of Silver, Copper and Zinc Nanoparticles from *Passiflora edulis*, *Cycas circinalis* and *Pouteria campechiana* Seeds and Evaluation of their Antibacterial Activity

Prem Jose Vazhacharickal* and Jismon Thomas

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

**Corresponding author*

Abstract

Nanotechnology is an emerging field of science. It has increased applications in diverse area for the development of new materials at nanoscale levels. Synthesis of nanoparticles using biological methods is referred as greener synthesis of nanoparticles. Seed extracts of Passion fruit (*Passiflora edulis*), Eenth (*Cycas circinalis*) and Egg fruit (*Pouteria campechiana*) are used for the synthesis of silver, copper, and zinc nanoparticles. These plants have medicinal as well as antibacterial activity. Nanoparticles prepared from these seed extracts have antibacterial activity. Synthesized nanoparticles were characterized by UV-VIS Spectrophotometry. Silver nanoparticles shows maximum peak at 385 nm. Copper nanoparticles shows maximum peak at 680 nm. Zinc nanoparticles shows maximum peak at 350 nm. Synthesized silver, copper and zinc nanoparticles shows antibacterial activity against *Salmonella species*, *Pseudomonas species*, *Staphylococcus species*, *E. coli* and *Klebsiella species*. Antimicrobial assay was performed by agar well diffusion method using Muller Hinton agar media. when antibacterial activity of silver, copper and zinc nanoparticles from 3 different concentrations were observed, nanoparticles have 60 µl concentration shows maximum activity against these microbes. Silver nanoparticles shows greater antibacterial activity compared to silver nitrate and seed extract. Copper nanoparticles shows greater antibacterial activity compared to copper Sulphate and seed extract. Zinc nanoparticles shows greater antibacterial activity compared to zinc Sulphate and seed extract. Maximum zone of inhibition was at 60 µl for all the bacterial cultures. This green synthesis method is alternative to chemical method, since it is cheap, pollutant free and eco-friendly.

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Keywords

Nanoparticles; *Passiflora edulis*; *Cycas Circinalis; Pouteria campechiana*; Antimicrobial assay; Muller Hinton agar.

Introduction

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).

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).

Seed extracts of Passion fruit (*Passiflora edulis*), Eenth (*Cycas circinalis*), Egg fruit (*Pouteria campechiana*) are used for the synthesis of silver, copper, and zinc nanoparticles. These plants have medicinal as well as antibacterial activity (Hernandez *et al.*, 2008; Prabu *et al.*, 2015; Vijaymeena *et al.*, 2013; Jageessar *et al.*, 2017; Peter *et al.*, 2014). Nanoparticles prepared from these seed extracts have antibacterial activity (Paul *et al.*, 2015; Showmya *et al.*, 2012).

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 tumor 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).

The main objectives of this study Synthesis of silver, copper and zinc nanoparticles using seed extract of three different plants (canistel, Eenth, passion fruit) determine the antibacterial properties of these nanoparticles against *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi* and *Klebsiella pneumoniae*.

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.

Taxonomical classification (Passion fruit)

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

Subkingdom: Tracheobiota

Superdivision: Spermatophyta

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Dileniidae

Order: Violales

Family: Passifloraceae

Genus: Passiflora L.

Species: *Passiflora edulis*

Taxonomical classification (Canistel; egg fruit)

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

Subkingdom: Tracheobiota

Super division: Spermatophyta

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Dilleniidae

Order: Ebenales

Family: Sapotaceae

Genus: Pouteria aubi.

Species: *Pouteria campechiana*

Taxonomical classification (*Cycas circinalis*; eenth)

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

Subkingdom: Tracheobiota

Superdivision: Spermatophyta

Division: Cycadophyta

Class: Cycadopsida

Order: Cycadales

Family: Cycadaceae

Genus: Cycas L.

Species: *Cycas circinalis* L.

Review of literature

Seed extracts of Passion fruit (*Passiflora edulis*), Eenth (*Cycas circinalis*), Egg fruit (*Pouteria campechiana*) are used for the synthesis of silver, copper, and zinc nanoparticles. These plants have medicinal as well as antibacterial activity (Hernandez *et al.*, 2008; Prabu *et al.*, 2015; Vijaymeena *et al.*, 2013; Jageessar *et al.*, 2017; Peter *et al.*, 2014).

Nanoparticles prepared from these seed extracts have antibacterial activity (Paul *et al.*, 2015; Showmya *et al.*, 2012). A nanoparticle has lot of scope for health care products such as burn dressings, antimicrobial applications, medical devises and scaffolds (Mishra *et al.*, 2014).

Passion fruit belongs to the paccifloraceae family and is originally from the tropical America, featuring over 500 species worldwide. The seeds of passion fruit contain large amount of fiber and oil (Malacrida *et al.*, 2012).

Eenth (*Cycas circinalis*) is commonly known as Queen sago, a member of the cycad family. The ovule of Queen sago has three layers. The outermost layer Sarcotesta, the middle layer Sclerotesta and the inner most layer endotesta. Ovule of endotesta showed antibacterial activity (Kalpashree *et al.*, 2013).

Canistel (*Pouteria campechiana*) is also known as yellow sapote or egg-fruit, native to central America but distributed to tropical and subtropical areas, which belongs to sapotaceae family. Dietary phytochemicals are found in its seeds (Mehraj *et al.*, 2015).

Seed extracts of Passion fruit (*Passiflora edulis*), Eenth (*Cycas circinalis*) and Egg fruit (*Pouteria campechiana*) are used for the synthesis of silver, copper, and zinc nanoparticles. Copper nanoparticles widely used due to their superior, optical, electrical, antifungal/antibacterial and biomedical applications. Copper nanoparticles have superior antibacterial activity as compared to silver nanoparticles. Because copper is highly toxic to microorganisms (Singh, 2017).

The antimicrobial activity mainly tested for drug discovery and prediction of therapeutic outcome. Agar disc diffusion and agar well diffusion are two methods used to evaluate antimicrobial activity (Balouiri *et al.*, 2016).

Feng *et al.*, (2000) conducted a study to observe the effects of silver ions on gram-positive (*Staphylococcus aureus*) and gram-negative bacteria (*Escherichia coli*). Under TEM they observed that cells exposed to the Ag^+ ions seemed to have activated a stress response that led to the condensation of DNA in the center of the cell. They also observed cell membrane detachment from the cell wall, cell wall damage, and electron dense granules outside and, in some instances, inside the cell. It was proposed that condensation of DNA occurred as a protective measure in order to protect the genetic information of the cell (Feng *et al.*, 2000), however condensation of DNA could also prevent cell replication by preventing the DNA from being accessed by transcriptional enzymes such as DNA polymerase. The electron dense granules that formed inside and outside the cell were extracted and subjected to X-ray microanalysis to determine their composition. It was found that the granules were in part composed of silver and sulfur. This finding supports the idea that silver inactivates proteins by binding to sulfur-containing compounds (Klueh *et al.,* 2000). It was also observed that when treated with Ag+, *E. coli*, a gram-negative bacterium, sustained more structural damages than the gram-positive *Staphylococcus aureus* (Feng *et al.,* 2000). It was also reported that treating cells with silver leads to cell shrinkage and dehydration (Guggenbichler *et al.,* 1999).

Studies shows that silver nanoparticles anchor to and penetrate the cell wall of Gram-negative bacteria (Morones *et al.,* 2005), it is reasonable to suggest that the resultant structural change in the cell membrane could cause an increase in cell permeability, leading to an uncontrolled transport through the cytoplasmic membrane, and ultimately cell death. It has also been proposed that the antibacterial mechanism of silver nanoparticles is related to the formation of free radicals and subsequent free radical-induced membrane damage (Danilczuk *et al.,* 2006; Kim *et al.,* 2007).

Novel wound dressings have been developed that use silver to help prevent wound infections (Joshua *et al.,* 2008). Silver nanoparticles are incorporated into the wound dressing, and the silver-enhanced wound dressings were found in vitro to consistently kill *Pseudomonas aeruginosa* cultures entirely and kill *Staphylococcus aureus* cultures with >99.99% efficiency (Ong *et al.,* 2008). In mice, the silver-enhanced wound dressings were also found to reduce mortality from *Pseudomonas aeruginosa* wound infections from 90% to 14.3% (Ong *et al.,* 2008).

Studies revealed the antibacterial properties of surgical masks coated with silver nanoparticles (Li *et al.,* 2006). Nanoparticle coated masks were capable of a 100% reduction in viable *Escherichia coli* and *Staphylococcus aureus* cells after incubation. Additionally, the study reported no signs of skin irritation in any of the persons wearing the masks (Li *et al.,* 2006).

Silver nanoparticles have been used to impart antimicrobial activity to cotton fibres. Cotton samples were immersed in silver nanoparticle solutions and then subjected to a curing process to allow the nanoparticles to adhere to the cotton (El-Rafie *et al.,* 2010). A chemical binder was then applied to the fabric to help maintain nanoparticle-cotton binding. Cotton samples prepared in this manner were able to reduce *Staphylococcus aureus* and *Escherichia coli* cell counts by 97% and 91% respectively. Even after subjecting the fabric to 20 laundry cycles, the cotton samples were still able to reduce *Staphylococcus aureus* and *Escherichia coli* cell counts by 94% and 85% respectively. Cotton prepared in this manner could be used by individuals working in the medical field or those who often work with microbes to prevent the spread of infectious bacteria (El-Rafie *et al.,* 2010).

In the past few decades, researchers are taking interest in the development of textile fabrics containing antibacterial agents. As, silver is non-toxic and posses antimicrobial properties it has encouraged workers to use silver nanoparticles in different textile fabrics. In this direction, silver nanocomposite fibres were prepared containing silver nanoparticles incorporated inside the fabric but from the scanning electron microscopic study it was concluded that the silver nanoparticles incorporated in the sheath part of fabrics possessed significant antibacterial property compared to the fabrics incorporated with silver nanoparticles in the core part (Yeo and Jeong, 2003).

Toxicity from silver is observed in the form of argyria, only when there is a large open wound and large amount of silver ions are used for dressing. There are no regular reports of silver allergy (Leaper, 2006). Silver nanoparticles in most studies are suggested to be non-toxic. But due to their small size and variable properties they are suggested to be hazardous to the environment (Braydich-Stolle *et al.,* 2005). Hussain *et al.,* (2005) studied the toxicity of different sizes of silver nanoparticles on rat liver cell line (BRL 3A) (ATCC, CRL-1442immortalized rat liver cells). The authors found that after an exposure of 24 hour the mitochondrial cells displayed abnormal size, cellular shrinkage and irregular shape. Cytotoxicity study of silver nanoparticle impregnated five commercially available dressings was undertaken by Burd *et al.,* (2007). In the study, it was found that three of the silver dressings depicted cytotoxicity effects in keratinocytes and fibroblast cultures. Braydich-Stolle *et al.,* (2005) reported the toxicity of silver nanoparticles on C18-4 cell, a cell line with spermatogonial stem cell characteristics. From the study, it was concluded that the cytotoxicity of silver nanoparticles to the mitochondrial activity increased with the increase in the concentration of silver nanoparticles.

Silver has been known to possess strong antimicrobial properties both in its metallic and nanoparticle forms hence; it has found variety of application in different fields. The Fe_3O_4 attached Ag nanoparticles can be used for the treatment of water and easily removed using magnetic field to avoid contamination of the environment (Gong *et al.,* 2007). Silver sulfadazine depicts better healing of burn wounds due to its slow and steady reaction with serum and other body fluids (Fox and Modak, 1974). The nanocrystalline silver dressings, creams and gels effectively reduce bacterial infections in chronic wounds (Richard *et al.,* 2002; Leaper, 2006; Ip*et al.,* 2006).

The silver nanoparticle containing poly vinyl nano-fibers also show efficient antibacterial property as wound dressing (Jun *et al.,* 2007). The silver nanoparticles are reported to show better wound healing capacity, better cosmetic appearance and scarless healing when tested using an animal model (Tian *et al.,* 2006). Silver impregnated medical devices like surgical masks and implantable devices show significant antimicrobial efficacy (Furno*et al.,* 2004). Environmental-friendly antimicrobial nanopaint can be developed (Kumar *et al.,* 2008). Inorganic composites are used as preservatives in various products (Gupta and Silver, 1998). Silica gel micro-spheres mixed with silica thio-sulfate are used for long lasting antibacterial activity (Gupta and Silver, 1998).Treatment of burns and various infections (Feng *et al.,* 2000). Silver zeolite is used in food preservation, disinfection and decontamination of products (Matsuura *et al.,* 1997; Nikawa *et al.,* 1997). Silver nanoparticles can be used for water filtration (Jain and Pradeep, 2005).

Hypothesis

The current research work is based on the following hypothesis

Seeds of Passion fruit (*Passiflora edulis*), Eenth (*Cycas circinalis*), Egg fruit (*Pouteria campechiana*) could be used as antibacterial agents.

These seed extracts could be used in formulating nanoparticles (silver, copper and zinc) and their antibacterial activity 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

Seeds of passion fruit, Eenth and canistel were collected from Thankamany, Idukki district of Kerala state, India. The seeds were thoroughly cleaned using double distilled water. The samples 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

The seeds of Eenth, egg fruit and passion fruit are powdered. Then 10g of each powdered seed sample is dissolved in 50 ml distilled water, the contents are mixed thoroughly using a mortar and pestle and filtered using a filter paper, thus filtered solution is taken as the extract. The extract was then stored at 4°C after covering the beaker with aluminum foil for further use. The obtained seed extract which appeared light yellowish 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.10 ml of seed extract of different plants (Eenth, canistel and passion fruit separately) was added to 90 ml of 1mM $AgNO_3$ solution and allowed to react at room temperature.

Copper nanoparticles

Stock solution was prepared by dissolving 2.49 g Copper sulphate ($CuSO_4$) and volume made up to 100 ml with distilled water. 10 ml of seed extract of different plants (Eenth, canistel and passion fruit separately) was added to 90 ml of 100 mM $CuSO_4$ and allowed to react at room temperature.

Zinc nanoparticles

Stock solution was prepared by dissolving 2.87 g Zinc sulphate ($ZnSO_4$) and volume made up to 100 ml with distilled water. 10 ml of seed extract of different plants (papaya, Mullatha, Eenth, canistel and passion fruit separately) was added to 90 ml of 100 mM $ZnSO_4$solution and allowed to react at room temperature.

Test microorganisms

The organism used comprise of 4 gram-negative organisms (*E. coli, Klebsiella, Salmonella and pseudomonas*) and one gram-positive organism (*Staphylococcus*). 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. *Staphylococus species* 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.

Salmonella typhi

Salmonella typhi is a rod shaped flagellated gram negative organisms, that causes systemic infections and typhoid fever in humans.

Pseudomonas aeruginosa

Pseudomonas aeruginosa is a common gram negative, rod shaped bacterium that cause disease in plants and animals. It is an opportunistic human pathogen.

Characterization of nanoparticles

UV-Vis spectroscopy

The periodic scans of the optical absorbance between 345 and 700 nm with a UV- Vis spectrophotometer (Model 118, Systronics, Mumbai, India) at a resolution of 1 nm were performed to investigate the reduction rate of green synthesised nanoparticles. 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 (seed extract). The plates were incubated at 37^0C for 24 hours after which the diameter of zones of inhibition were measured.

Statistical analysis

The 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

Silver nanoparticles

To synthesize silver nanoparticles, seed extracts of different plants (Egg fruit, Eenth and Passion fruit separately) was added to 1mM silver nitrate solution and kept to reaction takes place. A colour change was observed from colourless to dark brown. This occurred due to the reduction of silver ions present in the solution. Synthesized silver nanoparticles were characterized by UV-VIS Spectrophotometry.

The maximum peak was found to be 435 nm (λ max) for *Pouteria campechiana* and *Cycas circinalis.* 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 (λ max) for *Carica papaya*, and *Passiflora edulis.* The intensity of the peak at 385 nm was increased with time until the reduction completes.

Copper nanoparticles

To synthesize copper nanoparticles, seed extracts of different plants (Egg-fruit, Eenth and Passion fruit separately) was added to 100 mM Copper sulphate solution and kept to reaction takes place. A color change was observed from blue to pale yellow.

This occurred due to the reduction of copper ions present in the solution. Synthesized copper nanoparticles were characterized by UV-VIS Spectrophotometry.

The maximum peak was found to be 680 nm (λ max) for *Pouteria campechiana*, *Cycas circinalis* and *Passiflora edulis.* The intensity of the peak at 680 nm was increased with time until the reduction completes.

Zinc nanoparticles

To synthesize zinc nanoparticles, seed extracts of different plants (Egg-fruit, Eenth and Passion fruit separately) was added to 100 mM zinc Sulphate solution and kept at room temperature for reaction takes place. A colour change was observed from colourless to pale brown. This occurred due to the reduction of zinc ions present in the solution. Synthesized zinc nanoparticles were characterized by UV-VIS Spectrophotometry. The maximum peak was found to be 350 nm (λ max) for *Pouteria campechiana*, *Cycas circinalis*, and *Passiflora edulis*. The intensity of the peak at 350 nm was increased with time until the reduction completes.

Antibacterial assay

Seed extracts of Egg-fruit, Eenth and Passion fruit showed growth inhibitory effects against *Salmonella typhi*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *E. coli* and *Klebsiella pneumoniae.*

For *Pouteria campechiana*, the zone of inhibition showed for *Salmonella* species, *Pseudomonas* species, *Staphylococcus* species, *E. coli* and *Klebsiella* species by silver nanoparticles formed from 20 µl concentration of nanoparticles were 10 mm, 12 mm, 15 mm, 11 mm & 11 mm respectively; from 40 µl concentration of nanoparticles were 11 mm, 16 mm, 18 mm, 12 mm & 12 mm respectively; from 60 µl concentration of nanoparticles were 12 mm, 20 mm, 21 mm, 13 mm & 13 mm respectively. The zone of inhibition showed by *Salmonella* species, *Pseudomonas* species, *Staphylococcus* species, *E. coli* and *Klebsiella* species by $AgNO_3$ solution was 10 mm, 11 mm, 14 mm, 12 mm & 10 mm respectively; seed extract was 10 mm, 9 mm, 11 mm, 11 mm & 9 mm respectively.

The zone of inhibition showed by *Salmonella* species, *Pseudomonas* species, *Staphylococcus* species, *E. coli* and *Klebsiella* species by copper nanoparticles formed from 20µl concentration of nanoparticles were 11 mm, 30 mm, 13 mm, 10 mm & 10 mm respectively; from 40 µl concentration of nanoparticles were 12 mm, 34 mm, 18 mm, 11 mm & 12 mm respectively; from 60 µl concentration of nanoparticles were 14 mm, 37 mm, 21 mm, 12 mm & 13 mm respectively. The zone of inhibition showed by *Salmonella* species, *Pseudomonas* species, *Staphylococcus* species, *E. coli* and *Klebsiella* species by CuSO4 solution was 9 mm, 28 mm, 12 mm, 10 mm & 10 mm respectively.

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

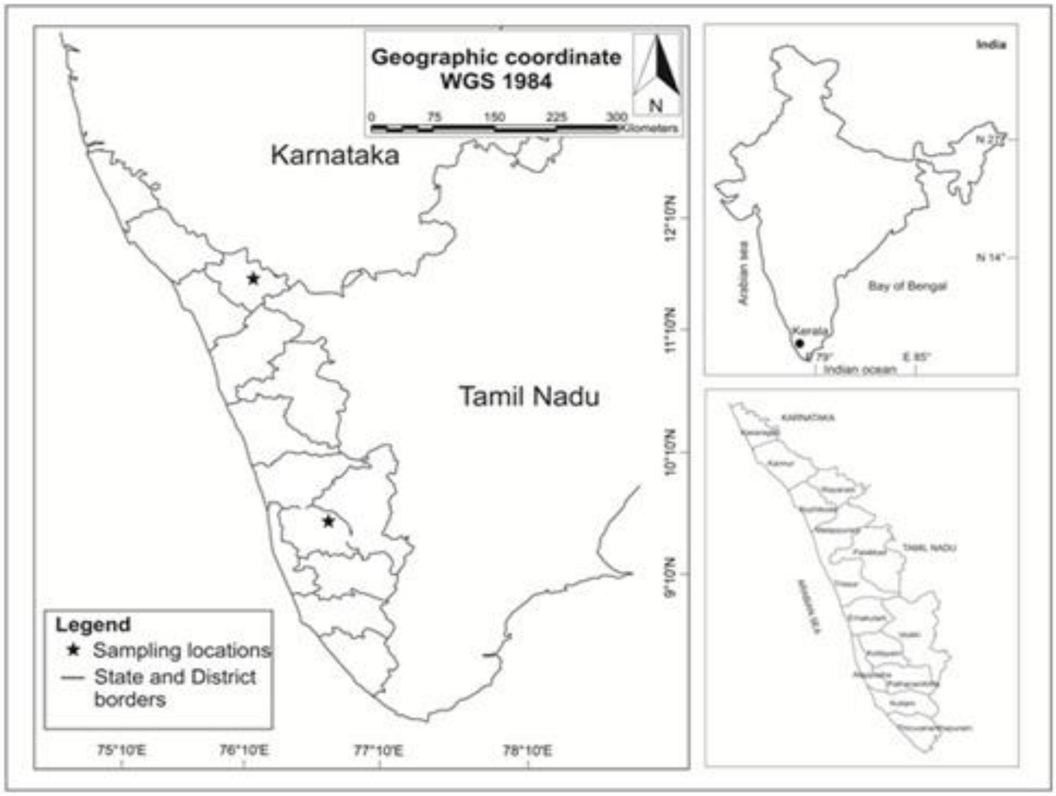


Table.1 Different vernacular names of Passion fruit (*Passiflora edulis*) around the globe and India.

Language	Names
Scientific names	*Passiflora edulis*
Name in various global languages	
French	Gouzou
German	Maracuja
English	Passion fruit
Name in various Indian languages	
Sanskrit	Mamataphala
Hindi	Krishna Fal
Urdu	
Marathi	
Kannada	
Gujarati	
Malayalam	
Tamil	Kotittotaippalam

Table.2 Different vernacular names of Egg fruit (*Pouteria campechiana*) around the globe and India.

Language	Names
Scientific names	*Pouteria campechiana*
Name in various global languages	
French	Jauned'oeuf
German	
English	Canistel
Name in various Indian languages	
Sanskrit	
Hindi	
Urdu	
Marathi	
Kannada	
Gujarati	
Malayalam	Muttappazham
Tamil	Muttaippazham

Table.3 Different vernacular names of Eenth (*Cycas circinalis*)around the globe and India.

Language	Names
Scientific names	*Cycas circinalis*
Name in various global languages	
French	Grand Rameau
German	
English	Queen Sago
Name in various Indian languages	
Sanskrit	Varaguna
Hindi	Jangli
Urdu	
Marathi	
Kannada	
Gujarati	
Malayalam	Eenthu
Tamil	EenthaPanai

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

Microorganism	Nanoparticles	Control	Sample	Measure of zone of inhibition in mm		
				20	40	60
E.coli	Silver	10	9	11	12	13
	Copper	10	9	12	16	17
	Zinc	19	11	19	22	23
Klebsiella species	Silver	11	10	12	15	16
	Copper	12	10	15	19	24
	Zinc	23	9	23	25	27
Pseudomonas aeruginosa	Silver	11	10	12	14	16
	Copper	10	9	19	28	32
	Zinc	21	12	25	28	29
Salmonella typhi	Silver	10	8	11	12	13
	Copper	10	9	11	12	13
	Zinc	18	10	20	22	24
Staphylococcus aureus	Silver	9	8	10	11	12
	Copper	9	8	11	12	13
	Zinc	12	10	13	21	23

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

Microorganism	Nanoparticles	Control	Sample	Measure of zone of inhibition in mm		
				20	40	60
E.coli	Silver	7	11	10	12	13
	Copper	13	12	15	18	21
	Zinc	17	12	20	22	25
Klebsiella species	Silver	10	8	10	11	12
	Copper	14	11	14	18	24
	Zinc	17	10	23	25	28
Pseudomonas aeruginosa	Silver	8	9	12	15	16
	Copper	10	8	13	14	17
	Zinc	11	9	11	14	16
Salmonella typhi	Silver	9	-	10	11	13
	Copper	15	9	14	17	21
	Zinc	18	12	21	24	26
Staphylococcus aureus	Silver	10	-	10	11	12
	Copper	20	13	24	27	30
	Zinc	20	9	22	26	30

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

Microorganism	Nanoparticles	Control	Sample	Measure of zone of inhibition in mm		
				20	40	60
E.coli	Silver	12	11	11	12	13
	Copper	10	9	10	11	12
	Zinc	12	9	13	16	18
Klebsiella species	Silver	10	9	11	12	13
	Copper	10	11	10	12	13
	Zinc	11	9	10	13	19
Pseudomonas aeruginosa	Silver	11	-	12	16	20
	Copper	28	14	30	34	37
	Zinc	10	9	11	12	13
Salmonella typhi	Silver	10	6	10	11	12
	Copper	9	10	11	12	14
	Zinc	11	10	10	11	13
Staphylococcus aureus	Silver	14	9	15	18	21
	Copper	12	10	13	18	21
	Zinc	10	11	11	12	13

Table.7 UV absorption spectrum of Silver nanoparticles formed from Egg fruit during different time of incubation.

Time	385 nm	435nm	560nm	680nm
½ hr	**1.074**	**1.350**	**0.530**	**0.272**
1 hr	**1.776**	**1.984**	**1.202**	**0.600**
1 ½ hr	**2.140**	**2.496**	**1.324**	**0.652**
2 hr	**2.488**	**2.872**	**1.420**	**0.698**
2 ½ hr	**2.672**	**3.100**	**1.812**	**0.884**
Blank	**0.414**	**0.354**	**0.208**	**0.108**

Table.8 UV absorption spectrum of *Copper nanoparticle* formed from Egg fruit during different time of incubation.

Time	385 nm	435nm	560nm	680nm
½ hr	**0.466**	**0.302**	**0.183**	**0.613**
1 hr	**0.515**	**0.302**	**0.188**	**0.597**
1 ½ hr	**0.565**	**0.341**	**0.205**	**0.624**
2 hr	**0.662**	**0.448**	**0.263**	**0.731**
2 ½ hr	**0.686**	**0.496**	**0.286**	**0.790**
Blank	**0.039**	**0.033**	**0.044**	**0.471**

Table.9 UV absorption spectrum of *Zinc nanoparticle* formed from Egg fruit during different time of incubation.

Time	350 nm	385 nm	435 nm	560 nm
½ hr	0.440	0.236	0.100	0.041
1 hr	0.502	0.301	0.155	0.075
1 ½ hr	0.554	0.314	0.161	0.077
2 hr	0.558	0.315	0.150	0.063
2 ½ hr	0.494	0.274	0.128	0.061
Blank	0.000	0.000	0.000	0.000

Table.10 UV absorption spectrum of Silver nanoparticles formed from Eenth during different time of incubation.

Time	385 nm	435nm	560nm	680nm
½ hr	0.538	0.576	0.416	0.302
1 hr	0.541	0.577	0.441	0.308
1 ½ hr	0.581	0.589	0.442	0.309
2 hr	0.583	0.603	0.450	0.334
2 ½ hr	0.588	0.642	0.467	0.348
Blank	0.414	0.354	0.208	0.108

Table.11 UV absorption spectrum of Copper nanoparticles formed from Eenth during different time of incubation.

Time	385 nm	435nm	560nm	680nm
½ hr	0.132	0.120	0.069	0.460
1 hr	0.170	0.128	0.109	0.501
1 ½ hr	0.201	0.146	0.116	0.503
2 hr	0.226	0.156	0.118	0.511
2 ½ hr	0.231	0.161	0.118	0.515
Blank	0.039	0.033	0.044	0.471

Table.12 UV absorption spectrum of Zinc nanoparticles formed from Eenth during different time of incubation.

Time	350 nm	385 nm	435 nm	560 nm
½ hr	0.124	0.092	0.069	0.037
1 hr	0.132	0.097	0.070	0.044
1 ½ hr	0.140	0.101	0.072	0.046
2 hr	0.147	0.108	0.089	0.071
2 ½ hr	0.217	0.161	0.125	0.082
Blank	0.000	0.000	0.000	0.000

Table.13 UV absorption spectrum of Silver nanoparticles formed from Passion fruit during different time of incubation.

Time	385 nm	435nm	560nm	680nm
½ hr	0.446	0.430	0.354	0.310
1 hr	0.663	0.606	0.489	0.388
1 ½ hr	0.706	0.626	0.520	0.416
2 hr	0.734	0.686	0.536	0.430
2 ½ hr	0.908	0.840	0.602	0.586
Blank	0.414	0.354	0.208	0.108

Table.14 UV absorption spectrum of Copper nanoparticles formed from Passion fruit during different time of incubation.

Time	385 nm	435nm	560nm	680nm
½ hr	0.142	0.136	0.150	0.628
1 hr	0.154	0.180	0.210	0.640
1 ½ hr	0.178	0.193	0.216	0.770
2 hr	0.202	0.202	0.221	0.862
2 ½ hr	0.351	0.263	0.277	0.872
Blank	0.039	0.033	0.044	0.471

Table.15 UV absorption spectrum of Zinc nanoparticles formed from Passion fruit during different time of incubation.

Time	350 nm	385 nm	435 nm	560 nm
½ hr	0.313	0.149	0.136	0.088
1 hr	0.336	0.188	0.137	0.092
1 ½ hr	0.340	0.204	0.140	0.096
2 hr	0.360	0.211	0.154	0.098
2 ½ hr	0.369	0.212	0.163	0.109
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

Fig.2 Passion fruit (*Passiflora edulis*) description a) pink coloured fruits, b) flower, c) pink coloured fruit cut opened, d) yellow coloured fruit, e) am made from passion fruit . Photo courtesy: Wikipedia.

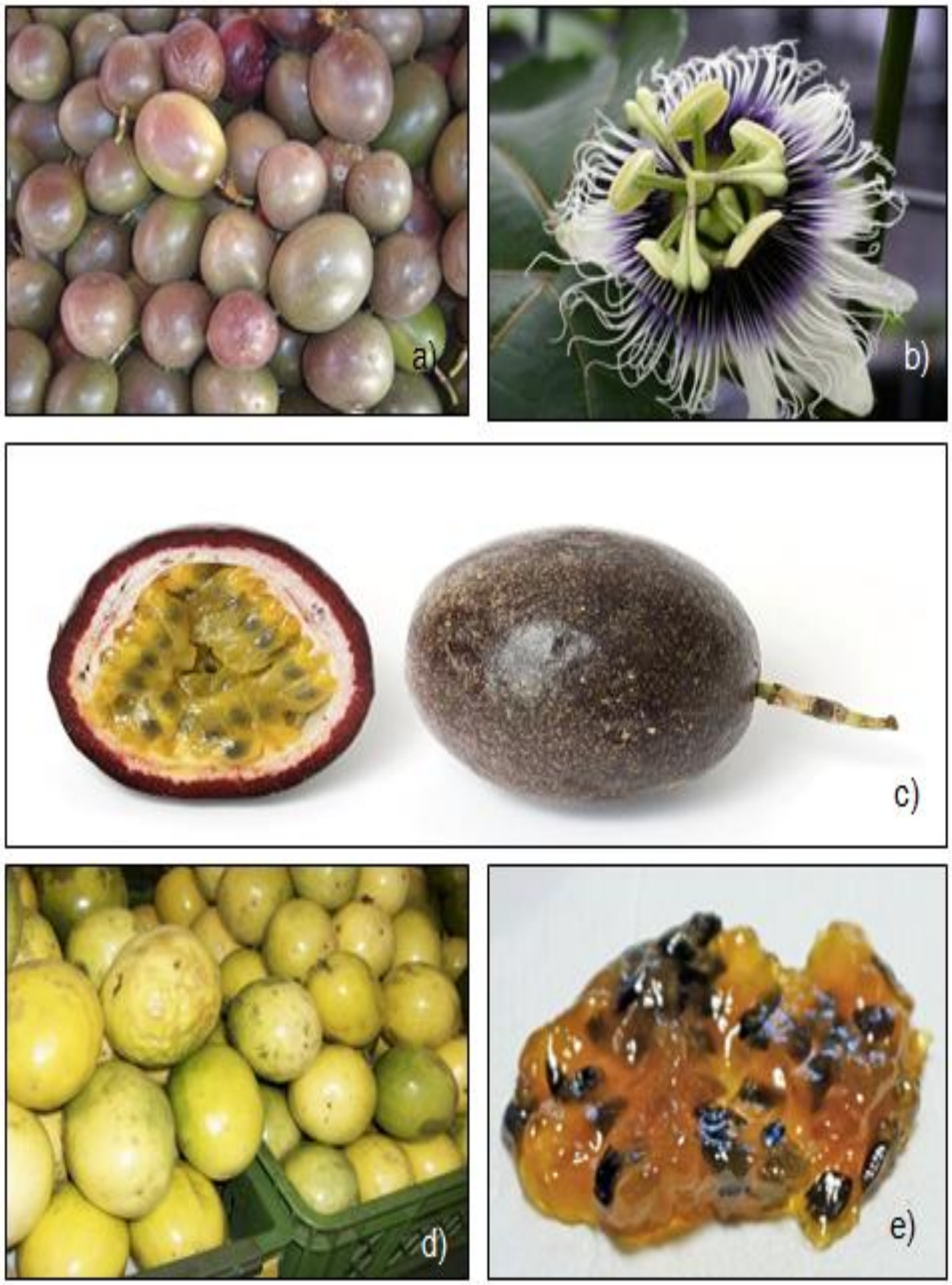


Fig.3 Eenth (*Cycas circinalis*)description a) tree in natural habitat, b) young plant in natural habitat, c) fruits harvested, d) male cone, e) mature leaf. Photo courtesy: Wikipedia.

Fig.4 Egg fruit (*Pouteria campechiana*) description a) collected leaves, b) single fruit, c) and d) fruit cut opened showing seeds, e) fruit sliced vertically, f) fruits sold on market. Photo courtesy: Wikipedia.

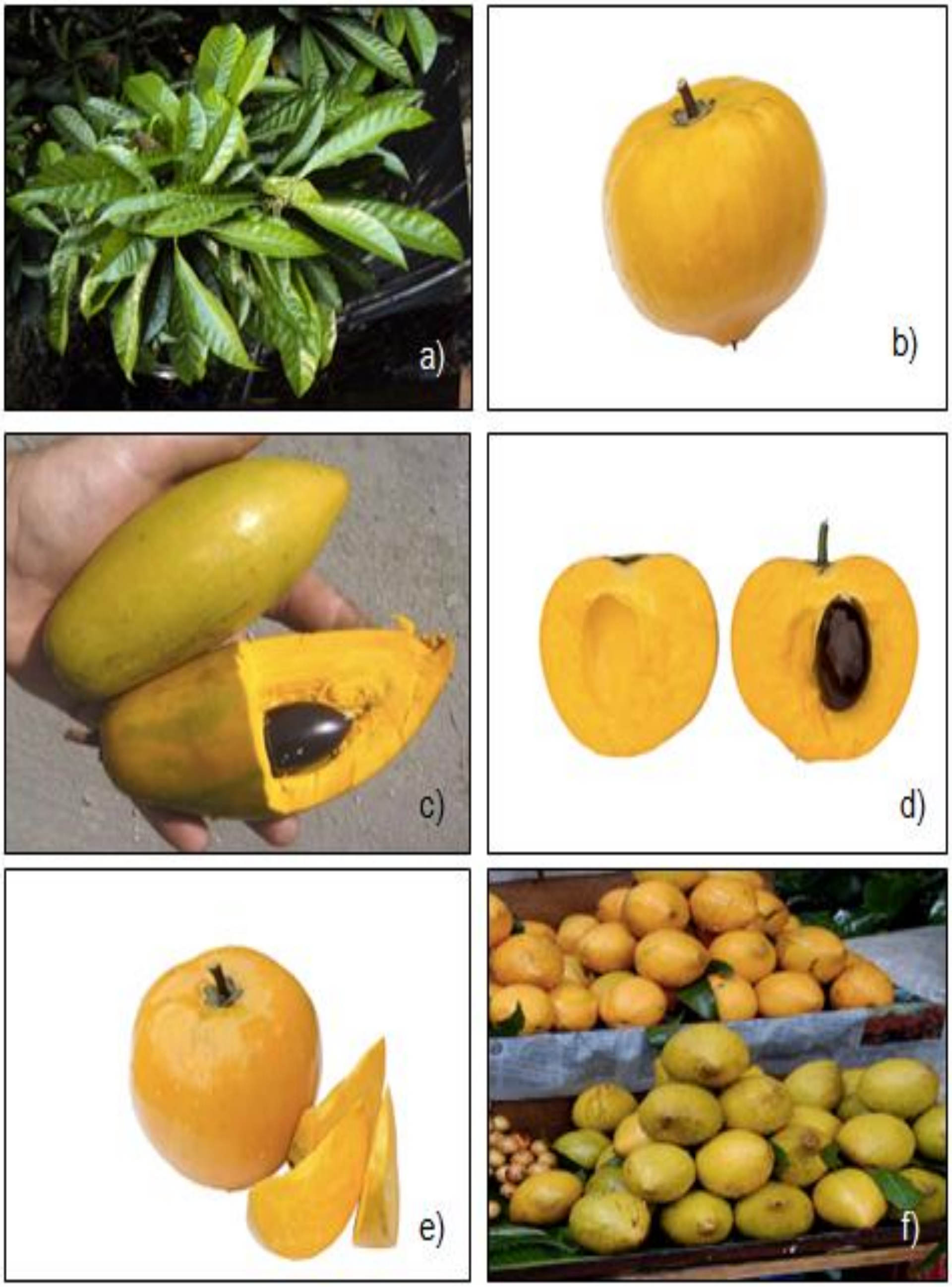


Fig.5 Description of the seeds used for making nanoparticles a) Mullatha (*Annona muricata*) seeds*, b) Passion fruit (*Passiflora edulis*) seeds, c) Eenth (*Cycas circinalis*)seeds, d) Papaya (*Carica papaya*) seeds*, e) Egg fruit (*Pouteria campechiana*) seeds.* data not provided.

Fig.6 Antibacterial activity study using well diffusion method of Passion fruit seed extract nanoparticles (Ag) and (Zn)a) *Salmonella typhi* control plate (Ag), b) *Salmonella typhi* green synthesised nanoparticle test plate (Ag; 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.

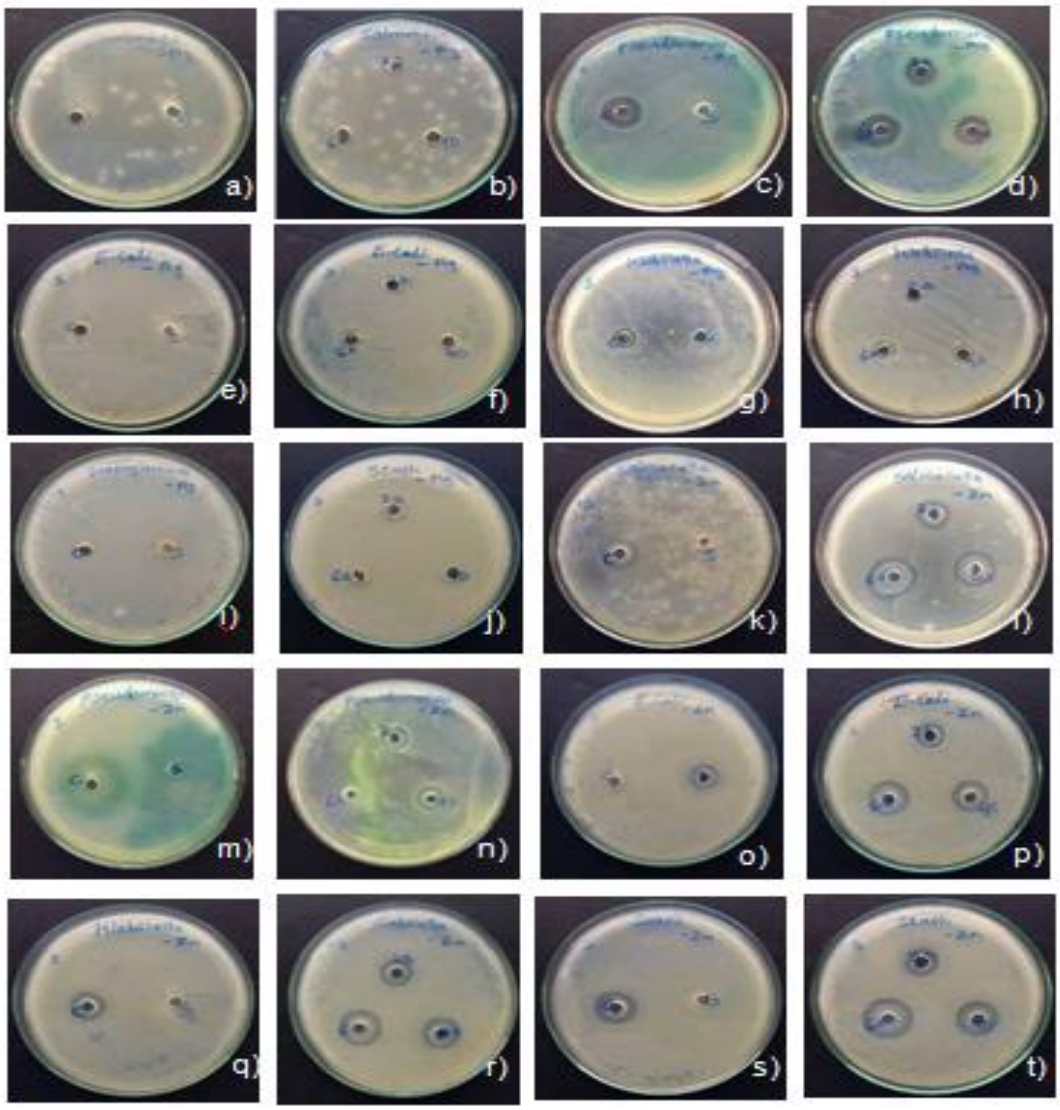


Fig.7 Antibacterial activity study using well diffusion method of Eenth seed extract nanoparticles (Cu) and (Ag)a) *Salmonella typhi* control plate (Cu), b) *Salmonella typhi* green synthesised nanoparticle test plate (Cu; 20, 40 and 60 µl), c) *Pseudomonas aeruginosa* control, d) *Pseudomonas aeruginosa*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

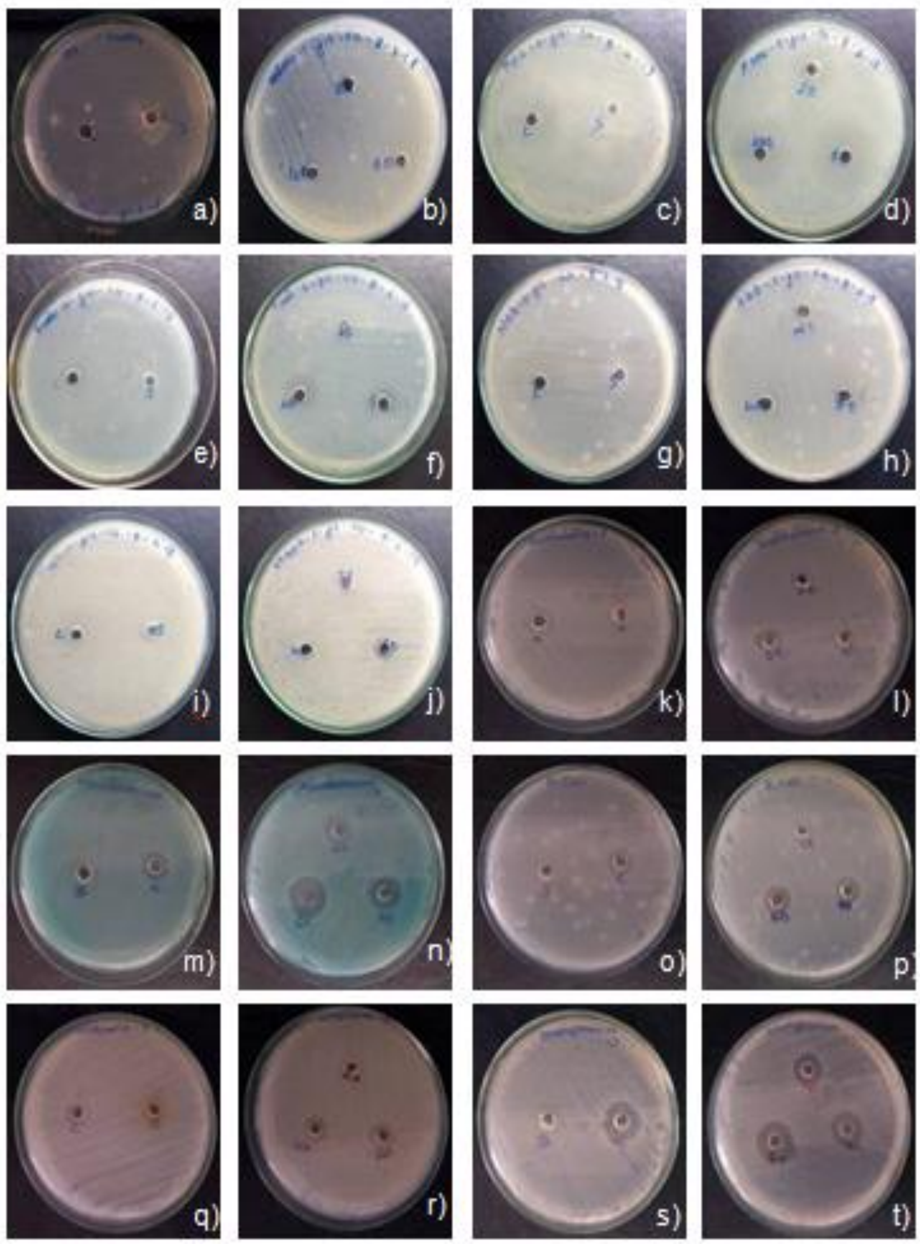


Fig.8 Antibacterial activity study using well diffusion method of Eenth and Egg fruit seed extract nanoparticles (Zn) and (Cu)a) *Salmonella typhi* control plate (Zn), b) *Salmonella typhi* green synthesised nanoparticle test plate (Zn; 20, 40 and 60 µl), c) *Pseudomonas aeruginosa* control, d) *Pseudomonas 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 Cu nanoparticles (Egg fruit).

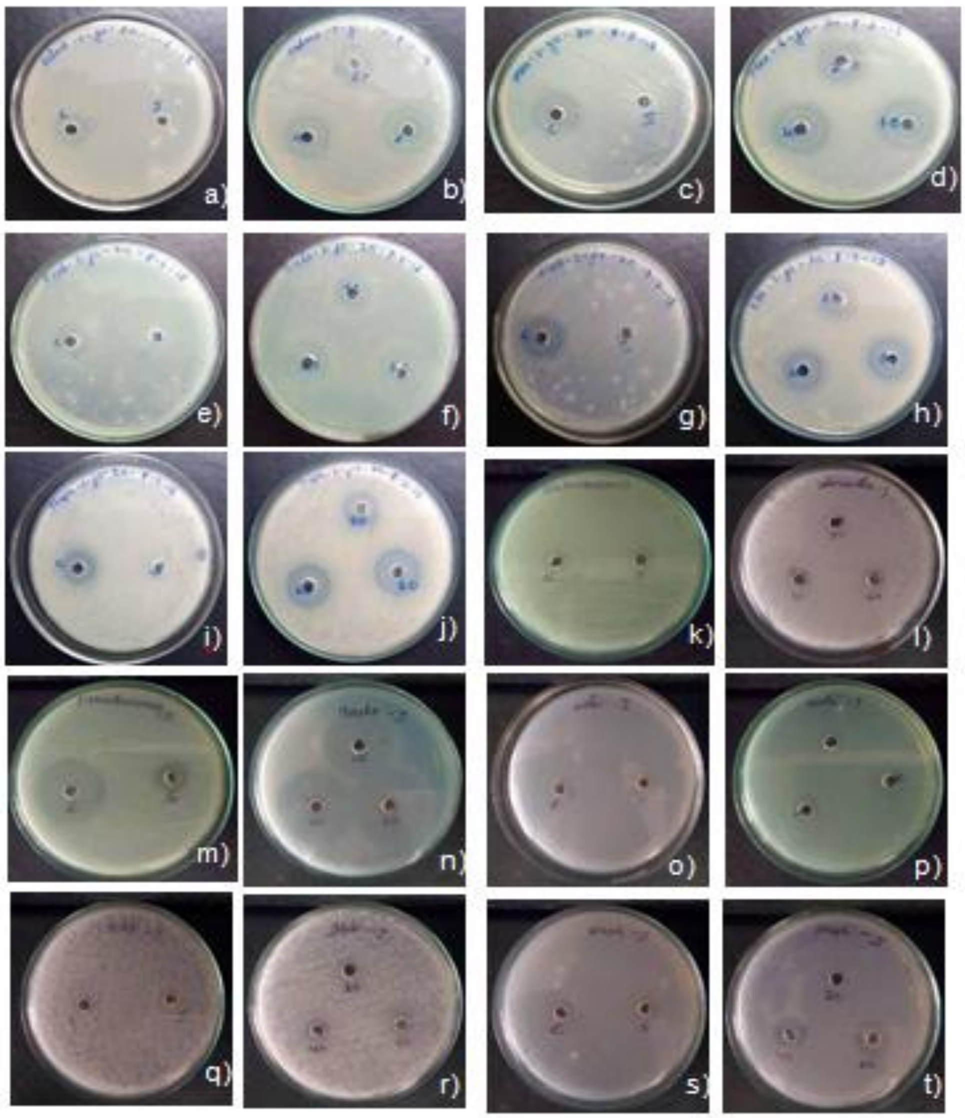


Fig.9 Antibacterial activity study using well diffusion method of Egg fruit seed extract nanoparticles (Ag) and (Zn)a) *Salmonella typhi* control plate (Ag), b) *Salmonella typhi* green synthesised nanoparticle test plate (Zn; 20, 40 and 60 µl), c) *Pseudomonas aeruginosa* control, d) *Pseudomonas aeruginosa*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

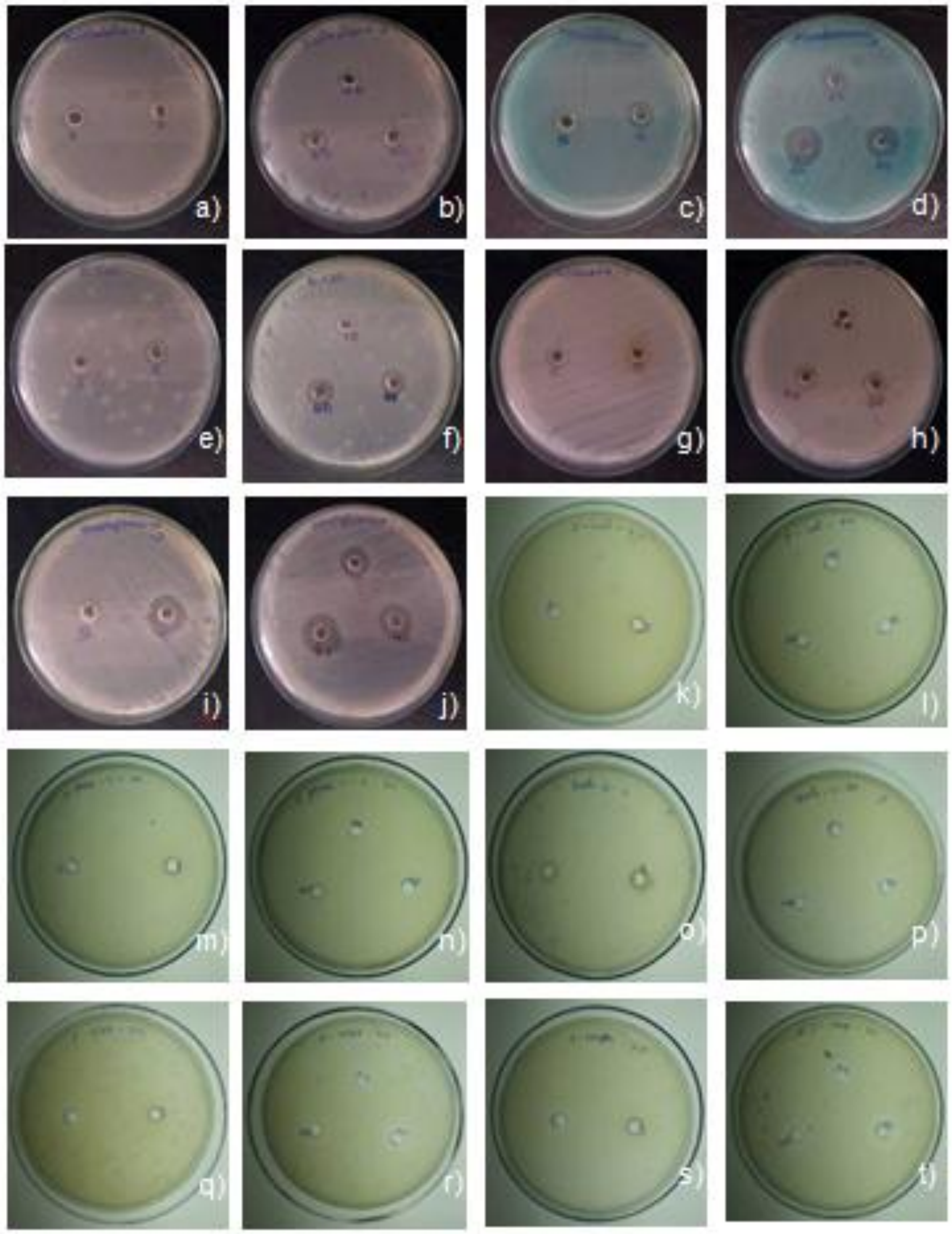


Fig.10 Colour change during nanoparticle formation, a) silver, copper and zinc nanoparticle formation in Egg fruit seed extract.

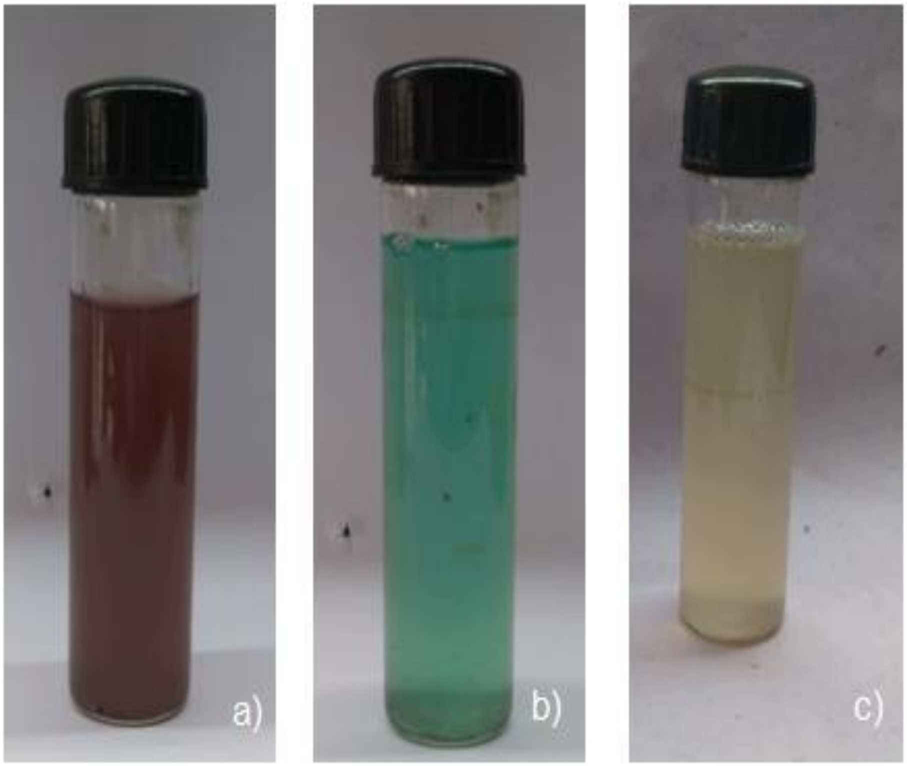


Fig.11 Silver nanoparticle formation of Egg fruit seed extract under SEM imaging system with various resolutions.

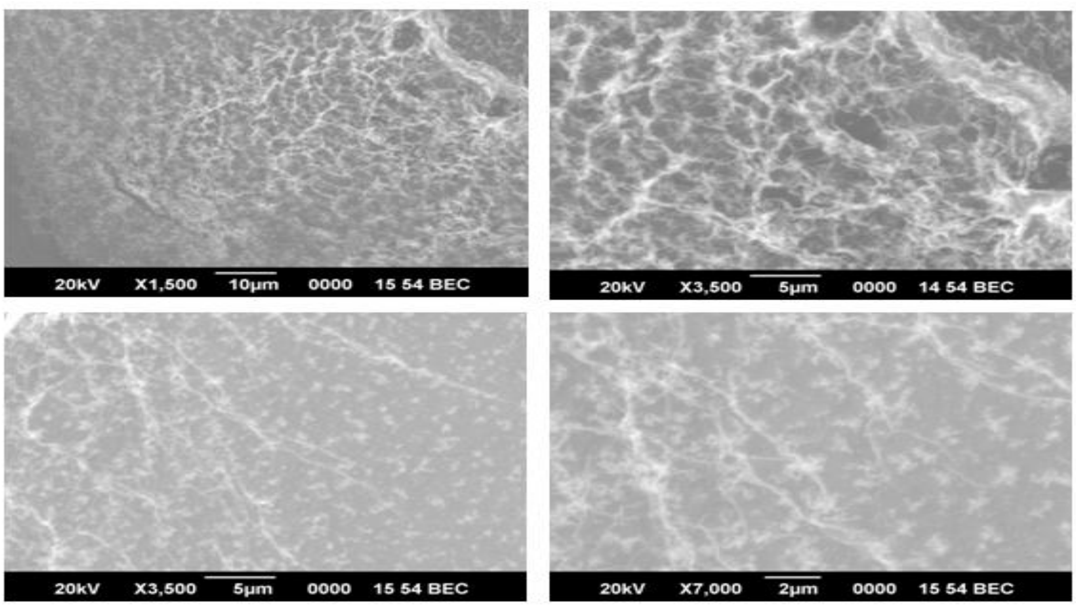


Fig.12 Copper nanoparticle formation of Egg fruit seed extract under SEM imaging system with various resolutions.

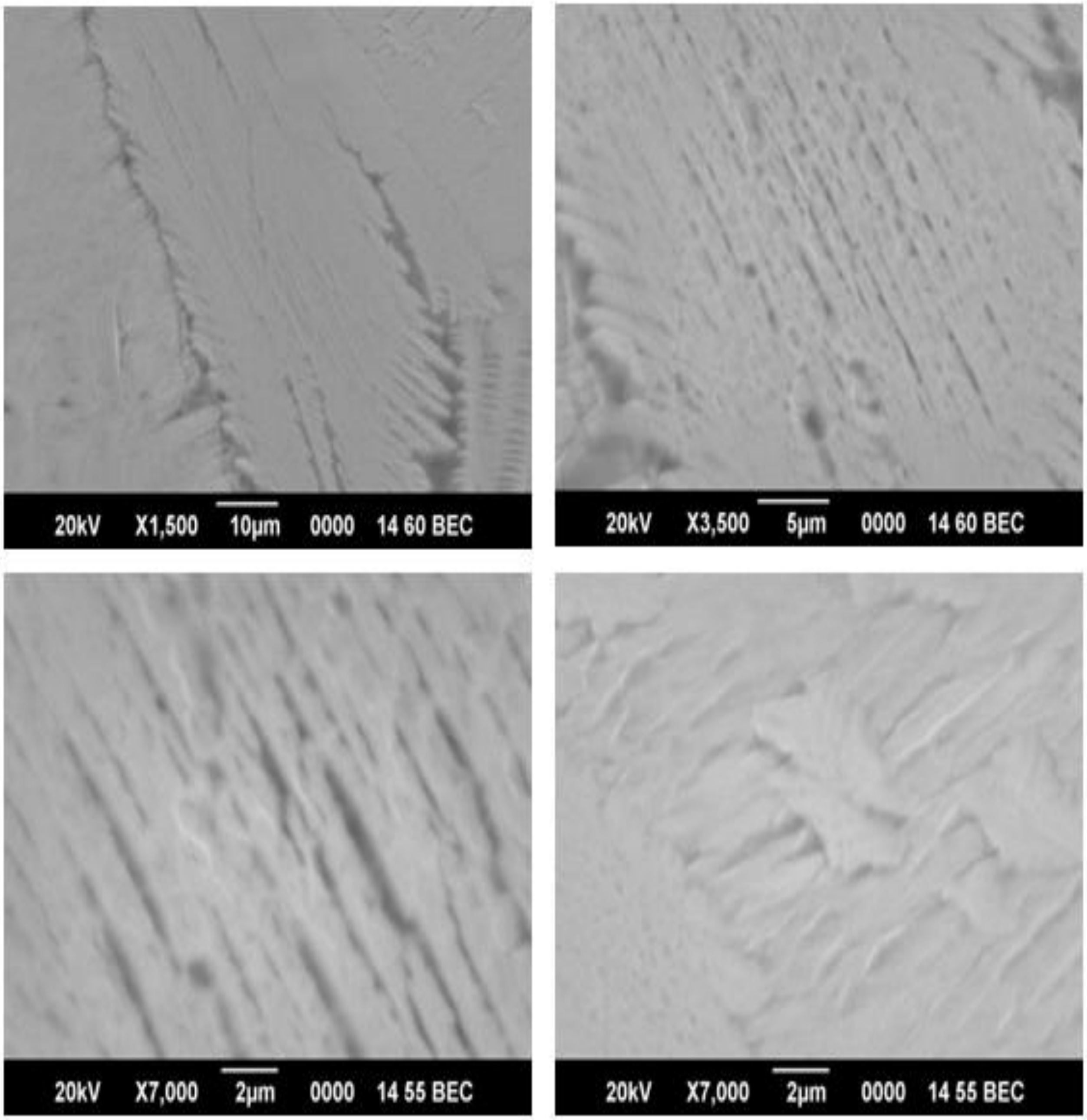


Fig.13 Zinc nanoparticle formation of Egg fruit seed extract under SEM imaging system with various resolutions.

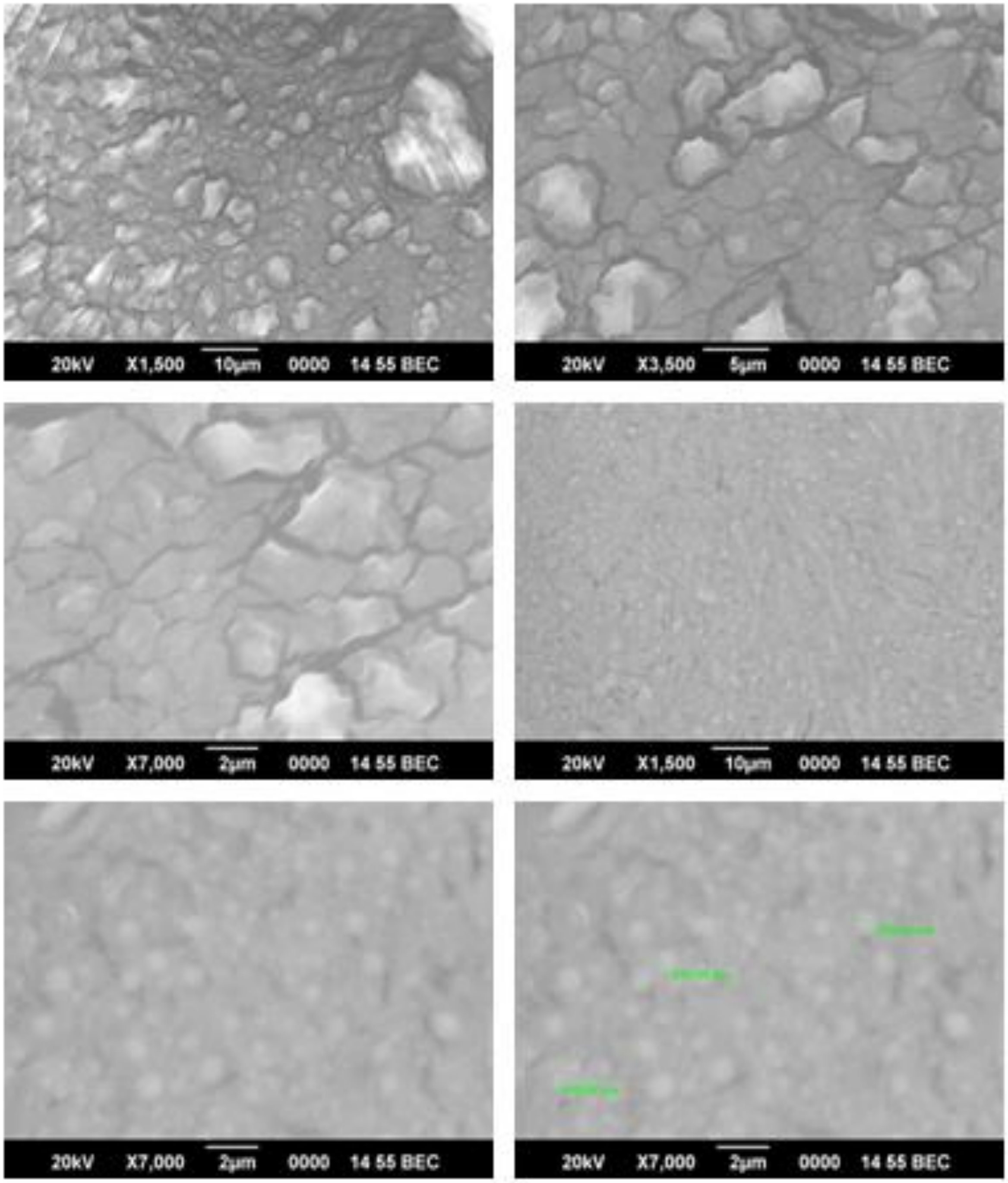
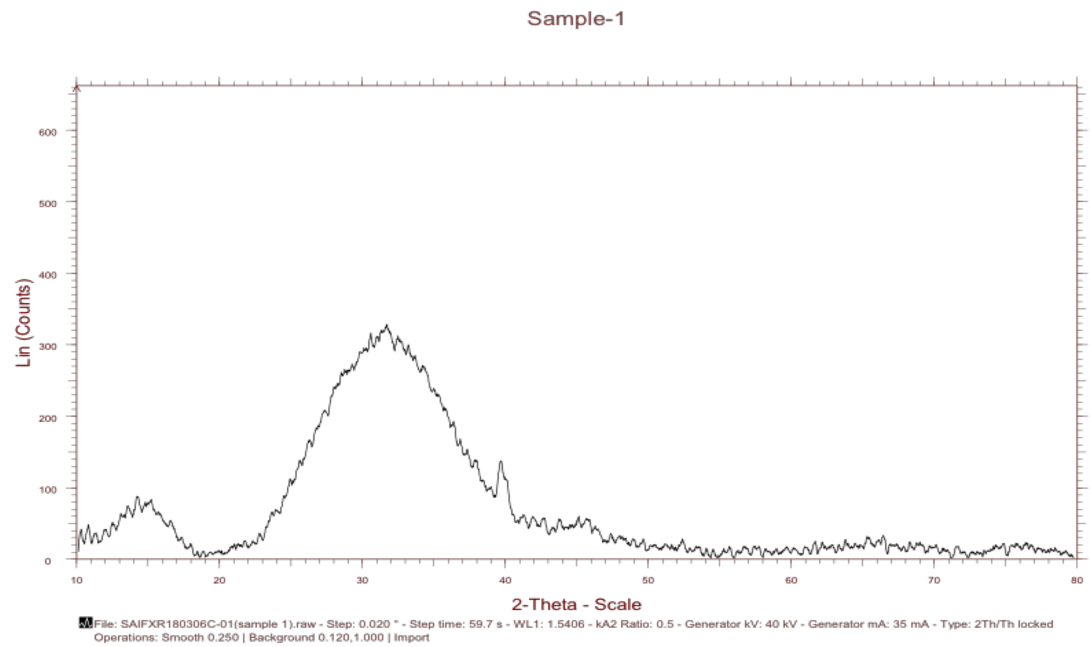


Fig.14 Silver nanoparticle formation of Egg fruit seed extract under XRD imaging system.



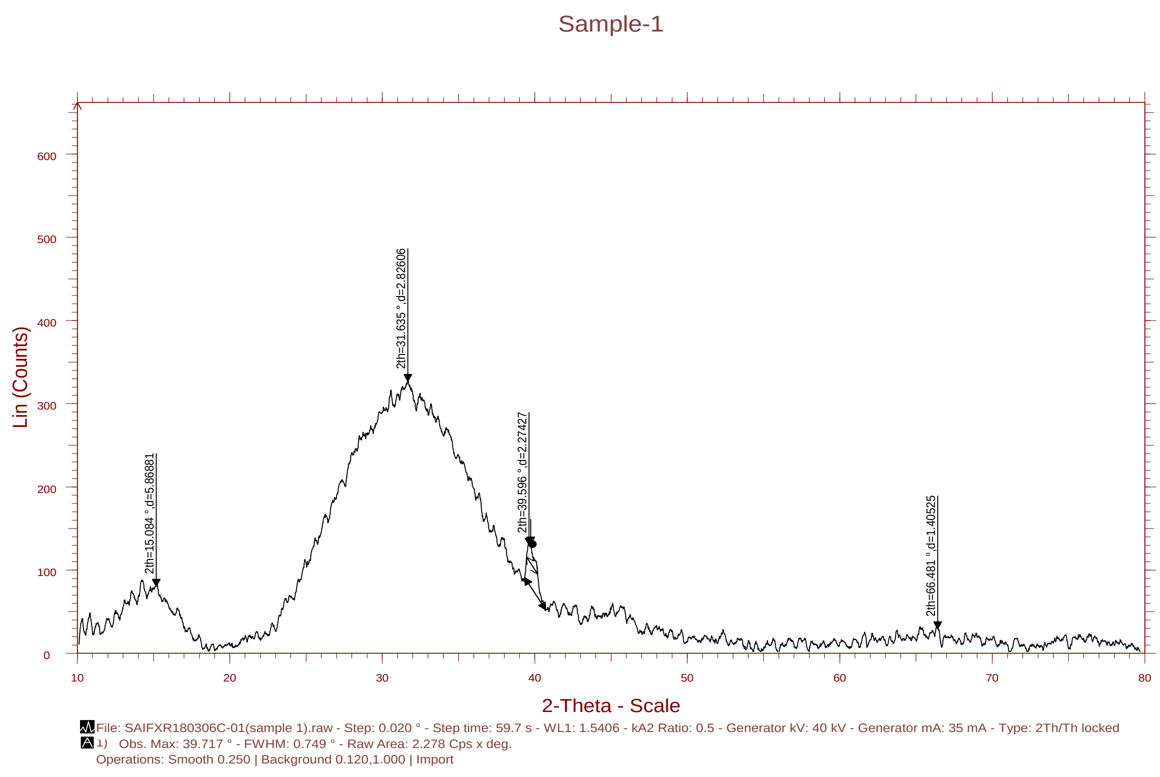
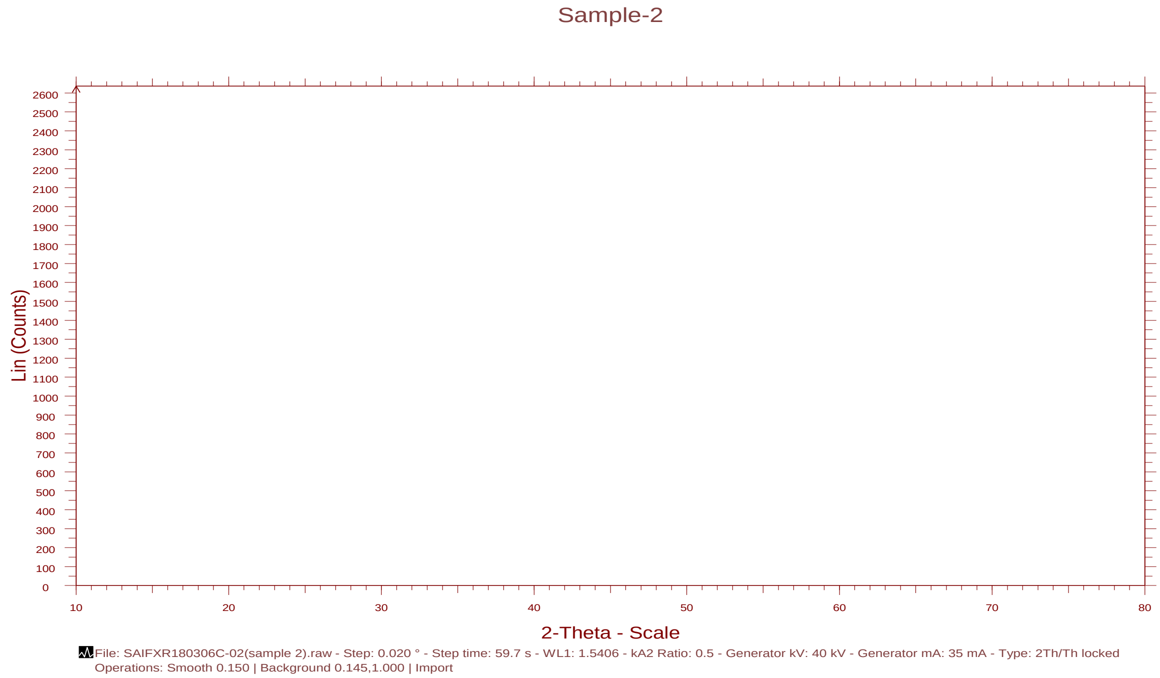


Fig.15 Copper nanoparticle formation of Egg fruit seed extract under XRD imaging system.



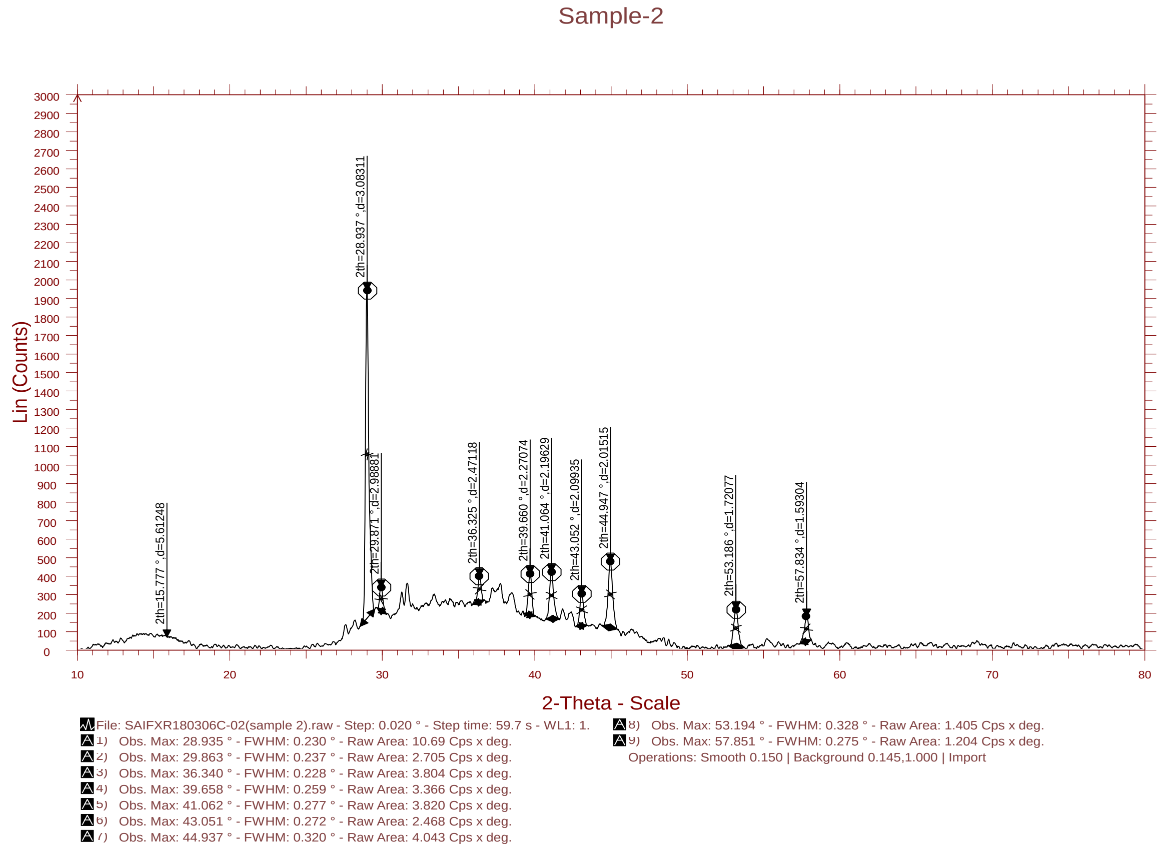
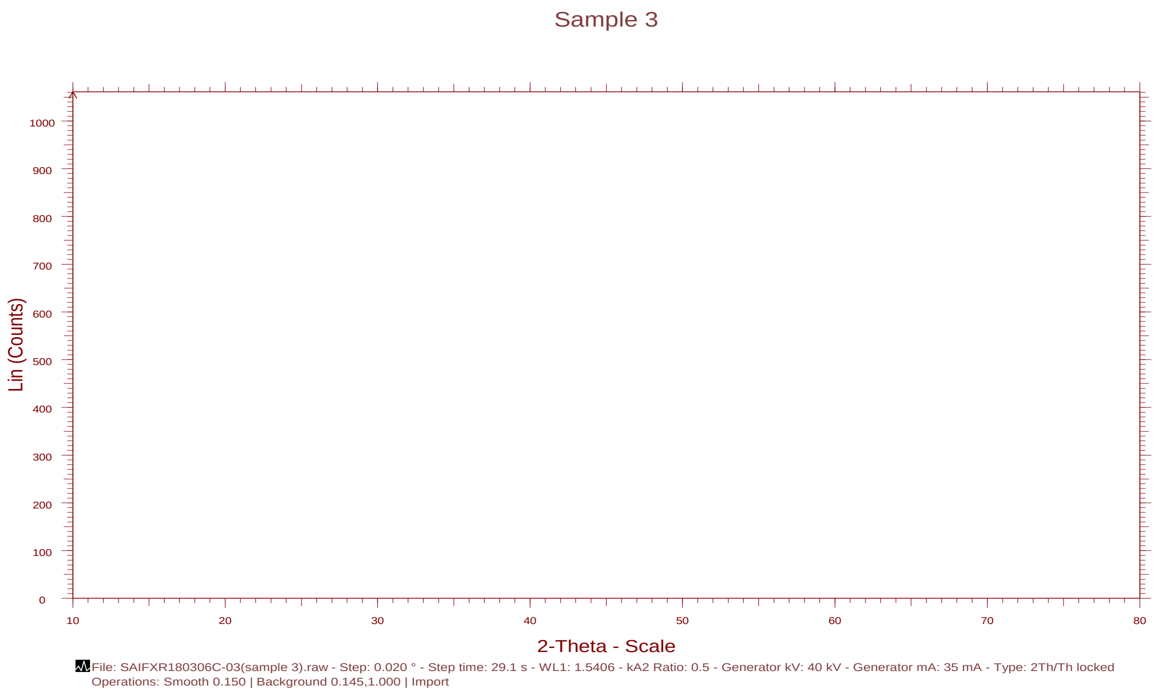
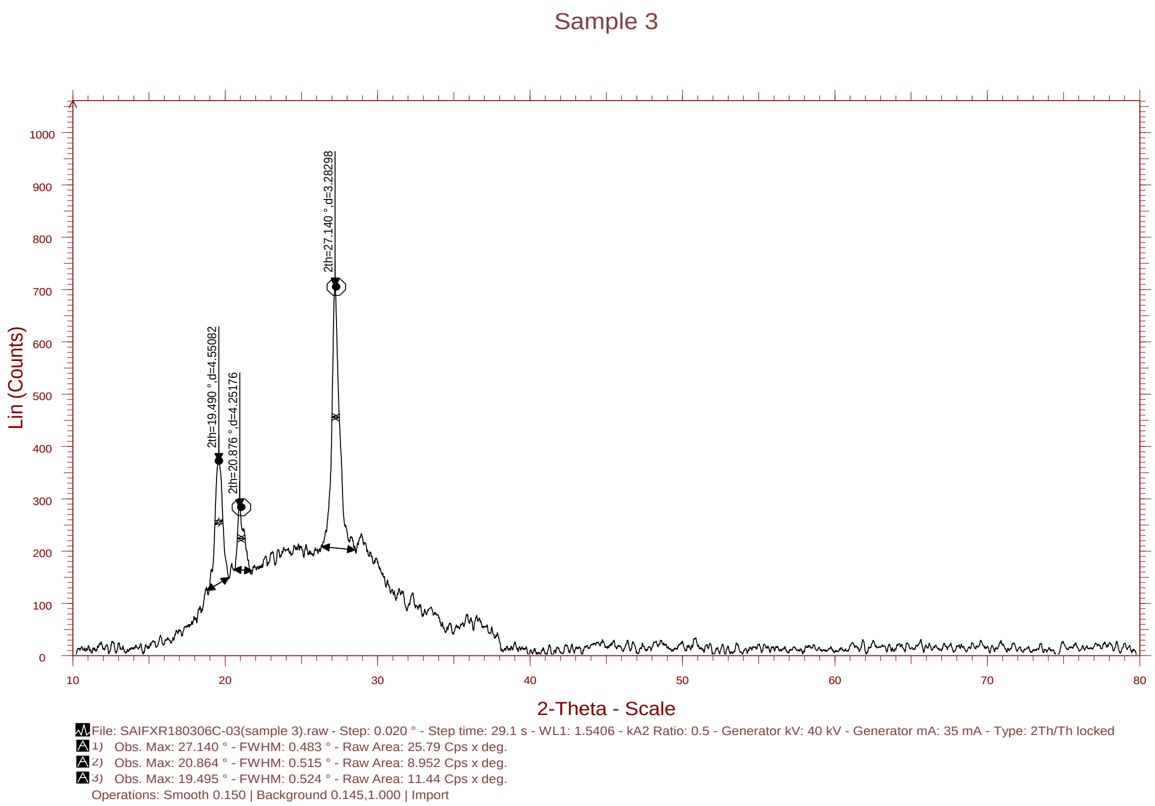


Fig.16 Zinc nanoparticle formation of Egg fruit seed extract under XRD imaging system.





The zone of inhibition showed by *Salmonella* species, *Pseudomonas* species, *Staphylococcus* species, *E. coli* and *Klebsiella* species by zinc nanoparticles formed from 20µl concentration of nanoparticles were 10 mm, 11 mm, 11 mm, 13 mm & 10 mm respectively; from 40 µl concentration of nanoparticles were 11 mm, 12 mm, 12 mm, 16 mm & 13 mm respectively; from 60 µl concentration of nanoparticles were 13 mm, 13 mm, 13 mm, 18 mm & 19 mm respectively.

The zone of inhibition showed by *Salmonella* species, *Pseudomonas* species, *Staphylococcus* species, *E. coli* and *Klebsiella* species by $ZnSO_4$ solution was 11 mm, 10 mm, 10 mm, 12 mm & 11 mm respectively.

For *Cycas circinalis*, the zone of inhibition showed for *Salmonella* species, *Pseudomonas* species, *Staphylococcus* species, *E. coli* and *Klebsiella* species by silver nanoparticles formed from 20 µl concentration of nanoparticles were 11 mm, 12 mm, 10 mm, 11 mm & 12 mm respectively; from 40 µl concentration of nanoparticles were 12 mm, 14 mm, 11 mm, 12 mm & 15 mm respectively; from 60 µl concentration of nanoparticles were 13 mm, 16 mm, 12 mm, 13 mm & 16 mm respectively.

The zone of inhibition showed by *Salmonella* species, *Pseudomonas* species, *Staphylococcus* species, *E. coli* and *Klebsiella* species by seed extract was 8 mm, 10 mm, 8 mm, 9 mm & 10 mm respectively.

The zone of inhibition showed by *Salmonella* species, *Pseudomonas* species, *Staphylococcus* species, *E. coli* and *Klebsiella* species by copper nanoparticles formed from 20µl concentration of nanoparticles were 11 mm, 19 mm, 11 mm, 12 mm & 15 mm respectively; from 40 µl concentration of nanoparticles were 12 mm, 28 mm, 12 mm, 16 mm & 19 mm respectively; from 60 µl concentration of nanoparticles were 13 mm, 32 mm, 13 mm, 17 mm & 24 mm respectively.

The zone of inhibition showed by *Salmonella* species, *Pseudomonas* species, *Staphylococcus* species, *E. coli* and *Klebsiella* species by zinc nanoparticles formed from 20µl concentration of nanoparticles were 20 mm, 25 mm, 13 mm, 19 mm & 23 mm respectively; from 40 µl concentration of nanoparticles were 22 mm, 28 mm, 21 mm, 22 mm & 25 mm respectively; from 60 µl concentration of nanoparticles were 24 mm, 29 mm, 23 mm, 23 mm & 27 mm respectively. For *Passiflora edulis*, the zone of inhibition showed for *Salmonella* species, *Pseudomonas* species, *Staphylococcus* species, *E. coli* and *Klebsiella* species by silver nanoparticles formed from 20 µl concentration of nanoparticles were 10 mm, 12 mm, 10 mm, 10 mm & 10 mm respectively; from 40 µl concentration of nanoparticles were 11 mm, 15 mm, 11 mm, 12 mm & 11 mm respectively; from 60 µl concentration of nanoparticles were 13 mm, 16 mm, 12 mm, 13 mm & 12 mm respectively.

The zone of inhibition showed by *Salmonella* species, *Pseudomonas* species, *Staphylococcus* species, *E. coli* and *Klebsiella* species by seed extract was 9 mm, 8 mm, 13 mm, 12 mm & 11 mm respectively.

The zone of inhibition showed by *Salmonella* species, *Pseudomonas* species, *Staphylococcus* species, *E. coli* and *Klebsiella* species by copper nanoparticles formed from 20µl concentration of nanoparticles were 14 mm, 13 mm, 24 mm, 15 mm & 14 mm respectively; from 40 µl concentration of nanoparticles were 17 mm, 14 mm, 27 mm, 18 mm & 18 mm respectively; from 60 µl concentration of nanoparticles were 21 mm, 17 mm, 30 mm, 21 mm & 24 mm respectively.

The zone of inhibition showed by *Salmonella* species, *Pseudomonas* species, *Staphylococcus* species, *E. coli* and *Klebsiella* species by zinc nanoparticles formed from 20µl concentration of nanoparticles were 21 mm, 11 mm, 22 mm, 20 mm & 23 mm respectively; from 40 µl concentration of nanoparticles were 24 mm, 14 mm, 26 mm, 22 mm & 25 mm respectively; from 60 µl concentration of nanoparticles were 26 mm, 16 mm, 30 mm, 25 mm & 28 mm respectively.

Silver, copper and zinc nanoparticles have antibacterial activity against *Salmonella species*, *Pseudomonas species*, *Staphylococcus species*, *E. coli* and *Klebsiella specie*s. When antibacterial activity of silver, copper and zinc nanoparticles from 3 different concentrations were observed, nanoparticles have 60 µl concentration shows maximum activity against these microbes.

Silver nanoparticles shows greater antibacterial activity compared to silver nitrate and seed extract. Copper nanoparticles shows greater antibacterial activity compared to copper Sulphate and seed extract. Zinc nanoparticles shows greater antibacterial activity compared to zinc Sulphate and seed 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.

SEM-XRD analysis

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

The results showed that seed extracts of *Pouteria campechiana*, *Cycas circinalis* and *Passiflora edulis* could be used to the synthesis of silver, copper and zinc nanoparticles. The synthesized silver, copper and zinc nanoparticles shows antibacterial activity on both Gram positive and Gram negative bacteria. This biosynthesis of nanoparticles is cost efficient, pollutant free and simpler to synthesize.

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