Int.J.Curr.Res.Aca.Rev.2022; 10(07): 1-41

International Journal of Current Research and Academic Review

ISSN: 2347-3215 (Online) Volume 10 Number 07 (July-2022)

Journal homepage: http://www.ijcrar.com

doi: https://doi.org/10.20546/ijcrar.2022.1007.001

Green Synthesis of Silver, Copper and Zinc Nanoparticles from Chick pea (*Cicer arietinum* L.), Black gram (*Vigna mungo* (L.) Hepper) Exudates and Evaluation of their Antibacterial Activity: An Overview

Prem Jose Vazhacharickal*and Gopika S. Krishna

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

**Corresponding author*

Abstract

Nanotechnology is an emerging field of science with 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. Pulses exudates of two different legumes; Chick pea (*Cicer arietinum* L.) and Black gram (*Vigna mungo* (L.) Hepper)were used for the synthesis of silver, copper, and zinc nanoparticles anddetermine the antibacterial properties of these nanoparticles against *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi* and *Klebsiella pneumoniae*. 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 the selected bacterial 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 150 µl concentration shows maximum activity against these microbes. Silver, Copper and Zinc nanoparticles showed greater antibacterial activity compared to silver nitrate, copper sulphate and zinc sulphate solution. This green synthesis method is alternative to chemical method, since it is cheap, pollutant free and eco-friendly.

Article Info

Received: 01 June 2022
Accepted: 28 June 2022
Available Online: 20 July 2022

Keywords

Nanoparticles; *Cicer arietinum*; *Vigna mungo*; 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; Vazhacharickal *et al.*, 2022). Nanoparticles bear antibacterial properties (Hajipour *et al.*, 2012).

Nanoparticles play important role in fighting against disease causing microbes. Nanoparticles are very minute particles. Due to large surface volume ratio; renewable surface and varying micro electrode potential values

nanoparticles are largely used as catalysts also (Din and Rehan, 2017). There are different types of nanoparticles including; silver, copper, zinc (metal nanoparticles).

Nowadays humans face dangers infections due to pathogenic microbes. Nanoparticles can overcome this problems. Nanoparticles have antibacterial property. Metal nanoparticles such as silver, copper and zinc has inhibitory effect on microorganisms.

Gram pulses are the legume family part. They grows in a pod with one to twelve seeds. Metal nanoparticles are recognised by Faraday and their colour was quantitatively explained by Mie. Metal nanoparticles have electronic, mechanical, optical, magnetic & chemical properties; different from bulk material (Mitiku and Yilma, 2017). Gram pulses are edible seeds. They have low fat content and rich in protein and fibre.

Pulses have nitrogen fixing capacity and there for they reduce the use of nitrogen fertilizers. Gram pulses also contain minerals like iron and zinc. Pulses have many other health benefit than other legumes. At maturity gram pulses are harvested and can be used as food. For their own needs gram pulses can fix atmospheric nitrogen.

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 (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, 2004).

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).

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 (Patravale *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 (Chen *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).

Antimicrobial 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 (Al Juhaiman *et al.,* 2010). 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.

Antibacterial 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 indiscriminate 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.

Objectives

Synthesis of silver, copper and zinc nanoparticles using pulses exudates of seven different legumes; Chick pea (*Cicer arietinum* L.) and Black gram (*Vigna mungo* (L.) Hepper) 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.

Review of literature

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 possess 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 varietyof 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).

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 scar less 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).

Cicer arietinum L. (Chick pea)

*Cicer arietinum*L.(Chick pea) is a legume of the family Fabaceae popularly known as grams.It is an important legume crop used as human food. Chick pea is grown up to 12 million hectares all over the world, annual production is about 10.9 million tons (www.faostat.org).

There are two categories of chick pea; Kabuli and Desi based on variation in their size, shape and colour (Singh *et al.*, 1991). Chick pea contains flavonoids and polyphenols in high amount exhibits high levels of antioxidant activity (Segev *et al.*, 2010).

Chick pea have many medicinal value such as; it helps to lowering cholesterol level, prevents neural tube defects, fights nervous system disorders, aids digestion, aids weight loss and improves heart health. The fibre content in the chick pea adsorbs the cholesterol content from other food in intestine. Chick also consists poly unsaturated fats which is also helps to the reduction of cholesterol level. Chick pea helps in vitamin D deficiency like nervousness and irritability. It also helps in case of depression and stress. The fibre content present in the chick pea helps to boost overall digestive function of the body and it produce digestive enzymes and also favour the growth of the beneficial microbes in gut.

Taxonomical classification (*Cicer arietinum*; Chick pea; Kadala)

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

Subkingdom: Viridiplantae

Superdivision: Embryophyta

Division: Tracheophyta

Class: Magnoliopsida

Order: Fabales

Family: Fabaceae

Genus: Cicer L.

Species: *Cicer arietinum* L.

Vigna mungo (L.) Hepper (Black gram)

Vigna mungo (L.) Hepper; black gram, urad bean, mungo bean is a bean grown in Indian subcontinent. The product is sold as black lentil is usually the whole urad bean, whereas the split bean is called white lentil. This is sometimes confused with the true black lentil (*Lens culinaris*) which is much smaller.

Taxonomical classification (*Vigna mungo* (L.) Hepper; Black gram; Uzhunnu)

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

Subkingdom: Viridiplantae

Superdivision: Embryophyta

Division: Tracheophyta

Class: Magnoliopsida

Order: Fabales

Family: Fabaceae

Genus: Vigna Savi

Species: *Vigna mungo* (L.) Hepper

Hypothesis

The current research work is based on the following hypothesis

Seeds exudates of Chick pea (*Cicer arietinum* L.) and Black gram (*Vigna mungo* (L.) Hepper)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 Chick pea (*Cicer arietinum* L.) and Black gram (*Vigna mungo* (L.) Hepper) were collected from Ramapuram, Kottayam district of Kerala State, India. The seeds were thoroughly cleaned using double distilled water. The samples were dried in hot air oven at 60ºC for 48hrs and later stored in air tight polyethylene zipper bag for analysis.

Extraction method

The seeds of Chick pea (*Cicer arietinum* L.) and Black gram (*Vigna mungo* (L.) Hepper) were soaked in 100 ml distilled water for 6, 12 and 24 hrs, the contents were mixed thoroughly using a glass road and filtered using a filter paper, thus filtered solution is taken as the extract (exudates). The obtained seed exudate which appeared light yellowish to grey in color was stored 4°C for further use.

Synthesis of nanoparticles

Silver 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 (Chick pea (*Cicer arietinum* L.) and Black gram (*Vigna mungo* (L.) Hepper)) 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 (Chick pea (*Cicer arietinum* L.) and Black gram (*Vigna mungo* (L.) Hepper)) 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 (Chick pea (*Cicer arietinum* L.) and Black gram (*Vigna mungo* (L.) Hepper)) 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 pneumonia, 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.

2SEM-EDX 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 EDX spectrum of the silver nanoparticles was performed to confirm the presence of elemental silver 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^{0}C 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 exudates of different legumes Chick pea (*Cicer arietinum* L.) and Black gram (*Vigna mungo* (L.) Hepper)) 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 Chick pea *(Cicer arietinum* L.). The intensity of the peak at 435nm was increased with time until the reduction completes. The maximum peak was found to be 385 nm (λ max) and the intensity of the peak at 385 nm was increased with time until the reduction completes.

Copper nanoparticles

To synthesize copper nanoparticles, seed exudates of different legumes (Chick pea (*Cicer arietinum* L.) and Black gram (*Vigna mungo*(L.) Hepper)) 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) and the intensity of the peak at 680 nm was increased with time until the reduction completes.

Zinc nanoparticles

To synthesize zinc nanoparticles, seed exudates of extracts of different legumes (Chick pea (*Cicer arietinum* L.) and Black gram (*Vigna mungo*(L.) Hepper)) 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 Chick pea (*Cicer arietinum* L.) and Black gram *(Vigna mungo* (L.) Hepper).The intensity of the peak at 350 nm was increased with time until the reduction completes.

Antibacterial assay

Seed exudates of Chick pea (*Cicer arietinum* L.) and Black gram (*Vigna mungo* (L.) Hepper) showed growth inhibitory effects against *Salmonella Typhi, Pseudomonas aeruginosa, Staphylococcus aureus, E. coli* and *Klebsiella pneumoniae.*

The exudates of Chick pea and Black gram show growth inhibitory effects against salmonella, Pseudomonas, Staphylococcus, *E.coli* and Klebsiella. These types of gram pulses show more activity at 24 hours than 12 and 6 hours. That means here antibacterial activity was increased with time.

In the case of Chick pea, Zinc nanoparticles showed upper hand in antimicrobial activity against *Staphylococcus aureus* (25 nm) and *Pseudomonas aeruginosa* (25 mm). Copper nanoparticles showed more antimicrobial activity against *E.coli* (25 mm). Here, fresh exudate showed less antimicrobial activity (low zone of inhibition is found. When antimicrobial activity of Copper and Zinc nanoparticles were observed, nanoparticles have 150 µl concentration showed maximum antimicrobial activity against *E.coli*, Klebsiella species, *Pseudomonas aureginosa*, *Salmonella typhi* and *Staphylococcus aureus*. In the case of *Vigna mungo*, Copper nanoparticles showed more antimicrobial activity with respect to Zinc nanoparticles against *E.coli* (32 mm). 150 µl concentration of nanoparticles showed higher growth inhibition zone against these five types of bacteria. Fresh exudate shows less antimicrobial activity. Zinc nanoparticles showed maximum antimicrobial activity against *Salmonella typhi* (28 mm) and *Staphylococcus aureus* (26 mm).

From the above results we can understand that, 24 hours old sample exudates showed maximum antimicrobial activity. 150 µl concentration of nanoparticles were more active against the bacteria than 50 and 150 µl. In the case of *Vigna mungo*, Copper nanoparticles showed more antimicrobial activity than Zinc nanoparticles. Zinc nanoparticles showed more activity against *E.coli*, Klebsiella species, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococcus aureus* in the case of Chick pea.

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 exudates.

Copper nanoparticles shows greater antibacterial activity compared to copper Sulphate and seed exudates. Zinc nanoparticles shows greater antibacterial activity compared to zinc Sulphate and seed exudates. Maximum zone of inhibition was at 150 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 Chick pea (*Cicer arietinum* L.) around the globe and India.

Language	Names
Scientific names	*Cicer arietinum* L.
Name in various global languages	
French	
German	
English	Chick pea
Name in various Indian languages	
Sanskrit	Jivana
Hindi	Chana
Urdu	Chana
Marathi	Harbhara
Kannada	Kadale
Gujarati	Chania
Malayalam	Kadala
Tamil	Katalai

Table.2 Different vernacular names of Black gram (*Vigna mungo* (L.) Hepper around the globe and India.

Language	Names
Scientific names	*Vigna mungo* (L.) Hepper
Name in various global languages	
French	
German	
English	Black gram
Name in various Indian languages	
Sanskrit	Mashah
Hindi	Urad
Urdu	Arad mung
Marathi	Masha
Kannada	Uddu
Gujarati	Adad
Malayalam	Uzhunnu
Tamil	Uluntu

Table.3 Zone of inhibition against various bacteria (*E. coli*, *Klebsiella species*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococus aureus*) using nanoparticles produced byChick pea (*Cicer arietinum* L.) 6 hrs seed exudate.

Microorganism	Nanoparticles	Control	Sample	Measure of zone of inhibition in mm		
				50	100	150
E.coli	Silver	11	7	10	12	13
	Copper	14	10	14	20	22
	Zinc	21	10	17	22	26
Klebsiella species	Silver	10	8	10	11	12
	Copper	14	10	17	18	20
	Zinc	20	10	17	20	24
Pseudomonas aeruginosa	Silver	9	8	12	15	16
	Copper	18	10	18	19	20
	Zinc	12	11	14	15	19
Salmonella typhi	Silver	9	-	10	12	13
	Copper	12	10	14	15	20
	Zinc	22	10	22	25	27
Staphylococcus aureus	Silver	10	-	10	11	12
	Copper	14	10	18	22	25
	Zinc	27	11	27	28	35

Table.4 Zone of inhibition against various bacteria (*E. coli*, *Klebsiella species*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococus aureus*) using nanoparticles produced byBlack gram (*Vigna mungo* (L.) Hepper6 hrs seed exudates.

Microorganism	Nanoparticles	Control	Sample	Measure of zone of inhibition in mm		
				50	100	150
E.coli	Silver	7	11	10	12	13
	Copper	18	10	19	23	24
	Zinc	22	10	22	24	28
Klebsiella species	Silver	10	8	10	11	12
	Copper	17	10	14	17	25
	Zinc	20	11	18	20	25
Pseudomonas aeruginosa	Silver	8	9	12	15	16
	Copper	18	10	18	23	25
	Zinc	14	11	14	16	17
Salmonella typhi	Silver	9	-	10	11	13
	Copper	14	11	11	14	16
	Zinc	20	11	20	25	26
Staphylococcus aureus	Silver	10	-	10	11	12
	Copper	24	10	17	23	24
	Zinc	20	10	20	28	30

Table.5 Zone of inhibition against various bacteria (*E. coli*, *Klebsiella species*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococus aureus*) using nanoparticles produced byBlack gram (*Vigna mungo* (L.) Hepper12 hrsseed exudate.

Microorganism	Nanoparticles	Control	Sample	Measure of zone of inhibition in mm		
				50	100	150
E.coli	Silver	7	5	9	11	13
	Copper	15	12	15	19	22
	Zinc	15	10	15	18	20
Klebsiella species	Silver	8	9	11	13	14
	Copper	15	11	15	18	19
	Zinc	14	11	11	16	18
Pseudomonas aeruginosa	Silver	9	6	12	13	16
	Copper	17	10	17	18	19
	Zinc	14	10	11	15	17
Salmonella typhi	Silver	8	6	9	11	13
	Copper	13	10	14	16	24
	Zinc	13	13	12	15	17
Staphylococcus aureus	Silver	12	9	13	14	16
	Copper	14	10	14	18	22
	Zinc	19	11	17	20	23

Table.6 Zone of inhibition against various bacteria (*E. coli*, *Klebsiella species*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococus aureus*) using nanoparticles produced by Chick pea (*Cicer arietinum* L.)12 hrsseed exudate.

Microorganism	Nanoparticles	Control	Sample	Measure of zone of inhibition in mm		
				50	100	150
E.coli	Silver	5	4	9	11	13
	Copper	22	11	19	24	28
	Zinc	20	11	18	23	23
Klebsiella species	Silver	10	9	11	12	13
	Copper	20	11	18	21	21
	Zinc	19	11	19	20	24
Pseudomonas aeruginosa	Silver	6	5	11	13	17
	Copper	17	10	18	20	25
	Zinc	20	13	18	20	22
Salmonella typhi	Silver	10	6	10	11	12
	Copper	20	11	15	18	19
	Zinc	19	10	15	20	22
Staphylococcus aureus	Silver	14	9	15	18	21
	Copper	21	10	15	20	23
	Zinc	16	11	17	20	22

Table.7 Zone of inhibition against various bacteria (*E. coli*, *Klebsiella species*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococus aureus*) using nanoparticles produced by Chick pea (*Cicer arietinum* L.) 24 hrs seed exudate.

Microorganism	Nanoparticles	Control	Sample	Measure of zone of inhibition in mm		
				50	100	150
E.coli	Silver	5	4	9	10	13
	Copper	12	10	15	20	25
	Zinc	23	10	18	24	25
Klebsiella species	Silver	7	6	9	12	13
	Copper	13	10	13	16	17
	Zinc	17	10	15	16	18
Pseudomonas aeruginosa	Silver	5	4	8	11	13
	Copper	17	10	15	17	18
	Zinc	18	10	19	23	25
Salmonella typhi	Silver	10	6	10	11	12
	Copper	17	10	13	15	17
	Zinc	20	10	18	21	23
Staphylococcus aureus	Silver	14	9	15	18	21
	Copper	15	10	13	14	16
	Zinc	18	10	20	25	25

Table.8 UV absorption spectrum of Sliver nanoparticles formed fromChick pea (*Cicer arietinum* L.) 6 hrs seed exudate during different time of incubation.

Time	385 nm	435nm	560nm	680nm
½ hr	**0.382**	**0.424**	**0.305**	**0.103**
1 hr	**0.438**	**0.488**	**0.332**	**0.113**
1 ½ hr	**0.453**	**0.498**	**0.365**	**0.120**
2 hr	**0.488**	**0.555**	**0.379**	**0.117**
2 ½ hr	**0.605**	**0.777**	**0.145**	**0.021**
Blank	**0.033**	**0.028**	**0.027**	**0.016**

Table.9 UV absorption spectrum of Sliver nanoparticles formed fromChick pea (*Cicer arietinum* L.) 12 hrs seed exudate during different time of incubation.

Time	350 nm	385 nm	435 nm	560 nm
½ hr	**0.552**	**0.667**	**0.178**	**0.027**
1 hr	**0.630**	**0.822**	**0.218**	**0.032**
1 ½ hr	**0.687**	**0.883**	**0.260**	**0.038**
2 hr	**0.605**	**0.777**	**0.145**	**0.021**
2 ½ hr	**0.642**	**0.810**	**0.160**	**0.025**
Blank	**0.333**	**0.273**	**0.202**	**0.016**

Table.10 UV absorption spectrum of Copper nanoparticles formed from Chick pea (*Cicer arietinum* L.) 6 hrs seed exudate during different time of incubation.

Time	385 nm	435nm	560nm	680nm
½ hr	0.142	0.089	0.041	0.434
1 hr	0.107	0.058	0.034	0.433
1 ½ hr	0.095	0.054	0.027	0.430
2 hr	0.095	0.050	0.024	0.429
2 ½ hr	0.088	0.046	0.023	0.432
Blank	0.076	0.054	0.016	0.365

Table.11 UV absorption spectrum of Copper nanoparticles formed from Chick pea (*Cicer arietinum* L.) 12 hrs seed exudates during different time of incubation.

Time	350 nm	385 nm	435 nm	560 nm
½ hr	0.148	0.092	0.042	0.437
1 hr	0.134	0.083	0.040	0.428
1 ½ hr	0.118	0.078	0.034	0.431
2 hr	0.087	0.050	0.025	0.432
2 ½ hr	0.080	0.039	0.022	0.425
Blank	0.025	0.015	0.019	0.411

Table.12 Description of UV absorption spectrum of Sliver nanoparticles formed from Black gram (*Vigna mungo* (L.) Hepper 6 hrs seed exudates during different time of incubation.

Time	350 nm	385 nm	435 nm	560 nm
½ hr	0.414	0.354	0.208	0.108
1 hr	0.429	0.377	0.233	0.125
1 ½ hr	0.858	0.788	0.53	0.296
2 hr	0.838	0.788	0.558	0.31
2 ½ hr	0.774	0.75	0.554	0.308
Blank	0.307	0.299	0.203	0.107

Table.13 Description of UV absorption spectrum of Sliver nanoparticles formed from Black gram (*Vigna mungo* (L.) Hepper, 12 hrs during different time of incubation.

Time	350 nm	385 nm	435 nm	560 nm
½ hr	1.074	1.350	0.530	0.272
1 hr	1.776	1.984	1.302	0.700
1 ½ hr	2.14	2.496	1.324	0.652
2 hr	2.488	2.872	1.42	0.698
2 ½ hr	2.672	3.1	1.812	0.884
Blank	0.307	0.298	0.203	0.109

Table.14 Description of UV absorption spectrum of Copper nanoparticles formed from Black gram (*Vigna mungo* (L.) Hepper 6 hrs seed exudates during different time of incubation.

Time	350 nm	385 nm	435 nm	560 nm
½ hr	0.066	0.053	0.055	0.505
1 hr	0.014	0.017	0.028	0.464
1 ½ hr	0.104	0.089	0.077	0.495
2 hr	0.072	0.055	0.058	0.489
2 ½ hr	0.063	0.055	0.056	0.498
Blank	0.039	0.033	0.025	0.417

Table.15 Description of UV absorption spectrum of Copper nanoparticles formed from Black gram (*Vigna mungo* (L.) Hepper 12 hrs seed exudates during different time of incubation.

Time	350 nm	385 nm	435 nm	560 nm
½ hr	0.466	0.302	0.183	0.613
1 hr	0.565	0.341	0.203	0.608
1 ½ hr	0.515	0.302	0.188	0.597
2 hr	0.731	0.448	0.263	0.662
2 ½ hr	0.790	0.496	0.286	0.686
Blank	0.039	0.033	0.025	0.417

Table.16 Description of UV absorption spectrum of Sliver nanoparticles formed from Black gram (*Vigna mungo* (L.) Hepper 24 hrs seed exudate during different time of incubation.

Time	350 nm	385 nm	435 nm	560 nm
½ hr	0.582	0.412	0.231	0.146
1 hr	0.615	0.486	0.291	0.202
1 ½ hr	0.559	0.439	0.273	0.188
2 hr	0.545	0.421	0.246	0.148
2 ½ hr	0.580	0.466	0.265	0.165
Blank	0.307	0.298	0.207	0.163

Table.17 Description of UV absorption spectrum of Sliver nanoparticles formed from Chick pea (*Cicer arietinum* L.) 24 hrs seed exudate during different time of incubation.

Time	350 nm	385 nm	435 nm	560 nm
½ hr	0.890	0.953	0.345	0.112
1 hr	1.057	1.369	0.548	0.157
1 ½ hr	1.248	1.376	0.495	0.171
2 hr	1.343	1.614	0.635	0.199
2 ½ hr	1.15	1.89	0.776	0.316
Blank	0.541	0.424	0.246	0.146

Table.18 Description of UV absorption spectrum of Copper nanoparticles formed from Black gram (*Vigna mungo* (L.) Hepper 24 hrs seed exudates during different time of incubation.

Time	350 nm	385 nm	435 nm	560 nm
½ hr	0.263	0.204	0.151	0.537
1 hr	0.198	0.151	0.133	0.525
1 ½ hr	0.270	0.202	0.139	0.529
2 hr	0.268	0.205	0.147	0.516
2 ½ hr	0.263	0.195	0.141	0.515
Blank	0.138	0.096	0.077	0.464

Table.19 Description of UV absorption spectrum of Copper nanoparticles formed from Chick pea (*Cicer arietinum* L.) 24 hrs seed exudate during different time of incubation.

Time	350 nm	385 nm	435 nm	560 nm
½ hr	0.262	0.209	0.131	0.498
1 hr	0.200	0.131	0.073	0.454
1 ½ hr	0.214	0.152	0.093	0.488
2 hr	0.225	0.158	0.103	0.483
2 ½ hr	0.215	0.153	0.101	0.496
Blank	0.138	0.096	0.077	0.464

Table.20 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.21 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.1 Map of Kerala showing the sample collection point.

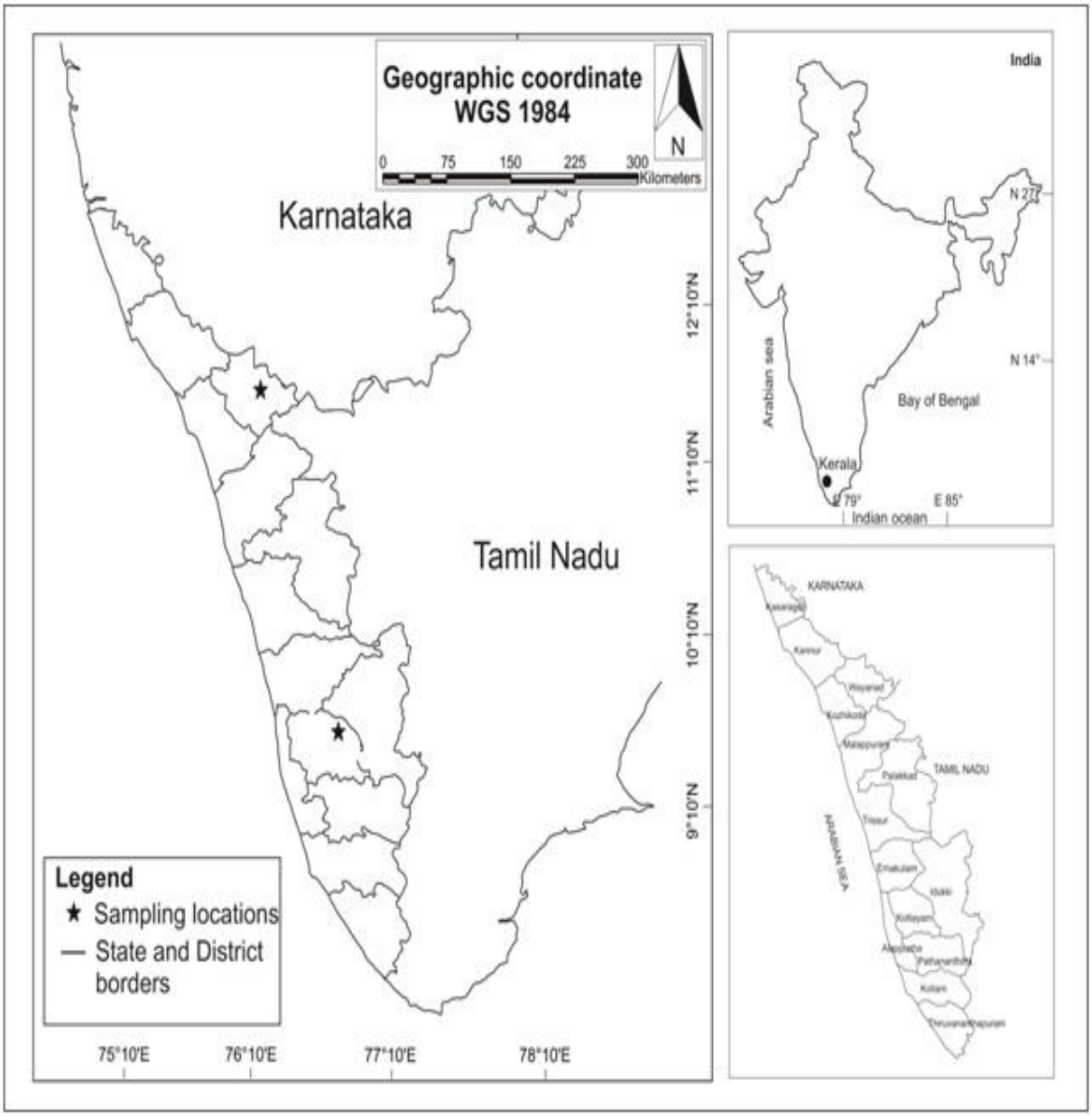


Fig.2 Chick pea (*Cicer arietinum* L.),description a) germinating seed, b) two different varieties of chick pea, c) mature pods and seeds, d) collecting fresh seeds, e) fresh seeds and pods opened, f) dried and split seeds ready for cooking. Photo courtesy: Wikipedia.

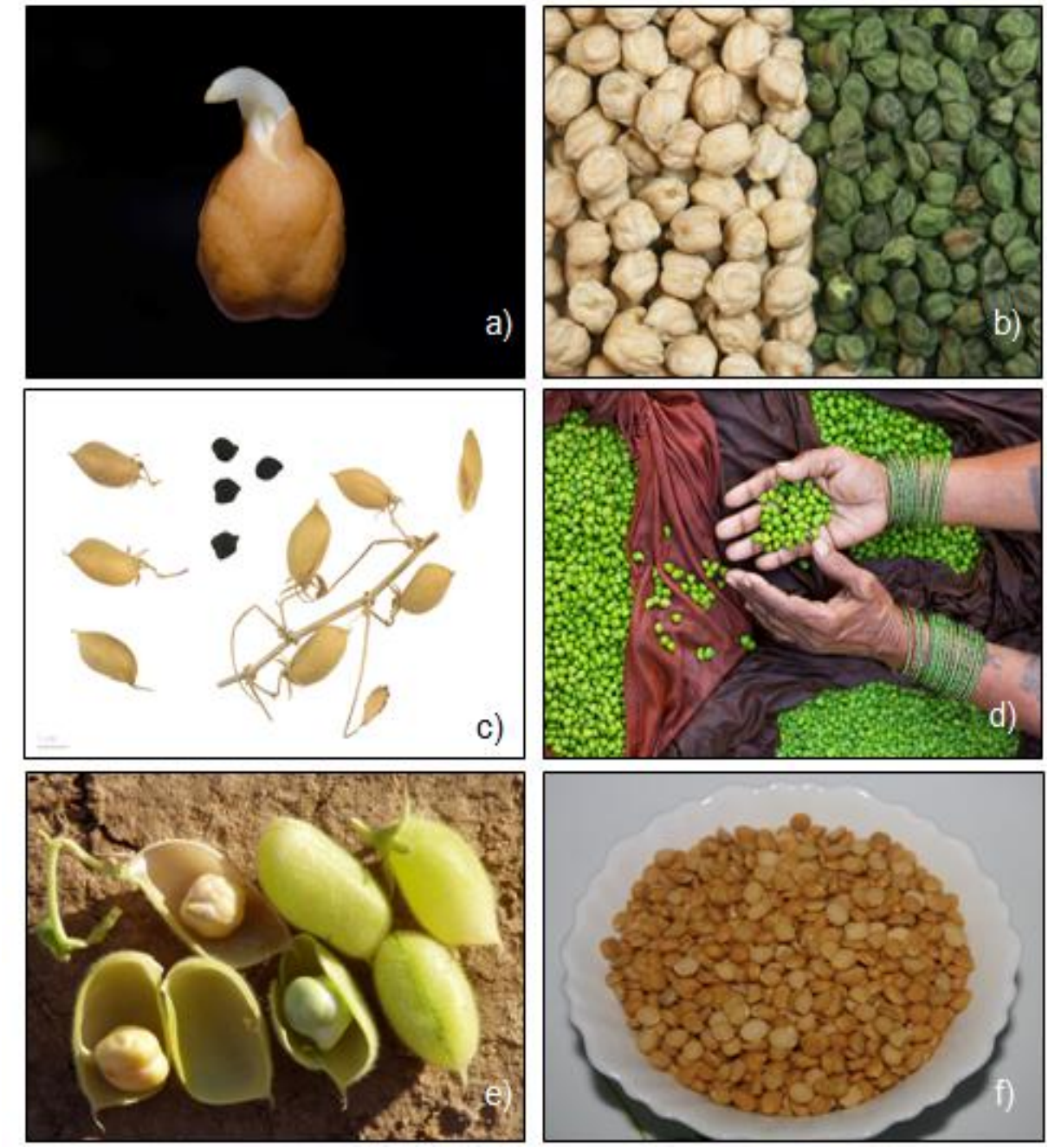


Fig.3 Black gram (*Vigna mungo* (L.) Hepper) description a) harvested seeds, b) vada (breakfast snacks) made with black gram powder, c) dosa (breakfast) made with black gram powder, d) mature pods on plant, e) chutney (curry) made with black gram, f) seeds with outer skin removed (polished). Photo courtesy: Wikipedia (a, b, c, d and f).

Fig.4 Description of the various seeds a) Cowpea* (*Vigna unguiculate*), b) Black gram (*Vigna mungo* (L.) Hepper, c) Peanut* (*Arachis hypogaea* L.), d) Chick pea (*Cicer arietinum* L.), e) Fenugreek* (*Trigonella foenum-graecum*), f) Mungbean* (*Vigna radiata*), g) Green peas* (*Pisum sativum* L.), h) all the seeds mixed together. * data not provided.

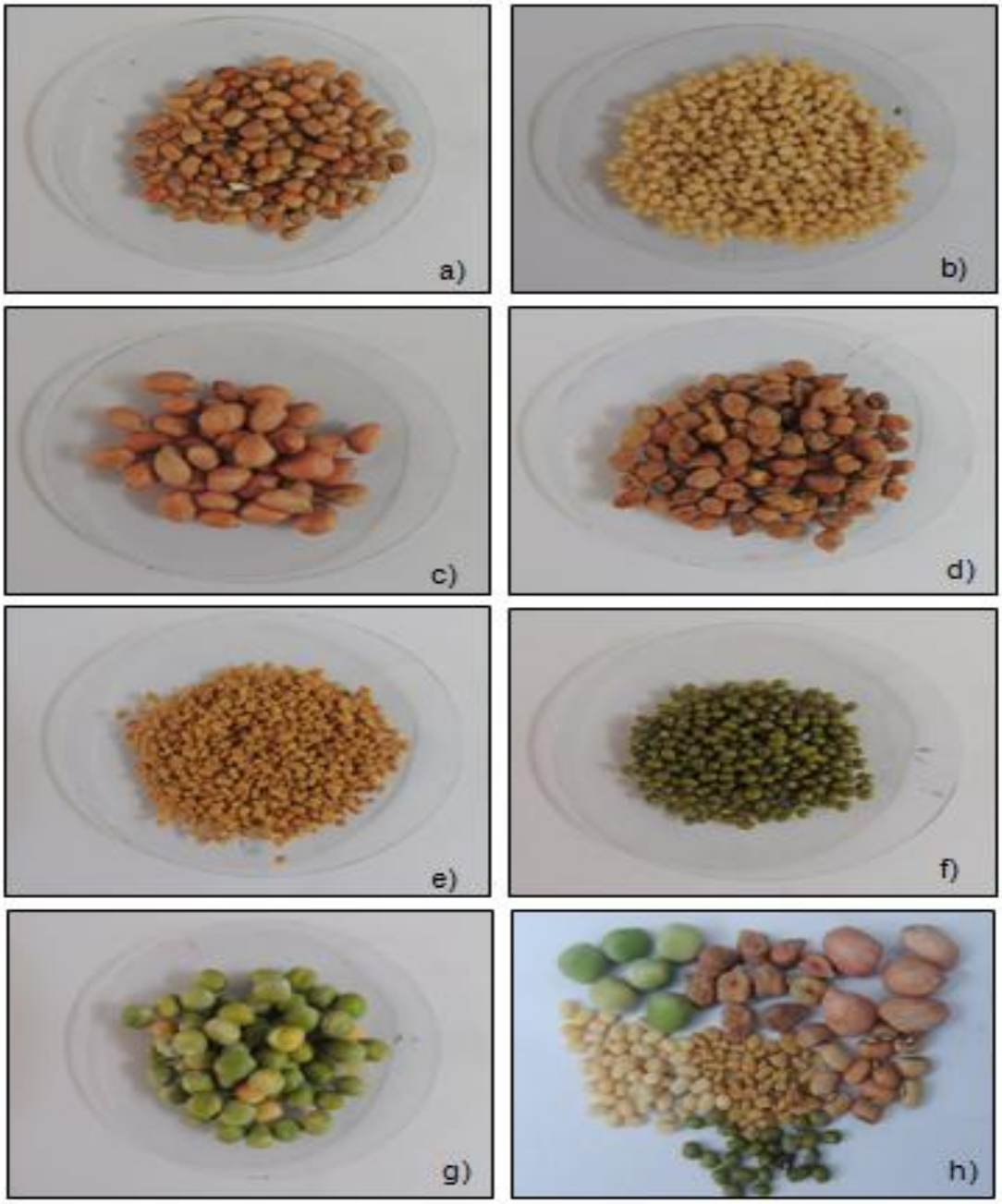


Fig.5 Description of the seed exudates used for making nanoparticles a) Cowpea* (*Vigna unguiculate*), b) Black gram (*Vigna mungo* (L.) Hepper, c) Peanut* (*Arachis hypogaea* L.), d) Chick pea (*Cicer arietinum* L.), e) Fenugreek* (*Trigonella foenum-graecum*), f) Mungbean* (*Vigna radiata*), g) Green peas* (*Pisum sativum* L.), h) all the seeds mixed together. * data not provided.

Fig.6 Seed exudates used for making nanoparticles a) Cowpea* (*Vigna unguiculate*), Black gram (*Vigna mungo* (L.) Hepper, c) Peanut* (*Arachis hypogaea* L.), d) Chick pea (*Cicer arietinum* L.), e) Fenugreek* (*Trigonella foenum-graecum*), f) Mungbean* (*Vigna radiata*), g) Green peas* (*Pisum sativum* L.), h) and i) all the seeds placed in different extraction bottles. * data not provided.

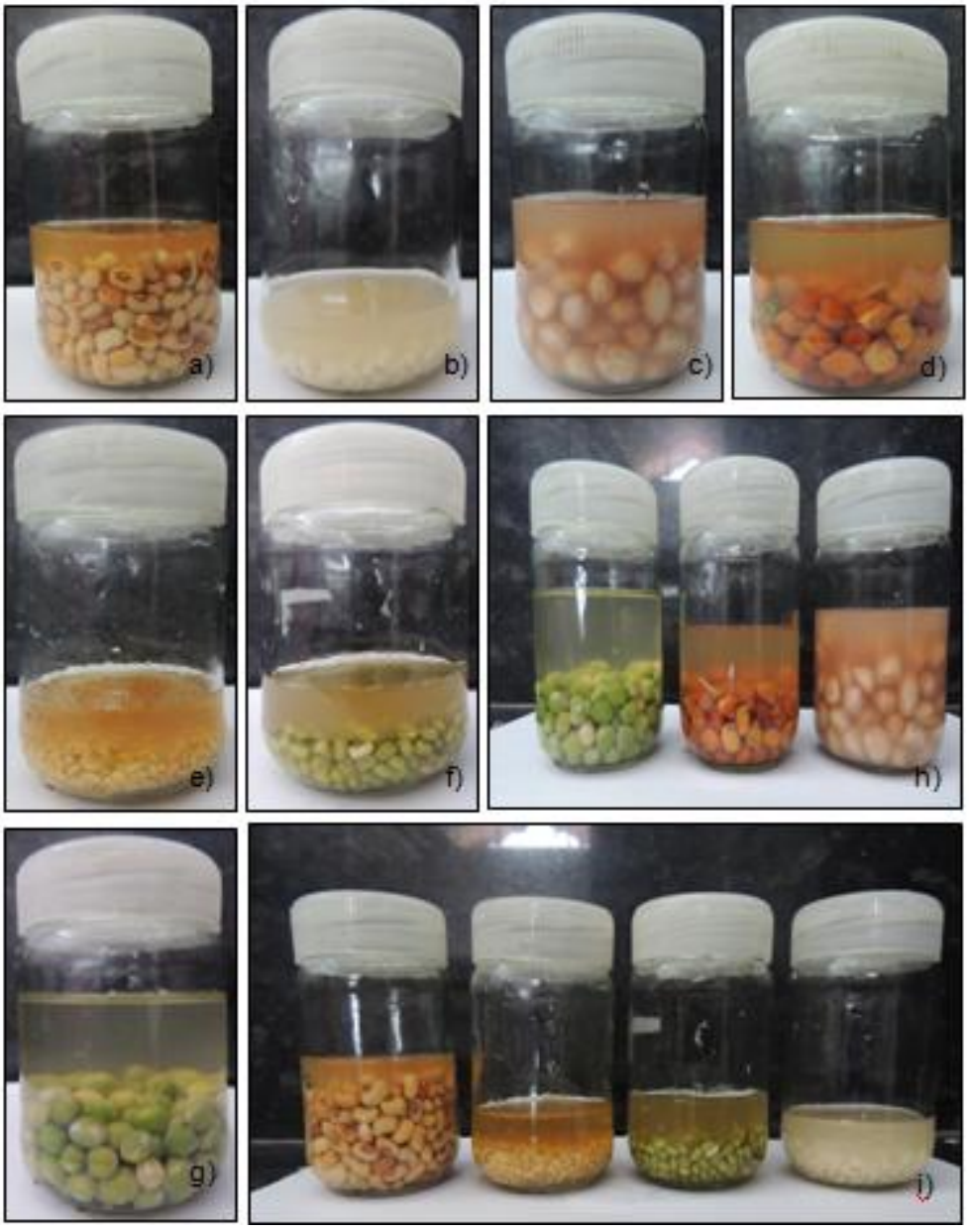


Fig.7 Description of the a) and b seed exudates used for making nanoparticles (Black gram and Chick pea), c) and d)green synthesized zinc nanoparticles, e) and f) green synthesized copper nanoparticles, g) and h)green synthesized silver nanoparticles.

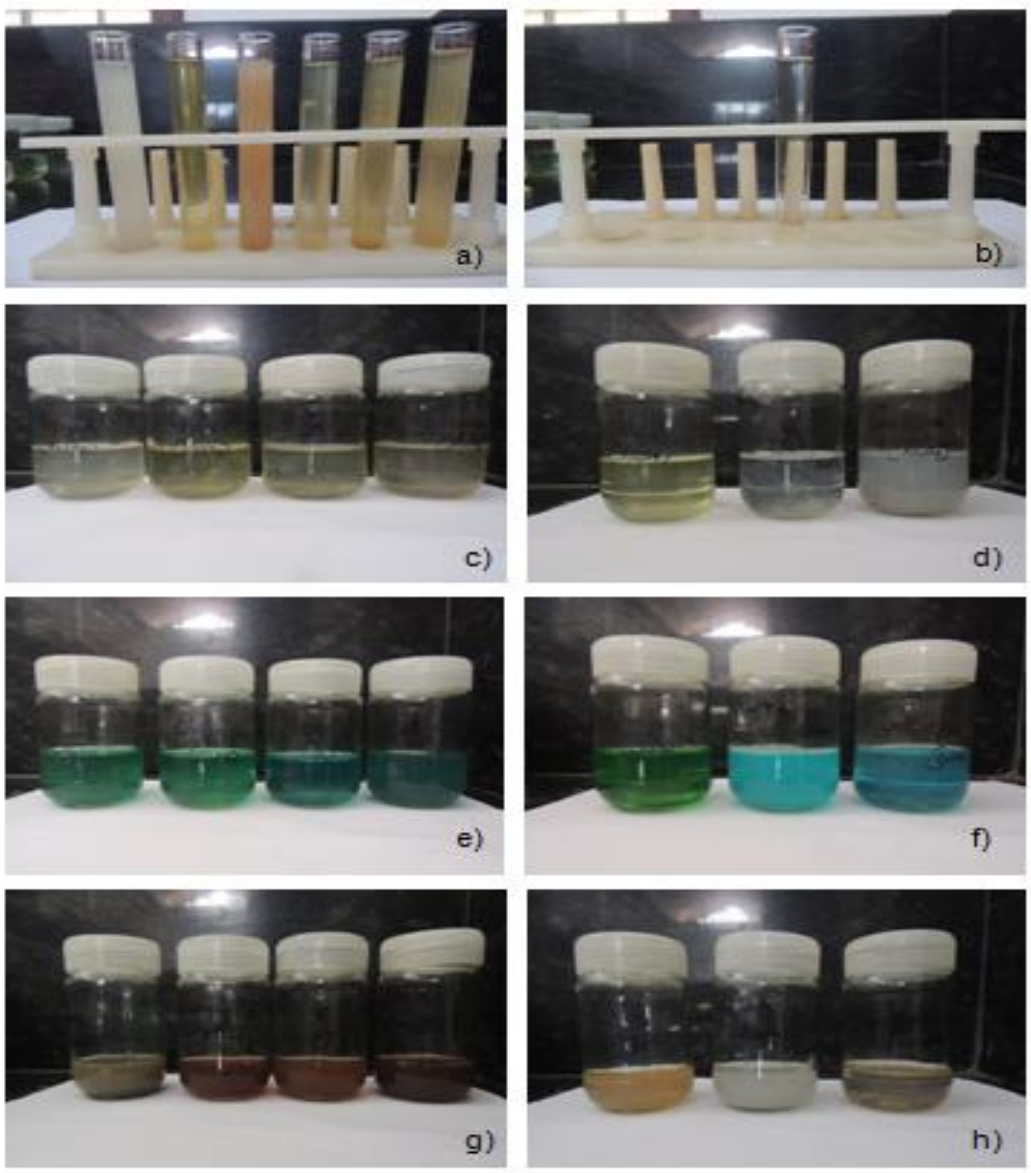


Fig.8 Antibacterial activity study using well diffusion method of Chick pea (*Cicer arietinum* L.) and Fenugreek* (*Trigonella foenum-graecum*) 12hrs seed exudates nanoparticles (Cu)a) *Salmonella typhi* control plate (Cu), b) *Salmonella typhi* green synthesised nanoparticle test plate (Cu; 50, 100 and 150 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 (Fenugreek (*Trigonella foenum-graecum*).* data not provided.

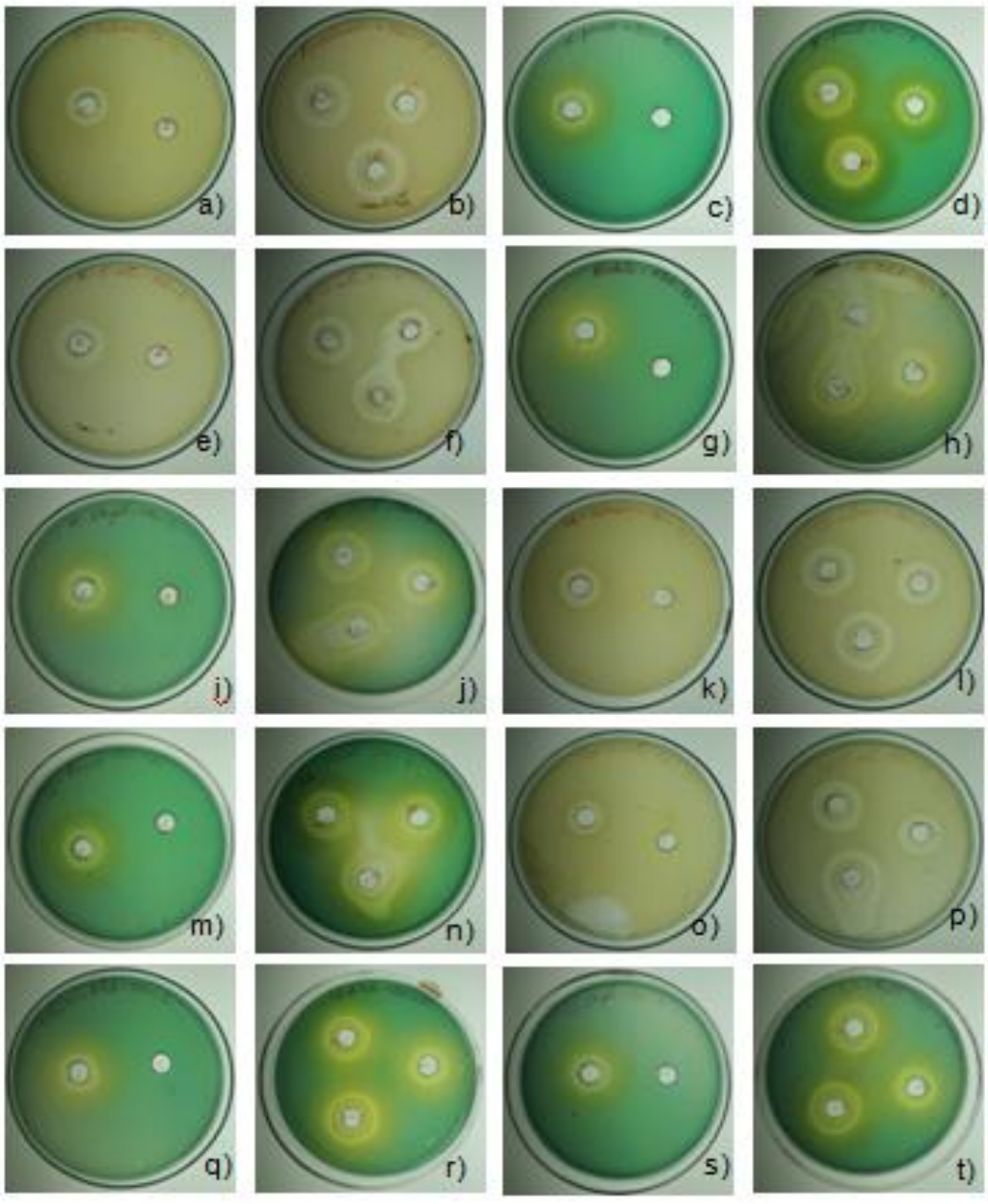


Fig.9 Antibacterial activity study using well diffusion method of Peanut* (*Arachis hypogaea* L.) and Chick pea (*Cicer arietinum* L.) 12 hrs seed exudates nanoparticles (Cu) a) *Salmonella typhi* control plate (Cu), b) *Salmonella typhi* green synthesised nanoparticle test plate (Cu; 50, 100 and 150 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 (Chick pea (*Cicer arietinum* L.).* data not provided.

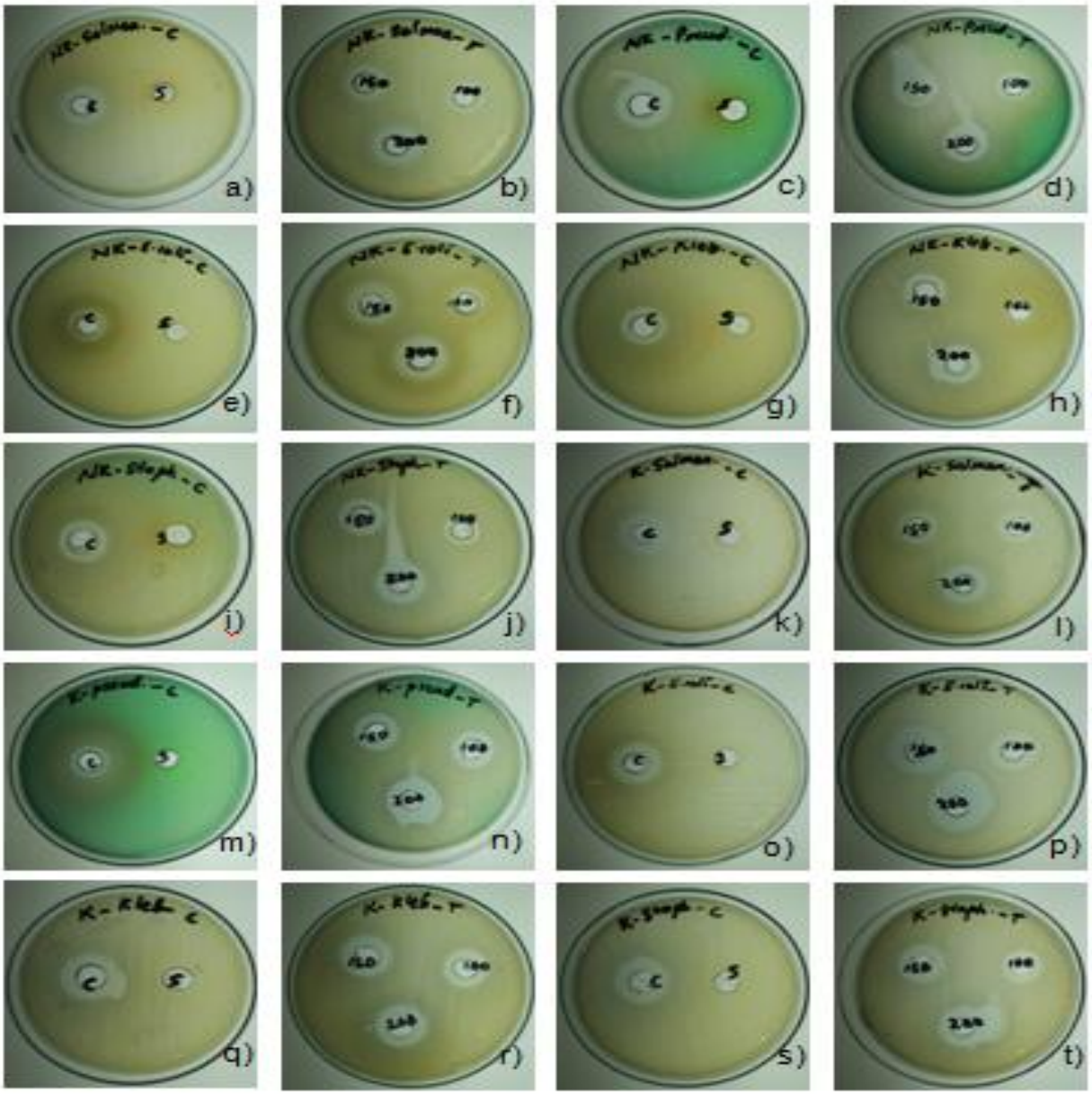


Fig.10 Antibacterial activity study using well diffusion method of Chick pea (*Cicer arietinum* L.) and Cowpea* (*Vigna unguiculate*) 12 hrs seed exudates nanoparticles (Zn)a) *Salmonella typhi* control plate (Zn), b) *Salmonella typhi* green synthesised nanoparticle test plate (Zn; 20, 40 and 60 µl), 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 (Cowpea (*Vigna unguiculate*).* data not provided.

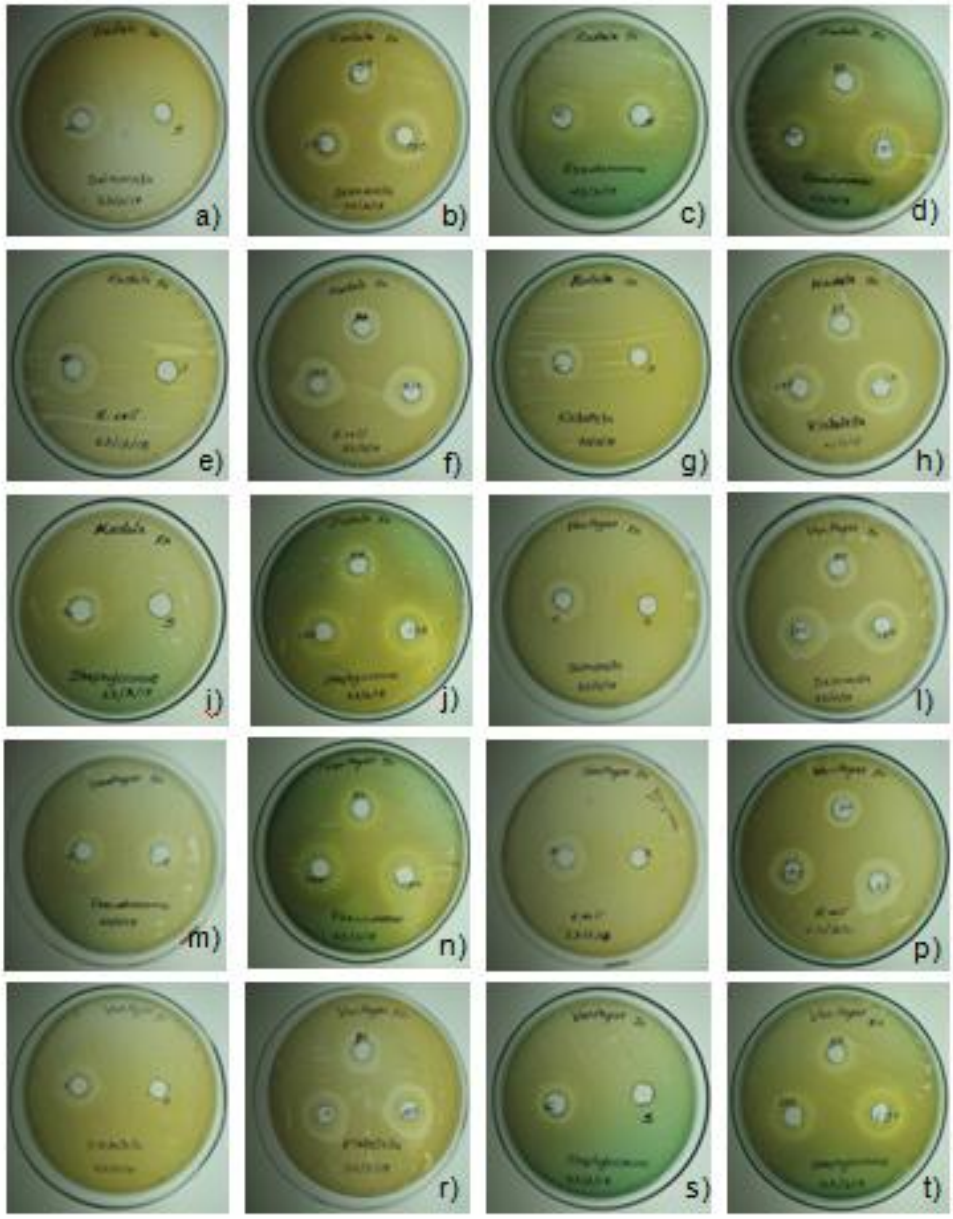


Fig.11 Antibacterial activity study using well diffusion method of Black gram (*Vigna mungo* (L.) Hepper and Fenugreek* (*Trigonella foenum-graecum*) 24 hrs seed exudates nanoparticles (Zn)a) *Salmonella typhi* control plate (Ag), b) *Salmonella typhi* green synthesised nanoparticle test plate (Zn; 50, 100 and 150 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 (Fenugreek (*Trigonella foenum-graecum*).

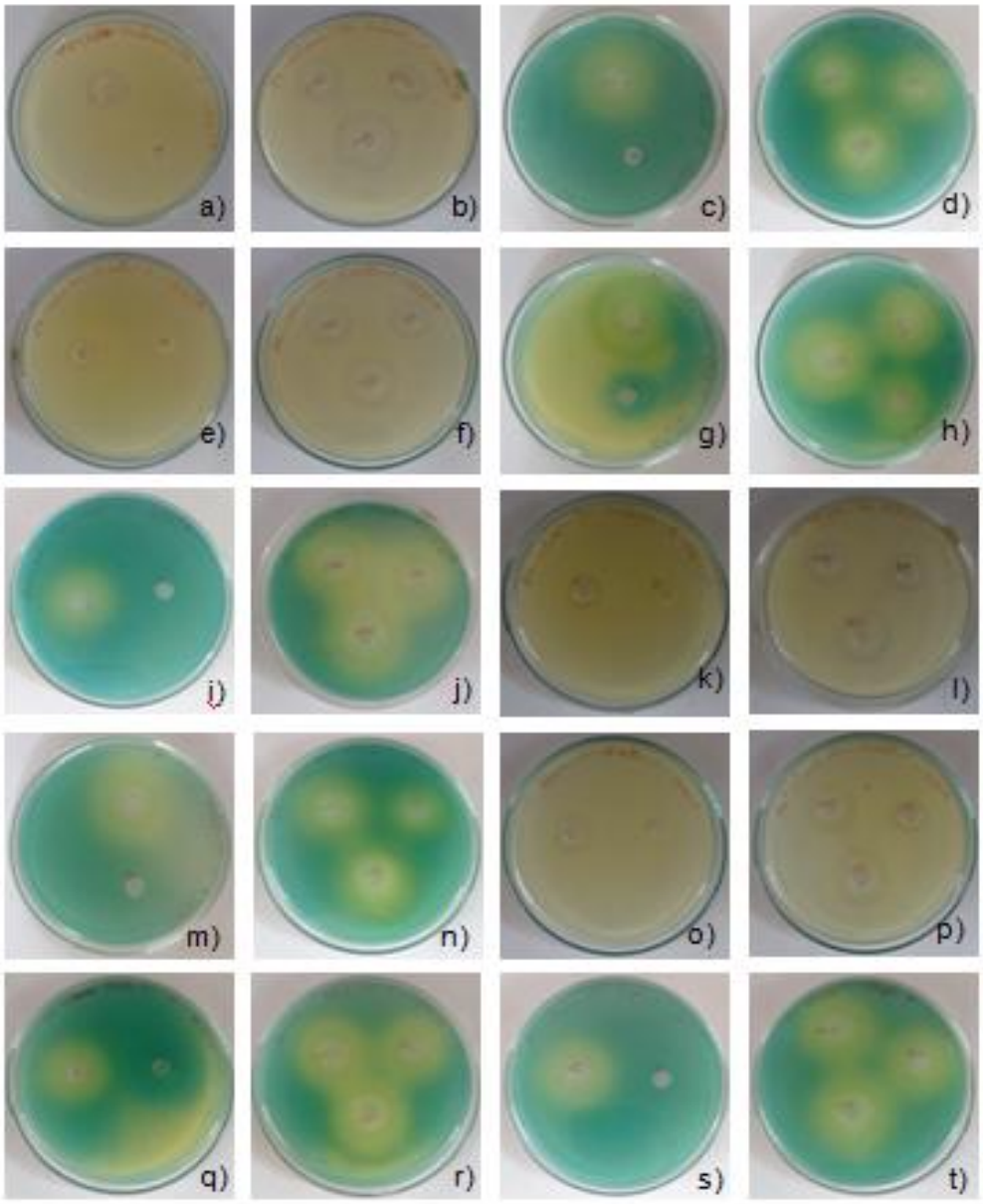


Fig.12 Antibacterial activity study using well diffusion method of Mungbean* (*Vigna radiata*) and Black gram (*Vigna mungo* (L.) Hepper 12 hrs seed exudates nanoparticles (Cu)a) *Salmonella typhi* control plate (Cu), b) *Salmonella typhi* green synthesised nanoparticle test plate (Cu; 50, 100 and 150 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 (Black gram (*Vigna mungo* (L.) Hepper.

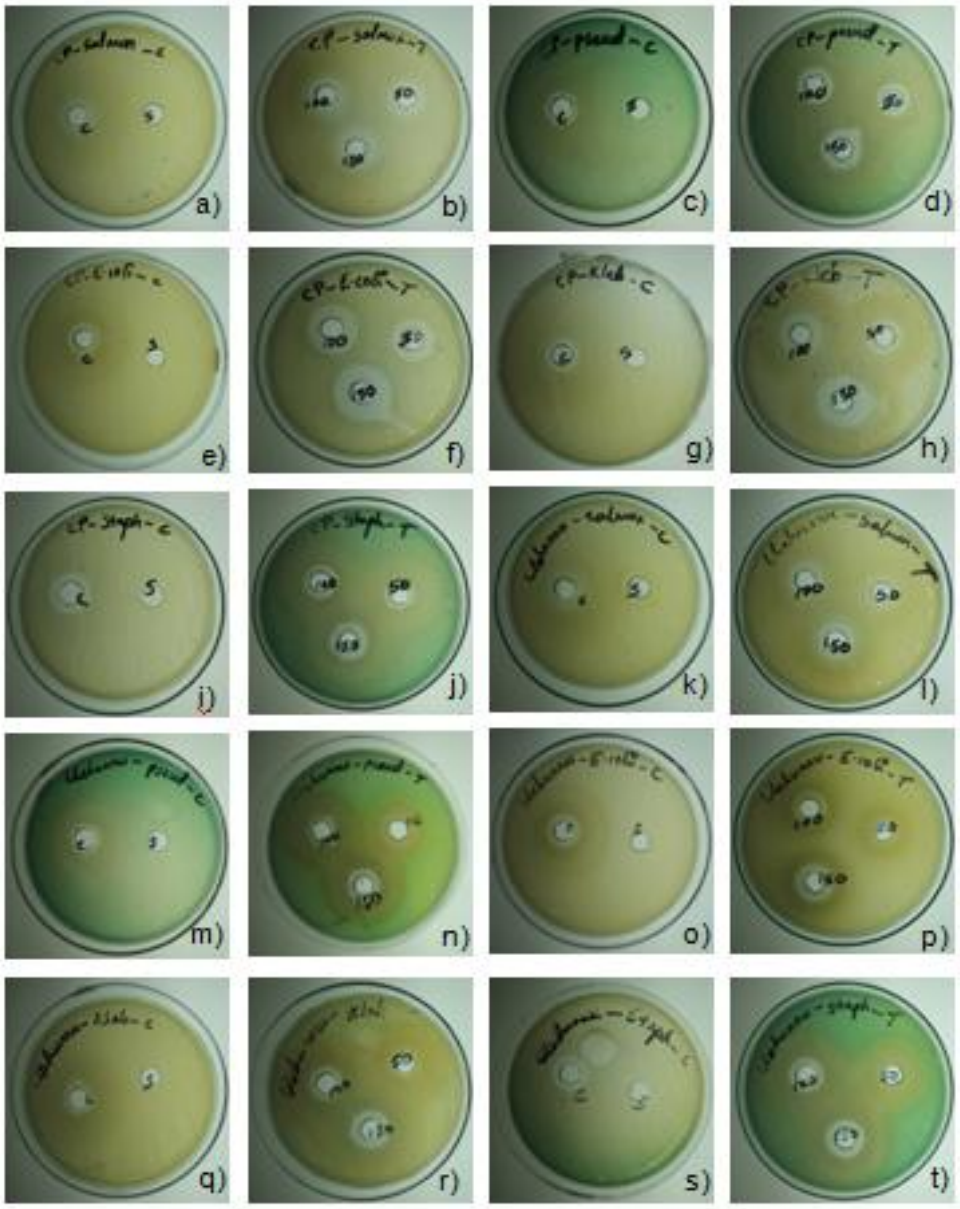


Fig.13 Antibacterial activity study using well diffusion method of Black gram (*Vigna mungo* (L.) Hepper 6 hrs seed exudates nanoparticles (Cu) and (Zn) a) *Salmonella typhi* control plate (Cu), b) *Salmonella typhi* green synthesised nanoparticle test plate (Cu; 50, 100 and 150 µl), c) *Pseudomonas 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 (Black gram (*Vigna mungo* (L.) Hepper.

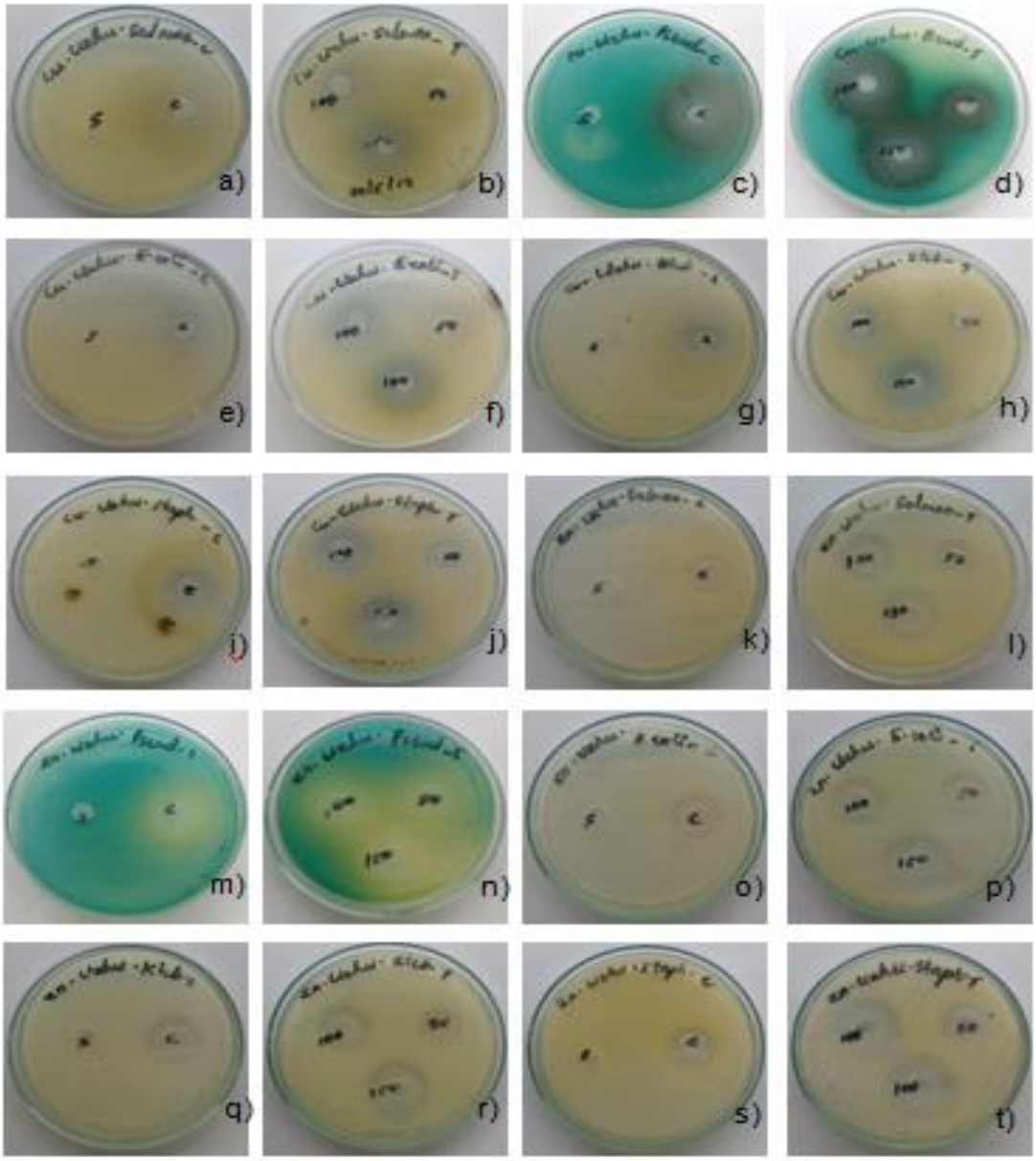


Fig.14 Antibacterial activity study using well diffusion method of Chick pea (*Cicer arietinum* L.) 6 hrs seed exudate nanoparticles (Cu) and (Zn) a) *Salmonella typhi* control plate (Cu), b) *Salmonella typhi* green synthesised nanoparticle test plate (Cu; 50, 100 and 150 µl), c) *Pseudomonas aeruginosa* 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 (Chick pea (*Cicer arietinum* L.).

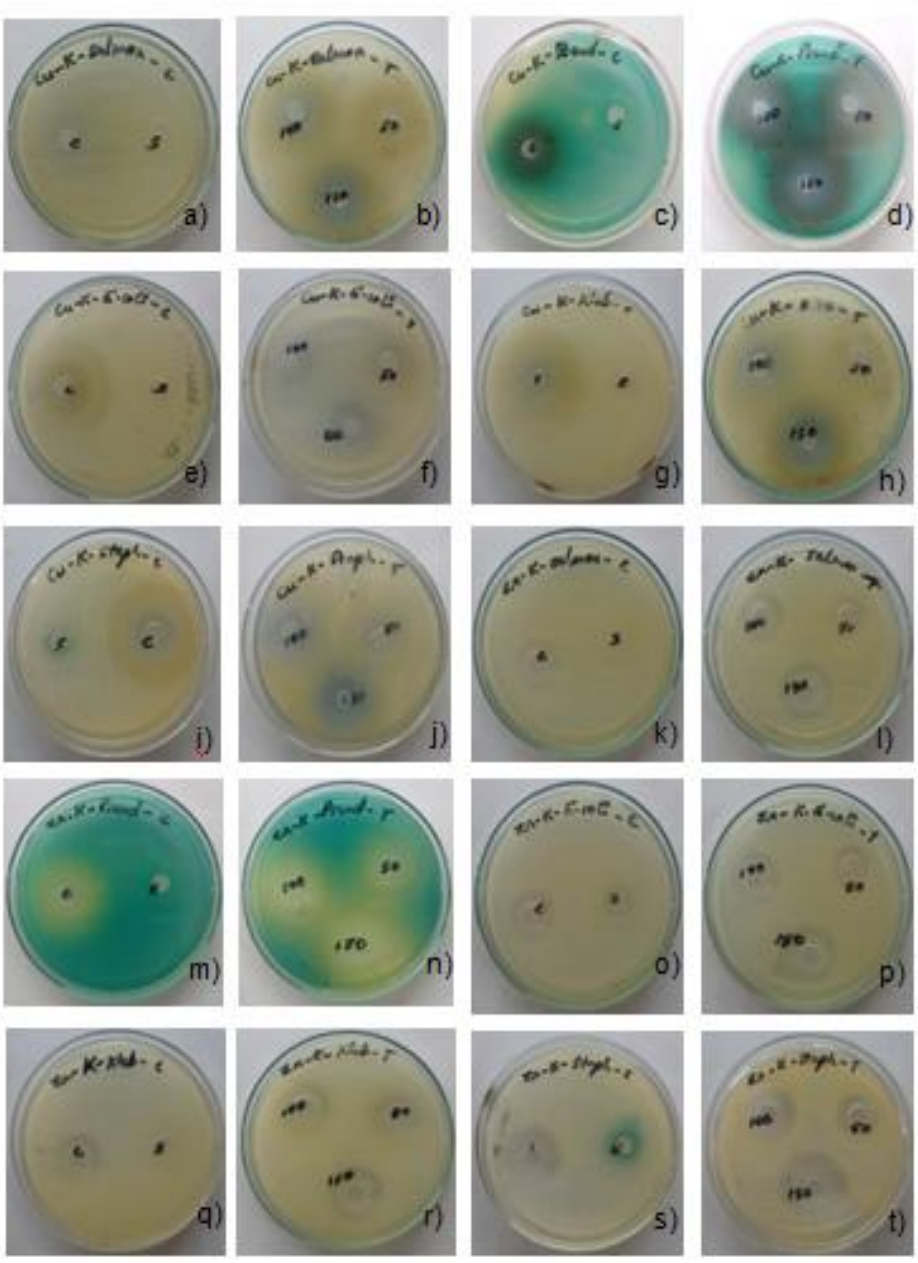


Fig.15 Antibacterial activity study using well diffusion method of Chick pea (*Cicer arietinum* L.) 24 hrs seed exudates nanoparticles (Cu) and (Zn) a) *Salmonella typhi* control plate (Cu), b) *Salmonella typhi* green synthesised nanoparticle test plate (Cu; 50, 100 and 150 µl), c) *Pseudomonas aeruginosa* 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 (Chick pea (*Cicer arietinum* L.).

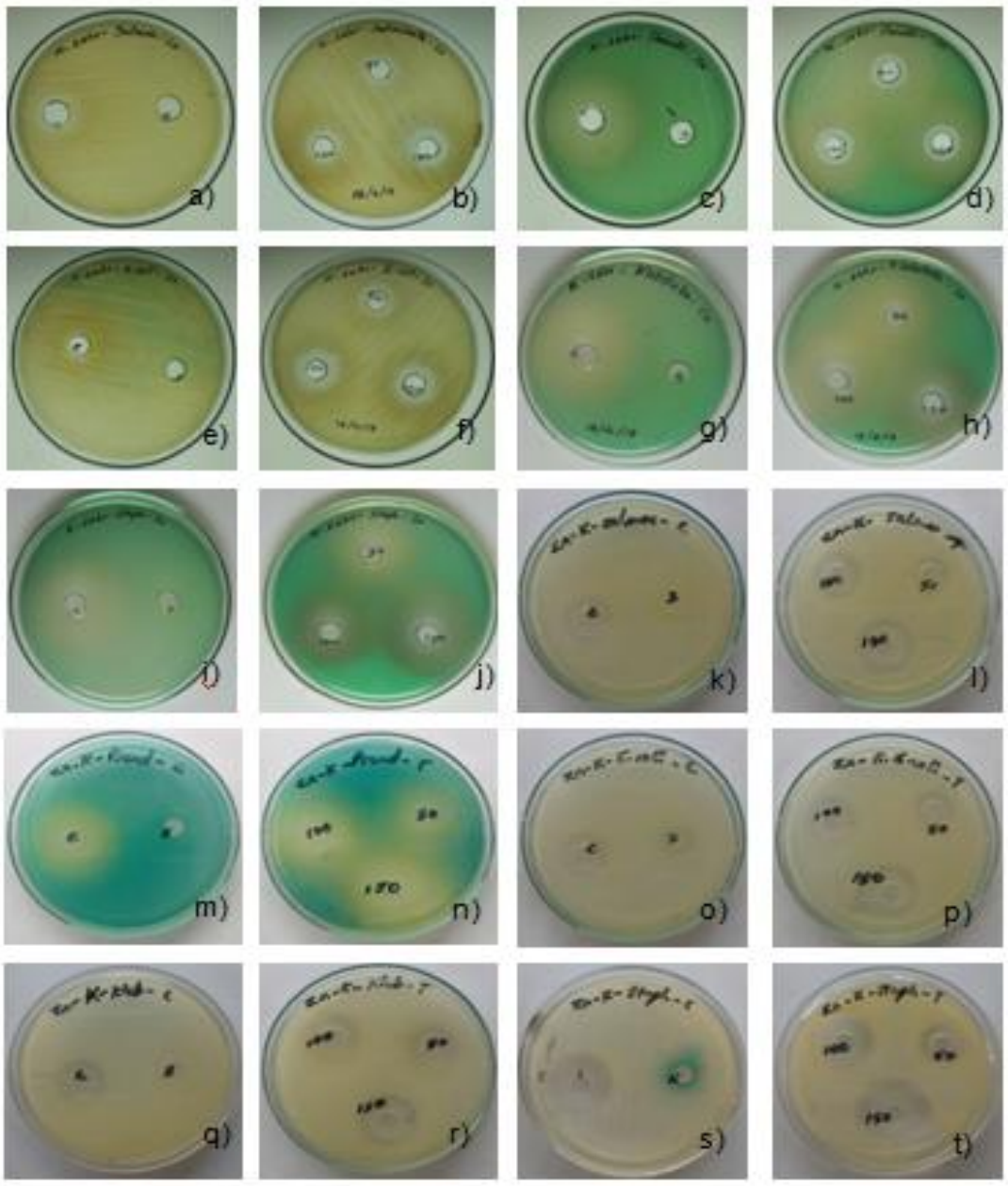


Fig.16 Antibacterial activity study using well diffusion method of Black gram (*Vigna mungo* (L.) Hepper and Fenugreek* (*Trigonella foenum-graecum*) 24 hrsseed exudates nanoparticles (Cu)a) *Salmonella typhi* control plate (Cu), b) *Salmonella typhi* green synthesised nanoparticle test plate (Cu; 50, 100 and 150 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 (Fenugreek (*Trigonella foenum-graecum*).

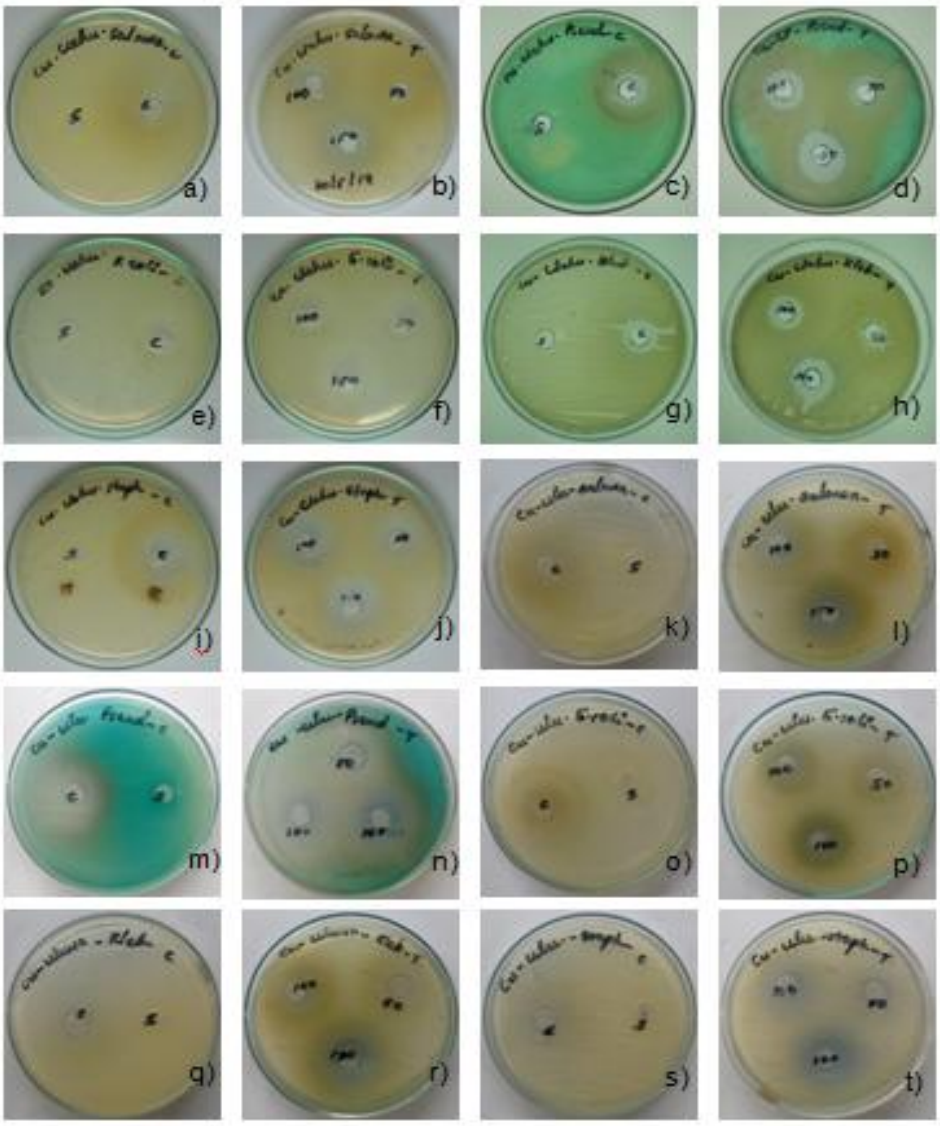


Fig.17 Antibacterial activity study using well diffusion method of Black gram (*Vigna mungo* (L.) Hepper 12 hrs seed exudates nanoparticles (Zn)a) *Salmonella typhi* control plate (Zn), b) *Salmonella typhi* green synthesised nanoparticle test plate (Zn; 50, 100 and 150 µl), c) *Pseudomonas aeruginosa* control, d) *Pseudomonas aeruginosa* test, e) *E. coli* control, f) *E. coli* test, g) *Klebsiella species* control, h) *Staphylococcus species* test, i) *Staphylococcus species* control, j) *Klebsiella species* test.

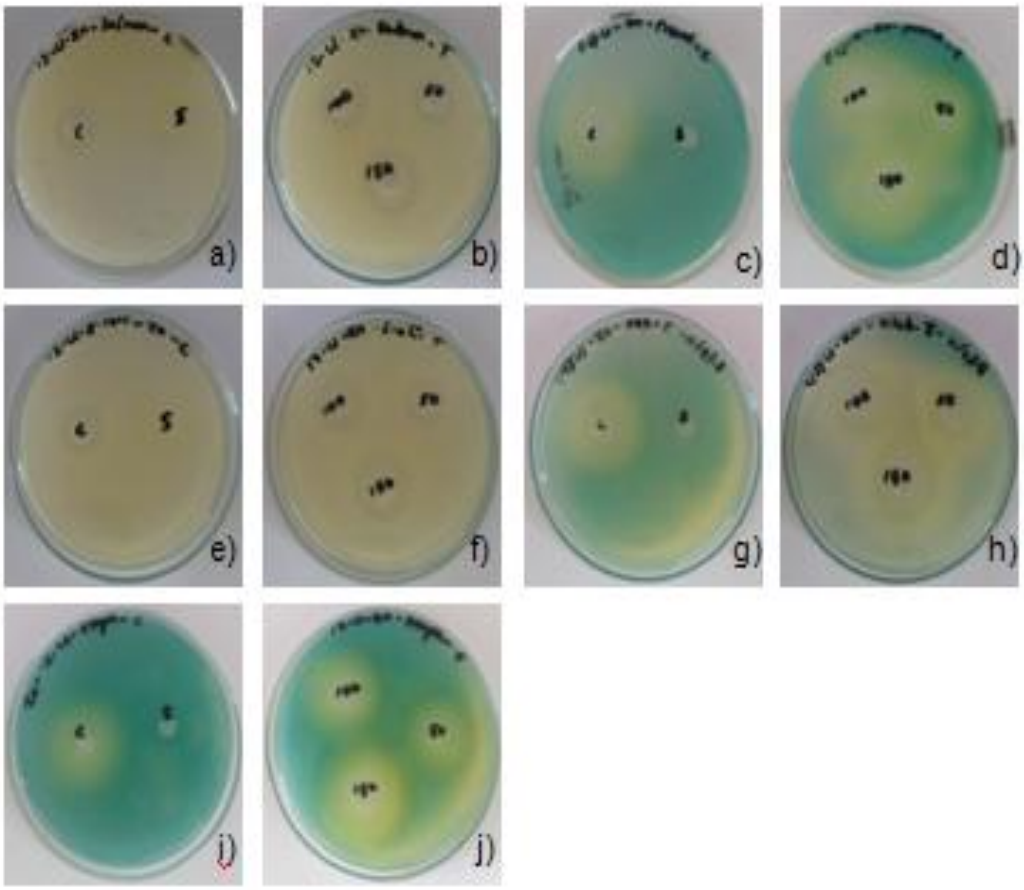


Fig.18 Silver nanoparticle formation of Black gram (*Vigna mungo* (L.) Hepper 12 hrs seed exudates under SEM imaging system with various resolutions.

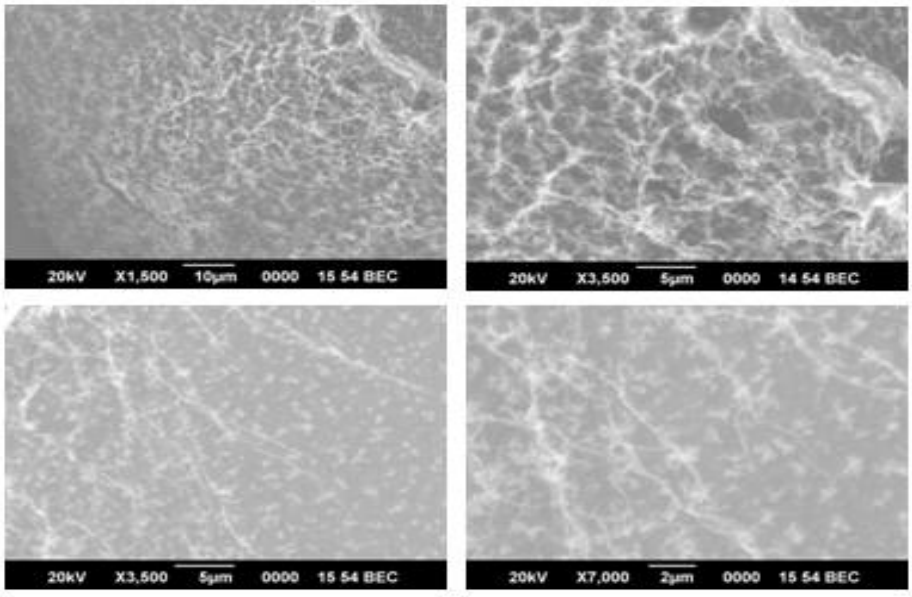


Fig.19 Copper nanoparticle formation of Black gram (*Vigna mungo* (L.) Hepper 12 hrs seed exudates under SEM imaging system with various resolutions.

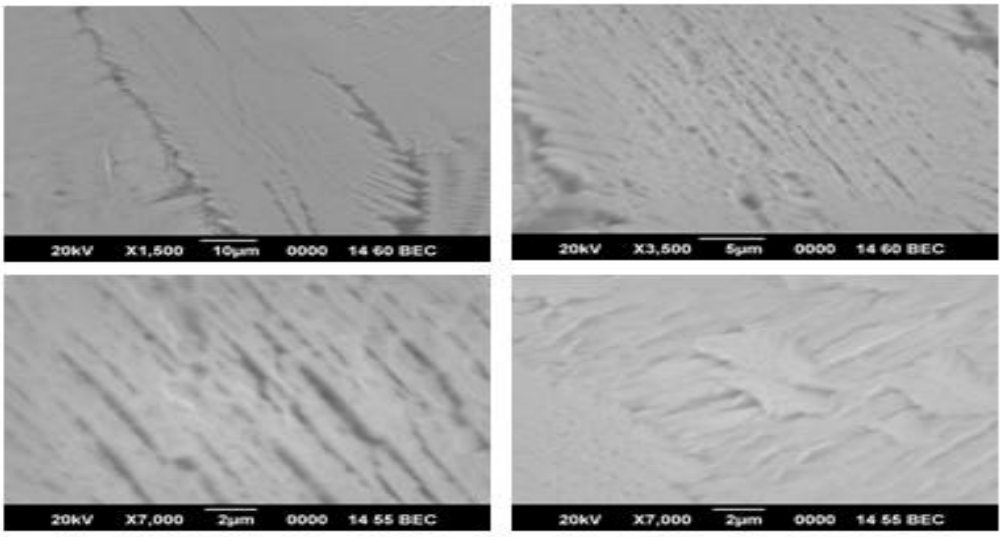


Fig.20 Zinc nanoparticle formation of Black gram (*Vigna mungo* (L.) Hepper 12 hrs seed exudates under SEM imaging system with various resolutions.

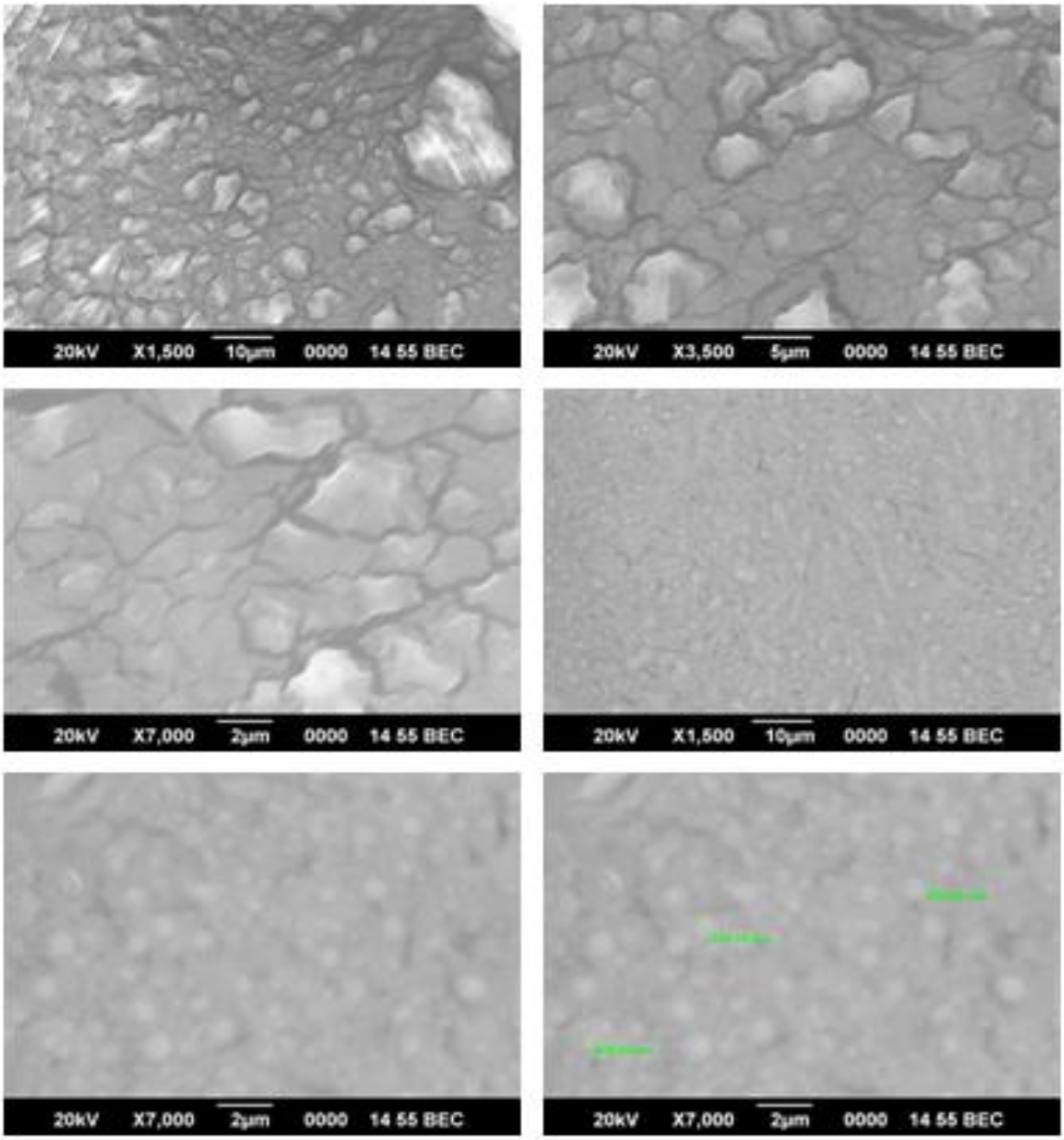


Fig.21 Silver nanoparticle formation of Black gram (*Vigna mungo* (L.) Hepper 12 hrs seed exudatesunder XRD imaging system.

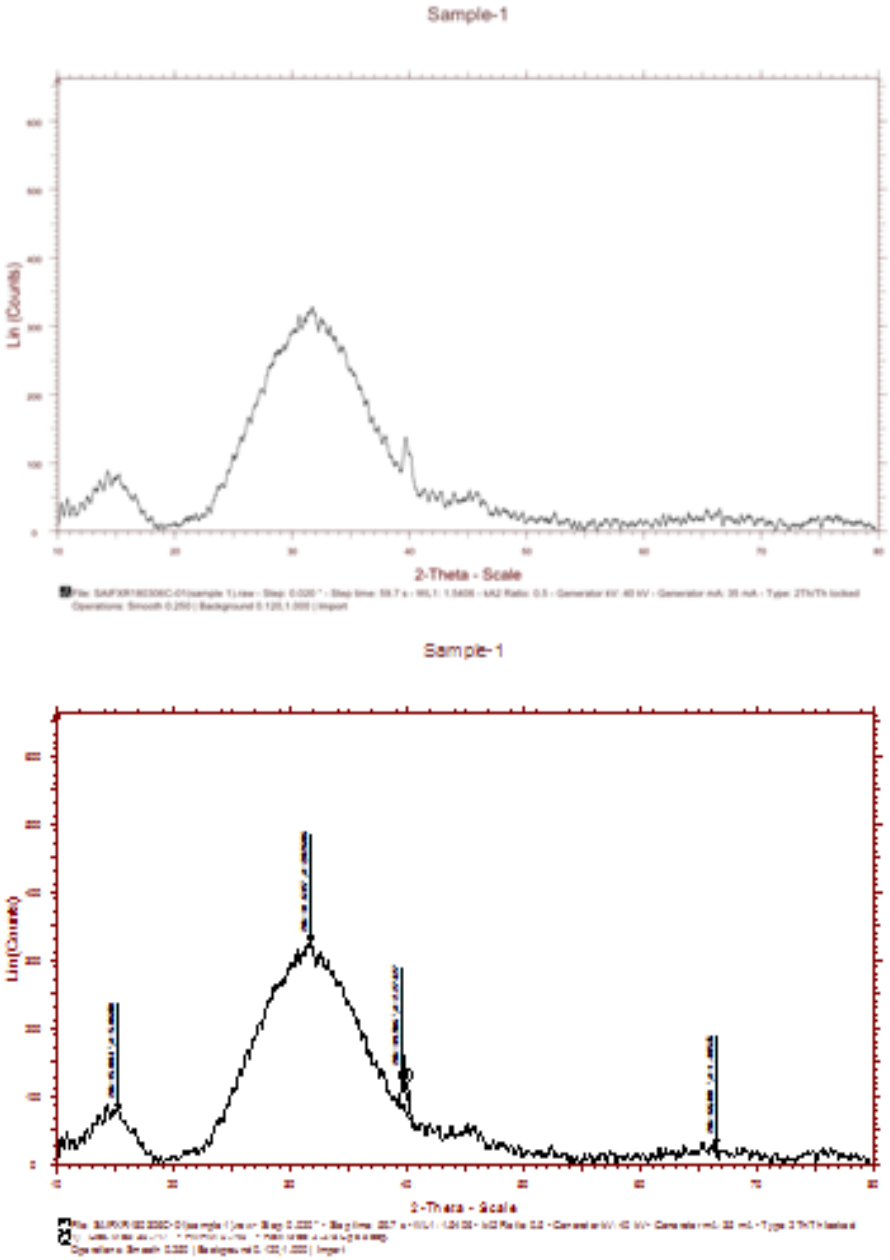


Fig.22 Copper nanoparticle formation of Black gram (*Vigna mungo* (L.) Hepper 12 hrs seed exudates under XRD imaging system.

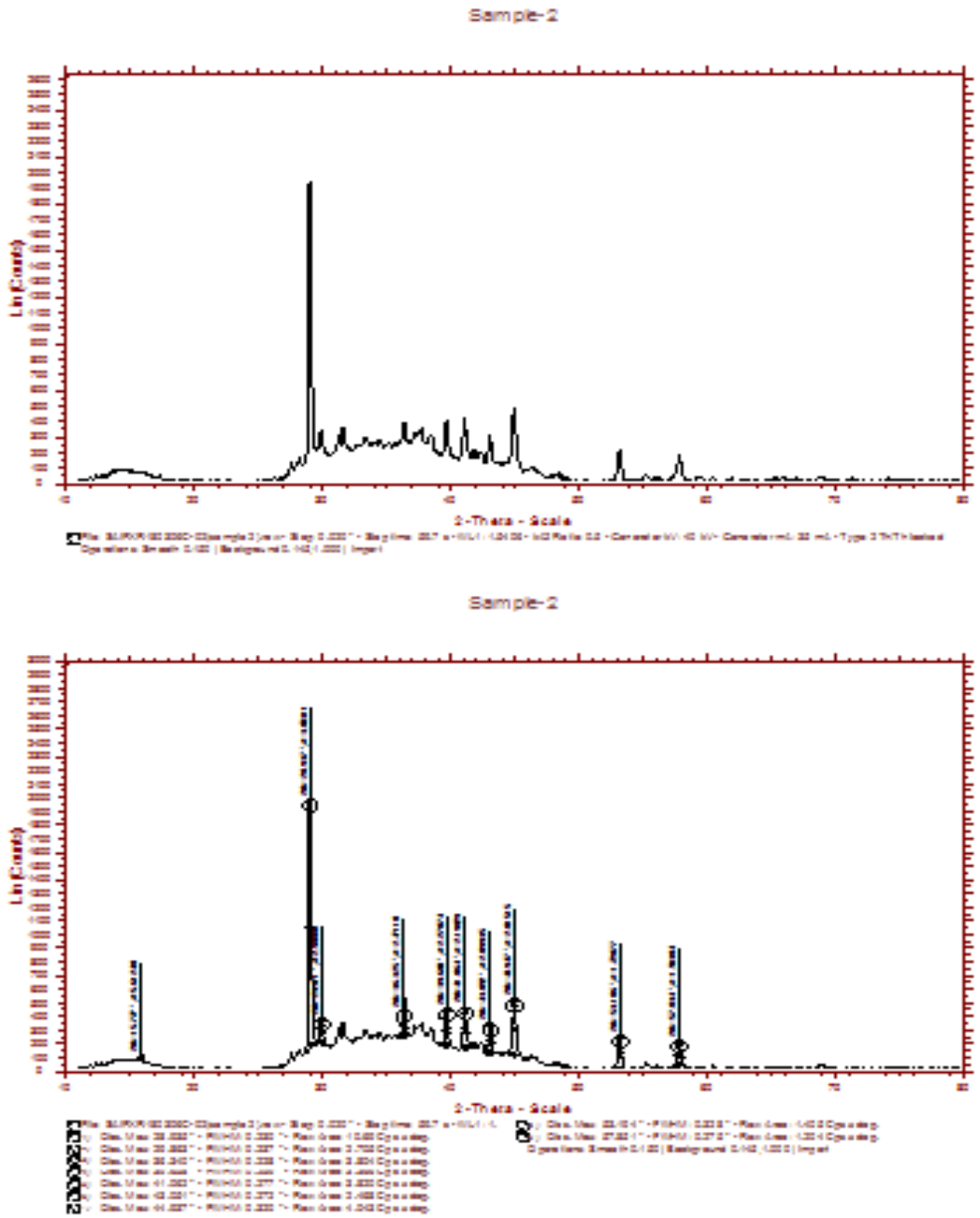
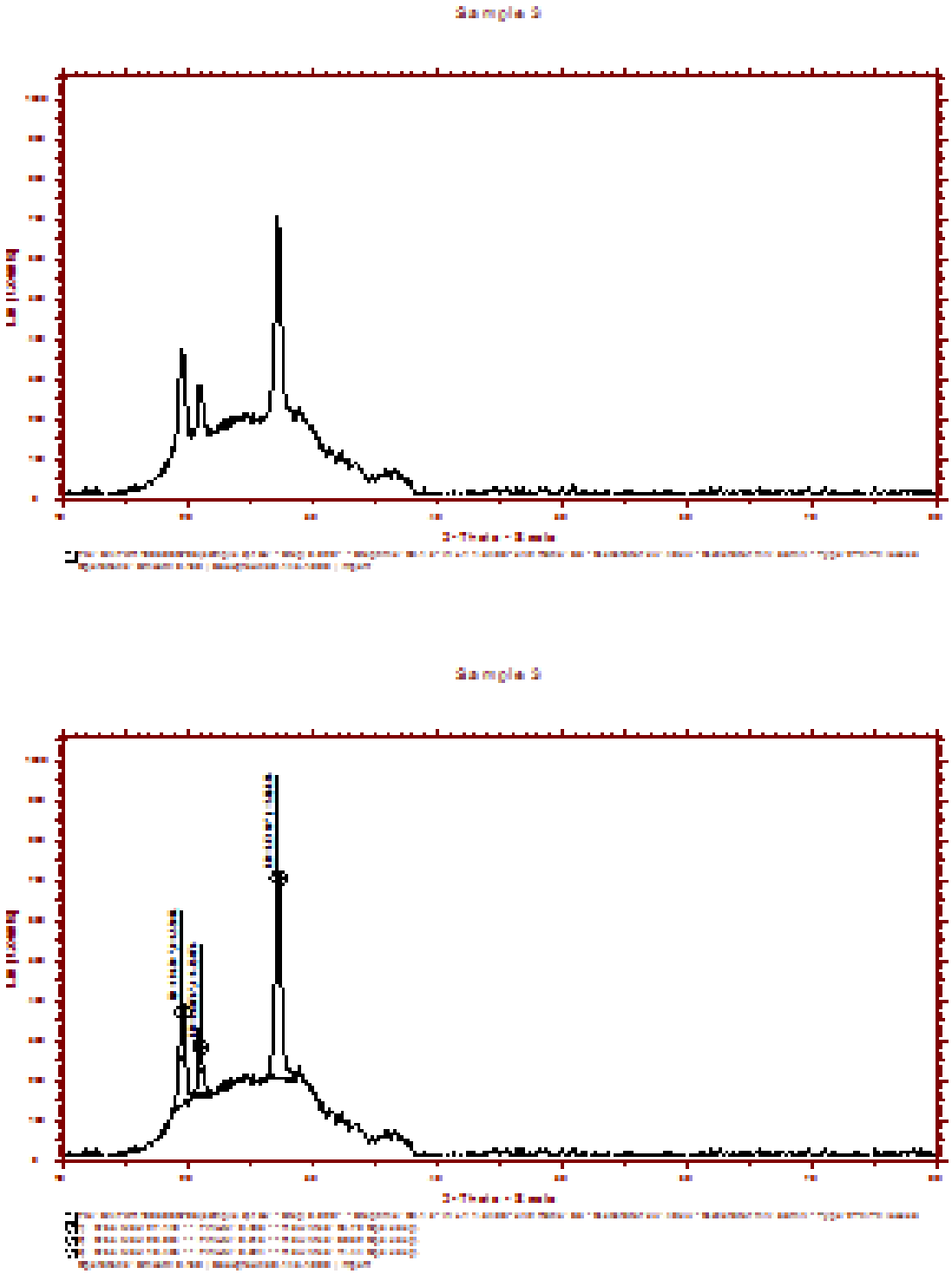


Fig.23 Zinc nanoparticle formation of Black gram (*Vigna mungo* (L.) Hepper 12 hrs seed exudates under XRD imaging system.



The results showed that seed exudates of Chick pea (*Cicer arietinum L.*) and Black gram (*Vigna mungo* (L.) Hepper) are 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.

Acknowledgements

The authors are grateful for the cooperation of the management of Mar Augusthinose college for necessary support. Technical support from Binoy A Mulanthra is also acknowledged.

References

Al Juhaiman, L., Scoles, L., Kingston, D., Patarachao, B., Wang, D., & Bensebaa, F. (2010). Green synthesis of tunable Cu (In1− xGax) Se2 nanoparticles using non-organic solvents. Green Chemistry, 12(7), 1248-1252.

Balouiri, M., Germano, M. P., & Nostro, A. R. (2016). Methods for in vitro evaluating antimicrobial activity. A review. *Journal of Pharmaceutical Analysis*, 6(2), 71-79.

Braydich-Stolle, L., Hussain, S., Schlager, J. J., & Hofmann, M. C. (2005). In vitro cytotoxicity of nanoparticles in mammalian germline stem cells. *Toxicological sciences*, *88*(2), 412-419.

Burd, A., Kwok, C. H., Hung, S. C., Chan, H. S., Gu, H., Lam, W. K., & Huang, L. (2007). A comparative study of the cytotoxicity of silver-based dressings in monolayer cell, tissue explant, and animal models. *Wound repair and regeneration*, *15*(1), 94-104.

Chen, P., Song, L., Liu, Y., & Fang, Y. E. (2007). Synthesis of silver nanoparticles by γ-ray irradiation in acetic water solution containing chitosan. Radiation Physics and Chemistry, 76(7), 1165-1168.

Danilczuk, M., Lund, A., Sadlo, J., Yamada, H., & Michalik, J. (2006). Conduction electron spin resonance of small silver particles. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *63*(1), 189-191.

Devasenan, S., Beevi, N. H., & Jayanthi, S. S. (2016). Synthesis and characterization of Copper Nanoparticles using Leaf Extract of *Andrographis paniculata* and their Antimicrobial Activities. International Journal of ChemTech Research, 9(04), 725-730.

Din, M. I., & Rehan, R. (2017). Synthesis, characterization, and applications of copper nanoparticles. Analytical Letters, 50(1), 50-62.

Duran, N., Marcata, P. D., Alves, O. L., & Esposito, E. (2017). Antibacterial effect of silver nanoparticles produced by fungal process on textile fabrics and their effluent treatments. *Journal of Biomedical Nanotechnology*,3(2), 203-208.

Duran, N., Marcato, P. D., De Souza, G. I., Alves, O. L., & Esposito, E. (2007). Antibacterial effect of silver nanoparticles produced by fungal process on textile fabrics and their effluent treatment. Journal of biomedical nanotechnology, 3(2), 203-208.

El-Rafie, M. H., Mohamed, A. A., Shaheen, T. I., & Hebeish, A. (2010). Antimicrobial effect of silver nanoparticles produced by fungal process on cotton fabrics. *Carbohydrate Polymers*, *80*(3), 779-782.

Elechiguerra, J. L., Burt, J. L., Morones, J. R., Bragado, A. C., Gao, x., Lara, H. L., & Yacaman, M. J. (2005). Interaction of silver nanoparticles with HIV-1. *Journal of Nanobiotechnology*,3(6), 1-10.

Feng, Q. L., Wu, J., Chen, G. Q., Cui, F. Z., Kim, T. N., & Kim, J. O. (2000). A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. *Journal of biomedical materials research*, *52*(4), 662-668.

Feng, Q. L., Wu, J., Chen, G. Q., Cui, F. Z., Kim, T. N., & Kim, J. O. (2000). A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. *Journal of biomedical materials research*, *52*(4), 662-668.

Fox, C. L., & Modak, S. M. (1974). Mechanism of silver sulfadiazine action on burn wound infections. *Antimicrobial agents and chemotherapy*, *5*(6), 582-588.

Furno, F., Morley, K. S., Wong, B., Sharp, B. L., Arnold, P. L., Howdle, S. M., & Reid, H. J. (2004). Silver nanoparticles and polymeric medical devices: a new approach to prevention of infection?. *Journal of Antimicrobial Chemotherapy*, *54*(6), 1019-1024.

Gong, P., Li, H., He, X., Wang, K., Hu, J., Tan, W.,... & Yang, X. (2007). Preparation and antibacterial activity of Fe3O4@ Ag nanoparticles. *Nanotechnology*, *18*(28), 285604.

Guggenbichler, J. P., Böswald, M., Lugauer, S., & Krall, T. (1999). A new technology of microdispersed silver in polyurethane induces antimicrobial activity in central venous catheters. *Infection*, *27*(1), S16-S23.

Gupta, A., & Silver, S. (1998). Molecular genetics: silver as a biocide: will resistance become a problem?. *Nature biotechnology*, *16*(10), 888.

Hajipour, M. J., Fromm, K. M., Ashkarran, A. A., de Aberasturi, D. J., de Larramendi, I. R., Rojo, T., & Mahmoudi, M. (2012). Antibacterial properties of nanoparticles. Trends in biotechnology, 30(10), 499-511.

Hernandez, C. L. C., Villasenor, I. M., Joseph, E., & Tolliday, N. (2008). Isolation and evaluation of antimitotic activity of phenolic compounds from *Pouteria Campechiana*. *Philippine Journal of Science*,137(1), 1-10.

Hussain, S. M., Hess, K. L., Gearhart, J. M., Geiss, K. T., & Schlager, J. J. (2005). In vitro toxicity of nanoparticles in BRL 3A rat liver cells. *Toxicology in vitro*, *19*(7), 975-983.

Ip, M., Lui, S. L., Poon, V. K., Lung, I., & Burd, A. (2006). Antimicrobial activities of silver dressings: an in vitro comparison. *Journal of Medical Microbiology*, *55*(1), 59-63.

Jagessar, R. C., Hafeez, A., Chichester, M., & Crepaul, Y. (2017). Antimicrobial activity of the ethanolic and aqueous extract of passion fruit (*Passiflora edulis SIMS)*, in the absence of $Zn(OAc)_2.2H_2O$. *World Journal of Pharmacy and Pharmaceutical Sciences*,6(9), 230-246.

Jain, P., & Pradeep, T. (2005). Potential of silver nanoparticle-coated polyurethane foam as an antibacterial water filter. *Biotechnology and Bioengineering*, *90*(1), 59-63.

Kale, R., Barwar, S., Kane, P., & More, S. (2018). Green synthesis of silver nanoparticles using papaya seed and its characterization. *International Journal for Research in Applied Science and Engineering Technology*, 6(2), 168-174.

Kalpashree, M. M., & Raveesha, K. A. (2013). Antibacterial activity of *Cycas circinalis* ovules-A naked seeded gymnosperm. *International Journal of Herbal Medicine*,1(3), 53-55.

Kim, J. S., Kuk, E., Yu, K. N., Kim, J. H., Park, S. J., Lee, H. J.,... & Kim, Y. K. (2007). Antimicrobial effects of silver nanoparticles. *Nanomedicine: Nanotechnology, Biology and Medicine*, *3*(1), 95-101.

Klueh, U., Wagner, V., Kelly, S., Johnson, A., & Bryers, J. D. (2000). Efficacy of silver-coated fabric to prevent bacterial colonization and subsequent device-based biofilm formation. *Journal of Biomedical Materials Research Part A*, *53*(6), 621-631.

Krishna, K. L., Paridhavi, M., & Patel, J. A. (2008). Review on nutritional, medicinal and pharmacological properties of papaya *(Carica papaya Linn*). *Natural Product Radiance*,7(4), 364-373.

Krishnakumar, K. N., Rao, G. P., & Gopakumar, C. S. (2009). Rainfall trends in twentieth century over Kerala, India. *Atmospheric Environment*, 43(11), 1940-1944.

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.

Leaper, D. J. (2006). Silver dressings: their role in wound management. *International Wound Journal*, *3*(4), 282-294.

Li, X. Q., Elliott, D. W., & Zhang, W. X. (2006). Zero-valent iron nanoparticles for abatement of environmental pollutants: materials and engineering aspects. *Critical reviews in solid state and materials sciences*, *31*(4), 111-122.

Lok, C. N., Ho, C. M., Chen, R., He, Q. Y., Yu, W. Y., Sun, H., & Che, C. M. (2007). Silver nanoparticles: partial oxidation and antibacterial activities. *JBIC Journal of Biological Inorganic Chemistry*, *12*(4), 527-534.

Luo, X., Morrin, A., Killard, A. J., & Smyth, M. R. (2006). Application of nanoparticles in electrochemical sensors and biosensors. *Willey Online Library*, 18(4).

Malacrida, C. R., & Jorge, N. (2012). Yellow passion fruit seed oil (*Passiflora edulis f. flavicarpa*): Physical and chemical characteristics. *Brazilian Archives of Biology and Technology*, 55(1), 127-134.

Mehraj, H., Sikder, R. K., Mayda, U., Taufique, T., & Uddin, J. (2015). Plant physiology and fruit secondary metabolites of canistel (*Pouteria*

Campechiana). *World Applied Sciences Journal*, 33(12), 1908-1914.

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.

Mitiku, A. A., & Yilma, B. (2017). Review Article A Review on Green Synthesis and Antibacterial Activity of Silver Nanoparticles. Int. J. Pharm. Sci. Rev. Res., 46, 52-57.

Morones, J. R., Elechiguerra, J. L., Camacho, A., Holt, K., Kouri, J. B., Ramírez, J. T., & Yacaman, M. J. (2005). The bactericidal effect of silver nanoparticles. *Nanotechnology*, *16*(10), 2346.

Muhamad, S. A. S., Jamilah, B., Russly, A. R., & Faridah, A. (2017). The antibacterial activities and chemical composition of extracts from *Carica papaya* cv. *Sekaki* / Hong Kong seed. *International Food Research Journal,* 24(2), 810-818.

Nikawa, H., Yamamoto, T., Hamada, T., Rahardjo, M. B., Murata, H., & Nakanoda, S. (1997). Antifungal effect of zeolite-incorporated tissue conditioner against *Candida albicans* growth and/or acid production. *Journal of oral Rehabilitation*, *24*(5), 350-357.

Ong, H. T., Loo, J. S., Boey, F. Y., Russell, S. J., Ma, J., & Peng, K. W. (2008). Exploiting the high-affinity phosphonate–hydroxyapatite nanoparticle interaction for delivery of radiation and drugs. *Journal of Nanoparticle Research*, *10*(1), 141-150.

Pankhurst, Q. A., Connolly, J., Jones, S. K., & Dobson, J. (2003). Application of magnetic nanoparticles in biomedicine. *Journal of Physics D: Applied Physics*, 36(13), 167-181.

Patravale, V. B., Date, A. A., & Kulkarni, R. M. (2004). Nanosuspensions: a promising drug delivery strategy. Journal of pharmacy and pharmacology, 56(7), 827-840.

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.

Paul, N. S., Sharma, R., & Yadav, R. P. (2015). Biological synthesis of antimicrobial silver nanoparticles by *Phaseolus vulgaris* seed extract. *MGM Journal of Medical Sciences,* 2(1), 1-6.

Peter, J. K., Kumar, Y., Pandey, P., & Masih, H. (2014). Antibacterial activity of seed and leaf extract of *Carica papaya var. Pusa dwarf* Linn. *IOSR Journal of Pharmacy and Biological Sciences*,9(2), 29-37.

Prabu, H. J., & Johnson, I. (2015). Antibacterial activity of silver nanoparticles synthesized from plant leaf extract of *Cycas Circinalis, Ficus amplissima, Commelina benghalensis and Lippia nodiflora* leaves. *Journal of Chemical and Pharmaceutical Research,* 7(9), 443-449.

Prasad, B. L. V., Sorensen, C. M., & Klabunde, K. J. (2008). Gold nanoparticle superlattices. *Chemical Society Reviews*, 37(9), 1871-1883.

Prasad, S., Singh, Mritunjai., Singh, Shinjini., & Gambhir, I. S. (2008). Nanotechnology in medicine and antibacterial effect of silver nanoparticles. *Digest Journal of Nanomaterials and Biostructures*, 3(3), 115-122.

Prema, P. (2011). Chemical mediated synthesis of silver nanoparticles and its potential antibacterial application. In *Progress in Molecular and Environmental Bioengineering-From Analysis and Modeling to Technology Applications*. InTech.

Rajeswari, D. V., Gajalakshmi, S., & Vijayalakshmi, S. (2012). Phytochemical and pharmacological properties of *Annona muricata*: A review. *International Journal of Pharmacy and Pharmaceutical Sciences*, 4(2), 3-6.

Ramesh, B., Vijayameena, C., Subhashini, G., & Loganayagi, M. (2013). Phytochemical screening and assessment of antibacterial activity for the bioactive compounds in *Annona muricata*. *International Journal of Current Microbiology and Applied Sciences*,2(1), 1-8.

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., & Sebastin, 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.

Salata, O. V. (2004). Applications of nanoparticles in biology and medicine. *Journal of Nanobiotechnology,* 2(1), 3-8.

Showmya, J. J., Harini, K., Pradeepa, M., Thiyagarajan, M., Manikandan, R., Venkatachalam, P., & Geetha, N. (2012). Rapid green synthesis of silver nanoparticles using seed extract of *Foeniculum vulgare* and screening of its

antibacterial activity. *Plant Cell Biotechnology and Molecular Biology*,13(1-2), 31-38.

Singh, S., (2017). Green approach towards synthesis of copper nanoparticles: A review. *International Journal of Innovative and Emerging Research in Engineering*, 4(7), 1-7.

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

Vazhacharickal, P. J., Joseph, N. M., Mathew, J. J., & Sajeshkumar, N. K. (2022). Green synthesis of copper and zinc nanoparticles from *Chromolaena odorata* and *Justicia adhatoda* and evaluation of their antifungal activity against *Fusarium oxysporum* cubense: An overview. Journal of Medicinal Plants, 10(2), 13-29.

Yeo, S. Y., Lee, H. J., & Jeong, S. H. (2003). Preparation of nanocomposite fibers for permanent antibacterial effect. *Journal of Materials Science*, *38*(10), 2143-2147.

How to cite this article:

Prem Jose Vazhacharickal and Gopika S. Krishna. 2022. Green Synthesis of Silver, Copper and Zinc Nanoparticles from Chick pea (*Cicer arietinum* L.), Black gram (*Vigna mungo* (L.) Hepper) Exudates and Evaluation of their Antibacterial Activity: An Overview. *Int.J.Curr.Res.Aca.Rev.* 10(07), 1-41.
doi: https://doi.org/10.20546/ijcrar.2022.1007.001