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Green Synthesis of Silver, Copper and Zinc Nanoparticles from Fenugreek (*Trigonellafoenum-graecum*), Green Peas (*Pisum sativum* L.) and Peanut (*Arachis hypogaea* L.) Exudates and Evaluation of their Antibacterial Activity

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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 seven different legumes; Fenugreek (*Trigonellafoenum-graecum*), Green peas (*Pisum sativum* L.) and Peanut (*Arachis hypogaea* L.) were used for the synthesis of silver, copper, and zinc nanoparticles and determine 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.

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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 grow 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 different legumes; Fenugreek (*Trigonellafoenum-graecum*), Green peas (*Pisum sativum* L.), Peanut (*Arachis hypogaea* L.) 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 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). 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).

Trigonellafoenum-graecum L. (Fenugreek)

Trigonellafoenum-graecum (Fenugreek) is an annual plant in the family Fabaceae cultivated worldwide as a semiarid crop. Fenugreek is a leguminous plant under the fabaceae family and cultivated throughout the world especially in Asia and North African countries. Fenugreek has many uses such as it is used as culinary spice, flavouring agent and an ingredient for food preparation. From ancient time fenugreek is considered as a medical plant. Their leaflets are about 2-2.5cm long. They are cultivated throughout the country.

Mainly Fenugreek is used in food for enhancing the colour and flavour, so, they modify the texture of the food. They also used against chronic cough, enlargement of the liver and spleen (Ghosh *et al.,* 2015). Fenugreek not only have beneficial effects but also have side effects such as it may increase the risk of bleeding, may reduce the blood potassium levels, numbness, facial swelling, difficulty in breathing, cause allergic reactions, diarrhoea and gastric problems (Yadav *et al.,* 2011).

In the diet, Fenugreek leaves are used as green leafy vegetable. Fresh Fenugreek leaves are useful for indigestion. If Fenugreek leaves use regularly, it helps hair growth, maintains natural colour and helps to keep the hair silky and removes the dandruff. Fenugreek leaves prevent the hair falling problem. Fenugreek leaves are also used for the burns, external and internal swellings also (Kritikar *et al.,* 1991; Prajapati *et al.,* 2003). Seeds of the Fenugreek is appears as solid-rhomboidal seeds, length is 3-5 cmand2mm thick. Hard and pebble like seed. Seeds are light brown or yellowish brown in colour. Seeds of the Fenugreek are rich with vitamin E. Their seeds have high medicinal value. Fenugreek helps to make the child birth easy and also it helps to increase the milk flow in the mother. Tourists are used this for remove the stomach problems. In the case of small pox patients, this seeds are given them as a cool drink (Kritikar *et al.,* 1991). Various bioactive compounds such as flavonoids, saponins and amino acids are present in the seed of Fenugreek. It is considered as a medicinal plant, they shows activity against allergies, cholesterol, diabetic retinopathy, gas, gastric disorders, lung infections, mucus excessive, throat/sore, abscesses, asthma, anaemia, ulcers, cancer, swollen eyes, uterine problems, fever etc. They have major role in reducing blood sugar and other harmful fats. Seeds of the Fenugreek are bitter in taste.

Fenugreek seeds helps to reduce the amount of calcium oxalate in kidney and so, it helps to escape from kidney stone. They block the action of some enzymes and thereby reducing the chance for developing colon cancer. Fenugreek shows; anti-diabetic activity, antiplasmodic activity, hypolipidemic activity, immunological activity, antibacterial activity, analgesic activity, anthelmintic activity, antioxidant activity and anti-inflammatory activity.

Now a days cancer plays a villain role over the world. The extract of the Fenugreek has protective activity against 7,12-dimethylbenz (a) anthracene (DMBA)-induced breast cancer in rats at 200mg/kg. Fenugreek seed extract has the power to prevent DMBA-induced mammary hyperplasia. Epidemiological study stated a mechanism which indicates apoptosis that also mediate the anti-breast cancer effects of Fenugreek (Amin *et al.,* 2005).

Apoptosis is a cell death. Previous studies shows that Flavonoids produced certain biological effects such as; apoptosis inducing activity (Chen *et al.,* 2003). Flavonoids and catechins were first shown to be

apoptotic in human carcinoma cells (Ahamed *et al.*, 2000). Similar observations has been developed to lung tumour cell lines (Valette *et al.*, 1984), breast cancer cells, colon cancer cells, prostate cancer cells (Hannan *et al.*, 2003), stomach cancer cells (Zia *et al.*, 2001). Already proved that other food flavonoids inhibits carcinogenesis in animal models and all of them induces apoptosis in tumour cells (Puri *et al.*, 2002; Devi *et al.*, 2003).

The leaf and seed extract of the Fenugreek when combined with various organic solvents shows effective activity against various types of bacteria and certain types of fungus also (Yadav *et al.*, 2011; Omezzine *et al.*, 2014).

Taxonomical classification (*Trigonellafoenum-graecum* L.; Fenugreek; Uluva)

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

Subkingdom: Viridiplantae

Superdivision: Embryophyta

Division: Tracheophyta
Class: Magnoliopsida

Order: Fabales

Family: Fabaceae

Genus: Trigonella L.

Species: *Trigonellafoenum-graecm* L.

***Pisum sativum* L. (Green peas)**

Pisum sativum L. (Green peas) is an annual plant with a life cycle of one year. These plants can withstand fairy cold conditions, because Green Peas are a cool-season crop. Their optimum growth temperature is between 15°C and 18°C. Below 5°C is unfavourable for growth. This crop can be grown over variety of soil types. Cool, well-drained, medium to heavy loam soils are more suitable for growth.

This plant is sensitive to soil acidity. For good growth adequate liming of soil is essential. The supply of nitrogen containing fertilizer can be avoided, when these seeds are inoculated with Rhizobium bacteria. The most common pea disease is powdery mildow, it is appears like a powdery substance on the upper surface of leaves, on stems and pods also.

Taxonomical classification (*Pisum sativum* L.; Green peas)

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

Subkingdom: Viridiplantae

Superdivision: Embryophyta

Division: Tracheophyta

Class: Magnoliopsida

Order: Fabales

Family: Fabaceae

Genus: Pisum L.

Species: *Pisum sativum* L.

***Arachis hypogaea* L. (Peanut)**

Arachis hypogeal L. (Peanut) also known as groundnut is a legume crop grown mainly for edible seeds. The pea nut plant is believed to originated in Peru or Brazil in South America, but there is no fossil evidences to prove this. About 3,500 years ago, South American peoples made pottery in the shape of peanuts or decorated jars with pea nuts (nationalpeanutboard.org). Peanut is a food and oil crop belongs to the leguminaceae family.

They are grown about approximately 42million acres all over the world. The maximum growth of the pea nut plant is reaches at 60 cm height. Ovary fertilization leads to the development of the elongated stalk called peg, that grows in the downward direction and the ovary is carried into the soil to 2-7 cm depth. When the penetration of the soil surface happened then the fruit enlargement proceeds at the peg tip with eventual formation of the pea nut pod.

Pods consists of about 1-6 seeds. When the pea nuts grows in soil, then that soil gets aeration and moderate amount of organic matter. Pea nuts have very low salt tolerance capacity so, is not suitable to grow in saline soils. Early and late leaf spot, southern blight, seedling disease, pea nut rust, pod rot and Aflatoxin are the diseases that affect on peanut.

Taxonomical classification (*Arachis hypogaea* L.; Peanut)

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

Superdivision: Embryophyta

Division: Tracheophyta

Class: Magnoliopsida

Order: Fabales

Family: Fabaceae

Genus: Arachis L.

Species: *Arachis hypogaea* L.

Hypothesis

The current research work is based on the following hypothesis

Seeds exudates of Fenugreek (*Trigonellafoenum-graecum*), Green peas (*Pisum sativum* L.), Peanut (*Arachis hypogaea* L.) 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 Fenugreek (*Trigonellafoenum-graecum*), Green peas (*Pisum sativum* L.) and Peanut (*Arachis hypogaea* L.) 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 Fenugreek (*Trigonellafoenum-graecum*), Green peas (*Pisum sativum* L.) and Peanut (*Arachis hypogaea* L.) 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 (Fenugreek (*Trigonellafoenum-graecum*), Green peas (*Pisum sativum* L.) and Peanut (*Arachis hypogaea* L.)) 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 (Fenugreek (*Trigonellafoenum-graecum*), Green peas (*Pisum sativum* L.) and Peanut (*Arachis hypogaea* L.)) 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 (Mungbean (*Vigna radiata*), Cowpea (*Vigna unguiculate*), Chick pea (*Cicer arietinum* L.), Black

gram (*Vigna mungo* (L.) Hepper), Fenugreek (*Trigonellafoenum-graecum*), Green peas (*Pisum sativum* L.) and Peanut (*Arachis hypogaea* L.)) 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.

SEM-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 37^0C 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 exudates of different legumes Fenugreek (*Trigonellafoenum-graecum*), Green peas (*Pisum sativum* L.) and Peanut (*Arachis hypogaea* L.)) 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) and 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) for Fenugreek (*Trigonellafoenum-graecum*), Green peas *(Pisum sativum* L.),and Peanut *(Arachis hypogaea* L.). 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 legumesFenugreek (*Trigonellafoenum-graecum*), Green peas (*Pisum sativum* L.) and Peanut (*Arachis hypogaea* L.)) 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 Fenugreek *(Trigonellafoenum-graecum)*, Green peas *(Pisum sativum* L.) and Peanut *(Arachis hypogaea* L.). 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 (Fenugreek (*Trigonellafoenum-graecum*), Green peas (*Pisum sativum* L.) and Peanut (*Arachis hypogaea* L.)) 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.

Antibacterial assay

Seed exudates of Fenugreek (*Trigonellafoenum-graecum*), Green peas (*Pisum sativum* L.) and Peanut (*Arachis hypogaea* L.) showed growth inhibitory effects against *Salmonella Typhi, Pseudomonas aeruginosa, Staphylococcus aureus, E. coli* and *Klebsiella pneumoniae.*

The exudates of Peanut, Fenugreek and Green peas 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 Pea nut, Fresh exudate showed more antimicrobial activity against *Salmonella typhi* (22 nm). Zinc nanoparticles showed more zone of growth inhibition against *Staphylococcus aureus* (18 mm) and *E.coli* (18 mm). Zinc nanoparticles showed more antimicrobial activity with respect to Copper nanoparticles. 150 µl concentration of nanoparticles showed maximum antimicrobial activity against these five types of bacteria. Copper nanoparticles showed higher length growth inhibition zone against *E.coli*. In the case of Fenugreek, Copper nanoparticles were more active against *E.coli*, Klebsiella species, *Pseudomona aeruginosa*, *Salmonella typhi* and *Staphylococcus aureus* than that of Zinc nanoparticles. Copper nanoparticles showed maximum antimicrobial activity against Klebsiella species (30 mm) and *Salmonella typhi* (30 mm). Fresh extract showed less antimicrobial activity indicated by the presence of small zone of inhibition. Zinc nanoparticles showed maximum antimicrobial activity against *E.coli* (26 mm). In the case of Green peas, Zinc nanoparticles showed more zone of growth inhibition than that of Copper nanoparticles *Staphylococcus aureus* (26 mm) and *E.coli* (24 mm). Copper nanoparticles were more active against *Pseudomonas aeruginosa* (21 mm).

Table.1 Different vernacular names of Fenugreek (*Trigonellafoenum-graecum*),around the globe and India.

Language	Names
Scientific names	*Trigonellafoenum-graecum*
Name in various global languages	
French	
German	
English	Fenugreek
Name in various Indian languages	
Sanskrit	Bhauparni
Hindi	Methi
Urdu	
Marathi	Methi
Kannada	Mente
Gujarati	
Malayalam	Uluva
Tamil	Vendayam

Table.2 Different vernacular names of Green peas (*Pisum sativum* L.)around the globe and India.

Language	Names
Scientific names	*Pisum sativum* L.
Name in various global languages	
French	
German	
English	Green peas
Name in various Indian languages	
Sanskrit	Renuka
Hindi	Matar
Urdu	
Marathi	Vatane
Kannada	Batgadle
Gujarati	Patana
Malayalam	Pattani
Tamil	Pattani

Table.3 Different vernacular names of Peanut (*Arachis hypogaea* L.) around the globe and India.

Language	Names
Scientific names	*Arachis hypogaea* L.
Name in various global languages	
French	
German	
English	Peanut
Name in various Indian languages	
Sanskrit	
Hindi	Chinabadam
Urdu	
Marathi	Badamchini
Kannada	Kadalekaayi
Gujarati	Magaphali
Malayalam	Nilakatala
Tamil	Manilakottai

Table.4 Zone of inhibition against various bacteria (*E. coli*, *Klebsiella species*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococus aureus*) using nanoparticles produced byFenugreek (*Trigonellafoenum-graecum*)6 hrs seed exudate.

Microorganism	Nanoparticles	Control	Sample	Measure of zone of inhibition in mm		
				50	100	150
E.coli	Silver	10	9	11	12	13
	Copper	11	10	15	17	19
	Zinc	20	10	20	21	25
Klebsiella species	Silver	11	10	12	15	16
	Copper	16	11	13	14	19
	Zinc	15	10	16	21	23
Pseudomonas aeruginosa	Silver	11	10	12	14	16
	Copper	14	10	14	18	20
	Zinc	12	11	15	17	18
Salmonella typhi	Silver	10	8	11	12	13
	Copper	13	10	13	15	17
	Zinc	23	10	19	25	29
Staphylococcus aureus	Silver	9	8	10	11	12
	Copper	17	10	16	17	22
	Zinc	17	10	27	28	29

Table.5 Zone of inhibition against various bacteria (*E. coli*, *Klebsiella species*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococus aureus*) using nanoparticles produced by Peanut (*Arachis hypogaea* L.) 6 hrs seed exudate.

Microorganism	Nanoparticles	Control	Sample	Measure of zone of inhibition in mm		
				50	100	150
E.coli	Silver	10	8	11	13	15
	Copper	17	11	17	19	22
	Zinc	25	10	20	21	23
Klebsiella species	Silver	9	-	11	12	14
	Copper	13	10	13	16	22
	Zinc	19	10	16	21	25
Pseudomonas aeruginosa	Silver	10	-	11	12	13
	Copper	16	10	17	19	20
	Zinc	14	10	14	16	17
Salmonella typhi	Silver	10	8	13	16	20
	Copper	13	10	14	16	19
	Zinc	20	10	21	23	25
Staphylococcus aureus	Silver	13	8	14	15	17
	Copper	17	10	16	20	22
	Zinc	19	10	26	30	35

Table.6 Zone of inhibition against various bacteria (*E. coli*, *Klebsiella species*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococus aureus*) using nanoparticles produced byGreen peas (*Pisum sativum* L.)6 hrs seed exudate.

Microorganism	Nanoparticles	Control	Sample	Measure of zone of inhibition in mm		
				50	100	150
E.coli	Silver	8	6	10	13	15
	Copper	18	10	15	24	25
	Zinc	24	12	25	27	30
Klebsiella species	Silver	7	6	10	13	14
	Copper	16	12	19	22	25
	Zinc	20	10	21	25	27
Pseudomonas aeruginosa	Silver	8	6	10	14	16
	Copper	15	13	15	18	19
	Zinc	14	10	12	15	16
Salmonella typhi	Silver	7	6	9	11	14
	Copper	19	10	19	21	22
	Zinc	24	10	24	29	30
Staphylococcus aureus	Silver	10	9	14	16	18
	Copper	12	12	12	16	19
	Zinc	18	11	16	20	22

Table.7 Zone of inhibition against various bacteria (*E. coli*, *Klebsiella species*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococus aureus*) using nanoparticles produced byGreen peas (*Pisum sativum* L.)12 hrs seed exudates.

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

Table.8 Zone of inhibition against various bacteria (*E. coli*, *Klebsiella species*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococus aureus*) using nanoparticles produced byFenugreek (*Trigonellafoenum-graecum*)12 hrs seed exudate.

Microorganism	Nanoparticles	Control	Sample	Measure of zone of inhibition in mm		
				50	100	150
E.coli	Silver	7	6	11	12	14
	Copper	11	11	15	15	17
	Zinc	16	10	18	21	27
Klebsiella species	Silver	5	7	9	11	13
	Copper	15	10	13	14	19
	Zinc	18	10	18	20	23
Pseudomonas aeruginosa	Silver	6	5	8	10	12
	Copper	14	10	14	17	18
	Zinc	18	10	18	23	25
Salmonella typhi	Silver	10	6	12	13	15
	Copper	13	10	11	15	17
	Zinc	18	11	19	22	23
Staphylococcus aureus	Silver	7	9	12	14	15
	Copper	17	10	17	18	22
	Zinc	18	10	20	22	26

Table.9 Zone of inhibition against various bacteria (*E. coli*, *Klebsiella species*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococus aureus*) using nanoparticles produced byPeanut (*Arachis hypogaea* L.)12 hrs seed exudate.

Microorganism	Nanoparticles	Control	Sample	Measure of zone of inhibition in mm		
				50	100	150
E.coli	Silver	6	4	9	10	13
	Copper	18	10	15	18	21
	Zinc	19	11	16	22	22
Klebsiella species	Silver	5	6	9	11	13
	Copper	16	10	17	18	21
	Zinc	18	11	15	18	21
Pseudomonas aeruginosa	Silver	7	3	9	12	14
	Copper	18	10	14	18	21
	Zinc	15	11	17	21	22
Salmonella typhi	Silver	6	6	10	11	14
	Copper	20	13	14	17	20
	Zinc	18	11	17	20	22
Staphylococcus aureus	Silver	7	5	9	13	15
	Copper	17	11	14	18	22
	Zinc	17	11	17	20	23

Table.10 Zone of inhibition against various bacteria (*E. coli*, *Klebsiella species*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococus aureus*) using nanoparticles produced byGreen peas (*Pisum sativum* L.) 24 hrs seed exudates.

Microorganism	Nanoparticles	Control	Sample	Measure of zone of inhibition in mm		
				50	100	150
E.coli	Silver	6	4	9	13	14
	Copper	14	11	15	17	18
	Zinc	16	10	20	23	24
Klebsiella species	Silver	10	9	11	12	13
	Copper	15	10	15	16	16
	Zinc	20	10	19	20	23
Pseudomonas aeruginosa	Silver	7	4	12	13	16
	Copper	14	11	18	20	21
	Zinc	18	10	18	22	25
Salmonella typhi	Silver	6	6	10	12	13
	Copper	13	10	16	19	19
	Zinc	19	10	20	20	22
Staphylococcus aureus	Silver	7	4	9	11	12
	Copper	12	10	13	19	20
	Zinc	18	11	18	22	26

Table.11 Zone of inhibition against various bacteria (*E. coli*, *Klebsiella species*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococus aureus*) using nanoparticles produced byPeanut (*Arachis hypogaea* L.) 24 hrs seed exudate.

Microorganism	Nanoparticles	Control	Sample	Measure of zone of inhibition in mm		
				50	100	150
E.coli	Silver	12	11	11	12	13
	Copper	14	10	13	15	18
	Zinc	18	10	16	21	25
Klebsiella species	Silver	10	6	11	12	13
	Copper	14	10	12	14	17
	Zinc	16	10	13	15	19
Pseudomonas aeruginosa	Silver	11	4	12	16	20
	Copper	14	10	14	14	16
	Zinc	19	10	16	22	22
Salmonella typhi	Silver	10	6	10	11	12
	Copper	14	12	14	15	17
	Zinc	17	12	18	20	22
Staphylococcus aureus	Silver	14	9	15	18	21
	Copper	14	10	12	15	18
	Zinc	18	10	19	21	26

Table.12 Zone of inhibition against various bacteria (*E. coli*, *Klebsiella species*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococus aureus*) using nanoparticles produced byFenugreek (*Trigonellafoenum-graecum*) 24 hrsseed exudate.

Microorganism	Nanoparticles	Control	Sample	Measure of zone of inhibition in mm		
				50	100	150
E.coli	Silver	12	11	11	12	13
	Copper	20	10	25	27	29
	Zinc	18	11	17	24	26
Klebsiella species	Silver	8	9	11	14	16
	Copper	20	11	26	28	30
	Zinc	16	10	16	20	21
Pseudomonas aeruginosa	Silver	7	8	12	16	17
	Copper	26	10	28	29	29
	Zinc	19	10	19	20	23
Salmonella typhi	Silver	10	6	10	11	12
	Copper	20	10	25	29	30
	Zinc	15	10	20	21	23
Staphylococcus aureus	Silver	14	9	15	18	21
	Copper	20	10	22	23	24
	Zinc	18	10	18	21	23

Table.13 Description of UV absorption spectrum of Silver nanoparticles formed from Fenugreek (*Trigonellafoenum-graecum*)6 hrs seed exudate during different time of incubation.

Time	385 nm	435nm	560nm	680nm
½ hr	0.436	0.413	0.207	0.142
1 hr	0.482	0.449	0.275	0.207
1 ½ hr	0.518	0.529	0.279	0.188
2 hr	1.191	1.738	0.599	0.283
2 ½ hr	0.564	0.563	0.253	0.170
Blank	0.155	0.022	0.152	0.014

Table.14 Description of UV absorption spectrum of Sliver nanoparticles formed from Fenugreek (*Trigonellafoenum-graecum*)12 hrs seed exudate during different time of incubation.

Time	385 nm	435nm	560nm	680nm
½ hr	0.391	0.369	0.231	0.169
1 hr	0.471	0.454	0.230	0.158
1 ½ hr	0.395	0.387	0.206	0.150
2 hr	0.397	0.407	0.208	0.140
2 ½ hr	0.446	0.453	0.220	0.148
Blank	0.155	0.023	0.152	0.014

Table.15 Description of UV absorption spectrum of Copper nanoparticles formed from Fenugreek (*Trigonellafoenum-graecum*)6 hrs seed exudate during different time of incubation.

Time	350 nm	385 nm	435 nm	560 nm
½ hr	0.164	0.092	0.028	0.446
1 hr	0.155	0.080	0.029	0.444
1 ½ hr	0.252	0.164	0.078	0.498
2 hr	0.190	0.122	0.052	0.500
2 ½ hr	0.207	0.124	0.057	0.492
Blank	0.027	0.017	0.019	0.411

Table.16 Description of UV absorption spectrum of Copper nanoparticles formed from Fenugreek (*Trigonellafoenum-graecum*)12 hrs seed exudate during different time of incubation.

Time	350 nm	385 nm	435 nm	560 nm
½ hr	0.215	0.151	0.093	0.487
1 hr	0.214	0.139	0.069	0.508
1 ½ hr	0.164	0.089	0.036	0.446
2 hr	0.163	0.089	0.033	0.449
2 ½ hr	0.258	0.161	0.078	0.477
Blank	0.026	0.017	0.019	0.412

Table.17 Description of UV absorption spectrum of Sliver nanoparticles formed from Peanut (*Arachis hypogaea* L.)6 hrs seed exudate during different time of incubation.

Time	350 nm	385 nm	435 nm	560 nm
½ hr	0.937	1.277	0.795	0.349
1 hr	1.121	1.632	0.619	0.269
1 ½ hr	0.977	1.555	0.523	0.283
2 hr	1.191	1.738	0.599	0.283
2 ½ hr	0.635	0.578	0.435	0.787
Blank	0.155	0.023	0.052	0.014

Table.18 Description of UV absorption spectrum of Sliver nanoparticles formed from Peanut (*Arachis hypogaea* L.)12 hrs seed exudate during different time of incubation.

Time	350 nm	385 nm	435 nm	560 nm
½ hr	2.002	2.4	1.632	0.856
1 hr	2.282	3.312	1.942	1.048
1 ½ hr	2.344	3.474	1.898	1.062
2 hr	2.32	3.452	1.828	1.00
2 ½ hr	0.952	3.532	1.962	1.126
Blank	0.155	0.025	0.053	0.015

Table.19 Description of UV absorption spectrum of Copper nanoparticles formed from Peanut (*Arachis hypogaea* L.)6 hrs seed exudate during different time of incubation.

Time	350 nm	385 nm	435 nm	560 nm
½ hr	0.164	0.092	0.028	0.446
1 hr	0.628	0.549	0.412	0.757
1 ½ hr	0.600	0.533	0.408	0.759
2 hr	0.511	0.523	0.415	0.763
2 ½ hr	0.635	0.578	0.435	0.787
Blank	0.026	0.017	0.019	0.411

Table.20 Description of UV absorption spectrum of Copper nanoparticles formed from Peanut (*Arachis hypogaea* L.)12 hrs seed exudate during different time of incubation.

Time	350 nm	385 nm	435 nm	560 nm
½ hr	0.401	0.308	0.228	0.586
1 hr	0.561	0.489	0.348	0.696
1 ½ hr	0.543	0.471	0.333	0.701
2 hr	0.458	0.385	0.281	0.646
2 ½ hr	1.173	0.407	0.300	0.663
Blank	0.026	0.016	0.019	0.412

Table.21 Description of UV absorption spectrum of Silver nanoparticles formed from Green peas (*Pisum sativum* L.)6 hrs seed exudates during different time of incubation.

Time	350 nm	385 nm	435 nm	560 nm
½ hr	0.175	0.172	0.296	0.157
1 hr	0.165	0.172	0.175	0.173
1 ½ hr	0.238	0.285	0.207	0.181
2 hr	0.205	0.205	0.185	0.159
2 ½ hr	0.140	0.145	0.183	0.148
Blank	0.150	0.125	0.118	0.108

Table.22 Description of UV absorption spectrum of Silver nanoparticles formed from Green peas (*Pisum sativum* L.)12 hrs seed exudates during different time of incubation.

Time	350 nm	385 nm	435 nm	560 nm
½ hr	0.376	0.335	0.400	0.233
1 hr	0.420	0.363	0.310	0.288
1 ½ hr	0.200	0.253	0.168	0.146
2 hr	0.187	0.175	0.158	0.138
2 ½ hr	0.237	0.218	0.128	0.106
Blank	0.150	0.125	0.118	0.108

Table.23 Description of UV absorption spectrum of Copper nanoparticles formed from Green peas (*Pisum sativum* L.)6 hrs seed exudates during different time of incubation.

Time	350 nm	385 nm	435 nm	560 nm
½ hr	0.057	0.056	0.171	0.442
1 hr	0.043	0.021	0.028	0.439
1 ½ hr	0.034	0.012	0.026	0.440
2 hr	0.035	0.023	0.022	0.439
2 ½ hr	0.144	0.095	0.057	0.475
Blank	0.039	0.033	0.046	0.471

Table.24 Description of UV absorption spectrum of Copper nanoparticles formed from Green peas (*Pisum sativum* L.)12 hrs seed exudates during different time of incubation.

Time	350 nm	385 nm	435 nm	560 nm
½ hr	0.116	0.090	0.217	0.498
1 hr	0.110	0.085	0.090	0.509
1 ½ hr	0.205	0.152	0.122	0.558
2 hr	0.197	0.138	0.112	0.515
2 ½ hr	0.095	0.056	0.076	0.484
Blank	0.039	0.033	0.046	0.471

Table.25 Description of UV absorption spectrum of Copper nanoparticles formed from Peanut (*Arachis hypogaea* L.) 24 hrs seed exudate during different time of incubation.

Time	350 nm	385 nm	435 nm	560 nm
½ hr	0.345	0.255	0.206	0.583
1 hr	0.493	0.405	0.288	0.637
1 ½ hr	0.470	0.397	0.271	0.616
2 hr	0.601	0.504	0.333	0.671
2 ½ hr	0.624	0.532	0.374	0.708
Blank	0.138	0.096	0.077	0.464

Table.26 Description of UV absorption spectrum of Copper nanoparticles formed from Fenugreek (*Trigonellafoenum-graecum*)24 hrs seed exudate during different time of incubation.

Time	350 nm	385 nm	435 nm	560 nm
½ hr	0.175	0.078	0.021	0.413
1 hr	0.300	0.210	0.082	0.480
1 ½ hr	0.208	0.106	0.028	0.430
2 hr	0.149	0.078	0.018	0.435
2 ½ hr	0.226	0.120	0.034	0.439
Blank	0.138	0.096	0.077	0.464

Table.27 Description of UV absorption spectrum of Copper nanoparticles formed from Green peas (*Pisum sativum* L.)24 hrs seed exudates during different time of incubation.

Time	350 nm	385 nm	435 nm	560 nm
½ hr	0.280	0.198	0.151	0.561
1 hr	0.185	0.107	0.098	0.523
1 ½ hr	0.282	0.214	0.154	0.555
2 hr	0.289	0.223	0.149	0.564
2 ½ hr	0.165	0.122	0.104	0.519
Blank	0.138	0.096	0.077	0.464

Table.28 Description of UV absorption spectrum of Sliver nanoparticles formed from Peanut (*Arachis hypogaea* L.) 24 hrs seed exudate during different time of incubation.

Time	350 nm	385 nm	435 nm	560 nm
½ hr	2.416	2.920	1.77	0.546
1 hr	2.56	3.472	1.596	0.432
1 ½ hr	2.614	3.334	2.006	0.806
2 hr	2.45	3.866	2.168	0.684
2 ½ hr	2.562	3.666	1.95	0.624
Blank	0.540	0.424	0.246	0.146

Table.29 Description of UV absorption spectrum of Sliver nanoparticles formed from Fenugreek (*Trigonellafoenum-graecum*)24 hrs seed exudate during different time of incubation.

Time	350 nm	385 nm	435 nm	560 nm
½ hr	0.780	0.691	0.235	0.096
1 hr	0.877	0.790	0.286	0.111
1 ½ hr	0.869	0.785	0.271	0.100
2 hr	0.989	0.908	0.341	0.149
2 ½ hr	1.042	0.978	0.406	0.178
Blank	0.540	0.424	0.246	0.146

Fig.1 Map of Kerala showing the sample collection point.

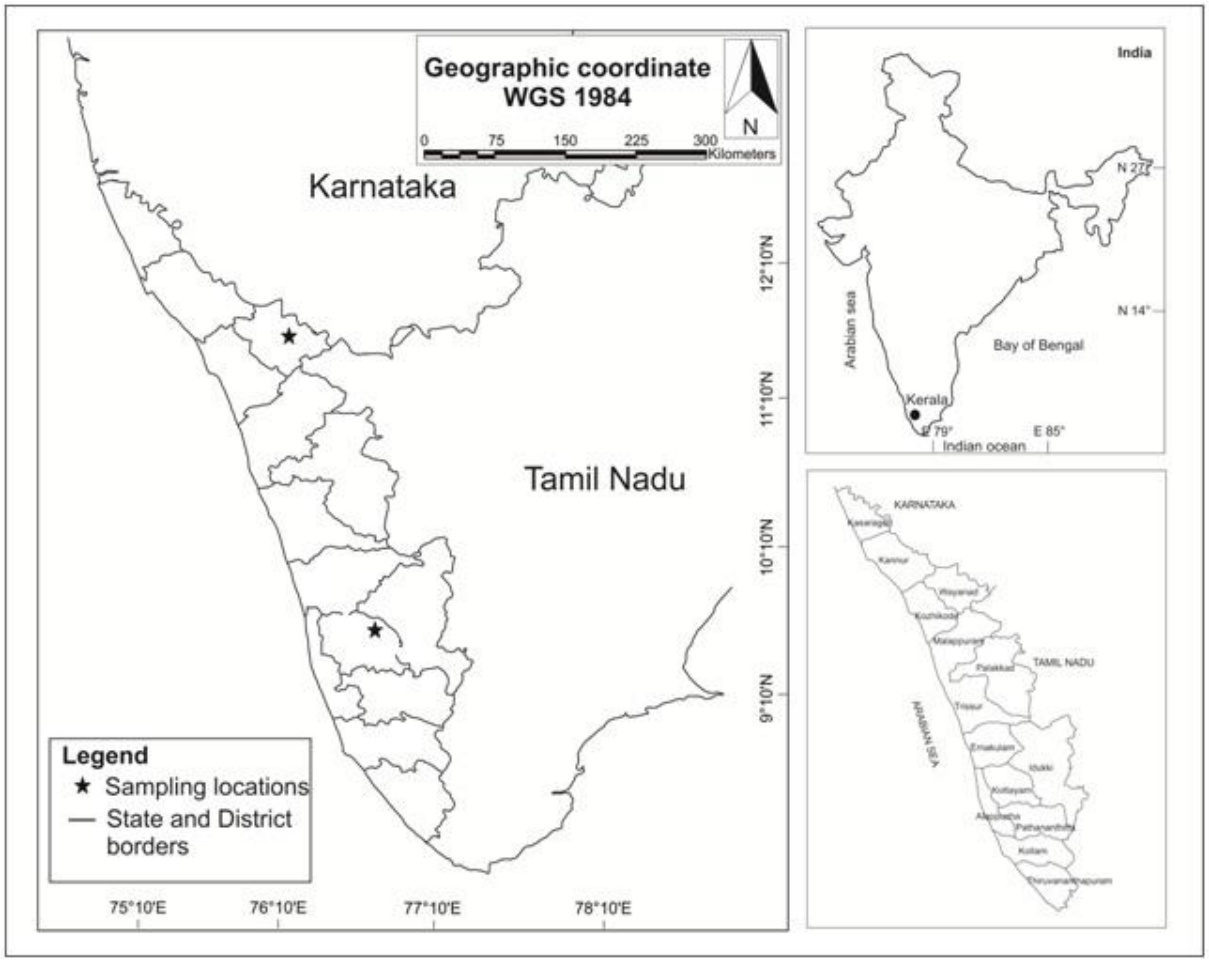


Table.30 Description of UV absorption spectrum of Sliver nanoparticles formed from Green peas (*Pisum sativum* L.)24 hrs seed exudates during different time of incubation.

Time	350 nm	385 nm	435 nm	560 nm
½ hr	0.230	0.166	0.118	0.106
1 hr	0.141	0.102	0.078	0.076
1 ½ hr	0.120	0.091	0.073	0.080
2 hr	0.248	0.266	0.254	0.248
2 ½ hr	0.257	0.210	0.264	0.228
Blank	0.541	0.424	0.246	0.146

Fig.2 Peanut (*Arachis hypogaea* L.) description a) mature pods opened with seeds, b) fully developed flower, c) split opened roasted seeds as snacks, d) plants grown on fields, e) cleaned seeds ready for sale, f) peanut butter. Photo courtesy: Wikipedia.

Table.31 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).

Fig.3 Fenugreek (*Trigonellafoenum-graecum*) description a) sketch of a full plant with parts, b) leaves harvested, c), d) and e) mature dried seeds. Photo courtesy: Wikipedia (a, b, c. and e).

Table.32 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.4 Green peas (*Pisum sativum* L.) description a) pods cut opened, b) mature pods in plants, c) mature pods and seeds, d) frozen seeds, e) dried seeds with outer skin removed and split (polished), f) fruits sold on market. Photo courtesy: Wikipedia.

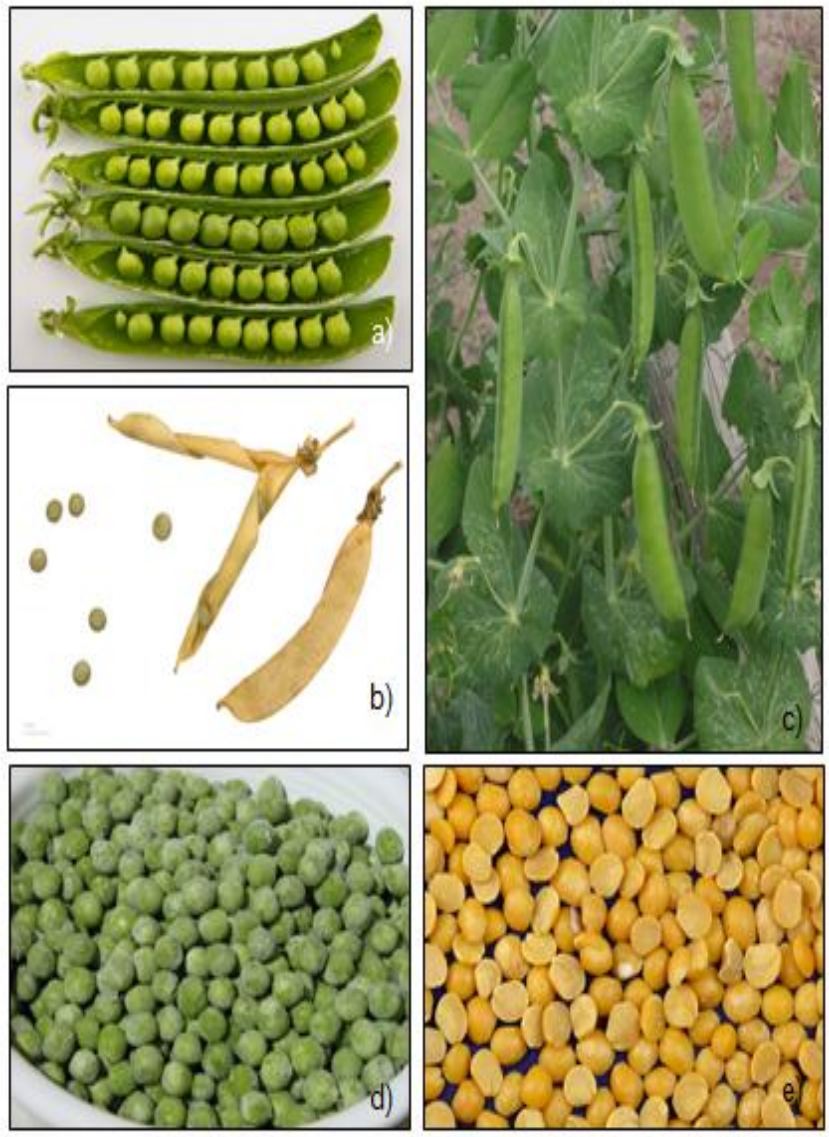


Fig.5 Description of the seed exudates used for making nanoparticles a) Cowpea* (*Vigna unguiculate*), b) Black gram* (*Vigna mungo* (L.) Hepper, c) Peanut (*Arachis hypogaea* L.), d) Chick pea* (*Cicer arietinum* L.), e) Fenugreek (*Trigonellafoenum-graecum*), f) Mungbean* (*Vigna radiata*), g) Green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

Fig.6 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 (*Trigonellafoenum-graecum*), f) Mungbean* (*Vigna radiata*), g) Green peas (*Pisum sativum* L.), h) all the seeds mixed together. * data not provided.

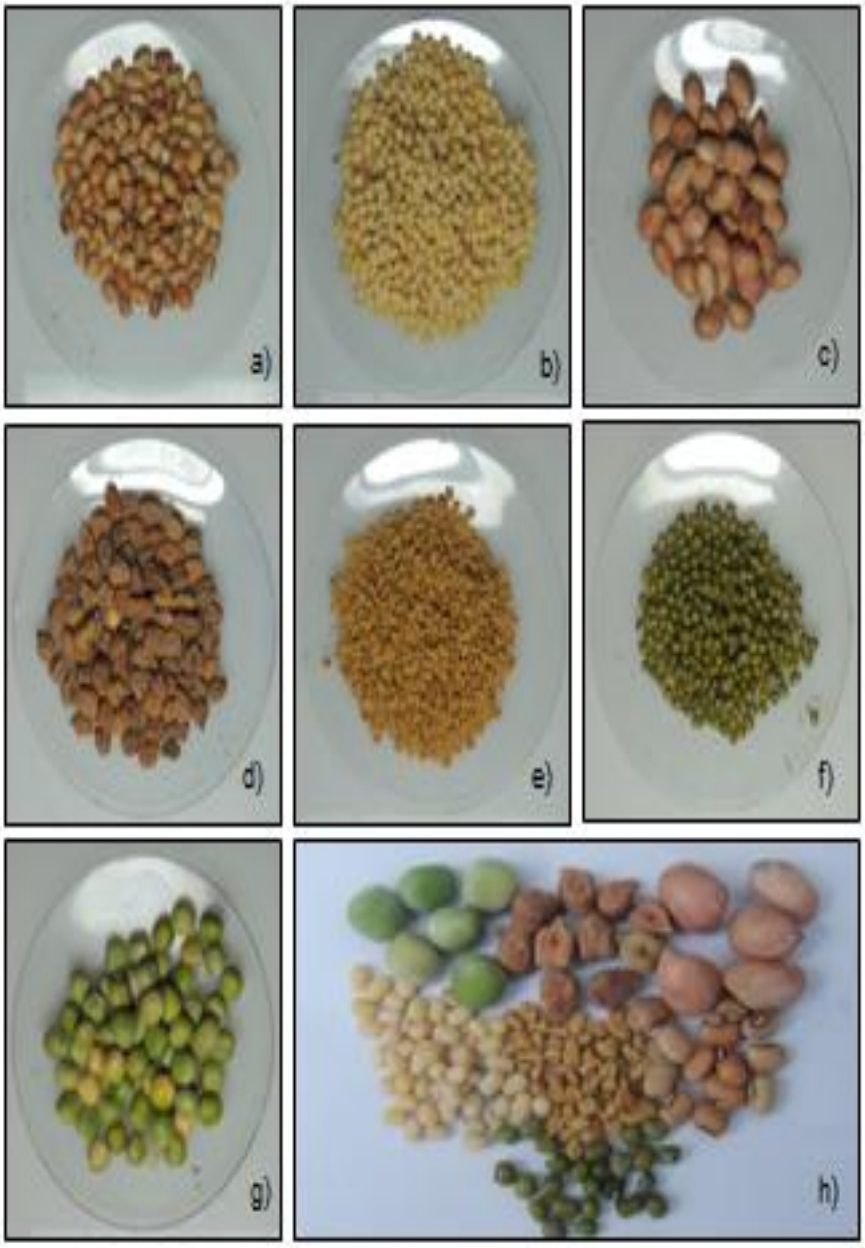


Fig.7 Seed exudates used for making nanoparticles a) Cowpea* (*Vigna unguiculate*), b) Black gram* (*Vigna mungo* (L.) Hepper, c) Peanut (*Arachis hypogaea* L.), d) Chick pea* (*Cicer arietinum* L.), e) Fenugreek (*Trigonellafoenum-graecum*), f) Mungbean* (*Vigna radiata*), g) Green peas (*Pisum sativum* L.), h) and i) all the seeds placed in different extraction bottles. * data not provided.

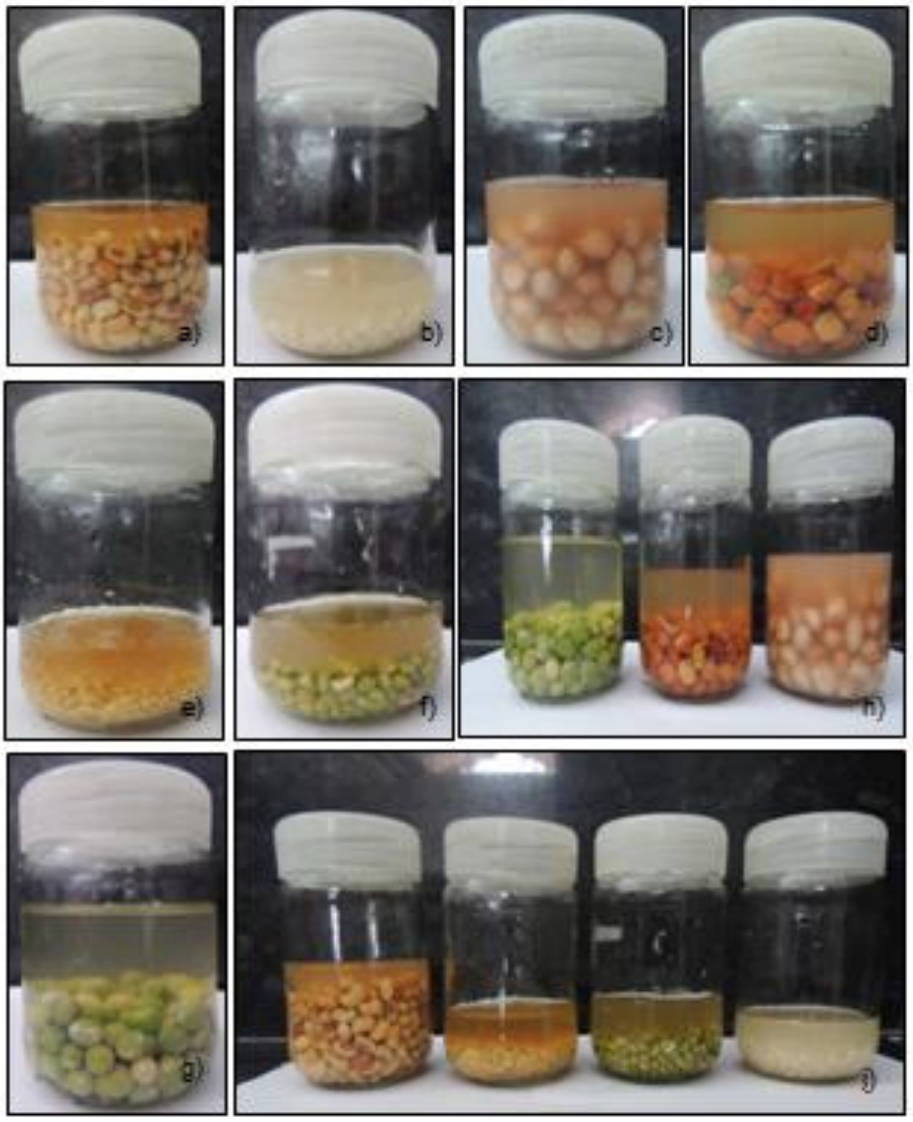


Fig.8 Description of the a) and b seed exudates used for making nanoparticles (Peanut, Fenugreek and Green peas), c) and d)green synthesized zinc nanoparticles, e) and f) green synthesized copper nanoparticles, g) and h)green synthesized silver nanoparticles.

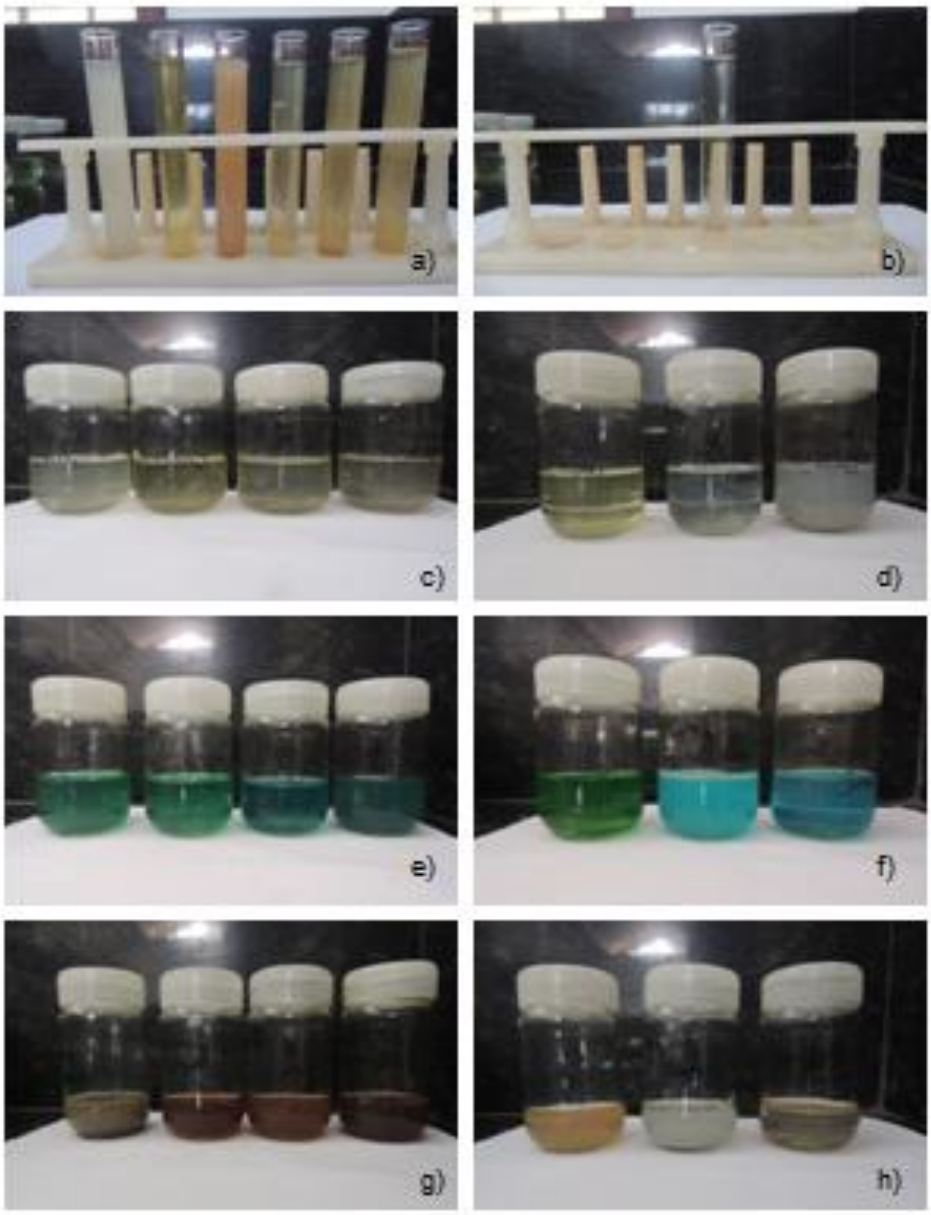


Fig.9 Antibacterial activity study using well diffusion method of Mungbean* (*Vigna radiata*) and Green peas (*Pisum sativum* L.)12 hrs seed exudates nanoparticles (Zn)a) *Salmonella typhi* control plate (Zn), b) *Salmonella typhi* green synthesised nanoparticle test plate (Zn; 50, 100 and 150 µl), c) *Pseudomonas aeruginosa* control, d) *Pseudomonas aeruginosa* test, e) *E. coli* control,f) *E. coli* test, g) *Klebsiella species* control, h) *Klebsiella species* test, i) *Staphylococcus species* control, j) *Staphylococcus species* test, k) to t) above mentioned organisms plate in same order for Zn nanoparticles (Green peas (*Pisum sativum* L.).

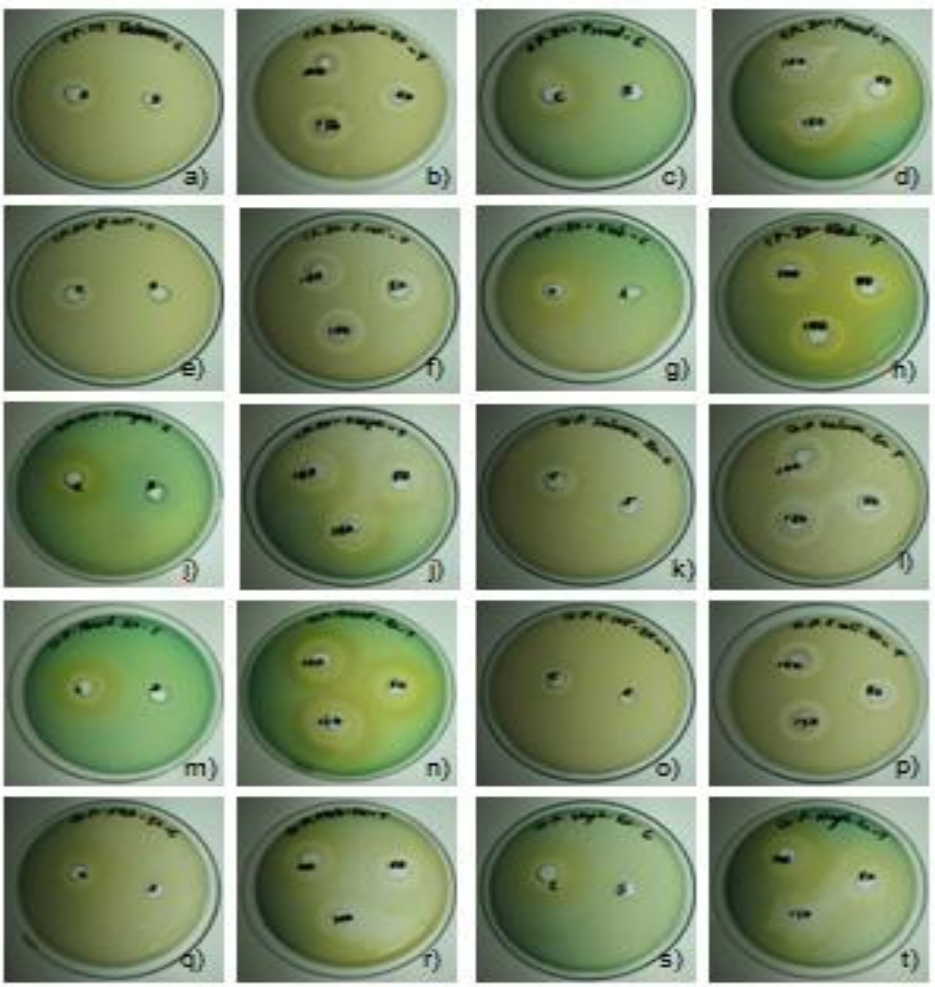


Fig.10 Antibacterial activity study using well diffusion method of Chick pea* (*Cicer arietinum* L.) and Fenugreek (*Trigonellafoenum-graecum*) 12hrs seed exudates nanoparticles (Cu)a) *Salmonella typhi* control plate (Cu), b) *Salmonella typhi* green synthesised nanoparticle test plate (Cu; 50, 100 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 (*Trigonellafoenum-graecum*).

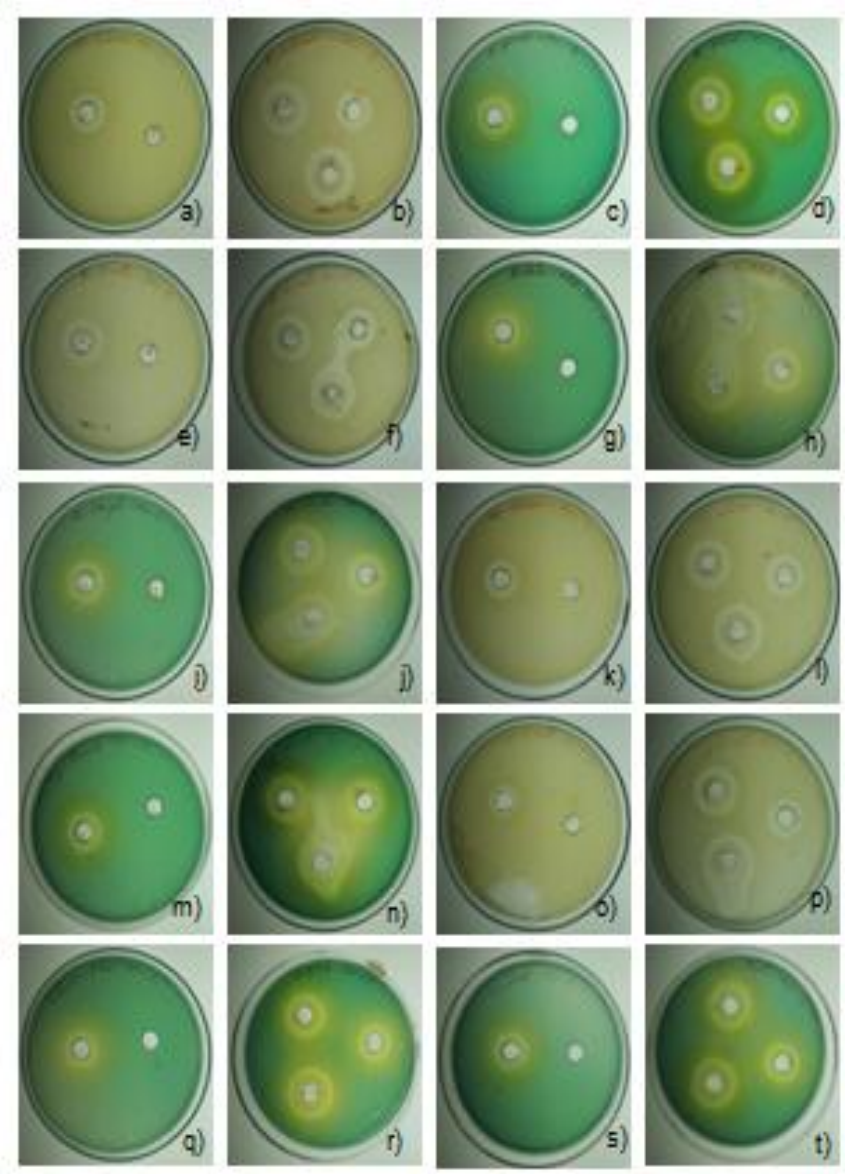


Fig.11 Antibacterial activity study using well diffusion method of Peanut (*Arachis hypogaea* L.) and Chick pea* (*Cicer arietinum* L.) 12 hrs seed exudates nanoparticles (Cu) a) *Salmonella typhi* control plate (Cu), b) *Salmonella typhi* green synthesised nanoparticle test plate (Cu; 50, 100 and 150 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.).

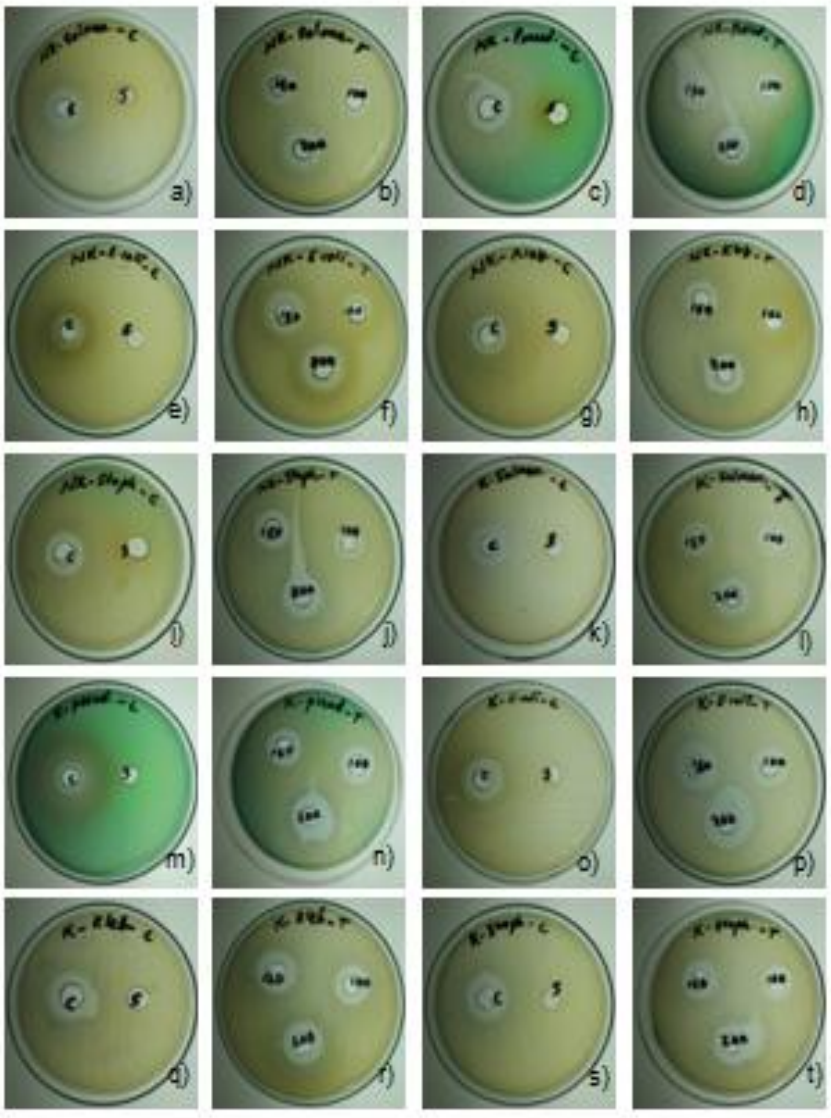


Fig.12 Antibacterial activity study using well diffusion method of Cowpea* (*Vigna unguiculate)*and Peanut (*Arachis hypogaea* L.) 12 hrs seed exudates nanoparticles (Cu) and (Zn)a) *Salmonella typhi* control plate (Cu), b) *Salmonella typhi* green synthesised nanoparticle test plate (Cu; 50, 100 and 150µl), 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 (Peanut (*Arachis hypogaea* L.).

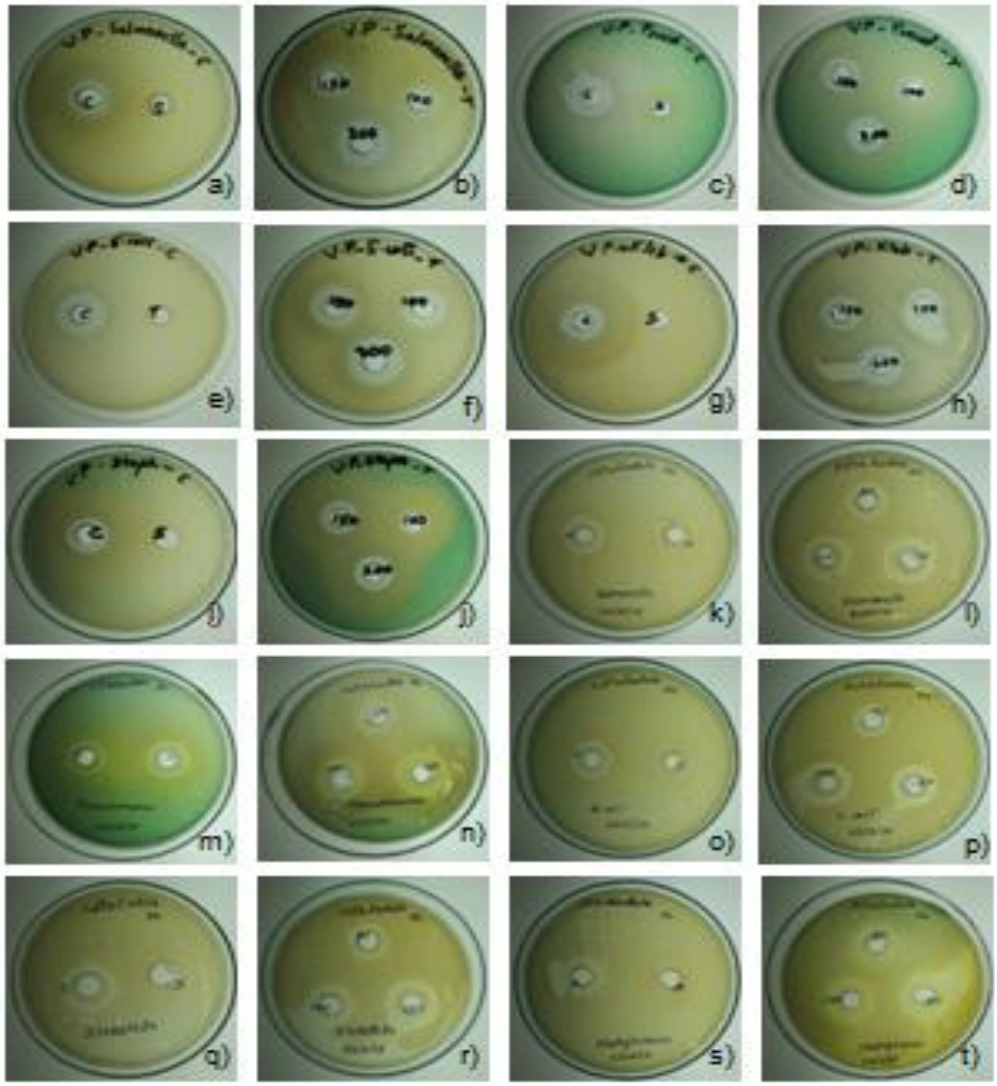


Fig.13 Antibacterial activity study using well diffusion method of Black gram* (*Vigna mungo* (L.) Hepper and Fenugreek (*Trigonellafoenum-graecum*) 24 hrs seed exudates nanoparticles (Zn)a) *Salmonella typhi* control plate (Ag), b) *Salmonella typhi* green synthesised nanoparticle test plate (Zn; 50, 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 (*Trigonellafoenum-graecum*).

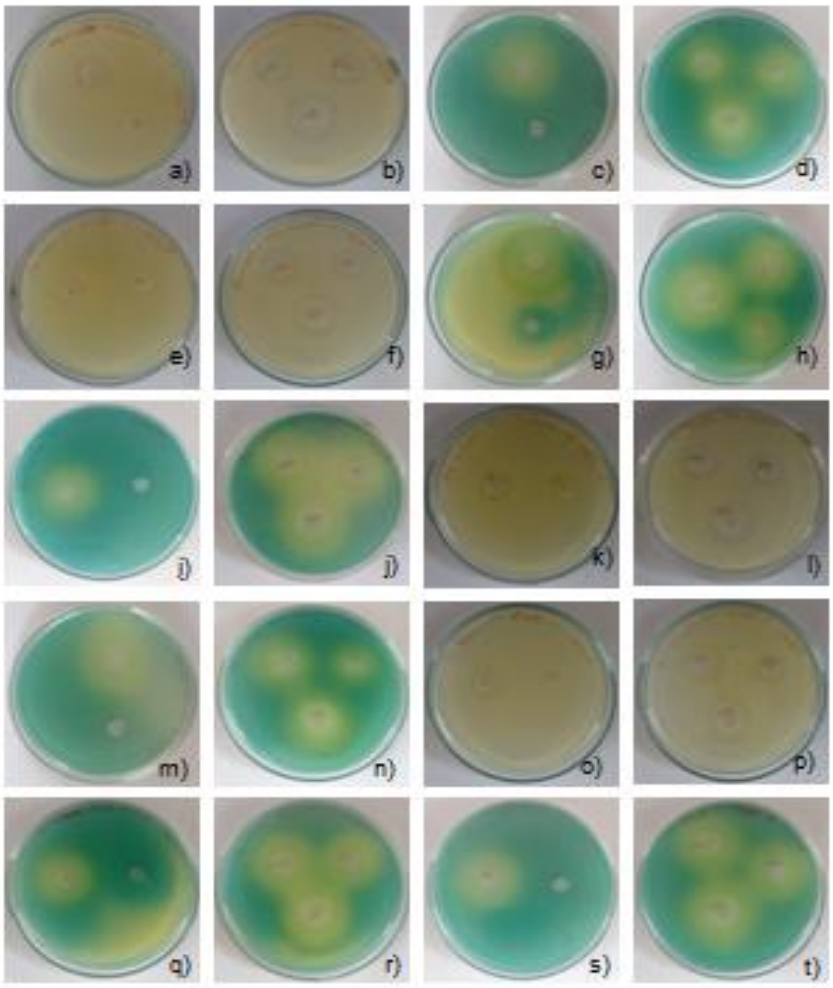


Fig.14 Antibacterial activity study using well diffusion method of Green peas (*Pisum sativum* L.)and Fenugreek (*Trigonellafoenum-graecum*) 12 hrsseed exudate nanoparticles (Cu) a) *Salmonella typhi* control plate (Cu), b) *Salmonella typhi* green synthesised nanoparticle test plate (Cu; 50, 100 and 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 (*Trigonellafoenum-graecum*).

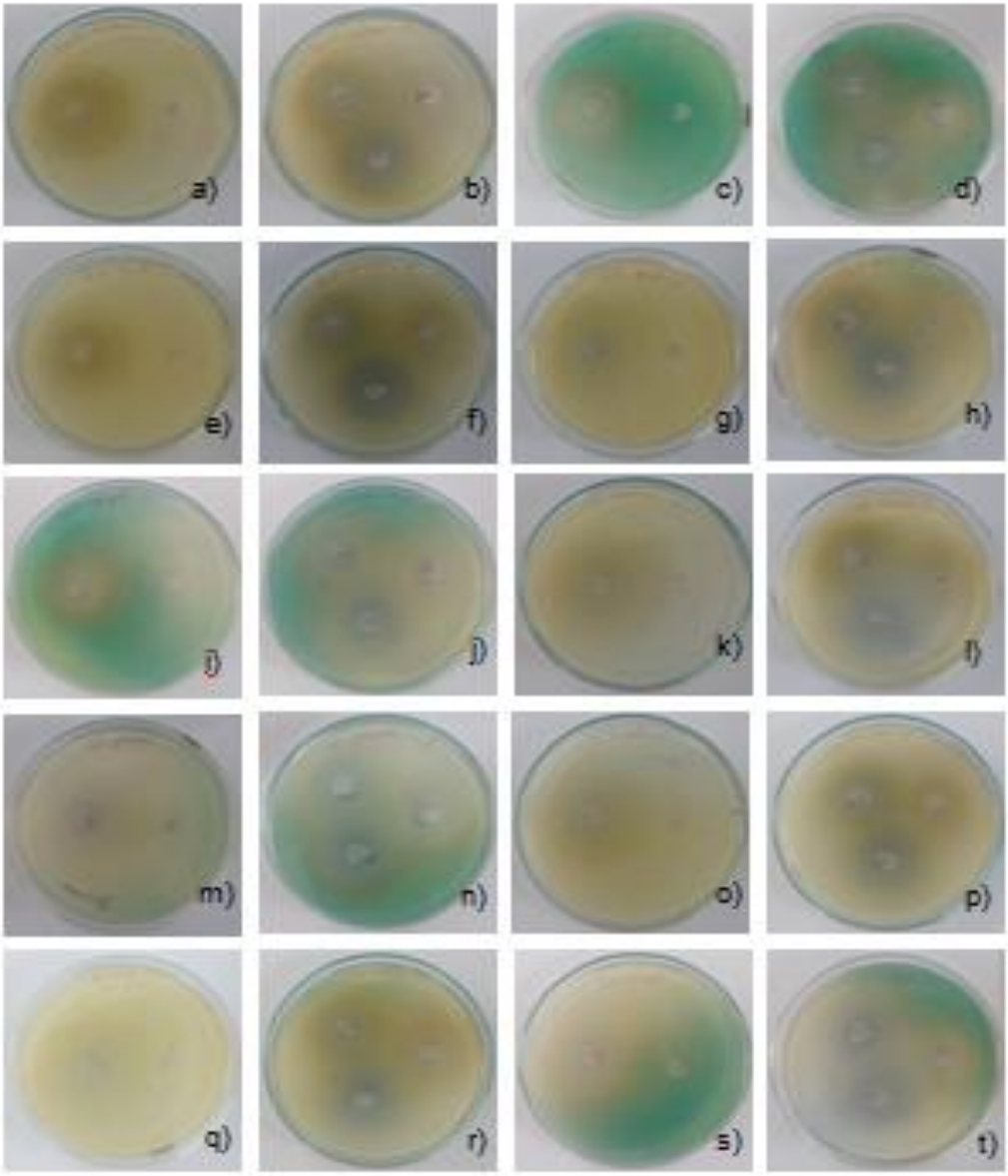


Fig.15 Antibacterial activity study using well diffusion method of Mungbean* (*Vigna radiata*) and Green peas (*Pisum sativum* L.)24 hrsseed exudates nanoparticles (Zn) a) *Salmonella typhi* control plate (Zn), b) *Salmonella typhi* green synthesised nanoparticle test plate (Zn; 50, 100 and 150 µl), c) *Pseudomonas aeruginosa* control, d) *Pseudomonas aeruginosa* test, e)*E. coli* control,f) *E. coli* test, g) *Klebsiella species* control, h) *Klebsiella species* test, i) *Staphylococcus species* control, j) *Staphylococcus species* test, k) to t) above mentioned organisms plate in same order for Zn nanoparticles (Green peas (*Pisum sativum* L.).

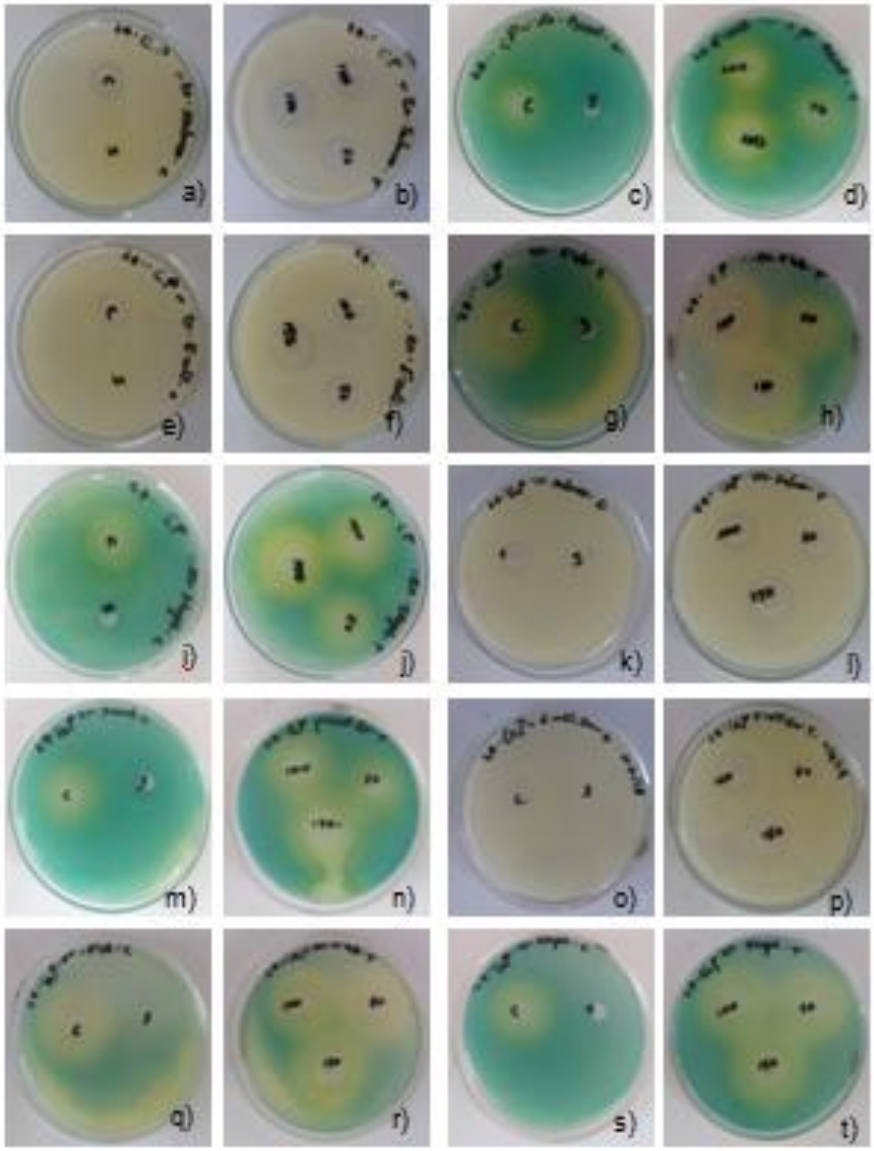


Fig.16 Antibacterial activity study using well diffusion method of Green peas (*Pisum sativum* L.)6 hrs seed exudate nanoparticles (Cu) and (Zn) a) *Salmonella typhi* control plate (Cu), b) *Salmonella typhi* green synthesised nanoparticle test plate (Cu; 50, 100 and 150 µl), c) *Pseudomonas aeruginosa* 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 (Green peas (*Pisum sativum* L.).

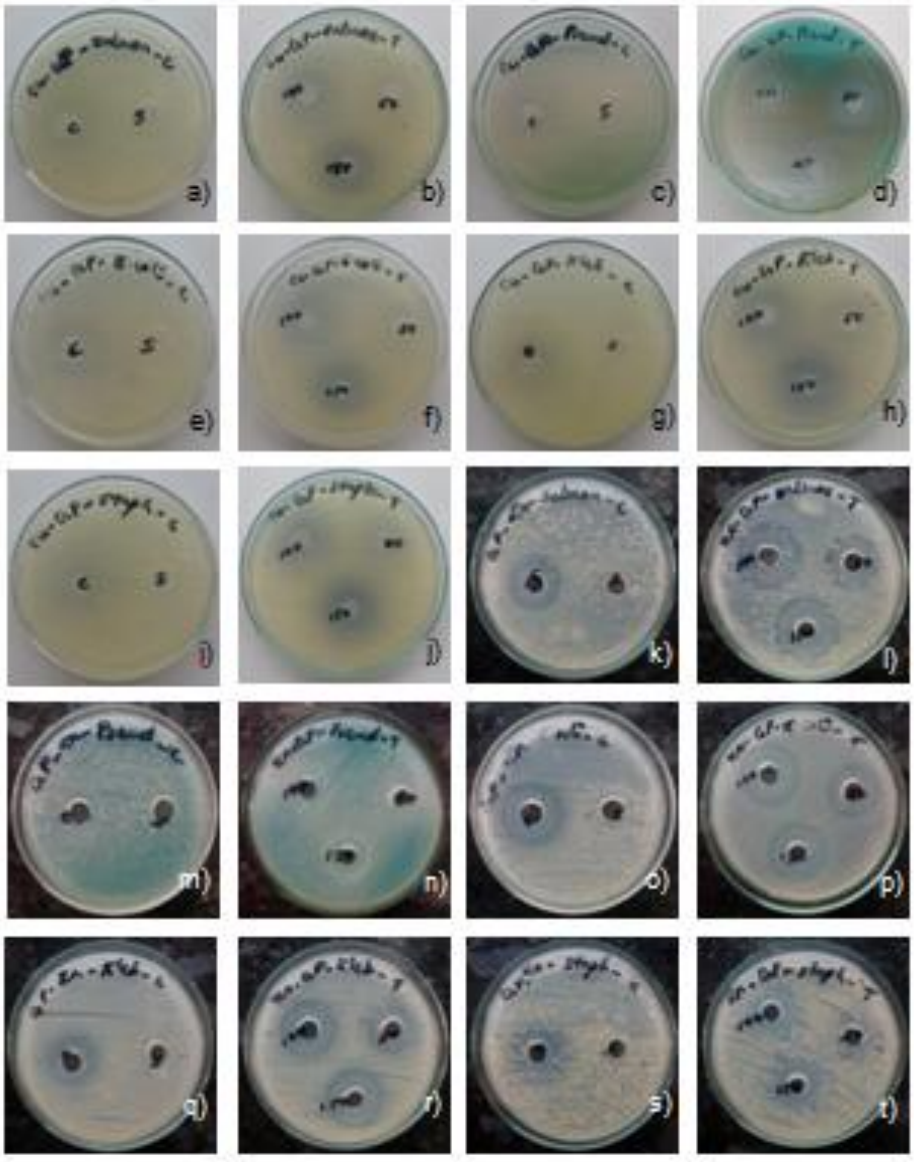


Fig.17 Antibacterial activity study using well diffusion method of Fenugreek (*Trigonellafoenum-graecum*)6 hrs seed exudate nanoparticles (Cu) and (Zn) a) *Salmonella typhi* control plate (Cu), b) *Salmonella typhi* green synthesised nanoparticle test plate (Cu; 50, 100 and 150 µl), c) *Pseudomonas aeruginosa* 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 (Fenugreek (*Trigonellafoenum-graecum*).

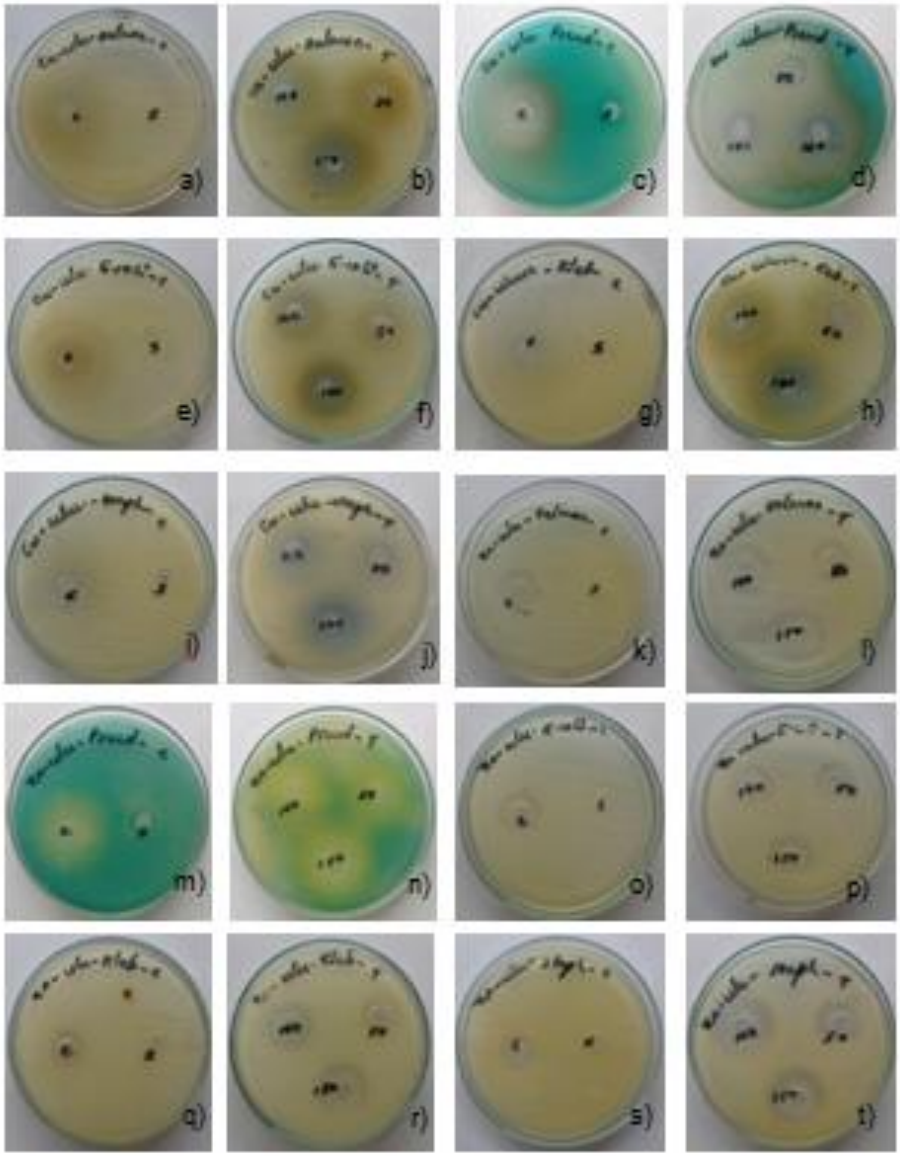


Fig.18 Antibacterial activity study using well diffusion method of Peanut (*Arachis hypogaea* L.)6 hrs seed exudate nanoparticles (Cu) and (Zn) a) *Salmonella typhi* control plate (Cu), b) *Salmonella typhi* green synthesised nanoparticle test plate (Cu; 50, 100 and 150 µl), c) *Pseudomonas aeruginosa* 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 (Peanut (*Arachis hypogaea* L.).

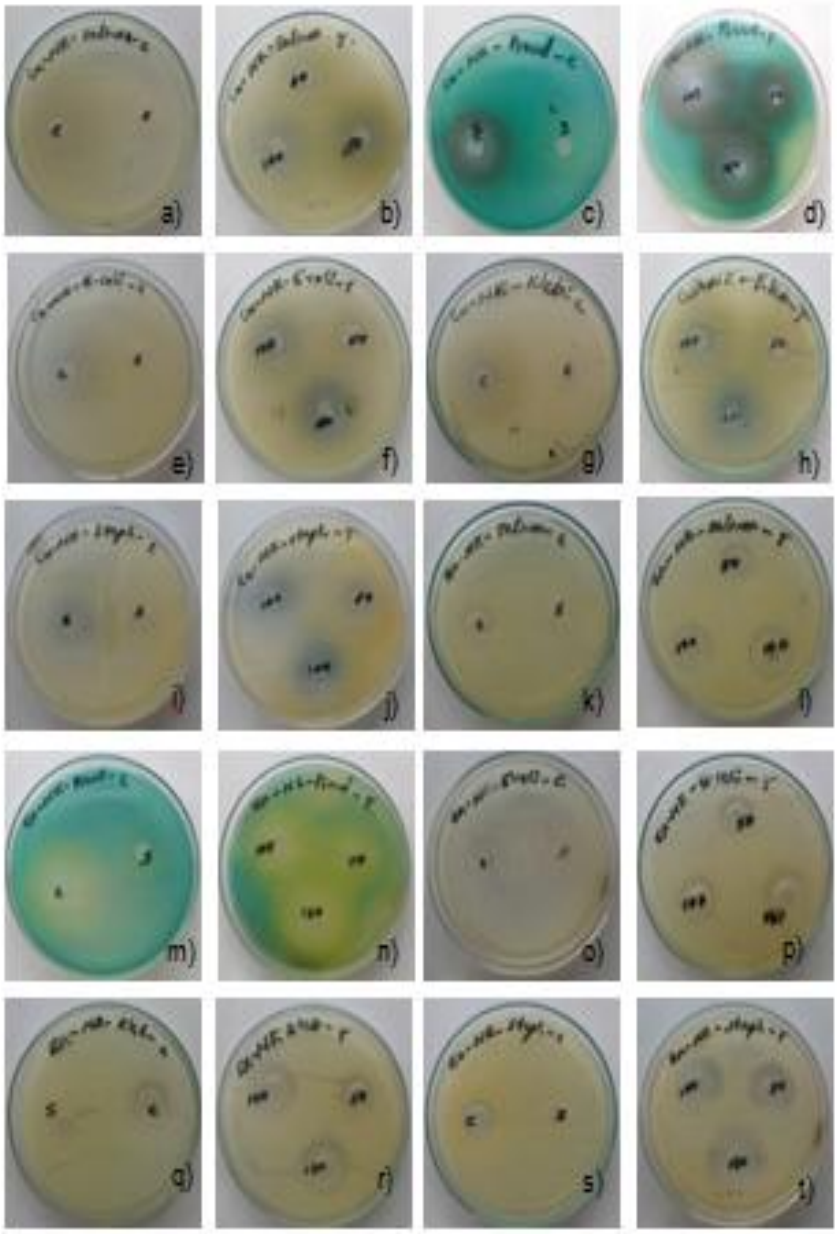


Fig.19 Antibacterial activity study using well diffusion method of Mungbean* (*Vigna radiata*)and Green peas (*Pisum sativum* L.)24 hrs seed exudates nanoparticles (Cu) a) *Salmonella typhi* control plate (Cu), b) *Salmonella typhi* green synthesised nanoparticle test plate (Cu; 50, 100 and 150 µl), 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 (Green peas (*Pisum sativum* L.).

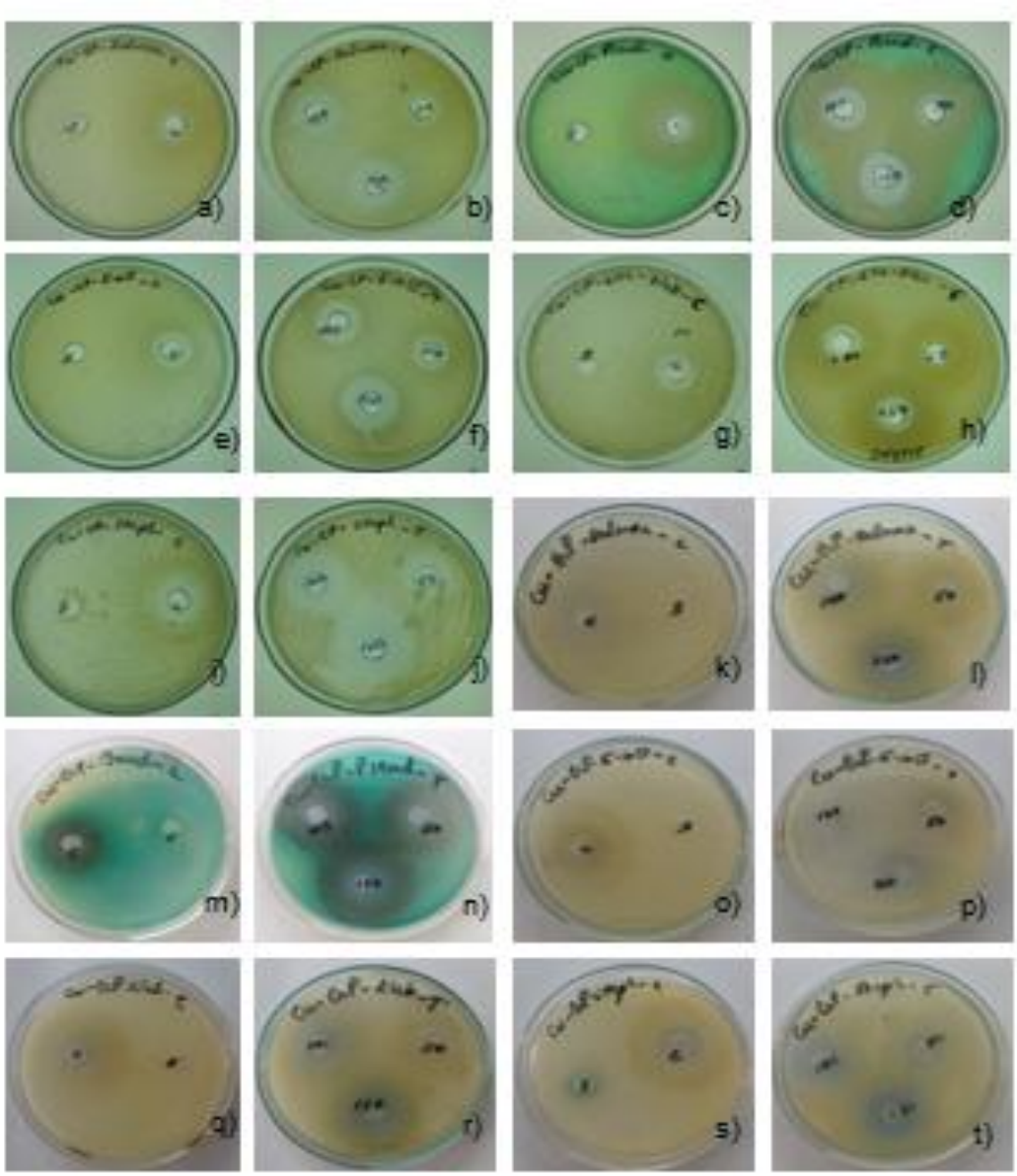


Fig.20 Antibacterial activity study using well diffusion method of Peanut (*Arachis hypogaea* L.) 24 hrs seed exudate nanoparticles (Cu) and (Zn) a) *Salmonella typhi* control plate (Cu), b) *Salmonella typhi* green synthesised nanoparticle test plate (Cu; 50, 100 and 150 µl), c) *Pseudomonas aeruginosa* 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 (Peanut (*Arachis hypogaea* L.).

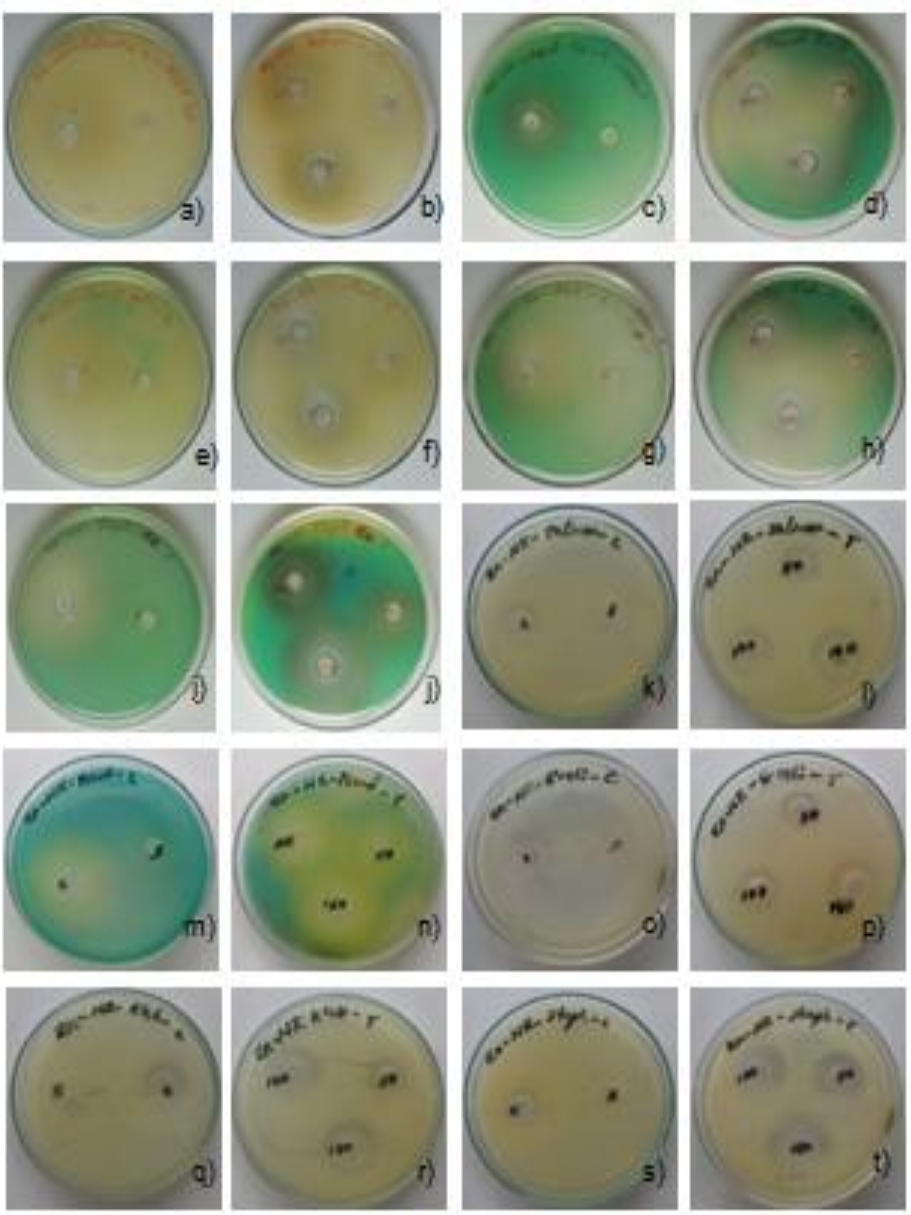
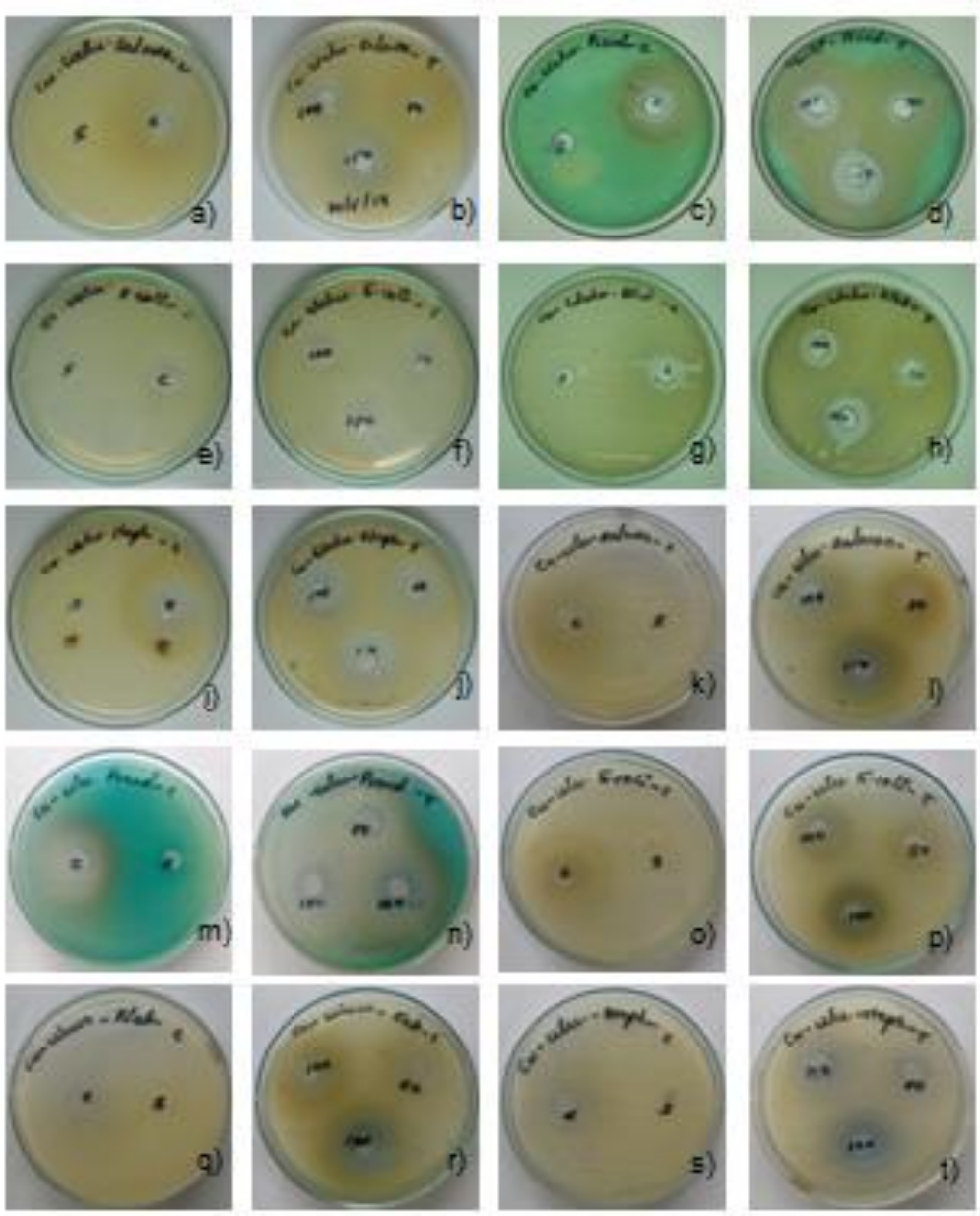


Fig.21 Antibacterial activity study using well diffusion method of Black gram* (*Vigna mungo* (L.) Hepper and Fenugreek (*Trigonellafoenum-graecum*) 24 hrs seed exudates nanoparticles (Cu)a) *Salmonella typhi* control plate (Cu), b) *Salmonella typhi* green synthesised nanoparticle test plate (Cu; 50, 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 (Fenugreek (*Trigonellafoenum-graecum*).



Fresh exudate of sample showed less antimicrobial activity. When antimicrobial activity of Copper and Zinc nanoparticles were observed, nanoparticles have 150 micro litre concentration showed maximum antimicrobial activity than that of 50 and 100 micro litre concentration. 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 Fenugreek, 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 the remaining pulses; Pea nut and Green peas.

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.

The results showed that seed exudates of Fenugreek (*Trigonellafoenum-graecum*), Green peas (*Pisum sativum* L.) and Peanut (*Arachis hypogaea* L.) 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.

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