



doi: <https://doi.org/10.20546/ijcrar.2022.1006.006>

Green Synthesis of Silver, Copper and Zinc Nanoparticles from Mung bean (*Vigna radiata*) and Cowpea (*Vigna unguiculata*) 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; Mung bean (*Vigna radiata*), Cowpea (*Vigna unguiculata*) 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.

Article Info

Received: xx May 2022

Accepted: 28 May 2022

Available Online: 20 June 2022

Keywords

Nanoparticles; *Vigna radiata*; *Vigna unguiculata*; 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 lymphatic 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 two different legumes; Mung bean (*Vigna radiata*) and Cowpea (*Vigna unguiculata*) 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 possesses 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-1442 immortalized 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 Fe₃O₄ 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 sulfadiazine 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 (Furnoet *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).

***Vigna radiata* (Mung bean)**

Vigna radiata the mung bean is alternatively known as green gram, mash or moong is a plant species in the legume family widely cultivated in India, Pakistan, Bangladesh, Nepal, China, Korea, South Asia. *Vigna* is a copper and iron rich pulse. It is a common food ingredient for Indians. *Vigna* is a leguminous plant have capacity for nitrogen fixing.

These plants are able to increase soil fertility. Mung bean is a pulse crop, which is a protein rich crop in the sub-tropical zones around the world. Mung bean consists of 51% carbohydrate, 24-26% protein, 4% mineral and 3% vitamins (Afzal *et al.*, 2008). The Mung bean was

domesticated in Persia where its progenitor occurs wild. It is used as an ingredient in savoury and sweet dishes.

Taxonomical classification (Mung bean; cherupayar)

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

Subkingdom: Viridiplantae

Superdivision: Embryophyta

Division: Tracheophyta

Class: Magnoliopsida

Order: Fabales

Family: Fabaceae

Genus: *Vigna* Savi

Species: *Vigna radiata*

***Vigna unguiculata* (Cowpea)**

Vigna unguiculata is an annual herbaceous legume from the genus *Vigna*. This is an essential crop in many developing countries such as; Africa, Asia and Latin America, here this crop is consumed as dry seeds, fresh green pods or leaves.

Cow pea is rich in proteins and it also contains high amount of proteins and minerals, so it plays an important role in human consumption and animal feeding (Singh *et al.*, 1997; Nielson *et al.*, 1997). Cow pea is a crop which is sensitive to soil water deficit. Cow pea is originated in Africa. These are used in the form of dry seeds, fodder, green pod, green manure and cover crops. They are also known as black eye pea due their appearance. Cow peas are ancient human food resource and it is one of the major food crop in many of the countries.

Taxonomical classification (Cowpea; Vanpayaru)

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

Subkingdom: Viridiplantae

Superdivision: Embryophyta

Division: Tracheophyta

Class: Magnoliopsida

Subclass: Magnoliidae

Order: Fabales

Family: Fabaceae

Genus: *Vigna Savi*

Species: *Vigna unguiculata*

Hypothesis

The current research work is based on the following hypothesis

Seeds exudates of Mung bean (*Vigna radiata*) and Cowpea (*Vigna unguiculata*) 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 km² with a population density of 859 per km² 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 Mung bean (*Vigna radiata*) and Cowpea (*Vigna unguiculata*) 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 60°C for 48hrs and later stored in air tight polyethylene zipper bag for analysis.

Extraction method

The seeds of Mungbean (*Vigna radiata*) and Cowpea (*Vigna unguiculata*) were soaked in 100 ml distilled water for 6, 12 and 24 hrs, the contents were mixed thoroughly using a glass rod 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 1mM silver nitrate (AgNO₃; Merck, Mumbai, India) and volume made up to 250 ml with distilled water. 10 ml of seed extract of different plants (Mungbean (*Vigna radiata*) and Cowpea (*Vigna unguiculata*)) was added to 90 ml of 1mM AgNO₃ solution and allowed to react at room temperature.

Copper nanoparticles

Stock solution was prepared by dissolving 2.49 g Copper sulphate (CuSO₄) and volume made up to 100 ml with distilled water. 10 ml of seed extract of different plants (Mungbean (*Vigna radiata*) and Cowpea (*Vigna unguiculata*)) was added to 90 ml of 100 mM CuSO₄ and allowed to react at room temperature.

Zinc nanoparticles

Stock solution was prepared by dissolving 2.87 g Zinc sulphate (ZnSO₄) and volume made up to 100 ml with distilled water. 10 ml of seed extract of different plants (Mungbean (*Vigna radiata*) and Cowpea (*Vigna unguiculata*)) was added to 90 ml of 100 mM ZnSO₄ 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. *Staphylococcus species* grow readily on ordinary media with a temperature range of 10 to 40°C, the optimum being 37°C and a pH of 7.4-7.6. *Staphylococcus 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⁺, Cu²⁺ and Zn²⁺ 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°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 Mung bean (*Vigna radiata*) and Cowpea (*Vigna unguiculata*) was added to 1mM silver nitrate solution and kept to reaction takes place. A colour change was observed from colourless to dark brown.

Table.1 Different vernacular names of Mung bean (*Vigna radiata*), around the globe and India.

Language	Names
Scientific names	<i>Vigna radiata</i>
Name in various global languages	
French	
German	
English	Mung bean
Name in various Indian languages	
Sanskrit	
Hindi	Moong
Urdu	Mash
Marathi	Moong
Kannada	
Gujarati	
Malayalam	Cherupayar
Tamil	Passi payaru

Table.2 Different vernacular names of Cowpea (*Vigna unguiculata*), around the globe and India.

Language	Names
Scientific names	<i>Vigna unguiculata</i>
Name in various global languages	
French	
German	
English	Cowpea
Name in various Indian languages	
Sanskrit	Mahamasah
Hindi	Lobiya
Urdu	
Marathi	Alasunda
Kannada	Alasabde
Telugu	Alasandalu
Malayalam	Vanpayar
Tamil	Kaattu ulundu

Table.3 Zone of inhibition against various bacteria (*E. coli*, *Klebsiella species*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococcus aureus*) using nanoparticles produced by Mungbean (*Vigna radiata*) 6 hrs seed exudate.

Microorganism	Nanoparticles	Control	Sample	Measure of zone of inhibition in mm		
				50	100	150
<i>E.coli</i>	Silver	12	11	11	12	13
	Copper	17	14	16	19	25
	Zinc	20	13	21	25	28
<i>Klebsiella species</i>	Silver	10	9	11	12	13
	Copper	15	12	12	16	16
	Zinc	20	13	22	25	26
<i>Pseudomonas aeruginosa</i>	Silver	11	-	12	16	20
	Copper	14	12	15	20	22
	Zinc	15	12	16	20	20
<i>Salmonella typhi</i>	Silver	10	6	10	11	12
	Copper	14	11	14	25	26
	Zinc	18	12	20	24	25
<i>Staphylococcus aureus</i>	Silver	14	9	15	18	21
	Copper	18	13	18	26	32
	Zinc	20	14	24	30	36

Table.4 Zone of inhibition against various bacteria (*E. coli*, *Klebsiella species*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococcus aureus*) using nanoparticles produced by Cowpea (*Vigna unguiculata*) 6 hrs seed exudates.

Microorganism	Nanoparticles	Control	Sample	Measure of zone of inhibition in mm		
				50	100	150
<i>E.coli</i>	Silver	7	6	10	13	17
	Copper	14	13	20	20	28
	Zinc	17	13	19	24	30
<i>Klebsiella species</i>	Silver	8	9	10	12	14
	Copper	14	13	16	18	20
	Zinc	19	13	19	22	24
<i>Pseudomonas aeruginosa</i>	Silver	9	4	11	14	16
	Copper	24	15	20	22	23
	Zinc	16	12	16	22	25
<i>Salmonella typhi</i>	Silver	7	5	10	13	14
	Copper	13	11	14	15	17
	Zinc	22	12	16	22	25
<i>Staphylococcus aureus</i>	Silver	8	9	15	17	20
	Copper	24	13	20	30	34
	Zinc	24	13	20	30	34

Table.5 Zone of inhibition against various bacteria (*E. coli*, *Klebsiella species*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococcus aureus*) using nanoparticles produced by Mungbean (*Vigna radiata*) 12 hrs seed exudates.

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

Table.6 Zone of inhibition against various bacteria (*E. coli*, *Klebsiella species*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococcus aureus*) using nanoparticles produced by Cowpea (*Vigna unguiculata*) 12 hrs seed exudates.

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

Table.7 Zone of inhibition against various bacteria (*E. coli*, *Klebsiella species*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococcus aureus*) using nanoparticles produced by Mungbean (*Vigna radiata*) 24 hrs seed exudates.

Microorganism	Nanoparticles	Control	Sample	Measure of zone of inhibition in mm		
				50	100	150
<i>E.coli</i>	Silver	4	6	9	11	12
	Copper	20	13	23	28	30
	Zinc	22	13	28	29	32
<i>Klebsiella species</i>	Silver	5	5	9	11	13
	Copper	20	14	32	33	36
	Zinc	18	12	23	28	34
<i>Pseudomonas aeruginosa</i>	Silver	6	5	11	12	14
	Copper	21	14	28	29	33
	Zinc	17	14	26	29	30
<i>Salmonella typhi</i>	Silver	7	5	10	11	13
	Copper	22	14	24	25	29
	Zinc	23	14	28	30	30
<i>Staphylococcus aureus</i>	Silver	5	7	9	11	12
	Copper	23	14	22	28	33
	Zinc	20	14	28	29	34

Table.8 Zone of inhibition against various bacteria (*E. coli*, *Klebsiella species*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococcus aureus*) using nanoparticles produced by Cowpea (*Vigna unguiculata*) 24 hrs seed exudates.

Microorganism	Nanoparticles	Control	Sample	Measure of zone of inhibition in mm		
				50	100	150
<i>E.coli</i>	Silver	7	6	11	12	13
	Copper	14	13	18	20	24
	Zinc	18	14	19	25	29
<i>Klebsiella species</i>	Silver	6	5	9	11	13
	Copper	14	12	18	24	27
	Zinc	16	12	19	25	28
<i>Pseudomonas aeruginosa</i>	Silver	4	5	8	11	12
	Copper	14	12	14	14	22
	Zinc	19	13	22	26	29
<i>Salmonella typhi</i>	Silver	5	6	9	10	11
	Copper	14	14	20	26	27
	Zinc	18	13	22	27	29
<i>Staphylococcus aureus</i>	Silver	5	7	9	11	13
	Copper	18	14	23	25	28
	Zinc	18	12	26	26	29

Table.9 UV absorption spectrum of Silver nanoparticles formed from Mungbean (*Vigna radiata*) 6 hrs seed exudate during different time of incubation.

Time	385 nm	435nm	560nm	680nm
½ hr	0.190	0.145	0.029	0.440
1 hr	0.239	0.228	0.074	0.020
1 ½ hr	0.226	0.193	0.051	0.009
2 hr	0.253	0.223	0.060	0.010
2 ½ hr	0.260	0.232	0.064	0.012
Blank	0.113	0.099	0.044	0.020

Table.10 UV absorption spectrum of Silver nanoparticle formed from Mungbean (*Vigna radiata*) 12 hrs seed exudates during different time of incubation.

Time	385 nm	435nm	560nm	680nm
½ hr	0.371	0.368	0.056	0.011
1 hr	0.422	0.444	0.095	0.012
1 ½ hr	0.401	0.419	0.064	0.012
2 hr	0.408	0.438	0.078	0.005
2 ½ hr	0.421	0.467	0.094	0.011
Blank	0.220	0.203	0.058	0.010

Table.11 UV absorption spectrum of Copper nanoparticle formed from Mung bean (*Vigna radiata*) 6 hrs seed exudates during different time of incubation.

Time	350 nm	385 nm	435 nm	560 nm
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	0.022	0.014	0.023	0.422

Table.12 UV absorption spectrum of Copper nanoparticles formed from Mungbean (*Vigna radiata*) 12 hrs seed exudates during different time of incubation.

Time	385 nm	435nm	560nm	680nm
½ hr	0.221	0.076	0.037	0.440
1 hr	0.218	0.075	0.038	0.424
1 ½ hr	0.226	0.079	0.038	0.448
2 hr	0.216	0.074	0.030	0.434
2 ½ hr	0.215	0.068	0.033	0.438
Blank	0.025	0.014	0.027	0.421

Table.13 UV absorption spectrum of Silver nanoparticles formed from Cowpea (*Vigna unguiculata*) 6 hrs seed exudates during different time of incubation.

Time	385 nm	435nm	560nm	680nm
½ hr	0.982	1.248	0.696	0.364
1 hr	1.449	1.774	0.982	0.514
1 ½ hr	1.672	2.06	0.996	0.440
2 hr	1.872	2.296	1.276	0.586
2 ½ hr	2.511	3.304	0.944	0.434
Blank	0.603	0.503	0.333	0.232

Table.14 UV absorption spectrum of Silver nanoparticles formed from Cowpea (*Vigna unguiculata*) 12 hrs seed exudates during different time of incubation.

Time	385 nm	435nm	560nm	680nm
½ hr	1.155	2.206	1.328	0.554
1 hr	1.491	3.556	1.514	0.732
1 ½ hr	1.623	3.992	1.700	0.856
2 hr	2.088	2.542	0.970	0.478
2 ½ hr	1.556	1.796	1.470	0.780
Blank	0.222	0.198	0.151	0.129

Table.15 UV absorption spectrum of Copper nanoparticles formed from Cowpea (*Vigna unguiculata*) 6 hrs seed exudates during different time of incubation.

Time	350 nm	385 nm	435 nm	560 nm
½ hr	0.024	0.010	0.024	0.455
1 hr	0.124	0.085	0.052	0.471
1 ½ hr	0.171	0.124	0.066	0.484
2 hr	0.282	0.222	0.132	0.536
2 ½ hr	0.255	0.205	0.127	0.528
Blank	0.023	0.013	0.023	0.458

Table.16 UV absorption spectrum of Copper nanoparticles formed from Cowpea (*Vigna unguiculata*) 12 hrs seed exudates during different time of incubation.

Time	385 nm	435nm	560nm	680nm
½ hr	0.134	0.081	0.186	0.579
1 hr	0.202	0.140	0.076	0.482
1 ½ hr	0.313	0.235	0.143	0.528
2 hr	0.340	0.267	0.165	0.551
2 ½ hr	0.330	0.246	0.190	0.563
Blank	0.052	0.056	0.072	0.491

Table.17 Description of UV absorption spectrum of Silver nanoparticles formed from Mungbean (*Vigna radiata*) 24 hrs seed exudates during different time of incubation.

Time	350 nm	385 nm	435 nm	560 nm
½ hr	0.627	0.403	0.201	0.115
1 hr	0.809	0.769	0.446	0.193
1 ½ hr	0.913	0.875	0.427	0.161
2 hr	0.942	0.916	0.465	0.198
2 ½ hr	0.945	0.912	0.447	0.200
Blank	0.541	0.424	0.246	0.146

Table.18 Description of UV absorption spectrum of Copper nanoparticles formed from Mungbean (*Vigna radiata*) 24 hrs seed exudates during different time of incubation.

Time	350 nm	385 nm	435 nm	560 nm
½ hr	0.287	0.124	0.056	0.442
1 hr	0.264	0.118	0.059	0.445
1 ½ hr	0.266	0.109	0.045	0.443
2 hr	0.290	0.135	0.073	0.440
2 ½ hr	0.290	0.136	0.070	0.453
Blank	0.138	0.096	0.077	0.464

Table.19 Description of UV absorption spectrum of Copper nanoparticles formed from Cowpea (*Vigna unguiculata*) 24 hrs seed exudates during different time of incubation.

Time	350 nm	385 nm	435 nm	560 nm
½ hr	0.231	0.177	0.124	0.508
1 hr	0.253	0.255	0.123	0.509
1 ½ hr	0.286	0.215	0.140	0.524
2 hr	0.300	0.246	0.750	0.544
2 ½ hr	0.520	0.399	0.221	0.589
Blank	0.138	0.096	0.077	0.464

Table.20 Description of UV absorption spectrum of Silver nanoparticles formed from Cowpea (*Vigna unguiculata*) 24 hrs seed exudates during different time of incubation.

Time	350 nm	385 nm	435 nm	560 nm
½ hr	1.515	3.708	0.728	0.281
1 hr	2.654	3.692	1.232	0.51
1 ½ hr	2.734	3.86	0.886	0.328
2 hr	1.146	3.956	1.168	0.446
2 ½ hr	2.556	1.951	1.134	0.408
Blank	0.540	0.424	0.246	0.146

Table.21 Biochemical characterization of the organisms used in the study.

Organisms	I	MR	VP	C	GS	U	O	CL	COG	NR
<i>Salmonella typhi</i>	-VE	+VE	-VE	-VE	-VE	-VE	-VE	+VE	_	+VE
<i>Pseudomonas aeruginosa</i>	-VE	-VE	-VE	+VE	-VE	-VE	+VE	+VE	-VE	+VE
<i>Staphylococcus aureus</i>	-VE	+VE	+VE	+VE	+VE	+VE	-VE	+VE	+VE	+VE
<i>E. coli</i>	+VE	+VE	-VE	-VE	-VE	-VE	-VE	+VE	_	+VE
<i>Klebsiella pneumoniae</i>	-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.22 Antibiotic susceptibility test of the organisms used in the study.

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

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

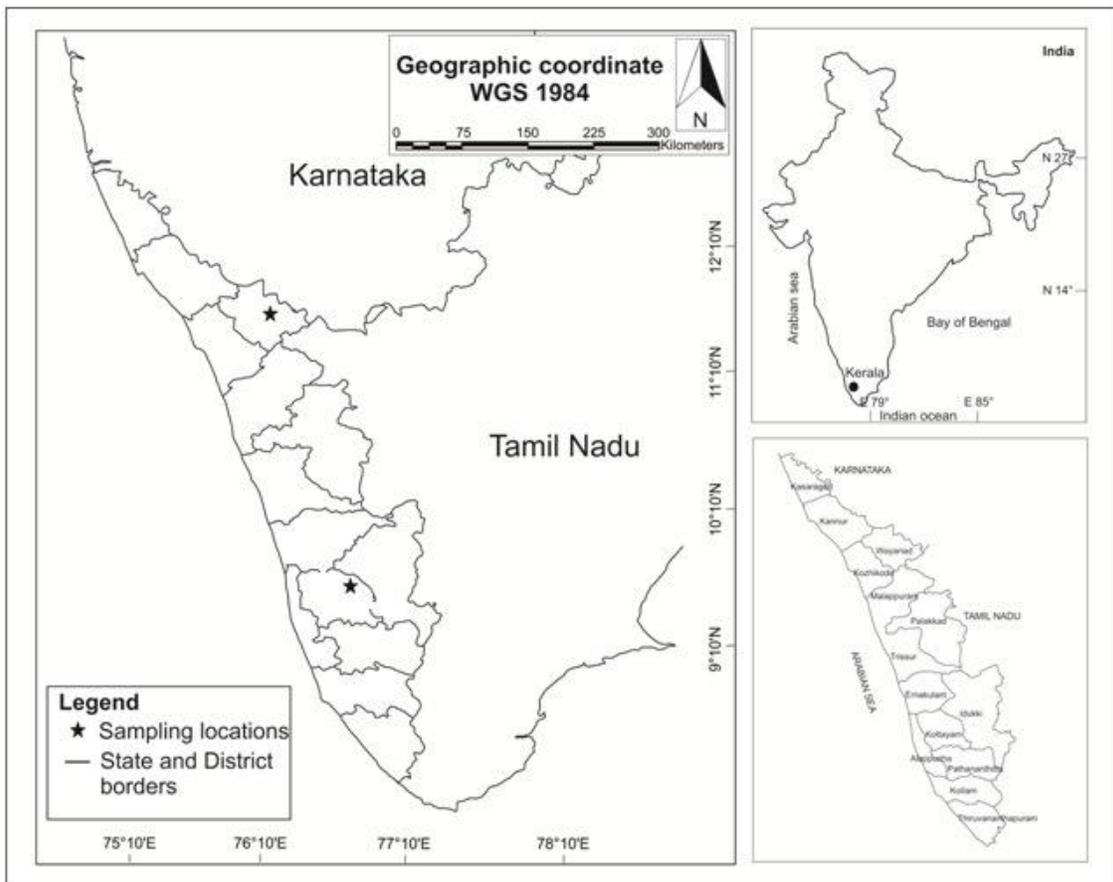


Fig.2 Description of Mungbean (*Vigna radiata*) a) dried and cleaned seeds, b) mature seeds in the plant, c) fried seeds as snacks, d) dried seeds unpolished and polished, f) mature pods and seeds. Photo courtesy: Wikipedia.



Fig.3 Cowpea (*Vigna unguiculata*),description a) mature pods and seeds, b) harvested whole pods for cooking, c) and d)dried seeds, e) pest attacking the stored seeds. Photo courtesy: Wikipedia.



Fig.4 Description of the various pulses seeds a) Cowpea (*Vigna unguiculata*), 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.

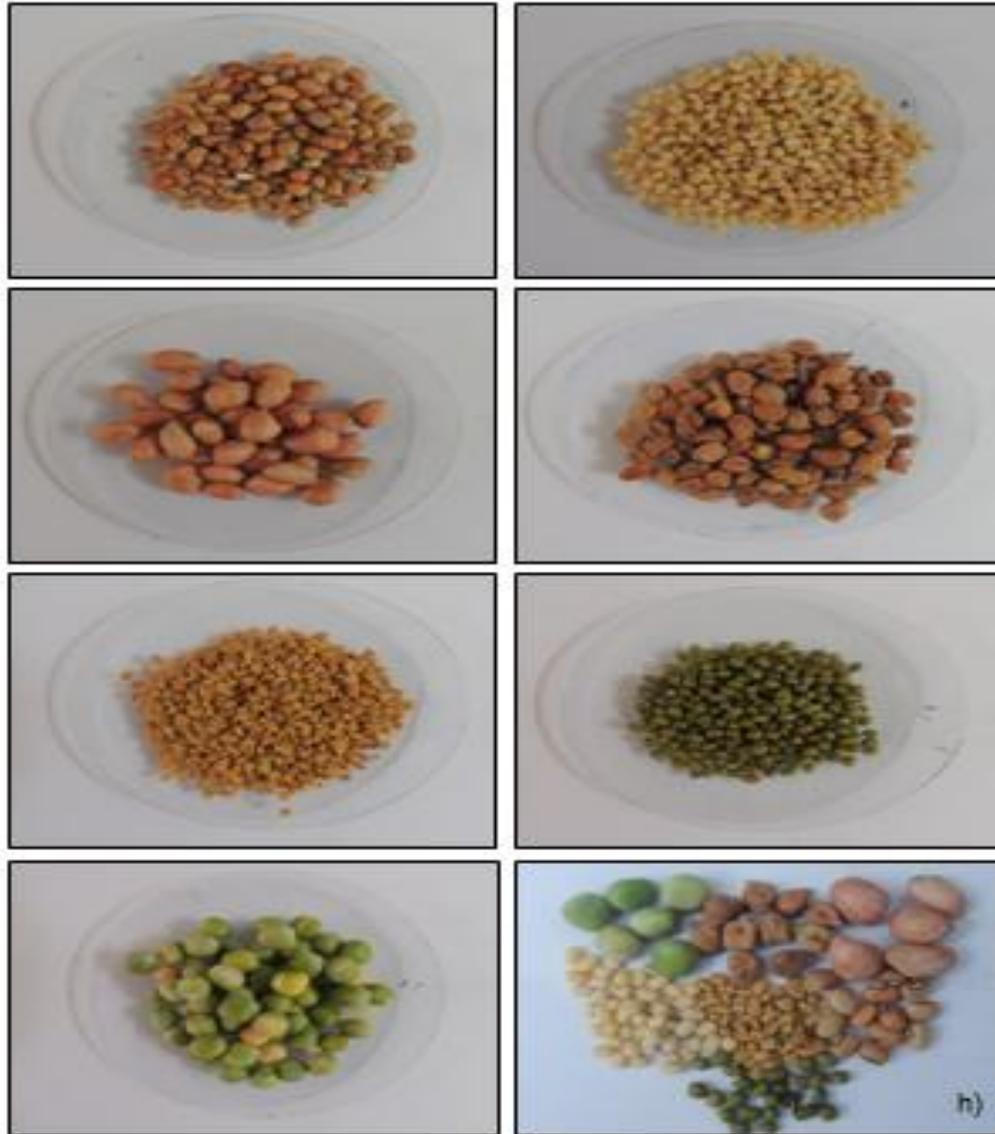


Fig.5 Description of the various pulses seeds a) Cowpea (*Vigna unguiculata*), b) Black gram (*Vigna mungo* (L.) Hepper, c) Peanut* (*Arachishypogaea* L.), d) Chick pea* (*Cicer arietinum* L.), e) Fenugreek* (*Trigonella foenum-graecum*), f) Mungbean* (*Vigna radiata*), g) Green peas* (*Pisum sativum* L.), h) all the seeds mixed together.* data not provided.

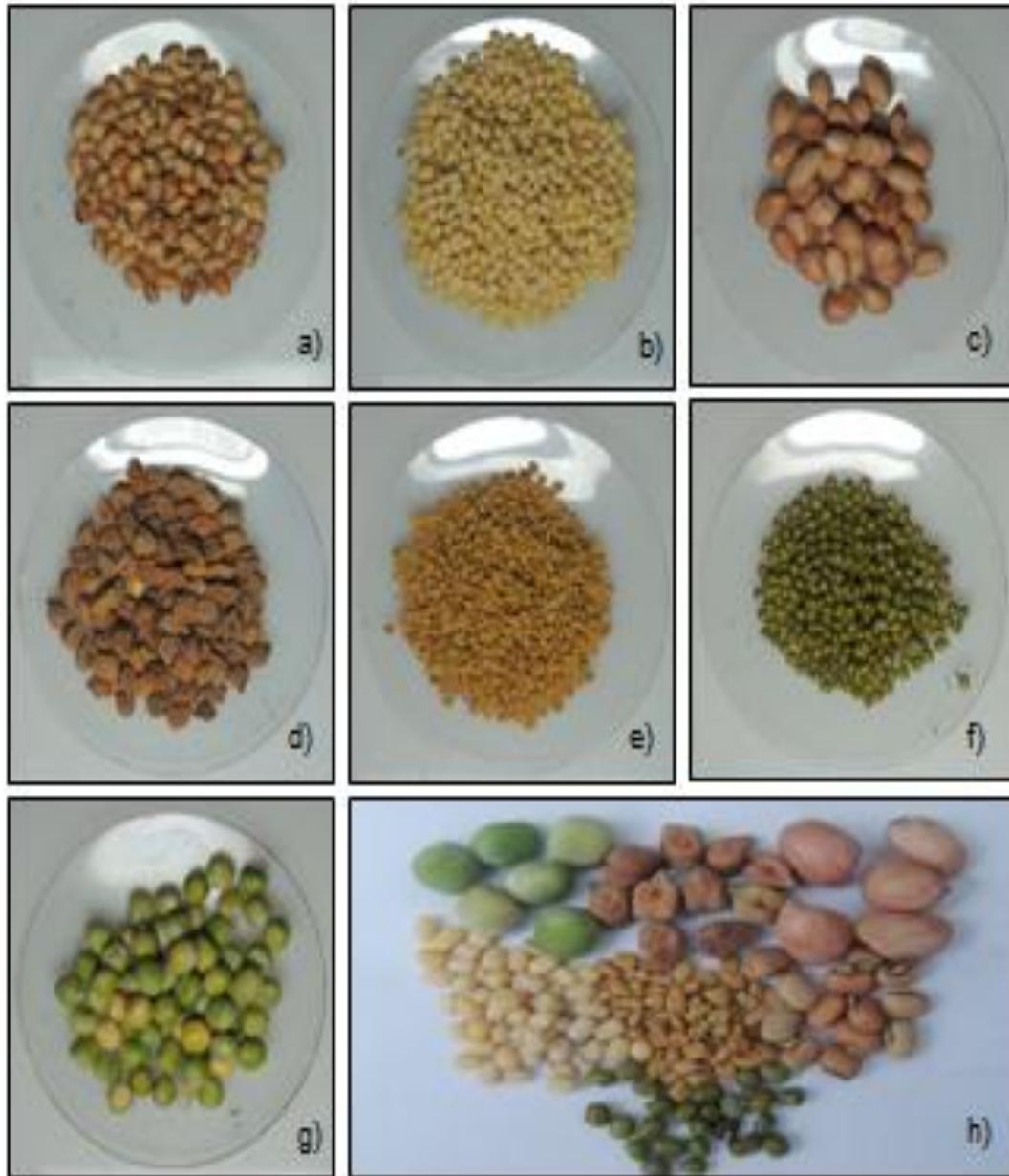


Fig.6 Seed exudates used for making nanoparticles a) Cowpea (*Vigna unguiculata*), b) Black gram (*Vigna mungo* (L.) Hepper, c) Peanut* (*Arachis hypogaea* L.), d) Chick pea* (*Cicer arietinum* L.), e) Fenugreek* (*Trigonella foenum-graecum*), f) Mungbean* (*Vigna radiata*), g) Green peas* (*Pisum sativum* L.), h) 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 (Cowpea and Mungbean), c) and d) green synthesized zinc nanoparticles, e) and f) green synthesized copper nanoparticles, g) and h) green synthesized silver nanoparticles.

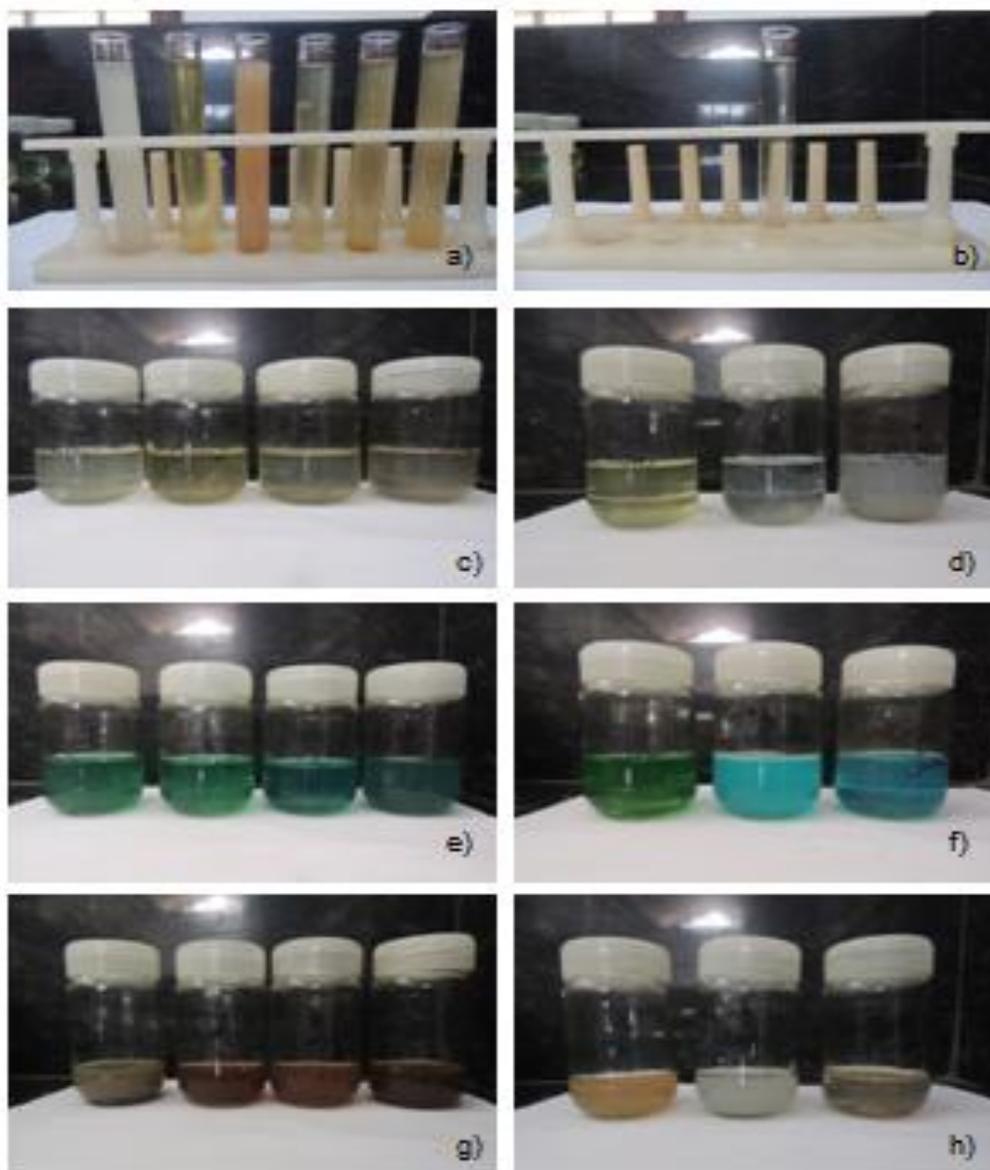


Fig.8 Antibacterial activity study using well diffusion method of Mungbean (*Vigna radiata*) and Green peas* (*Pisum sativum* L.) 12 hrs seed exudates nanoparticles (Zn) a) *Salmonella typhi* control plate (Zn), b) *Salmonella typhi* green synthesised nanoparticle test plate (Zn; 50, 100 and 150 μ l), 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.))* data not provided.

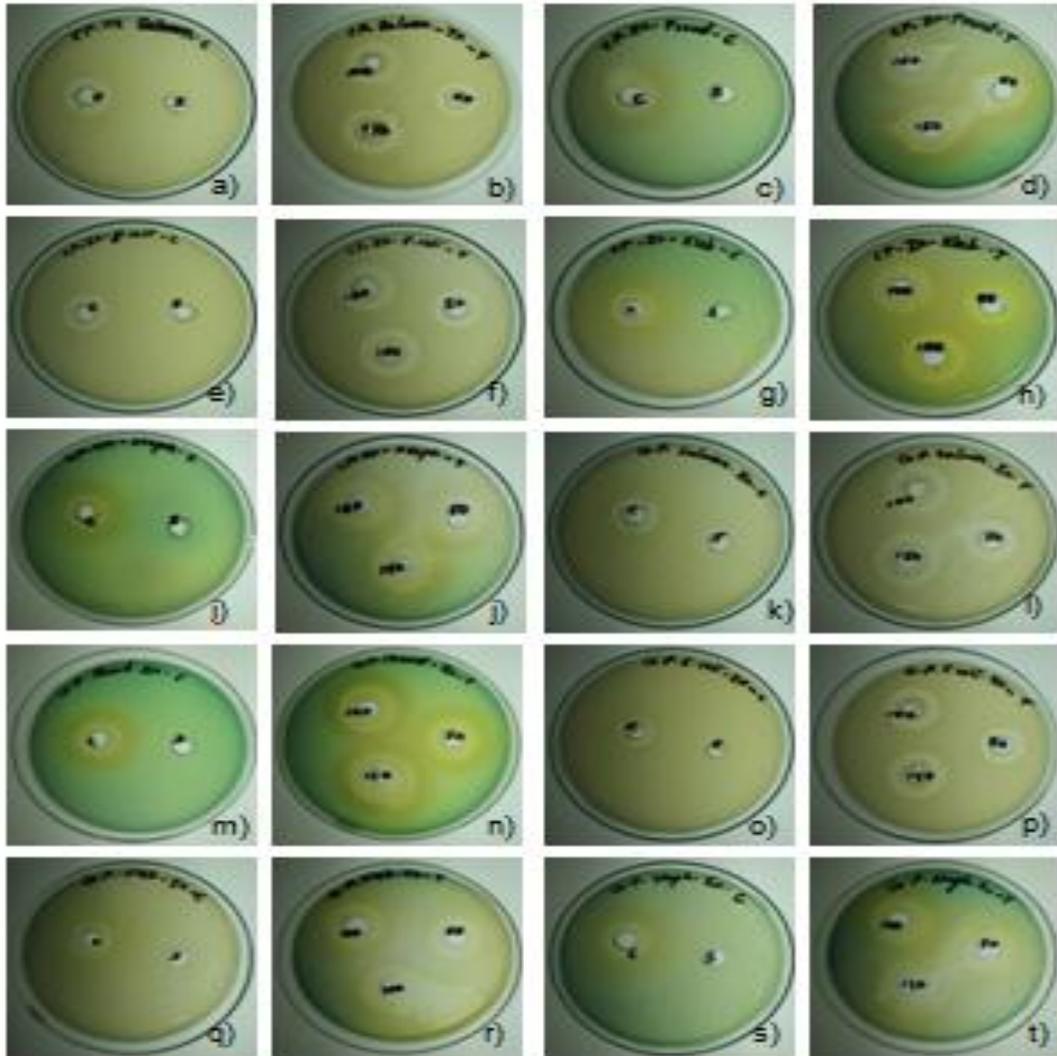


Fig.9 Antibacterial activity study using well diffusion method of Cowpea (*Vigna unguiculata*) and Peanut* (*Arachis hypogaea* L.) 12 hrs seed exudates nanoparticles (Cu) and (Zn) a) *Salmonella typhi* control plate (Cu), b) *Salmonella typhi* green synthesised nanoparticle test plate (Cu; 50, 100 and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



Fig.10 Antibacterial activity study using well diffusion method of Chick pea* (*Cicer arietinum* L.) and Cowpea (*Vigna unguiculata*) 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 μ l), 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 unguiculata*)).* data not provided.

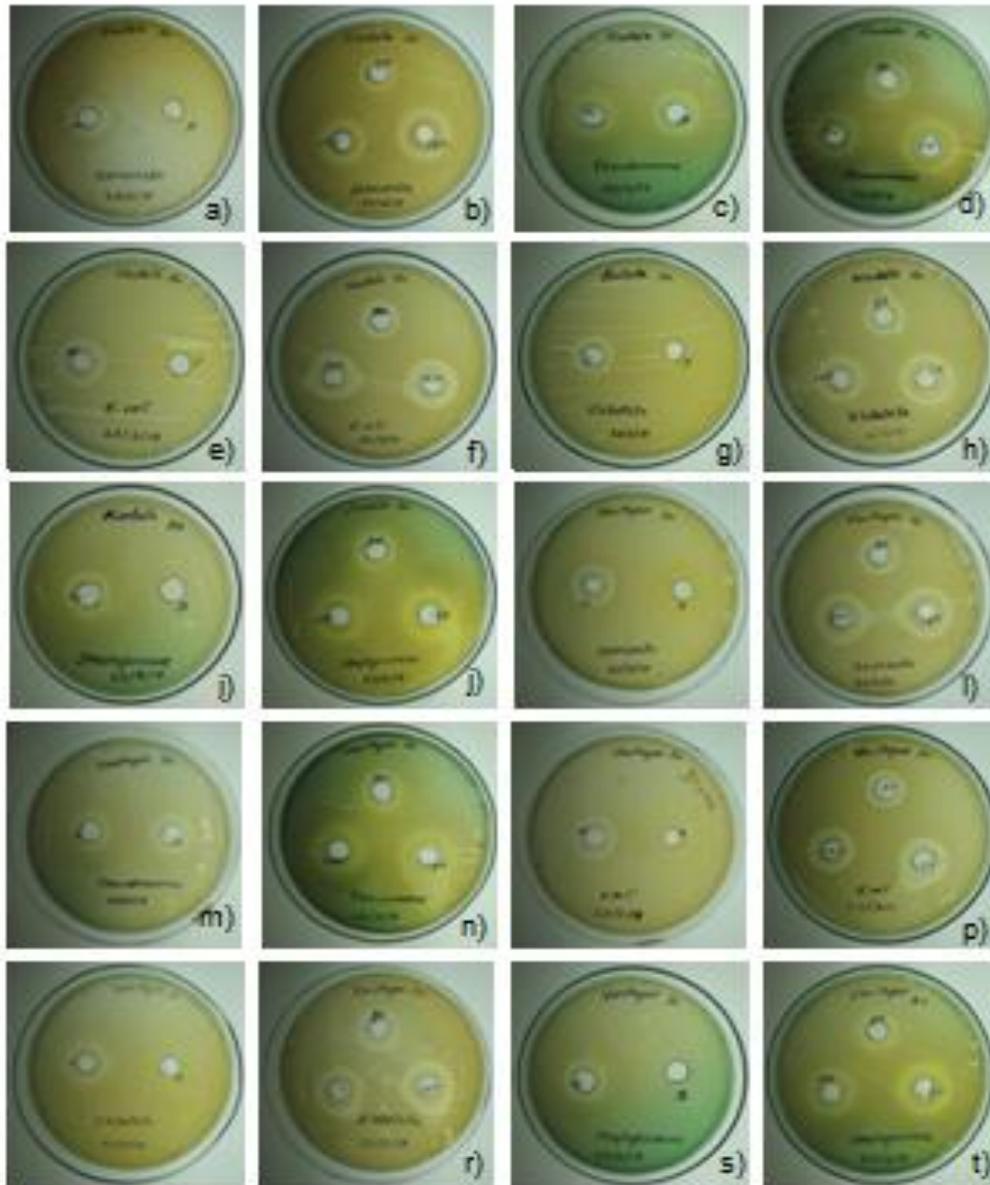


Fig.11 Antibacterial activity study using well diffusion method of Mungbean (*Vigna radiata*) and Black gram* (*Vigna mungo* (L.) Hepper 12 hrs seed exudates nanoparticles (Cu) a) *Salmonella typhi* control plate (Cu), b) *Salmonella typhi* green synthesised nanoparticle test plate (Cu; 50, 100 and 150 μ l), 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.* data not provided).

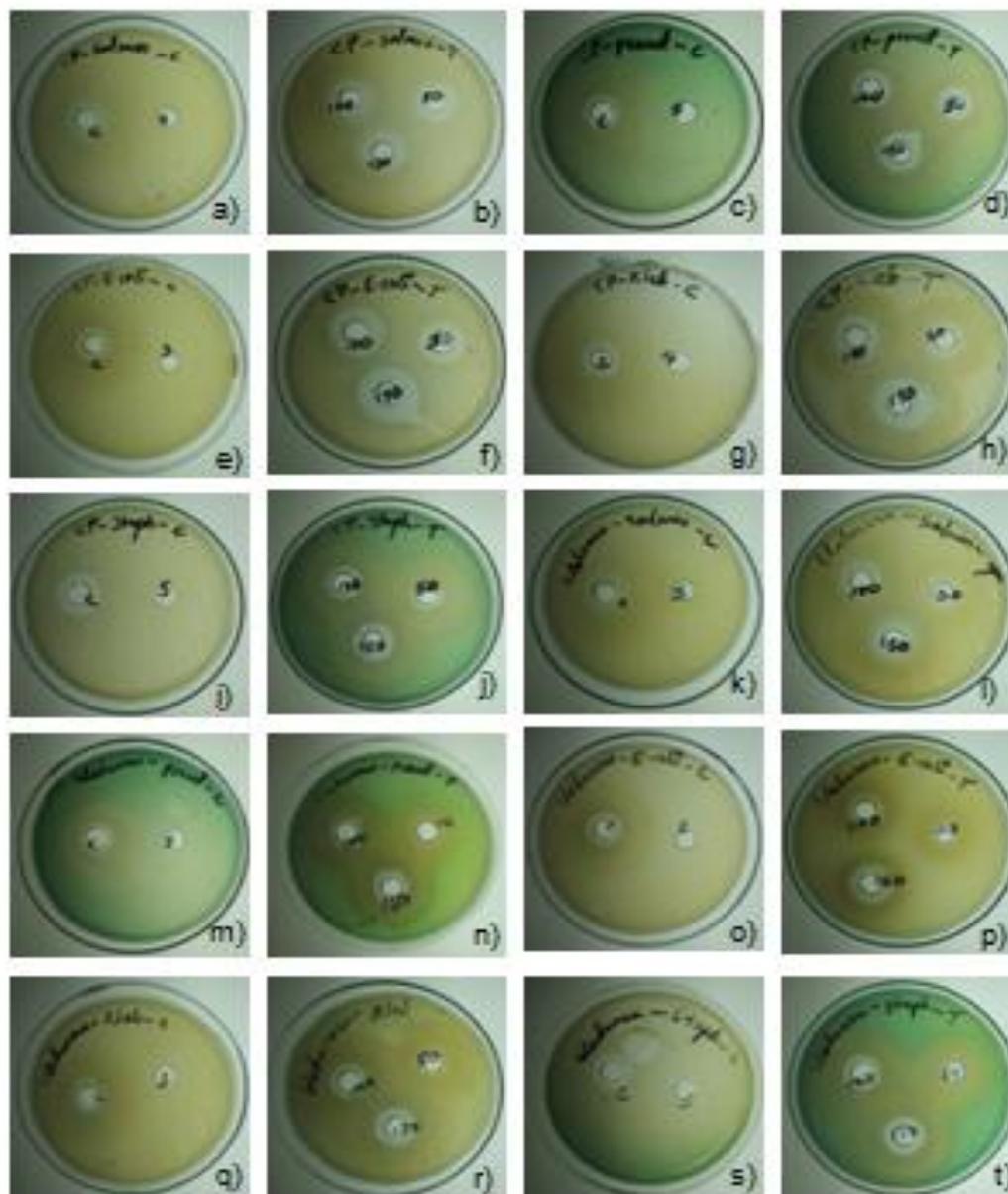


Fig.12 Antibacterial activity study using well diffusion method of Mungbean (*Vigna radiata*) and Green peas* (*Pisum sativum* L.) 24 hrs seed exudates nanoparticles (Zn) a) *Salmonella typhi* control plate (Zn), b) *Salmonella typhi* green synthesised nanoparticle test plate (Zn; 50, 100 and 150 μ l), 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.))* data not provided.

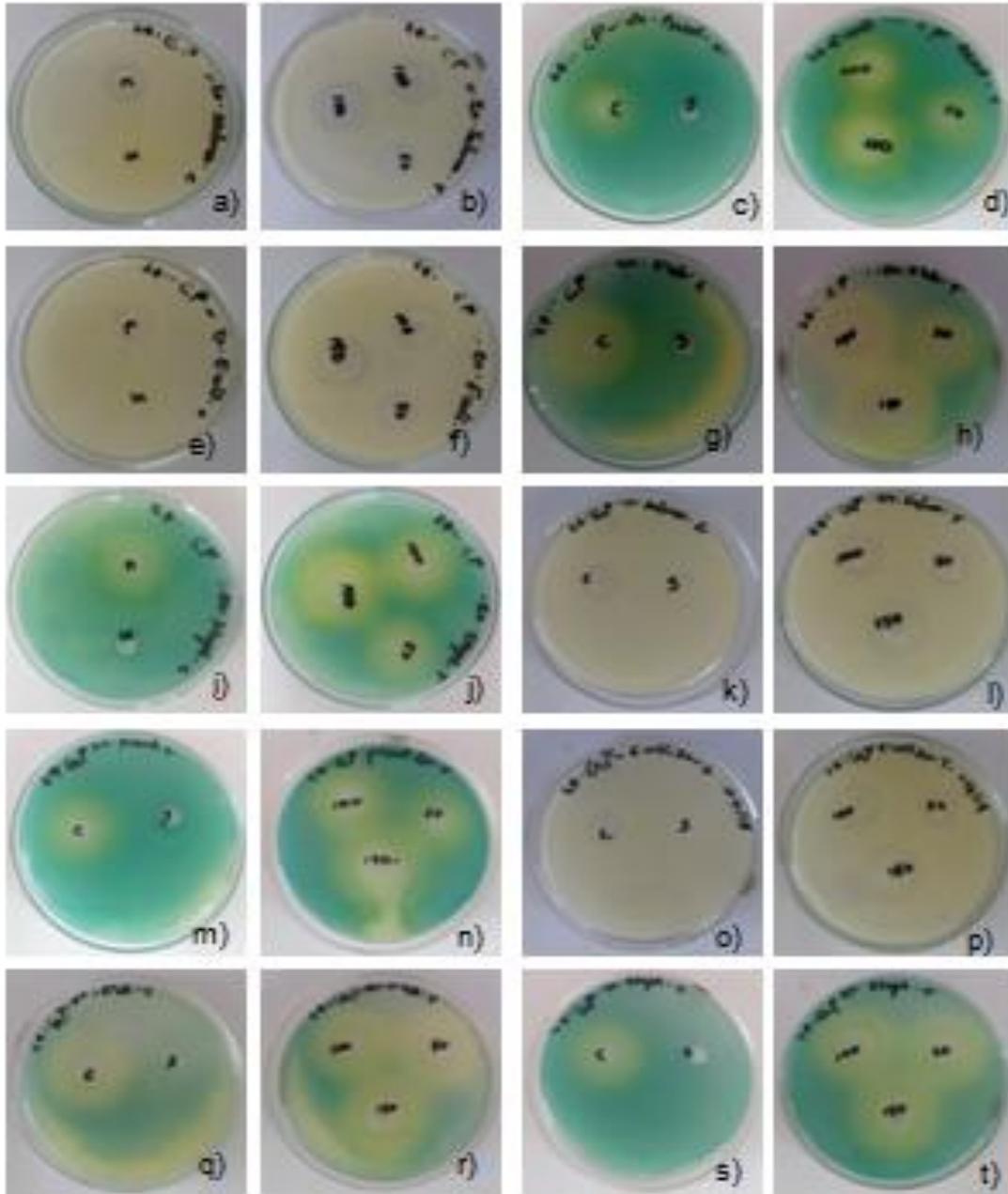


Fig.13 Antibacterial activity study using well diffusion method of Mungbean (*Vigna radiata*) 6 hrs seed exudates nanoparticles (Cu) and (Zn) a) *Salmonella typhi* control plate (Cu), b) *Salmonella typhi* green synthesised nanoparticle test plate (Cu; 50, 100 and 150 μ l), c) *Pseudomonas aeruginosa* 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 (Mungbean (*Vigna radiata*)).

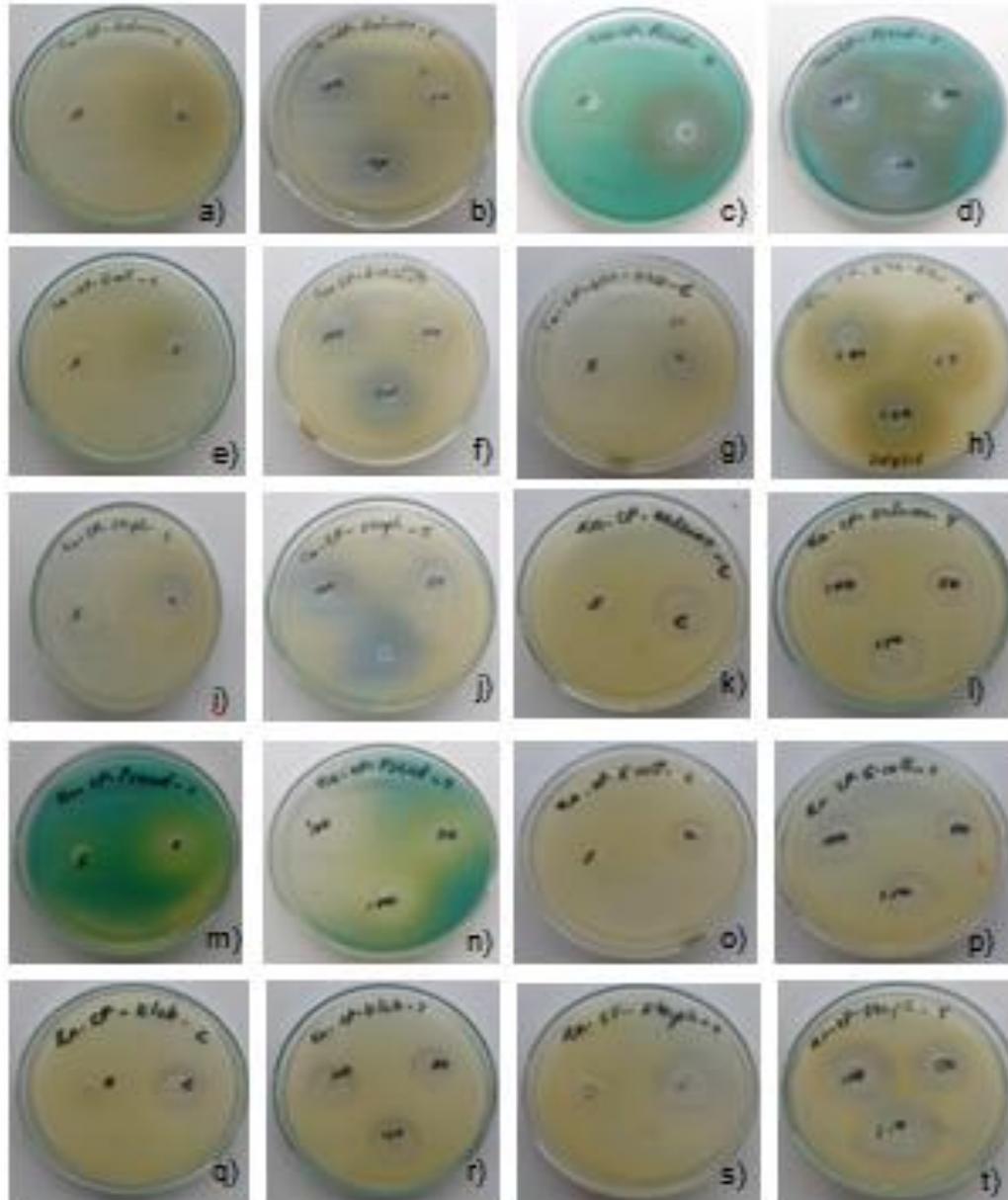


Fig.14 Antibacterial activity study using well diffusion method of Cowpea (*Vigna unguiculata*) 6 hrs seed exudate nanoparticles (Cu) and (Zn) a) *Salmonella typhi* control plate (Cu), b) *Salmonella typhi* green synthesised nanoparticle test plate (Cu; 20, 40 and 60 μ l), c) *Pseudomonas aeruginosa* 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 unguiculata*).

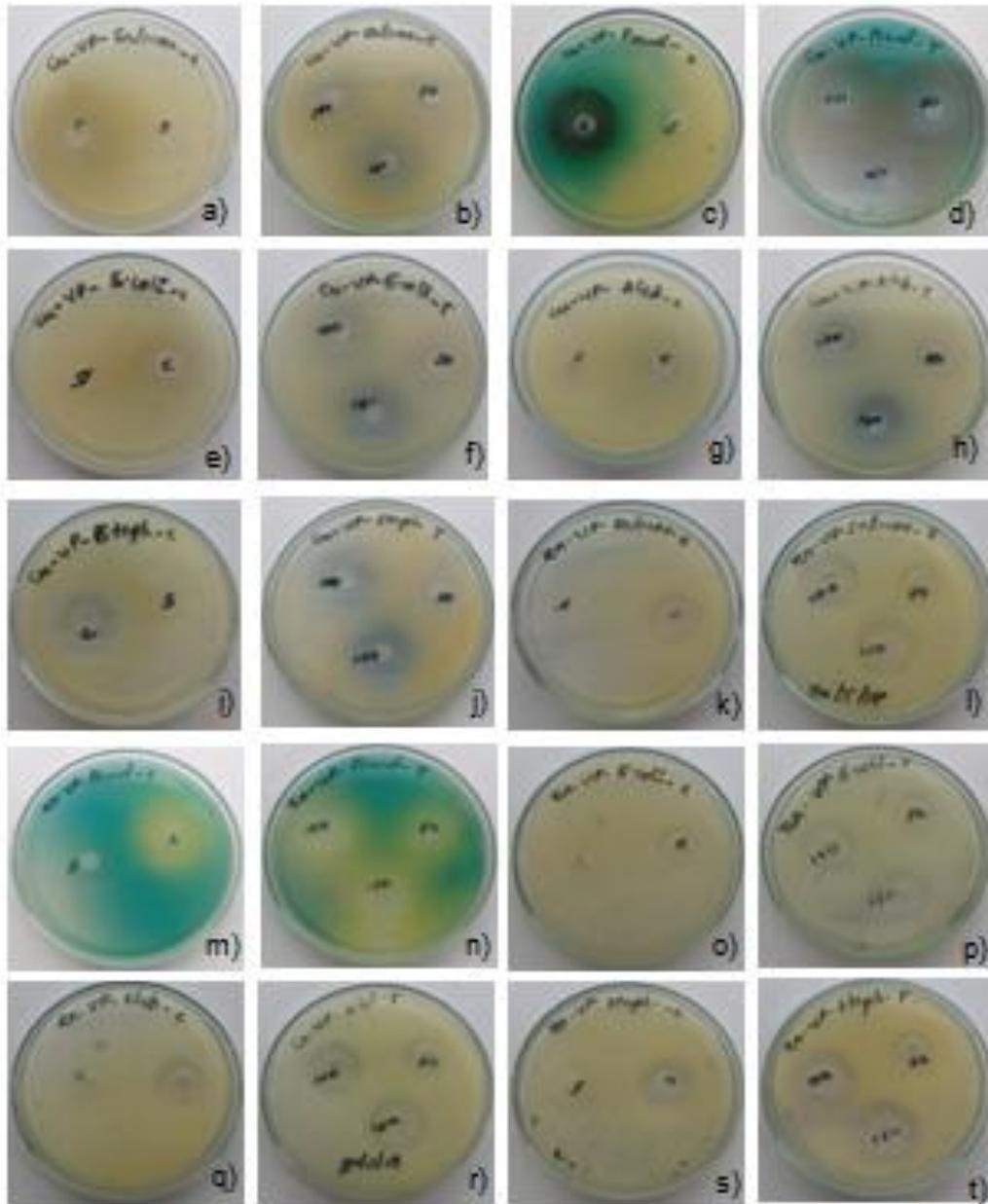


Fig.15 Antibacterial activity study using well diffusion method of Cowpea (*Vigna unguiculata*) 24 hrs seed exudate nanoparticles (Cu) and (Zn) a) *Salmonella typhi* control plate (Cu), b) *Salmonella typhi* green synthesised nanoparticle test plate (Cu; 20, 40 and 60 μ l), c) *Pseudomonas aeruginosa* control, d) *Pseudomonas aeruginosa* test, e) *E. coli* control, f) *E. coli* test, g) *Klebsiella species* control, h) *Klebsiella speciestest*, i) *Staphylococcus species* control, j) *Staphylococcus speciestest*, k) to t) above mentioned organisms plate in same order for Zn nanoparticles (Cowpea (*Vigna unguiculata*)).

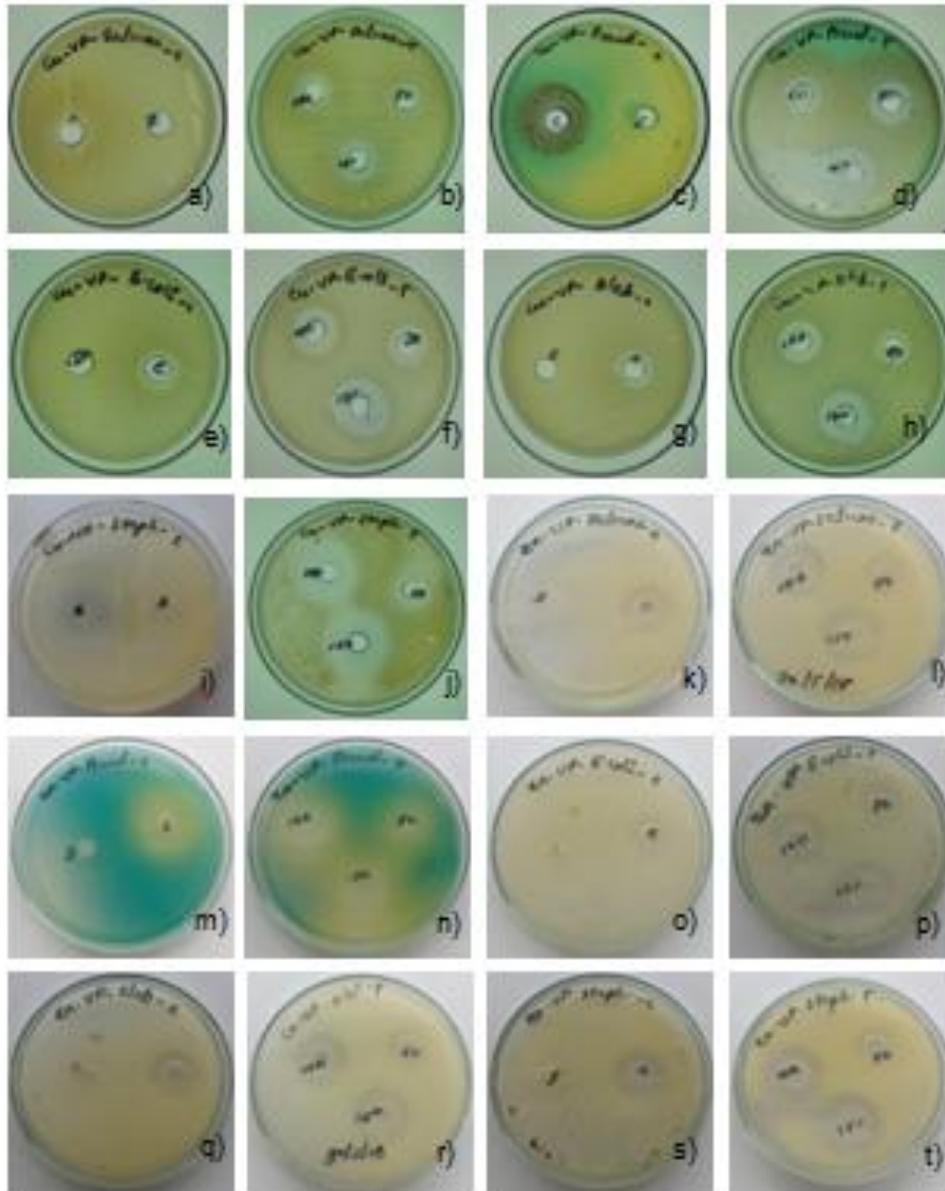
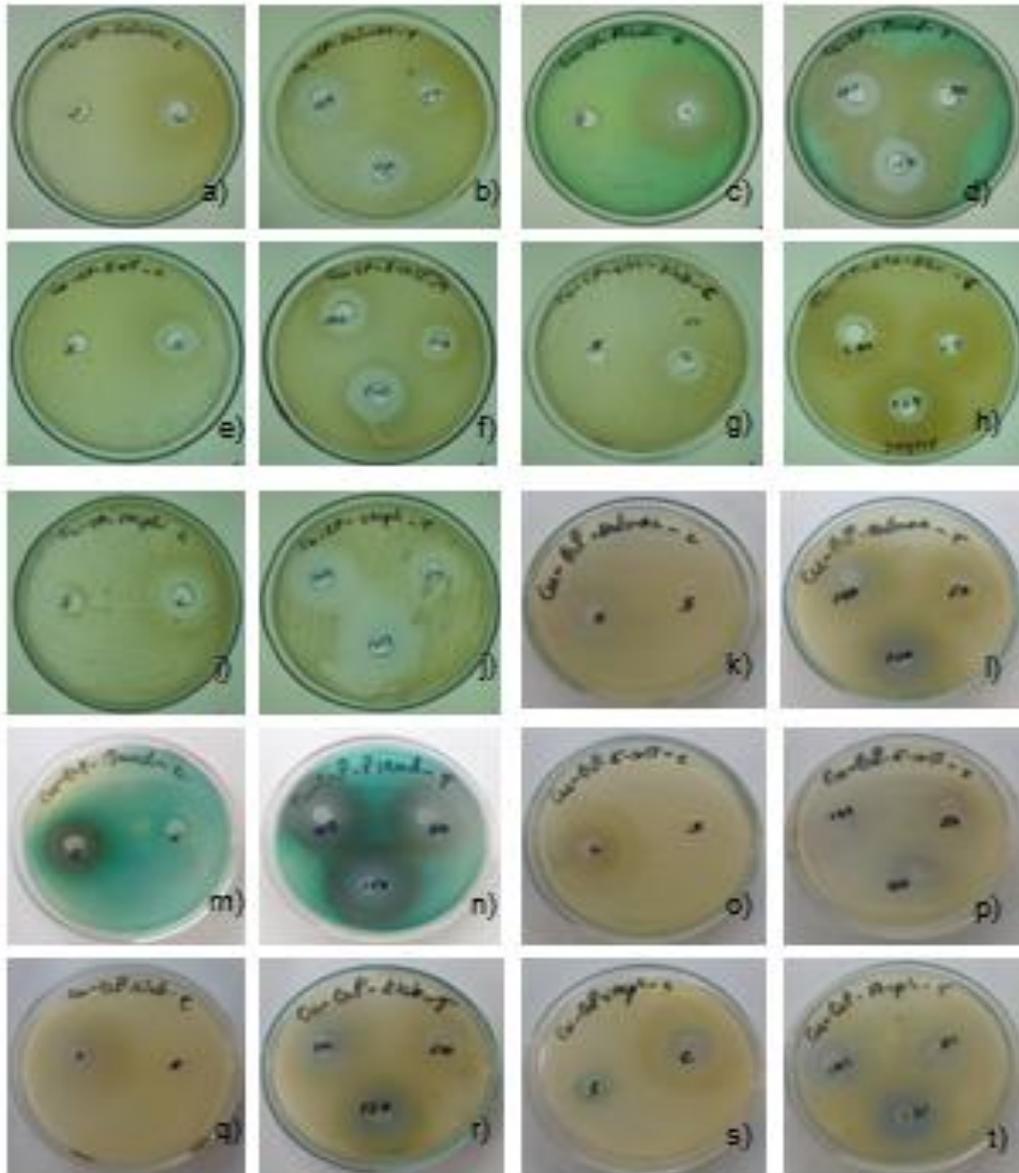


Fig.16 Antibacterial activity study using well diffusion method of Mungbean (*Vigna radiata*) and Green peas* (*Pisum sativum* L.) 24 hrs seed exudates nanoparticles (Cu) a) *Salmonella typhi* control plate (Cu), b) *Salmonella typhi* green synthesised nanoparticle test plate (Cu; 50, 100 and 150 μ l), 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.)).* data not provided.



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 Mung bean (*Vigna radiata*) and Cowpea (*Vigna unguiculata*). 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 (Mung bean (*Vigna radiata*) and Cowpea (*Vigna unguiculata*)) 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 (Mung bean (*Vigna radiata*) and Cowpea (*Vigna unguiculata*)) 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 Mung bean (*Vigna radiata*) and Cowpea (*Vigna unguiculata*). The intensity of the peak at 350 nm was increased with time until the reduction completes.

Antibacterial assay

Seed exudates of Mung bean (*Vigna radiata*) and Cowpea (*Vigna unguiculata*) showed growth inhibitory effects against *Salmonella Typhi*, *Pseudomonas Aeruginosa*, *Staphylococcus aureus*, *E. coli* and *Klebsiella pneumoniae*. 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 Mung bean, Copper nanoparticles showed more antibacterial activity than Zinc nanoparticles against *Klebsiella* species (36 mm). Copper nanoparticles showed higher zone than that of Zinc nanoparticles. They have antibacterial activity against *Salmonella* species, *Pseudomonas*, *Staphylococcus* species and *Klebsiella* species also. When antibacterial activity of different concentration were observed, nanoparticles have 150 micro litre concentration shows maximum activity against these microbes. Pure sample exudate of Mung bean also showed a zone of inhibition, that means the fresh exudate also have the capacity to act against microbes. Zinc nanoparticles showed more antimicrobial activity against *Klebsiella* species (34 mm) and *Staphylococcus aureus* (34 mm).

In the case of Cow pea, here Zinc nanoparticles showed higher zone of inhibition than that of Copper nanoparticles. It indicates Zinc nanoparticles have more antimicrobial activity. Here, Zinc nanoparticles was more active against *Salmonella typhi* (29 mm) and *Staphylococcus aureus* (29 mm). Fresh exudate was also shows growth inhibition zone against these five types of bacteria. Copper nanoparticles showed more antimicrobial activity against *Klebsiella* specie (27 mm). Nanoparticles have 150 μ l concentration were more active against these microbes.

From the above results we can understand that, 24 hours old sample exudates showed maximum antimicrobial activity. 150 μ l concentration of nanoparticles were more active against the bacteria than 50 and 150 μ l. In the case of Mung bean, 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 Cow pea.

Silver, copper and zinc nanoparticles have antibacterial activity against *Salmonella species*, *Pseudomonas species*, *Staphylococcus species*, *E. coli* and *Klebsiella species*. When antibacterial activity of silver, copper and zinc nanoparticles from 3 different concentrations were observed, nanoparticles have 60 μ l 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 Mung bean (*Vigna radiata*) and Cowpea (*Vigna unguiculata*) 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 Mulantra 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 antimutagenic 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)₂·2H₂O. *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., Ramirez, 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 Mung bean (*Vigna radiata*) and Cowpea (*Vigna unguiculata*) Exudates and Evaluation of their Antibacterial Activity: An Overview. *Int.J.Curr.Res.Aca.Rev.* 10(06), 48-81.
doi: <https://doi.org/10.20546/ijcrar.2022.1006.006>