To study antimicrobial and metal ion potential of Silver nanoparticles synthesized from *Zingiber officinale* using different solvents by EDS & TEM

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ABSTRACT: Nanotechnology is new form of technology which has produced a great development in various fields. Nanoparticles are of the great scientific interest as they are effectively a bridge between bulk material and atomic & molecular structures. Nanoparticles are the particle that have size 1 to 100 nm and possess due to large surface area to volume ratio & smaller size. Different types of nanomaterial such as Zinc, Copper, Gold, and Silver are available but silver nanoparticles have proven to be most effective. Silver nanoparticles can be synthesized from various conventional methods that are physical, chemical and biological. Physical and chemical method synthesis is expensive and that have toxic substances. To overcome biological method provides a feasible alternative. In present study it is reported that a cost effective, simple, environment friendly route of green synthesis of silver nanoparticles using extract of *Zingiber officinale* prepared from different solvents (double distilled water and 70% ethanol) by hot percolation method. Synthesized Silver nanoparticles were preliminary analysed by using UV-VIS spectrophotometer at 630 nm. Confirmatory analysis characterization Electron dispersive X-ray spectroscopy (EDS) for presence of true metal ion and Transmission electron microscopy (TEM) micrographs suggested the size using solvents ddw *(*double distilled water) 35(±5) nm and 70% ethanol 50(±5) nm in size. Synthesized Silver nanoparticles were characterized for their antimicrobial activity against gram positive (*Staphylococcus aureus)* and gram negative (*Escherichia coli, Pseudomonas aeruginosa)* using MIC. Here we purpose the application of synthesized silver nanoparticles from *Zingiber officinale* for the confirmation of presence of heavy metal ion. Metal nanoparticles have extensively used for presence of metal ion Hg^{2+} was detected by UV-VIS spectrophotometer at 630 nm.

Keywords: *Energy Dispersion X-Ray Spectroscopy (EDS), Metal ion detections, Minimum inhibitory concentration (MIC), Transmission Electron microscopy (TEM), Silver Nanoparticles, Zingiber officinale*

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INTRODUCTION

Nanotechnology has emerged as a rapidly growing field for the manufacture of new materials on the nanoscale level with frequent application of science and technology (Albrecht and Evan, 2006). The concept of nanotechnology was first begun with lecture delivered by Feymann in 1959 (Baker and Satish, 2012). Nanotechnology is the science, engineering, technology conducted at the nanoscale which is about 1 to 100 nm in size

(Gleiter, 2000). Nanotechnologies hold a promising future for the design and development of many types of novel product that are used in early detection, treatment and prevention of various diseases (EI-Nour, *et al.,* 2010). Nanobiotechnology is the combination ofengineering and molecular biology that is leading to a new class of multifunctional devices and systems for biological and chemical analysis with better sensitivity, specificity and a higher rate of recognition. Nanoparticles are the great scientific interest as they are effectively a bridge between bulk material and atomic & molecular structures. Nanoparticles can be engineered with different composition, size and shape and surface chemistries to enable novel technologies in wide range of biological applications (Edina C. Wang and Andrew Z. Wang, 2015). Nanoparticles are the particles that have size 1 to 100 nm in at least one dimension and possess due to large surface area to volume ratio &smaller size (Katoj, 2011). There are two basic approaches used in nanoparticles synthesis: Top down approach and Bottom up approach (Jeremy, 2009). Nanoparticles may or may not exhibit size related properties they differ from those observed fine particles or bulk materials (Buzea, *et al.,* 2007). Nanoparticles are finding their application in various fields such as biomedical, tissue engineering, health care, environmental, drug delivery, gene delivery, optics mechanics, nonlinear optical devices, food industry, and space industry (Mohanpuria, *et al.,* 2008). Nanoparticles are divided into various categories depending upon their size and shape, morphology and chemical devices such as Quantum based, Liposomes, Carbon nanotubes, Polymeric nanoparticles, Dendrimers, Nanoshell, Fullerenes (Ibrahim khan, *et al.,* 2017). Different types of nanomaterial like Titanium, Zinc (Retchkiman Schabes, *et al.,* 2006), Gold (Gu, *et al.,* 2003) and Silver has most effective as it has good antimicrobial actions against bacteria other microorganism (Gong, *et al.,* 2007). Nanoparticles are synthesized by physical, chemical, and biological methods. Various chemical and physical methods are proved to be quite expensive and potentially hazardous to the environments which involve the use of toxic and perilous chemicals that are responsible for various biological risks. This may be the reason for choosing biosynthesis of nanoparticles via green route that does not employ toxic chemicals and proved to be eco-friendly (Reddy, *et al.,* 2012). Biological method is more preferred over physical and chemical methods. Nanoparticles are biologically synthesized from the bacteria. Silver nanoparticles have been the subjects of researches because of their unique properties (e.g. size & shape) depending optical, antimicrobial and electrical properties (Iravani, *et al.,* 2014). Silver nanoparticles are one of the most vital and fascinating nanomaterial among several metallic nanoparticles that are involved in biomedical application. Silver nanoparticles play an imported role in nanoscience and nanotechnology, particular in nanomedicine. Silver (Ag) nanoparticles have focussed on potential application in cancer diagnosis & therapy (Zhang, *et al.,* 2016). Ag

nanoparticles were evaluated for their antimicrobial activity against the gram-positive *(Staphylococcus aureus)* and gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacteria. There are many method that perform antimicrobial activity, here we study that Minimum inhibitory concentration (MIC) was used to determine antimicrobial activity of plant by determining the MIC based on turbidity in each sample containing culturing microorganism in liquid media with plant extract at particular temperature. The size and shape of silver (Ag) nanoparticles are typically measured by analytical technique such as Energy dispersion X-ray spectrometer (EDS), Transmission electron microscopy (TEM) (Gurunathan, *et al.,* 2015). Here we perform detection of heavy metal ion using synthesized silver nanoparticles using *Zingiber officinale*. Heavy metal ion is non-biodegradable and contaminates most of natural resources occurring in the environment including water. Some of metal include Lead (Pb), Mercury (Hg), Arsenic (As), Chromium (Cr), Cadmium (Cd) are considered to be highly toxic and hazards to human health. To detect metal ion with inference such as microorganism, enzyme, and nanomaterial like gold and silver nanoparticles (Gumpu, *et al.,* 2015). Various applications of silver nanoparticles can be described as follow:

1.Diagnostic application: Ag nanoparticles are used in biosensors and numerous assays where silver nanoparticles material can be used as biological tags for quantitative detection.

2. Textile industry: Ag nanoparticles applications from disinfecting medical devices and home appliances to water treatment (Bosetti, *et al.,* 2003), Agnanoparticles can be used in textile industry for textile fabrics. The cotton fibre containing silver nanoparticles exhibited high antibacterial activity against *E.coli* (Yeo, *et al.,* 2003).

3. Antibacterial applications: Ag nanoparticles can be used for sterilizing nanomaterial in medical product, food storage bags.

4.Catalyst: Ag nanoparticles have been demonstrated to present catalytic redox properties for biological agents such as dyes as well as chemical agents such as benzene. In Ag nanoparticles mostly used with titanium dioxide as the catalyst for chemical reaction.

5.Optical probe: Ag nanoparticles are widely used as probes for surface enhanced Raman scattering (SERS), and metal enhanced fluorescence (MEF). Compared to other noble metal nanoparticles, silver nanoparticles exhibits more advantages for probe such as higher extinction coefficients, sharper extinction band. Ginger is a member of the family *Zingiberaceae.* Its common name is *Zingiber officinale.* Ginger is actually a thick, underground stem known as rhizome. *Zingiber officinale* is an herbaceous perennial plant. Ginger originated in the tropical rainforest from the Indian subcontinent to southern Asia where ginger plant show considerable genetic variation (Thomas and Everett, 1982). Ginger has many medicinal uses. The health benefits of ginger are likely due to antioxidants, anti-inflammatory properties and content of

therapeutic compounds like gingerol, shogaol, paradol, and *Zingerone* that has been liked to unique health benefits (Wang, *et al.,* 2014).

HEALTH BENEFITS OF GINGER:

1. Digestive disorders are the extremely useful herb to relieve patients suffering from Vomiting, dyspepsia other stomach problem.

2. Cough and Cold are the herb is used to relieve cough.

3. The plant is an excellent pain killer. Ointment made by ginger can help in relieving head pains.

4. Ginger can treat many forms of Nausea, especially morning sickness. Ginger appears to be highly effective against nausea and also relieve vomiting after surgery in cancer patient undergoing chemotherapy (Chaiyakunapruk, *et al.,* 2006).

5. Ginger may also reduce the muscle pain and soreness. The acute effect of ginger on muscle pain Inflammation and dysfunction induced by eccentric exercise were examined (Black and O'connor, 2010).

6. Ginger a substance that may help prevent cancer. Ginger extract has been studied as an alternative treatment for several form of cancer. Anticancer property of ginger includes Gingerol and the major pungent component of ginger, its impact on different steps of the metastatic process (Poltronieri, *et al.,* 2014).

7. Ginger can help fight infection. Gingerol, the bioactive substance in fresh ginger, can help lower the risk of infections .In fact, ginger extract can inhibit the growth of many different types of bacteria (Karuppiah and Rajaram, 2012).

MATERIAL AND METHODS

Herbaceous Material

The *Zingiberofficinale* was collected from the local market of Ludhiana. It was properly cleaned with running tap water and was used for experimental purpose.

Biosynthesis of Silver Nanoparticles from herbaceous material Zingiber officinale using different solvent

1. Preparation of herbaceous material *Zingiber officinale* of raw extract:

A. Preparation of aqueous extracts of *Zingiber officinale* paste using double distilled water as a solvent: *Zingiber officinale* was grinded in distilled water to form fine paste. 25g paste was diluted 5 times in double distilled water and then was subjected to hot percolation treatment. In hot percolation treatment diluted paste was heated at 40-50^{0}C for 2-3 hours till resultant mixture boils completely and then kept undisturbed for 10 minutes. The filtrate so obtained was kept in waterbath at 60^{0}C till reduced volume of filtrate was obtained. The resultant mixture was then filtered out using Whatman filter paper no.1 in conical flask and was used as raw extract for the synthesis of silver nanoparticles(Komal and Arya, 2013).

Different Reaction factor analysis silver nanoparticles using *Zingiber Officinale* paste:

- **H:** 2.5 ml raw extract was augmented with

50 ml of $AgNo_3$ solution. This reaction mixture was subjected to varied pH conditions i.e. pH 3, 7, 9. The incubation temperature of 37^0C was maintained for each flask. Change in colour was observed as preliminary observation. The optical density of sample at 630 nm on regular interval of 1 hour was recorded using UV-VIS spectrophotometer. Sample with maximum optical density at defined pH (9) was further used.

- **Temperature:** Sample at (pH-9) with maximum optical density at 630 nm was observed further subjected to different temperature conditions i.e. 0^0C, RT (22^0C), 37^0C, 60^0C, 100^0C.Change in colour was observed as preliminary observation. The Optical density of sample was observed at 630 nm using UV-VIS spectrophotometer.

B. Preparation of aqueous extract from *Zingiber officinale*paste using 70%Ethanol as a solvent: Hot percolation treatment was given to 4ml dried paste dissolved in 200ml of 70% ethanol in reflux condenser. In hot percolation method mixture was kept in Waterbath at 50-60^0 C for 3-4 hours. The resultant mixture was filtered out using Whatman filter paper no.1 in a conical flask and the filtrate so obtained from hot percolation treatment was used as raw extract for the synthesis of silver nanoparticles.

Different Reaction factor analysis silver nanoparticles using *Zingiber Officinale paste*:

- **PH:** 2.5 ml raw extract was augmented with 50 ml of $AgNo_3$ solution. This reaction mixture was subjected to varied pH conditions i.e. pH 3, 7, 9. The incubation temperature of 37^0C was maintained for each flask. Change in colour was observed as per preliminary observation. The optical density of sample at 630 nm on regular interval of 1 hour was recorded using UV-VIS spectrophotometer. Sample with maximum optical density at defined (pH= 9) was further used.

- **emperature:**Sample with (pH= 9) at maximum optical density at 630 nm was observed further subjected to different temperature conditions i.e. 0^0C, RT (22^0C), 37^0C, 60^0C, and 1000C. Change in colour was observed as per preliminary observation. The Optical density of sample was observed at 630 nm using UV-VIS spectrophotometer.

2. Characterizations of Energy Dispersive X-RAY Spectroscopy (EDS) and Transmission Electron Microscopy (TEM): The pellet of synthesized silver nanoparticles was used for the Transmission electron microscopic images were taken. Carbon coated grid of nanoparticles are placed inside a partly evacuated chamber connected to power supply. Nps were identified at areas of highest particle density to be viewed as images in order to collect more information possible from images. The elemental analysis or

chemical characterizations of a synthesized AgNps were carried out by Energy Dispersive Spectroscopy (EDS).

3. Antimicrobial activity of synthesized silver nanoparticles against pathogenic strains

The Antibacterial activity of synthesizedsilver nanoparticles were determined by using Minimum Inhibitory Concentration method. This method was employed against selected human pathogens i.e. *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* were obtained from Christian Medical college and Hospitality, Ludhiana. Antimicrobial activity was determined using the different *Zingiber Officinale* against three pathogenic bacteria (*Esherichia coli, staphylococcus aureus, pseudomonas aeruginase)*using Minimum Inhibitory Concentration (MIC) method. MIC can be determined by culturing microorganism in liquid media I.e, Mullar hinton broth. A lower MIC value indicates that less amount ofsample is required for inhibiting the growth of microorganism; therefore sample with lower MIC scores are more effective antimicrobial agents. Microbial strain and synthesized silver nanoparticles pH-9 at 60^0C were mixed together in 3 ratios i.e. 1:1, 1:2, 1:3 respectively. 3ml of microbial strain was mixed with 3ml of silver nanoparticles, 3 ml of microbial strain was mixed with 6ml silver nanoparticles and 3ml of microbial strain was mixed with 9ml silver nanoparticle.Sample test tubes so prepared were then incubated at 37^0C.At interval of 1 hour, MIC based on turbidity of sample in test tubes was determined using UV-VIS spectrophotometer at 630 nm.Plot determining antimicrobial activity of AgNps against pathogenic strains were examined.

4. Determination of metal ion detection using silver nanoparticles

Metal ion detection using Double distilled water and 70% ethanol as a solvent by sample *Zingiber officinale (*juice). Dirty or contaminated water was taken from the industrial area in Ludhiana. To detect the presence of metal ions in silver nanoparticles, contaminated water of 3ml and 1ml of silver nanoparticles was mixed and then the metal ion solution of varied concentrations was added in the above mixture i.e. 50µl, 45µl, 40µl and 35µl in each tube. The optical density at 630 nm was measured and analysed in dirty water with silver nanoparticles, using UV-VIS spectrophotometer, etc. Incubation was given to each test tube at room temperature for 2 hours. After incubation, the optical density at 630 nm of sample was observed.

RESULTS AND DISCUSSION

To study the biological synthesis of silver nanoparticles from the extract of Zingiber officinale:

In present study silver nanoparticles synthesized from the aqueous extract of Zingiber officinale (paste) using different solvent like double distilled water(DDW)and 70%ethanol.

Double distilled water as a solvent:

Effect of pH on the biosynthesis of silver nanoparticles using paste of Zingiber officinale: 2.5 ml raw extract was augmented with 50 ml of $AgNO_3$ solution. This reaction mixture was subjected to varied pH conditions i.e. pH 3, 7, 9. The incubation temperature of 37^0C was maintained for each flask. Change in colour i.e. yellow to white at pH -3, yellow to dark brown at pH-7, yellow to black was observed as preliminary observation. The optical density of sample at 630 nm on regular interval of 1 hour was recorded using UV-VIS spectrophotometer. Sample with maximum optical density at defined pH- 9 was further used for the experiment.

Effect of temperature on the biosynthesis of silver nanoparticles using paste of Zingiber officinale:Sample with pH-9 having maximum optical density at 630 nm was further subjected to different temperature conditions i.e. 0^0C, RT (22^0C), 37^0C, 60^0C, and 1000C. Change in colour was observed at different temperature i.e. yellow to black red at 0^0C,yellow to dark brown at (RT), yellow to black at 37^0C yellow to light grey60^0C, yellow to dark grey at indicating the optical density of the sample under different temperature conditions was measured at 630 nm using UV-VIS Spectrophotometer. Maximum absorbance was observed at temperature 60^0C.

CHARACTERIZATION OF SILVER NANOPARTICLES USING ENERGY DISPERSION X-RAY SPECTROMETER AND TRANSMISSION ELECTRON MICROSCOPY

A. Energy dispersion X-ray spectroscopy

Using double distilled water as a solvent, sample with optimum pH -9 and temperature 60^0C was chosen for the confirmatory analysis of silver as a true metal nanoparticle using *Zingiber officinale.*

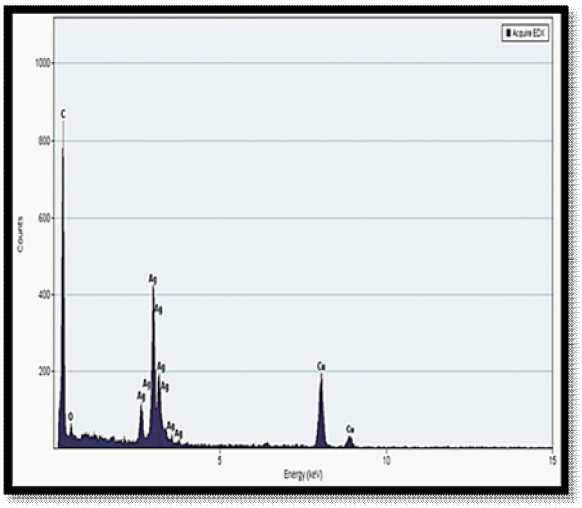


Fig. 1. Confirmatory analysis showing EDS spectra of synthesized silver nanoparticles from *Zingiber officinale* juice in ddw as a solvent.

In Fig. 1 EDS spectrum showing 4 peaks located before 5keV confirms presence of true metal ion. Carbon and copper peaks are shown in spectra because of carbon coated copper grid (used in sample preparation). Quantitative analysis proved presence of silver contents (31.67%) in an examined sample.

B. TRANSMISSION ELECTRON MICROSCOPY (TEM) ANALYSIS:

Using double distilled water as a solvent**,** sample with optimum pH -9 and temperature 60^0C was chosen for the confirmatory analysis of silver as a true metal nanoparticle using *Zingiber officinale.*

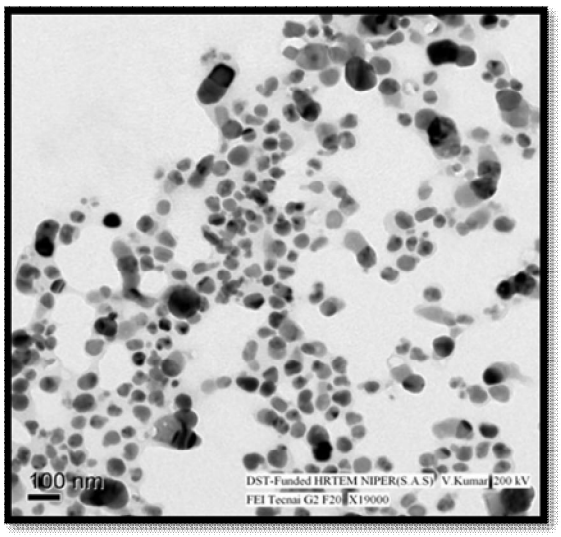


Fig. 2. Confirmatory analysis of synthesized silver nanoparticles using Transmission Electron Microscopy.

For the confirmatory analysis of synthesized silver nanoparticles from Zingiber *officinale* TEM was performed. The shape and size of the resultant nanoparticles were elucidated with the help of TEM (Fig. 2).

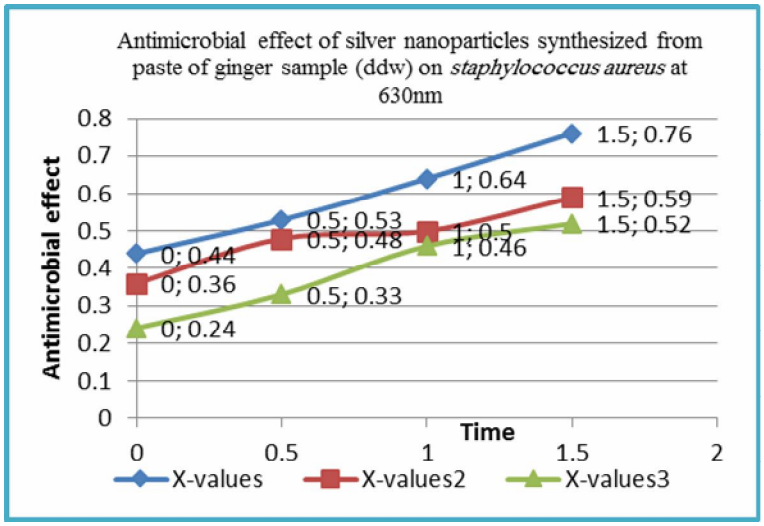
Aliquots of silver nanoparticles solution were placed on a carbon –coated copper grid and allowed to dry under ambient conditions and TEM image was recorded. The TEM micrographs suggest that the sizes of the nanoparticles were around 35(±5) nm and particles were of spherical in shape.

Antimicrobial activity of plant material *Zingiber officinale* paste using double distilled water as a solvent

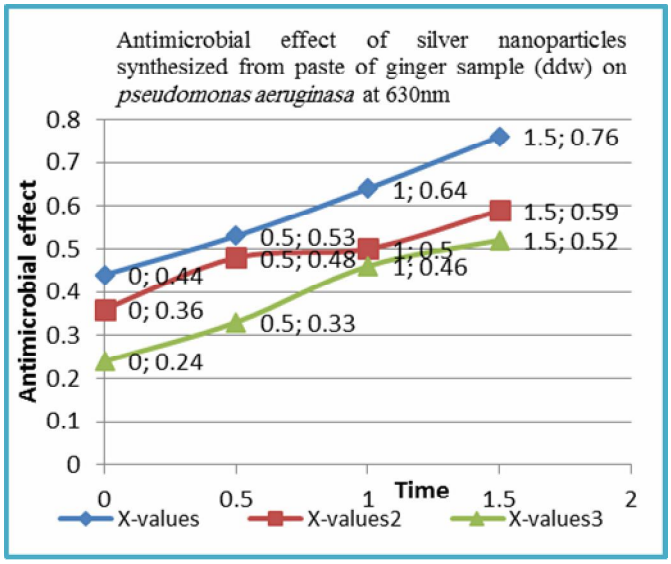
Table 1. Showing Optical density of MIC test against different pathogenic strains using *Zingiber officinale* paste of ddw depending upon different concentration at 630 nm.

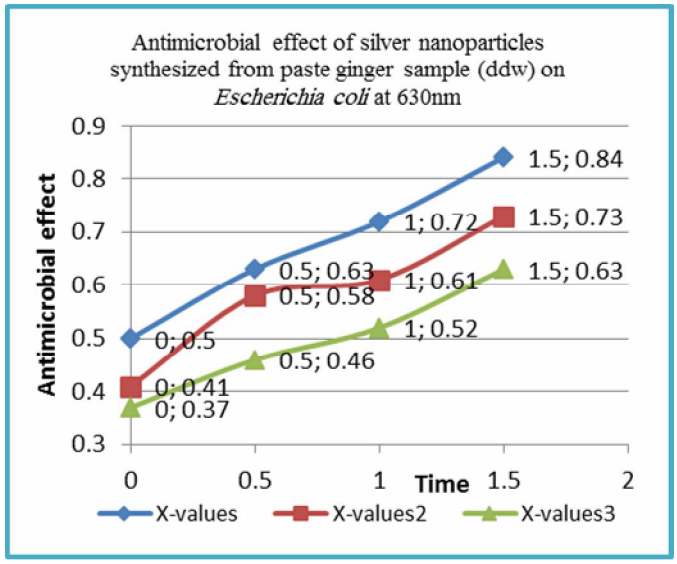
Minimum Inhibitory Concentration			
Antimicrobial effect			
	Optical density of concentration on nanoparticles+ MHB containing Microbial Strains at 630 nm		
Pathogenic strains	1:1 (3ml of MHB + 3mlof Np_s)	1:2 (3ml of MHB + 6mlof Np_s)	1:3 (3ml of MHB + 9mlof Np_s)
Staphylococcus aureus	0.592	0.482	0.387
Pseudomonas aeruginosa	0.677	0.592	0.487
Escherichia coli	0.672	0.582	0.495

Graphs showing that the antimicrobial effect of silver nanoparticles synthesized from ginger sample (paste) on different pathogenic strains i.e. *Staphlococcus aureus, Pseudomonas aeruginosa, Escherichia coli* using distilled water as a solvent. The antimicrobial effect depends on the MIC values of sample .The mean MIC values obtained was the highest for 1:1 sample and was the lowest 1:3. A lower MIC value indicates that less amount of sample is required for inhibiting the growth of microorganism: therefore sample with lower MIC concentration i.e. at 1:3 of sample is more effective antimicrobial agent.



(a)





(b) (c)

Fig. 3. Shows antimicrobial effect of silver nanoparticles using *Zingiber officinale (a) staphylococcus aureus* (b) *pseudomonas aeruginosa (c) staphylococcus aureus.*

Fig. 4. Antimicrobial effect of silver nanoparticles using *Zingiber officinale* paste using ddw on *Escherichia coli.*

Fig. 5. Antimicrobial effect of silver nanoparticles using *Zingiber officinale* paste of ddw on *Staphylococcus aureus.*

Fig. 6. Antimicrobial effect of silver nanoparticles using *Zingiber officinale* paste of ddw on *Pseudomonas aeruginosa.*

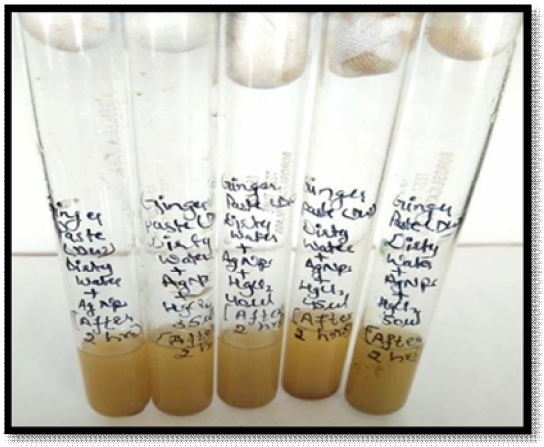


Fig. 7. shows the detection of metal ion in the synthesized silver nanoparticles from Zingiber officinale paste in DDW and C.W at 630 nm.

Table 2. Shows that optical density for the presence of metal ion in $AgNp_s$ from Zingiber officinale paste in ddw and C.W at 630 nm.

Optical density at 630 nm		
	Initial	**Final**
C.W+$AgNp_s$	0.30	1.15
C.W+$AgNp_s$+50µl metal ion	0.18	0.26
C.W+$AgNp_s$+45µl metal ion	0.16	0.22
C.W+$AgNp_s$+40µl metal ion	0.11	0.18
C.W+$AgNp_s$+35µl metal ion	0.17	0.23

METAL ION DETECTION

A. Detection of metal ion of plant Zingiber officinale juice using double distilled water as a solvent:

Absorbance of the metal ion by silver nanoparticles mixed with contaminated water (C.W) in ratio 1:3. As we know contaminated water consists of heavy metals like Hg^{2+}, Zn^{2+}. The addition of synthesized silver nanoparticles to this contaminated water can be used to detect the presence of added metal ions like Hg^{2+}, Zn^{2+}. The optical density of sample containing contaminated water with added metal ions and silver nanoparticles was noted using UV-VIS spectrophotometer at 630 nm before incubating the mixture for 2 hours. Upon incubating the mixture at room temperature, a significant change in optical density was observed hence the presence of metal ions i.e. Hg^{2+} was confirmed. With optical density in contaminated water and silver nanoparticle (0.30) at different concentration of metal ion are added 50µl, 45µl, 40µl, 35µl. With decreasing concentration of metal ion solution having maximum concentration (35µl) shows that interaction of C.W and $AgNp_s$ observed. Changes in optical density are evaluated (0.17).

Effect of pH on the biosynthesis of silver nanoparticles using paste of Zingiber officinale:

In this, 2.5ml raw extract was augmented with 50 ml of $AgNO_3$ solution. This reaction mixture was subjected to varied pH conditions i.e. pH 3, 7, 9. Change in colour i.e. yellow to white at pH -3, yellow to orange at pH-7, yellow to black was observed as preliminary observation. The incubation temperature of 37^0C was maintained for each flask on regular intervals of 1 hour. The optical density of sample was recorded at 630 nm using UV-VIS spectrophotometer. Sample with maximum optical density at pH-9 was used further for the experiment.

Effect of temperature on the biosynthesis of silver nanoparticles using paste of Zingiber officinale:

Sample with pH-9 having maximum optical density at 630 nm was further subjected to different temperature conditions i.e. 0^0C, RT (22^0C), 37^0C, 60^0C, and 100^0C. Change in colour was observed at different i.e. the optical density of the sample under different temperature conditions was measured. yellow to dark brown at 0^0C, yellow to black at RT, yellow to black at 37^0C, yellow to dark grey at 60^0C, yellow to grey at 100^0C as preliminary. Optical density of the sample at 630 nm using UV-VIS Spectrophotometer and Maximum Absorbance of *Zingiber officinale* was observed at temperature 60^0C at pH-9.

CHARACTERIZATION OF SILVER NANOPARTICLES USING ENERGY DISPERSION X-RAY SPECTROMETER AND TRANSMISSION ELECTRON MICROSCOPY

A. Energy dispersion X-ray spectroscopy (EDS)

Using 70% ethanol as a solvent, sample with optimum pH -9 and temperature 60^0C was chosen for the confirmatory analysis of silver

as a true metal nanoparticles using *Zingiber officinale*. In Fig. 8 EDS spectra showing 4 peaks located before 5keV confirms presence of true metal ion. Carbon and copper peaks are shown in spectra because of carbon coated copper grid (used in sample preparation). Quantitative analysis proved presence of silver contents (66.98%) in an examined sample.

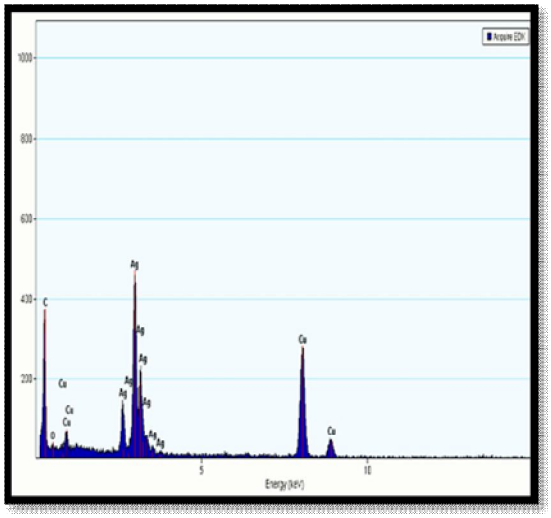


Fig. 8. Confirmatory analysis showing EDS spectra of synthesized silver nanoparticles from Zingiber officinale juice in 70%ethanol as a solvent.

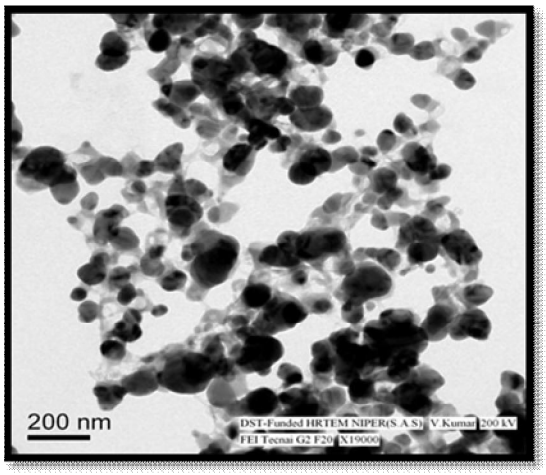


Fig. 9. Confirmatory analysis of synthesized silver nanoparticles using Transmission Electron Microscopy.

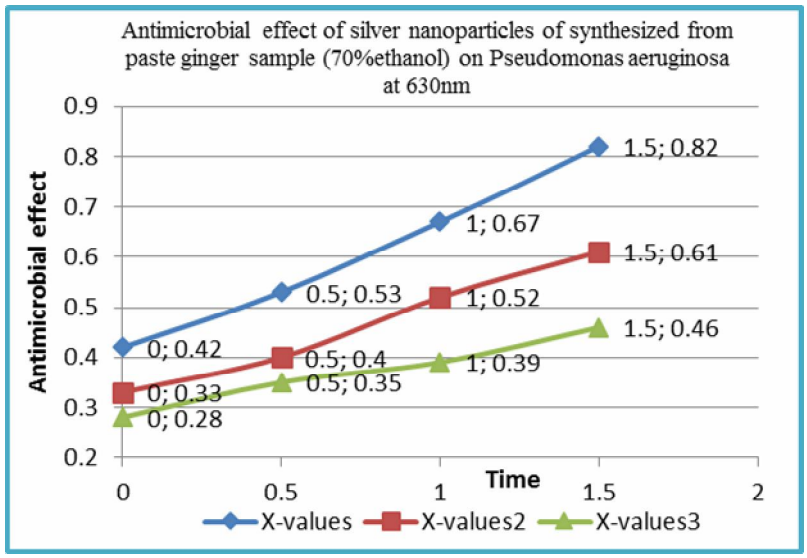
B. TRANSMISSION ELECTRON MICROSCOPY (TEM) ANALYSIS

Using 70% ethanol as a solvent**,** sample with optimum pH -9 and temperature 60^0C was chosen for the confirmatory analysis of silver as a true metal nanoparticle using *Zingiber officinale*. For the confirmatory analysis of synthesized silver nanoparticles from Zingiber *officinale* TEM was performed. The shape and size of the resultant nanoparticles were elucidated with the help of TEM (Fig. 9).Aliquots of silver nanoparticles solution were placed on a carbon –coated copper grid and allowed to dry under ambient conditions and TEM image was recorded. The TEM micrographs suggest that the sizes of the nanoparticles were around 50(±5) nm and particles were of spherical in shape.

Antimicrobial activity of plant material *Zingiber officinale* paste using 70%ethanol as a solvent

Table 3. Showing Optical density of MIC test against different pathogenic strains using *Zingiber officinale* paste of 70%ethanol depending upon different concentration at 630 nm.

Minimum Inhibitory Concentration			
Antimicrobial effect			
	Optical density of concentration on nanoparticles+ MHB containing Microbial Strains at 630 nm		
Pathogenic strains	1:1 (3ml of MHB +3ml of Np_s)	1:2 (3ml of MHB +6ml of Np_s)	1:3 (3ml of MHB +9ml of Np_s)
Staphylococcus aureus	0.975	0.77	0.12
Pseudomonas aeruginosa	0.61	0.46	0.37
Escherichia coli	0.977	0.682	0.585



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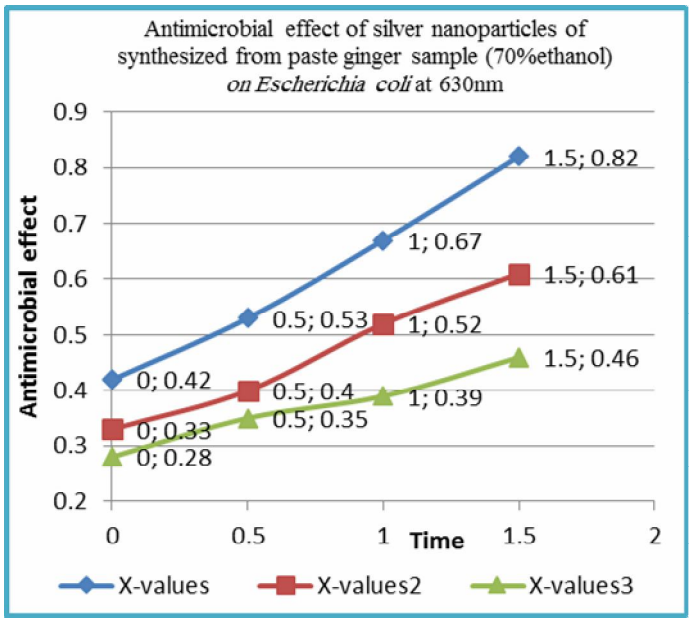

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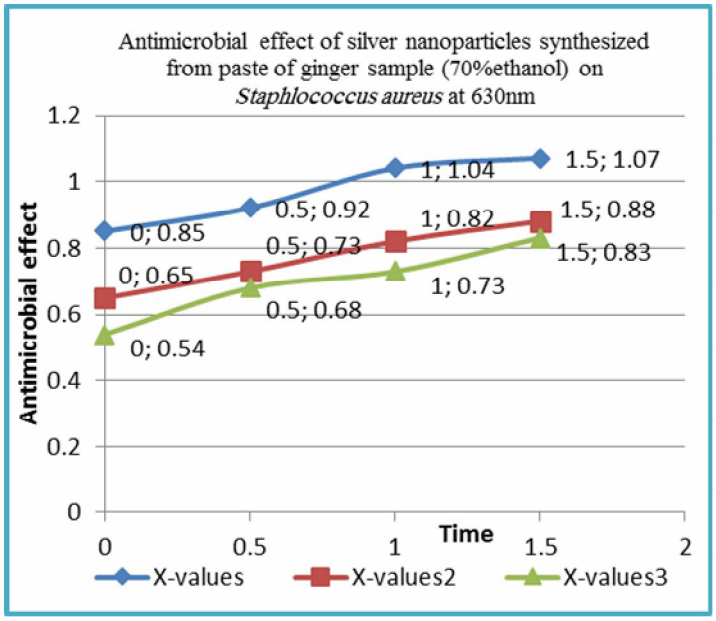

(c)

Fig. 10. Shows antimicrobial effect of silver nanoparticles using *Zingiber officinale (*a) *Escherichia coli* (b) *pseudomonas aeruginosa (c) staphylococcus aureus.*

Graphs showing that the antimicrobial effect of silver nanoparticles synthesized from ginger sample (paste) on different pathogenic strains i.e.*Staphlococcus aureus, Pseudomonas aeruginosa, Escherichia coli* using ethanol as a solvent. The antimicrobial effect depends on the MIC values of sample. The mean MIC value obtained was the highest for 1:1 sample and was the lowest 1:3. A lower MIC value indicates that less amount of sample is required for inhibiting the growth of microorganism: therefore sample with lower MIC concentration i.e. At 1:3 of sample is more effective antimicrobial agent.

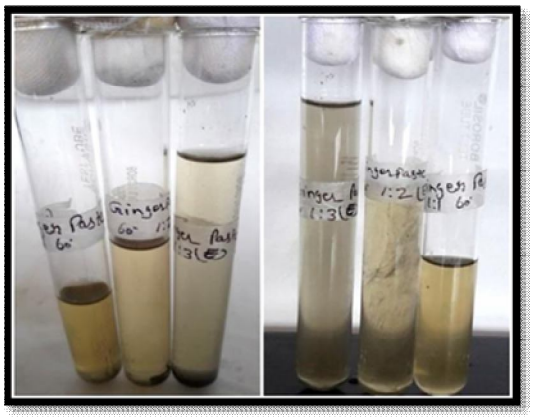

Fig. 11. Antimicrobial effect of silver nanoparticles using *Zingiber officinale* paste of 70%ethanolon *Escherichia coli.*

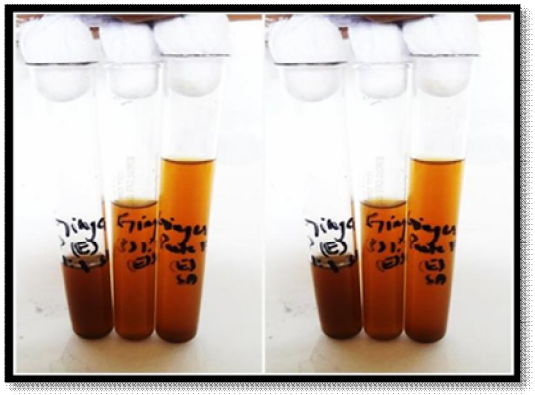

Fig. 12. Antimicrobial effect of silver nanoparticles using Zingiber officinale paste of 70% ethanol on Staphylococcus aureus.

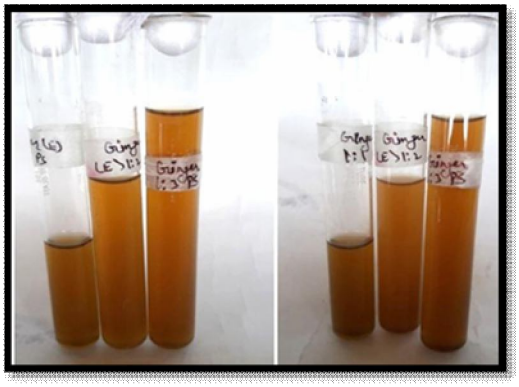

Fig. 13. Antimicrobial effect of silver nanoparticles using Zingiber officinale paste of 70%ethanolonPseudomonas aeruginosa.

Fig. 14. Shows the detection of metal ion in the synthesized silver nanoparticles from Zingiber officinale paste in 70%ethanol and C.W at 630 nm.

Table 4. Shows that optical density for the presence of metal ion in $AgNp_s$ from Zingiber officinale paste in 70%ethanol and C.W at630 nm.

Optical density at 630 nm		
	Initial	**Final**
C.W+$AgNp_s$	0.25	1.52
C.W+$AgNp_s$+50µl metal ion	0.14	0.30
C.W+$AgNp_s$+45µl metal ion	0.17	0.14
C.W+$AgNp_s$+40µl metal ion	0.19	0.27
C.W+$AgNp_s$+35µl metal ion	0.33	0.20

Metal ion detectionDetection of metal ion of plant *Zingiber officinale* juice using 70% ethanol as a solvent:

Absorbance of the metal ion by silver nanoparticles mixed with contaminated water (C.W) in ratio 1:3. As we know contaminated water consists of heavy metals like Hg^{2+}, Zn^{2+}. The addition of synthesized silver nanoparticles to this contaminated water can be used to detect the presence of added metal ions like Hg^{2+}, Zn^{2+}. The optical density of sample containing contaminated water with added metal ions and silver nanoparticles was noted using UV-VIS spectrophotometer at 630 nm before incubating the mixture for 2 hours. Upon incubating the mixture at room temperature, a significant change in optical density was observed hence the presence of metal ions i.e. Hg^{2+} was confirmed. With optical density in contaminated water and silver nanoparticle (0.25) at different concentration of metal ion are added 50µl, 45µl, 40µl, 35µl. With decreasing concentration of metal ion solution having maximum concentration (35µl) shows that interaction of C.W and $AgNp_s$ observed. Change in optical density is evaluated (0.33).

CONCLUTIONS

Green synthesis of nanoparticles has been exploring research topic in recent days due to their advanced use in biomedical, chemical and related fields. In present study silver nanoparticles were synthesized from plant extract of *Zingiber officinale*. This plant has been used extensively in the medicinal as well as Ayurveda value. The biosynthesis of silver nanoparticles from plant was prepared by hot percolation method. Bio reduction of Ag^{+} to Ag^{0} was observed when extract were augmented with $AgNO_3$ and kept under different pH (3, 7, and 9) and temperature (0^0C, RT (22^0C, 37^0C, 60^0C, 100^0C). Overall nanoparticles synthesized from Herbaceous material *Zingiber officinale* juice using 70%ethanol as a solvent show better result as compared to other solvent (DDW).

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