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RESEARCH PAPER

Characterization and Assessment of Antimicrobial Activity and Potential of Heavy Metal ion Detection of Silver Nanoparticles synthesized from Actinidia deliciosa paste using double distilled water and 70% ethanol as solvent

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ABSTRACT

Green synthesis of nanoparticles using plants as sources exhibiting superiority over the physical and chemical methods that are expensive and can involve the use of toxic, hazardous chemicals, which may pose biological and environmental risks. In present study silver nanoparticles were synthesized in single step by biological method using extracts of *Actinidia deliciosa* paste *as* reducing and stabilizing agent. Extracts were prepared using two different solvents i.e. double distilled water and 70% ethanol by Hot Percolation method. These extracts were augmented with $AgNO_3$ solution and subjected to different reaction conditions i.e. pH and temperature. Synthesized silver nanoparticles of *Actinidia deliciosa* paste samples were preliminary characterized by using UV-VIS spectrophotometer and also on basis of change in colour in samples. Confirmatory characterization of biologically synthesized silver nanoparticles was done by Energy-Dispersive X-ray Spectroscopy **(**EDS) for presence of true silver metal ions samples, Transmission Electron Microscopy (TEM) so as to find out the shape and size of silver nanoparticles. The MIC test was performed to evaluate antimicrobial activity of silver nanoparticles against *E.coli, Staphylococcus aureus, Pseudomonas aeruginosa.* Applicative test of biosynthesized silver nanoparticles for detection of minimum concentration of Hg^{2+} heavy metal ions in contaminated water sample was done by observing change in optical density with respect to fixed silver nanoparticles concentration and varied Hg^{2+} heavy metal ions concentration by using UV-VIS spectrophotometer at wavelength of 630 nm.

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INTRODUCTION

Nanotechnology deals with materials having dimensions in nanometre range. The conceptual underpinning of nanotechnologies was first laid out in 1957 by Physicist Richard Feynman in his lecture, "There is plenty of room at bottom". In 1974 term nanotechnology was firstly introduced by Norio Taniguchi, Japanese researcher at University of Tokyo to describe precision manufacturing of materials at nanoscale (1). Nanobiotechnology refers to the way that nanotechnology is used to create devices to study and manipulate biological systems. It provides a critical bridge between the physical sciences and engineering on one hand and molecular biology on other hand. Nanobiotechnology incorporates the synthesis of nanoparticles by using biological entities such as plants, fungi, bacteria, yeast, algae Nanobiotechnology ease many avenues of life sciences by integrating cutting-edge applications

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of information technology & nanotechnology into contemporary biological issues (2).

Metallic nanoparticles are of great interest due to their novel physio-chemical, magnetic and optical properties that are governed by their extremely small size, large surface area to volume ratio and shape distribution (3). Different types of noble metallic nanoparticles like copper, silver, gold, titanium, zinc etc have been produced which have attracted the attention of the scientific community and technologists due to their ever emerging, numerous and fascinating application in various fields such as medicine, cosmetics, renewable energies, environmental remediation biomedical sciences and engineering, pharmaceutical, food and agriculture sectors, targeted cancer remedies etc (4).

Synthesis of silver nanoparticles involves reduction of silver salt (Ag^{+}) to Ag^{o}. Reduction of silver salts involves two stages: Nucleation and Subsequent growth. Several methods are used for synthesis of nanoparticles such as physical, chemical, biological methods. Both physical and chemical methods have been using high radiation and highly concentrated reductants and stabilizing agents, generation of hazardous by-products, and high energy consumption that are harmful to environment and to human health (5). Biological synthesis of nanoparticles is a single step bioreduction method and less energy is used to synthesize eco-friendly nanoparticles. Biological entities such as plants extract (6), microorganisms, and enzymes are used as reducing agents for synthesis of nanoparticles. Among them the most important bioreductants are plant extracts which are relatively easy to handle, readily available, low cost, provides both reducing agent and stabilizing agent, compatible for pharmaceutical and biomedical applications have been well explored for green synthesis of nanoparticles (7). The reduction of metal ions is done by combination of biomolecules like proteins, enzymes, vitamins, amino acids, flavonoids (8).

The general techniques of plant extraction include maceration, influsion, hot percolation, soxhlet extraction etc. Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, fruits etc (9). For successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Alcohols, Water, Chloroform, Ether and Acetone are used as solvent. Sometime mixture of solvents also used to give better efficiency (10).

Among all noble metal nanoparticles silver nanoparticles are an arch product from the field of nanotechnology. Silver nanoparticles has unique non-linear optical, electrical, catalytical, surface enhanced raman scattering and thermal properties and are incorporated into products that range from photovoltaic to biological and chemical sensors. Examples include conductive inks, pastes and fillers which utilize silver nanoparticles for their high electrical conductivity, stability, and low sintering temperatures (11), molecular diagnostics, nano silver coated ceramic water filters, wound dressings and biodegradable poly fibre etc (12). The respiratory chain and cell division are affected by AgNps[1] which ultimately lead to cell death. AgNps broad spectrum bactericidal and fungicidal activity thus has wide range of applications from disinfecting medical devices and home appliances to water treatment. AgNps are extensively studied for detection of pesticides and heavy metal ions in drinking water (13).

Actinidia. deliciosa (kiwifruit or Chinese gooseberry) is the edible berries of several species of woody wines in the genus *Actinidia.* Compared with other commonly consumed fruit, kiwifruit are exceptionally high in vitamins C, folate, carotenoids, potassium, fiber, fructose, galactose, flavanoid, isoflavanoid, minerals and phytochemicals acting in synergy to achieve multiple health benefits such as support immune function, improve skin and bone health, limiting hypertension and high blood pressure, better sleep (14). Kiwi fruit also has different biological properties such as anti-oxidant, anti-allergic, Cardio-vascular defensive effect (15).

MATERIALS AND METHODS

Biological source (Fruit): Actinidia deliciosa

The *Actinidia deliciosa* 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 Actinidia deliciosa paste

Preparation of raw extracts of Actinidia deliciosa paste

A. Using double distilled water as solvent:

Dried Actinidia deliciosa was grinded in distilled water to form fine paste. 25 gm paste was diluted

1- AgNps- Silver nanoparticles

5 times in double distilled water to get final volume of about 125 ml 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 resultant mixture was then filtered out using what's man filter paper no.1 in conical flask. The filtrate so obtained was kept in water bath at 60^{0}C till reduced volume of filtrate was obtained and was used as raw extract for the synthesis of silver nanoparticles (16).

B. Using 70% ethanol as solvent

Hot percolation treatment was given to 4 gm dried paste which was diluted 5 times with ethanol and dissolved in 200 ml of 70% ethanol in reflux condenser. In hot percolation method mixture was kept in Water bath at 50-60^0 C for 3-4 hours. The resultant mixture was filtered out using Whatman's filter paper no.1 in a conical flask and the extract so obtained from hot percolation treatment was used as raw extract for the synthesis of silver nanoparticles.

Different reaction conditions for Actinidia deliciosa paste samples

- **pH:** 2.5 ml raw extracts were augmented with 50 ml of $AgNO_3$ solution and was subjected to varied pH conditions i.e. pH 3, 7, 9. The incubation temperature of 37^{0}C was maintained for each flask. Change in color was observed as preliminary observation. The optical density of double distilled water and 70% ethanol paste samples at 630 nm and 540 nm respectively were recorded on regular interval of 1 hour using UV-VIS spectrophotometer. Samples with maximum optical density at defined pH 7 were further used.
- **Temperature:** Samples with pH 7 having maximum optical density were observed and further subjected to different temperature conditions i.e. 0^{0}C, RT (22^{0}C), 37^{0}C, 60^{0}C, 100^{0}C. The optical density of double distilled water and 70% ethanol paste samples at 630 nm and 540 nm respectively were observed using UV-VIS spectrophotometer.

Characterization of Energy Dispersive X-Ray Spectroscopy (EDS) and Transmission Electron Microscopy (TEM)

Aliquots of nanoparticles dissolved in solvent used were placed on a carbon coated copper grid and allow drying under ambient conditions and then carbon coated grid of nanoparticles was placed inside a partly evacuated chamber connected to power supply. Presence of true silver metal ions was confirmed by EDS[2]. Nanoparticles were identified at areas of highest particle density to be viewed as images in order to collect more information possible from each image.

Antimicrobial activity of synthesized silver nanoparticles against pathogenic strains

- **Bacterial strains:** Three strains of pathogenic bacteria i.e. *Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus* were obtained from Christian Medical College and Hospitality Ludhiana.

Minimum Inhibitory Concentration (MIC) method

Muller Hinton broth was prepared. For subculturing 2-3ml of bacterial strain was mixed in 12 ml of Muller Hinton Broth. Microbial strain and synthesized silver nanoparticles were diluted together in 3 ratios 1:1, 1:2, 1:3 respectively i.e. 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^{0}C**.** 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. Test tubes with lowest microbial growth i.e. test tubes with less of either ratio (i.e. 1:1, 1:2, 1:3) were observed on regular interval of 1 hour incubation.

Metal ion detection using silver nanoparticles of Actinidia deliciosa paste

Contaminated water was taken from the industrial area in Ludhiana. 3ml of CW[3] 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. Incubation was given to each test tube at room temperature for 2 hours. The optical density at 630nm was measured using UV-VIS spectrophotometer.

RESULT AND DISCUSSION

In the present study, silver nanoparticles were synthesized from paste extracts of *Actinidia*

2- EDS- Energy Dispersive X-ray Spectroscopy
3- CW- Contaminated water

deliciosa prepared by using double distilled water and 70% ethanol as solvents by hot percolation method. Bioreduction of Ag^{+} to Ag^{0} was observed when extracts were augmented with $AgNO_3$ at different experimental conditions (temperature, pH). The synthesized silver nanoparticles were preliminary characterized by UV-VIS spectrophotometer at different wavelengths. These nanoparticles showed antibacterial activity against *Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa*. Biosynthesized silver nanoparticles were used for colorimetric detection of Hg^{2+} heavy metal ions in polluted water sample.

UV-VIS SPECTROPHOTOMETERIC ANALYSIS

Effect of pH: The effect of pH on synthesis of silver nanoparticles was studied under different pH conditions (3, 7, 9). With increase in pH of reaction mixture an increase in optical density was observed (as in Table 1, 2) it seems that pH affects synthesis of silver nanoparticles. Change in color was observed with change in pH as shown in Fig. 1. Paste extracts prepared by using double distilled water and 70% ethanol as solvent, showed maximum absorbance at pH 7 indicating maximum silver nanoparticles synthesized. These results are in good agreement with results reported by *Elias et al,* he reported that there is maximum synthesis

Table 1. Absorbance of $AgNO_3$ treated *Actinidia deliciosa* paste (double distilled water) extract at 630 nm due to pH variations.

pH	Optical density				Mean
	Initial	After 1 hour	After 2 hour	After 3 hour	
3	1.25	0.81	0.71	0.61	0.845
7	1.19	0.99	0.86	0.94	0.995
9	0.32	0.78	0.85	0.86	0.703

Table 2. Absorbance of $AgNO_3$ treated *Actinidia deliciosa* paste (70% ethanol) extract at 540 nm due to pH variations.

pH	Optical density				Mean
	Initial	After 1 hour	After 2 hour	After 3 hour	
3	0.16	0.29	0.32	0.45	0.31
7	0.24	0.30	0.34	0.46	0.34
9	0.18	0.20	0.28	0.36	0.26

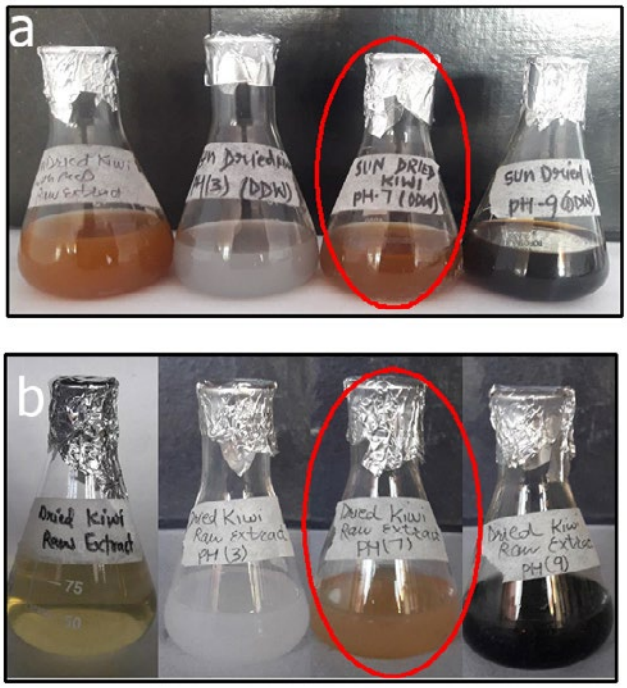

Fig. 1. Change in color of extracts augmented with silver nitrate after one hour of incubation at pH 3, 7, 9 indicating silver nanoparticles synthesis a) Actinidia deliciosa paste (double distilled water) extract b) Actinidia deliciosa paste (70% ethanol) extract

of spherical shape silver nanoparticles at neutral pH (6.8 to 7) (17).

Effect of temperature: The effect of temperature on synthesis of silver nanoparticles was studied under different temperatures 0^0C, RT (22^0C), 37^0C, 60^0C, 100^0C for one hour. An increase in optical density was observed with increase in temperature (as in Table 3, 4) it seems that temperature affects synthesis of silver nanoparticles. Change in color was observed with change in temperature as shown in Fig. 2. Paste extracts prepared by using double distilled water and 70% ethanol as solvent, showed maximum absorbance at 60°C indicating maximum silver nanoparticles synthesized. Similar findings were reported by *Darroudi et al*, in his study. He reported that a low temperature (~0°C) significantly slow down the formation and growth of silver nanoparticles, with increase in temperature upto 60°C there is increase in synthesis of silver nanoparticles beyond this temperature (60°C) bioreduction of silver metal ion ceases (18).

CHARACTERIZATION OF SILVER NANOPARTICLES USING ENERGY DISPERSIVE X-RAY SPECTROSCOPY AND TRANSMISSION ELECTRON MICROSCOPY

Presence of silver metal ions in *Actinidia*

Table 3. Absorbance of $AgNO_3$ treated *Actinidia deliciosa* paste (double distilled water) extract at 630 nm due to temperature variations.

Temperature	Optical density				Mean
	Initial	After 1 hour	After 2 hour	After 3 hour	
0°	1.03	1.04	1.06	1.08	1.053
22°	1.05	1.09	1.12	1.18	1.11
37°	1.12	1.16	1.26	1.27	1.203
60°	1.18	1.20	1.28	1.30	1.24
100°	1.03	1.08	1.16	1.18	1.113

Table 4. Absorbance of $AgNO_3$ treated *Actinidia deliciosa* paste (70% ethanol) extract at 540 nm due to temperature variations.

Temperature	Optical density				Mean
	Initial	After 1 hour	After 2 hour	After 3 hour	
0°	0.12	0.16	0.22	0.26	0.19
22°	0.02	0.10	0.17	0.20	0.123
37°	0.11	0.16	0.22	0.25	0.186
60°	0.28	0.45	0.56	1.08	0.59
100°	0.12	0.20	0.32	0.86	0.38

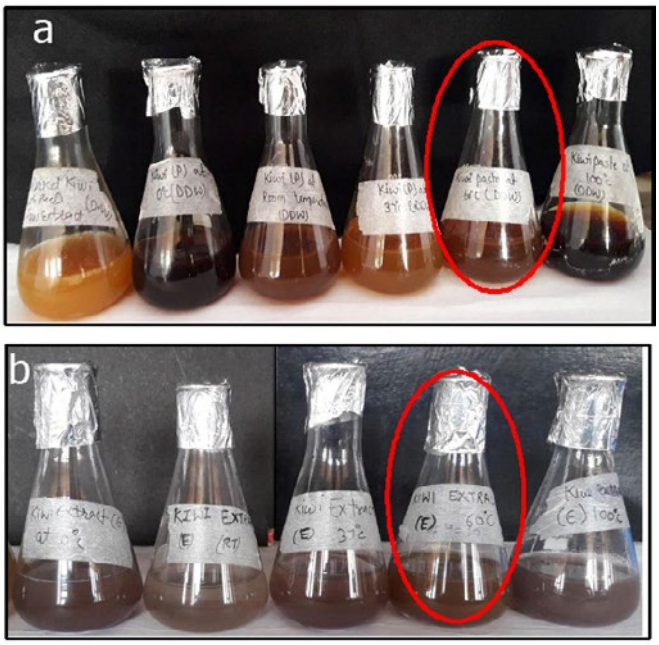

Fig. 2. Change in color of Actinidia deliciosa paste extracts augmented with silver nitrate after one hour of incubation at different temperatures 0oC, RT(22oC), 37oC, 60oC, 100oC indicating silver nanoparticles synthesis a) Actinidia deliciosa paste (double distilled water) extract b) Actinidia deliciosa paste (70% ethanol) extract

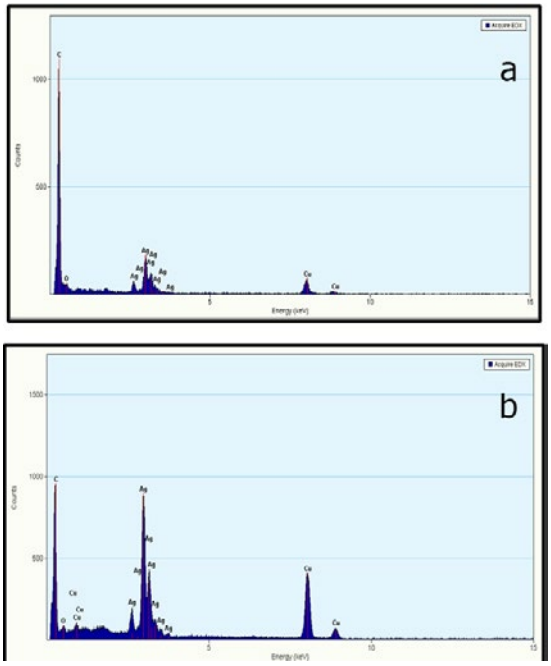

Fig. 3. EDS characteristic spectrum obtained for confirmatory analysis of silver nanoparticles a) Actinidia deliciosa paste (double distilled water) extract b) Actinidia deliciosa paste (70% ethanol) extract

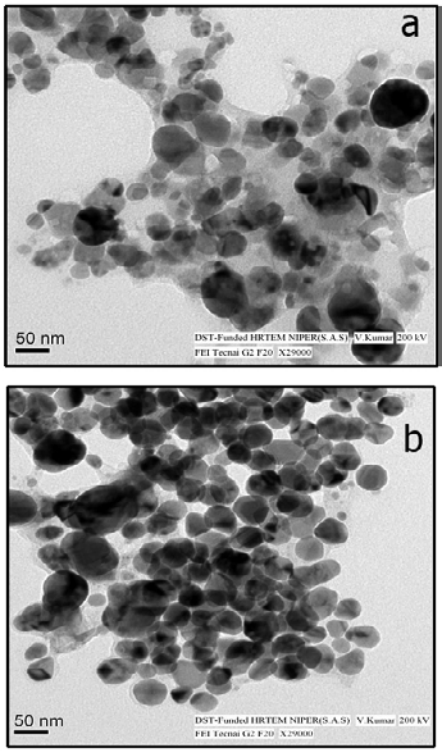

Fig. 4. TEM images of silver nanoparticles depicting spherical shape a) Actinidia deliciosa paste (double distilled water) extract b) Actinidia deliciosa paste (70% ethanol) extract

deliciosa paste samples was confirmed by EDS. EDS spectra of paste sample (double distilled water) showed 4 peaks located before 5 keV. While EDS spectra of paste sample (70% ethanol) showed 5 peaks as shown in Fig. 3. 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 12.4% and 47.7% in the examined paste samples of double distilled water and 70% ethanol respectively. TEM images were recorded which confirms presence of nanoparticles. TEM images of examined paste samples of double distilled water and 70% ethanol showed that nanoparticles produced are 38 ±5 nm and 48 ±5 nm in size and mostly are spherical in shape as shown in Fig. 4.

ANTIMICROBIAL ACTIVITY OF SYNTHESIZED NANOPARTICLES AGAINST PATHOGENIC BACTERIA

Double distilled water as solvent

Silver nanoparticles of paste sample (double distilled water) showed maximum, moderate and lowest antibacterial effect against *Staphylococcus aureus, Escherichia coli*, *Pseudomonas aeruginosa* (as in Table 5) respectively with MIC of 1:3 concentrations. Graphs showed maximum, moderate, minimum antibacterial effects of silver nanoparticles on different pathogenic strains with MIC concentration of 1:3, 1:2 and 1:1 respectively as shown in Fig. 5. Change in turbidity was observed due to antibacterial effect of silver nanoparticles on pathogenic bacteria as shown in Fig. 6.

Table 5. MIC observation for silver nanoparticles of *Actinidia deliciosa paste* (double distilled water)

MIC TEST			
	Antimicrobial effect		
Test Pathogen	Optical density		
	1:1 (3ml of MHB + 3ml of AgNps)	1:2 (3ml of MHB + 6ml of AgNps)	1:3 (3ml of MHB + 9ml of AgNps)
Escherichia coli	0.635	0.51	0.36
Pseudomonas aeruginosa	0.58	0.50	0.40
Staphylococcus aureus	0.53	0.43	0.31

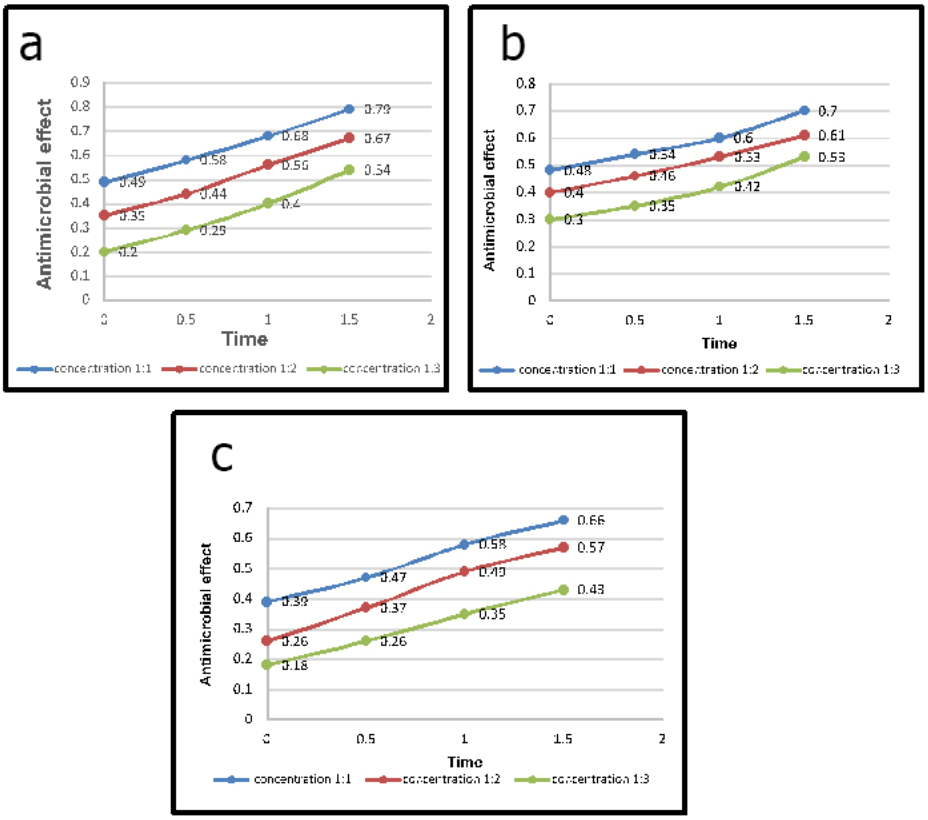

Fig. 5. Graphs showing antibacterial effect of silver nanoparticles of Actinidia deliciosa paste (double distilled water) against (a) Escherichia coli, (b) Pseudomonas aeruginosa, (c) Staphylococcus aureus at 360 nm

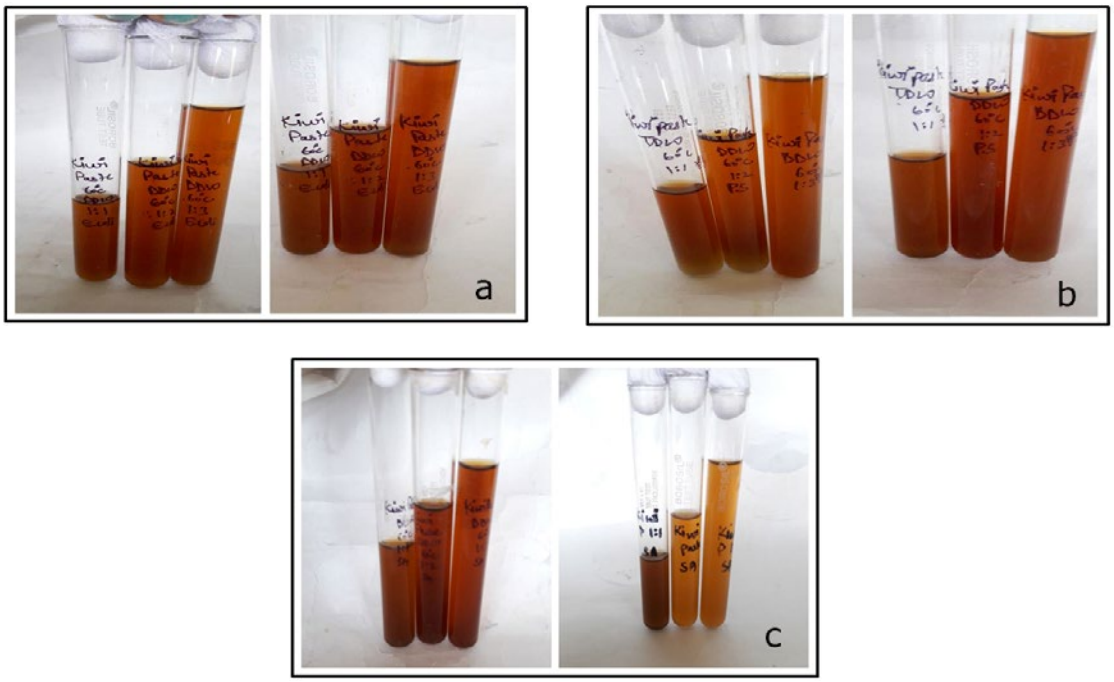

Fig.6. Antibacterial effect of silver nanoparticles of Actinidia deliciosa paste (double distilled water) on (a) Escherichia coli, (b) Pseudomonas aeruginosa, and(c) Staphylococcus aureus in Initial and after incubation for 1 hour at regular interval

70% ethanol as solvent

Silver nanoparticles of paste sample showed maximum, moderate and lowest antibacterial effect against *Staphylococcus aureus*, *Pseudomonas aeruginosa, Escherichia coli* respectively with MIC of 1:3 concentrations (as in Table 6). Graphs showed maximum, moderate, minimum antibacterial effects of silver nanoparticles on different pathogenic strains with MIC concentration of 1:3, 1:2, and 1:1 respectively as shown in Fig. 7. Change in turbidity was observed due to antibacterial effect of silver nanoparticles on pathogenic bacteria as shown in Fig. 8.

METAL ION DETECTION

Double distilled water as solvent

With optical density in CW and silver nanoparticles (0.35) at different concentration of metal ions solution are added with decreasing concentration of metal ion solution. Minimum concentration of metal ion solution showed interaction between silver nanoparticle and heavy metal ion Hg^{2+}. Change in optical density was observed (0.74) (as in Table 7).

Ethanol as solvent

With optical density in CW and silver nanoparticles (0.35) at different concentration of metal ions solution are added with decreasing concentration of metal ion solution. Minimum concentration of metal ion solution showed interaction between silver nanoparticle and heavy metal ion Hg^{2+}. Change in optical density was observed (0.09) (as in Table 8).

Table 6. MIC observation for silver nanoparticles of *Actinidia deliciosa paste* (70% ethanol)

MIC TEST			
Antimicrobial effect			
Test Pathogen	Optical density		
	1:1 (3ml of MHB + 3ml of AgNps)	1:2 (3ml of MHB + 6ml of AgNps)	1:3 (3ml of MHB + 9ml of AgNps)
Escherichia coli	0.76	0.59	0.38
Pseudomonas aeruginosa	0.65	0.48	0.30
Staphylococcus aureus	0.42	0.34	0.23

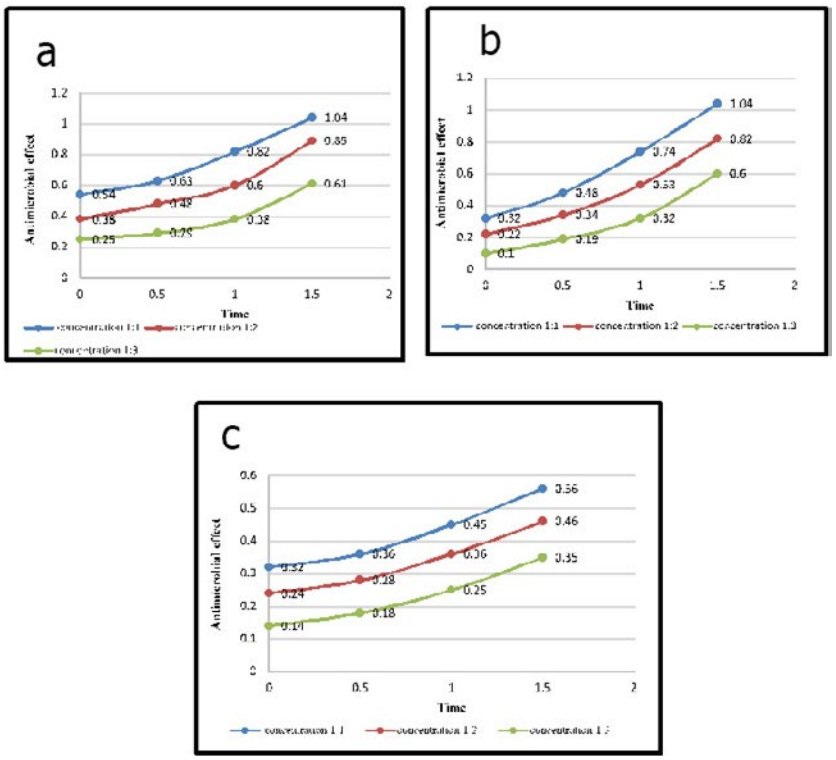

Fig. 7. Graphs showing antibacterial effect of silver nanoparticles of Actinidia deliciosa paste (70% ethanol) against (a) Escherichia coli, (b) Pseudomonas aeruginosa, (c) Staphylococcus aureus at 540 nm

Table 7. Metal ion detection observation of *Actinidia deliciosa paste* AgNps (double distilled water) for Hg^{2+} metal ions

	Optical density at 630 nm	
	Initial	After 2 hours
CW+AgNps	0.35	0.37
CW+AgNps+ 50µl Metal ions	0.76	0.30
CW+AgNps+ 45µl Metal ions	0.25	0.60
CW+AgNps+ 40µl Metal ions	0.46	0.11
CW+AgNps+ 35µl Metal ions.	0.74	0.43

Table 8. Metal ion detection observation of *Actinidia deliciosa* paste AgNps (70% ethanol) for Hg^{2+} metal ions

	Optical density at 630 nm	
	Initial	After 2 hours
CW+AgNps	0.35	0.39
CW+AgNps+ 50µl Metal ions	0.18	0.37
CW+AgNps+ 45µl Metal ions	0.39	0.50
CW+AgNps+ 40µl Metal ions	0.14	0.46
CW+AgNps+ 35µl Metal ions	0.09	0.03

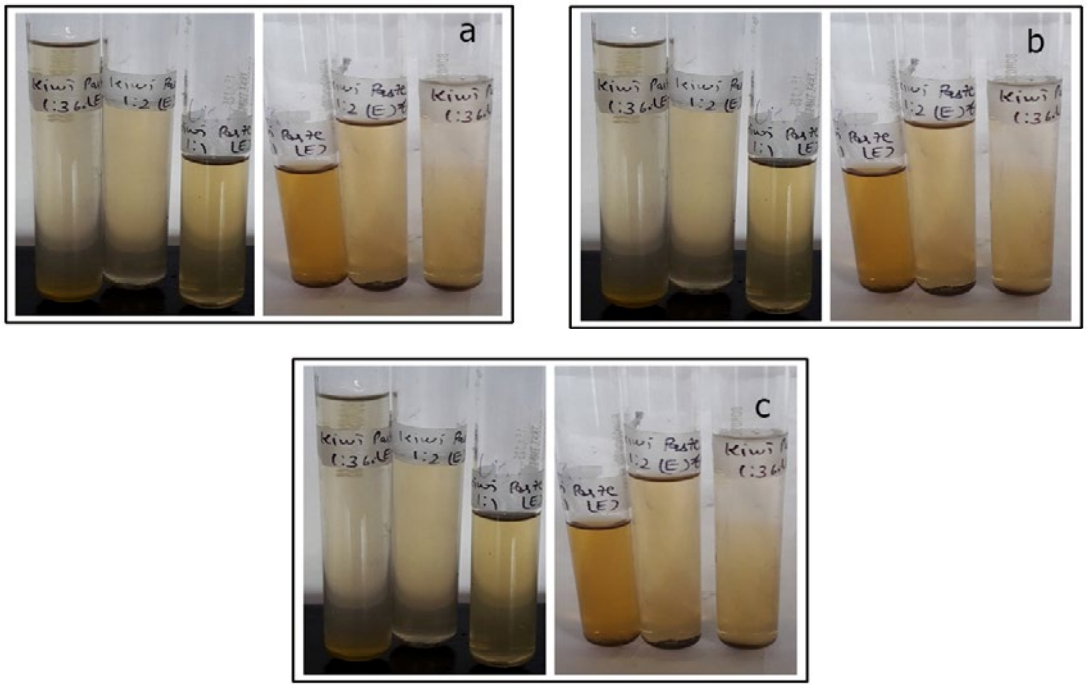

Fig. 8. Antibacterial effect of silver nanoparticles of Actinidia deliciosa paste (70% ethanol) on (a) Escherichia coli, (b) Pseudomonas aeruginosa, and(c) Staphylococcus aureus in Initial and after incubation for 1 hour at regular interval

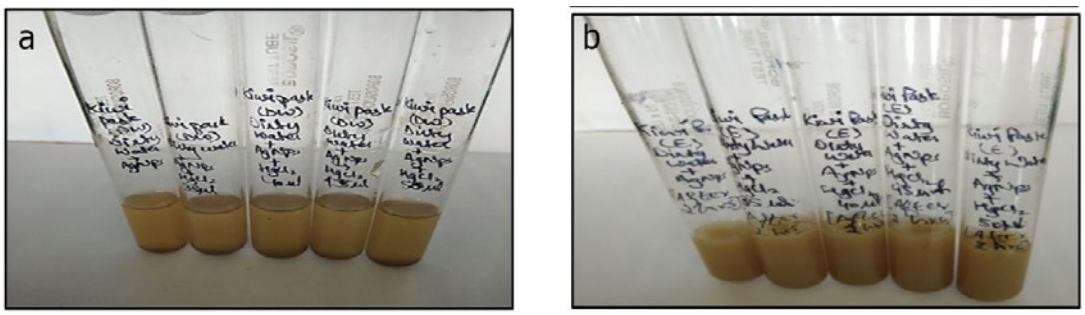

Fig. 9. Colorimetric response of AgNps incubated with various concentration of Hg^{2+} metal ion in contaminated water sample a) Actinidia deliciosa paste (double distilled water) extracted AgNPs b) Actinidia deliciosa paste (70% ethanol) extracted AgNPs

Colorimetric results showed that these biosynthesized AgNps can detect minimum concentration of Hg^{2+} heavy metal ions in contaminated water sample due to surface plasmon resonance, unique property of metallic nanoparticles (as shown in Fig. 9).

CONCLUSION

Silver nanoparticles attracted scientific community because of its strong antimicrobial activity. Biological method is simple, cost effective; eco-friendly method involves bioreduction of metal ions by combinations of biomolecules found in extract of biological entities. This study support successful biological synthesis of silver nanoparticles from *Actinidia deliciosa* paste extracts. *Actinidia deliciosa* was selected as biological source for silver nanoparticles synthesis because of its balanced nutritional composition and health benefits. Bioreduction of Ag^{+} to Ag^{0} was observed when paste extracts were augmented with certain concentration of $AgNO_3$ treatment and kept at different reaction parameters like pH (3, 7, 9) and temperature (0°C, RT, 37°C, 60°C, 100°C). Maximum absorbance was observed at pH 7 and temperature 60°C indicating optimal condition for silver nanoparticles biosynthesis. Biosynthesized silver nanoparticles showed antimicrobial activity and detected minimum concentration of 35 µl of Hg^{2+} heavy metal ions in contaminated water sample. Quantitative data of EDS proved presence of 12.4% and 47.7% silver content in samples *Actinidia deliciosa* paste (double distilled water) and *Actinidia deliciosa* paste (70% ethanol) respectively. Overall nanoparticles synthesized from *Actinidia deliciosa* paste using 70% ethanol as a solvent shows better result as compared to other solvent (double distilled water).

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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