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Synthesis of silver nanoparticles by aqueous extract of *Zingiber officinale* and their antibacterial activities against selected species

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Silver nanoparticles have special plasmonic and antibacterial characteristics that make them efficient in a variety of commercial medical applications. According to recent research, chemically synthesized silver nanoparticles are harmful even in low concentrations. It was crucial to identify appropriate synthesis methods that may have low costs and be nontoxic to the environment. Zingiber officinale (ginger) extracts used to prepare silver nanoparticles were inexpensive and environmentally friendly, and the best physicochemical characteristics were analyzed. Silver nanoparticles were characterized by using UV-visible spectroscopy, Scanning electron microscopy (SEM), and X-ray diffraction (XRD). The surface Plasmon resonance peak at 425 nm was observed using UV-Visible spectroscopy. Scanning electron microscopy observed that the nanoparticles were spherical and ranged in size from 5 to 35 nm. The XRD pattern values of 2θ: 38.2°, 46.3°, and 64.58° are used to determine the planes (111), (200), and (220). The silver nanoparticle's existence was verified by the face-centered cubic (FCC). Silver nanoparticles were found to have antibacterial efficacy against gram-positive *Staphylococcus* and gram-negative bacteria such as *Pseudomonas aeruginosa*, *Klebsiella Aerogenes*, *Salmonella*, *Staphylococcus* and *Escherichia coli*. The antibacterial activity of silver nanoparticles was observed using the agar well diffusion (AWD) method at three different concentrations (100 μg/ml, 75 μg/ml, and 50 μg/ml). The zone of inhibition measured against the bacterial strains *pseudomonas Aeruginosa*, *Klebsiella aerogenes*, *Escherichia coli*, *Salmonella* and *Staphylococcus* which were (18.4±1.25 mm, 16.9±0.74 mm, 14.8±1.25 mm), (16.8±0.96 mm, 14.6±0.76 mm, 14.0±1.15 mm), (19.7±0.76 mm, 18.2±0.66 mm, 15.4±1.15 mm), (16.6±0.67 mm, 14.2±0.23 mm, 12.8±0.78 mm) and (12±0.68 mm, 10±0.20 mm, 08±0.15 mm). These nanoparticles' potent antibacterial properties may enable them to be employed as nanomedicines for a variety of gram-negative bacterial illness treatments.

Keywords: Nanotechnology, Antibacterial activity, Silver-nanoparticles, green synthesis, Ginger .

INTRODUCTION

In the current era one of the most exciting and vast fields of study with great opportunities in Nanotechnolog[1], because of its idiosyncratic properties of nanoparticles including a high ratio of surface area to volume and magnetic, mechanical, optical, and chemical properties, they have remarkable prospects in emerging fields such as food, biomedicine, agriculture, and genetics[2–5]. Nobel metal NPs such as copper silver, platinum, gold, zinc, magnesium, and titanium have gained a lot of recognition for their multifunctional theragnostic abilities in biomedical applications[1]. American physicist Richard Feynman first invented the term "Nanoscience". It is the branch of science that was the first to do things, and it mainly focuses on producing nonmaterial and studying their characteristics. Currently, nanoscience provides the door to «nanotechnology,» a multidimensional and dynamic field[6–9]. It includes a wide range of subjects, including engineering, chemistry, physics, and biology[9–11].

Nanotechnology is now growing in every area of research and technology, including drug delivery science, chemical, physical sciences, electronics, and medical[12]. Nanotechnology is a field of science that synthesized and uses small particles with unique properties[13]. Nanotechnology brings up new possibilities for interesting research in the fields of nanomedicine, biomedical engineering, and drug delivery. It emphasizes the search for information to develop new technologies at atomic and subatomic scales and offers methods for understanding nanoscale materials with a size range of 1–100 nm[14]. A multidisciplinary branch of science and technology with rapid growth, nanotechnology expands the possibilities for controlling at the cellular level the interactions between synthetic materials and biological systems[15]. In the pharmaceutical industry, research organizations, and biomedical sciences, nanotechnology had been declared as a crucial strategy employed in battling numerous viral and microbial diseases[16].

The scientific community gives special attention to the production of nanoparticles because of their distinctive features and technical uses that have a favorable impact on various aspects of the economy, including energy, pharmaceutics, industry, agriculture, and cosmetics[17]. The primary benefit of nanoparticles is that they exhibit entirely new and improved characteristics depending on certain characteristics like size and shape owing to an increase in surface area[15]. The main effects of nanoparticles include their smaller size ratio, which severely impairs the functioning of cell membranes, their penetration into bacteria, which damages Sulphur and phosphorus compounds, particularly DNA, and their release of silver ions, which can have significant bactericidal effects[18]. The food industry, production of cosmetics, health business, and many other fields depend primarily on nanoparticles[19]. Nanoparticles have unique

physicochemical characteristics that aren't present in bulk material[20]. The ease of synthesis, environmental friendliness, and affordability of nanoparticles make them ideal possibilities[21].

The synthesis of silver nanoparticles uses silver the most commonly. Silver nanoparticles are well-established and commonly utilized in a variety of disciplines, including nanomedicine, anti-microbial products, drug delivery, Nano fertilizers, Nano pesticides and biomedical applications[22]. The production of bacterial biofilms was decreased by silver nanoparticles[23]. Silver nanoparticles are mixed in milk to reduce microbial activity. Silver nanoparticles attach to the cell wall and membrane of bacteria when they interact with them, preventing cell reproduction and resulting in cell death. The ionization of silver during its dissolution in the cytosol produces nanoparticles that enhance its bactericidal action. The best way to increase the use of silver nanoparticles in biomedicine is to create superficial, one-step, dependable, affordable, non-toxic, complex fibers, cryogenic superconducting materials, electronic components, and environmentally friendly methods[24]. The most prevalent and significant silver nanoparticles were demonstrated to exhibit a variety of actions, including antibacterial characteristics against a variety of human infections, antifungal, anticancer, larvicidal effects, antioxidant, anti-inflammatory, catalytic and antiviral[25].

There are several techniques to synthesis nanoparticles, like proteins, and extract of plant and microorganisms. These techniques have become widespread for synthesizing silver nanoparticles[26]. Silver nanoparticle synthesis from plants is relatively straightforward, nontoxic, quick, reliable, and environmentally friendly. The plant extracts used to preparation of silver nanoparticles have a benefit while biological synthesis techniques have difficulty maintaining microbes' cultures. After silver nanoparticles production, the characterization of the silver nanoparticles is significant for examining their distinctive properties, such as shape, aggregation, morphology, size, solubility, and surface area[27]. There are many different types of plant diseases, including fungus, algae, protozoa, and bacteria[28].

Ginger, also known as Zingiber officinale, is a plant of the *Zingiberaceae* family and one of the most popular species. Ginger has demonstrated a wide range of pharmacological properties, including gastroprotection, antimicrobial, anti-diabetic agent, anti-inflammatory and antioxidant[29]. There is a lot of interest in plant extracts that have known therapeutic benefits and no negative effects[30]. Zingiber officinale is frequently used to flavor food and beverages. Ginger is commonly recognized for its potential medicinal and food health advantages. Ginger is a popular herbal remedy for several infections like nausea, gastrointestinal issues, throat infections, inflammatory disorders, and arthritis[31]. It is a traditional spice that is used to flavor a variety of meals and beverages[32]. In addition to being a well-known spice Zingiber officinale is used in traditional Eastern therapy to treat a variety of illnesses, including tumors inflammation, rheumatism, heart abnormalities and nausea. Numerous bioactive substances have been recognized to be present in ginger including alkaloids, shogaols, flavonoids, gingerols and zingiberene[33]. The root extract contains volatile oils such as sesquiterpenes, terpenes, geraniols, zingiberene, beta bisabolene, terpineol, curcumin, farnesene, geranyl acetate, alpha-pinene, and limonene, as well as non-volatile substances such as ginerone, gingerols, paradole and shogaols. The secondary metabolites include amadaldehyde, Diterpense, gingerdiols, 6-gingerol sulfonic acid, ginger diacetates and ginger glycolipids A, B, and C[34]. The active ingredients in ginger include 6-gingerol, 6-shogaol and zingiberene[35]. The bioactive substances present in ginger like paradol, Zingiberol, zingiberone, zingerone, zingiberene, gingerol and shogaol[36].

In this work, eco-friendly techniques were used to fabricate silver nanoparticles, and Ginger extract was used as reducing agents. We investigated silver nanoparticles using ultraviolet-visible spectroscopy (UV-Vis), scanning electron microscopy (SEM), and X-ray diffractometer. The antibacterial properties of green-produced silver nanoparticles were examined against *Staphylococcus, Pseudomonas aeruginosa, Klebsiella aerogenes, Salmonella,* and *Escherichia coli.*

MATERIALS AND METHODS

Ginger and silver nitrate (99.9%) were purchased from the local market in Lahore, Punjab, Pakistan. The nutritional agar, Salmonella, Pseudomonas aeruginosa, Staphylococcus, Klebsiella aerogenes, and *Escherichia coli* were obtained by the Pakistan Council of Scientific and Industrial Research (PCSIR).

Ginger extract preparation

The ginger was properly cleaned with distilled water after being purchased from a local market in Lahore, Punjab, Pakistan. Fresh ginger was cleaned, then it was finely chopped and baked to dry. The small pieces of ginger were crushed by mortar pestle and the extract was prepared by adding 5 g of ginger powder in 100 mL of distilled water and heated for about 5 minutes at 30–50 °C. The magnetic stirrer with a hot plate was used for this purpose. Filtration was utilized to remove the insoluble material using Whatman No. 1 filter paper, and the filtrate was stored for later use in a refrigerator at 4 °C (Fig. 1).

Synthesis of silver nanoparticles

The silver nitrate used in this experiment was procured from a local market in Lahore, Punjab, Pakistan, and utilized directly after collection without additional purification. The first precursor stock solution (1 mM) was created by dissolving 0.0169 g of silver nitrate ($AgNO_3$) into 100 ml of distilled water to produce AgNPs utilizing extracts. Drop by drop, 5 mL of ginger extract was added to 50 mL of 1mM silver nitrate solution, and the mixture was then left to react at room temperature without being disturbed. The initiation of the reaction results in the production of yellowish-brown color in the aqueous solution of silver nitrate, which shows the synthesis of Ag nanoparticles. As the reaction progresses, the color of the Ag nanoparticles gradually changes to dark brown (Fig. 2). The reduction of Ag^+ to Ag was followed by UV-visible spectroscopy. Figure 3 presents a schematic drawing of AgNPs synthesis.

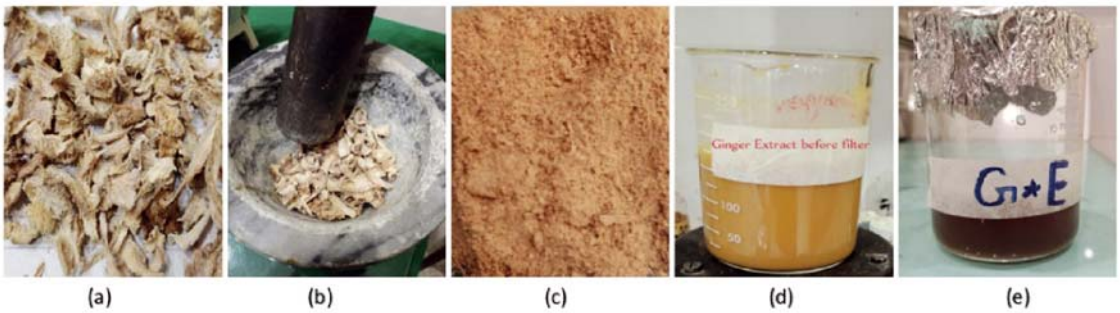

Figure 1. (a) shade dried ginger (b) grinding process (c) fine powder of ginger plant (d) extraction process (e) filtered aqueous extract

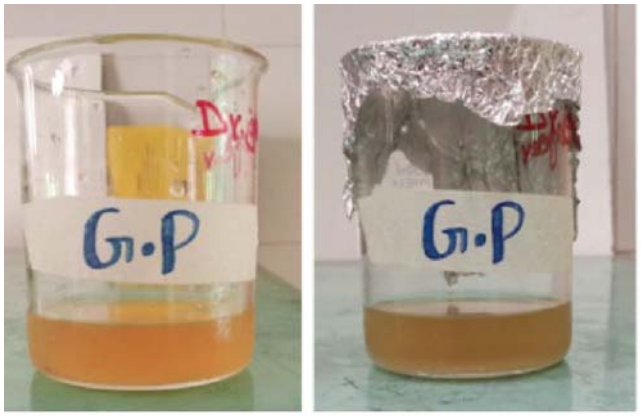

Figure 2. Color change of silver nanoparticles of ginger extract

Figure 3. Schematic drawing of AgNPs synthesis (ginger extract is used in the green synthesis of silver nanoparticles as both a coating agent and a reducing agent; silver nitrate solution ($AgNO_3/H_2O$) and ginger extract (5 mL) was mixed with stirred continuously; *UV-Vis* spectroscopy or color change of solution represented the formation of silver nanoparticles[38])

Ramzan *et al.*[37] reported that an efficient and environment-friendly method was used to fabricate silver nanoparticles to use the extract of ginger.

UV Vis spectroscopy

The UV-Visible and emission spectrum of Ag NPs were examined using a Shimadzu 1800 spectrophotometer[39]. A Shimadzu spectrophotometer (UV 1800) was used to measure the produced nanoparticles' absorption spectra in the 250–1100 nm range. The UV-vis absorption spectra were used to study the production of silver nanoparticles.

Scanning Electron Microscopy (SEM)

The sample is investigated using a field emission scanning electron microscope with X-ray energy-dispersive spectrometry system (Model: SIGMA VP, manufacturing company: Carl Zeiss Microscopy GmbH, Germany; operated at 15 kV)[40]. The scanning electron microscope was used to examine the size and morphology of nanoparticles (Nova Nano SEM 450, USA).

X-ray diffraction

The X-ray diffractometer (Bruker AXS, Germany) was used to obtain the XRD patterns of the green synthesis particles. The radiation used was Co Kα radiation with a 1.544 nm wavelength, at 40 kV and 15 mA[41]. An X-ray Diffraction experiment was used to check the structure of the silver nanoparticle. The sample was deposited on a glass plate, and the analysis was performed at a voltage and current of 40 kV and 40 mA, respectively.

Antibacterial Activity by Well Diffusion Method

The agar well diffusion technique was used to test the produced silver nanoparticles› antibacterial properties[33]. Silver nanoparticles' antibacterial resistance against *Salmonella Pseudomonas aeruginosa*, *Staphylococcus, Klebsiella aerogenes* and *Escherichia coli* was tested using the agar well diffusion technique. Müller-Hinton (MH) agar media was spread with bacteria at 0.5 McFarland turbidity. The agar plates were made with 5 wells that were each 6 mm in diameter. The wells were labeled with 50 µg/mL, 75 µg/mL and 100 µg/ mL of biosynthesized silver nanoparticles, 100 µg/ mL concentration of ginger extract and 100 µg/ mL concentration of distilled water. The zone of inhibition was measured after the 18-hour incubation period at 37 °C.

Green reducing and capping agents

Numerous environmentally friendly, cost-effective, biocompatible, and non-hazardous green reducing and capping agents have been studied by researchers. The biomolecules included in the plant extracts allow for the bio-reduction of silver nanoparticles. The reducing and capping agents are citric acid, phenolic, membrane proteins, sugars, tartaric acid, amino acids and functional groups (amines, aldehydes, alcohols, carboxylic acid ketones)[42].

Collection of microbial culture

The agar well diffusion technique was used to examine the antibacterial properties of fabricated silver nanoparticles. The bacterial strains were used in this work like *Klebsiella aerogenes Escherichia coli, Staphylococcus, Pseudomonas aeruginosa* and *Salmonella*. The Pakistan Council of Scientific and Industrial Research, Lahore, provided the pathogens.

Statistical analysis

All data were subjected to statistical analysis using the Advanced Models 16.0 software's one-way ANOVA (SPSS, Tokyo, Japan)[43]. The data were statistically examined using the statistical program SPSS, and mean, standard deviation, and ANOVA were carried out to elaborate the results' significance.

RESULTS AND DISCUSSION

UV–Vis analysis

The UV-vis absorption spectra of colloidal solutions of aqueous silver nitrate and leaf extract are shown in Figure 4. The addition of ginger leaf extract to the silver nitrate solution was changed colorless to brown color. The formation of silver nanoparticles was confirmed by the change in color of the silver nitrate solution. According to the addition of extract drop by drop of volume 5 mL in the reaction solution for 20 minutes, the reaction mixture's color changed to yellowish brown, brown, and deep brown. Silver ions are converted to silver nanoparticles at a faster rate as ginger extract volume increases. A prominent and singular surface Plasmon resonance (SPR) peak is produced at 425 nm in the absorption spectra as a result of the resonance of incident photon energy to surface electrons on the silver nanoparticles. There were no further resonance peaks present in the spectra. The emergence of a brown color caused by the activation of surface Plasmon vibrations in Nano-silver, showed the progressive production of silver nanoparticles. The instantaneous color variation that occurred after combining the silver nitrate and aqueous extract solution showed very quickly the reaction occurred. This color change shows the activity of a redox reaction, in which extract ingredients, which are oxidized to various species, decrease Ag^+ ions to Ag^0 [44]. Silver presents the surface plasmon resonance (SPR) phenomenon, silver nanoparticles have an absorption peak in their UV-Vis spectrum at around 425 nm, which is their unique absorption band[45].

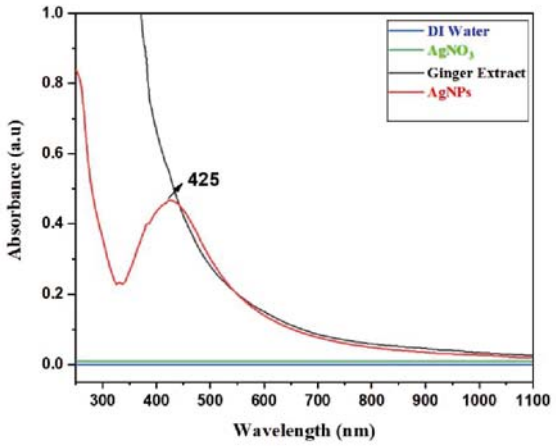

Figure 4. UV-Vis spectra show absorption peak for silver nanoparticle

Scanning electron microscopy (SEM)

A scanning electron microscope was used to assess the size and morphology of silver nanoparticles. As a result, SEM images are shown in Figure 5 and Table 1. The ginger extract was used to produce the silver nanoparticles, and the silver nanoparticle was spherical and ranged in size from 5 to 35 nm. Sukweenadhi *et al.*[46] reported that silver nanoparticles mediated by ginger extract were spherical.

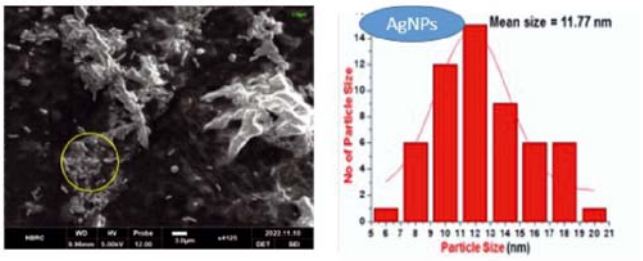

Figure 5. SEM image of silver nanoparticle and Histogram plot showing the distribution of sizes of the silver nanoparticles

Table 1. Summary of the UV-Vis and SEM results

Sample	Color	Absorbance peak	Shape
AgNPs	Dark brown	425nm	Spherical

X-Ray Diffraction

X-Ray Diffraction (XRD) was used to examine the crystalline properties of produced silver nanoparticles. The XRD pattern recorded in the range from 20° to 70° is shown in Figure 6. In the XRD pattern, three

diffraction maxima at 2θ = 38.2°, 44.3°, and 64.58° were observed. The diffraction maxima were indexed as (111), (200), and (222) plane of the face center cubic structure of Ag. Wisam *et al*.[47] reported that peaks at 2θ = 38.2°, 44.4°, and 64.6° are typical diffractions of face-centered cubic (FCC) structured Ag (JCPDS Card no. 04-0783). Additional unassigned peaks (marked stars) appeared in the recorded XRD pattern due to the presence of bioorganic or metalloproteins in the solution[48]. The weaker peaks relate to bio-organic matter found on the nanosilver surface[49]. Scherrer's formula was used to get the average particle size from the FWHM of the (111) plane:

$$D = k/\beta \cos\theta$$

Where λ is the employed X-ray wavelength (1.5406 Å), θ is Bragg's angle, K is the dimensionless form factorwith a value of 0.9, and is the full width at half maximum (FWHM) of the (111) peak[12]. Silver nanoparticles were an average crystallite size of 11.77 nm (Table 2).

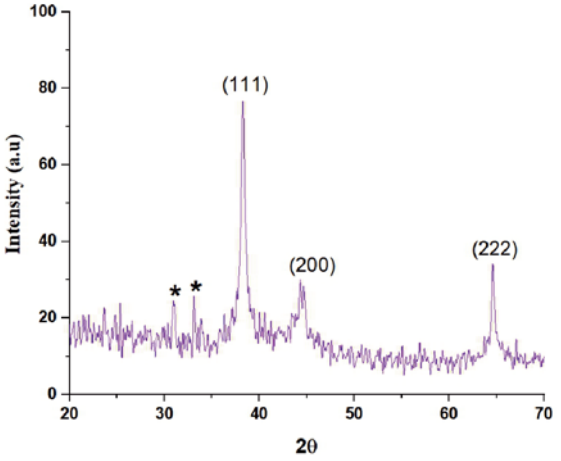

Figure 6. XRD spectrum of the synthesized silver nanoparticles

Table 2. Summary of silver nanoparticle obtained from XRD result

Sample	Peak position (2θ)	Diffraction plane (hkl)	FWHM (β) radiations	Lattice parameter(a), nm	Crystalline size (D), nm
AgNPs	38.2	(111)	0.0128	0.403	11.77

Zone of Inhibition of Nanoparticles on the Different Bacteria

It is necessary to find new antimicrobial medications using environmentally friendly and green methods due to the rise in antibiotic resistance among human infections. The rapid development of bionanotechnology stimulates considerable production of novel substances with potent antibacterial properties[50]. Plant phytochemical extracts may have the potential for application in allopathic medicine as sources of antiviral, antitumoral, and antibacterial drugs. The chemical components of medicinal plants are given their therapeutic and pharmacological significance. There are several recognized positive medicinal benefits of plants[51]. Agar well diffusion methods were used to test the antibacterial activity of silver nanoparticles against one strain of gram-positive bacteria (*Staphylococcus*) and four strains of gram-negative bacteria, including *Escherichia coli, Pseudomonas aeruginosa, salmonella*, and *Klebsiella aerogenes.* Significant antimicrobial activities shown in Figure 7 below resulted in the efficient potential of AgNPs for medical applications.

In Figure 8 and Table 3, the antibacterial effects of silver nanoparticles synthesized from ginger leaves on specific bacterial species are presented. Silver nanoparticle concentrations of 50 μg/ml, 75 μg/ml, and 100 μg/ml were prepared from ginger leaves. Better zones of inhibition were found against *Pseudomonas aeruginosa* (18.4±1.25 mm) at 100 μg/ml concentration, *Escherichia coli* (19.7±0.76 mm) at 100 μg/ml concentration, *Salmonella* (1 mm) at 100 μg/ml concentration, *Klebsiella aerogenes* (16.3±0.96 mm) at 100 μg/ml concentration and *Staphylococcus (*10.2±0.68 mm*)* at 100 μg/ml concentration. The growth of all the examined bacterial isolates was stopped by silver nanoparticles that were isolated from ginger leaves. At a silver nanoparticle concentration of 100 μg/ml, *Pseudomonas aeruginosa* growth was most inhibited (18.7± 1.25 mm zone of inhibition). *Pseudomonas aeruginosa* is renowned for having antimicrobial resistance. The antibacterial action of silver nanoparticles among synthetic metal nanoparticles has been thoroughly studied and experimentally verified[52]. The pathogen's sensitivity to silver nanoparticles is strongly affected by the structure of the bacterial cell surface[53–61].

Table 3. Antibacterial activity of AgNPs by different concentrations

Bacterial strain	Zone of inhibition of AgNPs against different bacteria at different concentrations.		
	100 μg/ml	75 μg/ml	50 μg/ml
Staphylococcus	12±0.68	10±0.20	08±0.15
Klebsiella aerogenes	16.8±0.96	14.6±0.75	14.0±1.15
Pseudomonas aeruginosa	18.4±1.25	16.9±0.74	14.8±1.25
Salmonella	16.6±0.67	14.2±0.23	12.8±0.78
Escherichia coli	19.7±0.76	18.2±0.66	15.4±1.15

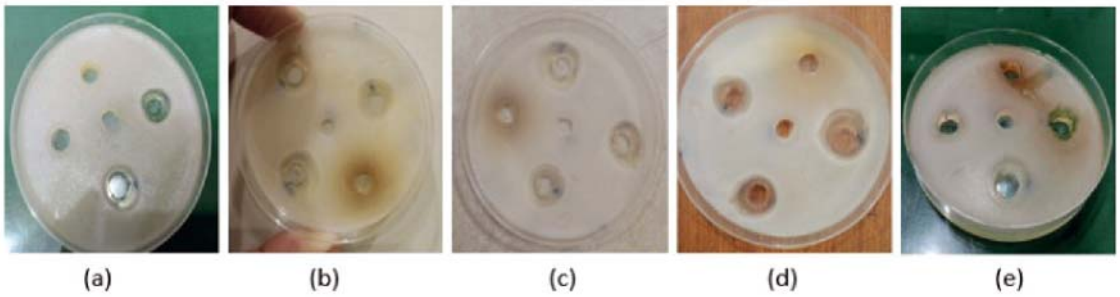

Figure 7. Bacterial colony formation of (a) *Staphylococcus* (b) *Klebsiella aerogenes* (c) *Pseudomonas aeruginosa* (d) *Escherichia coli* (e) *Salmonella*. In ager well diffusion method against silver nanoparticles extracts mint leaves

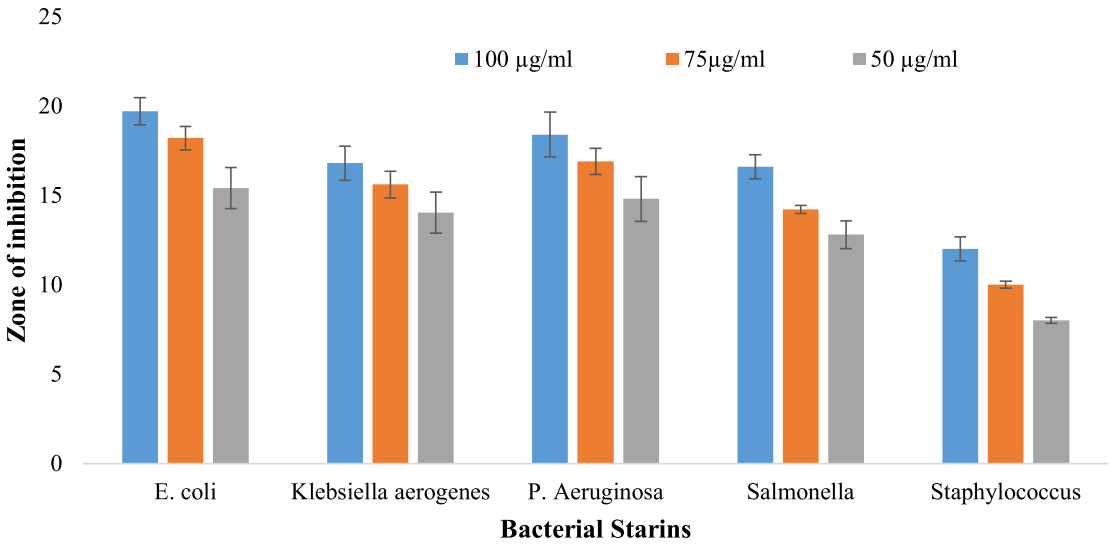

Figure 8. Antibacterial activity of different concentrations of ginger AgNPs against different gram-positive and gram-negative bacterial strains

CONCLUSION

Preparation of silver nanoparticles by green synthesis method is low cost, environment friendly, and non-toxic. We have successfully established a green method for synthesizing silver nanoparticles by using aqueous ginger extract. The biological approach utilized to synthesize silver nanoparticles is very successful since the biomolecules involved in the reduction process come from plants and are completely non-toxic to the environment. It was observed that the presence of biomolecules acting as stabilizing agents on the surface of the nanoparticles made them more stable. Numerous microscopic methods, such as scanning electron microscopy and UV-visible spectroscopy, have also been used to analyze the fresh aqueous extract of ginger silver nanoparticles. Electron scanning microscopy Confirm that the nanoparticles were spherical and ranged in size from 5 to 35 nm. Silver nanoparticles exhibited their anti-microbial activity against selected one strain of gram-positive bacteria (*Staphylococcus*) and selected four strains of gram-negative bacteria *Escherichia coli, Pseudomonas aeruginosa* and *salmonella.* Silver nanoparticles can permeate bacterial cell walls, affecting the composition of cell membranes and potentially causing cell death. Silver nanoparticles with improved antibacterial properties were produced using this technique, which also resulted in smaller, less agglomerated silver nanoparticles. Large-scale applications are possible for these biogenic synthesis-mediated anti-microbial capabilities. These silver nanoparticles worked well against several illnesses septic arthritis, endocarditis, osteomyelitis, pneumonia, abscesses and Skin infections are diseases caused by gram-positive bacterial strains while meningitis Pneumonia, wound or surgical site infections, and bloodstream infections are diseases caused by gram-negative bacteria**.** The successful use of nanoparticles in cancer treatment, immunization, and delivering genes to cells.

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