SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES MEDIATED BY THE *Mentha piperita* LEAVES EXTRACT AND EXPLORATION OF ITS ANTIMICROBIAL ACTIVITIES

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ABSTRACT

Medicinal use of nanotechnology included a significant contribution from the antibacterial activity of biologically produced silver nanoparticles (AgNPs). Scientists investigated an efficient and environmentally friendly way to make silver nanoparticles by extracting *Mentha piperita* leaves as well as using their antimicrobial properties. Green synthesis method was used to produce AgNps from extract of mint plant and characterization was done by XRD, SEM and UV Visible Spectroscopy. A peak at 440 nm, which corresponds to the plasmon absorbance of silver nanoparticles, was evident in the UV-visible spectra of the solution containing AgNPs. Scanning electron microscopy observed that the nanoparticles were spherical in shape and ranged in size from 20 to 50 nm. The planes (111), (200), and (220) were found using the XRD patterns and value 0f 2θ: 38.5^0, 46.3^0 and 64.7^0 are observed. The silver nanoparticle's existence was verified by the face-centered cubic (FCC). Silver nanoparticles were found to have antibacterial efficacy against both gram-positive Staphylococcus and gram-negative bacteria such *Pseudomonas aeruginosa, Klebsiella Aerogenes, Salmonella, Staphylococcus* and *E. coli.* The antibacterial activity of silver nanoparticles against bacterial strains were observed using the agar well diffusion (AWD) method at three different concentrations (100µgml^{-1}, 75 µgml^{-1}, and 50 µgml^{-1}). The zone of inhibition measured against the bacterial strains *pseudomonas Aeruginosa, Klebsiella aerogenes, E. coli, Salmonella* and *Staphylococcus* which were (18.7±1.25mm, 16.5±0.74mm, 14.0±1.25mm), (16.3±0.96mm, 14.5±0.76mm, 14.0±1.15mm), (16±0.76mm, 14.4±0.66mm, 14.0±1.15mm), (16.5±0.67mm, 14.5±0.23mm, 12.6±0.78mm) and (110.2±0.68mm, 8.8±0.20mm, 7.0±0.15mm). These nanoparticles' potent antibacterial properties may enable them to be employed as nanomedicines for a variety of gram-negative bacterial illness treatments.

Keywords: Antibacterial activity, Silver-nanoparticles, green synthesis, mint leaves, Gram negative and gram-positive bacteria.

1. INTRODUCTION

The word "Nano" comes from the Greek word "dwarf," which refers to something that is one billionth of a meter in size. [1]. Due to their special characteristics, nanoscale materials are useful in many industries, including agriculture, aquaculture, food production, and pharmaceuticals [2-6]. The large surface area, small particle size, thermal and optical characteristics, catalytic action, and electrical conductivity of nanomaterials make them extremely important. Nanotechnology, a recent subject that combines physics, biology, and chemistry, is one of the many branches of science [7-8]. Nanotechnology is growing rapidly on various industries and biomedical fields due to large surface area to volume ratio and their unique physical, chemical, and biological properties as compared to the bulk material [9]. (One of the most prominent study topics is nanotechnology, which significantly contributes to the production of nanomaterials with distinctive features in the disciplines of science and engineering [10]. Currently, nanotechnology is generating a lot of interest and is being promoted as a potential solution to issues in the scientific and technical fields [11]. The manipulation of matter on molecular, supramolecular and an atomic scale is known as nanotechnology. It is a branch of the applied sciences and engineering that includes a broad range of areas [12]. Construction of active packaged foods with qualities of both exterior barriers and antimicrobial is made possible by nanotechnology, which is one of options for increasing the shelf life of foods [13]. Nanotechnology able to develop a variety of new items, including gadgets and materials, for use in a wide range of industries, including energy generation consumer goods, electronics, biomaterials [14].

Nanoparticles have many important uses, including in the chemical, pharmaceutical, food, environmental, and electronics sectors [15]. The desire of researchers to develop new techniques for the preparation of nanoparticles has increased dramatically over the past ten years because of the widespread use of nanoparticles in a variety of fields, including agriculture, biomedicine, biosensors, cosmetics, drug delivery systems [16]. The development of low-cost, green nanoparticle synthesis methods that don't use dangerous chemicals in the synthesis process is becoming more and more important [17]. Researchers' interest has recently been drawn to the unique and distinctive, biological, chemical and physics features of nanoparticles. They are utilized in a variety of contexts, including coatings, medicine delivery, and cosmetic care items [18]. When compared to chemical and physical approaches, the production of nanoparticles by biosynthesis is beneficial and appealing. These efficient and environmentally syntheses don't need harmful chemicals, high temperatures, or pressure because they are affordable and use an eco-friendly process [19]. In comparison to organic antibacterial agents, metal nanoparticles have a wide spectrum of antimicrobial action against Gram-positive and Gram-negative bacteria, moulds, yeast, and viruses in addition to having improved chemical and thermal durability. Application for food products have received a lot of interest because to the simplicity and affordability of nanoparticle production. The significant antibacterial agent for the diagnosis of diseases and the storage of food, silver has been known antiquity. Due to their ease of penetration through. Gram-negative bacteria's thin cell walls, silver nanoparticles are more efficient against them than against Gram-positive bacteria [20]. Inhibiting the activities of bacteria and viruses is one of silver nanoparticles' well-known antibacterial characteristics [21].

Silver nanoparticles are said to be non-toxic to animals and effective against a variety of microbes at low concentrations and without side effects [22]. There are several more characteristics of silver nanoparticles like antiviral, antitumor antioxidant, and antimicrobial [23]. It is possible to produce silver nanoparticles utilizing a variety of chemical and physical processes. The chemical processes had high production costs and toxicity problems, which paved the way for a very efficient, dependable, and environmentally friendly plant - mediated technology [24]. Gram-negative and Gram-positive bacteria were significantly affected by the spectrum antibacterial properties of silver nanoparticles, which produced the size of nanoparticles at the range of 100nm [25]. Antimicrobial coatings, catalysis, bio sensing, chemical production, medication delivery and environmental health are areas where silver nanoparticles can be used [26]. The use of silver nanoparticles in paintings, textile and dye deterioration, toys for children, cosmetics, pharmaceuticals, bacterial contaminations, food storage, handling containers and other applications is commonplace [27]. For the manufacture of silver nanoparticles, several physical and chemical techniques, including thermal decomposition, microwave, electrocemical and laser ablation have been used, However, there are a number of drawbacks to the aforementioned techniques, including their high cost, toxicity of the solvents, chemical systems, environmental danger, hazardous by-products, greater temperature, and higher pressure [28], several advantages such as being non-toxic, having no unpleasant side effects, being environmentally friendly, clean, safe, and readily available, the production of nanoparticles utilizing natural sources is receiving increased attention [29].

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The "green" synthesis technique is one of the crucial methods for producing noble metal nanoparticles. The byproducts of plant metabolism are utilized in this procedure as reducing and stabilizing reagents [30]. Plants have secondary metabolites such phenolic chemicals, triterpenoids, alkaloids, steroid, quinines, coumarins, glycosides, flavonoids and anthocyanins that provide them resistance to pests, insects, drought stress and other stress situations. These substances have a wide range of biological properties including antiviral effects, anticancer, antioxidant, antidiabetic, antibacterial and anti-obesity making them highly significant for humans [31]. As a result of their antibacterial and antioxidant characteristics, several spices, including mint, clove, cinnamon, oregano, and thyme have been used as medicines [32]. The usage of Mentha in traditional medicine is the same as in contemporary herbal medicine. It can be used for a fever, common cold, loss of appetite, sinusitis, bronchitis, nausea, cough, vomiting, indigestion, and intestinal colic. It also has antibacterial and antioxidant properties. Additionally, it is utilized to flavor toothpaste, candy, chewing gum and medicinal preparations [33]. There are more than 4000 species in 200 genera in Lamiaceae family is among the most popular and attractive plants in the Lamiaceae family. Many locations of the world have moderate climates where mint may be cultivated. The Mentha plants *Mentha piperita* is significant for a variety of therapeutic purposes due to essential oils, the polyphenols and flavonoids which are contained mint leaves extract [34]. The unique composition aromatic, hydrophobic chemicals and volatile known as essential oils is derived from plant origins and has significant water vapor barrier qualities [35]. Infectious pathogens are currently the biggest concern facing the world, and they are the main cause of about 50,000 fatalities per day. Numerous harmful bacterial strains, including staphylococcus aureus, Klebsiella pneumonia and E. coli are causes infectious illnesses. The most typical pathogens are *vibrio cholera, Staphylococcus, Salmonella spp.* and *Shigella* spp. [36].

In this work, mint extract was used to produce silver nanoparticles and the technique's important parameters were improved to produce small-size silver nanoparticles. Researchers thoroughly examined the antibacterial potential of silver nanoparticles produced using green technologies against various gram-negative and gram-positive bacteria. According to the observations, using AgNPs of a smaller size was considerably more successful for antibacterial potential when using the green technique.

2. MATERIALS AND METHODS

In the local market of Lahore, Punjab, Pakistan, we found silver nitrate (99.9%) and mint leaves. *Pseudomonas aeruginosa, Escherichia coli, Salmonella, Staphylococcus,* and *Klebsiella aerogenes* were acquired from Pakistan Council of Scientific and Industrial Research, Lahore (Punjab, Pakistan)

2.1. Preparation of mint leaf extract

The usual method was used to make mint leaf extract as shown in **Figure 1**. Fresh mint leaves were taken from the Pakistan Council of Scientific and Industrial Research's Garden in Lahore, Punjab, Pakistan, and properly cleaned with double-distilled water. The mint leaves were dried in oven overnight at a temperature of 50 °C and crushed using a mortar and pestle. It was combined with 150 ml distilled water and 10g of powdered mint leaves. The mint leaves' components were removed by heating the solution to 100°C for 2 minutes, letting it cool to room temperature, and then filtering the mixture using Whatman 1 filter paper. At 4 degrees Celsius, the extracted solution was kept.

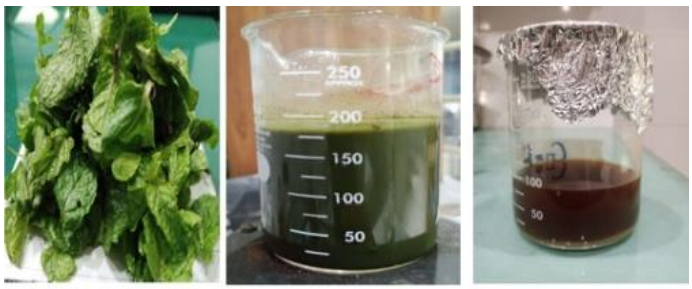

Figure 1: Preparation of bioactive plant extract (a) mint leaves (b) aqueous solution of shade dried and ground mint leaves (c) aqueous extract of mint leaves.

2.2 Preparation of silver nanoparticles

Silver nitrate was obtained from a local market in Lahore, Punjab, Pakistan, and utilized directly after collection without additional purification. First precursor stock solution (1mM) was produced by mixing 0.0169g of silver nitrate ($AgNO_3$) into distilled water containing 100 ml to prepare AgNPs utilizing extracts. Add 5mL of mint extract, drop by drop, to 50mL of a 1mM silver nitrate solution, and let the mixture react at 20°C without being disturbed. The commencement of the reaction results in the emergence of yellowish-brown color in the aqueous solution of silver nitrate, which signals the synthesis of Ag nanoparticles. As the reaction progresses, the color of the Ag nanoparticles gradually changes to dark brown. The reduction of Ag^+ to Ag was followed by UV-visible spectroscopy as shown in **Figure 2.** The detailed experimental conditions for the synthesis of AgNPs have been given in **Table 1.**

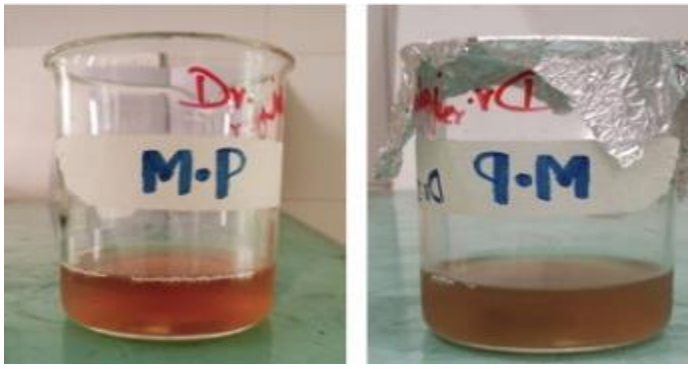

Figure 2: color change from yellow brown to dark brown represent synthesis of silver nanoparticles.

Table 1: Detailed experimental conditions for the synthesis of AgNPs using green synthesis method.

Sample	Precursor (1mM)	Reducing agent	Reduction time	Stirring	Temperature
AgNPs	50 ml	5 ml	20 minutes	Constant	20 ^{0}C

2.3 Green reducing and capping agents

Many green reductive and capping agents were studied by researchers, and they may be biocompatible, inexpensive, non-hazardous, and environmentally beneficial. The availability of biomolecules contained in the leaf extracts facilitates for the bio-reduction of silver nanoparticles. Sugars, Amino acids, tartaric acid, citric acid, membrane proteins, phenolic, and functional groups (amines, alcohol, carboxylic acid ketones, aldehydes) operate as the reducing and capping agents in this process, however the detailed mechanism is still not entirely understood [37].

2.4 UV –Visible spectra analysis

For the examination of silver nanoparticles, the UV-Vis spectrophotometer is a highly helpful, straightforward, and sensitive technique [38]. Utilizing a UV-Vis Shimadzu spectrophotometer (UV 1800), the absorption spectra were recorded across the range of 300-1100nm, and the reaction medium's UV-Vis spectrum was measured to measure the reduction of pure Ag^+ ions.

2.5 Scanning electron microscopy

The scanning electron microscope was used to examine the size and morphology of nanoparticles (Nova Nano SEM 450, USA)

2.6 X -Ray Diffraction (XRD)

The crystalline structure of the purified AgNPs was examined using X-ray diffraction analysis [39]. We employed wide-angle XRD to identify the specific position of AgNP formation in the mint extract. Using the Rigaku diffractometer (Cu Kα radiation, =0.1548 nm) at 40 kV and 50 mA, measurements were taken on dried and finely powdered materials.

2.7 Antibacterial assay

The antibacterial efficacy of biosynthesized AgNPs was evaluated using the Agar well diffusion approach [40]. According to the ager well diffusion method, the minimum inhibitory concentration (MIC) of as-synthesized AgNPs against four Gram-negative bacteria *(Escherichia coli, Pseudomonas aeruginosa, salmonella* and *Klebsiella aerogenes)* and one Gram-positive bacteria, *Staphylococcus*, was determined. To demonstrate the relevance of the synthesis technique and its effect on the antibacterial characteristics of the final AgNPs, the growth of each bacterium was monitored using a varied amount of AgNPs up to 24 hours at the determined MIC values for the best samples.

The identified pathogenic bacteria were grown in 24-hour active cultures for the antibacterial test. Then, on nutrient agar medium plates, wells (6 mm in diameter) were prepared and filled with various concentrations of AgNPs (50 µg/mL, 75 µg/mL, and 100 µg/mL) before being seeded with new cultures of pathogenic bacteria. Mint extract (100 µg/mL) and $AgNO_3$ (100 µg/mL) solution were used as control. The plates were incubated overnight at 37 °C. Zones of inhibition were computed following incubation to assess the antibacterial properties of the biosynthesized AgNPs.

2.8 Collection of microbial culture

The agar well diffusion technique was used to examine the antibacterial properties of synthetic silver nanoparticles. We utilize specific microbes for this aim, including *salmonella, Klebsiella aerogenes*, *Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus*. These microorganisms were acquired from Pakistan Council of Scientific and Industrial Research, Lahore (Punjab, Pakistan). The data were statistically analyzed by Statistical software SPSS and mean ±SD and ANOVA were performed to elaborate significances of results.

3. RESULTS AND DISCUSSION

3.1. Ultraviolet Visible Spectroscopy

The absorption spectra at wavelength ranges between 300-1100 nm were used to observe the biogenesis of the AgNPs in the aqueous solution. The reaction mixture's color changed after 30 minutes from yellow brown to dark brown. When mint extract was added to a silver nitrate solution, the color of the solution changed to a dark brown color after 30 minutes of incubation at room temperature, indicating the synthesis of AgNPs. In comparison, no color change was seen in the absence of plant extract. AgNP production in the colloidal solution was verified using *UV-Vis* spectra.

The synthesis of AgNPs was verified by a single, strong, and wide surface plasmon resonance (SPR) peak in the UV-vis spectrum at 440 nm as shown **Figure 3**. The change of the reaction mixture's color into a dark brown helped to identify the creation of Ag-NPs from the extract of mint leaves [41]. When added to plant extracts, the reduction of silver ion into silver nanoparticles causes a change in color. A UV-visible spectrophotometer was used to examine silver nanoparticles. Silver nanoparticles with specific sizes and shapes may absorb light in the visible spectrum because of SPR phenomenon [42].

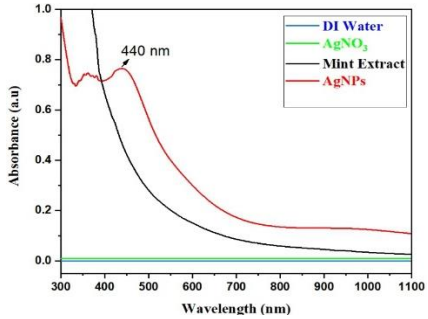

Figure: 3 *UV-Vis* spectra show absorption peak for silver nanoparticle.

3.2 Scanning Electron Microscopy Analysis

Scanning electron microscope was used to identify the shape and size of silver nanoparticles. As a result, we obtained the SEM images shown in **Figure 4** and **Table 2.** The silver nanoparticle prepared by using the mint leaves extract and observed that silver nanoparticles are different sizes ranges from 20-50nm. Reda et al., [43] discussed that the shape of silver nanoparticles prepared by the mint leaves extract are spherical. The biosynthesized AgNPs have spherical shape with a size range of 20–70 nm.

Table 2: Summary of the UV-Vis and SEM results

Sample	Color	Absorbance peak	Shape
AgNPs	Dark brown	440 nm	Spherical

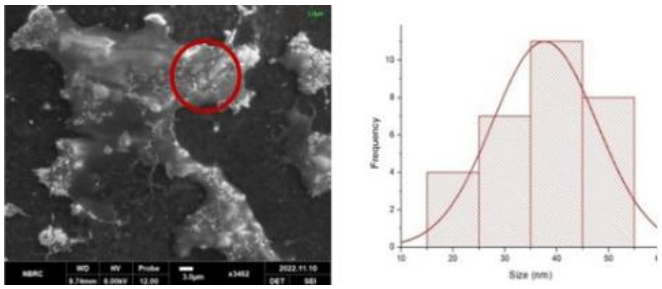

Figure 4: SEM image and graph of silver nanoparticle mediated mint leaves

3.3 X-Ray diffraction

The XRD technique was used to determine the crystalline structure of fabricated silver nanoparticles. These nanoparticles X ray Diffraction results were presented in the **Figure 5** and **Table 3**. The result observed that silver nano particles have hexagonal structure because silver has face centered cubic structure. According to the X-ray patterns, silver nanoparticles displayed sharp peak at 2θ of about 38.5°, 46.4°, and 64.4°, which were assigned to the (111), (200), and (220) orientations, respectively. These peaks can be significantly related to the face-centre cubic (fcc) silver data from the standard JCPDS analysis (JCPDS No. 04-0783). These peaks were produced by the organic substances in the leaves extract of mint which reduced Ag ions and produced silver nanoparticles. The presence of bioorganic or metallo-proteins in the solution, which are in responsible of stabilizing nanoparticles, caused additional unassigned peaks (marked stars) to appear in the recorded XRD pattern. Syed et al., [44] reported similar observations in majority of previous studies. Alkfaj et al., [45] discussed that the XRD pattern demonstrated the unique Bragg peaks of the (111), (200), (220), and (311) sides of the face centre cubic (fcc) silver nanoparticles, confirming that these nanoparticles are crystalline in shape. Rasool et al., [46] reported that the crystallization of the extract's bioorganic molecules on silver nanoparticles was causes the "star peaks" at 27.8° and 32.5°.

Scherer equation was used to determine the average crystallite size of silver nanoparticles:

$$d = K\lambda/\beta \cos\theta$$

Where K is the shape factor 0.9, k–incident X-ray wavelength (CuKα = 1.542Å), β–full width half-maximum in radians of the prominent line and θ–position of that line in the pattern [47].

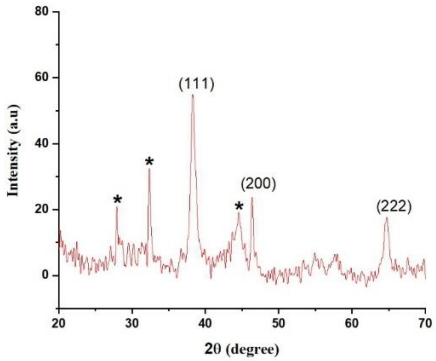

Figure 5: X-ray diffraction result for silver nanoparticles of Mentha piperita.

Table 03: Brief 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	38.5	(111)	0.0128	0.403	11.33

3.4 Inhibition of Nanoparticles on the Different Bacteria

The antibacterial activity of silver nanoparticles was examined using agar well diffusion techniques against one strain of gram-positive bacteria *(Staphylococcus)* and four strains of gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa, salmonella* and *Klebsiella aerogenes.* Significant antimicrobial activities shown in the **Figure 6** below resulted in the efficient potential of AgNPs for medical applications.

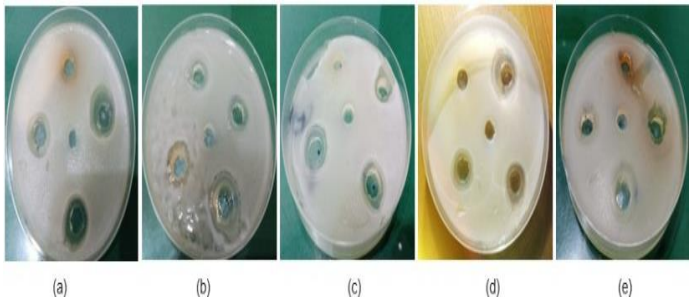

Figure 6: Bacterial colony formation of (a) *Escherichia coli* (b) *Klebsiella aerogenes* (c) *Pseudomonas aeruginosa* (d) *Salmonella* (e) *Staphylococcus*. In ager well diffusion method against silver nanoparticles extracts mint leaves.

The antibacterial properties of mint leaf-derived silver nanoparticles against certain bacterial species were shown in Figure 7 and listed in Table 4. Silver nanoparticle concentrations of 50 µg/ml, 75 µg/ml, and 100 µg/ml prepared from mint leaves. Better zones of inhibition were found against *Pseudomonas aeruginosa* (18.7±1.25 mm) at 100 µg/ml concentration, *Escherichia coli* (16±0.76 mm) at 100 µg/ml concentration, *Salmonella* (16.5±0.67mm) at 100 µg/ml concentration, *Klebsiella aerogenes* (16.3±0.96 mm) at 100 mg/ml concentration and *Staphylococcus (*10.2±0.68*mm) at 100* µg/ml concentration. Silver nanoparticles extracted from mint leaves inhibited the bacterial growth of all the selected bacterial isolates. Growth inhibition of *Pseudomonas aeruginosa* was highest (18.7±1.25 mm zone of inhibition) at 100 µg/ml concentration of silver nanoparticles. Antimicrobial resistance is a well-known characteristic of Pseudomonas aeruginosa. According to the literature, AgNPs effectively prevent Pseudomonas aeruginosa from forming biofilms, however, it only occurs when nanoparticles in liquid culture come into frequent contact with the bacterial cells [48]. Gram-negative bacteria are typically more resilient than Gram-positive bacteria, making them more challenging to deal with because their outer membrane covers the peptidoglycan cell wall [49]. The permeability and respiratory activity of the bacterial cells are unstable when silver nanoparticles are connected to the cell membrane's surface. The capacity of DNA to replicate is often lost when bacteria are exposed to silver ions. In contrast to Gram positive bacteria, Gram negative bacteria's cell wall construction makes it simpler for Ag^{+} to reach the cytoplasmic membrane [50].

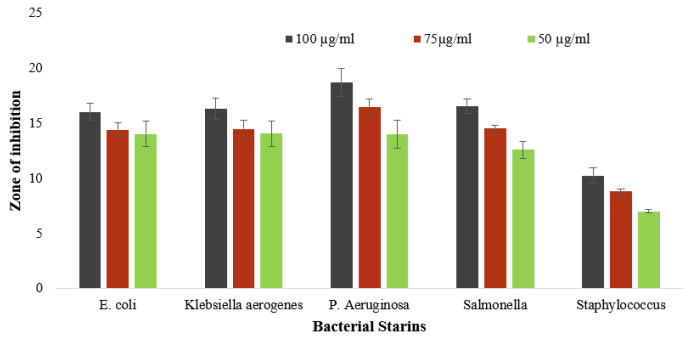

Figure 7: Antibacterial activity of different concentration of mint leaves AgNPs against different gram positive and gram negative bacterial strains.

Table 04: Antibacterial activity of AgNPs by different concentration of mint leaves

Bacteria name	Zone of inhibition of AgNPs against different bacteria at different concentration.		
	100 µg/ml	75µg/ml	50 µg/ml
E. coli	16±0.76	14.4±0.66	14.0±1.15
Klebsiella aerogenes	16.3±0.96	14.5±0.76	14.0±1.15
Pseudomonas aeruginosa	18.7±1.25	16.5±0.74	14.0±1.25
Salmonella	16.5±0.67	14.5±0.23	12.6±0.78
Staphylococcus	10.2±0.68	8.8±0.20	7.0±0.15

CONCLUSION

Aqueous plant extracts are being used to make silver nanoparticles instead of more conventional techniques of synthesis because they are simple to make, inexpensive, and toxic-free. The environmentally friendly technique to prepared silver nanoparticles from mint leaf extract. The precursor and reducing agent are silver nitrate and leaf extract. The synthesized silver nanoparticles were characterized using scanning electron microscopy, UV-visible spectroscopy and XRD analysis. *UV-Vis* microscopy was used to confirm the presence of silver nanoparticles, and a plasmon surface was observed at 440 nm. X-ray diffraction was utilized to verify the crystalline nature of silver nanoparticles. Silver nanoparticles produced by biosynthesis were spherical and ranged in size from 20 to 50 nm. The size of nanoparticles has a major effect on their antibacterial effectiveness and is depending on plant extracts. Smaller silver nanoparticles are more effective against germs because they can pass quickly through cell membranes. The gram-negative bacterial strains Klebsiella aerogenes, Pseudomonas aeruginosa, Salmonella and Escherichai coli were all successfully eradicated by the biosynthesized silver nanoparticles. In summary, these applications of biosynthesized silver nanoparticles may be used as powerful agent for future biomedical research.

AUTHOR CONTRIBUTIONS

Conceptualization, Z.H, M.J, A.S, N.U and T.A; methodology, Z.H, M.J, A.S, N.U and T.A;; software, M.A; validation, A.A.S; formal analysis, T.A.; investigation, Z.H, M.J, A.S, N.U and T.A; resources, M.A and A.A.S.; data curation, T.A.; writing—original draft preparation, T.A and M.J.; writing—review and editing, T.A and M.N; visualization, N.U; supervision, T.A and M.N.; project administration, A.A.S and M.A ; funding acquisition, T.A

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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