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EVALUATION OF ANTIBACTERIAL ACTIVITY OF GREEN-SYNTHESIZED SILVER NANOPARTICLES AGAINST PATHOGENIC BACTERIA

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ABSTRACT

There is an urgent need to find alternative therapeutic agents because pathogenic bacteria are becoming more resistant to antibiotics. The broad-spectrum antimicrobial qualities of silver nanoparticles (AgNPs) have gained interest. Plant extract-mediated reduction was utilized in this study to produce silver nanoparticles, giving a sustainable, economical, and environmentally friendly substitute for conventional chemical processes. To verify their formation, surface chemistry, and morphology, the synthesized AgNPs were examined using scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), and UV-Vis spectroscopy. Using the agar well diffusion method, the antibacterial activity of the green-synthesised AgNPs was assessed against a number of pathogenic bacterial strains, such as Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus, and Escherichia coli. The antimicrobial efficacy has been assessed by measuring the zones of inhibition. Significant antibacterial activity has been demonstrated by the results, and larger inhibition zones were seen for Gram-negative bacteria than for Gram-positive strains, indicating differences in cell wall susceptibility. Furthermore, it was discovered that the activity was dose-dependent, meaning that higher AgNP concentrations had greater antibacterial effects. The findings indicate that green-synthesised silver nanoparticles have powerful antibacterial qualities and show potential as a substitute for traditional antibiotics, particularly when addressing bacterial infections that are resistant to drugs. This study emphasizes the significance of combining nanotechnology and green chemistry for sustainable biomedical solutions and supports the use of biosynthesized nanoparticles in the fabrication of novel antimicrobial agents.

Introduction

Antibiotics have revolutionized the practice of medicine by enabling breakthroughs across the spectrum of clinical medicine, including safer childbirth, surgical procedures, organ transplantation, and myeloablative chemotherapy regimens. However, antimicrobial resistance (AMR) threatens to impede and even reverse some of this progress. In the United States, AMR organisms cause more than 2 million infections and are associated with approximately 23 000 deaths each year [1].In Europe, AMR is associated with approximately 25 000 deaths annually [2].The economic costs of AMR are substantial, estimated at $20 billion in excess medical spending each year in the United States. The full global effect of AMR is more difficult to quantify, as epidemiological data are sparse in many areas of the world. However, data that are available represent considerable concern. In this regard, the recent global emergence of resistance factors emanating from the United States (carbapenem-resistant Klebsiella pneumoniae), India (bacteria with the plasmid-mediated blaNDM-1 gene that confers resistance to carbapenems),[3] and elsewhere (Escherichia coli with the plasmid-mediated mcr-1 gene that confers resistance to colistin, originally described in China) demonstrate the widespread nature of the problem and the importance of improved global surveillance [4].

There is an urgent need to explore innovative alternatives to antibiotics acting differently by preventing infections, reducing the emergence of resistance by targeting different mechanisms of action (MOAs) or increasing the effectiveness of existing antibiotics [5]Use of these alternatives for community-acquired infections would ultimately reduce the dependency on antibiotics [6]. Alternative to antibiotics refers to products such as vaccines, antibodies, pattern recognition receptors (PRRs), probiotics, bacteriophages, peptides, phytochemicals, metals and antimicrobial enzymes7,8. The available literature on alternatives to antibiotics is scattered in terms of different focus areas, different development stages and restricted to either human or animal use [7]. This review is aimed to provide a holistic overview of various promising, potential or under-investigation alternatives to antibiotics with their MOA, current status, challenges associated in commercialization and future scope. Vaccine, probiotics, antimicrobial enzymes, heavy metals and nanoparticles are the alternatives [8]. The bactericidal action of silver nanoparticles along with amoxicillin on E. coli was studied. Silver nanoparticles (0-40 ug/ml) and amoxicillin (0-0.525 mg/ml) showed high antimicrobial effect in Luria Bertani medium. E. coli showed different bactericidal sensitivity to the silver nanoparticles. As compared to the individual treatment, when

amoxicillin and silver nanoparticles were combined, greater bactericidal activity of silver nanoparticles has been observed. Delay in synergistic effect of silver nanoparticles and amoxicillin and decrease in stationary and exponential phases were indicated in dynamic tests on bacterial growth on preincubating E. coli cells with silver nanoparticles antimicrobial effects were observed. Thus, solutions with more silver nanoparticles have showed better antimicrobial effect [9]. In a very interesting study, antibacterial effects of silver nanoparticles synthesized by the sodium borohydride method was evaluated on recombinant E. coli bacteria expressing green florescent protein (GEP) was used as the model system. It was observed that silver nanoparticles above a certain concentration were not only bactericidal but also found to reduce sizes of the treated bacteria compared to untreated ones. However, no direct effect on DNA/protein profile was observed in electrophorestic studies [5].Several antibiotics use silver compounds such as metallic silver, silver nitrate, silver sulfadizine for treatment of burns, wounds and several bacterial infections but has been declined remarkably [10]. Sharma and his co-workers in 2009 also observed the antibacterial activity of silver nanoparticles and their modified form by the surfactant and polymers against various gram positive and negative bacteria [11]. In another study, starch stabilized silver nanoparticles were deveopled from X- ray synthesis and they are found to possess antibacterial activity against E. coli. Their antibacterial property shown to be dependent on the X-ray doses [12]. Similarly, oleic acid stabilized silver nanoparticles were obtained by the simple green chemical synthetic methods, shown to possess high antibacterial activity against gram negative E. coli and gram-positive Staphylococcus aureus bacteria [10].

Silver nanoparticles which are biologically synthesized by Fusarium oxysporum were found to possess antibacterial properties. These nanoparticles were incorporated in materials and cloth, making them sterile and can be used in hospitals where often wounds are contaminated by micro-organisms. Marcato and his co-workers observed antibacterial effects when silver nanoparticles were incorporated in the cotton cloth. However, in silk cloth antibacterial effects was not observed due to less incorporation of silver nanoparticles because of less pore size [13]. The antimicrobial effect of biologically synthesized silver nanoparticles from Fusarium oxysporum was observed when incorporated in cotton fabrics against S. aureus [14]. Even though silver nanoparticles (AgNPs) are well known for their strong antibacterial abilities, the traditional processes used to create them frequently require hazardous chemicals, a lot of energy, and are not environmentally friendly. Green synthesis, which uses plant extracts, has become a viable,

reasonable, and environmentally friendly substitute in recent years. By using phytochemicals derived from plants as stabilizing and reducing agents, these biologically produced nanoparticles reduce the risks to the environment and human health. However, despite their potential, a thorough assessment of green-synthesised silver nanoparticles against clinically significant pathogenic bacteria remains lacking. Furthermore, comparative analysis can be difficult because current studies frequently lack standardization in synthesis processes and antibacterial testing. The current work aims to fill this gap by synthesizing silver nanoparticles using plant extracts in a sustainable way, characterizing the nanoparticles using suitable analytical methods, and assessing their antibacterial activity using the disc diffusion method against a subset of pathogenic bacteria, including Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa. The study also aims to assess the concentration-dependent effects of these nanoparticles on bacterial inhibition and compare their antibacterial efficacy with that of traditional antibiotics. It is expected that this research will aid in the development of sustainable nanomaterials with possible applications in biomedicine.

2 Materials and Methods

Fresh plant leaves, especially those from Ocimum sanctum or Azadirachta indica, were collected and thoroughly rinsed with distilled water. An accepted supplier supplied the analytical grade silver nitrate ($AgNO_3$). Before to use, all glassware was sterilized. The experiment was carried out employing distilled water. A microbiology lab culture collection offered the bacterial strains (Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Bacillus subtilis), which were subsequently maintained on nutrient agar slants.

2.1 Preparation of plant extract

Fresh Neem leaves were carefully sliced and rinsed with distilled water. Boil a known number of leaves in 100 mL of deionized water for 15 minutes in a water bath. The mixture was filtered to yield an aqueous extract of specific amounts. To create silver nanoparticles, a particular quantity of Neem leaf broth was mixed with 0.01 M AgNO3 solution in 250 mL Erlenmeyer flasks. Incubate the flasks in a rotary shaker at 120 rpm for the essential time at 28°C.

2.2 Preparation of plant extract

For the purpose to produce AgNPs, 90 mL of a 1 mM silver nitrate solution was stirred constantly at room temperature while 10 mL of the plant extract was added dropwise. The formation of silver nanoparticles was indicated by a change in color from pale yellow to dark

brown. To avoid photoreduction, the mixture was incubated in the dark for the entire day. Centrifugation at 10,000 rpm for 15 minutes was used to collect the nanoparticles, which were subsequently rinsed three times with distilled water and dried at 60°C.

3 Results and Discussion

3.1 Antibacterial activity of silver nanoparticles

The agar well diffusion method was used to evaluate the green-synthesised silver nanoparticles' antibacterial efficacy. In order to ensure uniformity in microbial density, bacterial cultures adjusted to the 0.5 McFarland standard were evenly inoculated onto Mueller-Hinton agar plates. 50 µL, 100 µL, and 150 µL, 200 µL of the silver nanoparticle suspension were added to wells that were carefully punched into the agar and had a diameter of 6 mm. After that, the plates were incubated for 24 hours at 37°C to promote bacterial growth and interaction with the nanoparticles. The antibacterial efficacy has been evaluated by measuring the zones of inhibition surrounding each well in millimeters after incubation. A 1 mM silver nitrate solution was employed as the positive control, and a plant extract devoid of silver nitrate was employed as the negative control for comparison. These controls allowed it to be easier to differentiate the antimicrobial effects of the produced nanoparticles from those of the plant extract and silver ions by themselves.

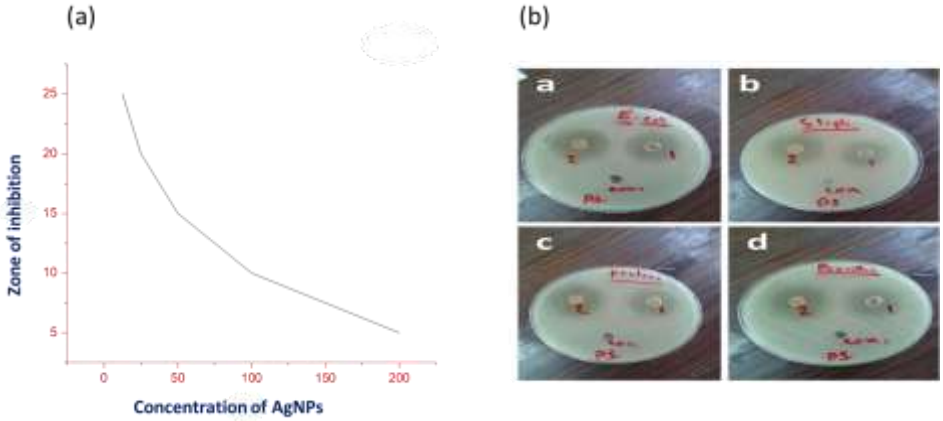

Figure 3.1: (a) line graph show the zone of inhibition (b)Antibacterial activity of bioemulsifier produced by P. aeruginosa against different bacteria species (a, E. coli; b, S. aureus; c, P. mirabilis; d, B. subtilis). Con, ontrol; 1, 100,000 µg/ml; 2, 200,000 µg/ml.

3.2 Disc diffusion method

In this study, the antibacterial properties of AgNPs were studied by cultivating Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus, and Escherichia coli pneumoniae colonies on Muller-Hinton agar plates containing AgNPs. Previous research findings indicate AgNPs' antimicrobial potential. Figure 3 depicts the zone of inhibition seen in the Muller-Hinton agar plate surrounding both the discs (AgNO3 and AgNPs). In both cases (AgNO3 and AgNPs), the inhibition zone for gm+ veB. subtilis and gm - K. pneumonia was identical. However, when comparing AgNO3 and AgNPs, AgNPs had a larger zone of bacterial inhibition than AgNO3. There was no zone of inhibition observed with the control Neem extract.

Figure 3.2: Showing Zone of Inhibition Around the Discs: Right (*K. pneumonia*), Left (*B. **subtilis***)

The zones of inhibition surrounding various discs in Figure 6 visually represent the outcomes of an antibacterial assay against Klebsiella pneumoniae (right plate) and Bacillus subtilis (left plate). As an essential negative control, the disc marked "-" on both plates displays no zone of inhibition, demonstrating that the disc material or solvent by itself does not prevent bacterial growth. A small degree of antibacterial activity linked to the material it contains—which, in the context of the earlier FTIR analysis, might be the Neem plant extract itself—is suggested by the

disc marked "Ex" showing a small but apparent zone of inhibition against both bacterial species. The "Ag" disc exhibits a distinct and significant zone of inhibition against both B. subtilis and K. pneumoniae, suggesting strong antibacterial activity that is probably caused by silver ions or a silver salt. Furthermore, the disc with the label "Nano" shows the biggest and clearest zones of inhibition on both plates, especially when it comes to K. pneumoniae. This demonstrates that, out of all the substances tested, the green-synthesised silver nanoparticles have the strongest antibacterial activity. According to the FTIR analysis, the nanoparticles' more effective effectiveness over the silver salt ("Ag") may be the result of their special qualities, which include a larger surface area for interaction with bacterial cells and possibly a synergistic effect with the biomolecules from the Neem plant extract that stabilize and cap them.

3.3 SEM Analysis

The surface morphology and size distribution of the green-synthesised silver nanoparticles (AgNPs) were examined using SEM analysis. According to the SEM micrographs, the AgNPs were mostly spherical in shape, had smooth surfaces, and ranged in size from about 20 to 50 nm. Given the high surface energy of nanoparticles, some degree of particle aggregation was expected; however, the bio-organic compounds from the plant extract likely served as effective stabilizers, decreasing excessive agglomeration.

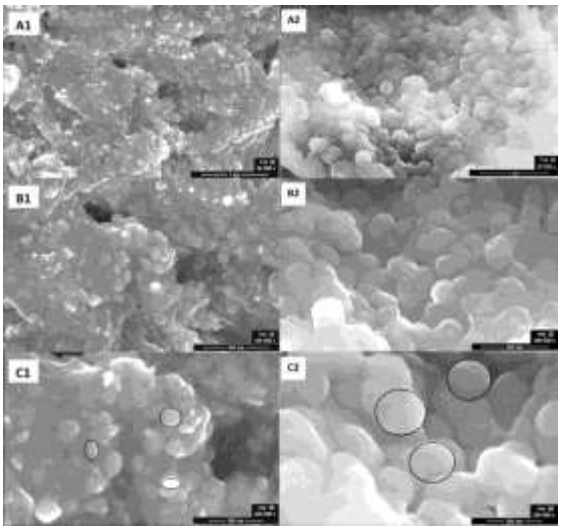

Figure 3.3: SEM of silver nanoparticles using Neem Extract

The morphology of green-synthesised silver nanoparticles (AgNPs) that were assessed for their antibacterial activity can be better understood from these Scanning Electron Microscopy

(SEM) pictures. The successful formation of nanoparticles is confirmed by the micrographs, which show a particulate nature at different magnifications. Higher magnification views (A2, B2, C2) show that the AgNPs are primarily spherical or nearly spherical in shape, a morphology frequently linked to potent antibacterial action because of the high nanoscale surface area to volume ratio. The scale bars in the images show that the produced particles are in the nanometer range, which is essential for their ability to interact with bacterial cell membranes. Although individual nanoparticles can be seen in the images, there is also evidence of agglomeration, or the clustering of nanoparticles, which may affect the particles' overall dispersion and possibly their antibacterial effectiveness. While individual nanoparticles at higher magnification show a relatively smooth surface, the surface texture of the clusters of nanoparticles appears somewhat irregular at lower magnification views. The size, shape, and aggregation state of the green-synthesised AgNPs are important factors in determining and correlating their antibacterial activity against pathogenic bacteria, and these SEM images help to characterise these physical attributes.

3.4 UV-Visible Spectroscopy

For different aqueous $AgNO_3$ concentrations, comparative studies were carried out to examine the effect of different amounts of leaf biomass on bioreduction rate of AgNPs. The quantity of leaf extract showed a significant role in size dispersion of AgNPs. The plant extract showed peak at 280 nm but no peak was observed between 400 nm and 500 nm. The reduction of the silver ions into AgNPs in the presence of plant extract can be observed through change in color. The leaf extract solution color changed from yellowish green to brown and became darkish brown gradually with time on addition of Ag^+ ions due to the surface plasmon resonance (SPR). Ag^+ ions reduction occurred rapidly in the presence of neem leaf extract and AgNP synthesis was completed in 1 h. The change in the absorbance was noted down every 10 minutes interval but there was no change in absorbance after 1 h indicating no further formation of nanoparticles as observed in Figure 1. The maximum absorption peak at 420 nm was observed in the UV-Vis spectrophotometer analysis indicating the formation of AgNPs. This broad SPR peak has been well studied for AgNPs with the size ranged from 10 to 100 nm [18].

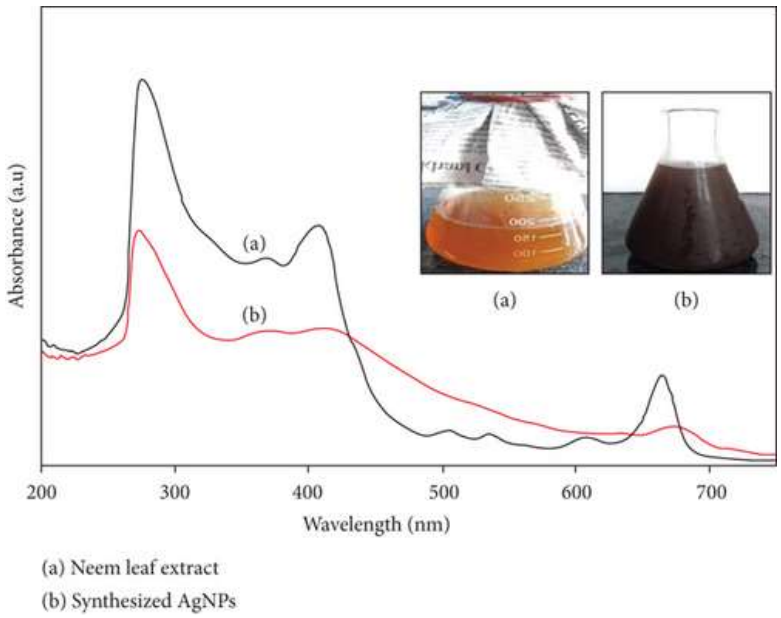

Figure 3.4: UV-Vis spectra of (a) neem leaf extract and (b) synthesized AgNPs

A vital first step in assessing the antibacterial activity of silver nanoparticles (AgNPs) is the green synthesis of the particles using neem leaf extract, which is confirmed by UV-Vis spectroscopy data and visual evidence in this figure. Numerous organic compounds that function as capping and reducing agents are visible in the neem leaf extract's UV-Vis spectrum (black line). The spectrum of the produced AgNPs (red line) shows a distinctive Surface Plasmon Resonance (SPR) band centered around 400–450 nm after the addition of the silver salt and the reaction is finished. This is a clear optical signature of silver nanoparticles. The formation of these nanoparticles is further supported by the visual shift from the translucent light brown of the neem leaf extract to the dark brown solution of the synthesized AgNPs, a color resulting from their distinct SPR properties. The size and size distribution of the AgNPs, which are known to affect their antibacterial efficacy, can be inferred from the position and broadness of the SPR peak. Thus, this first characterization using UV-Vis spectroscopy confirms that AgNPs were successfully synthesized using an environmentally friendly method, opening the door for a more thorough morphological examination and evaluation of their antibacterial potential against harmful bacteria.

3.5 FTIR Analysis

The functional groups involved in the reduction and stabilization process are identified using Fourier Transform Infrared (FTIR) spectroscopy in the environmentally friendly synthesis of silver nanoparticles. The presence of polyphenols is indicated by the broad absorption band in the 3200–3400 cm^{-1} range of the FTIR spectrum of silver nanoparticles, which corresponds to O–

H stretching vibrations of hydroxyl groups, which are frequently found in alcohols or phenolic compounds. C=C or C=O stretching vibrations are responsible for peaks seen between 1600 and 1650 cm^{-1}, which indicate the presence of aromatic or carbonyl groups. The bending vibrations of $-CH_2$ or $-CH_3$ groups are linked to absorption bands around 1400–1450 cm^{-1}. Moreover, peaks that show up between 1000 and 1100 cm^{-1} indicate C–O–C or C–N stretching, which usually comes from proteins, ethers, or esters. The successful synthesis of silver nanoparticles is confirmed by the presence of bands in the 600–800 cm^{-1} range, which are indicative of Ag–O or Ag–N bond formation. These functional groups collectively indicate that different secondary metabolites, including proteins, alkaloids, terpenoids, and flavonoids, are involved in the reduction of silver ions and act as capping agents to stabilize the nanoparticles.

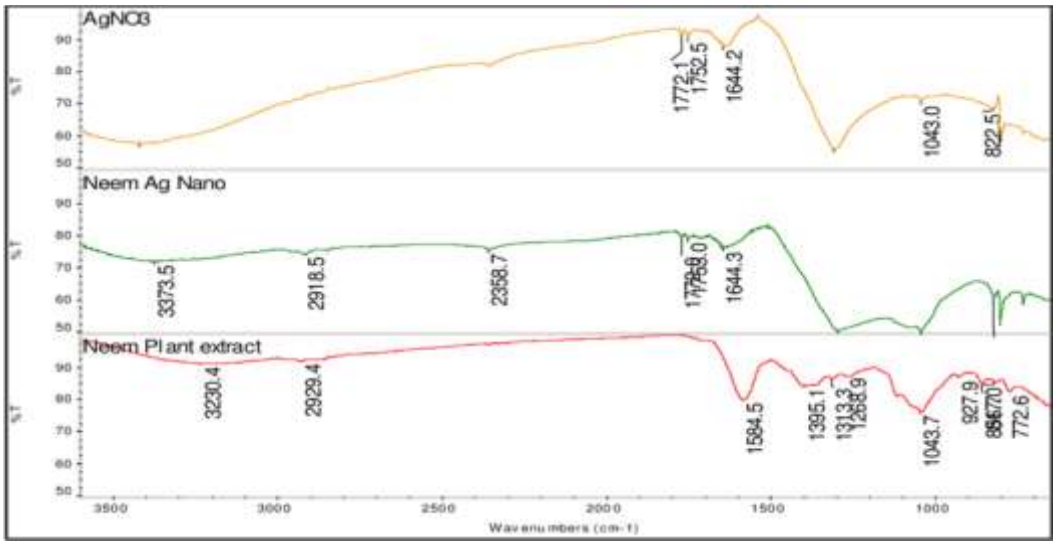

Figure 3.5: FTIR of AgNO3, Neem AgNPs and Neem plant extract

The presented FTIR spectra provide important information about the environmentally friendly synthesis of silver nanoparticles (AgNPs) using plant extract from neem. Prominent peaks in the spectrum of the Neem plant extract suggest a complex mixture of biomolecules that correspond to O-H stretching (alcohols, phenols, carboxylic acids), C-H stretching (alkanes, fatty acids), C=O stretching (carbonyl compounds), C-O stretching (alcohols, polysaccharides), and C-N stretching (amines, amides). These functional groups draw attention to the existence of substances that can stabilize the resultant nanoparticles and reduce silver ions. On the other hand, the silver nitrate spectrum, which acts as a baseline for the silver precursor, mostly indicates attributes connected to the nitrate ion. The spectrum of Neem AgNPs shows that biomolecules from the plant extract successfully cap silver nanoparticles. While there are some wavenumber and

intensity shifts, it still has the distinctive peaks from the Neem extract, which show interactions between the functional groups and the surface of the silver nanoparticle. O-H, C-H, C=O, and C-O stretching vibrations indicate that substances from the Neem extract, such as polysaccharides, alcohols, carboxylic acids, and perhaps proteins, are attached to the AgNPs, enhancing their stability and avoiding agglomeration. According to the context of the evaluation given, these surface-bound biomolecules—which may have intrinsic antimicrobial qualities—could work in combination to enhance the antibacterial activity of the green-synthesised silver nanoparticles against harmful bacteria.

Conclusion:

With the help of plant extracts, the current study effectively illustrated how to synthesize silver nanoparticles in an environmentally responsible manner. This process uses naturally occurring phytochemicals as stabilizing and reducing agents, offering a sustainable substitute for traditional chemical synthesis. The produced nanoparticles' suitability for biological applications was ensured by characterizing them to confirm their formation and evaluate their characteristics. Using the disc diffusion method, the antibacterial activity of these green-synthesised silver nanoparticles was assessed against common pathogenic bacteria, including Pseudomonas aeruginosa, Staphylococcus aureus, and Escherichia coli. With visible zones of inhibition surrounding the discs, the results clearly demonstrated the nanoparticles' strong antibacterial activity and verified their efficacy. There was a concentration-dependent relationship found, with larger zones of inhibition suggesting greater antibacterial potency at higher AgNP concentrations. In some cases, the green-synthesised AgNPs showed similar or even better efficacy than conventional antibiotics, especially when it was applied to gram-negative bacteria. In light of the developing antibiotic resistance, this study emphasizes the potential of green-synthesised silver nanoparticles as potent antimicrobial agents. According to the results, these nanoparticles may be improved for use in coatings, wound dressings, and other biomedical goods. This method offers an inexpensive and environmentally friendly way to produce nanoparticles, and it is also consistent with the ideas of green chemistry and sustainable development. To entirely investigate the clinical potential of these biologically produced nanoparticles, more research involving in vivo models, toxicity assessments, and mechanism of action is recommended.

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