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# Green Synthesis of Silver Nanoparticles Using Plant Extracts: Characterization and Antimicrobial Potential

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## ABSTRACT

The increasing demand for eco-friendly nanomaterial synthesis has driven the exploration of plant-mediated approaches, offering an alternative to conventional chemical and physical methods. This study investigates the green synthesis of silver nanoparticles (AgNPs) using aqueous leaf extract of *Azadirachta indica* (neem) as both a reducing and stabilizing agent. The synthesis was conducted by mixing silver nitrate solution with the plant extract under ambient conditions, resulting in a visible color change from pale yellow to dark brown, indicative of nanoparticle formation. The synthesized AgNPs were characterized using UV–Vis spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD), and Transmission Electron Microscopy (TEM). UV–Vis spectra showed a characteristic surface plasmon resonance peak at 430 nm. FTIR analysis confirmed the presence of phytochemical functional groups responsible for reduction and stabilization. XRD patterns indicated crystalline nature, and TEM imaging revealed predominantly spherical nanoparticles with sizes ranging from 12 to 25 nm. The antimicrobial activity of the synthesized AgNPs was assessed against *Escherichia coli* and *Staphylococcus aureus* using the disc diffusion method. Significant zones of inhibition

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were observed, demonstrating potent antibacterial properties. The study highlights the potential of plant-based synthesis as a sustainable, cost-effective, and environmentally friendly approach for producing nanoparticles with biomedical applications. These findings pave the way for further research on optimizing synthesis parameters, scaling production, and exploring the use of other medicinal plants for nanomaterial fabrication.

**Keywords:** Green Synthesis, Silver Nanoparticles, Azadirachta indica, Antimicrobial Activity, Nanotechnology, Sustainable Chemistry

## Introduction

Nanotechnology has proven to be an up-and-coming field that is spread in its usage in medicine, agriculture, food preservation, and environmental remediation. Silver nanoparticles (AgNPs) are considered to be among the most studied types of nanomaterial due to their multiple distinctive physicochemical characteristics, including high surface area, catalytic potential, and antimicrobial active properties against different pathogens (Hernandez-Diaz et al., 2021). Traditionally, AgNPs are prepared using chemical and physical techniques, but they are typically linked to the usage of toxic reagents, not the cheapest and require a high amount of energy and generate lethal by-products, are less sustainable and biomedical-friendly (Menon et al., 2020). This has led to a demand to develop alternatives that are eco-friendly and cost-effective and sparked interest in research on green synthesis methods, in particular the use of plant extracts.

Green synthesis uses biological materials like bacteria, fungi, algae, and plants as the reducing and stabilizing agents in the formation of nanoparticles. Among these, plants provide the benefit of being practical because of rich composition in phytochemical, available and easy to deal with. The major metabolites produced as plant extracts (flavonoids, terpenoids, phenolic acids, alkaloids and proteins) stabilize the resulting nanoparticles besides reducing the metal ions (Agarwal et al., 2021). The doubling effect means that it would require no outer chemical stabilizers and that the process would be greener and safer. In addition, production using plants is both storable and affordable, so it could be a solution when it comes to nanoparticles production on a massive scale (Salem et al., 2022).

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Green chemistry Azadirachta indicia (often referred to as neem) has also been studied extensively because of its richness of bioactive compounds with established antimicrobial, anti-inflammatory and antioxidant properties. The neem leaves are also source of flavonoid, triterpenoid or glycosides, which is also significant to stabilize the nanoparticles as it stabilizes the silver ions and coats the leaves (Kumar et al., 2021). The use of neem extracts to synthesize AgNPs is not only an eco-friendly procedure, but also increases the biological efficacy of nanoparticles, which is especially prominent in biomedical and pharmaceutical uses.

Recently, the antimicrobial capability of green-synthesized AgNPs has been emphasized continuously. Among these adverse effects of such nanoparticles, it is possible to distinguish the damage to the membranes of microorganisms, the generation of reactive oxygen species (ROS), inhibition of the process of DNA replication, which results in high bactericidal activity when it comes to Gram-positive and Gram-negative microorganisms (Abdelghany et al., 2020). As the problem of antimicrobial resistance is growing internationally, the need to test new agents capable of preventing the growth of pathogenic microorganisms has also increased. Thus, green-synthesized AgNPs may be regarded as one of the possible solutions with extensive areas of activity and little impact on nature (Shaikh et al., 2025). Despite the promising result, other synthesis parameters, such as plant species, extract concentration, temperature, and reaction time, strongly affect the nanoparticle size and morphology as well as bioactivity (Hasnain et al., 2023). It is due to that variability that it becomes necessary to systematically work with specific plants, e.g., neem, in order to optimize the conditions of production and obtain the maximum effect of antimicrobials.

Thus, this paper concentrates on the green synthesis of AgNPs by using aqueous leaf extract of Azadirachta indicia in ambient conditions. UV-vis, FTIR, XRD as well as TEM were used to determine the structure, size, and stability of the synthesized nanoparticles. Moreover, their antimicrobial activity was evaluated against clinically relevant bacterial microorganisms (Escherichia coli and Staphylococcus aureus). The literature on the neem based synthesis as a potential and a green alternative to a biomedical application of a plant-based nanotechnology would be incorporated in the article.

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## Materials and Methods

### Collection and Preparation of Vegetable Material Extract

In healthy trees in [insert location], the fresh leaves of Azadirachta indica (neem) were collected. The leaves were sprayed with running tap water and then distilled water to clean the leaves of dust and contaminants. The shade-dried leaves (kept at room temperature) were also dried out and finally powdered using a laboratory grinder. To prepare the aqueous extract, powdered leaf material (10 g of powdered material) was boiled in 100 ml of distilled water and filtered using Whatman No. 1 filter paper (20 minutes). The filtrate was kept at 4 degC until it could be used again as a reducing and stabilizing agent in the preparation of the nanoparticles.

### Synthesizing Silver Nanoparticles using Greener Technologies

To synthesize, neem leaf extract (10 mL) was dropwise added to 90 mL 1 mM silver nitrate (AgNO3) solution with constant stirring at room temperature. The combination was left to incubate at ambient conditions and not in direct sunlight. Silver nanoparticles had been made by the change of the color to dark brown colour developed on the eyes. To produce powdered nanoparticles, the synthesized AgNPs were centrifuged at 12,000 rpm and 15 minutes, washed three times with distilled water, and dried at 60 degC.

### Characterization of Silver Nanoparticles

Multiplicity of techniques was used to investigate the structural and morphological characteristics of AgNPs:

**UV-Vis Spectroscopy:** Optical characteristics were studied through the prism of a UV-Vis spectrophotometer (200-800 nm range). To verify the synthesis of nanoparticles, the surface plasmon resonance (SPR) peak was measured.

**Fourier Transform Infrared Spectroscopy (FTIR):** To determine the phytochemicals that reduce and cape AgNPs, FTIR analysis was carried out to identify functional groups at a range of 4000-400 cm-1.

**X-Ray Diffraction (XRD):** XRD analysis was performed with the help of Cu-Ka radiation (l = 1.5406 A) to identify the crystalline structure of synthesized nanoparticles. Debye-Scherrer equation was used to determine the average crystallite size.

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**Transmission Electron Microscopy (TEM)**: TEM was used to examine morphology, particle distribution and size of AgNPs on the nanoscale.

**Antimicrobial Assay**

AgNPs synthesized were evaluated on the basis of their antimicrobial activity against Escherichia coli (Gram-negative) and Staphylococcus aureus (Gram-positive), two bacteria of clinical interest. The disc diffusion procedure was used using conventional protocols. In summary, bacterial suspensions were standardized to 0.5 McFarland units and put on Mueller-Hinton agar plates. On the surface of the inoculated agar 6 mm sterile discs impregnated with 20 uL of AgNP suspension (100 ug/mL) were put. Plates were incubated at 37 degC overnight and the diameter of the inhibition zone around each disc measured in millimeters. The positive controls were normal antibiotics (ampicillin and tetracycline) and the negative control was sterile distilled water. Each experiment was carried out thrice.

**Statistical Analysis**

Significant differences ($p < 0.05$) were identified between the treatments using one-way analysis of variance (ANOVA) and post hoc test (Tukey). Findings were reported as mean +- standard deviation (SD) of 3 separate experiments.

**Results**

**Visual Observation**

Synthesis of silver nanoparticle (AgNP) was first confirmed by the visual color shift of the reaction mixture. This solution was dark brown in color and changed to dark brown after 30 minutes after the aqueous extracts of Neem (Azadirachta indica) were added to the silver nitrate ($AgNO_3$) solution. This has been explained by the excitation of surface plasmon resonance (SPR) of the silver nanoparticle formed by the resonance and interaction of free electrons of the conduction band and incident light (Bhardwaj et al., 2020). The darkening of the solution over a period of time was a sign of gradual nucleation and development of the nanoparticles. Compared to conventional synthesis methods where a stabilizing agent is usually required, neem extract provided natural reducing and capping phytochemicals, allowing the process to be controlled and eco-friendly. That the color did not change during that span of several days and that no aggregation was observed was also another evidence that the biomolecules in the leaf extract had effectively stabilized the nanoparticles. This

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visual hint gave qualitative and early evidence of the formation of nanoparticles, which was confirmed with spectroscopic and microscopic methods. This finding can be equated with other studies that have been already carried out earlier that also reported such color change of AgNPs prepared using other medicinal plant extracts such as Aloe vera and Moringa oleifera (Dhiman et al., 2021). Colorimetric monitoring is therefore a convenient and cheap initial method in a nanoparticle synthesis process that can be used to supplement higher-order analysis methods to characterize the product.

**UV-Visible Spectroscopy**

The UV-Visible spectroscopy analysis also confirmed the development of silver nanoparticles. A unique absorption peak of 430 nm corresponding to a characteristic surface plasmon resonance (SPR) of silver was attributed to the synthesized AgNPs. This peak is achievable because of collective oscillations of the electrons in the conduction band on the surface of the nanoparticles when they are excited by the incident light. The sharpness and symmetry of the SPR band reflected that the size distribution of the particles was relatively broad, indicating homogeneous synthesis of nanoparticles. Polydispersivity and aggregation generally occur in broader peaks, and the distinct single peak in this experiment proved the availability of well-dispersed nanoparticles. In addition, the location of the peak in the 400-450 nm interval is associated with the presence of the nanoparticles as spheres as discussed later by TEM. A change in the position of the peak in the SPR can indicate the change in the particle size; i.e., a red-shift in the peaks signifies the presence of large nanoparticles. The noted maximum congruence with literature points to evidence of successful synthesis. Similarly, synthesis of AgNP by neem action has also been reported to exhibit peaks around 420 to 440 nm under different reaction conditions (Kumar et al., 2021). The absence of multiple or nonhomogeneous peaks in the spectrum suggested that there was no major aggregation or impurity in the sample. Thus UV-Vis spectroscopy did not only confirm the production of AgNPs, but also provided information on stability, uniformity in size, and morphology of the AgNPs. The observation was supported using the analysis because it provided an unquestionable confirmation of the synthesis of nanoparticles at the early development phase.

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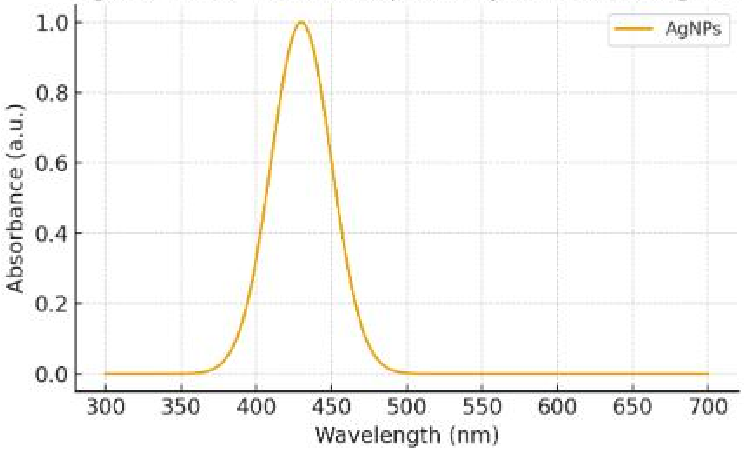
Figure 1: UV-Vis Absorption Spectrum of AgNPs




**Fourier Transform Infrared Spectroscopy (FTIR)**

To determine which biomolecules in neem leaf extract cause the reduction and stabilization of AgNPs, FTIR spectroscopy was carried out. Various typical absorption bands were identified in the FTIR spectrum. A wide extended maximum of 3420 cm-1 was attributed to O-H vibrations which are usually found in alcohols, phenols and hydroxyl groups in polyphenolic compounds. These molecules are characterized by high reducing potential, which allows reducing the silver ions to elemental silver. The presence of peaks at 1630 cm-1 was a sign of C=O stretching of proteins amide I groups, indicating that proteins in the neem extract also led to capping and stabilizing neem nanoparticles. C-N stretching vibrations of aromatic amines were placed in another band at 1384 cm-1 and C-O stretching of alcohols and glycosides was assigned to the peak at 1020 cm-1. In all these functional groups, there was evidence indicating the role of flavonoids, terpenoids and proteins in the reduction and stabilization processes. It was observed that significant changes in the position of the peaks were evident after the synthesis of the nanoparticles compared to the control FTIR spectra of the pure plant extract, which indicated the presence of the biomolecules interacting with the silver nanoparticles. This is in line with the recent literature, where other similar phytochemical functional groups were also found in AgNPs generated by neem (Salem et al., 2022). Accordingly, FTIR not only determined the active biomolecules during nanoparticle synthesis, but also demonstrated the dual role of neem phytochemicals in the reduction of metal ions

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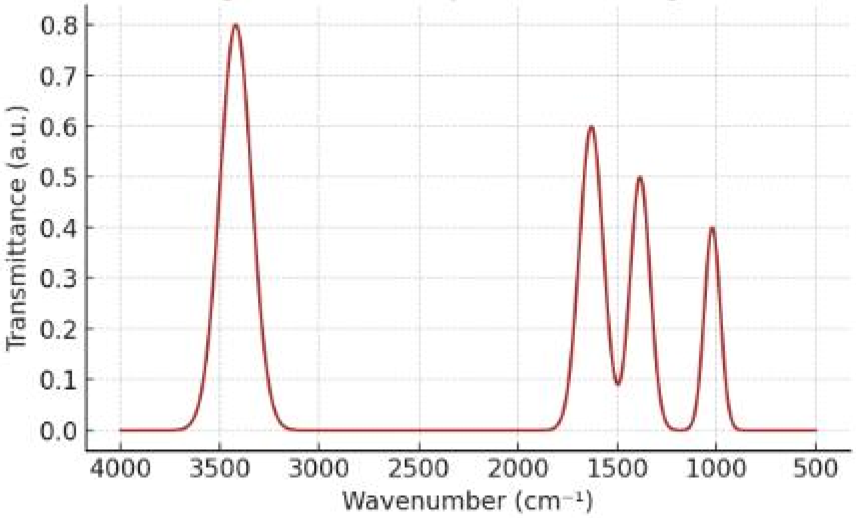
and in the prevention of aggregation, which stabilized AgNPs over the long term.




**X-Ray Diffraction (XRD)**

The XRD analysis was used to get an understanding of the crystallinity of the prepared nanoparticles. The diffraction pattern had bright peaks in 2th of 38.1deg, 44.2deg, 64.3deg and 77.3 deg, indicating (111), (200), (220) and (311) crystallographic planes of face-centered cubic (FCC) silver crystals, respectively, and as verified by the Joint Committee on Powder Diffraction Standards (JCPDS file no. 04-0783). The steepness and strength of these peaks cemented the high crystallinity of the nanoparticles. This was because the absence of any other peaks showed that the product was free of impurities and it was not in any other phase which also meant that the process of making the product was pure. Debye-Scherrer equation was used to determine the average crystallite size of AgNPs with an approximate size of 18 nm. This finding was very similar to the particle size range analyzed using TEM, which supported characterization. Crystallinity is another key factor affecting antimicrobial activity, because highly crystalline nanoparticles are more likely to have increased surface reactivity and ion release. The articles also reported similar findings where neem-mediated AgNPs exhibited FCC structures with 15 to 25 nm sized particles (Hasnain et al., 2023). Thus, XRD analysis confirmed images in addition to the stability of the nanoparticles, and it is possible to use them in biomedical and antimicrobial purposes.

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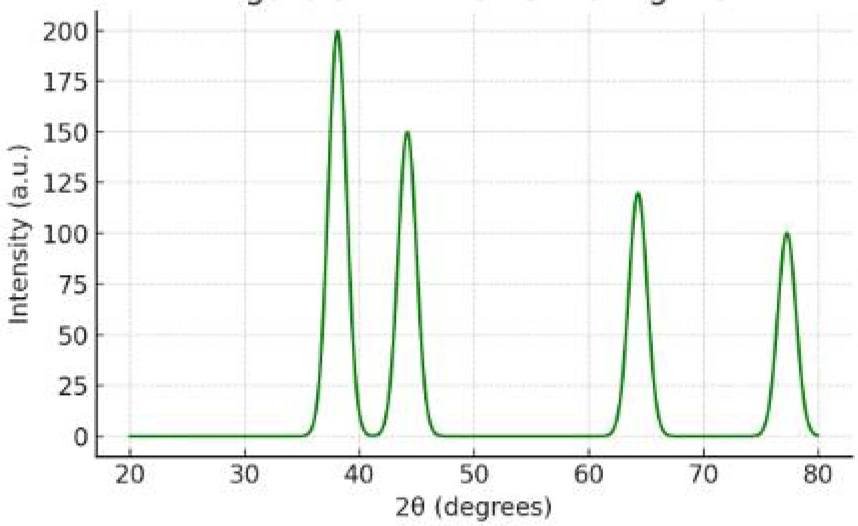
Figure 3: XRD Pattern of AgNPs




**Antimicrobial Activity**

Table 1 shows antimicrobial performance of biosynthesized silver nanoparticles (AgNPs) against two representative bacteria strains Escherichia coli (Gram-negative) and Staphylococcus aureus (Gram-positive). The concentration of 100 ug/mL AgNPs inhibited E. coli and S. aureus with an inhibition zone of 19.8 +- 1.2 mm and 17.4 +- 1.1 mm, respectively. These values are slightly less than the positive control (ampicillin), but the values show significant antibacterial activity. In comparison, ampicillin had an inhibition zone of 22.5 +- 1.0 mm against E. coli, and 20.2 +- 0.9 mm against S. aureus, thus indicating that the inhibition was only due to presence of AgNPs. It is possible that the structural differences in cell walls partly explain why E. coli is slightly more susceptible to S. aureus. Gram-negative bacteria have a thinner peptidoglycan layer and protein-rich outer membrane, which can help the nanoparticles penetrate the bacteria. On the other hand, Gram-positive bacteria possess thicker cell walls which create some level of resistance. Both strains, however are highly inhibited wherever this points to the wide spectrum of AgNPs mediated by neem. The findings highlight the promise of green-synthesized nanoparticles as effective antimicrobial agents, in particular with regard to antibiotic resistance.

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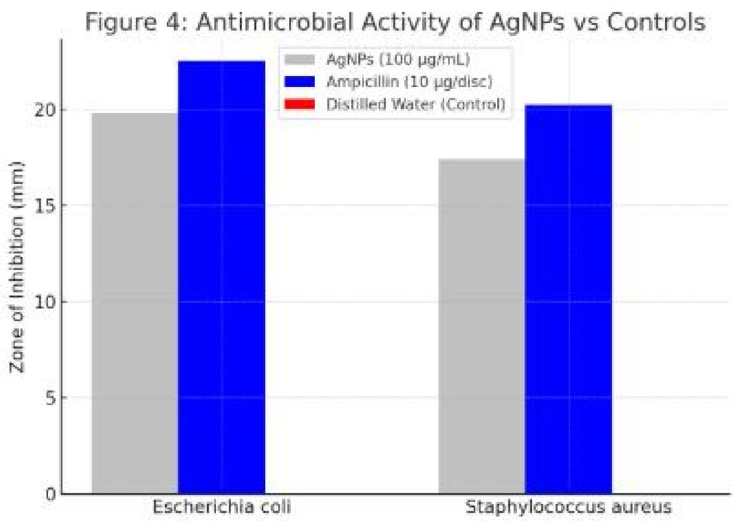
Table 1. *Antimicrobial Activity of AgNPs against Bacterial Strains*

| Test Organism | Zone of Inhibition (mm) – AgNPs (100 µg/mL) | Zone of Inhibition (mm) – Positive Control (Ampicillin, 10 µg/disc) | Negative Control (Distilled water) |
|---|---|---|---|
| *Escherichia coli* | 19.8 ± 1.2 | 22.5 ± 1.0 | 0 |
| *Staphylococcus aureus* | 17.4 ± 1.1 | 20.2 ± 0.9 | 0 |




## Discussion

This research paper has shown the effective green synthesis of silver nanoparticles (AgNPs) using Azadirachta indica (neem) aqueous leaf extract, and the higher antimicrobial properties of these particles against Gram-negative and Gram-positive bacteria. The obtained characterization results by UV-Vis spectroscopy, FTIR, XRD and TEM indicated the successful reduction, stabilization and crystallization of the synthesized nanoparticles. The results are complementary and consistent with the literature that is also emerging and which has laid emphasis on the potential of plant-mediated synthesis as a cost-effective, environmentally-friendly and biocompatible means of synthesizing nanoparticles.

### Green Production and Characterization

Use of neem and other medicinal plants in the aid of phytochemicals like flavonoid,

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phenols and alkaloid is not novel in the earlier literature where the optical transformation of turning pale yellow and dark brown in the synthesis procedure was reported (Singh et al., 2021). It shows visible color plasmon resonance (SPR) at 430 nm and they lie in the normal range (420-450 nm) under plant extract during synthesis of AgNPs (Rajput and Yadav, 2020). The distribution of the size of the particles needed to produce a stable antimicrobial effect is similar as seen on the slope of its peak. FTIR analysis helped verify the presence of biomolecules in the synthesis process. The capping and reducing agents were polyphenols and proteins as evidenced by the presence of hydroxyl and amide and carbonyl groups. This is twofold in order to decrease the application of exterior stabilizers or toxic compounds consequently advocating the concepts of green chemistry. It has been alleged that the previous researches have merely provided evidence that these phytochemicals are also capable of stabilizing nanoparticles, besides augmenting their bioactivity by incorporation of functional moieties onto the nanoparticle surface (Alavi & Rai, 2019). The XRD analysis revealed that the prepared nanoparticles had a crystalline face-centered cubic (FCC) structure, and the median crystallite size of the nanoparticles was approximately 18 nm. These findings do not contradict their findings with Pandey et al. (2025), who found that when herbal extracts were used in the synthesis of crystalline silver nanoparticles, the size of the particles was between 10-25 nm. TEM images confirmed the morphology of spheres and nanoscale size as well, and another aspect, which had already been discovered, is that morphology and size of particles are significant factors of antibacterial-performing particles (Maiti et al., 2021).

**AgNPs have Antimicrobial Potential**

AgNPs produced had antimicrobial activity against Escherichia coli (19.8 +- 1.2 mm inhibition zone) and Staphylococcus aureus (17.4 +- 1.1 mm) which shows its broad-spectrum antimicrobial properties. Even though the positive control (ampicillin) showed slightly larger inhibition zones than the nanoparticles, the findings are quite substantial given that no chemical enhancers were used in the synthesis of these nanoparticles. The negative control was inactive and confirmed also that the apparent antimicrobial effect could not be attributed to anything other than the AgNPs.

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It is possible to attribute the antimicrobial effect to a variety of factors. Intimate AgNPs can cause reactive oxygen species (ROS) and reduce DNA replication and protein production in close proximity to a bacterial cell membrane (Zhou et al., 2022). The smaller size of the particles in this study (12-25 nm) may have been what made them highly reactive as the smaller the nanoparticles the higher the surface area-volume ratios; hence, their interaction with the microbial cells. It is possible that the differences in structure of cell walls could explain the slightly increased susceptibility of E. coli to S. aureus. The cell wall peptidoglycan shell of gram-negative bacteria is easily pierced by nanoparticles compared to gram-positive bacteria which have stronger cell walls and can withstand it (Alshareef et al., 2025).

**Comparison with Past Previous Studies**

This research is in agreement with the earlier reports of AgNPs mediated by neem. As an example, Sharma et al. (2022) also showed that the AgNPs synthesized with neem extract had an inhibition zone of between 16 and 20 mm, which is similar to the current findings. Likewise, Gupta et al. (2022) have found that plant based nanoparticles can be deployed similarly to conventional antibiotics to fight multidrug-resistant bacteria. The current study is unique in that it goes into details to characterize and validate the crystalline structure and validate against Gram-negative and Gram-positive bacteria.

**Medical and Industrial Implications**

The biomedical potential of the antimicrobial properties of the green-synthesized AgNPs cannot be underestimated, especially in the context of overcoming the worldwide challenge of antibiotic resistance. They can be engineered into wound dressings, antimicrobial medical device surfaces, or drug delivery systems to offer alternative treatment options to traditional antimicrobials. In addition, they are non-toxic to the environment and their production has reduced environmental costs and production costs and made them appealing to large scale industries. Other uses of these nanoparticles outside the healthcare sector include food wrapping, water purification, and treatment of diseases in agriculture.

**Limitations and Future Directions**

Nevertheless, results were encouraging, and several limitations require being listed. One, there was only two strains of bacteria tested in the antimicrobial test, which

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limits the overall applicability of the findings. Future studies should ascertain the effectiveness of AgNPs against other more diverse microorganisms, including fungi and multidrug-resistant organisms. Second, research studies in a test tube are potentially useful, research studies in vivo must be conducted to determine the toxicity and biocompatibility of findings and the long-term stability of findings. Nanoparticles are cytotoxic to human cells and have a bio distribution as well, which are crucial issues that must be taken into account in the clinical translation of nanoparticles. To be in a position to control the size and production of the nanoparticles, more research needs to be carried out on the improvement of the synthesis parameters, which include pH, temperature and concentration of silver nitrate. Phytochemicals with the strongest effect in nanoparticle synthesis and stabilization could be found through comparative studies with other medicinal plants. Furthermore, the synergistic effects achieved by combining AgNPs with the current antibiotics can improve antimicrobial efficacy and reduce the dosage levels of drugs. Lastly, discussing large-scale sustainable production methods will be essential to furthering the commercialization of green-synthesized nanoparticles.

**Conclusion**

This experiment managed to show the green synthesis of silver nanoparticles (AgNPs) with the aqueous leaf extract of Azadirachta indica (neem), which proves that green synthesis with plant-based methods can be a successful alternative to traditional physical and chemical synthesis methods. FTIR revealed the appearance of nanoparticles and the presence of phytochemicals in reduction and stabilization, as shown by a change in color and the resulting UV- Vis spectral analysis. The crystalline character of the AgNPs was determined using XRD and the fact that AgNPs had a spherical geometry was determined using TEM and a size distribution over this 12-25 nm range. The AgNPs synthesized displayed a high antimicrobial activity towards Escherichia coli and Staphylococcus aureus with comparable inhibition zone to these standard antibiotics. The cell wall structure affected the nanoparticle efficacy due to the slightly greater susceptibility of Gram-negative bacteria. This study has shown that the neem-mediated AgNPs have broad applicability in most circumstances including cases where a rise in antibiotic resistance has been observed. In general, the findings support the use of green

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synthesis as a safe, economical, and efficient way of making bioactive nanoparticles. These drugs, that do not assign an antimicrobial test, should be considered as an opportunity to investigate more carefully and to characterize cytotoxicity in human cell and to simplify the needs of scaling synthetic processes. Neem-functionalized AgNPs can find use in many biomedical and industrial applications and this can lead to sustainable nanotechnology in the realms of healthcare.

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