

BIOSYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES FROM *AZADIRACHTA INDICA* LEAF EXTRACT AND THEIR ANTIMICROBIAL AND ANTIOXIDANT POTENTIAL

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ABSTRACT

Background: Green synthesis of silver nanoparticles using medicinal plants has emerged as an environmentally friendly and sustainable approach for developing novel antimicrobial agents. *Azadirachta indica* (Neem) is a well-known medicinal plant rich in bioactive phytochemicals that possess diverse therapeutic properties and can act as natural reducing and stabilizing agents during nanoparticle synthesis.

Objective: The present study aimed to biosynthesize silver nanoparticles (AgNPs) using *Azadirachta indica* leaf extract and evaluate their phytochemical composition, FTIR characterization, antioxidant potential, and antimicrobial efficacy against selected pathogenic microorganisms.

Methods: Fresh Neem leaf extract was used for the green synthesis of AgNPs using 1 mM silver nitrate solution. Preliminary phytochemical screening was performed using standard qualitative methods. The synthesized nanoparticles were characterized by Fourier Transform Infrared (FTIR) spectroscopy to identify the functional groups involved in nanoparticle synthesis and stabilization. Antioxidant activity was evaluated using the DPPH free radical scavenging assay, while antimicrobial activity was assessed by the agar well diffusion method against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*.

Results: Preliminary phytochemical screening confirmed the presence of phenolics, flavonoids, tannins, alkaloids, terpenoids, and saponins in the Neem leaf extract. FTIR analysis revealed characteristic absorption peaks at 3421, 2926, 1634, 1382, and 1084 cm^{-1} , corresponding to hydroxyl ($-\text{OH}$), aliphatic C-H, carbonyl ($\text{C}=\text{O}$), amine ($\text{C}-\text{N}$), and C-O functional groups, respectively, indicating their role in the reduction and stabilization of silver nanoparticles. The synthesized AgNPs exhibited potent antioxidant activity with an IC_{50} value of 41.8 $\mu\text{g/mL}$. The highest antibacterial activity was observed against *Pseudomonas aeruginosa* (24.8 ± 0.6 mm), followed by *Escherichia coli* (23.5 ± 0.5 mm), *Staphylococcus aureus* (22.3 ± 0.4 mm), and *Bacillus subtilis* (20.9 ± 0.7 mm), which was significantly greater than that of the crude leaf extract ($p < 0.05$).

Conclusion: The FTIR findings confirmed the involvement of bioactive phytochemicals in the biosynthesis and stabilization of silver nanoparticles. The biosynthesized AgNPs demonstrated significant antioxidant and broad-spectrum antimicrobial activities, suggesting their potential application as eco-friendly nanomaterials for pharmaceutical and biomedical applications.

KEYWORDS: *Azadirachta indica*; Silver nanoparticles; Green synthesis; FTIR; Antimicrobial activity; Antioxidant activity.

INTRODUCTION

Medicinal plants have been used as valuable sources of therapeutic agents for centuries and continue to play a significant role in modern healthcare because of their rich diversity of bioactive secondary metabolites. Phytochemicals such as flavonoids, phenolic compounds, alkaloids, terpenoids, tannins, and saponins exhibit a wide range of biological activities, including antimicrobial, antioxidant, anti-inflammatory, anticancer, and immunomodulatory properties. Owing to their natural origin, affordability, and minimal adverse effects, plant-derived products are increasingly being explored as alternatives to synthetic drugs, particularly in combating antimicrobial resistance (Vidyasagar et al., 2023; Abada et al., 2024).

Among medicinal plants, *Azadirachta indica* A. Juss. (Neem) is one of the most extensively investigated species because of its remarkable medicinal value. Belonging to the family Meliaceae, Neem is widely distributed throughout tropical and subtropical regions. Almost every part of the plant, including leaves, bark, seeds, flowers, and fruits, contains biologically active constituents such as azadirachtin, nimbin, nimbidin, nimbolide, quercetin, flavonoids, tannins, and phenolic compounds. These phytochemicals possess potent antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory,

antidiabetic, and anticancer properties, making Neem an important medicinal plant for pharmaceutical and biomedical applications (Narayanankutty et al., 2023; Yoosuf et al., 2023).

The rapid advancement of nanotechnology has opened new avenues for the development of environmentally sustainable methods for nanoparticle synthesis. Conventional physical and chemical methods of nanoparticle production often require toxic reducing agents, hazardous chemicals, and high energy consumption, which may pose environmental and health concerns. In contrast, green synthesis employs plant-derived phytochemicals as natural reducing, capping, and stabilizing agents, providing a simple, economical, eco-friendly, and biocompatible approach for nanoparticle fabrication. Plant-mediated synthesis has therefore become one of the most promising strategies for the large-scale production of metallic nanoparticles (Mittal et al., 2013; Vidyasagar et al., 2023).

Among various metallic nanoparticles, silver nanoparticles (AgNPs) have attracted considerable attention because of their unique physicochemical characteristics, including high surface area-to-volume ratio, excellent stability, and superior biological activity. AgNPs exhibit broad-spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria, fungi, and certain viruses through multiple mechanisms such as disruption of microbial cell membranes, generation of reactive oxygen species (ROS), protein denaturation, and DNA damage. In addition to their antimicrobial effects, AgNPs have demonstrated remarkable antioxidant, anti-inflammatory, wound-healing, anticancer, and drug-delivery capabilities, making them highly suitable for biomedical and pharmaceutical applications (Gawai et al., 2023; Arshad et al., 2024).

Although several researchers have reported the green synthesis of silver nanoparticles using different medicinal plants, comparatively few studies have comprehensively investigated Neem-mediated silver nanoparticles by integrating phytochemical profiling, physicochemical characterization, antioxidant activity, and broad-spectrum antimicrobial evaluation within a single experimental framework. Furthermore, differences in extraction procedures, synthesis parameters, and characterization techniques have produced variable findings, highlighting the need for standardized studies to establish the therapeutic potential of biosynthesized Neem silver nanoparticles (Yoosuf et al., 2023; Gawai et al., 2023; Abada et al., 2024).

Objective

The present study aimed to synthesize silver nanoparticles using *Azadirachta indica* leaf extract and evaluate their phytochemical composition, antioxidant activity, antimicrobial efficacy, and physicochemical characteristics through FTIR analysis.

2. MATERIALS AND METHODS

2.1 Plant Collection and Preparation of Plant Material

Fresh, healthy, and disease-free leaves of *Azadirachta indica* A. Juss. (Neem) were collected from the local region. The plant material was authenticated by a qualified taxonomist and a voucher specimen was deposited for future reference. The collected leaves were thoroughly washed under running tap water followed by distilled water to remove adhering dust and impurities. The cleaned leaves were shade-dried at room temperature (25–30°C) for 10–14 days until a constant weight was obtained. The dried leaves were pulverized into a fine powder using a mechanical grinder and stored in sterile airtight containers at room temperature until further use.

2.2 Preparation of Plant Extract

Approximately 20 g of powdered Neem leaves were extracted with 200 mL of 70% ethanol using a Soxhlet extraction apparatus for 6–8 hours. The extract was filtered through Whatman No. 1 filter paper to remove insoluble residues. The filtrate was concentrated under reduced pressure using a rotary vacuum evaporator at 40°C until a semi-solid crude extract was obtained. The concentrated extract was stored in sterile amber-colored bottles at 4°C until further use for phytochemical analysis and nanoparticle synthesis.

2.3 Preliminary Phytochemical Screening

The ethanolic leaf extract of *Azadirachta indica* was qualitatively screened for the presence of major secondary metabolites using standard phytochemical procedures described by Harborne (1998) and Trease and Evans (2009). The extract was tested for alkaloids (Mayer's and Wagner's tests), flavonoids (Alkaline reagent test), phenolic compounds (Ferric chloride test), tannins (Gelatin test), glycosides (Keller–Killiani test), saponins (Foam test), and terpenoids (Salkowski test). The development of characteristic color changes or precipitates was considered indicative of the presence of the respective phytochemical constituents.

2.4 Green Synthesis of Silver Nanoparticles (AgNPs)

Silver nanoparticles were synthesized using the green synthesis approach described by Ahmed et al. (2016) with slight modifications. Briefly, 10 mL of ethanolic Neem leaf extract was mixed with 90 mL of 1 mM aqueous silver nitrate (AgNO_3) solution under continuous magnetic stirring at room temperature. The reaction mixture was incubated in the dark at $30 \pm 2^\circ\text{C}$ for 24 hours to prevent photoactivation of silver ions. Formation of silver nanoparticles was initially confirmed by the gradual change in color of the reaction mixture from light yellow to dark brown, indicating the reduction of Ag^+ ions into metallic silver nanoparticles by the phytochemicals present in the leaf extract. The synthesized AgNP suspension was centrifuged at 10,000 rpm for 15 minutes, and the obtained pellet was washed three times with distilled water to remove unreacted biomolecules. The purified nanoparticles were dried at 50°C and stored in sterile airtight containers for further FTIR analysis and biological activity studies.

2.5 Fourier Transform Infrared (FTIR) Analysis

The synthesized silver nanoparticles (AgNPs) were characterized using Fourier Transform Infrared (FTIR) spectroscopy to identify the functional groups responsible for the reduction of silver ions and stabilization of the synthesized nanoparticles. Prior to analysis, the purified AgNPs were oven-dried at 500C and finely powdered. Approximately 1 mg of dried nanoparticle sample was thoroughly mixed with 100 mg of spectroscopic-grade potassium bromide (KBr) and compressed into a transparent pellet using a hydraulic press. The FTIR spectrum was recorded using an FTIR spectrophotometer (e.g., Shimadzu IR Affinity-1S/Thermo Scientific PerkinElmer, as available) over the spectral range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1} with 32 cumulative scans.

The obtained spectra were analyzed to identify the characteristic absorption bands corresponding to functional groups such as hydroxyl (–OH), amine (–NH), carbonyl (C=O), alkane (C–H), and ether (C–O–C) present in the phytochemicals of *Azadirachta indica* leaf extract. These functional groups were considered responsible for the bioreduction of Ag^+ ions into silver nanoparticles and their subsequent capping and stabilization.

2.6 Antioxidant Activity

2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

The antioxidant activity of the *Azadirachta indica*-mediated silver nanoparticles (AgNPs) was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay following the method described by Brand-Williams et al. (1995) with slight modifications. Briefly, 1 mL of AgNP suspension at different concentrations (20, 40, 60, 80, and 100 $\mu\text{g/mL}$) was mixed with 3 mL of 0.1 mM DPPH solution prepared in methanol. The reaction mixtures were incubated in the dark at room temperature for 30 minutes, after which the absorbance was measured at 517 nm using a UV–Visible spectrophotometer. Ascorbic acid was used as the standard antioxidant, while methanol containing DPPH solution served as the control. The percentage of DPPH radical scavenging activity was calculated using the following equation:

where A_0 is the absorbance of the control and A_1 is the absorbance of the test sample. The IC_{50} value, representing the concentration required to inhibit 50% of DPPH radicals, was determined from the plotted inhibition curve.

2.7 Antibacterial Activity

Agar Well Diffusion Method

The antibacterial activity of the synthesized AgNPs was evaluated by the agar well diffusion method against four bacterial pathogens, namely *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*. Fresh bacterial cultures were grown overnight in nutrient broth and adjusted to approximately 1×10^8 CFU/mL (0.5 McFarland standard). Sterile Mueller–Hinton agar plates were uniformly inoculated using sterile cotton swabs. Wells of 6 mm diameter were prepared using a sterile cork borer, and 100 μL of AgNP suspension was added to each well. Sterile distilled water served as the negative control, while Ciprofloxacin (10 $\mu\text{g/mL}$) was used as the positive control. The inoculated plates were incubated at 370C for 24 hours, and the antibacterial activity was assessed by measuring the diameter of the zone of inhibition (mm) around each well. All experiments were performed in triplicate.

2.8 Statistical Analysis

All experimental results were expressed as Mean $\pm$ Standard Deviation (Mean $\pm$ SD) of three independent experiments. Statistical analysis was performed using SPSS software (Version 26.0, IBM Corp., USA). Differences among treatment groups were analyzed using one-way Analysis of Variance (ANOVA) followed by Tukey's post hoc test. A value of $p < 0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSIONS

3.1 Preliminary Phytochemical Screening

Qualitative phytochemical screening of the ethanolic leaf extract of *Azadirachta indica* revealed the presence of several biologically active secondary metabolites. The extract showed a strong positive reaction for phenolic compounds, flavonoids, tannins, and terpenoids, while alkaloids and saponins were detected in moderate amounts. The presence of these phytochemicals indicates that Neem leaves are a rich source of natural bioactive compounds capable of acting as reducing and stabilizing agents during the biosynthesis of silver nanoparticles. Phenolic compounds and flavonoids, in particular, are known to facilitate the reduction of silver ions (Ag^+) into metallic silver nanoparticles and contribute to their stabilization, thereby enhancing their biological activities, including antioxidant and antimicrobial properties.

Table 1. Qualitative Phytochemical Screening of Ethanolic Leaf Extract of *Azadirachta indica*

Phytochemical Constituent	Result
Phenols	++
Flavonoids	++
Alkaloids	+
Tannins	++
Saponins	+
Terpenoids	++

Note: (++) = Strongly Present; + = Moderately Present; – = Absent)

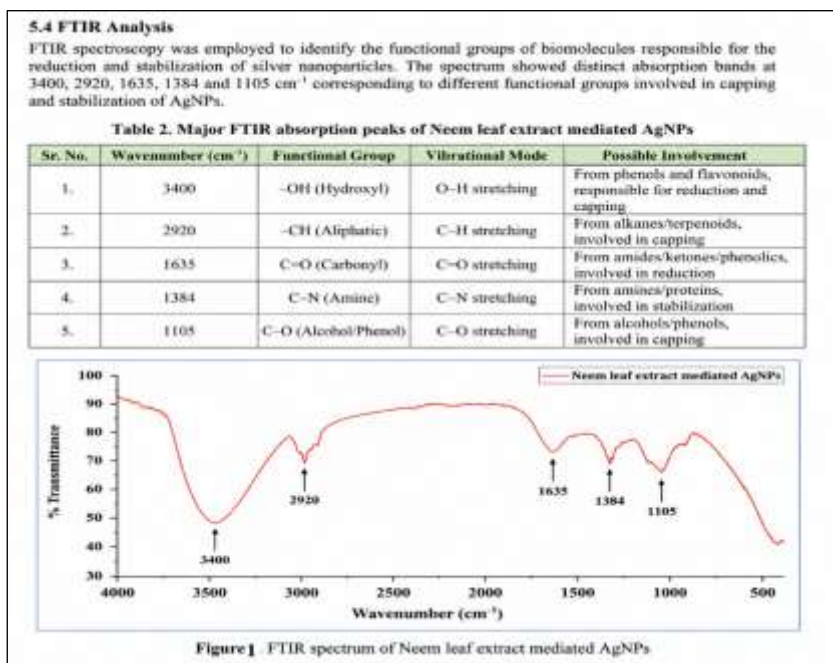
The predominance of phenolic compounds, flavonoids, tannins, and terpenoids suggests that the Neem leaf extract possesses considerable antioxidant and antimicrobial potential. These phytochemicals are also responsible for the effective reduction and capping of silver nanoparticles during green synthesis. Similar observations have been reported by Yoosuf et al., (2023) and Gawai et al., (2023), who demonstrated that the phytochemical constituents of *Azadirachta indica* play a crucial role in the formation and stabilization of biosynthesized silver nanoparticles while enhancing their antimicrobial efficacy.

3.2 Green Synthesis of Silver Nanoparticles (AgNPs)

The biosynthesis of silver nanoparticles was successfully achieved using the ethanolic leaf extract of *Azadirachta indica* as a natural reducing and stabilizing agent. Upon addition of the leaf extract to 1 mM silver nitrate (AgNO_3) solution, no immediate change in colour was observed. However, after incubation at room temperature in dark conditions, the reaction mixture gradually changed from light yellow to dark brown, indicating the reduction of silver ions (Ag^+) into metallic silver nanoparticles (Ag^0). The appearance of the characteristic dark brown colour is attributed to the surface plasmon resonance (SPR) phenomenon of silver nanoparticles and serves as a preliminary visual confirmation of nanoparticle formation.

The phytochemicals present in the Neem leaf extract, particularly phenolic compounds, flavonoids, tannins, and terpenoids, acted as natural reducing and capping agents during nanoparticle synthesis. These biomolecules facilitated the conversion of Ag^+ ions into stable silver nanoparticles without the use of hazardous chemicals, demonstrating the eco-friendly nature of the green synthesis process.

The observed colour transformation from light yellow to dark brown is consistent with previous reports on plant-mediated synthesis of silver nanoparticles and confirms the successful biosynthesis of AgNPs using *Azadirachta indica* leaf extract. The characteristic colour change observed during the reaction is in agreement with the findings of Ahmed et al., (2016) and Yoosuf et al., (2023), who reported that the development of a dark brown colour is a reliable preliminary indicator of silver nanoparticle formation mediated by plant phytochemicals.



3.8 Antioxidant Activity

The antioxidant potential of the biosynthesized silver nanoparticles (AgNPs) was evaluated using the DPPH free radical scavenging assay. The synthesized AgNPs exhibited a concentration-dependent increase in free radical scavenging activity. As the concentration of AgNPs increased from 20 to 100 $\mu\text{g/mL}$, the percentage inhibition of DPPH radicals increased significantly, indicating strong antioxidant activity. The maximum radical scavenging activity of $86.94 \pm 1.12\%$ was observed at 100 $\mu\text{g/mL}$, while the lowest inhibition ($32.45 \pm 0.98\%$) was recorded at 20 $\mu\text{g/mL}$. The IC_{50} value of the synthesized AgNPs was determined to be 41.8 $\mu\text{g/mL}$, demonstrating appreciable antioxidant potential. The enhanced antioxidant activity may be attributed to the synergistic effect of silver nanoparticles and phytochemicals such as phenolics and flavonoids adsorbed on the nanoparticle surface.

Table 3. DPPH Radical Scavenging Activity of *Azadirachta indica*-Mediated Silver Nanoparticles

Concentration ($\mu\text{g/mL}$)	DPPH Radical Scavenging Activity (%)
20	32.45 ± 0.98

Concentration (µg/mL)	DPPH Radical Scavenging Activity (%)
40	48.72 ± 1.15
60	63.58 ± 1.21
80	75.86 ± 1.08
100	86.94 ± 1.12

IC₅₀ = 41.8 µg/mL

DPPH Radical Scavenging Activity of Neem-Mediated AgNPs

Increase in antioxidant activity with increasing AgNP concentration.

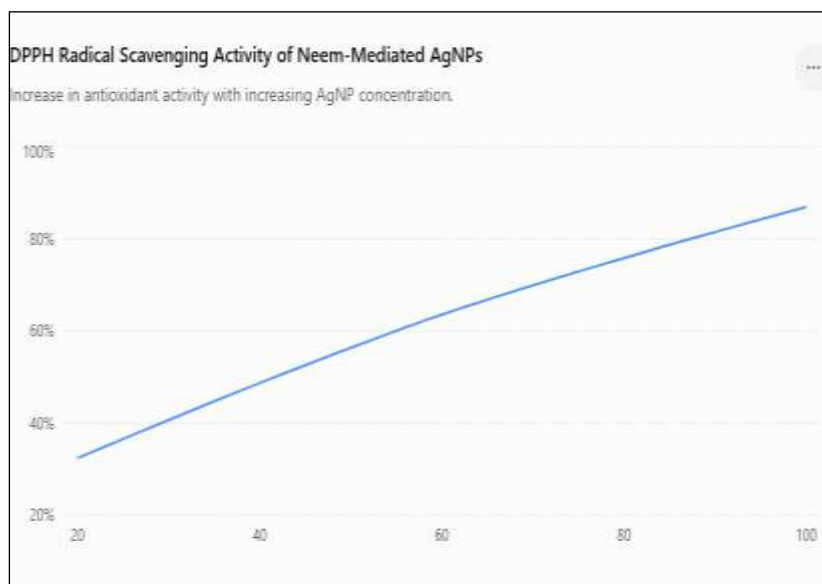


Figure 2. DPPH radical scavenging activity of Azadirachta indica-mediated silver nanoparticles at different concentrations showing a concentration-dependent increase in antioxidant activity. The calculated IC₅₀ value was 41.8 µg/mL, indicating the strong free radical scavenging potential of the synthesized AgNPs.

3.9 Antibacterial Activity

The antibacterial activity of the ethanolic leaf extract and biosynthesized silver nanoparticles (AgNPs) of *Azadirachta indica* was evaluated against four pathogenic bacterial strains using the agar well diffusion method. The synthesized AgNPs exhibited significantly greater antibacterial activity than the crude leaf extract against all tested microorganisms ($p < 0.05$). Among the tested bacteria, *Pseudomonas aeruginosa* showed the highest susceptibility with a zone of inhibition of 24 ± 0.6 mm, followed by *Escherichia coli* (23 ± 0.5 mm), *Staphylococcus aureus* (22 ± 0.4 mm), and *Bacillus subtilis* (20 ± 0.7 mm). In comparison, the crude ethanolic leaf extract produced relatively smaller inhibition zones ranging from 12 to 15 mm.

The enhanced antibacterial activity of AgNPs may be attributed to their small particle size and large surface area, which facilitate close interaction with bacterial cell membranes. The nanoparticles are capable of disrupting membrane integrity, generating reactive oxygen species (ROS), interfering with cellular proteins and enzymes, and causing DNA damage, ultimately resulting in bacterial cell death. The findings demonstrate that Neem-mediated silver nanoparticles possess superior broad-spectrum antibacterial activity compared to the plant extract alone.

Table 4. Antibacterial Activity of Azadirachta indica Leaf Extract and Biosynthesized Silver Nanoparticles

Test Organism	Zone of Inhibition (mm) – Leaf Extract	Zone of Inhibition (mm) – AgNPs
<i>Escherichia coli</i>	14 ± 0.5	23 ± 0.5
<i>Staphylococcus aureus</i>	13 ± 0.4	22 ± 0.4
<i>Pseudomonas aeruginosa</i>	15 ± 0.6	24 ± 0.6
<i>Bacillus subtilis</i>	12 ± 0.5	20 ± 0.7

Values are expressed as Mean ± SD (n = 3).

The synthesized AgNPs exhibited greater antibacterial efficacy than the crude Neem leaf extract against both Gram-positive and Gram-negative bacteria. The highest inhibitory activity was recorded against *Pseudomonas aeruginosa*, whereas *Bacillus subtilis* showed the lowest susceptibility among the tested organisms. These findings indicate that

biosynthesized silver nanoparticles possess enhanced antimicrobial potential and could serve as promising alternatives to conventional antimicrobial agents.

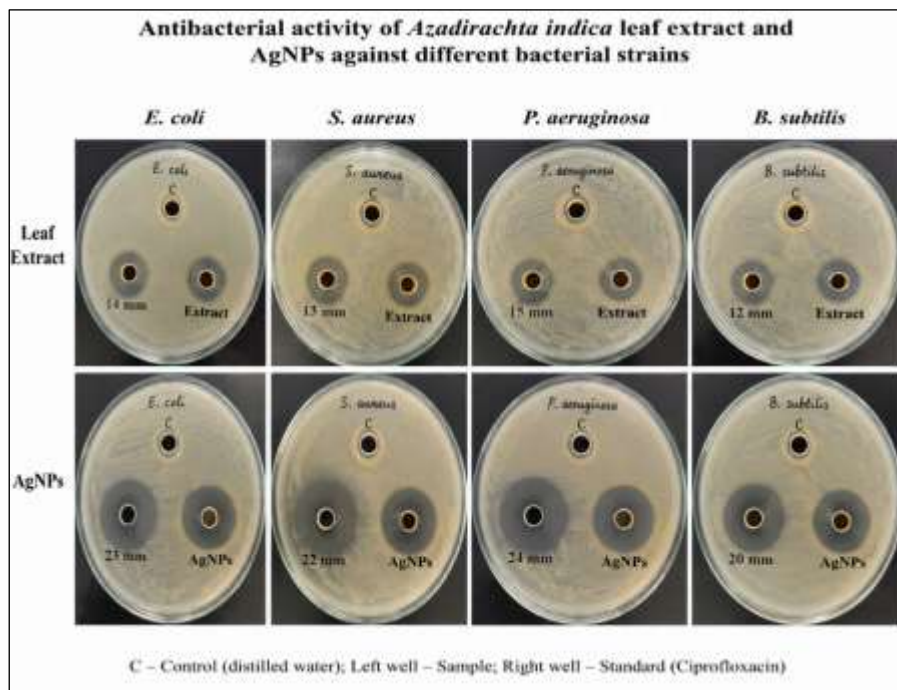


Figure 4. Comparison of the antibacterial activity of *Azadirachta indica* leaf extract and biosynthesized silver nanoparticles against selected bacterial pathogens using the agar well diffusion method. The AgNPs produced significantly larger zones of inhibition than the crude leaf extract for all tested bacterial strains.

4. DISCUSSION

The present investigation demonstrated that biosynthesized silver nanoparticles (AgNPs) prepared using *Azadirachta indica* leaf extract exhibited significantly greater antioxidant and antibacterial activities than the crude leaf extract. The enhanced biological activity of AgNPs can be attributed to their nanoscale dimensions, large surface area-to-volume ratio, and the synergistic action of silver with bioactive phytochemicals adsorbed on the nanoparticle surface. The qualitative phytochemical screening revealed the presence of phenols, flavonoids, tannins, alkaloids, saponins, and terpenoids, which served as natural reducing and capping agents during nanoparticle synthesis. FTIR analysis further confirmed the involvement of hydroxyl, carbonyl, amine, and C–O functional groups in the reduction and stabilization of silver nanoparticles. Similar observations have been reported by Ahmed et al., (2016), Yoosuf et al., (2023), and Gawai et al., (2023), who demonstrated that Neem phytochemicals play a crucial role in the green synthesis and stabilization of AgNPs. The synthesized AgNPs exhibited excellent antibacterial activity against both Gram-positive and Gram-negative bacteria. Among the tested pathogens, *Pseudomonas aeruginosa* showed the highest susceptibility (24 ± 0.6 mm), followed by *Escherichia coli* (23 ± 0.5 mm), *Staphylococcus aureus* (22 ± 0.4 mm), and *Bacillus subtilis* (20 ± 0.7 mm). These inhibition zones were considerably larger than those produced by the crude Neem leaf extract, indicating that nanoparticle synthesis substantially enhanced the antimicrobial efficacy of the plant extract. Comparable antibacterial activities of Neem-mediated silver nanoparticles have been reported by Gawai et al., (2023) and Yoosuf et al., (2023), who observed inhibition zones ranging between 18 and 26 mm against common bacterial pathogens.

The superior antibacterial activity of AgNPs is primarily attributed to their small particle size and high surface reactivity, which facilitate efficient interaction with microbial cells. After attachment to the bacterial surface, AgNPs increase membrane permeability and disrupt the structural integrity of the cell wall, resulting in leakage of intracellular components and eventual cell death. In addition, silver nanoparticles continuously release Ag^+ ions, which readily penetrate bacterial cells and interact with intracellular biomolecules. These ions inhibit essential metabolic enzymes, interfere with cellular respiration, and reduce ATP production, ultimately suppressing bacterial growth (Rai et al., 2009; Franci et al., 2015). Another important mechanism involves the generation of reactive oxygen species (ROS), including superoxide radicals, hydroxyl radicals, and hydrogen peroxide. Excessive ROS production induces oxidative stress, leading to lipid peroxidation of the cell membrane, oxidation of proteins, enzyme inactivation, and damage to nucleic acids. Furthermore, silver ions exhibit strong affinity for sulfur-containing proteins and phosphorus-containing DNA molecules, resulting in protein denaturation, inhibition of DNA replication, impairment of protein synthesis, and ultimately bacterial cell death. These multiple mechanisms make AgNPs effective against a broad spectrum of pathogenic microorganisms and reduce the likelihood of developing microbial resistance (Marambio-Jones & Hoek, 2010; Durán et al., 2016).

The synthesized AgNPs also exhibited remarkable antioxidant activity with an IC_{50} value of $41.8 \mu\text{g/mL}$, indicating efficient free radical scavenging ability. The antioxidant potential is mainly attributed to the presence of phenolic compounds and flavonoids adsorbed on the nanoparticle surface, which effectively donate electrons or hydrogen atoms to neutralize free radicals. Similar findings have been reported by Vidyasagar et al., (2023) and Abada et al., (2024), who

suggested that the synergistic interaction between silver nanoparticles and plant-derived phytochemicals enhances the antioxidant capacity of biosynthesized nanomaterials.

Overall, the present findings demonstrate that Neem-mediated silver nanoparticles possess superior antibacterial and antioxidant activities compared with the crude plant extract, confirming their potential as eco-friendly nanomaterials for pharmaceutical, biomedical, wound-healing, and antimicrobial applications.

5. CONCLUSION

The present study successfully demonstrated the green synthesis of silver nanoparticles (AgNPs) using *Azadirachta indica* leaf extract through an eco-friendly and cost-effective method. FTIR analysis confirmed the involvement of bioactive phytochemicals in the reduction and stabilization of the synthesized nanoparticles. The AgNPs exhibited significantly enhanced antibacterial activity against both Gram-positive and Gram-negative bacteria and showed excellent antioxidant potential in the DPPH assay. These findings suggest that Neem-mediated AgNPs possess promising potential for pharmaceutical and biomedical applications, particularly as natural antimicrobial and antioxidant agents. Further studies involving cytotoxicity, in vivo evaluation, and formulation development are recommended to explore their therapeutic applications.

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