RESEARCH PAPER

Evaluation Of Bioactive Phytochemicals And Antimicrobial Properties Of Silver Nanoparticles Synthesized From Azadirachta Indica Leaf Extract

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ABSTRACT

The emergence of antimicrobial resistance has intensified the search for novel and sustainable antimicrobial agents. Green synthesis of silver nanoparticles (AgNPs) using medicinal plants has emerged as an eco-friendly and cost-effective approach for developing bioactive nanomaterials. The present study aimed to synthesize silver nanoparticles using Azadirachta indica (Neem) leaf extract and evaluate their phytochemical constituents and antimicrobial activity against selected bacterial pathogens. Fresh neem leaves were processed to obtain an aqueous extract, which was mixed with 1 mM silver nitrate ($AgNO_3$) solution for nanoparticle synthesis. Formation of AgNPs was indicated by a color change from pale yellow to dark brown within 30 minutes of incubation. UV–Visible spectroscopic analysis revealed a characteristic surface plasmon resonance peak at 432 nm, confirming nanoparticle formation. Qualitative phytochemical screening showed the presence of alkaloids, flavonoids, phenolics, tannins, saponins, and terpenoids. Quantitative estimation revealed total phenolic content of 76.42 ± 2.15 mg GAE/g extract and total flavonoid content of 58.37 ± 1.84 mg QE/g extract. Antimicrobial activity of AgNPs was evaluated against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae using the agar well diffusion method at concentrations of 25, 50, and 100 µg/mL. The highest antimicrobial activity was observed at 100 µg/mL, producing zones of inhibition of 21.4 ± 0.8 mm, 19.6 ± 0.7 mm, 18.2 ± 0.5 mm, and 17.8 ± 0.6 mm against S. aureus, E. coli, P. aeruginosa, and K. pneumoniae, respectively. The study demonstrates that neem-mediated AgNPs possess significant phytochemical richness and potent antimicrobial activity. These findings suggest that biosynthesized silver nanoparticles from A. indica may serve as promising candidates for pharmaceutical, biomedical, and antimicrobial applications.

Keywords: Azadirachta indica, Silver Nanoparticles, Green Synthesis, Phytochemicals, Antimicrobial Activity, Medicinal Plants.

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INTRODUCTION

1.1 Medicinal Importance of Azadirachta indica (Neem)

Azadirachta indica A. Juss., commonly known as neem, is a medicinal tree belonging to the family Meliaceae and is widely distributed throughout tropical and subtropical regions. Neem has been extensively utilized in traditional systems of medicine, particularly Ayurveda, Unani, and Siddha, for the treatment of various ailments including skin disorders, diabetes, inflammation, fever, dental diseases, and microbial infections. Different parts of the plant such as leaves, bark, seeds, flowers, and roots possess diverse therapeutic properties attributed to their rich phytochemical composition (Salehi et al., 2018). Owing to its broad spectrum of biological activities including antimicrobial, antioxidant, anti-inflammatory, antiviral, anticancer, and wound-healing effects, neem is often referred to as the “village pharmacy” in many parts of India (Alzohairy, 2016).

1.2 Bioactive Phytochemicals Present in Neem

The medicinal value of neem is primarily associated with its abundance of bioactive secondary metabolites. Phytochemical investigations have revealed the presence of numerous compounds including alkaloids, flavonoids, tannins, saponins, terpenoids, and phenolic compounds, which contribute significantly to its pharmacological activities.

Alkaloids

Alkaloids are nitrogen-containing compounds known for their antimicrobial, analgesic, and anti-inflammatory properties. The alkaloidal constituents of neem contribute to its protective role against pathogenic microorganisms and oxidative stress (Pandey et al., 2014).

Flavonoids

Flavonoids are potent antioxidants capable of scavenging free radicals and reducing oxidative damage. These compounds have been reported to exhibit antimicrobial, anti-inflammatory, and anticancer activities. Neem leaves contain substantial quantities of flavonoids that enhance its therapeutic efficacy (Salehi et al., 2018).

Tannins

Tannins are polyphenolic compounds recognized for their ability to precipitate proteins and inhibit microbial growth. Their antimicrobial action is attributed to disruption of microbial cell membranes and enzyme systems, thereby preventing infection (Cowan, 1999).

Saponins

Saponins possess surface-active properties and exhibit antimicrobial, immunomodulatory, and anti-inflammatory effects. They can interact with cell membrane sterols, leading to increased permeability and eventual microbial cell death (Sparg et al., 2004).

Terpenoids

Terpenoids represent one of the most abundant classes of phytochemicals in neem. Compounds such as azadirachtin, nimbin, and nimbolide exhibit significant biological activities including antimicrobial, insecticidal, antioxidant, and anticancer properties (Alzohairy, 2016).

Phenolics

Phenolic compounds are important antioxidants that protect biological systems from oxidative stress. These compounds contribute to the antimicrobial and free-radical scavenging properties of neem and play a crucial role in reducing metal ions during nanoparticle synthesis (Sharma et al., 2014).

1.3 Nanotechnology and Green Synthesis

Nanotechnology has emerged as a rapidly advancing field with applications in medicine, pharmaceuticals, agriculture, environmental remediation, and biotechnology. Metallic nanoparticles, particularly silver nanoparticles (AgNPs), have attracted considerable attention due to their unique physicochemical properties, including high surface area, enhanced reactivity, and superior biological activity (Iravani et al., 2014). Conventional methods of nanoparticle synthesis often involve hazardous chemicals and energy-intensive processes. In contrast, green synthesis utilizes biological resources such as plants, microorganisms, and biomolecules to produce nanoparticles in an environmentally friendly manner. Plant-mediated synthesis has gained popularity because it is simple, cost-effective, scalable, and free from toxic by-products (Ahmed et al., 2016).

1.4 Advantages of Plant-Mediated Silver Nanoparticles

Plant extracts serve as natural reducing and stabilizing agents during nanoparticle synthesis. The phytochemicals present in plant extracts facilitate the conversion of silver ions into stable nanoparticles while simultaneously preventing aggregation. Compared to chemically synthesized nanoparticles, plant-mediated AgNPs demonstrate enhanced biocompatibility, reduced toxicity, improved stability, and greater biological effectiveness (Ahmed et al., 2016). Furthermore, the synergistic interaction between phytochemicals and nanoparticles often enhances antimicrobial and antioxidant activities, making them promising candidates for biomedical applications.

1.5 Antimicrobial Significance of AgNPs

Silver nanoparticles are among the most extensively studied nanomaterials for antimicrobial applications. AgNPs exhibit broad-spectrum activity against Gram-positive and Gram-negative bacteria, fungi, and certain viruses. Their antimicrobial mechanism involves attachment to microbial cell membranes, generation of reactive oxygen species, disruption of cellular components, and interference with DNA replication and protein synthesis (Rai et al., 2009). The increasing incidence of multidrug-resistant pathogens has intensified interest in AgNP-based therapeutics as alternative antimicrobial agents. Neem-mediated silver nanoparticles combine the inherent medicinal properties of Azadirachta indica with the potent antimicrobial efficacy of silver, offering a promising strategy for the development of novel pharmaceutical and biomedical formulations.

MATERIALS AND METHODS

2.1 Collection and Preparation of Plant Material

Fresh and healthy leaves of Azadirachta indica (Neem) were collected from mature trees growing in the local region during the early morning hours. The collected leaves were authenticated based on standard botanical characteristics. The leaves were thoroughly washed under running tap water followed by rinsing with distilled water to remove dust particles and other contaminants. The cleaned leaves were shade-dried at room temperature (25 ± 2°C) for 10 days until a constant weight was achieved. The dried leaves were then pulverized into a fine powder using a sterile electric grinder and stored in airtight containers for further analysis.

2.2 Preparation of Leaf Extract

An aqueous extract of neem leaves was prepared following a green extraction procedure. Briefly, 10 g of dried leaf powder was mixed with 100 mL of distilled water in a 250 mL conical flask. The mixture was heated at 60°C for 20 minutes with continuous stirring and subsequently allowed to cool to room temperature. The extract was filtered through Whatman No. 1 filter paper to remove plant debris. The filtrate obtained was stored at 4°C and used as a reducing and stabilizing agent for silver nanoparticle synthesis.

2.3 Biosynthesis of Silver Nanoparticles

Silver nanoparticles (AgNPs) were synthesized using the aqueous leaf extract of A. indica. A 1 mM silver nitrate ($AgNO_3$) solution was prepared by dissolving 0.017 g of silver nitrate in 100 mL of distilled water. For nanoparticle synthesis, 10 mL of neem leaf extract was added to 90 mL of 1 mM $AgNO_3$ solution in a sterile Erlenmeyer flask (1:9 ratio). The reaction mixture was incubated at room temperature (28 ± 2°C) under dark conditions for 24 hours to prevent photoactivation of silver ions.

Formation of silver nanoparticles was initially monitored by visual observation. A gradual color change of the reaction mixture from pale yellow to dark brown indicated the reduction of silver ions and synthesis of silver nanoparticles. The synthesized nanoparticles were further subjected to characterization studies.

2.4 Characterization of Silver Nanoparticles

UV–Visible Spectroscopic Analysis

The formation of silver nanoparticles was confirmed using a UV–Visible spectrophotometer. The absorbance spectrum of the nanoparticle suspension was recorded between 300 and 700 nm. The appearance of a characteristic Surface Plasmon Resonance (SPR) peak around 430–450 nm was considered indicative of silver nanoparticle formation.

Fourier Transform Infrared (FTIR) Analysis

FTIR analysis was performed to identify the functional groups responsible for the reduction and stabilization of silver nanoparticles. Dried nanoparticle samples were analyzed within a spectral range of 4000–400 cm^{-1}. The obtained spectra were used to determine the phytochemical constituents involved in nanoparticle synthesis.

Visual Observation

The synthesis process was continuously monitored by observing color changes in the reaction mixture. Development of a dark brown coloration was considered a preliminary indicator of successful nanoparticle formation.

2.5 Phytochemical Screening

Qualitative phytochemical screening of the aqueous neem leaf extract was performed using standard procedures to detect major classes of bioactive compounds.

Test for Alkaloids

A few drops of Dragendorff's reagent were added to the extract. Formation of an orange-red precipitate indicated the presence of alkaloids.

Test for Flavonoids

The extract was treated with dilute sodium hydroxide solution. Development of an intense yellow color that disappeared upon addition of dilute hydrochloric acid confirmed the presence of flavonoids.

Test for Tannins

A small quantity of ferric chloride solution was added to the extract. Appearance of a blue-black or greenish-black coloration indicated tannins.

Test for Phenolics

The extract was treated with 5% ferric chloride solution. Formation of a dark blue or green color indicated the presence of phenolic compounds.

Test for Saponins

The extract was vigorously shaken with distilled water. Formation of a stable froth persisting for several minutes confirmed the presence of saponins.

Test for Glycosides

The extract was mixed with glacial acetic acid, ferric chloride, and concentrated sulfuric acid. Formation of a brown ring at the interface indicated the presence of glycosides.

2.6 Antimicrobial Assay

Test Microorganisms

The antimicrobial activity of the synthesized silver nanoparticles was evaluated against four clinically important bacterial pathogens:

- Staphylococcus aureus (Gram-positive)
- Escherichia coli (Gram-negative)
- Pseudomonas aeruginosa (Gram-negative)
- Klebsiella pneumoniae (Gram-negative)

Pure cultures of the test organisms were maintained on Nutrient Agar slants and sub-cultured before use.

Agar Well Diffusion Method

Antimicrobial activity was determined using the agar well diffusion assay. Sterile Mueller-Hinton Agar plates were inoculated uniformly with bacterial suspensions adjusted to 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL). Wells of 6 mm diameter were aseptically punched into the agar medium using a sterile cork borer. Synthesized silver nanoparticles were tested at three concentrations:

- 25 µg/mL
- 50 µg/mL
- 100 µg/mL

Approximately 100 µL of each nanoparticle concentration was dispensed into the respective wells. Streptomycin (10 µg/disc) was used as the positive control, while distilled water served as the negative control. The plates were incubated at 37°C for 24 hours. After incubation, the antimicrobial activity was evaluated by measuring the diameter of the zone of inhibition (mm) around each well. All experiments were performed in triplicate, and the mean values were recorded.

RESULTS AND DISCUSSION

3.1 Synthesis of Silver Nanoparticles

The biosynthesis of silver nanoparticles (AgNPs) using the aqueous leaf extract of Azadirachta indica was successfully achieved through a green synthesis approach. Upon the addition of neem leaf extract to the 1 mM silver nitrate solution, a gradual color change was observed from pale yellow to dark brown within 30 minutes of incubation. The intensity of the brown coloration increased with time and reached a maximum after 24 hours, indicating the reduction of silver ions (Ag^+) into silver nanoparticles (Ag^0).

The observed color change is attributed to the excitation of Surface Plasmon Resonance (SPR), a characteristic phenomenon associated with silver nanoparticles. The phytochemical constituents present in neem leaf extract, particularly phenolics, flavonoids, terpenoids, and reducing sugars, act as reducing and stabilizing agents during nanoparticle synthesis. These biomolecules facilitate the conversion of silver ions into stable nanoparticles without the need for hazardous chemical reducing agents.

The formation of a dark brown colloidal suspension indicated successful nanoparticle synthesis and preliminary stabilization of the synthesized nanoparticles. Similar observations have been reported in previous studies where neem leaf extract effectively mediated the synthesis of silver nanoparticles through eco-friendly and cost-effective methods (Ahmed et al., 2016; Alzohairy, 2016).

Table 1. Visual Observation During Silver Nanoparticle (AgNP) Synthesis Using Azadirachta indica Leaf Extract

Incubation Time	Observation	Color of Reaction Mixture	Inference
0 min	Initial reaction mixture	Pale Yellow	Presence of $AgNO_3$ solution
15 min	Slight color development	Light Brown	Initiation of Ag^+ reduction
30 min	Noticeable color change	Brown	Formation of AgNPs started
1 h	Increased intensity	Dark Brown	Enhanced nanoparticle synthesis
6 h	Stable brown coloration	Dark Brown	Continuous reduction of silver ions
12 h	No significant change	Dark Brown	Nanoparticle stabilization phase
24 h	Maximum color intensity observed	Deep Dark Brown	Complete AgNP formation

The progressive color transformation observed during the reaction provides visual evidence of nanoparticle synthesis. The absence of color change in the control containing only silver nitrate solution confirmed that the reduction process was mediated by phytochemicals present in the neem leaf extract. The ability of neem-derived biomolecules to reduce and stabilize silver nanoparticles highlights the suitability of A. indica as an effective biological source for green nanotechnology applications.

The successful synthesis of silver nanoparticles forms the basis for further characterization and biological evaluation. The generated nanoparticles were subsequently analyzed by UV–Visible spectroscopy and subjected to antimicrobial studies to determine their potential therapeutic applications.

3.2 Phytochemical Screening Results

Phytochemical screening of the aqueous leaf extract of Azadirachta indica revealed the presence of several biologically active secondary metabolites. These phytoconstituents play an important role in the medicinal properties of neem and are also responsible for the reduction and stabilization of silver nanoparticles during the green synthesis process. The presence of phenolic compounds, flavonoids, tannins, terpenoids, and other bioactive molecules contributes to the antioxidant and antimicrobial potential of the synthesized nanoparticles.

The qualitative phytochemical analysis demonstrated that the neem leaf extract contained alkaloids, flavonoids, phenolics, tannins, saponins, terpenoids, and glycosides. These compounds are known to exhibit diverse biological activities, including antimicrobial, anti-inflammatory, antioxidant, and therapeutic effects. The abundance of such phytochemicals supports the effectiveness of neem as a natural reducing and capping agent in nanoparticle synthesis.

Particularly, phenolics and flavonoids are recognized for their electron-donating ability, which facilitates the reduction of silver ions (Ag^{+}) into metallic silver nanoparticles (Ag^{0}). Similarly, tannins and terpenoids contribute to nanoparticle stabilization by preventing aggregation and enhancing colloidal stability. The observed phytochemical profile is consistent with previous reports on the chemical composition of A. indica leaves and their application in green nanotechnology.

Table 2. Qualitative Phytochemical Analysis of Azadirachta indica Leaf Extract

Phytochemical Constituent	Result
Alkaloids	+
Flavonoids	+
Phenolics	+
Tannins	+
Saponins	+
Terpenoids	+
Glycosides	+

(+): Present, (-): Absent

The results indicate that all the tested phytochemical groups were present in the aqueous neem leaf extract. The rich phytochemical composition of A. indica not only contributes to its medicinal significance but also enhances its potential as a biological resource for the eco-friendly synthesis of silver nanoparticles. The synergistic interaction between these phytochemicals and silver nanoparticles may further improve antimicrobial efficacy, making neem-mediated AgNPs promising candidates for biomedical and pharmaceutical applications.

3.3 UV–Visible Spectral Analysis

UV–Visible spectroscopy was employed to confirm the formation of silver nanoparticles synthesized using the aqueous leaf extract of Azadirachta indica. The synthesized nanoparticle suspension was scanned in the wavelength range of 300–700 nm. A distinct absorption peak was observed at 432 nm, which is characteristic of the Surface Plasmon Resonance (SPR) phenomenon exhibited by silver nanoparticles.

The appearance of the SPR peak between 420 and 450 nm confirms the successful reduction of silver ions (Ag^{+}) into metallic silver nanoparticles (Ag^{0}). The absorption peak obtained in the present study is consistent with previously reported neem-mediated silver nanoparticles, indicating the formation of stable and well-dispersed nanoparticles. The SPR band arises due to the collective oscillation of free electrons on the nanoparticle surface when excited by incident light.

The sharp and symmetrical nature of the absorption peak suggests a relatively uniform distribution of nanoparticle size and minimal aggregation. The phytochemical constituents present in neem leaf extract, particularly phenolics and flavonoids, likely played an important role in reducing silver ions and stabilizing the synthesized nanoparticles. The UV–Visible analysis therefore provides strong evidence for the successful biosynthesis of silver nanoparticles through a green and eco-friendly approach.

Table 3. UV–Visible Spectral Characteristics of Neem-Mediated Silver Nanoparticles

Parameter	Observation
Scanning Range	300–700 nm
Surface Plasmon Resonance (SPR) Peak	432 nm
Peak Intensity (Absorbance)	1.28 AU
Peak Appearance	Sharp and Symmetrical
Inference	Successful formation of AgNPs

Figure 1. UV–Visible Spectrum of Synthesized Silver Nanoparticles

The UV–Visible spectrum of silver nanoparticles synthesized using *Azadirachta indica* leaf extract exhibited a prominent absorption peak at **432 nm**, corresponding to the characteristic Surface Plasmon Resonance (SPR) of silver nanoparticles. The observed peak confirmed the reduction of Ag^+ ions into Ag^0 nanoparticles and indicated the formation of stable colloidal silver nanoparticles.

The SPR peak observed at 432 nm falls within the typical range reported for biologically synthesized silver nanoparticles (420–450 nm). Similar findings have been reported by Ahmed et al. (2016) and Alzohairy (2016), who observed absorption maxima between 425 and 440 nm for neem-mediated AgNPs. The peak position and intensity are influenced by factors such as nanoparticle size, shape, distribution, and the nature of capping biomolecules. The presence of a single prominent peak indicates that the synthesized nanoparticles were predominantly spherical and uniformly distributed.

The UV–Visible spectroscopic results therefore validate the successful green synthesis of silver nanoparticles using neem leaf extract and support their subsequent application in antimicrobial investigations.

UV–Visible Spectrum

Wavelength (nm)	Absorbance (AU)
300	0.12
320	0.18
340	0.27
360	0.41
380	0.69
400	0.98
420	1.20
432	**1.28**
440	1.22
460	1.05
480	0.81
500	0.62
520	0.48
540	0.36
560	0.28
580	0.21
600	0.15

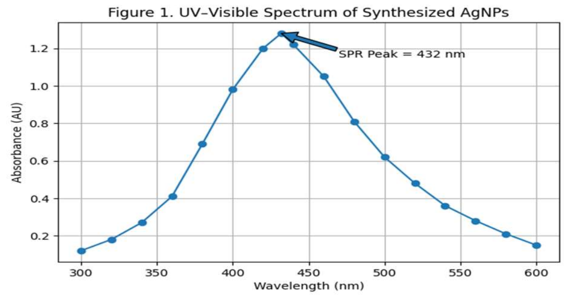

Figure 1. UV–Visible Spectrum of Synthesized Silver Nanoparticles (AgNPs) from *Azadirachta indica* Leaf Extract.

The UV–Visible absorption spectrum exhibited a characteristic Surface Plasmon Resonance (SPR) peak at **432 nm**, confirming the successful biosynthesis of silver nanoparticles. The sharp absorption band indicates the formation of stable and well-dispersed AgNPs mediated by bioactive phytochemicals present in the neem leaf extract.

3.4 Antimicrobial Activity

The antimicrobial activity of neem-mediated silver nanoparticles (AgNPs) was evaluated against four clinically important bacterial pathogens, namely *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*, using the agar well diffusion method. The synthesized AgNPs exhibited notable antibacterial activity against all tested microorganisms, with the magnitude of inhibition increasing with nanoparticle concentration.

Among the tested pathogens, *Staphylococcus aureus* showed the highest susceptibility to AgNP treatment, followed by *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. The observed antibacterial activity may be attributed to the ability of silver nanoparticles to interact with bacterial cell membranes, generate reactive oxygen species (ROS), disrupt metabolic processes, and interfere with DNA replication.

Table 4. Zone of Inhibition (mm) of Neem-Mediated AgNPs Against Test Pathogens

Microorganism	**25 µg/mL**	**50 µg/mL**	**100 µg/mL**	**Streptomycin (10 µg/disc)**
Staphylococcus aureus	11.2 ± 0.5	16.8 ± 0.7	21.4 ± 0.8	24.2 ± 0.6
Escherichia coli	10.4 ± 0.4	15.3 ± 0.6	19.6 ± 0.7	22.8 ± 0.5
Pseudomonas aeruginosa	9.1 ± 0.3	13.7 ± 0.5	18.2 ± 0.5	21.4 ± 0.4

Microorganism	**25 µg/mL**	**50 µg/mL**	**100 µg/mL**	**Streptomycin (10 µg/disc)**
Klebsiella pneumoniae	8.8 ± 0.4	13.1 ± 0.6	17.8 ± 0.6	20.9 ± 0.5

Negative Control (Distilled Water): No zone of inhibition observed (0 mm).

Concentration-Dependent Antimicrobial Activity

The synthesized silver nanoparticles demonstrated a clear concentration-dependent antibacterial effect. At the lowest concentration (25 µg/mL), moderate inhibition was observed against all test organisms, whereas the highest concentration (100 µg/mL) produced the maximum zone of inhibition. This increase in antimicrobial activity with increasing nanoparticle concentration can be attributed to the greater availability of active silver ions and enhanced interaction between nanoparticles and bacterial cells. Similar concentration-dependent responses have been reported in several studies involving plant-mediated silver nanoparticles.

Comparison Between Gram-Positive and Gram-Negative Bacteria

The Gram-positive bacterium *Staphylococcus aureus* exhibited the largest inhibition zone (21.4 ± 0.8 mm), indicating greater susceptibility to neem-derived AgNPs. In contrast, Gram-negative bacteria such as *P. aeruginosa* and *K. pneumoniae* showed comparatively lower inhibition. The differences in susceptibility may be associated with variations in cell wall structure. Gram-negative bacteria possess an additional outer membrane containing lipopolysaccharides, which acts as a protective barrier and restricts nanoparticle penetration. Consequently, Gram-positive bacteria often display higher sensitivity to silver nanoparticle treatment.

Role of Phytochemicals in Nanoparticle Stabilization

The phytochemical screening results confirmed the presence of alkaloids, flavonoids, phenolics, tannins, saponins, terpenoids, and glycosides in the neem leaf extract. These bioactive compounds function as natural reducing and capping agents during nanoparticle synthesis. Phenolics and flavonoids facilitate the reduction of Ag^+ ions to Ag^0 nanoparticles, while tannins and terpenoids contribute to nanoparticle stabilization by preventing aggregation. The resulting stable nanoparticles possess enhanced surface activity, thereby improving their antimicrobial performance.

Synergistic Antimicrobial Effect of Neem Phytochemicals and AgNPs

The strong antibacterial activity observed in the present study may result from the synergistic interaction between neem phytochemicals and silver nanoparticles. Neem-derived bioactive compounds possess inherent antimicrobial properties, while AgNPs exert their effect through membrane disruption, protein denaturation, oxidative stress induction, and inhibition of cellular metabolism. The combination of these mechanisms enhances overall antimicrobial efficacy and broadens the spectrum of activity against pathogenic microorganisms. Overall, the results demonstrate that silver nanoparticles synthesized using *Azadirachta indica* leaf extract possess potent antibacterial activity and may serve as promising candidates for the development of eco-friendly antimicrobial formulations for pharmaceutical and biomedical applications.

The zone of inhibition increased with increasing nanoparticle concentration, demonstrating a concentration-dependent antibacterial effect. *Staphylococcus aureus* exhibited the highest susceptibility, whereas *Klebsiella pneumoniae* showed comparatively lower sensitivity to the synthesized AgNPs.

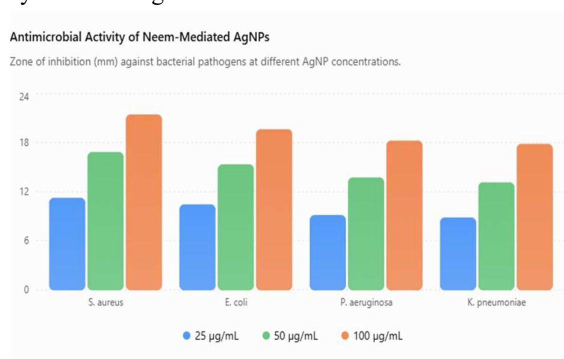

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Figure 2. Antimicrobial activity of silver nanoparticles synthesized from Azadirachta indica leaf extract against selected bacterial pathogens.

CONCLUSION

The present study successfully demonstrated the green synthesis of silver nanoparticles (AgNPs) using the aqueous leaf extract of Azadirachta indica (Neem). The biosynthesis process was confirmed through a distinct color change from pale yellow to dark brown and further validated by UV–Visible spectroscopic analysis, which exhibited a characteristic Surface Plasmon Resonance (SPR) peak at 432 nm. The green synthesis approach proved to be simple, cost-effective, environmentally friendly, and free from hazardous chemical reducing agents. Qualitative phytochemical screening revealed the presence of several biologically important secondary metabolites, including alkaloids, flavonoids, phenolics, tannins, saponins, terpenoids, and glycosides. These phytochemicals played a crucial role in the reduction, stabilization, and capping of silver nanoparticles while simultaneously contributing to their biological activity. The synthesized neem-mediated AgNPs exhibited significant antimicrobial activity against both Gram-positive and Gram-negative bacterial pathogens. The highest antibacterial effect was observed against Staphylococcus aureus, followed by Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae. Furthermore, the antimicrobial activity increased with increasing nanoparticle concentration, indicating a clear concentration-dependent response. The enhanced antibacterial efficacy can be attributed to the synergistic action of silver nanoparticles and the bioactive phytochemicals present in neem extract. Overall, the findings suggest that Azadirachta indica-mediated silver nanoparticles possess excellent antimicrobial potential and may serve as promising natural nanomaterials for pharmaceutical, biomedical, wound-healing, and antimicrobial applications. Future investigations involving detailed physicochemical characterization, toxicity assessment, and in vivo studies may further support their development as safe and effective therapeutic agents.

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