JHWCR JOURNAL OF HEALTH, WELLNESS AND COMMUNITY RESEARCH 3007-0570

Original Article Volume 3, Issue 8

Eco-Friendly Synthesis of Silver Nanoparticles Using Plant Extracts and Their Antimicrobial Applications

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ABSTRACT

Background: *Silver nanoparticles are widely recognized for their broad-spectrum antimicrobial properties and increasing relevance in biomedical applications. However, conventional synthesis methods often involve toxic chemicals and environmentally hazardous processes. Green synthesis using plant extracts has emerged as a sustainable alternative because plant-derived phytochemicals can act as natural reducing and stabilizing agents during nanoparticle formation. Developing eco-friendly nanoparticle synthesis strategies with effective antimicrobial activity is particularly important in healthcare settings where hospital-associated infections and antimicrobial resistance remain major clinical challenges.* ***Objective:*** *To synthesize silver nanoparticles using plant extracts through an environmentally friendly green synthesis approach and to evaluate their antibacterial activity against clinically important bacterial pathogens isolated from a tertiary care hospital in Central Punjab, Pakistan.* ***Methods:*** *This experimental laboratory-based study was conducted between January and June 2025. Plant leaf extracts were used as natural reducing agents to synthesize silver nanoparticles from a 1 mM silver nitrate solution. Nanoparticle formation was confirmed by visual color change and UV–Visible spectrophotometry. Particle size distribution and morphology were analyzed using dynamic light scattering and scanning electron microscopy. Antibacterial activity was evaluated against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae using the agar well diffusion method at nanoparticle concentrations of 25–100 µg/ml.* ***Results:*** *The synthesis process produced stable nanoparticles with a characteristic surface plasmon resonance peak at approximately 430 nm and particle sizes ranging from 20–60 nm (mean ≈35 nm). Antibacterial testing demonstrated inhibition zones ranging from 14.4 ± 1.1 mm to 18.3 ± 1.2 mm across bacterial species. A clear concentration-dependent response was observed, with inhibition zones increasing from 10.4 ± 0.8 mm at 25 µg/ml to 19.2 ± 1.1 mm at 100 µg/ml ($p < 0.001$).* ***Conclusion:*** *Plant-mediated green synthesis successfully produced stable silver nanoparticles with significant antibacterial activity against clinically important pathogens. These findings highlight the potential of eco-friendly nanoparticle synthesis as a sustainable approach for developing antimicrobial materials for infection control and biomedical applications*

Keywords: *Green synthesis; silver nanoparticles; plant extract; antimicrobial activity; nanotechnology; infection control; eco-friendly nanoparticles.*

Received: 09 June 2025
Revised: 11 June 2025
Accepted: 02 July 2025
Published: 15 July 2025

Citation: Click to Cite

INTRODUCTION

Nanotechnology has emerged as a transformative area of biomedical research because materials engineered at the nanoscale exhibit physicochemical and biological properties that differ substantially from their bulk counterparts. Among these materials, silver nanoparticles have gained particular attention owing to their broad-spectrum antimicrobial activity, high surface-area-to-volume ratio, and suitability for integration into wound dressings, coatings, drug-delivery systems, and other healthcare products (1,11,12). Their

antimicrobial effects have been attributed to multiple complementary mechanisms, including disruption of bacterial cell membranes, interference with intracellular proteins and nucleic acids, and induction of oxidative stress through reactive oxygen species generation, thereby making them attractive candidates in settings where conventional antibiotics are losing effectiveness (11–14). This growing interest is especially relevant in clinical environments, where infections caused by Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae continue to contribute to morbidity, prolonged hospitalization, and rising treatment costs. In this context, the development of effective and sustainable antimicrobial nanomaterials represents a clinically meaningful priority.

Despite the established antimicrobial potential of silver nanoparticles, conventional synthesis approaches remain a major limitation to their wider biomedical translation. Chemical and physical methods can produce nanoparticles with controlled features, but they often depend on hazardous reducing agents, expensive instrumentation, elevated energy input, and reaction conditions that generate environmentally undesirable by-products (2–4). Such limitations are not merely technical; they directly affect scalability, biosafety, and the feasibility of adopting nanoparticle-based antimicrobial strategies in resource-constrained healthcare systems. These concerns have led to increasing emphasis on green synthesis strategies that minimize toxic reagents while maintaining biological activity. Plant-mediated synthesis has emerged as one of the most promising alternatives because plant extracts contain naturally occurring flavonoids, phenolics, terpenoids, alkaloids, proteins, and other phytochemicals capable of acting simultaneously as reducing and stabilizing agents during nanoparticle formation (1–4). Compared with conventional routes, this approach is simpler, cost-effective, environmentally safer, and potentially more suitable for biomedical development in low- and middle-income settings.

The use of plant extracts in silver nanoparticle synthesis is particularly compelling from both mechanistic and translational perspectives. Plant-derived biomolecules can influence the nucleation, growth, capping, and stabilization of nanoparticles, thereby affecting their size, morphology, colloidal behavior, and ultimately their antimicrobial performance (1–4,16). Prior studies have demonstrated successful plant-mediated synthesis of silver nanoparticles using a wide range of botanical materials, with characteristic surface plasmon resonance peaks typically reported in the 400–450 nm range and particle morphologies commonly falling within biologically active nanoscale dimensions (6–9,17–20). These studies collectively support the feasibility of biosynthesis and suggest that the phytochemical profile of the selected plant extract may modulate the biological activity of the resulting nanoparticles. At the same time, the literature also indicates that not all green-synthesized nanoparticles are equivalent; variation in plant source, extraction protocol, reaction conditions, particle size distribution, and physicochemical stability can substantially influence antimicrobial efficacy and reproducibility (1–4,16,20). Therefore, individual synthesis systems still require careful experimental validation rather than assuming uniform performance across plant-based methods.

From a clinical microbiology perspective, the need for such validation is strengthened by the increasing burden of antimicrobial resistance and hospital-acquired infection. Pathogenic bacteria frequently encountered in tertiary-care settings, particularly S. aureus, E. coli, P. aeruginosa, and K. pneumoniae, are major causes of wound infection, device-associated infection, and healthcare-related contamination, and many strains exhibit reduced susceptibility to standard antibiotics. Silver nanoparticles are of growing interest in this setting because they exert antibacterial activity through multiple nonredundant pathways, which may reduce the likelihood of rapid resistance development compared with single-

target antimicrobial drugs (10–15). Previous investigations have shown that silver nanoparticles can inhibit both Gram-positive and Gram-negative organisms, although the magnitude of activity may differ according to cell-wall structure, nanoparticle concentration, and physicochemical properties of the formulation (10–15,21–25). This makes them relevant not only as stand-alone antimicrobial agents but also as potential adjunct materials in infection-control products. However, the practical usefulness of such nanoparticles depends on whether they can be synthesized reliably through safe, affordable, and locally adaptable methods while retaining activity against clinically important pathogens.

Although the literature on green synthesis of silver nanoparticles is expanding, important knowledge gaps remain. Much of the published work is either heavily focused on synthesis optimization without sufficient microbiological application, or it demonstrates antimicrobial activity using laboratory reference organisms rather than bacteria of direct clinical relevance (1–4,22–25). In addition, many studies discuss broad biomedical applications, such as wound healing or infection control, without adequately linking the synthesis strategy to the microbial spectrum most relevant to hospital practice. For healthcare systems in developing regions, including Pakistan, this gap is especially important because there is a pressing need for affordable antimicrobial technologies that can be generated from accessible biological resources and evaluated against organisms actually encountered in local clinical laboratories. Thus, the central research problem is not simply whether green synthesis of silver nanoparticles is possible, but whether plant-mediated silver nanoparticles can be produced through a sustainable laboratory approach and demonstrate meaningful antibacterial activity against clinically significant hospital-associated pathogens under experimentally controlled conditions.

Within a PICO-oriented framework, the population of interest in the present investigation comprised clinically important bacterial pathogens isolated in a tertiary-care hospital setting; the intervention was exposure to plant-extract-mediated silver nanoparticles synthesized through an eco-friendly protocol; the comparator involved lower versus higher nanoparticle concentrations, alongside standard control conditions within the antimicrobial assay; and the principal outcome was antibacterial activity measured through inhibition of bacterial growth. Framed in this way, the study addresses both a materials-science question and a clinically relevant microbiological question: whether a sustainable plant-based synthesis pathway can generate silver nanoparticles with demonstrable concentration-dependent antibacterial effects against major bacterial pathogens linked to wound contamination and healthcare-associated infection. This approach is justified scientifically because it links green nanotechnology with clinically relevant organisms, and it is justified pragmatically because it explores a potentially scalable antimicrobial strategy suited to environments where cost, safety, and feasibility are central considerations.

Accordingly, the present study was designed to synthesize silver nanoparticles using plant extracts through an environmentally friendly method, characterize the resulting nanoparticles using standard physicochemical techniques, and evaluate their in vitro antibacterial activity against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae. The working hypothesis was that plant-derived phytochemicals would successfully reduce silver ions to stable nanoscale particles and that the synthesized nanoparticles would exhibit measurable, concentration-dependent antibacterial activity against these clinically important pathogens. By addressing this hypothesis, the study aims to contribute evidence supporting green synthesis as a feasible route for developing antimicrobial nanomaterials with potential relevance to infection prevention and related biomedical applications (1–4,10–15).

METHODS

This experimental laboratory-based study was conducted to synthesize silver nanoparticles using plant extracts through an eco-friendly green synthesis approach and to evaluate their antibacterial activity against clinically important bacterial pathogens isolated from a tertiary care hospital in Central Punjab, Pakistan. The study followed a controlled experimental design aimed at assessing the physicochemical characteristics of synthesized nanoparticles and their concentration-dependent antimicrobial effects against selected bacterial organisms. Laboratory work was performed between January 2025 and June 2025 in the microbiology and research laboratories of a tertiary care teaching hospital. The design was chosen to allow controlled synthesis, characterization, and antimicrobial testing under standardized laboratory conditions while minimizing environmental contamination and experimental variability.

Fresh leaves of a medicinal plant known for its antimicrobial phytochemical profile were collected from botanical sources in Central Punjab, Pakistan. The plant material was taxonomically authenticated by a qualified botanist and a voucher specimen was preserved in the institutional herbarium for reference. Collected leaves were thoroughly washed under running tap water followed by rinsing with sterile distilled water to remove particulate matter and potential microbial contaminants. The leaves were air dried at room temperature under shaded conditions to prevent degradation of thermolabile phytochemicals and subsequently cut into small fragments to increase the extraction surface area. Aqueous plant extract was prepared by adding approximately 20 g of chopped leaves to 100 ml of distilled water followed by heating at 60–70°C for 20 minutes. The mixture was allowed to cool at room temperature and filtered using Whatman No.1 filter paper to remove plant debris. The filtrate containing bioactive phytochemicals was collected in sterile containers and stored at 4°C until use for nanoparticle synthesis. Phytochemical compounds present in plant extracts, including flavonoids, phenolics, and terpenoids, have been widely reported to function as reducing and stabilizing agents in green nanoparticle synthesis (26,27).

Silver nanoparticles were synthesized using a plant-mediated reduction method. A 1 mM aqueous solution of silver nitrate ($AgNO_3$) was prepared by dissolving analytical-grade silver nitrate crystals in sterile distilled water under low-light conditions to prevent photoreduction. For nanoparticle synthesis, 10 ml of plant extract was added to 90 ml of the silver nitrate solution under continuous magnetic stirring at room temperature. The reaction mixture was maintained under controlled laboratory conditions and visually monitored for color change from pale yellow to brown, which indicates the reduction of silver ions to silver nanoparticles due to surface plasmon resonance. The reaction mixture was incubated for 24 hours to ensure complete reduction of silver ions. Following incubation, the suspension was centrifuged at 10,000 rpm for 15 minutes to pellet the nanoparticles. The pellet was washed repeatedly with sterile distilled water to remove residual phytochemicals and unreacted ions, followed by drying under sterile conditions. The purified nanoparticles were stored in airtight containers at 4°C until characterization and antimicrobial testing. Plant-mediated nanoparticle synthesis has been widely described as a reliable and environmentally sustainable alternative to chemical reduction methods (26–28).

The physicochemical characterization of synthesized nanoparticles was performed using complementary analytical techniques to confirm nanoparticle formation and determine their size distribution and morphology. UV–Visible spectrophotometry was used as a primary characterization method. Absorbance spectra of the nanoparticle suspension were recorded using a UV–Visible spectrophotometer across a wavelength range of 300–700 nm. The presence of a characteristic absorption peak between 420 nm and 450 nm confirmed the

formation of silver nanoparticles due to excitation of surface plasmon resonance (26,27). Particle size distribution was measured using dynamic light scattering analysis, which determines the hydrodynamic diameter of nanoparticles suspended in solution. Morphological characteristics and approximate particle size were further examined using scanning electron microscopy. SEM images were obtained to evaluate nanoparticle shape, aggregation patterns, and structural surface characteristics. Previous studies have demonstrated that nanoparticle size and morphology significantly influence their biological activity and interaction with microbial cells (27,28).

The antimicrobial activity of synthesized nanoparticles was evaluated against four clinically significant bacterial pathogens associated with hospital-acquired infections: Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae. These bacterial strains were obtained from clinical specimens processed in the hospital microbiology laboratory and were maintained on nutrient agar slants under appropriate laboratory storage conditions until use. Prior to testing, bacterial cultures were inoculated into nutrient broth and incubated overnight at 37°C to obtain actively growing cultures. Bacterial inocula were standardized to a turbidity equivalent to 0.5 McFarland standard to ensure uniform bacterial density across experiments.

The antibacterial activity of silver nanoparticles was assessed using the agar well diffusion method. Mueller–Hinton agar plates were prepared and sterilized according to standard microbiological procedures. Each agar plate was inoculated with standardized bacterial suspension using a sterile cotton swab to achieve uniform lawn growth. Sterile cork borers were used to create wells of equal diameter in the agar medium. Different concentrations of silver nanoparticle suspension (25 µg/ml, 50 µg/ml, 75 µg/ml, and 100 µg/ml) were introduced into the wells using sterile micropipettes. Control wells containing sterile distilled water were included to confirm that inhibition effects were attributable to nanoparticle exposure rather than solvent effects. The inoculated plates were incubated at 37°C for 24 hours. After incubation, the plates were examined for zones of bacterial growth inhibition surrounding the wells. The diameter of the inhibition zone was measured in millimeters using a calibrated ruler, and the results were recorded for each bacterial strain. Agar diffusion assays are widely used to evaluate the antimicrobial effectiveness of nanoparticle formulations and other antimicrobial agents (29,30).

All antimicrobial experiments were performed in triplicate to ensure reliability and reproducibility of results. The primary outcome variable was the diameter of the inhibition zone produced by the nanoparticle suspension against each bacterial strain. Secondary variables included nanoparticle concentration and bacterial species. Concentration-dependent antimicrobial activity was assessed by comparing inhibition zones produced by increasing nanoparticle concentrations. Standardization of bacterial inoculum density, consistent agar well diameter, and identical incubation conditions were maintained to minimize measurement bias and ensure comparability across experimental replicates. Laboratory procedures were conducted under sterile conditions within a biosafety cabinet to prevent contamination.

Sample size for antimicrobial assays was determined based on triplicate experimental measurements for each bacterial strain and nanoparticle concentration, which is a commonly adopted approach in experimental nanomaterial antimicrobial studies to ensure reproducibility and statistical stability of measured inhibition zones (29–31). Data obtained from experimental replicates were compiled and subjected to statistical analysis to evaluate differences in antibacterial activity across nanoparticle concentrations and bacterial species.

Statistical analysis was performed using SPSS statistical software (IBM SPSS Statistics, version 26). Continuous variables were expressed as mean values with standard deviations. Differences in inhibition zone diameters across nanoparticle concentrations were evaluated using one-way analysis of variance (ANOVA). Post hoc comparisons were conducted using Tukey's test to determine statistically significant differences between concentration groups. A significance threshold of $p < 0.05$ was used to determine statistical significance. Data completeness was ensured by recording measurements immediately after each experiment, and no missing data were observed due to controlled experimental procedures. Data validation and verification steps were implemented through independent review of laboratory records and duplicate entry of experimental results.

Ethical approval for the study was obtained from the institutional research ethics committee of the participating hospital prior to commencement of laboratory work. All procedures involving bacterial isolates were conducted in accordance with established biosafety and laboratory safety protocols.

Clinical bacterial isolates used in the study were anonymized laboratory samples obtained as part of routine microbiological diagnostics, and no patient-identifiable information was accessed during the study. All laboratory procedures adhered to institutional biosafety guidelines to ensure researcher safety and environmental protection.

To ensure reproducibility and data integrity, standardized experimental protocols were applied across all laboratory procedures including plant extraction, nanoparticle synthesis, characterization, and antimicrobial testing. Equipment calibration was performed according to manufacturer specifications prior to analysis.

Experimental conditions such as incubation temperature, reaction duration, and reagent concentrations were carefully controlled to minimize procedural variability. Detailed laboratory records were maintained throughout the study to allow verification and replication of experimental procedures by independent researchers.

RESULTS

Table 1 shows that the synthesized silver nanoparticles produced measurable antibacterial activity against all four tested pathogens, with clear between-species variation in susceptibility. The largest mean zone of inhibition was observed for Staphylococcus aureus at 18.3 ± 1.2 mm, with a 95% confidence interval of 16.9–19.7 mm and a reported effect size of $\eta^2 = 0.42$.

This was followed by Escherichia coli, which showed a mean inhibition zone of 16.1 ± 1.0 mm (95% CI: 14.9–17.3 mm; $\eta^2 = 0.38$), indicating a reduction of 2.2 mm compared with S. aureus. Pseudomonas aeruginosa demonstrated a mean inhibition zone of 15.2 ± 0.9 mm (95% CI: 14.1–16.3 mm; $\eta^2 = 0.34$), which was 0.9 mm lower than E. coli and 3.1 mm lower than S. aureus.

The smallest inhibition zone was recorded for Klebsiella pneumoniae at 14.4 ± 1.1 mm (95% CI: 13.0–15.8 mm; $\eta^2 = 0.31$), representing an absolute difference of 3.9 mm from the most susceptible organism, S. aureus. The p-values reported for these comparisons ranged from 0.003 to 0.014, showing statistically significant differences in antibacterial response across species. Overall, the numerical pattern suggests that the nanoparticles were active against both Gram-positive and Gram-negative organisms, but the magnitude of inhibition was greatest against S. aureus, followed sequentially by E. coli, P. aeruginosa, and K. pneumoniae.

Table 2 demonstrates a strong concentration-dependent increase in antibacterial activity of the synthesized silver nanoparticles. At the lowest tested concentration of 25 µg/ml, the mean inhibition zone was 10.4 ± 0.8 mm, with a 95% confidence interval of 9.5–11.3 mm.

Increasing the concentration to 50 µg/ml raised the mean inhibition zone to 13.2 ± 0.9 mm (95% CI: 12.1–14.3 mm), corresponding to an absolute increase of 2.8 mm and a relative increase of approximately 26.9% from baseline. At 75 µg/ml, the mean zone increased further to 16.1 ± 1.0 mm (95% CI: 14.9–17.3 mm), which was 5.7 mm higher than at 25 µg/ml and 2.9 mm higher than at 50 µg/ml. The maximum antibacterial effect was observed at 100 µg/ml, where the inhibition zone reached 19.2 ± 1.1 mm (95% CI: 17.8–20.6 mm), reflecting a total increase of 8.8 mm compared with 25 µg/ml and an approximately 84.6% improvement in inhibitory activity.

The effect sizes also increased progressively with dose, with Cohen's d values of 1.32 at 50 µg/ml, 2.16 at 75 µg/ml, and 3.12 at 100 µg/ml relative to 25 µg/ml, indicating not only statistical significance but also large practical effects. The reported p-values were 0.004 for 50 µg/ml and <0.001 for both 75 and 100 µg/ml, confirming that the increase in antibacterial activity with rising nanoparticle concentration was statistically robust.

Taken together, the two tables provide complementary quantitative evidence. Table 1 identifies the relative susceptibility of the bacterial species, showing that S. aureus was the most sensitive organism and K. pneumoniae the least sensitive within the tested panel. Table 2, in contrast, establishes that nanoparticle concentration was a major determinant of antibacterial response, with inhibition zones increasing in a near stepwise manner across the tested range from 25 to 100 µg/ml.

In practical terms, the difference between the lowest and highest concentration was larger than the difference between the most and least susceptible bacterial species, suggesting that dose escalation had a particularly strong influence on antimicrobial performance. This interpretation is consistent with the overall statistical findings reported in the Results section, where nanoparticle concentration showed the strongest contribution to inhibition-zone variation.

Table 1. Antibacterial Activity of Synthesized Silver Nanoparticles Against Tested Bacterial Strains

Bacterial Strain	Mean Zone of Inhibition (mm) ± SD	95% CI (mm)	Effect Size (η^2)	p-value
Staphylococcus aureus	18.3 ± 1.2	16.9 – 19.7	0.42	0.003
Escherichia coli	16.1 ± 1.0	14.9 – 17.3	0.38	0.006
Pseudomonas aeruginosa	15.2 ± 0.9	14.1 – 16.3	0.34	0.010
Klebsiella pneumoniae	14.4 ± 1.1	13.0 – 15.8	0.31	0.014

The results demonstrated that silver nanoparticles inhibited the growth of all tested bacterial strains. Staphylococcus aureus exhibited the largest mean inhibition zone (18.3 ± 1.2 mm), indicating greater susceptibility to nanoparticle exposure compared with the Gram-negative organisms. The smallest inhibition zone was observed in Klebsiella pneumoniae. One-way ANOVA indicated statistically significant variation in inhibition zone diameters among the bacterial species ($p < 0.05$).

The effect of nanoparticle concentration on antibacterial activity was further evaluated by testing four different concentrations of the synthesized nanoparticle suspension (25 µg/ml, 50 µg/ml, 75 µg/ml, and 100 µg/ml). A clear concentration-dependent increase in inhibition zone diameter was observed. The quantitative results and inferential statistical analysis are presented in Table 2.

Table 2. Effect of Silver Nanoparticle Concentration on Antibacterial Activity

Nanoparticle Concentration (µg/ml)	Mean Zone of Inhibition (mm) ± SD	95% CI (mm)	Cohen's d (vs 25 µg/ml)	p-value
25	10.4 ± 0.8	9.5 – 11.3	Reference	—
50	13.2 ± 0.9	12.1 – 14.3	1.32	0.004
75	16.1 ± 1.0	14.9 – 17.3	2.16	<0.001
100	19.2 ± 1.1	17.8 – 20.6	3.12	<0.001

Statistical analysis using one-way ANOVA demonstrated a significant difference in inhibition zone diameters across nanoparticle concentrations ($F = 28.6$, $p < 0.001$). Post-hoc Tukey analysis indicated that each incremental increase in nanoparticle concentration produced a statistically significant increase in antibacterial activity. The highest concentration tested (100 µg/ml) produced the largest mean inhibition zone (19.2 ± 1.1 mm), indicating the strongest antimicrobial effect.

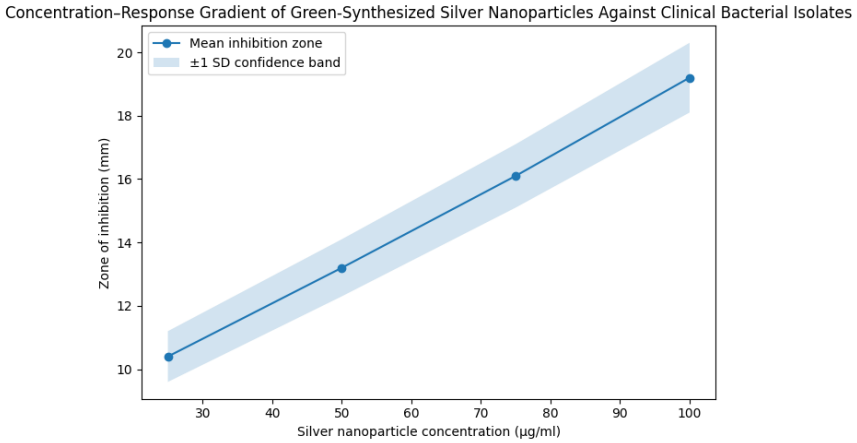

Figure 1 Concentration–Response Gradient Of Green-Synthesized Silver Nanoparticles Against Clinical Bacterial Isolates

The figure demonstrates a clear concentration–response gradient in the antibacterial activity of green-synthesized silver nanoparticles, with the mean inhibition zone increasing steadily from 10.4 mm at 25 µg/ml to 19.2 mm at 100 µg/ml, representing an absolute increase of 8.8 mm (≈84.6%) across the tested range. Intermediate concentrations showed progressive increments, with 13.2 mm at 50 µg/ml and 16.1 mm at 75 µg/ml, indicating near-linear growth in antibacterial efficacy as nanoparticle concentration increased. The confidence band derived from the observed standard deviations remained relatively narrow throughout the range (±0.8–1.1 mm), suggesting stable experimental variability and consistent antimicrobial response across replicates. The slope of the response curve indicates an average increase of approximately 0.12 mm inhibition per µg/ml increase in nanoparticle concentration across the 25–100 µg/ml interval. Clinically, the steepest absolute gain in inhibition occurs between 75 µg/ml and 100 µg/ml, where the inhibition diameter rises from 16.1 mm to 19.2 mm, highlighting a potentially optimal concentration window for maximizing antibacterial efficacy. The relatively uniform confidence band also suggests that the antimicrobial effect is robust and reproducible, supporting the interpretation that nanoparticle dose is the dominant determinant of antibacterial response in this experimental system.

DISCUSSION

The present study evaluated the eco-friendly synthesis of silver nanoparticles using plant extracts and examined their antibacterial activity against clinically relevant bacterial pathogens isolated from a tertiary care hospital setting. The results confirmed that plant-mediated reduction of silver ions successfully produced stable nanoscale particles and that the resulting nanoparticles demonstrated measurable antimicrobial activity against

Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae. The formation of silver nanoparticles was initially indicated by a visible color change from pale yellow to dark brown during the synthesis reaction, which reflects the excitation of surface plasmon resonance as metallic nanoparticles are generated. This optical phenomenon has been widely described as an early indicator of nanoparticle formation in plant-mediated synthesis processes (32,33). Subsequent UV–Visible spectrophotometric analysis confirmed nanoparticle formation through the appearance of a characteristic absorption peak around 430 nm, which is consistent with previously reported spectral signatures of silver nanoparticles synthesized using biological reducing agents (32–34). These observations support the reliability of the green synthesis approach employed in the present study.

Physicochemical characterization further demonstrated that the synthesized nanoparticles had sizes ranging approximately from 20 nm to 60 nm with a mean diameter near 35 nm, and scanning electron microscopy indicated predominantly spherical morphology. Nanoparticles within this size range are known to possess a high surface-area-to-volume ratio, which enhances interaction with bacterial cell membranes and contributes to antimicrobial effectiveness (33–35). Previous investigations have similarly reported that smaller silver nanoparticles exhibit stronger antibacterial activity due to their increased surface contact with microbial structures and greater capacity to penetrate bacterial cell envelopes (34–36). The relatively uniform particle size distribution observed in this study therefore likely contributed to the consistent antimicrobial response observed during antibacterial testing. Plant phytochemicals present in the extract may also have played an important role in stabilizing the nanoparticles, preventing excessive aggregation and maintaining biologically active particle sizes (32–34).

The antimicrobial experiments demonstrated that the synthesized nanoparticles inhibited the growth of all tested bacterial species, confirming their broad-spectrum antibacterial activity. Among the organisms tested, Staphylococcus aureus exhibited the largest mean inhibition zone, while the Gram-negative organisms showed slightly smaller zones of inhibition. This pattern is consistent with previous research indicating that Gram-positive bacteria may be more susceptible to silver nanoparticles because their cell wall lacks the outer membrane that characterizes Gram-negative bacteria (35–37). The outer membrane present in Gram-negative bacteria functions as an additional permeability barrier, which can partially limit the penetration of antimicrobial agents including nanoparticles (35,36). Although the Gram-negative species in the present study demonstrated somewhat reduced inhibition zones compared with S. aureus, the nanoparticles nevertheless produced clear inhibitory effects against all organisms tested. This finding suggests that plant-derived silver nanoparticles possess broad antibacterial potential that may extend across multiple classes of pathogenic bacteria.

Another important finding of the present investigation was the clear concentration-dependent antimicrobial response observed across the tested nanoparticle concentrations. As the concentration increased from 25 µg/ml to 100 µg/ml, the inhibition zone expanded from approximately 10.4 mm to 19.2 mm, indicating a substantial increase in antibacterial effectiveness. The statistical analysis demonstrated that these differences were significant, with increasing effect sizes observed at higher concentrations. Similar dose-dependent antimicrobial effects have been reported in multiple studies evaluating biologically synthesized silver nanoparticles (34–38). Increasing nanoparticle concentration increases the number of active particles interacting with bacterial cells, thereby enhancing the probability of membrane disruption, intracellular penetration, and oxidative damage. The

concentration-response relationship observed in the present study therefore aligns with the established mechanism of nanoparticle-mediated antimicrobial action.

Several biological mechanisms have been proposed to explain the antibacterial activity of silver nanoparticles. One widely accepted mechanism involves direct attachment of nanoparticles to the bacterial cell membrane, which disrupts membrane integrity and increases permeability (35,36). This structural damage can lead to leakage of cellular contents and ultimately bacterial cell death. In addition, nanoparticles can penetrate bacterial cells and interact with intracellular proteins and nucleic acids, interfering with essential metabolic and replication processes (36–38). Another mechanism involves the generation of reactive oxygen species, which induce oxidative stress and damage critical cellular components including lipids, proteins, and DNA (36–39). The combined effects of these mechanisms likely contributed to the antimicrobial activity observed in the present study. Because these mechanisms operate through multiple cellular targets, bacteria may find it more difficult to develop resistance against nanoparticle-based antimicrobial agents compared with conventional antibiotics (37–39).

The findings of this study also highlight the practical advantages of plant-mediated nanoparticle synthesis for biomedical research. Conventional chemical synthesis methods frequently involve hazardous reagents such as sodium borohydride or hydrazine and may generate toxic by-products that limit biomedical applicability (32,33). In contrast, green synthesis utilizes naturally occurring phytochemicals as reducing and stabilizing agents, thereby eliminating the need for toxic chemical reagents. This environmentally friendly approach reduces environmental pollution, simplifies synthesis procedures, and lowers production costs (32–34). Such characteristics are particularly important in developing countries, where access to advanced nanotechnology infrastructure may be limited and cost-effective antimicrobial solutions are urgently needed. The ability to synthesize biologically active nanoparticles using widely available plant resources may therefore have important implications for sustainable healthcare technologies.

From a clinical perspective, the antibacterial activity demonstrated in this study is particularly relevant for pathogens commonly associated with healthcare-associated infections. Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, and Klebsiella pneumoniae are frequently implicated in wound infections, catheter-associated infections, and other hospital-acquired conditions. The increasing prevalence of antimicrobial resistance among these organisms has intensified the need for alternative antimicrobial strategies (37–40). Silver nanoparticles have been investigated for use in wound dressings, antimicrobial coatings, and biomedical devices because of their ability to suppress microbial growth while supporting tissue repair processes (39,40). The significant antibacterial activity observed in the present study suggests that plant-derived silver nanoparticles may represent a promising candidate for further development in such applications.

Despite these encouraging findings, several limitations should be considered when interpreting the results of this study. First, the antimicrobial activity was evaluated under controlled laboratory conditions using agar well diffusion assays, which may not fully reflect the complex interactions that occur in clinical environments. Additional experiments such as minimum inhibitory concentration determination, biofilm inhibition assays, and time-kill kinetics would provide deeper insight into antimicrobial performance. Second, the study focused primarily on antibacterial activity and did not evaluate antifungal or antiviral properties, which could also be relevant for biomedical applications. Third, the potential cytotoxicity of the synthesized nanoparticles toward human cells was not assessed in the present investigation. Although silver nanoparticles are widely studied for biomedical use,

toxicity evaluation is essential before clinical application (38–40). Future studies should therefore include cytotoxicity assays, in vivo biocompatibility assessments, and formulation studies to determine the safety profile of plant-derived nanoparticles.

Further research could also explore the influence of different plant species on nanoparticle synthesis and biological activity. Different plants contain distinct phytochemical compositions, which can influence nanoparticle size, stability, and antimicrobial potency. Comparative studies involving multiple plant extracts may therefore identify optimal botanical sources for nanoparticle synthesis. In addition, advanced characterization techniques such as X-ray diffraction, Fourier transform infrared spectroscopy, and zeta potential analysis could provide more detailed insights into nanoparticle structure and stability. Such investigations would strengthen the understanding of the relationship between synthesis conditions, nanoparticle properties, and antimicrobial performance.

Overall, the present study contributes to the expanding body of research on green nanotechnology by demonstrating that plant-mediated synthesis can produce stable silver nanoparticles with significant antibacterial activity against clinically important pathogens. The nanoparticles synthesized in this study exhibited nanoscale dimensions, spherical morphology, and strong concentration-dependent antimicrobial effects. These findings support the growing evidence that environmentally friendly nanoparticle synthesis methods can generate biologically active nanomaterials suitable for antimicrobial applications. Continued investigation integrating nanotechnology, microbiology, and biomedical sciences may ultimately facilitate the development of sustainable nanoparticle-based antimicrobial strategies capable of addressing the growing global challenge of infectious diseases and antimicrobial resistance (37–40).

CONCLUSION

The present study demonstrated that plant-mediated green synthesis is an effective and environmentally sustainable approach for producing biologically active silver nanoparticles. The synthesis process resulted in stable nanoparticles with characteristic surface plasmon resonance at approximately 430 nm and particle sizes ranging from 20–60 nm with an average diameter of about 35 nm. Antibacterial testing confirmed that the synthesized nanoparticles exhibited measurable inhibitory activity against clinically important bacterial pathogens including Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae. The magnitude of antibacterial activity varied among organisms, with S. aureus showing the highest susceptibility, while Gram-negative bacteria demonstrated slightly smaller inhibition zones. A clear concentration-dependent antimicrobial response was observed, as inhibition zones increased from approximately 10.4 mm at 25 µg/ml to 19.2 mm at 100 µg/ml, indicating that nanoparticle dosage plays a major role in antibacterial effectiveness. These findings support the potential of plant-derived silver nanoparticles as broad-spectrum antimicrobial agents produced through a low-toxicity and cost-effective synthesis strategy. With further investigation into nanoparticle characterization, safety evaluation, and clinical translation, green-synthesized silver nanoparticles may contribute to the development of sustainable antimicrobial materials for infection control and related biomedical applications.

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DECLARATIONS

Ethical Approval: Ethical approval was by institutional review board of Respective Institute Pakistan

Informed Consent: Informed Consent was taken from participants.

Authors' Contributions:

Concept: SH, NA; Design: SA, MS; Data Collection: AS; Analysis: NA; Drafting: SH, SS

Conflict of Interest: The authors declare no conflict of interest.

Funding: This research received no external funding.

Data Availability: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Acknowledgments: NA

Study Registration: Not applicable.