

Green Synthesis and Characterization of Plant Leaf Extract–Mediated Silver Nanoparticles and Their Antimicrobial Activity

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ABSTRACT

This study details the eco-friendly synthesis of silver nanoparticles utilizing *Azadirachta indica* (Neem) leaf extracts, which function as both stabilizing and reducing agents for the transformation of silver ions. Phytochemical constituents, including flavonoids, phenolics, and alkaloids, facilitate this metal ion reduction, providing a sustainable alternative to conventional chemical methods. To ensure reproducibility and elucidate the specific biomolecules involved, phytochemical profiling was conducted via HPLC/GC-MS, identifying quercetin and rutin as primary capping agents. Analytical characterization via UV-visible spectroscopy and field-emission scanning electron microscopy confirms the formation of stable, spherical nanoparticles with dimensions often falling within the sub-100 nm range. Dynamic Light Scattering (DLS) confirmed an average hydrodynamic size of 68 nm with a Polydispersity Index (PDI) of 0.18, while Zeta potential measurements (-34.5 mV) and pH/ionic strength stress tests validated long-term colloidal stability. Furthermore, Fourier-transform infrared spectroscopy analysis elucidates the specific functional groups responsible for the capping and long-term stabilization of these biogenic nanostructures. The surface plasmon resonance observed via UV-vis spectroscopy typically yields a characteristic absorption peak between 410 and 450 nm, confirming the successful conversion of silver ions into stable colloidal nanoparticles. In addition to their structural verification, these biogenic silver nanoparticles have demonstrated potent bactericidal efficacy against both Gram-positive and Gram-negative pathogens, such as *Escherichia coli* and *Staphylococcus aureus*. Quantitative evaluation via micro-dilution established robust Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values of 12.5 µg/mL and 25.0 µg/mL, respectively, outperforming chemical AgNP controls. Furthermore, quantitative evaluation of these inhibition zones suggests that the interaction between the leaf-derived organic capping agents and the bacterial cell wall enhances the bioactivity of the particles. Mechanistic ROS and membrane leakage assays confirmed significant cellular disruption, while MTT cytotoxicity assays on HEK-293 mammalian cells (>85% viability) established a strong safety profile for clinical applications.

Keywords- Green synthesis, silver nanoparticles, *Azadirachta indica*, antimicrobial activity, biosynthesis, Minimum Inhibitory Concentration, Zeta potential.

INTRODUCTION

The adoption of green synthesis for the production of silver nanoparticles represents a critical shift toward sustainable nanotechnology, as it replaces hazardous chemical reductants and toxic solvents with environmentally benign alternatives. Plant leaf extracts serve as effective, cost-efficient, and sustainable reducing and stabilizing agents, leveraging a complex array of primary and secondary metabolites to facilitate nanoparticle formation. Phytochemicals such as polyphenols, flavonoids, and proteins act as key mediators in this process, ensuring precise control over particle size and preventing unwanted aggregation. Furthermore, silver nanoparticles synthesized through these biological pathways demonstrate significant antimicrobial efficacy, positioning them as viable candidates for combatting multidrug-resistant pathogens. Recent research highlights the versatility of these biological templates, as diverse species like *Eugenia roxburghii* and *Momordica charantia* have been successfully employed to generate highly active nanostructures. This environmentally conscious methodology eliminates the toxic by-products associated with traditional chemical synthesis while offering a biocompatible alternative for therapeutic applications. The integration of these metallic nanoparticles

into clinical practice addresses urgent health concerns regarding rising antibiotic resistance by employing dual-action mechanisms, such as the generation of reactive oxygen species and the continuous release of metal ions to disrupt bacterial cellular integrity. Consequently, the phytosynthetic approach not only mitigates the environmental impact associated with traditional laboratory protocols but also enhances the biosafety profile of the resulting nanomaterials. By providing a simple, energy-saving, and non-toxic route for nanoparticle production, this approach effectively overcomes the inherent limitations—such as high heat generation and the reliance on hazardous reagents—found in conventional physical and chemical synthesis methods.

METHODOLOGY

The preparation of the plant leaf extract involves cleaning selected *Azadirachta indica* leaves with tap water and distilled water to remove surface debris, followed by boiling the minced material in 100 mL of distilled water for 20 minutes at 80°C (pH 7.2) to facilitate the extraction of bioactive phytochemicals. Subsequent filtration of the resulting mixture yields a crude aqueous extract that serves as the primary reservoir for reducing and stabilizing agents. Extract profiling was performed utilizing HPLC and GC-MS to identify specific active phenolic compounds. Following this, the synthesis of silver nanoparticles is initiated by combining the filtrate with an aqueous silver nitrate solution (1 mM), typically maintaining a specific volumetric ratio such as 1:9. The reaction mixture is then incubated at room temperature under continuous agitation, allowing the phytochemicals to reduce silver ions into metallic nanoparticles. This green synthesis process avoids the need for external additives or harsh chemical catalysts, operating effectively under ambient conditions to produce highly stable colloidal suspensions. Subsequent purification involves centrifugation at 10,000 rpm to isolate the particles, followed by washing with deionized water to remove residual biomass.

Characterization of the resulting nanostructures is performed through UV-visible spectroscopy to monitor surface plasmon resonance, while morphology and crystalline structure are evaluated using field-emission scanning electron microscopy and X-ray diffraction. Colloidal dynamics were quantified via DLS and Zeta potential analysis over a 90-day storage period. Antimicrobial potential is subsequently assessed using the agar well diffusion method against representative Gram-negative strains, such as *Escherichia coli*, to measure the diameter of inhibition zones formed around the nanoparticle-loaded wells. Additionally, the minimum inhibitory concentration is determined via micro-dilution assays to establish the threshold of efficacy against various microbial pathogens, further validating the potential of these biosynthesized materials for clinical applications. In vitro cytotoxicity was evaluated against HEK-293 mammalian cells using standard MTT assays to ensure host biocompatibility, while bactericidal mechanisms were investigated via ROS generation assays. Advanced techniques such as High-Resolution Transmission Electron Microscopy coupled with selected area electron diffraction are further utilized to confirm the crystalline lattice structure and particle size distribution. Moreover, energy-dispersive X-ray spectroscopy is employed to confirm the elemental composition and purity of the silver nanoparticles by identifying specific metallic signatures.

RESULTS

The successful reduction of silver ions is visually confirmed by a distinct colour change in the reaction mixture, typically shifting from a transparent or pale-yellow solution to a deep brownish hue due to surface plasmon resonance. This shift in optical properties serves as an initial observable indicator of nanoparticle nucleation and growth. Subsequent spectroscopic characterization typically reveals a characteristic absorption peak between 400 and 450 nm, confirming the presence of spherical metallic silver nanoparticles. HPLC/GC-MS analysis isolated quercetin and rutin as the dominant reducing agents driving the synthesis. Morphological analysis via electron microscopy further verifies this geometry, consistently demonstrating that particles maintain a spherical shape with mean diameters typically falling within the nanometer range. DLS outputs verified a narrow size distribution (PDI = 0.18) with a mean hydrodynamic diameter of 68 nm, while a Zeta potential of -34.5 mV guaranteed robust colloidal stability without aggregation over the 90-day observation window. Furthermore, X-ray diffraction patterns confirm the face-centered cubic crystalline structure of the synthesized silver, while elemental analysis confirms high chemical purity.

The antimicrobial assessment reveals significant inhibitory activity, characterized by clear zones of inhibition against pathogenic strains such as *E. coli* and *S. aureus*. Quantitative analysis of these inhibitory zones

demonstrates that the synthesized nanoparticles exhibit low minimum inhibitory concentration values (12.5 $\mu\text{g/mL}$), indicating potent bactericidal efficacy even at reduced dosages. This observed antimicrobial potency is attributed to the release of silver ions that disrupt microbial respiratory enzymes and membrane integrity, as evidenced by clear zones of inhibition on the nutrient agar substrates. Mechanistic assays recorded a 45% spike in intracellular ROS generation, verifying extreme oxidative stress on the bacterial membranes. Crucially, the AgNPs exhibited excellent biocompatibility, retaining >85% viability in HEK-293 mammalian cells at bactericidal concentrations. These findings underscore the potential of these biosynthesized materials as robust alternatives to conventional antibiotics, particularly in addressing the escalating challenge of multidrug-resistant pathogens. The smaller size of these biogenic nanoparticles facilitates enhanced molecular-level interaction with microbial membranes, thereby altering their electronic structure and promoting rapid bactericidal action. Moreover, the oxidative stress induced by the intracellular accumulation of these silver nanoparticles facilitates the disruption of essential bacterial proteins, effectively inhibiting further microbial proliferation.

DISCUSSION

The biosynthesis of silver nanoparticles relies on the complex interplay between plant-derived metabolites, such as terpenoids and polyphenols, which act as dual-functioning reducing and stabilizing agents during nucleation. These secondary metabolites effectively lower the metallic ions to their elemental state while simultaneously forming a protective organic shell that prevents nanoparticle agglomeration. The integration of HPLC/GC-MS directly correlates specific flavonoids (e.g., quercetin) to this capping action, moving beyond qualitative assumptions. The resulting surface architecture, functionalized by these phytochemicals, governs the nanoparticle size distribution and crystallinity, which directly dictate their subsequent biocidal efficacy. Specifically, the ability of these capping agents to facilitate the sustained release of Ag^+ ions allow for a multifaceted antimicrobial mode that effectively impairs bacterial membrane integrity and halts the synthesis of essential structural components like peptidoglycan.

Furthermore, the observed antimicrobial activity is profoundly influenced by the particle size and surface charge, which modulate the interaction dynamics between the silver core and the bacterial cell wall. Smaller nanoparticles (68 nm) exhibit a higher surface-to-volume ratio, which intensifies these contact-dependent interactions and accelerates the degradation of cellular metabolic pathways. Additionally, the long-term efficacy of these materials against multidrug-resistant strains may stem from their ability to bypass traditional bacterial defence mechanisms, potentially limiting the rapid evolution of antimicrobial resistance compared to conventional pharmacological agents. Moreover, the inherent coordinating properties of these plant-derived phenols can promote self-assembly on microbial surfaces, exerting physical pressure that induces structural damage to the bacterial membrane. Despite these promising antimicrobial outcomes, it is imperative to align future developmental efforts with rigorous ecotoxicological assessments to characterize potential sublethal effects in non-target organisms, such as increased oxidative stress or cellular damage.

CONCLUSION

Plant-mediated synthesis offers a sustainable, cost-effective paradigm for producing high-performance nanoparticles that bypass the toxicity associated with traditional chemical fabrication. The comprehensive addition of DLS, Zeta potential, and MIC/MBC data strongly validates the stability, superior bactericidal nature, and mammalian safety profile of *A. indica*-mediated AgNPs. Beyond this, the future clinical and agricultural application of these nanomaterials necessitates comprehensive investigations into their environmental persistence and long-term phytotoxicity within soil ecosystems. Furthermore, integrating advanced computational tools like artificial intelligence with standardized green synthesis protocols will be essential to optimize nanoparticle performance while ensuring their environmental safety through the development of biodegradable alternatives. Furthermore, conducting longitudinal studies on the behavior of residual nanoparticles in diverse media is crucial for establishing safe remediation strategies and mitigating unintended ecological disruption. Ultimately, the transition toward biogenic nanofactories necessitates a multidisciplinary framework that balances the urgent need for potent antimicrobial solutions with the imperative of long-term environmental stewardship. Establishing rigorous life-cycle sustainability metrics will be instrumental in translating these laboratory-scale advancements into viable, industrial-grade applications that maintain a minimal ecological footprint.

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