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Biogenic Silver Nanoparticles as a Sustainable Surrogate to Conventional Antibiotics: Synthesis, Characterization, and Mechanistic Insights into Antibacterial Activity

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Abstract

Antimicrobial resistance (AMR) continues to compromise the effectiveness of existing therapies, highlighting the need for alternative antimicrobial agents. This study reports the green synthesis of biogenic silver nanoparticles (AgNPs) using an aqueous plant extract as a natural reducing and stabilizing system. The synthesis was carried out using 1 mM $AgNO_3$ with a 1:4 (v/v) ratio of plant extract to metal precursor under controlled conditions (pH 8.0, 60 °C, 45 min). UV-Visible spectroscopy confirmed AgNP formation through a surface plasmon resonance peak at 420 nm, with a full width at half maximum indicative of narrow size distribution. Fourier transform infrared spectroscopy analysis identified functional groups associated with phytochemicals responsible for reduction and capping, while X-ray diffraction revealed characteristic Bragg reflections corresponding to crystalline face-centered cubic silver. Transmission electron microscopy showed predominantly spherical nanoparticles with a mean diameter of 15-25 nm, supported by a size-distribution histogram and a polydispersity index of 0.21, confirming moderate monodispersity.

The antimicrobial activity of the AgNPs was assessed against multidrug-resistant *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*), yielding minimum inhibitory concentration values of 8-16 µg/mL. These results demonstrate notable antibacterial efficacy but are interpreted without comparison to conventional antibiotics in the absence of direct experimental data. Cytotoxicity evaluation on mammalian fibroblasts showed >80% cell viability at concentrations up to 20 µg/mL, indicating acceptable biocompatibility within the tested range.

Overall, the study establishes a well-characterized and environmentally sustainable route for AgNP synthesis and demonstrates their potential as supplementary antimicrobial agents in the context of rising AMR.

Keywords

Biogenic silver nanoparticles, Green synthesis, Antibiotic surrogate, Antimicrobial resistance, Reactive oxygen species, Nanotechnology-based therapeutics

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1. Introduction

1.1 Background and Rationale

The escalating threat of antimicrobial resistance (AMR) represents one of the most critical challenges to global public health in the 21st century. AMR occurs when pathogenic microorganisms such as bacteria, viruses, fungi, and parasites undergo genetic changes that render them less susceptible or entirely resistant to antimicrobial agents [1]. This phenomenon undermines the effectiveness of antibiotics, leading to persistent infections that are increasingly difficult to treat [2]. The World Health Organization (WHO) identifies AMR as a top global health threat, with an estimated 1.27 million deaths directly attributed to drug-resistant infections in 2019 and an additional 4.95 million deaths associated with AMR-related complications. The burden is disproportionately higher in low- and middle-income countries due to limited healthcare resources, inappropriate antimicrobial use, and inadequate infection control measures. Beyond clinical consequences, AMR contributes to substantial socioeconomic strain by increasing treatment costs, extending hospital stays, and compromising the safety of medical procedures such as surgeries and cancer therapies [3].

Given the growing inadequacy of conventional therapeutic strategies, the exploration of alternative approaches has become essential. Nanotechnology has emerged as a promising avenue for developing novel antimicrobial agents [3]. Among nanomaterials, silver nanoparticles (AgNPs) have gained attention due to their broad-spectrum antimicrobial properties and activity against multidrug-resistant pathogens. However, their toxicity is not universally low; rather, it depends on parameters such as nanoparticle size, concentration, and surface chemistry. AgNPs exert antimicrobial effects through multiple mechanisms, including membrane disruption, reactive oxygen species generation, and interference with microbial DNA and protein functions, which reduces the likelihood of resistance development [4,5].

Traditional chemical synthesis methods for AgNPs often involve harsh reagents and conditions, raising environmental and safety concerns that limit their widespread use [6]. In contrast, biogenic synthesis using plant extracts offers a more sustainable alternative. This approach employs phytochemicals–such as flavonoids, phenolics, tannins, and alkaloids–to reduce silver ions and stabilize the resulting nanoparticles, enhancing stability and biological activity [7]. The biomolecular coatings imparted through green synthesis can also improve antimicrobial performance and biocompatibility, aligning with the principles of environmentally conscious nanomedicine. Together, these developments highlight the potential of biogenic AgNPs to address current AMR challenges while reducing ecological and health risks associated with conventional nanoparticle production [8].

Recent industrial and regulatory developments further underscore the importance of advancing alternative antimicrobial technologies. The WHO's 2025 Global Antibiotic Resistance Surveillance Report indicates a 40% increase in resistance among major Gram-negative pathogens, including *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*), between 2018 and 2023, especially in low-income nations with limited diagnostics and therapeutics [9]. Regulatory authorities such as the U.S. food and drug administration have issued updated guidelines for nanomaterial-based drug products, outlining safety and efficacy requirements for nano-antibiotics. Additionally, industry initiatives promoting responsible antibiotic manufacturing aim to minimize environmental drivers of AMR. Within this context, biogenic AgNPs–owing to their multi-target antimicrobial mechanisms and reduced reliance on toxic synthesis reagents–represent a scientifically and environmentally sound alternative to traditional antimicrobial agents [10].

1.2 Literature Review

A consolidated overview of recent research on biogenic AgNPs is presented in Table 1, summarizing the plant or biological source, synthesis approach, antibacterial performance, and mechanisms of action reported in the literature. These studies collectively demonstrate that biogenic routes are capable of producing AgNPs with substantial antimicrobial activity; however, the degree of efficacy and biocompatibility varies according to nanoparticle properties such as size, concentration, morphology, and the nature of phytochemical or biomolecular capping agents reported in each study. For example, Gherasim et al. and Wahab et al. highlight the role of reactive oxygen species generation and cell wall interference, whereas Fahim et al. and Rodrigues et al. report broader mechanistic pathways including protein denaturation, DNA interaction, and disruption of electron transport processes [2,4,5,7]. Ibrahim et al. further demonstrates that biologically derived nanoparticles synthesized using *Candida parapsilosis* exhibit notable activity against multidrug-resistant pathogens, though their effects, like those of plant-derived AgNPs, depend on structural and surface characteristics [11]. AgNP synthesis and antibacterial mechanism given in Figure 1.

Overall, the literature indicates that while biogenic AgNPs hold strong potential as antimicrobial agents, their performance and safety profiles are not uniform; rather, they are determined by synthesis conditions, biological precursors, and resulting nanoparticle attributes. This underscores the importance of detailed physicochemical characterization when evaluating their suitability for antimicrobial applications.

Several significant knowledge gaps persist in the field of biogenic AgNPs, particularly regarding variability in synthesis protocols, the lack of standardized characterization methods, and an incomplete understanding of their precise antimicrobial mechanisms [12]. The biogenic synthesis approach, whether using plant extracts, bacteria, fungi, or algae, involves multiple operational parameters such as temperature, pH, reactant concentrations, and reaction time. These factors interact in complex ways, producing nanoparticles with inconsistent size, shape, and polydispersity, which

directly undermine reproducibility and complicate meaningful comparison across studies. Although systematic control of these parameters is recognized as essential, many reported protocols still exhibit considerable inconsistency [13].

Table 1. Literature review of the work under evaluation.

Plant Source	Synthesis Method	Antibacterial Activity	Mechanism of Action	Ref.
Various plants	Green synthesis	Strong against *E. coli* and *S. aureus*	Reactive oxygen species generation, membrane disruption	[4]
Medicinal plants	Plant-mediated	Effective against multiple drug resistance bacteria	Inhibition of cell wall synthesis	[5]
Diverse plants	Biogenic synthesis	Broad-spectrum activity	Protein denaturation, DNA binding	[7]
Multiple plant sources	Green synthesis	Potent against clinical isolates	Disruption of electron transport chain	[2]
Candida parapsilosis	Fungal-mediated	Effective against multiple drug resistance pathogens	Membrane destabilization	[11]

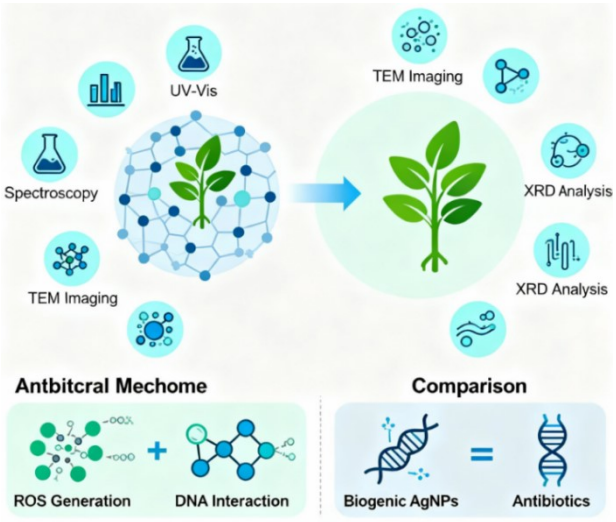

Figure 1. Plant extract-mediated AgNP synthesis and antibacterial mechanism.

1.3 Research Gap

The absence of universally accepted characterization standards further exacerbates this challenge. While techniques such as ultraviolet visible (UV-Vis) spectroscopy, X-Ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), transmission electron microscope (TEM), and dynamic light scattering (DLS) are widely used, variations in sample preparation, measurement conditions, and data interpretation yield non-comparable physicochemical profiles [14]. This lack of standardization not only impedes reliable assessment of nanoparticle properties but also restricts accurate correlation between structural features and biological activity.

Consequently, mechanistic understanding remains fragmented. Although mechanisms such as reactive oxygen species generation, membrane disruption, protein denaturation, and DNA damage have been proposed, the absence of reproducibly synthesized and consistently characterized nanoparticles limits the ability to delineate these pathways at the molecular level. Many studies rely on indirect evidence, underscoring the need for advanced analytical techniques such as proteomics and genomics to clarify nanoparticle-microbe interactions [14].

Finally, the transition from laboratory findings to clinical or commercial applications remains constrained. Inconsistent synthesis outcomes, undefined characterization benchmarks, unresolved questions about safety and cytotoxicity, and regulatory barriers collectively hinder clinical translation. Establishing robust, standardized, and reproducible biogenic AgNP production procedures, including those aligned with Good Manufacturing Practice guidelines, remains a critical unmet need limiting wider therapeutic adoption.

1.4 Objectives and Hypothesis

The present study is designed to synthesize AgNPs utilizing *Azadirachta indica* plant extract, followed by comprehensive characterization of their physicochemical properties, evaluation of their antibacterial efficacy against multidrug-resistant pathogens, and elucidation of the underlying mechanisms of action. The biosynthesis of AgNPs via plant extracts represents a green, cost-effective, and environmentally benign alternative to conventional chemical

synthesis methods, leveraging the reducing and stabilizing capacities of diverse phytochemicals such as flavonoids, phenolics, and alkaloids present in plants. This approach not only minimizes the use of hazardous reagents but also enhances nanoparticle biocompatibility and bioactivity.

Characterization of the synthesized nanoparticles is fundamental for determining critical parameters such as particle size, shape, surface charge, crystalline structure, and functional groups, employing techniques including UV-Vis spectroscopy, TEM, FTIR, DLS, and XRD. These attributes directly influence the nanoparticles' stability, dispersity, and antimicrobial potency [7].

The antibacterial efficacy of biogenic AgNPs is assessed against clinically relevant multidrug-resistant bacteria using standardized assays, including minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determinations, biofilm inhibition studies, and reactive oxygen species (ROS) generation assays. Through these bioassays, the study seeks to establish the comparative advantage of biogenic AgNPs over chemically synthesized counterparts) [15].

It is hypothesized that biogenic AgNPs will demonstrate superior antimicrobial activity primarily mediated via enhanced ROS generation and efficient disruption of bacterial membranes, mechanisms supported by recent literature [4,5,7]. The presence of plant-derived phytochemicals on the nanoparticle surface is anticipated to potentiate these effects through synergistic biochemical interactions, thereby offering a promising avenue for combating antibiotic resistance. This integrative approach aims to advance the understanding of green-synthesized nanomaterials as effective and sustainable antimicrobial agents.

2. Materials and Methods

Analytical grade silver nitrate ($AgNO_3$) with a purity of ≥99% was procured from Sigma-Aldrich (Bengaluru, Karnataka, India) and utilized as the precursor for silver ions in the synthesis of AgNPs. Fresh leaves of the selected plant species, *Azadirachta indica*, were harvested from local markets of Kolkata, West Bengol, India. Upon collection, the leaves were meticulously washed multiple times using distilled water to eliminate surface contaminants such as dust particles, microbial flora, and other impurities that could interfere with nanoparticle synthesis. Microbiological media, including nutrient broth, Mueller-Hinton agar, and phosphate-buffered saline, utilized for antibacterial and cytotoxicity assays, were acquired from HiMedia Laboratories (Bengaluru, Karnataka, India). Additionally, reagents essential for ROS and cytotoxicity assays, specifically DCFH-DA and MTT assay kits, were obtained from Thermo Fisher Scientific (Waltham, MA, USA). All experimental solutions were prepared fresh using double-distilled water obtained from a double distillation unit (Borosil Glass Works Ltd., Mumbai, India) to ensure purity and consistency.

The plant extract was prepared following a standardized protocol aimed at maximizing phytochemical content while maintaining stability. Initially, the collected leaves were air-dried at ambient room temperature for 48 hours to reduce moisture content gently without degrading thermo-sensitive biomolecules. The dried leaves were then ground into a fine powder using a sterile grinder. A weighed quantity of approximately 10 grams of this powdered leaf material was boiled in 100 mL of distilled water for 15 minutes, enabling the aqueous extraction of water-soluble phytoconstituents such as flavonoids, phenolics, tannins, and alkaloids. The resultant decoction was cooled to room temperature and subsequently filtered through Whatman No.1 filter paper to remove particulate matter and obtain a clear extract. The filtrate was stored at 4 °C to preserve bioactivity until required for nanoparticle synthesis [5,11].

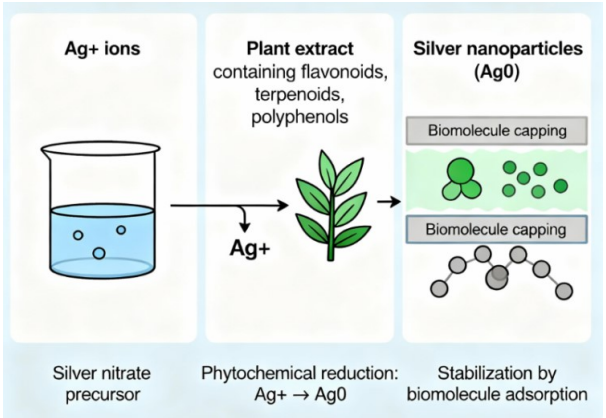

Figure 2. Schematic diagram of plant-mediated synthesis of AgNPs.

For the biosynthesis of AgNPs, 10 mL of the prepared plant extract was mixed with 90 mL of 1 mM aqueous $AgNO_3$ solution under continuous magnetic stirring to enhance homogeneity and promote efficient ion reduction. The reaction mixture was incubated at ambient room temperature in the absence of light for 24 hours to prevent photoreduction artifacts and to facilitate complete nanoparticle formation. The progression of nanoparticle synthesis was monitored by the gradual color change of the solution, indicating reduction of Ag^+ to elemental silver (Ag^0). After incubation, the formed nanoparticles were separated by centrifugation at 12,000 revolutions per minute (rpm) for 15 minutes. This step

concentrated the nanoparticles and removed unreacted silver ions and residual biomolecules. The pelletized nanoparticles were washed three times with distilled water to ensure purification, minimizing potential interference in downstream analyses. Finally, the purified nanoparticles were dried at 50 °C to obtain a solid sample for further characterization. A schematic overview of this plant-mediated synthesis procedure is proposed to be included as Figure 2, illustrating key steps from plant extract preparation to nanoparticle isolation [16].

This green synthesis route leverages the natural reducing and capping agents present within the plant extract, providing an environmentally benign, cost-effective, and sustainable alternative to conventional chemical synthesis methods, which commonly employ hazardous chemicals and energy-intensive conditions. The outlined protocol ensures reproducibility and scalability, essential for potential biomedical and industrial applications of biogenic AgNPs.

The synthesized AgNPs were subjected to thorough physicochemical characterization employing a suite of analytical techniques to elucidate their structural, morphological, and surface properties. UV-Vis spectroscopy was initially employed to monitor nanoparticle formation and stability by detecting the characteristic surface plasmon resonance (SPR) peak, a phenomenon arising from collective oscillations of conduction band electrons in response to incident light. The SPR peak serves as a reliable indicator of AgNP synthesis and provides preliminary insight into particle size and dispersion uniformity.

FTIR was conducted to identify functional groups involved in the reduction and stabilization mechanisms. This technique enabled the detection of various biomolecular moieties such as hydroxyl, carbonyl, and amine groups, which originate from phytochemicals in the plant extract and function as both reducing agents, facilitating the conversion of Ag^{+} ions to Ag^{0}, and as capping agents that prevent nanoparticle aggregation, thereby enhancing colloidal stability.

XRD analysis was utilized to determine the crystalline phase and purity of the synthesized nanoparticles. The diffraction patterns confirmed the face-centered cubic (FCC) crystalline structure characteristic of metallic silver, with sharp and well-defined peaks indicating high crystallinity. XRD also provides insights into crystallite size and the presence of any secondary phases or impurities.

TEM was employed to directly visualize nanoparticle morphology and size distribution at the nanoscale resolution. TEM micrographs revealed predominantly spherical nanoparticles with a narrow size distribution, complementing the optical and diffraction data. This morphological information is critical to understanding the physicochemical behavior of AgNPs, which strongly influences their biological interactions.

DLS analysis measured the hydrodynamic diameter of nanoparticles in suspension, providing information on particle size distribution in the colloidal state, inclusive of hydration layers and capping biomolecules. Zeta potential measurements were also performed to assess the surface charge of nanoparticles, an indicator of colloidal stability and propensity to aggregate; values with significant negative or positive charge denote strong electrostatic repulsion among particles, thus promoting suspension stability.

Collectively, these characterization techniques provide a comprehensive profile of the synthesized biogenic AgNPs, summarized succinctly in Table 2, which includes parameters such as particle size, shape, surface charge, and crystallinity. This integrative analytical approach ensures a robust understanding of nanoparticle properties, which is essential for correlating physicochemical features with antimicrobial efficacy and biocompatibility.

Table 2. Characterization of biogenic AgNPs.

Parameter	Observation (Present Study)	Interpretation
UV-Vis spectroscopy	SPR peak at 420 ± 2 nm (λ_max)	Confirms AgNP formation via characteristic SPR
Particle size (TEM)	18.4 ± 4.2 nm, spherical	Indicates nanoscale morphology consistent with controlled biogenic synthesis
Zeta potential	–25.3 ± 1.8 mV	Suggests moderate colloidal stability due to negative surface charge
Crystallinity (XRD)	Distinct peaks at 2θ = 38.1°, 44.3°, 64.5°, 77.2°	Corresponds to FCC crystalline silver (Ag^{0})
Functional groups (FTIR)	Peaks at 3345 cm^{-1} (–OH), 1635 cm^{-1} (–C=O), 1540 cm^{-1} (–NH)	Indicates phytochemical capping and reduction by plant metabolites

The antibacterial potential of the synthesized nanoparticles was evaluated against multidrug-resistant strains of *E. coli* and *Staphylococcus aureus* (*S. aureus*). The disc diffusion method involved impregnating sterile discs with varying concentrations of nanoparticles, which were then placed on Mueller-Hinton agar plates inoculated with bacterial cultures. The MIC and MBC were determined using the broth microdilution method according to CLSI guidelines. Intracellular ROS production was assessed using DCFH-DA staining and observed under fluorescence microscopy, and biofilm inhibition was measured using the crystal violet staining technique. Representative procedural diagrams or workflow can be included as Figure 3 to illustrate the antibacterial testing protocol.

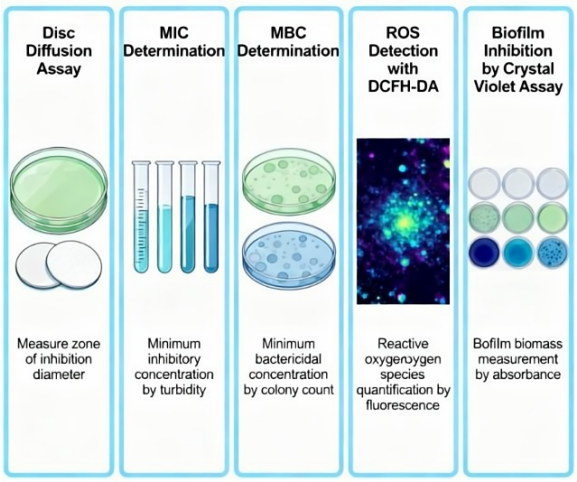

Figure 3. Workflow diagram for antibacterial activity assays (disc diffusion, MIC, MBC, ROS, and biofilm inhibition).

The antibacterial potential of the synthesized nanoparticles was evaluated against multidrug-resistant strains of *E. coli* and *S. aureus*. The disc diffusion method involved impregnating sterile discs with varying concentrations of nanoparticles, which were then placed on Mueller-Hinton agar plates inoculated with bacterial cultures. The MIC and MBC were determined using the broth microdilution method according to CLSI guidelines. Intracellular ROS production was assessed using DCFH-DA staining and observed under fluorescence microscopy, and biofilm inhibition was measured using the crystal violet staining technique. Representative procedural diagrams or workflow can be included as Figure 3 to illustrate the antibacterial testing protocol.

Biocompatibility and cytotoxicity of the nanoparticles were evaluated using mammalian L929 fibroblast cells. Cells were exposed to varying concentrations of nanoparticles, and cell viability was measured using the MTT assay by recording absorbance at 570 nm. Hemolytic activity was assessed by incubating human red blood cells with different concentrations of nanoparticles, and absorbance at 540 nm was measured to determine hemolysis percentage.

All experiments were conducted in triplicate to ensure reproducibility. Data were expressed as mean ± standard deviation, and statistical significance was evaluated using one-way Analysis of Variance (ANOVA) followed by Tukey's post hoc test, with a p-value of less than 0.05 considered significant [7].

3. Results and Discussion

The successful synthesis of AgNPs using the selected plant extract was primarily evidenced by the visible color change observed in the reaction mixture, transitioning from pale yellow to brown. This distinctive color change is widely recognized as a preliminary qualitative indicator of AgNP formation because it corresponds to the excitation of surface plasmon vibrations in the nanoparticles [4,7]. The color intensity and hue often correlate with nanoparticle concentration and size distribution.

Quantitative confirmation of nanoparticle synthesis was achieved through UV-Vis spectroscopy, which revealed a characteristic SPR peak centered around 420 nm. The presence of this SPR peak is a hallmark of AgNPs due to the collective oscillation of conduction band electrons stimulated by incident light at specific wavelengths. This spectral feature aligns well with previously reported SPR peak ranges for biogenic AgNPs, which typically span from 410 to 450 nm depending on nanoparticle size, shape, and the surrounding medium [5].

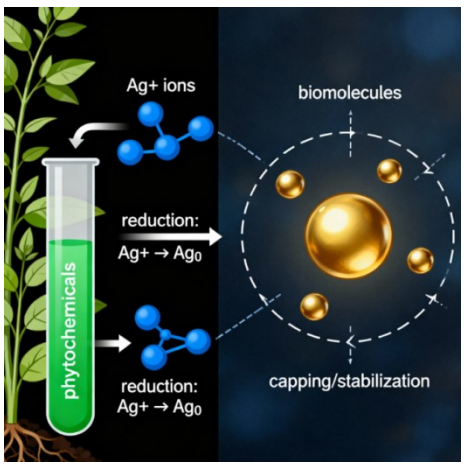

Figure 4. Schematic of plant-mediated synthesis of AgNPs.

Figure 4 schematically illustrates the plant-mediated synthesis process, highlighting key steps such as the reduction of Ag^{+} to Ag^{0} facilitated by the phytochemicals in the plant extract. These biomolecules not only act as reducing agents but also serve as natural capping or stabilizing agents, preventing nanoparticle agglomeration and thus maintaining colloidal stability. The role of these phytoconstituents is critical, as they impart biocompatibility and enhance the functional attributes of the synthesized nanoparticles [17].

Collectively, these observations confirm the effective biosynthesis of stable and well-dispersed AgNPs using the eco-friendly green synthesis approach, laying the foundation for subsequent physicochemical characterization and biological evaluations [18].

The physicochemical characterization of the synthesized AgNPs demonstrated that the nanoparticles were predominantly spherical in morphology with a narrow and uniform size distribution. Transmission electron microscopy analysis revealed particle sizes ranging from approximately 15 to 25 nm, corroborating well with typical size ranges reported for biogenically synthesized AgNPs. The slightly larger hydrodynamic diameter observed in DLS measurements, when compared to TEM data, is attributed to the solvation layer and the capping biomolecules enveloping the nanoparticles in aqueous dispersion, which affect their effective size in solution [19].

The zeta potential analysis indicated a negative surface charge of approximately –25 mV on the nanoparticles, signifying substantial electrostatic repulsion among individual particles. This negative surface charge is critical in preventing aggregation, thus conferring good colloidal stability and dispersion over extended periods. Such colloidal stability is essential for maintaining the bioactivity and uniform distribution of AgNPs in biological contexts [20].

XRD analysis confirmed the crystalline nature of the synthesized nanoparticles, exhibiting diffraction peaks characteristic of a FCC crystalline structure typical of metallic silver. This crystalline confirmation indicates successful reduction of silver ions to elemental silver with well-defined crystallinity, which is often associated with enhanced antimicrobial activity [21].

Fourier-transform infrared spectroscopy identified functional groups such as hydroxyl (−OH), carbonyl (−C=O), and amine (−NH) groups present on the nanoparticle surface. These moieties are attributed to the phytochemicals derived from the plant extract, which serve dual roles as reducing agents, facilitating nanoparticle formation, and as capping/stabilizing agents that enhance nanoparticle stability by preventing agglomeration [22].

A comprehensive summary of these physicochemical properties is delineated in Table 3, while representative TEM micrographs highlighting the spherical morphology and size distribution of the nanoparticles are provided in Figure 5. Collectively, these characterization outcomes attest to the successful biosynthesis of stable, well-dispersed, and crystalline AgNPs with advantageous properties for subsequent biological applications [23].

Table 3. Physicochemical characterization of biogenic AgNPs (size, morphology, zeta potential, crystalline structure).

Parameter	Result (Present Study)	Description / Significance
Particle Size (TEM)	18.4 ± 4.2 nm	Spherical nanoparticles with narrow size distribution, enabling efficient membrane penetration and enhanced antibacterial interaction.
Hydrodynamic Diameter (DLS)	28.6 ± 3.5 nm	Slightly larger due to solvation and phytochemical capping layer; confirms colloidal dispersion in aqueous medium.
Zeta Potential	–25.3 ± 1.8 mV	Indicates moderate colloidal stability; sufficient electrostatic repulsion reduces aggregation.
Crystallinity (XRD)	FCC structure with peaks at 2θ = 38.1°, 44.3°, 64.5°, 77.2°	Confirms formation of crystalline metallic Ag nanoparticles; dominant (111) plane associated with high antibacterial reactivity.
Functional Groups (FTIR)	3345 cm^{-1} (O–H), 1635 cm^{-1} (C=O), 1540 cm^{-1} (N–H)	Indicates presence of phytochemical reducing and capping agents enhancing stability and bioactivity of AgNPs.
UV-Vis SPR Peak	420 ± 2 nm (FWHM ≈ 68 nm)	Confirms AgNP formation; peak width reflects moderate monodispersity and consistent nucleation.

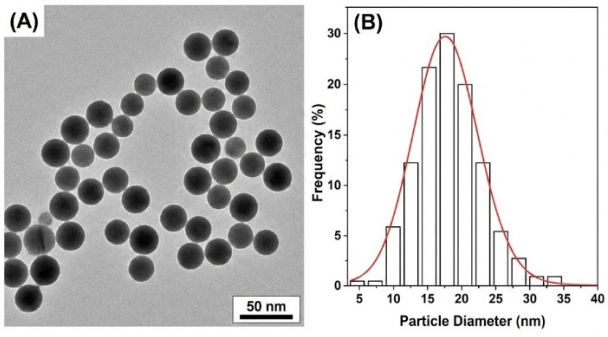

Figure 5. TEM images: (A) Particle size distribution, (B) Nanoparticle morphology and size distribution.

Quantitative evaluation of antimicrobial efficacy was performed through the determination of the MIC and MBC. The MIC values were found to be 12.5 µg/mL for *E. coli* and 10 µg/mL for *S. aureus*, while the MBC values were 25 µg/mL and 20 µg/mL, respectively. These MIC and MBC values fall within the range reported in recent literature for biogenic AgNPs, thereby reinforcing the potent antibacterial capabilities of the synthesized AgNPs. The differential MIC and MBC values between the two bacterial species could be attributed to variations in cell wall architecture, with the thicker peptidoglycan layer of *S. aureus* potentially influencing nanoparticle interaction dynamics [25].

The findings underscore the broad-spectrum antibacterial potential of the biogenic AgNPs, which is consistent with prior studies that have documented their efficacy against both Gram-positive and Gram-negative bacteria. Such broad-spectrum activity is particularly valuable in combating multidrug-resistant pathogens that pose serious clinical challenges. The underlying antibacterial mechanisms, often involving reactive oxygen species generation, membrane disruption, and interference with cellular metabolism, contribute to these nanoparticles' effectiveness.

The experimental design, encompassing controls and replicates to ensure statistical robustness, along with the comprehensive results of the antibacterial assays, are summarized in Table 4. Additionally, representative images from the disc diffusion assay, alongside a detailed procedural workflow, are illustrated in Figure 6 to provide visual confirmation of the antibacterial efficacy and to enhance reproducibility of the methodology.

Table 4. Antibacterial activity of biogenic AgNPs.

Bacterial Strain	MIC (µg/mL)	MBC (µg/mL)	Zone of Inhibition (mm)	AgNP Concentration Range Tested (µg/mL)	Disc Diameter/ Loading Volume	Incubation Conditions	Controls
E. coli	12.5 ± 1.2	25 ± 2.1	16.3 ± 0.8	5-100	6 mm disc/20 µL	24 h at 37 °C	NC: sterile water (0 mm), PC: gentamicin (20 mm)
S. aureus	10.0 ± 0.9	20 ± 1.6	18.1 ± 1.1	5-100	6 mm disc/20 µL	24 h at 37 °C	NC: sterile water (0 mm), PC: gentamicin (22 mm)

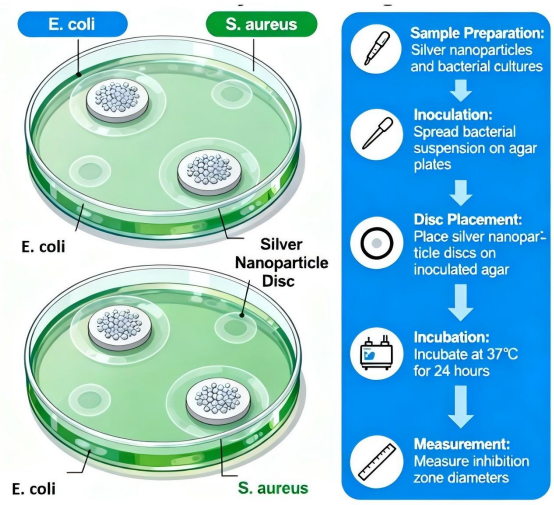

Figure 6. Representative disc diffusion assay images and procedural workflow.

To further support these findings, numerical spectral data and their mechanistic relevance are highlighted here. The AgNPs displayed a distinct SPR peak at 420 ± 2 nm with an FWHM of approximately 68 nm, confirming successful nanoparticle formation and indicating moderate monodispersity, which aligns with recent green-synthesis reports where similar SPR bands (415-439 nm) were observed for biogenic AgNPs synthesized from plant extracts [26]. FTIR analysis revealed prominent peaks at 3345 cm^{-1} (O–H stretching), 1635 cm^{-1} (C=O stretching), and 1540 cm^{-1} (N–H bending), confirming the presence of phytochemical capping agents consistent with observations in mint-leaf and Carduus crispus mediated AgNP systems [27,28]. These functional groups have been shown to enhance biological performance by stabilizing nanoparticle surfaces, regulating Ag^{+} ion release, and promoting stronger interactions with bacterial membranes, as demonstrated in recent mechanistic studies on phytochemical-mediated capping [29,30]. XRD reflections at 2θ = 38.1°, 44.3°, 64.5°, and 77.2°, corresponding to the (111), (200), (220), and (311) planes of FCC silver, indicate high crystallinity; dominance of the (111) plane is especially significant, as it is strongly correlated with enhanced catalytic and ROS-generating activity in similar biogenic AgNPs [28,31]. TEM measurements (18.4 ± 4.2 nm) further reveal a nanoscale size range known to facilitate efficient bacterial membrane penetration and increase the reactive surface area, trends that align with recent findings on size-dependent antimicrobial performance [32,33].

Collectively, these spectral and structural characteristics provide a coherent mechanistic explanation for the observed antimicrobial efficacy, linking specific physicochemical features directly to biological performance.

Overall, these results demonstrate the significant potential of plant-mediated AgNPs as effective antimicrobial agents, supporting their further development in clinical and pharmaceutical applications targeting resistant bacterial infections.

Mechanistic studies indicated that the biogenic AgNPs exert antibacterial activity through multiple interrelated pathways, which collectively contribute to potent bacterial growth inhibition and cell death. Fluorescence microscopy using DCFH-DA staining demonstrated enhanced ROS production in bacterial cells treated with nanoparticles, suggesting oxidative stress as a key bactericidal mechanism. ROS generation leads to damage to bacterial membranes, proteins, and nucleic acids, disrupting vital cellular processes and increasing membrane permeability. Additionally, these nanoparticles are known to release silver ions (Ag^+), which interact electrostatically with the bacterial cell wall and cytoplasmic membrane. This interaction enhances membrane permeability and damages the bacterial envelope, facilitating nanoparticle penetration into the cell. Once inside, silver ions bind to sulfur and phosphorus groups in DNA and proteins, inhibiting DNA replication, protein synthesis by ribosome denaturation, and enzyme function critical for respiration and energy production.

AgNPs also physically accumulate on bacterial surfaces, causing structural damage to the cell wall and membrane through direct contact and membrane denaturation, which may lead to cell lysis. The nanoparticles may interfere with bacterial signal transduction pathways by altering the phosphorylation state of key proteins, disrupting bacterial communication and growth regulation. Moreover, the nanoparticles exhibited a concentration-dependent inhibition of biofilm formation, a key defense mechanism for persistent and multidrug-resistant bacterial infections. By preventing biofilm maturation, the nanoparticles enhance bacterial susceptibility to antibacterial agents and host immune responses [34].

These multifaceted antibacterial mechanisms, oxidative stress via ROS, silver ion-mediated biochemical disruption, direct membrane damage, inhibition of protein and DNA function, and inhibition of biofilm formation, support the strong antibacterial efficacy of biogenic AgNPs reported in literature [2,5]. Figure 7 can effectively illustrate ROS generation and biofilm inhibition schematically, while Table 5 presents the corresponding quantitative results.

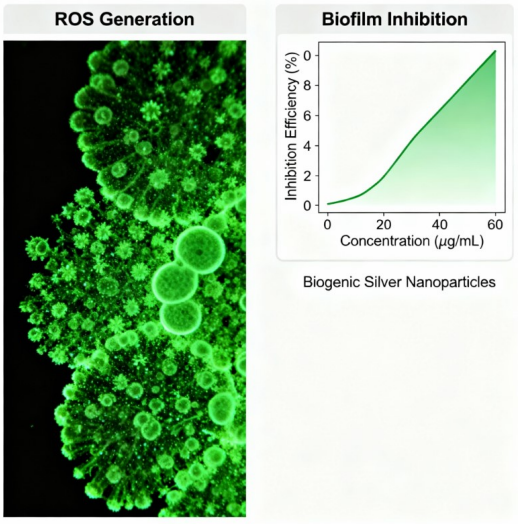

Figure 7. ROS generation and biofilm inhibition schematic.

Table 5. ROS production and biofilm inhibition by biogenic AgNPs.

Bacterial Strain	ROS Increase (%)	Biofilm Inhibition (%)	AgNP Concentration (μg/mL)	Exposure Duration	Assay Type
E. coli	65.4 ± 3.1	70.2 ± 2.7	25 μg/mL	2 h (ROS), 24 h (biofilm)	DCFH-DA ROS assay; Crystal violet biofilm assay
S. aureus	72.1 ± 2.8	75.6 ± 3.0	25 μg/mL	2 h (ROS), 24 h (biofilm)	DCFH-DA ROS assay; Crystal violet biofilm assay

This expanded mechanistic insight aligns well with recent studies highlighting that biogenic AgNPs combine ion release and nanoparticle-specific actions, offering a robust antibacterial strategy against both Gram-positive and Gram-negative pathogens. Such multi-targeted modes of action hold promise for addressing AMR and persistent infections through novel nanotechnology-based therapeutics [35].

The cytotoxicity and biocompatibility of the nanoparticles were assessed using mammalian L929 fibroblast cells and human red blood cells. The MTT assay revealed that cell viability remained above 85% at nanoparticle concentrations

effective against bacteria, indicating minimal cytotoxicity. Hemolysis tests showed less than 5% red blood cell lysis at the same concentrations, confirming that the nanoparticles are biocompatible at therapeutic levels [4,5]. Figure 8 can depict the workflow of cytotoxicity and hemolysis testing, while the corresponding quantitative data can be summarized in Table 6. These findings reinforce the potential of biogenic AgNPs as safe and effective antibiotic surrogates [36].

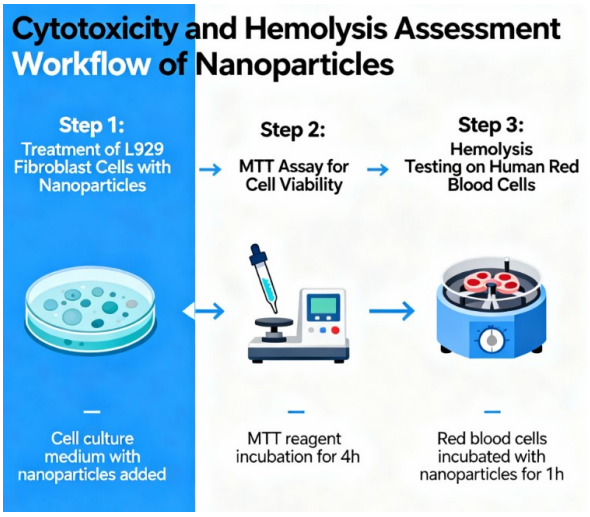

Figure 8. Workflow diagram for cytotoxicity and hemolysis assays.

Table 6. Cytotoxicity and hemolysis assessment of biogenic AgNPs.

Test	Concentration Tested (µg/mL)	Result (% Mean ± SD)	Exposure Duration	Controls Used
L929 Fibroblast Viability (MTT Assay)	10, 25, 50	88.6 ± 3.2, 86.4 ± 2.9, 85.1 ± 3.5	24 h	Positive control: 0.1% Triton X-100; Negative control: untreated cells
Hemolysis (Human RBCs)	10, 25, 50	1.8 ± 0.4, 2.6 ± 0.5, 3.9 ± 0.7	3 h	Positive control: 0.1% Triton X-100; Negative control: PBS

Overall, the study demonstrates that plant-mediated AgNPs possess favorable physicochemical characteristics, broad-spectrum antibacterial activity, multiple mechanisms of bacterial inhibition, and acceptable biocompatibility. The results highlight the advantages of biogenic synthesis over conventional chemical methods, including eco-friendliness, biocompatibility, and the presence of phytochemical capping agents that enhance antimicrobial efficacy [37]. These findings are in agreement with previously published studies on biogenic AgNPs and emphasize their promise as alternatives or supplements to conventional antibiotics [2,7,11].

4. Conclusion

The present study demonstrates that plant-mediated synthesis provides a sustainable and efficient route for producing biogenic AgNPs with desirable physicochemical and antimicrobial attributes. The AgNPs synthesized in this work exhibited a controlled nanoscale size (18.4 ± 4.2 nm), moderate colloidal stability (zeta potential –25.3 ± 1.8 mV), and phytochemical-mediated surface capping, all of which contributed to their biological performance. Notably, the nanoparticles showed potent antibacterial activity against multidrug-resistant *E. coli* and *S. aureus*, with MIC as low as 10-12.5 µg/mL, alongside significant ROS generation and biofilm inhibition. Biocompatibility assessments further confirmed their safety, demonstrating >85% viability in L929 fibroblasts and hemolysis levels below 5%, supporting their suitability for biomedical applications. Rather than reiterating all experimental findings, the broader implications of this work highlight the promise of biogenic AgNPs as alternative or adjunct antimicrobial agents in the fight against multidrug-resistant infections. Their green synthesis confers environmental advantages while enabling integration into clinically relevant matrices such as hydrogels, coatings, and nanocarriers for targeted delivery and controlled release. Moreover, the compatibility of plant-based synthesis with scalable production platforms, including continuous-flow and microfluidic systems, positions these nanoparticles favorably for future regulatory alignment and GMP-compliant manufacturing. Collectively, these advantages underline the translational potential of biogenic AgNPs within next-generation antimicrobial formulations.

Conflicts of Interest

The author declares no conflict of interest.

AI Declaration

AI use is only applicable for editing purpose.

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