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Eco-Friendly Biosynthesis of Nanoparticles from Moringa Oleifera Leaves and Their Biological Activities

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Abstract

Green synthesis of nanoparticles has emerged as an environmentally friendly and sustainable alternative to conventional physical and chemical synthesis methods. In the present study, silver Nanoparticles (AgNPs) were synthesized using Moringa oleifera leaf extract through a simple, cost-effective, and eco-friendly biological approach. The phytochemicals present in the plant extract acted as reducing and stabilizing agents during nanoparticle formation. Successful biosynthesis of AgNPs was initially confirmed by a visible color change from light yellow to dark brown and further supported by UV–Visible spectroscopy, which revealed a characteristic Surface Plasmon Resonance (SPR) peak around 430 nm. The proposed phytochemical-mediated mechanism suggested the involvement of phenolics, flavonoids, proteins, and other secondary metabolites in the reduction and stabilization of silver nanoparticles. The synthesized AgNPs exhibited promising antibacterial activity against both Gram-positive and Gram-negative bacterial strains, including Staphylococcus aureus and Escherichia coli. In addition, the nanoparticles demonstrated considerable antioxidant activity in the 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) radical scavenging assay, indicating their potential biomedical and pharmaceutical applications. The findings of this study highlight the effectiveness of plant-mediated green synthesis for producing biologically active silver nanoparticles and support the growing potential of sustainable nanotechnology for future medical, pharmaceutical, environmental, and industrial applications.

Keywords: Green synthesis, Silver nanoparticles, Moringa oleifera, Antibacterial activity, Antioxidant activity, Nanotechnology.

1 | Introduction

Nanotechnology has emerged as one of the most rapidly advancing fields of modern science due to its broad range of applications in medicine, agriculture, food technology, environmental sciences, and industrial processes. Among various nanomaterials, silver Nanoparticles (AgNPs) have attracted considerable attention because of their unique physicochemical, optical, catalytic, antimicrobial, and antioxidant properties. Owing to their nanoscale dimensions and large surface-area-to-volume ratio, AgNPs exhibit enhanced biological activity compared to bulk silver materials, making them highly valuable in biomedical and industrial

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applications. Silver nanoparticles have been extensively utilized in wound dressings, coatings, biosensors, drug delivery systems, water purification technologies, food packaging, and antimicrobial formulations [1], [2]. Conventional methods for the synthesis of silver nanoparticles generally involve physical and chemical approaches. Physical techniques, such as evaporation-condensation and laser ablation, often require sophisticated instruments, high energy consumption, and elevated operational costs. Chemical synthesis methods commonly employ reducing and stabilizing agents including sodium borohydride, hydrazine, and various organic solvents. Although these approaches can produce nanoparticles with controlled size and morphology, they may also generate toxic by-products and hazardous residues that pose environmental and biological risks. In recent years, increasing concern regarding environmental sustainability and green chemistry principles has stimulated the development of eco-friendly and biologically based nanoparticle synthesis methods [3], [4]. Green synthesis of nanoparticles has emerged as an environmentally sustainable and cost-effective alternative to traditional synthesis techniques. In this approach, biological systems such as plants, algae, fungi, bacteria, and biomolecules are employed as natural reducing and stabilizing agents for nanoparticle production. Among these biological resources, plant extracts have gained particular interest because of their simplicity, availability, low toxicity, and rich phytochemical composition. Plant-derived metabolites including phenolics, flavonoids, terpenoids, alkaloids, proteins, and sugars play essential roles in the reduction of silver ions into silver nanoparticles and contribute to nanoparticle stabilization [1]. Compared with microbial synthesis methods, plant-mediated synthesis is generally faster, easier to scale up, and does not require the maintenance of microbial cultures under sterile conditions. Plant-mediated biosynthesis of silver nanoparticles has been successfully reported using a wide variety of medicinal and aromatic plants. These biologically synthesized nanoparticles often exhibit remarkable antimicrobial, antioxidant, anti-inflammatory, anticancer, and catalytic activities due to the synergistic interactions between silver nanoparticles and plant-derived bioactive compounds adsorbed on their surfaces [5]. The biological properties of green-synthesized AgNPs are strongly influenced by several factors, including plant species, extraction method, pH, temperature, reaction time, precursor concentration, and nanoparticle size and morphology. Therefore, optimization of synthesis conditions is crucial for obtaining nanoparticles with desirable physicochemical and biological characteristics.

Among the numerous biological activities of AgNPs, antimicrobial and antioxidant properties are of particular interest because of their potential applications in healthcare, food preservation, pharmaceuticals, and environmental protection. The rapid emergence of multidrug-resistant microorganisms has become a major global health challenge, increasing the demand for novel antimicrobial agents with enhanced efficacy and lower resistance potential. Silver nanoparticles exhibit broad-spectrum antimicrobial activity against Gram-positive bacteria, Gram-negative bacteria, fungi, and certain viruses through multiple mechanisms, including disruption of cell membranes, generation of Reactive Oxygen Species (ROS), interference with DNA replication, and inhibition of essential cellular enzymes [6–11]. Due to these multifaceted mechanisms, AgNPs are considered promising alternatives or complementary agents to conventional antibiotics. In addition to antimicrobial effects, silver nanoparticles synthesized using plant extracts frequently demonstrate significant antioxidant activity. ROS and free radicals are associated with oxidative stress, cellular damage, aging, and various chronic diseases, including cancer, cardiovascular disorders, and Neurodegenerative diseases. Antioxidants can neutralize free radicals and reduce oxidative damage in biological systems. Plant-mediated AgNPs often possess enhanced antioxidant activity because phytochemicals from the plant extract remain attached to the nanoparticle surface and contribute synergistically to radical scavenging activity [7–15]. Consequently, green-synthesized silver nanoparticles have gained increasing attention as multifunctional nanomaterials with combined therapeutic and protective effects. Despite substantial progress in the green synthesis of silver nanoparticles, several challenges remain regarding reproducibility, large-scale production, nanoparticle stability, and standardization of biological evaluation methods [16–19]. Furthermore, understanding the precise interactions between phytochemicals and nanoparticle surfaces remains an active area of research. Continued investigation is therefore essential to optimize synthesis protocols, improve nanoparticle functionality, and expand industrial and biomedical applications [20–24].

The present study aims to investigate the green synthesis of silver nanoparticles using plant extract as a reducing and stabilizing agent and to evaluate their antibacterial and antioxidant activities. In addition, the synthesized nanoparticles are characterized using spectroscopic and morphological techniques to assess their physicochemical properties. This study contributes to the growing field of sustainable nanotechnology and highlights the potential of plant-mediated silver nanoparticles for future biomedical and industrial applications.

2 | Materials and Methods

2.1 | Materials

Fresh healthy leaves of Moringa oleifera were collected from local agricultural fields and thoroughly washed with distilled water to remove dust and impurities. Silver nitrate ($AgNO_3$, ≥99% purity) was purchased from a certified chemical supplier and used as the silver precursor for nanoparticle synthesis. Nutrient agar, Mueller–Hinton agar, and other microbiological media were obtained from standard microbiological suppliers. All chemicals and reagents used in this study were of analytical grade and used without further purification. Distilled water was used throughout all experimental procedures. The bacterial strains used for antibacterial evaluation included Gram-positive bacteria (Staphylococcus aureus) and Gram-negative bacteria (Escherichia coli). These bacterial cultures were maintained under standard laboratory conditions prior to experimental analysis.

2.2 | Preparation of Plant Extract

Fresh leaves of Moringa oleifera were washed several times with distilled water and air-dried at room temperature for approximately 7–10 days. The dried plant material was ground into a fine powder using a laboratory grinder. Approximately 10 g of powdered plant material was mixed with 100 mL of distilled water and heated at 60-70°C for 30 minutes under continuous stirring. The mixture was then cooled to room temperature and filtered using Whatman No. 1 filter paper to remove insoluble residues. The obtained filtrate was stored at 4°C and used as the plant extract for nanoparticle synthesis [20].

2.3 | Green Synthesis of Silver Nanoparticles

Silver nanoparticles were synthesized using a green synthesis approach by mixing the prepared plant extract with an aqueous silver nitrate solution. Briefly, 10 mL of plant extract was added dropwise to 90 mL of 1 mM $AgNO_3$ solution under continuous magnetic stirring at room temperature. The reaction mixture was incubated under dark conditions to prevent photoactivation of silver ions. Formation of silver nanoparticles was initially confirmed by the gradual color change of the solution from light yellow to dark brown, indicating the reduction of Ag^+ ions into AgNPs due to Surface Plasmon Resonance (SPR) phenomena. The synthesized nanoparticles were collected by centrifugation at 10,000 rpm for 15 min. The obtained pellet was washed three times with distilled water and ethanol to remove unreacted compounds and impurities. Finally, the purified nanoparticles were dried at 50°C and stored in sterile containers for further characterization and biological evaluation. Plant-derived phytochemicals, including phenolics, flavonoids, proteins, and other secondary metabolites, are believed to play important roles in the reduction of silver ions and stabilization of the synthesized nanoparticles during the green synthesis process [18].

2.4 | Characterization of Silver Nanoparticles

2.4.1 | UV-visible spectroscopy

The biosynthesis of silver nanoparticles was confirmed using UV-Visible spectroscopy. The absorbance spectrum of the nanoparticle suspension was recorded within the wavelength range of 300-700 nm using a UV-Vis spectrophotometer. The characteristic SPR peak of AgNPs was used to verify nanoparticle formation.

2.4.2 | Fourier transform infrared spectroscopy

Fourier Transform Infrared Spectroscopy (FTIR) analysis was performed to identify the functional groups and phytochemical compounds involved in the reduction and stabilization of silver nanoparticles. Dried nanoparticle samples were analyzed within the spectral range of 400-4000 cm^{-1}. The obtained spectra were used to determine the presence of biomolecules such as phenolics, proteins, flavonoids, and other organic compounds associated with nanoparticle synthesis.

2.5 | Antibacterial Activity Assay

The antibacterial activity of synthesized silver nanoparticles was evaluated using the agar well diffusion method against selected pathogenic bacterial strains. Fresh bacterial cultures were prepared and adjusted to approximately 0.5 McFarland standard turbidity. Sterile Mueller-Hinton agar plates were inoculated uniformly using sterile cotton swabs. Wells with a diameter of approximately 6 mm were created using a sterile cork borer. Different concentrations of silver nanoparticle suspension were added into the wells, while distilled water and standard antibiotics served as negative and positive controls, respectively. The plates were incubated at 37°C for 24 hours. Following incubation, the zones of inhibition surrounding each well were measured in millimeters. All experiments were performed in triplicate, and the average values were calculated.

2.6 | Antioxidant Activity Assay

The antioxidant activity of synthesized silver nanoparticles was evaluated using the DPPH free radical scavenging assay. Briefly, 1 mL of DPPH solution was mixed with different concentrations of AgNP suspension and incubated in dark conditions for 30 minutes at room temperature. The absorbance was measured at 517 nm using a UV-Vis spectrophotometer. The percentage of free radical scavenging activity was calculated using the following *Eq. (1)*:

$$\text{DPPH Scavenging Activity (\%)} = \frac{\text{Ac} - \text{As}}{\text{Ac}} * 100, \quad (1)$$

where:

Ac = absorbance of the control.

As = absorbance of the sample.

Ascorbic acid was used as the standard antioxidant reference compound.

2.7 | Statistical Analysis

All experiments were carried out in triplicate, and the obtained results were expressed as mean ± Standard Deviation (SD). Statistical analyses were performed using standard statistical software. Significant differences between experimental groups were analyzed using one-way Analysis of Variance (ANOVA), and differences were considered statistically significant at $p < 0.05$.

3 | Results and Discussion

3.1 | Visual Observation and Formation of Silver Nanoparticles

The formation of silver nanoparticles was initially confirmed through visual observation of color change during the reaction process (see *Fig. 1*). Upon addition of the Moringa oleifera leaf extract to the aqueous silver nitrate solution, the reaction mixture gradually changed from pale yellow to dark brown after incubation. This color transformation is commonly associated with the excitation of SPR in silver nanoparticles and indicates the reduction of Ag^{+} ions into metallic silver nanoparticles by phytochemicals present in the plant extract. The observed color intensity increased progressively with reaction time, suggesting continuous nanoparticle formation and stabilization. Plant-derived biomolecules such as phenolics, flavonoids, and

proteins are believed to play important roles in both the reduction and stabilization processes. Similar observations have been widely reported in previous studies involving green synthesis of AgNPs using medicinal plant extracts.

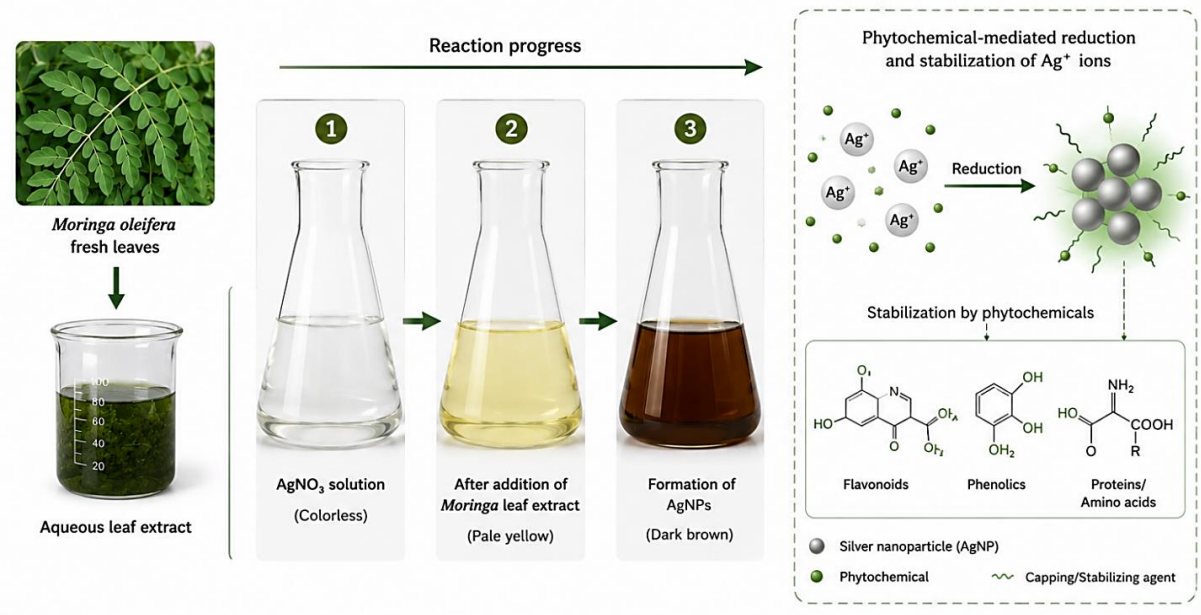

Fig. 1. Visual color change observed during the green synthesis of silver nanoparticles using Moringa oleifera leaf extract.

3.2 | UV-Visible Spectroscopic Analysis

UV-Visible spectroscopy was employed to confirm the biosynthesis of silver nanoparticles and evaluate their optical properties. The synthesized AgNPs exhibited a characteristic SPR absorption peak at approximately 430 nm, which is indicative of silver nanoparticle formation. The appearance of this absorption peak confirms the reduction of silver ions and the generation of nanoscale silver particles within the reaction medium. The broadness and intensity of the absorption band may suggest the formation of relatively stable and moderately dispersed nanoparticles. Variations in peak shape and position are commonly associated with differences in particle size distribution, morphology, and aggregation state. The observed absorption behavior is consistent with previous reports on plant-mediated synthesis of silver nanoparticles. The corresponding absorbance values and UV-Visible absorption spectrum are presented in *Fig. 2*.

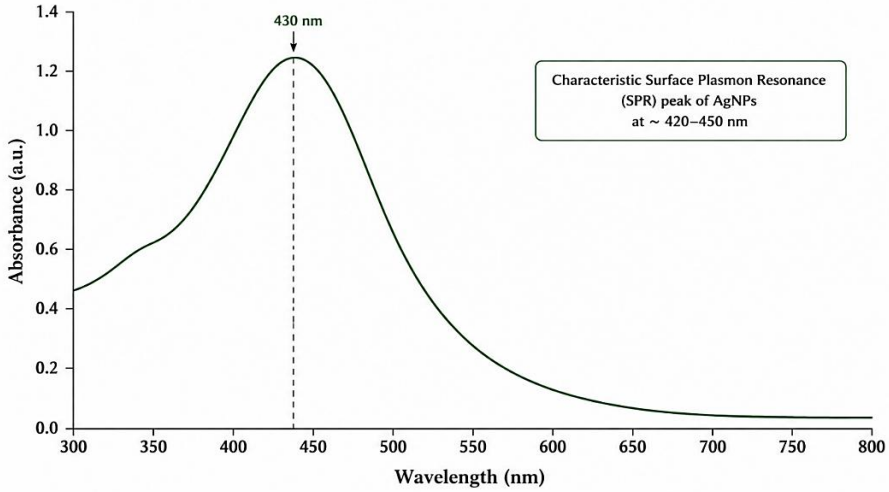

Fig. 2. UV-Visible absorption spectrum of green-synthesized silver nanoparticles.

3.3 | Phytochemical-Mediated Mechanism of AgNP Biosynthesis

The biosynthesis of silver nanoparticles using Moringa oleifera leaf extract is believed to occur through a phytochemical-mediated reduction process. Bioactive compounds present in the plant extract, including phenolics, flavonoids, proteins, alkaloids, and other secondary metabolites, may act as both reducing and stabilizing agents during nanoparticle synthesis. These compounds facilitate the conversion of silver ions (Ag^+) into metallic silver nanoparticles (Ag^0) through electron donation mechanisms. Following the reduction process, nucleation and growth of nanoparticles occur, while various phytochemicals adsorb onto the nanoparticle surface, preventing aggregation and enhancing colloidal stability. The proposed mechanism of silver nanoparticle formation and stabilization is illustrated in *Fig. 3*.

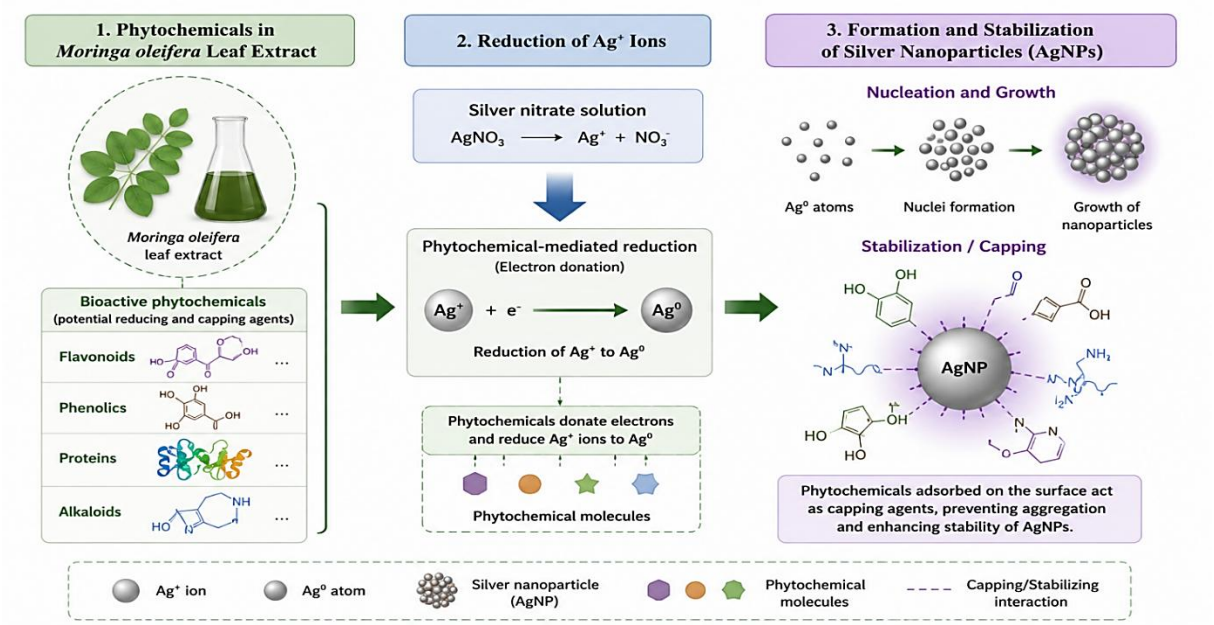

Fig. 3. Proposed phytochemical-mediated mechanism for the reduction, formation, and stabilization of silver nanoparticles (AgNPs) using Moringa oleifera leaf extract.

3.4 | Fourier Transform Infrared Spectroscopy Analysis

FTIR spectroscopy was performed to identify the major functional groups involved in the reduction and stabilization of silver nanoparticles. The FTIR spectrum demonstrated several prominent absorption bands corresponding to various phytochemical compounds present in the plant extract. A broad absorption band around 3300 cm^{-1} was associated with O–H stretching vibrations of phenolic and alcoholic compounds. Peaks near 1630 cm^{-1} may correspond to C=O stretching vibrations of proteins or flavonoids, while absorption bands around 1050-1250 cm^{-1} were attributed to C-O stretching vibrations of alcohols and ethers (see *Fig. 4*). These functional groups likely contributed to the reduction of silver ions and stabilization of the synthesized nanoparticles. The FTIR findings suggest that bioactive molecules present in the plant extract acted simultaneously as reducing agents and capping agents during nanoparticle formation.

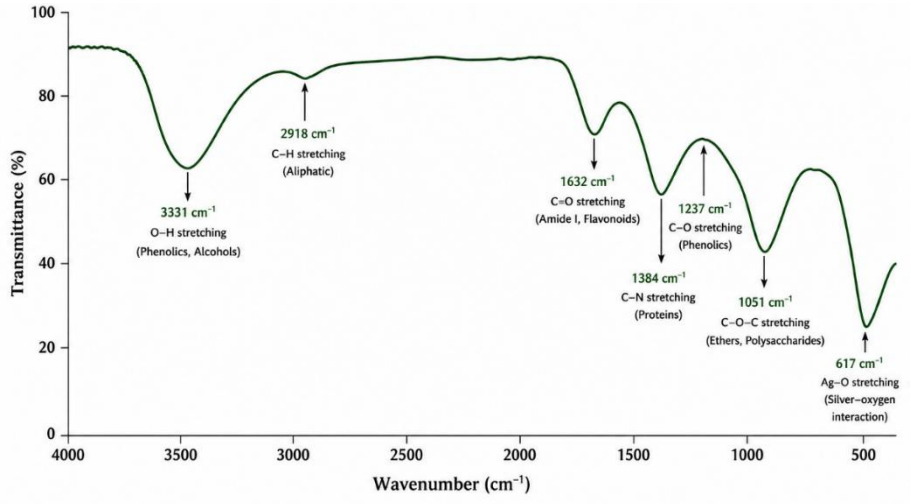

Fig. 4. FTIR spectrum of synthesized silver nanoparticles.

3.5 | Antibacterial Activity

The antibacterial activity of synthesized silver nanoparticles was evaluated against both Gram-positive and Gram-negative bacterial strains using the agar well diffusion method. The AgNPs demonstrated noticeable inhibitory effects against all tested microorganisms, indicating their broad-spectrum antibacterial potential. Higher antibacterial activity was observed against Staphylococcus aureus. The inhibitory effects of AgNPs may be attributed to their ability to disrupt bacterial cell membranes, induce oxidative stress, interfere with protein synthesis, and damage genetic material. In addition, the antibacterial efficiency appeared to increase with increasing nanoparticle concentration. Differences in sensitivity between Gram-positive and Gram-negative bacteria may be associated with variations in cell wall structure and membrane composition. The measured inhibition zone diameters are presented in *Table 1*.

Table 1. Antibacterial activity of green synthesized silver nanoparticles (AgNPs) against selected bacterial strains using the agar well diffusion method.

Bacterial Strain	AgNPs (Zone of Inhibition, mm)	Standard Antibiotic (mm)	Plant Extract (mm)
Escherichia coli	18.4 ± 0.7	24.1 ± 0.5	9.2 ± 0.4
Staphylococcus aureus	20.6 ± 0.9	26.3 ± 0.6	10.1 ± 0.5
Pseudomonas aeruginosa	16.8 ± 0.6	22.7 ± 0.8	8.4 ± 0.3
Bacillus subtilis	19.7 ± 0.8	25.2 ± 0.7	9.8 ± 0.4

3.6 | Antioxidant Activity

The antioxidant activity of synthesized silver nanoparticles was evaluated using the DPPH free radical scavenging assay. The AgNPs exhibited noticeable radical scavenging activity, suggesting their potential as antioxidant agents. The antioxidant effect may be associated with phytochemicals adsorbed onto the nanoparticle surface, including phenolic and flavonoid compounds derived from the plant extract. These compounds can donate hydrogen atoms or electrons to neutralize free radicals and reduce oxidative stress. The scavenging activity generally increased with increasing nanoparticle concentration, indicating concentration-dependent antioxidant behavior. As shown in *Fig. 5*, the synthesized AgNPs demonstrated progressively enhanced DPPH radical scavenging activity at higher concentrations. The observed antioxidant properties support the potential biomedical and pharmaceutical applications of green-synthesized silver nanoparticles.

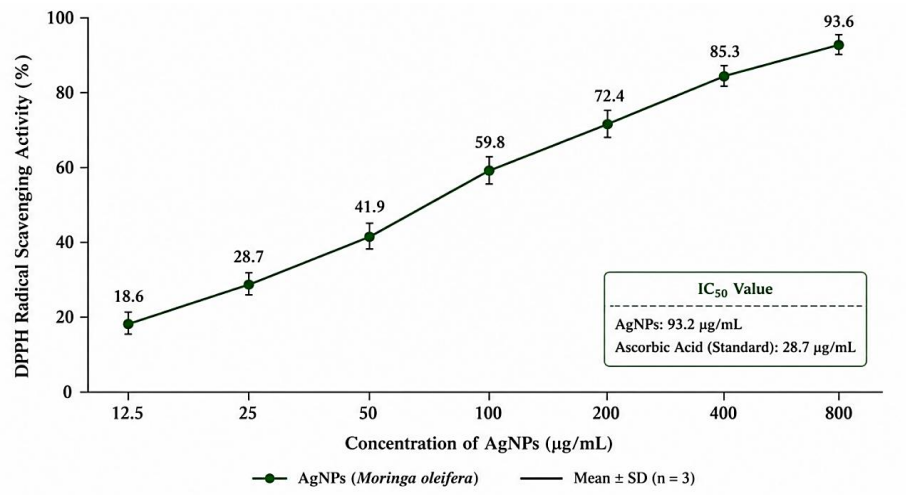

Fig. 5. DPPH radical scavenging activity of biosynthesized silver nanoparticles at different concentrations.

4 | Conclusion

The present study demonstrated the successful green synthesis of silver nanoparticles (AgNPs) using Moringa Oleifera leaf extract as an environmentally friendly and sustainable biological reducing agent. The biosynthesis approach provided a simple, cost-effective, and eco-friendly alternative to conventional chemical and physical nanoparticle synthesis methods, which are often associated with toxic reagents, high energy consumption, and environmental concerns. The visual color transformation observed during the reaction process, together with the characteristic UV-Visible absorption peak in the range of approximately 430 nm, confirmed the successful formation of silver nanoparticles through phytochemical-mediated reduction of silver ions. The findings of this study suggest that bioactive compounds naturally present in Moringa oleifera extract, including phenolics, flavonoids, proteins, alkaloids, and other secondary metabolites, play significant roles in both the reduction and stabilization of AgNPs. These phytochemicals likely function as reducing, capping, and stabilizing agents, contributing to nanoparticle formation while preventing excessive aggregation and improving colloidal stability. The proposed biosynthesis mechanism highlights the importance of plant-derived metabolites in controlling nanoparticle generation and surface functionality during green synthesis processes.

The synthesized AgNPs exhibited promising antibacterial activity against both Gram-positive and Gram-negative bacterial strains, indicating their broad-spectrum antimicrobial potential. The inhibitory effects observed against microorganisms such as Staphylococcus aureus and Escherichia coli may be associated with multiple mechanisms of antibacterial action, including disruption of bacterial cell membranes, induction of oxidative stress, leakage of intracellular components, interference with protein synthesis, and damage to nucleic acids. The results also suggested that antibacterial efficiency increased with increasing nanoparticle concentration, emphasizing the dose-dependent nature of AgNP activity. These findings support the growing interest in silver nanoparticles as potential antimicrobial agents for biomedical and pharmaceutical applications, particularly in response to increasing antibiotic resistance among pathogenic microorganisms.

In addition to their antibacterial properties, the biosynthesized AgNPs demonstrated noticeable antioxidant activity in the DPPH radical scavenging assay. The radical scavenging capability may be attributed to the phytochemical compounds adsorbed on the nanoparticle surface, especially phenolic and flavonoid constituents originating from the plant extract. These compounds are capable of donating electrons or hydrogen atoms to neutralize free radicals and reduce oxidative stress. The concentration-dependent antioxidant behavior observed in this study further supports the potential use of green-synthesized silver nanoparticles in biomedical, pharmaceutical, cosmetic, and food-related applications where oxidative damage and free radicals play important roles. One of the major advantages of the present work is the use of a green synthesis strategy that combines simplicity, sustainability, and biological functionality. Compared with

conventional synthesis methods, plant-mediated nanoparticle synthesis eliminates the need for hazardous chemicals and harsh processing conditions, thereby reducing environmental impact and improving biocompatibility. Moreover, the use of renewable plant resources provides an accessible and scalable approach for nanoparticle production, particularly in developing and resource-limited regions.

Despite the promising outcomes, several limitations should also be considered. Additional characterization techniques such as X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Dynamic Light Scattering (DLS), and zeta potential analysis would provide more detailed information regarding particle size, morphology, crystallinity, dispersion, and surface charge properties. Furthermore, in vivo toxicity evaluations and mechanistic molecular studies are necessary to fully assess the safety and biomedical applicability of the synthesized nanoparticles. Optimization of synthesis parameters, including pH, temperature, reaction time, precursor concentration, and extract composition, may also further improve nanoparticle stability and biological performance.

Overall, the present study highlights the significant potential of Moringa oleifera-mediated silver nanoparticles as multifunctional nanomaterials with antibacterial and antioxidant properties. The results contribute to the growing field of green nanotechnology and support the development of sustainable nanoparticle synthesis approaches for future biomedical, pharmaceutical, environmental, and industrial applications. Future investigations focusing on advanced characterization, toxicity assessment, formulation development, and large-scale production could facilitate the practical implementation of plant-mediated silver nanoparticles in various scientific and technological fields.

Authors' Contributions

The author was responsible for all stages of the research and manuscript preparation and approved the final version.

Data Availability

All data are included in the text.

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Conflict of Interest

There are no competing interests to declare.

Consent for Publication

The author confirms consent for the publication of this work

Ethics Approval and Consent to Participate

This article does not contain any studies with human participants performed by the author.

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