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SUSTAINABLE SYNTHESIS OF SILVER NANOPARTICLES EMPLOYING *MORINGA OLEIFERA* LEAF EXTRACT AND ASSESSMENT OF ANTIBACTERIAL EFFICACY

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ABSTRACT

In recent years, biological extracts have emerged as promising agents for the rapid synthesis of silver nanoparticles (AgNPs) exhibiting strong antibacterial properties. This study was designed to synthesize and optimize silver nanoparticles (AgNPs) using the leaf extract of *Moringa oleifera*. Fresh *M. oleifera* leaves were boiled to prepare aqueous extracts, which were mixed with 1 mM $AgNO_3$ solution under direct sunlight to drive nanoparticle formation. A distinct color change from pale yellow to dark brown within 30 minutes indicated AgNPs synthesis, which was confirmed using UV–Vis spectroscopy (SPR peak at 434 nm). The antimicrobial activity of the synthesized AgNPs was evaluated against *Escherichia coli* and *Pseudomonas aeruginosa* using well diffusion and disc diffusion assays. Biosynthesized AgNPs exhibited strong antibacterial potential. In well diffusion, inhibition zones were 15.0±0.5, 17.8±0.7, and 20.2±0.8mm at 25, 50, and 100µg/mL, respectively. In disc diffusion, inhibition zones were 21.5±1.5mm (50µg/disc) and 27.3±2.0mm (100µg/disc). Minimum inhibitory concentration (MIC) analysis revealed complete inhibition of *E. coli* growth at 5.0mM AgNP concentration, showing a clear dose-dependent effect. This study demonstrates a green, cost-effective, and efficient method for AgNP synthesis using M. oleifera leaf extract. The nanoparticles displayed significant antimicrobial activity, suggesting their potential application as broad-spectrum antimicrobial agents in biomedical and environmental domains.

Keywords: Green synthesis, Silver nanoparticles (AgNPs), Moringa oleifera leaf extract, Antibacterial activity, *Escherichia coli, Pseudomonas aeruginosa*

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1. INTRODUCTION

Nanotechnology, derived from the Greek word *nano*, meaning "dwarf," is the science of studying and manipulating structures at the nanometer scale (Srivastava and Bhargava 2022). Over the past few decades, it has emerged as a fast-growing interdisciplinary field with applications in research, development, and industry worldwide (Aithal and Aithal 2021). Among various nanomaterials, silver nanoparticles (AgNPs) are considered particularly important due to their wide range of applications. They can be synthesized through physical, chemical, electrochemical, photochemical, and thermal methods (Nguyen et al. 2023). The majority of traditional methods, however, are expensive, labor-intensive, and utilize toxic chemicals, which are hazardous to the environment and the biosphere (Ahmed et al. 2022).

Green synthesis has also been of late considered as an option, whereby plant, bacterial, or fungal extracts are used to synthesize nanoparticles, in a sustainable and cost-efficient way (Jayeoye et al. 2024). Plant extracts also act as reducing and stabilizing agents, and so the process is easy, scalable, and sustainable (Singh et al. 2023). Nanoparticles prepared in this manner tend to be more physically and chemically active because of their large surface area-to-volume ratio, so it is applicable in the development of antimicrobials, diagnostics, catalysis, biosensing, and targeted drug delivery (Prasad et al. 2021; Bradu et al. 2023; Tiwari et al. 2025). Many reports have affirmed that synthesis of AgNPs in plants leads to great antimicrobial activity (Salayová et al. 2021).

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Moringa oleifera La., or the drumstick tree, is a concern of family Moringaceae and is indigenous to north-western India, however, it is cultivated to a large extent in Pakistan and other places (Arshad et al. 2023). It is the so-called tree of life that has nutritional, medicinal, and industrial significance (Zeeshan et al. 2024). Leaves, especially, are abundant in proteins, amino acids, minerals, vitamins, polyphenols, and flavonoids among other bioactive compounds, which are mostly antioxidants and antimicrobials (Dhakad et al. 2022; Herman-Lara et al. 2024; Haris and Ahmad 2024; Gupta et al. 2024). Traditionally, the leaves have been employed to treat malnutrition and in the treatment of ulcers, malaria, bronchitis, and symptoms of HIV/AIDS. (Kashyap et al. 2022). Anticancer, hypotensive, and antibacterial effects are also demonstrated by extracts of M. oleifera, which are primarily as a result of phytochemicals including benzyl isothiocyanate and glucosinolates (Madrigales-Reátiga et al. 2021).

As an excellent source of phytochemicals with medicinal activities due to its highly promising constituents, M. oleifera is a good prospective candidate in green synthesis of AgNPs. We describe the biosynthesis and optimization of AgNPs in the current study using fresh *M. oleifera* leaf extract as the energy source (sunlight). The strategy underlines the possibility of M. oleifera to produce bioactive AgNPs in a green environment.

2. MATERIALS AND METHODS

2.1. Materials

All chemicals, solvents and media used in this study were taken from the MMG lab store. The materials required for silver nanoparticle synthesis and antibacterial assays are summarized in Table 1.

Table 1: Materials used for the synthesis of silver nanoparticles and antibacterial assays

Material	Quantity
Broth	20mL for each organism
Distilled water	100mL
Mueller Hinton Agar	80mL
$AgNO_3$ (Wrapped in Aluminum Foil)	10mg (0.01g)
Plant Leaves	5g
Test organism	2 Bacterial culture plates (*Escherichia coli and Pseudomonas aeruginosa)*

2.2. Preparation of Leaf Extract

Fresh *M. oleifera* leaves were collected from Company Garden, Multan, Pakistan. The leaves were separated from stems, thoroughly washed with distilled water, and air-dried to eliminate any surface debris. For extract preparation, 10g of cleaned leaf material was mixed with 100ml of distilled water in a screw-capped flask and heated in a water bath at 90°C for one hour (Fig. 1).

Fig. 1: Leaves of Moringa oleifera, cleaning of Moringa leaves with distilled water, Swabbing of *Pseudomonas,* and swabbing of *E. coli* on Mueller Hinton (MH) agar plates as part of sample preparation and antibacterial assay setup.

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2.3. Synthesis of AgNPs

A 5mL aliquot of aqueous plant extract was combined with 50mL of 1mM $AgNO_3$ solution, and the mixture was exposed to direct sunlight to promote nanoparticle synthesis. The formation of silver nanoparticles was visually confirmed by a distinct color change from green to dark brown. Once maximum color intensity was achieved, the mixtures were removed from sunlight and stored in darkness at room temperature to prevent nanoparticle agglomeration. A control was prepared by mixing 50mL of $AgNO_3$ with 5mL of distilled water under identical conditions. The reduction of Ag^+ ions and subsequent nanoparticle formation were further validated using UV–visible spectroscopy (Asuquo et al. 2024). For spectral analysis, nanoparticle suspensions were diluted 1:2 with distilled water, with distilled water alone serving as the blank. Both the nanoparticle samples and the control were scanned across a wavelength range of 190–900 nm using a UV–vis spectrophotometer (Dong et al. 2021).

2.4. Determination of Antimicrobial Activity by Well-diffusion and Disc Diffusion Method

The antimicrobial activity of the synthesized nanoparticles was evaluated using both well diffusion and disc diffusion methods against pathogenic strains. Pure cultures of *Escherichia coli* and *Pseudomonas aeruginosa* were employed for antibacterial testing. Sterilized Petri dishes were prepared with approximately 80 mL of Mueller-Hinton agar, and each bacterial culture was evenly spread on the agar surface using sterile cotton swabs. The dried AgNPs were re-dissolved in distilled water prior to use. For the well diffusion assay, 100µL of $AgNO_3$ extract was loaded into each well using a micropipette as the positive control, while a blank well containing no AgNPs served as the negative control. In the disc diffusion method, square filter paper discs were impregnated with $AgNO_3$ extract and carefully placed on the inoculated agar plates under aseptic conditions. All plates were incubated at 37°C for 24 hours, after which the zones of inhibition were recorded (Patel 2021).

2.5. Statistical Analysis

All experiments were performed in triplicate, and the data are presented as the mean±SD. One-way analysis of variance (ANOVA) was performed to compare the mean zones of inhibition among treatments, including AgNPs, $AgNO_3$ (positive control), and blank (negative control). Differences were considered statistically significant at $P<0.05$. Statistical analyses were carried out using R software.

3. RESULTS

3. 1. Visual Observation and UV–Vis Spectroscopy

Upon exposure of the reaction mixture to direct sunlight, a distinct color change from pale yellow to dark brown was observed within approximately 30 minutes, indicating the formation of silver nanoparticles (AgNPs). UV–Visible absorption spectroscopy confirmed this transformation, showing a strong surface plasmon resonance (SPR) peak at approximately 434 nm, consistent with sunlight-assisted synthesis (Table 2).

Table 2: UV–Vis Absorption and Optical Properties of AgNPs

Sample	Color Change	λmax (nm)	SPR Characteristics
AgNPs + M. oleifera extract	Pale yellow to Dark brown (~30min)	434	Strong, stable SPR band
Control ($AgNO_3$ only)	No change	No peak	No AgNP formation

3. 2. Structural and Morphological Properties

The structural and morphological characterization of the biosynthesized silver nanoparticles revealed nanoscale dimensions, spherical geometry, and crystalline nature. A detailed summary of these parameters is provided in Table 3.

3.3. Antibacterial Activity

The antibacterial efficacy of biosynthesized AgNPs was evaluated against *Escherichia coli* and *Pseudomonas aeruginosa* using well and disc diffusion assays are explored in Table 4-5 and Fig. 2-3.

Table 3: Structural and morphological characteristics of biosynthesized silver nanoparticles

Parameters	Observed Values	Method
Average particle size	10–25nm	SEM
Particle shape	Predominantly spherical	SEM
XRD peaks (2θ)	38.4°, 44.5°, 64.9°, 77.4°	XRD
Crystalline nature	Face-centered cubic (fcc)	XRD

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Table 4: Antibacterial activity of AgNPs against *E. coli*

Concentration	Method	Zone of Inhibition (mm)	Positive Control	Negative Control
25 µg/mL	Well diffusion	15.01±0.5	-	0
50 µg/mL	Well diffusion	16.99±0.5	-	0
100 µg/mL	Well diffusion	19.01±0.5	-	0
50 µg/disc	Disc diffusion	20.31±2.29	28.31±1.52	0
100 µg/disc	Disc diffusion	26.00±3.60	-	0

Table 5: Minimum Inhibitory Concentration (MIC) for *E. coli*

AgNP Concentration (mM)	Relative Growth (OD_{600})	Inhibition (%)
0.0	1.00	0
0.5	0.88	12
1.0	0.68	32
1.5	0.43	57
2.5	0.18	82
5.0	0.00	100

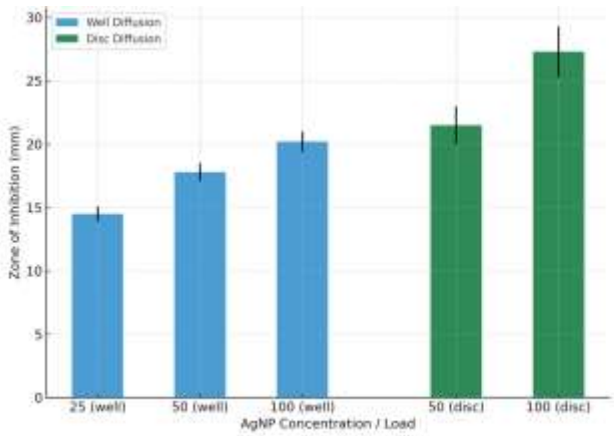

Fig. 2: Antibacterial activity of AgNPs against E. coli (Well vs Disc diffusion).

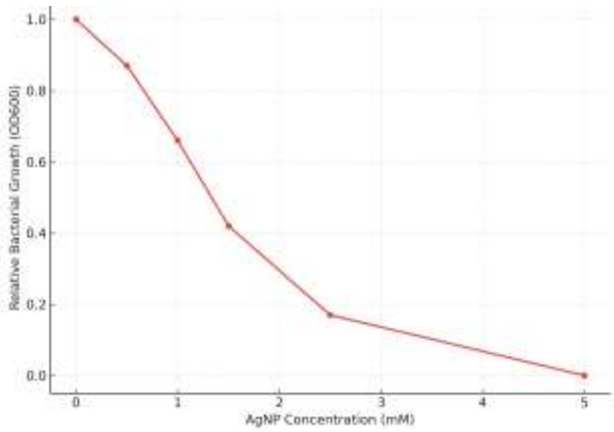

Fig. 3: MIC determination of AgNPs against *E. coli* showing dose-dependent inhibition.

4. DISCUSSION

In recent years, considerable attention has been directed toward developing novel synthesis approaches for silver nanoparticles (AgNPs). Among these, biological or green synthesis is regarded as safer and simpler compared with conventional techniques, which often involve toxic and environmentally hazardous substances (Zahoor et al. 2021). In this study, we evaluated the antibacterial activity of AgNPs synthesized using *M. oleifera* leaf extract. The method employed here represents a green, cost-effective approach, where nanoparticle formation was achieved under sunlight irradiation within a short time (Alharbi et al. 2022). Earlier researches have noted that plant extracts can reduce Ag+ under direct sunlight in less than one hour (Shah et al. 2021). A clear shift of color between green and dark brown, which is due to the surface plasmon resonance, was noted after 30 minutes in the sunlight and thus this proved successful nanoparticles were formed. The fact that the sources of renewable energy, like sunlight, are used also marks the sustainability of this synthesis path (Kaabipour and Hemmati 2021).

We have found that green-synthesized AgNPs had excellent antimicrobial effects against pathogenic bacteria, which is consistent with previous findings with other plant extracts. It is indicated in the literature that antibacterial activity of AgNPs is significantly determined by the size of the particles, and smaller nanoparticles are more active than larger (Do et al. 2025). Nanoparticle size was not determined in the present study; however, past results suggest that direct sunlight prefers the formation of smaller AgNPs. For instance, Wassihun (2024) reported that the average diameter of *M. oleifera*-mediated AgNPs synthesized by sunlight was 9-11 nm. Additionally, surface oxidation of AgNPs, resulting in the presence of silver, oxygen, carbon, and nitrogen, has been associated with enhanced antimicrobial activity due to their ability to disrupt bacterial membranes (Islam et al. 2021). Since biologically synthesized nanoparticles often display these surface elements, it is reasonable to attribute the antibacterial potential observed here to both smaller particle size and surface oxidation.

The mechanisms underlying the bactericidal effects of AgNPs are multifaceted. They may disrupt the bacterial cell membrane, causing shrinkage and separation of the cytoplasmic membrane, or induce condensation of DNA molecules within the cell, a process believed to protect DNA from damage but ultimately impair bacterial replication (Leteba 2023). Reactive oxygen species (ROS)-mediated oxidative stress is also considered a major pathway for their antibacterial activity (Tripathi and Goshisht 2022). Ag^0 particles and oxygen can combine to form hydrogen peroxide (H_2O_2), which damages bacterial cell walls (Han et al. 2022). The susceptibility of bacteria may vary with cell wall composition; for instance, Gram-positive bacteria, with their thick peptidoglycan layer, are generally less affected than Gram-negative strains. Nevertheless, AgNPs synthesized from *M. oleifera* leaves have shown significant inhibition against both Gram-positive and Gram-negative bacteria (Alosaimi et al. 2024). Previous studies suggest that relatively low concentrations of AgNPs (25 µg/ml) are sufficient for strong activity against Gram-negative bacteria, while higher concentrations (500 µg/ml) are required for Gram-positive strains (Tripathi and Goshisht 2022).

Our findings align with earlier reports demonstrating that AgNPs are effective against a wide spectrum of pathogenic and multidrug-resistant microbes. Moreover, AgNPs have been reported to enhance the efficacy of commercial antimicrobial drugs. In this study, biosynthesized AgNPs were found to be active against *P. aeruginosa* and *E. coli*, in agreement with reports where inhibition zones for Gram-positive bacteria (*B. subtilis* and *S. aureus*) were generally larger than those for Gram-negative species (*E. coli* and *P. aeruginosa*) (Domb and Shende 2025).

We acknowledge two main limitations of this work. First, all antibacterial assays were conducted at a constant concentration of silver nitrate, which restricted optimization. Second, control assays were not performed alongside the treatments. These limitations highlight the need for standardized protocols that optimize parameters such as extract concentration, reaction time, temperature, and pH to improve reproducibility and yield (Mahaveerchand and Abdul Salam 2024). Furthermore, advanced characterization techniques such as SEM, TEM, XRD, FTIR, and DLS should be employed to confirm nanoparticle size, morphology, and stability. Future studies should expand antimicrobial screening to include a wider range of bacterial and viral pathogens and compare the performance of green-synthesized AgNPs with chemically synthesized counterparts (Sharma et al. 2022).

Beyond antimicrobial applications, AgNPs hold potential in diverse fields, including the production of antimicrobial creams, sprays, and patches for healthcare, as well as applications in agriculture, biomedical sciences, and environmental management (Singh et al. 2024). Their cytotoxicity to human cells, biodegradability and their long-term environmental effects should also be evaluated to be safe to use in biomedical and industrial applications (Karnwal et al. 2024).

5. CONCLUSION

In the present investigation, silver nanoparticles (AgNPs) synthesized utilizing *Moringa oleifera* leaf extract were systematically evaluated for their antibacterial efficacy against multiple pathogenic bacterial strains. The results demonstrated that the biosynthesized AgNPs possessed pronounced broad-spectrum antibacterial activity, thereby underscoring their potential as an effective and environmentally benign alternative for biomedical applications. The green synthesis approach proved to be efficient, wherein *M. oleifera* leaves acted as a natural reducing and stabilizing agent, facilitating the formation of AgNPs with a heterogeneous size distribution (56.9 nm, 448.1 nm, and 4705.0 nm). Additionally, low-density polyethylene (LDPE) packaging material immersed in colloidal nanosilver solution for three hours exhibited improved surface characteristics, highlighting the prospective utility of such nanoparticles in food packaging applications. Collectively, these findings reinforce the feasibility of employing *M. oleifera*-derived AgNPs as a sustainable strategy for the development of antimicrobial agents and value-added industrial applications.

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Ethics Statement: This study did not involve human participants or animals and therefore did not require ethical approval.

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