



Green Synthesis of Silver Nanoparticles Using *Moringa oleifera* Leaf Extract: Antibacterial Efficacy and Sustainable Nanotechnology

Shoheli Ferdous¹, GM Shafiur Rahman¹, Jafor Rayhan¹, Md Zakir Hossain¹, Md Hasibur Rahman¹, Alam Khan¹, Md Shariful Islam^{1*}

Abstract

Background: The green synthesis of silver nanoparticles (AgNPs) is an emerging, environmentally friendly method that employs natural substances, such as plant extracts, to create nanoparticles without the use of harmful chemicals. In this study, we explored the synthesis of AgNPs using an aqueous extract of *Moringa oleifera* leaves, known for their antimicrobial properties. The objective was to evaluate the synergistic antibacterial effects of the synthesized AgNPs in combination with the plant extract. **Methods:** Silver nitrate (AgNO_3) was reduced by the plant extract at 65°C, resulting in the formation of AgNPs. Characterization of the nanoparticles was conducted using various techniques including X-ray diffraction (XRD), scanning electron microscopy (SEM), thermogravimetric analysis (TGA), Fourier-transform infrared (FTIR) spectroscopy, and UV-Vis spectroscopy. **Results:** XRD analysis confirmed the successful formation of silver nanostructures with average particle size ranging from 80 to 85 nm. SEM images revealed spherical nanoparticles, while TGA and FTIR data indicated the stability of the synthesized nanoparticles and the

presence of bioactive compounds in the leaf extract that facilitated the reduction and stabilization of AgNPs. Antibacterial activity was assessed against *Escherichia coli* using the disc diffusion method, and results demonstrated a concentration-dependent inhibition, with the 20 mg/mL M-AgNPs exhibiting a zone of inhibition of 13.33 mm, comparable to that of ciprofloxacin. **Conclusion:** This study demonstrated the potential of *Moringa oleifera* leaf extract in the eco-friendly synthesis of AgNPs with promising antibacterial properties. These findings suggest that green-synthesized AgNPs could serve as an alternative to traditional antibiotics, offering a sustainable approach for combating microbial infections. Further research is needed to optimize the synthesis process and assess the broader applications of these nanoparticles in medical and environmental fields.

Keywords: Green synthesis, silver nanoparticles, *Moringa oleifera*, antibacterial activity, sustainable nanotechnology.

Introduction

The study of environmentally friendly methods for producing metal nanoparticles, known as green synthesis, is rapidly gaining prominence. This approach utilizes natural substances such as vitamins, carbohydrates, plant extracts, biodegradable polymers, and microorganisms to create nanoparticles. Unlike traditional methods, green synthesis avoids the use of hazardous byproducts and toxic chemicals, making it a more sustainable and safer alternative (Kharissova et al., 2013). The process of synthesizing

Significance | This study demonstrates the eco-friendly synthesis of silver nanoparticles using *Moringa oleifera* extract, highlighting sustainable nanotechnology's potential in combating bacterial infections.

*Correspondence. Md Shariful Islam, Department of Veterinary and Animal Sciences, Rajshahi University, Rajshahi.
E-mail: msips06@ru.ac.bd

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Author Affiliation.

¹Department of Veterinary and Animal Sciences, Rajshahi University, Rajshahi.
² Bangladesh Agricultural University, Mymensingh 2202, Bangladesh.

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nanoparticles using plant extracts is straightforward: plant extracts act as reducing and stabilizing agents, reacting with metal salts to produce nanoparticles within a few minutes to hours at room temperature. This method is cost-effective, efficient, and simpler to characterize compared to conventional techniques, making it particularly effective for synthesizing silver nanoparticles (AgNPs) (Abdelghany et al., 2018; Mittal et al., 2013).

In green synthesis, plant extracts are employed to chemically reduce a silver nitrate (AgNO_3) solution, facilitating the formation of AgNPs (Carmona et al., 2017). The physical, optical, and catalytic properties of AgNPs are highly dependent on their size, distribution, and surface characteristics, which can be tailored using stabilizers, reducing agents, and specific synthesis techniques (Atwater & Polman, 2010; Abou El-Nour et al., 2010). Factors such as the synthesis method, choice of stabilizing and reducing agents, and the precursor salt play a crucial role in determining the size and shape of AgNPs (Mukherji et al., 2018). AgNPs have versatile applications, including sensing, catalysis, wound healing, and antibacterial treatments, showcasing the potent antimicrobial properties of Ag^+ ions. Their remarkable characteristics have enabled their widespread use in biomedical engineering, biosensing, nanomedicine, and pharmaceutical applications (Mukherji et al., 2018).

Moringa oleifera, often referred to as the "magic tree," has been widely used in traditional medicine. Various parts of the plant, including its leaves, flowers, seeds, and roots, have been reported to offer numerous health benefits (Patil et al., 2022). *Moringa* has been traditionally used to enhance energy, memory, alertness, and skin health while addressing ailments such as asthma, sore throats, fevers, urinary tract infections (UTIs), and diarrhea. It also exhibits anti-inflammatory, antibacterial, antidiabetic, anticancer, and tissue-protective properties (Leone et al., 2015; Dhakad et al., 2019; Mahmood et al., 2010; Bhattacharya et al., 2018). Specifically, cold aqueous extracts of *M. oleifera* leaves have demonstrated antibacterial activity through disk diffusion assays (Oluduro et al., 2012). Previous studies have shown that aqueous, acetone, and ethanolic extracts of *M. oleifera* leaves exhibit significant antibacterial effects (Bukar et al., 2010).

Building on this foundation, the current study employed an aqueous extract of *M. oleifera* leaves to synthesize AgNPs through a green synthesis approach. The study aimed to evaluate the synergistic antibacterial effects of *M. oleifera* leaf extract and the synthesized AgNPs. This research highlights the potential of plant-based synthesis for advancing sustainable nanotechnology and enhancing antibacterial treatments.

2. Materials and methods

2.1 Preparation of Plant Extract and Silver Nitrate Solution

The highest-quality chemicals for this study were sourced from Sigma Aldrich. Fresh *Moringa oleifera* leaves were collected from trees on the university campus. After collection, the leaves (50 g) were weighed using a digital balance. All unwanted materials, including dust, contaminants, and insects, were carefully removed. The leaves were then washed thoroughly, first with regular water (three to four times) and subsequently with distilled water (twice). The cleaned leaves were combined with 200 mL of distilled water and heated at 70–80°C for 25–30 minutes. After heating, the solution was filtered using Whatman filter paper, yielding a fresh extract of *Moringa oleifera* leaves (Figure 1a). The yellowish extract was allowed to cool to room temperature and stored for further use. Additionally, a 1 mM silver nitrate (AgNO_3) solution was prepared by dissolving 0.17 g of silver nitrate in 1000 mL of distilled water.

2.2 Synthesis of Silver Nanoparticles (M-AgNPs)

The synthesis of *Moringa*-based silver nanoparticles (M-AgNPs) was carried out using a standard procedure. Different ratios of *Moringa oleifera* leaf extract (MLE) and silver nitrate solution were mixed and heated to 65°C with continuous stirring for approximately 30 minutes until the mixture turned dark brown. The mixture was then centrifuged at 10,000 rpm for 15–20 minutes. The resulting sediment was washed with distilled water and centrifuged repeatedly (five to six times).

The final product, M-AgNPs, was collected and dried in an oven at 80°C for 24 hours (Figure 1b). The dried nanoparticles were stored for subsequent characterization and analysis.

2.3. Characterization and Analysis

The crystalline structure, phase, lattice parameters, and grain size of the synthesized M-AgNPs were analyzed using X-ray diffraction (XRD) with a Rigaku RINT-TTR III system, as described by Islam and Todo (Islam & Todo, 2016). The microstructures of the nanoparticles were characterized using field emission scanning electron microscopy (FE-SEM, Hitachi S-4100).

Thermal gravimetric analysis (TGA) was performed using a Perkin Elmer Simultaneous Thermal Analyzer 8000 to evaluate the phase transitions and thermal stability of the M-AgNPs. The vibrational characteristics and local structural features of the nanoparticles were examined using Fourier Transform Infrared (FTIR) spectroscopy (Perkin Elmer Spectrum 100) in the wavelength range of 500–4000 cm^{-1} .

The optical properties and absorbance of the M-AgNP solution were measured using UV-Visible spectroscopy (PG Instruments T60 UV-Visible Spectrophotometer). Finally, the antibacterial activity of M-AgNPs against *Escherichia coli* was assessed using the disc diffusion method and zone of inhibition measurements.

3. Results and discussion

The X-ray diffraction (XRD) pattern of M-AgNPs is presented in Figure 2(a). The unique diffraction peaks confirmed the successful

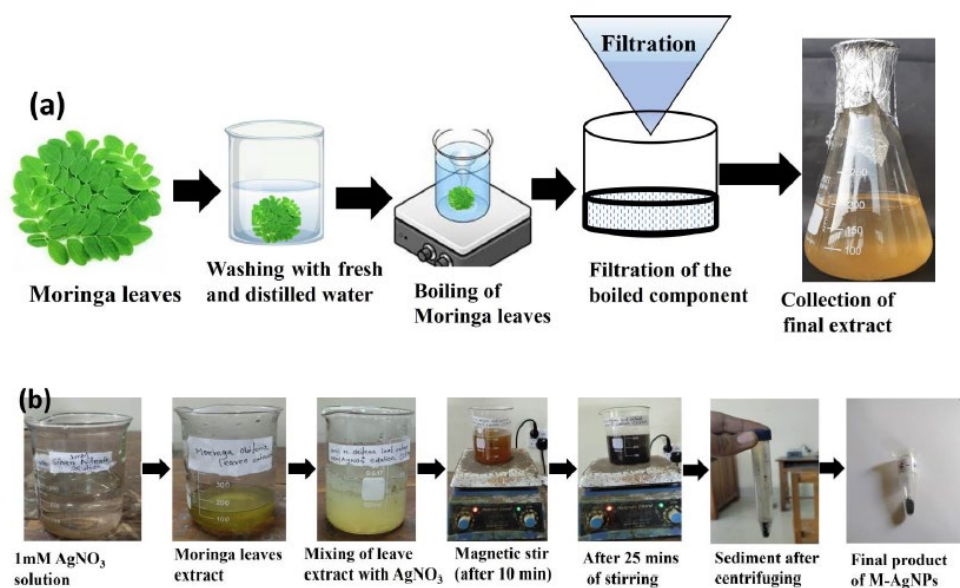


Figure 1. Schematic representation of the preparation of Moringa leaf extracts (a), and silver nanoparticles (b)

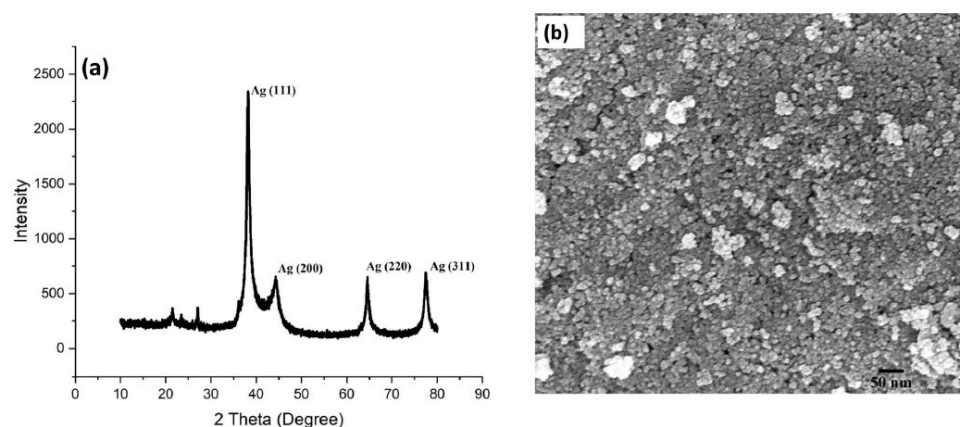


Figure 2. XRD pattern (a) and FE-SEM microstructure (b) of M-AgNPS powder

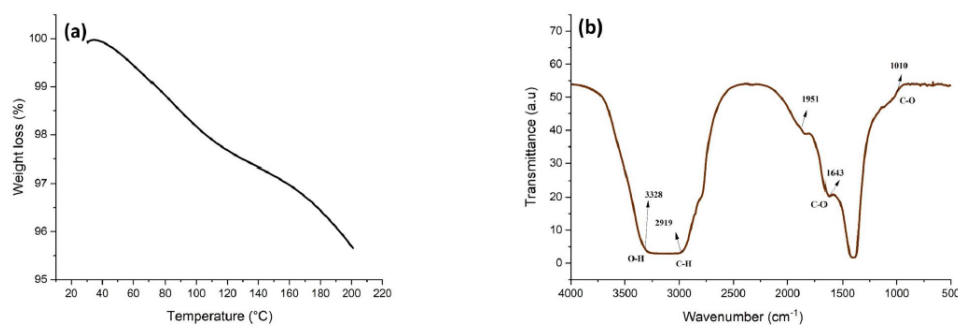


Figure 3. TGA (a) and FTIR (b) images of M-AgNPS powder.

synthesis of silver nanostructures using *Moringa oleifera* leaf extract. The characteristic peaks were observed at 2θ angles of 37.59° , 44.29° , 64.68° , and 76.98° , corresponding to the crystallographic planes (111), (200), (220), and (311), respectively. These results indicated a mixed-phase structure comprising cubic and hexagonal silver nanoparticles, consistent with findings reported by Shameli et al. (2012). The Scherrer formula, applied to the full-width at half-maximum (FWHM) data, revealed that the synthesized M-AgNPs had an average size of approximately 80–85 nm.

Scanning electron microscopy (SEM) images of the Ag/*Moringa oleifera* emulsion are shown in Figure 2(b). The SEM analysis, performed after 24 hours of stirring, demonstrated the successful modulation of Ag-NP size and shape by the *Moringa* leaf extract (MLE). The external surfaces of the Ag/MLE composite exhibited a bright and spherical morphology, attributable to the presence of uniformly distributed Ag-NPs. The particles were narrowly distributed, with diameters ranging from 80 to 100 nm, suggesting precise control over the synthesis process.

Thermogravimetric analysis (TGA) was employed to evaluate the thermal behavior of the synthesized nanoparticles, as shown in Figure 3(a). The weight loss of the AgNPs was monitored as the temperature increased. Initial decomposition commenced around 40°C , with substantial mass loss occurring between 40°C and 100°C , primarily due to the removal of moisture content. Further weight reduction observed between 100°C and 205°C was attributed to the thermal degradation of the capping agents. The negligible residual weight beyond 205°C , illustrated by the plateau of the TGA curve, supports the notion of complete decomposition of organic materials. These findings align with earlier studies (Vidhu et al., 2011; Khalil et al., 2014).

Fourier Transform Infrared (FTIR) spectroscopy was conducted to identify the biomolecules responsible for reducing Ag^+ ions and stabilizing the synthesized Ag-NPs. The FTIR spectrum (Figure 3(b)) of the MLE-AgNPs exhibited distinct transmission peaks at 3328, 2919, 1951, 1643, and 1010 cm^{-1} . The peak at 3328 cm^{-1} corresponded to hydroxyl (-OH) or amine (-NH) groups, while the 2919 cm^{-1} peak was associated with aliphatic C-H stretching. The peak at 1643 cm^{-1} was attributed to the stretching vibration of carboxyl groups (-C=O). Notably, the absorption bands at 1010 cm^{-1} indicated the stretching vibrations of amine (-C-N), ether (-C-O-C), or hydroxyl (-C-O) groups, likely originating from flavonoids or alkaloids in the leaf extract (Huang et al., 2007).

Residual nitrate ions ($-\text{NO}_3$) were detected in the range of $1300\text{--}1500\text{ cm}^{-1}$, confirming their presence in the synthesized AgNPs, as previously noted by Shameli et al. (2012). Peaks near $1004\text{--}1010\text{ cm}^{-1}$ were linked to the interaction of AgNPs with hydroxyl molecules in the *Moringa* compounds. The observed color change during the reaction provided visual evidence of nanoparticle

formation, indicating surface plasmon resonance (SPR) effects caused by the reduction of silver ions (Krishnaraj et al., 2010). The SPR peak, located at 430–440 nm in the UV-Vis spectrum, affirmed the role of reducing agents in the leaf extract in transforming silver ions (Ag^+) into silver nanoparticles (Ag^0) (Prasad & Elumalai, 2011).

The structural, thermal, and optical analyses of the synthesized M-AgNPs revealed the efficacy of *Moringa oleifera* leaf extract as a reducing and stabilizing agent in the green synthesis of silver nanoparticles. The results highlighted the formation of uniformly distributed, spherical nanoparticles with enhanced stability and surface plasmon resonance properties. The combined characterization techniques demonstrated the potential of MLE in producing high-quality silver nanoparticles for biomedical and environmental applications.

The antibacterial activity of *Moringa oleifera*-based silver nanoparticles (M-AgNPs) was evaluated using five different discs, as shown in Figure 4(a). Discs A, B, and C represent *Escherichia coli* (*E. coli*) cultures treated with 10 μL of M-AgNPs at concentrations of 5 mg/mL, 10 mg/mL, and 20 mg/mL, respectively. Disc D represents a control disc containing Ciprofloxacin. The visual images demonstrate the significant antibacterial activity of the synthesized AgNPs combined with *Moringa oleifera* leaf extracts. Figure 4(b) illustrates the average minimum zone of inhibition (mm) produced by these treatments at varying concentrations.

The inhibition zones observed in the treated samples revealed distinct antibacterial effects. Samples A, B, C, and D exhibited average minimum inhibition zones of 10.36 mm, 11.26 mm, 13.33 mm, and 21.29 mm, respectively (Figure 4(b)). Statistical analysis indicated a significant difference ($P < 0.05$) between the zones of inhibition produced by M-AgNPs and the Ciprofloxacin control. Notably, samples treated with 20 mg/mL M-AgNPs exhibited a moderate inhibition zone of 13.33 mm, which, although smaller than that of Ciprofloxacin, highlights the potential effectiveness of the green-synthesized nanomaterials in combating harmful pathogens such as *E. coli*.

The findings underscore the promise of M-AgNPs as eco-friendly antibacterial agents. The moderate efficacy observed at 20 mg/mL suggests that green nanomaterials derived from *Moringa oleifera* could serve as alternative antibacterial solutions, particularly in contexts where conventional antibiotics may not be preferred or accessible.

The medicinal properties of *Moringa oleifera* have been recognized for centuries in traditional medicine due to its diverse therapeutic benefits. In recent years, there has been growing interest in the antibacterial properties of *Moringa oleifera* extracts and their nanoparticle formulations. Particular attention has been given to their potential action against *E. coli*, a common pathogen known for its role in human infections. The current study contributes to this

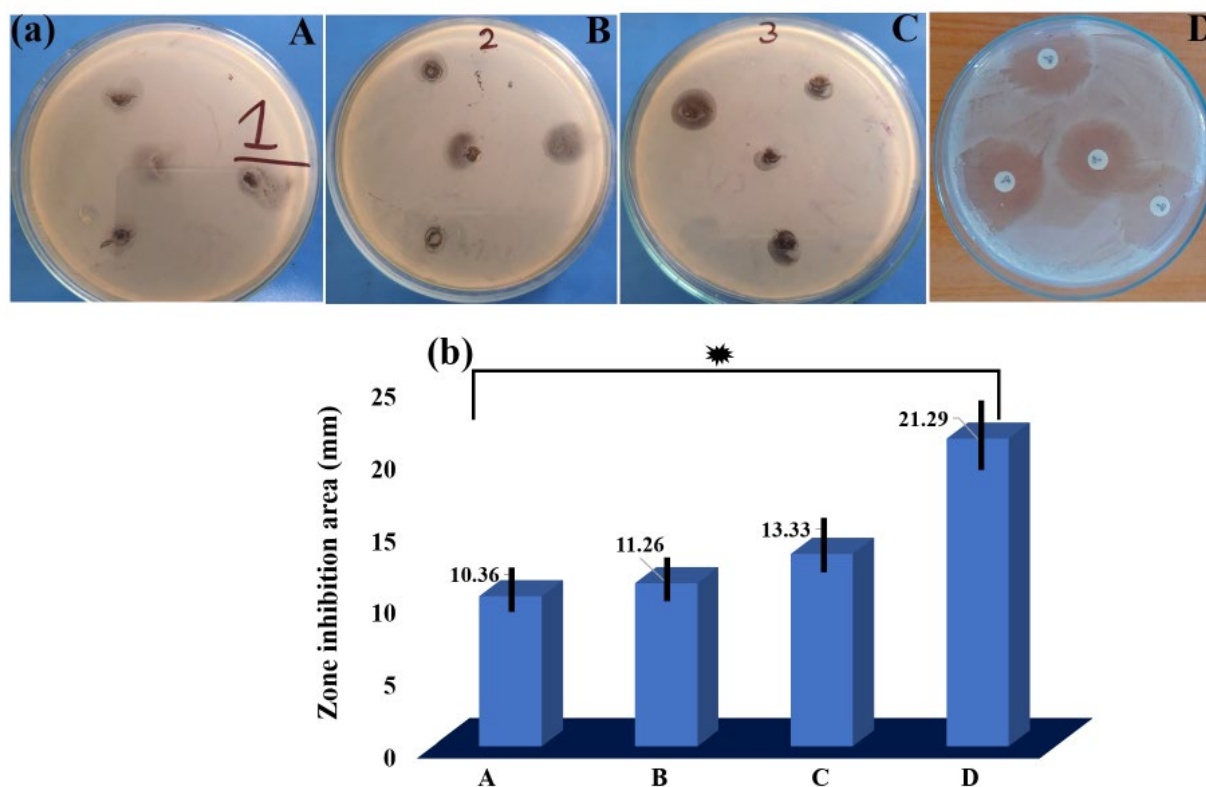


Figure 4. Antibacterial (*E. coli*) potential of M-AgNPS measured by disc diffusion (a) and zone inhibition (b) method.

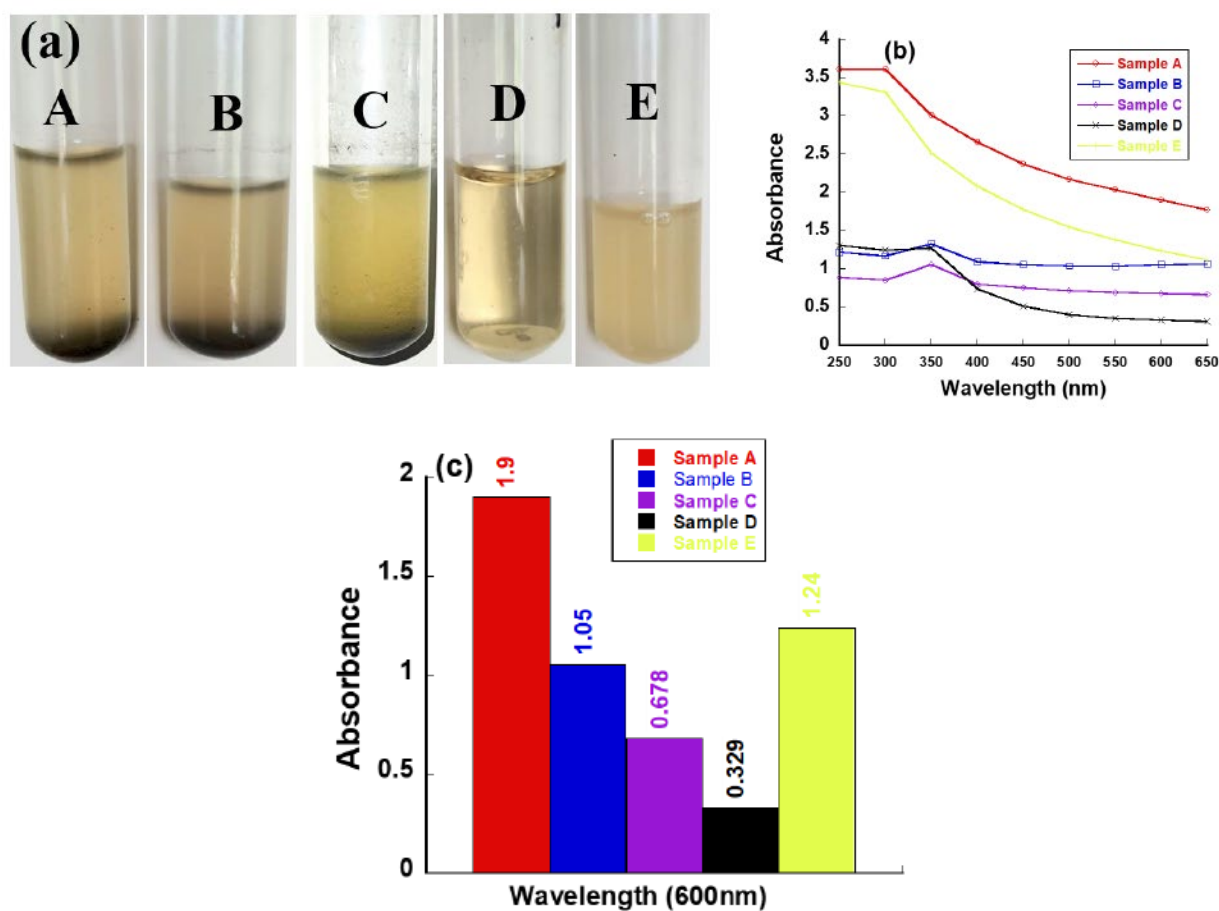


Figure 5. *E. coli* cell sediment transparency (a), optical density (b), and specific optical density at 600 nm (c) in various concentrations of M-AgNPs.

body of research, demonstrating the antibacterial efficacy of M-AgNPs synthesized using *Moringa oleifera* leaf extract.

The results align with previous research highlighting the antibacterial properties of plant-based silver nanoparticles. A study by Supriya and Chaitanya Kumari (2019) found that silver nanoparticles synthesized from *Aloe vera* extracts demonstrated significant antibacterial activity, with zones of inhibition of 25 mm and 20 mm against *Bacillus* sp. and the lowest antifungal activity of 11 mm against *Aspergillus* sp. These findings further support the potential of green-synthesized AgNPs as effective antimicrobial agents.

The antibacterial performance of the synthesized M-AgNPs is attributed to the unique properties of silver nanoparticles and the bioactive compounds present in *Moringa oleifera* leaf extracts. Silver nanoparticles are known for their potent antimicrobial properties, which involve multiple mechanisms, including disruption of microbial cell walls, generation of reactive oxygen species, and interference with cellular functions. The bioactive compounds in *Moringa oleifera*, such as flavonoids, tannins, and alkaloids, may enhance these effects by stabilizing the nanoparticles and contributing to their antibacterial activity.

This study's findings highlight the potential of green nanotechnology as a sustainable alternative for addressing microbial infections. The use of plant-based synthesis methods not only reduces environmental impact but also capitalizes on the natural bioactivity of plant extracts. The moderate inhibition zone of 13.33 mm observed at 20 mg/mL suggests that while M-AgNPs may not yet rival traditional antibiotics like Ciprofloxacin in efficacy, they hold promise as complementary or alternative agents, particularly in applications where sustainability and biocompatibility are prioritized.

In conclusion, the study demonstrates the antibacterial potential of *Moringa oleifera*-based silver nanoparticles against *E. coli*. The results support the broader applicability of green-synthesized nanomaterials in addressing microbial challenges, contributing to sustainable practices in nanotechnology and antimicrobial research. Future studies could focus on optimizing the synthesis process, exploring higher concentrations, and evaluating the nanoparticles' efficacy against a broader spectrum of pathogens to further validate their potential as antimicrobial agents.

The transparency of *Escherichia coli* (*E. coli*) cultures grown with different concentrations of Moringa-AgNPs is illustrated in Figure 5(a). Among the test tubes, samples treated with Ciprofloxacin (sample D), 20 mg/mL Moringa-AgNPs (sample C), and 10 mg/mL Moringa-AgNPs (sample B), as well as the untreated control (sample E), appeared the clearest and most transparent. The optical density of *E. coli* cultures across a broad wavelength range (250–650 nm) is presented in Figure 5(b), while Figure 5(c) highlights the

optical density at a fixed wavelength of 600 nm for varying concentrations of Moringa-AgNPs.

The lowest absorbance value (0.329) was recorded for sample D (Ciprofloxacin), followed by sample C (20 mg/mL Moringa-AgNPs) with 0.678, and sample B (10 mg/mL Moringa-AgNPs) with 1.05. The untreated control (sample E) exhibited an absorbance of 1.24, while sample A (5 mg/mL Moringa-AgNPs) showed the highest absorbance at 1.90. These results demonstrate that increasing concentrations of Moringa-AgNPs reduced bacterial growth, as indicated by lower optical density values, with sample C (20 mg/mL Moringa-AgNPs) exhibiting the most pronounced antibacterial effect compared to the untreated control (sample E).

Optical density is a widely used metric to estimate bacterial biomass in liquid culture. The relationship between optical density and bacterial concentration is influenced by the scattering and absorption of light by bacterial cells. Higher optical density values correspond to higher bacterial concentrations, as the increased number of cells scatters and absorbs more light, reducing the amount transmitted through the culture. Calibration curves tailored to the bacterial species, growth medium, and measurement wavelength are often employed to establish this relationship.

In this study, the antibacterial activity of Moringa-AgNPs was demonstrated to significantly reduce *E. coli* growth, particularly in the case of sample C (20 mg/mL Moringa-AgNPs). This reduction was comparable to the effect of Ciprofloxacin (sample D). The results align with previous findings, such as those by Kim et al. (2017), which demonstrated growth inhibition of bacteria treated with various AgNP shapes and sizes. Kim et al. observed that as the concentration of AgNPs increased, bacterial growth delays became more pronounced, with concentrations of 150 mg/mL and 1,500 mg/mL completely halting growth in spherical/plate-shaped and cubic AgNPs, respectively.

Further insights into the antibacterial efficacy of AgNPs were provided by Sondi and Salopek-Sondi (2004), who investigated their effects on *E. coli* as a model for Gram-negative bacteria. The study revealed that silver nanoparticles caused structural damage to *E. coli* cell walls, forming "pits" and leading to increased membrane permeability, ultimately resulting in cell death. The cytotoxicity of silver nanoparticles against *E. coli* was effective at concentrations as low as 60 µg/mL. Raffi et al. (2008) also highlighted that silver nanoparticles penetrate bacterial membranes, attaching to the cell walls and inhibiting cell growth and replication.

The findings from this study indicate that Moringa-AgNPs exhibit strong antibacterial activity against *E. coli*. These nanoparticles present a promising natural alternative to synthetic antibiotics, leveraging the antimicrobial properties of silver nanoparticles in combination with the eco-friendly synthesis facilitated by Moringa leaf extracts.

While the antibacterial potential of Moringa-AgNPs is significant, further investigations are necessary to assess their therapeutic applications, particularly regarding human safety and efficacy. Such research would provide a deeper understanding of the potential of these nanoparticles in biomedical applications and help establish their role as viable alternatives in combating antibiotic-resistant bacteria.

4. Conclusion

The study determines the potential of *Moringa oleifera*-based silver nanoparticles (M-AgNPs) synthesized through green methods as effective antibacterial agents. Using an eco-friendly approach, M. oleifera leaf extract acted as both reducing and stabilizing agents in the synthesis of AgNPs, yielding nanoparticles with uniform size and spherical morphology. Comprehensive characterization confirmed their stability, surface plasmon resonance properties, and antibacterial efficacy. The M-AgNPs demonstrated significant activity against *Escherichia coli* (E. coli), with higher concentrations (20 mg/mL) achieving pronounced growth inhibition, comparable to the antibiotic Ciprofloxacin.

The study underscores the role of bioactive compounds in M. oleifera, such as flavonoids and alkaloids, in enhancing nanoparticle stability and antimicrobial effects. These findings support the application of plant-based synthesis methods in sustainable nanotechnology, addressing microbial challenges while reducing environmental impact. Future research should focus on optimizing synthesis, expanding pathogen spectrum studies, and evaluating therapeutic safety to solidify M-AgNPs' role in combating antibiotic resistance.

Author contributions

SF synthesized nanomaterials and performed the experiment; GMSR supervised the research and revised the manuscript; JR wrote the manuscript; MZH managed and checked the citations; MHR and AK conducted the literature review and edited the manuscript, MSI designed the experiment, reviewed and submitted the manuscript. All authors reviewed and approved the final manuscript

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Competing financial interests

The authors have no conflict of interest.

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