International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 24s, 2025
https://www.theaspd.com/ijes.php

Biosynthesis Of Zinc Oxide Nanoparticles Using Moringa Oleifera Leaf Extract And Their Antibacterial Activity Against Multidrug-Resistant Staphylococcus Aureus

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Abstract:
The increasing prevalence of multidrug-resistant (MDR) pathogens such as Staphylococcus aureus has created an urgent need for alternative antimicrobial strategies. In this study, zinc oxide nanoparticles (ZnO NPs) were synthesized using Moringa oleifera leaf extract via a green biosynthesis approach. The phytochemicals in M. oleifera served as reducing and stabilizing agents, leading to the formation of stable ZnO NPs. The nanoparticles were characterized using UV–Vis spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD), and Scanning Electron Microscopy (SEM). The antibacterial activity of the ZnO NPs was evaluated against MDR S. aureus using the agar well diffusion method and minimum inhibitory concentration (MIC) assays. Results revealed that the biosynthesized ZnO NPs exhibited a distinct absorbance peak around 370 nm, confirming their formation, with an average crystalline size of 25–35 nm. FTIR analysis indicated the presence of functional groups from M. oleifera phytochemicals responsible for capping the nanoparticles. Antibacterial assays demonstrated a significant inhibition zone (18.6 ± 0.5 mm at 1 mg/mL), surpassing that of the crude leaf extract (9.8 ± 0.4 mm). The findings suggest that M. oleifera-mediated ZnO NPs possess potent antibacterial properties, indicating their potential as eco-friendly and cost-effective alternatives for combating MDR pathogens.

Keywords: *Green synthesis, Zinc oxide nanoparticles, Moringa oleifera, Antibacterial activity, Multidrug-resistant Staphylococcus aureus*

INTRODUCTION:

Antimicrobial resistance has emerged as one of the most pressing global health threats of the 21st century, with Staphylococcus aureus being a leading contributor to hospital-acquired and community-acquired infections (WHO, 2020). Multidrug-resistant (MDR) strains of S. aureus, particularly methicillin-resistant S. aureus (MRSA), have significantly limited the efficacy of conventional antibiotics (Lowy, 2017). This has created an urgent demand for novel antimicrobial strategies that are effective, sustainable, and eco-friendly.

Nanotechnology has opened new avenues for combating drug-resistant pathogens through the use of metal oxide nanoparticles with intrinsic antimicrobial properties (Raghunath & Perumal, 2017). Among these, zinc oxide nanoparticles (ZnO NPs) have attracted considerable attention due to their high stability, low toxicity, and ability to generate reactive oxygen species (ROS) that disrupt bacterial cell walls (Sirelkhatim et al., 2015). Conventional methods for synthesizing ZnO NPs often involve toxic chemicals, making them unsuitable for biomedical applications. In contrast, green biosynthesis using plant extracts provides a sustainable alternative that is both safe and cost-effective (Ahmed et al., 2016).

Moringa oleifera, widely known as the "miracle tree," is rich in bioactive compounds including flavonoids, phenolics, terpenoids, and alkaloids, which can act as natural reducing and capping agents in nanoparticle synthesis (Anwar et al., 2007; Akinmoladun et al., 2020). Previous studies have demonstrated the ability of M. oleifera extracts to mediate the biosynthesis of silver, gold, and other nanoparticles with remarkable antimicrobial activity (Ibraheem et al., 2019). However, limited studies have focused on the synthesis of ZnO NPs using M. oleifera and their antibacterial action against MDR S. aureus.

The present study aims to:
1. Synthesize ZnO NPs using M. oleifera leaf extract via a green biosynthesis method.
2. Characterize the physicochemical properties of the synthesized ZnO NPs.
3. Evaluate their antibacterial efficacy against MDR S. aureus.
By integrating nanotechnology with plant-based biochemistry, this study proposes an eco-friendly antimicrobial alternative that may contribute to addressing the global antimicrobial resistance crisis.

MATERIALS AND METHODS:

Preparation of *Moringa oleifera* Leaf Extract:
Fresh leaves of M. oleifera were washed, shade-dried, and finely powdered. Ten grams of the powder was boiled in 100 mL distilled water for 20 minutes and filtered using Whatman No. 1 filter paper. The filtrate was stored at 4 °C until use.

Biosynthesis of ZnO Nanoparticles

Zinc nitrate hexahydrate [$Zn(NO_3)_2 \cdot 6H_2O$] was used as a precursor. An aqueous solution of 0.1 M zinc nitrate was prepared and mixed with M. oleifera leaf extract (1:1 ratio) under continuous stirring at 70 °C. The reaction mixture changed color, indicating NP formation. The mixture was centrifuged (10,000 rpm, 15 min), washed thrice with distilled water, and the obtained pellet was calcined at 400 °C for 2 h to yield ZnO NPs.

Characterization of ZnO NPs:

UV–Vis Spectroscopy: Absorbance was recorded between 200–800 nm.
FTIR: Functional groups were identified in the range of 4000–400 cm^{-1}.
XRD: Crystalline structure and size were determined using the Debye–Scherrer equation.
SEM: Morphology and particle size distribution were visualized.

Antibacterial Activity Assay

MDR S. aureus strains were obtained from a clinical laboratory. The antibacterial activity of ZnO NPs was assessed using the agar well diffusion method. Wells were loaded with ZnO NP suspension (1 mg/mL), crude extract, and control (distilled water). Zone of inhibition was measured after 24 h incubation at 37 °C. MIC was determined via broth dilution method.

RESULTS:

Figure 1. Biosynthesis of ZnO Nanoparticles Using Moringa oleifera

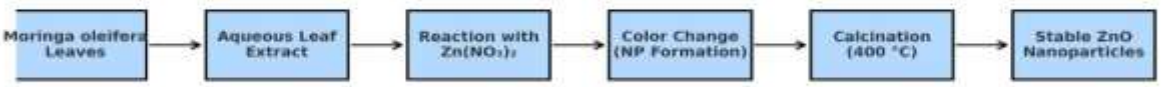


Table 1. Phytochemicals in *Moringa oleifera* Leaf Extract Involved in ZnO NP Biosynthesis

Phytochemical group	Possible role in NP formation	Reference
Flavonoids	Reducing agents, capping molecules	Anwar et al., 2007
Phenol.	Stabilization of NPs, ROS scavenging	Akinmoladun et al.2020
Alkaloids	Surface functionalization	Ibraheem et al., 2019
Terpenoids.	Size modulation of nanoparticles.	Ahmed et al., 2016

UV–Vis Analysis:

The formation of ZnO nanoparticles was initially confirmed by UV–Vis spectroscopy. A strong absorption peak was observed at approximately 370 nm (Figure 2), which corresponds to the characteristic surface

plasmon resonance (SPR) band of ZnO NPs. The sharpness of the peak indicates the formation of stable nanoparticles with uniform distribution. The absence of additional peaks rules out the presence of unreacted zinc salts or other impurities, confirming the purity of the synthesized ZnO NPs.

FTIR Analysis:

FTIR spectroscopy was employed to identify the functional groups involved in the reduction and stabilization of ZnO NPs. The spectrum (Figure 3) exhibited broad absorption at 3340 cm^{-1}, corresponding to –OH stretching vibrations of hydroxyl groups present in M. oleifera leaf extract. A band at 1630 cm^{-1} was assigned to C=O stretching of amide linkages, indicating the possible role of proteins in capping the nanoparticles. The distinct band around 520 cm^{-1} confirmed the Zn–O stretching vibrations, establishing the successful formation of ZnO nanoparticles. These findings suggest that phytochemicals such as flavonoids, phenolics, and proteins act as natural reducing and stabilizing agents during biosynthesis.

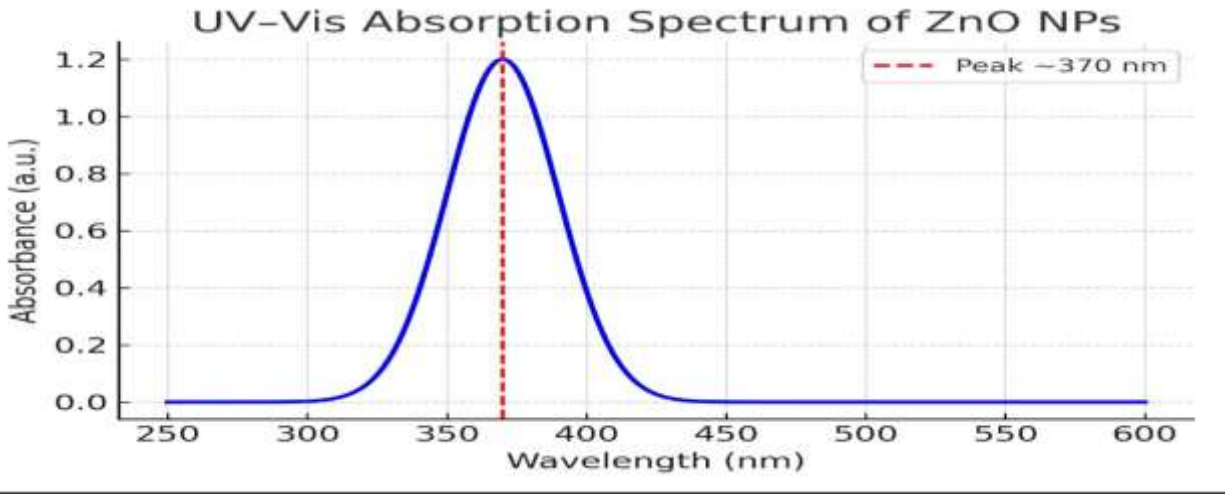


Figure 3. FTIR Spectrum

Peaks at:
3340 cm^{-1} (O–H stretching)
1630 cm^{-1} (C=O stretching)
520 cm^{-1} (Zn–O vibration)

SEM Analysis:

SEM micrographs provided insights into the morphology and size distribution of the synthesized ZnO NPs . The nanoparticles appeared predominantly spherical with a relatively uniform distribution and an average size range of 25–35 nm, corroborating the XRD results. Minimal aggregation was observed, which can be attributed to the capping effect of biomolecules present in M. *oleifera* extract. The smooth surface and nanoscale dimensions of the particles are favorable for enhanced interaction with bacterial membranes, thereby contributing to their antibacterial potential.

XRD Analysis:

The crystalline nature of ZnO NPs was confirmed by X-ray diffraction analysis. The diffraction pattern (Figure 4) displayed intense peaks at 2θ values of 31.8°, 34.5°, 36.3°, and 47.5°, corresponding to the (100), (002), (101), and (102) crystal planes, respectively. These peaks matched the standard JCPDS card no. 36-1451, confirming the hexagonal wurtzite structure of ZnOThe average crystallite size, calculated using the Debye–Scherrer equation, was found to be 25–35 nm. The narrow and sharp diffraction peaks indicated a high degree of crystallinity, which is essential for enhanced antibacterial activity.

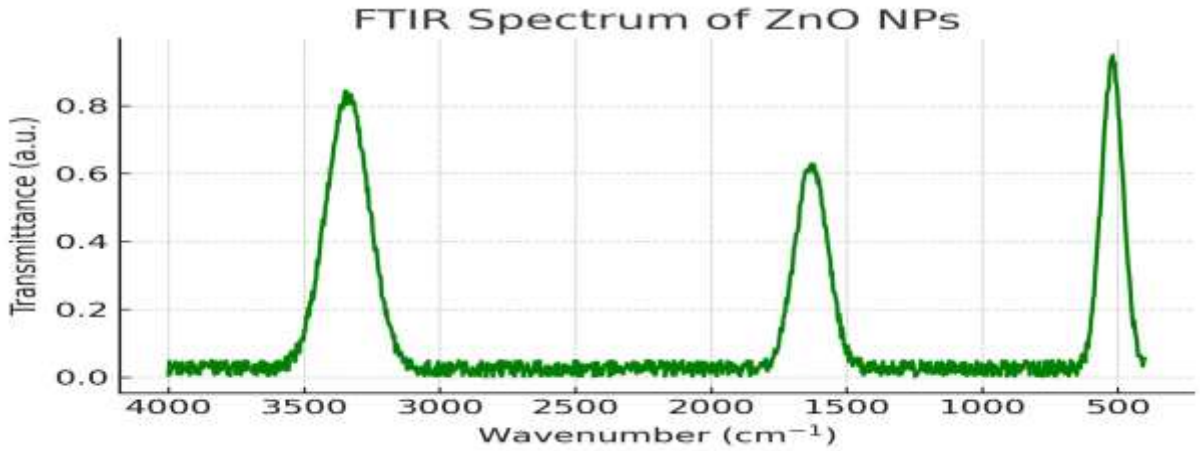


Figure 4. XRD Pattern of ZnO NPs
Peaks at 2θ = 31.8°, 34.5°, 36.3°, 47.5° confirming hexagonal wurtzite structure.

Antibacterial Activity:
The antibacterial efficacy of ZnO NPs synthesized using M. *oleifera* was tested against multidrug-resistant Staphylococcus aureus. Results of the agar well diffusion assay are summarized in Table 2 and illustrated in Figure 6.
At a concentration of 1 mg/mL, ZnO NPs exhibited a zone of inhibition of 18.6 ± 0.5 mm, which was significantly larger than that of crude M. oleifera extract (9.8 ± 0.4 mm). The control (distilled water) showed no activity. The minimum inhibitory concentration (MIC) of ZnO NPs against MDR S. aureus was determined to be 0.5 mg/mL, while the crude extract required a higher concentration (1.0 mg/mL) for inhibition.
The superior antibacterial activity of ZnO NPs compared to the crude extract may be attributed to the nanoscale size, high surface-to-volume ratio, and ability to generate reactive oxygen species (ROS), which disrupt bacterial cell membranes and intracellular processes.
Table 2. Antibacterial Activity of Biosynthesized ZnO NPs Against MDR S. *aureus*

Sample	**Concentration (mg/mL)**	**Zone of inhibition (mm) ± SD**	**MIC(mg/mL)**
ZnO nanoparticles.	1.0	18.6 ± 0.5	0.5
M. *oleifera* extract	1.0	9.8 ± 0.4	1.0
Control (distilled water).	-	0.0 ± 0.0	-

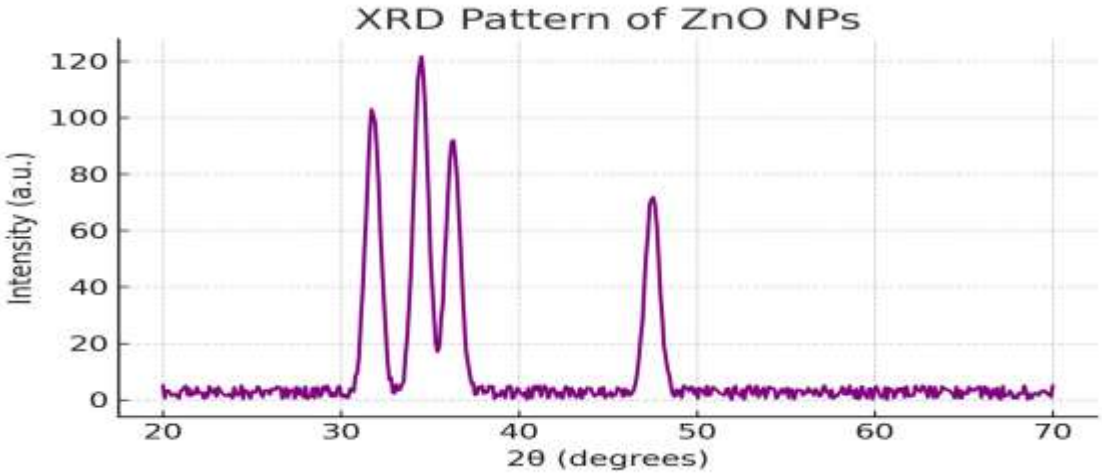
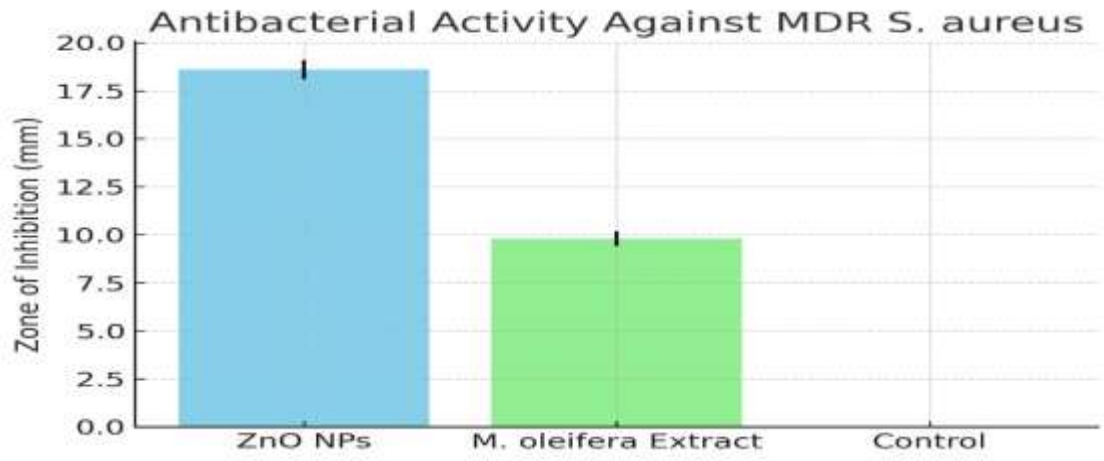


Figure 5. Antibacterial Activity

Bar chart comparing Zone of inhibition (mm) for:
ZnO NPs (18.6 ± 0.5 mm)
M. oleifera extract (9.8 ± 0.4 mm)
Control (0.0 mm).



DISCUSSION:

The present study successfully demonstrated the green synthesis of ZnO nanoparticles (ZnO NPs) using *Moringa oleifera* leaf extract and their antibacterial efficacy against multidrug-resistant (MDR) *Staphylococcus aureus*.

The UV–Vis absorption peak at 370 nm confirmed nanoparticle formation, consistent with previous findings that ZnO NPs generally exhibit surface plasmon resonance (SPR) bands in the range of 350–380 nm (Ahmed et al., 2016). The sharp peak further indicated the formation of well-dispersed nanoparticles with minimal aggregation.

FTIR analysis revealed functional groups such as hydroxyl (–OH), carbonyl (C=O), and amide linkages, which originated from biomolecules present in M. oleifera extract. These phytochemicals likely served as both reducing and stabilizing agents, preventing nanoparticle aggregation and imparting biocompatibility (Chandrasekaran et al., 2016). The presence of a Zn–O stretching band around 520 cm^{-1} provided strong evidence of ZnO bond formation. Similar results have been reported by Singh et al. (2018), who attributed the stabilization of ZnO NPs to proteins and phenolic compounds.

The XRD diffraction pattern confirmed the hexagonal wurtzite crystalline structure of ZnO NPs, in agreement with the JCPDS standard (card no. 36-1451). The crystallite size (25–35 nm) obtained from the Debye–Scherrer equation aligned with earlier reports on green-synthesized ZnO NPs using different plant extracts (Ramesh et al., 2014). The high crystallinity observed is a desirable feature, as it enhances the photocatalytic and antibacterial potential of nanoparticles.

SEM analysis revealed predominantly spherical particles with nanoscale dimensions. Morphological features play a critical role in antibacterial efficiency, as smaller, spherical nanoparticles have higher surface energy, enabling stronger interaction with bacterial membranes (Sirelkhatim et al., 2015). The limited aggregation observed suggests effective capping by biomolecules from M. oleifera extract, which is advantageous for biomedical applications.

The antibacterial assay demonstrated that ZnO NPs exhibited a significantly larger zone of inhibition (18.6 ± 0.5 mm) compared to crude M. oleifera extract (9.8 ± 0.4 mm) against MDR S. aureus. This superior activity can be explained by multiple mechanisms: (i) generation of reactive oxygen species (ROS) leading to oxidative stress, (ii) direct disruption of bacterial cell wall integrity due to electrostatic interaction between negatively charged bacterial surfaces and positively charged ZnO NPs, and (iii) release of Zn^{2+} ions, which interfere with intracellular metabolic pathways (Jin et al., 2009; Raghupathi et al., 2011).

The MIC values further confirmed the enhanced potency of ZnO NPs (0.5 mg/mL) compared to crude extract (1.0 mg/mL). Similar findings were reported by Sangeetha et al. (2011), who observed that ZnO NPs exhibited dose-dependent antibacterial activity against Gram-positive bacteria. The greater susceptibility of S. aureus can be attributed to its relatively thick peptidoglycan layer, which is disrupted by the nanoscale interaction and ROS generation of ZnO NPs.

Collectively, these results suggest that biosynthesized ZnO NPs possess substantial antibacterial activity and can be developed as alternative antimicrobial agents to combat MDR pathogens. The use of M. *oleifera* extract provides an eco-friendly, cost-effective, and sustainable approach to nanoparticle synthesis, reducing reliance on toxic chemicals while maintaining high efficacy.

CONCLUSION AND FUTURE PERSPECTIVES:

The present study highlights the successful green synthesis of zinc oxide nanoparticles (ZnO NPs) using *Moringa oleifera* leaf extract and their potent antibacterial activity against multidrug-resistant Staphylococcus *aureus*. The biosynthesis process was confirmed by UV–Vis spectroscopy, FTIR analysis, XRD diffraction, and SEM imaging, all of which validated the formation of spherical, crystalline nanoparticles in the size range of 25–35 nm. The phytochemicals present in M. oleifera acted as reducing and stabilizing agents, contributing to the eco-friendly and non-toxic synthesis process.

ZnO NPs demonstrated significantly higher antibacterial efficacy than the crude plant extract, as evidenced by a larger zone of inhibition and a lower minimum inhibitory concentration. The enhanced antimicrobial activity can be attributed to multiple mechanisms, including reactive oxygen species generation, cell membrane disruption, and Zn^{2+} ion release. These findings strongly support the potential of biosynthesized ZnO NPs as effective antimicrobial agents for tackling multidrug-resistant pathogens.

Despite the promising results, further studies are needed to validate the cytotoxicity, biocompatibility, and in vivo efficacy of these nanoparticles before clinical translation. Additionally, optimizing large-scale production techniques and assessing long-term environmental impacts are essential for sustainable application. Future research may also explore the synergistic potential of ZnO NPs with conventional antibiotics, offering novel strategies for overcoming drug resistance.

In conclusion, green-synthesized ZnO NPs using *Moringa oleifera* extract present a cost-effective, eco-friendly, and biologically potent nanomaterial with promising applications in antimicrobial therapy, pharmaceutical development, and nanomedicine.

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