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Phytochemical-Mediated Green Synthesis of Zinc Oxide Nanoparticles from *Azadirachta indica* Extract for Antimicrobial Wastewater Purification

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Abstract

*The development of sustainable synthetic routes for functional nanomaterials is essential for advancing environmentally benign technologies for water treatment. Herein, zinc oxide (ZnO) nanoparticles were synthesized through a green approach employing aqueous leaf extract of *Azadirachta indica* (neem) as both the reducing and stabilizing agent. The influence of precursor-to-extract ratio (1:1 and 1:2) and reaction temperature (room temperature and 60 °C) on nanoparticle formation and performance was systematically investigated. Formation of the nanoparticles was initially evidenced by colour change during synthesis and further characterized using Fourier-transform infrared (FTIR) spectroscopy, which revealed O–H, C=O, and C–N functional groups associated with phytochemicals involved in nanoparticle reduction and surface stabilization, together with Zn–O vibrational bands confirming ZnO formation. The synthesized materials exhibited pH values ranging from mildly acidic to alkaline depending on the synthesis conditions, indicating that reaction parameters significantly influenced nanoparticle stability. The antimicrobial performance of the ZnO nanoparticles was evaluated using contaminated wastewater containing aerobic mesophilic bacteria. Nanoparticles prepared using the higher neem extract ratio (1:2) and applied at 0.5 g demonstrated the greatest reduction in bacterial load, indicating concentration-dependent antimicrobial activity. The enhanced performance is attributed to the synergistic effects of phytochemical surface capping, high surface reactivity, and ZnO-mediated disruption of bacterial cells. These findings demonstrate that neem-mediated ZnO nanoparticles represent an environmentally sustainable material for wastewater disinfection and highlight the potential of plant-assisted nanomaterial synthesis for low-cost water purification technologies.*

KeyWords- AERONET, Green synthesis, Zinc oxide nanoparticles, *Azadirachta indica*, Wastewater purification, Antimicrobial activity.

INTRODUCTION

Water pollution has become a critical global environmental challenge due to rapid industrialisation, urbanisation, agricultural expansion, and population growth. The discharge of untreated wastewater containing heavy metals, pathogenic microorganisms, pharmaceuticals, dyes, and other emerging contaminants threatens aquatic ecosystems and public health, particularly in developing countries (Hussain et al., 2024). Conventional wastewater treatment technologies, such as

coagulation, chlorination, and membrane filtration, are effective for many pollutants but often suffer from high operational costs, energy demands, and limited removal efficiency for emerging contaminants (Hussain et al., 2024). Consequently, nanotechnology has emerged as a promising alternative for sustainable water treatment. Among metal oxide nanomaterials, zinc oxide (ZnO) nanoparticles have attracted significant attention because of their high surface area, excellent photocatalytic activity, chemical stability, and broad-spectrum antimicrobial properties. Furthermore, green

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synthesis using plant extracts offers an environmentally friendly, cost-effective, and sustainable approach for producing ZnO nanoparticles suitable for wastewater purification applications.

Zinc oxide (ZnO) nanoparticles are among the most extensively investigated metal oxide nanomaterials because of their excellent photocatalytic activity, chemical stability, biocompatibility, and broad-spectrum antimicrobial properties. These characteristics make ZnO nanoparticles suitable for applications in wastewater treatment, environmental remediation, and biomedicine (Mutukwa et al., 2024; El-Saadony et al., 2024). Conventional synthesis methods often require toxic chemicals and high energy input, whereas green synthesis employs plant extracts as natural reducing and stabilising agents, providing a safer, cost-effective, and environmentally sustainable alternative. Plant-derived phytochemicals, including flavonoids, phenolics, and terpenoids, facilitate nanoparticle formation while enhancing stability and biological activity (Gul et al., 2024; Al-Darwesh et al., 2024). Consequently, plant-mediated ZnO nanoparticle synthesis has become an attractive strategy for developing sustainable nanomaterials with enhanced antimicrobial and photocatalytic performance for wastewater purification.

Azadirachta indica (neem) is rich in flavonoids, phenolics, terpenoids, tannins, and proteins that function as natural reducing and stabilising agents during ZnO nanoparticle synthesis. These phytochemicals facilitate nanoparticle formation while improving stability and antimicrobial activity, making neem an effective, eco-friendly precursor for green nanotechnology applications (El-Beltagi et al., 2024).

Although green synthesis of ZnO nanoparticles using plant extracts has been widely reported, limited studies have investigated the combined effects of precursor-to-extract ratio and synthesis temperature on the physicochemical properties and antimicrobial performance of neem-mediated ZnO nanoparticles for wastewater treatment (Hussain et al., 2024; Mutukwa et al., 2024). Furthermore, there is limited information on the application of locally sourced *Azadirachta indica* in developing sustainable nanomaterials for water purification in Nigeria (El-Saadony et al., 2024). Therefore, this study aimed to synthesise ZnO nanoparticles using aqueous *Azadirachta indica* leaf extract as a natural reducing and stabilising agent. The study further evaluated the influence of zinc-to-neem extract ratio and reaction temperature on nanoparticle synthesis using pH and FTIR analyses, and assessed the antimicrobial effectiveness of the synthesised nanoparticles in contaminated wastewater. The findings are expected to support the development of eco-friendly, low-cost nanomaterials for sustainable wastewater treatment.

MATERIALS AND METHODS

A. Materials

The synthesis employed local Nigerian plant material, identified as *Azadirachta indica* (neem), along with distilled water, metal salts, a beaker, conical flask, measuring cylinder, hot plate, filter paper, centrifuge, UV-Vis spectrophotometer,

and, optionally, SEM/REM and FTIR for further characterisation. A contaminated water sample was also used for the purification test and pH and turbidity meters were noted as optional analytical tools.

B. Preparation of Neem Extract

Fresh neem leaves were collected, thoroughly washed, and dried. The dried plant material was then ground. Next, 10 g of the plant material was boiled in 100 mL of distilled water for 10–15 minutes, after which the mixture was filtered using Whatman No. 1 filter paper to obtain a clear aqueous extract.

C. Synthesis of Metal Nanoparticles

Zinc nitrate was dissolved to afford the metal precursor solution. Neem extract was introduced at 1:1 and 1:2 (v/v) ratios, and each reaction mixture was divided into two portions for incubation at ambient temperature and at 60 °C, respectively. Development of a visible colour change was used as an initial indicator of nanoparticle formation. The synthesised products were subsequently characterized using UV-Vis spectroscopy and Fourier-transform infrared (FTIR) spectroscopy (Alqahtani, et al., 2025).

D. Isolation and Recovery of Nanoparticles

After synthesis, the reaction mixture was centrifuged to separate the nanoparticles. The pellet was washed with distilled water, dried, and stored for subsequent use (Alqahtani, et al., 2025).

E. Wastewater Purification Test

Contaminated water samples were collected and divided into control and test groups. Different amounts of the synthesised nanoparticles, specifically 0.5 g, 0.25 g, or 1 g, were added to the test samples. The samples were stirred and allowed to stand for 1 hour. After treatment, the water was filtered and analysed for microbial load reduction using standard plate count methods.

F. Characterization and Analysis

Nanoparticle formation was assessed using UV-Vis spectroscopy and FTIR spectroscopy. FTIR was used to identify functional groups associated with the neem extract and to confirm ZnO-related vibrations. The results section also included pH measurements and microbial analysis of treated samples, indicating that these parameters were part of the evaluation of the synthesised nanoparticles.

RESULT AND DISCUSSION

The formation of zinc oxide (ZnO) nanoparticles using *Azadirachta indica* leaf extract was initially confirmed by a visible colour change observed during the reaction between the aqueous neem extract and zinc nitrate solution. The colour transition occurred under both room temperature and 60 °C synthesis conditions, indicating the reduction of zinc ions and the formation of ZnO nanoparticles. Following synthesis, the nanoparticles were recovered by centrifugation, washed with distilled water, dried, and stored for further characterisation and application studies.

A. pH of Synthesised Nanoparticles

The pH values of the synthesised nanoparticle suspensions are presented in Table 1. Sample A, prepared using a 1:2 zinc-to-neem ratio at 60 °C, exhibited an acidic pH of 4.90. In contrast, Samples A₁, B, and B₁ displayed alkaline pH values ranging from 7.92 to 8.18. These results indicate that the synthesis conditions—specifically the metal-to-extract ratio and reaction temperature—significantly influenced the pH of the nanoparticle suspensions.

The acidic pH observed for Sample A is consistent with reports that lower precursor concentrations and moderate temperatures can yield ZnO suspensions with more protonated surface hydroxyl groups, whereas alkaline conditions (pH ≈ 8–9) promote deprotonation and stabilisation of ZnO nanoparticles in aqueous media. Systematic optimisation studies similarly show that reaction pH and temperature jointly control nucleation, growth, and surface charge of biogenic ZnO nanoparticles, thereby modulating suspension pH and colloidal stability (Ngangom et al., 2025; Kumar et al., 2026; Zhang et al., 2026).

Table 1. pH of Synthesised ZnO Nanoparticle Samples.

Samples	Synthesis ondition	PH
A	1:2 ratio at 60 °C	4.90
A ₁	1:2 ratio at Room Temperature	8.18
B	1:1 ratio at 60 °C	8.10
B ₁	1:1 ratio at Room Temperature	7.92

B. Fourier Transform Infrared (FTIR) Analysis

The FTIR spectra of the synthesised nanoparticles are summarised in Table 2. All samples exhibited broad absorption bands in the range of 3260–3286 cm⁻¹ corresponding to O–H stretching vibrations. A strong absorption band at 1629 cm⁻¹ was observed in all samples and was assigned to C=O stretching vibrations. Additional bands between 1406 and 1491 cm⁻¹ were attributed to C–N stretching vibrations, while Zn–O stretching vibrations appeared between 713 and 743 cm⁻¹, confirming the formation of ZnO nanoparticles.

Table 1. pH of Synthesised ZnO Nanoparticle Samples.

Sample	O–H (cm ⁻¹)	C=O (cm ⁻¹)	C–N (cm ⁻¹)	C–H (cm ⁻¹)	Zn–O (cm ⁻¹)
A	3279	1629	1406	2929	713
A ₁	3286	1629	1406	2933	—
B	3272	1629	1451	2929	739
B ₁	3260	1629	1491	2992	743

Key A: 1:2 ratio (1 g Zn : 2 g neem) at 60 °C, A₁: 1:2 ratio at room temperature

B: 1:1 ratio (1 g Zn : 1 g neem) at 60 °C B₁: 1:1 ratio at room temperature

C. Antibacterial Activity of Synthesised Nanoparticles

The antimicrobial activity of the neem-mediated ZnO nanoparticles was evaluated using contaminated wastewater containing aerobic mesophilic bacteria. The bacterial counts obtained after treatment with different nanoparticle concentrations and synthesis ratios are presented in Table 3.

The 1:2 nanoparticle formulation applied at 0.50 g produced the lowest mean bacterial count (160×10^7 CFU g⁻¹), whereas the 1:1 formulation at 0.25 g produced the highest bacterial count (332×10^7 CFU g⁻¹). The remaining treatments showed intermediate values of 152×10^7 and 126×10^7 CFU g⁻¹, respectively these values are similar to those reported by Ferdush et al., 2025; Alqaffaf et al., 2026; Ngangom, et al., 2025).

Table 3: Microbial Analysis of Wastewater Treated with Neem-Synthesised ZnO Nanoparticles.

Sample	Zn:Neem Ratio	Nanoparticle Concentration (g)	Mean Bacterial Count ($\times 10^7$ CFU g ⁻¹)
A	1:1	0.50	152
A ₁	1:1	0.25	332
B	1:2	0.50	160
B ₁	1:2	0.25	126

D. Fourier Transform Infrared (FTIR) Spectral Analysis

The FTIR spectra of the synthesised ZnO nanoparticles are presented in Figures 1 and 2, illustrating the spectral profiles of samples prepared at both room temperature and 60 °C using zinc-to-neem extract ratios of 1:1 and 1:2. The spectra reveal characteristic absorption bands associated with the functional groups involved in nanoparticle synthesis and stabilisation. Although the major peaks were observed in all samples, slight variations in peak intensity and position were evident, indicating that synthesis temperature and precursor ratio influenced the surface chemistry of the nanoparticles. The presence of these characteristic bands confirms successful synthesis of ZnO nanoparticles and demonstrates the role of neem phytochemicals as reducing and capping agents during the green synthesis process.

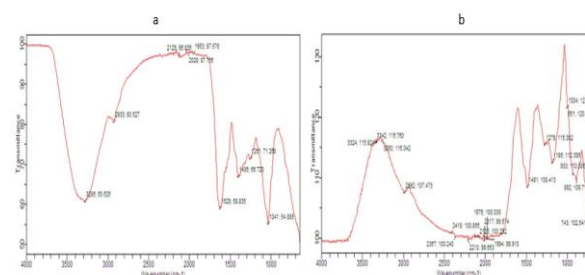


Figure 1. FTIR spectrum of ZnO nanoparticles synthesised using *Azadirachta indica* leaf extract at room temperature under (a) 1:2 Zn:neem ratio and (b) 1:1 Zn:neem ratio..

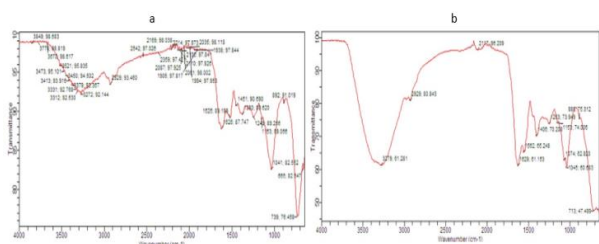


Figure 2. FTIR spectrum of ZnO nanoparticles synthesised using *Azadirachta indica* leaf extract at 60 °C under (a) 1:1 Zn:neem ratio and (b) 1:2 Zn:neem ratio.

CONCLUSION

The experimental results demonstrate successful synthesis of ZnO nanoparticles using *Azadirachta indica* leaf extract under both room temperature and elevated-temperature conditions. FTIR analysis confirmed the presence of functional groups associated with neem phytochemicals and Zn–O bonding, while pH measurements showed that synthesis conditions influenced the chemical environment of the nanoparticle suspensions. Antimicrobial evaluation indicated that the synthesised nanoparticles reduced the bacterial population in contaminated wastewater under the tested conditions. These findings support the potential application of neem-mediated ZnO nanoparticles as environmentally friendly materials for wastewater purification.

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