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ANTIBACTERIAL ASSAY OF PLANT-MEDIATED ZINC OXIDE NANOPARTICLES SYNTHESIZED FROM AQUEOUS ROOT EXTRACT OF NAUCLEA LATIFOLIA

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Abstract

Microbial resistance to conventional antibiotics is a growing global challenge. Multifaceted approaches are being developed to mitigate this concern, including the use of nanotechnology. Among nanomaterials of interest in this research area are metal nano-oxides. Here, zinc oxide nanoparticles (ZnO NPs) were successfully synthesized from aqueous root extract of *Nauclea latifolia* by a green synthetic method, to provide a simple, eco-friendly, and cost-effective method of nanoparticle production and assayed for antibacterial potency. The synthesized nanoparticles were characterized using UV–Vis spectroscopy, FTIR, XRD, DTA/TGA, BET, and assayed for their antibacterial activity. The UV–Vis spectrum showed an absorption peak at 267 nm, confirming nanoparticle formation, while the FTIR spectrum revealed a Zn–O band at 879 cm^{-1}. XRD analysis indicated a hexagonal wurtzite crystal structure with an average crystallite size of 21.7 nm. The DTA/TGA results demonstrated a multistep decomposition pattern characteristic of biosynthesized ZnO nanoparticles, while BET analysis confirmed a mesoporous structure with a high surface area. The antibacterial activity of the ZnO NPs was tested against *Klebsiella pneumoniae*, *Salmonella typhi*, and *Staphylococcus aureus*. The nanoparticles exhibited concentration-dependent inhibitory effects, ranging from slight to strong activity against the tested microorganisms. In comparison with amoxicillin, used as the standard antibacterial agent, the synthesized ZnO nanoparticles showed promising antibacterial potency. The findings demonstrates that *Nauclea latifolia* root extract is an effective biological resource for the green synthesis of ZnO nanoparticles. With further optimization and surface modification, these nanoparticles may have significant potential for antibacterial and other biomedical applications.

Keywords: Antibacterial assay, Plant-mediated, Zinc oxide nanoparticles, Nauclea latifolia

INTRODUCTION

The rapid rise in antibiotic-resistant bacteria has become a major global health concern, threatening the effectiveness of many conventional antimicrobial therapies. The continued emergence of multidrug-resistant pathogens has intensified the search for new and effective antimicrobial agents capable of overcoming resistance mechanisms. In recent years, nanotechnology has offered promising solutions to this challenge through the development of nanomaterials with unique physicochemical and biological properties that can be exploited for medical and pharmaceutical applications (Iravani, 2011).

Among various nanomaterials, zinc oxide nanoparticles have attracted considerable attention because of their remarkable antibacterial activity, chemical stability, biocompatibility, and relatively low production cost. Owing to their nanoscale dimensions and large surface area, ZnO nanoparticles exhibit enhanced interactions with microbial cells, enabling them to inhibit the growth of a broad range of pathogenic microorganisms. Several studies have shown that ZnO nanoparticles exert antibacterial effects through the generation of reactive oxygen species (ROS), disruption of bacterial cell membranes, leakage of intracellular contents, and the release of zinc ions that interfere with essential cellular functions (Sirelkhatim *et al*., 2015; Vijayakumar *et al*., 2018). These multiple mechanisms of action make ZnO nanoparticles attractive candidates for combating bacterial infections and reducing the development of antimicrobial resistance.

Traditionally, ZnO nanoparticles have been synthesized using physical and chemical techniques such as sol-gel processing, hydrothermal methods, chemical precipitation, laser ablation, and vapor deposition. While these approaches can produce nanoparticles with controlled sizes and morphologies, they often involve high energy requirements, expensive instrumentation, and hazardous chemicals that may pose environmental and health risks (Singh *et al*., 2018). Furthermore, residual toxic reagents can limit the biomedical applicability of nanoparticles produced through conventional methods. These concerns have stimulated interest in environmentally friendly and sustainable synthesis routes.

Green synthesis has emerged as a viable alternative to conventional nanoparticle production methods. This approach utilizes biological materials such as plant extracts, bacteria, fungi, algae, and other natural products as reducing and stabilizing agents during nanoparticle formation. Among these biological resources, plant extracts are particularly advantageous because they are readily available, cost-effective, biodegradable, and rich in phytochemicals that facilitate nanoparticle synthesis (Ahmed *et al*., 2016). Bioactive compounds such as flavonoids, phenolics, alkaloids, tannins, terpenoids, and saponins can participate in the reduction of metal precursors and simultaneously stabilize the resulting nanoparticles, thereby eliminating the need for harmful synthetic chemicals.

Nauclea latifolia, commonly known as African peach or pin-cushion tree, is a medicinal plant widely distributed across tropical regions of Africa. The plant has long been used in traditional medicine for the treatment of ailments such as malaria, fever, gastrointestinal disorders, skin infections, and microbial diseases. Scientific investigations have confirmed that *Nauclea latifolia* contains a wide range of bioactive phytochemicals, including alkaloids, flavonoids, tannins, phenolic compounds, saponins, terpenoids, and glycosides. These phytoconstituents are largely responsible for its diverse pharmacological activities, such as antioxidant, antimicrobial, anti-inflammatory, antimalarial, antidiabetic, and hepatoprotective effects (Balogun *et al*., 2016; Boumendjel *et al*., 2016; Iheagwam *et al*., 2020).The abundance of these bioactive compounds makes the plant an excellent candidate for the green synthesis of metal oxide nanoparticles.

The phytochemicals present in *N. latifolia* extract can act as natural reducing and capping agents during nanoparticle formation. Functional groups such as hydroxyl, carbonyl, and amino moieties interact with metal ions, facilitating nucleation and growth while preventing particle aggregation. Consequently, plant-mediated synthesis not only promotes environmentally sustainable nanoparticle production but can also enhance the biological activity and stability of the synthesized nanomaterials (Singh *et al*., 2018).

Additionally, the small size of ZnO nanoparticles enables close interaction with bacterial cell walls, leading to membrane disruption and eventual cell death (Sirelkhatim *et al.*, 2015).

Despite the growing number of studies on plant-mediated nanoparticle synthesis, there remains a need to explore underutilized medicinal plants with rich phytochemical profiles for the development of novel antimicrobial nanomaterials. Given its medicinal importance and abundance of bioactive constituents, *Nauclea latifolia* represents a promising biological resource for the green synthesis of ZnO nanoparticles. Therefore, the present study aims to synthesize zinc oxide nanoparticles using an aqueous root extract of *Nauclea latifolia*, characterize the resulting nanoparticles using appropriate analytical techniques, and evaluate their antibacterial activity against selected pathogenic bacterial strains. The findings of this study may contribute to the development of sustainable and effective antibacterial agents for potential biomedical and pharmaceutical applications.

MATERIALS AND METHODS

All materials and chemicals were used as purchased, no further purification was necessary. Materials include roots of *Nauclea latifolia* purchased from North Bank Market in Makurdi, Benue-Nigeria, zinc acetate dehydrate (> 99 %, Koch-Light), sodium hydroxide (> 99 %, BDH), and deionized water.

Preparation of Nauclea latifolia aqueous leaf extract

The roots of *Nauclea latifolia* bought from North Bank Market Makurdi was identified in the Department of Botany, Joseph Sarwuan Tarka University, Makurdi. To remove dust, the roots were washed multiple times under running water and rinsed in deionized water, after which they were shade dried for 48 days. The roots were pulverized in a grinder and stored in an air tight container for further use.

Approximately 5 g of the root powder were added to 100 mL deionized water in a beaker, the suspension was heated at 80 °C on a hot plate with continuous stirring for 2 h to facilitate the extraction of bioactive phytochemicals (Mohamed *et al*., 2019). After extraction, a bright yellow solution was obtained, allowed to cool to room temperature, and filtered. To ensure the removal of residual particulate matter and high-molecular-weight impurities, the cooled extract was subsequently filtered three times using Whatman filter paper. The resulting clear extract was collected in amber glass bottles and stored at 4 °C until further use.

Synthesis of Zinc Oxide Nanoparticles

A 0.5 mol dm^{-3} zinc acetate solution was prepared by dissolving 4.39 g of zinc acetate dehydrate in 40 mL of deionized water. Subsequently, 10 mL of the zinc acetate solution was mixed with 20 mL of freshly prepared plant extract under continuous stirring. The pH of the reaction mixture was adjusted and maintained at 10 using NaOH solution. The mixture was then heated on a hot plate at 60 °C and stirred continuously for 60 min (Din *et al*., (2024). During the reaction, a gradual colour change from yellow to white was observed, indicating the reduction of the zinc precursor and the formation of zinc oxide nanoparticles. The dried product was carefully pulverized into a fine powder and subsequently calcined at 450 °C for 2 h to improve its crystallinity and facilitate the formation of a well-defined zinc oxide phase. Following calcination, a characteristic white powder was obtained, indicating the successful formation of ZnO NPs. The synthesized nanoparticles were then collected and stored in an airtight container to prevent contamination and moisture uptake prior to further characterization and application studies.

Characterization

Following the green synthesis of zinc oxide nanoparticles (ZnO NPs), several characterization techniques were employed to evaluate their optical, structural, thermal, and surface properties. UV–Visible spectroscopy was used to investigate their optical behaviour and electronic transitions, while FTIR spectroscopy (4000–400 cm^{-1}) was used to identify the functional groups and phytochemicals involved in the reduction and stabilization of the nanoparticles. The crystalline structure and phase purity of the ZnO NPs were examined using X-ray diffraction (XRD), and the average crystallite size was estimated using the Debye–Scherrer equation. Thermal stability and decomposition behaviour were assessed through differential thermal analysis (DTA) and thermogravimetric analysis (TGA). In addition, Brunauer–Emmett–Teller (BET) analysis was carried out to determine the surface area, pore size, and porosity of the nanoparticles, providing valuable information on their potential applications in biological and catalytic systems.

Antibacterial Analysis

The antibacterial activity of the synthesized zinc oxide nanoparticles (ZnO NPs) was evaluated using the agar well diffusion method against *Klebsiella pneumoniae*, *Salmonella typhi*, and *Staphylococcus aureus*. Amoxicillin served as the standard antibacterial drug for comparison. ZnO NP suspensions (500 and 250 mg/mL) were prepared in dimethyl sulphoxide (DMSO) and introduced into wells bored into inoculated nutrient agar plates. The plates were allowed to stand for 15 minutes to facilitate diffusion and were subsequently incubated at 37 °C for 24 hours. Antibacterial activity was determined by measuring the diameters of the inhibition zones formed around the wells (Nasiri Sovari and Zobi, 2020).

All assays were conducted in triplicate, and results were expressed as mean ± standard deviation. The antibacterial effectiveness of the ZnO NPs was compared with that of amoxicillin. The observed activity may be attributed to the generation of reactive oxygen species, release of Zn^{2+} ions, and disruption of bacterial cell membranes, which ultimately lead to bacterial cell death (Sirelkhatim *et al.*, 2015). These properties highlight the potential of ZnO nanoparticles as promising antimicrobial agents.

RESULTS AND DISCUSSION

UV–Visible spectrophotometry analysis

The UV–visible absorption spectrum of the ZnO NPs within a wavelength range of 200 – 700 nm (Figure 1) was measured on a Jenway 7405 UV-Vis spectrophotometer.

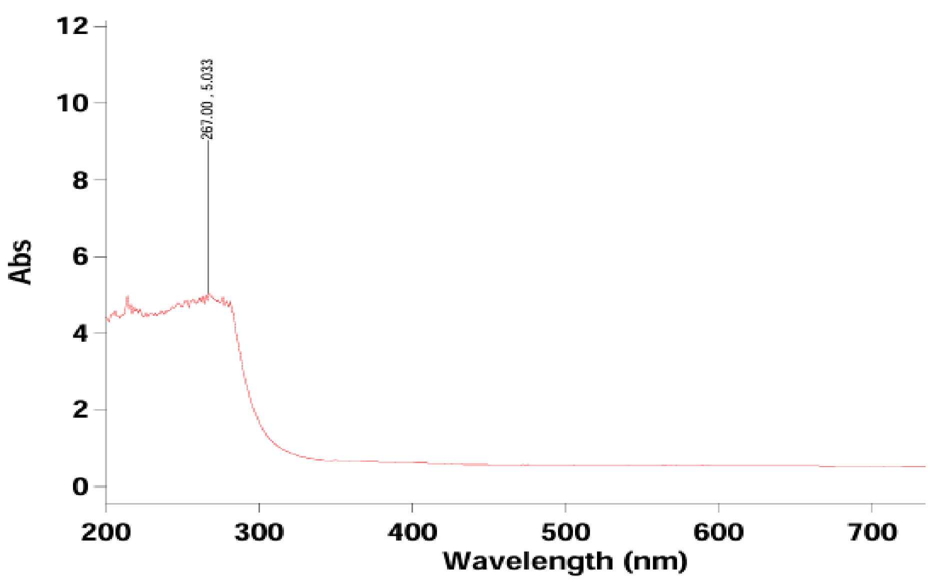

Figure 1: UV-Visible Spectrum of ZnO NPs

The sample had an absorption maximum at 267 nm which corresponds to $\pi \rightarrow \pi^*$ transition of the phytochemicals in the plant extract. The lack of other peaks at higher wavelength is attributed to the absence of *d-d* transitions in Zn(II) as a result of d^{10} electronic structure, this confirms the absence of ligand field absorptions within the ZnO nanoparticles system in the visible region. Hence, the optical property of this system is primarily controlled by charge transfer and band gap related absorptions. This agrees with reports by Mousa *et al*. (2024). Lack of other absorbance peak in the spectrum confirms the purity of the ZnO NPs.

FTIR spectral data

The FTIR spectrum of the green synthesized ZnO NPs (Figure 2) ran on a ThermoFisher Scientific NicoletTM iS50 spectrophotometer revealed the presence of organic functional groups arising from the *N. latifolia* root extract.

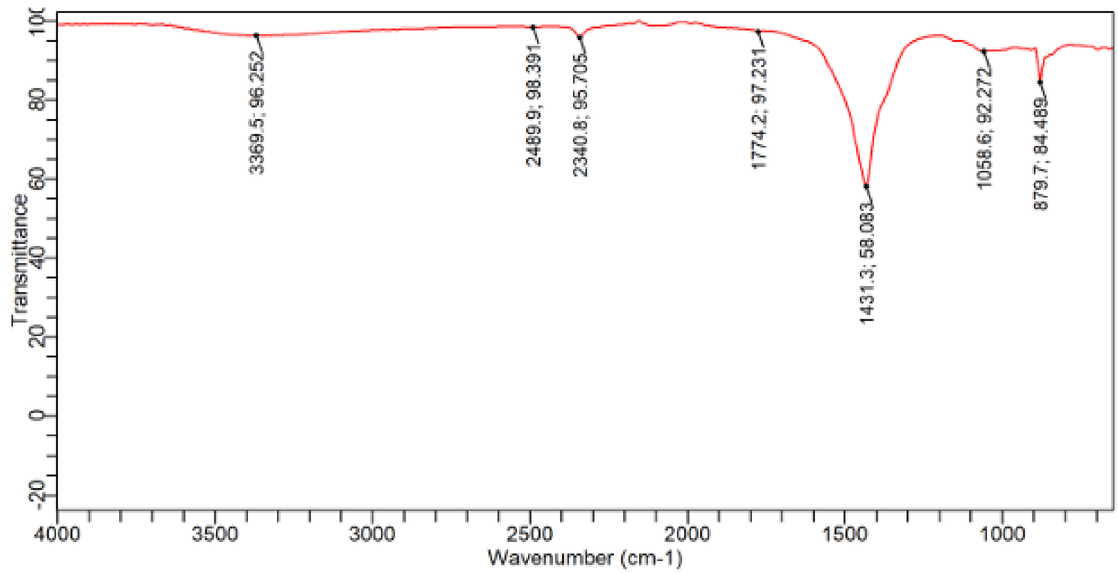

Figure 2: FTIR spectrum of ZnO NPs

The FTIR spectrum of the ZnO NPs displayed a broad absorption band at 3369 cm^{-1}, which is attributed to O–H stretching vibrations of hydroxyl groups originating from plant-derived polyphenols, alcohols, and adsorbed water molecules. The peak observed between 2340-2499 cm^{-1} corresponds to N–H stretching vibrations of amines in the plant extract involved as capping and stabilizing agents during the nanoparticles synthesis. The absorption band at 1774 cm^{-1} was assigned to C=O stretching vibrations of carbonyl-containing biomolecules, while the peak at 1431 cm^{-1} is associated with aromatic C–C stretching vibrations, suggesting the involvement of phenolic and other aromatic constituents from the plant extract in the nanoparticle formation process. The band located at 1058 cm^{-1} corresponds to C–O stretching vibrations of alcohols, ethers, or esters, further confirming the presence of oxygenated organic functional groups on the nanoparticle surface. Most importantly, the characteristic absorption band observed at 879 cm^{-1} is attributed to Zn–O stretching vibrations, which confirms the successful formation of zinc oxide nanoparticles. Similar Zn–O absorption bands in the fingerprint region have been reported for green-synthesized ZnO nanoparticles derived from plant extracts by Rajakumar *et al*. (2018) and Sharmila *et al*. (2018), who attributed these low-wavenumber bands to Zn–O lattice vibrations and nanoparticle formation. The retention of these functional groups after synthesis suggests that phytochemicals from the plant extract played significant roles in the reduction, stabilization, and surface functionalization of the ZnO nanoparticles.

XRD results analysis

The X-ray diffraction analysis performed on a **Thermo Scientific ARL'XTRA** model showed valuable information about the crystal structure and degree of crystallinity of the synthesized nanomaterials. The XRD pattern of the biosynthesized ZnO nanoparticles (Figure 3) exhibited prominent diffraction peaks at 2θ values of 31.92°, 34.64°, 36.46°, 47.67°, 56.70°, 62.97°, 68.04°, and 69.25°, corresponding to the (100), (002), (101), (102), (110), (103), (112), and (201) crystallographic planes, respectively. These reflections are characteristic of the hexagonal wurtzite crystal structure of ZnO and are in good agreement with standard ICDD/JCPDS data (Card No. 36-1451). The sharp and intense diffraction peaks indicate good crystallinity of the synthesized nanoparticles. Additional low-intensity peaks observed at 28.77°, 32.76°, 42.90°, and 60.70° may be attributed to lattice imperfections, residual phytochemical capping agents, or trace secondary phases introduced during the green synthesis process. These agree with data presented for the synthesis of ZnO nanoparticles using peels of *Passiflora foetida* (Khan *et al*., 2021).

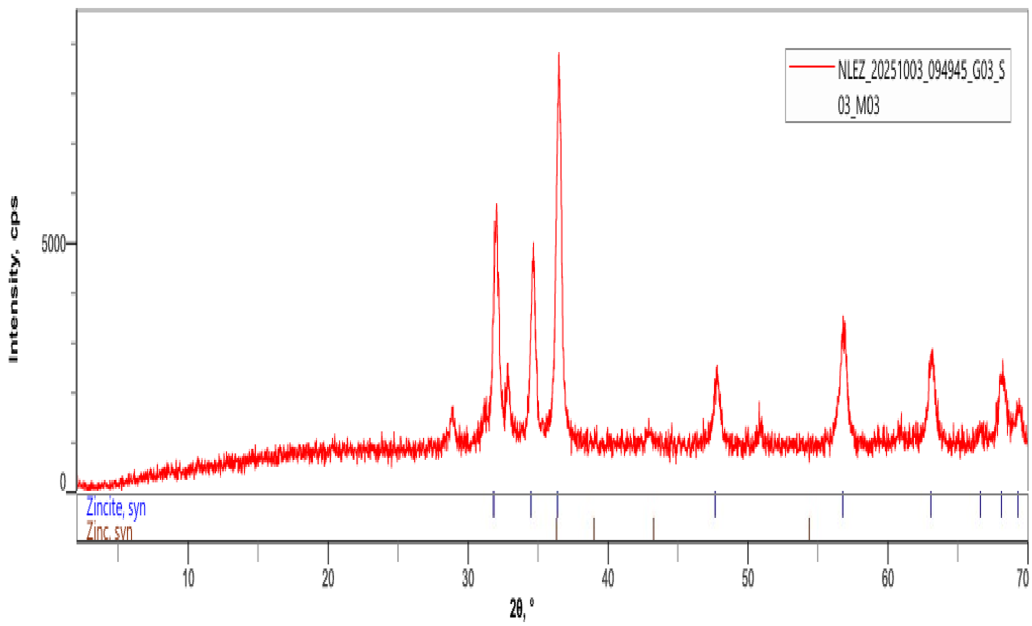

Figure 3: XRD Diffractogram of ZnO NPs

The average crystallite size of the biosynthesized ZnO nanoparticles, estimated from the major diffraction peaks of the XRD pattern, was approximately **21.7 nm,** indicating the formation of nanocrystalline ZnO with good crystallinity. The crystallite size was significantly affected by the synthesis parameters and post-synthesis annealing temperature, suggesting that careful control of these factors can be used to tailor the structural features of the nanoparticles. The study further confirms the potential of plant-mediated synthesis as a green and sustainable strategy for the production of high-quality zinc oxide nanoparticles.

Table 1: Comparison of Experimental d-spacing values with Standard d-spacing values for ZnO NPs

Standard ZnO (hkl)	Standard d (Å)	Experimental d (Å)
(100)	2.814	2.801
(002)	2.603	2.587
(101)	2.475	2.463
(102)	1.911	1.906
(110)	1.624	1.622
(103)	1.477	1.475
(112)	1.379	1.377

(201)	1.360	1.363

The excellent agreement between the experimental and standard d-spacing values confirm the formation of crystalline ZnO nanoparticles by the green synthetic process.

DTA/TGA results analysis

The Differential Thermal Analysis (DTA) profile of the biosynthesized zinc oxide nanoparticles performed on a TG analyzer (TGA Q50 series (Figure 4) showed a series of thermal changes. The DTA thermogram of the biosynthesized ZnO nanoparticles revealed a prominent decomposition peak at approximately 390 °C, indicating the temperature at which the maximum rate of mass loss occurred. This major thermal event can be attributed to the decomposition and removal of phytochemical compounds derived from the plant extract that remain adsorbed on the surface of the nanoparticles and acted as reducing and stabilizing agents during synthesis. Similar thermal behavior has been reported for green-synthesized ZnO nanoparticles, where the degradation of organic capping molecules occurred within the temperature range of 300–500 °C (Vijayakumar *et al*., 2018).

A less intense shoulder peak observed around 300 °C is likely associated with the decomposition of relatively unstable organic constituents, including low-molecular-weight phenolics, flavonoids, and other volatile biomolecules attached to the nanoparticle surface. The appearance of this feature suggests the stepwise degradation of organic matter present in the biosynthesized material. In addition, a secondary peak near 490 °C may be related to the decomposition of more strongly bound carbonaceous residues and the final conversion of intermediate zinc-containing species into a well-crystallized ZnO phase. This temperature could be inferred as the optimal calcination temperature for producing highly crystalline zinc oxide nanoparticles with improved structural stability. Such thermal events have been linked to the elimination of residual organic carbon and the enhancement of crystallinity in ZnO nanomaterials synthesized through plant-mediated routes (Zelekew *et al*., 2023).

Beyond 600 °C, no significant decomposition peaks or mass-loss events were observed, indicating that the organic components had been almost completely removed and that a thermally stable ZnO structure had been formed. The absence of further thermal degradation at higher temperatures confirms the excellent thermal stability of the zinc oxide nanoparticles, which is a characteristic feature of highly crystalline ZnO materials (Diallo *et al*., 2015)

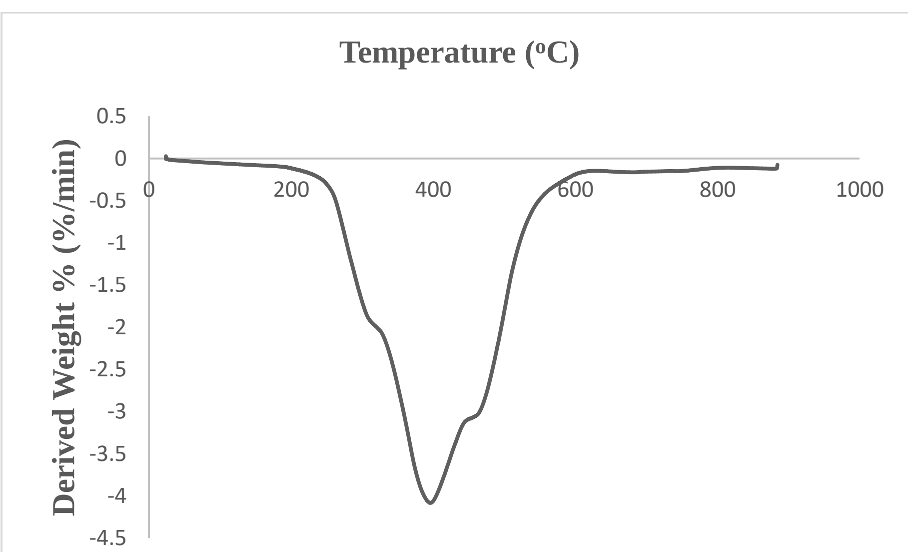

Figure 4: DTA Thermogram of the biosynthesized ZnO Nanoparticles

The thermogravimetric analysis (TGA) of the biosynthesized zinc oxide nanoparticles (Figure 5) revealed a multi-stage weight-loss profile, reflecting the progressive thermal transformations occurring within the material as the temperature increased.

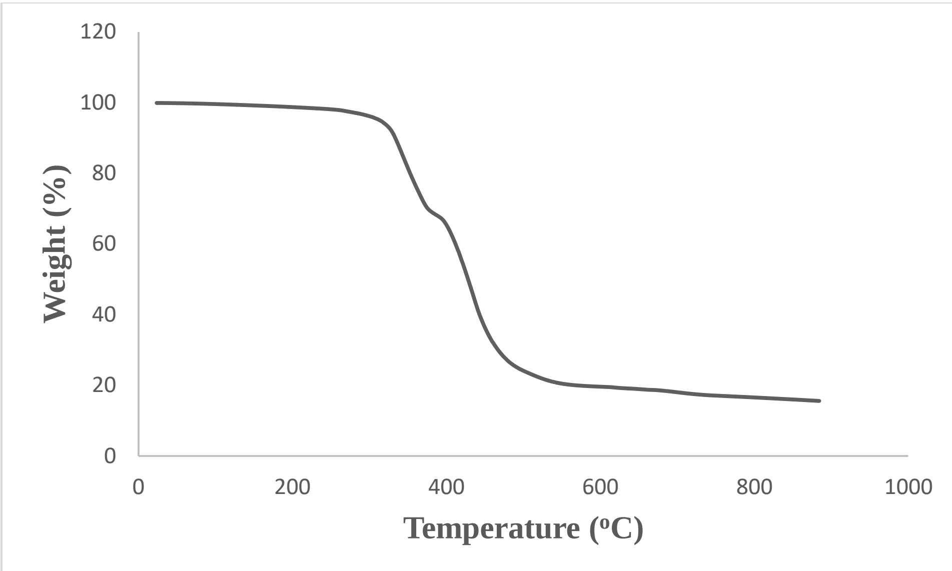

Figure 5: TGA Thermogram of the biosynthesized ZnO Nanoparticles

An initial weight loss of approximately 3.63 % was observed at lower temperatures and is attributed to the evaporation of physically adsorbed water molecules and other volatile phytochemical species present on the nanoparticle surface. Such a relatively small mass loss indicates low moisture content and suggests that the synthesized ZnO nanoparticles possessed good thermal stability and were adequately dried following synthesis (Nguyen *et al*., 2024; Al-Darwesh *et al*., 2024).

A more substantial weight loss of about 75.54 % occurred at elevated temperatures and can be associated with the thermal decomposition of more stable phytochemical constituents derived from the plant extract used during synthesis. These biomolecules, including phenolic compounds, flavonoids, tannins, alkaloids, and other secondary metabolites, serve as reducing, stabilizing, and capping agents during nanoparticle formation. Their degradation upon heating confirms their strong interaction with and adsorption onto the nanoparticle surface. Similar thermal behavior has been widely reported for green-synthesized ZnO, and other metal oxide nanoparticles, where significant mass losses between 200 and 600 °C are attributed to the decomposition of surface-bound organic compounds and carbonaceous residues originating from plant extracts (Osman *et al*., 2024).

The pronounced mass reduction further suggests that a considerable fraction of the as-synthesized material consisted of organic capping layers surrounding the zinc precursor and the developing ZnO nanostructures. As the temperature increased, these organic species gradually decomposed, resulting in the formation of a more purified and thermally stable oxide phase. Recent study has highlighted that plant-derived metabolites play a crucial role in controlling nanoparticle nucleation, growth, morphology, and colloidal stability, but are subsequently removed during thermal treatment, thereby improving the crystallinity and purity of the final ZnO product (Janani *et al*., 2024).

The residual mass of approximately 20.83 % observed at the end of the analysis corresponds to the inorganic zinc oxide component that remained after complete combustion of the organic matter. The persistence of this residue at high temperatures confirms the successful formation of ZnO nanoparticles, as zinc oxide is known for its excellent thermal stability and resistance to decomposition under normal TGA operating conditions. Similar observations have been reported for biosynthesized ZnO, Co_3O_4, Fe_2O_3, and TiO_2 nanoparticles, where the final residue represents the thermally stable metal oxide framework remaining after the elimination of organic constituents (Zelekew *et al*., 2023).

The DTA/TGA findings demonstrates that the synthesized nanoparticles were initially coated with substantial quantities of phytochemical capping agents. These organic materials gradually decomposed with increasing temperature, ultimately yielding a stable ZnO residue. This behavior is consistent with recent reports on green-synthesized metal oxide nanoparticles and provides additional evidence for the successful formation of thermally stable ZnO nanostructures through a plant-mediated synthesis route (Osman *et al*., 2024; Sharma *et al*., 2024; Al-Darwesh *et al*., 2024).

BET result analysis

The textural properties of the green-synthesized ZnO nanoparticles were investigated using Brunauer–Emmett–Teller (BET) analysis. The results revealed a specific surface area of **223.14 m²/g,** a pore diameter of **2.80 nm,** and a pore volume of **0.139 cm³/g,** indicating the formation of highly porous nanoparticles with a large accessible surface area. These characteristics are particularly important because the performance of metal oxide nanoparticles is strongly influenced by their surface properties and pore structure.

The exceptionally high surface area of **223.14 m²/g** suggests that the synthesized ZnO nanoparticles consist of very small particles with a large number of exposed surface atoms. As particle size decreases to the nanoscale, the proportion of atoms located on the surface increases significantly, resulting in greater surface reactivity. This high surface area provides more active sites for interactions with surrounding molecules, which can enhance adsorption, catalytic activity, and antimicrobial performance. Similar observations have been reported for green-synthesized metal oxide nanoparticles, where plant-derived phytochemicals help control particle growth and prevent excessive aggregation, leading to materials with enhanced surface characteristics (Lowell *et al*., 2012).

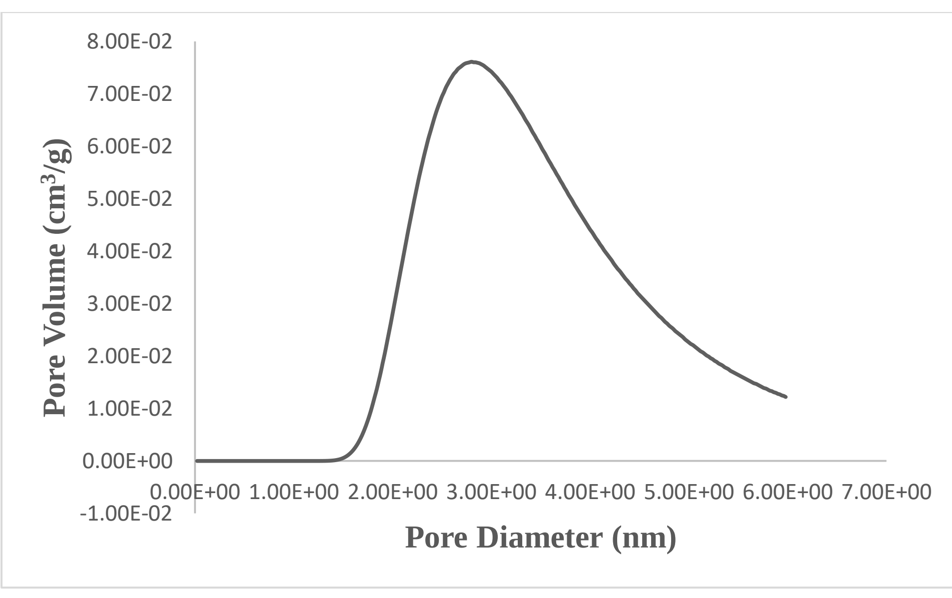

Figure 6: BET micropore analysis plot of the biosynthesized ZnO Nanoparticles

The average pore diameter of 2.80 nm (Figure 6) places the material within the mesoporous category according to the IUPAC classification, which defines mesoporous materials as those possessing pore diameters between 2 and 50 nm. Mesoporous structures are particularly advantageous because they facilitate the diffusion and transport of molecules into and out of the material, thereby enhancing their performance in applications such as catalysis, adsorption, and drug delivery (Li, 2023; Sotomayor *et al*., 2018). Such pore dimensions provide an ideal balance between high surface area and efficient mass transfer, enabling better contact between the ZnO surface and target molecules. This feature is particularly beneficial in applications involving adsorption, photocatalysis, and antimicrobial activity, where accessibility to active sites is essential.

The pore volume of **0.139 cm³/g** further confirms the porous nature of the synthesized ZnO nanoparticles. A relatively high pore volume indicates the presence of numerous internal voids capable of accommodating guest molecules. These pores can facilitate the diffusion and retention of reactants, pollutants, or microbial cells, thereby improving the overall efficiency of the material. In porous nanomaterials, larger pore volumes are often associated with improved adsorption capacity and enhanced surface interactions (Thommes *et al*., 2015).

The combination of a high surface area, mesoporous pore size, and appreciable pore volume suggests that the synthesized ZnO nanoparticles possess a highly accessible and reactive surface. These structural features are likely to contribute significantly to their biological and catalytic activities. In antimicrobial applications, for example, the large surface area can increase contact between the nanoparticles and microbial cell membranes, while the porous structure may facilitate the generation and diffusion of reactive oxygen species (ROS), which are known to play a major role in the antimicrobial mechanism of ZnO nanoparticles (Rasmussen *et al*., 2010). Likewise, in catalytic and environmental applications, the large number of available active sites can promote faster reaction rates and improved pollutant degradation.

The BET analysis demonstrates that the green-synthesized ZnO nanoparticles possess desirable textural properties characterized by a **high specific surface area (223.14 m²/g), mesoporous pore diameter (2.80 nm), and substantial pore volume (0.139 cm³/g).** These findings indicate the successful formation of a porous nanostructured material with excellent surface accessibility, which is expected to enhance its effectiveness in antimicrobial, catalytic, adsorption, and environmental remediation applications.

Antibacterial activity results

The antibacterial activity of the biosynthesized zinc oxide nanoparticles (ZnO NPs) was evaluated against two Gram-negative bacterial strains, *Klebsiella pneumoniae* and *Salmonella typhi*, and one Gram-positive bacterium, *Staphylococcus aureus*. The antimicrobial efficacy of the nanoparticles was assessed using the agar diffusion method, and the results were expressed as zones of inhibition.

The ZnO nanoparticles exhibited antibacterial activity against all the tested organisms, although the degree of inhibition varied with both bacterial species and nanoparticle concentration. Against *Klebsiella pneumoniae*, the nanoparticles demonstrated moderate activity at 500 mg/mL, producing a mean inhibition zone of 17.67 ± 0.58 mm. At the lower concentration of 250 mg/mL, the inhibition zone decreased to 12.67 ± 0.58 mm, indicating a concentration-dependent antibacterial effect. Similar observations have been reported for ZnO nanoparticles, where increased nanoparticle concentration resulted in enhanced bacterial growth inhibition due to greater nanoparticle–cell interactions and reactive oxygen species (ROS) generation (Chausov *et al*., 2021).

A stronger antibacterial effect was observed against *Salmonella typhi*. The ZnO NPs produced inhibition zones of 25.00 ± 0.82 mm and 20.67 ± 0.58 mm at concentrations of 500 and 250 mg/mL, respectively, suggesting high and moderate antibacterial activity. The greater susceptibility of *S. typhi* may be attributed to enhanced interactions between the nanoparticles and the bacterial cell membrane, resulting in membrane disruption, oxidative stress, leakage of intracellular components, and eventual growth inhibition. The antibacterial activity of ZnO nanoparticles has been widely linked to the generation of ROS, release of Zn^{2+} ions, and direct contact with bacterial cell surfaces, all of which contribute to cellular damage and bacterial death (Hadi *et al*., 2025).

Similarly, the nanoparticles showed appreciable antibacterial activity against *Staphylococcus aureus*, with inhibition zones of 20.67 ± 0.58 mm at 500 mg/mL and 12.67 ± 0.58 mm at 250 mg/mL. The larger inhibition zone recorded at the higher concentration indicates a concentration-dependent antibacterial effect, which has been widely reported for ZnO nanoparticles (Naqvi *et al*., 2019; Hadi *et al*., 2025). In comparison with 30 µg/mL of amoxicillin which showed high activity against all tested microbes, the biosynthesized ZnO NPs is a promising antibacterial agent.

The antibacterial activity observed in this study may be attributed to the unique physicochemical properties of ZnO nanoparticles, including their large surface-area-to-volume ratio, generation of reactive oxygen species (ROS), release of Zn^{2+} ions, and ability to disrupt bacterial cell membranes. These mechanisms can induce oxidative stress, damage cellular proteins, lipids, and nucleic acids, and ultimately lead to bacterial cell death (Dizaj *et al*., 2014; Siddiqi *et al*., 2018). Furthermore, residual phytochemicals from the plant extract used during synthesis may contribute synergistically to the antimicrobial activity of the nanoparticles (Nguyen *et al*., 2024; Janani *et al*., 2024).

The findings obtained in this study are consistent with previous reports demonstrating the broad-spectrum antibacterial properties of ZnO and other metal oxide nanoparticles against both Gram-positive and Gram-negative bacteria (Dizaj *et al*., 2014; Luo *et al*., 2024). The notable inhibition zones observed, particularly against *Salmonella typhi* and *Staphylococcus aureus*, suggest that the biosynthesized ZnO nanoparticles possess promising antibacterial potential. Therefore, further optimization of synthesis conditions, particle size, surface characteristics, and dosage could enhance their efficacy and support their application as alternative antimicrobial agents in biomedical and pharmaceutical fields.

CONCLUSION

In summary, ZnO nanoparticles with an average crystallite size of 21.7 nm have been successfully synthesized through a cost-effective and eco-friendly green process using aqueous root extract of *Nauclea latifolia*. The prepared nanoparticles were characterized through techniques such as UV, FTIR, XRD, DTA/TGA, BET, and were assayed for their antibacterial potency. The nanoparticles exhibited excellent-good antibacterial effect on the tested microbes in comparison with the standard drug. These biocidal properties may be linked to the high surface area of the nanoparticles and the bioactive phytochemicals associated with their surface.

The results of this study indicate that *Nauclea latifolia* is an effective and sustainable source for the green synthesis of zinc oxide nanoparticles. The synthesized ZnO NPs showed promising properties that could support their application in biomedical and environmental fields. Further optimization may enhance their potential use as antibacterial agents, catalysts, paint driers, and adsorbents for wastewater treatment and pollution remediation.

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