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GREEN SYNTHESIS AND CHARACTERIZATION OF ZINC OXIDE NANOPARTICLES USING *Euphorbia lateriflora* LEAF EXTRACT: EVALUATION OF ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES

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ABSTRACT

The biosynthesis of zinc oxide nanoparticles (ZnO NPs) using plant extracts has emerged as an environmentally friendly and cost-effective alternative to conventional chemical and physical methods. This study reports the green synthesis of ZnO NPs using aqueous leaf extract of *Euphorbia lateriflora* as both reducing and stabilizing agent. The biosynthesized nanoparticles were characterized using UV-visible spectroscopy, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and scanning electron microscopy (SEM). UV-Vis spectroscopy revealed a characteristic absorption peak at 260 nm, which may be attributed to the band edge absorption of very small ZnO nanoparticles or contributions from surface-bound phytochemicals. FTIR analysis identified functional groups responsible for the reduction and stabilization of nanoparticles. XRD patterns indicated a hexagonal wurtzite structure with diffraction peaks corresponding to JCPDS-36-1451. SEM analysis showed spherical morphology with polydispersed distribution. Phytochemical screening of *Euphorbia lateriflora* methanolic extract revealed the presence of alkaloids, steroids, cardiac glycosides, saponins, sterols, terpenes, terpenoids, and flavonoids. The biosynthesized ZnO NPs demonstrated significant antibacterial activity against Klebsiella pneumoniae, Escherichia coli, and Pseudomonas aeruginosa, with inhibition zones of 27.00 ± 2.42 mm, 21.00 ± 1.83 mm, and 17.00 ± 1.25 mm, respectively. Antioxidant assays showed DPPH radical scavenging activity with an IC_{50} value of 111.07 ± 15.72 µg/mL and FRAP value of 1.88 ± 0.06 µg/g. These findings demonstrate that Euphorbia lateriflora-mediated ZnO NPs possess promising antimicrobial and antioxidant properties suitable for biomedical applications.

Keywords: Green Synthesis, Zinc Oxide Nanoparticles, *Euphorbia Lateriflora*, Antimicrobial Activity, Antioxidant Activity, Phytochemicals

INTRODUCTION

Nanotechnology has transformed numerous fields of science by controlling and fabricating materials to the nanoscale size, usually less than 100 nm (Ma et al., 2024; Yusuf et al., 2025). Metal oxide nanoparticles are one of the nanomaterials that have attracted considerable interest because of their pronounced physicochemical characteristics such as high surface area, catalytic expansion, and quantum confinement (Harish *et al*., 2020). Zinc oxide nanoparticles (ZnO NPs) have become especially promising candidates due to their biocompatibility, safety to the environment, cost-effectiveness, and non-toxicity (Shaba *et al*., 2021; Jayachandran & Nair, 2021). The properties of ZnO NPs render them useful in various applications such as photocatalysis, antimicrobial agents, drug delivery systems, biosensors, and water purification (Islam et al., 2021).

Physical and chemical methods are traditional synthesis methods of nanoparticles. Physical techniques utilize top-down approaches, that is, mechanical separation of bulk materials, which uses special equipment and large amounts of energy (Akinsipo, 2025). Volatile organic solvents, harsh reducing agents, and toxic stabilizing compounds are frequently used in chemical synthesis, producing harmful byproducts that are both harmful to the environment and their health (Harsh *et al*., 2020; Akinsipo, 2025). Such constraints have given rise to the interest of finding alternative strategies in synthesis that are green chemistry principles.

The green synthesis of biological entities is an environmentally friendly and cost-efficient technology that causes minimal environmental harm and generates biocompatible nanoparticles (Martinez-Cabanas et al., 2021). Plant-mediated synthesis is associated with unique benefits in relation to biological systems, such as high rates of synthesis, scalability to large-scale production, and the production of a wide range of nanoparticle morphologies (Selim et al., 2020; Kuppusamy et al., 2016). Plant extracts are rich in secondary metabolites such as phenolics, flavonoids, terpenoids, and alkaloids, which are natural reducing agents and stabilizers in the development of nanoparticles (Azad et al., 2023). These phytochemicals also promote the reduction of metal ions and inhibit agglomeration to produce stable nanoparticle suspensions (Gopalan et al., 2021).

Euphorbia lateriflora (also called enu opiri in Yoruba and fidda sartse in Hausa) is a medicinal shrub that is traditionally used in African ethnomedicine to treat intestinal parasites, skin diseases, and convulsive fevers (Falana & Nurudeen, 2022). Phytochemical research has discovered several bioactive substances, such as flavonoids, steroids, alkaloids, saponins, and terpenoids in *Euphorbia lateriflora* extracts (Coker et al., 2021). These phytochemicals have antimicrobial effects against antibiotic-resistant bacteria and pathogenic fungi, which indicates the possibility of creating new therapeutic agents. Nonetheless, the use of *Euphorbia lateriflora* extract in the biosynthesis of zinc oxide nanoparticles is not studied.

The objective of the study was to synthesize zinc oxide nanoparticles by using *Euphorbia lateriflora* leaf extract as reducing and capping agent, characterize biosynthesized nanoparticles by various analytical methods, carry out a comprehensive phytochemical profiling of the plant extract and evaluate antimicrobial and antioxidant activity of plant extract and biosynthesized nanoparticles. The study meets the necessity of environmentally friendly methods of nanoparticle synthesis and the possible biomedical

application of ZnO NPs produced through *Euphorbia lateriflora.*

MATERIALS AND METHODS

Plant Material and Extract Preparation

Fresh leaves of *Euphorbia lateriflora* were collected from Ijagun, Ogun State, Nigeria. The plant material was authenticated at the herbarium of Tai Solarin University of Education. Collected leaves were thoroughly washed with distilled water to remove surface contaminants and air-dried at room temperature for seven days. Dried leaves were pulverized using mortar and pestle to obtain fine powder. For extract preparation, 20 g of leaf powder was mixed with 200 mL distilled water and boiled at 60°C for 30 minutes. The mixture was cooled to room temperature and filtered through Whatman No. 1 filter paper. The resulting aqueous extract was stored at 4°C for subsequent nanoparticle synthesis.

Synthesis of Zinc Oxide Nanoparticles

Zinc oxide nanoparticles were synthesized through bioreduction of zinc acetate dihydrate [$Zn(CH_3COO)_2 \cdot 2H_2O$] using *Euphorbia lateriflora* leaf extract. In a typical synthesis, 0.1 M zinc acetate dihydrate solution was prepared in distilled water. The leaf extract (50 mL) was added dropwise to 100 mL of a zinc acetate solution under constant magnetic stirring at 60°C, and the resulting mixture turned pale green. The reaction mixture was stirred continuously for 2 hours. Color change from an initial pale green to white indicated the formation of zinc oxide nanoparticles. The suspension was centrifuged at 5000 rpm for 15 minutes, and the precipitate was washed three times with distilled water, followed by absolute ethanol to remove unreacted precursors and organic residues. The purified nanoparticles were dried in an oven at 80°C overnight and calcined at 400°C for 3 hours to obtain crystalline ZnO NPs.

Characterization Techniques

UV-Visible Spectroscopy

Optical properties of biosynthesized ZnO nanoparticles were analyzed using UV-Visible spectrophotometer (Jenway 77428, UK) in wavelength range 200-800 nm. Nanoparticle suspension was diluted appropriately with distilled water, and absorbance spectrum was recorded against distilled water as blank reference.

Fourier Transform Infrared Spectroscopy (FTIR)

Functional groups present in leaf extract and on nanoparticle surface were identified using Shimadzu FTIR 8400 S spectrophotometer. Samples (0.01 g) were homogenized with anhydrous KBr (0.01 g) using agate mortar. Mixture was pressed into transparent pellets using vacuum hydraulic press at 1.2 psi. FTIR spectra were recorded in transmission mode over wavenumber range 600-4000 cm^{-1} with resolution of 4 cm^{-1}.

X-ray Diffraction (XRD)

Crystalline structure and phase purity of biosynthesized ZnO NPs were determined using X-ray diffractometer (EMPYREAN MALVERN PANALYTICAL) operating at 45 kV voltage and 40 mA current. Powder samples were scanned in 2θ range of 10-80° with step size of 0.02° and scan rate of 2° min^{-1}. Crystalline phases were identified by comparing diffraction patterns with standard JCPDS database. Average crystallite size was calculated using the Debye-Scherrer equation.

Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDX)

Surface morphology and particle size distribution were examined using a Scanning Electron Microscope (Phenom ProX, Netherlands). Dried nanoparticle samples were mounted on aluminum stubs using carbon adhesive tape, sputter-coated with gold for electrical conductivity, and examined at various magnifications (1000×-5000×) with an accelerating voltage of 15 kV. EDX spectra were acquired at the same accelerating voltage with 60 seconds acquisition time for quantitative elemental analysis.

Phytochemical Screening

Fresh leaves of *Euphorbia lateriflora* were shade-dried, pulverized, and subjected to methanolic extraction. Fifty grams of dried leaf powder were macerated in 500 mL of methanol for 72 hours at room temperature with intermittent agitation. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure using a rotary evaporator at 40°C to obtain the crude methanolic extract. Qualitative phytochemical screening of the extract was performed following standard protocols (Harborne, 1998; Trease and Evans, 2002) to identify major classes of secondary metabolites, including alkaloids, flavonoids, tannins, saponins, terpenoids, sterols, cardiac glycosides, anthraquinones, and phlobatannins. These preliminary tests were conducted to establish the phytochemical profile of the extract and to correlate the presence of bioactive compounds with the reducing and capping properties observed during ZnO nanoparticle synthesis.

Antimicrobial Activity Assessment

Antibacterial activity was evaluated using agar well diffusion method against three pathogenic bacteria: Klebsiella pneumoniae, Escherichia coli, and Pseudomonas aeruginosa. Bacterial cultures were adjusted to 0.5 McFarland standard and swabbed onto Mueller-Hinton agar plates. Wells (6 mm diameter) were bored, and test samples (biosynthesized ZnO NPs, methanolic extract, and zinc solution) were introduced into wells. Gentamycin served as positive control while n-hexane was negative control. Plates were incubated at 37°C for 24 hours, and zones of inhibition were measured in millimeters. Experiments were performed in triplicate.

Antioxidant Activity Evaluation

DPPH Radical Scavenging Assay

Free radical scavenging capacity was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Test samples at various concentrations (10-200 µg/mL) were mixed with 0.1 mM DPPH methanolic solution in 1:1 ratio. Mixtures were incubated in dark at room temperature for 30 minutes. Absorbance was measured at 517 nm using UV-Vis spectrophotometer. Ascorbic acid served as positive control. Percentage inhibition was calculated, and IC_{50} values were determined from dose-response curves.

Ferric Reducing Antioxidant Power (FRAP) Assay

FRAP assay measured reducing potential of test samples. FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ in ratio 10:1:1. Test samples (100 µL) were mixed with 3 mL FRAP reagent and incubated at 37°C for 4 minutes. Absorbance was measured at 593 nm against blank. Ascorbic acid standard curve was used to express results as micrograms per gram ascorbic acid equivalents (µg/g AAE).

Statistical Analysis

All experiments were conducted in triplicate, and results are expressed as mean ± standard deviation. Statistical significance was evaluated using one-way analysis of variance (ANOVA) followed by post-hoc tests. Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Characterization of Biosynthesized Zinc Oxide Nanoparticles

UV-Visible Spectroscopy Analysis

Initial validation of the formation of nanoparticles using UV-Visible spectroscopy is based on characteristic surface plasmon resonance peaks. The ZnO nanoparticles were biosynthesized with the highest absorption at 260 nm (Figure 1) with a strong blue shift over bulk ZnO (usually around 370 nm). The presence of such a strong blue shift (around 110 nm) is evidence of strong quantum confinement effects, which would imply creation of ultra-small ZnO nanoparticles or quantum dots, which will presumably be within the size range of less than 5 nm (Ahmed &Edvinsson, 2020).

The absorption band appears due to the electronic transitions of the large binding energy of the excitons of ZnO (60 meV) at room temperature (Ozgur et al., 2005). Nevertheless, the peak at 260 nm can also be due to charge-transfer reactions of surface-bound phytochemicals (especially flavonoids and phenolic compounds of the *Euphoria lateriflora* extract) and the surface of the ZnO nanoparticle (Suresh et al., 2015). The sharp absorption peak implies that the size distribution of nanoparticles is relatively uniform and that the crystallinity of the biosynthesized nanoparticles is good, and this is supported by XRD analysis.

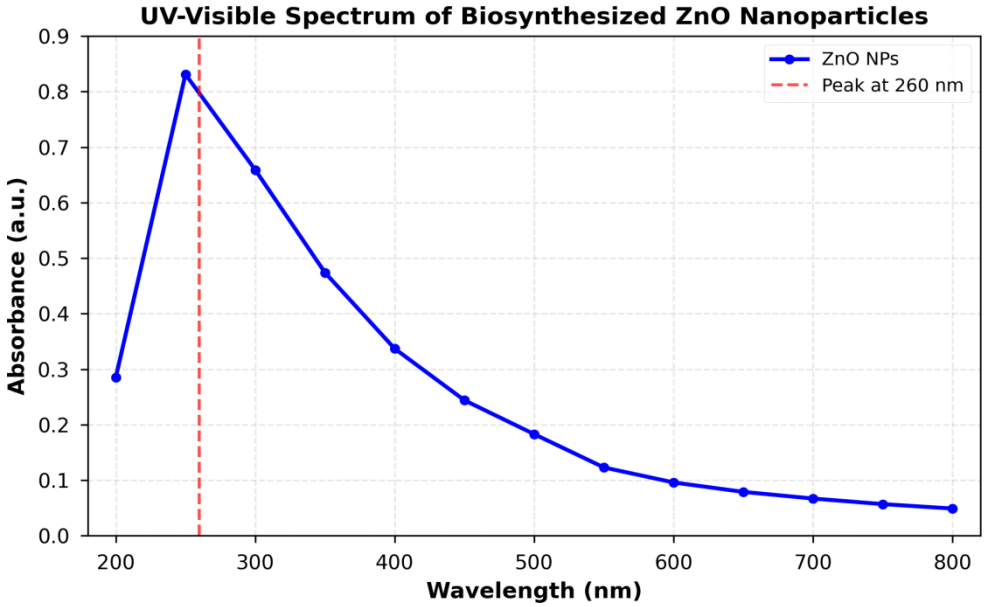
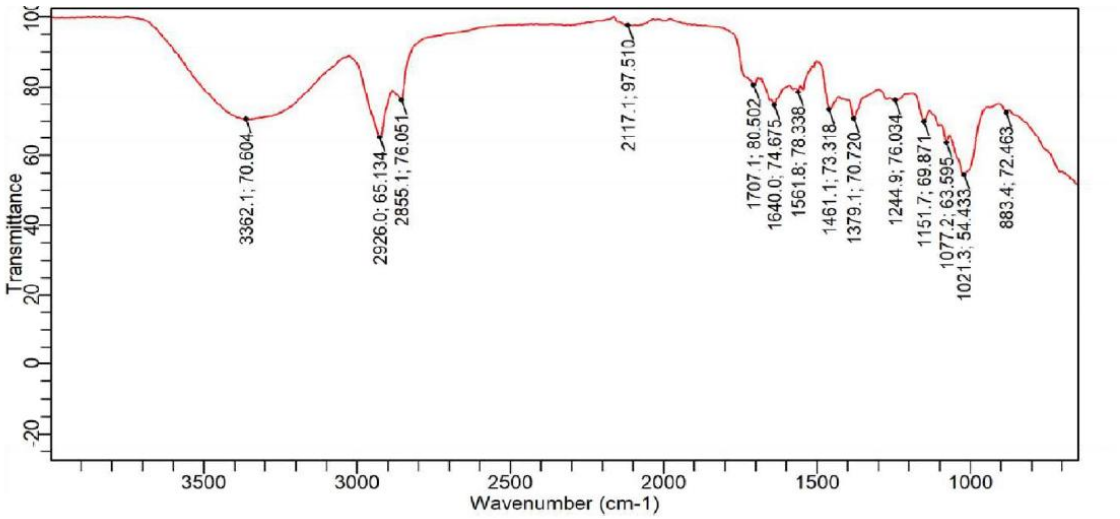


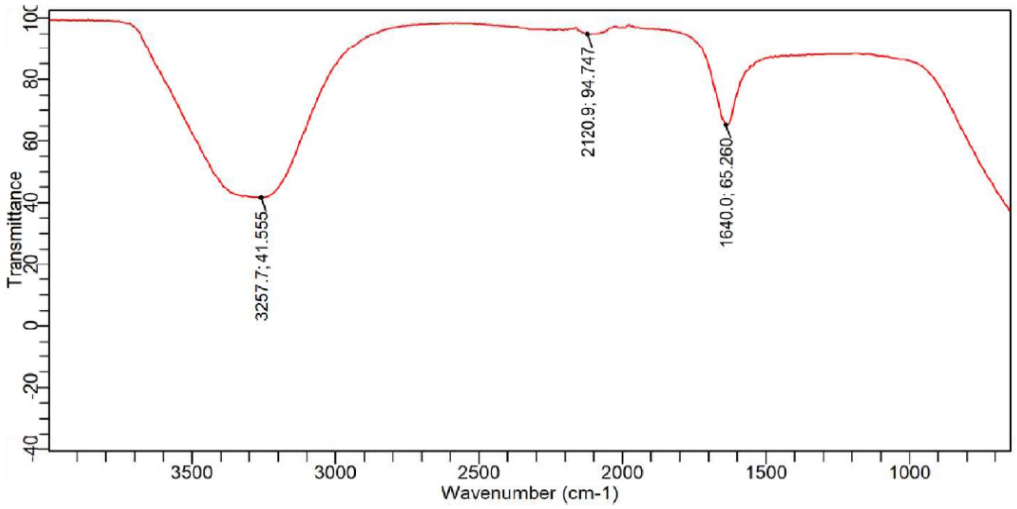
Figure 1: UV-Visible Absorption Spectrum of Biosynthesized ZnO Nanoparticles showing Characteristic Peak at 260 nm

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

FTIR spectroscopy elucidated functional groups present in *Euphorbia lateriflora* extract and their interaction with zinc oxide nanoparticles. The methanolic extract displayed prominent absorption bands at 3362, 2925, 2855, 1707, 1640, 1561, 1461, 1379, 1244, 1151, 1077, 1021, and 883 cm^{-1} (Figure 2A). The broad band around 3362 cm^{-1} corresponds to O-H stretching vibrations from phenolic compounds and alcohols. Peaks at 2925 and 2855 cm^{-1} indicate C-H stretching of aliphatic hydrocarbons. The absorption at 1707 cm^{-1} represents C=O stretching of carbonyl groups in ketones or carboxylic acids. Band at 1640 cm^{-1} signifies C=C stretching of aromatic rings or amide carbonyl groups. The presence of these functional groups confirms abundance of phytochemicals including flavonoids, phenolic acids, and terpenoids capable of reducing metal ions (Harish et al., 2020). Following nanoparticle synthesis, FTIR spectrum of ZnO NPs of showed modifications in peak intensities and positions, indicating interaction between phytochemicals and nanoparticle surface. The appearance of absorption band in region 400-600 cm^{-1} (typically around 450-500 cm^{-1}) confirms Zn-O bond formation (Figure 2B), characteristic of zinc oxide nanostructures (Bandeira et al., 2021). Retention of peaks corresponding to phenolic and flavonoid groups suggests these biomolecules remained adsorbed on nanoparticle surface, functioning as capping agents that prevent agglomeration and enhance stability.



(A)



(B)

Figure 2A-B: FTIR Spectra of (A) Methanolic Extract of Euphorbia lateriflora (MeEL) and (B) Euphorbia Lateroflora synthesized Zinc Oxide Nanoparticles (ZnONPs)

X-ray Diffraction (XRD) Analysis

The crystalline structure and the phase composition of the biosynthesized nanoparticles were determined by XRD analysis. The presence of zincite (ZnO) as the major nanomaterial phase was confirmed by qualitative phase analysis based on the JCPDS database (Figure 3). The shape of the diffraction pattern was broad, with large widths, typical of nanocrystalline materials with extremely small crystallite sizes. The phases that were identified were zincite (ZnO), traces of zinc carbonate (smithsonite, $ZnCO_3$), and other organic residues of the plant extract. The most common peaks were at 2Θ of 22.91º and 29.60º, and the Full Width at Half Maximum (FWHM) of 2.0º and 1.6º, respectively. The large broadening of the peaks is a marker of ultra-small nanoparticles approaching the quantum dot size regime. Using the Debye-Scherrer equation:

$$D = K\lambda / (\beta \cos \theta) \quad (1)$$

D is the crystallite size, K is the Scherrer constant (0.9 in the case of the spherical particle), 0.15406 A) or Cu Kα radiation, β is the FWHM in radians, and Θ is the Bragg angle. The average size of the crystallite was found to be about 4-5 nm. The small size of crystallites is correlated to the creation of ZnO quantum dots, and this is the reason why there is a huge blue shift in the UV-Vis absorption spectrum to 260 nm of ZnO compared to 370 nm of ZnO in bulk form. The effect of quantum confinement in the nanoparticles that have sizes less than 5 nm is an increase in the energy of the band gap and changes in optical absorption to lower wavelengths (Ahmed & Edvinsson, 2020; Wojnarowicz et al., 2020).

Small secondary phases (zinc carbonate and leftover organics) are typical of green synthesis techniques and are probably due to the capping of the sizable ZnO quantum dots by phytochemicals of the *Euphoria lateriflora* extract, which also offers a stabilizer and prevents aggregation (Matinise et al., 2017). The diffraction peaks are wide and diffuse, and the fact that there are no sharp, well-defined diffraction peaks, such as those of bulk ZnO, is yet another indication of the nanocrystalline nature and incredibly small size of the biosynthesized product.

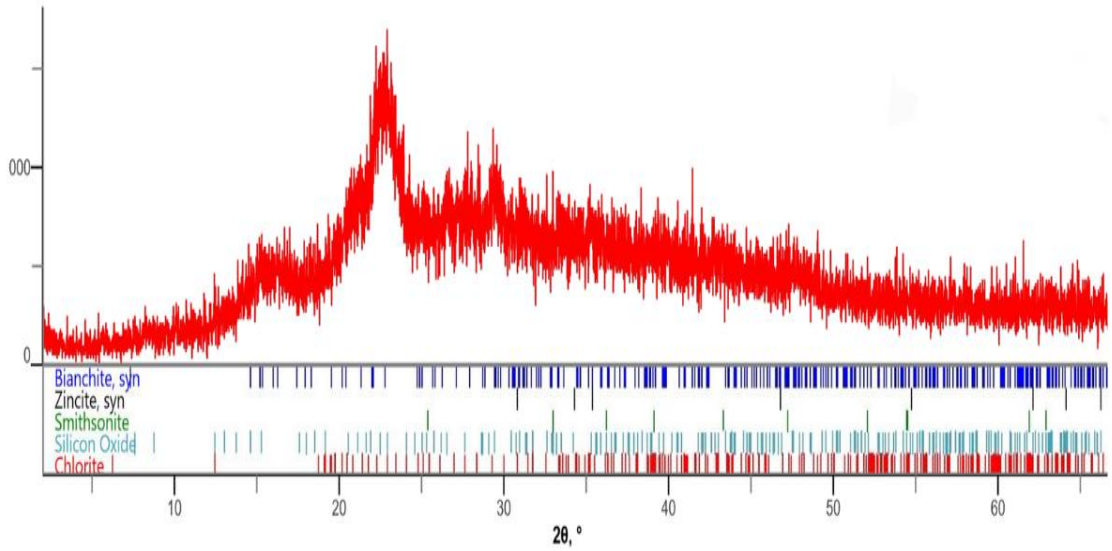


Figure 3: X-ray Diffraction Analysis of ZnONPs Synthesized from the Leaves of *Euphorbia Lateriflora*

Scanning Electron Microscopy (SEM) Analysis

SEM analysis revealed morphological characteristics of biosynthesized ZnO nanoparticles. Micrographs showed predominantly spherical particles with smooth surfaces distributed throughout the sample matrix. The nanoparticles exhibited polydisperse size distribution typical of biogenic synthesis, where multiple phytochemicals with varying reducing capabilities produce particles of different dimensions (Bandeira et al., 2021). Some degree of aggregation was observed, attributed to magnetic and van der Waals interactions between particles during the drying process. The relatively small particle size observed is consistent with the XRD analysis, which indicated crystallite sizes in the quantum dot range (4-5 nm). The apparent discrepancy between XRD crystallite size and SEM particle size suggests that the observed particles in SEM may consist of multiple crystalline domains or represent small agglomerates of individual quantum dots, which is typical for ultra-small nanoparticles (Ong et al., 2018). To ascertain the elemental composition of the nanoparticles biosynthesized, Energy Dispersive X-ray Spectroscopy (EDX) was conducted (Figure 4). The quantitative method showed that the most dominant metallic element is zinc (35.50 atomic %, 73.10 weight %), and the characteristic Zn peaks were 1.0, 8.6 and 9.6 keV. The presence of oxygen was also confirmed by the typical peak at approximately 0.5 keV, which is characteristic of oxygen but because of the limitation of the EDX to measure light elements, the oxygen concentration was not measured accurately but the presence of zinc oxide was confirmed. Also, a significant amount of carbon (40.20 atomic %), nitrogen (12.00 atomic %) and phytochemicals of the *Euphorbia lateriflora* extract in its form were identified as capping agents on the nanoparticle surface. Such a layer prevents aggregation and helps in preserving the stability of nanoparticles (Mousa et al. 2024). Trace elements such as phosphorus (0.20%), sulfur (0.18%), and calcium (0.07%) are due to the plant extract, while copper, nickel, aluminum, and silicon (<1.5% total) are due to sample preparation and instrumental background. The ZnO nanoparticles synthesized by aqueous extract of *Euphorbia lateriflora* are successfully functionalized on their surfaces using phytochemicals as confirmed in the EDX analysis.

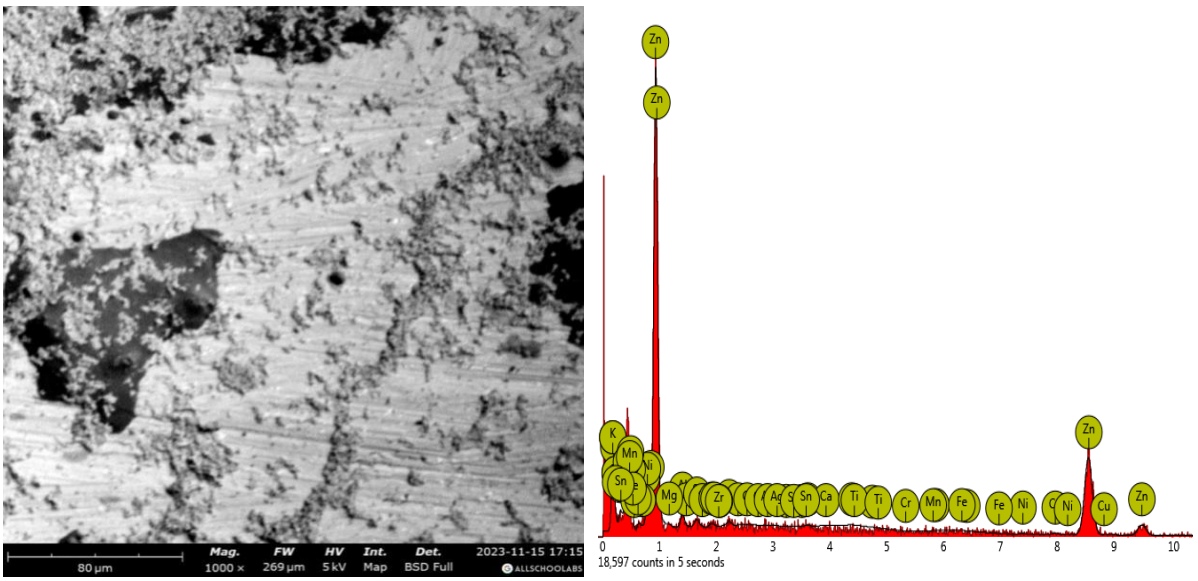


Figure 4: SEM Micrograph and EDX Results of ZnONPs Synthesized from the Aqueous Extract of *Euphorbia lateriflora*

Phytochemical Profiling of Euphorbia *lateriflora*

Qualitative phytochemical screening identified diverse secondary metabolites in *Euphorbia lateriflora* methanolic extract and biosynthesized ZnO nanoparticles (Table 1). Both samples tested positive for alkaloids, saponins, sterols, terpenes, terpenoids, flavonoids, and cardiac glycosides. Anthraquinones, tannins, and phlobatannins were absent in both samples. The presence of these phytochemicals explains the reducing and stabilizing capabilities of the plant extract during nanoparticle synthesis.

Alkaloids possess nitrogen-containing functional groups that can chelate metal ions and facilitate electron transfer during the reduction process (Akinfenwa and Hussein, 2023). Flavonoids contain multiple hydroxyl groups capable of donating electrons to reduce Zn^{2+} ions while simultaneously coordinating with the nanoparticle surface through oxygen atoms (Marslin et al., 2018). Terpenoids and sterols contribute to nanoparticle stabilization through hydrophobic interactions and steric hindrance effects. Saponins form protective layers around nanoparticles through their amphiphilic nature, preventing aggregation in aqueous medium. Cardiac glycosides may participate in surface functionalization, potentially enhancing the biocompatibility of synthesized nanoparticles (Agidew et al., 2022). The retention of an identical phytochemical profile in both extract and nanoparticles confirms that bioactive compounds remain associated with ZnO NPs post-synthesis, contributing to their biological activities.

Table 1: Phytochemical Constituents of *Euphorbia Lateriflora* Methanolic Extract and Biosynthesized ZnO Nanoparticles

Phytochemical Constituent	Methanol Extract	ZnEL
Alkaloids	+	+
Saponins	+	+
Anthraquinones	–	–
Sterols	+	+
Terpenes	+	+
Terpenoids	+	+
Flavonoids	+	+
Tannins	–	–
Phlobatannins	–	–
Cardiac glycosides	+	+

+ = Present; - = Absent

Antimicrobial Activity

Biosynthesized ZnO nanoparticles demonstrated significant antibacterial activity against all tested pathogens (Figure 5. Table 2). Against Klebsiella pneumoniae, ZnEL exhibited largest inhibition zone (27.00 ± 2.42 mm), comparable to gentamycin control (30.00 ± 1.49 mm). This represents superior activity compared to methanolic extract alone (18.00 ± 0.26 mm) and zinc solution (19.00 ± 1.11 mm), indicating synergistic effects between zinc oxide nanostructure and phytochemical coating. For Escherichia coli, zinc solution showed highest activity (26.00 ± 0.22 mm), followed by gentamycin (28.00 ± 0.70 mm) and ZnEL (21.00 ± 1.83 mm). Against Pseudomonas aeruginosa, methanolic extract demonstrated superior performance (22.00 ± 1.11 mm) compared to ZnEL (17.00 ± 1.25 mm), suggesting that certain phytochemicals may be more effective against this organism than nanoparticulate form.

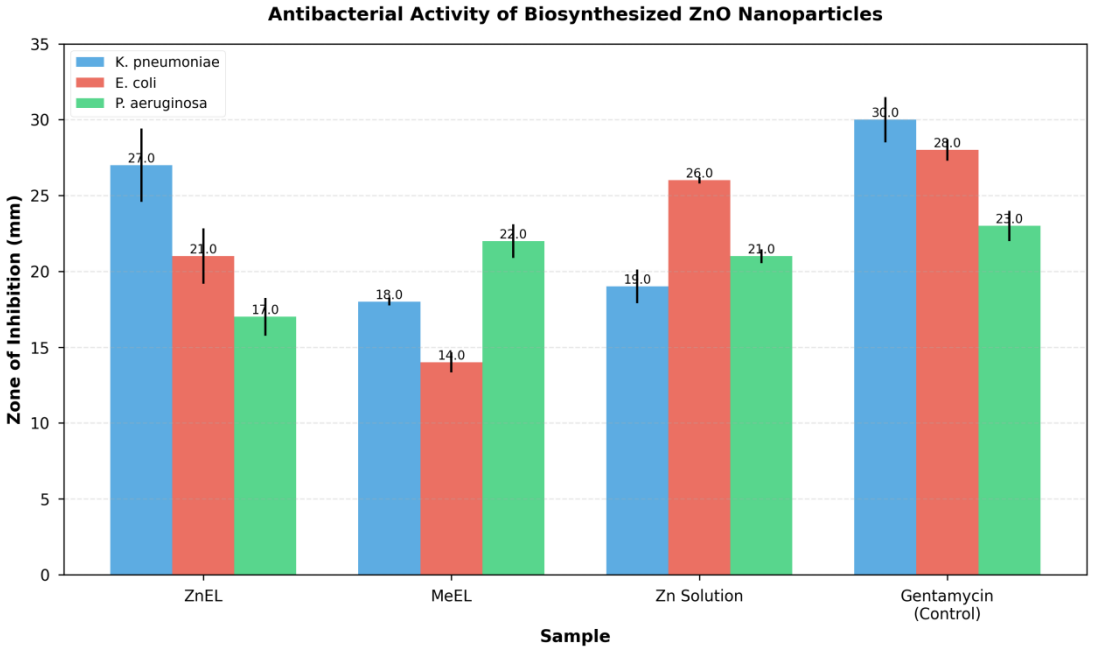


Figure 5: Antibacterial Activity of Biosynthesized ZnO Nanoparticles against Pathogenic Bacteria. Data Represent mean ± SD (n=3).

The antimicrobial mechanism of ZnO nanoparticles involves multiple pathways. Nanoparticles can penetrate bacterial cell walls through their small size, disrupting membrane integrity and causing leakage of cellular contents (Fahadul et al., 2021). ZnO NPs generate reactive oxygen species (ROS) including hydrogen peroxide, superoxide radicals, and hydroxyl radicals that induce oxidative stress, damaging proteins, lipids, and nucleic acids (Shaba et al., 2021). Release of Zn^{2+} ions interferes with enzymatic activities and protein synthesis. Additionally, phytochemicals coating nanoparticle surface contribute antimicrobial effects through their inherent bioactivities. The differential susceptibility of bacterial strains reflects variations in cell wall structure, with Gram-negative bacteria possessing outer membrane that provides additional protective barrier (Coker et al., 2021).

Table 2: Zones of Inhibition (mm) for Antimicrobial Activity of Biosynthesized ZnO Nanoparticles

Sample	*K. pneumoniae*	*E. coli*	*P. aeruginosa*
ZnEL	27.00 ± 2.42	21.00 ± 1.83	17.00 ± 1.25
MeEL	18.00 ± 0.26	14.00 ± 0.66	22.00 ± 1.11
Zn Solution	19.00 ± 1.11	26.00 ± 0.22	21.00 ± 0.46
Gentamycin (Control)	30.00 ± 1.49	28.00 ± 0.70	23.00 ± 1.00
n-hexane (Control)	–	–	–

Values represent mean ± standard deviation (n=3)

Antioxidant Activity

DPPH Radical Scavenging Activity

DPPH assay evaluates free radical scavenging capacity through reduction of stable DPPH radical to diphenylpicrylhydrazine, monitored by decrease in absorbance at 517 nm. Lower IC_{50} values indicate higher antioxidant activity. Results showed that zinc solution exhibited strongest activity (IC_{50} = 56.72 ± 12.26 µg/mL), approaching that of ascorbic acid standard (IC_{50} = 51.24 ± 10.22 µg/mL). Methanolic extract demonstrated IC_{50} value of 81.26 ± 13.90 µg/mL, while ZnEL showed IC_{50} of 111.07 ± 15.72 µg/mL (Figure 6, Table 3). The higher IC_{50} value for biosynthesized nanoparticles compared to extract alone suggests that immobilization of phytochemicals on nanoparticle surface may partially reduce their accessibility to DPPH radicals, though ZnEL still maintains significant antioxidant capacity (Martinez et al., 2021).

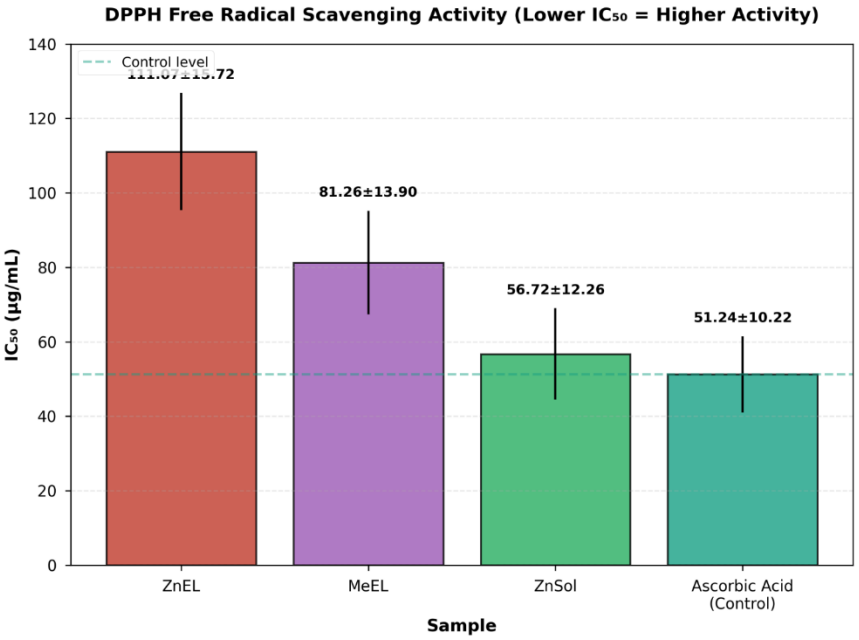


Figure 6: DPPH free Radical Scavenging Activity Expressed as IC_{50} values. Lower Values Indicate Higher Antioxidant Activity. Data Represent mean ± SD (n=3)

Ferric Reducing Antioxidant Power (FRAP)

FRAP assay measures reducing potential through ability to reduce Fe^{3+}-TPTZ complex to blue-colored Fe^{2+}-TPTZ, with higher absorbance indicating greater reducing power. Results showed methanolic extract possessed highest FRAP value among test samples (1.97 ± 0.02 µg/g AAE), followed by ZnEL (1.88 ± 0.06 µg/g AAE) and zinc solution (1.84 ± 0.08 µg/g AAE), though all values were significantly lower than ascorbic acid standard (4.22 ± 0.04 µg/g AAE) (Figure 7, Table 3). The comparable FRAP values between extract and nanoparticles indicate that reducing capacity of phytochemicals is largely preserved after nanoparticle synthesis.

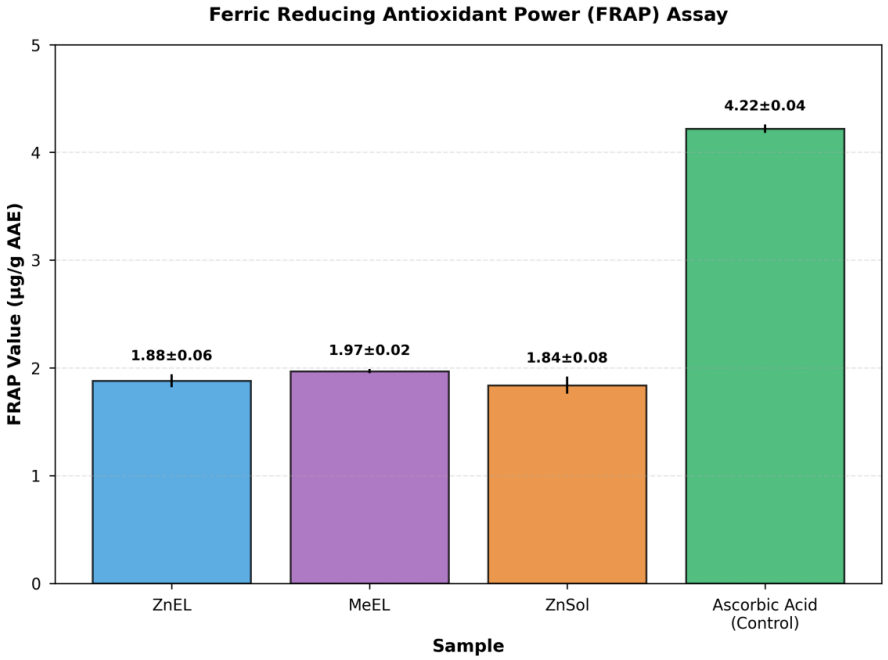


Figure 7: Ferric Reducing Antioxidant Power (FRAP) Expressed as Ascorbic Acid Equivalents (AAE). Data Represent mean ± SD (n=3)

The antioxidant properties of biosynthesized ZnO nanoparticles arise from dual contributions: intrinsic antioxidant activity of zinc oxide through electron donation and ROS scavenging, and extrinsic activity from surface-bound phytochemicals containing phenolic hydroxyl groups capable of hydrogen atom transfer. Flavonoids and phenolic compounds exhibit antioxidant activity through multiple mechanisms including free radical scavenging, metal chelation, and electron donation (Agidew et al., 2022). The presence of these compounds on nanoparticle surface, confirmed through FTIR and phytochemical screening, explains sustained antioxidant capacity of biosynthesized ZnO NPs. These properties suggest potential applications in oxidative stress-related conditions and as protective agents against cellular damage.

Table 3. Antioxidant Activity of Biosynthesized ZnO Nanoparticles Evaluated through DPPH and FRAP Assays

Sample	DPPH IC_{50} (µg/mL)	FRAP (µg/g AAE)
ZnEL	111.07 ± 15.72	1.88 ± 0.06
MeEL	81.26 ± 13.90	1.97 ± 0.02
Zn Solution	56.72 ± 12.26	1.84 ± 0.08
Ascorbic Acid (Control)	51.24 ± 10.22	4.22 ± 0.04

Values represent mean ± standard deviation (n=3); AAE = Ascorbic Acid Equivalents

CONCLUSION

This study successfully demonstrated green synthesis of zinc oxide nanoparticles using *Euphorbia lateriflora* leaf extract as both reducing and stabilizing agent. The biosynthesized nanoparticles exhibited a characteristic hexagonal wurtzite structure with spherical morphology and polydisperse size distribution, confirmed through comprehensive characterization using UV-Vis spectroscopy, FTIR, XRD, and SEM analyses. Phytochemical screening revealed abundant secondary metabolites, including alkaloids, flavonoids, terpenoids, and phenolic compounds that facilitate nanoparticle formation and contribute to biological activities.

Biological evaluation demonstrated that *Euphorbia lateriflora*-mediated ZnO nanoparticles possess significant antimicrobial activity against Gram-negative pathogenic bacteria, with particularly strong efficacy against Klebsiella pneumoniae. The nanoparticles also exhibited notable antioxidant properties through both DPPH radical scavenging and ferric reducing mechanisms, attributed to synergistic effects between zinc oxide nanostructure and surface-adsorbed phytochemicals. These findings validate *Euphorbia lateriflora* as an effective biogenic agent for synthesizing multifunctional zinc oxide nanoparticles.

The environmentally benign synthesis approach eliminates toxic chemicals while producing biocompatible nanoparticles suitable for biomedical applications. The demonstrated antimicrobial and antioxidant activities suggest potential applications in wound healing, food preservation, water treatment, and the development of therapeutic agents against bacterial infections and oxidative stress-related disorders. Future research should focus on optimizing synthesis parameters for enhanced uniformity, investigating cytotoxicity profiles, and exploring additional biological activities, including anticancer and anti-inflammatory properties. Mechanistic studies elucidating precise molecular interactions during nanoparticle formation and biological action would further advance understanding of this promising green nanotechnology platform.

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