RESEARCH ARTICLE

ECO-FRIENDLY SYNTHESIS AND CHARACTERIZATION OF ZINC OXIDE NANOPARTICLES DERIVED FROM *ZANTHOXYLUM ARMATUM* SEEDS WITH BIOLOGICAL APPLICATIONS

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Abstract:

The present study focuses on the green synthesis of zinc oxide nanoparticles (ZnO NPs) using *Zanthoxylum armatum* seed extract, emphasizing an eco-friendly and cost-effective approach. The phytochemicals present in the seed extract act as natural reducing and stabilizing agents, facilitating the formation of ZnO nanoparticles without the need for toxic chemicals. The successful synthesis of ZnO NPs was confirmed and characterized using a range of analytical techniques. Dynamic Light Scattering (DLS) analysis indicated the average particle size and distribution, while X-ray Diffraction (XRD) confirmed the crystalline nature and phase purity of the nanoparticles. Fourier Transform Infrared Spectroscopy (FTIR) analysis identified functional groups responsible for the reduction and capping of the nanoparticles. UV-Visible spectroscopy revealed a characteristic absorption peak, validating the formation of ZnO NPs. The antioxidant potential of the synthesized nanoparticles was evaluated using standard free radical scavenging assays, indicating significant antioxidant activity, likely attributed to the bioactive compounds from the plant extract adhered to the nanoparticle surface. Furthermore, the antibacterial activity of the ZnO NPs was assessed against selected gram-positive and gram-negative bacterial strains, showing promising results with effective inhibition zones. These findings suggest that ZnO nanoparticles synthesized using *Zanthoxylum armatum* seed extract possess strong antioxidant and antibacterial properties, making them suitable for potential biomedical and pharmaceutical applications. This study highlights the synergistic role of plant-based synthesis in nanotechnology, offering a sustainable alternative for nanoparticle fabrication with enhanced biological functionalities.

Keyword: *Zanthoxylum armatum*, UV, FTIR, DLS, XRD, Nanoparticles.

Introduction:

The deciduous, fragrant shrub or small tree *Zanthoxylum armatum*, a member of the Rutaceae family, is indigenous to Asia's subtropical and temperate zones, especially the Himalayan belt. Widely prevalent in India, Nepal, Bhutan, China, and Southeast Asia, *Z. armatum* is often referred to as toothache tree, timur, or winged prickly ash (Bhattarai *et al.,* 2006). It grows best at elevations between 900 and 2100 meters, frequently on open slopes on the edges of forests. *Zanthoxylum armatum* has long been used in a variety of ethnomedical procedures. Numerous illnesses, such as toothaches, fever, dyspepsia, and respiratory conditions, have been treated using the seeds, bark, fruit, and leaves (Kumar *et al.,* 2013). Its rich phytochemical composition, which contains lignans, alkaloids, flavonoids, coumarins, terpenoids, and essential oils, is largely responsible for its significance in traditional medicine (Ghimire *et al.,* 2015). According to phytochemical research, *Z. armatum* has high concentrations of bioactive substances such as limonene, sesamin, and xanthoxylin, which support its antibacterial, antifungal, antioxidant, and anti-inflammatory properties (Sharma & Kumar, 2011). These characteristics not only support its long-standing medical use but also point to possible uses in the biotechnological and pharmaceutical industries. The use of plant extracts for environmentally friendly nanoparticle production has drawn a lot of interest recently. Green synthesis provides a non-toxic, economical, and environmentally friendly substitute for traditional chemical and physical processes (Iravani, 2011).

Z. armatum, rich in secondary metabolites, is capable of reducing metal ions to their respective nanoparticles while simultaneously acting as capping and stabilizing agents. The phenolic compounds, flavonoids, and terpenoids present in the seed extract play a crucial role in the reduction and stabilization of nanoparticles (Ahmed *et al.,* 2016). Zinc oxide (ZnO) nanoparticles have attracted significant interest due to their unique optical, antibacterial, and photocatalytic properties. The green synthesis of ZnO nanoparticles using *Z. armatum* seed extract offers a promising and sustainable approach for developing nanomaterials suitable for applications in biomedicine, environmental remediation, and agriculture. Thus, given its rich phytochemical profile and strong reducing potential, *Zanthoxylum armatum* represents a valuable biological resource for the eco-friendly synthesis of zinc oxide nanoparticles. Based on the above information, we wished to develop an eco-friendly synthesis method for zinc oxide (ZnO) nanoparticles using *Zanthoxylum armatum* seed extract, characterize and evaluate its biological applications.

Materials and Methods:

Collection of Samples

The *Zanthoxylum armatum* Seed was collected from the market in Chennai district, Tamil Nadu.

Figure 1: *Zanthoxylum armatum* Seed

Extraction

For extraction, 10 g of the powdered *Zanthoxylum armatum* seed material was placed in a sterile conical flask containing 100 mL of hydroalcoholic solvent (ethanol:water in a 70:30 v/v ratio). The mixture was covered with aluminum foil and kept at room temperature for 48 hours. After the extraction period, the mixture was filtered using Whatman No. 1 filter paper. The obtained filtrate was allowed to air-dry at room temperature in a clean, dust-free environment until complete evaporation of the solvent. The resulting crude extract was scraped, collected in a sterile container, and stored at 4°C for further use in zinc oxide nanoparticle synthesis and biological activity evaluation.

Figure 2*: Zanthoxylum armatum Seed* extrac

Synthesis of Zinc Oxide Nanoparticles

Zinc oxide (ZnO) nanoparticles were synthesized via a green synthesis method using *Zanthoxylum armatum* seed extract as a reducing and stabilizing agent. 0.4069 g of zinc oxide (ZnO) powder was dispersed in 45 mL of distilled water in a beaker under continuous magnetic stirring to form a uniform suspension. After complete dispersion, 5 mL of the *Z. armatum* seed extract was added slowly to the ZnO suspension under constant stirring at room temperature. The reaction mixture was stirred continuously for 2–3 hours. A gradual change in the color and turbidity of the solution was observed, indicating the formation of ZnO nanoparticles. After completion of the reaction, the solution was allowed to settle and the product was collected by simple decantation. The material is dried in a hot air oven at 60°C for 6–8 hours to obtain dry ZnO nanoparticle powder. The nanoparticles were carefully collected, powdered, and stored in containers for further characterization and biological studies. This green synthesis method is simple, eco-friendly, and avoids the use of toxic chemicals.

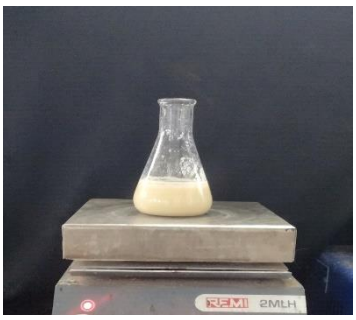

Figure 3: Synthesis of Zinc Oxide Nanoparticle

Characterization of Nanoparticles

UV–Visible Spectrophotometer:

A UV–Visible spectrophotometer was used to obtain the spectral response of ZnONPs. The

sample was monitored by absorbance measurements carried out on a UV-Visible spectrophotometer in the wavelength range of 200-800nm (Thermo Scientific—Evolution 201).

Dynamic Light Scattering (DLS):

Dynamic Light Scattering (DLS) was employed to determine the hydrodynamic size distribution and polydispersity index (PDI) of the synthesized zinc oxide nanoparticles. The analysis provides insight into the average particle size and uniformity of the nanoparticles in colloidal form. A low PDI value indicates a narrow size distribution, suggesting good stability and homogeneity of the nanoparticle suspension.

Fourier Transforms Infrared Spectroscopy (FTIR):

Fourier-transform infrared spectroscopy (FTIR) analysis was used for identifying the functional group of the nanoparticles. It was used to find an infrared spectrum of absorption or emission of a solid, liquid, or gas. FTIR spectrometer collects high-spectral-resolution data over a wide range of spectral. This confers a significant advantage over a dispersive spectrometer, which measures intensity over a narrow range of wavelengths at a time. It was analyzed by a SHIMADZU spectrometer in the range of 500–4000 cm−1.

Antioxidant Assay:

DPPH Scavenging Activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay is a widely employed method for evaluating the free radical scavenging ability of plant extracts, nanoparticles, and other bioactive compounds. DPPH is a stable free radical characterized by a deep violet color in solution, with a maximum absorbance at 517 nm. Upon interaction with an antioxidant, which can donate a hydrogen atom or an electron, the DPPH radical is reduced, leading to a noticeable discoloration of the solution from violet to yellow. The degree of discoloration is proportional to the antioxidant capacity of the sample. The antioxidant activity is quantified by measuring the decrease in absorbance at 517 nm using a UV-Visible spectrophotometer. According to Manzocco, Anese, and Nicoli (1998), the DPPH assay is based on the principle that antioxidants act either by donating electrons or hydrogen atoms to neutralize free radicals. Moreover, the kinetics of the reduction reaction can provide additional insight into the mechanism and efficiency of antioxidant compounds. The simplicity, rapidity, and reproducibility of the DPPH assay make it a valuable tool for preliminary screening of antioxidant properties in natural products and synthesized materials.

Antibacterial Activity Assay:

The antibacterial activity of the synthesized samples was evaluated using the agar well diffusion method, following the procedure described by Balouiri *et al.* (2016) with slight modifications. Escherichia coli (Gram-negative) and Staphylococcus aureus (Gram-positive) were employed as test organisms. The bacterial cultures were grown overnight in nutrient broth at 37°C with shaking at 100 rpm, and the inoculum was adjusted to approximately CFU/mL. Sterile nutrient agar plates were prepared, and the bacterial suspensions were evenly swabbed across the surface. Wells of 3 mm diameter were aseptically punched using a sterile cork borer. Different concentrations of the test sample (25, 50, 100, and 200 µg/mL) were prepared, and 20 µL of each concentration was loaded

into the respective wells. Additionally, 20 µL of gentamycin (as a positive control) was loaded into separate wells to compare antibacterial efficacy.The plates were incubated at 37°C for 24 hours. Following incubation, the antibacterial activity was assessed by measuring the zone of inhibition (ZOI) formed around each well, recorded in millimeters (mm). An increased diameter of the inhibition zone indicated a greater antibacterial effect. All experiments were performed in triplicate for accuracy.

Result and Discussion:

Extraction

The seeds after the extraction period, the mixture was filtered using Whatman No. 1 filter paper. The obtained filtrate was allowed to air-dry at room temperature in a clean, dust-free environment until complete evaporation of the solvent. The resulting crude extract was scraped, collected in a sterile container, and stored at 4°C for further use in zinc oxide nanoparticle synthesis and biological activity evaluation.

Characterization of Nanoparticles

UV–Visible Spectrophotometer:

The UV-Visible absorption spectrum of the synthesized ZnO nanoparticles was recorded in the wavelength range of 300–700 nm (Figure: 7). The spectrum shows a sharp and strong absorption peak at approximately 315–320 nm. UV-Visible spectroscopy results clearly confirm the successful green synthesis of stable, nanoscale ZnO particles using *Zanthoxylum armatum* seed extract.

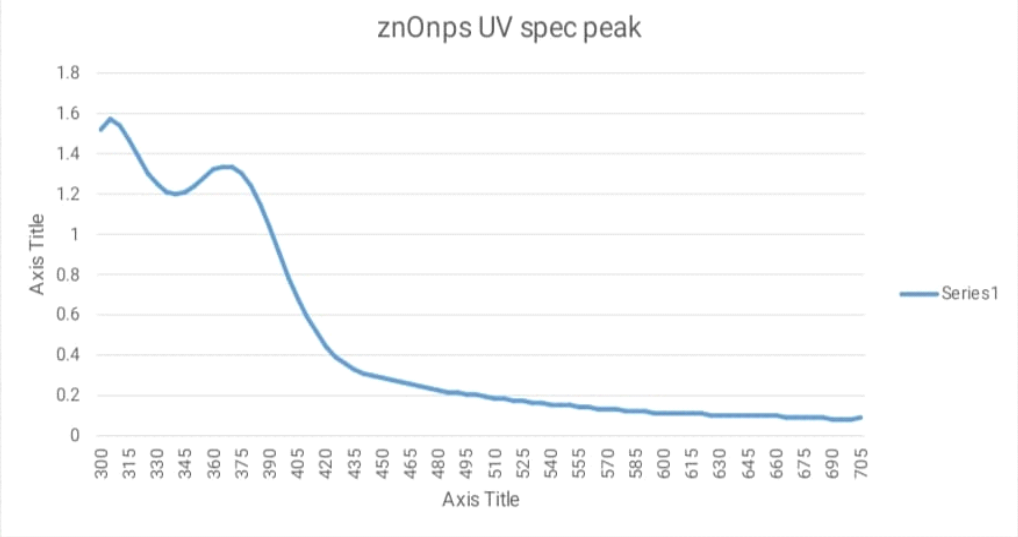

Figure 4: UV Visible spectra

The UV-Visible absorption spectrum of ZnO nanoparticles, with a sharp peak at 315–320 nm, is consistent with findings from other green synthesis studies. Bindhu and Umadevi (2013) reported a prominent absorption peak around 320 nm for ZnO nanoparticles synthesized using *Tridax procumbens* leaf extract, confirming their nanoscale properties. Similarly, Singh *et al.,* (2018) observed an absorption peak near 318 nm for ZnO nanoparticles synthesized with *Aloe vera* extract, highlighting its effectiveness in nanoparticle formation. Research by Ramesh *et al.,* (2015) demonstrated a characteristic absorption peak at 325 nm for ZnO nanoparticles synthesized using *Azadirachta indica*, validating the use of plant extracts for stable nanoparticle production.

Fourier Transforms Infrared Spectroscopy (FTIR):

FTIR analysis identified key functional groups involved in synthesizing zinc oxide nanoparticles using *Zanthoxylum armatum* seed extract. O–H stretching (~3410 cm^{-1}) suggests hydroxyl group interactions, showing phytochemical capping. C=O stretching or N–H bending (~1625 cm^{-1}) indicates proteins, flavonoids, or other reducing agents. C–H bending (~1450 cm^{-1}) confirms organic molecule interactions. Zn–O stretching (~450–500 cm^{-1}) finds successful ZnO nanoparticle formation.

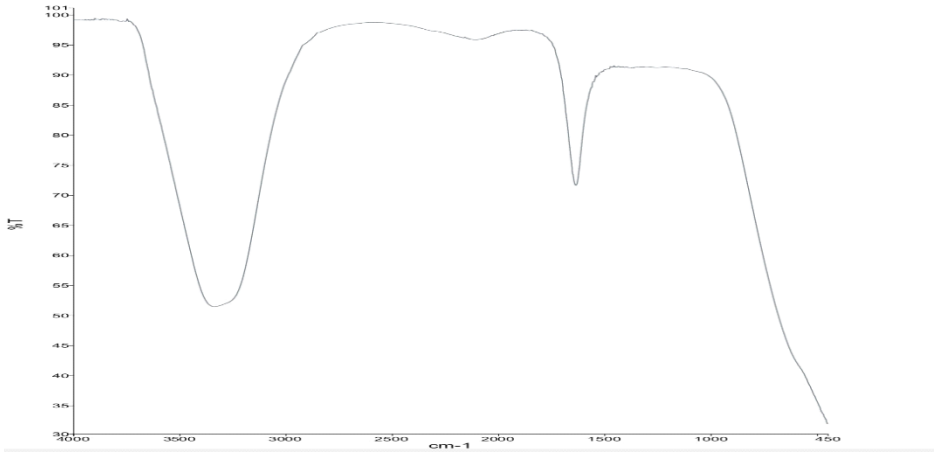

Figure 5: FTIR analysis of Zinc Oxide nanoparticles

Ramesh *et al.,* (2015) identified C–H bending vibrations (1450 cm^{-1}) in ZnO nanoparticles synthesized using *Azadirachta indica*, confirming organic molecule interactions. Zn–O stretching vibrations (450–500 cm^{-1}) were consistently reported by Goyal *et al.,* (2021) in ZnO nanoparticles synthesized using *Piper betle* extract, validating the formation of stable ZnO nanoparticles.

Dynamic Light Scattering (DLS) Analysis:

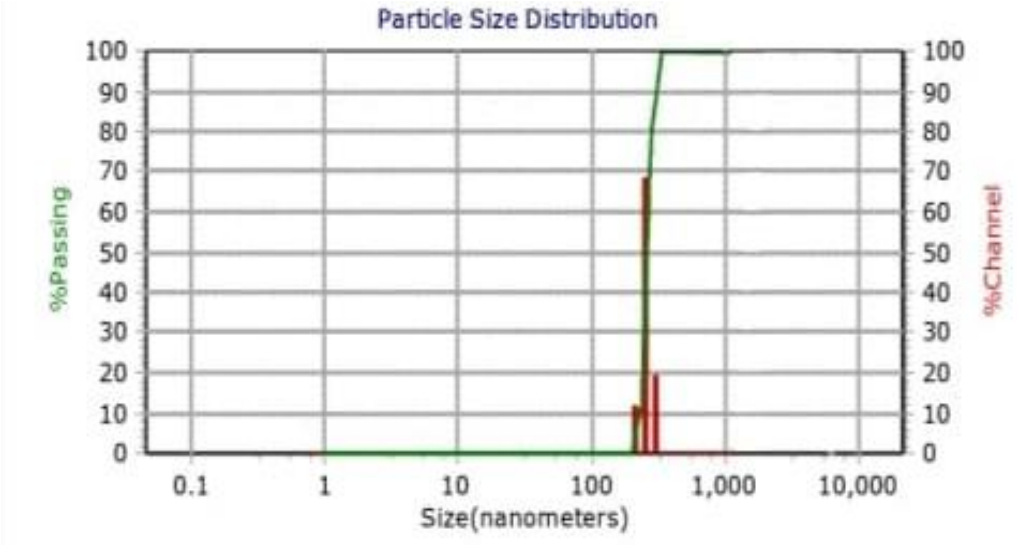

Figure 6: DLS of Zinc Oxide nanoparticles

Dynamic Light Scattering (DLS) measures the size distribution of nanoparticles in liquid. A laser beam interacts with particles undergoing Brownian motion, causing fluctuations in scattered light intensity. DLS provides rapid, non-invasive analysis, offering parameters like average particle size, size

distribution, and polydispersity index (PDI). Nanoparticles range from **100–500 nm,** showing uniformity and suitability for biological applications.

Hoo *et al.,* (2008) compared DLS with atomic force microscopy (AFM) and found DLS to be effective for analyzing monodisperse nanoparticles, though slightly skewed toward larger particle sizes. Their study also validated the use of DLS for determining the polydispersity index (PDI), a critical parameter for assessing nanoparticle uniformity.

X-Ray Diffraction (XRD) Analysis:

X-ray diffraction (XRD) is used to analyze the structure and purity of crystalline materials. When X-rays interact with a sample, they scatter, forming a peak pattern that reveals atomic planes and helps identify the crystal phases using reference data like JCPDS cards. Sharp peaks indicate high crystallinity, while broad ones suggest smaller particles or disorder. Using the Scherrer equation, crystallite size can be calculated. ZnO nanoparticles, peaks at typical positions (31.7°, 34.4°, 36.2°.) confirm a hexagonal structure. The absence of extra peaks suggests purity, while clean signals and low background noise validate quality.

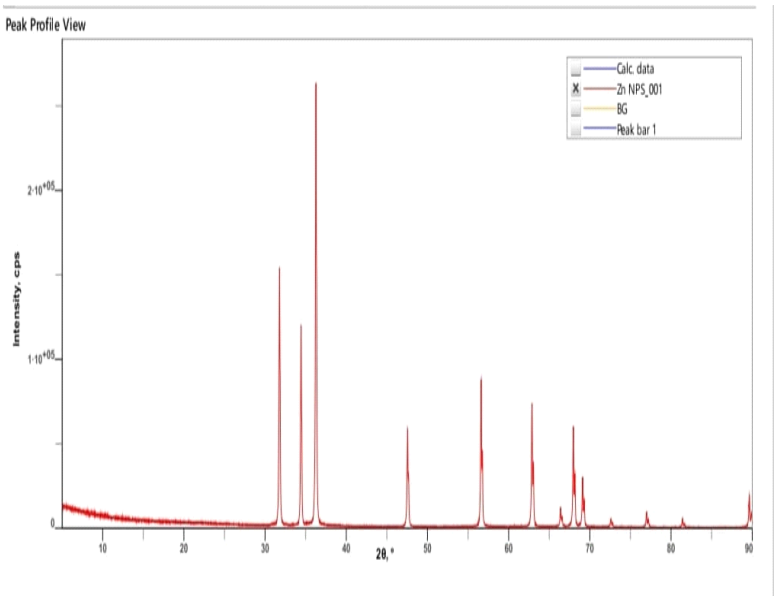

Figure 7: XRD of Zinc Oxide nanoparticles

Antioxidant Activity:

DPPH Scavenging Activity

The DPPH assay evaluates free radical scavenging ability of plant extracts, nanoparticles, and bioactive compounds. DPPH, a stable violet-colored free radical (absorbance at 517 nm), reduces to yellow upon interaction with antioxidants. The reduction, proportional to antioxidant activity, is measured via UV-Visible spectrophotometer. DPPH is a stable free radical characterized by a deep violet color in solution, with a maximum absorbance at 517 nm. The X-ray diffraction (XRD) analysis of ZnO nanoparticles, showing sharp peaks at typical positions (~31.7°, 34.4°, 36.2°, etc.), aligns with findings from other studies. Alange (2021) reported similar sharp peaks in ZnO nanoparticles synthesized via sol-gel auto-combustion, confirming their hexagonal wurtzite structure and high crystallinity. Christobel and Mahadevan (2015) also observed distinct peaks at these positions in ZnO

nanoparticles prepared by a solvothermal process, validating their structural integrity and purity.

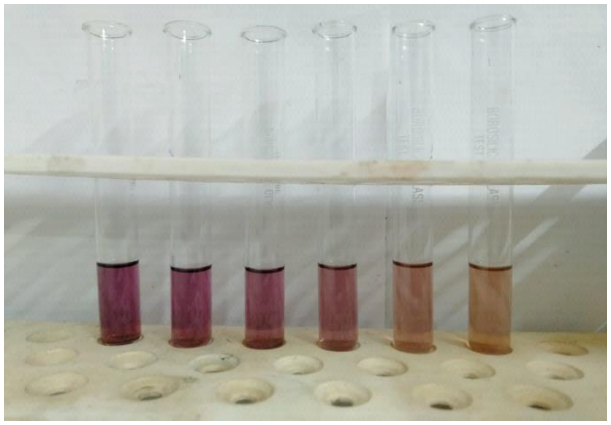

Figure 8: Antioxidant activity DPPH Assay

Antibacterial Activity:

The antibacterial activity of synthesized samples was tested using the agar well diffusion method with *E. coli* (Gram-negative) and *S. aureus* (Gram-positive) as test organisms. Bacterial cultures were grown overnight in nutrient broth at 37°C, and the inoculum was adjusted to CFU/mL. Sterile nutrient agar plates were swabbed with bacterial suspensions, and 3 mm wells were punched. Test samples (25–100 µg/mL) and gentamycin (positive control) were loaded into wells. Plates were incubated at 37°C for 24 hours, and zones of inhibition (ZOI) were measured in millimeters to assess antibacterial effects. Larger ZOI diameters indicated stronger activity.

Figure 9: *Staphylococcus aureus* (Gram-positive) & *Escherichia-coli* (gram-negative)

Table 1: Antibacterial activity

Micro organisms	Zone of Inhibition (mm)				
	Positive control (c)	25µl	50µl	75µl	100µl
S. aureus	20	9	11	15	18
E.coli	23	7	9	11	13

Mudenda *et al.* (2023) demonstrated the antibacterial efficacy of honey against *E. coli* and *S. aureus* using the agar well diffusion method, showing dose-dependent zones of inhibition. Ansari *et al.,* (2021) it highlights the effectiveness of plant extracts in inhibiting bacterial growth, with larger ZOI diameters correlating to higher concentrations of the test samples. Chaudhary (2023) also reported the utility of the agar well diffusion method for evaluating antimicrobial activity, its reliability in assessing the inhibitory effects of natural and synthetic compounds.

Conclusion:

In the present study, zinc oxide (ZnO) nanoparticles were synthesized via an eco-friendly approach utilizing *Zanthoxylum armatum* seed extract as a natural reducing and capping agent. This green synthesis method offered a sustainable, cost-effective, and non-toxic alternative to conventional chemical and physical methods. The successful synthesis of ZnO nanoparticles was confirmed through comprehensive characterization techniques, including UV-Vis spectroscopy, FTIR, XRD, SEM, and other relevant analyses, which revealed the nanoparticles' nanoscale size, high crystallinity, and the presence of bioactive functional groups from the plant extract.

The biologically synthesized ZnO nanoparticles exhibited remarkable antioxidant activity, as evidenced by their effective free radical scavenging potential in standard assays. Additionally, the nanoparticles demonstrated significant antibacterial activity against both Gram-positive and Gram-negative bacterial strains, suggesting their promising application in antimicrobial formulations. These biological activities are attributed to the synergistic effects of the intrinsic properties of ZnO nanoparticles and the phytochemicals associated with the *Z. armatum* extract.

The outcomes of this research validate the potential of *Zanthoxylum armatum* seeds as a valuable bio-resource for the sustainable synthesis of functional nanomaterials. This study contributes to the growing field of green nanotechnology and highlights the possibility of integrating plant-based synthesis routes for biomedical and environmental applications. Scaling up the synthesis process, evaluating the biocompatibility and cytotoxicity through in vivo models, and exploring applications such as wound healing, drug delivery would enhance the practical utility of the synthesized ZnO nanoparticles.

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