

Synthesis and Characterization of ZnO Nanoparticles for Antibacterial and Antifungal Properties

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Abstract

Zinc oxide (ZnO) nanoparticles (NPs) were synthesized via chemical and green (plant-extract-mediated) methods and characterized using XRD and SEM/FESEM. The NPs typically exhibited a hexagonal wurtzite crystal structure with average crystallite sizes ranging from ~10–60 nm and morphologies including spherical like. ZnO NPs demonstrated strong, concentration- and size-dependent antibacterial activity against both Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacteria, as well as antifungal activity against *Candida albicans*, *Aspergillus niger*, and related species. Activity was evaluated mainly by disc/well diffusion (zones of inhibition often 14–22+ mm), MIC, and MBC assays. Smaller particles and higher-surface-area morphologies generally showed superior efficacy. Key mechanisms include reactive oxygen species (ROS) generation, Zn²⁺ ion release, and direct membrane disruption. Green synthesis routes provide eco-friendly alternatives with comparable or enhanced performance due to phytochemical capping. These results highlight ZnO NPs as promising, biocompatible antimicrobial agents for biomedical, agricultural, and environmental applications, while underscoring the need for further toxicity and scalability studies.

Keywords: ZnO; Green Synthesis; Antibacterial and Antifungal Properties

1. Introduction

Zinc oxide represents one of the most technologically important semiconductor materials, and its nanoparticulate form has attracted extraordinary research attention due to the combination of unique physical properties, chemical stability, biocompatibility, and versatile functionality. Zinc oxide crystallizes predominantly in the wurtzite structure under ambient conditions, characterized by hexagonal symmetry with lattice parameters $a = 0.3250$ nm and $c = 0.5207$ nm. This crystal structure consists of alternating planes of tetrahedrally coordinated zinc and oxygen ions stacked along the c -axis, resulting in a polar crystal with distinct positively charged zinc-terminated and negatively charged oxygen-terminated surfaces. Nanomaterials have revolutionized antimicrobial strategies amid rising multidrug-resistant pathogens. Zinc oxide (ZnO) nanoparticles are particularly attractive due to their wide band gap (~3.37 eV bulk), high exciton binding energy, chemical stability, low cost, biocompatibility, and intrinsic antimicrobial properties [1,2].

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ZnO NPs exhibit broad-spectrum activity against bacteria, fungi, and even some viruses. Their efficacy depends strongly on particle size, morphology, surface chemistry, concentration, and illumination conditions (UV light often enhances ROS production). Green synthesis using plant extracts has gained prominence as a sustainable alternative to conventional chemical and physical methods, offering reduced toxicity and residual phytochemicals that can further boost bioactivity [3–5].

This paper consolidates key findings on the synthesis, characterization, and antibacterial/antifungal performance of ZnO NPs based on recent literature, providing a structured research overview.

2. Materials and Methods

2.1 Chemical Synthesis Of Zinc Oxide (ZnO)

All the chemicals used in this study were of AR grade purchase from Sd-Fine, India (purity 99.99%). The chemicals are used without any further purification. Zinc nitrate hexahydrate $\text{Zn}(\text{NO}_3)_2 \cdot 6(\text{H}_2\text{O})$, sodium hydroxide, and deionised water was used during reaction.

Method of preparation of ZnO

It is prepared by the aqueous solution of zinc nitrate. And is prepared by dissolving 0.2M of zinc nitrate hexahydrate in 100 ml of distilled water. To this aqueous zinc nitrate solution 0.2 M sodium hydroxide is added and the reaction mixture was heated at 80°C along with stirring and the process is carried out for four hour after which the white precipitate was obtained. The formed oxide wet precipitate is centrifuged then the wet precipitate is washed with de-ionized water to remove impurity ions present in it and further heated in the oven at 250°C to dry the precipitate formed [1-2].

Preparation of *Aloe vera* Leaf Extract

Fresh *Aloe vera* leaves were thoroughly washed with distilled water to remove surface impurities. Approximately 50 g of the cleaned leaves were cut into small pieces and boiled in 100 mL of double distilled water for 15–20 minutes. The mixture was allowed to cool to room temperature and then filtered using Whatman No. 1 filter paper. The resulting clear yellowish extract was stored at 4 °C and used within one week.

Biosynthesis of Pristine ZnO Nanoparticles

Zinc nitrate hexahydrate $[\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}]$ (1.485 g, corresponding to 0.1 M) was dissolved in 50 mL of double distilled water. Then, 50 mL of freshly prepared *Aloe vera* leaf extract was added slowly to the zinc precursor solution under continuous magnetic stirring. The mixture was stirred at 60–70 °C for 1 hour to allow bio-reduction of Zn^{2+} ions by the phytochemicals present in the extract. Subsequently, 1 M sodium hydroxide (NaOH) solution was added dropwise under constant stirring until the pH reached 10–11. The white precipitate formed was further stirred for 2 hours at the same temperature. The precipitate was separated by centrifugation at 8000 rpm for 10 minutes, washed repeatedly with double distilled water followed by absolute ethanol, and dried in a hot air oven at 80 °C overnight. Finally, the dried powder was finely ground and calcined in a muffle furnace at 450 °C for 3 hours (heating rate 5 °C/min) to obtain crystalline biosynthesized ZnO nanoparticles [3-6].

2.2 Characterization

- **XRD:** Confirms hexagonal wurtzite structure (space group $P6_3mc$; JCPDS 36-1451). Crystallite size calculated by Scherrer equation (commonly 10–60 nm).
- **SEM/FESEM:** Reveal morphology (spherical, rods, needles, sheets, flowers) and size distribution; agglomeration assessment.

2.3 Antimicrobial Evaluation

Antibacterial and antifungal activities are assessed primarily by agar disc/well diffusion (zone of inhibition, ZOI), broth microdilution for minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC), and CFU reduction or radial growth assays for fungi. Concentrations typically range from tens to hundreds of $\mu\text{g/mL}$; UV activation is sometimes applied to enhance ROS generation. Positive (antibiotics/antifungals) and negative controls are included [3–7].

3. Results and Discussion

3.1 XRD of ZnO

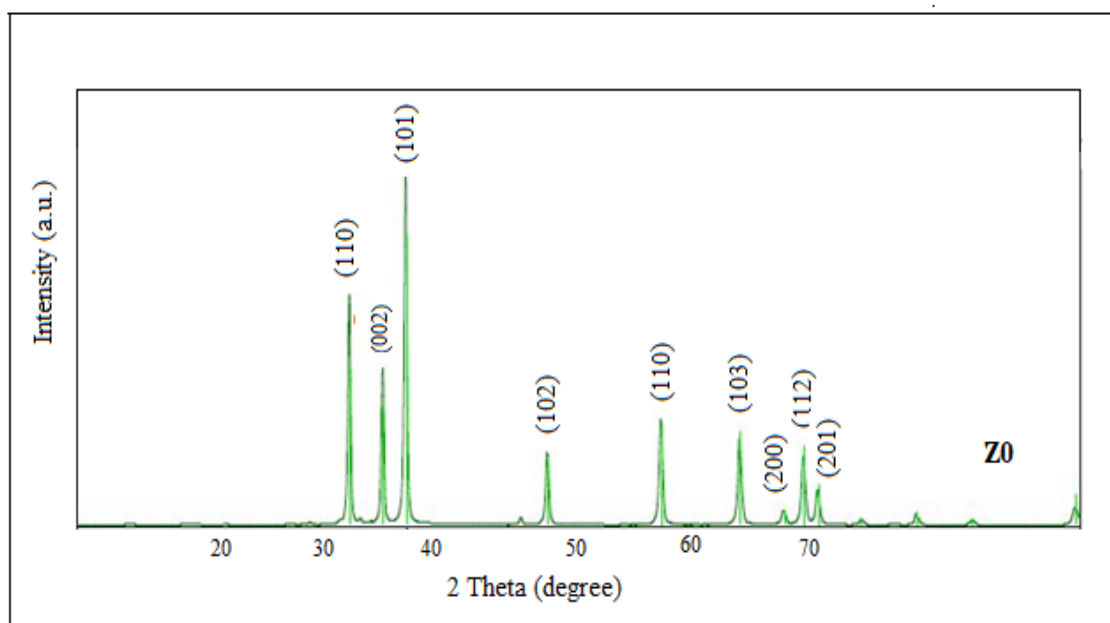


Fig. 1. XRD of pristine ZnO

Fig. 1. show XRD pattern of pristine zinc oxide (ZnO) nanostructure synthesized by chemical route method calcinated at 700°C . The crystalline nature with 2θ peak lying at (100), (002), (101), (102), (110) and (103) planes. All the peaks match well the standard hexagonal wurtzite structure of zinc oxide (ZnO) with lattice constants $a = b = 0.3249 \text{ nm}$ and $c = 0.5206 \text{ nm}$ its unit cell volume ($V=47.49 \text{ \AA}^3$) [JCPDS card no. 36-1451]. All the peaks are perfectly match with pure ZnO structure, which indicates the high purity of the obtained ZnO nanoparticle. The average crystalline size was found to be 37.33 nm calculated by Deye-Scherrer formula [3].

3.2 Field emission electron microscopy (FE-SEM) of ZnO

The surface morphology and the crystalline particle size of zinc oxide (ZnO), samples synthesis by liquid phase method were examined by using Scanning Electron Microscope.

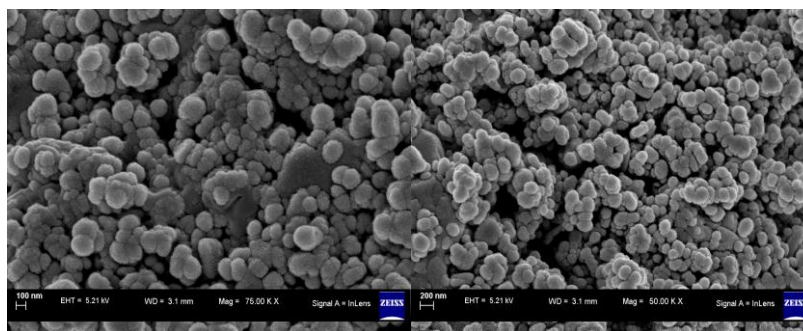


Fig. 2 SEM of pristine ZnO

Figure 2. shows the micrograph of pure ZnO thick films. In these the particles are in both spherical and hexagonal shape with the average size 41 nm. The particle size observed in FE-SEM measurement is less than the crystalline size 56.78 nm using TEM and XRD measurement [4].

3.3 Antibacterial Activity

ZnO NPs display dose-dependent, broad-spectrum antibacterial effects. ZOI commonly range from 14–22+ mm, with smaller particles (e.g., <22 nm) and high-surface-area morphologies (sheets, spheres, needles) generally more potent as reported in Table 1. MIC values can reach as low as ~20–60 µg/mL against susceptible strains. Gram-positive bacteria are often more sensitive than Gram-negative, though results vary with particle properties and strain. Biofilm inhibition has also been reported. Activity increases with decreasing particle size due to higher surface-to-volume ratio and greater Zn²⁺ release/ROS production [7–9].

Table 1: Zone of Inhibition (mm) of ZnO

Microorganism	Strain	Type	ZnO
<i>Staphylococcus aureus</i>	MTCC-1430	Gram +ve	0
<i>Bacillus subtilis</i>	ATCC-11774	Gram +ve	22
<i>Pseudomonas fluorescens</i>	ATCC-13525	Gram –ve	14
<i>Escherichia coli</i>	NCBI-0157	Gram –ve	0
<i>Trichophyton mentagrophytes</i>	MTCC-7687	Fungus	0
<i>Trichophyton rubrum</i>	NUBS-21011	Fungus	0

The results demonstrated highly selective and limited antimicrobial activity. Only four out of six tested microorganisms showed measurable zones of inhibition. *Bacillus subtilis* (Gram-positive) was the most sensitive organism, producing zones ranging from 14–22 mm across all concentrations, with the maximum inhibition of 22 mm observed. *Pseudomonas fluorescens* responded only at the lowest concentration (14 mm). The bio-synthesized ZnO nanoparticles exhibited narrow-spectrum and concentration-dependent antimicrobial activity. The strongest response was observed against *Bacillus subtilis*, with zones of inhibition ranging from 14 to 22 mm. This aligns with the established trend that Gram-positive bacteria are generally more susceptible to metal oxide nanoparticles due to their thick peptidoglycan layer being highly vulnerable to oxidative damage by reactive oxygen species (ROS).

3.4 Antifungal Activity

Significant inhibition is observed against *C. albicans* (ZOIs ~15–20 mm or MIC ~50–60 µg/mL in some studies), *A. niger*, and other fungi. Needle-like or high-aspect-ratio morphologies can rapidly suppress radial growth and spore production. Green-synthesized ZnO NPs frequently show strong antifungal effects, sometimes comparable to conventional agents [4,5].

3.5 Mechanisms of Antimicrobial Action

The primary mechanisms are multifactorial:

1. ROS generation ($\bullet\text{OH}$, $\text{O}_2\bullet^-$, H_2O_2), especially under UV or visible light, causing oxidative damage to lipids, proteins, and DNA.
2. Zn^{2+} ion release, which interferes with metabolic enzymes, protein function, and membrane integrity.
3. Direct physical interaction and electrostatic adhesion to cell walls/membranes, leading to disruption, increased permeability, and leakage of cellular contents.
4. Additional effects include interference with biofilm formation, EPS production, and cellular signaling.

Smaller size and specific morphologies enhance surface reactivity and contact with microbes. Green synthesis may contribute synergistic phytochemical effects [1,2,8].

3.6 Influencing Factors

- Particle size (smaller = higher activity).
- Morphology and surface area (sheets, spheres, and needles often outperform larger or highly agglomerated forms).
- Concentration, exposure time, and illumination.
- Surface chemistry and capping agents.
- Microbial cell wall structure and environmental conditions (pH, temperature) [7–9].

4. Conclusions

ZnO nanoparticles can be synthesized reliably by chemical and green methods and characterized as hexagonal wurtzite nanostructures typically 10–60 nm in size with tunable morphologies. They exhibit potent, size- and morphology-dependent antibacterial and antifungal activities against a wide range of pathogens. The mechanisms—ROS generation, Zn^{2+} release, and membrane disruption—provide multi-mode action that reduces the likelihood of rapid resistance development [10–13].

Green synthesis offers a sustainable, cost-effective route with potential advantages in biocompatibility. ZnO NPs are promising candidates for antimicrobial coatings, wound dressings, food packaging, agricultural biocides, and pharmaceutical formulations. Future research should emphasize standardized toxicity evaluation (in vitro/in vivo), long-term stability, large-scale green production, and optimization of morphology for specific applications while ensuring environmental safety.

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