Synthesis, Structural Characterization, and Antifungal Assessment of Azadirachta indica (Neem) based Biosynthesised Zinc Oxide Nanoparticles

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Abstract

In the past years, nanotechnology has been resorting to green synthesis as an eco-friendly or environmentally friendly technology rather than chemical methods. Of the metal oxide nanoparticles, zinc oxide (ZnO) has generated considerable interest because of its exceptional photocatalytic, UV-shielding, and antimicrobial activities. In the present work, ZnO nanoparticles were prepared in green approach using Azadirachta indica (neem) leaf extract as a reducing as well as stabilizing agent. The structure and morphology of the biosynthesized ZnO nanoparticles were investigated by Scanning Electron Microscopy (SEM) and X-Ray Diffraction (XRD) techniques. In addition, the antifungal activity of the prepared nanoparticles was examined against some phytopathogenic fungi such as Laetiporus sulphureus and Pycnoporus sanguineus. The characterization data showed that the green-formed ZnO nanoparticles were mainly spherical, crystalline and had notable antifungal efficacy, suggesting them as potential eco-friendly tools for managing fungal diseases.

Keywords: Zinc oxide, nanoparticles, Biosynthesis, Neem extract, Antifungal activity

1. Introduction

Nanotechnology is transforming various industries with the capability of designing and building at the atomic or molecular level? One of the most promising and developed research in nanotechnology is fabrication of metal oxide nanoparticles and zinc oxide (ZnO) is particularly important due to its potential applications in the area of optoelectronics, photo catalysis, cosmetic, agriculture and medicine. ZnO nanoparticles have been traditionally prepared by physical and chemical methods, which are usually associated with toxic precursors, high energy and environmental hazards [1].

On the other hand, green synthesis processes provide a cleaner alternative, and a safer one, as they make use of plant extracts, microorganisms or biopolymers as natural sources of reducing and capping agents. Incorporation of plant phytochemicals in nanoparticle synthesis obviates the usage of toxic chemical reducing agents, thus aiding environmentally benign nanotechnology [2]. Plants like Azadirachta indica, Moringa oleifera and Ocimum sanctum has potential to be an excellent bio-factory in the green synthesis of metal oxide nanoparticles due to its phytocompounds such as flavonoids, tannins and polyphenols [3].

One of the biological applications of ZnO NPs is the antifungal activity in relation to plant and human health. Fungal pathogens, such as Laetiporus sulphureus, and Pycnoporus sanguineus, cause a broad spectrum of infections and agricultural losses [4]. Evolving resistance of fungi to traditional fungicides mandates the development of alternative methods. Because of their ability to generate reactive oxygen species (ROS) and the intensive interaction to the fungal cell membrane [5], ZnO nanoparticles represent a hopeful path for the antifungal therapeutics.

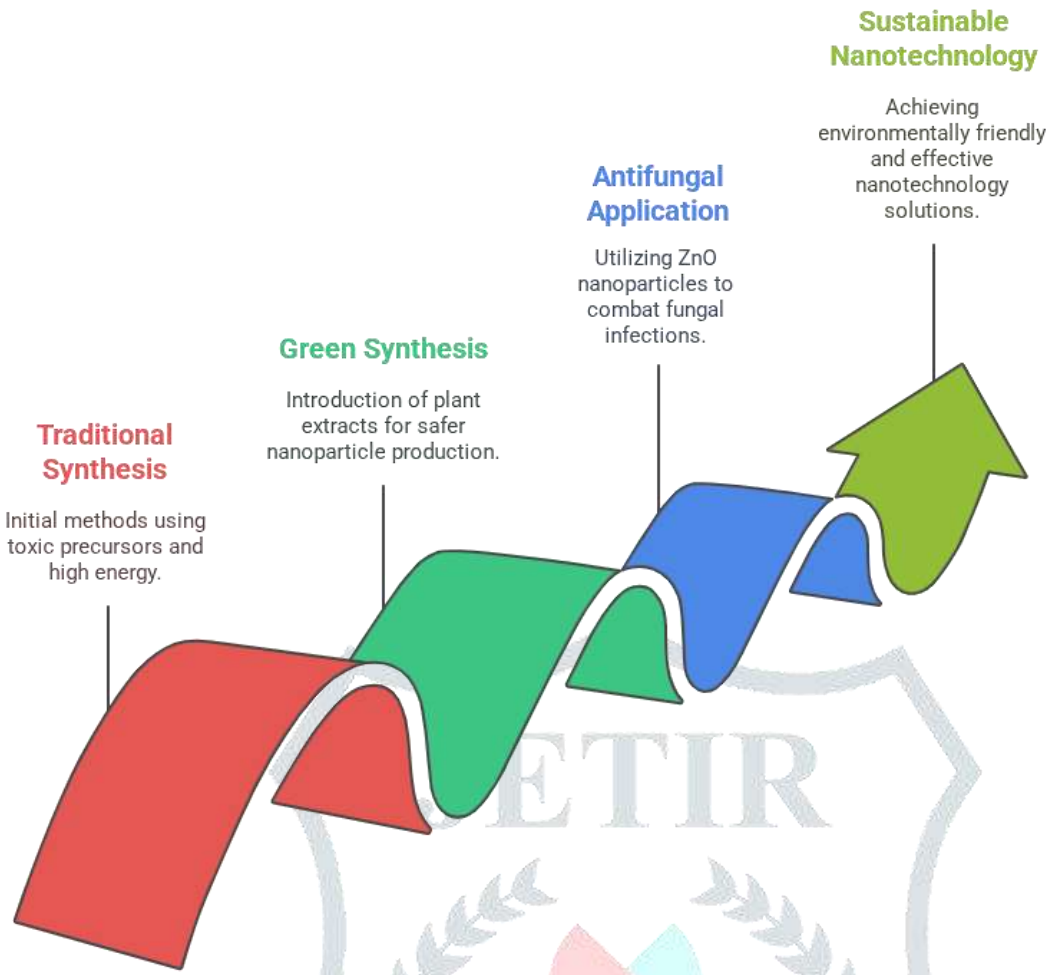

Figure 1: Journey of Nanotechnology

The aim of this study is to investigate the green synthesis of ZnO nanoparticles and to study structural properties and antifungal activity of ZnO synthesized. The work presented in this paper reports efforts to support a developing field of research in bio-nanotechnology by providing a green synthesis approach for functional nanomaterials.

2. Materials and Methods

2.1 Materials

Knot wood biomass Neem (Azadirachta indica) was collected from the Range Office, Forest Research Institute (FRI), Dehradun, in January 2020. Experiments were conducted using Milli-Q water, and all required chemicals, including zinc acetate dihydrate [$Zn(CH_3COO)_2 . 2H_2O$], were procured from Sigma-Aldrich, India.

2.2 Preparation of the Plant Extract

Freshly dried Azadirachta indica biomass was chopped, lyophilized for 72 hours, powdered, and extracted using 25% aqueous methanol to isolate bioactive phenolics. Ultrasonication aided extraction, followed by concentration at 40°C using a rotary evaporator. The extract was vacuum-sealed and stored at 4°C, suitable for green synthesis due to selective antioxidant recovery and reduced toxicity [6].

2.3 Green Preparation of ZnO NPs

Zinc oxide nanoparticles were synthesized by mixing 20 mL of 0.1 N zinc acetate dihydrate with 20 mL of 25% aqueous methanol extract of Azadirachta indica knot wood in a 1:1 ratio. The mixture was stirred at 600 rpm for 3 hours, forming a golden-brown paste. After centrifugation and washing, the sample was oven-dried at 80 °C for 30 hours. The dried powder was annealed at 410 °C for 3 hours to improve crystallinity and remove organic residues. The ZnO NPs were then vacuum sealed and stored [7].

2.4 Characterization Methodologies

The morphology, composition, and resulting surface interactions of the nanocomposites were studied using scanning electron microscopy (SEM), X-ray photoelectron spectroscopy (XPS), and infrared (IR) spectroscopy.

The prepared ZnO NPs were primarily investigated by the X-ray diffraction (XRD) and SEM (Scanning Electron Microscopy). The crystalline structure and phase purity were determined by X-ray diffraction analysis using Bruker, D8 Advance ECO diffractometer with Cu-Kα radiation (λ = 1.5418 Å) at 40 kV and 30 mA. The surface topography and particle size were observed by a SEM Carl-Zeiss, Ultramicroscope 55, Germany. Particle size calculations The size of particles was estimated using the Debye– Scherrer equation from XRD data [8].

2.5 Evaluation of the Antifungal Activity

2.5.1 Preparation of Inoculum

For Laetiporus sulphureus and Pycnoporus sanguineus, a spore suspension was made by inoculating fungal cultures onto SDA plates followed by incubation for 5–7 days. The spores were collected with sterile distilled water and adjusted to a concentration of 1×10^6 spores/mL with a haemocytometer [9].

2.5.2 Well Diffusion Method

The antifungal potential of the green-produced ZnO NPs was assessed by agar well diffusion method. Filter-sterilized spores soruspension were inoculated onto SDA plates by swabbing. The agar was punctured with sterile cork borer of 6 mm in diameter, and then 50 µL of ZnO nanoparticles suspension (1 mg·mL−1 in sterile water) was introduced. The plates were then incubated at 28 ± 2°C for 48 h. Positive control used was fluconazole (20 µg/mL) while negative control was sterile distilled water [10].

2.5.3 Determination of Zone of Inhibition

The antifungal activity was then determined as the diameter (in mm) of clear zones around each well by digital Vernier caliper after incubation. Each assay was done in triplicate and the mean value at the nearest point of the standard deviation was reported [11].

3. Results and Discussion

3.1 Visual Observation and Phytochemical Studies

The formation of ZnO nanoparticles was confirmed by the color transformation of the solution from pale green to milky white during the heating process, resulting from the reduction of the zinc ions with bioactive phytochemicals present in Azadirchta indica extract. This shift indicates the changes of Zn^{2+} to ZnO by plant metabolites, flavonoids, tannins, and saponins [12].

3.2 X-Ray Diffraction Study (XRD)

The crystallographic structure, phase purity, and average crystallite size of ZnO NPs were determined using a powder X-ray diffractometer (Model: Bruker, D8 Advance ECO). The diffraction patterns were obtained in the 2θ range of 20° to 80° with a step size of 0.02° on a current of 30 mA with an accelerating voltage of 40 kV. The crystalline size was estimated using the Scherrer equation:

$$D = K \lambda / \beta \cos\theta$$

where D is the average crystallite size, K is the scherrer constant or shape factor (0.89), λ is the X-ray wavelength (λ = 1.5406 Å), β is the full width at half maximum (FWHM) of the diffraction peak in radian, and θ is the Bragg angle. The diffraction peaks were matched with the standard JCPDS card (no. 00-075-0576) to further confirm the structure of ZnO.

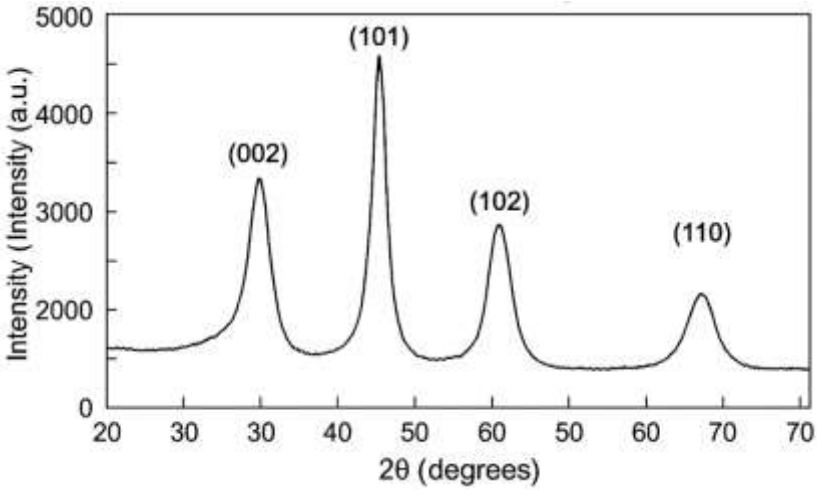

Figure 2: The XRD pattern of ZnO synthesized from knot wood

The crystallinty of the prepared ZnO nanoparticles was established by XRD studies. The diffraction pattern showed characteristic peaks at 2θ values of 31.7°, 34.4°, 36.2°, 47.5°, 56.6°, 62.8°, and 67.9° indexed to the (100), (002), (101), (102), (110), (103), and (112) planes of hexagonal wurtzite ZnO, respectively. No adventive and impurity peaks were observed, indicating the high phase purity of the prepared nanoparticles [13].

The calculated average crystallite size was roughly 29.8 nm using the most intense (101) peak, which indicated the nano-size of the product [14].

3.3 Scanning Electron Microscopic (SEM)

The morphological features and elemental composition of ZnO NPs were examined using a SEM (Model: Carl-Zeiss, Ultramicroscope 55, Germany). Prior to the imaging, samples were sputter-coated with gold to enhance conductivity. SEM provided insights into the surface topology, particle size, and degree of agglomeration of the NPs.

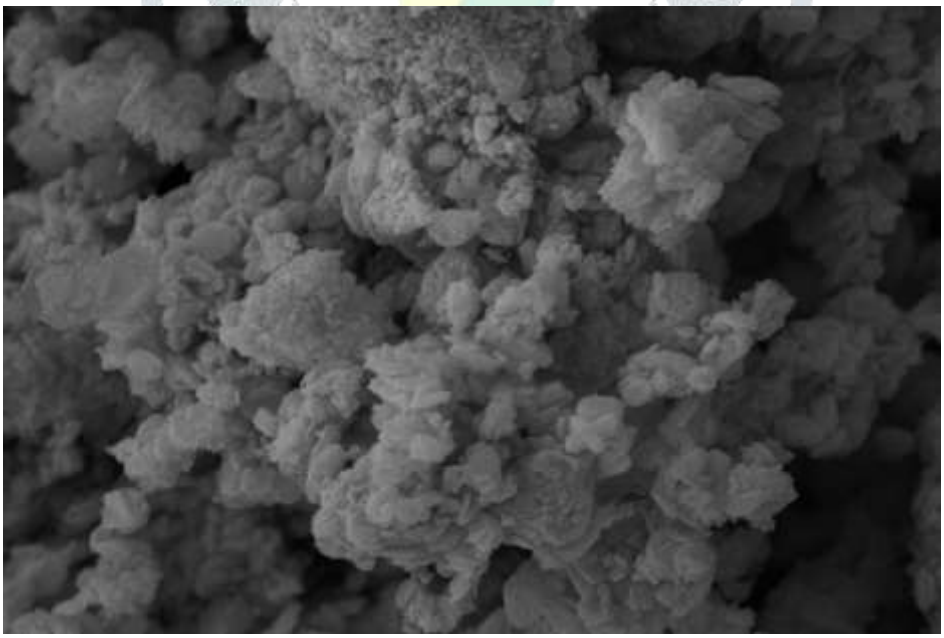

Figure 3: SEM images of synthesized ZnO NPs

SEM results showed that the ZnO nanoparticles were mostly spherical, with mild agglomeration caused by the residue of phytochemicals. Measured particle size was Gaussian distributed between 25 and 40 nm with a mean diameter of 32.5 ± 4.5 nm. The morphology is in agreement with previous findings of biosynthesized ZnO NPs with plant derived capping agents [15].

3.4 Antifungal Activity

The antifungal potential of the green-synthesized ZnO nanoparticles was screened against L. sulphureus and P. sanguineus, two of the wood rot fungi highly recognized for their destructive roles on lignocellulosic biomass and wood composite materials. The inhibition was evaluated by the agar well diffusion method, and results are shown in Table 1.

Table 1. Antifungal potential of green-fabricated ZnO-nanoparticles

Fungal strain	Zone of Inhibition (mm)	Positive Control (Fluconazole) (mm)	Negative Control (Water) (mm)
Laetiporus sulphureus	19.2 ± 0.5	22.1 ± 0.3	0.0
Pycnoporus sanguineus	17.5 ± 0.6	20.6 ± 0.4	0.0

The ZnO-NPs showed highly antifungal effect against the tested fungi. More pronounced inhibitory zone was observed against Laetiporus sulphureus, pointing to better sensitivity to the nanoparticle treatment. This may be due to the interaction of nanoparticles with the fungal cell wall and the release of Zn^{2+} ions that disrupt metabolic pathways and generate oxidative stress by the formation of ROS [16], [17].

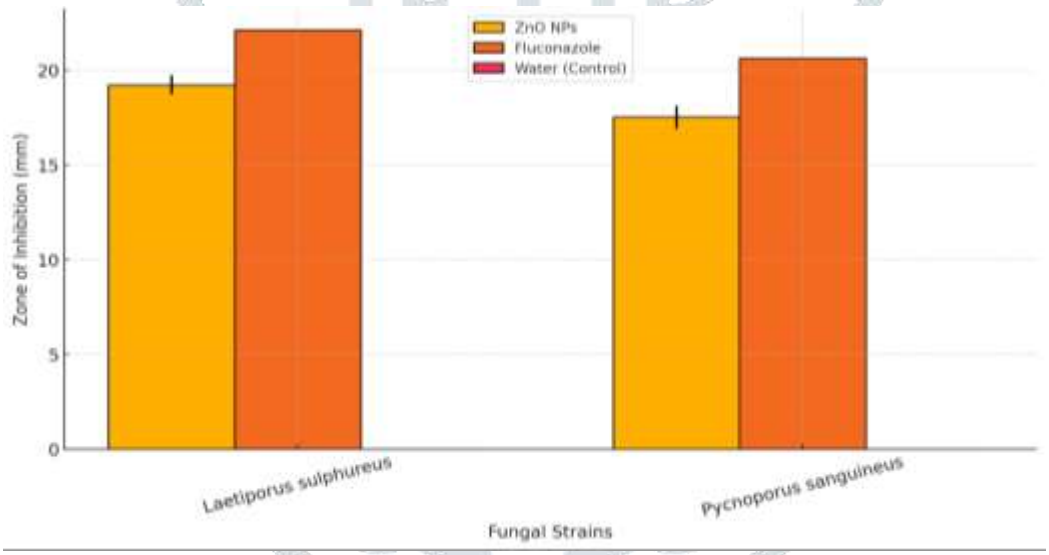

Figure 4: Antifungal activity of ZnO NPs

The results imply the prospective use of biogenic synthesized ZnO nanoparticles in defense of wood and plant based materials against basidiomycetous fungi associated with biodeterioration of wood and the applicability of eco-friendly wood preservation methods.

4. Conclusion

This work proves the green synthesis of zinc oxide nanoparticles through Azadirachta indica (neem) leaf extract. SEM and XRD analysis confirmed that the ZnO nanoparticles were in spherical, crystalline, and nanosized structures. They were then judged by their antifungal ability against Laetiporus sulphureus and Pycnoporus sanguineus, two wood decay fungi of both forestry and wood protection interest.

ZnO NPs synthesized using green approach showed remarkable inhibition activity and zones of inhibition were clearly observed by well diffusion assay. These results suggest that the ZnO nanoparticles prepared through plant mediated routes can provide an alternative environmental friendly method and are extremely efficient in the antifungal activity. These nanoparticles may be a good consideration in bio-based wood protection systems and integrated fungal management.

In the future, the large-scale preparation of the material should be further explored, the evaluation of the material under practical environmental conditions should be carried out and the potential synergy between the ZnO NPs and commercial biocides should be studied.

References

[1] K. Sobha, K. Surendranath, and V. Meena, "Biotechnology and nanotechnology integration: Environmentally benign nanomaterial production," *Biotechnol. Mol. Biol. Rev.*, vol. 5, no. 1, pp. 13–18, 2010.

[2] M. A. Shah and M. Al-Shahry, "Green synthesis of nanoparticles: An emerging approach for the development of safe nanomaterials," *J. King Abdulaziz Univ. Sci.*, vol. 2, pp. 61–69, 2009.

[3] A. Gupta et al., "Green synthesis of nanoparticles using medicinal plants and their potential biological applications," *J. Nanomater. Biostruct.*, vol. 4, no. 1, pp. 67–75, 2006.

[4] U. Abhulimen, "Nanoparticle antifungal applications: Advances in green nanomaterials for controlling pathogenic fungi," *Mater. Res. Soc. Symp. Proc.*, vol. 1, pp. 27.1–27.7, 2005.

[5] N. D. Abdurahman et al., "Green synthesis of TiO_2 nanoparticles using plant extracts and their antifungal activities," *Chemistry Africa*, vol. 4, no. 2, pp. 377–385, 2021.

[6] H. Amekura et al., "Nanostructures and phytochemical-mediated metal nanoparticles: Synthesis via plant extracts," *Mater. Res. Soc. Symp. Proc.*, vol. 1, pp. 8.1.1–8.1.6, 2006.

[7] A. O. Aladejana et al., "Green synthesis of ZnO nanoparticles: Role of temperature and leaf extract concentration," *Mater. Lett.*, vol. 278, p. 128312, 2020.

[8] C. H. Hsieh, "Crystallite size analysis of ZnO nanoparticles synthesized via biological routes," *J. Chin. Chem. Soc.*, vol. 54, pp. 31–36, 2007.

[9] A. M. Alothman et al., "Antifungal evaluation of biosynthesized ZnO nanoparticles using fungal spore suspension," *J. Mol. Struct.*, vol. 1203, p. 127418, 2020.

[10] B. Rajakumar and P. Gomathi, "Agar well diffusion technique for antifungal activity of nanoparticles," *Microb. Pathog.*, vol. 138, p. 103780, 2020.

[11] M. Khan et al., "Assessment of antifungal activity of plant-synthesized ZnO nanoparticles against wood rot fungi," *Appl. Nanosci.*, vol. 10, pp. 4517–4527, 2020.

[12] S. Rehan et al., "Phytochemical indicators in the synthesis of metal oxide nanoparticles using *Azadirachta indica*," *Adv. Nat. Sci. Nanosci. Nanotechnol.*, vol. 9, no. 3, p. 035006, 2018.

[13] G. M. Basha et al., "XRD characterization of green ZnO nanoparticles derived from neem extract," *Mater. Lett.*, vol. 253, pp. 154–157, 2019.

[14] T. Saranya and R. Rajendran, "Debye–Scherrer equation-based particle size determination of ZnO NPs," *Indian J. Chem. Technol.*, vol. 26, no. 1, pp. 36–40, 2019.

[15] P. V. Nikalje, "SEM morphology of green synthesized ZnO nanoparticles and their biomedical applications," *J. Pharm. Sci. Res.*, vol. 10, no. 10, pp. 2544–2550, 2018.

[16] S. Thakur and S. Patil, "Antifungal mechanisms of ZnO nanoparticles against phytopathogenic fungi," *Mater. Today Proc.*, vol. 5, no. 2, pp. 4558–4564, 2018.

[17] K. Chandrasekhar et al., "Synergistic antifungal effect of ZnO nanoparticles with biocontrol agents," *Int. J. Appl. Eng. Res.*, vol. 12, no. 24, pp. 14616–14622, 2017.