



Research article

Antifungal activity of zinc oxide nanoparticles synthesised by a novel green method using waste biomass residue of *Eucalyptus tereticornis*

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Abstract: In this study, we introduce an eco-friendly approach to synthesizing zinc oxide nanoparticles (ZnO NPs) using *Eucalyptus tereticornis* knot wood waste, an abundant yet underutilized biomass residue from plantations and wood industries. *E. tereticornis*, valued in traditional medicine for its bioactive compounds, acts as a natural chelating and reducing agent under ambient conditions. The extraction process yielded 7.50% *E. tereticornis* extract with a total phenolic content of 530.20 mg GAE/g, leading to a 27.52% yield of ZnO NPs. Physicochemical characterization confirmed the formation of highly crystalline ZnO NPs with a wurtzite structure (~29 nm), as validated by powder X-ray diffraction (XRD). UV-Vis spectroscopy revealed a characteristic absorption peak at 369 nm, closely matching bulk ZnO (~377 nm). Further structural and compositional analysis through scanning electron microscopy (SEM), transmission electron microscopy (TEM), energy dispersive X-ray (EDX), and Fourier-transform infrared (FTIR) confirmed their purity, morphology, and functional group interactions. The ZnO NPs exhibited predominantly spherical structures ranging from 20–40 nm, in close agreement with XRD results. Moreover, antifungal studies have demonstrated the significant bioactivity of ZnO NPs against common wood-decaying fungi, *Laetiporus sulphureus* (brown rot) and *Pycnoporus sanguineus* (white rot). Mycelial growth inhibition at different ZnO NP concentrations (10, 30, and 50 ppm) was recorded at 47.14%, 72.86%, and 98.57% for *L. sulphureus*, and 41.54%, 75.38%, and 98.46% for *P. sanguineus*, respectively. This study highlights the potential of *Eucalyptus tereticornis* knot wood waste as a sustainable resource for ZnO NP synthesis, offering a cost-effective and environmentally friendly alternative for wood protection against fungal decay.

Keywords: nanoparticles; bio synthesis; zinc oxide; industrial waste; *Eucalyptus tereticornis*; knot wood extract

1. Introduction

The distinctive properties of ZnO, along with its applications in photocatalysis, pharmaceuticals, cosmetics, and photonics, have established it as one of the most widely utilized materials globally [1–6]. Moreover, it has multifunctional audio-visual, photonic, and spintronic properties that make it a highly exploited n-type semiconducting metal oxide [7–9].

Many physiochemical processes are used to synthesize ZnO NPs, including ball milling, laser vaporization, sol-gel processes, micro emulsions, and co-precipitation [10,11]. In general, these techniques are highly energy-intensive, requiring a large amount of space for equipment setup, the high cost of equipment, the use of harsh chemicals, stabilizers, and capping agents, and have many restraints that are environmentally unfriendly, which constraints their potential biological applications [12]. Thus, greener approaches are being explored to overcome the limitations of hazardous wastes generated and energy imbalances by these two major processes.

One of the major approaches to nanoparticle synthesis involves the use of non-toxic plant extracts. Due to its safe and environmentally friendly nature, it has engrossed significant scientific courtesy and provided an effective new path in synthesizing metal oxide nanoparticles [13]. This was inferred from the literature that plant extracts and biomass residues have significant potential as a green synthesis medium for creating metal nanoparticles, offering notable advantages such as easy availability, simple handling, cost-effectiveness, fast kinetics, low maintenance of cell culture, enhanced stability, controlled NP size and shape, and enabling large-scale environmental friendly biosynthesis over the conventional methods of NP synthesis [14–16]. Despite considerable research, it has not been possible to synthesize ZnO NPs using biomass residue (knot wood) extracts of plants and their application in wood preservation. Moreover, some of the studies highlighted antifungal potential of other nanoparticles, which have demonstrated promising activity against brown- and white-rot fungi, thereby enhancing the durability and resistance against biodeterioration [17,18]. While green synthesis of ZnO NPs is known, using industrial/agricultural wood waste biomass makes the process scalable, low-cost, and environmentally impactful, which will open green novel routes for producing safe and effective NP-based wood preservatives. Researchers have demonstrated the feasibility of utilizing lignocellulosic waste such as *Shorea robusta* sawdust for ZnO NP synthesis [19]; however, comparative understanding of different biomass residues remains unexplored.

Eucalyptus, commonly called Safeda, Nilgiri, has around 400 species. It belongs to the family Myrtaceae and is found in many southeastern countries. It is an evergreen tree growing to 45 m at an altitude up to 1700 m. It prefers dry moist, wet, and mildly acidic to mildly alkaline soil and can tolerate drought. Of the total *Eucalyptus* plantations around the globe, India has ~10% of it, which is second most widely planted species after teak, and it is distributed/planted all over India except in the Northeastern states [3]. Agro/farm forestry plantations contribute around 93% of the total industrial wood requirement (~70% is *Eucalyptus*) in the country [4]. In Ayurveda and traditional medicines, these plants' leaves are widely used for cold and respiratory infections because of their health-promoting properties [5]. It is the first time green reducing and capping agents have been used to synthesize ZnO NPs using *Eucalyptus tereticornis*. High amounts of natural antioxidants, mainly

flavonoids and polyphenols, which contain large numbers of hydroxyl, carboxyl, and carbonyl groups, considered suitable stabilizing agents are found in Eucalyptus knot wood [5]. It also contains biomolecules required to reduce metal ions and stabilize nanoparticles. Because of anticancer, anti-inflammatory, and antioxidant agents, these compounds can positively affect human health. Moreover, in place of expensive and hazardous chemical surfactants, it could be a good alternative due to its natural origin, abundant availability, easy collection, underutilized condition, and outstanding natural properties. Unlike our other studies utilizing *Shorea robusta* sawdust [19,20], we demonstrate here a higher phenolic-rich *E. tereticornis* knotwood extract that enables enhanced reduction efficiency and improved antifungal performance, highlighting biomass-specific synthesis behavior.

Wood deterioration is primarily caused by fungi and insects, with bacteria playing a minor role [21–23]. For decay to occur, four key factors are required: Moisture content below the fiber saturation range (typically 25–30%), a temperature range of 2 °C to 45 °C, oxygen availability (as wood-decaying fungi are aerobic), and a suitable substrate like wood to sustain fungal growth. Disrupting any of these factors halts the decay process [22–26]. Among wood-decay fungi, brown and white-rot species are the most aggressive and destructive.

In this study, we demonstrate knot wood extract of abundantly available yet underutilized biomass residue of *E. tereticornis* from plantations and wood-based industries since waste is successfully used as a natural surfactant for the novel green synthesis of ZnO NPs. To confirm the synthesized ZnO NPs, a detailed characterization was carried out. Additionally, its antifungal efficacy against most common wood decaying brown rot fungi (*Laetiporus sulphureus*) and white rot fungi (*Pycnoporus sanguineus*) for different concentrations of NPs were also performed. Altogether our findings will open new sustainable strategies to upgrade the difficult to manage biomass residues (i.e., converting underutilized biomass residues into functional nanomaterials), which would go a long way in boosting the environmental and economic profiles of these industries, not only in terms of waste valorization but also a step toward circular economy.

2. Materials and methodology

2.1. Materials

Knot wood biomass residue of *E. tereticornis* was sourced from the Range Office, Forest Research Institute (FRI), Dehradun, in February 2024. All experiments were conducted using deionized Milli-Q water (dH₂O). The necessary experimental setup was utilized, and Zinc acetate dihydrate [Zn(CH₃COO)₂·2H₂O], along with the other required chemicals, were procured from Sigma-Aldrich, India.

2.2. Preparation of extract

Recently dried biomass of *E. tereticornis* tree were chopped and flaked in small pieces, then lyophilized (Allied Frost FD 5 model) for 72 hours. The lyophilized sample was then crushed finely into powder form using an electric mixer or sawdust maker. The obtained dried knot wood powder was extracted with 25% aqueous methanol (aq. MeOH) using an electronic ultrasonicate water bath (Model: RK510 H; Brandlin, Berlin, Germany) set at 35KHz. These extracts were concentrated at 40 °C with a vacuum of 218 mbar using a rotatory evaporator (Model: Buchi R-100), and its yield (%) was determined (mentioned in Table 1). For further use, the sample extract was vacuum sealed and refrigerated at 4 °C.

The schematic flow chart used for the preparation of industrial knot wood waste extract of *E. tereticornis* tree is shown in Figure 1.

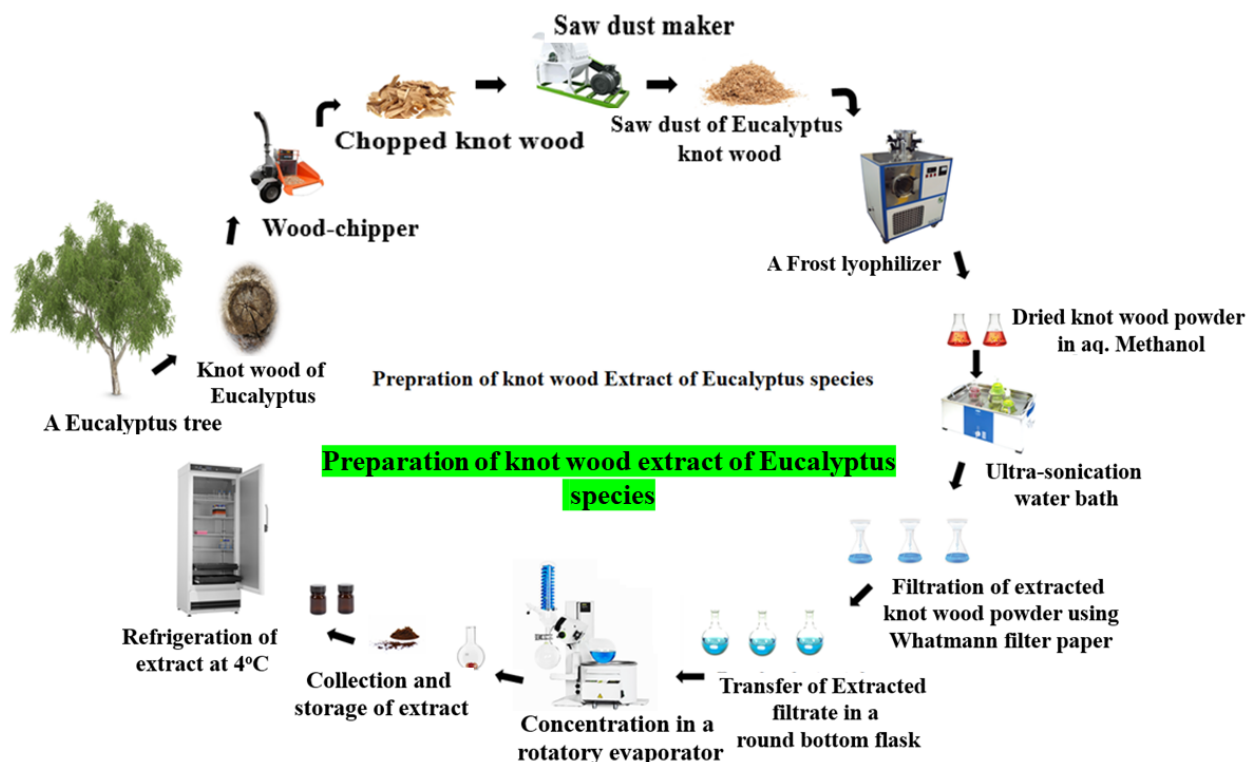


Figure 1. Schematic illustration of the extraction process from knot wood of *Eucalyptus tereticornis*.

2.3. Total phenolic contents

Phenolics are secondary plant metabolites with one or more aromatic rings or compounds having complex polymers like tannins and lignans. Flavonoids and tannins possess a high amount of chemicals and show biological properties. Folin Ciocalteu (FC) reagent determines the total phenolic content, which reacts with the phenolic compound under basic conditions. A 1:10 ratio of FC reagent with water was prepared, which was used with extract and solvent to make different concentrations. After 5 minutes, Na_2CO_3 was added and incubated for 1.5 hours at 50–60 °C. Extract-solvent was replaced with Gallic acid-water to make a standard. After taking absorbance at 740 nm, the first concentration and total phenolic contents (mg GAE/g extract) were calculated.

2.4. Novel Green synthesis of zinc oxide nanoparticles

The innovative ZnO NP synthesis involved preparing 20 mL of 0.1 N zinc acetate dihydrate $[\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}]$ in dH_2O . After heating and continuous stirring on a hot plate using a magnetic stirrer, 20 mL precursor solution (i.e., 25% aqueous methanol extracts of knot wood waste of *E. tereticornis*) was mixed, drop by drop, at an equal ratio (1:1 v/v). This was stirred continuously at 600 rpm for three hours until a golden brown paste was formed. The sample was subjected for drying in a hot air oven (at 80 °C for 30 hours) after centrifugation (at 6000 rpm for 15 minutes) and thorough

washing (twice). The resulting dried ZnO NPs powder was then annealed in a muffle furnace (at 410 °C for 3 hours) to produce highly crystalline particles. For further examination, the dried ZnO NPs were vacuum sealed and kept. A detailed schematic representation of the green synthesis of ZnO NPs is shown in Figure 2.

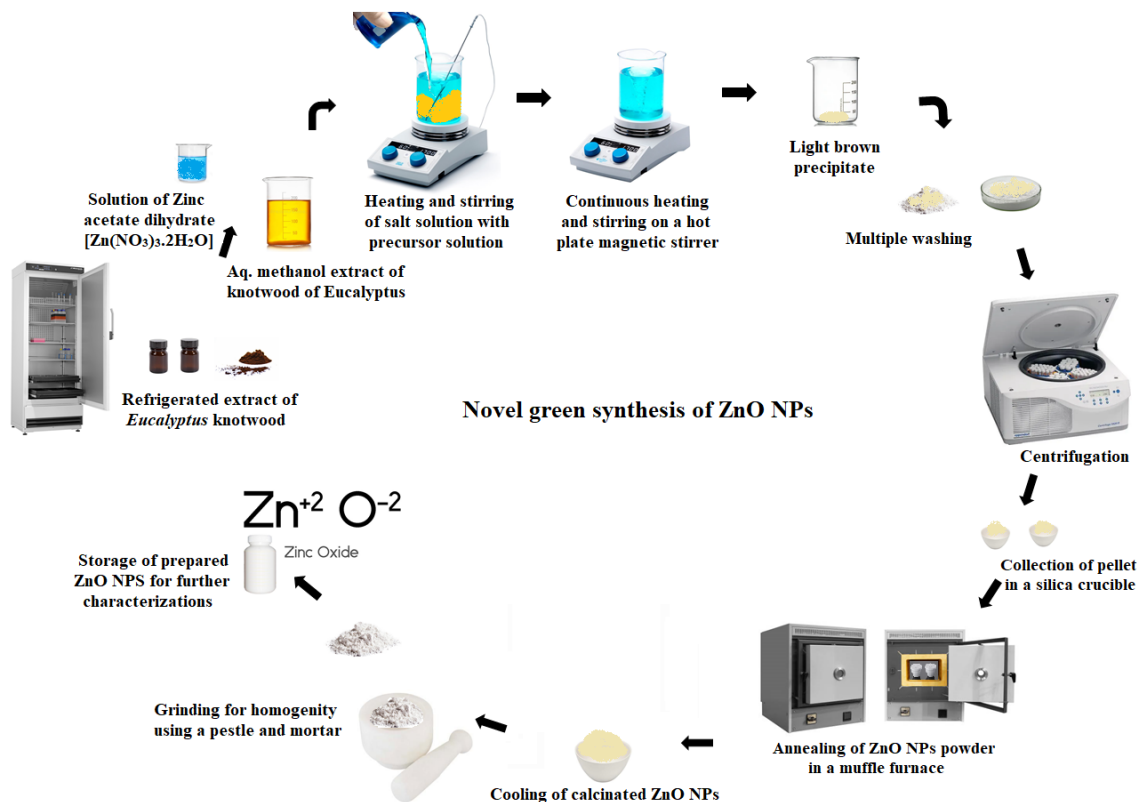


Figure 2. Schematic illustration of the novel green synthesis of ZnO NPs.

2.5. Characterization of synthesized ZnO NPs

The physicochemical properties of the green synthesized ZnO NPs were systematically analyzed using a suite of advanced characterization techniques, which provided comprehensive insights into the structural, morphological, optical, and elemental properties of the synthesized ZnO NPs. The characterization techniques included Ultra violet-visible (UV-Vis) spectroscopy, XRD analysis, FTIR spectroscopy, SEM coupled with EDX spectroscopy, and TEM. Each characterization method is detailed below:

(a) UV-Vis spectroscopy

The optical properties and bandgap estimation of the biosynthesized ZnO NPs were assessed using a UV-visible spectrophotometer (Model: Biogen, BGS-UV Plus). The powdered ZnO NPs were dispersed in deionized Milli-Q water (dH_2O) and subjected to ultrasonication to ensure uniform dispersion before measurement. The spectral absorbance was recorded at room temperature within the wavelength range of 300 to 600 nm, with a resolution of 1 nm. The characteristic absorption peak of ZnO NPs, typically observed around 360–380 nm, was analyzed to confirm the formation of nanoparticles [6,7].

(b) XRD analysis

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 ($\lambda = 1.5406 \text{ \AA}$), β is the full width at half maximum (FWHM) of the diffraction peak in radian, and θ is the Bragg angle [8,9]. The diffraction peaks were matched with the standard JCPDS card (no. 00-075-0576) to further confirm the structure of ZnO [10].

(c) FTIR spectroscopy

The identification of the functional groups and the metal-oxygen bonding interactions in the ZnO NPs was confirmed by FTIR (Model: Frontier FT-IR/FIR, Perkin Elmer) analysis in the wavenumber range of $4000\text{--}400 \text{ cm}^{-1}$ at a resolution of 4 cm^{-1} . The formation of ZnO NPs was validated by the presence of Zn–O stretching vibrations in the low-wavenumber region, consistent with published spectra of green-synthesized ZnO nanoparticles [27,31,33]. Other functional-group assignments associated with plant-derived biomolecules involved in capping and stabilization were made by comparison with published FTIR literature on plant-mediated ZnO synthesis [27,31,33].

(d) SEM and EDX spectroscopy

The morphological features and elemental composition of ZnO NPs were examined using a SEM (Model: Carl-Zeiss, Ultramicroscope 55, Germany). Prior to 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. Additionally, EDX was employed at an electron beam energy of 30 kV to analyze the purity and elemental composition of the synthesized ZnO NPs, ensuring the absence of unwanted impurities [13].

(e) TEM

To elucidate the size, shape, and structural integrity of the synthesized ZnO NPs, TEM was carried out at an accelerating voltage of 200 kV. The samples were prepared by dispersing ZnO NPs in ethanol, followed by ultrasonication and deposition onto a carbon-coated copper grid for analysis. The TEM results, in correlation with XRD findings, approved the presence of quasi-spherical ZnO NPs with a 20–40 nm of size distribution range [14].

2.6. Antifungal analysis

The antifungal efficacy of synthesized ZnO NPs (using knot wood extract of *E. tereticornis*) was evaluated *in vitro* against most common wood decaying brown rot fungi (*L. sulphurous*) and white rot fungi (*P. sanguineus*) for different concentrations of NPs by the Agar Plate Test (poison food technique), as shown in Figure 4. The complete antifungal analysis consisted of the following steps:

(a) Purification and inoculation of fungal culture on agar media

National Type Culture Collection (NTCC), Forest Research Institute, Dehradun held 1000 live fungal cultures. The NTCC procured cultures of *L. sulphurous* (NTCC 1268) and *P. sanguineus* (NTCC 677) were purified by the “single hyphal tip isolation method” and maintained in pure state on

Potato Dextrose Agar (PDA) slants with appropriate labels. The cultures were inoculated in PDA medium and incubated at 28 °C before use for the revival of the fungal strains.

(b) Confirmation of fungal culture

Further confirmation of the fungal cultures were done through microscopy. The microscopy was performed to confirm the fungus species and for reassurance of the desired fungus. This was done using a 1% potassium hydroxide (KOH) solution. The slides were prepared by adding 1 drop of 1% KOH solution on a clean sterile glass slide and then a spore was placed on the drop, and with the help of a sterile teasing needle, the spore was teased, followed by placing a clean coverslip over the solution carefully and avoiding bubbles. The spores were visualized under the compound microscope at 40X resolution, as shown in Figure 3.

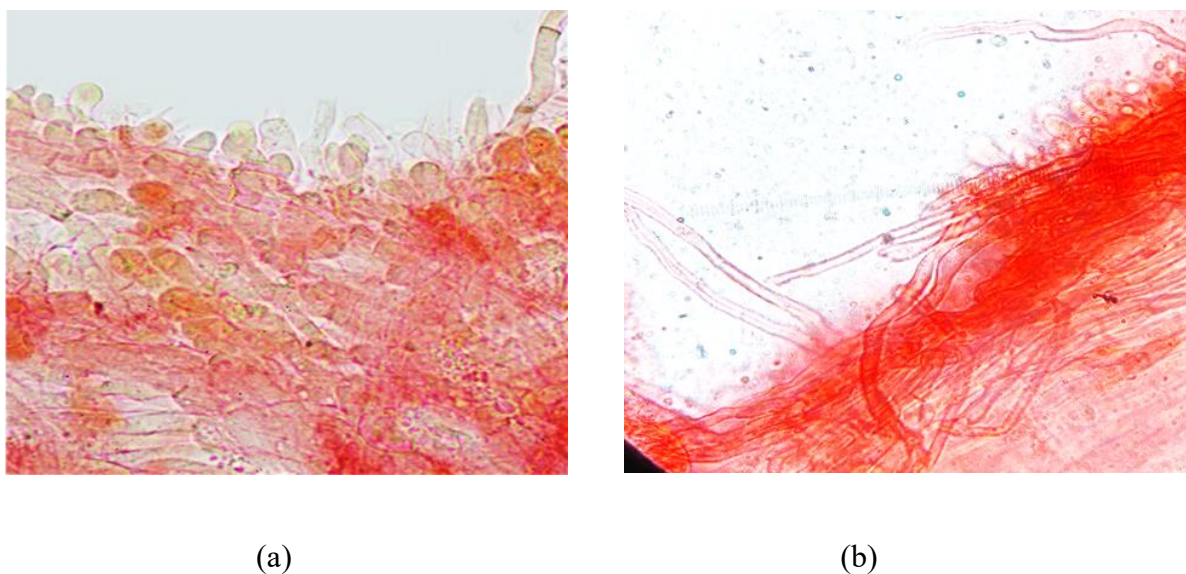


Figure 3. Microscopic picture showing mycelia of (a) *Laetiporus sulphureus* (brown rot) and (b) *Pycnoporus sanguineus* (white rot).

(c) Preparation of nutrient media

PDA was selected as the nutrient medium to support fungal growth throughout the study. A precise quantity of 39 g of PDA powder (Himedia Laboratories, India) was dissolved in 1 L of sterile dH₂O with continuous stirring to prevent clumping and ensure homogeneity in a 2 L Erlenmeyer flask. The pH of the medium was adjusted and maintained at 5.6 ± 0.2 , which is optimal for fungal cultivation [15]. The flask was subsequently covered with a cotton plug, wrapped in aluminum foil, and subjected to sterilization at 121 °C and 15 psi pressure in an autoclave for 30 minutes to eliminate microbial contaminants [16]. Following sterilization, the medium was cooled to approximately 45 °C before further processing.

(d) Incorporation of ZnO Nanoparticles into nutrient culture media

To evaluate the antifungal efficacy of ZnO NPs, three concentrations, 10, 30, and 50 ppm, were prepared using a stock solution (0.10 mg/mL) of ZnO NPs. These concentrations were thoroughly mixed with the sterilized PDA medium before pouring. Approximately 20 mL of the ZnO NP-supplemented PDA medium was dispensed into pre-sterilized Petri plates (90 mm diameter) and

allowed to solidify under aseptic conditions inside a laminar airflow chamber to minimize contamination risks [17,18].

For comparison, two control sets were prepared:

- Positive control: PDA medium containing a standard antifungal agent, fluconazole (50 ppm), as a reference inhibitor.
- Negative control: PDA medium without any antifungal agent or ZnO NPs.

The prepared Petri plates were left undisturbed overnight in the laminar airflow chamber to ensure complete solidification and to prevent microbial contamination before fungal inoculation.

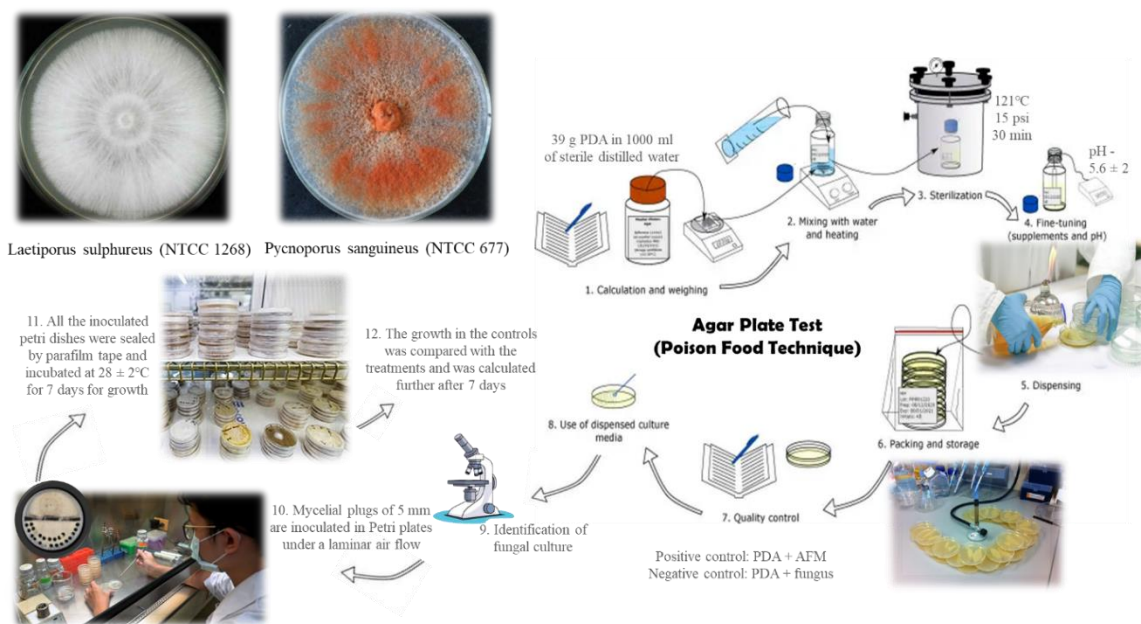


Figure 4. The schematic representation of complete antifungal analysis.

(e) Fungal inoculation and incubation

Mycelial plugs (5 mm in diameter) were excised from actively growing fungal cultures using a sterile cork borer under aseptic conditions. Each fungal plug was carefully placed at the center of ZnO NP-treated and control Petri plates with the mycelium facing downward [17,18]. The Petri plates were subsequently sealed with parafilm tape to prevent desiccation and contamination.

The inoculated plates were incubated in a biological incubator at 28 ± 2 °C for 7 days under controlled environmental conditions (relative humidity ~70%) to facilitate fungal growth. These incubation conditions were selected for the present agar-plate assay; IS 4873 (Part 1): 2008 was consulted as a general reference for laboratory testing of wood preservatives against fungi [36]. The experiment was conducted in triplicate for each treatment, and the procedure was repeated twice to ensure reproducibility.

(f) Measurement and calculation of mycelial growth inhibition

Fungal growth was monitored daily, and the radial expansion of the mycelium was measured using a digital Vernier caliper to ensure accuracy [19,20]. On the seventh day, the antifungal efficacy of ZnO NPs was quantified by comparing the radial mycelial growth in treated plates to that in the control plates.

The percentage inhibition of fungal growth was calculated using the following formula [21,22]:

$$\text{Percentage inhibition of mycelial growth} = (D_c - D_t/D_c)100,$$

where:

D_c = Mean mycelial diameter in the control group (mm), D_t = Mean mycelial diameter in the treated group (mm).

This calculation provided a quantitative measure of the antifungal activity of ZnO NPs across the tested concentrations.

3. Results and discussion

3.1. Total phenolic content (TPC) and yield

The polyphenolic phytochemicals are believed to act as potential bioactive compounds, acting as reducing and main stabilizing agents [23] during NP biosynthesis. The value of TPC of knot wood extract of abundantly available yet underutilized biomass residue of *E. tereticornis* generated from plantations and wood-based industries was 530.20 mg GAE/g extract. The higher TPC suggests stronger reducing capability compared to previously reported biomass systems [19]. The corresponding yield of extract and ZnO NPs were also determined (Table 1).

Table 1. Percentage yield of extracts and synthesized NPs.

S.No.	Source material	Percentage yield
1.	Extract of <i>Eucalyptus tereticornis</i>	07.50
2.	ZnO NPs	27.52

3.2. Band gap energy analysis

The optical properties of the biosynthesized ZnO NPs were characterized using UV-Visible (UV-Vis) spectroscopy, a widely used technique for validating nanoparticle synthesis [24]. Several factors influence ZnO NPs' absorption characteristics, including particle size, shape, synthesis method, and temperature, which all influence absorption spectrum patterns.

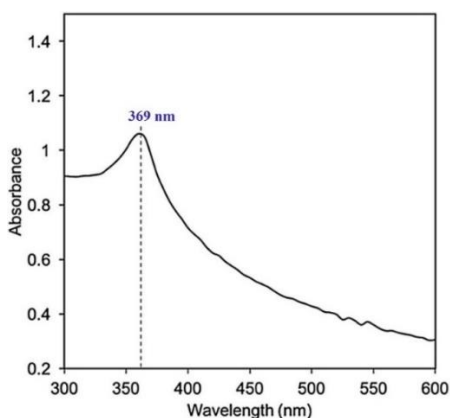


Figure 5. UV-Visible Absorption Spectrum of biosynthesized ZnO NPs.

As shown in Figure 5, the synthesized ZnO NPs exhibited a strong absorption band centered at 369 nm, corresponding to the electronic transition within the conduction band of ZnO. This observed absorption peak is blue-shifted relative to the bulk ZnO absorption value of 377 nm, confirming nanoscale formation [25]. This shift can be attributed to the quantum confinement effect, which occurs due to the reduction in particle size, enhancing the surface area-to-volume ratio of the nanoparticles [26,27]. Quantum confinement significantly alters the electronic structure of nanoparticles, leading to bandgap widening and modified optical properties.

To further assess the optical behavior of the biosynthesized ZnO NPs, Tauc's plot method was used to determine the optical band gap energy (E_{gE_gEg}). The extrapolation of the linear region in the Tauc plot revealed an estimated band gap energy of 3.15 eV, which is in close agreement with other reported values for ZnO NPs synthesized via green chemistry approaches [28]. The characteristic peak position in the UV-Vis spectrum, along with the estimated band gap, strongly confirmed the successful formation of ZnO NPs with nanoscale properties [29–32].

3.3. Elemental & structural analysis

The structural properties of the biosynthesized ZnO NPs were analyzed using X-ray diffraction (XRD), and the corresponding diffraction pattern is presented in Figure 6. The XRD profile exhibits distinct, sharp peaks of high intensity, indicative of the formation of nanocrystalline ZnO with well-defined lattice planes. The observed Bragg peaks acquired at 31.74° , 34.43° , 36.26° , 47.53° , 56.61° , 62.77° , 67.93° , and 69.11° show that the observed pattern corresponds to the (100), (002), (101), (102), (110), (103), (200), and (201) lattice planes, respectively. These values align precisely with the standard wurtzite ZnO phase (JCPDS card no. 00-075-0576), confirming the hexagonal crystal structure of the synthesized nanoparticles. Further, an average crystallite size of 29 nm was estimated using the Debye-Scherrer equation for ZnO NPs, indicating nanoscale characteristics and excellent crystallinity.

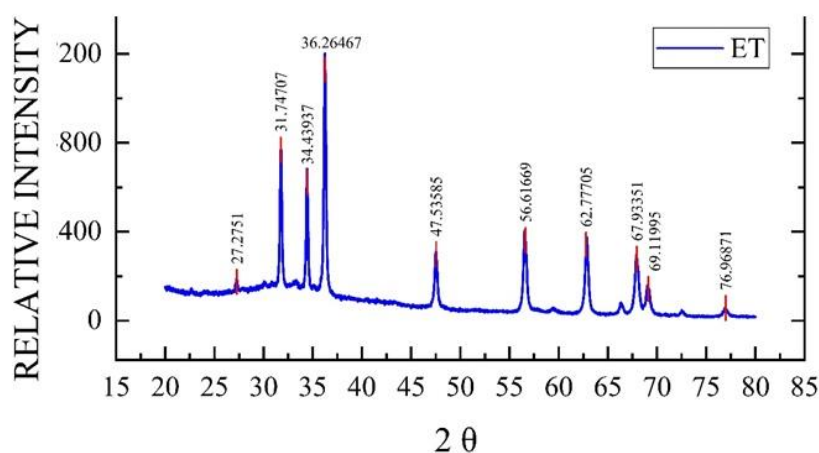


Figure 6. The XRD pattern of ZnO synthesized from knot wood of *Eucalyptus tereticornis*.

The relatively broad diffraction peaks observed in the XRD pattern suggest a high surface area-to-volume ratio, a characteristic feature of nanostructured materials, which enhances their functional properties, particularly in catalysis, optoelectronics, and biomedical applications [31,33]. Additionally,

the phase purity of the biosynthesized NPs was confirmed by the absence of secondary or impurity peaks in the diffraction spectrum, validating the effectiveness of the green synthesis approach using sawdust extract from *E. tereticornis*, an underutilized biomass residue. These findings corroborate reports on biosynthesized ZnO nanoparticles, demonstrating that the proposed synthesis route produces highly crystalline, phase-pure ZnO NPs with controlled morphology and structural stability, which is suitable for environmental, electronic, and biomedical applications [33–35].

3.4. Vibrational properties

FTIR analysis was carried out to confirm the zincite phase of the synthesized ZnO NPs, which enabled an identification of functional groups in plant extracts that supported the bonding mechanism. In addition to being packed with polyphenolic derivatives, *E. tereticornis* extract also contains many carbonyls, carboxyls, and hydroxyls. These compounds are believed to act as potential bioactive compounds that can provide therapeutic effects and act as reducing agents [23]. The FTIR spectrum (Figure 7) ZnO NPs synthesized using extract of abundantly available yet underutilized biomass residue (knot wood) of *E. tereticornis* was recorded in the 4000–400 cm^{-1} spectral range. To exclude the absorptions observed at lower wavelengths, the IR transmission was plotted. Consequently, several peaks are unveiled at 3438.72, 2923.72, 2346.80, 1910.04, 1639.50, 1104.95, and 827.90 cm^{-1} from the spectrum of extracts. Alkaloids, phenolic compounds, and flavonoids are also within the peaks at 3439 (O-H), 2924 (C-H), 1105 (C-O) or 1046 (RCOO), and 1640 (N-H) cm^{-1} , respectively [14,27]. The observed broad stretching band at 3439 cm^{-1} coincides with the O-H stretching mode of hydroxyl groups, typically associated with polyphenols, acting as the main stabilizing agents during NP synthesis. In this study, the *E. tereticornis* knot wood extract contained flavonoid derivatives. It is possible that flavonoids and phenols may interact with zinc NPs, causing their positions or intensities in a sample spectrum to change. Moreover, the functional groups that are present in the extract of biomass residue may act as electron donors, enabling the reduction of zinc ions (Zn^{2+} to Zn^{+1}) and subsequently leading to the formation of zinc NPs (ZnO).

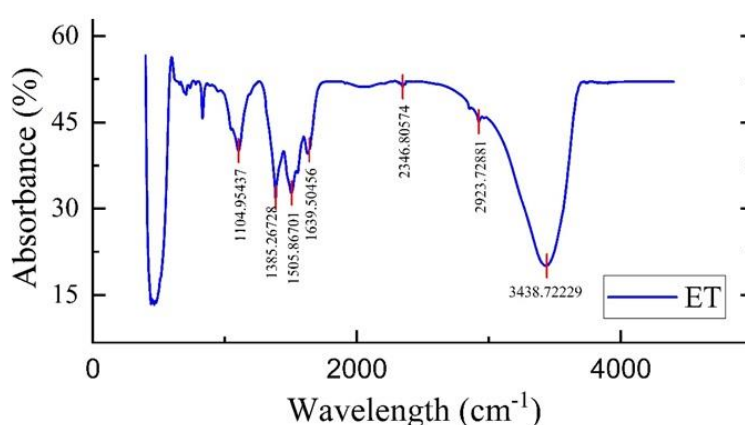


Figure 7. FTIR spectrum depicting ZnO synthesized from knot wood extract of *E. tereticornis*.

In addition, the extract may have a stabilizing effect because of its negative functional groups. The FTIR spectrum showed a slight shift and changes in related peak intensities, indicating that the

major biomolecules were capped on ZnO NP surfaces. As a result, ZnO NPs became bent out of plane by C-H and C=C, respectively, by the appearance of new peaks at 2340 cm^{-1} . Comparable Zn–O absorption bands in the low-wavenumber region have been reported for plant-mediated ZnO nanoparticles [27,31,33,35]. The higher relative intensity of Zn–O modes compared with adsorbed OH compounds is consistent with formation of crystalline ZnO. For optimal functionality in their pure chemical form, ZnO NPs should be composed predominantly of Zn and O.

3.4. Surface morphology and structural analysis

The surface morphology of the biosynthesized ZnO nanoparticles (ZnO NPs) was examined using SEM (Figure 8). The SEM micrographs revealed that the ZnO NPs exhibit predominantly spherical and hexagonal shapes with well-defined separation between individual particles. Comparable morphology has been reported for plant-mediated ZnO nanoparticles [31,33,35].

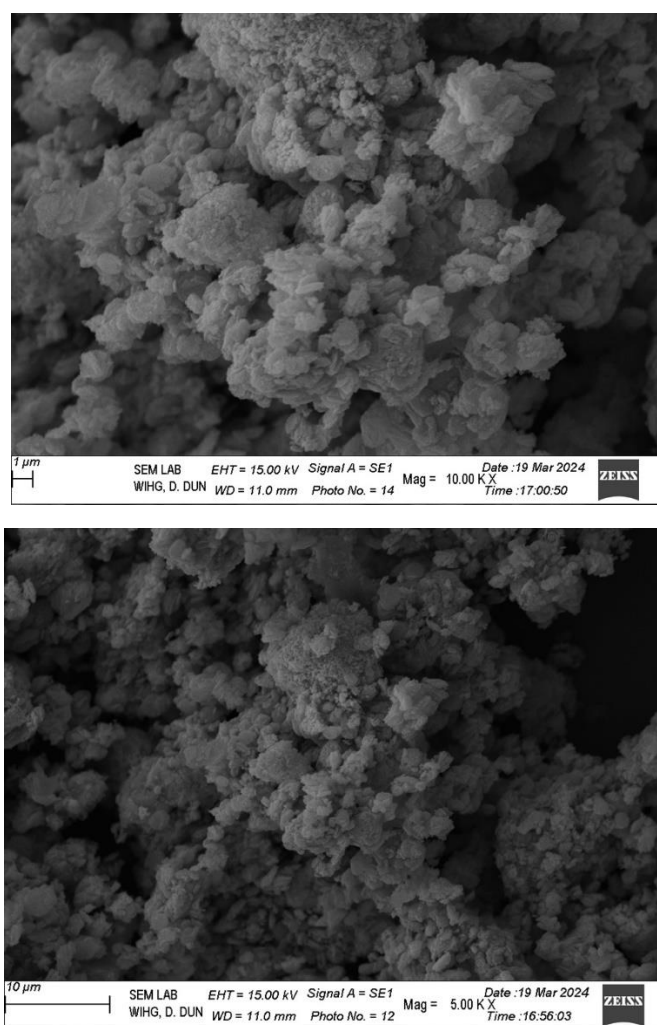


Figure 8. SEM images of synthesized ZnO NPs at different magnifications.

Energy dispersive X-ray spectroscopy (EDX) analysis was conducted to further confirm the elemental composition and purity of the synthesized ZnO NPs. The EDX spectrum (Figure 9) of a densely

populated region of ZnO NPs during spot analysis displayed characteristic strong signals for zinc (Zn) and oxygen (O), with only a minimal carbon (C) presence at very low intensity, thereby confirming a good purity of the synthesized NPs. The atomic and weight percentages of Zn, O, and C were determined to be 40.21 (74.43%), 46.42 (21.03%), and 13.3 (4.55%), respectively, affirming that the pure ZnO NPs were synthesized successfully.

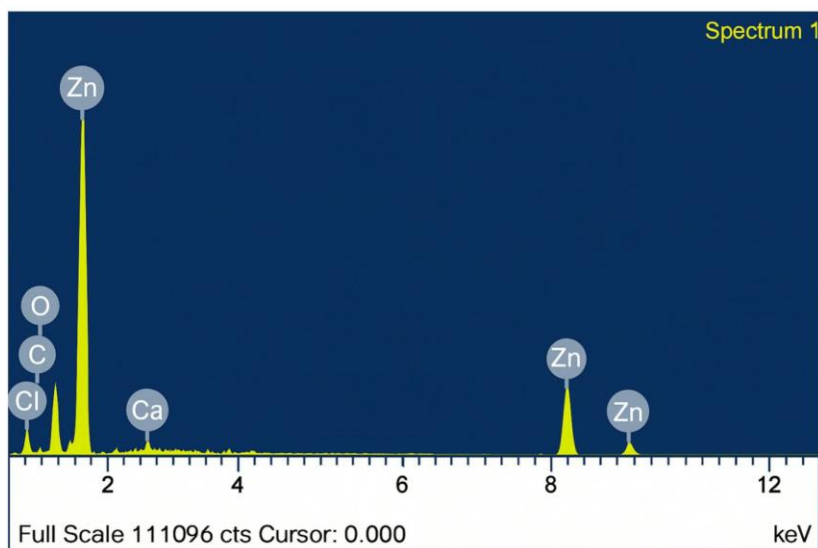


Figure 9. EDX spectra of synthesized ZnO NPs.

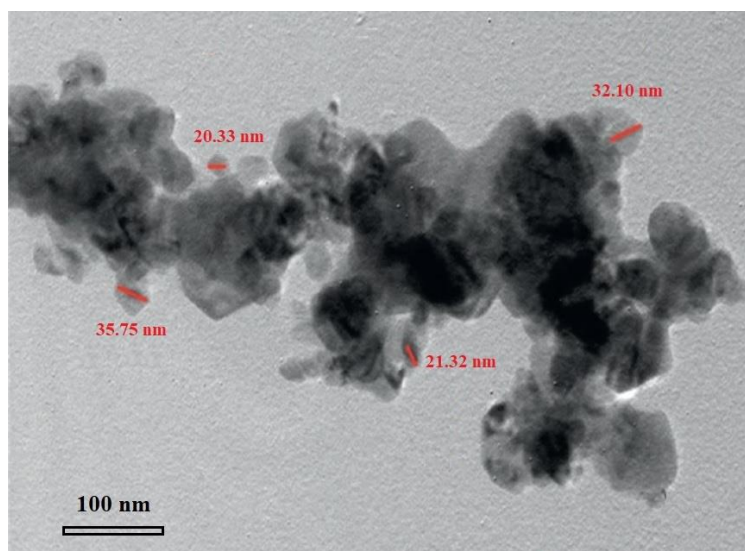


Figure 10. TEM image of synthesized ZnO NPs.

Additionally, TEM was employed to analyze the size, shape, and detailed morphological characteristics of the synthesized ZnO NPs. TEM images captured at different magnifications (Figure 10) provided clear insights into the agglomeration behavior, revealing that the nanoparticles maintained a predominantly spherical and hexagonal wurtzite structure. The mean particle size was found 27.37 nm,

which aligned closely with the crystallite size estimated from X-ray diffraction (XRD) analysis, further corroborating the accuracy and reliability of the synthesis method.

4. Antifungal activity

The antifungal efficacy of synthesized ZnO NPs (using knot wood extract of *E. tereticornis*) was evaluated *in vitro* against most common wood decaying brown rot (*L. sulphureus*) and white rot (*P. sanguineus*) fungi for different NP concentrations. It was observed that increasing the concentration of ZnO NPs led to a significant reduction in the viability of mycelium in both fungal species, confirming the dose dependent antifungal activity (Figure 11). As the growth of fungi is very slow, more inhibition was observed. There was no growth in the plate containing standard antifungal agent, fluconazole. The results in term of percentage inhibition of growth of fungal mycelia of fungi over PDA medium containing different concentration of NPs are shown in Table 2.

Table 2. The percentage of mycelial growth inhibitions for brown rot fungi (*Laetiporus sulphureus*) and white rot fungi (*Pycnoporus sanguineus*).

S.No	Fungi	Concentration of NPs (ppm)		
		10	30	50
1.	<i>Laetiporus sulphureus</i>	47.14	72.86	98.57
2.	<i>Pycnoporus sanguineus</i>	41.54	75.38	98.46

The study showed that at 10 ppm, ZnO NPs had a mild inhibitory effect on fungal growth (*L. sulphureus*: 47.14 %, *P. sanguineus*: 41.54 %). However, at 50 ppm, inhibition was nearly complete, reaching 98.57 % and 98.46 % for *L. sulphureus* and *P. sanguineus*, respectively.

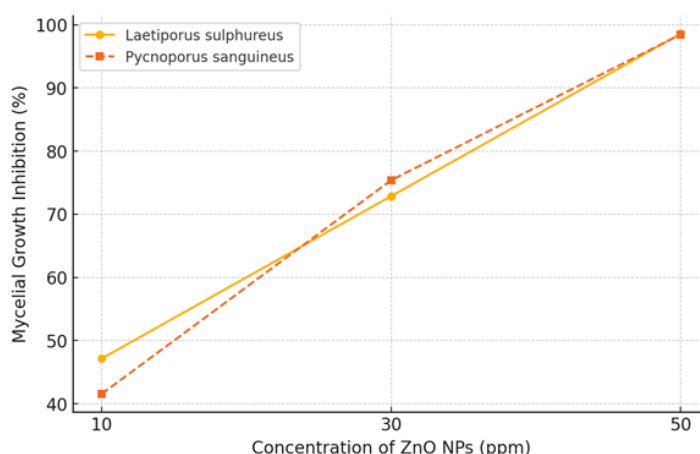


Figure 11. Antifungal efficacy of ZnO NPs against *Laetiporus sulphureus* and *Pycnoporus sanguineus*.

The percentage of mycelial growth inhibitions for 10 ppm, 30 ppm, and 50 ppm were 47.14%, 72.86%, and 98.57%, respectively for *L. sulphureus* and were 41.54%, 75.38%, and 98.46%, respectively, for *P. sanguineus*. The greater susceptibility of *L. sulphureus* indicates that brown rot fungi may be more sensitive to ZnO NPs than white rot fungi.

The observed antifungal activity of ZnO NPs can be attributed to multiple interacting mechanisms reported in the literature. A primary pathway involves the generation of reactive oxygen species (ROS), which induces oxidative stress and damages cellular components [37,38]. Microscopic and physiological studies have also reported deformation and structural damage of fungal cells or hyphae following ZnO NP exposure [38,39]. Dissolution of Zn^{2+} from ZnO can further contribute to toxicity by disturbing cellular homeostasis and metabolic processes [39,41]. Together, ROS-mediated oxidative injury, particle-associated structural damage, and released Zn^{2+} provide a mechanistically plausible basis for the concentration-dependent fungal inhibition observed in this study [37–41].

5. Conclusions

In this study, we present an eco-friendly and innovative approach toward the green synthesis of ZnO NPs using sawdust waste extract from *E. tereticornis*, an abundant yet underutilized biomass residue generated from plantations and wood-based industries. This is the first time when an extract of biomass residue (knot wood) of *E. tereticornis* has effectively reduced chemical agents, showing interesting properties. The FTIR analysis confirmed the crucial role of polyphenolic compounds in the bioreduction and stabilization of ZnO NPs. Under controlled thermal annealing at 410 °C for approximately three hours, highly crystalline ZnO NPs were obtained, as validated by XRD. Further, UV-Vis spectroscopy analysis revealed a distinct absorption band at 369 nm, exhibiting a slight blue shift compared to bulk ZnO, which is indicative of the influence of plant-derived biomolecules and the quantum confinement effect. A hexagonal wurtzite ZnO (of 29 nm) phase was confirmed by an XRD pattern. Morphological characterization via SEM revealed predominantly spherical and hexagonal ZnO NPs with clear separation, while TEM confirmed their size distribution, ranging between 20–40 nm. The EDX analysis corroborated an elemental purity of the synthesized ZnO NPs, consistent with XRD findings, highlighting their well-defined physicochemical properties.

The synthesized ZnO NPs showed potent antifungal activity, with a notable inhibition of *L. sulphureus* and *P. sanguineus*, demonstrating their potential as an eco-friendly alternative to conventional chemical fungicides. The enhanced antifungal efficacy, superior biocompatibility, and reduced environmental footprint of these NPs position them as a promising candidate for sustainable wood protection and preservation applications. In a follow-up study, we will investigate the interaction mechanism between ZnO nanoparticles and fungal cell structures to understand their inhibitory effects at the cellular level. Additionally, for long-term studies, we will evaluate the stability and efficacy of ZnO NPs when applied to wood surfaces, ensuring sustained antifungal protection. Furthermore, potential synergistic effects of ZnO NPs in combination with natural antifungal agents will be explored to enhance their bioactivity and broaden their scope of application in the real-world.

Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

Conflict of interest

The authors declare there is no conflict of interest.

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