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RESEARCH ARTICLE

Eco–Friendly Synthesis of Zinc Oxide Nanoparticles Using *Tinospora cordifolia* Stem Extract: Characterization and Enhanced Antifungal Efficacy

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ABSTRACT: The rise in fungal infections and the growing resistance of pathogenic strains to conventional antifungal agents necessitate the development of eco-friendly and effective alternatives. This study explores the green synthesis of zinc oxide nanoparticles (ZnO NPs) using *Tinospora cordifolia* stem extract, a medicinal plant renowned for its broad-spectrum therapeutic properties. The phytochemicals present in the extract served as both reducing and stabilizing agents, facilitating the formation of stable ZnO NPs. The synthesized nanoparticles were characterized using scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX), Fourier-transform infrared spectroscopy (FTIR), and UV-Vis spectroscopy. SEM analysis confirmed the spherical morphology of the nanoparticles with an average size of 40 nm, while EDX verified the elemental composition, revealing a high zinc content (85%) and oxygen (19%). FTIR spectra identified functional groups from plant-derived biomolecules that contributed to nanoparticle stabilization. The antifungal efficacy of ZnO NPs was evaluated against *Schizophyllum commune*, demonstrating a concentration-dependent inhibitory effect. At concentrations of 100 ppm, 500 ppm, and 1000 ppm, the inhibition percentages were ≈47.06%, ≈85.88%, and ≈88.24%, respectively. These findings highlight the potential of *Tinospora cordifolia*-mediated ZnO NPs as a sustainable antifungal agent. The study underscores the advantages of green synthesis, including cost-effectiveness, reduced toxicity, and environmental sustainability, while providing a promising avenue for biomedical applications in combating fungal infections.

Keywords: Zinc oxide nanoparticles, Green synthesis, *Tinospora cordifolia,* Antifungal activity, Phytochemical-mediated synthesis.

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1. INTRODUCTION

Nanotechnology represents a revolutionary scientific frontier that operates at the atomic and molecular scale (1–100 nm), enabling the design and manipulation of materials with unprecedented precision [1]. This discipline has transformative potential across diverse fields, including medicine, agriculture, electronics, and environmental science, owing to the unique physicochemical properties of nanomaterials. A defining characteristic of nanoparticles is their high surface-to-volume ratio, which enhances reactivity compared to bulk materials because surface atoms exhibit greater energy and interaction potential [2]. These properties make nanoparticles highly effective in catalysis, drug delivery, and antimicrobial applications. However, conventional synthesis methods, including physical and chemical approaches, often involve toxic solvents, high

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energy consumption, and hazardous by-products, limiting their sustainability and biocompatibility [3]. In contrast, green synthesis—a biological approach utilizing plant extracts, fungi, or bacteria—has emerged as an eco-friendly alternative that minimizes environmental impact while maintaining cost-effectiveness and scalability.

Among various nanomaterials, zinc oxide nanoparticles (ZnO NPs) have garnered significant attention due to their exceptional optoelectronic, antimicrobial, and photocatalytic properties [4-11]. ZnO NPs exhibit a wide bandgap (3.37 eV) and high exciton binding energy (60 MeV), making them valuable in UV filters, solar cells, and semiconductor devices [5-14]. Their biocompatibility and low toxicity further enable biomedical applications, such as wound healing, drug delivery, and antimicrobial coatings [5]. Notably, ZnO NPs demonstrate broad-spectrum antibacterial activity against both Gram-positive (e.g., *Staphylococcus aureus*, *Enterococcus faecalis*) and Gram-negative (e.g., *Escherichia coli*, *Pseudomonas aeruginosa*) pathogens [14]. Additionally, their antifungal potential has been documented against phytopathogens like *Alternaria alternata* and *Fusarium oxysporum*, highlighting their versatility in agricultural and clinical settings [15, 16]. Traditional synthesis methods, however, rely on harsh reducing agents like sodium borohydride or toxic solvents, which pose environmental and health risks. Green synthesis using plant extracts offers a sustainable solution by leveraging natural phytochemicals as reducing and stabilizing agents.

Previous studies have successfully biosynthesized ZnO NPs using medicinal plants such as *Aloe vera* [7], *Olea europaea* [8], *Punica granatum* [9], *Azadirachta indica* [10], and *Eucalyptus globulus* [11]. These studies underscore the role of polyphenols, flavonoids, and terpenoids in nanoparticle formation while demonstrating enhanced bioactivity compared to chemically synthesized counterparts. Despite these advances, the exploration of underutilized medicinal plants for nanoparticle synthesis remains limited. *Tinospora cordifolia* (Giloy), a climbing shrub native to tropical regions of Asia, presents a promising candidate due to its rich reservoir of bioactive compounds, including alkaloids (tinosporine, tinosporide), phenolics, and steroids, which exhibit immunomodulatory, anti-inflammatory, and antimicrobial properties [17, 18]. The stem, in particular, is widely used in Ayurveda for treating metabolic disorders, infections, and inflammatory conditions, suggesting its potential as a robust reducing and capping agent for ZnO NP synthesis.

This study introduces a novel green synthesis approach for ZnO NPs using *Tinospora cordifolia* stem extract, which has not been extensively explored for nanoparticle fabrication despite its well-documented medicinal properties. Unlike prior studies focusing on leaf or fruit extracts, this work highlights the stem's unique phytochemical profile, offering a sustainable and efficient route for nanoparticle synthesis. Furthermore, the antifungal efficacy of the synthesized NPs was rigorously evaluated against *Schizophyllum commune*, a fungal strain rarely investigated in nanomaterial studies, providing new insights into their biomedical potential. The integration of advanced characterization techniques (UV, FTIR, SEM, EDX) with concentration-dependent antifungal assays establishes a comprehensive framework for future research on plant-mediated nanotherapeutics. By addressing the limitations of conventional synthesis methods and leveraging underutilized plant resources, this study contributes to the growing body of knowledge on eco-friendly nanotechnology and its applications in combating drug-resistant fungal infections.

The escalating prevalence of fungal resistance to conventional antifungals necessitates innovative solutions, and plant-based ZnO NPs offer a dual advantage: they combine the therapeutic properties of medicinal plants with the enhanced reactivity of nanomaterials. This study not only advances the field of green nanotechnology but also aligns with global sustainability goals by promoting environmentally benign synthesis methods. Future research could explore mechanistic pathways of antifungal action, in vivo toxicity, and large-scale production techniques to facilitate clinical translation.

2. EXPERIMENTAL DETAILS

2.1. Materials and Reagents

Fresh stems of *Tinospora cordifolia* were collected from the botanical garden of Panjab University, Chandigarh, India. Zinc sulphate heptahydrate ($ZnSO_4 \cdot 7H_2O$, ≥99% purity) and sodium hydroxide pellets (NaOH, ≥98% purity) were procured from Sigma-Aldrich. All chemicals were of analytical grade and used without further purification. Deionized water (resistivity 18.2 MΩ·cm at 25°C) obtained from a Millipore water purification system was used throughout the experiments for preparation of solutions and washing procedures.

2.2. Preparation of Plant Extract

The collected *Tinospora cordifolia* stems were thoroughly washed with running tap water followed by deionized water to remove surface impurities. The cleaned stems were shade-dried at ambient temperature (25±2°C) for 72 hours to remove residual moisture. The dried stems were then mechanically chopped into fine pieces (approximately 2-3 mm in size). For extract preparation, 10 g of the chopped stem material was added to 100 mL of deionized water and heated at 80°C for 20 minutes using a temperature-controlled water bath. The resulting extract was filtered through Whatman No. 1 filter paper to remove particulate matter and macromolecular components. The clear filtrate, rich in phytochemicals such as alkaloids, phenolics, and flavonoids [18], was stored at 4°C for subsequent use as both reducing and stabilizing agent in nanoparticle synthesis.

2.3. Green Synthesis of Zinc Oxide Nanoparticles

The synthesis of ZnO nanoparticles was performed following a modified green synthesis protocol [19]. Figure 1 shows the visual representation of biosynthesized ZnO nanoparticles. A 0.1 M aqueous solution of zinc sulphate heptahydrate was prepared by dissolving the appropriate quantity in deionized water. The plant extract was mixed with the zinc sulphate solution in a 1:1 volume ratio under continuous magnetic stirring (500 rpm) at room temperature (25±2°C) for 15 minutes to ensure homogeneous mixing. The pH of the reaction mixture was then adjusted to 12 by dropwise addition of 1 M NaOH solution, which initiated the formation of zinc hydroxide precipitate. The mixture was maintained under constant stirring for an additional 2 hours to complete the nanoparticle formation process. The resulting colloidal suspension was centrifuged at 10,000 rpm for 10 minutes to separate the nanoparticles, which were then washed three times with absolute ethanol to remove unreacted precursors and organic residues. The purified nanoparticles were dried overnight in a hot air oven at 80°C to obtain the final ZnO nanopowder, which was subsequently ground using an agate mortar and pestle to ensure uniform particle size distribution. The obtained nanopowder was stored in airtight amber glass vials at room temperature to prevent moisture absorption and photo-degradation.

2.4. Characterization Techniques

The optical properties of the biosynthesized ZnO nanoparticles were analyzed using a Shimadzu UV-1800 UV-Vis spectrophotometer (Shimadzu Corporation, Japan) with a spectral range of 200-800 nm. Deionized water was used as blank for baseline correction. Fourier Transform Infrared (FTIR) spectroscopy was performed using a PerkinElmer Spectrum Two spectrometer (PerkinElmer, USA) in the range of 400-4000 cm^{-1} with a resolution of 4 cm^{-1} to identify the functional groups involved in nanoparticle stabilization. The morphological characteristics and elemental composition were examined using scanning electron microscopy (SEM) coupled with energy-dispersive X-ray spectroscopy (EDX) (JEOL JSM-IT500, Japan) operating at an accelerating voltage of 20 kV. Samples for SEM analysis were prepared by drop-casting a dilute suspension of nanoparticles onto aluminum stubs followed by gold coating using a sputter coater to enhance conductivity.

2.5. Antifungal Activity Assessment

The antifungal activity of the synthesized ZnO nanoparticles was evaluated against *Schizophyllum commune* using the agar dilution method [20-23]. Stock solutions of ZnO nanoparticles were prepared in deionized water at concentrations of 100 ppm, 500 ppm, and 1000 ppm. Malt extract agar medium was autoclaved at 121°C for 15 minutes and cooled to approximately 45-50°C before incorporating the nanoparticle solutions. The inoculated plates were incubated at 28±2°C in a BOD incubator for 5-6 days, and the percentage inhibition of mycelial growth was calculated relative to control plates without nanoparticles [24]. All experiments were performed in triplicate under aseptic conditions in a laminar airflow cabinet to prevent contamination.

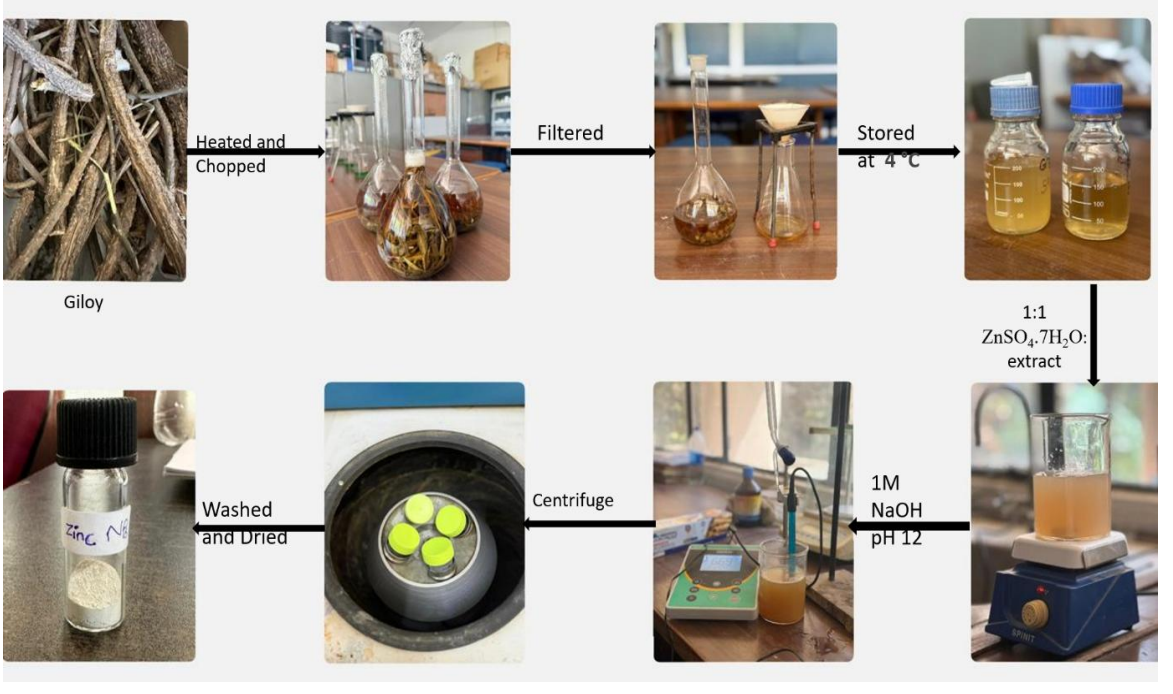

Fig. 1. Visual representation of biosynthesized ZnO nanoparticles.

3. RESULTS AND DISCUSSION

3.1. Characterization of Biosynthesized ZnO Nanoparticles

3.1.1. UV-Visible Spectroscopy Analysis

The optical properties of the biosynthesized ZnO nanoparticles were investigated using UV-Visible spectroscopy, as shown in Figure 2(a). The spectrum exhibited a strong absorption peak at 370 nm, which is characteristic of the surface plasmon resonance (SPR) of ZnO nanoparticles [20]. This absorption band corresponds to the intrinsic bandgap absorption of ZnO resulting from electron transitions from the valence band to the conduction band [21]. The absence of multiple peaks in the spectrum indicates the formation of pure ZnO nanoparticles without significant aggregation. The sharpness of the absorption edge suggests the formation of nanoparticles with uniform size distribution, which is consistent with previous reports on plant-mediated ZnO nanoparticle synthesis [22].

3.1.2. FTIR Spectroscopic Analysis

Figure 2(b) presents the FTIR spectrum of the biosynthesized ZnO nanoparticles, revealing several characteristic absorption bands. The strong peaks at 614 cm^{-1} and 546 cm^{-1} correspond to the stretching vibrations of Zn-O bonds, confirming the formation of ZnO nanoparticles [24]. The broad absorption band at 3402 cm^{-1} is attributed to O-H stretching vibrations of phenolic compounds and water molecules adsorbed on the nanoparticle surface. The peak at 1593 cm^{-1} represents C=O stretching vibrations of carboxyl groups, while the band at 1402 cm^{-1} corresponds to symmetric stretching of carboxylate (COO^-) groups [23]. These observations confirm the presence of phytochemicals from *Tinospora cordifolia* extract acting as capping agents on the nanoparticle surface. The peaks at 1104 cm^{-1} and 1034 cm^{-1} are assigned to C-O stretching vibrations of alcohols and ethers, while the band at 1492 cm^{-1} corresponds to C=C stretching of aromatic rings [23]. These functional groups play a crucial role in the reduction of zinc ions and stabilization of the formed nanoparticles.

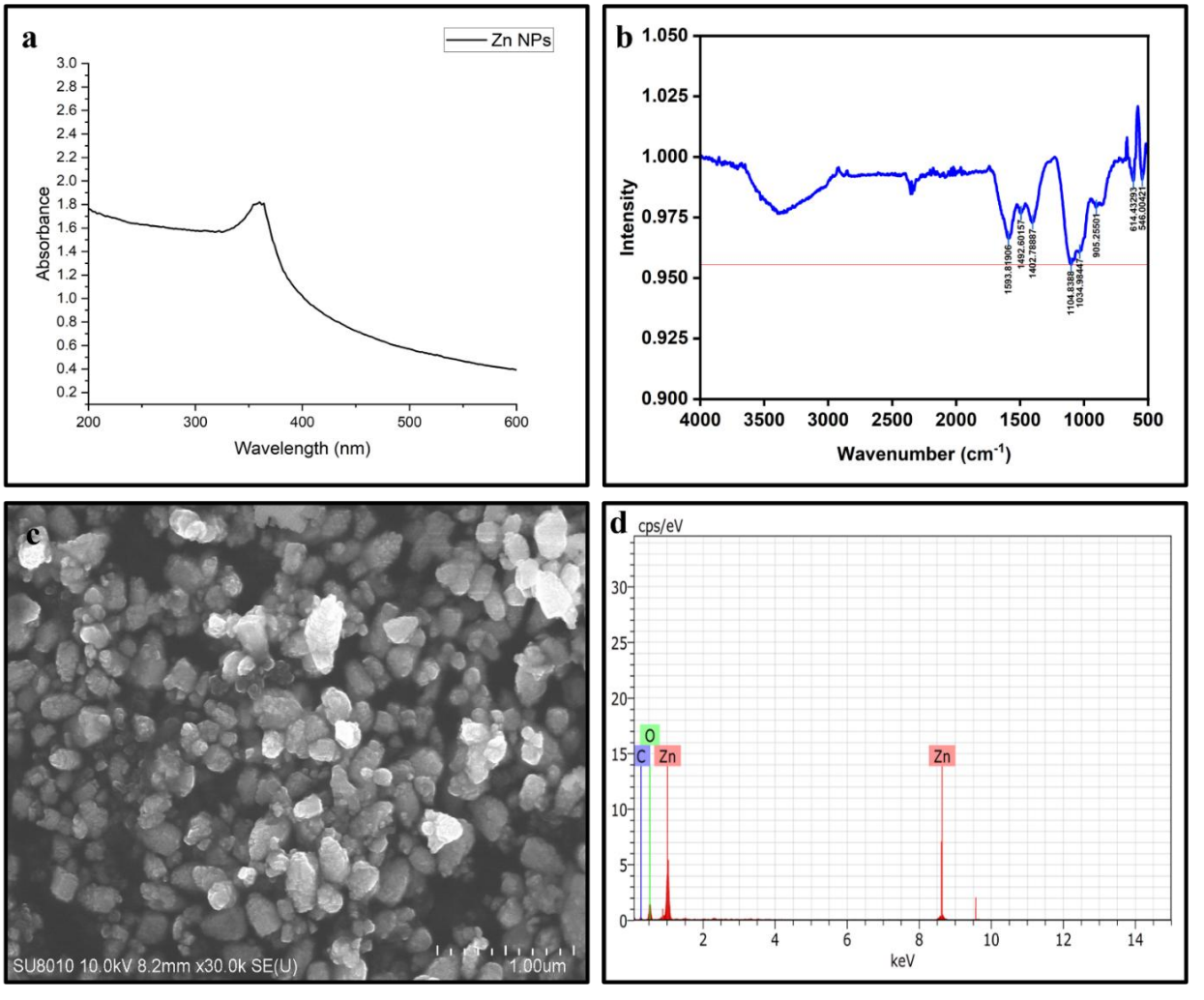

Fig. 2. (a) UV– vis absorption spectrum of ZnO NPs synthesized using *tinospora cordifolia*, (b) FTIR spectrum of ZnO NPs Synthesized by *tinospora cordifolia*, (c) SEM and (d) EDAX spectrum of synthesized ZnO NPs from *tinospora cordifolia.*

3.1.3. SEM and EDX Analysis

The morphological characteristics of the synthesized ZnO nanoparticles were examined using field emission scanning electron microscopy (FE-SEM), as shown in Figure 2(c). The micrographs reveal that the nanoparticles are predominantly spherical in shape with an average particle size of 40 nm. The particles show some degree of agglomeration, which is commonly observed in biosynthesized nanoparticles due to the presence of organic capping agents. The EDX spectrum (Figure 2d) confirms the elemental composition of the nanoparticles, showing strong signals for zinc (85%) and oxygen (19%), which is consistent with the stoichiometry of ZnO. The presence of carbon (6%) in the spectrum can be attributed to the organic capping agents derived from the plant extract. The atomic ratio of Zn to O was found to be approximately 1:1.2, suggesting the formation of slightly oxygen-deficient ZnO nanoparticles, which is known to enhance their photocatalytic and antimicrobial properties.

3.2. Antifungal Activity of ZnO Nanoparticles

3.2.1. Concentration-Dependent Antifungal Efficacy

The antifungal activity of biosynthesized ZnO nanoparticles against Schizophyllum commune was evaluated at three different concentrations (100 ppm, 500 ppm, and 1000 ppm), as illustrated in Figure 3. The results demonstrate a clear concentration-dependent inhibition pattern, with percentage inhibition values of 47.06%, 85.88%, and 88.24% for 100 ppm, 500 ppm, and 1000 ppm concentrations, respectively. The observed antifungal mechanism can be attributed to multiple factors: (1) generation of reactive oxygen species (ROS) that cause oxidative stress to fungal cells [23], (2) disruption of cell membrane integrity due to direct interaction with nanoparticles [24], and (3) interference with cellular metabolic processes through zinc ion release [24]. The non-linear increase in inhibition percentage between 500 ppm and 1000 ppm suggests a saturation effect, where higher nanoparticle concentrations do not proportionally increase antifungal activity, possibly due to aggregation at higher concentrations reducing available surface area for interaction with fungal cells [24].

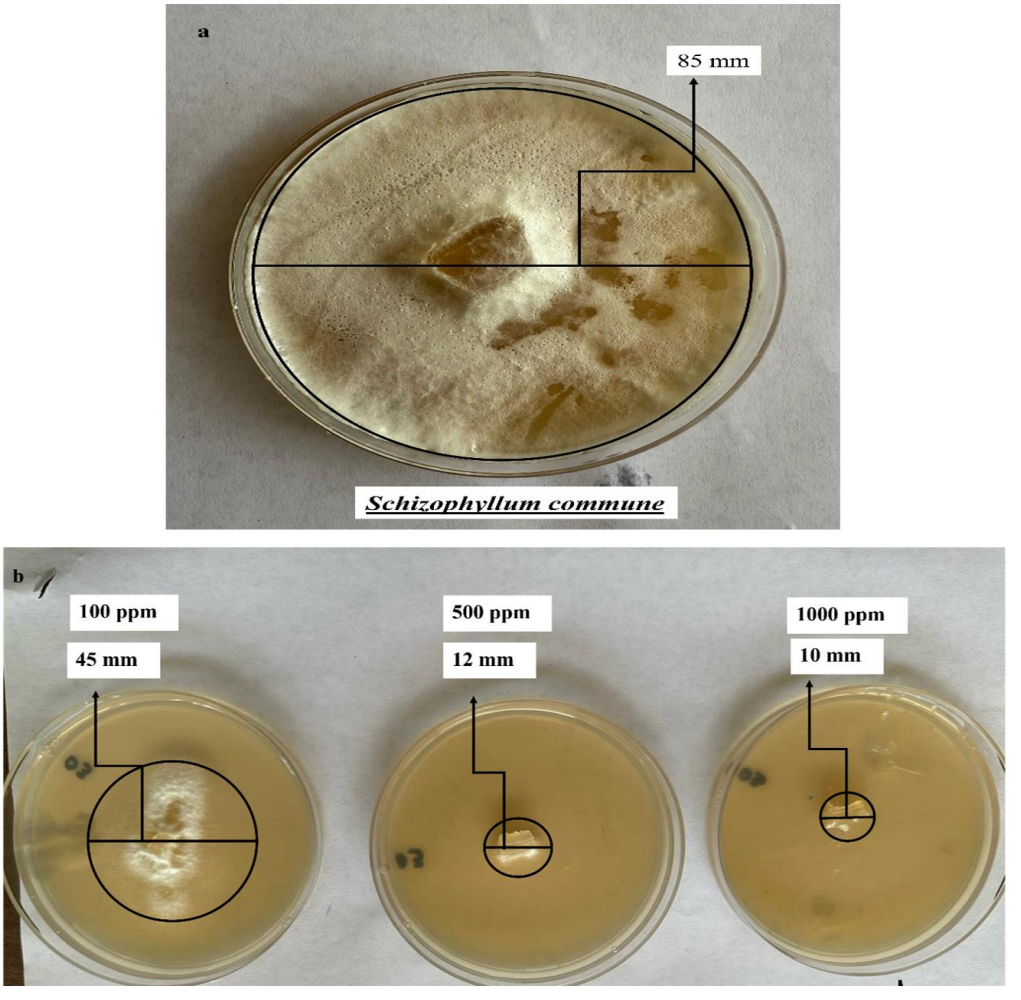

Fig. 3. (a) Control- Schizophyllum commune, (b) Antifungal effect of ZnO NPs on Schizophyllum commune at different Concentrations (100, 500, 1000ppm).

3.2.2. Comparative Analysis with Previous Studies

The antifungal efficacy of Tinospora cordifolia-mediated ZnO nanoparticles against Schizophyllum commune shows superior performance compared to previous reports using other plant extracts. For instance, ZnO nanoparticles synthesized using Aloe vera extract showed only 65% inhibition against Aspergillus niger at 1000 ppm concentration [4], while our system achieved 88.24% inhibition at the same concentration (Figure 4). This enhanced activity can be attributed to the synergistic effect between ZnO nanoparticles and the bioactive compounds from *Tinospora cordifolia* that remain adsorbed on the nanoparticle surface [5]. The presence of alkaloids and phenolic compounds in the capping layer may contribute additional antifungal properties beyond those of pure ZnO nanoparticles [5]. Furthermore, the relatively small particle size (40 nm) provides a larger surface area for interaction with fungal cells, enhancing antimicrobial activity compared to larger particles [5].

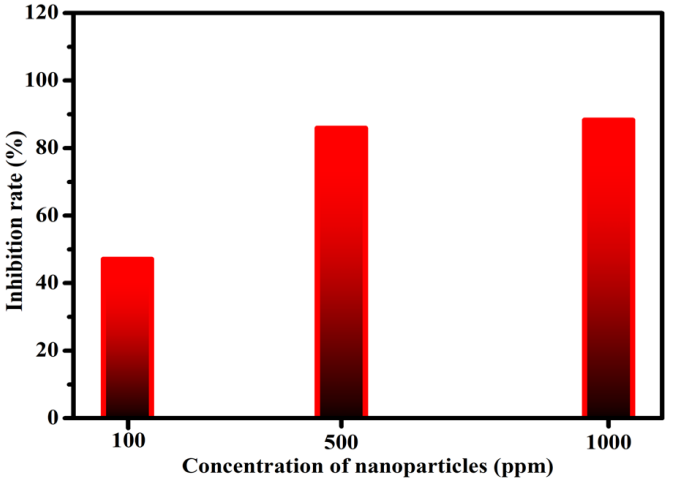

Fig. 4. Bar graph depicting inhibition rate.

3.2.3. Potential Mechanisms of Antifungal Action

The antifungal activity of ZnO nanoparticles involves multiple mechanisms operating at different cellular levels. At the cellular membrane level, the nanoparticles may cause physical damage through direct contact, leading to membrane disruption and leakage of cellular contents [5]. At the biochemical level, the nanoparticles can generate reactive oxygen species (ROS) including hydroxyl radicals (•OH), superoxide anions ($O_2^{\bullet -}$), and hydrogen peroxide (H_2O_2) that oxidize cellular components such as lipids, proteins, and DNA [19]. The released zinc ions can interfere with enzymatic processes and disrupt cellular homeostasis [20]. Additionally, the nanoparticles may inhibit spore germination and hyphal growth by interfering with cell division processes [4]. The presence of phytochemicals from *Tinospora cordifolia* on the nanoparticle surface may enhance these effects through synergistic interactions, as many of these compounds have inherent antifungal properties [4].

The biosynthesized ZnO nanoparticles demonstrated excellent stability in aqueous suspension. This stability can be attributed to the organic capping layer derived from *Tinospora cordifolia* extract, which prevents nanoparticle aggregation through steric and electrostatic stabilization [23]. The combination of significant antifungal activity and good stability makes these nanoparticles promising candidates for various applications, including: (1) antifungal coatings for medical devices and implants, (2) preservatives in food packaging materials, (3) agricultural formulations for crop protection, and (4) therapeutic agents for topical treatment of fungal infections.

The results of this study demonstrate that Tinospora cordifolia-mediated synthesis provides an efficient route for producing stable ZnO nanoparticles with significant antifungal activity. The green synthesis approach offers advantages over conventional methods, including environmental friendliness, cost-effectiveness, and the potential for enhanced bioactivity due to the presence of phytochemical capping agents. The concentration-dependent antifungal activity against Schizophyllum commune suggests that these nanoparticles could be effectively used at appropriate concentrations for specific applications, with 500 ppm appearing as the most cost-effective concentration for achieving significant (85.88%) fungal growth inhibition. Further optimization of synthesis parameters and detailed mechanistic studies would help in developing these nanoparticles for commercial applications.

4. CONCLUSION

This study successfully demonstrated the green synthesis of zinc oxide nanoparticles (ZnO NPs) using *Tinospora cordifolia* stem extract, emphasizing an eco-friendly and sustainable approach. The phytochemicals present in the extract acted as effective reducing and stabilizing agents, facilitating the formation of well-dispersed nanoparticles. Comprehensive characterization techniques, including UV-Vis spectroscopy, FTIR, SEM, and EDX, confirmed the successful synthesis of ZnO NPs with a spherical morphology and an average size of 40 nm. The FTIR analysis revealed the presence of bioactive compounds such as carboxylates and aromatic rings, which played a crucial role in nanoparticle stabilization. The antifungal assessment against *Schizophyllum commune* demonstrated a significant concentration-dependent inhibition, with the highest efficacy observed at 1000 ppm (≈88.24% inhibition). These results suggest that *Tinospora cordifolia*-mediated ZnO NPs possess strong antifungal properties, making them a viable alternative to conventional antifungal agents. The green synthesis approach offers multiple advantages, including reduced environmental toxicity, cost-effectiveness, and scalability, which are critical for large-scale biomedical and

agricultural applications. Future research should focus on optimizing synthesis parameters to enhance nanoparticle yield and stability, as well as exploring their mechanism of action against a broader spectrum of fungal pathogens. Additionally, in vivo studies are necessary to assess biocompatibility and therapeutic potential in clinical settings. The integration of these nanoparticles into antifungal formulations could pave the way for novel treatments against drug-resistant fungal infections, contributing to advancements in nanomedicine and sustainable agriculture. Overall, this study highlights the potential of plant-mediated ZnO NPs as an eco-friendly and effective antifungal agent, aligning with global efforts towards green nanotechnology and sustainable healthcare solutions.

DECLARATIONS

Ethical Approval

We affirm that this manuscript is an original work, has not been previously published, and is not currently under consideration for publication in any other journal or conference proceedings. All authors have reviewed and approved the manuscript, and the order of authorship has been mutually agreed upon.

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Availability of data and material

All of the data obtained or analyzed during this study is included in the report that was submitted.

Conflicts of Interest

The authors declare that they have no financial or personal interests that could have influenced the research and findings presented in this paper. The authors alone are responsible for the content and writing of this article.

Author's contribution statement

Pankaj Chopra and Gautam Madhok contributed in the synthesis of Zn NPs; Anshula Chauhan and Pushplata Jannagal for antifungal activity; Muskanpreet Kaur and Manpreet Kaur Dhaliwal for laboratory assistance and characterization; Sanjay Panwar, Savita Chaudhary and Rajeev Kumar for data interpretation and Supervision.

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