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Green Synthesis, Characterization, and Antifungal Activity of Propolis and Pomegranate Peel-Mediated Zinc Oxidenanoparticles Against *Candida Albicans*

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ABSTRACT

This study involving the green synthesis of zinc oxide nanoparticles by using the propolis and pomegranate peel, the nanoparticles showed superior efficacy, zinc oxide nanoparticles was prepared electrochemically by using Zn metal as positive electrode and Graphite as negative electrode, all electrodes was immersed in 200ml solution in a 200 mL solution containing 5 mL of potassium chloride (KCl) as the electrolyte, 10 mL of a stabilizer (polyvinyl alcohol (PVA)), and 185 mL of distilled water. A green synthesis approach is commonly used to coat zinc oxide nanoparticles with propolis and pomegranate peel extract. The plant extract acts as both a reducing agent and a coating agent, forming a bio-organic coating around the nucleus of the zinc oxide nanoparticles. Ultrasonic treatment was used to increase the surface area, and the pH was adjusted to the alkaline range to facilitate coating and precipitation. Candida albicans was readily cultured using Sabouraud Dextrose Agar (SDA) cultures at 35–37°C for 24 hours or by incubation. Gribkov 0.5 suspensions are used in standard McFarland tubes, including 1×10^6 CFU/ml. The XRD analysis of zinc oxide nanoparticles showed the values 72.58°, 69.191°, 68.051°, 66.52°, 62.983°, 56.683°, 47.645°, 36.374°, 34.552° and 31.873°, related to the Miller indices ((202), (004), (201), (112), (200), (110), (102), (101), (002) and (100), (002), (101), (102), (110), (103), (200), (112), (201), (004) and (202), also the analysis of zinc oxide nanoparticles using Transmission Electron Microscopy (TEM) and Field Emission Scanning Electron Microscopy (FESEM). Propolis and propolis are zinc oxide-enhanced (ZnO NPs) with anti-Candida albicans activity. They achieve a high concentration with a significant thermogenic effect. The tablets are available in 2 TB packs, with dosages of 50 µg/ml (16 mm - 20 mm), 75 µg/ml (moisture-activated area), and 100 µg/ml (23 mm diameter), with an external control for swelling (0 mm).

KEYWORDS: nanoparticles, zinc oxide, Miller indices, Candida albicans activity

INTRODUCTION

Fungal infections have become a major global healthcare challenge due to the increasing prevalence of opportunistic pathogens and the emergence of resistance to conventional antifungal agents[1]. Among these pathogens, Candida albicans remains the most common causative agent of superficial and systemic candidiasis, particularly in immunocompromised patients[2]. The extensive use of antifungal drugs has accelerated the development of resistant strains, reducing treatment efficacy and emphasizing the urgent need for innovative, safe, and environmentally sustainable antifungal strategies[3].

Zinc oxide nanoparticles (ZnO NPs) have attracted considerable attention because of their unique physicochemical characteristics, including high surface area, excellent chemical stability, biocompatibility, and intrinsic antimicrobial activity[4]. Numerous studies have demonstrated that ZnO nanoparticles exert antifungal effects through multiple mechanisms, such as the generation of reactive oxygen species (ROS), disruption of fungal cell membrane integrity, release of Zn^{2+} ions, and induction of oxidative stress[5]. These properties make ZnO nanoparticles promising candidates for biomedical, pharmaceutical, and antimicrobial applications.

Recently, green synthesis has emerged as an environmentally friendly alternative to conventional chemical and physical methods for nanoparticle production[6]. Plant-derived materials serve as natural reducing, stabilizing, and capping agents, eliminating the need for hazardous chemicals while improving nanoparticle biocompatibility[7]. Propolis is a natural resinous substance rich in flavonoids, phenolic acids, caffeic acid derivatives, and aromatic compounds with well-documented antimicrobial and antioxidant activities[8]. Likewise, pomegranate (Punica granatum) peel is an abundant agricultural by-product containing high concentrations of polyphenols, tannins, ellagic acid, gallic acid, and other bioactive compounds that exhibit potent antioxidant and antifungal properties[9]. The combination of these natural products with ZnO nanoparticles is expected to produce synergistic antimicrobial effects while enhancing nanoparticle stability and reducing aggregation.

Therefore, the present study aimed to synthesize zinc oxide nanoparticles through an environmentally friendly electrochemical approach followed by green coating using propolis and pomegranate peel extracts. The synthesized nanocomposite was characterized by X-ray diffraction (XRD), transmission electron microscopy (TEM), field emission scanning electron microscopy (FESEM), and energy-dispersive X-ray spectroscopy (EDX) to evaluate its structural and morphological properties. Furthermore, its antifungal activity against Candida albicans was investigated using the agar well diffusion method at different concentrations to assess its potential as a novel, eco-friendly antifungal nanomaterial for future pharmaceutical and biomedical applications.

2. MATERIALS AND METHODS

Experimental Procedure for Detecting Antifungal Activity

2.1 Study Design

An experimental laboratory study was applied to investigate the antimicrobial activity of Propolis and Pomegranate Peel after coating with zinc oxide nanoparticles against fungal isolates.

2.2 Collection of Microbial Isolates

Isolates of Candida albicans were used in this study. The isolates were obtained from laboratory cultures and maintained under appropriate microbiological conditions.

2.3Electrochemical Preparation of Zinc Oxide Nanoparticles (NPs)

The electrochemically prepared zinc oxide nanoparticles consist of two electrodes: a pure metal (Zn) for synthesizing ZnO nanoparticles, which serves as the positive electrode, and a graphite electrode as the negative electrode. All electrodes are connected to a DC power source. Both the positive and negative electrodes are washed with ethanol and acetone, followed by deionized water. The electrodes were immersed in a 200 mL solution containing 5 mL of potassium chloride (KCl) as the electrolyte, 10 mL of a stabilizer (polyvinyl alcohol (PVA)), and 185 mL of distilled water, as shown in Figure 1. Upon application of a voltage, the reaction began. The solution was stirred for 1 hour at approximately 30 °C, resulting in the formation of a white precipitate of zinc hydroxide (ZnOH) nanoparticles. After centrifugation, the precipitate was washed several times with deionized water, acetone, and ethanol, then poured into a drying vessel and dried at 100 °C for 1 hour, followed by calcination at 700 °C for 1 hour[10, 11].

Figure 1. Electrochemical preparation of zinc oxide (NPs)

2.4 Preparation and Extraction

Initially, the mixture was prepared by combining 15 grams of raw propolis with 5 grams of pomegranate peel. This mixture was then dissolved in 100 ml of 70% ethanol and stirred with a magnetic stirrer for one hour. For extraction, the solution was stored in a dark-colored bottle in a dark place for ten days. Afterward, the mixture was filtered using filter paper, and the resulting filtrate was concentrated in a water bath until a thick, waxy, viscous mass was obtained. This mass was left at room temperature for two additional days to ensure the ethanol had completely evaporated. The final formulation was obtained by adding 50 ml of olive oil to every 5 grams of the extracted waxy material[12].

Figure 2. Extraction process of Propolis and Pomegranate Peel

2.5 Coating Zinc Oxide Nanoparticles with Propolis and Pomegranate Peel Extract

A green synthesis approach is commonly used to coat zinc oxide nanoparticles with propolis and pomegranate peel extract. In this process, the plant extract acts as both a reducing agent and a coating agent, forming a bio-organic coating around the nucleus of the zinc oxide nanoparticles. Initially, an ultrasonic treatment is used to increase the surface area, preventing the agglomeration of the zinc oxide nanoparticles, propolis, and pomegranate peel extract for 5 minutes. The zinc oxide nanoparticles are then added to the extract with continuous stirring. To facilitate coating and precipitation, the pH of the mixture is typically adjusted to the alkaline range. As the reaction progresses, the phytochemicals bind to the surface of the formed nuclei, preventing agglomeration and stabilizing the particles. The resulting precipitate is then separated by centrifugation, washed with water or ethanol to remove unbound sediment, and dried at a low temperature to preserve the organic coating. The final product consists of nanoparticles coated with a layer of natural antioxidants[13].

Figure 3. Sonication process of Propolis and Pomegranate Peel

2.6Antifungal Activity of Propolis and Pomegranate Peel–ZnO Nanoparticles

Preparation of fungal inoculum

Candida albicans can be readily cultured using Sabouraud Dextrose Agar (SDA) cultures at 35–37°C for 24 hours or by incubation. Gribkov 0.5 suspensions are used in standard McFarland tubes, including 1×10^6 CFU/ml.

Test Conditions: Propolis containing sterile distilled zinc oxide (e.g., 5% DMSH) is dispersed in water. Dacian Buona concentrations:

- 50 µg/ml
- 75 µg/ml
- 100 µg/ml. A 10–15 minute suspension can be ultrapure and remains so even after dispersal.

Clear Diffuse Agar Analysis: Candida albicans is inoculated into a sterile plastic container with Candida albicans. A sterile tube is used for thorough inoculation. Sterile 1 mm diameter boric acid is used as a sterile salt. 100 ml of purified water for the suspension.

Quality Control:

Distillation Control: Sterile distiller (e.g., a still).

Environmental Control: Standard preservative preparation, used for fluconazole (25 micrograms/tablet), such as nystatin.

2.7 Incubation

Plastic incubator, 35-37°C temperature, 24-48 hours in hot water.

2.8 Mirtan

The incubator is a high-tension incubator with a severe support diameter of mm (mm). A great outdoor experiment is more than wonderful, resulting in good results outdoors or at home ± standard deviation.

3 RESULTS AND DISCUSSION

3.1 XRD Analysis of zinc oxide nanoparticles.

The values 72.58°, 69.191°, 68.051°, 66.52°, 62.983°, 56.683°, 47.645°, 36.374°, 34.552° and 31.873°, related to the Miller indices ((202), (004), (201), (112), (200), (110), (102), (101), (002) and (100), (002), (101), (102), (110), (103), (200), (112), (201), (004) and (202), indicate that the phase of the zinc oxide nanoparticles is wurtzite, as shown in Figure 4 [14]

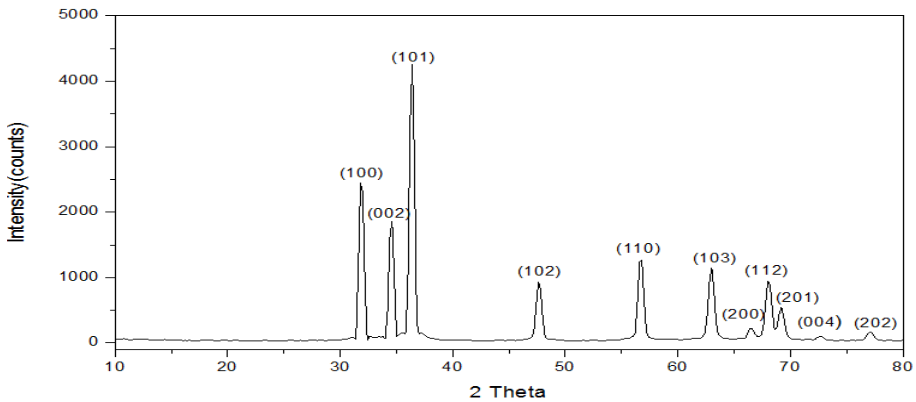

Figure 4. X-Ray Diffraction pattern of ZnO NPs.

3.2 Analysis of Zinc Oxide Nanoparticles Using Transmission Electron Microscopy (TEM).

The three-dimensional images of the zinc oxide nanoparticles show spherical and cubic shapes at the nanoscale. Both individual and aggregated zinc oxide nanoparticles are observed, as illustrated in Figure 5. The zinc oxide nanoparticles were synthesized using electrochemical methods[15].

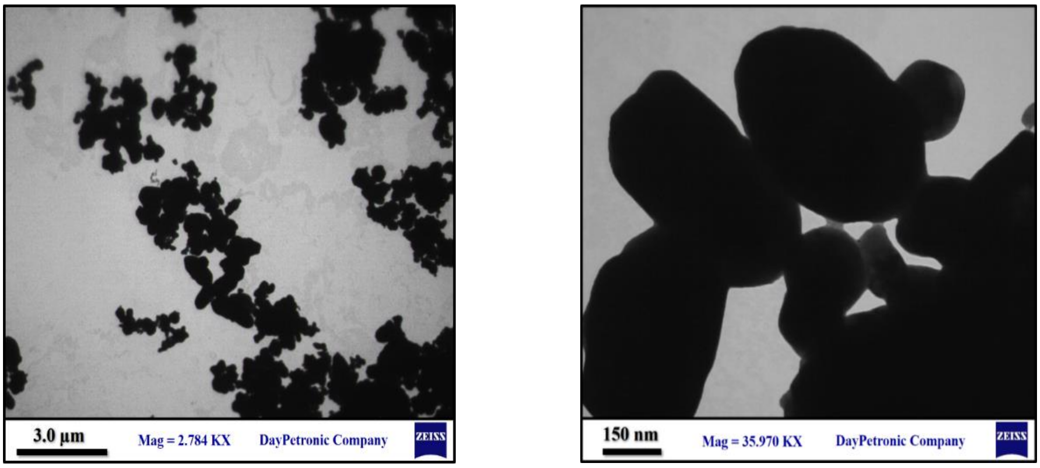

Figure 5. TEM images of ZnO NPs.

3.3 Analysis of Zinc Oxide Nanoparticles Using Field Emission Scanning Electron Microscopy (FESEM).

The morphology, size, and shape of zinc oxide nanoparticles were characterized using field emission scanning electron microscopy (FESEM), which provides high-resolution images of the zinc oxide nanoparticle surface. The FESEM images of the zinc oxide nanoparticles show a rice-grain-like shape[16], and agglomeration is also observed, as illustrated in Figure 6.

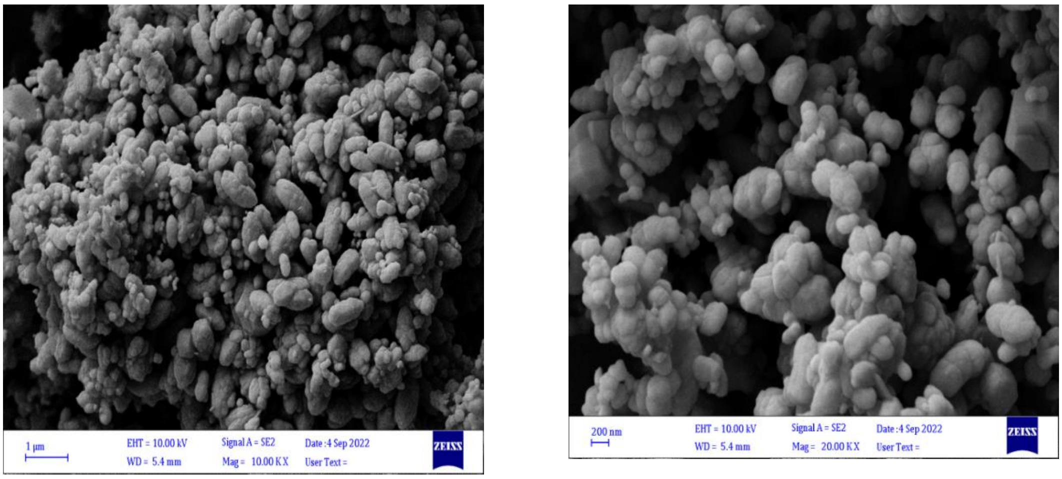

Figure 6. FESEM images of ZnO NPs.

3.4 EDX Analysis of Zinc Oxide Nanoparticles

The purity and composition of the electrochemically synthesized zinc oxide nanoparticles were determined using EDX measurements, as shown in Figure 5. Oxygen and zinc signals are present, indicating the purification of the zinc oxide nanoparticles from impurities. The composition of the zinc oxide nanoparticles was as follows: oxygen = 11.6%, zinc = 88.4%. Gold, used as a coating material according to the measurements, was also detected[14] .

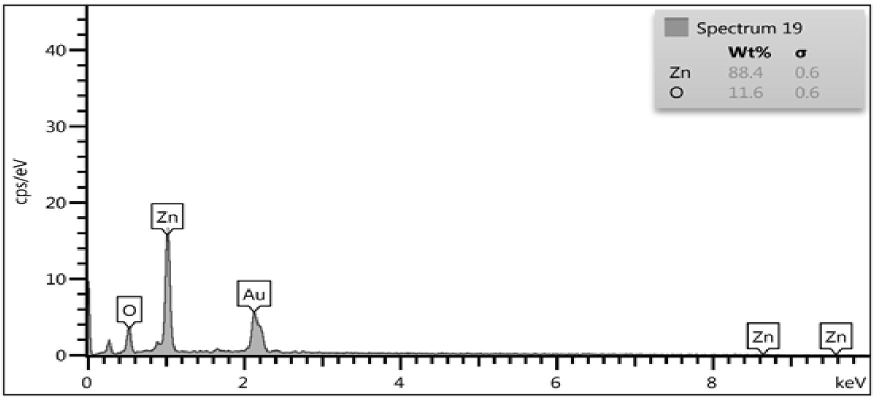

Figure 7. EDX -ray of ZnO NPS.

3.5 Discussion of the Antifungal Activity

Propolis and propolis are zinc oxide-enhanced (ZnO NPs) with anti-Candida albicans activity. They achieve a high concentration with a significant thermogenic effect. The tablets are available in 2 TB packs, with dosages of 50 µg/ml (16 mm - 20 mm), 75 µg/ml (moisture-activated area), and 100 µg/ml (23 mm diameter), with an external control for swelling (0 mm). In scrofula, the accumulated zinc oxide leads to a concentration ofCandida albicans spores, exhibiting remarkable antibacterial efficacy.

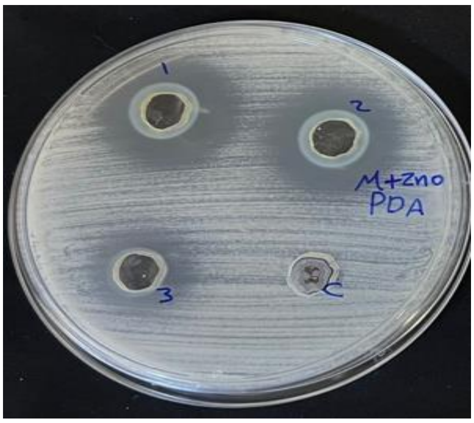

Figure 8. Representative agar well diffusion assay showing the antifungal activity of propolis and pomegranate peel-coated ZnO nanoparticles against *Candida albicans* at different concentrations.

The agar plate image shown in Figure 8 confirms the quantitative results listed in Table 2. Clear and distinct inhibition halos appear around the wells containing the tested formulation, while no inhibition is observed around the control well. The gradual expansion of inhibition zones with increasing concentration indicates better diffusion of biologically active compounds and greater antifungal activity. This correspondence between the photographic evidence and the measured inhibition diameters supports the reliability of the experimental results.

The enhanced antifungal activity may be attributed to the synergistic interaction between zinc oxide nanoparticles, propolis, and pomegranate peel extract. Zinc oxide nanoparticles possess intrinsic antifungal properties through multiple mechanisms, including generation of reactive oxygen species, disruption of fungal cell membrane integrity, changes in membrane permeability, release of zinc ions ($Zn2+$), and their degradation in oxidative stress and stress. The fungal cell. Zinc oxide nanoparticles are characterized by a high surface area-to-volume ratio, increasing their interaction with fungal cells and enhancing their antimicrobial efficacy.

Propolis provides additional antifungal activity given its richness in flavonoids, phenolic acids, caffeic acid derivatives, and aromatic compounds. These phytochemicals interfere with fungal cell wall construction, inhibit membrane-bound enzymes, disrupt ergosterol synthesis, and inhibit fungal growth. Pomegranate leaf extract also contains large amounts of soluble tannins, ellagic acid, gallic acid, catechins, and other polyphenols, which damage fungal membranes, precipitate cell proteins, and inhibit essential metabolic enzymes. These natural compounds combined provide multiple mechanisms of antifungal action complementary to the efficacy of zinc oxide nanoparticles.These natural compounds combined provide multiple mechanisms of antifungal action that complement the efficacy of zinc oxide nanoparticles. The encapsulation of zinc oxide nanoparticles with a natural extract may contribute to increase their stability, prevent their excessive agglomeration, improve their dispersion in biological media, and increase the content of active phytochemical compounds on their surface. Thus, fungal cells are simultaneously subjected to oxidative damage induced by zinc oxide nanoparticles, and biochemical inhibition induced by phytopolyphenols, resulting in a synergistic antifungal effect that exceeds the effect of each component separately.

The increase of inhibition zones with increasing concentration indicates that higher concentrations provided a larger number of active nanoparticles and phytochemical compounds molecules capable of interacting with fungal cells. And as the concentration increased from 50 to 100 µg/mL, the diameter of the inhibition zone increased by approximately 44% (from 16 mm to 23 mm), indicating a remarkable improvement in antifungal efficacy. This study shows that the antifungal effect is dose-dependent, supporting the use of this nanocomposite as an effective antifungal formulation.The graphical representation of these results also shows the positive correlation between concentration and antifungal activity. And the ascending curves, corresponding to increasing concentrations, clearly indicate that the fungal inhibition increases gradually with increasing nanocomposite concentration. These graphical trends provide immediate visual confirmation of the quantitative data presented in Table 2 , and the reproducibility of the observed biological response is highlighted.

Overall, these results indicate that zinc oxide nanoparticles coated with propolis and pomegranate peels represent a promising green nanomaterial with significant antifungal activity against Candida albicans. The combination of nanoparticle-induced oxidative stress and the rich phytochemical composition of propolis and pomegranate peels provides multiple additive effects that reduce the likelihood of resistance emergence while increasing antifungal efficacy. These results support the application of this eco-friendly nanocomposite in pharmaceutical and biomedical formulations, wound healing, and topical antifungals.

Table 1. Antifungal activity of propolis and pomegranate peel-coated zinc oxide nanoparticles against *Candida albicans* at different concentrations.

Sample	Fungal	100	75	50	control
Propolis and Pomegranate Peel -zinc oxide nanoparticles	Candida	23	20	16	0

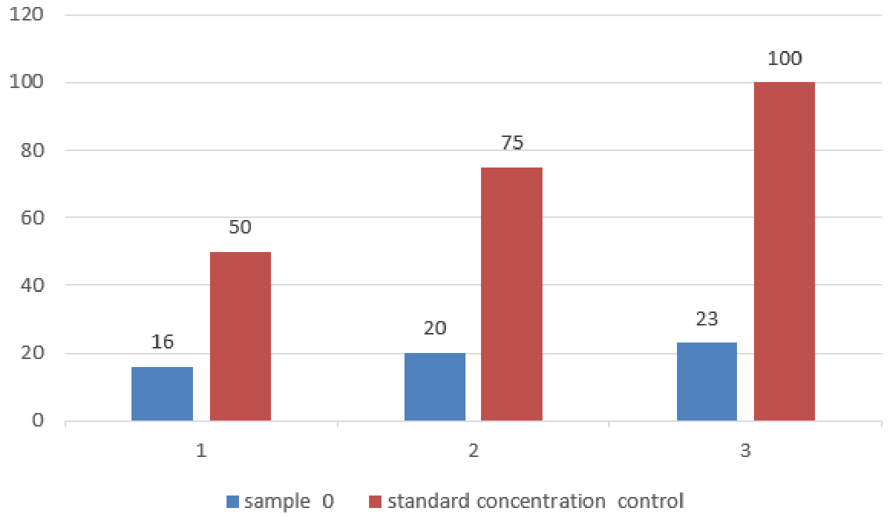

Figure 9. Effect of different concentrations of propolis and pomegranate peel-coated ZnO nanoparticles on the inhibition zone diameter of *Candida albicans*.

CONCLUSION

The present study successfully demonstrated the green synthesis of zinc oxide nanoparticles (ZnO NPs) using propolis and pomegranate peel extracts as environmentally friendly reducing and stabilizing agents. The synthesized nanoparticles were successfully characterized using physicochemical techniques, confirming the formation of ZnO nanoparticles with desirable structural and functional properties. The incorporation of bioactive phytochemicals from propolis and pomegranate peel enhanced the biological performance of the synthesized nanocomposite.

The antifungal evaluation revealed that the synthesized propolis and pomegranate peel-coated ZnO nanoparticles exhibited significant inhibitory activity against *Candida albicans*. A concentration-dependent increase in the inhibition zone was observed, with inhibition diameters increasing from 16 mm at 50 µg/mL to 23 mm at 100 µg/mL, indicating enhanced antifungal efficacy at higher concentrations. This activity is likely attributed to the synergistic effects of ZnO nanoparticles and the phenolic and flavonoid compounds present in the plant extracts, which collectively promote oxidative stress, disrupt fungal cell membranes, and inhibit fungal growth.

Overall, the findings indicate that the green-synthesized ZnO nanoparticles possess promising antifungal potential and may serve as a safe, cost-effective, and eco-friendly alternative to conventional antifungal agents. These results support the potential application of this nanocomposite in pharmaceutical, biomedical, and topical antifungal formulations. Further investigations involving cytotoxicity assessment, mechanism of action, and in vivo studies are recommended to validate their safety and therapeutic efficacy before clinical application.

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