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Eco-Friendly Fabrication of Olive Oil-Coated Zinc Oxide Nanoparticles and Their Antifungal Activity Against *Candida Albicans*

Zaher Kadhim Abbas[1*], Israa Sami Hadi[2], Wisam Taher Muslim[3]

[1,2,3]Department of Pharmaceutical Chemistry, College of Pharmacy, University of Kufa, Najaf, Iraq

ABSTRACT: The development of environmentally friendly nanomaterials with potent antimicrobial properties has garnered significant attention in biomedical applications. In this study, zinc oxide nanoparticles (ZnO NPs) were successfully synthesized using a simple electrochemical method and then coated with virgin olive oil using an environmentally friendly surface modification technique to enhance their stability and biological activity. The synthesized nanoparticles were characterized using X-ray diffraction (XRD), transmission electron microscopy (TEM), field emission scanning electron microscopy (FESEM), and energy dispersion analysis. The antifungal activity of the olive oil-coated ZnO NPs against Candida albicans was evaluated using a diffusion-well method on agar medium at concentrations of 50, 75, and 100 µg/ml. The nanocomposite exhibited concentration-dependent antifungal activity, producing inhibition zones with diameters of 17, 23, and 27 mm, respectively, while no inhibition was observed in the control group. The enhanced antifungal efficacy is attributed to the synergistic effect between zinc oxide nanoparticles and bioactive phytochemicals present in olive oil. These compounds promote the production of reactive oxygen species, release zinc ions, disrupt fungal cell membranes, and inhibit fungal growth. Furthermore, the olive oil coating improved nanoparticle dispersion and reduced aggregation, thereby enhancing the interaction between the nanoparticles and fungal cells. These findings demonstrate that olive oil-coated zinc oxide nanoparticles are a promising and environmentally friendly nanomaterial with potent antifungal activity and potential applications in biomedicine and antimicrobial pharmaceuticals.

KEYWORDS: Zinc oxide nanoparticles; Olive oil; Green synthesis; *Candida albicans*; Antifungal activity; Nanocomposite.

1. INTRODUCTION

Natural products continue to provide new molecular models for the discovery of synthetic drugs, including anticancer agents[1]. Extra virgin olive oil has garnered significant attention due to its antimicrobial activity against a wide range of pathogens. This premium olive oil is extracted directly from the fruit of the olive tree (Olea europaea) and cold-pressed without the use of chemicals, retaining most of its original bioactive components[2]. This unique combination of antioxidants, monounsaturated fatty acids, and polyphenols distinguishes extra virgin olive oil from other vegetable oils, undoubtedly contributing to its health benefits[3]. Fungal infections are a leading cause of infectious disease-related deaths worldwide. Among these species, Candida is the most common cause of invasive fungal diseases, with Candida albicans being the primary causative agent[4]. In healthy individuals, Candida albicans is often present as a harmless commensal organism in the oral cavity or gastrointestinal tract, except in severely immunocompromised patients. Systemic candidiasis can also lead to inflammation of the intestinal organs. Fungal hyphae are a key component contributing to the toughness of this yeast[5]. Here, the ability to transform from a yeast-like form to a silken form is associated with increased toughness and greater tissue penetration. Candida albicans is not inherently lethal, but it can become so as a result of dietary changes, antibiotic treatment, pH shifts, or immune system dysfunction, leading to proliferative overgrowth. In severe cases, vaginal, oropharyngeal, and intestinal candidiasis causes symptoms and skin disorders, representing an invasive multi-organ infection[6]. Candida albicans biofilms are characterized by regular incubation periods, and the long yeast cells release layers, allowing the bacteria to colonize new sites of infection[7]. The proliferative phase ensures the repetition of the "molecular life cycle" [8]. Despite the negative impact of fungi on human health, only a few antifungal drugs are available to treat this potentially fatal disease[8]. The nature of fungal cells as having a complete nucleus and the difficulty for drugs to bind to the fungal cell wall and membrane likely contribute to this. Antifungal drugs are also slowed down in elimination[9].

2. MATERIALS AND METHODS

Experimental Procedure for Detecting Antifungal Activity

2.1 Study Design
An experimental laboratory study was conducted to investigate the antimicrobial activity of olive oil coated 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.

2.3 Electrochemical 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].

Figure 1. Electrochemical preparation of zinc oxide (NPs)

2.5 Coating Zinc Oxide Nanoparticles with Olive Oil
A green synthesis approach is commonly used to coat zinc oxide nanoparticles with Olive Oil
. 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, Olive Oil for 5 minutes. The zinc oxide nanoparticles are then added to the Olive Oil 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[11].

2.6 Antifungal Activity of Olive Oil –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 2[12].

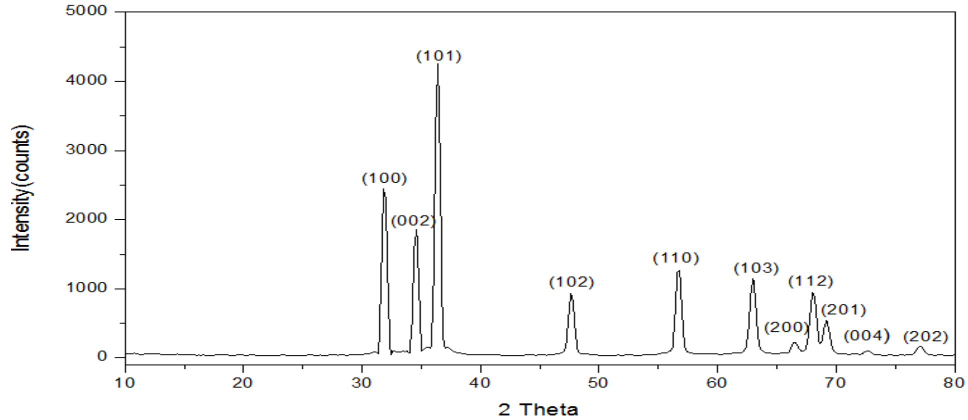

Figure 2. X-Ray Diffraction pattern of ZnO NPs.

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 3. The zinc oxide nanoparticles were synthesized using electrochemical methods[13].

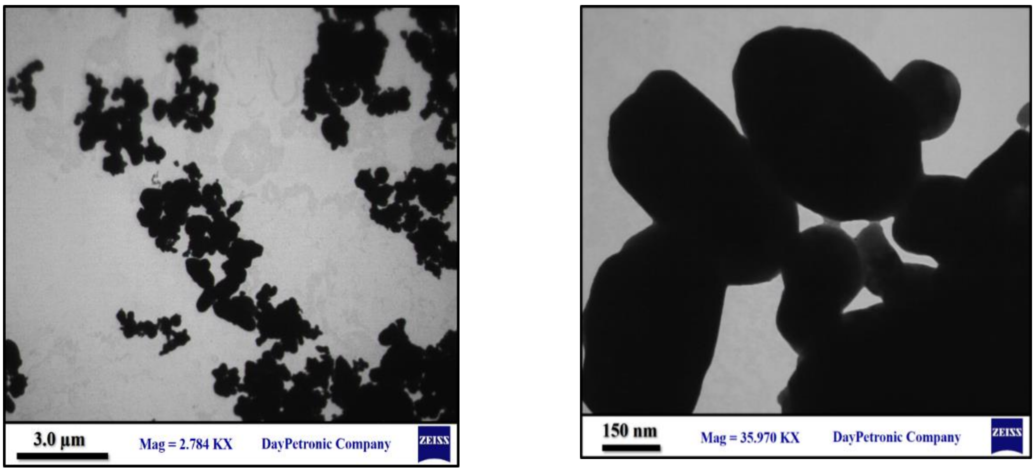

Figure 3. TEM images of ZnO NPs.

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, and agglomeration is also observed, as illustrated in Figure 4[14].

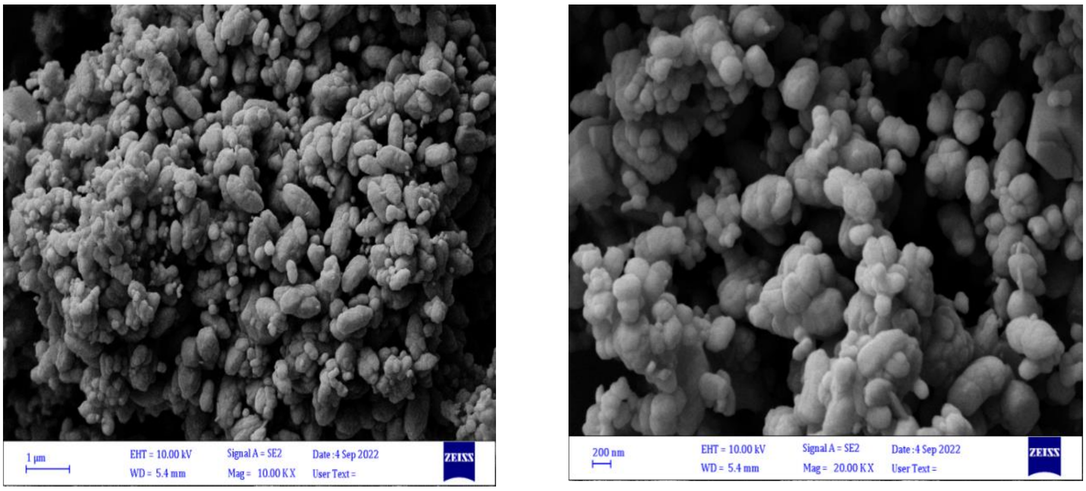

Figure 4. FESEM images of ZnO NPs.

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 (14). 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[15].

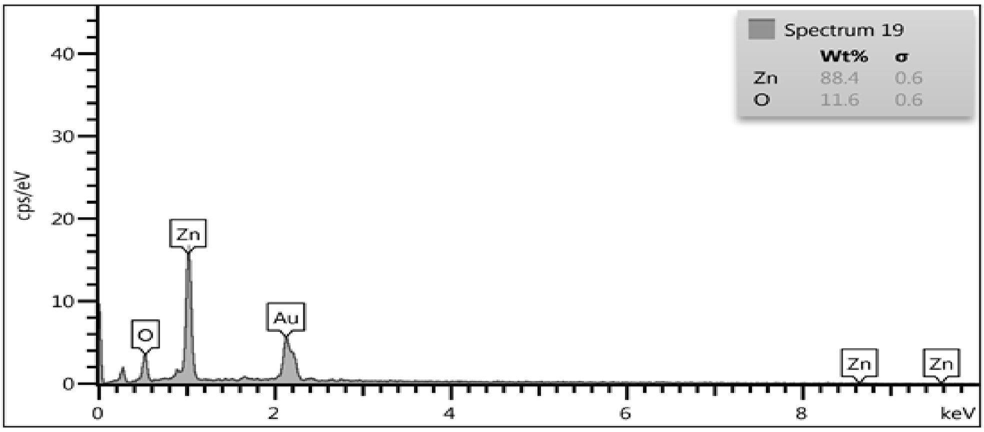

Figure 5. EDX -ray of ZnO NPS.

3.5 Discussion of the Antifungal Activity

Olive oil-coated zinc oxide nanoparticles as an antifungal agent against Candida albium

The antifungal activity of zinc oxide nanoparticles (ZnO NPs) coated with olive oil against Candida albium was evaluated by the agar-agar diffusion method. The results, shown in Table 2, demonstrated a clear concentration-dependent inhibitory effect. The diameter of the inhibition zone increased from 17 mm at 50 µg/ml to 23 mm at 75 µg/ml, and reached 27 mm at 100 µg/ml, while no inhibition was observed in the negative control sample (0 mM). These results indicate that increasing the concentration of zinc oxide nanoparticles coated with olive oil significantly potentiates their antifungal activity against Candida albium.

The photograph of Figure 7 confirms the quantitative data obtained in the diffusion test on agar. Clear and well-defined zones of inhibition were observed around the wells containing olive oil-coated zinc oxide nanoparticles, while no visible inhibition was observed in the control well.

Table 2. Antifungal activity of olive oil -coated zinc oxide nanoparticles against *Candida albicans* at different concentrations.

Sample	Fungal	100	75	50	control
Olive oil -zinc oxide nanoparticles	Candida	27	23	17	0

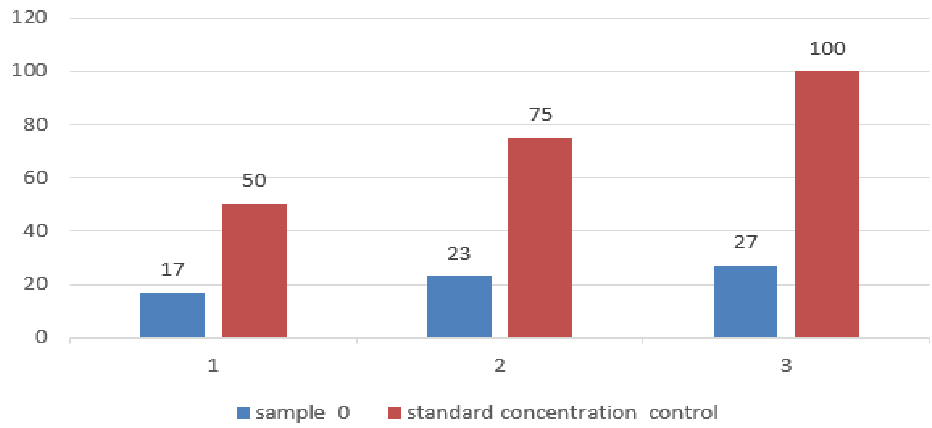

Figure 6. Effect of different concentrations of olive oil -coated ZnO nanoparticles on the inhibition zone diameter of *Candida albicans*.

The plot of Figure 6 illustrates the concentration-dependent antifungal response. The gradual increase in the diameter of the inhibition zone, from 17 mm to 27 mm, clearly demonstrates a direct correlation between nanoparticle concentration and antifungal activity. This trend indicates that higher concentrations provide a greater number of bioactive nanoparticles able to interact with the fungal cells, thus increasing cellular damage and reducing the viability of the fungus.

The observed antifungal activity can be attributed to the synergistic interaction between zinc oxide nanoparticles and bioactive components of olive oil, the generation of reactive oxygen species (ROS) in zinc oxide nanoparticles, the release of zinc ions ($Zn2+$), the alteration of the fungal membrane, and the increase in membrane permeability. The increase and stimulation of oxidative stress are antimicrobial properties that, ultimately, lead to fungal cell death. The nanometric size and large surface area of zinc oxide nanoparticles potentiate their interaction with fungal cells, thus improving their biological activity.

Olive oil contributes to potentiating antifungal effects thanks to its numerous bioactive compounds, among which are phenolic compounds such as hydroxytyrosols, tyrosols, oleuropein derivatives, flavonoids, tocopherols, and other antioxidant molecules. In addition to its effect on fungal cell membrane stability, inhibition of key metabolic enzymes, reduction of biofilm formation, and amelioration of oxidative damage caused by zinc oxide nanoparticles, the organic layers contributed by olive oil improve nanoparticle dispersion and stability, reducing their aggregation. This increases the effectiveness between the nanoparticles and the fungal cells, thus increasing the surface contact.

The broadened zones of inhibition observed in this study, compared to uncoated zinc oxide nanoparticles reported in prior studies, indicate that olive oil not only acts as a natural stabilizer but also as a source of antifungal phytochemicals that act synergistically with zinc oxide nanoparticles. This synergy improves the performance of nanoparticles, while simultaneously maintaining an environmentally friendly manufacturing methodology, in accordance with the principles of green nanotechnology. Overall, the results show that zinc oxide nanoparticles coated with olive oil present a promising antifungal activity against Candida albicans. The increase in inhibition zones, together with the pronounced inhibition halos observed in the agar diffusion assay, suggest that this green nanocomposite could be an effective alternative to conventional antifungal agents. The therapeutic efficacy of nanocomposites should be evaluated in greater depth.

Figure 7. Representative agar well diffusion assay showing the antifungal activity of olive oil -coated ZnO nanoparticles against *Candida albicans* at different concentrations.

4. CONCLUSION

This study successfully demonstrated the environmentally friendly synthesis of olive oil-coated zinc oxide nanoparticles using a sustainable manufacturing process. The structure and morphology were characterized using X-ray diffraction (XRD), transmission electron microscopy (TEM), phase-emission scanning electron microscopy (FESEM), and X-ray electrophoresis. The zinc oxide nanoparticles produce reactive oxygen species (ROS), release zinc ions (Zn^{2+}), and disrupt fungal cell membranes. Olive oil provides phenolic compounds and natural antioxidants that further damage fungal cells, inhibit biofilm formation, and enhance nanoparticle stability. These mechanisms collectively significantly enhance the susceptibility of Candida albicans to the nanoparticles. Overall, olive oil-coated zinc oxide nanoparticles represent a promising antifungal and environmentally friendly nanomaterial with potential biomedical applications. Its simple preparation method, biocompatible coating, and potent antifungal activity suggest that it may be used as an effective alternative or adjunctive therapy to conventional antifungal drugs. However, further studies are still needed to evaluate its cytotoxicity, biocompatibility, long-term stability, pharmacokinetics, and in vivo therapeutic efficacy before clinical application.

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