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Evaluation of the effect of green synthesis of Zinc oxide nanoparticles on Candida albicans

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ABSTRACT

The application of metal oxide nanoparticles, particularly zinc oxide, in medicine is essential, especially due to their physical and chemical characteristics and their antifungal potential. By the biological method, the zinc oxide nanoparticles were synthesized. This study aimed to investigate the in vitro activity generated by zinc oxide nanoparticles using a biological technique against Candida albicans in leukemia patients. The zinc oxide nanoparticles underwent characterization using Fourier transform infrared spectroscopy and XRD. The ability of the synthesized nanoparticles to inhibit human Candida albicans isolated from leukemia patients could be achieved by the Sabouraud Dextrose Agar method and differentiated using CHROM candida agar, germ tube test, and API Candida albicans. The antifungal activity of zinc oxide was investigated against Candida albicans. After assessing its susceptibility to several types of fungal antibiotics, thirty-three samples of Candida albicans developed resistance to fungal activity. Candida albicans inhibition zone diameters were 11, 12, 12, and 10 mm at zinc oxide nanoparticle concentrations of 400, 200, 100, and 50 mg/ml, respectively, while no inhibition zones occurred at low values of 25 mg/ml.

Keywords: Biosynthesis, Candida albicans, Nanomedicine, Nanoparticles; Zinc oxide.

ZnO NPs	Zinc Oxide Nanoparticles
C.albicans	Candida Albicans
SDA	Sabouraud Dextrose Agar
API	Analytical Profile Index
PDA	Potato Dextrose Agar

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1. INTRODUCTION

Leukemia is a malignancy that specifically targets the blood and bone marrow, which are responsible for the production of blood cells. Leukemia encompasses various forms, which are determined by the specific blood cell that undergoes aberrant changes and the rate at which the disease advances. Leukemia can manifest with symptoms such as hemorrhaging, contusions, exhaustion, elevated body temperature, and susceptibility to infections. Leukemia can also impact several organs, including the spleen, liver, and lymph nodes [1]. Leukemia is a serious and life-threatening disease that requires prompt diagnosis and treatment. Treatment methods for leukemia vary depending on the type, stage, and patient's condition. Some of the common treatments include chemotherapy, radiation therapy, targeted therapy, and bone marrow transplants. Nanotechnology is a new and promising field that uses tiny particles to deliver drugs or other substances to specific cells or tissues. Nanotechnology can help improve the effectiveness and reduce the side effects of leukemia treatment by targeting only the cancer cells and sparing the normal cells [2,3].

Leukemia can have a significant impact on the community and society, as it affects people of all ages and backgrounds. Leukemia can cause emotional, physical, and social challenges for the patients and their families, as they cope with the uncertainty, isolation, and distress of the disease. Leukemia can also impose a financial burden on the health care system and society, as the treatment and follow-up care can be costly and prolonged. Leukemia can also reduce the quality of life and productivity of the patients and their caregivers [4].

In the bone marrow of a child with leukemia, a large number of abnormal white blood cells are produced [5]. Leukemia is a prevalent cancer in young children, and acute lymphoblastic leukemia accounts for 75% of all cases. These children are more susceptible to bacterial and fungal infections, as well as the reactivation of viral diseases, due to immunosuppression caused by disease and treatment [6]. Candida species are typically harmless yeasts that exist in a mutually beneficial relationship with healthy individuals. However, they have the potential to cause infections throughout the body in immunocompromised individuals [7,8]. Candida albicans is a yeast that inhabits the digestive, respiratory, and female reproductive tracts of the human body and the skin [9]. Candida albicans is a prevalent fungal pathogen that is responsible for a significant number of superficial or invasive nosocomial infections [10,11]. Candida albicans candidiasis has been associated with a mortality rate of 40 percent [12] Candida albicans is simple to cultivate in the laboratory and both in vitro and in vivo can be studied [13].

On the other hand, Nanotechnology refers to the scientific investigation of materials at the nanoscale scale, which involves studying and characterizing elements such as fibers, particles, and grains with diameters smaller than 100 nm [14]. Nanomaterials are characterized as zero, one, two, or three-dimensional [15] according to their dimensions. The characteristics of nanoparticles vary from those of comparable atoms linked together to produce bulk materials and nanoparticles that are smaller than bulk materials but larger than atoms and molecules [16]. Nanoscale materials exhibit distinct behaviors in comparison to their macroscopic counterparts. Nanomaterials have been synthesized through a range of techniques, including precipitation, chemical vapor deposition, hydrothermal synthesis, milling, etching, sputtering, and laser ablation. These methods have been employed to take advantage of the larger surface-to-volume ratio exhibited by nanoparticles compared to bulk materials. Additionally, biological approaches involving bacteria and plants have also been utilized in the creation of nanomaterials [17]. ZnO NPs are considered to be a significant class of metal oxide nanoparticles. Zinc

oxide nanoparticles exhibit distinctive characteristics as an inorganic material, characterized by a white, insoluble powder and possessing an energy gap of 3.37 electron volts at standard ambient temperature [18,19]. In addition to industrial (rubber, concrete, and textile) and antibacterial and antifungal biological uses, they have been applied in a large array of sectors [20].

The rising incidence of fungal infections, namely those induced by Candida albicans, presents a substantial obstacle to public health. Conventional antifungal agents often exhibit limitations, including resistance development and adverse side effects. In this context, there is a growing need for innovative and sustainable approaches to combat fungal infections. The Biosynthesis of ZnO NPs presents an intriguing avenue, leveraging environmentally friendly methods to enhance the antifungal arsenal. Understanding the impact of these green-synthesized zinc oxide nanoparticles on Candida albicans is crucial for advancing the knowledge in the field of nanomedicine and developing alternative therapeutic strategies. The objective of the current research is to investigate the in vitro activity generated by zinc oxide nanoparticles using a biological technique against Candida albicans in leukemia patients. The antifungal efficacy of zinc oxide nanoparticles synthesized through green methods against Candida albicans has been systematically evaluated through clinical measurements.

2. MATERIALS AND METHODS

2.1 Collation of simple

Thirty-three (33) swabs were collected from hospitalized children with oral thrush, oral ulceration, microsites, mucosal ulceration, and white plaques who undergoing chemotherapy for acute leukemia, and these swabs were cultured on SDA and differentiated using (CHROM candida agar), germ tube test, and API candida.

2.2 Germ Tube test (GTT)

The ability of Candida albicans to produce short germ tubes after two hours of incubation in human blood serum at 37 °C makes this test a quick way to distinguish it from other species. In contrast to pseudo hyphae, which undergo origin-based contractions, germ tubes are extensions of the mother cell's daughter cells [21].

2.3 CHROM agar:

By streaking a loop of culture onto CHROMO agar Candida media and incubating for 48 hours at 37 °C, all Candida colonies on SDA, and PDA were sub-cultured. Based on colony color and phenotype, this selective and differential medium enables quick isolation and presumptive identification of numerous clinically significant Candida species. According to the description given, the CHROM agar medium contains a chromogenic substrate that reacts with the enzymes secreted by Candida species to produce colonies with distinct pigmentation [22].

2.4 Identifying Candida albicans

Candida albicans has resistance to various selected antifungal antibiotics. Candida albicans resistance patterns were identified using the Disk Diffusion Test (DDT). Fluconazole (25 mg), Ketoconazole (10 mg), and Itraconazole (50 mg) were the antifungal disks that were tested (25 mg). All of these antifungal antibiotics were ineffective against the Candida albicans isolates used in this study.

2.5 Antifungal of zinc oxide nanoparticles:

After pouring the PDA into a petri dish, a little swab of the most resistant Candida albicans sample was collected and dispersed uniformly over the surface of the PDA with a special sterile borer (5 mm in diameter). The PDA surface was then used to make wells in a petri dish. Different concentrations of zinc oxide nanoparticles (50, 100, 200, and 400 Ug/mL) were dissolved in sterilized deionized water and poured into the wells in 0.1 mL volumes, along with deionized water as a

control. Finally, in the incubator for 72 hours the dishes were placed. The formation of growth inhibitory zones was investigated at 37 °C. The diameter was measured in millimeters when it formed (mm) of the growth inhibition zone.

2.6 Preparation of bacterial suspension

The selected isolate was used to inoculate 250 ml of heart-brain infusion medium in a flask. A sterile conical flask test was then incubated for 60 hours at 37 °C in a vibrating incubator with 150 vibrations per minute. The sample was centrifuged for 25 minutes at 5000 cycles before being filtered. After centrifugation with filter paper and a filter, the sample was used to prepare zinc oxide nanoparticles.

2.7 Biosynthesis of ZnO NPs

The modified procedure [23] was used in the biosynthesis of zinc oxide nanoparticles. According to the following steps: First, take 25 ml of culture to contain bacteria Pseudomonas, put ida, and dilute with 75 ml D.W. The culture fluid was supplemented with 100 mg of $Zn(NO_3)_2$ (Sigma Aldrich) and maintained at a temperature of 37°C with a rotational speed of 50 rpm. After 24 hours, a white deposition started to form at the bottom of the flask, indicating the transformation of the ion. Third: The culture fluid was permitted to undergo incubation at ambient temperature and subsequently chilled. Following a period of two days of incubation on a rotary shaker, the reaction mixture had discernible accumulations of white clusters that had settled at the bottom of the flask. Fourth: Following the incubation period, the broth culture sample was subjected to centrifugation at a speed of 5000 revolutions per minute (rpm) for 15 minutes. The pellet and supernatant fractions were separated and subsequently subjected to a drying process. Next, the liquid portion was transferred onto a Petri dish and subjected to a drying process for two hours at a temperature of 400 °C in a hot air furnace, resulting in the formation of a solid powder specimen.

2.8 Nano characterization

Nano characterization refers to the comprehensive analysis and knowledge of structures and materials on the nanoscale, usually between 1 and 100 nanometers in diameter. Materials' characteristics and behaviors at this scale can vary greatly from those at the macroscopic level, making precise characterization essential for various scientific, industrial, and technological applications. To achieve this, a range of sophisticated instruments and techniques have been developed, collectively known as nano-characterization instrumentation.

2.8.1 Scanning Electron Microscopy (SEM)

SEM is a powerful imaging technique that utilizes electrons to scan the surface of a specimen, providing high-resolution, three-dimensional images. It is particularly effective for imaging surfaces and structures at the nanoscale, offering magnifications up to several hundred thousand times. SEM is essential for studying the topography, morphology, and composition of nanomaterials.

2.8.2 Transmission Electron Microscopy (TEM)

TEM takes imaging to the next level by transmitting electrons through a thin specimen, allowing for detailed examination of internal structures at the atomic scale. This technique is crucial for understanding the crystallography, defects, and chemical composition of nanomaterials.

2.8.3 Atomic Force Microscopy (AFM)

By methodically gliding a sharp tip over a specimen's surface, AFM can measure the forces exerted by the tip's interaction with the material. This technique provides topographical information at an extremely high resolution, allowing for the visualization of individual atoms. AFM is valuable

for studying surface roughness, mechanical properties, and molecular interactions of nanomaterials.

2.8.4 X-ray Photoelectron Spectroscopy (XPS)

XPS is a surface-sensitive technique that analyzes the elemental composition and chemical state of materials. By irradiating a sample with X-rays, the emitted photoelectrons are measured, providing information about the binding energies of atoms in the material. XPS is essential for characterizing the surface chemistry and electronic structure of nanomaterials.

2.8.5 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR is a spectroscopic technique employed to measure the amount of infrared radiation absorbed by a certain substance. It is commonly employed to identify functional groups, molecular vibrations, and chemical bonding in nanomaterials. FTIR is particularly valuable for studying the chemical composition and structure of organic and biological nanomaterials.

3. Result and Discussion

3.1 Antifungal susceptibility test

In this study, three popular antifungal medications (Terbinafine, Itraconazole, and Fluconazole) were utilized to determine the minimum inhibitory concentration (MIC) for antifungals using the excellent diffusion approach**.** Candida albicans were evaluated for their in-vitro susceptibility to certain antifungal medications. Table (1) reveals that Candida albicans have a minimum inhibitory concentration (MIC) of 50 g/ml and resistance at 25 g/ml. This research concurs with [24]. The primary allyl amine agent is terbinafine. Ergo sterol, a crucial sterol in the fungal cell's plasma membrane, is suppressed in its production. The enzyme squalene epoxidase, which catalyzes the transformation of squalene into squalene - 2, 3 epoxides, which is a precursor to lanosterol, which is a direct precursor of ergosterol, is inhibited by terbinafine. It can be used orally and topically [25] MIC of Itraconazole against Candida albicans was determined to be 25 g/ml. Our findings are consistent with those of stably [26]. Itraconazole is a member of the Azole family and is active against a broad range of pathogenic fungi, including Candida and dermatophytes. Candida albicans demonstrated MIC at (25 g /ml) when exposed to fluconazole. In contrast, research conducted by [27] in Baghdad indicated that fluconazole is ineffective against Candida species. Fluconazole's antifungal range is narrower than that of other azoles.

Table 1. MIC of Antifungal

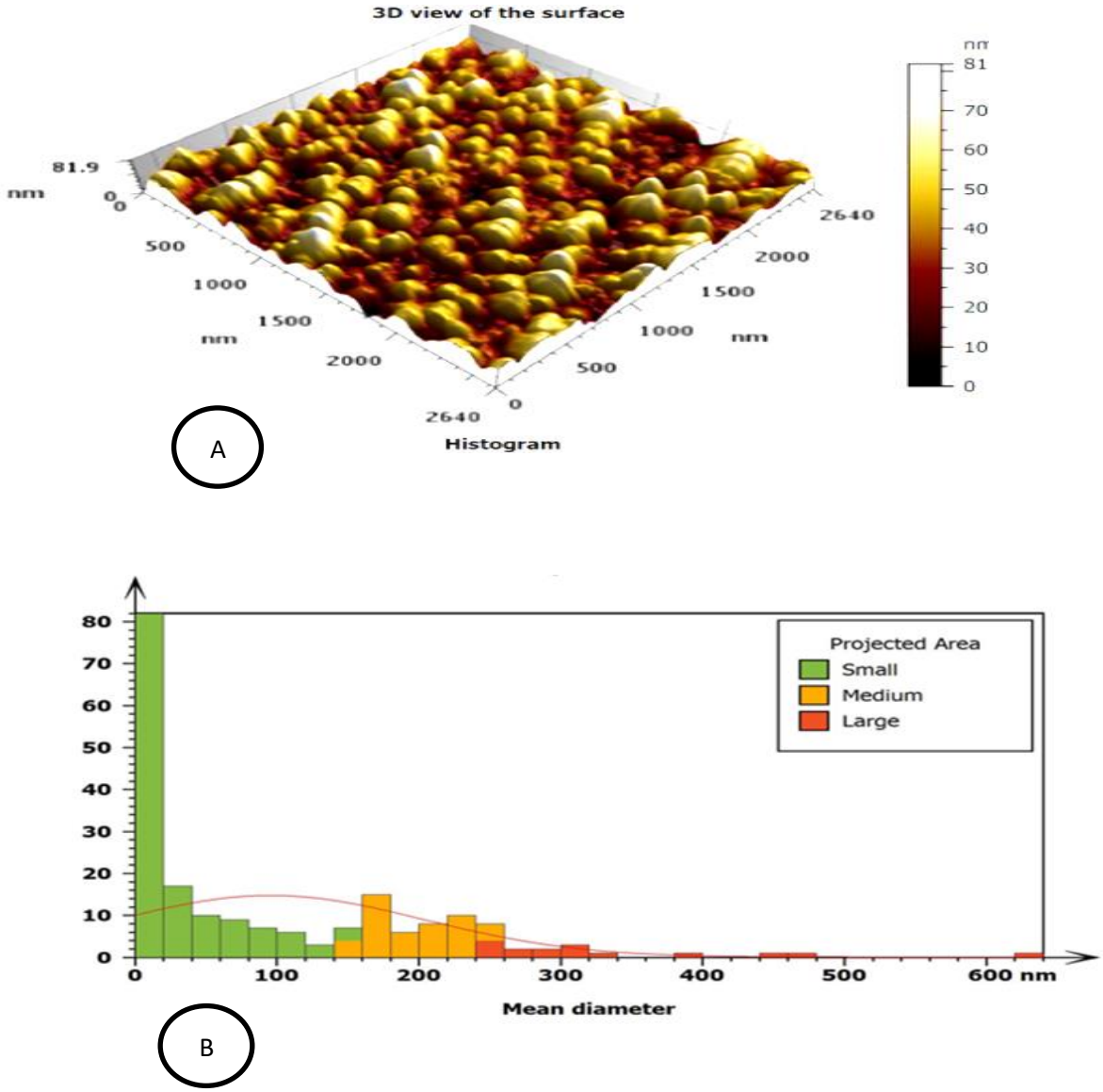
Fungal isolate	MIC of terbinafine (µg /ml)	MIC of Iitraconazole (µg /ml)	MIC of Fluconazole (µg /ml)
Candida alcicans	50	25	25

3.2 Biosynthesis of zinc oxide nanoparticles.

The color change could be correlated with the reduction of Zn^{+} into zinc oxide NPs during exposure to *Pseudomonas putida* culture filtrate. The freshly filtered culture was a dark yellow color However, the emulsion turned Wight after the addition of Zn $(NO_3)_2$ and 24 hours of incubation at a temperature of 37°C with a shaking speed of 50 rpm. Surface-plasmon resonance (SPR) is responsible for the alterations in color observed in aqueous solutions.

3.3 Characterization of ZnO NPs

A scanning probe microscope was employed to ascertain the topography and surface morphology. The atomic force microscope (AFM) offers both two-dimensional and three-dimensional depictions of the surface of the nanoparticle, as shown in Figure -1A. The particles were determined to have an average diameter in the nanoscale range. The size of zinc oxide nanoparticles was determined using AFM-SPM, and the findings indicate that the average size of the nanoparticles was 69 nm., as illustrated in (Fig.-1B).



Fig(1)**:** AFM characterization of zinc oxide nanoparticles. (A) Surface morphology**,** (B) Granularity distributed zinc oxide nanoparticles chart.

X-ray diffraction apparatus to obtain crystal and average particle size examined biologically created nanoparticles by *Pseudomonas putida* culture filtrate. (Fig. 2) The XRD spectrum displayed in the image shows the zinc oxide nanoparticles that were produced using a culture filtrate of Pseudomonas putida. The spectrum reveals the main peaks at 100, 002, 101, 102, 110, 103, 112, 004, and 104, which correspond to reflections with 2θ values of the Bragg angles 31.70°, 34.34°, 36.16°, 47.54°, 56.48°, 62.78°, 67.66°, 72.53°, and 76.58°, respectively. The results validate or substantiate that the material tested is zinc oxide nanoparticles and are of high purity. The average size of zinc oxide nanoparticles was extracted using the Dubai-sheerer equation and the average particle size was 20-40 nm [28].

The results of FTIR analysis showed two vertices which are 445.57cm. This refers to the transfer of bonds between the oxygen molecule and the zinc molecule resulting in the occurrence of two distinct forms of vibrations., as well as we note there is a weak peak and strong 1508.38 cm with a range (1500 to 1600 cm $^{-1}$) that indicates a carbon-carbon group (Alkane group or an aromatic ring). The peaks between (1500 -1700) cm^{-1} indicate the functional groups corresponding to the symmetrical and asymmetric expansion of the carbon-oxygen group (C-O) and the expansion mode 2850.88 cm^{-1}. This range represents the relationship between carbon and hydrogen (C-H) and the expansion mode in the presence of this group represents the presence of water molecules on the surface of zinc oxide nanoparticles.

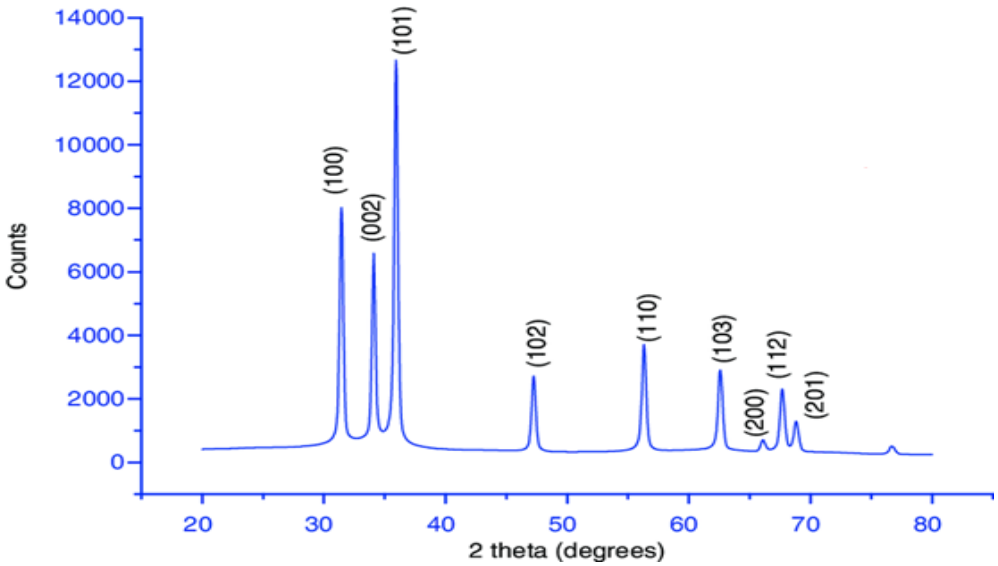


Fig 2. XRD of ZnO NPs.

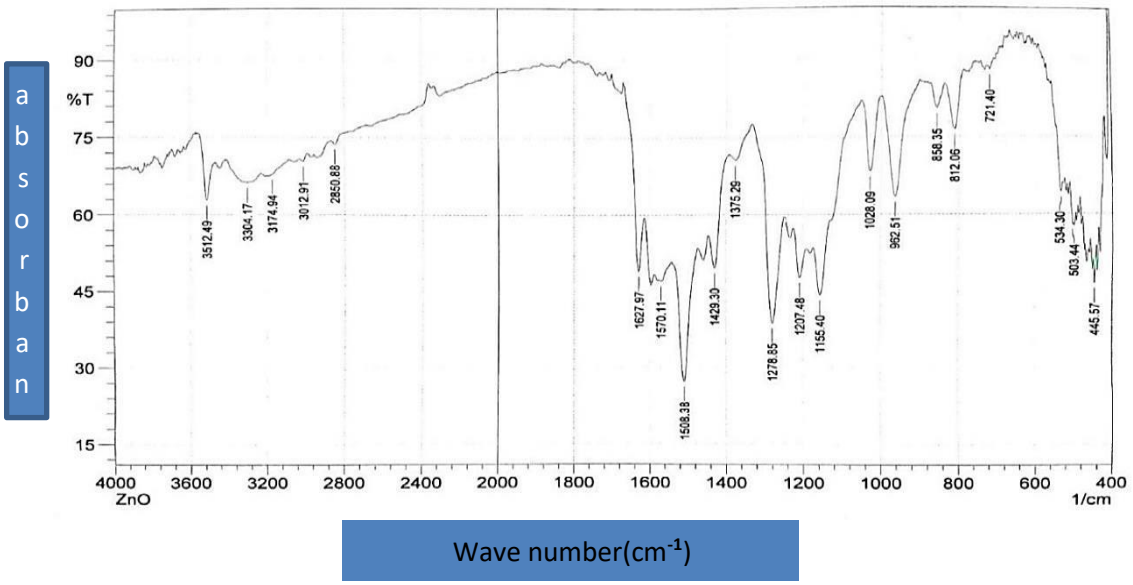


Fig 3. FTIR of biological zinc oxide nanoparticles.

3.4 Zinc oxide nanoparticles exhibit antifungal properties.

Using the agar well-diffusion method [29], Zinc oxide nanoparticles were tested for their antifungal activity against Candida albicans (Fig. 4). According to the findings, the inhibition zone had a diameter of (18 mm) at (400 µg /ml) of zinc oxide nanoparticles, as shown in (Fig. 4A) 11mm. At (200 µg /ml) of zinc oxide nanoparticles as shown in (Fig 4B), while the inhibition zone was (100 µg /ml) has a diameter of (10 mm) as in (Fig. 4C), However, when the other concentrations (12.5 µg /ml.) were used, no inhibition zones were seen (Fig. 4D). The precise molecular mechanisms underlying zinc oxide nanoparticles' antifungal activities still need to be clarified. In this research, we suggested a few likely mechanisms. Zinc oxide nanoparticles surface, which interacts with the fungal cell wall, can release Zn^{2+}, which can then pass through and accumulate in the cytoplasm [10]. Due to the disruption of cell metabolism, irreversible adherence of the nucleic acids, ribosome disassembly, protein denaturation, and electron chain disruption, all of these factors ultimately lead to cell death, Zn^{2+} can cause the fungal cell wall to become more permeable, which could then cause leakage of the plasma fluid and cellular organelles [29].

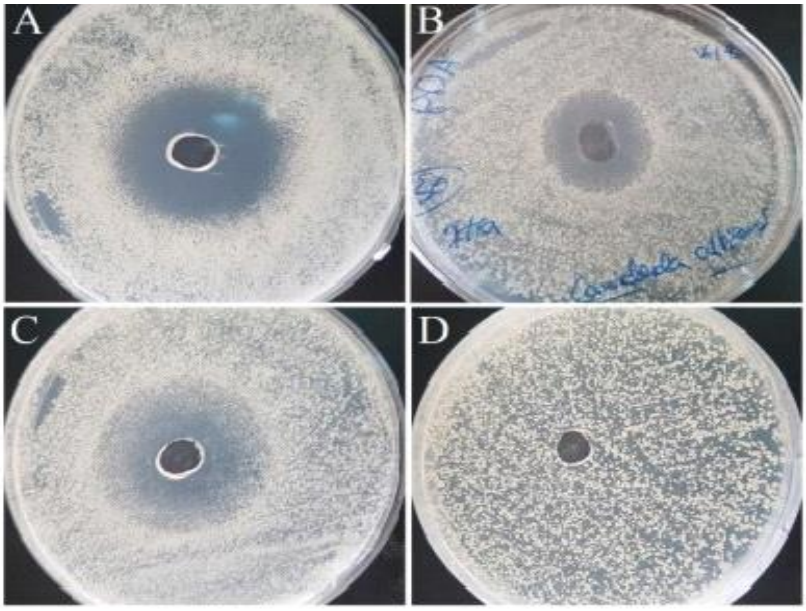


Fig. 4. Susceptibility test results of (A) 400 µg /ml, (B) 200µg /ml, (C) 100µg /ml, and (D) 50 µg /ml.

4. Conclusions

This study has elucidated the potential of green-synthesized zinc oxide nanoparticles zinc oxide nanoparticles as effective antifungal agents against Candida albicans. Through a comprehensive exploration of the synthesis process, physicochemical properties, and biological activities of the nanoparticles, several key findings have emerged. The green synthesis method utilizes successfully produced zinc oxide nanoparticles with well-defined characteristics, including size, shape, and surface morphology. The characterization techniques confirmed the presence of bioactive compounds from the green source, contributing to the unique properties of the synthesized nanoparticles. The findings of this study contribute to the broader field of nanomedicine and underscore the advantages of green synthesis in producing nanoparticles with enhanced biological activities. Future research should focus on refining the synthesis process, investigating in vivo efficacy, and exploring the potential application of green-synthesized zinc oxide nanoparticles in clinical settings. Ultimately, this work lays the foundation for the development of sustainable and effective antifungal agents, addressing the urgent need for alternative treatments against Candida albicans infections.

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Ethical approval

The project received approval from the local ethical commission at the University of Technology, Iraq.

Conflict of Interest:

All authors declare that they have no conflict of interest.

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