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RESEARCH PAPER

Synergistic Antifungal Activity of Zinc Nanoparticles Combined with Garlic Extract Against Clinical Isolates of Candida albicans from Immunocompromised Patients

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ABSTRACT

Candida albicans is the leading opportunistic fungal pathogen affecting immunocompromised patients, and the increasing emergence of antifungal resistance has highlighted the need for alternative therapeutic strategies. This study evaluated the antifungal activity of green-synthesized zinc nanoparticles (ZnNPs), garlic extract, and their combination against clinical Candida albicans isolates. A total of 126 clinical specimens were collected from immunocompromised patients in Wasit Province, Iraq. Candida isolates were identified using conventional microbiological methods, CHROMagar Candida, germ tube testing, and the VITEK 2 Compact system. Zinc nanoparticles were biosynthesized using garlic extract and characterized by UV-Vis spectroscopy, SEM, DLS, zeta potential, and FTIR analyses. Antifungal activity was evaluated using agar well diffusion and broth microdilution assays to determine inhibition zones, minimum inhibitory concentration (MIC), minimum fungicidal concentration (MFC), and the fractional inhibitory concentration index (FICI). Sixty-five Candida isolates were recovered, of which C. albicans was the predominant species (49.2%). Green-synthesized ZnNPs exhibited spherical morphology with an average particle size of 25.74 ± 8.75 nm. The ZnNPs-garlic extract combination demonstrated significantly greater antifungal activity than either ZnNPs or garlic extract alone ($P < 0.001$), producing the largest inhibition zone (28 ± 2.00 mm at 100% concentration). The combination also showed the lowest MIC (5 mg/mL) and MFC (10 mg/mL) values, with a FICI of 0.375, indicating a synergistic interaction. The combination of green-synthesized zinc nanoparticles and garlic extract exhibited superior antifungal activity against clinical Candida albicans isolates compared with either treatment alone. These findings suggest that this combination represents a promising eco-friendly antifungal strategy and warrants further in vivo and clinical investigations for the management of candidiasis

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INTRODUCTION

Candidiasis is one of the most important opportunistic fungal infections affecting humans, particularly immunocompromised patients. In recent years, fungal infections have become a major global health concern because they are associated with high morbidity and mortality rates. Recent studies estimate that fungal diseases are responsible for more than 1.5 million deaths annually, emphasizing their significant impact on public health [1]. The incidence of candidiasis has increased markedly owing to the growing number of immunocompromised patients, in addition to advances in medical treatments such as organ transplantation, chemotherapy, and the widespread use of immunosuppressive drugs. Furthermore, the COVID-19 pandemic increased the susceptibility of critically ill patients to secondary fungal infections, including candidiasis.

Candida albicans is considered the most common pathogenic species causing candidiasis in humans. Although it normally exists as a commensal microorganism in the oral cavity, gastrointestinal tract, and genitourinary tract of healthy individuals, alterations in the host immune system allow it to transform into an opportunistic pathogen capable of causing infections ranging from superficial mucosal lesions to life-threatening systemic infections [2]. Systemic candidiasis caused by C. albicans remains associated with a high mortality rate despite advances in diagnosis and antifungal therapy.Therefore, C. albicans continues to be the predominant fungal pathogen isolated from clinical specimens and represents a major challenge in immunocompromised patients [3].

Patients with diabetes mellitus, chronic kidney disease, malignancies, HIV infection, prolonged use of broad-spectrum antibiotics, corticosteroid therapy, and organ transplantation are more susceptible to C. albicans infection because of impaired immune function.In addition, the pathogenicity of C. albicans is enhanced by its ability to adhere to host tissues, invade epithelial cells, produce hydrolytic enzymes, and form biofilms, all of which contribute to persistence of infection and reduced susceptibility to antifungal agents .The treatment of candidiasis mainly depends on three major classes of antifungal agents, namely azoles, polyenes, and echinocandins. Although these antifungal drugs have markedly improved the management of Candida infections, their effectiveness has declined because of the increasing emergence of antifungal resistance, particularly among clinical isolates obtained from immunocompromised patients [4]. In addition, the ability of C. albicans to form biofilms further limits the efficacy of antifungal therapy by reducing drug penetration and increasing fungal tolerance, resulting in persistent infections and treatment failure.

Because of these limitations, considerable attention has been directed toward the use of natural products as alternative antifungal agents. Garlic (Allium sativum) is one of the oldest medicinal plants and has been extensively used in traditional medicine because of its antimicrobial properties. Garlic contains several bioactive organosulfur compounds, particularly allicin, in addition to various phenolic compounds, which have demonstrated significant antifungal activity against C. albicans. These compounds interfere with essential metabolic enzymes, alter cell membrane permeability, induce oxidative stress, and inhibit biofilm formation, thereby reducing fungal growth and virulence [5,6].

Recently, nanotechnology has become a promising approach for developing new antimicrobial agents. Zinc nanoparticles have attracted considerable attention because of their small particle size, large surface area, and enhanced biological activity compared with bulk materials. These unique properties increase their interaction with microbial cells and contribute to their antifungal activity through disruption of the fungal cell membrane, induction of reactive oxygen species, and interference with intracellular metabolic processes [7,8]. Furthermore, green synthesis of zinc nanoparticles using plant extracts has received increasing interest because it represents an environmentally friendly approach that combines the biological activity of medicinal plants with the beneficial properties of nanoparticles.

Recently, several studies have suggested that combining natural products with nanomaterials may improve antimicrobial efficacy through complementary mechanisms of action. Garlic extract contains a wide range of biologically active compounds, while zinc nanoparticles possess unique physicochemical characteristics that enhance their interaction with microbial cells. Therefore, combining garlic extract with zinc nanoparticles may provide greater

antifungal activity than either agent alone. Although numerous studies have investigated the antifungal activity of garlic extract or zinc nanoparticles separately, only limited studies have evaluated and compared the activity of garlic extract, zinc nanoparticles, and their combination against clinical isolates of Candida albicans recovered from immunocompromised patients.

Accordingly, the present study was designed to evaluate the antifungal activity of garlic extract, zinc nanoparticles, and their combination against clinical Candida albicans isolated from immunocompromised patients.

MATERIALS AND METHODS

Materials

The present study was carried out using standard microbiological and molecular laboratory equipment required for fungal isolation and antifungal susceptibility testing. The culture media used included Sabouraud's Dextrose Agar (SDA), Sabouraud's Dextrose Broth (SDB), HiCrome Candida Differential Agar, and Mueller Hinton Agar (MHA). Identification of Candida albicans isolates was confirmed using the VITEK 2 Compact identification system (bioMérieux, France) according to the manufacturer's instructions [9].

Methods

Location of the Study

This study was conducted in the Department of Biology, College of Science, University of Wasit, in collaboration with Al-Zahra Hospital, Al-Kut Hospital, the Public Health Laboratory, and Sheikh Saad Primary Health Center during the period from 1 August 2025 to 1 August 2026.

Sample Collection

A total of 126 clinical samples, including urine samples, vaginal swabs, oral swabs, and interdigital foot swabs, were collected from immunocompromised patients, including patients with diabetes mellitus, hemodialysis patients, elderly patients, and other immunocompromised cases, as well as pregnant women. Samples were collected using sterile swabs, transferred in transport media, and cultured on Sabouraud's Dextrose Agar (SDA) for the isolation of Candida species. Clinical information, including age, diabetes status, antibiotic use, clinical manifestations, and physician diagnosis, was recorded using astandardized questionnaire [10].

Isolation and Identification of Candida albicans

Culture media were prepared according to the manufacturers' instructions. Sabouraud's Dextrose Agar was prepared by dissolving 65 g/L of dehydrated medium in distilled water and sterilized by autoclaving at 121°C for 15 min. After cooling, chloramphenicol was added as an antibacterial agent before pouring into sterile Petri dishes. Clinical specimens were inoculated onto SDA plates and incubated aerobically at 37°C for 24-48 h. HiCrome Candida Differential Agar was prepared according to the manufacturer's instructions without autoclaving and used for differentiation of Candida species according to colony colour [11].

Presumptive C. albicans isolates were identified by colony morphology and microscopic examination. Gram staining was performed for preliminary identification, and the germ tube test was used as a rapid confirmatory test by incubating fresh colonies in human serum at 37°C for 2-3 h. Final confirmation of the isolates was carried out using the VITE 2 Compact identification system, in which standardized yeast suspensions (0.5 McFarland) were prepared in sterile saline and processed according to the manufacturer's instructions. Mueller Hinton Agar was prepared according to the manufacturer's recommendations and used for subsequent antifungal susceptibility testing [12].

Preparation of Garlic Extract

Fresh garlic (Allium sativum) bulbs were purchased from the local market, washed thoroughly, and dried at 60°C in the absence of light. The dried material was ground into a fine powder using an electric grinder. For extraction, 100 g of garlic powder was mixed with 100 mL of 30% ethanol and left for maceration at room temperature for 48 h with continuous stirring. The extract was then sonicated to improve the release of bioactive compounds and centrifuged at 10,000 rpm for 10 min. The supernatant was filtered through Whatman No. 2 filter paper, concentrated using a rotary evaporator at 60°C under reduced pressure, and finally freeze-dried to obtain a dry extract, which was stored at 4°C until further use [13].

Green Synthesis of Zinc Nanoparticles (ZnNPs)

Green synthesis of zinc nanoparticles was carried out using garlic extract as a reducing

and stabilizing agent. Different concentrations of garlic extract (2.5-10%, w/v) were mixed with 10 mL of 1 mM zinc sulfate (ZnSO4) solution and heated at 80°C for 0.5, 1, and 2 h to determine the optimum synthesis conditions. A control containing zinc sulfate solution without garlic extract was prepared under the same conditions. The formation of ZnNPs was indicated by a visible colour change of the reaction mixture, after which the synthesized nanoparticles were purified by dialysis against ultrapure water overnight to remove unreacted compounds. Based on the preliminary observations, nanoparticles synthesized using 10% garlic extract after 1 h were selected for further characterization.

Characterization of ZnNPs

The synthesized ZnNPs were characterized to evaluate their physicochemical properties. UV-Visible spectroscopy was used to determine the optical characteristics, whereas Fourier Transform Infrared Spectroscopy (FTIR) was performed to identify the functional groups involved in nanoparticle formation. The crystalline structure was determined by X-ray Diffraction (XRD), while particle morphology and size were examined

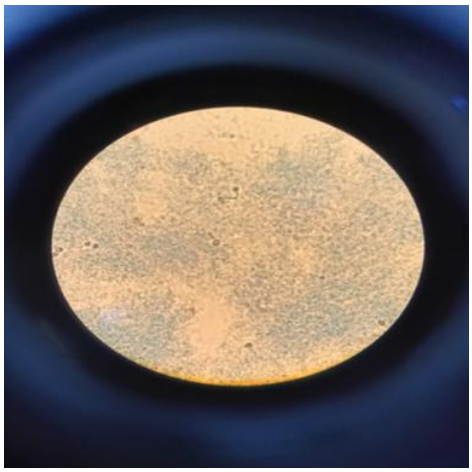

Fig. 1. Germ-tube formation in human blood serum after 3 hours incubation, which is a unique feature of c. albicans.

Fig. 2. Colony morphology and color change of *Candida albicans* on chromogenic medium.

using Field Emission Scanning Electron Microscopy (FESEM). In addition, the elemental composition was analyzed using Energy-Dispersive X-ray Spectroscopy (EDS), and particle size distribution was evaluated by Dynamic Light Scattering (DLS) [14].

Preparation of McFarland Standard

A 0.5 McFarland turbidity standard was used to standardize the yeast inoculum before antifungal susceptibility testing. The standard corresponded to approximately 1.5×10^8 CFU/mL and was prepared according to the recommendations of the Clinical and Laboratory Standards Institute [15].

Antifungal Activity Assessment

The antifungal activity of garlic extract, green synthesized zinc nanoparticles (ZnNPs), and their combination against Candida albicans isolates was evaluated using the broth microdilution method to determine the minimum inhibitory concentration (MIC). Fresh C. albicans colonies grown on Sabouraud's Dextrose Agar for 24 h were used for inoculum preparation. Three to five colonies were transferred into sterile tubes containing 4-5 mL of broth medium, and the suspension turbidity was adjusted to 0.5 McFarland standard before being diluted according to the Clinical and Laboratory Standards Institute (CLSI) recommendations [15].

Serial two-fold dilutions of garlic extract, ZnNPs, and their combination were prepared in sterile broth medium. Equal volumes of the standardized yeast inoculum were added to each dilution and incubated at 35°C under appropriate conditions. Growth inhibition was evaluated by visual examination, and the MIC was recorded as the lowest concentration showing complete inhibition of visible fungal growth [15].

The antifungal activity of the three tested preparations was compared by evaluating their inhibitory effects against C. albicans isolates. The obtained MIC values were used to determine the relative antifungal efficacy of garlic extract, ZnNPs, and their combination under the same experimental conditions. All experiments were carried out under aseptic laboratory conditions using standardized microbiological procedures to ensure the reliability and reproducibility of the obtained results [9].

RESULTS AND DISCUSSION

Isolation and Identification of Candida albicans

A total of 126 clinical samples were collected from immunocompromised patients suspected of candidiasis. Fungal culture confirmed 65 positive Candida isolates. Among these isolates, Candida albicans was the predominant species, representing 32 (49.2%) of the total isolates.

Therefore, C. albicans was selected for

Table 1. Comparative results of *Candida albicans* isolated from Candidiasis patients between Vitek 2 system and Candida chromogenic agar.

Candida isolates	No Candida	Candida chromogenic agar	Vitek 2 identification
		No Candida	No Candida
C. albicans	32	30	32

Table 2. Antifungal susceptibility pattern of Candida albicans.

Type antifungal	*C.albicans* n=32	
	S	R
amphotericin B	68.7%	31.3%
Itraconazole	53.1%	46.9%
Fluconazole	28.9%	71.1%
Nystatine	12.5%	87.5%
X^2	25.134	
Calculated P	0.000(S)	

subsequent evaluation of the antifungal activity of garlic extract, zinc nanoparticles (ZnNPs), and their combination. The remaining isolates included C. parapsilosis (24.6%), C. krusei (12.3%), C. tropicalis (9.2%), and C. famata (4.6%) [16].

Direct microscopic examination of the collected clinical specimens revealed the presence of yeast cells and pseudohyphae indicating Candida infection.

On Sabouraud Dextrose Agar (SDA), the isolates initially appeared as smooth, cream-colored colonies. After prolonged incubation, the colonies became wrinkled, whitish-creamy, and exhibited the characteristic yeasty odor of Candida species as shown in Fig. 1.

The germ tube test demonstrated positive germ tube formation in all Candida albicans isolates, whereas the remaining Candida species did not produce germ tubes, confirming the reliability of this method for the presumptive identification of C. albicans [17].

Subculture on Candida Chromogenic Agar differentiated the isolates according to colony color. Candida albicans produced characteristic green colonies, whereas the remaining species showed their specific chromogenic appearance as shown in Fig. 2 and Table 1. Identification using chromogenic agar was further confirmed by the VITEK 2 Compact identification system, which identified all 32 isolates as Candida albicans, with no significant difference between the two identification methods [18].

The antifungal susceptibility profile of the clinical Candida albicans isolates demonstrated

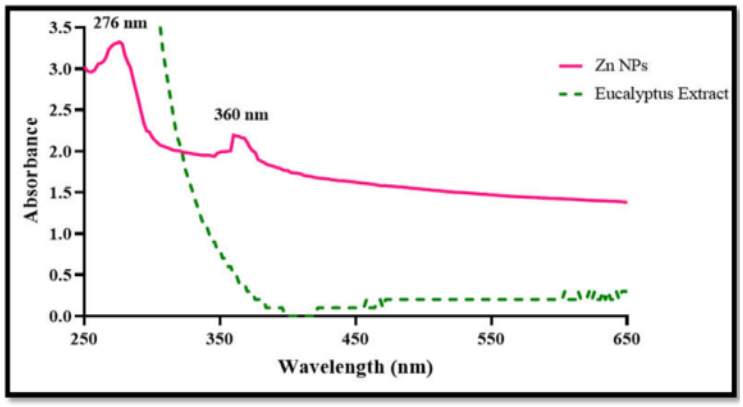

Fig. 3. Ultraviolet-Visible (UV-Vis) Spectroscopy.

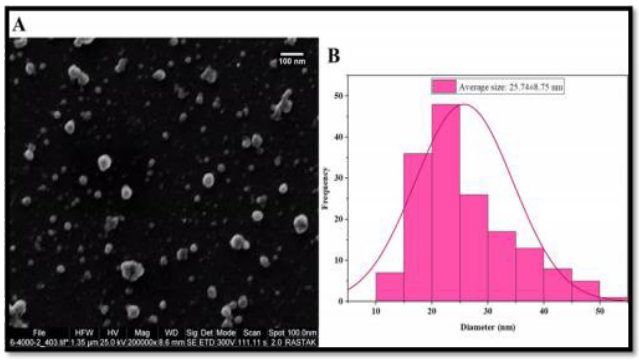

Fig. 4. SEM micrographs of biosynthesized ZnNPs: (A) SEM image of ZnNPs; (B) Statistical distribution of ZnNPs (25.74 ± 8.75 nm). Scale bars correspond to 100 nm.

that amphotericin B showed the highest sensitivity (68.7%), followed by itraconazole (53.1%). In contrast, high resistance rates were observed against fluconazole (71.1%) and nystatin (87.5%) as shown in Table 2. These findings indicate reduced susceptibility of the tested isolates to commonly used antifungal agents and support the evaluation of alternative antifungal agents such as garlic extract and ZnNPs [19].

The predominance of Candida albicans among the clinical isolates and its antifungal susceptibility profile supported its selection as the representative isolate for evaluating the antifungal activity of garlic extract, zinc nanoparticles, and their combination in the present study.

Characterization of Green-Synthesized Zinc Nanoparticles

UV-Visible spectroscopy showed two characteristic absorption peaks at 276 nm and 360 nm as shown in Fig. 3, indicating the successful reduction of zinc ions and the formation of ZnNPs [20].

Scanning Electron Microscopy (SEM) analysis demonstrated that the synthesized ZnNPs were predominantly spherical with a relatively uniform morphology as shown in Fig. 4. The average particle size was 25.74 ± 8.75 nm, confirming the successful formation of nanoparticles within the nanoscale range [21].

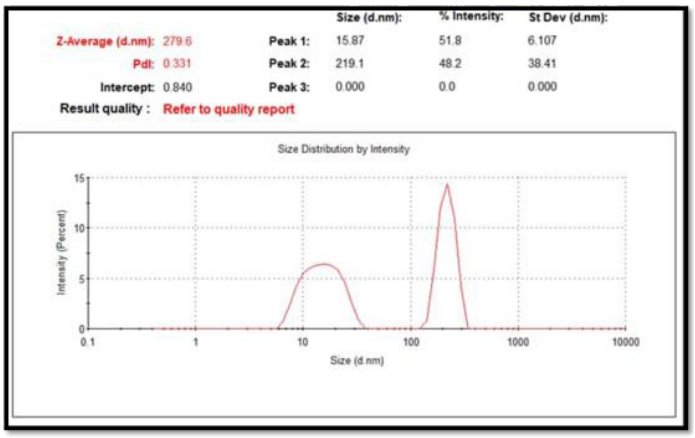

Fig. 5. Particle size distribution and polydispersity index (PDI) analysis of synthesized zinc nanoparticle.

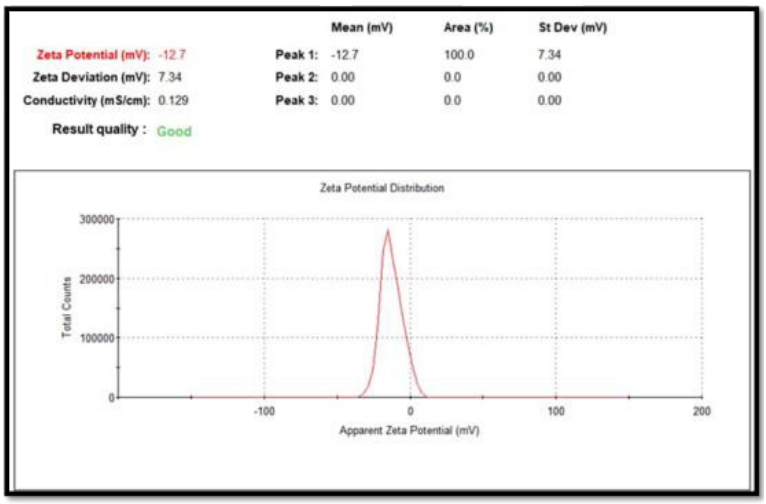

Fig. 6. The zeta potential of the ZnNPs.

Dynamic Light Scattering (DLS) analysis revealed that the synthesized nanoparticles had a hydrodynamic particle size of 279 nm with a polydispersity index (PDI) of 0.331 as shown in Fig. 5, indicating a relatively homogeneous nanoparticle population and acceptable size distribution for biological applications [21].

The zeta potential of the synthesized ZnNPs was -12.7 mV, suggesting acceptable colloidal stability and moderate nanoparticle dispersion as shown in Fig. 6.

FTIR analysis demonstrated changes in several functional groups after nanoparticle synthesis, indicating that garlic phytochemicals participated in the reduction and stabilization of ZnNPs as shown in Fig. 7. In addition, the appearance of Zn-O absorption bands confirmed the successful formation of zinc nanoparticles [20].

Overall, the characterization results confirmed the successful green synthesis of ZnNPs using garlic extract. The synthesized nanoparticles exhibited spherical morphology, nanoscale particle size,

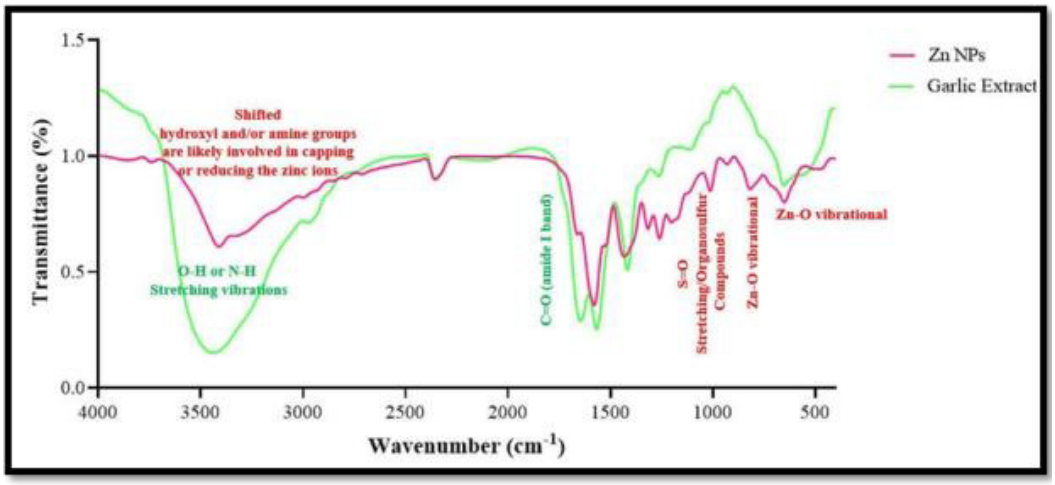

Fig. 7. FTIR spectrum of synthesized zinc nanoparticles.

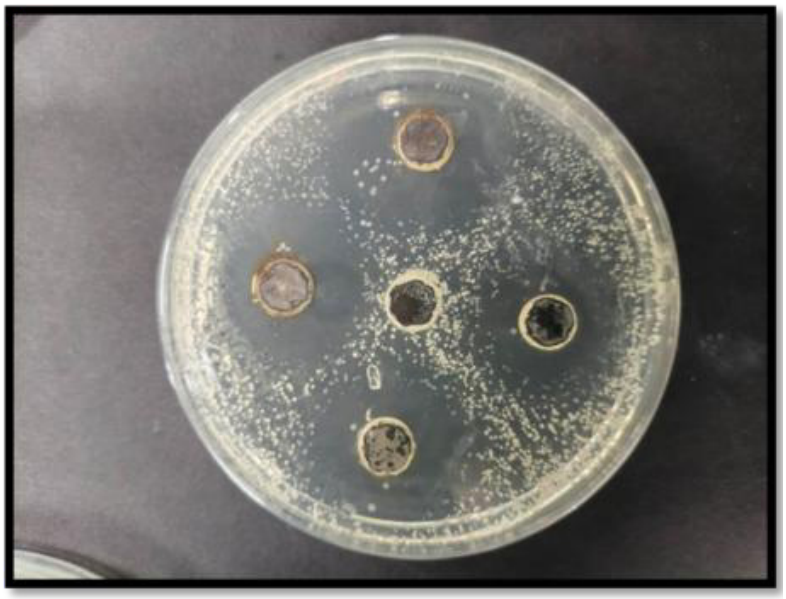

Fig. 8. Zinc NPs Susceptibility Test.

acceptable stability, and suitable physicochemical properties, supporting their application for evaluating antifungal activity against clinical Candida albicans isolates [22].

Antifungal Activity of Garlic Extract, Zinc Nanoparticles, and Their Combination Against Candida albicans

The antifungal activity of garlic extract, zinc nanoparticles (ZnNPs), and their combination against clinical Candida albicans isolates was evaluated using the agar well diffusion method at four concentrations (25%, 50%, 75%, and 100%).

Antifungal Activity of Zinc Nanoparticles

ZnNPs exhibited a concentration-dependent antifungal activity against *Candida albicans*.

The inhibition zone increased gradually with increasing nanoparticle concentration. The average inhibition zones were 15 ± 0.50 mm, 16 ± 1.00 mm, 20 ± 0.50 mm, and 23 ± 0.40 mm at 25%, 50%, 75%, and 100% concentrations, respectively as shown in Fig. 8. These findings indicate that increasing ZnNP concentration enhanced its inhibitory effect against C. albicans, which is consistent with previous reports describing the antifungal activity of ZnNPs [23,22].

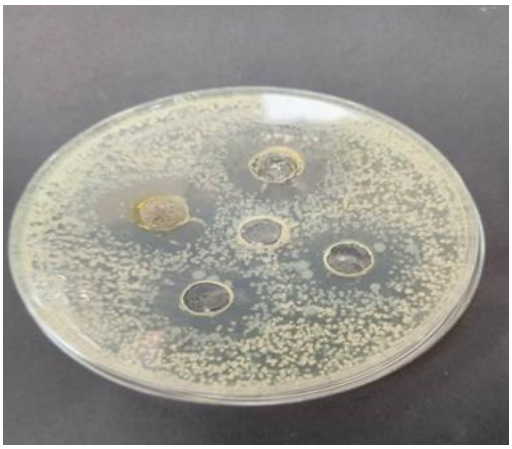

Fig. 9. Garlic Extract Susceptibility.

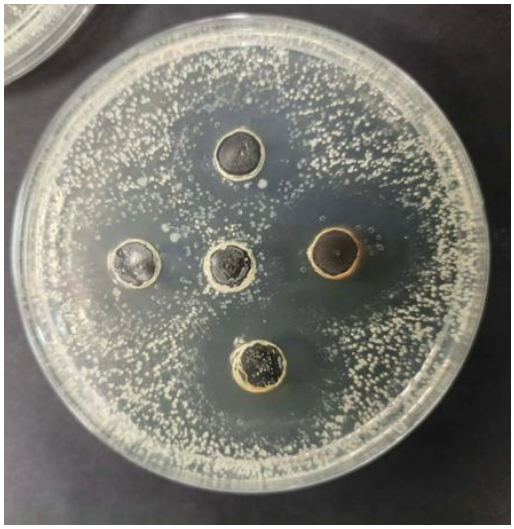

Fig. 10. ZnNPs –Garlic Extract mixture Susceptibility Test.

Antifungal Activity of Garlic Extract

Garlic extract also demonstrated antifungal activity against Candida albicans, although its inhibitory effect was lower than that of ZnNPs. The average inhibition zones were 9 ± 1.30 mm, 11 ± 0.20 mm, 12 ± 1.00 mm, and 14 ± 0.50 mm at 25%, 50%, 75%, and 100% concentrations, respectively as shown in Fig. 9. The observed antifungal activity is mainly attributed to sulfur-containing compounds, particularly allicin, which interfere with essential metabolic processes required for fungal growth [24].

Antifungal Activity of the ZnNPs-Garlic Extract Combination The combination of ZnNPs and garlic extract exhibited the highest antifungal activity among all tested preparations.

The average inhibition zones reached 15 ± 0.50 mm, 19 ± 1.00 mm, 23 ± 1.50 mm, and 28 ± 2.00 mm at 25%, 50%, 75%, and 100% concentrations, respectively as shown in Fig. 10. The largest inhibition zone was observed at the 100% concentration (28 ± 2.00 mm), demonstrating greater antifungal efficacy than either ZnNPs or garlic extract used alone.

Comparison of the three tested preparations demonstrated that the ZnNPs-garlic extract combination produced significantly larger inhibition zones than ZnNPs alone or garlic extract alone (P = 0.000) as shown in Table 3. These findings suggest that the combined treatment enhanced the antifungal activity against clinical Candida albicans isolates [20].

Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC)

The Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC)

of Garlic Extract, Zinc Nanoparticles, and Their Combination Against Candida albicans The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of garlic extract, ZnNPs, and their combination were determined against clinical Candida albicans isolates using the broth microdilution method.

The results demonstrated that ZnNPs exhibited greater antifungal activity than garlic extract alone. The MIC and MFC values of ZnNPs were 10 mg/mL (10,000 µg/mL) and 20 mg/mL (20,000 µg/mL), respectively. In comparison, garlic extract showed MIC and MFC values of 20 mg/mL (20,000 µg/mL) and 40 mg/mL (40,000 µg/mL), respectively as shown in Fig. 11.

The ZnNPs-garlic extract combination exhibited the strongest antifungal activity, with the MIC and MFC values reduced to 5 mg/mL (5,000 µg/

Table 3. The average of Antifungal Activity of Zinc nanoparticles, Garlic Extract and Zinc nanoparticles –Garlic Extract Mixture on the *Candida albicans* Growth.

Concentration	ZnNPs Susceptibility test	Garlic Extract Susceptibility test	ZnNPs Garlic Extract Mixture Susceptibility test
25%	15±0.50mm	9±1.30mm	15±0.50mm
50%	16±1.00mm	11±0.20mm	19±1.00mm
75%	20±0.50mm	12±1.00mm	23±1.50mm
100%	23±0.40	14±0.50mm	28±2.00mm
LSD 0.005		1.69	

Table 4. The MIC and MFC values (mg/ml) of ZnNPs, Garlic Extract and ZnNPs-Garlic Extract Mixture against *Candida albicans*.

C.albicans in wells	µg/ml	mg/ml	Result			Control -ve (DW)	Control +ve
			ZnNPs	Garlic Extract	ZnNPs –Garlic Extract Mixture		
1	40000	40	_	_	_	_	+
2	20000	20	_	_	_	_	+
3	10000	10	_	+	_	_	+
4	5000	5	+	+	_	_	+
5	2500	2.5	+	+	+	_	+
6	1250	1.25	+	+	+	_	+

mL) and 10 mg/mL (10,000 µg/mL), respectively, indicating greater inhibitory and fungicidal activity than either treatment alone as shown in Table 4.

The enhanced antifungal activity of the combined treatment was further confirmed by the calculated Fractional Inhibitory Concentration Index (FICI = 0.375), indicating a synergistic interaction between ZnNPs and garlic extract. The synergistic effect may be attributed to the complementary mechanisms of action of both agents, where ZnNPs disrupt fungal cell integrity while the bioactive sulfur compounds of garlic, particularly allicin, interfere with essential metabolic pathways, resulting in enhanced inhibition of Candida albicans growth [24,20,22].

The present study demonstrated that Candida albicans was the predominant species isolated from immunocompromised patients, representing 49.2% of the total Candida isolates. This finding is consistent with previous studies, which reported that C. albicans remains the most frequently isolated species because of its strong adherence to epithelial cells, biofilm formation, and production of several virulence factors that facilitate host tissue invasion [25]. In addition, the identification results obtained by direct microscopy, germ tube test, chromogenic agar, and the VITEK 2 Compact system confirmed the reliability of these methods for laboratory identification of C. albicans [17,18].

The characterization of the green-synthesized ZnONPs confirmed the successful preparation of nanoparticles using garlic extract.

The UV-Visible spectrum showed characteristic absorption peaks at276 and 360 nm, while SEM analysis demonstrated predominantly spherical nanoparticles with an average particle size of 25.74 ± 8.75 nm. Furthermore, DLS, zeta potential, and FTIR analyses confirmed acceptable nanoparticle stability and the participation of garlic phytochemicals in the reduction and stabilization process. These findings are in agreement with previous studies describing the successful green synthesis of ZnNPs using plant extracts [21,20].

The antifungal activity assay demonstrated that ZnNPs exhibited greater inhibitory activity against clinical Candida albicans isolates than garlic extract alone. The inhibition zones increased with increasing ZnNP concentration, indicating a concentration-dependent antifungal effect. The enhanced activity of ZnNPs may be attributed to their nanoscale size and large surface area, which increase their interaction with fungal cells, resulting in membrane disruption, oxidative stress, and inhibition of fungal growth [23,22].

Garlic extract also demonstrated antifungal activity against Candida albicans, although its inhibitory effect was lower than that of ZnNPs. This activity is mainly attributed to allicin and other sulfur-containing compounds that interfere with essential enzymes required for fungal metabolism and growth. Similar observations have been reported previously, supporting the use of garlic as a natural antifungal agent [24].

The combination of ZnNPs and garlic extract exhibited the highest antifungal activity among all tested preparations, producing the largest inhibition zones together with the lowest MIC and MFC values. In addition, the calculated FICI

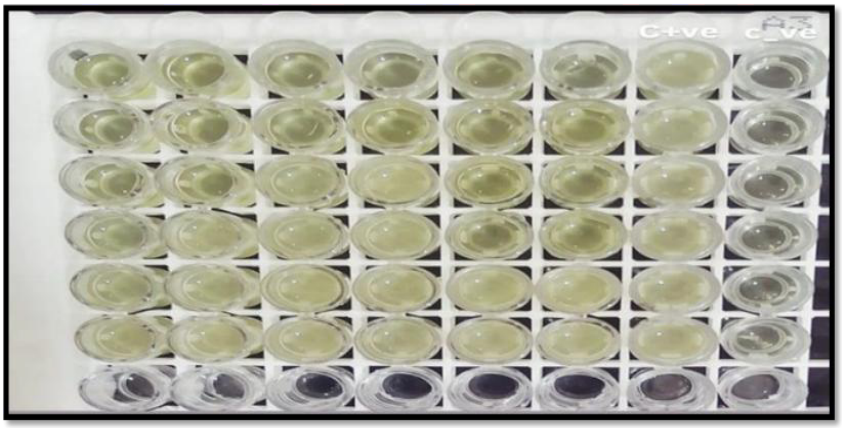

Fig. 11. Microtiter plate assay used for determination of MIC and MFC values of ZnNPs, garlic extract and ZnNPs-garlic extract mixture against Candida albicans showing serial dilutions with positive and negative controls.

value (0.375) confirmed a synergistic interaction between the two agents. This enhanced activity may result from the complementary mechanisms of action of ZnNPs and garlic bioactive compounds, leading to greater inhibition of fungal growth than either treatment alone [20,22].

Overall, the findings of the present study indicate that the green-synthesized ZnNPs-garlic extract combination exhibited superior antifungal activity against clinical Candida albicans isolates compared with ZnNPs or garlic extract alone. Therefore, this combination may represent a promising alternative strategy for the control of candidiasis, particularly against isolates showing reduced susceptibility to conventional antifungal drugs [23,22].

The antifungal susceptibility profile of the clinical Candida albicans isolates demonstrated relatively high resistance to fluconazole and nystatin, whereas amphotericin B remained the most effective antifungal agent. These findings are comparable with previous reports showing that the extensive use of azole antifungal agents has contributed to the emergence of resistant Candida isolates, emphasizing the need to investigate alternative therapeutic strategies [19,26].

Green synthesis of nanoparticles using plant extracts has attracted considerable attention because it provides an environmentally friendly and cost-effective approach compared with conventional chemical methods. In the present study, garlic extract served as both a reducing and stabilizing agent during ZnNP synthesis, resulting in nanoparticles with suitable physicochemical characteristics and enhanced biological activity. Similar observations have been reported in previous studies using medicinal plant extracts for nanoparticle biosynthesis [20,21].

The synergistic interaction observed between ZnNPs and garlic extract may provide an important advantage for antifungal therapy. Combining nanomaterials with natural bioactive compounds may enhance antimicrobial efficacy while reducing the concentration required for each individual agent. Such an approach could minimize toxicity and delay the development of antifungal resistance.

Similar synergistic effects have been described in previous investigations evaluating combinations ofnanoparticles with plant-derived compounds against fungal pathogens [24,22].

Although the present study demonstrated promising in vitro antifungal activity, further investigations are required to evaluate the cytotoxicity, pharmacological safety, and in vivo therapeutic efficacy of the ZnNPs-garlic extract combination before clinical application. Additional studies involving larger numbers of clinical isolates and different Candida species are also recommended to validate the reproducibility of these findings and to better understand the underlying antifungal mechanisms [27,20]. In conclusion, the findings of the present study demonstrated that the combination of green-synthesized zinc nanoparticles and garlic extract exhibited significantly greater antifungal activity against clinical Candida albicans isolates than either treatment alone. The reduction in MIC and MFC values together with the synergistic FICI result supports the potential application of thiscombination as a promising antifungal candidate for future experimental and clinical investigations.

CONCLUSION

Candida albicans was the predominant fungal species isolated from immunocompromised patients among the recovered Candida isolates. Zinc nanoparticles exhibited greater antifungal activity against Candida albicans than garlic extract when each material was tested separately. The antifungal activity of both zinc nanoparticles and garlic extract increased with increasing concentration, as demonstrated by the larger inhibition zones. The combination of zinc nanoparticles and garlic extract showed significantly higher antifungal activity than either treatment alone. The largest inhibition zone (28 mm), was recorded for the zinc nanoparticles-garlic extract combination, indicating a strong synergistic effect against Candida albicans. The findings suggest that combining zinc nanoparticles with garlic extract represents a promising strategy for enhancing antifungal activity against Candida albicans isolated from immunocompromised patients.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

REFERENCES

1. Walsh TJ, Katragkou A, Chen T, Salvatore CM, Roilides

E. Invasive Candidiasis in Infants and Children: Recent Advances in Epidemiology, Diagnosis, and Treatment. Journal of Fungi. 2019;5(1):11.
2. Mayer FL, Wilson D, Hube B. Candida albicans pathogenicity mechanisms. Virulence. 2013;4(2):119-128.
3. Barantsevich N, Barantsevich E. Diagnosis and Treatment of Invasive Candidiasis. Antibiotics. 2022;11(6):718.
4. Costa-de-Oliveira S, Rodrigues AG. Candida albicans Antifungal Resistance and Tolerance in Bloodstream Infections: The Triad Yeast-Host-Antifungal. Microorganisms. 2020;8(2):154.
5. Borlinghaus J, Albrecht F, Gruhlke M, Nwachukwu I, Slusarenko A. Allicin: Chemistry and Biological Properties. Molecules. 2014;19(8):12591-12618.
6. Sorlozano-Puerto A, Albertuz-Crespo M, Lopez-Machado I, Gil-Martinez L, Ariza-Romero JJ, Maroto-Tello A, et al. Antibacterial and Antifungal Activity of Propyl-Propane-Thiosulfinate and Propyl-Propane-Thiosulfonate, Two Organosulfur Compounds from Allium cepa: In Vitro Antimicrobial Effect via the Gas Phase. Pharmaceuticals. 2020;14(1):21.
7. Grigore M, Biscu E, Holban A, Gestal M, Grumezescu A. Methods of Synthesis, Properties and Biomedical Applications of CuO Nanoparticles. Pharmaceuticals. 2016;9(4):75.
8. Agarwal H, Shanmugam V. A review on anti-inflammatory activity of green synthesized zinc oxide nanoparticle: Mechanism-based approach. Bioorg Chem. 2020;94:103423.
9. Noble MA. Bailey and Scott's Diagnostic Microbiology, Eleventh Edition. Betty Forbes, Daniel F. Sahm, and Alice S. Weissfeld. St. Louis, MO: Mosby, 2002, 1069 pp. ISBN 0-323-01678-2. Clin Chem. 2002;48(10):1816-1816.
10. Cheesbrough M. District Laboratory Practice in Tropical Countries: Cambridge University Press; 2006 2006/03/02.
11. Odds FC, Bernaerts R. CHROMagar Candida, a new differential isolation medium for presumptive identification of clinically important Candida species. J Clin Microbiol. 1994;32(8):1923-1929.
12. Kanungo R. Jawetz, Melnick, and Adelberg's Medical Microbiology: Edited by Brooks GF, Butel JS, Morse SA: International Edition; 22nd edition, 2001, Lange Medical Books/McGraw - Hill, New York. Indian J Med Microbiol. 2002;20(4):231.
13. El-Saadony M, Fang G, Yan S, Alkafaas S, El Nasharty M, Khedr S, et al. Green Synthesis of Zinc Oxide Nanoparticles: Preparation, Characterization, and Biomedical Applications - A Review. International Journal of Nanomedicine. 2024;Volume 19:12889-12937.
14. Jayachandran A, T.R A, Nair AS. Green synthesis and characterization of zinc oxide nanoparticles using Cayratia pedata leaf extract. Biochemistry and Biophysics Reports. 2021;26:100995.
15. Fothergill AW. Antifungal Susceptibility Testing: Clinical Laboratory and Standards Institute (CLSI) Methods. Interactions of Yeasts, Moulds, and Antifungal Agents: Humana Press; 2011. p. 65-74. http://dx.doi.org/10.1007/978-1-59745-134-5_2
16. Talapko J, Juzbašić M, Matijević T, Pustijanac E, Bekić S, Kotris I, et al. Candida albicans—The Virulence Factors and Clinical Manifestations of Infection. Journal of Fungi. 2021;7(2):79.
17. Kidd S, Halliday C, Ellis D. Descriptions of Medical Fungi. CABI; 2022. http://dx.doi.org/10.1079/9781800622340.0000
18. Nadeem SG, Hakim ST, Kazmi SU. Use of CHROMagar Candida for the presumptive identification of Candida species directly from clinical specimens in resource-limited settings. Libyan J Med. 2010;5(1):2144.
19. Pfaller MA, Diekema DJ, Turnidge JD, Castanheira M, Jones RN. Twenty Years of the SENTRY Antifungal Surveillance Program: Results for Candida Species From 1997–2016. Open Forum Infectious Diseases. 2019;6(Supplement_1):S79-S94.
20. Khashan KS, Sulaiman GM, Hussain SA, Marzoog TR, Jabir MS. Synthesis, Characterization and Evaluation of Anti-bacterial, Anti-parasitic and Anti-cancer Activities of Aluminum-Doped Zinc Oxide Nanoparticles. Journal of Inorganic and Organometallic Polymers and Materials. 2020;30(9):3677-3693.
21. Rajeshkumar S, Lakshmi T, Naik P. Recent advances and biomedical applications of zinc oxide nanoparticles. Green Synthesis, Characterization and Applications of Nanoparticles: Elsevier; 2019. p. 445-457. http://dx.doi.org/10.1016/b978-0-08-102579-6.00019-8
22. Yassin MT, Mostafa AA-F, Al-Askar AA, Al-Otibi FO. Facile Green Synthesis of Zinc Oxide Nanoparticles with Potential Synergistic Activity with Common Antifungal Agents against Multidrug-Resistant Candidal Strains. Crystals. 2022;12(6):774.
23. Raghupathi KR, Koodali RT, Manna AC. Size-Dependent Bacterial Growth Inhibition and Mechanism of Antibacterial Activity of Zinc Oxide Nanoparticles. Langmuir. 2011;27(7):4020-4028.
24. Ankri S, Mirelman D. Antimicrobial properties of allicin from garlic. Microb Infect. 1999;1(2):125-129.
25. Calderone RA, Fonzi WA. Virulence factors of Candida albicans. Trends Microbiol. 2001;9(7):327-335.
26. Overman L. [PDF, EPUB] Download Goodman and Gilman's The Pharmacological Basis of Therapeutics, 14th Edition by Laurence Brunton, Bjorn Knollmann Full Book. ResearchHub Technologies, Inc.; 2026 2026/05/02.
27. Camp I, Spettel K, Willinger B. Molecular Methods for the Diagnosis of Invasive Candidiasis. Journal of Fungi. 2020;6(3):101.