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COMPARING THE ANTI-CANDIDA ALBICANS EFFECT OF ZINGIBER OFFICINALE WITH COMMON ANTIFUNGAL DRUGS

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Abstract

Candida albicans (C. albicans) is considered one of the most opportunistic fungal agents and the most common cause of fungal diseases that causes candidiasis in humans and manifests in different clinical forms ranging from simple superficial infection to severe systemic. The study aimed to investigate the inhibitory and lethal effects of Zingiber officinale (Z. officinale) ethanolic extract and common antifungal chemical drugs on C. albicans. Ethanol extract of Z. officinale was prepared at 2 to 20 mg/ml concentrations. Using the microbroth dilution method, the minimum inhibitory concentration and minimum fungicidal concentration of the extract and chemical antifungal drugs were determined. The disk diffusion method and Sabourud Dextrose Agar culture medium were used to evaluate the inhibition zone diameters. The results showed that with the increase in the concentration of the ethanolic extract of Z. officinale, the inhibition rate of C. albicans increased. Ketoconazole had the highest anti-C. albicans effects. The ginger extract at 20 mg/ml concentration had a higher anti-Candida inhibitory activity than nystatin and fluconazole. It was comparable to amphotericin. The current results revealed that the ethanolic extract of Z. officinale had a growth-inhibitory impact on C. albicans and can be used as a safe antifungal therapy.

Keywords: candidiasis, minimum fungicidal concentration, mnimum inhibitory concentration, ginger, ketoconazole.

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Introduction

The effects of infectious diseases have had a substantial impact on public health systems around the world [*Baker et al., 2022*]. In recent years, fungal infections have been linked to significant diseases and death in immunosuppressed or vulnerable populations, causing a broad range of symptoms in different ways, from mild to severe [*Gavanji, Larki, 2017; Pagano, Mayor, 2018; Gavanji et al., 2024b*]. *Candida*, a fungus, causes candidiasis, the most severe fungal infection [Bongomin et al., 2017; de Oliveira Santos et al., 2018; *Zilfyan A et al., 2020; Zilfyan A et al., 2025*]. Candida albicans (C. albicans) can have an impact on the oral cavity, gastrointestinal tract, skin, and reproductive systems [*Gavanji et al., 2015; Zilfyan et al., 2021; Nurdiana et al., 2023*]. This opportunistic fungus causes two clinical consequences, including temporary and fatal systemic infections [*Kumar et al., 2019; Mavor et al., 2005*]. The development of new classes of broad-spectrum antifungal drugs with pharmacological properties and powerful therapies has attracted interest because of the threat of drug resistance and the different side effects of synthetic antifungal medicines [*Murphy, Bicanic, 2021; Gavanji et al., 2023c*]. Traditional medicine has a prominent role in medication development and has been frequently used to treat fungal infections because of its efficiency and fewer side effects [*Aschale et al., 2021; Bakhtari, 2022*]. Over ancient times, folks have employed plant-based natural chemicals as pharmaceutical drugs [*Dzobo, 2022; Gavanji et al., 2024a*]. According to the research, traditional medicine served the primary healthcare needs of over 80% of the global population in 2008 [*Mbali et al., 2021*].

On the other hand, the growing concern of microbial resistance caused researchers to investigate and focus on natural compounds and herbal medicine, which have antimicrobial potential as the future supply of antimicrobial agents. *Zingiber officinale* (*Z. officinale*), also called as ginger, is a member of the *Zingiberaceae* family and is a significant medicinal plant used to treat various diseases [*Ali et al., 2008; Gavanji S.Et al., 2023*]. Many phytochemical compounds found in *Z. officinale*, such as zingiberene, gingerol, paradol, starch, and shogaol, have been demonstrated to have potent antifungal, antibacterial, and antiviral effects. Numerous disorders have historically been treated using the plant, including fever, colic, stomach ulcers, constipation, and lung disorders [A*pariman et al., 2006; Chaiyakunapruk et al., 2006; Chen et al., 2007; Fuhrman et al., 2000*].

Materials and methods

Extract preparation: The fresh *Z. officinale* rhizomes were prepared from the market and approved by the Institute of traditional medicine and herbal plants of Iran. In the next step, the rhizomes were washed and dried in the shade at room temperature and oven drying (35-45 °*C*). Dried rhizomes were thoroughly powdered by an electric blender and passed from mesh (100 sizes). Then, 10 *g* of ginger powder was dissolved in 100 *ml* of ethanol in a sterile dark conical flask for 72 *h* at room temperature (33 ± 2 ˚*C*). The ginger extract was filtered and the liquid part was separated using the Whatman filter paper (Pore size: 2.5 μm). The extract was concentrated using rotary evaporation (45 °*C*) and stored at 4 °*C* in darkness for further use [*Gavanji et al., 2014, Gavanji S. et al.,2023a*].

Antifungal activity assays

Standard strains: The standard strain of *C. albicans* (ATCC10231) was used to evaluate the antimicrobial assay. For this, the lyophilized strains of *C. albicans* were grown on Sabouraud Dextrose Agar (SDA) and incubated for two days at 25 °*C*.

Determining the inhibitory concentration and fungicide concentration: The minimum inhibitory concentration (MIC) and minimum fungicide concentration **(**MFC) of *Z. officinale*, amphotericin B, Nystatin, ketoconazole, and Fluconazole on *C. albicans* were evaluated using a microbroth dilution procedure. For this, a diluted extract of *Z. officinale* in DMSO at concentrations ranging from 0.062 to 20 *mg/mL* was prepared. The liquid media used was the SDA. Then, 100 *mL* of each dilution was added to each well of a 96-well microplate, followed by a *C. albicans* microbial suspension at a concentration of 10^4–10^5 *CFU/mL*. The MIC and MFC of *Z. officinale*, amphotericin B, nystatin, ketoconazole, and fluconazole were determined after incubation for 24 hours at 35 °*C*.

Antifungal activity test: The SDA was used to estimate the antifungal effects of *Z. officinale* on *C. albicans*. In the present study, *C. albicans* was cultivated and incubated on Sabouraud agar me-

dium for 48 *h* at 37 °*C*. The selected colonies (two to three colonies) were added to sterile saline and 0.5 McFarland 1 × 10^6 colony forming units CFU mL set for turbidity. The selected suspension was cultivated and developed on SDA or dextrose agar medium at the following step. The Blank disks (6.4 *mm*) contained an alcoholic extract of *Z. officinale* at concentrations of 0.062 to 20 *mg/ml* dissolved in dimethyl sulfoxide (DMSO). Positive control included amphotericin B, Nystatin, ketoconazole, and Fluconazole discs. Dimethyl sulfoxide (DMSO) was considered the negative control. All plates have been incubated at 37 °*C* for 72 hours. The diameters of the inhibition zones are assessed after 24, 48, and 72 hours.

Statistical analysis: Data were analyzed using a one-way ANOVA by GraphPad Prism 6 software (GraphPad Software, La Jolla, CA, USA). Means were compared using Tukey's multiple comparison tests. At p< 0.05, differences were considered significant.

RESULTS

Table 1 displays the effects of various *Z. officinale* extract concentrations on C. albicans at 24, 48, and 72 hours. The results showed that the antifungal activity of this extract is dose-dependent; a higher antifungal effect was observed at higher concentrations.

The findings of the MIC and MFC assays are shown in Figures 1 and 2, respectively. Ketoconazole and amphotericin revealed the lowest MIC (p0.05<). However, there was no significant difference between *Z. officinale* extract and nystatin. Compared to fluconazole, the ginger extract exhibited a lower MIC (p <0.05). The results showed ketoconazole had the lowest MFC against C. albicans (p <0.05). However, the *Z. officinale* extract had a better fungicidal effect than fluconazole.

The effect of different treatments on the inhibition zone of C. albicans at various times is shown in Figure 3. Overall, the treatments effects were not affected by the time. Nystatin and fluconazole exhibited the lowest inhibitory zones, whereas ketoconazole had the highest (p <0.05). There was no significant difference between amphotericin and *Z. officinale* extract in the inhibition zone at various times.

TABLE 1.

Inhibition zone diameters of Zingiber officinale extract against C. albicans using disk diffusion

Concentrations of ZO (mg/disc)	*Taim of treatment (Hours))*		
	24h	*48h*	72h
2	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
4	1.87±1.76^{ab}	2.63±1.10^{b}	2.63±1.10^{b}
6	4.60±2.29^{bc}	5.67±1.53^{c}	6.13±1.70^{c}
8	6.33±2.52^{c}	7.60±1.77^{c}	8.23±1.54^{c}
10	10.40±0.92^{d}	10.83±0.80^{d}	10.87±0.81^{d}
12	11.97±0.06^{d}	12.60±0.53^{de}	12.73±0.30^{de}
14	12.53±0.40^{d}	13.53±0.46^{e}	13.53±0.46^{e}
16	12.83±0.29^{d}	13.67±0.21^{e}	14.17±0.29^{ef}
18	13.50±0.50^{d}	14.4±0.36^{ef}	14.50±0.50^{ef}
20	15.87±0.85^{e}	16.43±0.40^{f}	16.50±0.50^{f}

Notes: *ZO - Zingiber officinale*, $^{a\text{-}f}$ In each column, different letters represent significantly difference at p < 0.05

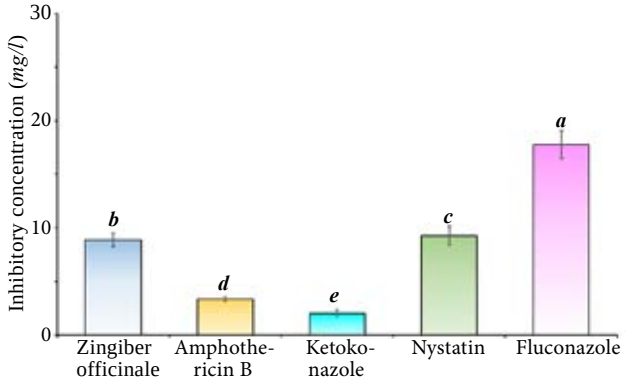

FIGURE 1. *Minimum inhibitory concentration (MIC) of Z. officinale extract (mg/ml) and common chemical antifungal agents (µg/ml) against C. albicans.* $^{a\text{-}c}$ *At P < 0.05, different letters represent significant differences.*

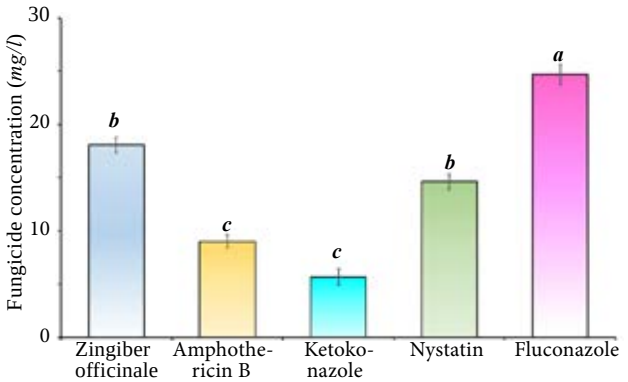

FIGURE 2. *Minimum fungicidal concentration (MFC) of Z. officinale extract (mg/ml) and common chemical antifungal agents (µg/ml) against C. albicans.* $^{a\text{-}d}$ *At P < 0.05, different letters represent significant differences.*

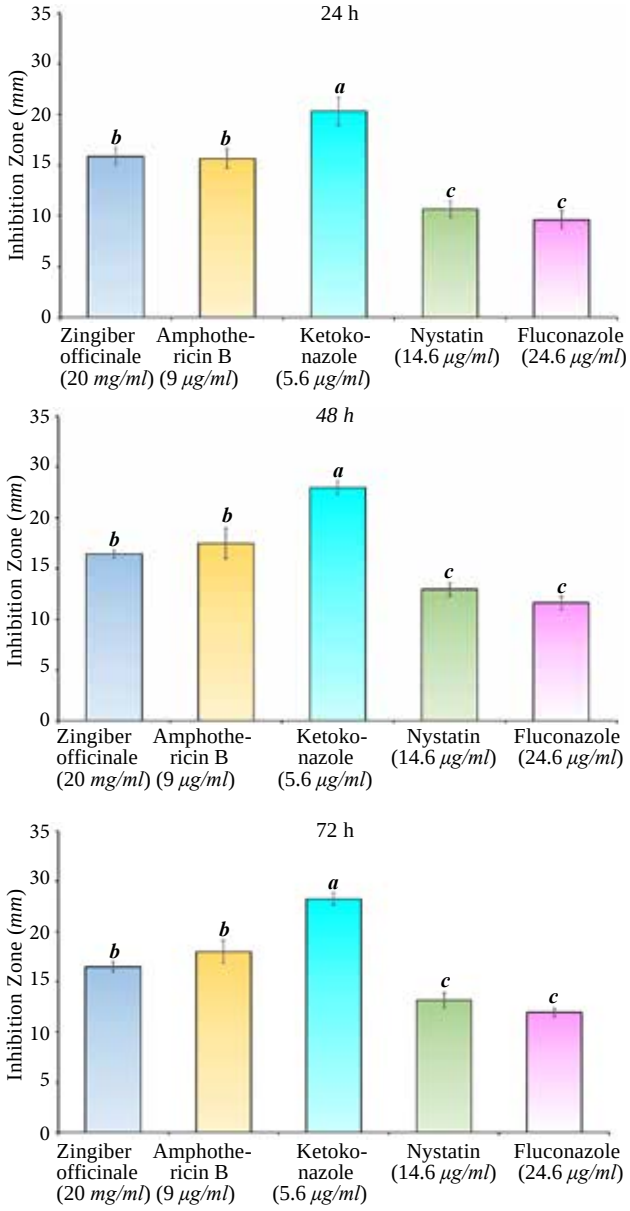

Figure 3. *Diameters of zones of inhibition of Z. officinale extracts (mg/ml) and common chemical antifungal agents (μg/ml) against C. albicans.*
$^{a-c}$ At $P < 0.05$, different letters represent significant differences.

Discussion

Because of the increase in drug resistance, researchers have concentrated their attention on finding novel compounds that might prevent the development of microorganisms. According to this study, ethanol extract of *Z. officinale* was more effective in preventing *C. albicans* growth than nystatin and fluconazole, although it exhibited similar anti-*C. albicans* properties to those of amphotericin. In previous research, the antifungal properties of *Z. officinale* have been shown on some fungi, such as *Fusarium oxysporum*, *Fusarium verticillioides*, *Fusarium moniliforme*, *Aspergillus flavus*, and *Aspergillus fumigatus* [*Nguefack et al., 2004; Wang and Ng, 2005; Yamamoto-Ribeiro et al., 2013*]. *Ficker et al. (2003)* also evaluated the antifungal properties of 33 plant extracts on 13 known human fungal infections and reported that ginger extracts have inhibitory effects on different fungal species [*Ficker et al., 2003; Gavanji et al., 2024a*]. In agreement with this present study, *Lee et al. (2018)* showed that 6-gingerol, one of the most important natural compounds isolated from *Z. officinale* rhizomes, at a concentration of 10 *μg/ml* reduced *C. albicans* biofilm formation [*Lee et al., 2018*]. A study on the effects of alcoholic *Z. officinale* extract on *C. albicans* isolated from patient mouths revealed that the concentration of the extract, between 50 and 150 *mg/ml*, inhibits the development of *C. albicans*, with a minimum inhibitory concentration (MIC) of 25 *mg/ml* [*Khalaf et al., 2020*]. In another study, *Z. officinale* extract was shown to have strong antifungal properties against fluconazole-resistant *C. albicans* strains isolated from patients with genital candidiasis [*Mohammadi and Moatar, 2007*]. It has been suggested that the antifungal activity of *Z. officinale* extracts may be attributed to their natural hydrophobic compounds. These compounds can adhere to the fungi plasma membrane and prevent fungi proliferation by increasing the membrane permeability or preventing the germination of spores and cellular respiration [*Kim et al., 2009*].

Conclusion

The findings of this study suggest that the ethanol extract of *Z. officinale* at 20 *mg/ml* concentration would be comparable to amphotericin and more potent than nystatin and fluconazole against *C. albicans*.

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