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Evaluation of the antifungal efficacy of *Salvia officinalis* extract against some clinical oral candidiasis isolates: a comparative study with standard antifungals

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Abstract

Background: Oral candidiasis is an opportunistic fungal infection caused by *Candida* spp. Although these yeasts are a normal inhabitant of the mouth in a large percentage of people, they can become pathogenic under suitable conditions. With increasing resistance to conventional antifungals, the need to discover effective natural alternatives has become apparent.

Objective: This study aimed to evaluate the inhibitory efficacy of an ethanolic extract of *S. officinalis* against three *Candida* isolates from patients with oral candidiasis.

Materials and Methods: involved preparing an ethanolic extract of *S. officinalis* and testing the susceptibility of the isolates using the disc diffusion method, comparing the results with the efficacy of some standard antifungal agents.

Results: The results showed that *S. officinalis* extract inhibited the growth of *C. albicanis*, with an average aura diameter of 19.5 ± 1.18 at 100% concentration. Compared to standard antifungal agents, the extract demonstrated superior efficacy, surpassing amphotericin B, which showed the highest effect on all three isolates. The average aura diameter for *C. tropicalis* was 25.17 ± 1.86 at 100%, while *C. parapsilosis* was the least affected by the extract.

Conclusions: The study concludes that *S. officinalis* extract possesses antifungal properties, making it a promising alternative for developing topical treatments for oral fungal infections.

Keywords: *Salvia officinalis*, *Candida albicans*, Antifungal activity, Oral candidiasis

Introduction

Salvia officinalis L. is an evergreen perennial shrub belonging to the Lamiaceae family Native to the Mediterranean region, it is widely cultivated worldwide for its medicinal and aromatic properties. This plant grows to about 60 cm in height and is characterized by its woody stems and strongly aromatic gray-green leaves. *S. officinalis* blooms in late spring and summer, and its flowers are purple or blue[1].

S. officinalis has been traditional use in treating various health conditions, such as gastrointestinal disorders, sore throat, and excessive sweating[2]. Contemporary research indication that *S. officinalis* may contribute to improving memory and cognitive function, alleviating menopausal symptoms, and lowering blood sugar and cholesterol levels[3]. It

bioactivity is attributed to a range of compounds, including volatile oils such as thujone, cineole, and borneol, flavonoids, and phenolic acids[4]. Numerous studies have demonstrated that sage has antioxidant, anti-inflammatory, and antimicrobial properties, making it of great importance in both traditional and modern medicine[5].

Oral candidiasis is one of the most common opportunistic infections of the oral cavity, caused by fungi of the genus *Candida* spp. These yeasts are commensal inhabitants of the oral microbiome in a significant proportionof healthy individuals. However, an imbalance resulting from factors such as compromised host immunity, HIV infection, chemotherapy or corticosteroid use, xerostomia, or prolonged administration of broad-spectrum antibiotics can lead to fungal overgrowth and the appearance of clinical symptoms[6, 7]

The clinical manifestations of the disease vary, the most prominent of which is pseudomembranous lesions, characterized by the formation of removable white plaques or patches on the mucous membrane, typically accompanied by a burning sensation, pain, and impaired sense of taste[8]. Conventional treatment relies on topical antifungal agents, with common therapeutic options including nystatin, clotrimazole, and Miconazole[9, 10].

However, the widespread and frequent use of these agents has led to the emergence and alarming increase in drug resistance of *Candida* spp., which poses a major therapeutic challenge and leads to treatment failure, prolonged disease duration, and increased healthcare costs, creating an urgent need to search for new and effective therapeutic alternatives[11].

In this context, *S. officinalis* extracts emerge as a promising source for the development of new therapeutic agents with different mechanisms of action that may overcome the problem of resistance. Several studies have shown that *S. officinalis* essential oil possesses antifungal activity against *Candida*, reducing its cells' ability to adhere to acrylic surfaces. This suggests its potential as a natural disinfectant for dentures to prevent *Candida*-related oral infections[12,13]. More recent studies aim to explore the effect of *S. officinalis* extract on clinical isolates of *Candida albicans*, with its ability to inhibit its growth and biofilm formation. This may contribute to the development of effective natural treatments for oral infections[14, 15].

This study aims to evaluate the antifungal activity of ethanolic extract of *S. officinalis* against some *Candida* species that cause oral thrush and compare it with the effectiveness of antifungal agents used in treatment.

Materials and Methods

Plant sample collection.

S. officinalis leaves were collected from the city of Zintan in August 2025 figure (1). To remove dust, the leaves were washed with sterile distilled water and then dried at room temperature for one week. To obtain plant powders, the air-dried plant materials was ground in a Silver Crescent SC-1589 blender and stored at 4°C until further tests.

Figure (1): *Salvia officinalis* L

Preparation of the ethanolic extract.

According to the modified method[16], 10 grams of leaf powder was mixed with 200 ml of 96% ethanol and the mixture was shaken for 24 hours. The extracts were dried in a rotary evaporator at 60°C and stored in an opaque container at 4°C.

Fungal strains

Three *Candida* isolates: *Candida albicans*, *Candida tropicalis*, and *Candida parapsilosis* were used, isolated from individuals with Oral candidiasis. These isolates were identified using chromium agar *Candida* and preserved in the herbarium of the Medical Mycology Laboratory, Faculty of Medical Technology, University of Zintan. Preparation steps began with activating the *Candida* by culturing it in SDA medium for three days at 37°C.

Effect of alcoholic extract of *S. officinalis* on *Candida* spp.

The effect of *S. officinalis L* extracts on *Candida* species was tested using the disc diffusion method[17]. Mueller-Hinton plates were inoculated using the agar stripping method. Sterile filter paper discs (6 mm in diameter) were placed on the media in Petri dishes using sterile forceps. Plant extracts were applied at 50% and 100% concentrations, with 50 µL of each concentration placed on the discs. A plate containing *Candida* was used as a negative control without the addition of antibiotics or extracts. Six replicates were performed for each species. The inoculated plates were stored at 37°C± 2 for 3 days. The inhibition zones were recorded in millimeters around the discs on each plate.

Effect of antifungal agents on *Candida* species

The disk diffusion method was used to test antifungal agents on isolated *Candida* spp. In this method, Mueller-Hinton agar plates approximately 4 mm thick were prepared. The surface of the medium was covered with the fungal suspension using a sterile cotton swab. After the medium had dried for 5–15 minutes, Liofilchem discs pre-saturated with three antifungals (Amphotericin B 20mg, Miconazole 10mg, and Nystatin 100mg) used in treatment were placed on the agar surface. According to the method[18], the plates were incubated under appropriate conditions at 37±2°C for 3 days. After incubation, the diameters of the inhibition zones surrounding the discs were measured.

Statistical analysis

Statistical analysis was performed using two-way ANOVA followed by Tukey's multiple comparison test when comparing the effects of the antifungal agents on the *Candida* species under study. Two-way ANOVA followed by SIDAC's multiple comparison test was also used when comparing the extract and the antifungal agents.

Results

Antifungal activity of antifungal agents

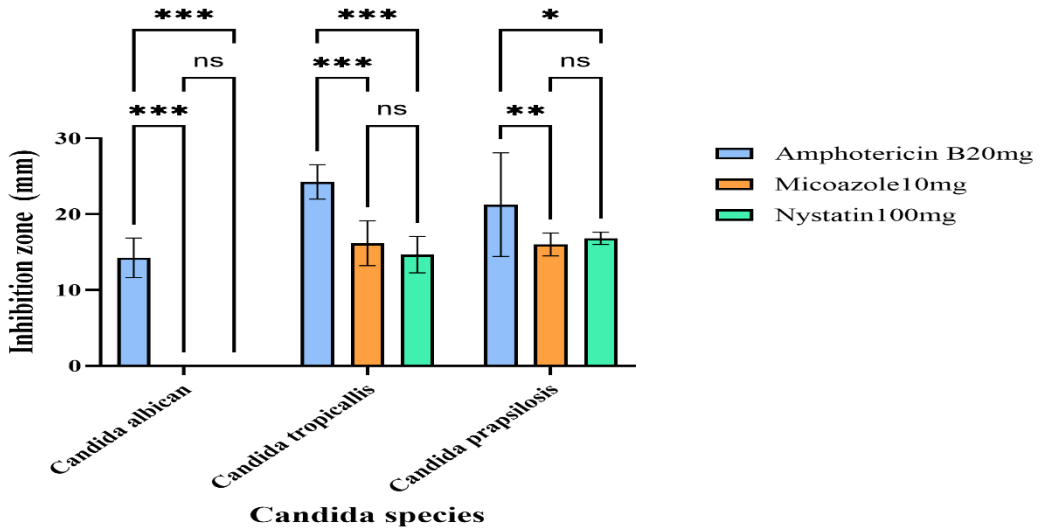
The antifungal activity of the tested antibiotics differed markedly among the *Candida* species, as reflected by variations in inhibition zone diameters (mm)as shown in Figure(2).

For *C. albicans*, Amphotericin B was the only antibiotic that showed antifungal activity, producing an inhibition zone of 14.25 ± 2.60 mm. In contrast, Miconazole and Nystatin did not exhibit any detectable inhibitory effect against this species (0.00 ± 0.00 mm for both), indicating a lack of antifungal activity under the tested conditions.

In *C. tropicalis*, Amphotericin B exhibited the strongest antifungal effect, yielding the largest inhibition zone (24.25 ± 2.25 mm). Both Miconazole (16.17 ± 2.96 mm) and Nystatin (14.67 ± 2.40 mm) also inhibited fungal growth, although their effects were markedly lower than that observed for Amphotericin B and were comparable to each other.

Similarly, *C. parapsilosis* showed the highest sensitivity to Amphotericin B, with an inhibition zone of 21.25 ± 6.85 mm. Moderate inhibition was observed with Miconazole (16.00 ± 1.52 mm) and Nystatin (16.83 ± 0.82 mm), while the inhibitory effects of these two antibiotics remained closely comparable.

Overall, Amphotericin B was the most effective antifungal agent against all tested *Candida* species, consistently producing the largest inhibition zones. Miconazole and Nystatin exhibited weaker and comparable antifungal activity, with particularly limited effectiveness against *C. albicans*.



Figure(2): Effect of different antibiotics on the growth of *Candida*s pecies**.**

Inhibition zone diameters are expressed as mean ± SD (mm). Statistical analysis was performed using two-way ANOVA followed by Tukey's multiple comparisons test. *p < 0.05, **p < 0.01, ***p < 0.001; ns, not significant.

Antifungal activity of *Salvia officinalis* extract

The antifungal activity of *S. officinalis* extract was evaluated against three *Candida* species at two concentrations (50% and 100%), revealing a concentration- and species-

dependent response. Overall, the higher extract concentration (100%) exhibited enhanced inhibitory effects compared to 50% for most *Candida* species.

In *C. albicans*, treatment with 100% *S. officinalis* extract resulted in a marked increase in antifungal activity, yielding a mean inhibition zone of 19.5 ± 1.18 mm, compared to 10.33 ± 0.68 mm at 50%, indicating a clear dose-dependent enhancement. A similar trend was observed for*C. tropicalis*, which showed the strongest response among the tested species. The 100% extract produced a substantial inhibition zone (25.17 ± 1.86 mm), whereas the 50% concentration displayed minimal activity (5.00 ± 1.61 mm), highlighting the pronounced sensitivity of this species to higher extract levels.

In contrast, *C. parapsilosis* exhibited a relatively modest and less concentration-dependent response. Comparable inhibition zones were observed at both concentrations, with mean values of 7.25 ± 3.22 mm for 100% and 7.92 ± 2.18 mm for 50%, suggesting limited enhancement with increased extract concentration.

Collectively, these findings demonstrate that *S. officinalis* extract exerts species-specific antifungal effects, with the 100% concentration showing superior activity against *C. albicans* and *C. tropicalis*, while *C. parapsilosis* appears less responsive to concentration-dependent increases. As shown in table(1)

Table (1): Evaluation of *S. officinalis*extract effect against selected *Candida* spp.

***Candida* species**	**Extract concentration**	**Mean ± sd**
C. albicans	**100%**	19.5 ± 1.18
	50%	10.33 ± 0.68
C. tropicalis	**100%**	25.17 ± 1.86
	50%	5.00 ± 1.61
C. parapsilosis	**100%**	7.25 ± 3.22
	50%	7.92 ± 2.18

Comparison between the most effective antibiotic and *S. officinalis* extract

The antifungal efficacy of Amphotericin B20mg was compared with the optimal concentration (100%) of *S. officinalis* extract across three *Candida* species Figure(3). Overall, both treatments demonstrated species-dependent inhibitory effects,

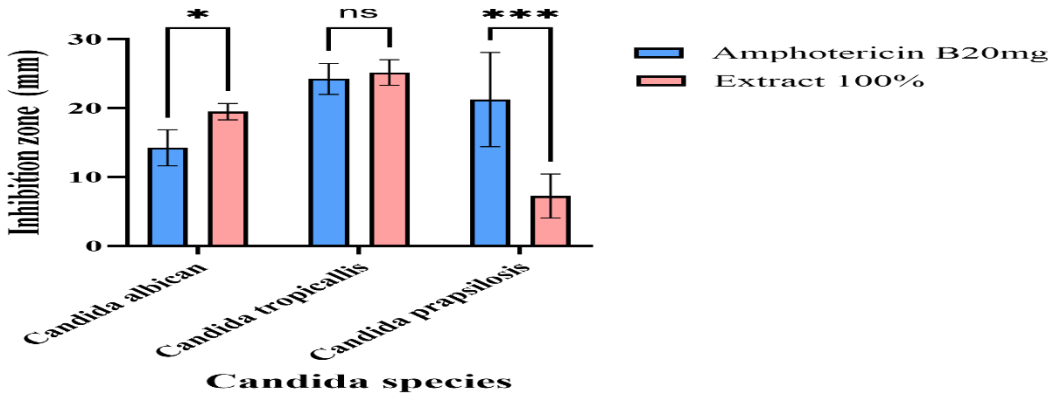
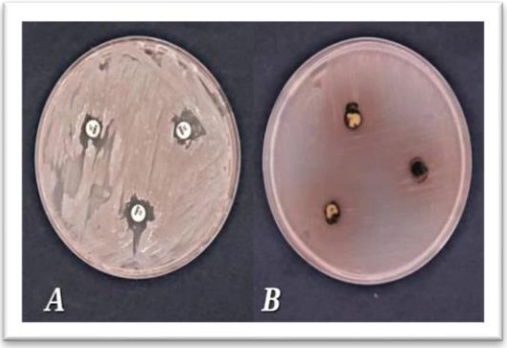


Figure (3). Comparison between the most effective antibiotic (AmphotericinB20mg)

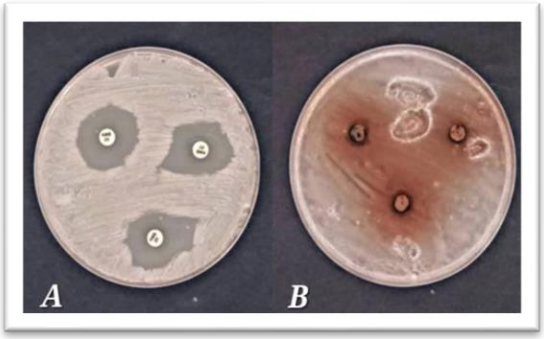
with distinct response patterns observed among the tested strains.

In *C. albicans*, the plant extract exhibited a stronger inhibitory activity than amphotericin B20mg, producing a larger mean inhibition zone, indicating a pronounced sensitivity of this species to *S. officinalis* at the optimal concentration Figure (4)A similar trend was observed in *C. tropicalis*, where the where the extract showed

comparable or slightly enhanced antifungal activity relative to amphotericin B20mg, highlighting its potent effect against this species Figure (4).



Figure(4) The image compares the effects of *S. officinalis* extract (B)and the antifungal amphotericin B(A) on *C. albicans*.



Figure(5) The image compares the effects of *S. officinalis* extract(B) and the antifungal amphotericin B(A) on *C. tropicalis*.

In contrast, *C. parapsilosis* displayed a different response profile. Amphotericin B20mg demonstrated superior antifungal activity compared to the plant extract, suggesting that this species remains more responsive to conventional antifungal therapy than to the tested natural extract.

Collectively, these findings indicate that while amphotericin B20mg remains highly effective against certain *Candida* species, *S. officinalis* extract at 100% concentration exhibits strong and, in some cases, superior antifungal activity, particularly against *C. albicans* and *C. tropicalis*, supporting its potential as a complementary or alternative antifungal agent, and *Salvia officinalis* extract (the most effective 100%) against *Candida* species. Inhibition zone diameters are expressed as mean ± SD (mm). Statistical analysis was performed using two-way ANOVA followed by Sidak's multiple comparisons test. $p < 0.05$, $*p < 0.01$, $**p < 0.001$; ns, not significant.

Discussion

The study results show a disparity in the efficacy of the tested antifungal agents, with amphotericin B emerging as the most effective antibiotic against all strains. These results are consistent with those[19,20,31,22], but differ from those of[23]. The effect of amphotericin B is attributed to its mechanism of action, which targets ergosterol in the fungal cell membrane, leading to the formation of pores and cell death[2]. The results also

showed complete resistance in the *C. albicans* strain to miconazole and nystatin, findings that are consistent with those of[25,26.27]. This resistance can be explained by the increased prevalence of azole-resistant strains resulting from the excessive and repeated use of these antibiotics, as well as the ability of these fungi to form biofilms that act as a barrier preventing the drug from reaching lethal concentrations[28,29].

On the other hand, the study provided strong evidence of the inhibitory potential of the alcoholic extract of *S. officinalis*, especially at its full concentration (100%), as several studies agreed on this effect, including the study{30,31]. The 100% concentration of the extract also outperformed the 50% concentration in the *C. albicans* and *C. tropicalis* strains. This activity confirms the existence of a direct relationship between the concentration of phenolic compounds and terpenes present in sage and the antifungal activity[32].

In comparing the efficacy of antifungals and extracts, the most notable result is that the extract, at a concentration of 100%, statistically outperformed amphotericin B when tested against *C. albicans*; and its efficacy against *C. tropicalis* was comparable. This suggests that the extract may contain active compounds whose mechanism of action surpasses that of the traditional antibiotic against these strains, opening up prospects for its use as a promising natural alternative[33.34].

While the *C. parapsilosis* strain showed mixed results, exhibiting a weak effect of the extract that was unaffected by changes in concentration[35], this weak effect, compared to the high efficacy of amphotericin B against the same strain, suggests structural differences in the *C. parapsilosis* cell wall that may inhibit the permeability of the active compounds present in *S. officinalis*.[36,37]

The results of this study reinforce the trend towards exploring plant alternatives to compensate for the lack of efficiency in traditional antibiotics, with the need to conduct future studies to identify the precise chemical compounds in *S. officinalis* extract responsible for this selective activity.

Conclusion

This study concludes that the *Candida* strains tested exhibit considerable variation in their sensitivity to antifungal agents, both chemical and natural. a 100% extract of *S. officinalis* demonstrated superior inhibitory efficacy, significantly outperforming amphotericin B in inhibiting *C. albicans*. These results suggest that highly concentrated plant extracts can provide effective solutions against strains that have developed resistance to conventional antibiotics, although their efficacy varies depending on the specific fungal strain.

Recommendations

Studying the effect of *S. officinalis* extract on a wider range of clinical fungal strains to validate the results and Performing GC-MS analysis of sage extract to identify the precise active compounds responsible for inhibiting *C. albicans* and characterizing their molecular mechanism of action.

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