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RESEARCH ARTICLE

# *Syzygium Aromaticum* (Clove) Extracts Demonstrate Superior Antifungal Activity Compared to Conventional Drugs Against Clinical Oral *Candida* Isolates from Gharyan, Libya: A Comparative *In Vitro* Study

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## Abstract

**Background:** The escalating challenge of antifungal resistance and the side effects associated with conventional drugs have intensified the search for natural alternatives. This study investigated the efficacy of *Syzygium aromaticum* (clove) extracts compared to standard antifungal agents against clinical oral *Candida* isolates from Libyan patients.

**Methods:** Five *Candida* species (*C. albicans, C. glabrata, C. krusei, C. parapsilosis, C. tropicalis*) were isolated from patients in Gharyan City, Libya. The antifungal susceptibility to Amphotericin B, Miconazole and Nystatin was tested using the disc diffusion method. The activity of aqueous and alcoholic clove extracts (25%, 50%, 100%) and pure clove oil (Eugenol) was evaluated using a well-diffusion assay.

**Results:** Among conventional antifungals, Amphotericin B was the most effective (Mean Inhibition Zone: 27.83 ± 5.22 mm), followed by Miconazole (22.43 ± 6.50 mm) and Nystatin (20.60 ± 3.70 mm). Remarkably, clove extracts demonstrated superior activity. The 100% aqueous clove extract showed the highest overall efficacy (53.93 ± 27.82 mm), significantly outperforming all conventional drugs ($p<0.001$). The 100% alcoholic extract was also highly effective (41.40 ± 9.83 mm). Clove oil showed no inhibitory activity. Efficacy was concentration-dependent and species-specific. For instance, *C. glabrata* was highly susceptible to aqueous extracts (90.00 mm

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inhibition at 100%), while *C. krusei* showed relative resilience, responding similarly to both clove extracts and conventional drugs.

**Conclusion:** Clove extracts, particularly aqueous preparations at high concentrations, exhibit potent and broad-spectrum antifungal activity against clinical oral *Candida* isolates, surpassing standard antifungals. These findings position *Syzygium aromaticum* as a highly promising, naturally sourced candidate for developing new therapeutic or preventive strategies against oral candidiasis, especially in regions where access to conventional medicine is limited.

**Key Words:** *Syzygium aromaticum; Clove; Antifungal; Oral candidiasis; Candida; Eugenol; Natural products; Libya*

## Introduction

The human oral cavity harbors one of the most diverse microbial environments in the body, second only to the gastrointestinal tract, containing over 700 bacterial species alongside numerous fungi, viruses and protozoa [1]. This complex ecosystem, with its warm, humid and nutrient-rich conditions on teeth and soft tissue surfaces, provides an ideal habitat for various microorganisms that have co-evolved with humans, developing symbiotic relationships crucial to maintaining health [2]. The fungal component of this ecosystem the oral mycobiome plays a vital role in maintaining homeostasis, with colonization beginning shortly after birth and progressing with age [3,4].

Among the fungal inhabitants of the oral cavity, *Candida* species predominate, particularly *Candida albicans*, followed by *Cladosporium*, *Aureobasidium* and *Saccharomycetes* [5]. Under normal physiological conditions, these organisms exist as harmless commensals; however, when the oral environment is perturbed, they can transition into opportunistic pathogens, causing oral candidiasis the most prevalent fungal infection of the oral mucosa [6,7]. This transition from commensal to pathogen involves complex virulence mechanisms, including the yeast-to-hyphal morphological switch, which enables tissue invasion; secretion of hydrolytic enzymes such as Secreted Aspartyl Proteinases (SAPs) and phospholipases; and robust biofilm formation on both mucosal surfaces and prosthetic devices [8,9]. These biofilms not only protect fungal cells from host immune responses but also significantly reduce susceptibility to antifungal agents, often requiring concentrations many times higher than those effective against planktonic cells [10,11].

Oral candidiasis manifests in several clinical forms pseudomembranous, erythematous, chronic hyperplastic and angular cheilitis with symptoms including white plaques, erythema, burning sensations, altered taste and pain that can compromise nutritional intake [6,12]. The condition is particularly prevalent among immunocompromised individuals, including those with HIV/AIDS, cancer patients undergoing chemotherapy, organ transplant recipients and individuals with diabetes mellitus. Additional risk factors include poor oral hygiene, malnutrition, high-carbohydrate diets, xerostomia, pregnancy, corticosteroid therapy and broad-spectrum antibiotic use.

The significance of oral fungal infections extends beyond local morbidity. They may serve as indicators of systemic disease, with recurrent or persistent candidiasis often signaling underlying immune dysfunction or

endocrinopathies. In vulnerable populations, oral lesions may represent the first manifestation of systemic mycoses, as demonstrated in a 25-year retrospective study from Brazil where oral involvement was the initial presentation in para coccidioidomycosis, histoplasmosis and disseminated candidiasis. Furthermore, oral *Candida* carriage has been associated with dental caries, periodontal disease and even systemic conditions such as Metabolic Dysfunction-Associated Fatty Liver Disease (MAFLD), where fungal dysbiosis correlates with elevated inflammatory markers and disease severity.

The current antifungal arsenal, while effective, faces significant limitations. Polyenes such as Amphotericin B and Nystatin and azoles including Miconazole and Fluconazole, are associated with dose-limiting toxicities, adverse effects and increasing antimicrobial resistance. Amphotericin B, though broad-spectrum and fungicidal, carries substantial nephrotoxicity risk [13]. Nystatin, while safer for topical oral use, exhibits instability in light, heat and humidity, limiting its practical application [14]. The emergence of resistant strains particularly among non-*Candida albicans* species such as *C. glabrata*, *C. krusei* and *C. tropicalis* has further complicated management [15,16]. These species often demonstrate intrinsic or acquired resistance to azole antifungals, form robust biofilms and are increasingly prevalent in clinical isolates worldwide.

The situation is particularly challenging in resource-limited settings. In Libya, recent studies have documented high oral *Candida* carriage rates, with *C. albicans* predominating but significant proportions of NCA species including *C. glabrata* and *C. tropicalis* [17]. Among Libyan diabetic patients, angular cheilitis prevalence exceeds 35%, strongly associated with poor glycemic control and *Candida* colonization. A local study in Musrata identified 13 *Candida* species from diabetic patients, underscoring the diversity of fungal pathogens in the region. These epidemiological realities, combined with limited access to advanced diagnostics, empirical treatment practices and the rising costs of conventional antifungals, necessitate exploration of alternative therapeutic approaches that are effective, affordable and culturally acceptable.

Medicinal plants have served as foundational elements of traditional medicine for centuries and represent a rich reservoir of novel bioactive compounds. Among these, *Syzygium aromaticum* (L.) Merr. & L.M. Perry, commonly known as clove, has garnered particular attention for its potent pharmacological properties, including antimicrobial, antioxidant, anti-inflammatory and analgesic activities. The primary bioactive constituent, Eugenol (4-allyl-2-methoxyphenol), comprises 70%-90% of clove essential oil and exerts antifungal effects through multiple mechanisms: disruption of fungal cell membrane integrity, inhibition of ergosterol synthesis, interference with enzymatic processes and suppression of biofilm formation and hyphal development. Additional compounds such as β-caryophyllene, eugenyl acetate and α-humulene may contribute synergistically to its antimicrobial efficacy.

The antifungal potential of clove has been increasingly documented. Studies have demonstrated its efficacy against *Candida albicans* and NCA species, with mechanisms including membrane permeabilization, inhibition of germ tube formation and disruption of mature biofilms [18]. Nanoemulsified clove oil formulations have shown enhanced stability, bioavailability and sustained release, further

improving antifungal activity. Comparative investigations have revealed that clove extracts exhibit comparable or superior activity to conventional antifungals, with the advantage of natural origin and minimal toxicity. Clove-based nanoparticles synthesized from *S. aromaticum* have demonstrated broad-spectrum antimicrobial, anticancer and antioxidant properties, suggesting potential for multifaceted therapeutic applications.

However, despite this growing body of evidence, direct comparative data evaluating various clove preparations aqueous extracts, alcoholic extracts and pure oil against a comprehensive panel of clinical oral *Candida* isolates alongside standard antifungal agents remain limited, particularly from North African populations. The present study addresses this gap by conducting a comparative *in vitro* evaluation of the efficacy of conventional antifungal drugs (Amphotericin B, Miconazole, Nystatin) and various concentrations of *S. aromaticum* extracts against clinically confirmed oral *Candida* isolates obtained from patients in the Gharyan region of Libya. By integrating species-specific susceptibility testing and statistical comparisons, this investigation aims to establish the potential of clove extracts as viable alternatives or adjuncts to conventional therapy, particularly relevant for resource-constrained healthcare settings where antifungal resistance and treatment access pose significant challenges.

## Materials and Methods

### Study design and setting

This comparative *in vitro* study was designed to evaluate the antifungal efficacy of *Syzygium aromaticum* (clove) extracts against clinical oral *Candida* isolates. Sample collection was conducted among patients attending the outpatient clinics at Gharyan Central Hospital and three primary healthcare centers in the Gharyan region, Libya. All mycological analysis, including culture, identification and antifungal susceptibility testing, was performed at the Fungi Laboratory, Faculty of Medical Technology Alriyaina, University of Zintan, Libya.

### Ethical considerations

The study protocol was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine, University of Gharyan (Approval No.: UOG/MED/2025/08, dated 25 January 2025). Written informed consent was obtained from all adult participants prior to sample collection. For minor participants (under 18 years of age), consent was obtained from their parents or legal guardians. All procedures were conducted in accordance with the ethical standards of the Declaration of Helsinki of 1975, as revised in 2013.

### Study population and sample collection period

Sample collection was carried out over a period from 2 February 2025 to 20 April 2025. A total of 140 patients attending the outpatient clinics for various medical complaints were enrolled in the study. To ensure anonymity and proper tracking throughout the laboratory procedures, each patient was assigned a unique serial number upon enrollment.

### Inclusion and exclusion criteria

**Inclusion criteria:** Patients of both genders, aged between 2 and 78 years, who presented with clinical signs suggestive of oral fungal infection (e.g., white plaques, erythema, burning sensation, angular cheilitis) and were willing to provide informed consent were included in the study.

**Exclusion criteria:** Patients were excluded if they had received systemic or topical antifungal therapy within the preceding two weeks, were on immunosuppressive therapy, had a known diagnosis of HIV/AIDS or other severe immunocompromising conditions, or were unable to cooperate with the sample collection procedures.

**Sample collection procedure**

Under strict aseptic conditions and following a standardized precautionary protocol to avoid contamination, samples were collected from multiple oral sites depending on the clinical presentation. These sites included carious lesions or supragingival plaque on tooth surfaces; the buccal mucosa, palate, tongue dorsum and floor of mouth; the fitting surface of removable dentures (if present); and deep periodontal pockets using sterile curettes.

For each patient, three sterile swabs were used to collect specimens from the affected sites: the first swab was used for direct microscopic examination, the second for primary culture and the third for subculture and maintenance.

**Sample transport and storage**

Immediately after collection, each swab was placed into a sterile container and maintained at 4°C-8°C in a chilled, insulated transport box. All samples were transported to the Fungi Laboratory within 4-6 hrs of collection for immediate processing. Samples that could not be processed immediately were stored at 4°C for a maximum of 24 h before culture.

**Direct microscopic examination**

**Gram staining:** Smears prepared from the first swab were heat-fixed and stained using the standard gram staining procedure. The smears were then examined under light microscopy with a 100 × oil immersion objective for the presence of budding yeast cells, pseudohyphae characteristic of *Candida* species.

**Lactophenol Cotton Blue (LPCB) staining:** A second smear from the first swab was mounted in LPCB stain and examined under low power (10 × and 40 ×) to observe fungal morphological features, including chlamydospores, blastoconidia and pseudohyphae.

**Culture media and isolation**

**Primary culture:** The second sterile swab was streaked onto petri dishes containing Sabouraud Dextrose Agar (SDA) (Oxoid, UK). The medium was supplemented with chloramphenicol (0.05 g/L) to inhibit bacterial growth and cycloheximide (0.5 g/L) to suppress saprophytic fungi, thereby promoting the selective isolation of pathogenic *Candida* species. Inoculated plates were incubated aerobically at 37°C for 24-48 hrs. Plates showing no growth after 48 hrs were re-incubated at room temperature (25°C-30°C) for an additional 5-7 days to allow for the growth of slow-growing fungi.

**Subculture:** Suspected *Candida* colonies, identified by their creamy, smooth and pasty appearance, were subcultured onto fresh SDA plates to obtain pure cultures. The pure isolates were then maintained on SDA slants at 4°C for subsequent species identification and antifungal susceptibility testing.

**Fungal isolates and preparation**

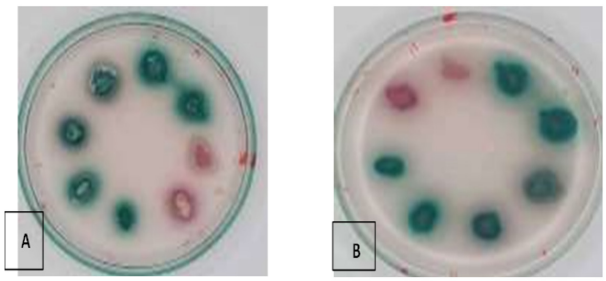
For this study, five clinically confirmed *Candida* species (*C. albicans, C. glabrata, C. krusei, C. parapsilosis and C. tropicalis*) were obtained from the stock cultures preserved from the patient samples described above. For susceptibility testing, fresh subcultures were prepared on SDA and incubated at 37°C for 24-48 hrs. A standardized

inoculum suspension equivalent to a 0.5 McFarland standard was prepared in sterile saline for each isolate.

**Identification of *Candida* isolates**

**Identification:** Phenotypic identification of the isolated *Candida* spp., was based on macroscopic and microscopic features [19-22].

**Growth on specific media (chrome agar media):** Chrome-agar is selective medium that allow the rapid isolation of yeasts from mixed cultures and rapidly identify of *Candida* spp. This medium was prepared according to the manufacturer's instructions (Chrome-agar Company. Liofilchem. Italy). A culture media utilized for rapidly identify many common *Candida* species [23]. Chemical colorimetric reaction on agar that allows distinction between *C. albicans, C. glabrata, C. krusei, C. tropicalis* and other non-*Candida albicans* species. The medically important *Candida* species appear as different colored colonies due to differential uptake of these chromogenic compounds. After incubation at 37ºC for 24-48 hrs *C. albicans* produces green colonies, while *C. tropicalis* produces blue colonies, *C. glabrata* produces dark pink colonies and *C. parapsilosis* produces cream to pale pink colonies, *C. Krusei* produce pink colure with pale edge. Chrome-agar can be helpful in detecting the presence of mixed cultures, as well as providing a preliminary species identification (Figure 1).




**Figures 1)** *A and B Candida species identified by using Chrome-agar media.*

**Preservation of fungi on agar slants**

Test tubes with a 15mL capacity were used to prepare and distribute Sabouraud Dextrose Agar (SDA). The fungal strains that needed to be preserved were inoculated in the agar slants and sterilized at 121°C for 20 mints before being incubated at 37°C for 7 days. The developed fungal slants were then kept in a frigid at 4°C for preservation [24].

**Conventional antifungal agents**

Commercially available antifungal discs (Liofilchem, Italy) were used: Amphotericin B (20 µg), Miconazole (10 µg) and Nystatin (100 IU). These were selected as representatives of major antifungal classes used in clinical practice.

**Preparation of clove extracts and oil**

**Alcoholic extract:** Dried clove buds (50 g) were ground and macerated in 500 mL of 96% ethanol for 48 hrs in a dark environment with continuous shaking. The mixture was filtered through sterile gauze and filter paper. The filtrate was concentrated using a rotary evaporator and the resulting extract was dried in an oven at 40°C ± 2°C to obtain a powder.

**Aqueous extract:** The same process was repeated using 500 mL of sterile distilled water as the solvent.

**Extract concentrations:** Both the alcoholic and aqueous clove extracts were reconstituted to final concentrations of 25%, 50% and 100% (w/v) using their respective solvents.

**Clove oil:** Commercially available Eugenol oil (DHARMA research, USA) was used directly.

**Determination of Minimum Inhibition Concentration (MIC) and Minimum Fungicidal Concentration (MFC)**

The minimum Inhibitory Concentration (MIC)

and Minimum Fungicidal Concentration (MFC) of the aqueous clove (*Syzygium aromaticum*) extract against *Candida* isolates were determined using the broth microdilution method as previously described by with minor modifications [25,26]. Fungal suspensions were prepared from 3-day-old cultures and adjusted to 0.5 McFarland standard, followed by dilution to obtain a final inoculum of approximately $1 \times 10^4$–$5 \times 10^4$ CFU/mL.

Serial two-fold dilutions of the clove extract were prepared in Sabouraud Dextrose Broth (SDB) to obtain concentrations ranging from 10%-0.039%. Each tube was inoculated with the standardized fungal suspension and incubated at 37°C for 48 h. Positive, negative and solvent controls were included to validate the assay.

The MIC was defined as the lowest concentration of the extract that completely inhibited visible fungal growth after incubation. To determine the MFC, aliquots from tubes showing no visible growth were subcultured onto Sabouraud Dextrose Agar (SDA) plates and incubated at 37°C for an additional 72 h. The MFC was recorded as the lowest extract concentration that resulted in no fungal colony growth on the agar plates.

**Antifungal susceptibility testing**

**Disc diffusion for conventional drugs:** The assay was performed on Mueller Hinton Agar (MHA) supplemented with 2% glucose and 0.5 µg/mL methylene blue, as recommended [8]. The standardized fungal suspension was swabbed uniformly onto the agar plates. Antifungal discs were placed on the inoculated surface and plates were incubated at 37°C for 24-48 hrs. The Zones of Inhibition (ZOI) were measured in millimeters (mm). All tests were performed in triplicate.

**Well diffusion for cove extracts/oil:** After swabbing the agar plates with the fungal inoculum, wells (10 mm diameter) were punched into the agar using a sterile cork borer. Each well was filled with 50 µL of the respective clove extract concentration or pure clove oil. Plates were incubated as above and the ZOI was measured. Tests were performed in triplicate for each isolate and concentration.

**Statistical analysis**

Data were analyzed using SPSS Statistics version 27. Descriptive statistics (mean ± standard deviation) were calculated. The significance of differences in the mean ZOI among different treatments and across *Candida* species was determined using a one-way analysis of variance (ANOVA), followed by post-hoc Tukey's HSD test for multiple comparisons. A p-value of less than 0.05 was considered statistically significant.

## Results

**Efficacy of conventional antifungal agents**

All tested *Candida* species were susceptible to the conventional antifungal agents, but with varying degrees of efficacy (Table 1). Amphotericin B demonstrated the strongest inhibitory effect, with a mean ZOI of 27.83 ± 5.22 mm. This was significantly greater than the ZOIs produced by Miconazole (22.43 ± 6.50 mm) and Nystatin (20.60 ± 3.70 mm) ($F=6.796$, $p=0.004$).

**Activity of antifungal aquas clove extract**

The antifungal activity of the aqueous clove extract against the tested *Candida* species was evaluated by determining the Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC). The obtained values are presented in Table 2. The extract exhibited varying degrees of antifungal activity among the tested isolates, with *C. parapsilosis* showing the greatest susceptibility and *C. albicans* and *C. tropicalis* requiring higher concentrations for growth inhibition.

**TABLE 1**

**Efficacy of conventional antifungal agents against oral *Candida* isolates (Mean ZOI in mm ± SD).**

| Antibiotics | N | Mean ± SD | F value | P value |
|---|---|---|---|---|
| Amphotericin B | 15 | $27.83^{a}$ ± 5.216 | 6.796 | 0.004 |
| Miconazole | 15 | $22.43^{b}$ ± 6.502 | | |
| Nystatin | 15 | $20.60^{b}$ ± 3.699 | | |

Different superscript letters (a, b) within a column indicate statistically significant differences ($p<0.05$).

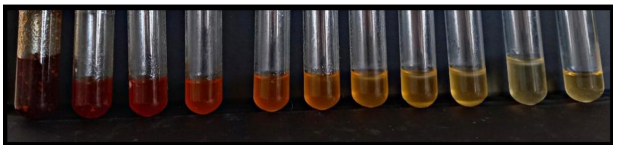
**TABLE 2**

**Values of Minimum Inhibitory Concentration (MIC) and Minimum Inhibitory Concentration (MFC) of clove aqueous extract (%) against different *Candida* isolates.**

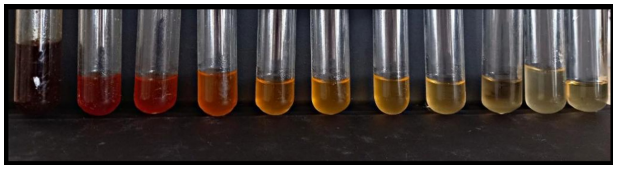
| *Candida* spp. | MIC (%) | MFC (%) |
|---|---|---|
| *C. albicans* | 5.00 | 10.00 |
| *C. glabrata* | 2.50 | 8.00 |
| *C. parapsilosis* | 1.25 | 7.00 |
| *C. tropicalis* | 5.00 | 10.00 |

The mark (>10%) indicates that no pesticide effect is recorded within the highest tested concentration (10%).

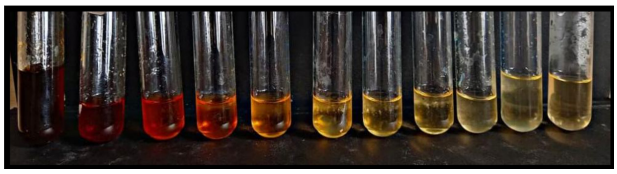
The MIC values expressed by (%) varied according to the fungal species. The lowest inhibitory concentration (MIC=1.25%) was recorded for *C. parapsilosis,* while the highest value (MIC=5%) was recorded for *C. tropicalis, C. albicans* and *C. glabrata* showed MIC values of 5% and 2.50%, respectively (Figures 2-5).



**Figures 2)** *Figure 2 Shows the results of the MIC test on Candida parapsilosis.*

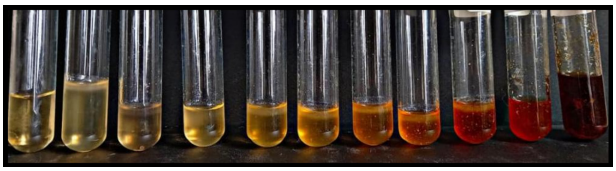


**Figures 3)** *Figure 3 shows the results of the MIC test on Candida glabrata.*



**Figures 4)** *Figure 4 shows the results of the MIC test on Candida albicans.*

The extract also showed a clear fungicidal effect against only two of the tested species, with an MFC value against *C. tropicalis* (5%), a concentration similar to its inhibitory value (MFC/MIC=1). The MFC value against *C. albicans* was (10%). In contrast, pesticide effect was recorded against *C. glabrata* and *C. parapsilosis* isolates concentration range (8% and 7% respectively). All control groups

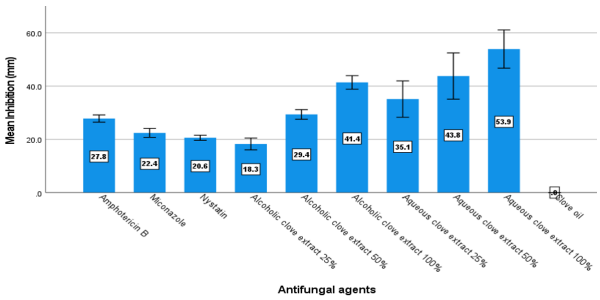


**Figures 5)** *Figure 5 shows the results of the MIC test on Candida tropicalis.*

(positive control, negative control) gave the expected results, confirming the validity of the test results and their absence of any inhibitory effect of the solvent used.

**Comparative efficacy of all antifungal agents**

A comprehensive comparison of all tested agents revealed highly significant differences (F=152.69, p<0.001) (Table 3). The 100% aqueous clove extract was the most effective treatment overall, producing a mean ZOI of 53.93 ± 27.82 mm. This was followed by the 50% aqueous extract (43.80 ± 33.67 mm) and the 100% alcoholic extract (41.40 ± 9.83 mm). All three of these natural preparations were significantly more effective than the conventional drugs Amphotericin B, Miconazole and Nystatin. Pure clove oil showed no measurable antifungal activity under the test conditions. Following graph displays different antifungal agents used in this study (Figure 6).




**Figures 6)** *Efficacy of antifungal agents to inhibition of oral fungal infections.*

**TABLE 3**

**Comparative efficacy of all antifungal agents and clove extracts (Mean ZOI in mm ± SD).**

| Antifungal agents | N | Mean ± SD | F Value | P Value |
|---|---|---|---|---|
| Amphotericin B | 15 | 27.83a ± 5.216 | | |
| Miconazole | 15 | 22.43b ± 6.502 | | |
| Nystatin | 15 | 20.60bd ± 3.699 | 152.692 | <0.001 |
| Alcoholic clove extract 25% | 15 | 18.27d ± 8.610 | | |
| Alcoholic clove extract 50% | 15 | 29.37 ± 7.005 | | |
| Alcoholic clove extract 100% | 15 | 41.40e ± 9.832 | | |
| Aqueous clove extract 25% | 15 | 35.13f ± 26.457 | | |
| Aqueous clove extract 50% | 15 | 43.80e ± 33.671 | | |
| Aqueous clove extract 100% | 15 | 53.93g ± 27.82 | | |
| Clove oil | 15 | 0.00 ± 0.000 | | |

Different superscript letters (a-g) within a column indicate statistically significant differences (p<0.05).

**Species-specific antifungal response**

The efficacy of the agents varied considerably depending on the *Candida* species (Table 4). Aqueous clove extracts were exceptionally effective against *C. albicans* and *C. glabrata*, with ZOIs exceeding 80 mm at 50% and 100% concentrations. In contrast, *C. krusei* and *C. parapsilosis* were less susceptible to the aqueous extracts, with the highest ZOIs being 32.00 mm and 33.33 mm, respectively. For these species, the conventional antifungals, particularly Amphotericin B and Miconazole, showed comparable or slightly better activity than the clove extracts. *C. tropicalis* was most effectively inhibited by the 100% alcoholic clove extract (45.61 mm).

**TABLE 4**

**Species-specific response: mean ZOI (mm) of antifungal agents against different *Candida* species.**

| *Candida* spp. | Treatment (most effective) | Mean ZOI ± SD | Treatment (conventional) | Mean ZOI ± SD |
|---|---|---|---|---|
| *C. albicans* | Aq. Extract 100% | 83.33 ± 3.06 | Amphotericin B | 26.33 ± 10.61 |
| *C. glabrata* | Aq. Extract 100% | 90.00 ± 0.00 | Amphotericin B | 31.00 ± 1.80 |
| *C. krusei* | Aq. Extract 100% | 32.00 ± 2.00 | Miconazole | 31.17 ± 0.29 |
| *C. parapsilosis* | Alc. Extract 100% | 43.44 ± 8.95 | Amphotericin B | 25.17 ± 4.81 |
| *C. tropicalis* | Alc. Extract 100% | 45.61 ± 2.97 | Amphotericin B | 28.33 ± 4.54 |

## Discussion

The search for effective and safe antifungal agents is a persistent priority in medical mycology. Our findings demonstrate that crude extracts of *Syzygium aromaticum* possess remarkable antifungal activity, significantly surpassing that of commonly used conventional drugs against clinical oral *Candida* isolates.

The superior performance of Amphotericin B over the azole (Miconazole) and the other polyene (Nystatin) is consistent with its broad-spectrum, fungicidal nature [27]. However, its clinical use is often hampered by nephrotoxicity and other side effects [10].

The aqueous extract of *Syzygium aromaticum* (clove) exhibited considerable antifungal activity against all tested *Candida* species, although susceptibility varied among the isolates. The lowest MIC value was observed for *Candida parapsilosis* (1.25%), indicating the highest sensitivity to the extract, whereas *C. albicans* and *C. tropicalis* required higher concentrations (5%) to achieve complete growth inhibition. *C. glabrata* demonstrated intermediate susceptibility with an MIC value of 2.5%.

The fungicidal activity of the clove extract was likewise species-dependent. MFC values ranged from 7%-10%, with the lowest fungicidal concentration recorded for *C. parapsilosis* (7%) and the highest for *C. albicans* and *C. tropicalis* (10%). The higher MFC values relative to the corresponding MIC values indicate that concentrations greater than those required for growth inhibition were necessary to achieve complete fungal eradication. These differences in susceptibility may reflect variations in cell

wall structure, membrane composition and intrinsic resistance mechanisms among the tested *Candida* species.

The antifungal activity observed in this study agrees with previous reports demonstrating the broad-spectrum antimicrobial properties of clove extracts [11,13]. This activity has been primarily attributed to eugenol, the major bioactive constituent of clove, which is known to disrupt fungal cell membranes, increase membrane permeability, inhibit ergosterol biosynthesis and interfere with essential cellular functions, ultimately resulting in fungal growth inhibition and cell death [11,12]. Furthermore, the expected outcomes obtained from the positive, negative and solvent controls confirmed the reliability of the assay and excluded any inhibitory effect of the DMSO solvent.

Overall, the findings suggest that aqueous clove extract possesses promising antifungal activity against clinically important *Candida* species and may represent a potential natural source of antifungal agents for future therapeutic applications. Further studies involving larger numbers of clinical isolates and investigations into the extract's active compounds and mechanisms of action are warranted [12].

The most striking result of this study is the potent efficacy of clove extracts. The 100% aqueous extract emerged as the most powerful agent overall. This was an unexpected but significant finding, as alcoholic extracts are typically more efficient at extracting non-polar bioactive compounds like Eugenol. The superior performance of the aqueous extract in our assay could be due to better diffusion of its constituents through the aqueous-based agar medium, leading to a larger observable zone of inhibition. Both aqueous and alcoholic extracts exhibited a clear dose-dependent response, with 100% concentrations being vastly more effective than 25% concentrations, underscoring the concentration of active antifungal components.

The failure of pure clove oil (Eugenol) to produce an inhibition zone was paradoxical. This could be attributed to its high volatility and hydrophobic nature, preventing its effective diffusion from the well into the agar matrix. In clinical dental applications, Eugenol is often used in a paste form with zinc oxide, which may facilitate its release and activity, unlike the pure oil in a well-diffusion test.

The species-specific variation in susceptibility is clinically crucial. The high susceptibility of *C. albicans* and *C. glabrata* to aqueous clove extracts is promising, given their high clinical prevalence. In contrast, the relative resilience of *C. krusei*, which is intrinsically less susceptible to fluconazole, to both clove extracts and conventional drugs highlight its challenging nature. This suggests that while clove extracts are a powerful broad-spectrum alternative, species identification remains important for tailoring therapy, especially in recalcitrant cases.

The mechanism of action is widely attributed to Eugenol, which can integrate into and disrupt the fungal cell membrane, leading to increased permeability and cell death [7]. Additionally, clove extracts are known to inhibit germ tube and biofilm formation, key virulence factors of *Candida*. The presence of other synergistic compounds in the crude extract, such as β-caryophyllene, may further enhance its antifungal potency beyond that of pure Eugenol alone.

## Limitations and future directions

Further *in vivo* studies and the development of stable formulations (e.g., mouthwashes, gels) are essential next steps to translate these findings into clinical practice.

## Conclusion

This study provides compelling evidence that *Syzygium aromaticum* (clove) extracts, particularly aqueous extracts at high concentrations, are potent inhibitors of clinical oral *Candida* species, exhibiting efficacy that surpasses standard antifungal drugs. Given their natural origin, anticipated low cost and reduced potential for side effects, clove extracts represent a highly promising alternative or adjunctive therapy for managing oral candidiasis. This is especially relevant for resource-limited settings like Libya, where access to conventional antifungals may be constrained. We recommend further phytochemical analysis and clinical trials to develop these findings into tangible therapeutic options.

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