# Effect of Clove Extract on Some Oral Fungi Isolated from Human Oral Cavities

Reham Ismail Ibrahim
Riham.Ibrahim929@st.tu.edu.iq
Roaa Hassan Latif
roaahassan@tu.edu.iq
Tikrit University / College of Education for Women / Department of Biology

**Abstract**

phytomedicinal plants are increasingly recognized for their rich content of pharmacological compounds that offer diverse pharmacological benefits. Among these, eugenol specific (*Syzygium aromaticum*) is prominent due to its elevated concentration of eugenol, which exhibits potent antifungal activity. This study was designed to examine the antifungal effects of *Syzygium aromaticum* extracts on fungi isolated from the oral cavities of both infants and elderly individuals. The research was conducted between October 2025 and January 2026 at Tikrit Teaching Hospital. Buccal swab samples were collected and subsequently incubated on Sabouraud Dextrose Agar (SDA) to identify dominant fungal strains. Various concentrations of clove extract were employed to determine their growth-inhibiting influence on fungal growth. A total of 150 oral swabs were obtained from infants and elderly individuals to assess the inhibitory capacity of clove (Syzygium aromaticum) extracts against oral fungi. Direct microscopic visualization demonstrated a higher colonization rate among infants compared to elderly individuals, with confirmed cases accounting for 87.6% and 54.1%, respectively. Likewise, culture results on Sabouraud Dextrose Agar (SDA) disclosed positive proliferation rates of 84.3% in infants and 55.7% in the elderly.Five fungal species were recovered and characterized: Candida albicans, Candida tropicalis, Candida ciferrii, Trichosporon mucoides, and Cryptococcus laurentii. Candida albicans was the dominant organism (42.20%), followed by Cryptococcus laurentii (22.02%). Candida albicans demonstrated the ability to produce hyphal projections and chlamydospores, whereas Cryptococcus laurentii was distinguished by polysaccharide capsule production.Aqueous, alcoholic, and glycerinic clove extracts were formulated at four different dosages (25%, 50%, 75%, and 100%) and examined against the recovered fungi. The results indicated that antimycotic efficacy rose in a dose-dependent manner. The alcoholic extract displayed the most potent suppressive action, followed by the glycerinic extract, whereas the aqueous extract exhibited the weakest antimycotic effect. Candida ciferrii was the most susceptible species to the extracts, while Candida albicans showed reduced susceptibility. These findings support the potential of clove extracts, particularly the alcoholic extract, as efficacious plant-derived antifungal substances against oral fungal infections.These findings indicate that clove extract may serve as a plant-based antifungal substance capable of reducing oral fungal infections, particularly in populations predisposed to such infections, such as infants and the elderly. Further research is recommended to investigate the therapeutic potential of clove extract in therapeutic settings.

**Keywords: Clove extract, Oral fungi, Antifungal activity.**

## Introduction

Oral candidiasis is a prevalent fungal infection in the oral cavity, typically induced by yeast species including *Candida albicans*, Candida krusei,

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Candida parapsilosis, Candida tropicalis, and Candida glabrata [1]. They typically reside on the skin and within the body without adverse effects; however, alterations in the oral, pharyngeal, or esophageal environment that promote their proliferation might result in an oral candidiasis infection Oropharyngeal candidiasis, commonly referred to as oral thrush, represents one of the most prevalent mycotic infections affecting the oral mucosa region. It is characterized by the presence of whitish lesions or pseudomembranous patches on the mucosal lining, tongue, palate, and inner cheeks, frequently accompanied by oral soreness, mucosal irritation, altered taste perception, and dysphagia in severe cases. Infants, elderly individuals, immunosuppressed individuals, and patients undergoing extended antimicrobial treatment are considered high-risk populations for developing this condition. The disease may arise as a consequence of oral microbial flora dysbiosis, xerostomia, inadequate oral care, or systemic disorders that promote candidal proliferation within the oral environment.[2]. The increasing microbial resistance to conventional antimicrobial agents has led to growing interest in exploring plant-derived natural compounds with antimicrobial activity [3]. Historically, numerous civilizations have employed plants to address various ailments, including microbial infections. Plants have served as a viable alternative and prospective source of novel antimicrobial compounds, utilized for centuries in traditional medicine across numerous countries to address diverse ailments, including microbial infections[4]. These phytogenic resources contain numerous biologically active compounds that demonstrate antimicrobial activity against a broad spectrum of microbial pathogens[5].Including bacterial pathogens, fungal pathogens, and viral pathogens. Botanical antimicrobial compounds have attracted considerable attention as potential alternatives to chemically synthesized pharmaceuticals due to their minimal adverse reactions, cost-effectiveness, enhanced availability, and superior safety profile. Consequently, plant-origin therapeutic substances are increasingly recognized as promising candidates for the prevention and management of microbial infections. [6]. Intrinsic and acquired resistance to numerous antifungal agents has been thoroughly documented in various Candida species, thereby facilitating the exploration of alternative drugs and treatments that may prove more effective than current options[7]. Clove (*Syzygium aromaticum*) is a fragrant flower that is part of the Myrtaceae family. Clove has been utilized in traditional medicine as an antiseptic for various diseases, including those in the oral cavity caused by numerous yeasts and bacteria[8].

**Materials and Methods**

In the present investigation, the study was carried out in the laboratories of Tikrit University / College of Education for Women / Department of Biology from October 2025 to January 2026. A total of 150 oral specimens were obtained from participants clinically confirmed with oral candidiasis, including both outpatients and admitted individuals at Tikrit Teaching Hospital. The diagnosis was validated by physicians. The ages of the participants ranged from 1 day to 100 years, and both genders were included. The participants had various predisposing health conditions.

**Isolation and identification**

*Candida spp* isolation based on culture Sabouraud Dextrose Agar (SDA) (SDA),

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Distinguish by chrom agar and germ tube formation as described by [9]The identification confirmed by germ tube formation, chlamydospore formation, and finally by **Vitek2 System[10]**.

**Preparation of Aqueous Extract:**

The aqueous extract was formulated by solubilizing 50 g of dried clove powder in 500 mL of purified water in a dark glass flask. The preparation was left at room temperature for three days, then clarified, and various dilutions were thereafter prepared the unrefined plant extract was subjected to a filtration process to eliminate botanical particulates and insoluble residues. The resulting filtered solution was obtained under aseptic conditions and further subjected to purification to ensure complete separation of residual particulates and leftover impurities. The clarified liquid layer was carefully collected and designated as the primary stock solution. Subsequently, the purified extract was preserved in aseptic amber-colored containers under refrigeration conditions at 4°C until use. A graded concentration series was then prepared through a sequential dilution technique for subsequent antifungal activity evaluation. [11].

**Results**

A total 150 samples were taken from oral, 109(**72.7**%) were positive isolated, while 41(**27.3%**) were negative isolated.

**Table (1): Distribution of positive and negative cases among samples**

| Result | Number of Ssamples | Percentage(%) |
|---|---|---|
| Positive | | 72.7% |
| Negative | 30 | 27.3% |
| Total | 109 | 100% |

Microscopic examination and laboratory culture of the fungi revealed three species belonging to the genus Candida spp. The most frequently isolated species across both age groups was *Candida albicans*, with 46 isolates (42.20%). These results align with numerous studies focusing on oral candidiasis, consistently ranking *Candida albicans* as the most prevalent species. While 24 samples of *Cryptococcus laurentii* were isolated, representing 22.02%, and Twenty isolates of *Trichosporon mucoides* were identified, representing 18.35% of total isolates, while 2.75% remained unidentified by the VITEK system due to identification limitations.. This may be attributed to the system's limitations in identifying some fungal species, as shown in Figure.(1) . This result agree with[12].

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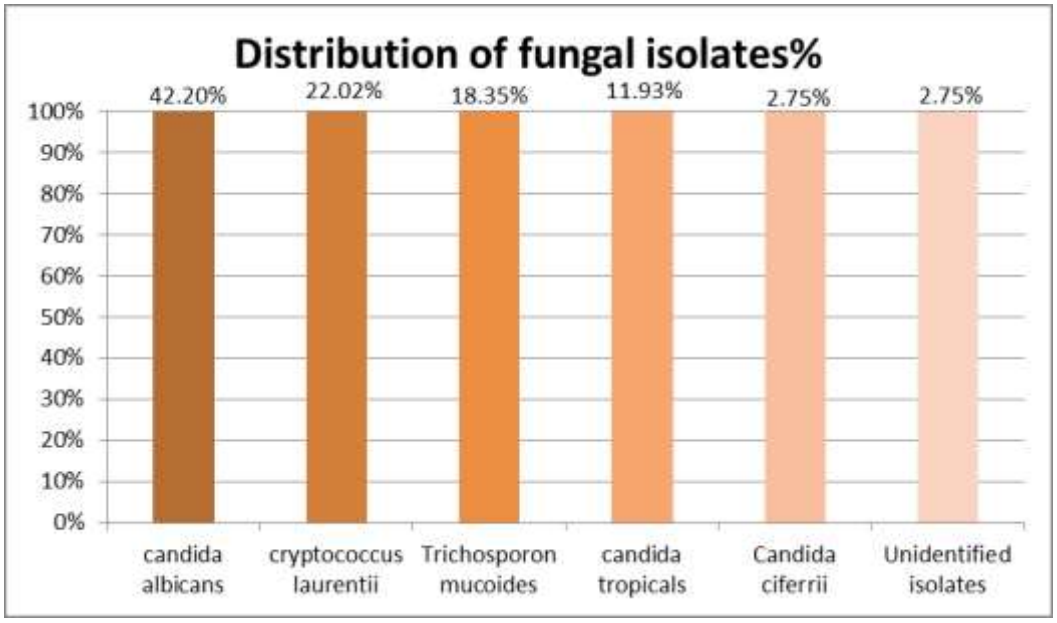


**Figure (1) shows the percentages of isolated fungal species**

Following

Following cultivation of the specimens on Sabouraud Dextrose Agar (SDA) at 37 °C for 24–48 hours, growth units were observed. They demonstrated cream-colored to milky pigmentation with a smooth, homogeneous surface topography and a soft, waxy consistency. Morphologically, the isolates ranged from round to ovoid forms and exhibited a prominent, convex-profiled appearance accompanied by a characteristic yeast-like aroma. These observations are consistent with [13], who reported that Candida spp. form glistening, creamy colony formations with an elevated colony morphology.

**Figure (2) illustrates fungal growth on SDA medium.**

The test results showed that colonies appeared in different colors on the medium (Chrom Agar), where the *C.albicans* colony appeared in a light

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green color , whereas *Candida krusei* displayed deep rose pigmentation. *Candida tropicalis* exhibited a bluish chromogenic expression. These findings are in accordance with those documented by[14] .

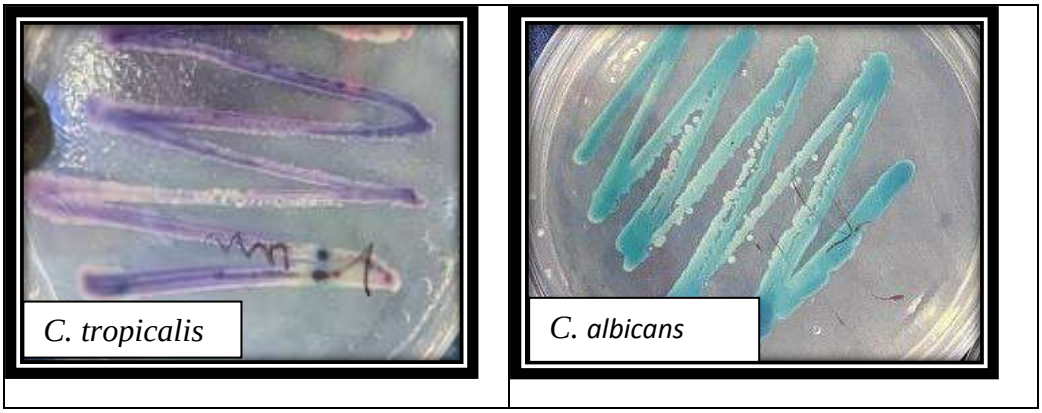


Figure (3) illustrates the types of yeasts growing on CHROMagar medium.

findings revealed that C. albicans developed hyphal extensions whereas the remaining species were unable to produce germ tubes under the same experimental settings. These results are in agreement with those reported [23].

**Figure (4) illustrates germ tube formation.**

The results revealed that the aqueous clove extract exhibited antifungal activity against the tested fungi, which rose consistently with increasing concentration, indicating a direct concentration-dependent correlation between extract level and antifungal effect. C. ciferrii had the highest sensitivity, with a zone of inhibition of 14.83 mm at 100% concentration, whereas C. albicans had the lowest susceptibility among the species. Higher concentrations resulted in a significant increase in inhibitory zones for all fungus, with Trichosporon mucoides exhibiting the weakest sensitivity at certain levels. while Trichosporon mucoides showed the poorest sensitivity at certain concentrations.

The antifungal effect of the aqueous extract is linked to its hydrophilic active constituents; however, its effectiveness was comparatively reduced than that of the alcoholic extract, presumably attributed to the poor dissolving capacity of major phenolic compounds, such as eugenol, in water, leading to decreased extraction efficiency and consequently reduced antimicrobial potency. The main active compound in clove extract is eugenol, a phenolic compound containing a hydroxyl group that is responsible for

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its antimicrobial activity against a wide range of microorganisms.

**Table (3) Inhibition zone diameters of aqueous clove extract against oral fungi**

| **Different concentrations of aqueous clove extract Mg/ml** | | | | **Types of funges** |
|---|---|---|---|---|
| 100 | 75 | 50 | 25 | |
| 7.33 ± 0.33 A b | 6.33 ±0.33 B b | 6.00± 0.29 B b | 2.67± 0.17 C b | *C.albicans* |
| 8.17 ± 0.33 A b | 6.50 ±0.50 B b | 5.50 ± 0.50 B b | 3.17 ±0.15 C b | *T.mucoides* |
| 8.83 ± 0.17 A a d | 7.83 ±0.44 B a b | 6.33 ± 0.17 B b | 2.83 ±0.09 C b | *C.tropicalis* |
| 12.50 ± 0.29 A a b | 11.50±0.50 A a | 8.17 ± 0.17 B a | 4.83 ±0.19 C a | *Cr.laurentii* |
| 14.83 ± 0.44 A a | 10.17±0.17 B a | 8.50 ± 0.29 B a | 5.17 ±0 .12 C a | *C. siferrii* |

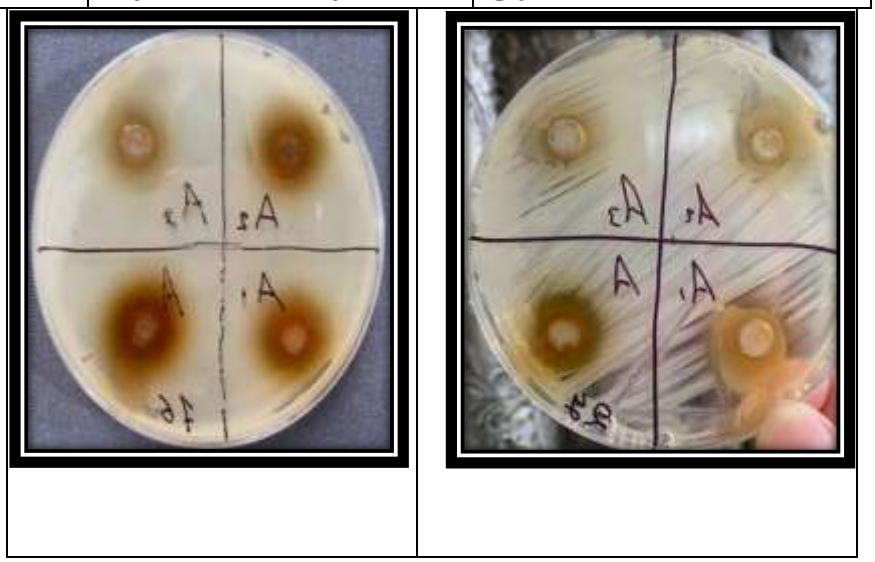

**Figure (5) illustrates the effect of the aqueous clove extract on oral fungi.**

## Discussion

The alcoholic clove extract (Syzygium aromaticum) exhibited strong antifungal activity against all tested oral fungi, with inhibition zones increasing in a concentration-dependent manner. The highest antifungal activity was recorded at 100% concentration, where *Candida ciferrii* showed the greatest sensitivity (27.67 mm), while *Candida albicans* demonstrated the lowest response across all concentrations as shown in Table (3). A clear dose–response pattern was observed, as inhibition zones progressively increased from 25% to 50% and further at higher concentrations, although a slight plateau was noted at 75% and 100% as shown in Figure (5).. Variations in susceptibility among fungal species indicate differential resistance mechanisms,

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likely associated with differences in cell wall and membrane composition. *C. ciferrii* was identified as the most sensitive species, whereas *C. albicans* represented the least affected isolate, suggesting higher tolerance in this species. These findings may be attributed to the high content of bioactive phytochemicals in clove extract, particularly eugenol, which is responsible for its antifungal activity. Eugenol disrupts fungal cell membrane integrity, interferes with enzymatic systems, and inhibits ergosterol synthesis, leading to impaired cellular function and fungal cell death. These mechanisms explain the observed concentration-dependent increase in antifungal activity.The results of the present study are consistent with previous studies [19–21], which reported strong antifungal effects of clove extracts against different *Candida species*. These studies confirmed that plant-derived phenolic compounds exhibit significant inhibitory activity due to their ability to damage fungal cell membranes and metabolic pathways.

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**Effect of alcoholic clove extract on fungi isolated from the oral cavity**

The alcoholic clove extract (Syzygium aromaticum) exhibited strong antifungal activity against all tested oral fungi, with inhibition zones increasing in a concentration-dependent fashion. The highest activity was recorded at 100% concentration, where *Candida ciferrii* demonstrated the greatest sensitivity (27.67 mm), while *Candida albicans* showed the lowest response across all concentrations as shown in Table (4), indicating comparatively greater tolerance.A distinct direct dose–effect pattern was detected, as inhibition zones showed marked enlargement from 25% to 50% and further elevated at higher concentrations, although with a diminished pace at 75% and 100%, suggesting a plateau phenomenon as shown in Figure (6). Variations in susceptibility among fungal species are probably associated with differences in cellular membrane composition and sensitivity to bioactive constituents, particularly eugenol.Collectively, *Candida ciferrii* was identified as the most sensitive organism, whereas *Candida albicans* represented the least affected isolate. These findings substantiate the dosage-related antimycotic capability of alcoholic clove extract against orally recovered fungal species.

**Table (4):Effect of different concentrations on inhibition zone diameters (mm) of the studied fungi (mean ± standard error)**

| **Different concentrations of ethanolic clove extract Mg/ml** | | | | **Types of funges** |
|---|---|---|---|---|
| **100** | **75** | **50** | **25** | |
| 12.33±0.58 A c | 11.50±0.87 A c | 9.50±0.50 B c | 7.17±0.29 C c | *C.albicans* |
| 21.67±1.53 A b | 19.83±0.76 A a b | 16.50±0.50 B b | 12.67±0.58 C b | *T.mucoides* |
| 22.00±0.50 A b | 18.33±0.29 A B a b | 16.83±0.76 B b | 11.67±0.58 C b | *C.tropicalis* |

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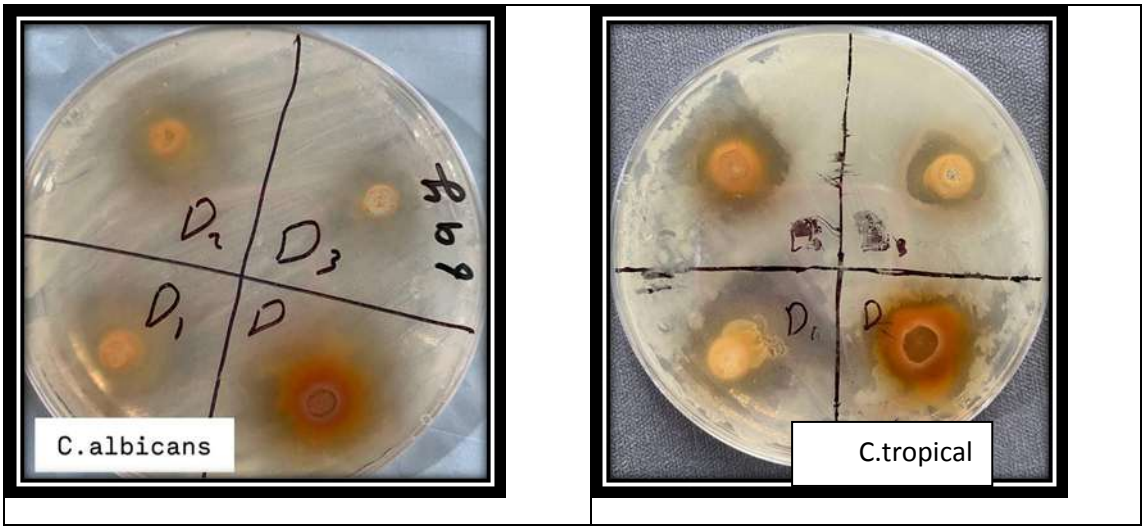
| | | | | |
|---|---|---|---|---|
| 22.83±0.76 A b | 17.83±0.76 B b | 18.50±0.50 B a b | 12.67±0.58 C b | *Cr.laurentii* |
| 27.67±2.52 A a | 22.50±0.50 B a | 21.17±1.53 B a | 19.83±0.76 C a | *C. siferrii* |




The results of this study are in accordance with several previous investigations that confirmed the antifungal activity of clove extract, including (19), who documented that plant extracts abundant in polyphenolic constituents, including clove, exhibit potent suppressive actions against various Candida species due to their ability to compromise fungal cellular integrity. The antimycotic efficacy is chiefly linked to eugenol, as(17) affirmed that it suppresses intracellular enzymatic pathways and disrupts vital physiological functions, resulting in growth inhibition or fungicidal outcomes. These findings are also consistent with study [21], which reported that alcoholic clove extract exhibits antifungal activity against different Candida species, including Candida tropicalis and *Candida albicans*, as evidenced by increased inhibition zones in a concentration-dependent manner.

These findings are also aligned with (21), who indicated that alcoholic clove extract shows antifungal activity against different Candida species such as *Candida tropicalis* and *Candida albicans*, with suppression diameters enlarging in a dose-responsive fashion, supporting its potential as a natural antifungal reservoir. In contrast, (20) reported reduced zones of inhibition (11–14 mm) against *Candida albicans* relative to the current investigation, which might be ascribed to variations in isolation procedures, employed solvent systems, or tested mycotic isolates . Overall, the alcoholic clove extract constitutes a promising reservoir of antifungal compounds, especially considering the escalating tolerance of fungi to traditional azole-based

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therapeutics, with its activity being evidently dosage-related, thereby reinforcing its prospective use in formulating plant-derived antimycotic compounds for clinical and pharmacological applications.

## Conclusion

The clove-derived ethanolic extract exhibited marked mycological suppressive activity against clinically derived oral fungal specimens in a dose-dependent fashion. The highest susceptibility strain was Candida ciferrii, whereas Candida albicans demonstrated reduced vulnerability and the poorest inhibitory response. These findings indicate the promising therapeutic application of clove phytochemical extract as a plant-derived antimycotic substance.

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