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Evaluation of the Antifungal Activity of Clove and Cinnamon Essential Oils against *Candida Albicans*

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Abstract

Fungal infections, particularly those caused by Candida albicans, pose a significant global health challenge, with rising incidences and emerging drug resistance complicating treatment strategies. This study investigates the antifungal potential of clove (Syzygium aromaticum) and cinnamon (Cinnamomum zeylanicum) essential oils against Candida albicans isolated from oral swabs of children in selected communities in Maiduguri. Phytochemical analysis revealed the presence of bioactive compounds, such as eugenol and cinnamaldehyde, in both oils, which are known for their antimicrobial properties. The antifungal activity was assessed using disk diffusion, minimum inhibitory concentration (MIC), and minimum fungicidal concentration (MFC) tests. Results showed that clove oil contained flavonoids, saponins, tannins, and phytosterols, while cinnamon oil exhibited phlobatannins and phytosterols. Both the plants' essential oils exhibited antifungal effects, with cinnamon oil demonstrating slightly higher potency than clove oil, particularly at higher concentrations (100% and 75%). The susceptibility testing also revealed that clove oil exhibited a minimum inhibitory concentration (MIC) of 50%, whereas cinnamon required a full 100% concentration to inhibit the growth of Candida albicans. At lower concentrations (<50%), both oils lost efficacy. The study also explored the concentration-dependent nature of their antifungal activities, indicating that these oils could serve as promising alternative therapies, especially in the context of antifungal resistance. The findings highlight the potential of clove and cinnamon essential oils as cost-effective, natural antifungal agents that could contribute to more sustainable treatment options for Candida albicans-related infections. Further research into their mechanisms of action, including effects on biofilm formation and cell membrane integrity, is warranted.

Key words: Candida albicans, Antifungal, Clove, Cinnamon

INTRODUCTION

Infections caused by unicellular fungal species present complex and under-documented health issues, particularly in developing countries (Bongomin and Fayemiwo, 2021). Invasive candidiasis, caused by *Candida albicans* and increasingly by non-*albicans Candida* species, is a significant concern, often stemming from endogenous human reservoirs and triggered by impaired host defenses (Lass-Flörl *et al.*, 2024). The actual burden of fungal diseases in Africa is not fully understood due to challenges in accessing and utilizing fungal diagnostics, as well as a lack of trained personnel in clinical and diagnostic mycology (Bongomin and Fayemiwo, 2021). This is further exacerbated by the disproportionately limited access to quality healthcare and the unavailability of effective antifungal drugs, placing immunocompromised individuals at heightened risk (Dangarembizi *et al.*, 2022). In Nigeria, a study in Benin City highlighted the rising incidence of candida infections, emphasizing the need for routine speciation and susceptibility testing (Oladugba, 2022).

Invasive candidiasis, a serious and potentially deadly infection, affects various organs and is associated with factors such as indwelling medical devices, prolonged hospital stays, and broad-spectrum antibiotic use, especially in immunocompromised patients (Okoye *et al.*, 2022). A gap analysis survey of a Nigerian tertiary hospital revealed significant deficiencies in laboratory capacity for diagnosing invasive fungal infections, indicating a critical need for improved diagnostic infrastructure. The situation in Nigeria mirrors broader trends across Africa, where fungal infections often go undiagnosed and

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underreported, contributing to preventable mortality (Mohamed *et al.*, 2022). The estimated annual global incidence of candidemia is high, reaching approximately 1500 thousand cases (Denning, 2024).

Candida albicans is a prevalent opportunistic fungal pathogen that poses a significant threat to human health, causing a range of infections from superficial mucosal infections to life-threatening systemic candidiasis (Bona *et al.*, 2016; El-Baz *et al.*, 2021). The increasing incidence of *Candida* infections, coupled with the emergence of drug-resistant strains, necessitates the exploration of alternative therapeutic strategies (Goel *et al.*, 2016; Man *et al.*, 2022; Parker *et al.*, 2022). Conventional antifungal agents, such as azoles, polyenes, and echinocandins, are often associated with toxicity, drug interactions, and the development of resistance, highlighting the urgent need for novel and effective antifungal agents (Goel *et al.*, 2016; Mandras *et al.*, 2016; Atron *et al.*, 2022).

In recent years, there has been a growing interest in the potential of plant-derived essential oils (EOs) as alternative therapeutic options for managing fungal infections (Prajapati *et al.*, 2021; Ben-Ami *et al.*, 2024) . Essential oils, known for their diverse chemical compositions and broad-spectrum antimicrobial properties, have demonstrated promising antifungal activity against various *Candida* species (Mandras *et al.*, 2016; Man *et al.*, 2022). Among these, clove (*Syzygium aromaticum*) and cinnamon (*Cinnamomum zeylanicum*) essential oils have garnered significant attention due to their potent antifungal properties and long history of traditional use (Shahina et al., 2022; Ali and Ibrahim, 2023).

Clove essential oil, derived from the flower buds of the clove tree, is rich in eugenol, a phenolic compound known for its strong antimicrobial and antioxidant properties (Pinto *et al.*, 2009; Alshaikh and Perveen, 2017; Shahina *et al.*, 2022). Studies have shown that clove oil exhibits significant antifungal activity against *C. albicans* by disrupting cell membrane integrity, inhibiting biofilm formation, and suppressing virulence factors (El-Baz *et al.*, 2021; Palaskar *et al.*, 2024). Similarly, cinnamon essential oil, extracted from the bark or leaves of the cinnamon tree, contains cinnamaldehyde as its primary active component, which possesses potent antifungal properties (Shahina *et al.*, 2018). Cinnamon oil has been shown to inhibit the growth of *C. albicans* by interfering with cell wall synthesis and inducing cell cycle arrest (Shahina *et al.*, 2018).

The synergistic potential of combining different essential oils has also been investigated as a strategy to enhance antifungal activity and overcome drug resistance (Purkait *et al., 2020;* Sethunga *et al.*, 2023). Several studies have reported that combinations of clove and cinnamon essential oils exhibit synergistic antifungal effects against *C. albicans*, suggesting that these oils may have complementary mechanisms of action. For example, Sethunga et al. (2023) demonstrated the zone of inhibition of different essential oils and combinations against microbial strains, including *Candida albicans*. The exploration of such synergistic combinations represents a promising avenue for developing more effective antifungal therapies.

Given the increasing prevalence of *Candida* infections and the limitations of conventional antifungal agents, this study aims to evaluate the *in vitro* antifungal activity of clove and cinnamon essential oils against *Candida albicans*. The objective of this study was to determine the minimum inhibitory concentrations (MICs) of clove and cinnamon essential oils against *C. albicans* isolates. The findings of this study will provide valuable insights into the potential of clove and cinnamon essential oils as natural antifungal agents for treating *Candida* infections, with implications for the development of novel therapeutic strategies to combat drug-resistant fungal pathogens.

MATERIALS AND METHODS

Study area

The study was conducted in Maiduguri (Lat.: 11° 49' 51.9528" N; Long.: 13° 9' 3.4812" E), the capital city of Borno State, Nigeria. Maiduguri is located in the northeastern region of the country and experiences a semi-arid climate. Due to the prevalence of fungal infections, particularly candidiasis, and the increasing number of immunocompromised individuals in the region, the city provides a relevant backdrop for the study.

Sampling

Analytical-grade clove and cinnamon essential oils were obtained from [Sigma-Aldrich, USA] and stored at 4 °C in amber vials to prevent photodegradation. All reagents used were of

analytical grade. Dimethyl sulfoxide (DMSO) was used as a diluent for oils when required. A community-based sampling of children with oral thrush was carried out using a sterile swab stick. The samples were transported to the laboratory following aseptic techniques, and they were processed immediately.

Isolation and Identification of *Candida albicans*

Swab samples from the oral cavity of the children were inoculated on freshly prepared Sabouraud Dextrose Agar (SDA) and incubated for 48 hour at 37 °C. After incubation, slightly doomed, opaque, creamy-colored smooth colonies were selected and sub-cultured on SDA. The isolate was maintained on Sabouraud Dextrose Agar (SDA) at 4 °C and sub-cultured onto fresh plates every two weeks. For the experiments, cells were grown overnight in Sabouraud Dextrose Broth (SDB) at 37°C under shaking conditions (150 rpm) until the mid-logarithmic phase.

Preparation of Essential Oil Dilutions

Essential oils were serially diluted using DMSO (to make a 0, 25, 50, 75 and 100%(v/v) final concentrations) to achieve test concentrations. Dilutions were prepared freshly before each assay. Control wells containing equivalent DMSO without essential oil were included to ensure that no solvent-induced inhibition occurred.

Antifungal Susceptibility Testing

a. Broth Microdilution Assay (CLSI M27-A3)

The minimum inhibitory concentration (MIC) was determined using the broth microdilution method, as described in the Clinical and Laboratory Standards Institute (CLSI) M27-A3 guidelines, according to Espinel-Ingroff *et al.* (2009). Briefly, essential oils were initially prepared as stock solutions in dimethyl sulfoxide (DMSO) and subsequently diluted in RPMI-1640 medium buffered with 0.165 M MOPS (pH 7.0) to obtain two-fold serial dilutions. A standardized suspension of *Candida albicans* was prepared from 24-hour-old cultures grown on Sabouraud Dextrose Agar (SDA). The yeast cells were adjusted spectrophotometrically to match the turbidity of a 0.5 McFarland standard, corresponding to approximately 1×10^6 CFU/mL. This suspension was further diluted in RPMI-1640 medium to yield a final inoculum of $0.5–2.5 \times 10^3$ CFU/mL.

In sterile, flat-bottomed 96-well microtiter plates, 100 µL of each essential oil dilution was dispensed into designated wells, followed by the addition of 100 µL of the prepared yeast inoculum, resulting in a total volume of 200 µL per well. Each plate included a positive control (inoculated wells without essential oil), a negative control (uninoculated medium), and a solvent control (medium with DMSO alone). All treatments were tested in triplicate. Plates were incubated at 35°C for 48 hours under aerobic conditions. After incubation, MIC values were determined visually as the lowest concentration of essential oil that completely inhibited visible growth, defined by the absence of turbidity when compared to the growth control.

b. Minimum Fungicidal Concentration (MFC)

From wells showing no visible growth, 10 µL aliquots were spot-inoculated onto fresh SDA plates and incubated for 48 hours. The MFC was defined as the lowest concentration resulting in no visible colony growth, indicating fungicidal activity.

Statistical Analysis

All experiments were conducted in triplicate ($n = 3$) and data are expressed as mean ± standard deviation (SD). Statistical analysis was performed using GraphPad Prism v9.0.

RESULTS

In this study, the phytochemical analysis of the ethanol extracts of *Syzygium aromaticum (clove)* and *Cinnamomum zeylanicum* (cinnamon) revealed distinct profiles (Table 1). Clove extract demonstrated the presence of flavonoids, saponins, tannins, and phytosterols, while cinnamon extract contained phlobatannins and phytosterols. Volatile oils were not detected in either extract, possibly due to the limitations of the ethanol extraction method in recovering thermolabile or highly volatile constituents. The presence of flavonoids and tannins in clove is notable given their known bioactivities, including membrane disruption and protein denaturation in fungal cells.

Results of antifungal susceptibility testing revealed a clear concentration-dependent antifungal activity for both oils. Cinnamon oil exhibited a larger inhibition zone (22.0 mm) at 100% concentration compared to clove (19.0

mm) (Table 2). However, both oils lost efficacy entirely at or below 50%, with no measurable inhibition zones.

The broth microdilution assay revealed that clove oil exhibited a minimum inhibitory concentration (MIC) of 50%, while cinnamon required a full 100% concentration to inhibit *Candida albicans* growth (Table 3). Moreover, the minimum fungicidal concentration (MFC) for clove oil was 50%, compared to 100% for cinnamon oil (Table 3). These results demonstrate a superior fungicidal potency of clove oil compared to that of cinnamon.

Table 1: Phytochemical Constituents of Clove and Cinnamon Essential Oils

Phytochemical	Clove	Cinnamon
Flavonoids	+	-
Saponins	+	-
Tannins	+	-
Volatile oils	-	-
Phlobatannins	-	+
Phytosterols	+	+

Table 2: Antifungal Activity of Clove and Cinnamon Essential Oil against *Candida albicans*

Concentration (%)	Zone of Inhibition (Mean ± SD mm)	
	Cinnamon Essential Oil	Clove Essential Oil
100	22.0± 0.5	19.0± 2.3
75	18.0± 1.8	14.0± 1.6
50	0.00	0.00
25	0.00	0.00
Control (C)	0.00	0.00

Table 3: Minimum Inhibitory Concentration (MIC) of Clove and Cinnamon Essential Oil against *Candida albicans*

Concentration (%)	Growth of *Candida albicans*	
	Clove	Cinnamon
100	-	-
50	-	+
25	+	+
12.5	+	+
6.5	+	+

Table 4: Minimum Fungicidal Concentration (MFC) of Clove and Cinnamon Essential Oils against *Candida albicans*

Concentration (%)	Growth of *Candida albicans*	
	Clove	Cinnamon
100	-	-
50	-	+
25	+	+
12.5	+	+
6.5	+	+

Agar diffusion assays revealed concentration-dependent antifungal activity against *Candida albicans*. At 100% concentration, cinnamon oil demonstrated the largest mean zone of inhibition (22.0 ± 0.5 mm), followed by clove oil (19.0 ± 2.3 mm). Both oils exhibited diminishing activity at 75% concentration and complete loss of activity below 50% (Table 2). The diffusion-limited antifungal performance at lower concentrations suggests that physicochemical constraints on compound availability or volatility-related evaporation during incubation are present.

DISCUSSION

The phytochemical disparity between clove and cinnamon oils appears to be a major determinant of their differing antifungal profiles. Clove oil is enriched with flavonoids and tannins—polyphenolic compounds widely recognized for their antimicrobial properties through mechanisms such as destabilizing

microbial membranes, denaturing surface proteins, and precipitating intracellular components. These interactions often culminate in loss of membrane integrity, disruption of metabolic flux, and eventual cell death. Their absence in cinnamon oil might explain its comparatively limited antifungal performance, particularly at submaximal concentrations.

A key active compound in clove oil, eugenol, is a lipophilic phenylpropanoid with multifaceted antifungal mechanisms. It inserts into the lipid bilayers of fungal membranes, increasing fluidity and permeability, thereby promoting efflux of ions and intracellular metabolites. Additionally, eugenol exhibits pro-oxidant behavior, which enhances oxidative stress within fungal cells, ultimately leading to mitochondrial dysfunction and apoptosis. These properties not only establish eugenol as a potent fungicide but also render clove oil effective even at lower concentrations (Ahmad *et al.*, 2005; Pinto *et al.*, 2009).

On the other hand, the antifungal capacity of cinnamon oil is predominantly ascribed to cinnamaldehyde, an aromatic aldehyde that interferes with fungal respiration by targeting mitochondrial enzymes and altering redox balance. It also impedes morphogenetic switching—a key virulence determinant in *Candida albicans*. However, cinnamaldehyde's high volatility, limited solubility in aqueous environments, and moderate cell membrane permeability may collectively reduce its bioavailability at lower concentrations. This is in line with findings by Gucwa *et al.* (2018), who demonstrated that its fungistatic activity is more pronounced than its fungicidal activity, particularly in resistant fungal strains.

Notably, phytosterols were detected in both oils and may function synergistically with flavonoids or cinnamaldehyde to compromise ergosterol biosynthesis—a pathway critical to fungal membrane structure and fluidity. Ergosterol depletion results in membrane disintegration, inhibition of nutrient uptake, and increased susceptibility to osmotic stress (Tran *et al.*, 2020). Such synergy is more pronounced in clove oil due to its richer polyphenolic matrix.

Therapeutically, clove oil's robust efficacy at lower doses makes it a promising candidate for antifungal drug development, particularly in topical creams, oral rinses, or suppositories. Nonetheless, the path from bench to bedside remains complex, as issues like volatility, potential mucosal irritation, host cytotoxicity, and formulation stability must be addressed. Strategies such as nanoencapsulation, emulsion-based delivery, or polymeric matrices could enhance the stability and bioavailability of these volatile compounds.

Cinnamon oil, despite its requirement for higher doses, may offer complementary benefits in prophylactic applications or in formulations targeting biofilm prevention, where sustained fungistatic pressure is advantageous. Taken together, both oils provide valuable scaffolds for antifungal therapeutics, with distinct yet complementary mechanistic profiles that warrant further pharmacological and toxicological investigation.

CONCLUSION

This study demonstrates that while both clove and cinnamon essential oils possess notable antifungal activity against *Candida albicans*, clove oil exhibits superior fungicidal potency at lower concentrations, making it a more promising candidate for therapeutic development. These results align with contemporary literature and support further research into synergistic formulations and clinical trials.

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