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Assessment Of Efficacy Of Coleus Amboinicus In The Prevention Of Candida Colonization

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ABSTRACT

Candida species are opportunistic fungal pathogens responsible for a wide range of infections, particularly in immunocompromised individuals. Increasing resistance to conventional antifungal drugs has created a need for alternative therapeutic agents. Coleus amboinicus, a medicinal plant widely used in traditional medicine, contains various bioactive phytochemicals with antimicrobial properties. The present study aimed to evaluate the antifungal activity of ethanolic leaf extract of Coleus amboinicus against Candida parapsilosis, Candida tropicalis, and Candida albicans. Antifungal activity was assessed using the Kirby–Bauer disk diffusion method, and the minimum inhibitory concentration (MIC) was determined using a microdilution assay. The extract demonstrated antifungal activity against C. tropicalis and C. albicanswith zones of inhibition of 17 mm and 19 mm respectively at 50 µL concentration, while no activity was observed against C. parapsilosis. MIC analysis showed maximum inhibition (91.9%) at dilution 10^{-1}. These findings suggest that Coleus amboinicus possesses promising antifungal potential against selected Candida species and may serve as a natural alternative for managing Candida-related infections.

KEYWORDS : Anti-fungal activity, canidiasis, Coleus amboinicus, Candida colonization, Herbal medicine..

INTRODUCTION

Oral candidiasis is one of the most common opportunistic fungal infections affecting the oral cavity and is predominantly caused by Candida albicans. Although Candida species exist as commensal microorganisms in healthy individuals, alterations in host immunity, prolonged antibiotic therapy, diabetes mellitus, xerostomia, denture use, and immunosuppressive conditions may facilitate their transition to pathogenic forms, resulting in oral and systemic infections.[1,2]

The increasing prevalence of antifungal resistance among Candida species has emerged as a significant public health concern. Conventional antifungal agents such as fluconazole, amphotericin B, and echinocandins have demonstrated reduced efficacy against certain Candida strains owing to biofilm formation and the development of resistance mechanisms.[3] Consequently, the exploration of alternative therapeutic approaches capable of preventing Candida colonization and reducing fungal burden has gained considerable attention.

Medicinal plants have historically served as valuable sources of antimicrobial compounds and continue to play an important role in the development of novel therapeutic agents.[4] Plant-derived bioactive constituents including flavonoids, phenolic compounds, terpenoids, alkaloids, and essential oils have demonstrated broad-spectrum antimicrobial activities against bacterial and fungal pathogens.[5]

Coleus amboinicus Lour. (synonym: Plectranthus amboinicus), belonging to the family Lamiaceae, is a perennial aromatic herb widely distributed in tropical and subtropical regions. Traditionally, the plant has been employed in folk medicine for the management of respiratory disorders, inflammatory conditions, skin infections, and gastrointestinal ailments.[6] Phytochemical investigations have revealed the presence of several biologically active compounds, including carvacrol, thymol, rosmarinic acid, β-caryophyllene, and various phenolic constituents that contribute to its antimicrobial, antioxidant, and anti-inflammatory properties.[7,8].

Previous studies have reported the antibacterial and antifungal activities of *C. amboinicus* against a variety of clinically relevant microorganisms.[9,10] However, evidence regarding its effectiveness against oral Candida species remains limited. Therefore, the present study was undertaken to evaluate the antifungal efficacy of ethanolic leaf extract of *Coleus amboinicus* against *Candida albicans*, *Candida tropicalis*, and *Candida parapsilosis* using the Kirby–Bauer disk diffusion method and minimum inhibitory concentration assay.

Figure 1. Fresh leaves of Coleus amboinicus used for preparation of ethanolic extract.

MATERIALS AND METHODS:

Plant Material

The plant material used for this study was *Coleus amboinicus*. Fresh leaves of the plant were collected and thoroughly washed with distilled water to remove dirt and other contaminants. The cleaned plant material was then air-dried in the shade to preserve the phytochemical constituents. In some cases, drying was performed in an oven at a low temperature to remove residual moisture. After complete drying, the leaves were ground into a fine powder using an electric grinder.

Preparation of Ethanolic Extract

The powdered plant material was transferred into a suitable beaker for extraction. Ethanol was added to the plant powder in a ratio of 1:2 to facilitate the extraction of bioactive compounds. The beaker containing the mixture was placed on a magnetic stirrer or shaker and allowed to mix continuously. The mixture was maintained at room temperature for 24 hours, allowing ethanol to extract the desired phytochemicals from the plant material.

After the 24-hour incubation period, the mixture was filtered using Whatman filter paper to separate the liquid ethanolic extract from the solid plant residues. To concentrate the extract, the filtrate was subjected to evaporation using a water bath maintained at approximately 50°C. Once the extract was sufficiently concentrated, it was transferred into a clean sterile glass bottle. The extract was then stored in a cool and dark place to protect it from light and heat, which could degrade the active compounds. The bottle was tightly sealed to prevent evaporation and contamination.

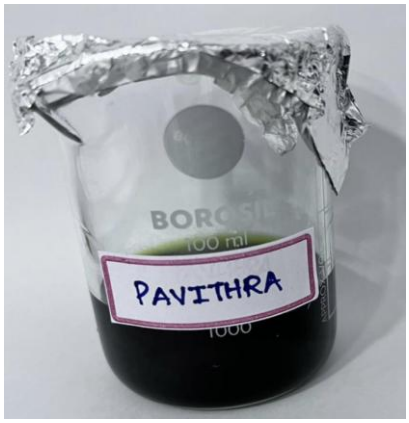

Figure 2. Ethanolic extract of Coleus amboinicus prepared for antifungal evaluation.

Antifungal Activity Assay

The antifungal activity of the ethanolic extract of *Coleus amboinicus* was evaluated against *Candida parapsilosis*, *Candida tropicalis*, and *Candida albicans* using the Kirby–Bauer disk diffusion method.

Using the lawn culture technique, fungal suspensions of *Candida parapsilosis*, *Candida tropicalis*, and *Candida albicans were* inoculated onto Muller Hinton Agar (MHA) plates to obtain a uniform microbial lawn. After inoculation, two wells were created on each agar plate using a sterile well cutter.

Each well was filled with 50 µL and 100 µL of the plant extract respectively. In addition, fluconazole was used as the standard antifungal control and was placed on the agar plates. The plates were allowed to stand for approximately 2 hours to permit diffusion of the extract into the agar medium.

The inoculated plates were then incubated at 37°C for 24 hours. Following incubation, the diameter of the zone of inhibition around each well was measured using a HiAntibiotic zone scale. The measured zones indicated the antifungal activity of the plant extract against the tested Candida species.

Minimum Inhibitory Concentration (MIC) Assay

The minimum inhibitory concentration (MIC) of *Coleus amboinicus* extract was determined using a 96-well microplate dilution method.

Initially, the wells of the microplate were labeled according to the experimental design, including wells for sample dilutions, positive controls, and negative controls. A volume of 160 µL of sterile broth was added into each well using a micropipette.

Subsequently, 20 µL of each sample dilution was added into the respective wells containing sterile broth. The contents were mixed gently by pipetting up and down several times to ensure thorough mixing. Serial dilution was then performed by transferring 20 µL from one well to the next well across the microplate, creating a gradient of extract concentrations. This process resulted in a final volume of 200 µL in each well.

Following dilution, 20 µL of standardized fungal culture was added into each well containing broth and sample dilution. The mixture was gently pipetted to ensure even distribution of the fungal cells.

Positive control wells were prepared by adding 20 µL of fungal culture into wells containing only sterile broth without plant extract. Negative control wells were prepared by adding 20 µL of sterile broth into wells containing only broth without fungal cultures or plant extracts.

The microplate was then incubated for 24 hours at the appropriate temperature to allow fungal growth and interaction with the plant extract. After the incubation period, the optical density of each well was measured at 600 nm (OD600) using a spectrophotometer to determine the extent of fungal growth inhibition.

RESULTS:

The antifungal activity of the ethanolic leaf extract of *Coleus amboinicus* was evaluated against three Candida species: *Candida parapsilosis*, *Candida tropicalis*, and *Candida albicans*. The activity was determined using the Kirby–Bauer disk diffusion method, and the effectiveness of the extract was assessed by measuring the zone of inhibition (ZOI) surrounding the wells containing the extract.

Figure 3. Antifungal activity of Coleus amboinicus against (A) Candida parapsilosis, (B) Candida tropicalis, and (C) Candida albicans using Kirby–Bauer disk diffusion method.

The results demonstrated that the ethanolic extract of *Coleus amboinicus* exhibited antifungal activity against *Candida tropicalis* and *Candida albicans*, while no inhibitory activity was observed against *Candida parapsilosis*. The standard antifungal drug fluconazole produced zones of inhibition of 27 mm, 30 mm, and 31 mm against *C. parapsilosis*, *C. tropicalis*, and *C. albicans*, respectively.

At a concentration of 50 µL, the *Coleus amboinicus* extract produced a zone of inhibition of 17 mm against *Candida tropicalis* and 19 mm against *Candida albicans*. However, no inhibition was observed against *Candida parapsilosis*. At 100 µL concentration, the extract showed a zone of inhibition of 17 mm against *Candida albicans*, while no inhibition was

observed against *Candida tropicalis* and *Candida parapsilosis*.

These findings indicate that the ethanolic extract of *Coleus amboinicus* possesses antifungal activity against selected Candida species, particularly *Candida tropicalis* and *Candida albicans*.

Table 1. Zone of inhibition of *Coleus amboinicus*' extract against *Candida* species.

Organism	Standard antifungal (Fluconazole)	50 µL extract	100 µL extract
Candida parapsilosis	27 mm	0 mm	0 mm
Candida tropicalis	30 mm	17 mm	0 mm
Candida albicans	31 mm	19 mm	17 mm

In addition to the disk diffusion assay, the minimum inhibitory concentration (MIC) of the ethanolic extract was determined using a microdilution method. The results showed that the inhibitory activity of the extract decreased with increasing dilution.

The highest inhibition of fungal growth (91.9%) was observed at a dilution of 10^{-1}. As the dilution increased, the percentage of inhibition gradually decreased to 88.8% at 10^{-2}, 70% at 10^{-3}, 59.4% at 10^{-4}, and 32.3% at 10^{-5}.

These results indicate that the antifungal activity of the *Coleus amboinicus* extract is concentration dependent, with higher concentrations showing stronger inhibitory effects on Candida growth.

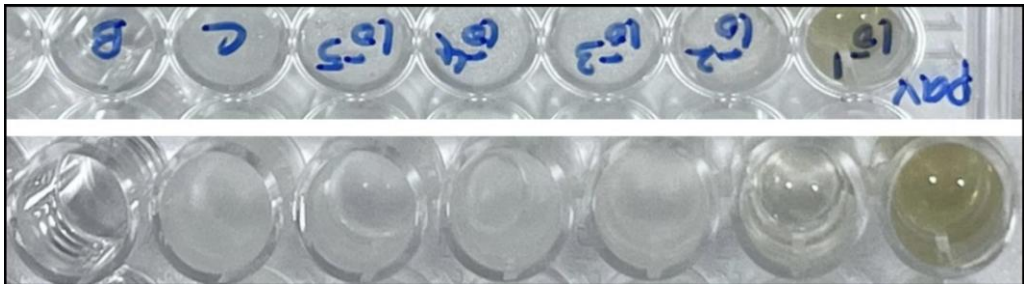

Figure 4. Determination of minimum inhibitory concentration of Coleus amboinicus ethanolic extract against Candida species.

Table 2. Minimum inhibitory concentration (MIC) of *Coleus amboinicus*' extract

Dilution	Percentage inhibition
10^{-1}	91.9%
10^{-2}	88.8%
10^{-3}	70%
10^{-4}	59.4%
10^{-5}	32.3%

Overall, the results demonstrate that *Coleus amboinicus* ethanolic leaf extract exhibits measurable antifungal activity against

Candida tropicalis and *Candida albicans*, suggesting its potential as a natural antifungal agent. Finally this study concludes that phytochemicals present in the *Coleus amboinicus* leaves extract play a crucial role the treatment of dental pathogens *Candida tropicalis* and *Candida albicans.*

DISCUSSION:

The present study evaluated the antifungal efficacy of *Coleus amboinicus* ethanolic leaf extract against *Candida parapsilosis, Candida tropicalis,* and *Candida albicans*. The findings demonstrated that the extract exhibited antifungal activity against *C. tropicalis* and *C. albicans*, while no inhibitory effect was observed against *C. parapsilosis*. These findings are consistent with previous studies that have reported the broad-spectrum antimicrobial and antifungal properties of *C. amboinicus*, which are attributed to the presence of phenolic compounds, flavonoids, terpenoids, thymol, and carvacrol that disrupt fungal cell membrane integrity and inhibit microbial proliferation [11,12].

Among the tested organisms, *C. albicans* demonstrated the highest susceptibility to the plant extract. This observation is in accordance with previous reports indicating that *C. albicans* is generally more susceptible to plant-derived antifungal agents than non-*albicans Candida* species [13,14]. The greater susceptibility may be associated with differences in cell wall composition, membrane permeability, and virulence factors. Conversely, the absence of antifungal activity against *C. parapsilosis* may be attributed to species-specific resistance mechanisms that reduce the effectiveness of phytochemical compounds present in the extract [15].

The minimum inhibitory concentration assay further supported the antifungal potential of *C. amboinicus*. The highest inhibition was observed at the 10^{-1} dilution, indicating a concentration-dependent antifungal effect. Similar findings have been reported in studies evaluating medicinal plant extracts against oral *Candida*species, where increased extract concentrations resulted in enhanced fungal growth inhibition [16,17].

The antifungal activity observed in this study may be attributed to the synergistic action of multiple phytochemicals present in *C. amboinicus*. Essential oil constituents such as thymol and carvacrol have been shown to alter fungal membrane permeability, induce leakage of intracellular components, and interfere with metabolic pathways essential for fungal survival [18]. These mechanisms may explain the significant inhibitory effects observed against *C. tropicalis* and *C. albicans* in the present study.

The increasing prevalence of antifungal resistance among *Candida* species has stimulated interest in alternative therapeutic approaches. Plant-derived compounds have emerged as promising candidates because of their antimicrobial efficacy, low toxicity, and reduced likelihood of promoting resistance [19]. The findings of the present study support the potential application of *C. amboinicus* as a natural antifungal agent for preventing oral candidal colonization and as an adjunct in the management of oral fungal infections [20].

However, the present study was conducted under in vitro conditions and therefore may not completely represent the complex oral environment. Furthermore, only a limited number of *Candida* strains were evaluated. Future studies involving larger sample sizes, detailed phytochemical characterization, toxicity assessments, and clinical trials are required to validate these findings and establish their clinical applicability [21].

CONCLUSION:

Coleus amboinicus exhibits strong antifungal properties comparable to other plant-derived essential oils and conventional antifungal drugs. Its ability to inhibit *Candida* biofilm formation and adherence, combined with a favorable safety profile, positions it as a promising candidate for further research and potential clinical application. The results from this study contribute to the growing body of evidence supporting the use of plant-based agents in the prevention and treatment of *Candida* infections, especially in the context of increasing antifungal resistance.

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