Evaluation of Antifungal Efficacy of *Vitex Negundo* against *Candida Albicans* and *Candida Glabrata*: An In Vitro Study

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ABSTRACT

Introduction: Oral candidiasis is an opportunistic disease commonly caused by Candida albicans. Immunocompromised patients are more prone to candidal opportunistic infections. Vitex negundo, is a well-recognised medicinal plant with a wide range of pharmacological properties, including anti-inflammatory, antioxidant, and antimicrobial activity. The present study aimed to evaluate the antifungal efficacy of leaves of Vitex negundo against oral candidal species.

Methods: Leaves of Vitex negundo were procured, dried and ground into fine powder. Dimethyl sulfoxide was used as the solvent. The antifungal efficacy of ethanolic leaf extract was evaluated at a concentration of 50mg/ml. The zone of inhibition was evaluated to study the antifungal efficacy of Vitex negundo against Candida albicans and Candida glabrata.

Results: The zone of inhibition against C. albicans and C. glabrata was 25 ± 0.8 mm and 29 ± 0.9 mm, respectively. Clotrimazole (positive control) produced a zone of inhibition measuring 30 ± 0.5 mm against C. albicans and 30.5 ± 0.7 mm against C. glabrata

Conclusion: The present in vitro study demonstrated the antifungal efficacy of Vitex negundo against Candida albicans and Candida glabrata. However, further clinical studies are necessary to establish the efficacy, safety, and sustainability as an alternative to conventional antifungal agents.

Keywords: Antifungal efficacy; Candida albicans; Candida glabrata; Vitex negundo

INTRODUCTION

Most of the mucocutaneous opportunistic infections in humans are caused by species of Candida. Oral candidiasis is commonly seen in the elderly population, denture wearers, diabetics, patients on long-term steroids, and immunocompromised patients. C. albicans is one of the most common mycotic infections in human oral and vaginal mucosa. Phenotypic switching, polymorphism and thigmotropism are responsible for the transition from commensal to pathogenic form in the human microbiome. Other non-albicans species include C. glabrata, C. krusei, C. tropicalis, C. parapsilosis, C. dubliniensi, and C. auris.[1] Recent studies have documented a surge in the prevalence of non-albicans over albicans.[2] C. glabrata is an opportunistic yeast that has emerged as a clinically important pathogen due to increasing antifungal resistance and ability to cause invasive infections, particularly in immunocompromised individuals. Both C. albicans and C. glabrata differ in their pathogenic virulence mechanism. C. glabrata evades the host immune response and proliferates within the macrophages, contributing to decreased susceptibility to antifungal agents. C. glabrata, belonging to the genus Nakaseomyces, lacks the

Conflict of Interest: None

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transition from yeast to hyphae form.[3-5] On the other hand, C. albicans exhibits morphological plasticity and switches from yeast (commensal) to hyphae (pathogenic). Currently, major challenges in the treatment of fungal infections include pathogen resistance, prolonged treatment duration, high cost, adverse effects, and long-term complications; therefore, researchers are exploring effective alternatives, particularly those derived from medicinal herbs.[6]

Phytoproducts have been proven to have inhibitory effects on microbes and are effective in the treatment of a variety of diseases as they enhance the host defence system. *Vitex negundo (*Nochi*)*, also known as Negundi or five-leaved chaste tree, is a member of the Verbenaceae family. All the parts of the plant have medicinal uses and are recommended for the treatment of various diseases. Anti-inflammatory, antioxidant, antidiabetic, anticancer, and antibacterial properties are a few of the properties of *Vitex negundo* from its active ingredients, namely flavonoids, terpenoids, lignans, and steroids. This shrub is also known as "sarvaroga nivarani" in the traditional medical system of India.[7] Several studies have reported the antifungal potential of *V. negundo* leaf extracts against a variety of pathogenic microorganisms, including dermatophytes and opportunistic fungi. Notably, phenolic extracts have demonstrated inhibitory effects against C. albicans.[8,9] Despite these findings, limited studies have evaluated the antifungal efficacy of V. negundo leaf extract against C. glabrata. Therefore, the present study was undertaken to assess the in vitro antifungal efficacy of V. negundo leaf extract against C. albicans and C. glabrata.

METHODS

Collection of leaves and preparation of extract

The leaves of V. negundo were collected and authenticated by a botanist. The leaves were shade-dried for a week and powdered. Ethanolic extract was prepared by the cold maceration technique. Twenty-five grams of the powder was mixed with 50 ml of 96% ethanol and was left for 3 days with intermittent shaking at room temperature. This was then filtered with Whatman filter paper no. 1. The solvent in the filtrate was allowed to evaporate by means of a hot water bath to yield crude extract. The dry concentrate was dissolved in dimethyl sulfoxide (DMSO) to obtain a working concentration of 50 mg/ml (Figure 1).

Determination of antifungal efficacy

The strains of C. albicans and C. glabrata were obtained from HiMedia Laboratories Pvt. Ltd., Mumbai, India. The strains were revived by subculturing on Sabouraud dextrose agar (SDA) and incubated at 35-37°C for 48 hours to obtain fresh growth. Following incubation, 3-5 well isolated colonies were aseptically picked and suspended in normal saline and adjusted to a concentration of 0.5 McFarland standard (1.5 x 10^8 CFU/mL). The standardized inoculum was spread on SDA plates and antifungal efficacy was determined using the agar well diffusion method. In each plate, a well of diameter 6-8 mm was punched with a sterile tip. 50 µL of the extract was added to the well and incubated at 37°c for 48 hours. Clotrimazole and DMSO were used as positive and negative controls, respectively. Zone of inhibition of growth of C. albicans and C. glabrata was seen as a pale halo around the well (Figure 2a, 2b, 2c). The diameter of the inhibition zone was measured in millimeters using a vernier caliper. The experiment was repeated three times under identical conditions to ensure reproducibility and the results were expressed as mean ± standard deviation of three independent experiments. Further, the culture was streaked onto CHROMagar to differentiate both species and the plates were incubated at 37°C for 48 hours. Colonies of both C. albicans and C. glabrata were identified based on the colour of the colonies (Figure 3a, 3b).

RESULTS

Colonies of C. albicans were apple green, and C. glabrata were pale white colonies on CHROMagar. Clotrimazole (positive control) produced a zone of inhibition measuring 30 ± 0.5 mm against C. albicans and 30.5 ± 0.7 mm against C. glabrata, while no inhibitory effect was observed with negative control, DMSO. V. negundo extract exhibited mean inhibition zone of 25 ± 0.8 mm against C. albicans and 29 ± 0.9 mm against C. glabrata. Both species were resistant to the negative control, DMSO. This proved that the zone of inhibition was due to the antifungal action of the *V. negundo* extract and not from the solvent. Mean and standard deviation of three independent experiments are shown in Table 1.

Figure 1: *Pure extract of Vitex negundo dissolved in dimethyl sulfoxide (DMSO)*

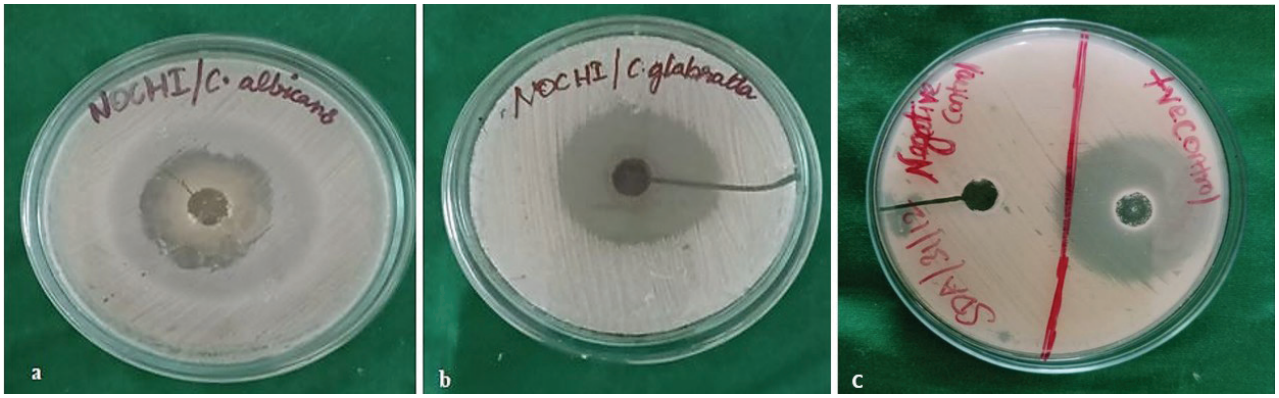

Figure 2: *a – Zone of inhibition against Candida albicans, b - Zone of inhibition against Candida glabrata; c- Zone of inhibition around positive control (clotrimazole) and negative control (DMSO)*

Figure 3: *a – Apple green colonies of Candida albicans on CHROMagar; b - Pale white colonies of Candida glabrata on CHROMagar;*

Table 1: Mean zone of inhibition (mm) produced by *Vitex negundo* leaf extract against *Candida albicans* and *Candida glabrata*

Test/Control	Zone of Inhibition for *Candida albicans* (Mean ± SD)	Zone of Inhibition for *Candida glabrata* (Mean ± SD)
Vitex negundo leaf extract (test)	25 ± 0.8	29 ± 0.9
Clotrimazole (positive control)	30 ± 0.5	30.5 ± 0.7
Dimethyl sulfoxide (negative control)	0	0

DISCUSSION

Oral candiadiasis is the most common opportunistic fungal infection frequently encountered in immunocompromised patients. *C. albicans* remains the predominant etiologic agent; however, an increasing prevalence of non-albicans candida species has been reported. The incidence of antifungal resistance, recurrence, and adverse effects associated with conventional antifungal therapy underscores the need for alternative or adjunctive treatment approaches.

In the present study, the antifungal activity of V. negundo leaf extract was evaluated against C. albicans and C. glabrata using the agar well diffusion method. The antifungal efficacy observed in this study may be attributed to the presence of bioactive phytochemicals in V. negundo leaves, particularly phenolic compounds and flavonoids. Sathiamoorthy et al. evaluated the antifungal activity of V. negundo with the flavone glycoside isolated by bioactive fractionation of ethanolic extract of V. negundo leaf. Their study assessed the antimicrobial efficacy, along with other active compounds, and reported that the flavone glycoside demonstrated notable antifungal efficacy against *Trichophyton mentagrophytes* and *Cryptococcus neoformans*.[8] A study by Jaiswal and Kumar identified that eighteen phenolic compounds from V. negundo exhibited antifungal activity against planktonic and biofilm forms of candida. This identification highlights the potential contribution of phenolic constituents in suppressing C. Albicans, which is clinically relevant given the inherent resistance of biofilms to conventional antifungal agents.[9] The inhibition zones demonstrated in our in vitro assay are consistent with these earlier reports identifying compounds from V. negundo with significant activity against candida species.

These compounds are found to disrupt cell membrane integrity, inhibit ergosterol synthesis, and interfere with enzyme systems essential for fungal growth and adhesion. The total phenolic content of V. negundo leaf extract was quantified using Folin-Ciocalteu reagent and expressed in terms of gallic acid equivalent in a study by Jaiswal and Kumar. Among the phenolic constituents examined, caffeic acid was identified as the most potent anticandidal compound exhibiting both fungistatic and fungicidal effects causing extensive membrane damage and complete disruption of biofilms.[10]

Swati Chainani et al.[11] evaluated the antimicrobial activity of Triphala on Lactobacilli and Candida albicans. The study showed that triphala demonstrated antimicrobial property against Lactobacilli and C. albicans with a maximum zone of inhibition of 22 mm at 6% and 20 mm at 9%. Antifungal efficacy of *Wrightia tinctoria* on candida species from the oral cavity was reported to be effective for ethyl acetate extract than acetone and chloroform extracts in a study by Devika et al.[12] Pai et al.[13] investigated the

antifungal properties of Punica granatum, Acacia nilotica, Cuminum cyminum, and Foeniculum vulgare against Candida albicans and reported that the inhibition zone was 4 mm for the ether extracts of Cuminum cyminum, whereas our study, based on *Vitex negundo*, showed a zone of inhibition of 25 ± 0.8 mm for C. albicans and 29 ± 0.9 mm for C. glabrata. Although V. negundo extract antifungal activity was comparable to clotrimazole, the absence of dose-response testing limits conclusions regarding the relative potency.

In a previous study by Nagarsekar et al, the antimicrobial activity of superficial extract, ether, and ethanol extract of V. negundo leaves was evaluated and was found to have antimicrobial property against Bacillus subtilis and Staphylococcus aureus, with the superficial extract showing comparatively superior antibacterial efficacy.[14] A broad review of scientific literature, comprising 104 publications from databases by Zheng et al. has detailed the phytochemical diversity and pharmacological relevance of V. negundo. Around 120 constituents identified from this plant have revealed a wide spectrum of biological activities such as analgesic, anti-inflammatory, antioxidant, antimicrobial, antiandrogenic, anticataract, anticancer, anti-hyperglycemic, hepatoprotective, and anti-osteoporotic effects. [(15)] In a related study by Zareshahrabadi et al., essential oils obtained from V. pseudo-negundo was reported to inhibit yeast growth and exert antifungal activity against filamentous fungi strains. Additionally, the study also documented that the biofilm formation of C. albicans was inhibited by the flower, leaf, and fruit.[16]

However, the present study has certain limitations as follows: assessed antifungal efficacy solely by the zone of inhibition method and quantitative parameters such as the minimum inhibitory concentration and minimum fungicidal concentration were not determined. The study did not use varying concentrations of the leaf extract. Furthermore, only one strain of C. albicans and C. glabrata was used in the study. As a result, the precise antifungal efficacy of the extract was not established. Additionally, the present study did not involve the isolation of individual bioactive compounds, nor was detailed phytochemical characterisation performed to identify the specific compounds responsible for the observed antifungal efficacy. However, these findings are preliminary and should be validated by further in-silico and clinical research before therapeutic use can be recommended.

CONCLUSION

Within the limitations of this study, *V. negundo* leaf extract demonstrated appreciable in vitro antifungal activity against *C. albicans* and *C. glabrata*. While these findings underscore the promising antifungal efficacy of V. negundo, further preclinical and in vivo studies are necessary to elucidate the mechanism of action, efficacy, safety, sustainability and therapeutic applicability, as an alternative to conventional antifungal agents.

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