



Antifungal effect of Nirgundi (*Vitex nigundo*) crude extracts on medically important *Candida* spp.

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Abstract:

Fungal infections are emerging as major threat especially in immune compromised patients in developing countries including India. Availability, effectiveness and cost are the major constraints in effective treatment of fungal infections in modern medicines. Nirundi (*Vitex nigundo*) is one of the anti-infective medicinal herb mentioned in ayurvedic literature. Antifungal effect of aqueous, ethanol, methanol and chloroform extract of *V. nigundo* dried leaf powder was investigated against clinical isolates of *Candida albicans* and *Candida tropicalis*. It was observed in the study that *C. albicans* could be effectively inhibited by ethanol, methanol and chloroform extracts while growth of *C. tropicalis* isolates could be effectively inhibited only by the chloroform extract of the *V. nigundo*. Results of the study suggest that medicinal plant mentioned in ayurvedic literature *V.nigundo* has the potential to be developed as antifungal drug.

Introduction:

In developing countries like India infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health, due to poor health care facilities especially in rural areas. Recent emergence of antimicrobial resistance has created a renewed interest in researchers for identification of newer antimicrobial molecules from natural sources. Though several methods have suggested for finding newer anti-infective drugs, use of ethnopharmacological knowledge can reduce the time and energy with enhanced probability of identification of novel antimicrobial molecules. Ancient Indian literatures have detailed description of several medicinal plant materials with their antimicrobial effect in various disease conditions.

In addition to viral and bacterial infections, fungal infestations are also considered major health hazard in under developed world and in developing countries like, India. Application of topical antifungal agents, like, nystatin, miconazole, fluconazole, itraconazole and amphotericin B is the most common approach to treat fungal infections. Less number of effective antifungal agents, toxicity, resistance and cost are the major issues faced during the antifungal therapy. Hence, the effort to identify newer antifungal agents is the urgent priority for effective treatment and management of infections due to fungal pathogens. Medicinal plants having antifungal activity have been extensively reported in ayurvedic literature and these substances need to be evaluated using *in vitro* antimicrobial assays as per the modern test requirements.

In ayurvedic literature drugs used in treatment of diseases conditions like, Kushta, Krimija shira shoola, krimija rhudroga etc have been described as Krimighna Dravyas (Charka samhita vimana sthana chapter 7, Shuruta Samhita utara tantra chapter 54, Astanga Hruda chapter 20) Bathing, douching and topical preparations of krimighna dravyas have been described for treatment of various infectious diseases.

Nirgundi (*Vitex nigundo*) swarasa along with arenda thail is indicated in katishoola (Vaidhyamanorama). Nirgundi thail is indicated in nadi vhrana, pama, apache, sarva vhrana (Chakradatta). Ghee cooked with nirgundi leaves said to cures kaphaga swasa and kphag kasa^{1,2}. Recent studies have reported the anti-inflammatory and analgesic³, cytotoxic⁴, antifeedant and antibacterial⁵, anticonvulsant⁶, estrogenic⁷,

antifungal⁸, hepatoprotective⁹, scavenging¹⁰, apoptotic¹¹ activity in different extracts of *Vitex nigundo*. However reports on antifungal activity of this plant against clinical isolates of fungal pathogens are scanty.

MATERIALS AND METHODS

Preliminary phytochemical analysis

Preliminary phytochemical analysis like, detection of tannins, alkaloids, saponins, cardiac glycosides, anthraquinone glycosides, steroids, resins and volatile oils was done for qualitative assessment of phytoconstituents as per the standard protocols mentioned in Trease and Evans (1983)¹².

Extraction drug from the powdered plant material

Extraction of active constituent from powdered *Vitex nigundo* was as per the standard procedures published elsewhere. Aqueous extraction was done using 20 grams of air dried powder in soxhlet apparatus for 12 hrs, while ethanolic, methanolic and chloroform extraction was accomplished by immersing each of 100 grams of powdered drug in ten times the volume of 80% ethanol, methanol or chloroform respectively and allowed for cold percolation on magnetic stirrer for 24 hrs. The extracts were first filtered through double layer of muslin cloth and then with Whatman filter paper No.1. The filtrate was air dried under low heat of 50⁰ C once entire diluent was evaporated the powder was weighted to calculate the yield. The extract diluted to predetermined concentration was stored at -20⁰ C till further use.

Isolation and identification of fungal pathogens

Candida albicans and *Candida tropicalis* were isolated from the clinical samples submitted to Department of Medical Microbiology, Christian Medical College (CMC), Ludhiana, Punjab. Isolates were identified based on colony morphology and staining characters as per the standard protocols mentioned in Berge's Manual of Systematic Bacteriology (1984)¹³.

Testing the antifungal activity of plant extracts

Antifungal activity of the plant extracts was done following the standard disc diffusion method or dilution method mentioned elsewhere. Fungal isolates were aseptically inoculated on Sabouraud dextrose agar plates and incubated at 31⁰C for 48 h. The colonies were suspended in sterilized 0.9% sodium chloride solution (normal saline), which was compared with McFarland solution. One ml of yeast suspension in normal saline was added to 74 ml of sterile medium, to give concentration of 2x10⁷cells/ml.

Viability count

Antifungal activity of different extracts was determined by a viable colony count. Nine hundred microliter of a suspension of 10⁸ fungi/ml was added to 100 µl of plant extract and incubated at 37⁰C in shaker incubator for 60 min. Similarly, positive bacteria control was kept in each experiment in which isolates were diluted in DMSO. Following incubation serial ten fold dilution was made in Nutrient Broth (10⁻¹ through 10⁻⁴) and suspension from the last dilution was spread on to SDA plates and incubated as described above. Following incubation number of colonies in each plate was counted colony forming unit (CFU) per plate. The effectiveness of the plant at killing fungal isolates was expressed as percentage inhibition of colony growth (i.e. percentage of fungi killed) compared to the control.

RESULTS AND DISCUSSION

Qualitative phytochemical analysis for tannins alkaloids, saponins, cardiac glycosides, steroids: trpenoids and flavonoids showed negative results (data not shown). These test results confirm the authenticity of the drugs collected for the present study and the results were in accordance with the previously published standard observations.

Aqueous extract of nirgundi showed moderate inhibition on *C. albicans* while showing mild inhibition of *C. tropicalis* isolates tested. Ethanol extract showed significant inhibition of *C. albicans* while no inhibition was observed on *C. tropicalis* fungal isolates. Methanol extract showed significant inhibition of *C. albicans* while showed mild inhibition of *C.tropicalis* fungal isolates. Chloroform extract showed complete inhibition of both *C.albicans* and *C.tropicalis* fungal isolates tested (Table 1).

In immunocompromised patients (with AIDS, cancer, or organ transplant) opportunistic fungal infections, mainly resulting from *Candida*, *Cryptococcus*, and *Aspergillus* are life-threatening. In the present study based on ayurvedic literatures Nirgundi (*Vitex nigundo*) was selected to study its effect on important fungi of medical importance. Being a significant cause of hospital-acquired infection, *Candida sp* have also showing increased resistance to traditional antifungal agents in recent times. Identification of newer phytoconstituents with antifungal activity will help for the effective therapy of drug resistant and newly emerging fungal pathogens.

Though *in vitro* results of the present study suggest the potential of the drug as antifungal agent the same may not be translated into clinical effectiveness. Additionally, studies are needed to understand the specific phytoconstituents responsible for the observed antifungal activity to understand the mechanism behind the observed anti-candida activity.

Table 1: Antifungal activity (percentage inhibition) of different crude extracts of Nirgundi (*Vitex nigundo*)

Sl.No.	Plant material	<i>Candida albicans</i>	<i>Candida tropicalis</i>
1.	Aqueous	50%	25%
2.	Ethanol	75%	0%
3.	Methanol	75%	25%
4.	Chloroform	100%	100%
5.	Fungi control	0%	0%
6.	DMSO control	0%	0%



Fig.1. Chloroform extract of Nirgundi (*Vitex nigundo*) showing 100 % inhibition compared to control



Fig.2. Methanol extract of Nirgundi (*Vitex nigundo*) showing 75 % inhibition of *Candida albicans* compared to control



Fig.3. Ethanol extract of Nirgundi (*Vitex nigundo*) showing 75 % inhibition of *Candida albicans* compared to control



Fig.4. Chloroform extract of Nirgundi (*Vitex nigundo*) showing 100 % inhibition of *Candida tropicalis* compared to control



Fig.5. Methanol extract of Nirgundi (*Vitex nigundo*) showing 25 % inhibition of *Candida tropicalis* compared to control

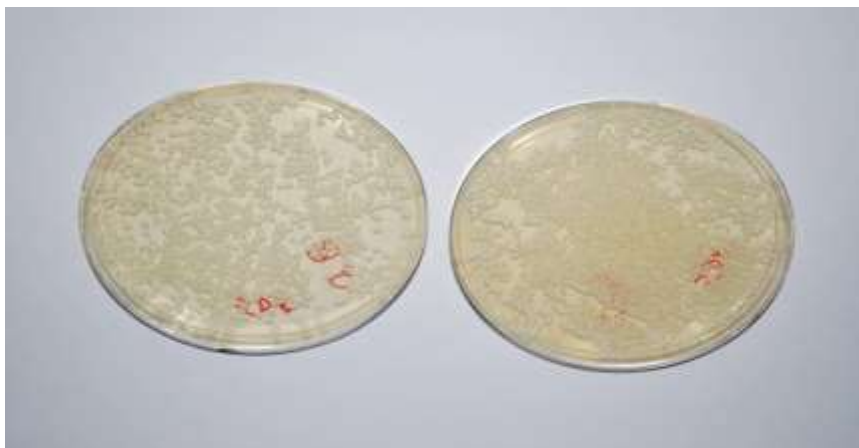


Fig.6. Ethanol extract of Nirgundi (*Vitex nigundo*) showing no inhibition of *Candida tropicalis* compared to control



Fig.7. Methanol extract of Nirgundi (*Vitex nigundo*) showing 75 % inhibition of *Candida albicans* compared to control

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