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**ORIGINAL ARTICLE**

# Antifungal Activity of Some Natural Essential Oils against *Candida* Species Isolated from Blood Stream Infection

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## Abstract:

*Background: Candida* is a part of normal microflora of human body and exists as an opportunistic pathogen as it attacks immunocompromised patients. *Aims and Objectives: Candida* is the most commonly isolated organism from blood stream infections. Fluconazole is the major antifungal drug used for treatment of *Candida*. Resistance to fluconazole has been increasing in recent years so there should be search of some other alternative. To find out these alternatives, anti candidial activity of some natural essential oils was studied. *Materials and Methods:* In the present study nine *Candida* strains isolated from blood stream infections were collected from National Culture Collection of Pathogenic Fungi (N.C.C.P.F.) P.G.I.M.E.R Chandigarh India and the antifungal activity of some natural essential oils such as lemongrass oil, coconut oil, almond oil and clove oil was checked by using agar diffusion method.

*Result:* All oils have shown a significantly *anti-candidal* activity. However, the antifungal activity was maximum in lemongrass oil. *Conclusion:* Our study may help to design new chemotherapeutic strategies against *Candidal* infections.

**Key Words:** *Candida,* Blood stream, Lemon grass oil, Antifungal activity.

## Introduction:

*Candida* is a genus of yeast, eukaryotic in nature. Many species of *Candida* are endosymbionts of animal's hosts including human [1]. *Candida* appears as large, round, white or cream colonies on agar plate [2]. The clinical manifestations include candidaemia, vulvovaginal infections (which affects women of all age groups), oropharyngeal infections and infections among persons with Human Immuno Viruses (HIV) deficiency or full blown disease of Acquired Immuno Deficiency Syndrome (AIDS) patients. The high risk category of patients reported are individuals with lowered immunity due to infections such as HIV, transplant recipients, patients on prolonged steroid or antibiotic therapy and patients with a prolonged stay in the intensive care units on central line [3,4,5]. Fluconazole and *Amphotericin B* are generally used against human pathogenic fungi but these show some side effects and toxicity. Thus, there is a need for better antifungal agent which can overcome these side effects. In last few years some researchers have focused on using herbal components such as essential oils which show antimicrobial activity [6]. Essential oils can be used against skin problems, sleep and nervous disorders. Beside this, oils from some grasses

are used by perfume and cuisine dye industries. Lemon grass oil as an essential oil is characterized for presence of monoterpen compounds which have antimicrobial activity [7]. The treatment of fungal disease is limited due to a narrow spectrum of the currently used antifungal drugs and expensive preparations. The indiscriminate use of antimicrobial drugs has led to resistance in uropathogens. Tyagi and Malik (2010) have evaluated essential oils for minimum inhibitory concentration (MIC) against *candida* species [8]. In the present study we have tried to evaluate the anti-candidal activity of some natural oils against *candida* species isolated from blood stream infection.

## Material and methods:

*Candida* isolates used in the present study were collected from National Culture Collection of Pathogenic Fungi (N.C.C.P.F) Post Graduate Institute of Medical Education and Research (P.G.I.M.E.R.) Chandigarh, India.

Four natural essential oils viz., lemongrass oil, coconut oil, almond oil and clove oil were obtained from various manufacturers and tested for their anticandidal activity. The antifungal activity of these oils against yeasts was determined using the disk diffusion method [9]. Nine strains of *Candida* species were used: *Candida albicans* B-1622/09 *Candida albicans* B-1599/09, *Candida tropicalis* B-1410/09, *Candida parapsilosis* B-1597/09, *Candida tropicalis* B-1389/09. *Candida glabrata* B-1366/09, *Candida guillermondi* B-1343/09, *Candida guillermondi* B-1418 /09 and *Candida glabrata* B-1303 /09. The cultures of *Candida* spp. were cultivated on Sabouraud dextrose agar (HiMedia) at 25ºC for 48 hours. Suspensions of yeasts were prepared in saline solution of 25% ± 2% turbidity, obtained at 580 nm, using a suitable spectrophotometer (Systronics). Seeded agar plates were prepared by pouring 20 mL of SDA into each sterile plate. After solidification of medium, each plate was overlaid with 5 mL of SDA, which was previously inoculated with 1% (v/v) of the suspensions of yeasts. Afore said natural essential oils i.e Lemongrass oil, Coconut oil, Almond oil and Clove oil were applied on filter paper (2.0 and 4.0 µL/disk) disks of 6 mm in diameter separately. 0.3 mg/mL of a Fluconazole was used as positive control. These disks were placed on the surface of seeded agar plates at equal distance. All plates were incubated at 25ºC for 24 hours. All the experiments were carried out in triplicate.

## Results:

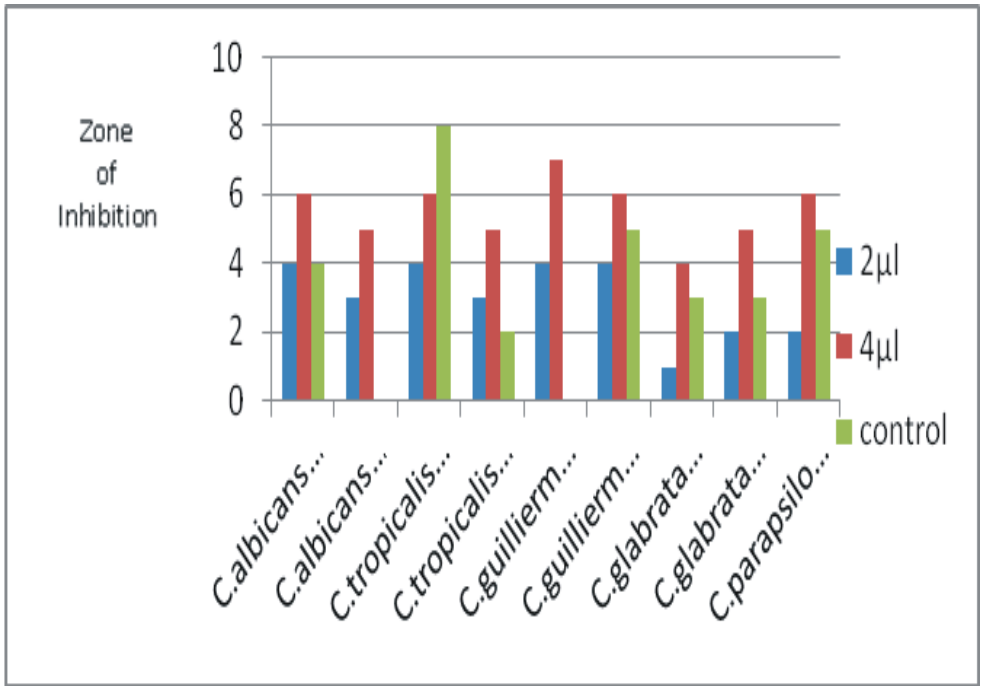
In this study antifungal activity of four different oils were observed. All oils have showed significant antifungal activity but a best result was obtained with lemongrass oil (Table-1).

The antifungal assay was performed by using disk diffusion method. Lemongrass oil showed maximum activity against *C. tropicalis* B-14100/09 (10 mm zone) followed by C. tropicalis B-1389/09 (9 mm zone) at 2 µl volume tested. Whereas, at 4 µl volume the lemongrass showed highest anticandidal activity against *C. tropicalis* B-1389/09 (16 mm zone) followed by *C. tropicalis* B-14100/09 (12 mm zone). The strain *C. guilliermondii*

| Table 1:- Diameter of Inhibition zone for Lemongrass Oil against Candida species | | | |
|---|---|---|---|
| Test Strain | Diameter of Inhibition Zone (mm) | | Fluconazole (0.3 mg/ml) 4µl |
| | Lemongrass Oil (µl) 2 µl | 4µl | |
| *C. albicans* B-1622/09 | 4 mm | 7 mm | 6 mm |
| *C. albicans* B-1599/09 | 5 mm | 10 mm | R |
| *C. tropicalis* B-1389/09 | 9 mm | 16 mm | 10 mm |
| *C. tropicalis* B-14100/09 | 10 mm | 12 mm | R |
| *C. guilliermondii* B-1343/09 | R | R | R |
| *C. guilliermondii* B-1418/09 | 4 mm | 6mm | R |
| *C. glabrata* B-1366/09 | 3mm | 5 mm | R |
| *C. glabrata* B-1303/09 | 3 mm | 10 mm | 13 mm |
| *C. parapsilosis* B-1597/09 | 3 mm | 7 mm | 5 mm |
| CD(5%) 0.002 | | | |

**Figure1: Zone of inhibition for lemongrass oil against Candida species among all oils tested during the study.**

Lemongrass oil



B-1343/09 was found to be resistant whereas, rest strains were found to be sensitive against lemongrass oil. It is evident from Table No. 1, that synthetic drug i.e. Fluconazole showed anticandidal activity against only four Candida species whereas, five strains were found resistant to the tested antibiotic.

Clove oil showed maximum activity against *C. albicans* B-1622/09, C. tropicalis B-1389/09 and *C. guilliermondii* B-1418/09 (4 mm zone at 2 µl volume and 6 mm zone at 4 µl volume). Coconut oil and almond oil were also tested for antifungal activity. Coconut oil showed maximum activity against *C. albicans* B-1622/09 (4 mm zone at 2 µl volume and 6 mm zone at 4 µl volume). In case of almond oil maximum activity was reported against *C. guilliermondii* B-1418/09 (3 mm zone at 2 µl volume and 5 mm zone at 4 µl volume). Among all the oils tested, lemongrass oil showed maximum antifungal activity followed by clove oil and coconut oil whereas, almond oil showed least activity.

## Discussion:

Over the last decade fungal infections are increasing at an alarming rate [10, 11]. This increase in incidence of fungal infections poses a great challenge to healthcare professionals. The rise in number of fungal infections is directly related to the increasing number of immunocompromised individuals due to use of extensive chemotherapy and other immunosuppressive drugs. Many diseases including HIV have contributed to this problem [11]. *Candida* is a genus of yeast having eukaryotic nature. *Candida* exists as opportunistic human pathogen in nature. *Candida* is responsible for causing candidiasis. Candidal infection in humans ranges from skin infection to the deep tissue infection. In the present study we used four essential oils: lemongrass oil, clove oil, almond oil and coconut oil. These oils were tested *in vitro* for their antifungal activity against *Candida* species isolated from bloodstream infection. It has been reported that lemongrass oil inhibits the growth of *C. albicans,* this study shows potential antifungal activity of lemongrass oil and its future use as antifungal agent against candidal infection such as cutaneous candidiasis [12]. In past two decades numerous essential oils have been tested for *in-vivo* and *in-vitro* antifungal activity and some oils were found to be potential anti-fungal agents. These essential oils attack on fungal cell membrane, disrupts its structure causing leakage and cell death, stop the membrane synthesis, inhibit spore germination, fungal proliferation and cellular respiration [13]. Lemongrass oil showed greater antifungal activity against *Candida* strains in the present study. This oil has higher volatility and lipohilicity, it can easily attache and penetrate in to cell membrane and cause cell lysis [14]. Similar study was conducted by Cristiane et al, and they found that lemon grass oil to be showing higher anti candidal activity against strains isolated from superficial mycoses as compared to citral [9]. But in the present study the strains tested were isolated from the blood stream infection and they also were sensitive to lemon grass oil. Clove oil is also known to have antifungal activity [15]. Pina-vaz et al,

found that carvacrol in clove oil has been able to kill *C. albicans* by producing lesions in the plasma membrane as a result the organism dies. Clove oil, coconut oil and almond oil has also showed significant antifungal activity in the present study, this reflects the potential use of these oils as antifungal agents [16]. Such type of study is necessary in order to find newer combination of antifungal agents, overcoming drug resistance and to avoid side effects associated with the currently used antifungal drugs, so that clinically safe and more effective antifungal agents can be provided to patients suffering from *Candida* infection.

In the present study lemon grass oil has good antifungal activity against all candidal stains tested, except *C. guilliermondii* B -1343/09 which showed resistance to it; where as almond oil has good antifungal activity against it.

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