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## Research Article

### ANTIFUNGAL ACTIVITY OF EUCALYPTUS OIL AGAINST CLINICAL ISOLATES OF CANDIDA SPECIES

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#### ABSTRACT

The majority of mycotic infections are due to Candida species, out of which Candida albicans is being the most frequently isolated species. Eucalyptus is one of the very important and most widely planted genera across the world. It is a tall, evergreen tree, native to Australia and Tasmania, successfully introduced worldwide, now extensively planted in many other countries. Thus, the aim of the present study was to determine the antifungal activity of eucalyptus oil against clinical isolates of Candida spp. We have observed that, clinical isolates of Candida species were not inhibited in any of these dilutions of this oil. The eucalyptus oil does not have antifungal activity against Candida species. However, it is important to include more number of isolates and different dilutions to validate the results.

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#### INTRODUCTION

The majority of mycotic infections are due to Candida species, out of which Candida albicans is being the most frequently isolated species<sup>[1]</sup>. Various virulence factors plays a crucial role in the pathogenicity of several microorganisms including Candida albicans.<sup>[2,3,4]</sup>

Eucalyptus is one of the very important and most widely planted genera across the world.<sup>[6]</sup> It is a tall, evergreen tree, native to Australia and Tasmania, successfully introduced worldwide, now extensively planted in many other countries.<sup>[5]</sup> It was introduced in Algeria in 1854 by Ramel.<sup>[8]</sup> Eucalyptus species are well known as medicinal plants due to their biological and pharmacological properties. In the international pharmacopeia, the most important and represented species, however, is *Eucalyptus globulus* (*E. globulus*) which is the main furnisher of essential oils. These essential oils have different applications as anesthetic, anodyne, antiseptic, astringent, deodorant, diaphoretic, disinfectant, expectorant, febrifuge, fumigant, hemostat, inhalant, insect repellent, preventative, rubefacient, sedative yet stimulant, vermifuge, for a folk remedy for abscess, arthritis, asthma, boils, bronchitis, burns, cancer, diabetes, diarrhea, diphtheria, dysentery, encephalitis, enteritis, erysipelas, fever, flu, inflammation, laryngalgia, laryngitis, leprosy, malaria, mastitis, miasma,

pharyngitis, phthisis, rhinitis, sores, sore throat, spasms, trachalgia, worms, and wounds. Sometimes their demand is also high in the soap and cosmetic industries.<sup>[7]</sup> Thus, the aim of the present study was to determine the antifungal activity of eucalyptus oil against clinical isolates of Candida spp.

#### MATERIALS AND METHODS

##### Candida isolates

A total of 20 non repetitive clinical isolates of *Candida* species were collected from different samples of immunocompromised individuals attending Saveetha Medical College, Thandalam. They were characterized by carbohydrate fermentation and assimilation tests and confirmed.<sup>[7]</sup> Isolates were preserved in semisolid Sabouraud chloramphenicol semi solid stock and stored at 4°C until further use.

##### Characterization of Candida species

*Candida* species were further characterized by using Hichrom agar (HiMedia, Mumbai).

##### Preparation of Hichrom agar

CHRO Magar *Candida* (HiMedia, Mumbai) was prepared following manufacturer's instructions. About 21.02 gram of Hi Chrome *Candida* differentiation agar base (modified) was suspended in 500 ml of distilled water. It was heated to boiling

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gently to dissolve the medium completely. Then it was allowed to cool to 50°C and rehydrated (one vial) contents of Hichrome *Candida* selective supplement was added under aseptic precautions. It was mixed well and poured into petridishes. Isolates were identified on Hichrome agar based upon the characteristic color of the colony by subculturing from Sabouraud's chloramphenicol agar plates and the *Candida* Hichrome plates were incubated at 37°C for 24- 48 hours<sup>[9]</sup>. Based on colour produced by the isolates speciation have been made.

<b>Candida species</b>	<b>colour</b>
C.albicans	Green
C.tropicalis	Blue
C.krusei	Pink dry colonies
C.kefyr	Pale
C.parapsilosis	Pale

**Detection of antibacterial activity of eucalyptus oil against clinical isolates of Candida spp**

Antifungal activity of eucalyptus oil was tested against *Candida* spp isolates by minimum inhibitory concentration method. Mueller Hinton broth was supplemented with 0.002% (V/V) tween 80 (HiMedia, Mumbai) to enhance the dispersion of the essential oil. Agar dilution method was performed to attain the different concentrations of essential oils such as 0.03%, 0.06%, 0.125%, 0.25%, 0.5%, 1% and 2% in Mueller Hinton Agar (MHA).

Media containing various concentrations of essential oils were poured over the sterile petridishes and allowed to dry. Media without essential oil was served as control plate. Spot inoculation of 0.5

McFarland standard turbidity adjusted isolates were made on the plates and incubated at 37°C for overnight. The lowest concentration of the essential oils that completely inhibited the growth of isolates was considered as MIC. [10]

**RESULTS**

**Characterization of Candida species by Hichrom Candida agar**

A total of 20 clinical isolates of *Candida*spp were seeded on to Hichrom *Candida* agar and results were tabulated based on pigment production.

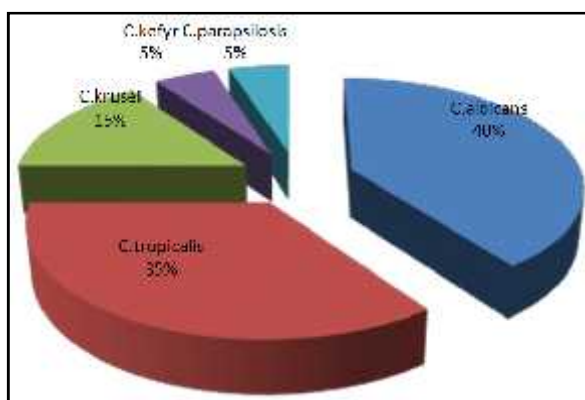


Figure 1 Pie chart showing distribution of Candida species

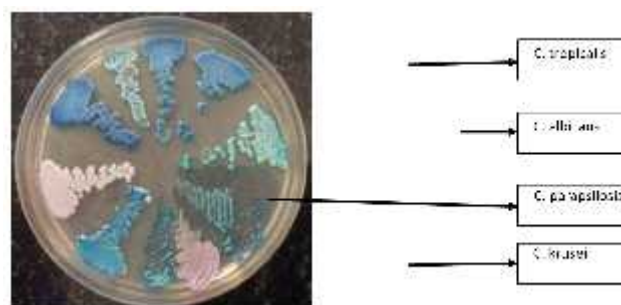


Figure 2 Representative picture showing *Candida* species on Hichrom *Candida* agar

**Sample wise distribution of Candida species**

Of the 20 clinical isolates of *Candida* spp, 6/20 (30%) were from oral thrush, 5/20 (25%) from urine, 3/20 (15%) from sputum, 3/20 (15%) from vaginal swab, 2/20 (10%) from ear swab and one (5%) from wound swab.

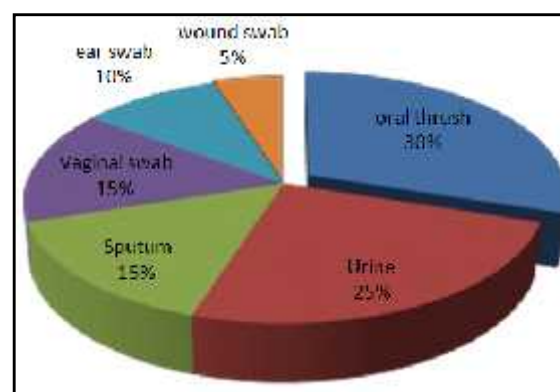


Figure 3 Pie chart showing the sample wise distribution of *Candida* species

**Result of antifungal activity of eucalyptus oil against clinical isolates of Candida species**

We have observed that, clinical isolates of *Candida* species were not inhibited in any of these dilutions of this oil.

**DISCUSSION**

Study conducted by Prakasam *et al* from Chennai in 2014 demonstrated that, *Acinetobacter* strains were inhibited from 0.06 to 0.25%, 0.25-1% and 0.125-1% for clove, peppermint and eucalyptus oils respectively. In clove oil, 14/50 (28%) isolates were inhibited at 0.06%, 25/50 (50%) at 0.125% and 11/50 (22%) at 0.25% of clove oil. In peppermint oil, 34/50 (68%) isolates were inhibited at 0.25%, 12/50 (24%) and 4/50 (8%) were at 0.5% and 1% concentrations of peppermint oil respectively. In eucalyptus oils, 10/50 (20%) isolates were inhibited at 0.125%, 18/50 (36%) at 0.25%, 16/50 (32%) and 6/50 (12%) were at 0.5% and 1% respectively. Thus, the MIC of clove oil was found to be 0.06%, 0.25% for peppermint oil and 0.125% for eucalyptus oil. [10]. In contrast, our isolates did not show any inhibitory activity against eucalyptus oil with these dilutions.

**CONCLUSION**

The eucalyptus oil does not have antifungal activity against *Candida* species. However, it is important to include more number of isolates and different dilutions to validate the results.

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