



Anti-*Candida* Activity of Essential Oils from *Eucalyptus* species. A Preliminary Study



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Abstract

Members of the genus *Eucalyptus* belonging to the *Myrtaceae* family originate in Australia but have been naturalized on most continents. They are popularly used as an anesthetic, expectorant, anti-inflammatory and antiseptic, because they present antibacterial, antifungal, anti-inflammatory and analgesic properties through their essential oils. Faced with the increase resistance of microorganisms to antimicrobials has increased the studies of medicinal plants as an alternative agent for treatments and prevention of infections. Candidiasis is an infection caused by *Candida* spp. Some strains are already reported as resistant to traditional therapies. Therefore, the present study evaluated the antifungal activity of two species of *Eucalyptus* spp. through the microdilution method in broth determining the MIC (Minimum Inhibitory Concentration) and determining the CFM (Minimum Fungicidal Concentration). The essential oils of *Eucalyptus citriodora* and *Eucalyptus globulus* are biologically active against *Candida* spp. tested, exhibiting promising antifungal properties and can be used in combination with other traditional antimicrobials.

Keywords: *Eucalyptus* spp; Essential oil; Antifungal; *Candida* spp

Introduction

The use of medicinal plants is an age-old practice where man sought use natural resources to improve their health. The beginning of the use of plants as a resource for the treatment of diseases was empirical, with a view that plants could be an alternative against the cure of diseases [1].

This practice has grown significantly throughout the world in recent years. Some factors favored the increase of its use, such as the option of natural therapies and a greater interest as an alternative of anti-microorganisms. The erroneous belief that natural products are superior to conventional drugs without causing harm to health, associated to the no satisfaction with pharmaceuticals results and self-medication [2], has led researches to work in the scientific search and validation of medicinal plants in the development of new medicinal products or therapeutic combinations due to the resistance of antimicrobial drugs already in use [3,4]. Antimicrobial resistance is reported as one of the greatest threats to human health [5]. This alarming growth of multi resistant bacterial infections due to the excessive use of antimicrobials spectrum has been a factor that has favored the dissemination, mainly of microorganisms of the genus *Candida*, increasing their chances of causing diseases [6].

The incidence of *Candida* strains spp. resistance to antifungal treatment has increased in the last decades, being a concern for professionals of health [6]. The most commonly used antifungal

for the treatment of candidoses belong to the azoles family (fluconazole, ketoconazole and itraconazole), and polyenes (nystatin and amphotericin B). Excessive use of these drugs is one of the factors that may favor the development of resistant strains to antifungals [7]. Candidiasis is a most commonly diseases caused by *Candida albicans* species. These infections are of broad spectrum and affect mainly immunodepressed patients, HIV-positive, cancer patients, patients who make excessive use of antibiotics and those who use immunosuppressors therapies as in the case of transplants [8].

C. albicans is a pleomorphic fungus, presenting in the yeast form, or as filamentous with the production of hyphae. Such morphological transition capacity is known as polymorphism due to the formation of germ tubes and the consequent development of this form is considered a virulence factor of this specie, which also has the capacity of chlamydo-spores formation. The two forms are related to the infectious process, and the yeast form has a greater ability to human dissemination while the hyphae have the ability to invade tissues of the host phagocytic cells [9,10].

Resistance to antifungals treatment has becoming a serious public health problem, which justifies the search for new alternatives with antifungal potential [11], and through numerous plant efficiency and reliability as new drugs [12].

The members of the genus *Eucalyptus*, belonging to the *Myrtaceae* family is originated from Australia, but has been found on most continents. The *Eucalyptus* is popularly used as anesthetic, expectorant, anti-inflammatory and antiseptic [13]. They are known in medicine as antibacterial, antifungal and having inflammatory activities, beside analgesic properties through their essential oils. Essential oils from members of the genus *Eucalyptus* are also used in the pharmaceutical and cosmetics industries due to their biological activities [14].

In view of the increase in antimicrobial resistance cases, studies looking for new drugs to treat infections having medicinal plants as adjuvants or a promising source for new treatment has been an alternative of research. The objective of this study was to preliminarily evaluate the antifungal activity of *Eucalyptus* spp essential oils against *Candida yeasts* using the microdilution method (MIC), minimal inhibitory concentration in broth M27-A3 [15], and the determination of the MFC (Minimum Fungicidal Concentration).

Materials and Methods

Essential oils

The essential oils of *Eucalyptus citriodora* (Lot: C064/16) and *Eucalyptus globules* (Lot: L173) were purchased commercially from the company TERRA FLOR.

Yeast Samples

Reference strains: *Candida rugosa* (IZ 12), *Candida lusitanae* (IZ 06), *Candida glabrata* (IZ 07), *Candida utilis* (CBS 5609), *Candida krusei* (CBS 573), *Candida guilliermondii* (CBS 566), *Candida tropicalis* (CBS 94), *Candida albicans* (CBS 562), *Candida parapsilosis* (CBS 604), *Candida albicans* (ATCC 90028), *Candida albicans* (ATCC MYA-2876), *Candida glabrata* (ATCC 5207) *Candida parapsilosis* (ATCC 22019), *Candida lusitanae* (ATCC 200950), *Candida tropicalis* (ATCC 750). (CBS - Centraalbureau voor Schimmelcultures; IZ - Instituto Zimotécnico - ESALQ/USP; ATCC -The Global Bioresource Center).

Antifungal activity of essential oils. Minimum Inhibitory Concentration (MIC)

Samples of the essential oils were tested for their activity on *Candida* spp strains determining the Minimum Inhibitory Concentration (MIC) following the recommendations of the M27-A3 protocol [15].

Inoculum Adjustment: It was prepared in saline solution, equivalent to 5.0×10^6 CFU/mL in spectrophotometer. The inoculum was standardized at 2.5×10^3 by serial dilution. In a sterile microplate, 100 μ l of RPMI was distributed in all wells, the first column added 100 μ l of the essential oil at the initial concentration, and then the serial microdilution of the essential oils was carried out. After the dilution process was added 100 μ l of the adjusted inoculum, giving the following groups:

a) Positive control group: culture medium and inoculum, for observation of yeast growth)

b) Negative control group 1: culture medium only, for observation possible contamination

c) Negative control group 2: culture medium, essential oil and diluent, the purpose of observing possible contaminations in the microdilution)

d) Negative control group 3: culture medium and diluent, for the purpose to observe possible toxic effects of the diluent on the yeast

e) Test group: *Eucalyptus citriodora* essential oil (Concentration initial 16 mg/mL), or *Eucalyptus globulus* essential oil (16mg/ml initial concentration) or antifungal treatment commercial Fluconazole (initial concentration 64 μ g/ml) or Amphotericin B (Initial concentration 16 μ g/ml).

The plates were incubated for 24 hours at 37°C in an aerobiose oven, and visual reading was performed [15]. The tests were performed in triplicate.

Determination of minimum fungicidal concentration - MFC

The determination of the Minimum Fungicidal Concentration (MFC) was made in Sabouraud Dextrose Agar plates - SDA (KASVI). The 96 wells were homogenized by pipetting, and an aliquot of 10 μ l was transferred to the Petri dish. After incubation at 37°C for 48h, the lowest fungicidal concentration was established. MFC was determined as the lowest concentration of essential oils, which does not allow the growth of any fungal colony in solid culture through visual reading to confirm the fungicidal and fungistatic effects of the essential oils tested [16]. The assays were performed in triplicate.

Results

MIC of *Candida* spp

The essential oils were tested against *Candida* reference strains in order to determine their inhibitory effect through broth microdilution technique. Both oils tested showed inhibitory activity on the planktonic cells of *Candida* spp (Table 1).

Inhibitory activities of up to 8 mg/mL were detected, and inhibitory concentrations ranged from 0.5 mg/ml and 0.125 mg/ml for *Eucalyptus citriodora* essential oil and 8 mg/ml to 1 mg/ml for essential oil of *Eucalyptus globulus*.

Fluconazole and amphotericin B antifungal agents were also tested against *Candida* spp. in order to determine the minimum inhibitory concentration through broth microdilution technique. Fluconazole showed lower inhibitory activity when compared to the antifungal Amphotericin B, which demonstrated inhibitory activity at concentrations between 0.25 μ g/ml and 1 μ g/ml (Table 1).

After determining the MIC values of the essential oils, an aliquot of the susceptibility assay was used to determine the Minimum Fungicidal Concentration (MFC) against the strains of *Candida* spp. The essential oil of *E. citriodora* demonstrated

fungicidal activity, varying its effect at concentrations between 0.25 mg/mL and 0.5mg/mL. The *E. globulus* essential oil showed fungicidal activity at concentrations of 8 mg/mL and 1 mg/ml (Table 1).

Discussion

In recent years, treatment against candidiasis remains a concern due to toxicity and the development of resistance to commercial antifungal agents [17]. The secondary metabolism of plants promotes the production of active compounds that are involved in their defense system. These metabolites are extracted in different ways and different parts of the plant, but mainly make up the essential oils and extracts produced with different vehicles.

The genus *Eucalyptus* was cultivated and exploited on a large scale for many years. Several species of the genus are used in folk medicine as an antiseptic and for infections treatments of the upper respiratory tract. The essential oils extracted from the

leaf of this plant exhibit various biological effects as antibacterial, antifungal and antioxidant activity. The antimicrobial, analgesic and anti-inflammatory properties of *E. citriodora* and *E. globulus* species have been reported [18].

In the present study, the data presented through the microdilution method [15] showed antifungal activity of the oils tested against planktonic cells of *Candida* spp. For *E. citriodora* essential oil, MIC concentrations ranged from 0.5mg/mL to 0.125mg/mL among the strains tested. *E. globulus* oil showed MIC from 2mg/mL to 1 mg/mL for the strains tested, except for the *Candida rugosa* strain IZ 12, which had its inhibited growth only at 8 mg/mL concentration (Table 1).

The minimal fungicidal concentration (MFC) of *E. citriodora* essential oil in the *Candida* strains tested was between 0.25 mg/ml and 0.5 mg / ml. The *E. globulus* essential oil had MFC concentrations between 1 mg/mL and 8mg/mL (Table 1).

Table 1: Visual reading results of *Candida* spp. (MIC).

Reference strains	<i>E. citriodora</i>		<i>E. globulus</i>		<i>Fluconazole</i>	<i>Amphotericin B</i>
	MIC	MFC	MIC	MFC	MIC	MIC
<i>C. albicans</i> ATCC 90028	0,25	0,25	2	2	1	1
<i>C. lusitaniae</i> IZ 06	0,25	0,25	2	2	1	0,5
<i>C. parapsilosis</i> CBS 5604	0,125	0,25	1	4	2	0,25
<i>C. rugosa</i> IZ 12	0,25	0,5	8	8	1	0,25
<i>C. glabrata</i> ATCC 5207	0,25	0,5	1	2	8	0,25
<i>C. guilliermondii</i> CBS 566	0,125	0,125	1	1	1	0,25
<i>C. albicans</i> CBS 562	0,25	0,25	4	4	1	0,25
<i>C. krusei</i> CBS 573	0,125	0,25	2	2	8	0,25
<i>C. tropicalis</i> CBS 94	0,5	0,5	4	4	2	0,25
<i>C. glabrata</i> IZ 07	0,25	0,25	1	2	8	0,25
<i>C. utilis</i> CBS 5609	0,125	0,125	1	1	4	0,25
<i>C. parapsilosis</i> ATCC 22019	0,25	0,25	2	2	1	0,25
<i>C. lusitaniae</i> ATCC 200950	0,25	0,5	4	4	1	1
<i>C. tropicalis</i> ATCC 40281	0,25	0,5	1	1	1	0,25
<i>C. albicans</i> ATCC MYA 2876	0,25	0,25	2	2	1	0,25
<i>C. krusei</i> CBS 6258	0,25	0,25	2	4	32	0,25

These data corroborate with the literature indicating that *Eucalyptus* species show antimicrobial activity by the presence of bioactive compounds in their essential oil (Luqman et al) [18]. According to Tyagi et al. [19] *E. globulus* oil showed antimicrobial activity against *B. subtilis* and *S. aureus*. *E. globulus* oil also showed activity against yeast cells, inhibiting the growth of *Candida albicans* and *Saccharomyces cerevisiae*. Antibacterial activity against the species *Fusobacterium nucleatum* and *Porphyromonas gingivalis* were also observed by Harkat-Madouri et al. [20]. In a study by Dhakad et al. [21], *E. globulus* exhibited antibacterial and antifungal activity. *E. citriodora* essential oil has also demonstrated antifungal activity against filamentous fungi species [22]. Luqman et al. [18] also reported the antimicrobial

activity of *E. citriodora* against *Candida albicans*, *Aspergillus flavus* and *Aspergillus niger*, and against the bacterial strains of *Escherichia coli* and *Mycobacterium smegmatis*. In addition, oil from *E. citriodora* exhibited antifungal activity against the dermatophytes *Microsporum canis*, *Trichophyton rubrum* and *Trichophyton mentagrophytes* [23].

All these data, when analyzed together, show that *Eucalyptus* spp species are potentially antibacterial and antifungal species, exhibiting action against these microorganisms in different ways. Different methodologies of extraction from different parts of the plants can exhibit different bio components with various capacity of microorganism's inhibition suggesting that more researches to look for understand such phenomena must be taken in account.

Since there are several reports in the literature indicating the antimicrobial action of these plant species, the phenomena of microbial resistance to commercial antifungal, and a smaller possibility to synthesizing new drugs, together with the increase of infections by these microorganisms, justify more extensive studies in the attempt to evaluate the antifungal potential of essential oils using lower concentrations in the synergistic combination with conventional drugs in an attempt to decrease or avoid side effects of these drugs, potentializing their antifungal activity, should be carried out as part of our future researchs, added of cytotoxicity and anti-cancer tests of such bio-activity plant components, including other parts of these plants.

This research refers to a preliminary study about antifungal potential of the *Eucalyptus* spp. essential oils against the *Candida* genus. New investigations as to evaluate the effect of the essential oils of the biofilm of *Candida albicans*; to evaluate the effect of essential oils on *Candida albicans* biofilm through Scanning Electron Microscopy; to evaluate the possible cytotoxic effect of essential oils on epithelial cells of the *HaCat* strain and also to evaluate the possible cytotoxic effect of essential oils “*in vivo*” in *Galleria mellonella* larvae are being carried out in an attempt to amplify the knowledge about this plants and its antifungal potential.

Conclusion

- a) The essential oil (EO) from *Eucalyptus citriodora* and *Eucalyptus globulus* leaves are biologically active in a dose dependent manner against *Candida* spp. tested in planktonic cells.
- b) The EO from both *Eucalyptus* species show fungicidal dose dependent activity against yeasts.

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