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Susceptibility of *Candida albicans* and *Candida* non-*albicans* Strains to Essential Oils

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ABSTRACT

Candida albicans is the most prevalent species in fungal infections. Substances contained in plants may have effects similar to synthetic drugs against pathogenic microorganisms. The essential oils (EO) of five plants (*Cymbopogon winterianus, Mentha arvensis, Pimpinella anisum, Eucalyptus citriodora* and *Baccharis trimera*) were tested against standard *Candida albicans*, *C. tropicalis* and *C. krusei* strains. The antifungal activity was determined based on the Minimum Inhibitory Concentration (MIC) and Minimum Fungicide Concentration (MFC) performed by the microdilution method using 96-well microplates and sowing in Petri dishes, respectively. EOs presented antifungal activity, with MIC between 625 and 10,000ìg / mL, especially EO obtained from *Cymbopogon winterianus*, which presented better performance, representing a potential natural product with anti-Candida activity.

Keywords: Candida albicans, Plants, Medicinal, Phytotherapy.

INTRODUCTION

Candidosis is characterized as an opportunistic infection that can affect skin and mucosa, being caused by yeast of the genus *Candida*, having *C. albicans* as the main pathogen that affects humans[1]. Antibiotic therapy with broad spectrum agents, use of corticosteroids, immunosuppression, parenteral nutrition and exposure to invasive medical procedures such as intravascular catheter insertion, hemodialysis and abdominal surgery are considered risk factors for the progressive increase of their frequency[2].

Several studies have indicated that although *C. albicans* is the most prevalent species in fungal infections, there has been an increase in the infection rates by non-*albicans* species such as *C. parapsilosis, C. tropicalis, C. glabrata*, and *C. krusei*[3-6]. However, the change in the proportion of infections between *C. albican* and *C. non-albican* species is still unclear[7].

A global public health concern is the increased resistance of bacteria and fungi to antimicrobial drugs[8,9] and the increasing number of immunocompromised patients undergoing fungal infections[10,11], which are a major cause of morbidity and mortality in the general population[6]. In this sense, the production of new drugs by the pharmaceutical industry has been stimulated[12,13].

Evidence on the biological properties of essential oils and extracts from various plants has

led to the search for potentially active compounds as alternative solutions for the treatment of infectious diseases[9]. Numerous compounds present in plants are capable of promoting protection especially against pathogenic microorganisms[12,14]. In addition, the enormous production of drugs from biomolecules present in plants is an important economic factor[15]. Thus, the use of essential oils for the control of yeast growth has gained importance due to the resistance acquired by pathogens to a series of widely used drugs[16].

This study aimed to evaluate the antifungal activity of five essential oils against different *Candida albicans* and *Candida* non-*albicans* species.

MATERIALS AND METHODS

Essential Oils

Essential oils of five plant species belonging to five distinct botanical families: *Baccharis trimera* (Family Asteraceae), *Cymbopogon winterianus* (Family Poaceae), *Eucalyptus citriodora* (Family Myrtaceae), *Mentha arvensis* (Family Lamiaceae) and *Pimpinella anisum* (Family Apiaceae) were commercially obtained (Quinarí Fragrâncias e Cosméticos Ltd., Ponta Grossa, PR, Brazil).

Fungal Strains

The antifungal activity was tested against *Candida albicans* (ATCC 289065), *Candida Krusei* (ATCC 40042) and *Candida tropicalis* (ATCC 40147) species. Microorganisms were provided by the Laboratory of Oral Microbiology of the Department of Tropical Medicine - Health Sciences Center - Federal University of Paraíba, Brazil. The storage and viability of strains were obtained by preservation under refrigeration at 4 ° C and periodic peaks.

Solutions of Essential Oils

The density of each essential oil, calculated from the quotient between weight (in g) and volume (in mL), was verified using a precision digital scale Kern® model PCB 1000- 2 (Ziegelei, Balingen, Germany). Then, an emulsion of oils was obtained in the proportion of 0.4 mL of essential oil, 5 mL sterile distilled water and 0.04 mL TWEEN 80[17]. The mixtures were homogenized for 5 minutes with the aid of a PHOENIX® Vortex type tube shaker model AP 56 (Araraquara, São Paulo, Brazil).

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Fungicide Concentration (MFC)

The Minimal Inhibitory Concentration (MIC) was determined by microdilution technique[13,18] in 96-well plates (ALAMAR®, Diadema, São Paulo, Brazil) divided into eight columns (A to H) and 12 lines. Each column corresponded to an essential oil, columns F, G and H were represented by positive control (Nystatin), sterility control and microbial growth control, respectively. The concentration of plant products ranged from 40,000 ìg / mL (line 1) to 19.5 ìg / mL (line 12).

Each well was added of 100ìL of doubly concentrated Sabouraud-Dextrose broth (DIFICO®, Detroit, MI, USA), 100ìL of essential oil emulsion and 10ìL of fungal inoculum. Plates were then placed in bacteriological oven at 37°C for 24 hours. MIC was determined by the visual method with the aid of the addition of 10ìL of 2, 3, 5 triphenyl chloride tetrazolium dye (Sigma-Aldrich®, St. Louis, MO, USA), where the formation of agglomerates of cells in the well concavity was considered. Thus, the lowest concentration of the test product capable of producing visible inhibition on the growth of yeast strains used in microbiological assays was considered as MIC[19].

Aliquots of 10ìL corresponding to MIC and the two previous concentrations were sown in Petri dishes containing SD agar medium (DIFICO®, Detroit, MI, USA) in order to obtain the Minimum Fungicide Concentration (MFC). Subsequently, they were incubated for 24 hours in bacteriological oven at 37°C. Concentrations capable of completely preventing microbial growth or less than three colony forming units (CFU) were considered as fungicides. Assays were performed in triplicate.

RESULTS

The susceptibility of strains to the essential oils is presented in Table 1.

Table 1: Minimum Inhibitory Concentration (MIC) and Minimum Fungicide Concentration (MFC) of the test substances against *Candida albicans* and *Candida* non-*albicans* species

Essential Oils and Positive Control	*C. albicans*		*C. krusei*		*C. tropicalis*	
	CIM*	CFM*	CIM*	CFM*	CIM*	CFM*
Mentha arvensis	1.25	2.5	2.5	2.5	2.5	2.5
Pimpinella anisum	-	-	5	20	10	40
Eucalyptus citriodora	1.25	2.5	5	5	2.5	10
Baccharis trimera	-	-	-	-	-	-
Cymbopogon winterianus	625	1.25	2.5	2.5	1.25	1.25
Nistatina®	3	6	6	12	3	3

*µg/mL

DISCUSSION

The extensive use of antifungal agents may select *Candida* species that are less sensitive to these substances[10]. The number of fungal infections by species of the genus *Candida* has increased and the widespread use of synthetic antifungals seems to be associated with the increased resistance of yeasts to these important agents[20].

This panorama supports the conduction of studies aimed to evaluate the antifungal activity of alternative substances, since it is necessary to scientifically investigate plants that have been indicated in traditional medicine to improve the quality of therapies[13,18,21,22], as the potential of plants as an alternative in the development of new drugs is still little explored, although substances used in the treatment of various diseases are part of the composition of these plants[23].

In this context, the antimicrobial activity of essential oils has been studied by several researchers to evaluate the viability of their use in human models[13,14,24-27]. However, the observation of the antimicrobial activity of essential oils is influenced by the physicochemical properties of molecules, such as solubility and volatility, which may facilitate or hinder the chemical interaction with their probable pharmacological receptors[28].

Based on the results observed in the present study, the essential oil of *Cymbopogon winterianus* was considered, among those evaluated, as the compound with the highest anti-yeast potential. Regarding the action of the essential oil of *Cymbopogon winterianus* and *Mentha arvensis* on *Candida albicans* species, MICs of 625 ìg / mL and 1,250 ìg / mL, respectively, have been observed. Other authors also confirmed the anti-candida activity of *Cymbopogon winterianus* (MIC = 600 ìg / mL) and *Mentha arvensis* (MIC = 1,100 ìg / mL), corroborating our findings.

The biological activity of natural products is influenced by their chemical composition, which may present variability due to the use of different methods for collecting the botanical material, plant part, climatic conditions of the collection region, type of technique for extracting the essential oil, and the method used to verify their pharmacological activity.

Although MIC values between 0.6 and 1.5 mg / mL are classified as moderate inhibitory effect[29], the results obtained suggest an advance in scientific research in order to identify the chemical compounds responsible for this effect. When evaluated in isolation, they may exhibit potent effects on *Candida* strains.

From the point of view of the systemic effects of *Cymbopogon winterianus*, some researchers observed hypotensive and vasorelaxant effect on rat[30], but doses of 20 mg / kg induced bradycardia and transient arrhythmia. In another study, essential oil extracted from the leaf of the same species was

able to produce effects on the Central Nervous System (CNS) of mice at doses of 25, 50 and 100 mg / kg[31].

Mentha arvensis was identified as a plant with antifungal activity that represents an interesting alternative in efforts to combat infectious diseases such as candidiasis[32]. MFC similar to Nystatin against *C. tropicalis* presented by the essential oil from this plant found in the present study confirms this condition. Some researchers have isolated Menthol[33], also present in *Mentha Arvensis*, which presented MICs against *Candida albicans* of 125.0 ìg / mL, which suggests that isolated compounds may offer an antimicrobial activity with greater potential, since in the present study, the essential oil of this plant showed MIC for the same species of fungus ten times higher than that observed by that study. Most of the times, isolated substances have less action potential, which justifies the use of extracts, since synergisms among phytoconstituents are considered.

In the present study, the essential oil of *Eucalyptus citriodora* showed MIC against *Candida Krusei*, corroborating the results described in previous studies that reported antifungal activity of this essential oil[34,35].

Some researchers found susceptibility of *C. albicans* to the methanolic extract of *Pimpinella anisum* (MIC = 16mg / mL and MFC = 256mg / mL)[36] and also to clinically isolated (MIC = 25mg / mL) *C. albicans, C. Glabrata* and *C. krusei*[37]. Alcoholic extracts obtained from a specific part of the same botanical species may exhibit different biological activities, since the type of solvent used in the extraction procedure also influences the pattern of the chemical composition of the products obtained due to differences in solubility. In the present study, although the MIC and MFC results were considerably higher when compared to the other substances analyzed, *Pimpinella anisum* presented antifungal action against *C. krusei* and *C. tropicalis*.

The antiseptic activity is one of the popular indications of *Baccharis trimera*[38]. Monotherpenic and sesquiterpene compounds are the main secondary metabolites present in the essential oils of plants of the genus *Baccharis*[39].

The results of this research pointed that only *B. trimera* showed no activity against all *Candida* species studied. However, some researchers who used other parts of the same plant[38,40] obtained positive results against some bacteria and fungi, including *C. albicans*. Therefore, in addition to the analysis of the chemical composition of this oil, it is necessary to verify the susceptibility to other microorganisms, of clinical origin or not, as well as to consider the same part of the investigated plant, making possible comparisons among studies and advancements in the scientific knowledge related to the medicinal properties of plant species.

CONCLUSION

The essential oil of citronella (*Cymbopogon winterianus*) is a potential anti-yeast agent that causes oral infections. However, cytotoxicity analyzes and clinical trials are required to ensure its use in humans.

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