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Antifungal Activity of the Essential Oil of *Pogostemon cablin* (Lamiaceae) against *Candida tropicalis* Strains

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Joyce Natielle Miranda Cavalcante[1]; Daniele De Sousa Siqueira[1], José Lucas Soares Ferreira[1], Raquel Vieira Bezerra[1], Letícia Ataíde Delgado[1], Rebeca Cícera Mendes De Oliveira Silva[1], Rafael Cartaxo Figueira[1], Daniele De Figueredo Silva[2], Heloísa Mara Batista Fernandes De Oliveira[3], Edeltrudes De Oliveira Lima[2], Abrahão Alves De Oliveira Filho*[1]

[1] Academic Unit of Biological Sciences, Federal University of Campina Grande, Patos-PB, Brazil

[2] Health Sciences Center, Federal University of Paraiba, João Pessoa-PB, Brazil

[3]Ana Bezerra University Hospital, Federal University Rio Grande do Norte, Santa Cruz-RN, Brazil

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ABSTRACT

Candida yeast is very common in the oral mucosa of healthy individuals. However, when there is a breakdown of host defense mechanisms, this yeast may cause a proliferation or infection of the oral cavity. Conventional methods for the treatment of candidiasis are based on the use of antifungal agents, however, a number of drawbacks are observed when using them, represented by toxicity, antagonistic drug-drug interactions, lack of fungicidal efficacy, high cost and emergence of resistant species caused by frequent use of some of them. In the search for new therapeutic strategies for oral candidiasis, studies using medicinal plants are being increasingly performed. This work aims to analyze the possible antifungal activity of *Pogostemon cablin* essential oil against *Candida tropicalis* strains. For the determination of MIC (Minimum Inhibitory Concentration) of the extract, the broth microdilution technique was performed. Viability control of the strains tested, as well as sensitivity control of these strains, was performed on the antimicrobial Nystatin. In view of the results, it was observed that for the ATCC type 13803 the MIC was 128 µg / ml, for the LM 64 it was 32 µg/ml; however, for the LM 04 and 20 strains, the extract presented a MIC of > 1024 µg/mL. For the Minimum Fungicide Concentration (CFM), the values were the same as the MIC, respecting their respective strains. It can be concluded that the essential oil of *Pogostemon cablin* has a strong fungicidal antimicrobial activity against strains of *Candida tropicalis.*

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INTRODUCTION

Candida yeast is very common in the oral mucosa of healthy individuals; its prevalence rate varies from 20 to 70%. However, when there is a breakdown of host defense mechanisms, this yeast may cause a proliferation or infection of the oral cavity, observed mainly in early childhood, senescence and immunocompromised patients[1].

Oral candidiasis was described as a disease associated with the first cases described in the AIDS (acquired immunodeficiency virus) literature, constituting the most frequent fungal infection in HIV-positive patients. It is estimated that up to 90% of HIV-infected individuals will experience at least one episode of oropharyngeal candidiasis [2].

According to Pfaller and Diekema (2007), more than 17 different species of the genus Candida are known as etiological agents of infections in humans, however, more than 90% of the invasive infections are caused by *C. albicans, C. glabrata, C. parapsilosis, C tropicalis,* and *C. krusei.*[3]

Conventional methods for the treatment of candidiasis, superficial or systemic, are based on the use of antifungal agents, such as nystatin, amphotericin B, miconazole, ketoconazole, among others. However, several drawbacks are observed when using them, represented by toxicity, antagonistic drug-drug interactions, lack of fungicidal efficacy, high cost and emergence of resistant species caused by the frequent use of some of them[4,5].

In addition, despite the introduction of new antifungal drugs, they are still in limited numbers, thus evidencing a great demand for new, more effective and less toxic antifungal agents. In this context, the use of metabolites extracted from medicinal plants appears for this purpose[4,5].

About 80% of the population of developing countries use medicinal plants as a way of treating their diseases. This fact broadens the perspective for the development of research with plants with the purpose of proposing alternatives that contribute to the qualification and expansion of primary health care. The high cost of industrialized medicines and the lack of access of the population to health services also drive the increasing use as alternative and complementary methods[6,7].

In the search for new therapeutic strategies for oral candidiasis, studies using essential oils of plants are being increasingly performed. These substances consist of complex mixtures (mainly formed by mono- and aliphatic or aromatic sesquiterpenes) that present in different proportions [8].

Based on the information on the therapeutic potential of essential oils of medicinal plants and the importance of combating yeast infections of the genus Candida, this work aims to evaluate the possible antifungal activity of the essential oil of *Pogostemon cablin* (Lamiaceae) (OE- *Pogostemon cablin*) against different strains of *Candida tropicalis*.

MATERIALS AND METHODS

In vitro assay

Substance test

The product submitted to the biological test was coded by OE-*Pogostemon cablin*. It was suitably solubilized in 0.02% tween 80, dimethyl sulfoxide (DMSO) in a proportion of up to 0.5% and supplemented with sterile distilled water (qsp 6 mL) to obtain a 1024 µg / mL [9,10].

Fungal Species

For the biological activity assay of OE, the following yeasts of the genus *Candida* were used: *Candida tropicalis (C. t. ATCC 13803, C. t.LM-04; C. t.LM-20 and C. t.LM-64).* These belong and are kindly provided by the MICOTECA of the Mycology Laboratory, Department of Pharmaceutical Sciences (DCF), Health Sciences Center (CCS) of the Federal University of Paraiba. All strains were maintained in media Sabouraud dextrose (ASD) at 4 °C. The assays were run for 24-48 hours in ASD, incubated at 35 ± 2 °C.

Inoculum

For the preparation of the inoculum, colonies obtained from cultures of *Candida* spp., maintained in ASD, were suspended in sterile 0.85% NaCl solution and adjusted according to the McFarland 0.5 standard to first obtain an inoculum of 10^6 CFU/mL. And then diluted in saline in a ratio of 1:9, finally resulting in a fungal suspension containing 10^5 CFU/mL which was used in the assays[11,12].

Determination of Minimum Inhibitory Concentration (MIC)

The antifungal activity assays were performed according to the [13, 14] and [15]. The determination of the MIC of the substances on strains of *Candida* was carried out using the 96-well microdilution technique in broth in cell culture plate. Initially, 100 µL of doubly concentrated RPMI 1640 was distributed into the holes of the microdilution plates. Then, 100 µL of the emulsion of the dual concentrate test products were dispensed into the wells of the first row of the plate. And by serial dilution at a ratio of two, concentrations of 1024 µg / mL to 4 µg / mL were obtained. Finally, 10 µL of *Candida spp*. in the wells, where each column of the plaque refers to a fungal strain, specifically. At the same time, the control of the inoculum (RPMI 1640 + yeasts) was carried out; and standard drug control (RPMI 1640+ inoculum + amphotericin B 100µg). The prepared and aseptically closed plates were incubated at a temperature of 35 ± 2 ° C for 24-48 hours. CIM was defined as the lowest concentration of the product, capable of producing visible inhibition on the fungal growth observed in the orifices when compared to its controls. The result was expressed by the arithmetic mean of the MICs obtained in the test performed in duplicate.

The antifungal activity of the products was interpreted and considered as active or inactive, according to the following criteria: 50-500 µg / mL = strong / optimal activity; 600-1500 µg / mL = moderate activity; > 1500 µg / mL = weak activity or inactive product [16].

Determination of Minimum Fungicide Concentration (CFM)

After reading the CIM, aliquots of 10 µL of the supernatant from the wells where complete inhibition of fungal growth (MIC, CIM x 2 and CIM x 4) was observed in the microdilution plates, were subcultured in 100 µL of RPMI 1640 contained in new plates cell culture. Subsequently, the same duly prepared were incubated at 35 ± 2 ° C for 24-48 hours. CFM was considered the lowest concentration in which there was no yeast growth in the culture medium. The tests were performed in duplicate and the result expressed by the arithmetic mean of the CFM's obtained in the two tests [17].

RESULTS AND DISCUSSION

Minimum Inhibitory Concentration (MIC) in liquid medium was tested and determined for the essential oil of *Pogostemon cablin* at the different concentrations suggested in the methodology and determined by the lowest concentration capable of visually inhibiting

fungal growth, as shown in the table. The results for the oil tested ranged from 1024 to 32 µg / mL.

CIM50 is said to be the lowest concentration capable of inhibiting 50% of the strains during the experiment; Following the premise, it is observed that the CIM_{50} for *Candida tropicalis* was 128 µg / mL.

However, the Minimum Bactericidal Concentration (MBC) was determined by the lower concentration of oil that resulted from the visible inhibition of microorganism growth. The values obtained were the same as the MIC, varying between 1024 and 32 µg / mL.

Table 1. Minimum inhibitory concentration (MIC) and Minimum Fungicidal Concentration (MFC) in µg / mL of essential oil of *Pogostemon cablin* against different strains of *Candida tropicalis*

	Pogostemon cablin	
Microorganisms	MIC	MFC
Candida tropicalis ATCC 13803	128	128
Candida tropicalis LM 04	>1024	>1024
Candida tropicalis LM 20	>1024	>1024
Candida tropicalis LM 64	32	32
Positive control	+	
Negative control	-	

(-) = There was no apparent inhibition of the strain

(+) = visible inhibition of the strain

Medicinal plants are important because they provide the raw material for drug synthesis, as well as being used as alternative therapeutic agents. The use of plants is overvalued in traditional use based on their medicinal benefits. Thus, it is essential to know the dose and the employed part of the plant, in addition to its therapeutic properties, since there are those that are highly toxic, even in small doses [18].

Candida species are part of the oral microbiota of approximately 50% of the population. However, under certain conditions, they may behave as opportunistic pathogens, producing

infections ranging from superficial mucosal lesions to serious and invasive systemic dissemination, potentially fatal in immunocompromised patients. In addition to various forms of yeast infection, these yeasts may be involved in persistent endodontic lesions and periodontal diseases. The most isolated species of these infections is *C. albicans,* followed by species *C. glabrata, C. tropicalis, C. parapsilosis* and *C. krusei* [19].

The patchouli, *Pogostemon cablin Benth. (*Lamiaceae*),* produces an essential oil extracted by steam distillation of its dry leaves, which has several properties, among them aromatherapy, antibacterial, antifungal, antioxidant, insecticide, and insect repellent activity[20].

Sartoratto et al. (2004) suggest that an antimicrobial activity is classified as strong when, for essential oils, they have MICs up to 500 µg / mL, moderate for MICs of 600 to 1500 µg / mL and weak for MICs above 1500 µg / mL [16].

Thus, according to the result of the oil, *Pogostemon cablin* can be considered a strong inhibitor against the strains of *Candida tropicalis* since it presented a MIC (Minimum Inhibitory Concentration capable of inhibiting the growth of 50% of the strains) of 128 µg / ml.

According to Hafidh et al. (2011) for a compound to be considered fungicidal or fungistatic according to the Minimum Fungicidal Concentration (MBC) should be equal to or twice as large as the MIC or the MFC should be greater than twice the MIC. Analyzing the CFM result it can be seen that *Pogostemon cablin* has fungicidal activity since the MIC was equal to MFC[21].

The results found in this study reinforce the data found by the Das et al. (2013) and Wang et al. (2012) studies demonstrating the antifungal potential of *P. cablin* against *Candida* strains[22,23].

CONCLUSION

In view of the results obtained, it was observed that the essential oil of *Pogostemon cablin* tested presented relevant results. In view of this, the natural product can be considered as promising against the strains of *Candida tropicalis*, however further studies are necessary to elucidate mechanisms and standards of efficiency and effectiveness.

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