**In vitro** anti-Candida effect of (S)-(−)-citronellal

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**ABSTRACT**

The terpenoids forms a group of natural compounds with the great potential to develop the products for disease control. In this study, the monoterpene (S)-(−)-citronellal was evaluated for its antifungal effects. The five *Candida albicans* strains and five *Candida tropicalis* strains were used in the study. All the microorganism strains were obtained from the Laboratory of Mycology. The Microdilution method was used for antifungal assay of the monoterpene and the Nistatin (100 UI/mL) was used as standard drug. The results showed that the monoterpene presented MIC50 and MFC50 the values of 256 µg/mL, and 512 µg/mL, respectively for both species of fungi. The obtained results showed activity fungicide against both fungal strains.

**INTRODUCTION**

Essential oils are natural plant products that have a large potential to replace the synthetic fungicides, due to their antifungal, antibacterial and insecticidal properties (Feng et al., 2007; Lee et al., 2008; Knaakand Fiuza, 2010). Essential oils are complex mixtures of volatile organic substances, consisting of oxygenated compounds and hydrocarbons such as monoterpenes and sesquiterpenes (Prabuseenivasan et al., 2006; Nerio et al., 2010). The terpenoids and phenolic compounds are responsible for the antimicrobial activity of essential oils, which accumulate on the membranes, with energy loss by microbial cells. Thus, the terpenoids that comprise essential oils forms a group of natural compounds with the great potential for developing products for disease control (Feng et al., 2007; Knaakand Fiuza, 2010).

Citronellal is a monoterpen, found in more than 50 essential oils of various plant species. Citronellal is a constituent of several essential oils used to treat various pathological conditions in different parts of the world, such as South America and Asia (Nerio et al., 2010). Based on this information, this work is aimed to evaluate the antifungal potential of monoterpene (S)-(−)-citronellal.

**MATERIALS AND METHODS**

The monoterpene (S)-(−)-citronellal was purchased from Sigma-Aldrich® (SãoPaulo-SP). To achieve the pharmacological tests, the substance was solubilized in DMSO and diluted in distilled water. The concentration of DMSO was less than 0.1% v/v.

**Determination of the minimum inhibitory concentration (MIC)**

The antifungal activity was performed on five selected strains of *Candida albicans* (ATCC 76485, LM62, LM106, LM...
108, LM 122) and five strains of Candida tropicalis (ATCC 13803, LM 06, LM 14, LM 31, LM 36). All the microorganism strains were obtained from the Laboratory of Mycology collection.

The antifungal activity was carried out according to the protocols described by Cleeland and Squires (1991), Hadacek and Greger (2000) and CSLI (2008).

The MICs of the monoterpene was determined against Candida strains by broth microdilution technique. Initially 100 µL of Sabouraud dextrose broth was added to the wells of microdilution plate. Then, 100 µL of the emulsion products, were dispensed in the wells of the first row. A ratio of two concentrations were obtained 1024 µg/mL to 2 µg/mL, so that the first line of the plate was meet the highest concentration and last, the lowest concentration. Finally, 10 µL of the inoculum was added to the cavities, where each plate column referred to a fungal strain, in particular.

In parallel, the feasibility control of the tested strains was carried out along with the sensitivity control, these strains to antifungal action considered standards in clinical use (Nistatin100 UI/mL). To verify the absence of interference of solvent (DMSO) in the results, a control was placed in the cavities 100 µL of the double-concentrated broth, 100 µL of DMSO and 10 µL of the suspension was made.

The plates were sealed aseptically and incubated at 35 °C for 24 - 48 hours. Testing was performed in duplicate and the result expressed by the arithmetic mean of the MIC's obtained in the two tests.

**Determination of the minimum fungicide concentration (MFC)**

A 20µL aliquot of each pit growth fungal (MIC, MIC x 2, MIC x 4) was grown in a plate with Sabouraud Dextrose Agar. It was then incubated at 35-37 °C for 24 hours. The MFC was considered the lower concentration in Sabouraud Dextrose Agar planted where there were 3 lower growth units forming colonies (UFC) (Espinel-Ingroff et al., 2002).

**RESULTS AND DISCUSSION**

The evidence of microbial resistance is of great importance in treatment of candidiasis. Researches are going on for the search of new substances and combinations of drugs to treat microbial infections (Candido et al., 2000). The interest in plants with healing properties has grown considerably due to the prospect of isolating substances showing significant efficacy and lower rate of disadvantages, for example, the monoterpenes (Souza-Júnior et al., 2011).

The results of MIC (Minimum Inhibitory Concentration) and MFC (minimum fungicidal concentration) of monoterpene against the Candida albicans strains are shown in Table 1 and 2. The MIC and MFC of monoterpene against the Candida tropicalis are shown in Table 3 and 4. The monoterpene presented MIC50 and MFC50 values of 256µg/mL, and 512µg/mL, respectively for both species of fungi.

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(-) No inhibition (+) inhibition

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The results showed that monoterpene (S)-(−)-citronellal presents the strong effect against *C. albicans* and *C. tropicalis* strains with MFC = 256 μg/mL. These results are in agreement with the data obtained by Mirona et al. (2014) in their study using several monoterpens against various strains of *Candida*. Analyzing the results of the MFC can be seen that the monoterpene have fungicidal activity against both species of *Candida*, these results are also in agreement with Hafidh et al. (2011) when the ratios of MFC/MIC were 1 or 2, indicating that the effect of the compound was fungicidal in nature (and not fungistatic).

**CONCLUSION**

After analyzing the results of the monoterpene and comparison with other results already reported in the literature, it can be said that the (S)-(−)-citronellal is a promising compound to combat diseases caused by fungi of the genus *Candida*.

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**Conflict of Interests:** There are no conflicts of interest.

**REFERENCES**


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