

Antifungal effect of 7-hydroxycitronellal against *Candida albicans* strains

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This word was carried out in collaboration between all authors. Authors AAOF, JPSJ, HMBFO and EDOL designed in this study, performed the statistical analysis, wrote the protocol and managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Monoterpenes, the main constituents of essential oils, are associated with antifungal, analgesic, antiepileptic, anti-inflammatory, anxiolytic and gastroprotective activities. The aim of this study was to evaluate the antifungal effects of the monoterpene 7-hydroxycitronellal against five *Candida albicans* strains using microdilution method. All the strains were obtained from the Laboratory of Mycology collection. Nistatin (100 UI/mL) was used as the standard drug. The monoterpene showed strong antifungal activity with MIC₅₀ and MFC₅₀ values of 256 and 512 µg/mL respectively. The results showed fungicidal potential of 7-hydroxycitronellal against *C. albicans* strains.

Key words: Monoterpene; Nistatin; Antifungal; *Candida albicans*.

1. INTRODUCTION

Candidiasis or thrush is a fungal infection caused by *Candida* yeasts, where the injury can be mild, acute or chronic, superficial or deep and quite variable clinical spectrum (1).

In conventional manner, the treatment of candidiasis has not been shown to be comprehensive in its entirety by the constant emergence of barriers caused mainly by the reduced amount of antifungal agents available for systemic treatment, as well as the high toxicity of these and the increasing resistance of fungi to antifungals (2,3).

Faced with the excessive use of synthetic antifungal medications, as well as on the resistance to these products, several alternatives are being made to control diseases caused by *Candida*. One of those alternatives is the search for natural products with antifungal effective against resistant microorganisms (4).

Among natural products with biological activity are the monoterpenes, main constituents of essential oils, associated with antifungal, analgesic, antiepileptic, anti-inflammatory, anxiolytic and gastroprotective activities (5,6).

In this context this work aimed to evaluate the antifungal potential of monoterpene 7-hydroxycitronellal, that presents few studies of its kind against *Candida* (Figure 1).

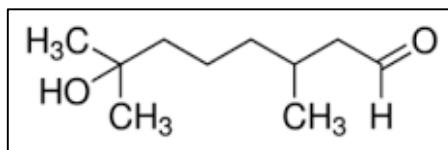


Figure 1- Chemical Structure of the 7-hydroxycitronellal

2. MATERIALS AND METHODS

2.1 Source and preparation of monoterpenes

The monoterpenes 7-hydroxycitronellal was purchased from Sigma-Aldrich® Industry (São Paulo-SP). For pharmacological testing, the substance was solubilized in cremophor and diluted in distilled water. The concentration of cremophor was less than 0.1% v/v. Concentrations were used in 1024 µg/mL to 2 µg/mL.

2.2 Determination of the minimum inhibitory concentration (MIC)

The strains of *C. albicans* (ATCC 76845, LM62, LM106, LM 108, LM 122) selected for the antifungal activity were obtained from the Laboratory of Mycology collection and were kept on Nutrient Agar (NA) slants at 4 °C.

Inocula were obtained from overnight cultures grown on NA slants at 37 °C and diluted in sterile saline solution (NaCl 0.85 % w/v) to provide a final concentration of approximately 10^6 count forming unit per mL (cfu/mL) adjusted according to the turbidity of 0.5 McFarland standard.

The antifungal activity assays were carried out according to the protocols Cleeland and Squires (1991) (7), Hadacek and Greger (2000) (8) and CLSI (2008) (9).

The MICs of the monoterpenes was determined against *Candida* strains by broth microdilution technique. Initially was distributed 100 µL of Sabouraud dextrose broth doubly concentrated in the wells of microdilution plates. Then, 100 µL of the emulsion products also doubly concentrated, were dispensed in the wells of the first row of the plate. And by means of a serial dilution at 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, it was added 10 µL of the

inoculum of the species in the cavities, where each plate column referred to a fungal strain, in particular.

In parallel, it was carried out feasibility control of the tested strains. Also sensitivity control these forward strains to antifungal action considered standards in clinical use. To verify the absence of interference in the results for the solvent used in the preparation of the substance in the event the cremophor. In which a control was placed in the cavities 100 μ L of the double-concentrated **broth**, 100 μ L of cremophor and 10 μ L of the suspension was made. The Nistatin (100 UI/mL) was the standard drug.

The plates were sealed aseptically and incubated at 35 ° C for 24 - 48 hours to the reading performed. MIC was defined for the products tested as the lowest concentration able to produce inhibition of visible fungal growth recorded in the holes, compared with the control growth. Testing was performed in duplicate and the result expressed by the arithmetic mean of the MIC's obtained in the two tests.

2.3 Determination of the minimum fungicide concentration (MFC)

A 20 μ L 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 was 3 lower growth units forming colonies (10).

3. RESULTS

The results of antifungal activity to determine the MIC (Minimum Inhibitory Concentration) and MFC (minimum fungicidal concentration) of monoterpenes front of the *C. albicans* strains are shown in Table 1 and 2. Observing these results can be seen

that the monoterpenes presented MIC₅₀ and MFC₅₀ the values of 256 µg/mL, and 512 µg/mL, respectively.

Table 1- Antifungal activity for determination of the MIC of the 7-hydroxycitronellal (OC).

Fungal strains/	<i>Candida</i>	<i>Candida</i>	<i>Candida</i>	<i>Candida</i>	<i>Candida</i>
Substance	<i>albicans</i>	<i>albicans</i>	<i>albicans</i>	<i>albicans</i>	<i>albicans</i>
	ATCC 76845	LM 62	LM 106	LM 108	LM 122
OC (1024 µg/mL)	+	+	+	+	+
OC (512 µg/mL)	+	+	+	+	+
OC (256 µg/mL)	+	+	+	+	-
OC (128 µg/mL)	-	-	-	-	-
OC (64 µg/mL)	-	-	-	-	-
OC (32 µg/mL)	-	-	-	-	-
OC (16 µg/mL)	-	-	-	-	-
Negative control	-	-	-	-	-
Positive control	+	+	+	+	+

(-) No inhibition (+) inhibition

Table 2- Antifungal activity for determination of the MFC of the 7-hydroxycitronellal (OC).

Fungal strains/	<i>Candida</i>	<i>Candida</i>	<i>Candida</i>	<i>Candida</i>	<i>Candida</i>
Substance	<i>albicans</i>	<i>albicans</i>	<i>albicans</i>	<i>albicans</i>	<i>albicans</i>
	ATCC 76845	LM 62	LM 106	LM 108	LM 122
OC (1024 µg/mL)	+	+	+	+	+
OC (512 µg/mL)	+	+	+	+	+
OC (256 µg/mL)	-	-	-	-	-
OC (128 µg/mL)	-	-	-	-	-
OC (64 µg/mL)	-	-	-	-	-
OC (32 µg/mL)	-	-	-	-	-

OC (16 µg/mL)	-	-	-	-	-
Negative control	-	-	-	-	-
Positive control	+	+	+	+	+

(-) No inhibition (+) inhibition

4. DISCUSSION

Regarding the treatment of candidiasis, lots of drugs obtained by organic synthesis have it has been used in the treatment of mycotic infections such as antiseptics to iodine-based, gentian violet, salicylic acid and benzoic acid, sulphonamide derivatives, dyes, quinones and antifungal polyenic (nystatin, amphotericin). In addition to these, also are used as antifungal azoles (ketoconazole, econazole, sulconazole, miconazole, clotrimazole and fluconazole) and amphotericin B. However, infections yeast are difficult to treat, due to emergence of resistance in *Candida* strains agaisnt conventionally used antifungals (11).

Due to the occurrence of undesirable factors such as the emergence of resistance of some strains to conventional antifungal - especially in individuals immunocompromised patients and the presence of these toxic effects, the study of plants with therapeutic properties, including those with antimycotic activity has grown considerably (12).

In parallel, the search for antimicrobial of natural origin that have activity on broad spectrum of microorganism which can be used as an alternative to conventional antibiotics It has aroused the interest of the scientific class, especially in the molecules of vegetable origin, such as the monoterpenes (13).

According with Sartoratto et al (2004) (14) results strong activity is for MIC values between 0.05 – 0.50 mg/mL, moderate activity MIC values between 0.6 – 1.50

mg/mL and weak activity above 1.50 mg/mL. The results showed that monoterpene 7-hydroxycitronellal present the strong effect against *C. albicans* strains with MIC₅₀ for monoterpene is 256 µg/mL. These results are in agreement with the data obtained by Trindade et al. (2015) (15) in their study using the monoterpene citronellal against various strains of *Candida*.

Analyzing the results of the MFC can be seen that the monoterpene does have fungicide activity against *C. albicans* species, because according Hafidh et al. (2011) (16) when the ratios of MFC/MIC were 1 or 2, indicating that the effect of the compound was fungicide in nature (and not fungistatic).

5. CONCLUSION

Therefore, with the analysis of results obtained in this research can be seen that the monoterpene has a strong antifungal effect against strains of *C. albicans*, a causative fungus of various infections in the human body. Thus making it a choice for the treatment of diseases caused by this microorganism.

CONSENT

All the authors declare that no consent was obtained for this study.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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