

Original Research Article

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

ABSTRACT

Monoterpenes, main constituents of essential oils, associated with antifungal, analgesic, antiepileptic, anti-inflammatory, anxiolytic and gastroprotective. In the study, the monoterpene 7-hydroxycitronellal was evaluated for its antifungal effects. IN the methodology five *Candida albicans* strains were used in the study for activities. All the microorganism strains were obtained from the Laboratory of Mycology collection. The Microdilution method was used for antifungal assay of the monoterpene. The Nistatin (100 UI/mL) was the standard drug. The obtained results showed activity fungicid against *Candida 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 specimens to antifungals (2,3).

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

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

31 In this context this work aimed to evaluate the antifungal potential of
32 monoterpene 7-hydroxycitronellal.

33 2. MATERIALS AND METHODS

34 2.1 Obtained of monoterpene

35 The monoterpene 7-hydroxycitronellal was purchased from Sigma-Aldrich®
36 Industry (São Paulo-SP). To achieve the pharmacological tests, the substance was
37 solubilized in cremophor and diluted in distilled water. The concentration of cremophor
38 was less than 0.1% v/v.

39 2.2 Determination of the minimum inhibitory concentration (MIC)

40 For methodology by antifungal activity, were selected five strains of *Candida*
41 *albicans* strains (ATCC 76845, LM62, LM106, LM 108, LM 122). All the
42 microorganism strains were obtained from the Laboratory of Mycology collection.

43 The antifungal activity assays were carried out according to the protocols
44 Cleeland and Squires (1991) (7), Hadacek and Greger (2000) (8) and NCCLS (2002)
45 (9).

46 The MICs of the monoterpenes were determinated of the against *Candida* strains
47 by broth microdilution technique. Initially was distributed 100 µL of Sabouraud

dextrose broth doubly concentrated in the holes of microdilution plates. Then, 100 μ L 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 μ g/mL to 2 μ g/mL, so that the first line of the plate was meet the highest concentration and last, the lowest concentration. Finally, it was added 10 μ L of the inoculum of the species in the cavities, where each plate column refered 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 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.

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2.3 Determination of the minimum fungicide concentration (MFC)

A 20 μ L aliquot of each pit growth fungal (CIM, CIM 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 (UFC) (10).

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3. RESULTS

The results of antifungal activity to determine the MIC (Minimum Inhibitory Concentration) and CFM (minimum fungicidal concentration) of monoterpene front of the *Candida albicans* strains are shown in Table 1 and 2. Observing these results can be seen that the monoterpene presented MIC₅₀ and CFM₅₀ 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/ Substance	<i>Candida albicans</i> ATCC 76845	<i>Candida albicans</i> LM 62	<i>Candida albicans</i> LM 106	<i>Candida albicans</i> LM 108	<i>Candida albicans</i> 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/ Substance	<i>Candida albicans</i> ATCC 76845	<i>Candida albicans</i> LM 62	<i>Candida albicans</i> LM 106	<i>Candida albicans</i> LM 108	<i>Candida albicans</i> 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	+	+	+	+	+

84 (-) No inhibition (+) inhibition

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86 4. DISCUSSION

87 Regarding the treatment of candidiasis, lots of drugs obtained by organic synthesis
88 have it has been used in the treatment of mycotic infections such as antiseptics to
89 iodine-based, gentian violet, salicylic acid and benzoic acid, Sulphonamide derivatives,
90 dyes, quinones and antifungal polyenic (nystatin, amphotericin). In addition to these,
91 also are used as antifungal azoles (ketoconazole, econazole, sulconazole, miconazole,
92 clotrimazole and fluconazole) and amphotericin B. However, infections yeast are
93 difficult to treat, elevated to the related fact resistance Candida to the action of some
94 antifungals conventional (11).

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

100 In parallel, the search for antimicrobial of natural origin that have activity on

101 broad spectrum of microorganism which can be used as an alternative to conventional
102 antibiotics It has aroused the interest of the scientific class, especially in the molecules
103 of vegetable origin, such as the monoterpene (13).

104 According with Sartoratto et al (2004) (14) results strong activity is for MIC
105 values between 0.05 – 0.50 mg/mL, moderate activity MIC values between 0.6 – 1.50
106 mg/mL and weak activity above 1.50 mg/mL. The results showed that monoterpene 7-
107 hydroxycitronellal present de strong effect against *C. albicans* strains with MIC₅₀ for
108 TMF is 256 µg/mL. These results are in agreement with the data obtained by Trindade
109 et al. (2015) (15) in their study using the monoterpene citronellal against various strains
110 of *Candida*.

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

115 5. CONCLUSION

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

120 CONSENT

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

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