

Original Research Article

Antifungal effect of flavonoid 5,7,4'-trimethoxyflavone against *Candida*

***krusei* strains**

ABSTRACT

Most of the flavonoids are considered as constitutive antimicrobial ingredients, especially those belonging to prenylated flavonoids, flavones and isoflavones. In the study, the flavonoid 5,7,4'-trimethoxyflavone was evaluated for its antifungal effects. Four fungal strains were used in the study for activities, *Candida krusei* – LM 9700, *Candida krusei* – LM 656, *Candida krusei* – LM 13 and *Candida krusei* – LM08. All the microorganism strains were obtained from the Laboratory of Mycology collection. Microdilution method was used for antifungal assay of the flavonoid. The results were also compared with the standard drug, Nistatin (100 UI/mL). The obtained results showed activity fungistatic against *Candida krusei* strains.

Key words: Flavonoid; Nistatin; Antifungal; *Candida krusei*.

1. INTRODUCTION

Flavonoids are low molecular weight phenolic compounds, secondary metabolites found in fruits, vegetables, nuts, seeds, herbs, spices, stems and flowers as well as in tea and red wine [1].

Structurally, flavonoids can be grouped according to the presence of different substituents on the rings and to the degree of benzo-g-pyrone saturation. The most common classes are the flavones, flavanones and flavonols [2].

For centuries, preparations that contain flavonoids as the principal physiologically active constituents have been used by physicians and lay healers in attempts to treat human diseases [3]. Most of the flavonoids are considered as

25 constitutive antimicrobial ingredients, especially those belonging to prenylated
26 flavonoids, flavones and isoflavones [4,5].

27 Owing to the widespread ability of flavonoids to inhibit spore germination of
28 plant pathogens, they have been proposed for use against fungal pathogens of man [6].
29 For example, the flavonoid 7-hydroxy-3',4'-(methylenedioxy)flavan, isolated
30 from *Terminalia bellerica* fruit rind, has also been shown to possess activity
31 against *Candida albicans* [7].

32 Considering the few studies on the antifungal effects of this flavonoid 5,7,4'-
33 trimethoxyflavone isolated of *Praxelis clematidea* (TMF), the aim of the present study
34 was to evaluate the activities for the compost against different strains of the
35 fungus *Candida krusei*.

36 2. MATERIALS AND METHODS

37 2.1 Isolated of the flavonoid

38 The aerial parts of *Praxelis clematidea* R.M. King & Robinson were collected in
39 Lagoa do Paturi, a municipality of Santa Rita, in the state of Paraíba (Brazil), in May
40 2008. The identification of the botanical material was performed by Prof. Dr. Maria de
41 Fátima Agra, Botany Sector, Laboratory of Pharmaceutical Technology/UFPB
42 "Professor Delby Fernandes de Medeiros". Exsiccates of the plant are deposited in the
43 Prof. Lauro Pires Xavier (JPB) Herbarium, Paraíba Federal University, under the code
44 M. F. Agra et al. 6894 (JPB). Maia et al (2010) describe the method of obtaining the
45 flavonoid [8].

46 2.2 Fungal strains

47 For antifungal activity assays, were selected 4 strains of fungi (*Candida krusei* –
48 LM 9700, *Candida krusei* – LM 656, *Candida krusei* – LM 13 and *Candida krusei* –

LM08). All the microorganism strains were obtained from the Laboratory of Mycology collection. Fungi 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⁻¹) adjusted according to the turbidity of 0.5 McFarland scale tube.

2.3 Determination of the minimum inhibitory concentration (MIC)

The microplate bioassay was used to determine the minimum inhibitory concentration (MIC) of flavonoid [9,10].

The antifungal activity was detected using the colorimetric method by adding 200 µL of resazurin staining (0.1 g.100 mL⁻¹) aqueous solution in each well at the end of the incubation period. MIC was defined as the lowest flavonoid concentration able to inhibit the bacterial or fungi growth as indicated by resazurin staining (dead cells were not able to change the staining color by visual observation – blue to red) [11]. The results were also compared with the standard drug, Nistatin (100 UI/mL). All experiments were carried out at least twice with consistent results.

2.4 Determination of the minimum fungicide concentration (MFC)

A 20 µL 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) [12, 13, 14].

70

3. RESULTS

The results for antifungal activity for determination of the minimum inhibitory concentration (MIC) of the flavonoid TMF are shown in Table 1. The activity, in both

74 cases, was measured in terms of presence of microorganism growth, and demonstrate that
 75 MIC₅₀ (minimum inhibitory concentration able to inhibit 50% of the fungal strains) for
 76 TMF is 32 µg/mL.

77 Table 1- Antifungal activity for determination of the MIC of the TMF.

Fungal strains/ Substance	<i>Candida</i> <i>krusei</i> LM 9700	<i>Candida</i> <i>krusei</i> LM 656	<i>Candida</i> <i>krusei</i> LM 13	<i>Candida</i> <i>krusei</i> LM 08
TMF (1024 µg/mL)	+	+	-	+
TMF (512 µg/mL)	+	+	-	+
TMF (256 µg/mL)	+	+	-	+
TMF (128 µg/mL)	+	+	-	+
TMF (64 µg/mL)	-	+	-	+
TMF (32 µg/mL)	-	+	-	+
TMF (16 µg/mL)	-	-	-	+
Negative control	-	-	-	-
Positive control	+	+	+	+

78 (-) No inhibition (+) inhibition

79 The results for antifungal activity for determination of the minimum fungicide
 80 concentration (MFC) of the flavonoid TMF are shown in Table 2.

81 Table 2- Antifungal activity for determination of the MFC of the TMF.

Fungal strains/ Substance	<i>Candida</i> <i>krusei</i> LM 9700	<i>Candida</i> <i>krusei</i> LM 656	<i>Candida</i> <i>krusei</i> LM 13	<i>Candida</i> <i>krusei</i> LM 08
TMF (1024 µg/mL)	-	-	-	+
TMF (512 µg/mL)	-	-	-	-
TMF (256 µg/mL)	-	-	-	-
TMF (128 µg/mL)	-	-	-	-
TMF (64 µg/mL)	-	-	-	-
TMF (32 µg/mL)	-	-	-	-

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

(-) No inhibition (+) inhibition

4. DISCUSSION

Candidiasis are fungal infections predominantly endogenous and character opportunistic infections caused by yeasts belonging to *Candida*, resulting in only a number they are reduced, associated with proven human disease process, highlighting: *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. glabrata*, *C. krusei*, *C. kefyr*, *C. lusitaniae*, *C. rugosa*, *C. guilliermondii* and *C. dubliniensis* [15, 16].

Increased resistance to fungal classical drugs, their unwanted actions and the high cost treatment, justify the investigation of new strategies. The use of plants in the treatment of infections is being shown along the human history [17, 18, 19].

Increasingly, flavonoids are becoming the subject of medical research. They have been reported to possess many useful properties, including anti-inflammatory activity, oestrogenic activity, enzyme inhibition, antimicrobial activity [3, 20].

According with literature 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 [21]. The results showed that TMF present de strong effect against *C. krusei* strains with MIC₅₀ for TMF is 32 µg/mL. These results are in agreement with the data obtained by Oliveira-Filho et al (2012) [22] in their study using the flavonoid TMF against various strains of *Candida*.

Analyzing the results of the MFC can be seen that the flavonoid does not have fungicide activity against *C. krusei* species, because when the ratios of MFC/MIC were

104 1 or 2, indicating that the effect of the compound was fungicide in nature (and not
105 fungistatic) [23].

106

107 5. CONCLUSION

108 The results of this study reveal that the flavonoid TMF showed the strong effect
109 against *Candida krusei* strains, but this effect is not fungicidal and fungistatic, thus, its
110 use as a prospective source of antimicrobial agents could be eventually of interest for
111 pharmacological applications.

112 CONSENT

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

114 ETHICAL APPROVAL

115 Not applicable.

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