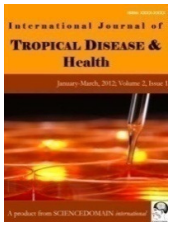

International Journal of TROPICAL DISEASE & Health
5(2): 136-140, 2015, Article no.IJTDH.2015.014
ISSN: 2278–1005

SCIENCEDOMAIN *international*
www.sciencedomain.org

Antifungal Effect of Flavonoid 5, 7, 4'-Trimethoxyflavone against *Candida krusei* Strains

Abrahão Alves de Oliveira Filho[1*], Heloísa Mara Batista Fernandes[1], Janiere Pereira de Sousa[1], Gabriela Lemos de Azevedo Maia[2], José Maria Barbosa Filho[1], Thais Josy Castro Freire de Assis[3], Hilzeth Luna Freire Pêssoa[1] and Edeltrudes Oliveira Lima[1]

[1]Programin Natural Products and Synthetic Bioactive, Federal University of Paraiba, Paraiba, Brazil.
[2]Department of Pharmacy, Federal University of São Francisco Valley, Pernambuco, Brazil.
[3]Academic Health Unit, Federal University of Campina Grande, Paraiba, Brazil.

Authors' contributions

This word was carried out in collaboration between all authors. Authors AAOF, HMBF, JPS, TJCFA and EDOL designed in this study, performed the statistical analysis, wrote the protocol and managed the analyses of the study. Authors GLAM and JMBF isolated the flavonoid. Authors AAOF and HLFP managed the literature searches and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJTDH/2015/14060
Editor(s):
(1) Mochammad Hatta, Department of Molecular Biology & Immunology, Hasanuddin University, Indonesia.
Reviewers:
(1) Natthanej Luplertlop, Department of Microbiology and Immunology, Mahidol university, Bangkok, Thailand.
(2) Anonymous, Ege University, Turkey.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=848&id=19&aid=6948

Original Research Article

Received 16th September 2014
Accepted 30th October 2014
Published 15th November 2014

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.

**Corresponding author: Email: abrahao.farm@gmail.com;*

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

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

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

2. MATERIALS AND METHODS

2.1 Isolation of the Flavonoid

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

2.2 Fungal Strains

For antifungal activity assays, 4 strains of fungi were selected (*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. Fungi was 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 106 count forming unit per mL (cfu.mL^{-1}) adjusted according to the turbidity of0.5 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 resauzurin 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 resauzurin 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 20µL 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 (cfu) [12,13,14].

3. RESULTS

The results for antifungal activity for determination of the minimum inhibitory concentration (MIC) of the flavonoid TMF are

show in Table 1. The activity, in both cases, was measured in terms of presence of microorganism growth, and demonstrate that MIC_{50} (minimum inhibitory concentration able to inhibit 50% of the fungal strains) for TMFis 32 µg/mL.
The results for antifungal activity for determination of the minimum fungicide concentration (MFC) of the flavonoid TMF are show in Table 2.

4. DISCUSSION

Candidiasis are fungal infections predominantly endogenous and characteristic 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 between0.05 – 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 MIC_{50} for TMF is 32 µg/mL. These results are in agreement with the data obtained by Oliveira-Filho et al. [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 1 or 2, indicating that the effect of the compound was fungicide in nature (and not fungistatic) [23].

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

Fungal strains/ Substance	***Candida krusei* LM 9700**	***Candida krusei* LM 656**	***Candida krusei* LM 13**	***Candida krusei* 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

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

Fungalstrains/ Substance	***Candida krusei* LM 9700**	***Candida krusei* LM 656**	***Candida krusei* LM 13**	***Candida krusei* 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

5. CONCLUSION

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

CONSENT

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

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. JrMiddleton E, C Kandaswami, Theoharides TC. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol. Rev. 2000;52(4):673–751.
2. Romano B, Pagano E, Montanaro V, Fortunato AL, Milic N, Borrelli F. Novel Insights into the Pharmacology of Flavonoids. Phytother. Res. 2013;27:1588–1596.
3. Havsteen B. Flavonoids, a class of natural products of high pharmacological potency. Biochem. Pharmacol. 1983;32:1141–8.
4. Örner MW, Jha HC. Antifungal activity of flavonoids and their mixtures against different fungi occurring on grain. Pestic. Sci. 1993;38(4):347-351.
5. Mukne AP, Viswanathan V, Phadatare AG. Structure pre- requisites for isoflavonesas effective antibacterial agents. Pharma-cogn. Rev. 2011;5(9):13–18.
6. Harborne JB, Williams CA. Advances in flavonoid research since 1992. Phytochemistry. 2000;55:481–504.
7. Wachter GA, Hoffmann JJ, Furbacher T, Blake ME, Timmermann BN. Antibacterial and antifungal flavanones from *Eysenhardtia texana*. Phytochemistry. 1999;52:1469–71.
8. Maia GLA, Falcão-Silva VS, Aquino PGV, Araújo-Júnior JX, Tavares JF, Silva MS, Rodrigues LC, Siqueira-Júnior JP, Barbosa-Filho JM. Flavonoids from *Praxelis clematidea* R.M. King and Robinson Modulate Bacterial Drug Resistance. Molecules. 2011;16:4828-4835.
9. Viljoen A, Vuuren SV, Ernst E, Lepser M, Demirci B, Baser H, Van Wyk BE. *Osmitopsis astericoides* (*Asteraceae*) – the antimicrobial activity and essential oil composition of a Cape-Dutch remedy. J. Ethnopharmacol. 2003;88:137-143.
10. Sahin F, Güllüce M, Daferera D, Sökmen A, Sökmen M, Polissiou M, Agar G, Özer H. Biological activities of the essential oils and methanol extract of *Origanum vulgare* ssp. vulgare in the Eastern Anatolia region of Turkey. Food Control. 2004;15:549-557.
11. Burt SA, Reinders RD. Antibacterial activity of selected plant essential oils against *Escherichia coli* O157:H7. Lett. Appl. Microbiol. 2003;36:162-167.
12. Klepser ME, Ernst EJ, Ernst ME, Messer SA, Pfaller MA. Evaluation of endpoints for antifungal susceptibility determinations with LY303366. Antimicrob. Agents Chemother. 1998;42:1387–1391.
13. Ernst EJ, Klepser ME, Ernst ME, Messer SA, Pfaller MA. In vitro pharmacodynamic properties of MK-0991 determined by time- kill methods. Mycology. 1999;33:75- 80.
14. Correa-Royero J, Tangarife V, Durán C, Stashenko E, Mesa-Arango A. *In vitro* antifungal activity and cytotoxic effect of essential oils and extracts of medicinal and aromatic plants against *Candida krusei* and *Aspergillus fumigatus.* Braz. J. Pharmacogn. 2010;20:734-741.
15. Bruder-Nascimento A, Camargo CH, Sugizaki MF, Sadatsune T, Montelli AC, Mondelli AL, Bagagli E. Species distribution and susceptibility profile of Candida species in a Brazilian public tertiary hospital. BMC Res. Notes. 2010;3:1-5.
16. Colombo AL, Guimarães T. Epidemiology of hematogenous infections *Candida* spp. Rev. Soc. Bras. Med. Trop. 2003;36:599-607.
17. Rates SMK. Plants as source of drugs. Toxicon. 2001;39:603-613.
18. Bansod S, Rai M. Antifungal activity of essential oils from indian medicinal plants against human pathogenic *Aspergillus*

fumigatus and *A. niger*. World J. Med. Sci. 2003;3:81-88.

19. Saad A, Fadli M, Bouaziz M, Benharref A, Mezrioui NE, Hassani L. Anticandidal activity of these sentialoils of *Thymus maroccanus and Thymus broussonetii* and their synergism with amphotericin Band fluconazol. Phytomedicine. 2010;17:1057-1060.
20. Harborne JB, Baxter H. The handbook of natural flavonoids, Vols 1 and 2. Chichester, UK: John Wiley and Sons; 1999.
21. Sartoratto A, Machado ALM, Delarmelina C, Figueira GM, Duarte MCT, Rehder VLG. Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil. Braz. J. Microbiol. 2004;35:275-280.
22. Filho AAO, Fernandes HMB, Sousa JP, Maia GLA, Tavares JF, Barbosa-Filho JM, Lima EO, Oliveira TL. Antifungal Potential of the Flavonoid Isolated from *Praxelis clematidea* R.M. King & Robinson. Lat. Am. J. Pharm. 2012;31(7):1064-6.
23. Hafidh RR, Abdulamir AS, Vern LS, Bakar FA, Abas F, Jahanshiri F, Sekawi Z. Inhibition of growth of highly resistant bacterial and fungal pathogens by a natural product. Open Microbiol. J. 2011;5:96–106.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=848&id=19&aid=6948