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Antifungal activity of the extract of *Curcuma zedoaria* against yeasts of the genus *Candida* isolated from the oral cavity of patients infected with the human immunodeficiency virus

Cristiane S. Shinobu-Mesquita, Thâmara A. Bertoni, Eliana Guilhermetti, Terezinha I. E. Svidzinski*

Laboratório de Micologia Médica, Departamento de Análises Clínicas e Biomedicina, Universidade Estadual de Maringá, Brazil.

Abstract: Oropharyngeal candidiasis is the most common fungal infection among patients infected with the human immunodeficiency virus (HIV), and is treated empirically with topical or systemic antifungals. The objective of the present study was to investigate the possible antifungal action of the hydroalcoholic extract of *Curcuma zedoaria* (Christm.) Roscoe, Zingiberaceae, on yeasts in this population. Samples were collected from HIV-positive patients who attended the Laboratory for Teaching and Research in Clinical Analysis at the Universidade Estadual de Maringá for routine exams. The isolated yeasts were identified at the genus and species levels through classical methodology. Next, tests of microdilution in broth were carried out to determine the profile of susceptibility of these yeasts towards the hydroalcoholic extract of *C. zedoaria*, following methodology standardised by the CLSI (2002). A total of 53 yeasts were identified, 49 of them *C. albicans,* two *C. tropicalis* and two *C. glabrata*. These yeasts were inhibited by low concentrations of the extract of *C. zedoaria* (between 1.95 and 15.63 µg/mL). In addition, 7.82 µg/mL inhibited 90% of the yeasts. Our results indicate a potent antifungal action for *C. zedoaria* and suggest more detailed studies with a view towards the practical application of this phytomedicine in topical pharmaceutical forms for the treatment of oral candidosis or candidiasis.

Introduction

Popularly known as zedoaria, *Curcuma zedoaria* (Christm.) Roscoe is a plant of the family Zingiberaceae, whose origin is Indian (Nadkarni, 1999), although today it is cultivated in Brazil. It is widely grown as a kind of pepper in countries of South and Southwest Asia, and in these countries, as well as in India, the rhizomes of the plant are used popularly in the treatment of stomatitis, as a stimulant, anti-flatulent, diuretic, anti-diarrheic, antiemetic, antipyretic, purgative, and also to clean and heal ulcers, wounds and other kinds of skin disorders (Matsuda et al., 2001).

Significant antifungal activities have been demonstrated in extracts of members of the Zingiberaceae, particularly *Alpinia galanga, C. zedoaria, Zingiber purpureum* and *Z. officinale* (Ficker et al., 2003). Investigation of the antifungal action of the volatile oil of *C. zedoaria* revealed that a concentration of 2000 ppm is sufficient to cause complete inhibition of the mycelium of the fungus *Colletotricum falcatum*. This concentration also brought about partial mortality of other fungi tested, but was inefficient against *Aspergillus niger* (Singh et al., 2002).

Oropharyngeal candidiasis is the most common fungal infection among patients infected with the human immunodeficiency virus (HIV), and is often detected in recurrent episodes, mainly when the CD4 lymphocyte count falls below 200 cells/mm^3 (Campos et al., 2002, Fidel, 2002). The drug treatment employed in oropharyngeal candidiasis, in patients infected with HIV, is empirical. This, together with the fact that antifungals are most often used for extended periods, contributes to the appearance of resistant species of yeasts (Powderly et al., 1993).

Generally, treatment to control the recurrence of these episodes in small lesions is exclusively topical, employing nystatin in an oral suspension or other options such as clotrimazole tablets which dissolve in the mouth, and miconazole gel (Schechter & Rachid, 2004). The use of phytomedicines containing zedoaria is aimed at, among other applications, the prevention and combat of problems of the oral mucosa including gingivitis, periodontitis and mouth ulcers (Antunes Jr, 2002). Aqueous solutions of 7.1%, obtained from the hydroalcoholic extract of *C. zedoaria*, are used as mouthwashes as a chemical adjuvant for the mechanical control of dental bacterial plaque and

gingivitis, as they present a significant anti-inflammatory and antimicrobial action (Sandrini et al., 1997).

The increase in resistance to antifungals and the slow delivery of new therapeutic options from the pharmaceutical industry have lead to various studies being carried out with the aim of examining the activity of natural products against fungi that cause infections, mainly in immunocompromised individuals (Rex et al., 1995, Bachmann et al., 2002). The objective of the present work was to determine the antifungal action of the extract of *C. zedoaria* against yeasts collected from the oral cavity of patients carrying the human immunodeficiency virus, as part of the search for possible new alternatives, based on popular culture, for the treatment of oral candidiasis.

Materials and Methods

Collection and identification of samples

Samples were collected from the mouthwash of patients who attended the Clinical Analysis Laboratory of the Universidade Estadual de Maringá for periodic examinations to monitor their disease (HIV/AIDS). The patients included in the study had a confirmed diagnosis of HIV infection or were already suffering from AIDS. These definitions followed the 1998 revision of the national definition of AIDS cases in people aged 13 and above for the purposes of epidemiological surveillance (Campos et al., 2002). The participating patients were aged over 18 years and were being monitored by the Specialised Treatment Service for HIV-infected patients of the 15th Regional Health Authority of Paraná and the Infectious Disease Outpatient Clinic at the Hospital Universitário Regional de Maringá. Those individuals undergoing immunosuppressant therapy, as well as those with oncological disease, were excluded from the study. The patients who took part in the study received an explanation of its aims and were invited to participate voluntarily after signing a term of free and informed consent approved by the Permanent Committee for Ethics in Human Research at the Universidade Estadual de Maringá.

The samples were obtained by rinsing the mouth, for 30 s, with 20 mL of a sterilised sodium chloride solution. The liquid resulting from rinsing was recovered and centrifuged for 30 s; the resulting pellet was resuspended in 1 mL of buffered saline and inoculated in CHROMágar® *Candida* selective differential medium (CHROMagar Company, Paris, France) before being incubated at 25 ºC for up to five days. The genus and species of individual colonies of each sample were identified by means of the classical manual system, which consists of: micromorphological characteristics in fubá agar with 1% Tween 80, capacity to produce a germinative tube and physiological profile (zymogram and auxanogram), performed according to the method described by Larone (1995) and modified by Kurtzman & Fell (1998). Following identification, these clinical isolates were stored in Sabouraud dextrose broth (SDB; Difco) with 10% glycerol under refrigeration at -20 °C until the experiment was performed.

Preparation of extract

The extract of *C. zedoaria* was prepared with approximately 95 g of dry rhizome, obtained commercially from Santosflora Comércio de Ervas Ltda. The extract was obtained by maceration in 96º GL alcohol. The proportions used were one part plant to three parts extraction liquid. The preparation was macerated, without stirring, for five days and then filtered. This liquid extract was concentrated in a rota-evaporator at 45 ºC and then lyophilised to obtain a hydroalcoholic extract with a concentration of 250 µg/mL. The aerial parts of *Curcuma zedoaria* (Christm.) has been deposited at the Herbarium of the Universidade Estadual de Maringá.

Tests of susceptibility to antifungals

The antifungal activity of the hydroalcoholic extract of *C. zedoaria* was compared to the *in vitro* action of nystatin. Nystatin was used in powdered form (Sigma Pharma, St Louis, MO, USA) prepared at ten concentrations varying from 0.125 to 64 µg/mL, according to Pádua et al. (2003).

The minimum inhibitory concentration (MIC) for nystatin was determined by the method of microdilution in broth, following the standards recommended by the Clinical and Laboratory Standards Institute (CLSI, 2002), published in document M27-A2; the same methodology was followed for the extract of *C. zedoaria*, although with some modifications to adapt the method to the study of natural products.

The tests of susceptibility were performed after reactivating the yeasts in SDB for subsequent culture in Sabouraud dextrose agar for 24 or 48 h at 30 ºC. This growth was used to prepare an inoculum in sterile saline, the cell density being adjusted with the aid of a spectrophotometer at 530 nm with 90±2% of transmittance. This turbidity resulted in 1.0 to 5.0 x10^6 CFU/mL which was used to prepare further dilutions in RPMI to obtain the desired final inoculum, containing 0.5 to 2.5 x10^3 CFU/mL. The test was carried out in sterilised plastic 96-well microplates, and a reference yeast *C. albicans* (ATCC 90028) was added to all the tests to ensure the reproducibility of the results. The microplates were incubated in a humid chamber at 35 ºC for 48 h. The test was carried out in triplicate on three different days, and the results presented refer to the mean of the values obtained. Following incubation, reading was performed

by visual comparison through reflection in a mirror.

MIC was the concentration of extract of *C. zedoaria* or of nystatin that inhibited 100% of the growth of each yeast. The MIC50 and MIC90 were defined as the MIC that inhibited 50% and 90% of the isolates, respectively.

Results and Discussion

Of the 100 patients infected with HIV who participated in this study, 53 presented positive cultures for *Candida* spp. Their ages ranged from 19 to 69 years, with a mean of 39 years. Of the 53, twelve (21%) reporting having had at least one previous episode of oral candidiasis. Thirteen patients (22%) had already used antifungals. With regard to the agents, in 49 (92.45%) of the 53 positive cultures, *Candida albicans* was identified, and there were two isolates each of *C. tropicalis* and *C. glabrata. C. albicans* was the most commonly isolated species, as observed in other studies where the frequency was above 90% (Laet Sant'Ana et al., 2002, Queiroz-Telles et al., 2002).

C. zedoaria exhibited an important antifungal activity against yeasts isolated from the oral cavity of HIV-positive patients. Concentrations between 1.95 and 15.63 µg/mL impeded the growth of all the yeasts examined (Figure 1). Such concentrations can be regarded as low, since according to Duarte et al. (2004) it has been established that when dealing with plant extracts, without any kind of purification, minimum inhibitory concentrations below 2.0 mg/mL can be considered to reflect potential antifungal properties, confirming the antifungal action of the extract of *C. zedoaria.*

The results of the present study are not very favourable to nystatin, one of the most widely employed antifungals for the treatment of oral candidoses, since 47 isolates (88.7%) were sensitive depending on the dose (SDD), besides one isolate that was resistant (Figure 2). The frequency of SDD obtained for nystatin was similar to that found by Ferrazza et al. (2005) and Dota et al. (2008) and for vaginal yeasts (79.3% and 52.8% respectively), supporting the requirement for higher concentrations of this antifungal already described in the literature. None of the isolates presented an MIC of 32 µg/mL and 64 µg/mL of nystatin.

It is important to highlight the fact that the SDD suggests the need for escalation of doses, which should be higher in order to exert fungicidal activity. Unfortunately, however, this is not taken into account in daily therapy, which is always empirical.

On the other hand, the results obtained with the extract of *C. zedoaria* are a cause for optimism, since there was an antifungal action with low concentrations of the extract (Figure 1). Gupta et al. (1976) isolated three antimicrobial components, extracted from dried rhizomes of *C. zedoaria* with ethanol, all of which were active against the fungi *Trichophyton rubrum, Aspergillus niger* and *Saccharomyces cerevisae*. According to Table 1, both nystatin and the extract of *C. zedoaria* presented the same concentrations for MIC50 and MIC90, which suggests a drug with a relatively uniform spectrum, with no specificity in terms of species and clinical isolates, something that is confirmed by the variation in the concentration of the extract, which was lower than the variation found with nystatin. This is very relevant, since the extract used was crude; in other words, the active principals that endow it with an antifungal action were not isolated. It is possible that, after a purification step, the concentration required for an antifungal action would be lower than that obtained in the present work. The concentration of the *C. zedoaria* extract that inhibited the growth of the isolates was lower than that of nystatin.

There should be encouragement for the assessment of phytomedicine products, represented in this study by the extract of *C. zedoaria,* which can be considered a good candidate for a medicine for

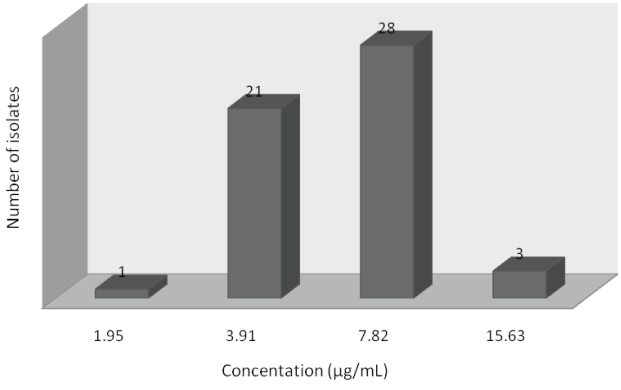

Figure 1. Distribution of mean values for the minimum inhibitory concentrations (µg/mL) of the alcoholic extract of *C. zedoaria* to obtain 100% inhibition in 53 yeasts isolated from the oral cavity of HIV-positive individuals.

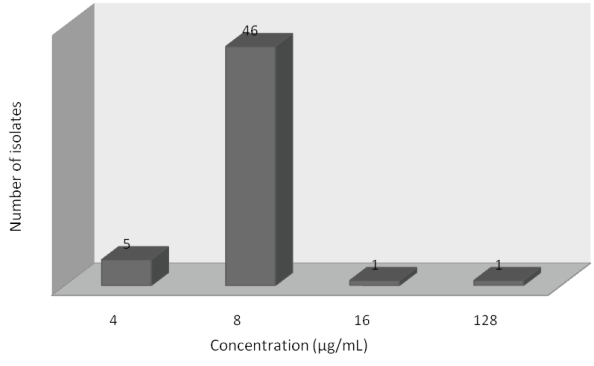

Figure 2. Distribution of mean values for the minimum inhibitory concentrations (µg/mL) of nystatin to obtain 100% inhibition in 53 yeasts isolated from the oral cavity HIV-positive individuals.

Table 1. Distribution of 53 yeasts isolated from the oral cavity of HIV-positive patients, according to the variation of MIC (μg/mL) for nystatin and the extract of *C. zedoaria* by species.

Species	N	Extract of *C. zedoaria* (μg/mL)			Nystatin (μg/mL)		
		CIM50	CIM90	CIM Range	CIM50	CIM90	CIM Range
C. albicans	49	7,82	7,82	1,95 – 15,63	8	8	4-128
C. glabrata	2	7,82	15,63	7,82 – 15,63	8	8	8
C. tropicalis	2	7,82	7,82	7,82	8	8	8

N: Number of isolates; MIC: Minimum inhibitory concentration; lowest concentration of each drug able to inhibit 100% of the growth of each yeast. MIC50 and MIC90: MIC of each drug able to inhibit 50% and 90% respectively of the number of isolates tested. The results presented are the mean obtained from three tests carried out on different days.

topical use in the form of mouthwashes, bearing in mind the antifungal performance demonstrated in vitro. Furthermore, the low toxicity (Lobo et al., 2009) and low cost are important points in favour of this development. As observed by Bugno et al. (2007), the antimicrobial efficacy of the *C. zedoaria* extract was similar to that of other commercial oral antiseptics, and its addition to this group of products may create an alternative to increase the antimicrobial efficacy of products for oral use.

Thus, further studies are necessary for the isolation of the active principle(s) and the purification of possible fractions or components that are responsible for the antifungal activity, followed by new tests of susceptibility, in an attempt to find a new drug to add to the arsenal of antifungals available to the population. Finally, the treatment of oral candidoses would probably be more effective if it was based on the laboratory confirmation of the fungal aetiology, determination of the species involved and the profile of susceptibility to antifungals.

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References

Antunes Jr D 2002. *Farmácia de Manipulação, Noções básicas*. 1° Ed. São Paulo: Tecnopress, p.117.

Bachmann SP, Patterson TF, Ribot JL 2002. *In vitro* activity of caspofungin (MK-0991) against *Candida albicans* clinical isolates displaying different mechanisms of azole resistance. *J Clin Microb 40*: 2228-2230.

Bugno A, Nicoletti MA, Almodóvar AAB, Pereira TC, Auricchio MT 2007. Antimicrobial efficacy of *Curcuma zedoaria* extract as assessed by linear regression compared with commercial mouthrinses. *Braz J Microbiol 38*: 440-445.

Campos J, Del Romero J, Castilha J, Garcia S, Rodriguez C, Bascones A 2002. Oral candidiasis as a clinical marker related to viral load, CD4 lymphocyte count and CD4 lynphocyte percentage in HIV-infected patients. *J Oral Pathol Med 31*: 5-10.

CLSI 2002. M27-A2 NCCLS. *Método de Referência para Testes de Diluição em Caldo para a Determinação da Sensibilidade a Terapia Antifúngica das Leveduras; Norma Aprovada* - 2ª. Ed. NCCLS document M27-A2 [ISBN 1-56238-469-4]. NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 Estados Unidos, 2002.

Dota KFD, Shinobu CS, Patussi EV, Consolaro MEL, Svidzinski TIE 2008. Susceptibilidad de levaduras vaginales a los antifúngicos más utilizados em Maringá, Paraná, Brasil. *Acta Bioquím Cliín Latinoam 42*: 561-566.

Duarte MT, Figueira M, Sartoratto A, Rehder VL, Delarmina C 2004. Anti-*Candida* activity of Brazilian medicinal plants. *J Ethnopharmacol 97*: 305-311.

Ferrazza MHSH, Maluf MLF, Consolaro MEL, Shinobu CS, Svidzinski TIE, Batista MR 2005. Caracterização de leveduras isoladas da vagina e sua associação com candidíase vulvovaginal em duas cidades do sul do Brasil. *Rev Bras Ginecol Obstet 27*: 58-63.

Ficker CE, Smit, ML, Susiarti S, Leaman DJ, Irawati Ç, Arnason JT 2003. Inhibition of human pathogenic fungi by members of Zingiberaceae used by the Kenyah (*Indonesian Borneo*). *J of Ethnopharmacol 85*: 289-293.

Fidel PL Jr 2002. Immunity to *Candida. Oral Dis 8 Suppl 2*: 69-75.

Gupta SK, Baneriee AB, Achari B 1976. Isolation of Ethyil p-methoxycinnamate, the major antifungal principle of *Curcuma zedoaria. Lloydia 39*: 218-222.

Kurtzman CP, Fell JW 1998. *The Yeast. A taxonomic study.* Amsterdam: Elsevier.

Laet Sant'Ana P, Milan EP, Martinez R, Queiroz-TellesTelles FQ, Ferreira MS, Alcântara MTC, Carvalho MT, Colombo AL.et al 2002. Multicenter Brazilian study of oral *Candida* species isolated from Aids patients. *Mem I Oswaldo Cruz 97*: 253-257.

Larone DH 1995. *Medically important fungi. A guide to identification.* Washington: AMS Press.

Lobo R, Prabhu KS, Shirwaikar A, & Shirwaikar A 2009.

Curcuma zedoaria Rosc. (with turmeric): a review of its chemical, pharmacological and ethnomedicinal properties. *J Pharm Pharmacol 61*: 13-21.

Matsuda H, Morikawa T, Ninomiya K, Yoshikawa M 2001. Absolute stereostructure of carabrane-type sesquiterpene and vasorelaxant-active from Zedoariae Rizoma. *Tetrahedron 57*: 8443-8453.

Nadkarni KM 1999. Indian Materia Medica, 3rd edn. Mumbai, India: Popular Prakashan Private Limited.

Pádua RAF, Guilhermetti E, Svidzinski TIE 2003. *In vitro* activity of antifungal agents on yeasts isolated from vaginal secretion. *Acta Scientiarum 25*: 51-54.

Powderly WG, Robinson K, Keath EJ 1993. Molecular epidemiology of recurrent oral candidiasis in human immunodeficiency virus-positive patients: evidence for two patterns of recurrence. *J Infect Dis 168*: 463-466.

Queiroz-Telles F, Silva N, Carvalho MM, Alcântara AP, da Matta D, Barberino MG, Bartczak S, Colombo AL. 2002. Evaluation of efficacy and safety of itraconazole oral solution for the treatment of oropharyngeal candidiasis in AIDS pacients. *Braz J of Infec Dis 5*: 60-66.

Rex JH, Rinald MG, Pfaller MA 1995. Resistance of *Candida* species to fluconazole. *Antimicrob Agents Chemother 39*: 1-8.

Sandrini JC, Navarro DF, Rocha JCF, Ribeiro PG, & Kozlowski Jr VA 1997. Efeitos do extrato de *Curcuma zedoaria* sobre a placa dental e gengivite em humanos - Avaliação clínica. *Revista Periodontia 6*: 3-6.

Schechter M, Rachid M 2004. *Manual de HIV/Aids*. Rio de Janeiro. Revinter.

Singh G, Singh OP, Maurya S 2002. Chemical and biocidal investigations on essencial oils of some Indian Curcuma species. *Prog Cryst Growth Charact Mater 45*: 75-81.

***Correspondence**

Terezinha I. E. Svidzinski
Laboratório de Micologia Médica, Departamento de Análises Clínicas e Biomedicina, Universidade Estadual de Maringá, Av. Colombo, 5790, Bloco J90, Sala 11, 87020-900 Maringá-PR, Brazil
tiesvidzinski@uem.br
Tel. +55 44 3011 4809
Fax: +55 44 3011 4959