

Experimental Report

Candida parapsilosis culture extracts: *in vitro*, antagonistic action against *Candida albicans*, *Candida auris* and *Candida parapsilosis*

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Abstract: Fungal infections caused by *Candida* species has increased significantly in recent years. Additionally, resistance to conventional therapy is an aggravating factor causing high morbidity and mortality, especially in immunocompromised patients or those undergoing treatment with another antimicrobials. During the infection process, *Candida* species produces metabolites which can self-regulate population density, in addition to controlling several virulence factors and exerting action on other microorganisms. In view of this fact, the present study evaluated the *in vitro* antifungal activity of the pure culture extract of *Candida parapsilosis* against *Candida albicans*, *Candida auris* and *Candida parapsilosis*. Fungal culture extracts of *C. parapsilosis* were prepared in Sabouraud Dextrose Broth, extracted with ethyl acetate and dried in a rotary evaporator. Subsequently, tests were performed to determine the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC). The antifungal activity of the extracts against *Candida* species showed values of MIC ranging between 500 - 2000 µg/mL and a MFC range of 1414 µg/mL - 2000 µg/mL. Future investigations for the identification and composition of this fungal culture extract will provide insights about metabolites are present and involved in the antifungal activity shown here, contributing for the future to new therapeutic approaches in the control of infections caused by *Candida*.

Keywords: Fungal infections; *Candida* spp.; Antifungal activity.

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1. Introduction

Candida species are the most common causal agents of infections in hospitalized patients with high rates of morbidity and mortality. The main etiologic agent of these infections is the *Candida albicans* yeast, however, in recent years, there has been an increasing incidence of infections caused by non-*albicans* species such as *Candida parapsilosis* [1]. The yeast *Candida auris* has gained prominence, as a pathogen of great concern for public health services, responsible for invasive infections with a high mortality rate. In addition, its rapid dispersion in healthcare environments, and its profile of resistance to multiple classes of conventional antifungals [2,3], impact all health area. The multidrug resistance associated with virulence and prolonged survival on surfaces makes this microorganism gain considerable attention from the scientific community [4]. The treatment for infections

fungal agents consists of the use of topical or systemic antifungal agents or a combination of both, whose mechanism of action involves the main structure as cell wall or membrane constituents [5]. In recent years, the incidence of recurrent infections, with the emergence of strains resistant to the drugs available for treatment, has increased and become a challenge for the control of these infections [6,7]. Therefore, the search for new compounds with antifungal action has gained considerable scientific interest over the years, essential for new therapeutic practices in the control of these infections. The present study evaluated the antifungal effect of the fungal culture extract of *Candida parapsilosis* against *Candida albicans*, *Candida auris* and *Candida parapsilosis*.

2. Material and Methods

2.1 Microorganisms

Clinical isolates of *C. parapsilosis* (CP2 – CP6), *C. albicans* (CA2 – CA6), *C. auris* (CAU2), from collection culture of Laboratory of Microbiology of São José do Rio Preto Medical School (FAMERP) were used. The isolates were registered in the Brazilian National System of Genetic Resource Management and Associated Traditional Knowledge (SisGen), under the protocol number AF41CDD. As control, reference strains from the American Type Culture Collections of *C. parapsilosis* 22019 (CPATCC), *C. albicans* 90028 (CAATCC) and from the Centraalbureau voor Schimmelcultures of *C. auris* 10913 (CAUCBS) were used.

2.2 Preparation of Extracts

To obtain the *C. parapsilosis* (CP) culture extract, colonies were subcultured on Sabouraud agar (DIFCO®) incubated at 35°C for 24 hours. After growing, new inoculation was performed into 500 mL of Sabouraud Dextrose broth (DIFCO®), following Mac Farland scale turbidity adjustment 10, and incubation at 35°C for 72 hours.

Subsequently, filtration was performed on a millipore 0.2 µm membrane and liquid-liquid extraction, using ethyl acetate as counter-phase. The procedure was repeated three times to allow the total extraction of fungal metabolites from the culture media. The ethyl acetate phase was submitted to a rotary evaporator for drying. The resulting bulk compound was solubilized in sterile distilled water solution with 10% dimethyl sulfoxide (Synth®).

2.3 Susceptibility profile *Candida* spp.

The susceptibility profile of *Candida* spp. against drugs and extract was determined by microdilution technique. For *Candida* species, fluconazole was used according to the protocol described by document M27-Ed4 2017 of the Clinical Laboratory Standard Institute (CLSI) in sterile 0.85% saline [8]. Subsequently, dilutions were performed at 1:100 – 1:20 in RPMI – 1640 medium (Sigma-Aldrich® - Rosen Park Media Institute), obtaining a concentration of 5×10^2 – 2.5×10^3 CFU/mL. The fungal inoculum, 0.1 mL, was plated into 96-well polystyrene plates containing RPMI-1640 medium under different concentrations of fluconazole (128 – 0.25 µg/mL) and extract (2000 – 15.6 µg/mL).

Sterile control (containing 0.2 mL of RPMI) and growth control (containing only inoculum and RPMI) were prepared. The plates were incubated at 35°C for 24 hours. The minimum inhibitory concentration (MIC) of fluconazole was determined as the lowest concentration capable to inhibiting 70% of fungal growth (MIC70), and for the extract, the concentration capable to inhibiting 100% (MIC100). To determine the minimum fungicide concentration (MFC), an aliquot from each well was plated in Sabouraud Agar medium. The CFM was defined as the lowest concentration of the extract to inhibit visible growth in solid medium. All tests were performed in triplicate.

3. Results

MIC values for fluconazole of *Candida* species are described in table 1. *C. albicans* and *C. parapsilosis* strains showed values of inhibition between 0.25 and 2 µg/mL. For *C. auris* strains, the higher MIC value was observed for the clinical strain (> 128 µg/mL) when compared to the reference strain (8 µg/mL).

Table 1. Values of minimum inhibitory concentration (MIC) of fluconazole against *Candida* spp.

<i>C. albicans</i>	MIC (µg/mL)	<i>C. parapsilosis</i>	MIC (µg/mL)	<i>C. auris</i>	MIC (µg/mL)
CAATCC	1	CPATCC	0.5	CAUCBS	8
CA2	1	CP2	1	CAU2	> 128
CA3	1	CP3	1		
CA4	0.25	CP4	2		
CA5	0.25	CP5	1		
CA6	0.5	CP6	2		

CA: *C. albicans*; CP: *C. parapsilosis*; CAU: *C. auris*.

The antifungal activity of the extracts against *Candida* species showed MIC values between 500 – 2000 µg/mL (Table 2). *C. parapsilosis* strains showed greater sensitivity to the extract when compared to *C. albicans* strains. Regarding to CFM, for *C. albicans* and *C. parapsilosis* strains MIC value was 2000 µg/mL. The highest CFM value was observed for clinical *C. auris* (4000 µg/mL) and the lowest for reference *C. auris* (500 µg/mL) (Figure 1).

Table 2. Values of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of *C. parapsilosis* extracts against *Candida* spp.

CAATCC			CPATCC			CAU-500		
MIC (µg/mL)		MFC (µg/mL)	MIC (µg/mL)		MFC (µg/mL)	MIC (µg/mL)		MFC (µg/mL)
CAATCC	2000	2000	CPATCC	1000	2000	CAU-500	500	
CA2	2000	2000	CP2	1000	2000	CBS		
CA3	2000	2000	CP3	1000	2000	CAU2	2000	4000
CA4	2000	2000	CP4	1000	2000			
CA5	2000	2000	CP5	2000	2000			
CA6	2000	2000	CP6	2000	2000			

CA: *C. albicans*; CP: *C. parapsilosis*; CAU: *C. auris*.

Considering geometric mean of MIC values, the higher inhibition values were observed for *C. albicans* (2000 µg/mL), followed by *C. parapsilosis* (1260 µg/mL) and *C. auris* (1000 µg/mL). Regarding CFM, both *C. albicans* and *C. parapsilosis* had a higher geometric mean (2000 µg/mL), when compared to *C. auris* strains (1414 µg/mL) (Table 3).

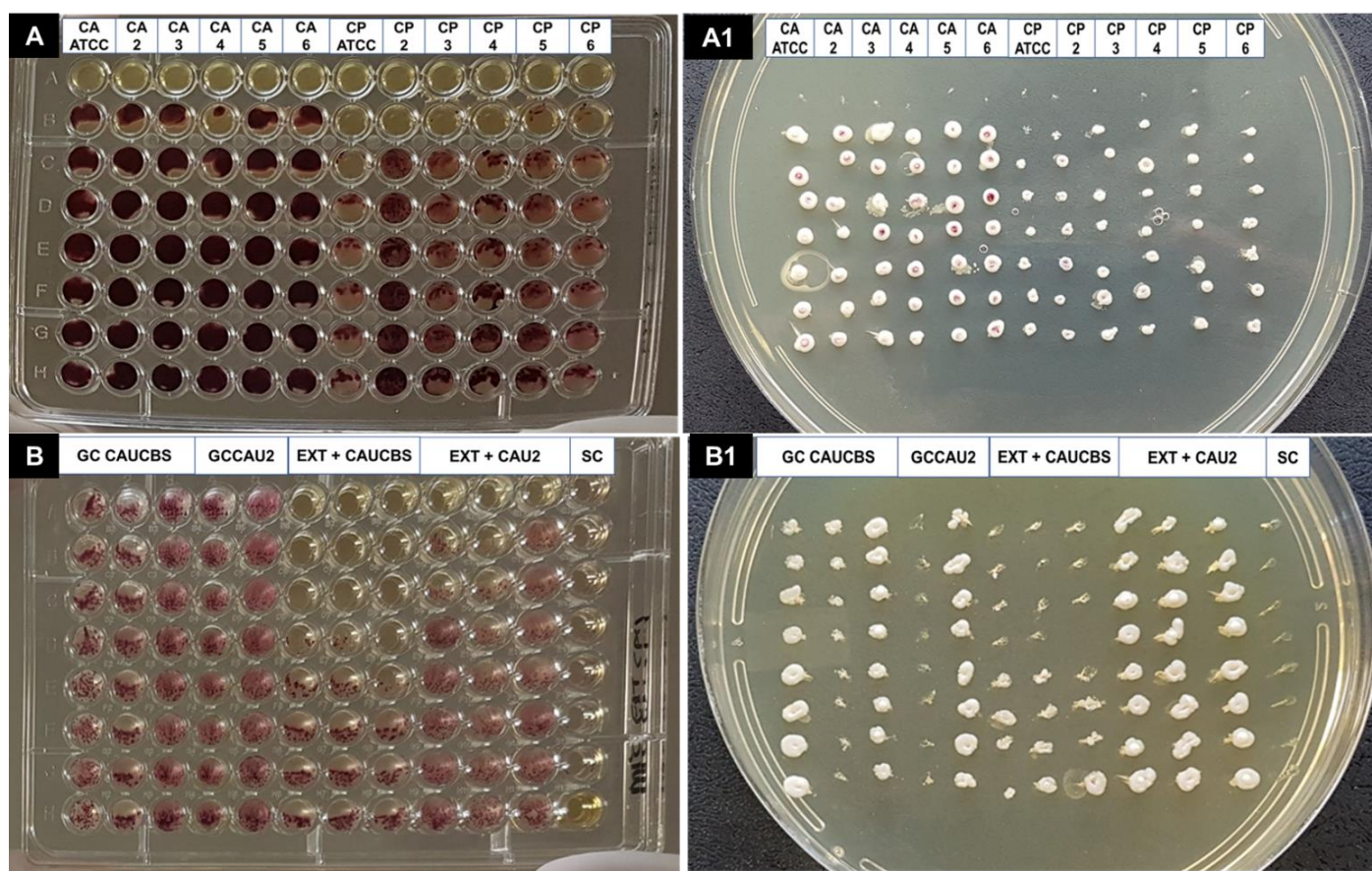


Figure 1. Photographic image of culture plates after microdilution of *C. parapsilosis* extracts against *Candida* species: A. MIC *Candida albicans* and *Candida parapsilosis*; B. MIC *Candida auris* and A1. MFC *Candida albicans* and *Candida parapsilosis*; B1 MFC *Candida auris*.

Table 3. Geometric means values of minimum concentration inhibitory (MIC) and minimum fungicidal concentration (MFC) of *Candida parapsilosis* culture extracts against *Candida* spp.

Geometric means		
	MIC (µg/mL)	MFC (µg/mL)
CA	2000	2000
CP	1260	2000
CAU	1000	1414

CA: *C. albicans*; CP: *C. parapsilosis*; CAU: *C. auris*.

3. Discussions

The incidence of fungal infections increases worldwide, especially in immunocompromised people or those undergoing treatment with antimicrobials. Yeasts of the *Candida* genus are the most common microorganisms involved in this process, with high rates of resistance to antifungal therapy [9]. The *C. albicans* species is a commensal pathogen of the oropharyngeal cavity, skin, and human gastrointestinal tract, but in homeostasis disorders they evolve to clinical manifestations, which comprise superficial to systemic infections [10]. In this context, a wide variety of virulence factors such as polymorphism, protease lipases and biofilm formation are produced for colonization and invasion of the host tissue [11]. Despite *C. albicans* being the main etiologic agent in this category, the growing

increases in species such as *C. parapsilosis* with strains resistant to antifungal drugs has changed this epidemiological profile [12]. The particular importance of the increased incidence of *C. parapsilosis* is due to its ability to form biofilms on intravascular devices and prosthetic materials, gastrointestinal colonization and transmission by colonized hands of health professionals, making it difficult to control [13].

Epidemiological data reported by Dizbay et al., showed a high incidence of candidemia by *C. parapsilosis* in non-neutropenic critical patients from a tertiary hospital in Turkey. At this reported data, *C. parapsilosis* corresponded to 77% of the cases against 23% of *C. albicans* with a high mortality rate (65%) [14]. Corroborating with these data, Mesini et al. presented a study about epidemiology changes of candidemia, reporting the increases cases of fluconazole-resistant by *C. parapsilosis* strains with mortality rates of 33% [15]. In contrast to the Mesini study, here the *C. albicans*, *C. parapsilosis* strains showed fluconazole-sensitive susceptibility profiles.

The MIC of extracts slightly lower for *C. parapsilosis* (1260 µg/mL) when compared to *C. albicans* (2000 µg/mL). In addition to the rise of *C. parapsilosis*, the emergence of *C. auris* and its resistance profile to available antifungal agents is a concern for global public health. Since 2009 infections caused by *C. auris* have been reported in more than 40 countries with strains isolated predominantly resistant to one or more classes of antifungal drugs [16]. Studies by Maphanga and colleagues revealed that up to 90% of strains isolated in South Africa were resistant to fluconazole [17].

In the present study the reference strain of *C. auris* presented a profile sensitive to fluconazole and, additionally to the extract (500 µg/mL) compared to the same clinical strain (2000 µg/mL). The differences in MICs values can be explained by individual biological characteristics of these strains, with previous exposure to environments that demand physiological changes as a defense mechanism for tolerance and survival of these microorganisms.

During the process of invasion and infection, *Candida* species generate metabolites in competitive environments. Such metabolites can self-regulate population density and controlling several virulence factors [18]. Oliver et al. showed in study the metabolic profiles of *Candida* spp. with 66 metabolites identified and quantified, associated with glycolysis or gluconeogenesis, tricarboxylic acid cycle, nucleic acid synthesis, amino acid and lipid metabolism [19]. The antifungal action of *C. parapsilosis* extract against strains of *C. parapsilosis* itself species when administered exogenously, are observed in other situations, such as farnesol, a molecule produced by *C. albicans* that regulates population density and several virulence factor [20]. However, in exogenous administration against strains of *C. albicans* present a high antimicrobial and antibiofilm potential [21, 22].

Although the composition of the *C. parapsilosis* extract obtained is unknown, its antifungal potential against *Candida* spp is evident. Future investigations to identify the composition of this extract will allow further clarification of which metabolites involved in the antifungal activity shown here, contributing to new therapeutic approaches in infections control of yeasts, mostly the genus *Candida*.

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Supplementary Materials: None.

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